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Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Programa de Doctorat de Biologia Vegetal

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Memòria presentada per l'Alba Anadon Rosell per optar al grau de
doctora per la Universitat de Barcelona

Barcelona, gener de 2017

La doctoranda:
Alba Anadon Rosell

Vist-i-plau del tutor i els directors de la tesi:

Josep M. Ninot Sugrañes	Salvador Nogués Mestres	Sara Palacio Blasco
Tutor	Director	Co-directora

Agraïments / Acknowledgements

Després de tots aquests anys de feina, són moltes les persones a qui estic sincerament agraïda. Des de la vostra ajuda al camp, al despatx, amb l'estadística, amb les correccions lingüístiques, amb petits o grans suggeriments, simplement amb discussions interessants, amb consells, amb els vostres ànims, les vostres preguntes, l'interès, les visites durant les meves estades, les trobades per la facultat, la vostra companyia... per tots els granets de sorra que heu aportat, pocs o molts, moltíssimes gràcies!

Pep, en primer lloc vull agraïr-te tot el teu esforç, les teves ganes, la teva paciència, la teva ajuda al camp, al despatx i on fos (ara mateix em ve al cap la teva imatge portant una cambra transparent de metacrilat per sobre el cap tot baixant de les parcel·les de Filià enmig de la boira i la pluja), la teva il·lusió, la teva confiança, la teva bondat i tantes altres coses més. Gràcies per ser un director i tutor amb qui sempre he pogut comptar, sempre amb un somriure. I per haver confiat sempre en mi i en el meu criteri, fins i tot quan tot just començava en el món de la recerca... Crec que hem fet un bon equip, no? Moltes gràcies!

Sara, muchas gracias por tu ayuda y tus consejos. Gracias por ser siempre tan rápida en responder mis (infinitos) mails, por trabajar siempre tan minuciosamente, por confiar en mí y por enseñarme tantas cosas.

Salvador, gràcies per haver acceptat dirigir aquesta tesi i per haver-me introduït en el món dels isòtops.

Empar, tu no has dirigit aquesta tesi, però hem compartit molts moments bonics a la Val d'Aran, a Enseu (fins i tot el dia en què no podíem entrar a casa!) i a Filià. Han sigut molts mostrejos! Mica inclosa! Gràcies per acollir-me i per ajudar-me sempre que has pogut, fos amb els inventaris al camp, amb la paperassa de la facultat o el que calgués.

Moltes gràcies als companys de la Unitat (ara Secció) i del COFFEA pels riures, els mostrejos compartits, les reunions, les sortides botàniques, les converses al passadís i els partits de bàsquet! Aaron, Estela, José M., Arnau, Albert, Roser, Paola, Agnès, Eulàlia, Marian, Alex, David, Jaume, Efrem, Noelia. Espero no deixar-me ningú! Oriol, tu has estat un exemple a seguir, m'has obert camí en el món de les OTC i dels projectes a Grenlàndia (ara mateix una mica frustrats)! A més, ets tot un expert en l'excavació de rases! Moltes gràcies per la teva ajuda, els teus consells i el teu exemple!

No em puc deixar la penya dels dinars! Ha sigut un plaer compartir totes aquestes estones a la gespa, prenent el solet tot dinant i xerrant de política, sèries de televisió, articles flipants,... i les nostres muntanyades! Berta, David, Eudald, Marc, Demi, Mays, Nora, moltes gràcies! Gràcies, a més, per la vostra ajuda quan ha calgut. Eudald sort dels teus testos de permutes! A més, la teva motivació sempre ha sigut una font inspiradora, tant a nivell científic com assambleari! I Marc, no sé on seria ara mateix el pluviòmetre sense les teves habilitats escaladores!

Moltes gràcies als companys i companyes de fisiologia: Claudia, Elena, Gladys, Camilo, Xavi, Eduardo i Rut. Merci per la vostra ajuda i per haver-me ensenyat a triturar, encapsular i a fer servir el licor! També a la Sílvia Poblador, el Biel Obrador i l'Emilia Gutiérrez d'Ecologia, que no heu dubtat en donar-me un cop de mà amb aparellatges i tècniques diverses. A la Pili Rubio, la Pilar Teixidor i el Rubén dels CCiT, que sempre m'heu donat un cop de mà i m'heu ensenyat el que calia per poder treballar amb l'espectrometria de masses de relació isotòpica. Y a Elena y Victoria del IPE, por vuestro trabajo y vuestra ayuda.

I also want to thank all the people I met in Davos, with whom I shared so many special moments in such an amazing place! Ellinor, it was so great to count on you, on your help and your patience when you had to translate German for me! It was really nice going hiking with you and sharing the room! Roland and Albena, we had an amzaing time in the hut, right?! Remember hiking to the plots every morning at high speed? Thanks to Stefan for showing me the "Murmeltiere" colony in Dischamatal and showing me how to improve my photography skills! Jonas, Thomas, Lukas, Magali, Julia, Jojo, Christina, and so many others: thank you! Christian and Sonja, thanks for being always so kind and for teaching me so many things! Melissa, it was amazing meeting you. I learnt so many things from you! And you always helped me so much! I am really glad we could kept on collaborating after the first paper!

And then, after Davos, another great experience happened (two times actually!) at WSL in Birmensdorf! Lena, Diego, Lucía, Quim, Simon, Stefan, Florian, Jodi, Maria, Giulia, Anne, Ulf, Dani, Felix and so many others, thank you! We had such a great time! Georg, thank you for introducing me to ROXAS and for introducing me to the world of wood anatomy. Patrick, Paolo, Loïc, thanks a lot for your help both times I came to WSL. Loïc it was very nice to stay at your place in Arni! Uri i Rosa, què bé haver pogut comptar amb vosaltres durant els mesos que he passat a Suïssa, i haver pogut compartir tants moments amb vosaltres i l'Alba i el Pol. Feia segles que no esquiava sobre gel! Laura Armengot, quina il·lusió coincidir a Suïssa amb tu també, les passejades, les visites a Frick i a Basilea, les cervesetes...

My stay in Innsbruck was also amazing thanks to all the people I met there. Roland and Johnny, you were such a support for me, and you taught me so many things! Thank you, really! Johnny you made me an expert on NEE measurements! Alex, thanks for your help during my experiment, for the nice discussions, for your positive attitude! Sarah, it was great to share fieldwork with you (especially that car "incident"... at least we had lots of fun!). Marine, Angela and Sefan, thanks a lot for the nice discussions, drinks, field trips and all the moments we shared! Stefan Mayr, I am very glad we managed to collaborate and it was very nice of you to teach me how to use the Scholander bomb and to make all those nice drawings so I could understand what goes on inside a plant. Michael, thanks a lot for accepting me in your team, for your time and for letting me carry out my own experiment, which was a challenge. Virginia, fue genial poder contar contigo, ir al festival de cine, salir por allí, ir de excursión... una suerte!

No em voldria deixar per res les tres estudiants que han fet el TFG amb nosaltres. Clara, Noelia, Irene, moltes gràcies pel vostre entusiasme, les vostres ganes i la vostra feina! Irene, sense tu no sé què hauria fet a Filià! Ha sigut un plaer poder comptar amb la teva ajuda i poder confiar en tu! I compartir els banys a l'estany de Montcortés després dels llargs dies de mostreig! I al super equip del marcatge de Filià: Nil, Marc, Isa, Zoë, moltes gràcies!

Vic, gràcies pel teu suport incondicional quan semblava que no hi havia manera d'obtenir una beca per fer el doctorat. I gràcies per tots els moments que hem compartit. Al final resulta que tu també t'has embrancat en aquest món de la recerca! A les amigues de la carrera, que també heu acabat totes liades en un doctorat! Hem pogut compartir les queixes del sistema, les aventures diàries, els problemes burocràtics... I hem pogut visitar-nos i trobar-nos allà on fos! Va ser genial la vostra visita a Innsbruck! Isa, Laura Baiges, Laura Vidal, Alba, gràcies! Luz i Julieth, qué contenta estoy de haber compartido el máster con vosotras! Fue un placer y el inicio de una gran amistad!

Julieth, ánimo que a ti también te falta muy poco! Gràcies Paula, Eli, Ona i Uri per preguntar sempre com em va, donar-me ànims en aquesta etapa final i venir a visitar-me a Anglaterra i a Suïssa! Som la resistència Barcelonina! Guerau, moltes gràcies per fer unes aquarel·les tan xules dels Vacciniums i deixar-me-les posar a la portada!

Clara, llàstima que al final no hagis pogut venir a veure els "tingladus" de Filià! Però moltes gràcies pel teu suport, per venir-me a veure allà on he anat i per fer de germana gran i preocupar-te sempre per mi! I a l'àvia, a qui li hagués fet molta il·lusió poder veure aquesta tesi.

Per acabar, Elias, mil gràcies per tot. Pels moments que compartim cada dia, pels projectes, pels viatges, per les incerteses, pels riures, pels mostrejos a 4 graus en ple agost immersos en la boira tirolesa, pels 500 viatges carregats amb bidons d'aigua pendent amunt-pendent avall a Filià per regar les parcel·les, per venir-me a veure allà on sigui, per aguantar-me... Gràcies!

Però sobretot, sobretot, gràcies als meus pares. Per poder comptar sempre amb vosaltres, pel vostre exemple, per ajudar-me sempre, pels papers que m'envies cada setmana papa, per venir al camp a ajudar-me, per preguntar-me sempre com em va, per interessar-vos pel que faig, per ajudar-me amb la tesi, per fer tràmits per mi quan era fora i semblava que (finalment sí!) em donaven la beca, per inculcar-me la passió per la natura (i per la ciència! Recordo quan t'ajudava a fer uns mostrejos d'aigua en unes basses de camí a Montcortés...) i l'amor pel Pallars i el Pirineu.

Moltes gràcies!

Alba

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Resum

Els ecosistemes d'elevades altituds i latituds són especialment vulnerables al canvi global. Les espècies subarbustives poden presentar recobriments elevats en moltes zones de muntanya, i l'augment de la temperatura i els canvis en l'ús del sòl n'afavoreixen l'expansió. Les espècies subarbustives coexistents podrien respondre de manera diferent al canvi climàtic, cosa que podria tenir grans efectes en les seves interaccions, així com en el seu creixement i el seu funcionament i, en última instància, comportar canvis dràstics en les comunitats vegetals. En aquesta Tesi Doctoral s'han dut a terme experiments d'escalfament, d'augment de la concentració de CO_2 de l'aire i de sequera a l'ecotò entre els estatges subalpí i alpí en diferents zones del Pirineu i dels Alps. L'objectiu era comprendre com aquests factors afecten les interaccions entre espècies subarbustives, el seu creixement, l'anatomia del xilema i la distribució de carboni (C). Els resultats d'aquesta Tesi mostren respostes específiques als tractaments. A més, aquestes respostes diferien en magnitud i direcció en funció del tractament aplicat. *Vaccinium myrtillus* va respondre positivament a l'escalfament, mentre que les espècies coexistents *Vaccinium uliginosum* i *Empetrum hermaphroditum* no van mostrar cap resposta. El creixement i funcionament de *V. myrtillus* era similar en claps amb diferent composició d'espècies arbustives i no es van trobar evidències d'alteracions en les interaccions planta-planta amb l'escalfament. *Vaccinium myrtillus* va respondre positivament a l'augment de CO_2 amb un augment de l'àrea basal i un augment retardat en la mida dels vasos conductors i la conductivitat hidràulica específica. Tanmateix, l'eficiència hidràulica es va reduir amb l'escalfament. Tot i que es va trobar una correlació entre creixement i l'anatomia en termes de conductivitat hidràulica acumulada en *V. myrtillus,* aquesta relació va canviar amb els tractaments d'escalfament i augment de CO_2, i el creixement va augmentar sota aquests tractaments sense que es produïssin canvis immediats en l'anatomia del xilema que l'acompanyessin. La sequera experimental no va tenir grans efectes en el potencial hídric ni en la distribució de C recentment assimilat de *V. myrtillus*. Tanmateix, *V. uliginosum* va presentar lleugers signes d'estrès amb la sequera, ja que la seva conductància estomàtica i el seu potencial hídric es van veure reduïts, així com també la velocitat de transferència de C cap a òrgans subterranis. Tots aquests resultats suggereixen que el canvi climàtic actua de diferent manera sobre espècies coexistents, cosa que podria comportar canvis en la composició de les comunitats, així com en la seva estructura i funcionament en el futur.

Summary

High-elevation and high-latitude ecosystems are expected to be especially vulnerable to global change. Dwarf shrub species cover extensive areas in high mountain ecosystems, and warmer temperatures and land-use change have favoured their expansion. Co-occurring dwarf shrub species may respond differently to climate change, which can have major effects on their interactions, as well as on their growth and performance, and ultimately lead to drastic changes in plant communities. In this Thesis, warming, CO_2 enrichment and drought experiments have been carried out at the ecotone between the subalpine and alpine belts in different study sites across the Pyrenees and the Alps. The objective was to assess how these factors may influence dwarf shrub species interactions, growth, xylem anatomy and C allocation dynamics. Results of this Thesis show species-specific responses to the treatments, and these responses differed in magnitude and direction depending on the treatment applied. *Vaccinium myrtillus* responded positively to increased temperatures, whereas the co-occurring *Vaccinium uliginosum* and *Empetrum hermaphroditum* showed no response. *Vaccinium myrtillus* growth and functioning was similar across stands differing in neighbouring shrub species and we did not find evidence of alterations in plant-plant interactions with warming. *Vaccinium myrtillus* responded positively to CO_2 enrichment with an increased stem basal area and a lagged increased vessel lumen size and xylem-specific hydraulic conductivity, but hydraulic efficiency decreased with warming. Although there was a correlation between growth and accumulated hydraulic conductivity, the growth-anatomical relationship changed with soil warming and CO_2 enrichment, and growth stimulation occurred without immediate accompanying changes in the xylem anatomy. We did not find major effects of the experimental summer drought on *V. myrtillus* water potential and its allocation of recently assimilated C. However, *V. uliginosum* showed evidence of mild stress, since its stomatal conductance, water potential and speed of C transfer to belowground organs were reduced under drought. All these results suggest that climate change acts differently on co-occurring species, which may lead to future changes in plant community composition, structure and function.

Introducció general

General Introduction

Introducció general

Subarbusts d'alta muntanya

Els subarbusts són espècies vegetals llenyoses que assoleixen uns 50 cm d'alçada, de port reptant o prostrat fins a erecte (Körner 2003). A elevades altituds, les dures condicions climàtiques suposen un risc per a la reproducció de les plantes, ja que fases crítiques com la floració o la producció, dispersió i germinació de les llavors es poden veure interrompudes per fortes ventades, glaçades o nevades en qualsevol moment de l'any. Per evitar aquest risc, moltes espècies han adoptat mecanismes de propagació vegetativa, els quals permeten la persistència i expansió de les seves poblacions independentment de les condicions adverses (Körner 2003). Els subarbusts clonals es propaguen vegetativament a través dels seus rizomes subterranis o tiges prostrades, des dels quals emergeixen peus aeris, els ramets. Per tant, una unitat genètica o genet pot incloure nombrosos ramets. Aquests ramets poden estar connectats pels rizomes (cas en què ens referim a un veget) o no. En estudis ecològics i ecofisiològics els ramets normalment es consideren com a unitats d'estudi a causa de les dificultats que implica identificar diferents vegets al camp, cosa que requeriria desenterrar els rizomes subterranis i, per tant, implicaria una pertorbació important del sòl. A més, la identificació de diferents genets només és possible mitjançant tècniques genètiques, com l'ús de marcadors microsatèl·lits (Mizuki et al. 2005; Araki et al. 2009; Lembicz et al. 2011). Tot i la utilització dels ramets com a unitats d'estudi, no són individus independents i no s'haurien de considerar com a tals (tot i que poden arribar a funcionar de manera força autònoma).

A l'alta muntanya pirinenca i alpina, els subarbusts clonals són presents al sotabosc en boscos subalpins (formats per *Pinus mugo* Turra subsp. *mugo* i subsp. *uncinata* (Ramond ex DC.), *Larix decidua* Mill., *Pinus cembra* L. o *Picea abies* (L.) Karsten), formant claps en prats o constituint comunitats arbustives denses en l'estatge subalpí i les zones inferiors de l'alpí. Així, juguen un paper important en l'ecotò de transició entre els boscos subalpins i els prats alpins o ecotò del límit supraforestal (Körner 2003; Ninot et al. 2008; Vigo 2008).

El canvi global a l'alta muntanya

Amb el concepte de canvi global ens referim a tots aquells processos de canvi que tenen lloc, en l'actualitat, a escala planetària. Aquests inclouen transformacions ambientals, així com aquelles activitats antropogèniques que causen aquestes transformacions, com ara l'economia, la utilització dels recursos, els usos del sòl o el desenvolupament, entre d'altres (Steffen et al. 2004). El canvi climàtic, en concret, fa referència als canvis en les condicions climàtiques associats al canvi global. Si bé és cert que els canvis en el clima són intrínsecs al nostre planeta i han ocorregut periòdicament al llarg dels temps geològics, les taxes de canvi actuals no semblen tenir precedents en les passades dècades, centúries i, fins i tot, mil·lennis (IPCC 2014).

Canvis en l'ús del sòl i emmatament

Al llarg del darrer segle, els canvis socioeconòmics i demogràfics en zones d'alta muntanya han dut a un progressiu declivi i abandonament de les pràctiques agrícoles tradicionals, la ramaderia extensiva i l'explotació de la fusta (MacDonald et al. 2000; Roura-Pascual et al. 2005). Els canvis en l'ús del sòl han incentivat l'expansió i colonització per part d'espècies arbustives de pastures abandonades i prats subalpins i alpins (Dullinger et al. 2003; Anthelme et al. 2007; Prévosto et al. 2011; Brandt et al. 2013), cosa que també es veu afavorida per l'escalfament climàtic (Rundqvist et al. 2011; Komac et al. 2013; Myers-Smith et al. 2015). Aquest procés es coneix com a emmatament.

Diversos estudis han donat a conèixer els impactes negatius de l'emmatament en l'estructura, funcionament i biodiversitat dels ecosistemes, incloent la pèrdua de la biodiversitat, l'alteració de la distribució espacial dels recursos, la reducció de les funcions del sòl (com el cicle de nutrients o l'estabilitat) i el repte que suposa per les activitats pastorals i ramaderes (Wilson i Nilsson 2009; van Auken 2000; Eldridge et al. 2011; Myers-Smith et al. 2011; Pajunen et al. 2011; Ratajczak et al. 2012; Brandt et al. 2013; Koch et al. 2015). Alguns estudis indiquen que l'emmatament també pot tenir efectes positius depenent del recobriment dels arbustos o dels trets funcionals de les espècies involucrades (Soliveres et al. 2014; Eldridge i Soliveres 2014), però aquests estudis s'han dut a terme en ecosistemes àrids i semiàrids, on la història de la vegetació i els mecanismes causants de l'emmatament poden diferir dels de les regions fredes (Myers-Smith et al. 2011; O'Connor et al. 2014).

L'emmatament pot causar una retroalimentació positiva de l'escalfament global. En primer lloc, les capçades arbustives absorbeixen més radiació que les plantes herbàcies i, per tant, redueixen l'albedo (Sturm et al. 2005a); en segon lloc, fan de trampa de neu, incrementant el gruix de neu sota seu i, per tant, fan augmentar la temperatura del sòl (Sturm et al. 2005b); i, per últim, fan incrementar l'escalfament nocturn a través de la reflexió de l'energia tèrmica emesa pel sòl (D'Odorico et al. 2013). Tot i això, els arbustos també poden provocar una retroalimentació negativa de l'escalfament, ja que les taxes de descomposició es redueixen a causa de la seva fullaraca més recalcitrant comparada amb la de les espècies herbàcies (Cornelissen et al. 2007), i així augmenten el carboni orgànic del sòl (Montané et al. 2010). A més, a l'estiu, les temperatures del sòl sota els arbustos poden baixar gràcies a la seva pròpia ombra (Myers-Smith i Hik 2013). Així, l'emmatament té implicacions importants en el cicle de carboni (C) i de nutrients de l'ecosistema (Wookey et al. 2009; Li et al. 2016), i està molt estès a la tundra, tal com ho han plasmat Myers-Smith et al. (2011) en la seva extensa revisió sobre l'expansió d'arbustos a la tundra i els seus impactes.

Així, l'estudi del creixement i el funcionament de les espècies arbustives és crucial per entendre el funcionament i dinàmica de les comunitats arbustives i els patrons d'emmatament. Per això, l'interès en la recerca sobre aquestes formes de creixement ha crescut en les últimes dècades.

Escalfament global

A causa de les emissions de gasos d'efecte hivernacle, la temperatura de la Terra està augmentant, i les prediccions climàtiques apunten cap a un increment més gran de cara al futur. Cap a finals del s. XXI, la temperatura mitjana global en superfície podria incrementar-se entre 0,3 i 4,8 °C respecte el període 1986-2005, depenent del nivell de les emissions i, per tant, de les futures concentracions dels gasos d'efecte hivernacle (Fig. 1; IPCC 2014). A elevades latituds i altituds està previst un increment de les taxes d'escalfament especialment acusat com a conseqüència, entre d'altres factors, de l'augment de les radiacions d'ona per l'increment del vapor d'aigua, i d'una major absorció solar causada per la reducció de la coberta de neu (Nogués-Bravo et al. 2007; Collins et al. 2013; Rangwala et al. 2013).

A elevades altituds, el creixement vegetal està limitat per les baixes temperatures i la curta durada de l'estació de creixement, marcada pels patrons nivals. A més, les plantes poden patir gelades durant tot l'any. Així, petits canvis de temperatura poden tenir efectes dràstics en les espècies i les comunitats vegetals en aquestes zones. A les serralades europees, especialment al Pirineu, on les projeccions indiquen un augment de l'escalfament i una reducció de la precipitació estivals, les espècies podrien estar amenaçades com a conseqüència de la pèrdua del seu hàbitat (Engler et al. 2011).

Hi ha força estudis sobre els efectes de l'escalfament (tant estival com hivernal) en comunitats vegetals alpines i àrtiques, i molts d'ells han demostrat que les respostes de les plantes a l'escalfament són específiques (és a dir, pròpies de cada espècie), fins i tot per espècies coexistents (Klanderud 2008; Hoffman et al. 2010; Bokhorst et al. 2011; Dawes et al. 2011; Little et al. 2015; Yang et al. 2015). Això posa de manifest la necessitat d'avaluar la resposta de les espècies al canvi climàtic de manera individual per tal d'entendre la dinàmica de comunitats i ecosistemes, i la seva direcció en un futur.

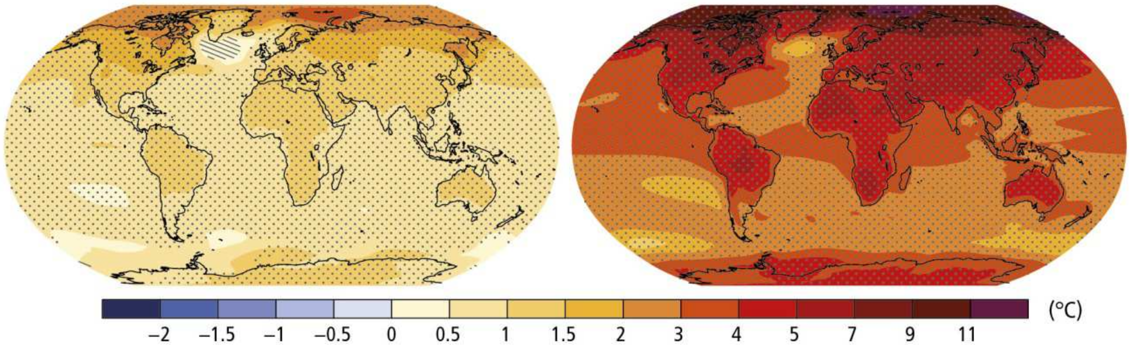

Figura 1. Canvi previst en la temperatura mitjana de la superfície per al període 2081-2100 respecte el 1986-2005 basat en projeccions de les mitjanes multimodel sota els escenaris RCP2.6 (mitigació estricta, esquerra) i RCP8.5 (molt elevada emissió de gasos d'efecte hivernacle, dreta). Font: IPCC 2014.

Increment de les concentracions de CO_2

La concentració dels gasos d'efecte hivernacle a l'atmosfera s'ha incrementat progressivament des del 1750 a causa d'activitats humanes com la crema de combustibles fòssils i els canvis en l'ús del sòl. Avui en dia, les concentracions atmosfèriques de CO_2, CH_4 i N_2O són considerablement més elevades que les concentracions més altes registrades en testimonis de gel en els darrers 800.000 anys. L'augment de les concentracions de CO_2 és la contribució més gran al forçament radiatiu antropogènic total, que és una mesura de la capacitat dels gasos o altres agents per alterar el balanç d'energia de la Terra. Les concentracions atmosfèriques de CO_2 s'han incrementat des de prop de 280 ppm en l'època preindustrial fins al voltant de 400 ppm en l'actualitat (Fig. 2; IPCC 2014).

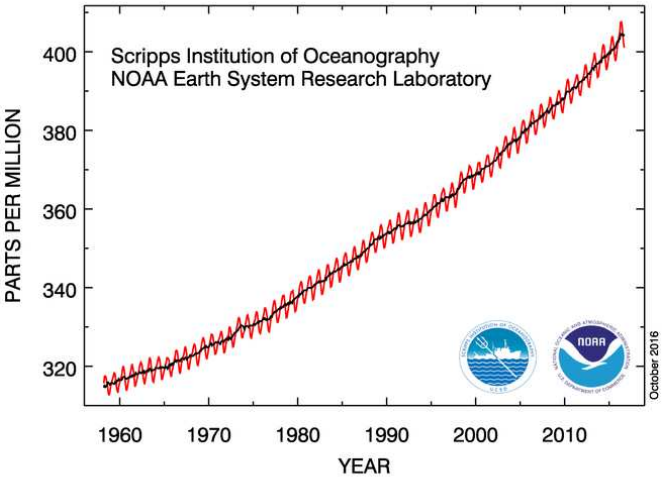

Figura 2. Augment de la concentració atmosfèrica de CO_2 des de finals dels anys 1950 fins al present (2016) a l'Observatori de Mauna Loa, Hawai. Font: ESRL, NOAA (http://www.esrl.noaa.gov/gmd/obop/mlo/, accedit el 28 d'octubre del 2016).

Força experiments han demostrat que un augment de la concentració de CO_2 de l'aire pot tenir un efecte positiu en el creixement vegetal, però aquest efecte de fertilització tan sols es produeix quan altres factors com la temperatura, la llum o els nutrients no són limitants (Prentice et al. 2001; vegeu revisions d'Ainsworth i Long 2005, i de Norby i Zak 2011). Experiments de fumigació amb CO_2 a l'aire lliure, anomenats *free-air* CO_2 *enrichment* en anglès (FACE), sovint han trobat una aclimatació fotosintètica a l'enriquiment de CO_2, associada a un augment de les concentracions foliars de carbohidrats no estructurals (CNE) i una reducció de les concentracions foliars de nitrogen (N) (Hättenschwiler i Körner 1996; Handa et al.

2005; Inauen et al. 2012; Bader et al. 2013). El mecanisme que explicaria l'aclimatació fotosintètica seria una regulació negativa de les quantitats de Rubisco i/o de la seva activitat (Rogers i Ellsworth 2002). En experiments FACE, sovint s'ha trobat un augment de l'assignació de biomassa a òrgans subterranis, que s'ha relacionat amb la necessitat d'invertir en òrgans que es dediquin a buscar nutrients limitants i d'ajustar la descompensació en el balanç font/embornal de C causada per un augment de l'assimilació d'aquest (Hättenschwiler i Körner 1998; Suter et al. 2002; Inauen et al. 2012).

En comparació amb la gran quantitat d'experiments centrats en els efectes de l'escalfament en espècies vegetals d'ecosistemes d'elevades altituds i latituds, els experiments *in situ* de fumigació amb CO_2 a l'aire lliure en aquests ecosistemes són escassos, ja que impliquen grans inversions logístiques i econòmiques (vegeu Tissue i Oechel 1987; Gwynn-Jones et al. 1997; Körner et al. 1997; Inauen et al. 2012; Dawes et al. 2015). Contràriament a la hipòtesi inicial segons la qual el creixement vegetal es veuria encara més estimulat per l'augment de les concentracions de CO_2 en ecosistemes d'elevades altituds a causa de les seves baixes pressions parcials de CO_2 (vegeu Körner 2003), la majoria d'aquests estudis van trobar que l'enriquiment de CO_2 no estimulava el creixement vegetal (Körner et al. 1997; Hättenschwiler i Körner 1998; Inauen et al. 2012; Dawes et al. 2015). Tanmateix, alguns estudis sí que han trobat efectes positius en el creixement, tot i que sovint petits i temporals (Gwynn-Jones et al. 1997; Dawes et al. 2015). De tots els experiments FACE realitzats a elevades altituds i latituds, només els estudis de Dawes et al. (2011) i Gwynn-Jones et al. (1997) s'han centrat en arbustos. Això emfasitza la necessitat de més treballs centrats en l'estudi de l'efecte de l'augment de les concentracions de CO_2 en aquestes formes vitals.

Canvis en els règims de precipitació

Si bé els models de predicció de temperatura per a aquest segle són prou congruents entre ells, les prediccions dels règims de precipitació són més incertes i més variables. Tanmateix, aquestes coincideixen en assenyalar un augment en la variabilitat dels patrons de precipitació i una intensificació dels episodis climàtics extrems (IPCC 2014). Per al sud i el centre d'Europa es prediu una reducció de la precipitació i un augment d'episodis extrems de sequera a l'estiu (Fig. 3; López-Moreno i Benniston 2009; Dai 2013; Osca et al. 2013, Roudier et al. 2016). Aquests episodis de sequera podrien tenir greus impactes en la fotosíntesi i la respiració de les plantes i, en última instància, comportar canvis dràstics en el cicle terrestre del C (Reichstein et al. 2013; Frank et al. 2015; Yuan et al 2016).

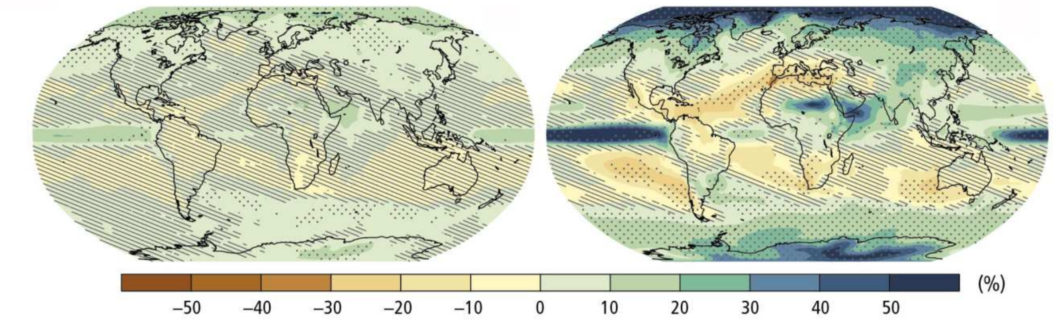

Figura 3. Canvi previst en la precipitació mitjana per al període 2081-2100 respecte el 1986-2005, basat en projeccions de les mitjanes multimodel sota els escenaris RCP2.6 (mitigació estricta, esquerra) i RCP8.5 (molt elevada emissió de gasos d'efecte hivernacle, dreta). Font: IPCC 2014.

Tot i que força estudis s'han centrat en els efectes de la sequera en prats i pastures a elevades altituds (Fuchslueger et al. 2014, 2016; Hasibeder et al. 2015; Leitinger et al. 2015; De Boeck et al. 2016, entre altres), són pocs els estudis enfocats a les comunitats arbustives en aquestes zones. Estudis en matollars d'ericàcies al llarg d'un gradient ambiental a Europa van mostrar que la sequera experimental tenia efectes negatius en la floració, biomassa, taxa d'intercanvi de gasos i concentració de fòsfor en els teixits de les espècies vegetals (Llorens et al. 2004; Peñuelas et al. 2004). A més, la sequera tenia efectes negatius en processos edàfics com ara la respiració o la nitrificació, tot i que no van trobar tendències consistents en la descomposició de la fullaraca (Emmett et al. 2004). Tanmateix, la magnitud dels efectes de la sequera en aquests treballs diferia entre espècies i llocs d'estudi dins el gradient. En un altre estudi en un matollar temperat, Albert et al. (2011) van trobar que la capacitat fotosintètica de *Calluna vulgaris* es veia reduïda per la sequera. Tots aquests treballs assenyalen els efectes negatius de la sequera en el funcionament dels arbustos, però un experiment al nord de Gal·les (Regne Unit) va mostrar que les taxes d'intercanvi de gasos del subarbust *Vaccinium myrtillus* no es veien alterades pel dèficit hídric (Llorens et al. 2002). Cal, doncs, una millora en el coneixement dels efectes de la sequera en comunitats arbustives de zones temperades i de l'alta muntanya.

Interaccions planta-planta

Les interaccions planta-planta es troben entre els principals factors que determinen la dinàmica de les comunitats vegetals (Callaway i Walker 1997). Aquestes interaccions poden ser neutres, positives (p. ex. facilitació) o negatives (p. ex. competència o al·lelopatia) i no són exclusives entre elles, ja que entre els individus hi té lloc una complexa xarxa d'interaccions. Les interaccions planta-planta estan estretament lligades a l'ambient i poden determinar les respostes de les comunitats vegetals al canvi climàtic (Brooker 2006). Poden tenir lloc entre plantes veïnes de la mateixa espècie (interaccions intraespecífiques) o entre plantes veïnes d'espècies diferents (interaccions interespecífiques). Tal com diu la teoria de la coexistència, la competència intraespecífica hauria de ser més gran que la competència interespecífica, entre altres coses perquè els individus de la mateixa espècie mostren una superposició completa del nínxol (Chesson 2000). Tanmateix, hi ha estudis que demostren que la facilitació intraespecífica pot ser més important que la competència a nivells moderats-elevats d'estrès (Chu et al. 2008; Fajardo i McIntire 2011). En plantes clonals, les interaccions intraespecífiques es poden donar entre ramets del mateix clon o ramets de clons diferents. Dins un mateix veget hi ha una integració fisiològica entre ramets, la qual permet que aquells ramets que ocupen zones més riques en recursos els comparteixin amb els ramets de zones més pobres (vegeu la revisió de Hutchings i Wijesinghe 1997 i la meta-anàlisi de Song et al. 2013). D'aquesta manera, les interaccions entre ramets del mateix veget es poden confondre amb els mateixos patrons fisiològics de distribució dels recursos (Fajardo i McIntire 2011).

La hipòtesi del gradient d'estrès (HGS, Bertness i Callaway 1994) postula que les interaccions positives són dominants en ambients estressants, mentre que les negatives dominen en condicions més benignes o favorables. Molts estudis al llarg de gradients ambientals en zones fredes o a través de manipulacions experimentals de les condicions ambientals han evidenciat canvis en les interaccions entre plantes, des d'interaccions de facilitació cap a competència i viceversa, donant suport a la HGS (Choler et al. 2001;

Klanderud 2005; Pugnaire et al. 2015 i Wheeler et al. 2015, entre d'altres). No obstant això, altres estudis realitzats en diversos tipus d'ecosistemes no han trobat evidència per recolzar la hipòtesi, cosa que s'explicaria perquè la naturalesa del gradient ambiental, l'ontogènia de l'espècie o la magnitud de l'estrès també influeixen en les interaccions entre plantes (Tielbörger i Kadmon 2000; Miriti 2006; Maestre i Cortina 2004; Maestre et al. 2009; Armas et al. 2013; Le Roux et al. 2013, Grau 2014). A més, les espècies que coexisteixen poden presentar diferents adaptacions a l'ambient, de manera que condicions relativament estressants per certes espècies, poden no ser-ho per d'altres. En la seva revisió sobre interaccions planta-planta, Soliveres i Maestre (2014) suggereixen que un sol model conceptual difícilment podria explicar les interaccions entre plantes al llarg de gradients ambientals significativament diferents, i que cal una major comprensió de la influència relativa de la facilitació com a modulador ecosistèmic. En qualsevol cas, podríem esperar canvis en les interaccions entre espècies vegetals si es produeixen canvis ambientals que siguin favorables per certes espècies però no ho siguin per les espècies veïnes (Kudo i Suzuki 2003; Dormann et al. 2004; Niu i Wan 2008; Little et al. 2015; vegeu la revisió de Tylianakis et al. 2008). Això podria comportar canvis en la dominància de les espècies i, finalment, canvis en la composició, estructura i funcionament de les comunitats (Dormann et al. 2004; Stevens et al. 2004; Walker et al. 2006; Wookey et al. 2009).

Objectius de la Tesi Doctoral

L'**objectiu principal** d'aquesta Tesi és aprofundir en el coneixement de les respostes fisiològiques, fenològiques i de creixement de subarbusts d'alta muntanya, especialment *Vaccinium myrtillus* i *Vaccinium uliginosum*, dues espècies molt comunes, davant el canvi climàtic. Aquesta Tesi també pretén assolir un coneixement més ampli d'aquestes respostes a través del seu estudi en dues serralades europees importants, el Pirineu i els Alps. En particular, les àrees d'interès es troben en la zona superior de l'estatge subalpí, comprenent també l'ecotò del límit supraforestal. L'estudi de les respostes de les espècies vegetals al canvi climàtic, amb la implícita influència antropogènica, és el primer pas per comprendre millor i predir la dinàmica de les comunitats vegetals i ecosistemes davant les futures condicions ambientals. Aquests objectius principals es poden dividir en els **objectius específics** següents:

1. Determinar si l'estructura i funcionament del subarbust *Vaccinium myrtillus* es veuen afectats per la coexistència amb les espècies *Vaccinium uliginosum* i *Rhododendron ferrugineum* i si l'escalfament té un efecte en les interaccions entre aquestes espècies.
2. Analitzar els efectes de l'escalfament en la fenologia i creixement dels subarbusts coexistents *V. myrtillus*, *V. uliginosum* i *Empetrum hermaphroditum*.
3. Avaluar els efectes de l'escalfament i l'augment de la concentració de CO_2 en el creixement i l'anatomia del xilema de *V. myrtillus*, i en la relació entre aquests paràmetres.
4. Entendre els patrons de distribució de C a curt termini en les espècies coexistents *V. myrtillus* i *V. uliginosum* i analitzar els efectes de la sequera en aquests patrons.

Estructura de la Tesi Doctoral

Aquesta Tesi s'estructura en tres parts diferents, que coincideixen amb diferents àrees d'estudi.

1. Efectes de l'escalfament de l'aire en les interaccions planta-planta a l'ecotò del límit supraforestal

La primera part se centra en les interaccions planta-planta, en particular en les interaccions entre *V. myrtillus* i dues espècies arbustives veïnes, *Vaccinium uliginosum* i *Rhododendron ferrugineum*, així com en els efectes de l'escalfament sobre aquestes interaccions. El **capítol 1** és un estudi de caire més descriptiu, on vam avaluar els efectes de la coexistència amb els arbustos veïns en el creixement i el funcionament de *V. myrtillus*. Aquest treball, en el qual no vam dur a terme cap manipulació de les condicions ambientals, és una introducció del capítol següent. El **capítol 2** correspon a un estudi experimental amb cambres *open-top* (OTC), que incrementen la temperatura entre 1 i 2 °C (Fig. 4). Aquest capítol se centra en els efectes de l'escalfament en les interaccions entre *V. myrtillus* i les dues espècies veïnes ja esmentades. Vam mesurar la biomassa aèria i subterrània, i la concentració de C i N i la seva composició isotòpica en diferents òrgans de *V. myrtillus* sota els diferents escenaris de coexistència i els tractaments d'escalfament. En aquelles parcel·les en què *V. uliginosum* era present, també vam avaluar-ne paràmetres similars (biomassa aèria, concentracions de C i N i la seva composició isotòpica). Vam fer un seguiment fenològic de *V. myrtillus* i vam avaluar possibles canvis en la diversitat funcional de la comunitat microbiana. Tots dos estudis es van realitzar a Eth Corrau des Machos, la Val d'Aran, a la zona perifèrica del Parc Nacional d'Aigüestortes i Estany de Sant Maurici, al Pirineu.

Figura 4. Cambra *open-top* (OTC).

2. Efectes de l'escalfament del sòl i l'augment de CO_2 en la fenologia, el creixement i l'anatomia de la fusta de subarbusts al límit supraforestal

El **capítol 3** se centra en l'estudi dels efectes de l'escalfament del sòl (+ 4 °C) amb cables elèctrics en la fenologia i el creixement de *V. myrtillus*, *V. uliginosum* i *Empetrum hermaphroditum* coexistint en les mateixes parcel·les sota la capçada de dues espècies arbòries diferents (*Pinus mugo* subsp. *uncinata* i *Larix decidua*). El **capítol 4** pretén avaluar l'efecte de l'escalfament del sòl i l'augment de la concentració del CO_2 de l'aire (es tracta d'un experiment FACE, + 200 ppm) en el creixement i l'anatomia de la fusta de *V. myrtillus*, així com en la relació entre aquests dos paràmetres. Aquests dos capítols corresponen a la mateixa àrea d'estudi, la zona experimental de Stillberg, a Davos, als Alps suïssos, on un experiment d'escalfament del sòl i un experiment d'augment de la concentració del CO_2 es van dur a terme durant 12 anys (Fig. 5). L'experiment d'augment de CO_2 es va realitzar del 2001 al 2009 i el d'escalfament del sòl del 2007 al 2012.

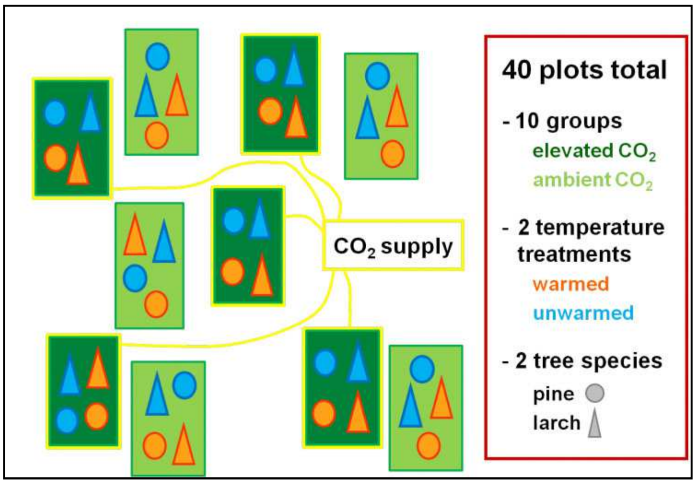

Figura 5. Disseny de l'experiment d'augment de CO_2 i escalfament del sòl a Stillberg, Davos. Font: Melissa Dawes

3. Dinàmica de la distribució de C recentment assimilat en subarbusts alpins i la seva resposta a una sequera estival experimental

Finalment, el **capítol 5** se centra en la distribució de C recentment assimilat en subarbusts. Correspon a un doble experiment de marcatge isotòpic amb $^{13}CO_2$ (experiments de pols i seguiment), amb l'objectiu d'estudiar la distribució de C a curt termini en diferents òrgans de *V. myrtillus* i *V. uliginosum*. Vam combinar dos experiments, un a la vall d'Stubai, als Alps tirolesos, i l'altre a la vall de Filià, al Pirineu català. En tots dos experiments vam comparar els patrons de distribució de C entre les dues espècies en condicions naturals a l'estatge subalpí. A més, a Stubai vam realitzar el marcatge isotòpic després d'un experiment de sequera, per tal de comparar la dinàmica de distribució del C entre plantes en condicions naturals d'irrigació i plantes en condicions de sequera (Fig. 6, 7). Mitjançant anàlisis d'isòtops estables vam traçar el ^{13}C assimilat pels dos arbusts tant en la matèria orgànica total de diversos òrgans com en la respiració.

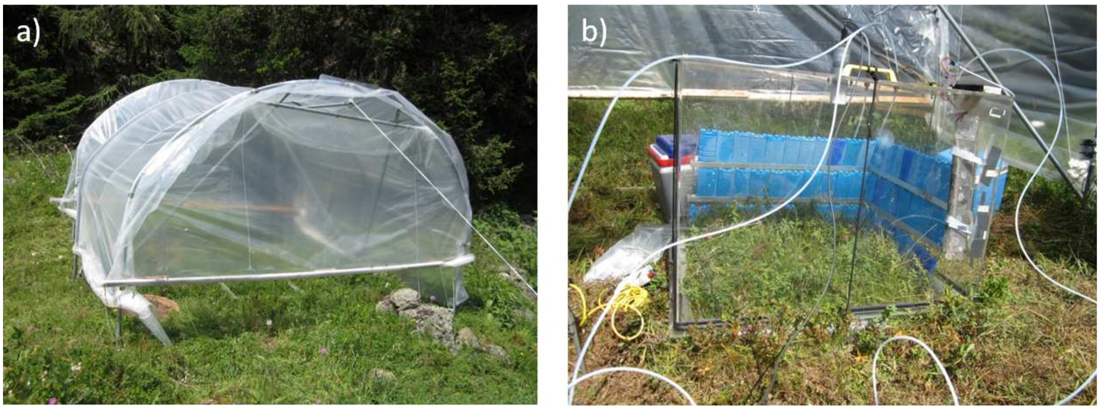

Figura 6 (a) Caseta d’exclusió de pluja i (b) cambra de marcatge isotòpic.

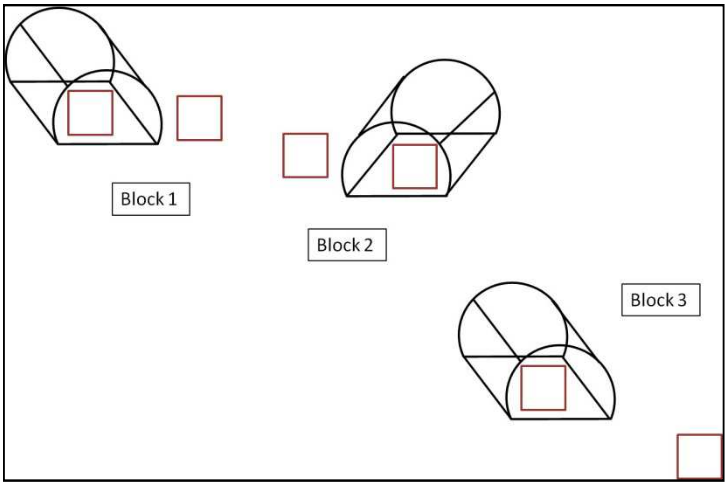

Figura 7. Disseny de l'experiment de sequera a Stubai el 2014.

Els diferents capítols d'aquesta Tesi corresponen a estudis que s'han dut a terme en localitats diferents i en grups de recerca diferents. Això explica que alguns capítols estiguin escrits en anglès americà mentre d'altres estan escrits en anglès britànic. Altres petites formalitats poden diferir també entre els capítols. Un resum dels principals tractaments emprats i els paràmetres estudiats és inclòs a la Fig. 8.

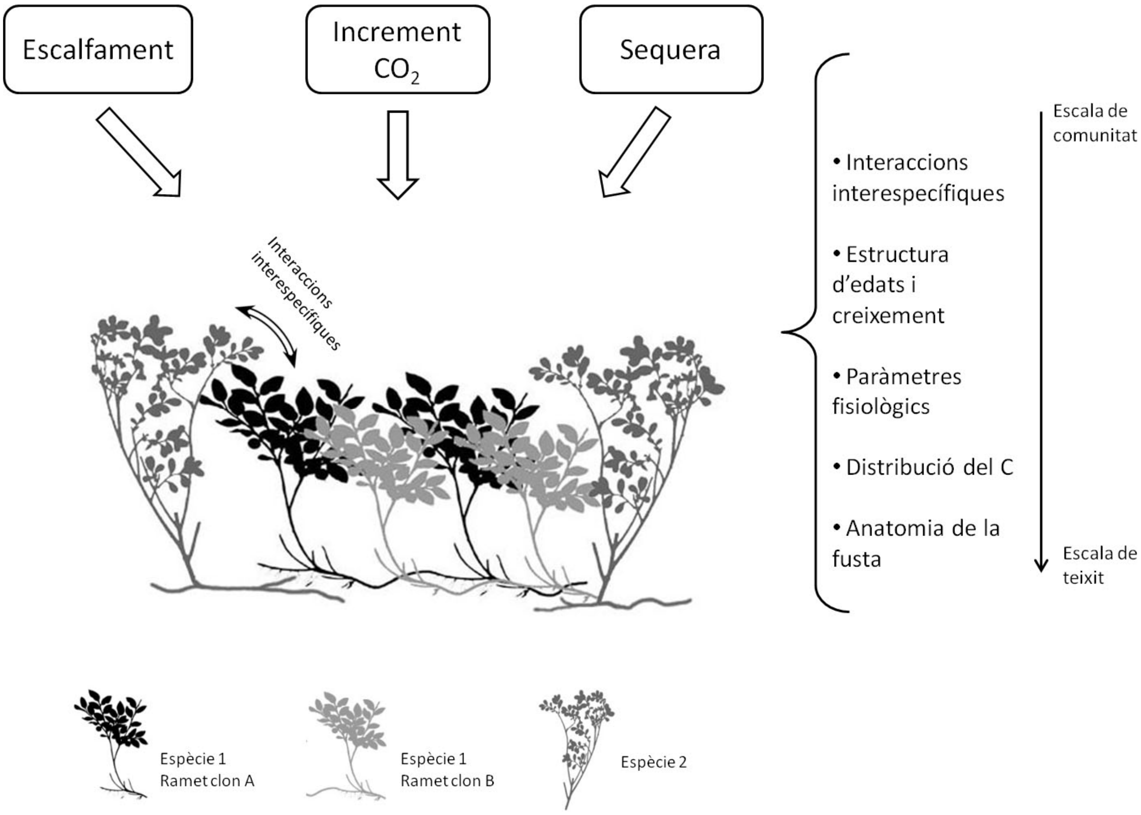

Figura 8. Tractaments aplicats i principals paràmetres i processos analitzats en aquesta Tesi Doctoral.

Espècies, àrees d'estudi i metodologies

Espècies d'estudi

La principal espècie d'estudi d'aquesta Tesi és *Vaccinium myrtillus* L., el nabiu (Fig. 9). Es tracta d'una ericàcia subarbustiva clonal, de fulla caduca, que assoleix uns 10-60 cm d'alçada (Flower-Ellis 1971). Les tiges joves de *V. myrtillus* són fotosintètiques fins, aproximadament, els 4 anys d'edat, moment en què perden el color verd. Aquestes tiges fotosintètiques tenen un paper molt important, ja que representen una proporció elevada de l'àrea fotosintètica total d'aquesta espècie, i li permeten fixar C quan les fulles ja han caigut. Els seus rizomes es troben a uns 15-20 cm de profunditat de sòl (Ritchie 1956) i poden assolir diversos metres en longitud (Flower-Ellis 1971). L'espècie es distribueix al llarg de les zones boreals i temperades de la regió Holàrtica. Al Pirineu, es troba des de la part inferior de l'estatge montà (al voltant dels 900 m) fins les zones més elevades de l'estatge subalpí, fins i tot arribant a l'alpí (aprox. 2450 m; Bolòs i Vigo 1995; Bolòs et al. 2005). Als Alps, creix des de l'estatge basal fins l'alpí (Aeschimann et al. 2004). Així, *V. myrtillus* troba el seu límit altitudinal en l'ecotò de transició entre l'estatge subalpí i l'alpí. Al nord d'Europa es troba en altituds més baixes (Popova 1972). Creix en substrat àcid, des d'ecosistemes oberts fins a matollars i boscos.

Figura 9. Imatge de *Vaccinium myrtillus*.

La segona espècie més estudiada en aquesta Tesi és l'espècie congenèrica subarbustiva clonal *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. (a partir d'aquí, *V. uliginosum*), el nabiu uliginós (Fig. 10). Igual que *V. myrtillus*, és rizomatós i de fulla caduca, però aquestes tenen un color més fosc i no presenta tiges fotosintètiques. Assoleix 5-20 cm d'alçada (Aeschimann et al. 2004; Bolòs et al. 2005) i té una distribució articoalpina. Arriba a altituds més elevades que *V. myrtillus*, des de l'estatge subalpí fins l'alpí, tot i que també se'l pot trobar a l'estatge montà (Bolòs i Vigo 1995; Aeschimann 2004; Bolòs et al. 2005). També creix en substrats àcids, des de prats oberts fins a landes. És més heliòfil que l'espècie anterior (Jacquemart 1996). Als Alps, s'empra el sinònim *Vaccinium gaultherioides* Bigelow per aquest tàxon. En aquesta Tesi, s'empra el nom acceptat a Flora Europaea (*V. uliginosum* subsp. *microphyllum*, Popova 1972) en la Introducció i Discussió generals, en els capítols corresponents als experiments duts a terme al Pirineu (capítols 1 i 2) i en el capítol 5, que combina un experiment al Pirineu i un als Alps, mentre que el sinònim *V. gaultherioides* s'utilitza en els capítols corresponents als experiments desenvolupats als Alps suïssos (capítols 3 i 4).

Figura 10. Imatge de *Vaccinium uliginosum*.

La tercera espècie d'estudi, només present en un capítol, és *Empetrum nigrum* L. subsp. *hermaphroditum* (Hagerup) Böcher (a partir d'aquí, *Empetrum hermaphroditum*), l'èmpetrum (Fig. 11). Es tracta d'una altra ericàcia subarbustiva clonal, però de fulla perenne. Tradicionalment s'havia classificat dins la família Empetraceae. Presenta fulles ericoides i assoleix 15-30 cm d'alçada. Es distribueix al llarg de les zones boreals i temperades de la regió Holàrtica. Al Pirineu és força rara, i creix des de les parts més elevades de l'estatge subalpí fins l'estatge alpí (entre 2100 i 2850 m; Bolòs i Vigo 1995; Bolòs et al. 2005). Als Alps també es troba entre els estatges subalpí i alpí, i és més comuna (Aeshimann et al. 2004). També creix en substrat àcid.

Figura 11. Imatge d' *Empetrum hermaphroditum*.

Finalment, val la pena mencionar l'ericàcia *Rhododendron ferrugineum* L., el neret (Fig. 12). No s'ha estudiat directament en cap dels experiments duts a terme en aquesta Tesi, però és una de les espècies que coexisteixen amb *V. myrtillus* en els capítols 1 i 2, en els quals s'ha avaluat la seva interacció. *Rhododendron ferrugineum* és un arbust més gran que els anteriorment esmentats, d'uns 30-70 (150) cm d'alçada. És un arbust clonal, amb unes fulles gruixudes de color verd fosc i perennes. Creix en àrees freqüentment cobertes per neu a l'hivern, ja que necessita la seva protecció (Neuner et al. 1999; Komac et al. 2016). Es distribueix al llarg dels sistemes muntanyencs del sud d'Europa (Aeshimann et al. 2004).

Figura 12. Imatge de *Rhododendron ferrugineum*.

Àrees d'estudi

En aquesta Tesi Doctoral hi ha incloses quatre àrees d'estudi. Dues d'elles, Eth Corrau des Machos i Filià, es troben al Pirineu, i les altres dues, Stillberg i Stubai, als Alps (Fig. 13).

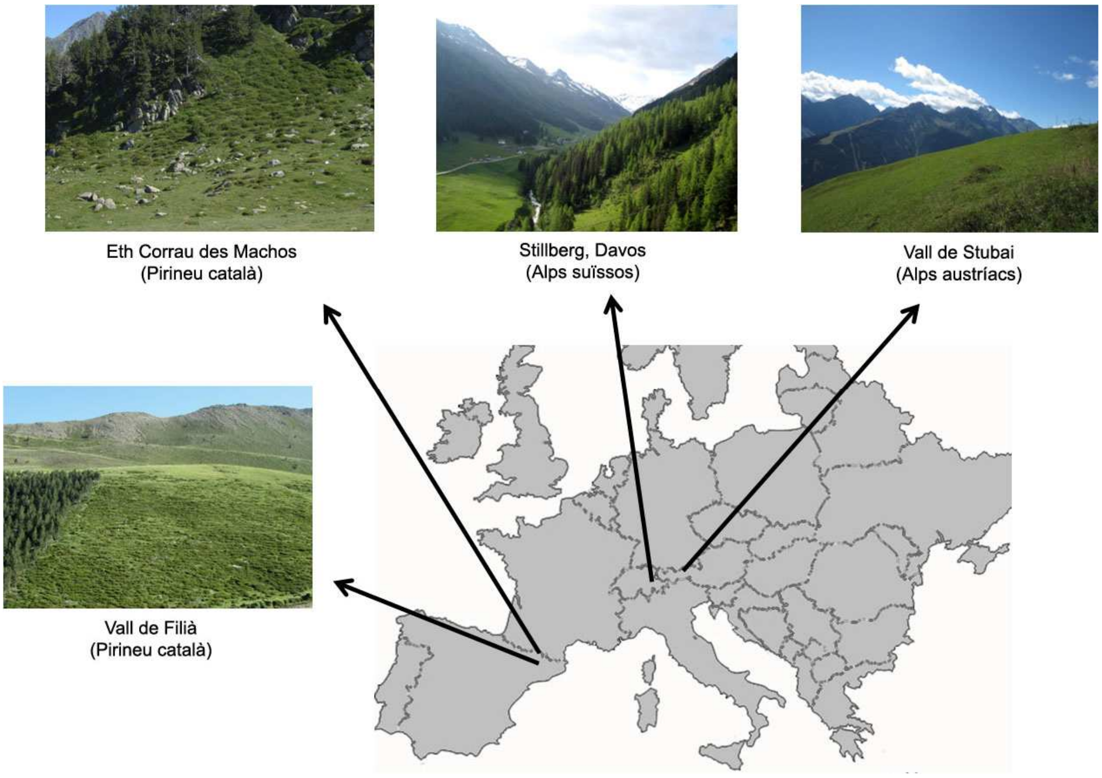

Figura 13. Mapa de la localització i fotografies de les zones d’estudi.

Eth Corrau des Machos se situa al Pirineu central, a la zona perifèrica del Parc Nacional d'Aigüestortes i Estany de Sant Maurici, a La Val d'Aran, Catalunya (42° 36' 48'' N, 0° 54' 54'' E). En aquesta zona d'estudi, en un vessant nord d'uns 10-25°, a 2.250 m d’altitud, el sòl és poc desenvolupat, ric en humus i àcid, derivat de la meteorització de granodiorites. La vegetació consisteix en prats de *Festuca eskia* Ramond ex DC. i *Nardus stricta* L. (Selino-Festucetum eskiae) barrejats amb claps de matollar subarbustiu, dominats per *V. myrtillus, V. uliginosum* i *R. ferrugineum*. Per al període 2001-2013, la precipitació anual mitjana va ser de 1.146 mm i la temperatura mitjana anual de 3 °C (dades d'una estació meteorològica propera a La Bonaigua, a 2.266 m, proporcionades pel Servei Meteorològic de Catalunya).

L'àrea d'estudi de Filià es troba al Pirineu central, a la vall de Filià, Pallars Jussà, Catalunya (42° 27' 41" N, 0° 57' 40" E). Correspon a un matollar subarbustiu en un vessant nord d'uns 28°, a 1.900 m. El sòl és rocós, ric en humus i àcid, sobre esquistos del Cambro-Ordovicià. Les espècies arbustives dominants són *V. myrtillus*, *V. uliginosum, R. ferugineum* i *Calluna vulgaris*. També hi abunden gramínies i fòrbies, entre les quals les més comunes són *Festuca nigrescens*, *Deschampsia flexuosa*, *Anthoxantum odoratum*, *Nardus stricta*, *Luzula multiflora*, *Alchemilla* gr. *alpina*, *Galium verum, Trifolium alpinum* i *Achillea millefolium*. Des de 1951 fins el 2002, la temperatura mitjana anual va ser de 3,7 °C i la precipitació anual mitjana de 1.297 mm. Pel període 2013-2015, la temperatura mitjana anual va ser de 5,5 °C i la precipitació anual mitjana de 1.259 mm (dades d'una estació meteorològica a l'Estany Gento, 2.120 m, proporcionades per Meteopirineus i l'Agencia Estatal de Meteorología).

La zona experimental de Stillberg es troba als Alps centrals, prop de Davos, Suïssa (46° 46' N, 9° 56' E). Stillberg és una zona de recerca a llarg termini, d'unes 5 hectàrees, on el Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) va realitzar una aforestació mitjançant la plantació de plançons el 1975. L'àrea corresponent a l'experiment presentat en aquesta Tesi constava de 2.500 m^2 en un vessant NE de 25-30°, a 2.180 m, lleugerament per sobre del límit supraforestal a la zona (Dawes et al. 2015). El sòl presenta una capa orgànica gruixuda (5-20 cm) i és derivat de paragneis silícics (Hagedorn et al. 2010). En cadascuna de les parcel·les que formaven part dels experiments d'augment de CO_2 i escalfament del sòl, s'hi trobava plantat un sol arbre de l'espècie *Pinus mugo* subsp. *uncinata* (DC.) Domin o *Larix decidua* Mill. La vegetació del sotabosc consistia en una coberta densa dels subarbusts *V. myrtillus*, *V. uliginosum* i *E. hermaphroditum*, així com nombroses espècies herbàcies, entre les quals *Deschampsia flexuosa*, *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus* i *Melampyrum pratense* (Dawes et al. 2011). Des de 1975 fins el 2012, la precipitació anual mitjana va ser de 1.155 mm i la temperatura mitjana anual de 2,1°C (dades extretes d'una estació micrometeoròlogica de l'Institut WSL a l'àrea d'estudi).

L'àrea d'estudi de Stubai es troba en un prat subalpí colonitzat per claps subarbustius a 1.820 m d'altitud, sobre material silícic, a la vall de Stubai, als Alps austríacs centrals (47° 7' 45" N, 11° 18' 20" E). La vegetació està composta pels subarbusts dominants *V. myrtillus* i *V. uliginosum*, així com per altres espècies subarbustives i herbàcies, entre les quals *Calluna vulgaris*, *Vaccinium vitis-idaea*, *Agrostis capillaris*, *Festuca ovina*, *Deschampsia flexuosa*, *Briza media*, *Luzula multiflora*, *Campanula scheuzeri*, *Chaerophyllum hirsutum*, *Crepis conyzifolia*, *Hypericum maculatum* i *Potentilla erecta*. La temperatura mitjana anual és de 3 °C i la precipitació anual mitjana 1100 mm (dades provinents d'una estació meteorològica a la zona d'estudi).

Metodologies emprades en l'estudi de les respostes dels subarbusts al canvi climàtic

Les diferents aproximacions a l'estudi de les respostes dels subarbusts d'alta muntanya al canvi climàtic realitzades en aquesta Tesi expliquen que s'hagin emprat diferents metodologies. Cada capítol inclou nombrosos paràmetres d'estudi i tècniques per les quals han calgut diferents protocols. En aquesta secció hi ha breus explicacions de les principals metodologies emprades per resoldre les qüestions relacionades amb la fenologia, la fisiologia, l'anatomia i el creixement dels subarbusts davant els diferents tractaments aplicats.

Seguiments fenològics

La fenologia és l'estudi del curs temporal dels fenòmens relacionats amb el desenvolupament del cicle vital, en aquest cas, dels vegetals. Entre aquests fenòmens hi trobem, per exemple, la brotació o la floració. L'estudi fenològic implica l'observació i el registre del moment en què aquests fenòmens tenen lloc, o dels fenòmens que tenen lloc durant un període de temps concret. El cicle vital dels vegetals és molt sensible a la temperatura (Walther 2003), especialment els esdeveniments fenològics que tenen lloc al principi de l'estació de creixement (Sparks i Menzel 2002). En ecosistemes freds, l'augment de la temperatura també pot tenir efectes indirectes en el cicle fenològic de les plantes a principis de l'estació de creixement a través d'una fosa avançada de la neu

(Wipf et al. 2009; Lambert et al. 2010; Livensperger et al. 2016; Semenchuk et al. 2016). L'avançament de la fenologia vegetativa amb l'escalfament s'ha relacionat amb un augment del creixement en espècies vegetals de zones alpines i àrtiques (Arft et al. 1999), tot i que altres estudis no han trobat aquesta relació (Livensperger et al. 2016). A més, una brotació avançada després d'una fosa de la neu primerenca podria comportar un risc de danys per gelades tardanes en un estadi del desenvolupament tan crític, cosa que podria tenir efectes negatius en el creixement i desenvolupament de la planta (Wipf et al. 2009; Wheeler et al. 2014).

En estudis comparatius centrats en la fenologia de plantes sota diferents condicions ambientals és especialment important captar diferències que poden ser subtils i ocórrer en només un o dos dies. Per aquesta raó, el seguiment fenològic s'hauria de dur a terme com a mínim unes tres vegades per setmana, especialment al començament de l'estació de creixement, just en fondre's la neu. Si un seguiment tan exhaustiu no és viable, caldria escollir una altra aproximació; enlloc de perseguir canvis al llarg del temps, seria més idoni anotar les diferències trobades a cada moment de mostreig.

El seguiment fenològic es pot realitzar seguint diferents metodologies. Un exemple és la identificació i seguiment al llarg del temps d'individus concrets. Un altre cas seria l'avaluació de l'estat fenològic mitjà de totes les plantes d'una parcel·la, i una altra aproximació seria registrar el moment en què el primer individu de cada parcel·la presenta un canvi fenològic. En aquesta Tesi, els seguiments fenològics s'han fet en els mateixos ramets al llarg del temps, cosa que proporciona un major detall dels canvis fenològics en una unitat funcional. La fenologia vegetativa s'ha estudiat amb més detall que la reproductiva per diverses raons. En primer lloc, com a conseqüència de la importància del comportament vegetatiu en les espècies clonals estudiades. La floració i la fructificació són essencials per la variabilitat genètica de les poblacions i, per tant, la seva adaptació i supervivència a llarg termini (Holsinger 2000; Jump i Peñuelas 2005), però la capacitat dels subarbusts clonals de propagar-se vegetativament confereix al seu aparell vegetatiu una importància cabdal per la dinàmica poblacional. En segon lloc, a Eth Corrau des Machos la fructificació va ser anecdòtica els dos anys d'estudi (2011-2012).

Biomassa i mesures de creixement

Els estudis sobre arbusts clonals solen estar centrats tan sols en les parts aèries. Això s'explica per les dificultats d'analitzar la biomassa, el creixement i el comportament de les parts subterrànies, ja que els rizomes formen una xarxa complexa que pot expandir-se força lluny del centre del clap. A més, no sempre és viable desenterrar rizomes i arrels, especialment en estudis a mitjà i llarg termini, en els quals una pertorbació massa gran del sòl impediria o perjudicaria futurs mostrejos. Per realitzar mesures de biomassa, cal recollir les plantes, assecar-les a l'estufa i pesar-les. Amb això podem obtenir informació de l'estat de la planta i, fins i tot, podem estimar la biomassa poblacional. Però aquestes mesures només ens proporcionen informació d'un moment del temps concret, i les taxes de creixement o la productivitat s'han de mesurar d'altres maneres. En aquesta Tesi, els paràmetres relacionats amb el creixement que s'han avaluat són: la producció de fulles i tiges noves (relativa a la biomassa total del ramet), l'alçada dels ramets i els increments de l'amplada dels anells de creixement (vegeu la següent secció). A més, una mesura interessant relacionada amb el creixement dels arbusts que no implica tècniques destructives és l'elongació de la tija. Hi ha força estudis que han emprat aquest paràmetre com una aproximació al creixement de la planta (Bret-Harte et al. 2002; Kudo i Suzuki 2003; Dawes et al. 2011; Campioli et al. 2012).

Dendrocronologia i anatomia quantitativa del xilema en subarbusts

La dendroecologia és la disciplina de la dendrocronologia que utilitza anells de creixement datats per estudis ecològics (Kaennel i Schweingruber 1995). Com que els subarbusts tenen una mida petita, el comptatge i mesura de l'amplada dels anells de creixement s'ha de fer a partir de preparacions microscòpiques de seccions transversals de tiges o rizomes. Aquestes seccions, d'uns 10-20 µm, es realitzen amb un micròtom de lliscament (Fig. 14a) i posteriorment es tenyeixen amb una barreja de safranina i blau astra per tal d'emfasitzar l'estructura dels anells de creixement. Per la seva preservació, és necessari deshidratar les mostres rentant-les amb etanol en concentracions creixents (75%, 96%, 100%) i, finalment, amb xilol (Fig. 14b). Per acabar de fixar la preparació, es fa una immersió en bàlsam del Canadà i es posa a assecar durant 24h a 60 °C (Gärtner & Schweingruber 2013).

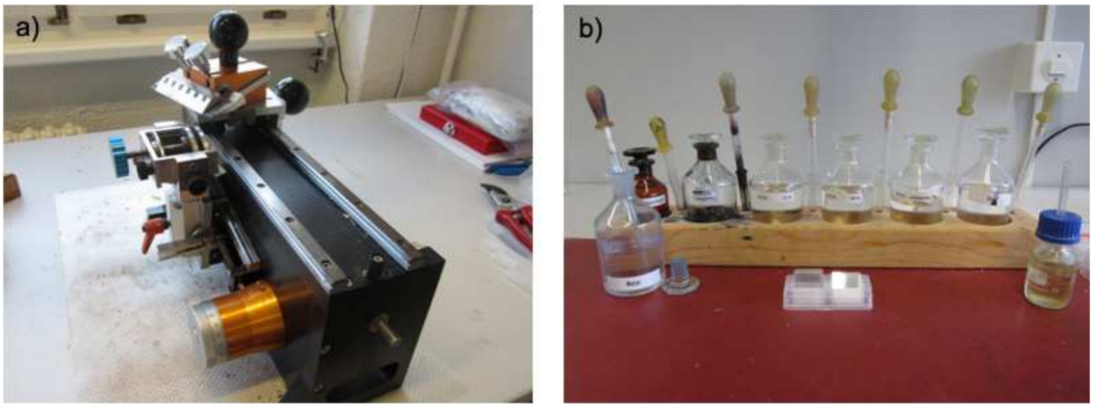

Figura 14 (a) Micròtom de lliscament a les instal·lacions del Swiss Federal Institute for Forest, Snow and Landscape Research (WSL, Birmensdorf, Suïssa). (b) Material per realitzar la deshidratació i fixació de les microseccions vegetals.

Un cop la preparació microscòpica està llesta, cal fotografiar-la amb una càmera incorporada al microscopi. Quan ja s'ha fet la fotografia i l'escala de la imatge està controlada, es pot procedir a comptar els anells de creixement i fer les anàlisis desitjades amb els programaris adequats. Hi ha programaris especialitzats per realitzar datacions creuades de les mostres, que permeten corregir la manca de sincronia entre sèries temporals i, per tant, trobar anells absents. Però quan la sèrie temporal és massa curta no es recomana utilitzar aquests programes, ja que les correlacions que estableixen podrien ser incorrectes (Grissino-Mayer 2001). En aquests casos, com passa en aquesta Tesi, en què la majoria de ramets de *V. myrtillus* eren més joves de 20 anys (i molts, fins i tot no arribaven als 12 anys) la datació creuada s'ha de fer manualment.

Les mateixes imatges utilitzades per fer datacions creuades i comptar anells de creixement es poden emprar per anàlisis anatòmiques quantitatives. L'anatomia de la fusta quantitativa permet estudiar la relació entre la variabilitat quantitativa de trets anatòmics del xilema i el creixement i funcionament de la planta, i així entendre com les relacions entre estructura i funció es veuen afectades per les condicions ambientals (Fonti et al. 2010; Fonti and Jansen 2012; Schweingruber et al. 2013; von Arx et al. 2016). L'avaluació quantitativa de la mida del lumen dels vasos conductors, la distribució espacial i densitat d'aquests o la quantificació del parènquima radial poden aportar un coneixement molt valuós sobre la capacitat conductora hidràulica i de reserva de la planta, i de com aquests paràmetres s'ajusten a les condicions ambientals.

L'anatomia quantitativa doncs, és una eina molt útil per l'estudi de les respostes de les plantes als canvis ambientals al llarg del temps. En aquesta Tesi, hem emprat el programa WinCELL per mesurar les amplades dels anells de creixement (capítol 3) i el programa ROXAS (von Arx et al. 2013; Wegner et al. 2013) per l'anàlisi quantitativa dels trets anatòmics de la fusta (capítol 4, Fig. 15).

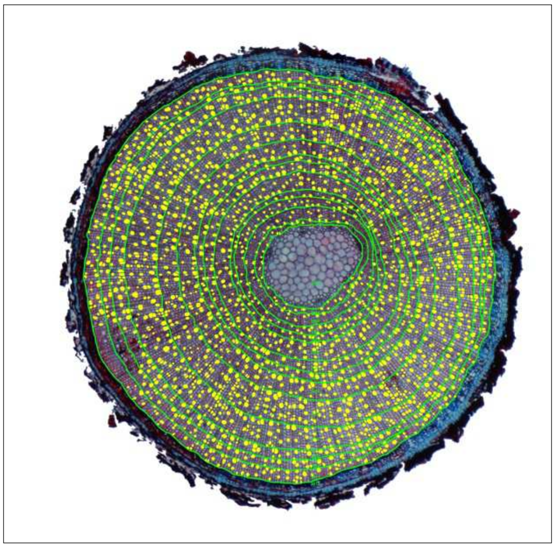

Fig 15. Imatge digital d'una secció transversal de *V. myrtillus* durant la seva anàlisi amb el programari ROXAS. Els vasos conductors són de color groc i el límit de cada anell de creixement és de color verd. Manca l'escala de la imatge perquè aquesta no es mostra durant el procés d'anàlisi.

Utilització d'isòtops estables i marcatge isotòpic per l'estudi de la dinàmica de distribució del C

Les tècniques que utilitzen isòtops estables són eines molt potents per l'estudi de la relació entre les plantes i l'ambient. Aquestes serveixen, per exemple, per traçar el C assimilat o les dinàmiques nutricionals dins la planta (Dawson et al. 2002). Els isòtops del mateix element són àtoms que tenen el mateix nombre atòmic però masses atòmiques diferents (és a dir, diferent nombre de neutrons). El fraccionament isotòpic fa referència al canvi en la partició dels isòtops pesants i lleugers entre una font i un substrat, mentre que la discriminació isotòpica és el fraccionament isotòpic provinent d'una reacció biològica (Dawson et al. 2002). El fraccionament té lloc perquè els isòtops pesants reaccionen de manera més lenta que els lleugers i tenen enllaços més difícils de trencar. Per exemple, en el procés de fotosíntesi té lloc una discriminació en contra del ^{13}C durant la difusió del CO_2 a través dels estomes i durant la carboxilació de la Rubisco (Farquhar et al. 1989; Lambers et al. 2008). Això explica que la matèria orgànica sigui empobrida en ^{13}C respecte el CO_2 atmosfèric. Els isòtops estables més emprats en estudis ecofisiològics són ^{13}C, ^{15}N, ^{18}O i ^{2}H, cosa que s'explica per la seva importància en processos fisiològics.

La relació isotòpica (R) és el quocient entre el contingut en l'isòtop pesant i el lleuger:

$$R = \frac{^{13}C}{^{12}C}$$

La composició isotòpica d'una mostra (δ) és la diferència relativa de la relació isotòpica de la mostra comparada amb la d'un estàndard, i s'expressa en tant per mil (‰):

$$\delta = \frac{R_{mostra}}{R_{estàndard}} - 1 * 1000$$

L'abundància natural dels isòtops estables és la quantitat en la qual es troben en la natura. Aquesta pot proporcionar informació ecofisiològica molt interessant, ja que és una integradora natural de processos ecològics (Dawson et al. 2002). Per exemple, l'abundància natural del δ^{13}C és una aproximació a l'eficiència en l'ús de l'aigua (EUA) i també pot proporcionar informació sobre vies metabòliques (p. ex. metabolisme C_3 *vs.* C_4; Brüggemann et al. 2011). L'abundància natural del δ^{15}N és una integradora de múltiples processos, i pot estar influenciada per la font de N (Michelsen et al. 1998), la disponibilitat d'aquest (Dawson et al. 2002), o per les associacions micoríziques i l'activitat d'aquestes (Hobbie et al. 2000; Emmerton et al. 2001), entre d'altres (vegeu la revisió feta per Evans 2001).

De vegades, els isòtops estables es poden emprar com a marcadors o traces per estudiar el destí d'una substància dins la planta (és a dir, el seu transport, distribució o transformació). Per aquest propòsit, les metodologies que utilitzen isòtops artificialment alterats són especialment útils. Una tècnica molt estesa per a l'estudi de la dinàmica de la distribució de C són els experiments de pols i seguiment (en anglès *pulse-chase*), en què C enriquit o empobrit en ^{13}C (en qualsevol cas, significativament diferent de l'abundància natural) és proporcionat a les plantes perquè l'assimilin, de manera que la seva destinació i la seva abundància es poden traçar posteriorment a través dels assimilats i altres components (vegeu la revisió d'Epron et al. 2012). Una aproximació força utilitzada és l'ús del CO_2 enriquit en ^{13}C. Un cop les plantes l'han assimilat durant un període de temps determinat (fase de pols), es traça dins seu (fase de seguiment). En aquest cas, ens podem referir a l'expressió de la composició isotòpica en percentatge d'excés atòmic. Per això, el δ^{13}C s'ha d'expressar en percentatge d'àtoms ^{13}C de la mostra (àtom%):

$$\text{àtom\%} = 100 * \frac{0.0111802 * (\frac{\delta}{1000} + 1)}{1 + 0.0111802 * (\frac{\delta}{1000} + 1)}$$

Per calcular l'excés atòmic de ^{13}C en cada compartiment o òrgan de la planta, s'empra l'equació següent:

$$\text{percentatge d'excés atòmic } ^{13}\text{C (\%)} = \text{àtom\%}_m - \text{àtom\%}_a$$

on àtom$\%_m$ és el percentatge d'àtoms de ^{13}C de la mostra i àtom$\%_a$ és el percentatge d'àtoms de ^{13}C de la mostra en abundància natural (mesurada en mostres no marcades).

L'espectrometria de masses de relació isotòpica (en anglès *Isotope Ratio Mass Spectrometry*, o IRMS) s'empra per l'anàlisi de la composició isotòpica (δ^{13}C) de la matèria orgànica. Breument, en la font d'ions de l'IRMS, molècules de CO_2 són ionitzades per l'impacte d'un flux d'electrons. Llavors, aquests ions són separats electromagnèticament per les seves masses atòmiques i recollits en col·lectors de Faraday. Finalment, el seu senyal és amplificat per a la detecció. El mateix s'aplicaria per als isòtops de nitrogen i oxigen (gasos N_2 i O_2).

Quan s'acobla un analitzador elemental (*Elemental Analyzer* o EA) amb un IRMS (EA-IRMS) es pot mesurar el contingut de C i N i la seva composició isotòpica (i, en el mode piròlisi, també es poden mesurar H i O) a través de la combustió-reducció de la matèria orgànica. En l'IRMS cal obtenir gasos a partir de la combustió de la matèria orgànica. Els gasos obtinguts amb aquesta combustió són reduïts i, finalment, s'allibera CO_2, N_2 i SO_2. Mitjançant la cromatografia de gasos, aquests gasos se separen a partir del seu temps de retenció i llavors entren a l'IRMS. Les mostres sòlides (matèria orgànica) han de ser preparades abans de poder ser analitzades: cal assecar-les a l'estufa, triturar-les fins obtenir-ne una pols fina i pesar-les en petites càpsules d'estany (aproximadament 1 mg). Aquestes càpsules s'introduiran al mostrejador automàtic de l'EA i llavors començarà l'anàlisi (Ghashghaie i Tcherkez 2013).

Les mostres gasoses no s'han de processar abans de la seva anàlisi. Es poden injectar en un cromatògraf de gasos de combustió acoblat a un espectròmetre de masses de relació isotòpica (en anglès *Gas Chomatography-Combustion-Isotope Ratio Mass Spectrometry*, GC-C-IRMS) que, principalment, s'utilitza per l'anàlisi de compostos específics de C. Si s'utilitza per l'anàlisi de mostres gasoses, la fase de combustió no és necessària (Ghashghaie i Tcherkez 2013).

General introduction

High mountain dwarf shrubs

Dwarf shrubs are woody plant species that reach up to 50 cm height, with creeping or prostrate to erect habit (Körner 2003). At high elevations, harsh climate conditions entail a risk for plant reproduction, since critical phases such as flowering or seed production, dispersal and germination can be interrupted by strong winds, freezing episodes or snowfall at any time during the year. To avoid this risk, many species have adopted mechanisms of vegetative propagation that allow their populations to persist and expand regardless of the adverse conditions (Körner 2003). Clonal dwarf shrubs propagate vegetatively through their subterranean rhizomes or prostrate twigs, from which individual aerial shoots arise, i.e. ramets. Thus, a genetic unit, or genet, may include numerous ramets. These may be connected by rhizomes (i.e. veget), or not. In ecological and ecophysiological plant studies, ramets are usually considered as study units due to the difficulties for identifying different vegets in the field, which would require the digging up of belowground rhizomes and thus, imply severe soil disturbance. Moreover, the identification of different genets would only be possible through the use of genetic techniques, such as those involving microsatellite markers (Mizuki et al. 2005; Araki et al. 2009; Lembicz et al. 2011). Although ramets are used as study units, they do not function as fully independent individuals (even if they may be relatively autonomous), and should not be considered as such.

At the Pyrenean and Alpine high mountain areas, clonal dwarf shrubs are either present in the understorey of subalpine forests (formed by *Pinus mugo* Turra subsp. *mugo* and subsp. *uncinata* (Ramond ex DC.), *Larix decidua* Mill., *Pinus cembra* L. or *Picea abies* (L.) Karsten), forming patches within grasslands or constituting dense shrub communities in the subalpine and lower alpine belts. Thus, they play an important role in the transitional ecotone between subalpine forests and alpine grasslands, the treeline ecotone (Körner 2003; Ninot et al. 2008; Vigo 2008).

Global change at high mountain ecosystems

The concept of global change refers to all the processes of change that are currently taking place at planetary scale. These include environmental transformations and also the human-related activities that are causing these transformations, such as economy, resource use, land use or development, amongst others (Steffen et al. 2004). Climate change specifically refers to changes in climate conditions associated to global change. Although changes in the climate system are intrinsic to our planet and have occurred periodically through geologic time, change rates occurring at present have no precedent in the past decades, centuries and millennia (IPCC 2014).

Land-use changes and shrub encroachment

In the last century, socio-economic and demographic changes at high mountain areas have led to the progressive decline and abandonment of agricultural practices, extensive livestock grazing and tree logging (MacDonald et al. 2000; Roura-Pascual et al. 2005). Land-use change has enhanced shrub species expansion and colonization of subalpine and alpine abandoned pastures and grasslands (Dullinger et al. 2003; Anthelme et al. 2007; Prévosto et al. 2011; Brandt et al. 2013), which are also favoured by climate warming (Rundqvist et al. 2011; Komac et al. 2013; Myers-Smith et al. 2015). This process is known as shrub encroachment.

Several studies have reported negative impacts of shrub encroachment on ecosystem function, structure and biodiversity including loss of species diversity, alteration of the spatial distribution of resources, reduced soil function (such as nutrient cycling or stability) and a challenge for pastoral and livestock activities (Wilson and Nilsson 2009; van Auken 2000; Eldridge et al. 2011; Myers-Smith et al. 2011; Pajunen et al. 2011; Ratajczak et al. 2012; Brandt et al. 2013; Koch et al. 2015). Some studies indicate that outcomes of shrub encroachment may also be positive depending on the shrub cover or the functional traits of the species involved (Soliveres et al. 2014; Eldridge and Soliveres 2014), but they have been performed in arid and semi-arid ecosystems, where vegetation history and mechanisms driving encroachment may differ from those in cold regions (Myers-Smith et al. 2011; O'Connor et al. 2014).

Shrub encroachment can have positive feedbacks to global warming, since shrub canopies absorb more radiation and thus decrease albedo (Sturm et al. 2005a), they trap snow underneath, increasing the snowpack and thus rising soil temperatures (Sturm et al. 2005b), and they increase nocturnal warming through the reflection of the thermal energy emitted by the soil (D'Odorico et al. 2013). However, shrubs can also have negative feedbacks to warming, since decomposition rates are slowed down due to their more recalcitrant litter respect to herbaceous species (Cornelissen et al. 2007), leading to increased soil organic carbon (Montané et al. 2010). Moreover, in summer soil temperatures under shrubs can be lowered by their own shade (Myers-Smith and Hik 2013). Thus, shrub encroachment has drastic implications for the ecosystem C and nutrient cycling (Wookey et al. 2009; Li et al. 2016) and it is very extended in tundra ecosystems, as reported by an extensive review on tundra shrub expansion and its impacts by Myers-Smith et al. (2011).

Therefore, the understanding of shrub species growth and performance is crucial for the understanding of the functioning and dynamics of shrub communities, and the encroachment patterns. Consequently, researchers' interest on these growth forms has increased in the last decades.

Global warming

Due to the emission of greenhouse gases, Earth average temperature is rising, and it is predicted to increase further in the future. By the end of the 21st century, global mean surface temperature could increase between 0.3 and 4.8 °C respect to the period 1986-2005, depending on the extent of emissions and thus on the future greenhouse gas concentrations (Fig. 1; IPCC 2014). At high-altitude and high-latitude ecosystems, warming rates are predicted to be especially large due to increased longwave radiation in response to increased water vapour, and a larger surface absorption of solar radiation due to a reduced snow cover, amongst other factors (Nogués-Bravo et al. 2007; Collins et al. 2013; Rangwala et al. 2013).

At high elevations, plant growth is constrained by low temperatures and short growing season lengths, which are delimited by snow patterns. Moreover, plants may suffer from freezing events throughout the year. Thus, small changes in temperature can have drastic impacts on plant species and plant communities in these areas. In European mountain ranges, especially in the Pyrenees, where projections point to increased warming and decreased summer precipitation, plant species are predicted to be especially threatened by their habitat loss (Engler et al. 2011).

There are numerous studies on the effects of warming (both summer and winter warming) on alpine and Arctic plant communities, and many of them have shown that plant responses are species-specific, even for coexisting species (Klanderud 2008; Hoffman et al. 2010; Bokhorst et al. 2011; Dawes et al. 2011; Little et al. 2015; Yang et al. 2015). This calls for the need of individually assessing species responses to environmental change to understand community and ecosystem dynamics and their future direction.

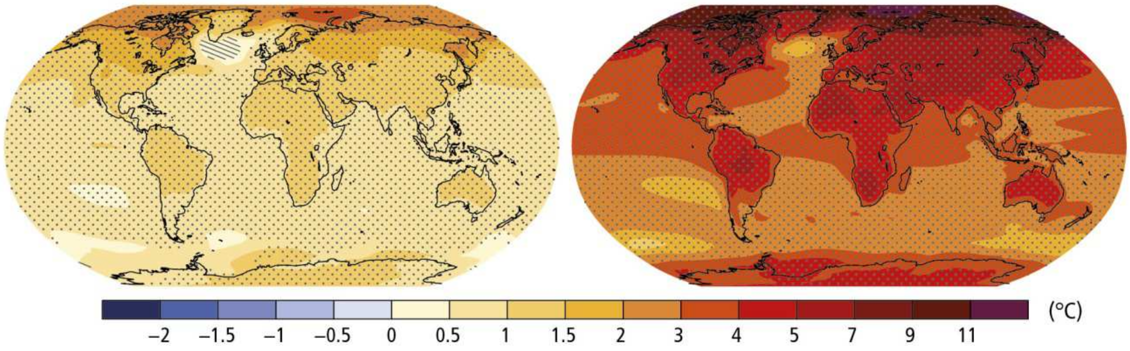

Figure 1. Projected change in the average surface temperature for the period 2081-2100 respect to 1986-2005 based on multi-model mean projections under the scenarios RCP2.6 (stringent mitigation, left) and RCP8.5 (very high greenhouse gas emission, right). Source: IPCC 2014.

Increase of CO_2 concentrations

Atmospheric greenhouse gas concentrations have progressively increased since 1750 due to anthropogenic activities such as fossil fuel combustion or land-use change. Current atmospheric CO_2, CH_4 and N_2O concentrations are considerably higher than the highest concentrations recorded in ice cores for the last 800,000 years. The increase in CO_2 concentrations is the largest contribution to the total anthropogenic radiative forcing, which is a measure of the capacity of gases or other forcing agents to affect Earth energy budget. Atmospheric CO_2 concentrations have increased from *ca*. 280 ppm at pre-industrial times to *ca*. 400 ppm at present (Fig. 2; IPCC 2014).

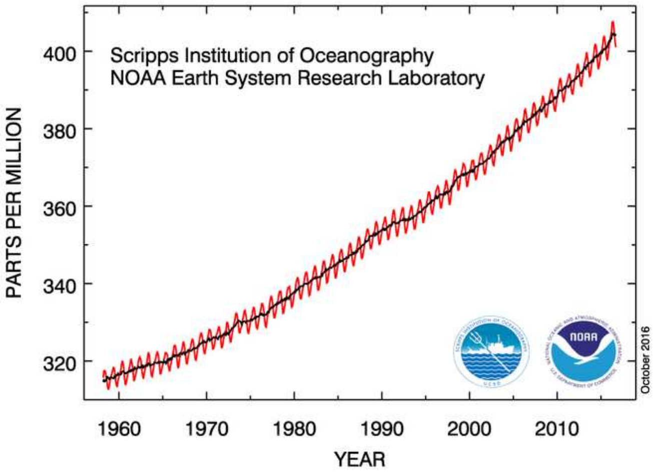

Figure 2. Atmospheric CO_2 concentration increase since the late 1950s to present at the Mauna Loa Observatory, Hawaii. Source: ESRL, NOAA (http://www.esrl.noaa.gov/gmd/obop/mlo/, accessed 28th October 2016).

Many experiments have shown that increased CO_2 concentrations may have a positive effect on plant growth, but this fertilization effect takes place only if other factors such as temperature, light or nutrients are not limiting (Prentice et al. 2001; see reviews by Ainsworth and Long 2005 and Norby and Zak 2011). Free-air CO_2 enrichment (FACE) studies have often reported a photosynthetic acclimation to CO_2 enrichment associated with increased leaf non-structural carbohydrate (NSC) concentrations and reduced leaf nitrogen (N) concentrations (Hättenschwiler and Körner 1996; Handa et al. 2005; Inauen et al. 2012; Bader et al. 2013). The mechanism explaining photosynthetic acclimation is the down-regulation of Rubisco amounts and/or activity (Rogers and Ellsworth 2002). Increased biomass allocation to belowground organs has also been frequently reported in FACE experiments, and has been related to the need for the investment in organs foraging for growth-limiting nutrients and to adjust the C source/sink imbalance caused by an increased assimilation (Hättenschwiler and Körner 1998; Suter et al. 2002; Inauen et al. 2012).

Compared to the large amount of experiments focusing on warming effects on plant species at high-latitude and high-elevation ecosystems, *in situ* experiments on CO_2 enrichment in these areas are scarce, as they involve large economic and logistic investments (see Tissue and Oechel 1987; Gwynn-Jones et al. 1997; Körner et al. 1997; Dawes et al. 2011; Inauen et al. 2012). Contrary to the initial hypothesis that plant growth would be even more stimulated at high elevations under increased CO_2 concentrations due to the lower partial pressure of CO_2 (see Körner 2003), most of these studies found that CO_2 enrichment did not stimulate plant growth (Körner et al. 1997; Hättenschwiler and Körner 1998; Inauen et al. 2012; Dawes et al. 2015). However, positive effects (although sometimes small and transient) have also been reported (Gwynn-Jones et al. 1997; Dawes et al. 2015). Of all FACE experiments performed at high-elevation and high-latitude ecosystems, only those by Dawes et al. (2011) and Gwynn-Jones et al. (1997) have focused on shrubs. This highlights the need for further studies focusing on CO_2 enrichment effects on these growth forms.

Change in precipitation regimes

While temperature projections for this century are consistent, predictions in precipitation regimes are far more uncertain and vary largely between models. Nevertheless, precipitation projections coincide in pointing towards an increased variability of precipitation patterns and an intensification of extreme events (IPCC 2014). In southern and central Europe, a reduction of summer precipitation and an increase in extreme events has been forecasted (Fig. 3; López-Moreno and Benniston 2009; Dai 2013; Osca et al. 2013, Roudier et al. 2016). Drought episodes could have strong impacts on plant photosynthesis and respiration and ultimately lead to drastic changes in the terrestrial C cycle (Reichstein et al. 2013; Frank et al. 2015; Yuan et al 2016).

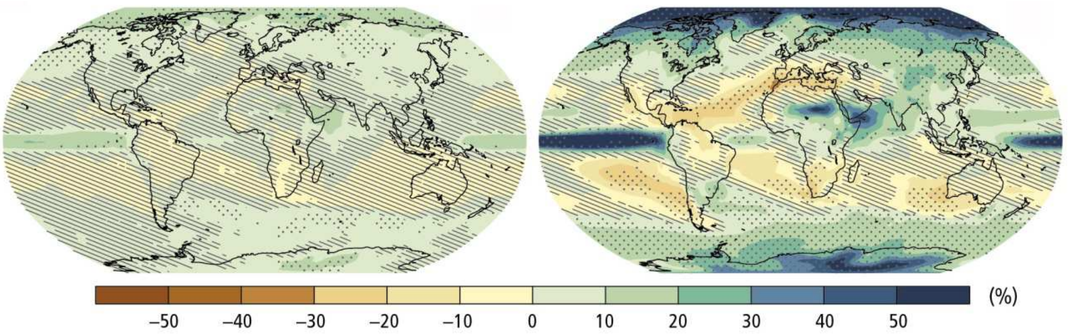

Figure 3. Projected average precipitation change for the period 2081-2100 respect to 1986-2005, based on multi-model mean projections under the scenarios RCP2.6 (stringent mitigation, left) and RCP8.5 (very high greenhouse gas emission, right). Source: IPCC 2014.

While numerous recent studies have focused on the effects of drought on grassland ecosystems at high elevations (Fuchslueger et al. 2014, 2016; Hasibeder et al. 2015; Leitinger et al. 2015; De Boeck et al. 2016, amongst others), few studies have dealt with drought effects on shrubs at these areas. In fact, only few studies have focused on the effects of drought on shrub species at cold ecosystems. Studies on ericaceous shrublands across an environmental gradient in Europe showed that experimental drought had negative effects on flowering, plant biomass, leaf gas exchange rates and tissue phosphorous concentrations (Llorens et al. 2004; Peñuelas et al. 2004). Moreover, drought had negative effects on soil processes such as respiration or nitrification, whereas consistent trends on litter decomposition were not found (Emmett et al. 2004). However, the magnitude of the drought effects reported in these studies differed between plant species and study sites within the gradient. Another study in a temperate heath found that the photosynthetic capacity of *Calluna vulgaris* was reduced under drought (Albert et al. 2011). All these studies point to the negative effects of drought on shrub performance, but a drought experiment in northern Wales (UK) showed that leaf gas exchange rates of the dwarf shrub *Vaccinium myrtillus* were not altered by drought (Llorens et al. 2002). Thus, further knowledge on the response to drought of temperate and high-altitude shrubland communities is needed.

Plant-plant interactions

Plant-plant interactions are one of the main factors influencing plant community dynamics (Callaway and Walker 1997). These interactions may be neutral, positive (e.g. facilitation) or negative (e.g. competition, allelopathy), and they are not exclusive, since there is a complex net of processes occurring between different individuals. Plant-plant interactions are strongly linked with the environment and can determine the response of plant communities to climate change (Brooker 2006). Plant interactions may occur between neighbours of the same species (intraspecific interactions) or neighbours from different species (interspecific interactions). Following the coexistence theory, intraspecific competition is expected to be stronger than interspecific competition, as individuals from the same species show a complete niche overlap (Chesson 2000). However, there are studies that have found that conspecific facilitation can supersede competition at moderate to high levels of stress (Chu et al. 2008; Fajardo and McIntire

2011). In clonal plants, intraspecific interactions may occur between ramets of different clones and ramets of the same clone. Within a veget, there is a physiological integration between ramets, which allows ramets occupying resource-rich patches to share these resources with those growing in resource-poor patches (see the review by Hutchings and Wijesinghe 1997 and the meta-analysis by Song et al. 2013). Indeed, interactions between ramets of the same veget may be confounded with mere physiological resource allocation patterns (Fajardo and McIntire 2011).

The stress-gradient hypothesis (SGH, Bertness and Callway 1994) postulates that positive interactions are dominant in stressful environments, whereas negative interactions dominate in more benign conditions. Many studies across environmental gradients in cold regions or through experimental manipulations of environmental conditions have evidenced shifts from facilitation to competition, and vice versa, in support of the SGH (Choler et al. 2001; Klanderud 2005; Pugnaire et al. 2015 and Wheeler et al. 2015, amongst others). However, other studies in a wide range of ecosystem types have not found evidence to support it, since the nature of the environmental gradient, the species ontogeny or the magnitude of the environmental stress also have a strong influence on the interaction between plant species (Tielbörger and Kadmon 2000; Miriti 2006; Maestre and Cortina 2004; Maestre et al. 2009; Armas et al. 2013; Le Roux et al. 2013, Grau 2014). Moreover, coexisting species might show different adaptations to the environment, and particular conditions that might be relatively stressful for some species might not be so for others. In their review on plant-plant interactions, Soliveres and Maestre (2014) suggested that a single conceptual model could difficultly explain plant-plant interactions across significantly different environmental gradients. Additionally, they acknowledged that the relative influence of facilitation as an ecosystem driver needs to be better understood. In any case, shifts in plant species interactions may be expected if changes in environmental conditions are favourable for one species but neutral or detrimental for coexisting neighbours (Kudo and Suzuki 2003; Dormann et al. 2004; Niu and Wan 2008; Little et al. 2015; and see review by Tylianakis et al. 2008), leading to changes in plant dominance and ultimately in plant community composition, structure and function (Dormann et al. 2004; Stevens et al. 2004; Walker et al. 2006; Wookey et al. 2009).

Objectives of the Thesis

The **main objective** of this Thesis is to gain insight on the growth, physiological and phenological responses of high mountain dwarf shrubs, especially the widespread species *Vaccinium myrtillus* and *Vaccinium uliginosum*, to the ongoing and projected climate change. This Thesis also aims to have a wider view of their responses through their study at two important mountain ranges in Europe, the Pyrenees and the Alps. In particular, areas of interest are comprised within the upper subalpine belt, including the treeline ecotone. The understanding of plant species responses to environmental change, with its implicit anthropogenic influence, is the first step for further understanding and predicting community dynamics and ecosystem responses to future environmental conditions.

These main objectives may be subdivided into the following **specific objectives**:

1. To determine whether the structure and functioning of the subalpine dwarf shrub *Vaccinium myrtillus* are affected by the co-occurrence with the shrub species *Vaccinium uliginosum* and *Rhododendron ferrugineum* and whether warming has an effect on the interaction between these species.
2. To analyse warming effects on the phenology and growth of the co-occurring dwarf shrub species *V. myrtillus*, *V. uliginosum* and *Empetrum hermaphroditum*.
3. To evaluate warming and CO_2 enrichment effects on *V. myrtillus* growth and xylem anatomy, as well as on the links between these parameters.
4. To gain insight into the short-term C allocation of the co-occurring *V. myrtillus* and *V. uliginosum* and to analyse the effects of drought on this function.

Structure of the Thesis

This Thesis is structured in three different parts, which coincide with the different study areas.

1. Effects of air warming on plant-plant interactions at the treeline ecotone

The first part focuses on plant-plant interactions, in particular on the interactions between *V. myrtillus* and two neighbour shrubs, *V. uliginosum* and *R. ferrugineum*, and on the effects of warming on these interactions. **Chapter 1** is a more descriptive study, where the effects of the coexistence with the two neighbour shrubs on *V. myrtillus* growth and performance have been assessed. This work, in which environmental variables were not manipulated, is an introduction for the following chapter. **Chapter 2** corresponds to an experimental study with open-top chambers (OTC), which increased air temperature by 1-2 °C (Fig. 4). This chapter focuses on the effects of warming on the interaction between *V. myrtillus* and the two neighbour shrubs. We measured aboveground and belowground biomass, C and N concentrations and their isotope composition in different organs of *V. myrtillus* under the different coexistence scenarios and warming treatments. In plots where *V. uliginosum* was present, we evaluated similar parameters in this species (biomass, C and N concentration and isotope composition). We also monitored *V. myrtillus* phenology and possible changes in the functional diversity of the microbial community with warming. Both studies were carried out at Eth Corrau des Machos, la Val d'Aran, in the peripheral area of the Aigüestortes i Estany de Sant Maurici National Park, in the Pyrenees.

Figure 4. Open-top chamber (OTC).

2. Effects of soil warming and CO_2 enrichment on dwarf shrubs phenology, growth and xylem anatomy at the treeline

Chapter 3 focuses on the study of the effects of soil warming (+ 4 °C) with heating wires on the phenology and growth of *V. myrtillus*, *V. uliginosum* and *E. hermaphroditum* co-occurring in the same plots in the understorey of two different tree species (*Pinus mugo* subsp. *uncinata* and *Larix decidua*). **Chapter 4** deals with the effect that soil warming and a free-air CO_2 enrichment (FACE) experiment (+ 200 ppm) had on the xylem anatomy of *V. myrtillus*. These two chapters correspond to the same experimental site, Stillberg, Davos in the Swiss Alps, where a soil warming and CO_2 enrichment experiment were set up for 12 years (Fig. 5). The CO_2 enrichment experiment run from 2001 to 2009 and the soil warming experiment run from 2007 to 2012.

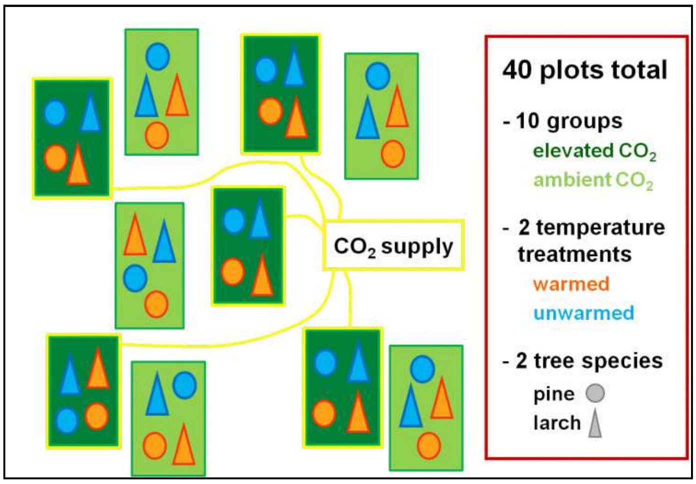

Figure 5. Experimental design of the CO_2 enrichment and soil warming experiments in Stillberg, Davos. Source: Melissa Dawes.

3. Short-term C allocation dynamics in subalpine dwarf shrubs and their response to experimental summer drought

Finally, **chapter 5** focuses on the C allocation in dwarf shrubs. It corresponds to a double labelling experiment with $^{13}CO_2$ (pulse-chase), which was performed to study the short-term C allocation in *V. myrtillus* and *V. uliginosum*. Two experiments were combined, one in the Stubai valley, in the Tyrolean Alps, and the other one in the Filià valley, in the Catalan Pyrenees. In both experiments we compared the C allocation dynamics between *V. myrtillus* and *V. uliginosum* at natural environmental conditions in the subalpine belt. Moreover, in Stubai the labelling was applied after a drought experiment with rain-out shelters, and we compared the C allocation dynamics of these two species between plants under a water shortage and plants under natural irrigation (Fig. 6, 7). With the use of stable isotope analyses we chased the ^{13}C assimilated by the two shrubs in their bulk plant material and in their respiration.

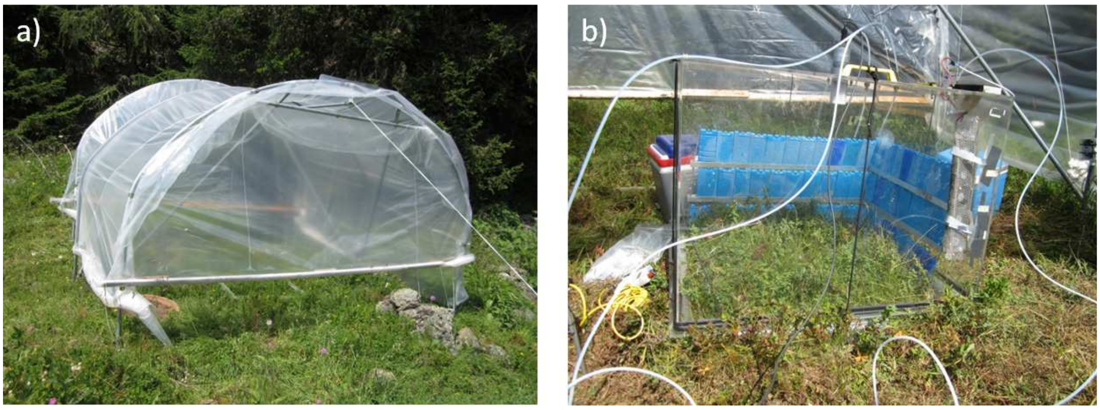

Figure 6. (a) Rain-out shelter and (b) pulse-labeling chamber.

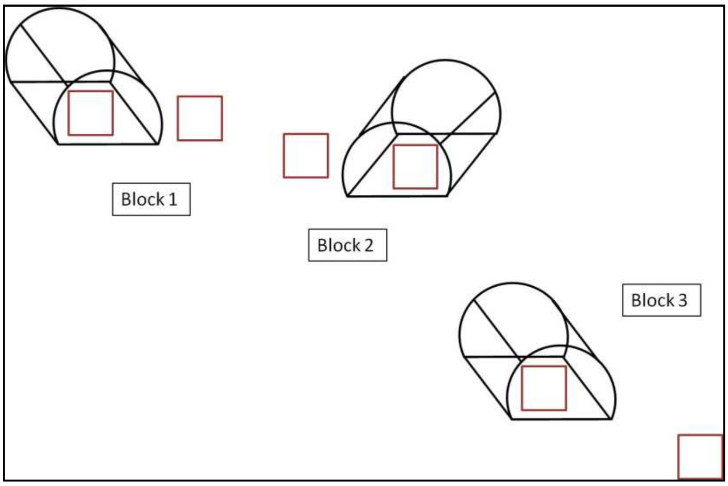

Figure 7. Experimental design of the drought experiment in Stubai in 2014.

The different chapters of this Thesis have been carried out in different locations with different research groups. This explains that some chapters are written in American English whereas others are written in British English. Other small formalities might also differ between chapters. A summary of the main treatments applied and variables studied is included in Fig. 8.

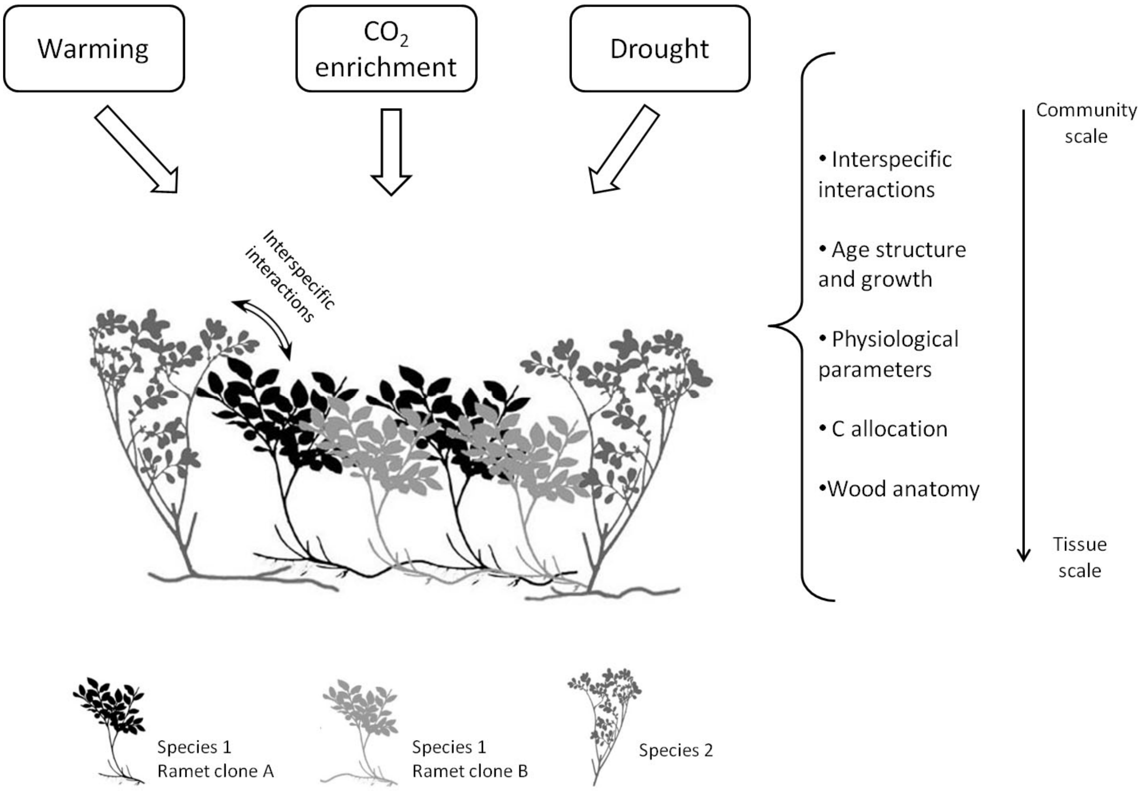

Figure 8. Applied treatments and main parameters and processes analysed in this Thesis.

Study species, sites and methodologies

Study species

The main study species in this Thesis is *Vaccinium myrtillus* L., the bilberry (Fig. 9). It is a deciduous ericaceous clonal dwarf shrub that reaches 10-60 cm height (Flower-Ellis 1971). *Vaccinium myrtillus* new shoots are photosynthetic until they reach *ca*. 4 years old, when they lose their green colour. These photosynthetic shoots have an important role, as they represent a large portion of the total photosynthetic area and allow *V. myrtillus* to fix C when leaves are already shed. Its rhizomes are found 15-20 cm deep in the soil (Ritchie 1956) and can expand several meters in length (Flower-Ellis 1971). It is distributed through the boreal and temperate zones of the Holarctic region. In the Pyrenees, its distributional area ranges from the lower montane belt (*ca*. 900 m a.s.l.) until the highest areas of the subalpine belt, sometimes reaching the alpine belt (*ca*. 2450 m a.s.l.; Bolòs and Vigo 1995; Bolòs et al. 2005). In the Alps, it grows from the colline to the alpine belts (Aeschimann et al. 2004). Therefore, it encounters its upper distributional limit at the transitional ecotone between the subalpine and the alpine belts. In northern Europe it is present at lower altitudes, in heaths, moors and woods (Popova 1972). It grows in acid substrates from open ecosystems to shrublands and forests.

Figure 9. Image of *Vaccinium myrtillus*.

The second most studied species in this Thesis is the congeneric ericaceous clonal dwarf shrub *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. (hereafter *V. uliginosum*), the bog blueberry (Fig. 10). Like *V. myrtillus* it is a rhizomatous and deciduous species. However, leaves have a darker green colour and its shoots are not photosynthetic. It reaches 5-20 cm height (Aeschimann et al. 2004; Bolòs et al. 2005) and has an Arctic-alpine distribution. It grows at higher elevations than *V. myrtillus*, from the subalpine to the alpine belts, although it may also be found in the montane belt (Bolòs and Vigo 1995; Aeschimann 2004; Bolòs et al. 2005). It also grows in acid substrates, ranging from open grasslands to heathlands. It is more heliophilous than the preceding congeneric species (Jacquemart 1996). In the Alps, the synonym *Vaccinium gaultherioides* Bigelow is used for this taxon. In this Thesis, the name accepted in Flora Europaea (*V. uliginosum* subsp. *microphyllum*, Popova 1972) is used in the Introduction and Discussion sections, in chapters corresponding to experiments performed in the Pyrenees (chapters 1 and 2) and in chapter 5, which combines experiments in the Pyrenees and the Alps. The name *V. gaultherioides* is used in chapters corresponding to experiments performed in the Swiss Alps.

Figure 10. Image of *Vaccinium uliginosum*.

The third study species, only present in one chapter, is *Empetrum nigrum* L. subsp. *hermaphroditum* (Hagerup) Böcher (hereafter *Empetrum hermaphroditum*), the mountain crowberry (Fig. 11). It is an evergreen ericaceous clonal dwarf shrub, which was traditionally classified within the family Empetraceae. It has ericoid leaves and reaches 15-30 cm height. It is distributed through the boreal and temperate zones of the Holarctic region. In the Pyrenees it is quite rare, and grows from the upper parts of the subalpine to the alpine belt (between 2100 and 2850 m a.s.l.; Bolòs and Vigo 1995; Bolòs et al. 2005). In the Alps it also ranges from the subalpine to the alpine belt, and it is more common (Aeshimann et al. 2004). It also grows in acid substrates.

Figure 11. Image of *Empetrum hermaphroditum*.

Finally, it is worth mentioning the ericaceous *Rhododendron ferrugineum* L., the alpenrose (Fig. 12). It has not directly been studied in any of the experiments carried out throughout this Thesis, but it is a companion species of *V. myrtillus* in chapters 1 and 2, in which their interaction is assessed. *Rhododendron ferrugineum* is a bigger shrub, of 30-70 (150) cm height. It is an evergreen species, with thick dark green leaves, and it is also clonal. It grows in areas frequently covered by snow in winter, as it relies on snow protection (Neuner et al. 1999; Komac et al. 2016). It is distributed through the South European mountain systems (Aeshimann et al. 2004).

Figure 12. Image of *Rhododendron ferrugineum*.

Study sites

Four different study sites were included in this Thesis. Two of them, Eth Corrau des Machos and Filià, are located in the Pyrenees, and the other two, Stillberg and Stubai, in the Alps (Fig. 13).

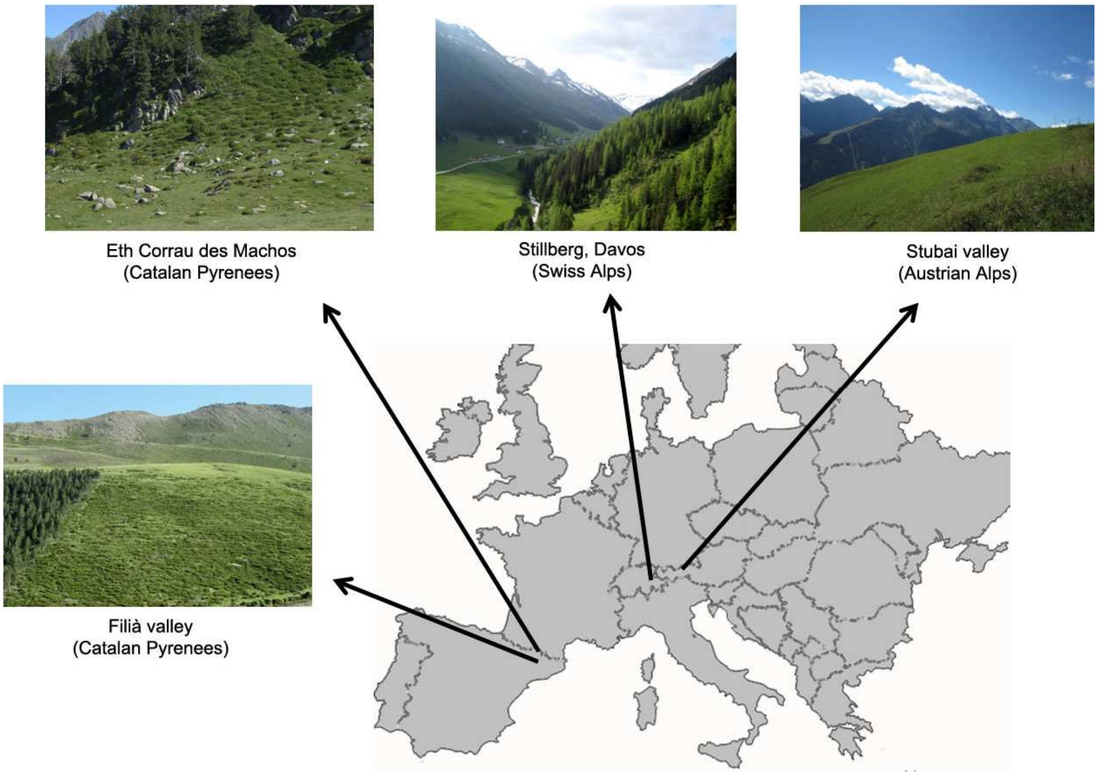

Figure 13. Location map and pictures of the study areas.

Eth Corrau des Machos lies in the Central Pyrenees, in the buffer zone of the Aigüestortes i Estany de Sant Maurici National Park, in La Val d'Aran, Catalonia (42° 36' 48'' N, 0° 54' 54'' E). At this site, located on a N-facing 10-25° steep slope at 2250 m a.s.l., soil is poorly developed, hummus-rich and acid, derived from granodiorite outcrops and debris. Vegetation is composed of *Festuca eskia* Ramond ex DC. and *Nardus stricta* L. grasslands (Selino-Festucetum eskiae) mixed with patches of dwarf shrub heath, dominated by *V. myrtillus*, *V. uliginosum* and *R. ferrugineum*. For the period 2001-2013, mean annual precipitation was 1146 mm and mean annual temperature was 3.0 °C (data from a nearby climate station at La Bonaigua, 2266 m a.s.l., provided by the Meteorological Service of Catalonia).

The study site Filià is also located in the Central Pyrenees, in the Filià valley, Pallars Jussà, Catalonia (42° 27' 41" N, 0° 57' 40" E). It corresponds to a dwarf shrub heath on a N-facing 28° steep slope, at 1900 m a.s.l. Soil is rocky, hummus-rich and acid, on Cambro-Ordovician schist. The dominant shrub species are *V. myrtillus*, *V. uliginosum, R. ferugineum* and *Calluna vulgaris*. Forbs and grasses are also abundant, amongst which the most common are *Festuca nigrescens*, *Deschampsia flexuosa*, *Anthoxantum odoratum*, *Nardus stricta*, *Luzula multiflora*, *Alchemilla* gr. *alpina*, *Galium verum*, *Trifolium alpinum* and *Achillea millefolium*. From 1951 to 2002, mean annual temperature was 3.7 °C and mean annual precipitation was 1297 mm. For the period 2013-2015, mean annual temperature was 5.5. °C and mean annual precipitation 1259 mm (data from a nearby climate station at Estany Gento, 2120 m a.s.l., provided by Meteopirineus and the Spanish National Institute of Meteorology).

The Stillberg treeline site is located in the Central Alps, near Davos, Switzerland (46° 46' N, 9° 56' E). Stillberg is a 5-ha long-term afforestation research area where tree seedlings were planted in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). The area corresponding to the experiment presented in this Thesis reached 2500 m^2 on a NE-facing 25-30° steep slope at 2180 m a.s.l., slightly above the current treeline in the region. Soil has a thick organic layer (5-20 cm), and is derived from siliceous paragneis parent material (Hagedorn et al. 2010). The tree species were *Pinus mugo* subsp. *uncinata* (DC.) Domin and *Larix decidua* Mill. The understorey vegetation consisted of a dense cover of the dwarf shrub species *V. myrtillus*, *V. uliginosum* and *E. hermaphroditum*, and numerous grasses and forbs, including *Deschampsia flexuosa*, *Gentiana punctata*, *Homogyne alpina*, *Leontodon helveticus* and *Melampyrum pratense* (Dawes et al. 2011). From 1975 to 2012, the mean annual precipitation was 1155 mm and the mean annual air temperature was 2.1 °C (data from a WSL micrometeorological station at the study site).

The Stubai study site is located at a subalpine grassland colonized by dwarf shrub patches (1820 m a.s.l.) on siliceous bedrock in the Stubai valley, Austrian Central Alps (47° 7' 45" N, 11° 18' 20" E). Vegetation is composed of the dominant dwarf shrub species *V. myrtillus* and *V. uliginosum* and other dwarf shrub species (i.e. *Calluna* *vulgaris* and *Vaccinium vitis-idaea*), as well as numerous grasses and forbs: *Agrostis capillaris*, *Festuca ovina*, *Deschampsia flexuosa*, *Briza media*, *Luzula multiflora*, *Campanula scheuzeri*, *Chaerophyllum hirsutum*, *Crepis conyzifolia*, *Hypericum maculatum* and *Potentilla erecta*, amongst others. Mean annual temperature is 3 °C and mean annual precipitation is 1100 mm (data from a meteorological station at the study site).

Methodologies used for the study of dwarf shrub responses to environmental change

The distinct approaches for the study of dwarf shrub responses to climate change in this Thesis explain that many different methodologies have been used. Each chapter includes several study parameters and techniques for which different protocols were followed. In this section, brief explanations of the main methodologies used to answer the principal questions related to the dwarf shrub phenological, physiological, anatomical and growth responses to environmental change are included.

Phenological surveys

Plant phenology is the study of the time course of developmental events that take place during the life cycle of a plant. It involves the observation and recording of the timing of their life history events, or the events occurring during a certain period of time. Phenology is highly responsive to temperature changes (Walther 2003), and early-season phenology has been shown to be more responsive than other phenological events taking place later in the growing season (Sparks and Menzel 2002). At high-elevation ecosystems, warmer temperatures can also have indirect effects on early-season phenology through an earlier snowmelt (Wipf et al. 2009; Lambert et al. 2010; Livensperger et al. 2016; Semenchuk et al. 2016). The advancement of vegetative phenology with warming has been related to growth increases in Arctic and alpine plant species (Arft et al. 1999), although other studies have not found such relationship (Livensperger et al. 2016). Moreover, an earlier bud burst after earlier snowmelt dates may induce plants to suffer from early-season frosts in such a critical developmental stage, which may have negative effects on plant growth and performance (Wipf et al. 2009; Wheeler et al. 2014).

In comparative studies dealing with plant species phenology under different environmental conditions it is especially important to capture differences between treatments that might take place within one or two days only. Therefore, phenological surveys should take place at least *ca*. three times a week, especially in spring. If such an exhaustive phenological survey is not possible, a different approach should be taken. Then, instead of tracking changes in phenology through time, differences at specific visit dates should be recorded.

Phenology may be assessed following different methodologies, such as marking specific individuals and subsequently monitoring them through time, recording the average phenological status of all plants within a plot or recording the first date when an individual within a plot shows a phenological change. In this Thesis, phenological assessments were made on the same ramets, which provides more detail on phenological changes within a functional unit through time. Vegetative phenology has been studied in more detail for two reasons. First, due to the importance of vegetative performance in the clonal dwarf shrub species studied. Flowering and fruiting are essential for population genetic variability and thus, for its adaptation and survival at the long term (Holsinger 2000; Jump and Peñuelas 2005), but the ability of clonal shrubs to propagate vegetatively confers their vegetative apparatus a great importance for population dynamics. Second, at Eth Corrau des Machos, fruit setting was anecdotal throughout the two years of study (2011-2012).

Biomass and growth measurements

Studies on clonal shrubs usually focus on their aboveground parts only. This is explained by the difficulties on assessing belowground biomass, growth and performance, as rhizomes form a complex network that expands several meters away from the centre of the stand. It is not always feasible to unbury belowground rhizomes and roots, especially in studies lasting for several years, in which disturbances caused in the soil would prevent us from taking future measurements. For biomass measurements, plants must be harvested, oven-dried and weighed. This provides information on plant status and allows inferring population aboveground biomass. Nevertheless, since biomass measurements only provide information at a single time point, growth rates or productivity should be evaluated by other measurements. Growth parameters measured in this Thesis are leaf and current-year shoot production (relative to total aboveground biomass or to total ramet biomass), shrub height and xylem ring width increments (see next section). Moreover, an interesting measure for shrub growth that does not involve destructive techniques is the annual shoot elongation. Several studies on dwarf shrub species have used shoot elongation measurements as plant growth proxies (Bret-Harte et al. 2002; Kudo and Suzuki 2003; Dawes et al. 2011; Campioli et al. 2012).

Dendrochronology and quantitative wood anatomy in dwarf shrubs

Dendroecology is the discipline within dendrochronology that uses dated tree rings for ecological studies (Kaennel and Schweingruber 1995). Due to the small size of dwarf shrubs, ring counting must be performed on microscope slides from cross-sections of stems or rhizomes carefully prepared. Stem/rhizome cross-sections of 10-20 µm can be cut with a sledge microtome (Fig. 14a) and stained with a mixture of safranin and astrablue to emphasize the structure of the growth rings. For their preservation, it is necessary to dehydrate the samples by rinsing them with increasingly concentrated ethanol solutions (75%, 96%, 100%) and Xylol (Fig. 14b). Finally, to mount them, they are imbedded in Canada-Balsam and dried for 24h at 60 °C (Gärtner & Schweingruber 2013).

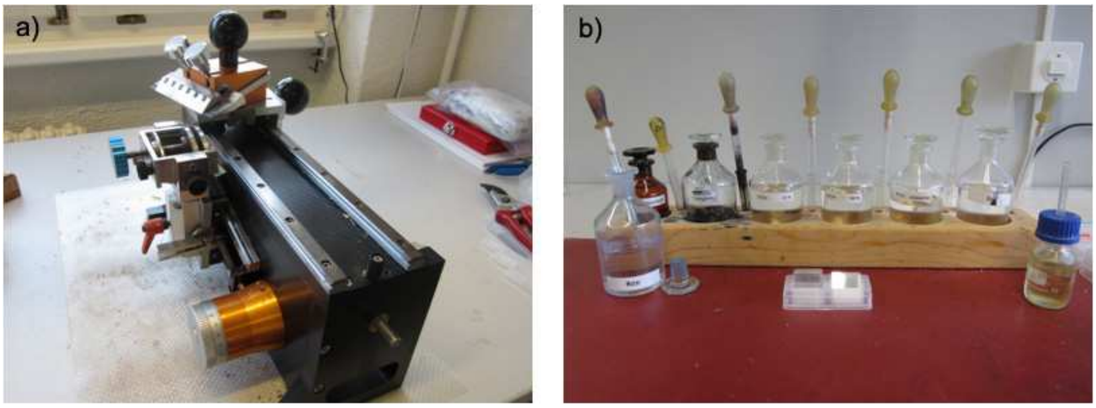

Figure 14 (a) Sledge microtome at the Swiss Federal Institute for Forest, Snow and Landscape Research facilities (WSL, Birmensdorf, Switzerland). (b) Material for dehydration of plant sample cross-sections.

Once microscope slides are prepared, it is necessary to photograph them for posterior analyses. For this, a digital camera should be mounted on a microscope. Once pictures are prepared and scaling is under control, these images can be used for ring counting. Specialized software is used to digitally cross-date samples and find missing rings. However, when series are too short, using software is not recommended, since correlations may be wrong (Grissino-Mayer 2001). In these cases, such as in this Thesis, where most of *V. myrtillus* ramets were younger than 20 years old (and many of them younger than 12), cross-dating must be performed manually.

The same digital pictures may be used for quantitative wood anatomy measurements. Quantitative wood anatomy can provide insights into the relationship between the quantitative variability in xylem anatomy and plant growth and function, and how the structure-function relationships are affected by the environment (Fonti et al. 2010; Fonti and Jansen 2012; Schweingruber et al. 2013; von Arx et al. 2016). Quantitative assessments of vessel lumen size, spatial arrangement and density, and quantification of ray parenchyma can provide knowledge on plant water conductive and storage capacity and how these parameters adjust to changes in the environment. Thus, quantitative wood anatomy is a valuable approach for the study of plant responses to environmental change over time. In this Thesis, I used the software WinCELL to measure ring widths (chapter 3) and ROXAS (von Arx et al. 2013; Wegner et al. 2013) for the analysis of quantitative wood anatomy (chapter 4, Fig. 15).

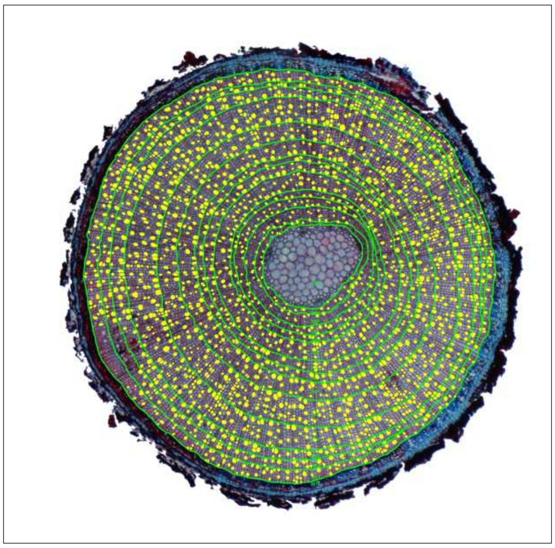

Fig 15. Digital image of *V. myrtillus* cross-section during its analysis with the software ROXAS. Vessels are filled in yellow and annual ring borders are drawn in green. Scale is not shown during the analysis process.

Use of stable isotopes and pulse labelling for the study of C allocation dynamics

Stable isotopes techniques are a powerful tool for the study of the relationship between plants and the environment. They may serve as tracers of assimilates or nutrient dynamics within plants and may be used as integrators of plant interactions with the environment (Dawson et al. 2002). Isotopes of the same element are atoms with the same atomic number but different atomic masses (thus, different neutrons). Isotope fractionation is the change in the partitioning of heavy and light isotopes between a source and a substrate, and isotope discrimination is the biologically mediated isotope fractionation (Dawson et al. 2002). Fractionation occurs because the heavy isotopes react at slower rates than light isotopes and have bonds that are more difficult to break. For example, in the photosynthetic process, discrimination against the heavy isotope ^{13}C occurs during diffusion of CO_2 through stomata and during carboxilation by Rubisco (Farquhar et al. 1989; Lambers et al. 2008). This explains that plant organic matter is ^{13}C depleted respect to atmospheric CO_2. The most common stable isotopes used in ecophysiological studies are ^{13}C, ^{15}N, ^{18}O and ^{2}H, due to their relevance in plant physiological processes.

The isotope ratio (R) is the quotient of the content in the heavy isotope to that in the light isotope:

$$R = \frac{^{13}C}{^{12}C}$$

The stable isotope composition of a sample (δ) is the relative difference of the sample isotope ratio compared to those of a standard, and is expressed in per mil (‰):

$$\delta = \frac{R_{sample}}{R_{standard}} - 1 * 1000$$

Stable isotopes at naturally occurring levels are called stable isotopes at natural abundance. Stable isotope composition at natural abundance can provide interesting ecophysiological information as they are natural integrators of ecological processes (Dawson et al. 2002). For example, natural abundance $\delta^{13}C$ is a proxy for intrinsic water use efficiency and can also give information on metabolic pathways (e.g. C_3 *vs*. C_4 plants; Brüggemann et al. 2011). Natural abundance $\delta^{15}N$ is an integrator of multiple processes, and can be influenced by the N source (Michelsen et al. 1998), N availability (Dawson et al. 2002) or mycorrhizal associations and their fractionation activity (Hobbie et al. 2000; Emmerton et al. 2001), amongst others (see review by Evans 2001).

Sometimes, stable isotopes may be used as tracers to study the fate of a substance within the plant (i.e. its transport, allocation or transformation). For this purpose, methodologies involving artificially altered isotopes are a powerful tool. A common technique for the study of C allocation dynamics is the pulse labelling, in which C either enriched or depleted in ^{13}C (significantly different than the naturally occurring level) is supplied to the study plants, so its fate and abundance within the plant can be traced through the assimilates and other components (see review by Epron et al. 2012). A common approach is the use of ^{13}C-enriched CO_2, where plants are left to assimilate this enriched C during a period of time (pulse phase), and later on it is traced through the plant (chase phase). In this case, the expression of the isotopic composition may be referred to in atom% excess. For this, $\delta^{13}C$ needs to be expressed in atom% of the sample:

$$\text{atom\%} = 100 * \frac{0.0111802 * (\frac{\delta}{1000} + 1)}{1 + 0.0111802 * (\frac{\delta}{1000} + 1)}$$

To calculate the ^{13}C atom% excess in each compartment, the following equation is used:

$$\text{atom\% excess } ^{13}\text{C} = \text{atom\%}_s - \text{atom\%}_b$$

where atom%$_s$ is the atom% of the sample and atom%$_b$ is the atom% of the sample at natural abundance (measured in unlabelled samples).

Isotope Ratio Mass Spectrometry, or IRMS, is used for the analysis of the isotope composition (δ^{13}C) of plant organic matter. In brief, in the source of IRMS, CO_2 molecules are ionised by the impact of an electron flow. Then, these ions are electromagnetically separated by their isotopic masses and collected in Faraday cups. Finally, their signal is amplified for detection. The same would apply for nitrogen and oxygen isotopes (gases N_2 and O_2, respectively).

When coupling an Elemental Analyser (EA) with an IRMS (EA-IRMS) we can measure the C and N content and their isotope composition (and in the pyrolysis mode, also H and O) through the combustion-reduction of the organic matter. Inside IRMS, a pure gas must be obtained by the combustion of organic matter. Gases obtained by this combustion are reduced and, finally, CO_2, N_2 and SO_2 are released. With the use of gas chromatography, these gases are separated based on their retention time, and then they enter the source of the IRMS. Solid samples (i.e. organic matter) must be prepared before analysing them: they need to be oven-dried, ground to a fine powder and weighed (*ca*. 1 mg) in small tin capsules. These capsules will be introduced into an autosampler in the EA, and then the analysis will start (Ghashghaie and Tcherkez 2013).

Gas samples do not need to be processed before their analysis. They may be injected into a Gas-Chromatography-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS), which is mainly used for compound-specific isotope ratio analyses. If it is used for the analysis of gas samples, the combustion phase will not take place (Ghashghaie and Tcherkez 2013).

Capítols

Chapters

Informe dels directors del factor d’impacte de les publicacions presentades

El Dr. Salvador Nogués i la Dra. Sara Palacio, directors de la Tesi Doctoral de l'Alba Anadon Rosell, presenten el següent informe sobre el factor d’impacte de les publicacions que formen part de la present memòria.

Capítol 1

Vaccinium myrtillus stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline

Anadon-Rosell A., Palacio S., Nogués S., Ninot J.M.

Plant Ecology (2016) 217: 1115-1128, doi: 10.1007/s11258-016-0637-2

Factor d’impacte (2015): 1.49

Posició dins l’àrea: Plant Sciences 92/209 (segon quartil)

Capítol 2

Four years of experimental warming do not modify the interaction between subalpine shrub species

Anadon-Rosell A., Ninot J.M., Palacio S., Grau O., Nogués S., Navarro E., Sancho M.C., Carrillo E.

Acceptat a *Oecologia* per a la seva publicació.

Factor d’impacte (2015): 2.902

Posició dins l’àrea: Ecology 44/150 (segon quartil)

Capítol 3

Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline.

Anadon-Rosell A., Rixen C. , Cherubini P., Wipf S., Hagedorn F., Dawes M.A.

PLoS ONE (2014) 9: e100577, doi:10.1371/journal.pone.0100577

Factor d'impacte (2014): 3.234

Posició dins l'àrea: Multidisciplinar 9/57 (primer quartil)

Els tres primers capítols de la Tesi Doctoral de l'Alba Anadon Rosell han estat publicats o acceptats en revistes científiques d'àmbit internacional que consten al Science Citation Index (SCI). Els capítols 4 i 5, que encara no han estat enviats, també s'enviaran en revistes científiques d'àmbit internacional que constin en el SCI.

Barcelona, gener de 2017

Salvador Nogués Mestres	Sara Palacio Blasco
Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals	Instituto Pirenaico de Ecología (IPE-CSIC)
Universitat de Barcelona	

Informe dels directors de la participació de la doctoranda en les publicacions

El Dr. Salvador Nogués i la Dra. Sara Palacio, directors de la Tesi Doctoral de l'Alba Anadon Rosell, presenten el següent informe sobre la contribució de la doctoranda en cadascuna de les publicacions que formen part de la present memòria.

Capítol 1

Vaccinium myrtillus stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline

Anadon-Rosell A., Palacio S., Nogués S., Ninot J.M.

Plant Ecology (2016) 217: 1115-1128, doi: 10.1007/s11258-016-0637-2

La doctoranda va participar activament en el disseny experimental de l’estudi i en l’execució del treball de camp. A més, va liderar el treball de laboratori, l’elaboració i l'anàlisi de les dades, la redacció del manuscrit i les revisions posteriors.

Capítol 2

Four years of experimental warming do not modify the interaction between subalpine shrub species

Anadon-Rosell A., Ninot J.M., Palacio S., Grau O., Nogués S., Navarro E., Sancho M.C., Carrillo E.

Acceptat a *Oecologia* per a la seva publicació

La doctoranda va participar activament en el disseny experimental de l’estudi. A més, va liderar l'elaboració dels protocols de mostreig, l’execució del treball de camp, el treball de laboratori, l’elaboració i l'anàlisi de les dades, la redacció del manuscrit i les revisions posteriors.

Capítol 3

Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline.

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PLoS ONE (2014) 9: e100577, doi:10.1371/journal.pone.0100577

La doctoranda va participar activament en el disseny experimental de l’estudi. A més, va liderar l’execució del treball de camp, el treball de laboratori, l’elaboració i l'anàlisi de les dades, la redacció del manuscrit i les revisions posteriors.

Capítol 4

Linking above-ground growth and functional xylem anatomy in the dwarf shrub *Vaccinium myrtillus* in a CO_2 enrichment and soil warming experiment at treeline

Anadon-Rosell A., Dawes M.A., Fonti P., Hagedorn F., Rixen C., von Arx G.

La doctoranda va participar activament en el disseny experimental de l’estudi. A més, va liderar l’execució del treball de camp, el treball de laboratori, l’elaboració i l'anàlisi de les dades, la redacció del manuscrit i les revisions posteriors.

Capítol 5

Short-term carbon allocation dynamics in subalpine dwarf shrubs and their response to experimental summer drought

Anadon-Rosell A., Hasibeder R., Palacio S., Ingrisch J., Mayr S., Nogués S., Ninot J.M., Bahn M.

La doctoranda va liderar el disseny experimental de l’estudi, l’execució del treball de camp, el treball de laboratori, l’elaboració i l'anàlisi de les dades, la redacció del manuscrit i les revisions posteriors.

Finalment, certifiquem que cap dels coautors dels articles abans esmentats ha utilitzat de manera implícita o explícita aquests treballs per a l’elaboració d’una altra tesi doctoral.

Barcelona, gener de 2017

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Capítol 1 / Chapter 1

Vaccinium myrtillus stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline

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Plant Ecology (2016) 217: 1115-1128

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Citation: Anadon-Rosell A., Palacio S., Nogués S., Ninot J.M. (2016) *Vaccinium myrtillus* stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline. *Plant Ecology* 217: 1115-1128

Resum

Les interaccions planta-planta són factors clau en la dinàmica del límit supraforestal. En aquestes zones, al Pirineu, el subarbust *Vaccinium myrtillus* creix formant claps purs o formant claps mixtos amb *Vaccinium uliginosum* o *Rhododendron ferrugineum*. Formen claps esparsos que colonitzen prats subalpins, i tenen un fort impacte en la seva estructura i funcionament. Vam investigar el paper dels dos arbusts coexistents com a possibles moduladors de l'estructura i funcionament de *V. myrtillus* al Pirineu Central. Vam analitzar la biomassa, creixement, paràmetres funcionals, distribució d'edats i concentracions de C i N i la seva composició isotòpica ($\delta^{13}C$, $\delta^{15}N$) en ramets de *V. myrtillus* de claps purs, claps mixtos amb *V. uliginosum* i claps mixtos amb *R. ferrugineum*. El volum, la biomassa aèria i l'edat dels ramets no diferia entre tipus de clap. Vam trobar valors de $\delta^{13}C$ més petits en fulles i tiges noves de *V. myrtillus* en claps amb *R. ferrugineum*, indicatius d'una menor eficiència en l'ús de l'aigua (EUA). El contingut de N i $\delta^{15}N$ de fulles i tiges noves de *V. myrtillus* de claps purs eren més grans que en claps mixtos, suggerint una competència pel N en aquests últims. Els nostres resultats semblen indicar que *V. myrtillus* competeix pels nutrients amb les espècies veïnes, però que ni aquesta competència ni la menor EUA afecten el funcionament de les parts aèries. Així, la interacció amb els arbustos veïns no té un gran efecte en l'estructura i funcionament de *V. myrtillus* al límit supraforestal i, en conseqüència, no hauria de ser considerada un factor clau en de la dinàmica d'aquesta espècie en l'emmatament de prats subalpins.

Abstract

Plant-plant interactions are key drivers of treeline dynamics. At the Pyrenean treeline, the dwarf shrub *Vaccinium myrtillus* grows in pure stands or in mixed stands with *Vaccinium uliginosum* or *Rhododendron ferrugineum*. They form sparse shrub patches that colonize subalpine grasslands, having dramatic impacts on their structure and functioning. We investigated the role of the two co-occurring shrubs as possible modulators of the structure and performance of *V. myrtillus* in the Central Pyrenees. We analysed biomass, growth, functional parameters, age distribution, N and C concentrations and isotope compositions ($\delta^{15}N$ and $\delta\ ^{13}C$) of *V. myrtillus* ramets in pure stands, and stands mixed with *V. uliginosum* or *R. ferrugineum*. Volume, above-ground biomass and age of the ramets did not differ between stand types. We found lower $\delta^{13}C$ values, indicative of lower water-use efficiency (WUE), in leaves and shoots of *V. myrtillus* in stands with *R. ferrugineum* than in pure stands. The N content and $\delta^{15}N$ of *V. myrtillus* leaves and shoots in pure stands were higher than in mixed stands, pointing to a competition for N in mixed stands. Our results indicate that *V. myrtillus* competes for nutrients with its neighbours, but neither this competition nor the lower WUE affect its above-ground performance. Therefore, the interaction with co-occurring shrubs does not have a major effect on *V. myrtillus* structure and functioning at treeline and, consequently, should not be considered as a key driver on the dynamics of this species in the encroachment of subalpine grasslands.

Keywords: biomass partition, competition, dwarf shrubs, stable isotope composition, treeline, xylem growth rings

Abbreviations: IRMS (isotope ratio mass spectrometry), SPA (shoot photosynthetic area), TOM (total organic matter), $\delta^{13}C$ (carbon isotope composition), $\delta^{15}N$ (nitrogen isotope composition)

Introduction

Plant-plant interactions represent one of the major selective forces driving population and community dynamics (Callaway and Walker 1997). At the treeline ecotone, shrubs have been identified as modulators of ecosystem dynamics (Batllori et al. 2009b; Grau et al. 2012). However, the role of plant-plant interactions in shrub communities has often been disregarded in favour of climate modulators (especially temperature; see the numerous warming experiments carried out at treeline areas, e.g. Michelsen et al. 1996; Xu et al. 2009; Kaarlejärvi et al. 2012; Anadon-Rosell et al. 2014). Shrubs can facilitate tree seedling survival and recruitment because they can exert a nurse effect when tree species are in their most vulnerable life stage (Gómez-Aparicio et al. 2008; Batllori et al. 2009b; Grau et al. 2012). Moreover, interactions between woody species can have a key role in the encroachment processes that have been reported during the last decades in alpine and Arctic tundra ecosystems (Eldridge et al. 2011; Myers-Smith et al. 2011).

In the last decades, the increase in temperature and the progressive abandonment of traditional land use practices (Tappeiner and Cernusca 1993; Gellrich et al. 2008; IPCC 2013) have caused a densification of woody plants at European alpine treelines (Stöcklin and Körner 1999; Camarero and Gutiérrez 2004; Kullman 2005; Batllori and Gutiérrez 2008) and the re-colonization of abandoned pastures by shrubs (Didier 2001; Vicente-Serrano et al. 2004; Albert et al. 2008). This shrub encroachment, which is predicted to increase in response to the higher temperatures forecasted for this century (Walker et al. 2006; Meehl et al. 2007; IPCC 2013), plays an important role in the first stadia of tree re-colonization at treeline (Tasser and Tappeiner 2002; Targetti et al. 2010; Ninot et al. 2011). Therefore, studies focusing on shrub species interactions at treeline can provide essential understanding of the functioning of treeline ecosystem dynamics (Smith et al. 2003; Batllori et al. 2009b; Grau et al. 2012; Llambí et al. 2013).

In the subalpine belt of the Pyrenees, *Vaccinium myrtillus* has a substantial role in the transitional ecotone between *Pinus uncinata* forests and alpine grasslands (see Batllori et al. 2009a). In these areas it grows close to the highest altitudinal limit of its distribution (Bolòs et al. 2005), where stressful conditions related to low temperatures, wind abrasion, early-season frost and short growing seasons could lead to a prevalence of facilitative plant-plant interactions (Batllori et al. 2009b; Fajardo and McIntire 2011). Numerous studies in alpine and Arctic ecosystems have reported facilitative interactions between *Vaccinium myrtillus* and co-occurring species (Maillette 1988; Shevtsova et al. 1995), although allelopathic effects of *V. myrtillus* on some conifer species have also been reported (Mallik & Pellissier 2000; Talavera & Ninot, unpublished). At treeline in the Central Pyrenees, *Vaccinium myrtillus* forms sparse patches together with the similarly-sized shrub *Vaccinium uliginosum* ssp. *microphyllum* and the taller shrub *Rhododendron ferrugineum*. These shrub communities have an important role on treeline dynamics due to their potential for colonizing subalpine grasslands. However, the role of *V. uliginosum* and *R. ferrugineum* as modulators of *V. myrtillus* population structure and performance and, ultimately, the effects that these interactions can have on treeline dynamics are poorly understood. Furthermore, studies on shrub interactions involving *Vaccinium* species have usually considered neighbour shrubs of similar dimensions (Maillette 1988; Shevtsova et al. 1995, 1997; Gerdol et al. 2000; Brancaleoni and Gerdol 2006), whereas the effect of taller shrubs remains mostly unexplored (but see Pornon et al. 2007).

Carbon (C) and nitrogen (N) isotope compositions (i.e. δ^{13}C and δ^{15}N) of plant tissues can be used to determine plant nutrient and/or water status. Natural abundance δ^{13}C values have been used to assess plant water-use efficiency (Farquhar et al. 1982) and δ^{15}N values may provide insight into the use of different sources of N (Ghashghaie et al. 2003, Tcherkez and Hodges 2008), the niche partitioning for N acquisition (Pornon et al. 2007; Gundale et al. 2012) and differential isotopic fractionation of N in response to different mycorrhizal N uptake mechanisms (Emmerton et al. 2001). Moreover, C and N mass-based concentrations can be used as a measure of nutrient availability and dynamics (Aerts 1996; Killingbeck 1996; Palacio et al. 2007; Baptist et al. 2009; 2013; Muller et al. 2011; Vergutz et al. 2012). Previous studies have shown differences in the N-acquisition and N-use strategies between *V. myrtillus* and coexisting species such as *R. ferrugineum* or *Vaccinium vitis-idaea* (Pornon et al. 2007; Gundale et al. 2012). Nevertheless, although studies on congeneric species have often shown that trait divergence and niche separation are strategies for coexistence (Maillette 1988; Vander Kloet and Hill 2000; Beltrán et al. 2012; Gundale et al. 2012), this has not been assessed for the co-occurring *V. myrtillus* and *V. uliginosum*.

We aimed to determine whether structure and functioning of *V. myrtillus* stands at the treeline ecotone are affected by the coexistence with *Vaccinium uliginosum* ssp.

microphyllum (*V. uliginosum* hereafter) and *Rhododendron ferrugineum* and to find out the primary plant-plant interactions driving the performance of *V. myrtillus* in the area. To do so, we measured functional, growth and demographical parameters using growth measures, isotopic analyses and dendroecological techniques. We specifically aimed to answer the following questions: (i) does *V. myrtillus* compete for light with the taller shrub *R. ferrugineum* and, thus, presents greater spread (vertically and horizontally) when co-occurring with it?; (ii) does *R. ferrugineum* exert a facilitative effect on *V. myrtillus*, protecting it from environmental damage (e.g. wind damage and early-spring frost), translating into older, more vigorous *V. myrtillus* ramets in these mixed stands?; (iii) are growth and survival of *V. myrtillus* ramets from stands mixed with *V. uliginosum* comparable to those from monospecific stands due to the structural similarity between these two species?; and finally, (iv) does *V. myrtillus* compete for N with the other two shrubs despite the putative different N-acquisition strategy from *R. ferrugineum* and the suggested niche separation from *V. uliginosum*?

Materials and methods

Study area and species

The study site was located at Eth Corrau des Machos, in the buffer zone of the Aigüestortes and Estany de Sant Maurici National Park (Central Pyrenees, Catalonia, 31N 329, 472). Sampled plots were located on a N-facing 10-25° steep slope at 2250 m a.s.l., slightly above the current treeline in the area, but with some trees scattered above. Climatic data from a nearby location (La Bonaigua, located 6.3 km away from the study site and at similar altitude) for the period 2007-2012 are: mean annual precipitation 1154.9 mm and mean annual temperature 2.7 °C (Meteorological Service of Catalonia, see detailed climatic data for the study period 2010-2012 in Fig. S1).

At this site, vegetation was composed of *Festuca eskia* Ramond ex DC. and *Nardus stricta* L. grasslands (Selino-Festucetum eskiae) mixed with patches of dwarf shrub heath dominated by *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. and *Rhododendron ferrugineum* L. These communities are considered as serial stages in the succession to mountain pine (*Pinus uncinata* Ramond ex DC.) forests (Rhododendro-Pinetum uncinatae; i.e. grassland-shrubland-woodland-forest succession). These forests were formerly extensive in the area, but are reduced at present due to anthropogenic deforestation.

Vaccinium myrtillus and *V. uliginosum* are both deciduous dwarf shrubs that reach *ca*. 15-35 cm height at our study site, whereas *R. ferrugineum* is a taller evergreen shrub, that reaches 40-50 cm height.

Sampling design

In mid-September 2010, we evaluated the performance of *V. myrtillus* in three different stand types: pure stands (M stands), stands with *V. myrtillus* co-occurring with *V. uliginosum* (U stands) and stands with *V. myrtillus* co-occurring with *R. ferrugineum* (R stands). The percentage cover of each shrub species in the different stand types is shown in Table S1. For each stand type we established five 20 cm x 20 cm plots, accounting for the small size but high density of the ramets. The distance between two plots ranged from one to a few meters (< 20 m), but always ensuring that different plots belonged to different patches. We carried out this study on a ramet basis due to the clonal structure of *V. myrtillus*, which consists of a horizontal network of subterranean rhizomes from which aerial shoots arise (Ritchie 1956; Flower-Ellis 1971). We measured and clipped at ground level all *V. myrtillus* ramets grounded within each plot boundaries, and we subsequently stored them in sealed plastic bags kept in a portable cooler until we arrived at the laboratory.

Stand structure, above-ground growth and plant performance

To have an estimate of the ability of plants to compete for space at a stand scale, we calculated the volume of all *V. myrtillus* ramets present in each plot. To do so, we measured their height and their canopy diameter (i.e. mean between the maximum and the minimum canopy diameters) and estimated the theoretical volume occupied for each ramet as a canopy cylinder ($V= \pi r^2 h$, Johnson et al. 1988).

To study above-ground biomass partition of individual ramets, we selected 12 ramets per plot when possible (except for one M plot, which only had 11 ramets, and two R plots, which only had 8 and 10 ramets), representative of the wide range of ramet sizes in the plots. We used the last three cohorts of shoots as the sampling unit for biomass allocation comparisons between stand types. Ramets younger than three years old were not used for biomass allocation measurements. The following fractions were detached and counted: leaves, new shoots (formed in 2010, Sh1), one-year-old shoots (formed in 2009, Sh2), two-year-old shoots (formed in 2008, Sh3) and main stem (> 3 years old). We identified the different cohorts of shoots by the scars left by bud scales after shoot elongation. Before drying, we cut a 1.5 cm segment at the base of the stem of each ramet for counting xylem growth rings to determine ramet age (see below). We dried all the fractions at 70 °C for 72 h and weighed them to the nearest 0.001 g (with a Mettler Toledo PB303 Delta Range scale). We corrected the weight of the main stems by adding the weight of segments of similar dimensions to the part previously cut. For each ramet, we calculated the dry mass of Sh1, Sh2 and Sh3 relative to the two-year-old shoot unit, and we also calculated the total above-ground biomass. Moreover, for each ramet (except for ramets younger than three years), we measured the main stem diameter as the mean between the maximum and minimum diameters measured at the base of the stem.

We randomly selected one of the 12 ramets described above in three plots of each stand type and measured length and width of all shoots from the different cohorts to calculate shoot photosynthetic area (SPA). For this, we assumed shoot surface area as that of a tetrahedron, because young shoots of *V. myrtillus* show a tetrahedral shape. The greenness of the shoots gradually decreases as ramets age, but the three last shoot cohorts still show a clear green photosynthetic colour. We carried out linear regressions between photosynthetic area and shoot dry mass separately for each cohort and found positive significant correlations (Sh1: $R^2 = 0.96$, $P < 0.001$; Sh2: $R^2 = 0.91$, $P < 0.001$; Sh3: $R^2 = 0.85$, $P < 0.001$, Fig. S2). Consequently, we used the obtained regression equations to estimate the photosynthetic area of each shoot cohort per two-year-old shoot unit for the rest of ramets.

Ramet age distribution

The structure and growth of *V. myrtillus* ramets depend on age (Flower-Ellis 1971). Thus, it is important to know and consider ramet age when performing comparative studies between *V. myrtillus* stands. To estimate the age of ramets and to identify differences in the above-ground age structure of different stands, we made cross-sections of 20-25 µm thickness from the basal segments of *V. myrtillus* stems using a sledge microtome. Sections were stained with Safranin to emphasize the growth-ring structure, subsequently rinsed with ethanol (75%) for dehydration (Schweingruber and

Poschold 2005, modified) and mounted in DPX. We counted xylem growth-rings under the microscope (Olympus CH2) to assess ramet age.

C and N concentration and isotope composition ($\delta^{13}C$ and $\delta^{15}N$)

At the time of harvest, leaves were already senescent, therefore we established another sampling period in August 2012. Then, we collected *V. myrtillus* leaf and new shoot samples in fifteen plots similar to those established in 2010, i.e. five plots for each stand type, and analysed their C and N concentrations and isotopic compositions. We sampled leaves and new shoots of five ramets per plot and pooled together the material of these ramets for each plot separately, both for leaves and new shoots. Thus, we had five replicates for each stand type and plant fraction. Samples were dried at 60 ºC for 72 h, and subsamples were weighed in small tin capsules.

Samples were analysed to determine the C and N isotope compositions using a Flash 1112 Elemental Analyzer (Carbo Erba, Milan) coupled to an IRMS Delta C isotope ratio mass spectrometer through a Conflo III Interface (Thermo-Finnigan, Germany). Results of C isotope analyses are reported in per thousand (‰) on the relative δ-scale as δ^{13}C, and refer to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} \right) - 1 \qquad \text{(Eq. 1)}$$

where *R* is the ^{13}C/^{12}C ratio.

C isotope discrimination (Δ^{13}C) of shoot TOM (total organic matter) was calculated from δ_a and δ_p (Farquhar et al. 1989) as:

$$\Delta^{13}C = \frac{\delta_a - \delta_p}{\delta_p + 1} \qquad \text{(Eq. 2)}$$

where *a* and *p* refer to air and plant, respectively.

N results are also expressed in δ notation (δ^{15}N) using international secondary standards of known ^{15}N/^{14}N ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) relative to N_2 in air:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} \right) - 1 \qquad \text{(Eq. 3)}$$

where *R* is the ^{15}N/^{14}N ratio.

N and C contents were determined using an elemental analyzer (EA1108, Series 1; Carbo Erba Instrumentazione, Milan, Italy).

In August 2013, we collected air samples with a 50 ml syringe (SGE, Ringwood, Australia) and kept them in 10 ml vacutainers (BD vacutainer, Plymouth, UK) to analyze the $^{13}CO_2$ isotopic composition at the study site by gas-chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) according to Nogués et al. (2004). The $\delta^{13}C$ of CO_2 of the air at the site in the Pyrenees was *ca*. -10.91‰. We also collected soil samples to determine soil N isotope composition at the study site and analysed them following the same technique as mentioned above for plant material. Soil $\delta^{15}N$ was *ca*. 7.33 ‰.

All the EA-IRMS and GC-C-IRMS analyses were performed at the CCiT of the University of Barcelona.

Statistical Analyses

We used linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML) to test the effects of the different scenarios of coexistence on *V. myrtillus* volume, canopy height and width, biomass partitioning, total above-ground biomass, SPA of each cohort of shoots, ramet age and stem diameter. We included stand type as a fixed factor and plot as a random factor. To account for the possible age effects on response variables, we included ramet age as a covariate in the analyses of the effects of stand type on individual ramet traits when it was significant, that is for total above-ground biomass, stem diameter and SPA in Sh3. For the N and C concentrations and isotope compositions, we evaluated differences between stand type, plant fraction (leaves and shoots) and their interaction with Type III analysis of variance using the linear model function. We tested for Pearson’s correlations between ramet age, stem diameter and total above-ground biomass.

For all statistical analyses, we visually evaluated the assumptions of normality and homoscedasticity of residuals (Zuur et al. 2009) and transformed response variables when necessary to reach these assumptions. When homoscedasticity of residuals was not met, we used the varIdent structure (Zuur et al. 2009) to account for the heterogeneity of variances among factor levels. Effects were considered significant at $P < 0.05$ and marginally significant at $0.05 < P < 0.10$ to account for the relatively low replication. When there were significant differences between stand types, we performed

Tukey HSD post hoc tests to determine which factor level means differed significantly. All analyses were performed with R 2.15.2 (R Core Team 2012), using the nlme package (Pinheiro et al. 2008) for linear mixed effects models, the lattice package (Sarkar 2008) for graphical evaluation of the assumptions of the models, the multcomp package (Hothorn et al. 2008) for multiple comparisons on linear mixed effects models and the agricolae package (de Mendiburu 2010) for multiple comparisons on simple linear models.

Results

Stand structure, above-ground growth and performance

Ramet volume (calculated as an elliptical cylinder) did not differ between stand types (Tables 1 and 2). Despite the average values for R stands were much higher than for the other two stand types, the great data dispersion in these R stands masked possible significant differences. The average ramet height and ramet canopy width did not differ between the three stand types either (Tables 1 and 2), although the trend of higher values but higher dispersion in R stands was also evident.

Similarly, total above-ground ramet biomass did not differ significantly between the three stand types. For ramets in M stands was 868.7 ± 195.9 mg (mean ± SD), in R stands 761.9 ± 635.1 mg and in U stands 507.1 ± 123.6 mg (Table 3). However, we found significant differences in the biomass allocation patterns. *Vaccinium myrtillus* ramets growing with *R. ferrugineum* showed a lower biomass allocation to Sh3 than ramets in the other stand types (Table 3, Fig. 1). Although the biomass allocation to Sh1 and Sh2 tended to be lower too, differences between stand types were not significant for these two cohorts. Biomass allocation to Sh1 was 14% higher than to Sh2, which was 9% higher than Sh3 averaged across all stand types. Stem diameter did not differ between stand types either (Table 3).

SPA is related to the biomass allocation to each fraction. Therefore, it showed a similar pattern than biomass allocation: it was greater in Sh1, followed by Sh2 and Sh3 for the three stand types (Fig. 2). Moreover, SPA of Sh3 was higher in M and U stands than in R stands (Table 3, Fig. 2).

Table 1. Mean (and standard deviation) for the parameters studied at plot scale (all the ramets in the plot). M: *V. myrtillus* pure stands, R: *V. myrtillus* mixed with *R. ferrugineum* stands, U: *V. myrtillus mixed* with *V. uliginosum* stands.

Stand type	Ramet volume (cm^3)	Ramet height (cm)	Canopy width (cm)
M	223.04 (111.62)	14.46 (2.71)	3.24 (0.71)
R	1074.93 (1691.77)	15.57 (5.76)	5.89 (4.16)
U	137.07 (66.16)	12.21 (1.41)	3.02 (0.66)

Table 2. Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* volume, height and canopy width studied in all ramets within each plot. No significant differences were found.

Variable	df	*F*	*P*
Ramet volume	2, 12	2.07	0.17
Ramet height	2, 12	1.05	0.38
Ramet canopy width	2, 12	2.38	0.14

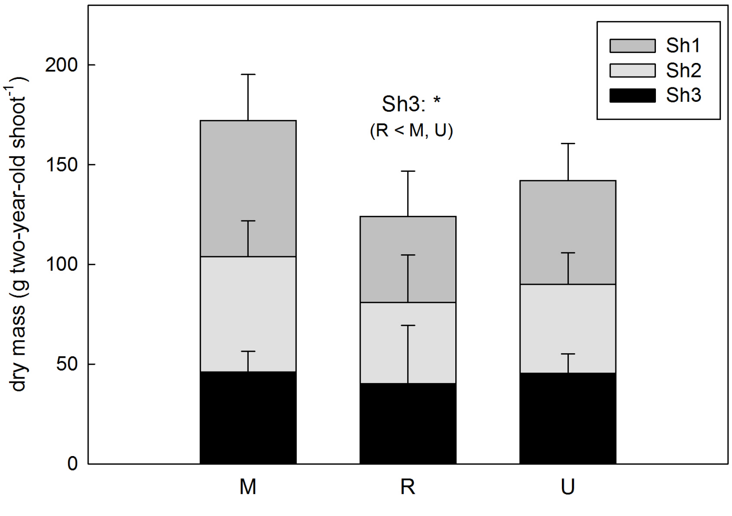

Fig. 1 *Vaccinium myrtillus* biomass allocation to the last three shoot cohorts in the three stand types. The asterisk shows significant differences between stand types ($0.01 < P < 0.05$), referred to Sh3 between R stands and M and U. Data are means + SD, $n = 5$. M: *V. myrtillus* pure stands, R: *V. myrtillus* mixed with *R. ferrugineum* stands, U: *V. myrtillus mixed* with *V. uliginosum* stands. Sh1: new shoots, Sh2: one-year-old shoots, Sh3: two-year-old shoots.

Table 3. Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* biomass partitioning, total above-ground (AG) biomass, shoot photosynthetic area (SPA), age and stem diameter in selected ramets per plot. Significant differences (*P* < 0.05) in bold. Sh1: new shoots, Sh2: one-year-old shoots, Sh3: two-year-old shoots.

Variable	Factor/Covariable	df	*F*	*P*
Biomass partition				
Sh1	Stand type	2, 12	2.39	0.13
Sh2	Stand type	2, 12	1.86	0.20
Sh3	Stand type	2, 12	3.96	**0.04**
Total AG biomass	Age covariable	1, 152	100.08	**<0.01**
	Stand type	2, 12	1.92	0.19
SPA				
Sh1	Stand type	2, 12	2.31	0.14
Sh2	Stand type	2, 12	2.72	0.11
Sh3	Age covariable	1, 139	5.26	**0.02**
	Stand type	2, 12	4.53	**0.03**
Age	Stand type	2, 12	0.20	0.82
Stem diameter	Age covariable	1, 123	57.91	**<0.01**
	Stand type	2, 12	0.89	0.44

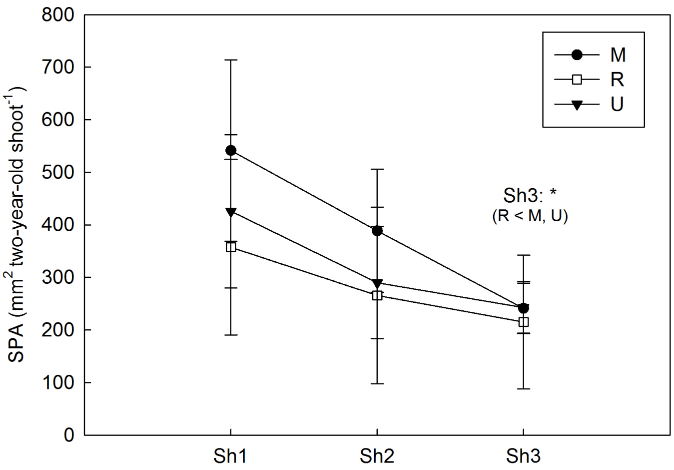

Fig. 2 Shoot photosynthetic area (SPA) of the last three shoot cohorts of *V. myrtillus* ramets in the three stand types. The asterisk shows significant differences between stand types (0.01 < *P* < 0.05), referred to Sh3 between R stands and M and U. Data are means + SD, *n* = 5. M: *V. myrtillus* pure stands, R: *V. myrtillus* mixed with *R. ferrugineum* stands, U: *V. myrtillus mixed* with *V. uliginosum* stands. Sh1: new shoots, Sh2: one-year-old shoots, Sh3: two-year-old

Ramet age distribution

Ramet age did not differ between stand types (Table 3, Fig. 3). The oldest ramet was 12 years old (U stand), but the majority of the ramets of each stand type were younger than 7 years old (70 % in M stands, 68 % in U stands and 61 % in R stands). Ramet age was positively correlated with stem diameter ($R^2 = 0.55$, $P < 0.001$) and total above-ground biomass ($R^2 = 0.53$, $P < 0.001$).

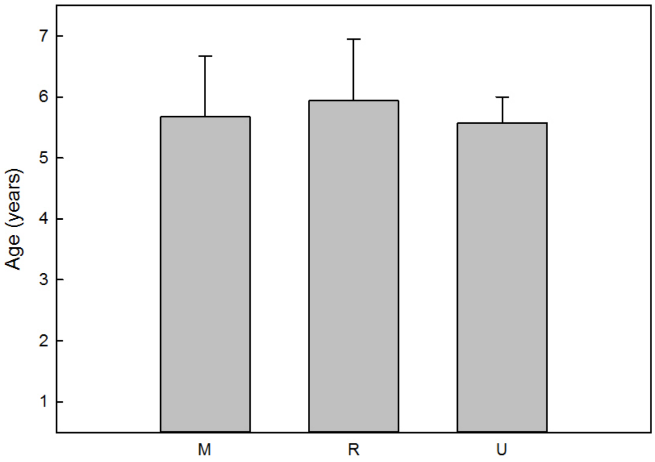

Fig. 3 Ramet age in the three stand types. No significant differences were found. Data are means + SD, *n* = 5. M: *V. myrtillus* pure stands, R: *V. myrtillus* mixed with *R. ferrugineum* stands, U: *V. myrtillus mixed* with *V. uliginosum* stands.

C and N concentrations and isotope compositions ($\delta^{13}C$ and $\delta^{15}N$) of leaves and new shoots

We found significant differences in the C and N isotopic composition and N concentration of leaves and new shoots of *V. myrtillus* between stand types. $\delta^{13}C$ values of leaves and new shoots of *V. myrtillus* in pure stands and U stands were higher than those of ramets from R stands (marginally significant, Table 4, Fig. 4a; see Table S2 for ^{13}C discrimination values, $\Delta^{13}C$) and $\delta^{13}C$ values of new shoots were higher than values for leaves. The C content of leaves and new shoots did not differ between stand types, and it was higher in new shoots than in leaves (Table 4, Fig. 4b). The N content and $\delta^{15}N$ of leaves and new shoots of pure stands were higher than those of mixed stands (Table 4, Fig. 4c, d) and the N content was higher in leaves than in new shoots. The interaction between stand type and plant fraction did not have a significant effect on any of the parameters analysed.

Table 4. Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) and plant fraction (leaves and new shoots) on *V. myrtillus* C and N isotope compositions ($\delta^{13}C$, $\delta^{15}N$) and concentrations. Significant differences ($P < 0.05$) in bold, and marginally significant differences ($0.10 > P < 0.05$) in bold in parentheses.

Variable	Factor	df	*F*	*P*
$\delta^{13}C$	Stand type	2, 24	2.93	**(0.07)**
	Fraction	1, 24	26.39	**<0.01**
	Stand type x Fraction	2, 24	0.20	0.82
$\delta^{15}N$	Stand type	2, 24	39.09	**<0.01**
	Fraction	1, 24	1.30	0.27
	Stand type x Fraction	2, 24	0.01	0.99
C concentration	Stand type	2, 24	0.86	0.44
	Fraction	1, 24	45.48	**<0.01**
	Stand type x Fraction	2, 24	1.58	0.23
N concentration	Stand type	2, 24	6.67	**<0.01**
	Fraction	1, 24	35.78	**<0.01**
	Stand type x Fraction	2, 24	0.19	0.83

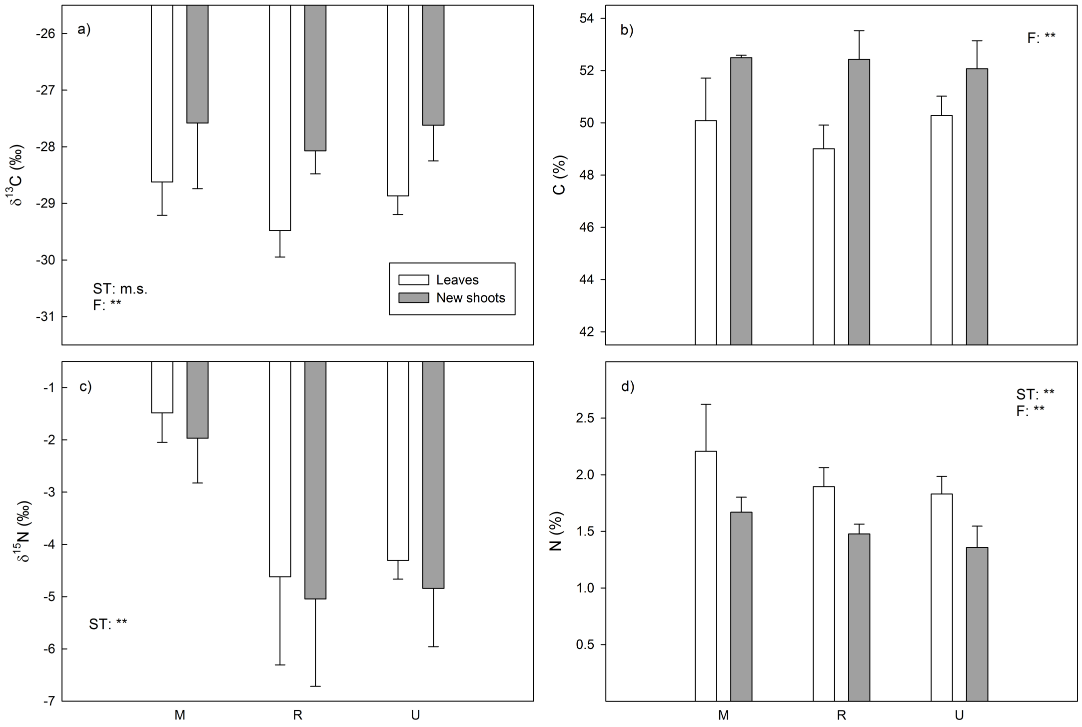

Fig. 4 C and N isotopic compositions (a, c) and C and N concentrations (b, d) of leaves and new shoots of *V. myrtillus* in the three stand types. Data are means + (b, d) or - (a, c) SD. Asterisks (**) show significant differences between situations of coexistence ($P < 0.01$), and marginally significant differences are indicated by m.s. M: *V. myrtillus* pure stands, R: *V. myrtillus* mixed with *R. ferrugineum* stands, U: *V. myrtillus mixed* with *V. uliginosum* stands, ST: stand type, F: plant fraction. For detailed explanation on the differences between factor level means see the main text.

Discussion

Above-ground growth and performance

We did not find major differences in *V. myrtillus* volume, biomass or SPA between the different stand types, which indicates that *V. myrtillus* above-ground growth and performance is similar when it grows in pure and in mixed stands.

Nevertheless, the higher values and greater spread of *V. myrtillus* volumes when growing with *R. ferrugineum* as compared to ramets growing with *V. uliginosum* or in pure stands, suggest that *V. myrtillus* finds more heterogeneous conditions when growing with *R. ferrugineum*. *Vaccinium myrtillus* might face some competition for light when growing with the taller shrub *R. ferrugineum*, which is the reason why *V. myrtillus* would spread its shoots both laterally and upwards to occupy more space, a plasticity that would allow it to favourably compete to intercept more light (van Kleunen and Fisher 2001; Mourelle et al. 2001; Callaway et al. 2003; Lepik et al. 2005).

Although *V. myrtillus* ramets tended to show a reduced biomass allocation to the three last shoot cohorts in plots with *R. ferrugineum*, differences were only significant in two-year-old shoots, and they were not maintained in the next two shoot cohorts. These results could indicate that *V. myrtillus* experienced less favourable conditions in stands with *R. ferrugineum* in 2008 that caused a lower investment in shoot growth that year. Despite these differences, results for the younger shoot cohorts and total above-ground biomass suggest that the coexistence with the other shrub species does not have major effects on the above-ground growth of *V. myrtillus* in the study area.

The photosynthetic potential of *V. myrtillus* shoots is especially important during the cold season, when ramets are leafless, because the green shoots of this species remain partly active in autumn and spring, and can photosynthesize even under the snow cover (Körner 2003). SPA increased with shoot biomass, and therefore, shoots produced in 2008 also had a lower photosynthetic area in ramets growing with *R. ferrugineum*. However, apart from two-year-old shoots, *V. myrtillus* SPA was similar across stand types, indicating a similar productive potential irrespective of the coexistence with *V. uliginosum* and *R. ferrugineum*.

The lack of major significant differences in these functional and growth parameters, however, needs to be carefully interpreted. Although important differences between stand types were not occurring, some differences could be masked by the high dispersion in some of the studied parameters and the relatively low replication.

Ramet age distribution

The lack of differences between the three stand types in ramet age, together with the above-ground biomass results, indicate that this species does not encounter a different environment for its ramets growth or turnover when coexisting with the other two shrubs than when growing in pure stands. We did not expect to find differences in the mean ramet age between *V. myrtillus* pure stands and stands with *V. uliginosum,* because the effects of the intraspecific interaction between *V. myrtillus* ramets would be functionally and structurally similar to the effects from the interspecific interaction between ramets of the two *Vaccinium* species (i.e. we did not expect *V. uliginosum* to exert any protection to *V. myrtillus*). However, our results indicate that we cannot assume that the taller *R. ferrugineum* exerts any facilitative effect on *V. myrtillus* ramets development at our study site either. Grau et al. (2013) found that *R. ferrugineum* reduced winter damage to tree seedlings at treeline sites in the Pyrenees. Also in the Pyrenees, we observed that *R. ferrugineum* had some kind of nurse effect on *V. myrtillus* in subalpine grasslands colonized by shrubs and in open shrublands, since the density of ramets growing next to *R. ferrugineum* was higher than far from it (unpublished data). Consequently, we expected to find older *V. myrtillus* ramets in plots with *R. ferrugineum*, but such a nurse effect was not detected at our study site.

All the stands analysed were formed by relatively young ramets, the oldest ones being only 12 years old. This indicates that we are dealing with patches where *V. myrtillus* has recently established or patches where a high above-ground turnover by environmental constrictions or disturbance maintains ramets at a young stage. The below-ground network of *V. myrtillus* rhizomes can spread and expand clonally and thus, be much older than the above-ground ramets (Flower-Ellis 1971). Therefore, only a long-term monitoring or a below-ground dendrochronological survey could corroborate whether we are dealing with a recent encroaching area where *V. myrtillus* could expand in the near future, or with older populations with a high above-ground turnover.

C and N concentration and isotope composition

Our results on C and N concentrations and isotope compositions showed differences between stand types, which evidence that competition occurred between *V. myrtillus* and its neighbours.

First, our results suggest that the WUE of *V. myrtillus* was reduced in the presence of *R. ferrugineum*. Several studies have reported lower values of $\delta^{13}C$ in leaves under shaded conditions than under full light (Gebauer and Schulze 1991; Berry et al. 1997; Le Roux et al. 2001; Duursma and Marshall 2006; Kranabetter et al. 2010). However, stomatal conductance can be reduced under shade (Gross et al. 1996; Forseth et al. 2001), leading to a lower discrimination against ^{13}C and higher $\delta^{13}C$ values in shaded plants. The observed lower $\delta^{13}C$ values in ramets coexisting with *R. ferrugineum* are probably explained by a greater difference in the assimilation rates between open and shady stands than the difference in stomatal conductance between these two situations

(Carelli et al. 1999; Forseth et al. 2001). When growing with *R. ferrugineum, V. myrtillus* might have encountered more shade, which probably reduced its photosynthetic rate to a greater extent than the stomatal conductance (i.e. leading to lower WUE values). A study in the semiarid Colorado Plateau showed that shading by neighbour shrub species reduced the performance of the herbaceous perennial *Cryptantha flava* through a reduction of its photosynthetic rate and a non-proportional reduction of its transpiration and stomatal conductance, thus reducing its WUE (Forseth et al. 2001). In our study, we also observed a reduction of the WUE of *V. myrtillus* growing with *R. ferrugineum*, but its performance was not affected. As suggested by Forseth et al. (2001), temperature, vapour pressure deficit and transpiration rates may have also been lower under the shade of *R. ferrugineum*, which would explain why *V. myrtillus* performance was not altered. As expected, the C content was higher in new shoots than in leaves, since the former are permanent structures whereas leaves are shed every year in this species.

The lower N content and the lower δ^{15}N values of leaves and new shoots in mixed stands than in pure stands suggest that *V. myrtillus* encountered a deficit in available N when growing with *R. ferrugineum* and *V. uliginosum* compared to pure stands (Olsrud et al. 2004; Craine et al. 2009). Wang and Schjoerring (2012) found a positive correlation between δ^{15}N and N concentration in leaves of ryegrass from intensively managed fields in Scotland, and Craine et al. (2009) also found evidence that foliar δ^{15}N increased with increasing N supply at a local and regional scale after reviewing data from 11,000 plants worldwide. These studies showed that foliar δ^{15}N values and foliar N concentrations correlate and that they can both give information on the N availability.

The lower N content values in leaves and new shoots of *V. myrtillus* in mixed plots are probably explained by the competition for N with its neighbours, which might have limited the amount of N that *V. myrtillus* ramets could invest in their tissues. *Vaccinium myrtillus* and *R. ferrugineum* have shown different N-acquisition strategies in an experiment in a subalpine community in the French Pyrenees (Pornon et al. 2007), where *V. myrtillus* took up N early in the growing season, whereas *R. ferrugineum* showed a slower N uptake rate, which was maintained over the growing season. Moreover, a study on *V. myrtillus* and *V. vitis-idaea* in a boreal shrub community (Gundale et al. 2012) showed that the removal of one shrub species did not affect the isotopic signal of the other coexisting shrub, demonstrating that the niches of these species were not affected by the presence of their neighbours. Differences in the study species, community composition and soil N pools (both content and dynamics) between these studies and ours might explain the contrasting results. A N shortage can have dramatic impacts on plant performance, since the majority of the leaf N is allocated to the photosynthetic apparatus and leaf N content and photosynthetic capacity are well correlated (Evans 1986). Thus, competition for N can have negative impacts on plant growth, as it has been reported in previous studies (Wilson and Tilman 1991; Li et al. 2015). However, despite the evidence of an existing competition for N in our study, *V. myrtillus* could clearly counteract the effects of this competition and grow as vigorously as in pure stands. These results suggest that competition did not lead to a sufficient N shortage that could negatively affect *V. myrtillus* growth. Further studies should be carried out to determine whether the lower $\delta^{15}N$ values in leaves and shoots of *V. myrtillus* from mixed populations were due to higher ^{15}N fractionation by mycorrhiza (Emmerton et al. 2001), to an increased proportion of N obtained by mycorrhiza (Hobbie et al. 2000), or to the use of different N sources (Michelsen et al. 1998) as a result of the competition with the other shrub species.

Concluding remarks

We did not find evidence that the performance of *V. myrtillus* at our study site was affected by the presence of *R. ferrugineum* or *V. uliginosum*. Although *V. myrtillus* seems to compete for N with both species, it can counterbalance these competition effects without compromising its growth. This is especially important to understand and predict landscape dynamics at treeline: the possible expansion of *V. myrtillus* at this treeline site would not be tightly related to the presence of *V. uliginosum* and *R. ferrugineum*, but mostly driven by the own population dynamics, mediated by other biotic interactions and environmental factors.

Acknowledgements

We are especially grateful to Empar Carrillo, Oriol Grau, Marc Talavera, Claudia Arias and Elena Sánchez for their field and laboratory assistance, and to CCiT of the University of Barcelona for the use of their facilities and their technical assistance. We also thank Jonathan Mitchley for his comments and corrections in an early version of this work, Emilia Gutiérrez for the use of the sledge microtome and Fritz Schweingruber for his support in the research of bibliographical resources. AAR was funded by a grant provided by the Fundació Agustí Pedro i Pons and an FPU grant (Ministerio de Educación, Cultura y Deporte, Spain) and SP was funded by a Ramón y Cajal contract (RYC-2013-14164, Ministerio de Economía y Competitividad, Spain). This work has been partly funded by Conselh Generau d'Aran, the European Research Project OPTIMA and the projects CGL2011-26654 (Ministerio de Ciencia e Innovación, Spain) and ARBALMONT/786-2012 (Organismo Autónomo de Parques Nacionales, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain).

References

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: Are there general patterns? J Ecol 84:597-608. doi: 10.2307/2261481

Albert C, Thuiller W, Lavorel S, Davies ID, Garbolino E (2008**)** Land use change and sub-alpine tree dynamics: colonisation of *Larix decidua* in French sub-alpine grasslands. J Appl Ecol 45:659-669. doi: 10.1111/j.1365-2664.2007.01416.x

Anadon-Rosell A, Rixen C, Cherubini P, Wipf S, Hagedorn F, Dawes MA (2014) Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. PLoS ONE 9. doi: 10.1371/journal.pone.0100577

Baptist F, Secher-Fromell H, Viard-Cretat F, Aranjuelo I, Creme A, Desclos M, Laine P, Nogués S, Lavorel S (2013) Carbohydrate and nitrogen stores in *Festuca paniculata* under mowing explain dominance in subalpine grasslands. Plant Biol 15:395-404. doi: 10.1111/j.1438-8677.2012.00652.x

Baptist F, Tcherkez G, Aubert S, Pontailler J-Y, Choler P, Nogués S (2009) ^{13}C and ^{15}N allocations of two alpine species from early and late snowmelt locations reflect their different growth strategies. J Exp Bot 60:2725–2735. doi: 10.1093/jxb/erp128

Batllori E, Blanco-Moreno JM, Ninot JM, Gutiérrez E, Carrillo E (2009a) Vegetation patterns at the alpine treeline ecotone: the influence of tree cover on abrupt change in species composition of alpine communities. J Veg Sci 20:814-825. doi: 10.1111/j.1654-1103.2009.01085.x

Batllori E, Camarero JJ, Ninot JM, Gutiérrez E (2009b) Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* treelines. Implications and potential responses to climate warming. Global Ecol Biogeogr 18:460-472. doi: 10.1111/j.1466-8238.2009.00464.x

Batllori E, Gutiérrez E (2008) Regional treeline dynamics in response to global change in the Pyrenees. J Ecol 96:1275–1288. doi: 10.1111/j.1365-2745.2008.01429.x

Beltrán E, Valiente-Banuet A, Verdú M (2012) Trait divergence and indirect interactions allow facilitation of congeneric species. Ann Bot 110:1369-1376. doi: 10.1093/aob/mcs089

Berry SC, Varney GT, Flanagan LB (1997) Leaf $\delta^{13}C$ in *Pinus resinosa* trees and understory plants: Variation associated with light and CO_2 gradients. Oecologia 109:499-506. doi: 10.1007/s004420050110

Bolòs O, Vigo J, Masalles RM, Ninot JM (2005) *Flora manual dels Països Catalans*. 3^{a} ed. rev. i amp. Ed. Pòrtic SA, Barcelona.

Brancaleoni L, Gerdol R (2006) Recovery of subalpine dwarf shrub heath after neighbour removal and fertilization. Plant Ecol 183:227-235. doi: 10.1007/s11258-005-9020-4

Callaway RM, Pennings SC, Richards, CL (2003) Phenotypic plasticity and interactions among plants. Ecology 84:1115-1128. doi: 10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2

Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958-1965. doi: 10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Camarero JJ, Gutierrez E (2004) Pace and pattern of recent treeline dynamics: response of ecotones to climatic variability in the Spanish Pyrenees. Climatic Change 63:181-200. doi: 10.1023/B:CLIM.0000018507.71343.46

Carelli MLC, Fahl JI, Trivelin PCO, Queiroz-Voltan RB (1999) Carbon isotope discrimination and gas exchange in *Coffea* species grown under different irradiance regimes. Rev Bras Fisiol Veg 11:63-68.

Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen

A, MacK MC, McLauchlan KK Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol 183:980-992. doi: 10.1111/j.1469-8137.2009.02917.x

Didier L (2001) Invasion patterns of European larch and Swiss stone pine in subalpine pastures in the French Alps. Forest Ecol Manag 145:67-77. doi: 10.1016/S0378-1127(00)00575-2

Duursma RA, Marshall JD (2006) Vertical canopy gradients in $\delta^{13}C$ correspond with leaf nitrogen content in a mixed-species conifer forest. Trees 20:496-506. doi: 10.1007/s00468-006-0065-3

Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JR, Whitford WG (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. Ecol Lett 14:709-722. doi: 10.1111/j.1461-0248.2011.01630.x

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. New Phytol 151:513-524. doi: 10.1046/j.1469-8137.2001.00179.x

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78: 9-19. doi: 10.1.1.463.5412

Fajardo A, Mc Intire EJB (2011) Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. J Ecol 99:642-650. doi: 10.1111/j.1365-2745.2010.01771.x

Farquhar GD, O’Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121-137. doi: 10.1071/PP9820121

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Phys 40:503-537. doi: 10.1146/annurev.pp.40.060189.002443

Flower-Ellis JGK (1971) Age, structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). Department of Forest Ecology and Forest Soils, Research Note 9. Royal College of Forestry, Stockholm, Sweden.

Forseth IN, Wait DA, Casper BB (2001) Shading by shrubs in a desert system reduces the physiological and demographic performance of an associated herbaceous perennial. J Ecol 89:670-680. doi: 10.1046/j.0022-0477.2001.00574.x

Gebauer G, Schulze ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. Oecologia 87:198-207. doi: 10.1007/BF00325257

Gellrich M, Baur P, Robinson BH, Bebi P (2008) Combining classification tree analyses with interviews to study why sub-alpine grasslands sometimes revert to forest: A case study from the Swiss Alps. Agr Syst 96:124-138. doi: 10.1016/j.agsy.2007.07.002

Gerdol R, Brancaleoni L, Menghini M, Marchesini R (2000) Response of dwarf shrubs to neighbour removal and nutrient addition and their influence on community structure in a subalpine heath. J Ecol 88:256-266. doi: 10.1046/j.1365-2745.2000.00445.x

Ghashghaie J, Badeck F-W, Lanigan G, Nogués S, Tcherkez G, Deléens E, Cornic G, Griffiths H (2003) Carbon isotope fractionation during dark respiration and photorespiration in C_3 plants. Phytochem Rev 2:145–161. doi: 10.1023/B:PHYT.0000004326.00711.ca

Grau O, Ninot JM, Blanco-Moreno JM, van Logtestijn RSP, Cornelissen JHC, Callaghan TV (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. Oikos 121:1680–1690. doi: 10.1111/j.1600-0706.2011.20032.x

Grau O, Ninot JM, Cornelissen JC, Callaghan TV (2013) Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. Plant Ecol Divers 6:329–342. doi: 10.1080/17550874.2013.810311

Gómez-Aparicio L, Zamora R, Castro J, Hódar JA (2008) Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores? J Veg Sci 19:161-172. doi: 10.3170/2008-8-18347

Gross K, Homlicher A, Weinreich A, Wagner E (1996) Effect of shade on stomatal conductance, net photosynthesis, photochemical efficiency and growth of oak saplings. Ann For Sci 53:279-290. doi: 10.1051/forest:19960211

Gundale MJ, Hyodo F, Nilsson M-C, Wardle DA (2012) Nitrogen niches revealed through species and functional group removal in a boreal shrub community. Ecology 93:1695-1706. doi: 10.1890/11-1877.1

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate-plant-mycorrhizal interactions. Oecologia 122:273-283. doi: 10.1007/PL00008856

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346-363. doi: 10.1002/bimj.200810425

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker T.F., Qin D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels A., Xia Y., Bex V., Midgley P.M. (Eds.). Cambridge University Press, Cambridge, UK and New York, USA.

Johnson PS, Johnson CL, West NE (1988) Estimation of phytomass for ungrazed crested wheatgrass plants using allometric equations. J Range Manage 41:421-425.

Kaarlejärvi E, Baxter R, Hofgaard A, Hytteborn H, Khitun O, Molau U, Sjögersten S, Wookey P, Olofsson J (2012) Effects of warming on shrub abundance and chemistry drive ecosystem-level changes in a forest-tundra ecotone. Ecosystems 15:1219-1233. doi: 10.1007/s10021-012-9580-9

Killingbeck KT (1996) Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. Ecology 77:1716-1727. doi: 10.2307/2265777

van Kleunen M, Fisher M (2001) Adaptive evolution of plastic foraging responses in a clonal Plant. Ecology 82:3309-3319. doi: 10.2307/2680154

Körner C (2003) Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. 2nd ed. Springer, Berlin.

Kranabetter JM, Simard SW, Guy RD, Coates KD (2010) Species patterns in foliar nitrogen concentration, nitrogen content and ^{13}C abundance for understory saplings across light gradients. Plant Soil 327:389-401. doi: 10.1007/s11104-009-0062-6

Kullman L (2005) Pine (*Pinus sylvestris*) treeline dynamics during the past millennium - A population study in west-central Sweden. Ann Bot Fenn 42:95-106.

Lepik M, Liira J, Zobel K (2005) High shoot plasticity favours plant coexistence in herbaceous vegetation. Oecologia 145:465-474. doi: 0.1007/s00442-005-0142-0

Li X, Rennenberg H, Simon J (2015) Competition for nitrogen between *Fagus sylvatica* and *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. Front Plant Sci 6:302. doi: 10.3389/fpls.2015.00302

Llambí LD, Puentes Aguilar J, García-Núñez C (2013) Spatial relations and population structure of a dominant tree along a treeline ecotone in the Tropical Andes: interactions at gradient and plant neighbourhood scales. Plant Ecol Divers 6: 343-353. doi: 10.1080/17550874.2013.810312

Maillette L (1988) Apparent commensalism among three *Vaccinium* species on a climatic gradient. J Ecol 76:877–888. doi: 10.2307/2260579

Mallik AU, Pellissier F (2000) Effects of *Vaccinium myrtillus* on Spruce regeneration: testing the notion of coevolutionary significance of allelopathy. J Chem Ecol 26:2197-2209. doi: 10.1023/A:1005528701927

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global climate projections. In: Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., Miller H.L. (Eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.

de Mendiburu F (2010). agricolae: Statistical Procedures for Agricultural Research. R package version 1.0-9. URL http://CRAN.R-project.org/package=agricolae.

Michelsen A, Jonasson S, Sleep D, Havström M, Callaghan TV (1996) Shoot Biomass, $\delta^{13}C$, nitrogen and chlorophyll responses of two arctic dwarf shrubs to in situ shading, nutrient application and warming simulating climatic change. Oecologia 105:1-12. doi: 10.1007/BF00328785

Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115:406-418. doi: 10.1007/s004420050535

Mourelle C, Kellman M, Kwon L (2001) Light occlusion at forest edges: an analysis of tree architectural characteristics. For Ecol Manage 154:179-192. doi: 10.1016/S0378-1127(00)00624-1

Muller O, Hirose T, Werger MJA, Hikosaka K (2011) Optimal use of leaf nitrogen explains seasonal changes in leaf nitrogen content of an understory evergreen shrub. Ann Bot 108:529-536. doi: 10.1093/aob/mcr167

Myers-Smith IH, Forbes BC, Wilmking M, et al. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. Environ Res Lett 6. doi: 10.1088/1748-9326/6/4/045509

Ninot JM, Batllori E, Carrillo E, Carreras J, Casals P, Casas C, Grau O, Gutiérrez E, Montané F., Puente A. (2011) Reforestación natural en el dominio de *Pinus uncinata*. In: Ramirez L., Asensio B. (eds.) Proyectos de investigación en Parques Nacionales: 2007- 2010. Organismo Autónomo de Parques Nacionales, pp 139-158.

Nogués S, Tcherkez G, Cornic G, Ghashghaie J (2004) Respiratory carbon metabolism following illumination in intact French bean leaves using $^{12}C/^{13}C$ labelling. Plant Physiol 136:3245-3254. doi: 10.1104/pp.104.048470

Olsrud M, Melillo JM, Christensen TR, Michelsen A, Wallander H, Olsson PA (2004) Response of ericoid mycorrhizal colonization and functioning to global change factors. New Phytol 162:459-469. doi: 10.1111/j.1469-8137.2004.01049.x

Palacio S, Millard P, Maestro M, Montserrat-Martí G (2007) Non-structural carbohydrates and nitrogen dynamics in Mediterranean sub-shrubs: An analysis of the functional role of overwintering leaves. Plant Biology 9:49-58. doi: 10.1055/s-2006-924224

Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2008) NLME: linear and nonlinear mixed effects models. R package version 3.1-89.

Pornon A, Escaravage N, Lamaze T (2007) Complementarity in mineral nitrogen use among dominant plant species in a subalpine community. Am J Bot 94:1778-1785. doi: 10.3732/ajb.94.11.1778

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Ritchie JC (1956) *Vaccinium myrtillus* L. J Ecol 44:291-299.

Le Roux X, Bariac T, Sinoquet H, Genty B, Piel C, Mariotti A, Girardin C, Richard P (2001) Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant Cell Environ 24:1021-1032. doi: 10.1046/j.0016-8025.2001.00756.x

Sarkar D (2008) Lattice: Multivariate Data Visualization with R. Springer, New York.

Schweingruber FH, Poschlod P (2005) Growth rings in herbs and shrubs: Life span, age determination and stem anatomy. Forest Snow and Landscape Research 79:195-415.

Shevtsova A, Haukioja E, Ojala A (1997) Growth response of subarctic dwarf shrubs, *Empetrum nigrum* and *Vaccinium vitis-idaea*, to manipulated environmental conditions and species. Oikos 78:440-458. doi: 10.2307/3545606

Shevtsova A, Ojala A, Neuvonen S, Vieno M, Haukioja E (1995) Growth and reproduction of dwarf shrubs in a subarctic plant community: Annual variation and above-ground interactions with neighbours. J Ecol 83:263-275. doi: 10.2307/2261565

Smith WK, Germino MJ, Hancock TE, Johnson DM (2003) Another perspective on altitudinal limits of alpine timberlines. Tree Physiol 23:1101-1112. doi: 10.1093/treephys/23.16.1101

Stöcklin J, Körner C (1999) Recruitment and mortality of *Pinus sylvestris* near the arctic treeline: the role of climatic change and herbivory. Ecological Bull 47:168-177.

Tappeiner U, Cernusca A (1993) Alpine meadows and pastures after abandonment. Pirineos 141-142:97-118. doi: 10.3989/pirineos.1993.v141-142.166

Targetti S, Staglianò N, Messeri A, Argenti G (2010) A state-and-transition approach to alpine grasslands under abandonment. iForest 3:44-51. doi: 10.3832/ifor0525-003

Tasser E, Tappeiner U (2002) The impact of land-use changes in time and space on vegetation distribution in mountain areas. Appl Veg Sci 5:173-184. doi: 10.1111/j.1654-109X.2002.tb00547.x

Tcherkez G, Hodges M (2008) How stable isotopes may help to elucidate primary nitrogen metabolism and its interaction with (photo)respiration in C_3 leaves. J Exp Bot 59:1685-1693. doi: 10.1093/jxb/erm115

Vander Kloet SP, Hill NM (2000) Bacca quo vadis: Regeneration niche differences among seven sympatric *Vaccinium* species on headlands of Newfoundland. Seed Sci Res 10:89-97. doi:10.1017/S0960258500000106

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Monogr 82:205-220. doi: 10.1890/11-0416.1

Vicente-Serrano SM, Lasanta T, Romo A (2004) Analysis of spatial and temporal evolution of vegetation cover in the Spanish Central Pyrenees: Role of human management. Environ Manage 34:802-818. doi: 10.1007/s00267-003-0022-5

Walker MD, Wahren CH, Hollister RD, Henry GHR, Ahlquist LE, Alatalo JM, Bret-Harte MS, Calef MP, Callaghan TV, Carroll AB, Epstein HE, Jónsdóttir IS, Klein JA, Magnússon B, Molau U, Oberbauer SF, Rewa SP, Robinson CH, Shaver GR, Suding KN, Thompson CC, Tolvanen A, Totland Ø, Turner PL, Tweedie CE, Webber PJ, Wookey PA (2006) Plant community responses to experimental warming across the tundra biome. P Natl Acad Sci USA 103:1342-1346. doi: 10.1073/pnas. 0503198103

Wang L, Schjoerring JK (2012) Seasonal variation in nitrogen pools and $^{15}N/^{13}C$ natural abundances in different tissues of grassland plants. Biogeosciences 9:1583-1595. doi: 10.5194/bg-9-1583-2012

Wilson S, Tilman D (1991) Components of plant competition along an experimental gradient of nitrogen availability. Ecology 72: 1050-1065 doi: 10.2307/1940605

Xu Z-F, Hu T-X, Wang K-Y, Zhang Y-B, Xian J-R (2009) Short-term responses of phenology, shoot growth and leaf traits of four alpine shrubs in a timberline ecotone to simulated global warming, Eastern Tibetan Plateau, China. Plant Spec Biol 24:27-34. doi: 10.1111/j.1442-1984.2009.00229.x

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York.

Supporting information

Table S1. Percentage cover (%) of the shrub species in each stand type. Data are means ± 1 SE (*n* = 5). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum*; U: *V. myrtillus* mixed with *V. uliginosum*.

Stand type	*V. myrtillus*	*R. ferrugineum*	*V. uliginosum*
M	100 ± 0	-	-
R	48 ± 2.0	62 ± 5.8	-
U	49 ± 6.0	-	60 ± 5.5

Table S2. δ^{13}C and Δ^{13}C of leaves and new shoots TOM (total organic matter) for the three stand types studied. M: monospecific stands of *V. myrtillus*, R: stands of *V. myrtillus* mixed with *R. ferrugineum*, U: stands of *V. myrtillus* mixed with *V. uliginosum*. Values represent means ± 1 SE (*n* = 5). The δ^{13}C of the CO_2 of the air was -10.91‰.

Stand type	Fraction	δ^{13}C (‰)	Δ^{13}C
M	Leaf	-28.62 ± 0.26	17.71 ± 0.26
	Shoot	-27.58 ± 0.52	16.67 ± 0.51
R	Leaf	-29.48 ± 0.21	18.57 ± 0.21
	Shoot	-28.07 ± 0.18	17.16 ± 0.18
U	Leaf	-28.87 ± 0.15	17.96 ± 0.15
	Shoot	-27.62 ± 0.28	16.71 ± 0.28

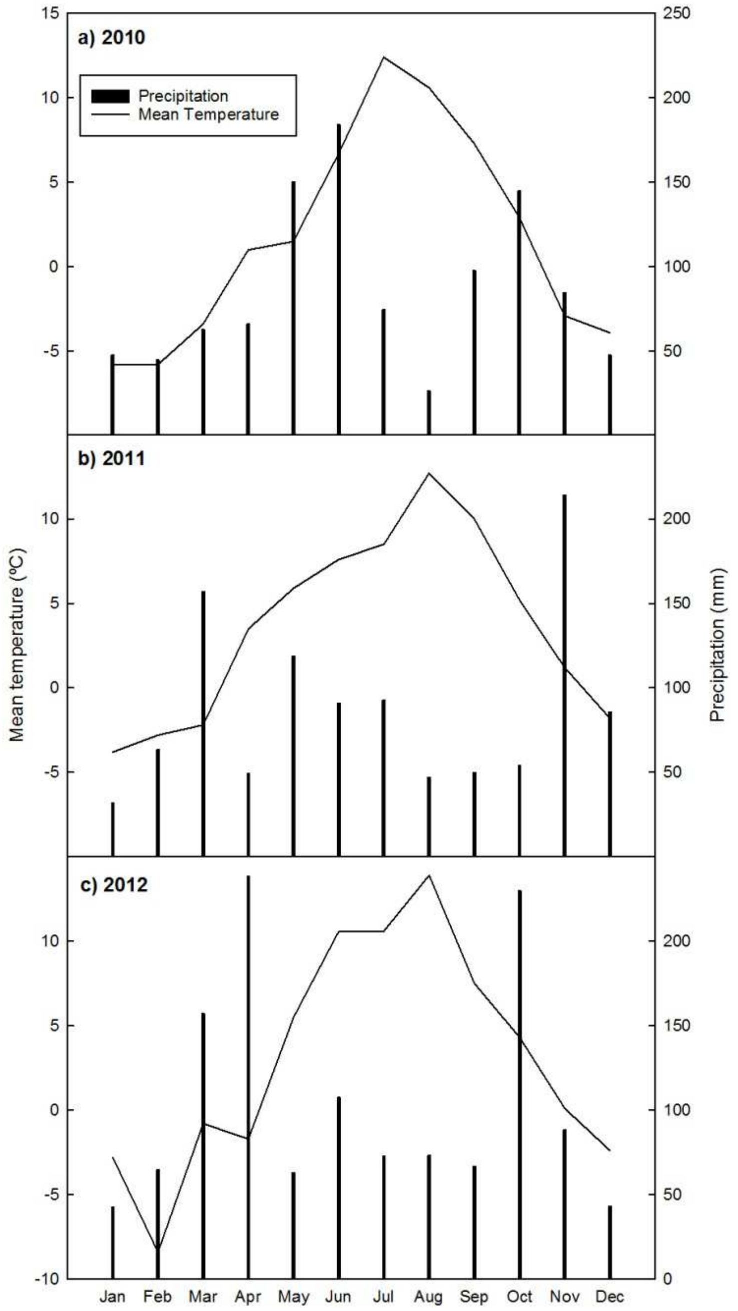

Figure S1. Monthly mean temperature (°C) and precipitation (mm) from 2010 to 2012 at La Bonaigua Station (Meteorological Service of Catalonia), located at 2266 m a.s.l., 6.3 km from the study site.

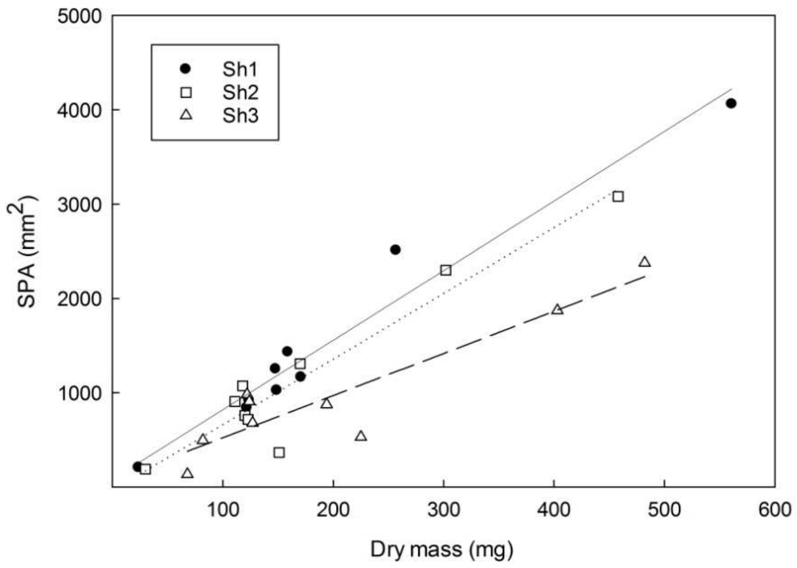

Figure S2. Correlations between shoot photosynthetic area (SPA, mm^2) and shoot dry mass (mg) of new shoots (Sh1), one-year-old shoots (Sh2) and two-year-old shoots (Sh3) of *V. myrtillus*. We used the obtained regression equations to estimate SPA of the rest of the study ramets: y = 7.38x + 79.66 (Sh1), y = 6.98x – 39.46 (Sh2), y = 4.48x + 73.26 (Sh3).

Capítol 2 / Chapter 2

Four years of experimental warming do not modify the interaction between subalpine shrub species

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Resum

L'escalfament global pot comportar canvis en la interacció entre espècies d'alta muntanya a través de modificacions en les condicions ambientals, cosa que podria provocar canvis dràstics en les comunitats vegetals. Vam explorar els efectes de quatre anys d'escalfament experimental amb cambres *open-top* (OTC) en el funcionament de *Vaccinium myrtillus* i en la seva interacció amb els arbusts veïns a l'ecotò del límit supraforestal al Pirineu. Vam examinar els efectes de l'escalfament en l'alçada, la biomassa aèria i subterrània, i en les concentracions de C i N i la seva composició isotòpica en *V. myrtillus* en claps purs o en claps mixtos amb *Vaccinium uliginosum* o amb *Rhododendron ferrugineum*. També vam analitzar variacions en les concentracions de N del sòl, relacions C/N en la rizosfera i la diversitat funcional de la comunitat microbiana; i vam avaluar si l'escalfament havia afectat la biomassa i les concentracions de C i N i la seva composició isotòpica en *V. uliginosum* en claps mixtos. Els nostres resultats van mostrar que l'escalfament havia estimulat el creixement de les parts aèries però no les subterrànies en *V. myrtillus*, mentre que *V. uliginosum* no va respondre a l'escalfament. El funcionament de *V. myrtillus* no va diferir entre tipus de clap sota el tractament d'escalfament, cosa que suggereix que l'escalfament no va induir canvis en la interacció entre *V. myrtillus* i les espècies veïnes. Aquests resultats contrasten amb els d'estudis previs, on les interaccions entre plantes havien canviat amb la modificació de la temperatura. Els nostres resultats mostren que les interaccions entre espècies poden reaccionar a l'escalfament en menor mesura en comunitats vegetals naturals que no pas en experiments d'extracció, cosa que emfasitza la necessitat de més estudis basats en comunitats naturals a l'hora d'explorar l'efecte dels canvis ambientals en les interaccions planta-planta.

Abstract

Climate warming can lead to changes in alpine plant species interactions through modifications in environmental conditions, which may ultimately cause drastic changes in plant communities. We explored the effects of four years of experimental warming with open-top chambers (OTC) on *Vaccinium myrtillus* performance and its interaction with neighbouring shrubs at the Pyrenean treeline ecotone. We examined the effects of warming on height, above-ground (AG) and below-ground (BG) biomass and the C and N concentration and isotope composition of *V. myrtillus* growing in pure stands or in stands mixed with *Vaccinium uliginosum* or *Rhododendron ferrugineum*. We also analysed variations in soil N concentrations, rhizosphere C/N ratios and the functional diversity of the microbial community, and evaluated whether warming altered the biomass, C and N concentration and isotope composition of *V. uliginosum* in mixed plots. Our results showed that warming induced positive changes in the AG growth of *V. myrtillus* but not BG, while *V. uliginosum* did not respond to warming. *Vaccinium myrtillus* performance did not differ between stand types under increased temperatures, suggesting that warming did not induce shifts in the interaction between *V. myrtillus* and its neighbouring species. These findings contrast with previous studies in which species interactions changed when temperature was modified. Our results show that species interactions can be less responsive to warming in natural plant communities than in removal experiments, highlighting the need for studies involving the natural assembly of plant species and communities when exploring the effect of environmental changes on plant-plant interactions.

Keywords: dwarf shrub, plant interactions, Pyrenees, *Rhododendron ferrugineum*, treeline, *Vaccinium myrtillus*, *Vaccinium uliginosum*, passive warming

Abbreviations: AG (above-ground), BG (below-ground), $\delta^{13}C$ (carbon isotope composition), $\delta^{15}N$ (nitrogen isotope composition)

Introduction

In recent decades, climate warming and land-use change (i.e. abandonment of extensive livestock grazing and tree logging) have led to shrub encroachment processes in the alpine treeline ecotone worldwide (Dullinger et al. 2003; Rundqvist et al. 2011; Ropars and Boudreau 2012). The forecasted global air temperature increase of 1.0–3.7 ºC by the end of the century could accelerate these processes; especially at high elevations and latitudes, where change is predicted to be larger (Nogués-Bravo et al 2007; Collins et al. 2013; Rangwala et al. 2013). This could have a dramatic impact on alpine and Arctic tundra ecosystems due to shifts in community composition and potential feedbacks to warming, such as decreasing albedo due to the higher radiation absorption by shrub canopies, reducing radiative cooling at night through the reflection of the thermal energy emitted by the soil, or through the inputs of more recalcitrant litter in the ecosystem (Hobbie 1996; Cornelissen et al. 2007; Myers-Smith et al. 2011; D’Odorico et al. 2013).

Many studies in Arctic and alpine ecosystems have shown the need to conduct species-specific studies to understand vegetation changes with warming, since coexisting species may differ in their responses to increasing temperatures (Kudo and Suzuki 2003; Klanderud 2008; Anadon-Rosell et al. 2014; Little et al. 2015; Yang et al. 2015). However, it is also important to consider plant–plant interactions, since they are crucial for plant community dynamics (Callaway and Walker 1997). The stress-gradient hypothesis (Bertness and Callaway 1994) postulates that competition is the major selective force in habitats with more benign environmental conditions, whereas facilitation dominates in more severe environments. Many studies in cold regions across the globe have shown that plant interactions shift from facilitation to competition as temperature increases, or in the opposite direction when temperature decreases (Shevtsova et al. 1997; Choler et al. 2001; Klanderud 2005; Pugnaire et al. 2015; Wheeler et al. 2015; Olsen et al. 2016). Nevertheless, most of these studies involved plant removal experiments, and studies focusing on the effects of temperature changes on plant interactions within natural communities are scarce (but see Dormann et al. 2004).

Shrubs are major components of alpine and Arctic tundra ecosystems. Amongst them, clonal dwarf shrub species are of great importance in terms of vegetation cover, structure and functionality. They present a complex network of subterranean rhizomes bearing fine roots, and producing individual above-ground (AG) ramets. Thus, the below-ground (BG) system of clonal shrubs is essential for their persistence and vegetative expansion, as well as an important source of soil carbon (C) (Cornelissen et al. 2014). Changes in the BG structure of dominant clonal shrubs could translate into major changes in the community and ecosystem functioning. Consequently, the study of BG responses to warming is an essential part of the complex responses to temperature increase in Arctic and alpine areas. However, the destructive nature of BG sampling and the difficulty to identify and separate roots from different species, together with the compromise of having studies running for the longest term possible, explain why warming experiments including both AG and BG plant measurements are infrequent (but see Hollister and Flaherty 2010 and Yang et al. 2015, amongst others).

Global warming may also induce shifts in the composition and function of the soil microbial community (Streit et al. 2014; Classen et al. 2015; DeAngelis et al. 2015), which can have strong impacts on ecosystem functioning (Schimel and Schaeffer 2012). For instance, rising temperatures can alter nitrogen (N) mineralization, with effects on N availability and, ultimately, plant growth (Bardgett and Wardle 2010). Several studies in cold ecosystems have found an increase in the soil N pool size with warming (Chapin et al. 1995; Hartley et al. 1999; Dijkstra et al. 2010; Dawes et al. 2011; Bai et al. 2013), which has been related to a stimulation of mineralization and decomposition processes. Since coexisting species show different N preferences and N-acquisition strategies (Körner et al. 2003; Pornon et al. 2007), shifts in N pools may affect interspecific interactions by altering relative niche and fitness differences between species (Chesson 2000; Tilman and Lehman 2001).

Vaccinium myrtillus L. forms shrub patches that colonize subalpine and alpine grasslands in the Pyrenees, where it grows close to the upper altitudinal limit of its distribution (Bolòs et al. 2005), subjected to low temperatures and short growing seasons. Warmer temperatures could favour its growth at the treeline ecotone, as has been reported in warming experiments in the Alps (Dawes et al. 2011; Anadon-Rosell et al. 2014) and in the Arctic tundra (Rinnan et al. 2009; Taulavuori et al. 2013). However, co-occurring species such as *Vaccinium uliginosum* or *Empetrum hermaphroditum* have not been found to respond to temperature increase (Richardson et al. 2002; Kudo and

Suzuki 2003; Anadon-Rosell et al. 2014) and, consequently, interactions between these species might shift with warming. On the other hand, in line with the stress-gradient hypothesis, a modification of the environment through air temperature increase could induce changes in the interaction between this species and its neighbours towards increased competition. Despite the numerous studies focusing on *V. myrtillus* in tundra ecosystems, to our knowledge the potential effects of warming on the interaction with its neighbours have not been reported. Moreover, the previously mentioned experiments on *V. myrtillus* have mainly focused on its AG responses to warming, whereas BG effects have not been assessed.

At the treeline ecotone in the Central Pyrenees, *V. myrtillus* grows in pure patches (stands hereafter) or in mixed stands together with *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. (hereafter *V. uliginosum*), or *Rhododendron ferrugineum* L. A previous study did not find evidence that the co-occurrence with these neighbouring shrubs had major effects on *V. myrtillus* structure and functioning (Anadon-Rosell et al. 2016). The objective of the present study was to investigate the AG and BG effects of four years of passive warming on *V. myrtillus*, and whether warming induced changes in interactions between *V. myrtillus* and its neighbouring species: a shrub of a very similar size (*V. uliginosum*) and a taller shrub (*R. ferrugineum*). For this purpose we assessed *V. myrtillus* phenology, AG and BG biomass, C and N concentration and isotopic signature (δ^{13}C and δ^{15}N), soil inorganic N concentrations (NO_3^- and NH_4^+) and C and N availability in the rhizosphere in different types of *V. myrtillus* stands subjected to warming treatments. We also assessed the effects of warming on *V. uliginosum* AG biomass and C and N concentration and isotopic signature in mixed stands. Moreover, we evaluated the effects of warming on the functional diversity of the microbial community in *V. myrtillus* pure stands. We hypothesized that warming will (i) benefit *V. myrtillus* AG and BG performance (i.e. biomass and physiological traits), but that it will also (ii) modify interactions with co-occurring species, which will be manifested as different responses to warming of *V. myrtillus* when growing in pure stands than when growing in mixtures. We also hypothesized that (iii) the soil inorganic N pool will increase under warming with different magnitude across stand types.

Materials and methods

Study area

The study site was located at Eth Corrau des Machos (Val d'Aran), in the buffer zone of the Aigüestortes and Estany de Sant Maurici National Park (Central Pyrenees, Catalonia, UTM coordinates 31N 329, 472), on a N-facing 10-15º steep slope at 2250 m a.s.l. The vegetation consisted of *Festuca eskia* Ramond ex DC. and *Nardus stricta* L. grasslands mixed with patches of dwarf shrub heath dominated by *V. myrtillus*, *V. uliginosum* and *R. ferrugineum*. For the period 2001-2013, the mean annual precipitation and mean annual temperature (± SD) were 1146.4 (± 58.3) mm and 3.0 (± 0.2) °C, respectively. For the study period (2010-2013) the mean annual precipitation and mean annual temperature were 1223.1 (± 244.8) mm and 2.7 (± 0.9) °C, respectively. The mean monthly precipitation and the mean temperature for the main months of the growing season (June–August) were 99.1 (± 28.5) mm and 10.2 (± 1.0) ºC (obtained from a meteorological station at a nearby location: La Bonaigua, 6.3 km away from the study site and at a similar altitude, run by the Meteorological Service of Catalonia, www.meteo.cat, accessed in May 2014).

Experimental design

In July 2010 we established 30 plots of 1.1 m^2 combining a stand type and a warming treatment. We selected 10 pure stands of *V. myrtillus* (M stands), 10 mixed stands of *V. myrtillus* and *V. uligiosum* subsp. *microphyllum* (U stands), and 10 mixed stands of *V. myrtillus* and *Rhododendron ferrugineum* (R stands). In each stand, shrubs were the dominant species (Table S1), but other grasses and forbs were also present. Amongst them, the most abundant species were *Festuca eskia*, *Festuca nigrescens*, *Nardus stricta*, *Trifolium alpinum*, *Phleum alpinum* and *Meum athamanticum*. The distance between two plots ranged from one to a few metres (< 20 m), always ensuring that the studied patches were independent from each other. Soil organic matter and organic C concentrations, pH and colour (according to Munsell System) were similar across plots (Table S2). In half of the plots (i.e. 15 plots, five for each stand type) we placed an open-top chamber (OTC) made of transparent polycarbonate, similar to the model used in the International Tundra Experiment (ITEX; Marion et al. 1997). The other 15 plots served as ambient air temperature controls. The air temperature increase inside the

OTCs in summer was 1.1 ºC, which we measured with temperature loggers (*iButton* 1-wire Thermochron, Embedded Data Systems, USA) placed at ground level in two plots of each stand type x warming combination during the growing season 2013 (recording every hour). The snow accumulation was high and homogenous along the study site and our phenological survey did not reveal substantial irregularities in the snowmelt pattern, even despite the presence of OTCs. Thus, we left the OTCs in place throughout the experiment.

Phenology and community composition

In 2011 we labelled six *V. myrtillus* ramets per plot, which we monitored during the growing seasons of 2011 and 2012 for a phenological survey. We recorded the following phenophases: winter state, bud swelling, bud bursting, leaf expansion, shoot elongation, vegetative state, leaf colour change, leaf shedding, leafless state and shoot winter colouring (brown-red coloration). We visited the plots *ca.* once a month starting after snowmelt until late Autumn, when ramets were leafless, and we recorded the presence of different phenophases in the six marked ramets. We assigned an ordinal numeric code to all phenophases and calculated the average numeric code per plot as the average score of the six ramets at each visit.

Plant community composition within the study plots was first recorded in 2011, by estimating the percentage cover of the main plant groups in each plot, i.e. shrubs and grasses. This was re-assessed in September 2013 before the end of the experiment (Table S1). Lichen and bryophyte cover was very low and was not recorded.

AG and BG biomass

On the 3rd September 2013 we harvested five *V. myrtillus* ramets per plot (not corresponding with those phenologically surveyed) plus five *V. uliginosum* ramets in U plots, avoiding sampling close to the edges of the OTCs. We also dug out their rhizomes (down to *ca.* 20 cm long) and the roots attached, and collected six soil cores of 12 cm length x 4 cm diameter in each plot (corresponding to 0-15 cm depth), which were kept in sealed plastic bags in a cool box until they arrived to the lab. Two of these cores were kept frozen and were used for BG biomass measurements at the plot scale. The rest were kept refrigerated at 4 ºC and were used for measurements of soil NO_3^- and NH_4^+ concentration and analyses of the functional diversity of the microbial community (two cores); and for rhizosphere C and N concentration and isotope composition analyses (two cores). Soil cores obtained for the same purpose from each plot were pooled together. Therefore, we had one composite soil sample per plot for each type of measurement.

Once in the lab, we measured the ramet height of both *Vaccinium* species and counted the scars left by the buds in each ramet to estimate their age. Then, we separated leaves, new shoots (i.e. shoots grown in 2013), rhizomes and roots of each species, and dried them at 60 ºC for 48 hours. Leaves and new shoots were weighed for AG biomass measurements and subsequently used for N and C concentration and isotope composition analyses. Rhizomes and roots were only used for N and C concentration and isotope composition analyses because BG biomass was measured at the plot scale on material obtained from the soil cores. We conducted BG biomass measurements referring to a specific soil volume to make comparisons between warming treatments and stand types possible. Soil cores for BG biomass measurements were sieved to separate rhizomes, coarse roots ($\geq$ 1 mm diameter) and fine roots ($<$ 1 mm diameter). We dried them in the oven at 60 ºC for 48 h and weighed them for BG biomass analyses.

Carbon and nitrogen concentration and isotope composition

For the analyses of C and N concentration and isotope composition of leaves, new shoots, rhizomes and roots, we pooled together the material from all the harvested ramets of each plot for each plant part of each *Vaccinium* species. Then we ground the material and weighed *ca.* 1 mg subsamples in small tin capsules. The C and N concentrations of samples were determined using an Elemental Analyzer Flash 1112 (Carbo Erba, Milan). The C and N isotope composition of samples were determined using the Elemental Analyzer coupled to an IRMS Delta C isotope ratio mass spectrometer through a Conflo III Interface (Thermo-Finnigan, Germany). The results of C isotope analyses are reported in per thousand (‰) on the relative δ-scale as $\delta^{13}C$, and refer to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} \right) - 1 \qquad \text{(Eq. 1)}$$

where R is the $^{13}C/^{12}C$ ratio.

N isotopic composition results are also expressed in δ notation ($\delta^{15}N$) using international secondary standards of known $^{15}N/^{14}N$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) relative to N_2 in air:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} \right) - 1 \qquad \text{(Eq. 2)}$$

where R is the $^{15}N/^{14}N$ ratio.

We used C and N concentrations to calculate C/N ratios of each plant part of the two *Vaccinium* species. We calculated total N pools for all *V. myrtillus* plant parts (for BG parts we only used data from pure stands, referred to soil volume) and for *V. uliginosum* AG plant parts. All EA-IRMS analyses were performed at the Scientific and Technological Centers (CCiT) of the University of Barcelona. The $\delta^{13}C$ of CO_2 of the air and the $\delta^{15}N$ of the bulk soil were analysed in 2013 and were *ca*. -10.91‰ and *ca*. 7.33 ‰, respectively (see Anadon-Rosell et al. 2016 for more information on air and soil sampling).

Soil inorganic N concentrations

NO_3^- concentrations were measured following the UV method described by Kaneko et al. (2010) by measuring the absorbance of KCl extracts from soils at 220 nm and 260 nm wavelengths. Soil NH_4^+ concentrations were measured by the conversion of ammonium into the intense blue indophenol complex (IPC) using salicylate, following the methods used by Kempers and Kox (1989).

Rhizospheric soil analyses and functional diversity of the microbial community

We carefully selected rhizomes and roots from the two soil cores collected for rhizosphere analyses and separated the soil that was attached using a small paint brush. We ground the soil and weighed *ca.* 3.5 mg subsamples in small tin capsules and analysed its C and N concentration and isotope composition following the same procedure as for plant tissues.

We assessed the impact of the warming treatment on the use of different C sources by soil microbial communities using Biolog EcoPlates (Insam 1997). Every plate had 96 wells containing 31 different C sources plus a blank well, in three replications. The rate of utilization of the C sources by microorganisms results in the increase of the optical density (OD_{590}) (Pohland and Owen 2009). We analysed the use of C by soil microbial communities in *V. myrtillus* pure stands only, using three replicates per warming treatment. Soils were sieved at 2 mm before extracting the bacterial community. Bacterial cells were extracted by mixing 10 g fresh soil with 95 ml of sterilized Milli-Q water inside 100 ml Erlenmeyers (see details in Muñiz et al. 2014). The mixture was magnetically shaken for 30 minutes, followed by one-hour rest. Afterwards, 10 ml of the soil suspension was put into 50 ml Falcon tubes and, after one-minute sonication, the tubes were centrifuged (1000 g, 10 minutes). 9.5 ml of the supernatant were separated and the remaining was resuspended after adding 9.5 ml of water. 47.5 ml of soil extract was obtained from each sample after five cycles of sonication-centrifugation. The extracts were kept at 4 °C for a few hours. Just before inoculating the Biolog plates, soil particles were removed by a low-speed centrifugation (500 g, 2 minutes). 150 µl of the soil extract were put into each well of the plates by a multipipete. All the laboratory material was sterile or it was previously autoclaved (121 °C, 20 minutes) and the operations were made inside a biological laminar flow chamber. The plates were incubated in the dark at 25 °C for 120 hours. The OD_{590} of each well was measured just after the inoculation (at 0, 5, 72, 96, 101 and 120 h) using the Anthos 2010 microplate reader and ADAP 2.0 Software (Biochrom, Ltd. Cambridge Science Park, Cambridge, CB4 0FJ. England).

Statistical analyses

We tested the effects of warming and stand type on *V. myrtillus* phenology, ramet height and AG biomass using linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML). We included warming and stand type as fixed factors and plot as a random factor to account for the multiple sampling within a plot. We used the same models for *V. uliginosum* variables, but in this case we only used warming as a fixed factor. To test the effects of warming and stand type on the C and N concentration and isotope composition of the different AG and BG tissues, BG biomass, soil NO_3^- and NH_4^+ concentration, rhizosphere C and N concentration and isotope composition we used simple linear model functions. We included ramet age as a covariate when it significantly contributed to improve model fits (which we tested with likelihood ratio tests) to account for possible age effects on our growth-related response variables. This was the case in the models for *V. myrtillus* height and AG biomass, plus the models for height, number of shoots and dry weight per shoot unit for *V. uliginosum*. We tested for significance with analysis of variance tests and graphically evaluated the assumptions of normality and homoscedasticity of residuals (Zuur et al. 2009). We double-checked with Shapiro and Bartlett tests when the visual evaluation of graphs was difficult. We log-transformed data when necessary to satisfy these assumptions. Moreover, when homoscedasticity of residuals was not met, we used the varIdent structure (Zuur et al. 2009; Pinheiro et al. 2016) to account for the heterogeneity of variances among factor levels. In *V. uliginosum* analyses, when both normality and homoscedasticity were not met, we used the non-parametric Wilcoxon test. We considered effects significant at $P < 0.05$ and trending towards significance at $0.05 > P < 0.10$ to account for the relatively low replication. When we found significant differences between stand types, we run Tukey HSD post hoc tests to determine those factor levels that differed significantly. We performed all the analyses with R 3.1.2 (R Core Team, 2015). For linear mixed effects models we used the *nlme* package (Pinheiro et al. 2016); for graphical evaluation of model assumptions we used the *lattice* package (Sarkar 2008); and for multiple comparisons we used the *multcomp* package on linear mixed effects models (Hothorn et al. 2008) and the *agricolae* package on simple linear models (de Mendiburu 2010).

The microbial activity of each microplate was expressed as the average well-colour development (AWCD) and was determined as previously described (Garland and Mills 1991) as follows:

$$\text{AWCD} = \sum ODi / 31 \qquad \text{(Eq. 3)}$$

where OD_i is the optical density value from each well at any given time. The AWCD curves were adjusted to a log-logistic 4-parameters model using R Software. This was done first for each replicate and also for the warming treatment levels, each including all replicates (in this case with the raw data of OD_{590}). Then curve parameters (curve slope -slop-, maximal degradative capacity -max- and time to reach half of the slope -TM50-) were compared using the *CompParm* function in *drc* package (Ritz and Streibig 2005): the null hypothesis was that the ratio between parameters equalled 1. The ratio was obtained by dividing the same parameters from each curve by the other parameters; if the ratio significantly differed from 1, the null hypothesis was rejected, meaning that values were significantly different ($P < 0.05$). *P*-values were adjusted using Bonferroni correction for multiple T-tests.

Results

Phenology

Warming advanced early-season vegetative phenology through an earlier onset of bud burst and leaf expansion (Fig. S1). On the 21st May 2011 (day of year, DOY, 141), *V. myrtillus* ramets in unwarmed plots were at the bud swelling phase, whereas in warmed plots had already started bursting ($F_{1,24} = 3.92$, $P = 0.059$). In 2012, *V. myrtillus* ramets in warmed plots were expanding their leaves on the 14th June (DOY 166), whereas ramets in unwarmed plots were still in the bud burst phenophase ($F_{1,24} = 6.59$, $P = 0.017$). Monitoring later in the season for both years did not show any other significant differences between warming treatments (see Fig. S1 for visit dates). We only found significant differences between stand types (regardless of the warming treatment) in September 2011, when ramets in M stands were already shedding their leaves whereas ramets in the other two stand types had just started changing their colour prior to leaf shedding ($F_{2,24} = 9.31$, $P = 0.001$). This advancement in senescence in M stands with respect to the other stand types was especially obvious in warmed plots (the interaction stand type x warming trended towards significance, $F_{2,24} = 3.13$, $P = 0.062$).

Age and AG biomass of Vaccinium *species*

Our age analyses confirmed that there were no differences in *V. myrtillus* ramet age between warming treatments ($F_{1,24} = 0.16$, $P = 0.696$) nor between stand types ($F_{2,24} = 1.89$, $P = 0.173$). Likewise *V. uliginosum* did not show differences in ramet age between warming treatments ($F_{1,6} = 0.01$, $P = 0.930$).

After four years of warming, *V. myrtillus* ramets were 15% taller in warmed plots than in unwarmed plots. There were no differences in ramet height between stand types or an interaction between warming and stand type (Table 1). Similarly, there was no warming effect on *V. uliginosum* height ($F_{1,6} = 0.08$, $P = 0.784$).

Vaccinium myrtillus leaf biomass per ramet did not differ between warming treatments (Table 1, Fig. 1a) but new shoot biomass was higher under warming than in control plots (Fig. 1b). The total above-ground biomass per ramet was also higher in warmed plots than in unwarmed plots (Table 1, Fig. 1c). There were no differences between stand types or a stand type x warming interaction for *V. myrtillus* AG biomass (Table 1). There were no differences between warming treatments in terms of *V. uliginosum* leaf biomass ($F_{1,6}$ = 2.77, P = 0.147), new shoot biomass ($F_{1,6}$ = 0.04, P = 0.849) or total AG biomass ($F_{1,6}$ = 0.39, P = 0.554, Fig. S2), but we found contrasting effects of warming on the dry weight per shoot and the number of new shoots. Dry weight per shoot in *V. uliginosum* was higher inside the OTCs than in control plots ($F_{1,6}$ = 6.42, P = 0.044), whereas the number of new shoots was higher in ramets from unwarmed plots ($F_{1,6}$ = 14.81, P = 0.009).

Table 1. Results of ANOVA for effects of warming and stand type on *Vaccinium myrtillus* height and above-ground (AG) biomass parameters at the ramet scale. Significant effects (P < 0.05) and effects trending towards significance (0.1 > P > 0.05) are in bold.

AG Variable	Factor/covariable	df	*F*	*P*
Height	Age	1, 118	26.47	**<0.001**
	Warming	1, 24	5.33	**0.030**
	Stand type	2, 24	1.89	0.173
	Stand type x warming	2, 24	0.16	0.857
Leaf biomass	Age	1, 118	12.00	**0.001**
	Warming	1, 24	2.38	0.136
	Stand type	2, 24	1.49	0.246
	Stand type x warming	2, 24	1.20	0.320
New shoots biomass	Age	1, 118	8.68	**0.004**
	Warming	1, 24	5.02	**0.035**
	Stand type	2, 24	1.38	0.271
	Stand type x warming	2, 24	0.37	0.693
Total AG biomass	Age	1, 118	32.41	**<0.001**
	Warming	1, 24	4.74	**0.040**
	Stand type	2, 24	1.77	0.193
	Stand type x warming	2, 24	0.16	0.857
No. of shoots	Age	1, 114	23.45	**<0.001**
	Warming	1, 23	0.26	0.613
	Stand type	2, 23	0.84	0.443
	Stand type x warming	2, 23	0.83	0.450
Dry weight/shoot	Age	1, 114	2.87	0.093
	Warming	1, 23	3.88	**0.061**
	Stand type	2, 23	2.24	0.129
	Stand type x warming	2, 23	0.55	0.584

Vaccinium myrtillus *BG biomass*

There were no effects of warming on *V. myrtillus* BG biomass (Fig. 1d, e, f). We only found differences in rhizome and coarse root biomass between stand types. R stands showed lower rhizome biomass per soil volume than in the other two stand types ($F_{2,24} = 6.93$, $P = 0.004$, Fig. 1d). U stands showed a trend towards significantly greater coarse root biomass than M stands ($F_{2,19} = 3.04$, $P = 0.071$, Fig. 1e). Fine root biomass did not differ between stand types ($F_{2,24} = 0.41$, $P = 0.667$, Fig. 1f). We did not find any warming x stand type interaction for any of the BG plant parts analysed (Table S3).

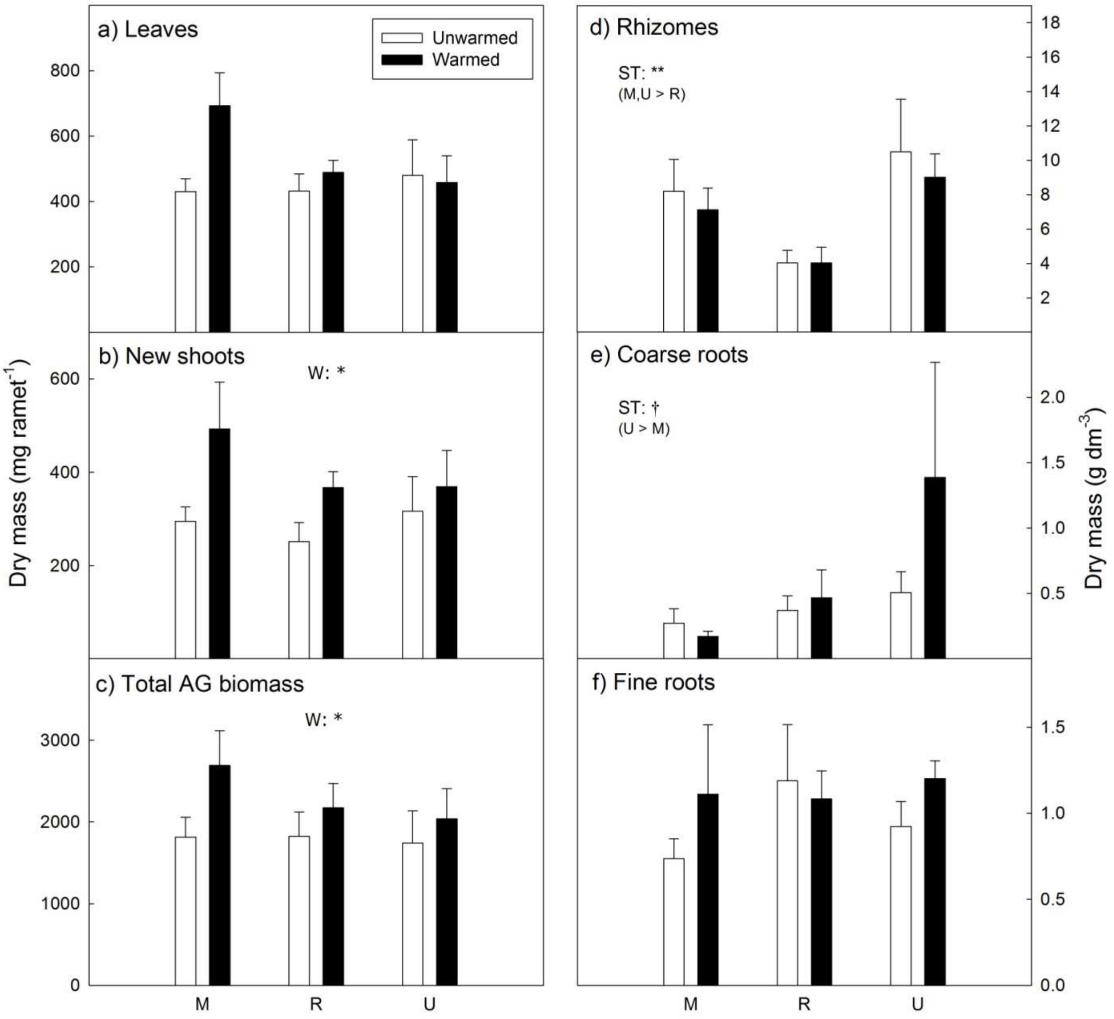

Figure 1. *Vaccinium myrtillus* AG biomass (a,b,c) and BG biomass (d,e,f) in different stand types (ST) and warming treatments in September 2013 (W; $n = 5$, means + 1 SE are shown). Note that BG biomass data are referred to unit volume of soil at the plot scale. Symbols show significant differences († trend towards significance at $0.1 > P > 0.05$; * significant differences at $0.05 > P > 0.01$; ** significant differences at $P < 0.01$). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

C and N concentration and isotope composition of AG and BG plant fractions

C concentration in *V. myrtillus* organs was similar across warming treatments and stand types for leaves, shoots and roots. Rhizomes, however, had greater C concentration under warming than in control plots (Table 2, Fig. 2), which was not related to any rhizome biomass increase under warming (see above). C concentration values of *V. uliginosum* new shoots, rhizomes and roots did not show any response to warming, but there was a trend towards a significantly positive effect of warming on leaf C concentration (Table 3, Fig. 3).

The δ^{13}C of *V. myrtillus* and *V. uliginosum* tissues did not differ between warming treatments (Fig. 2, 3) but we found significant differences in the δ^{13}C of *V. myrtillus* tissues between stand types. *Vaccinium myrtillus* δ^{13}C was lower in R stands than in the other two stand types for leaves (only trending towards significance), shoots and rhizomes. There were no significant differences between stand types for the δ^{13}C composition of roots (Table 2, Fig. 2), or any warming x stand type interaction.

There was no warming effect on the N concentration and δ^{15}N of any of the *V. myrtillus* organs, and only a very marginal trend towards significance of the interaction between warming and stand type in the N concentration of *V. myrtillus* rhizomes, which was higher in control plots than in warmed plots in U stands (Table 2, Fig. 2). However, we found significant differences between stand types. Leaf N concentration was higher in R stands than in U stands, but this was not the case for any of the other plant organs. Leaf and shoot δ^{15}N values were higher in M stands than in the other two stand types. Finally, rhizome δ^{15}N values were also higher in M stands than in the other two stand types, but only significantly higher than in R stands (Table 2, Fig. 2). The N pool in *Vaccinium myrtillus* rhizomes (0.037 ± 0.006 mg/cm^3) and roots (0.010 ± 0.002 mg/cm^3) did not differ between warming treatments ($F_{1,7} = 0.51$, $P = 0.497$; $F_{1,8} = 0.60$, $P = 0.462$, respectively). Leaf N pools did not differ between warming treatments ($F_{1,24} = 2.21$, $P = 0.150$) or stand types ($F_{2,24} = 1.34$, $P = 0.282$) (average across warming and stand type treatments of 9.16 ± 0.65 mg). The N pool in *V. myrtillus* new shoots was higher in warmed (5.46 ± 0.62 mg) than in unwarmed plots (3.72 ± 0.38 mg) ($F_{1,24} = 5.82$, $P = 0.024$), but did not differ between stand types ($F_{2,24} = 0.93$, $P = 0.408$). We did not find any warming effect on C/N ratios, only a trend towards significance for rhizomes in U stands, which showed higher values under warming than in unwarmed plots (Table 2, Fig. S3). We only found significant differences in C/N ratios between stand types in leaves, which showed higher values in U stands than in the other two (Table 2, Fig S3). New shoots and roots did not show significant differences in their C/N ratios for any of the treatments.

Vaccinium uliginosum shoots showed significantly lower N concentrations under warming than in unwarmed plots. This seemed associated with an increase in leaf N concentrations under warming (although the latter was not significant; Table 3). $\delta^{15}N$ values did not differ significantly between warming treatments (Table 3, Fig. 3). Leaf and new shoot N pools in *V. uliginosum* did not differ between warming treatments either ($F_{1,6} = 1.74$, $P = 0.235$; $F_{1,6} = 0.04$, $P = 0.843$, respectively). The total amount of N in leaves was 9.25 ± 0.78 mg and in new shoots 1.92 ± 0.23 mg (averaged across warming treatments). C/N ratios did not differ significantly between warming treatments in leaves (24.58 ± 0.74, $F_{1,6} = 1.59$, $P = 0.254$), rhizomes (93.12 ± 6.30, $F_{1,6} = 0.03$, $P = 0.874$) or roots (53.87 ± 1.96, $F_{1,6} = 0.90$, $P = 0.379$) (averages across warming treatments shown). However, in new shoots C/N values were higher under warming (52.37 ± 0.95) than in unwarmed plots (46.28 ± 1.84; $F_{1,6} = 8.65$, $P = 0.026$).

Table 2. Results of ANOVA for the effects of warming (W) and stand type (ST) on the C and N concentration and isotope composition ($\delta^{13}C$, $\delta^{15}N$) of *Vaccinium myrtillus* leaves, new shoots, rhizomes and roots. *F*-values and *P*-values (in parentheses) are given. Significant effects ($P < 0.05$) and effects trending towards significance ($0.1 > P > 0.05$) are in bold. Between-groups degrees of freedom were 1 for W, 2 for ST and 2 for ST x W. Within-groups degrees of freedom were 24, except for root N concentration and C/N (22), rhizome C and N concentration (22), root $\delta^{15}N$ (23) and rhizome C/N (21).

Organ	Variable	W	ST	W x ST
Leaves	C concentration	0.93 (0.344)	1.51 (0.242)	1.57 (0.228)
	N concentration	0.02 (0.884)	4.93 (**0.016**)	0.07 (0.931)
	$\delta^{13}C$	0.90 (0.352)	2.72 (**0.086**)	0.85 (0.441)
	$\delta^{15}N$	0.08 (0.780)	10.28 (**0.001**)	0.04 (0.960)
	C/N	0.06 (0.796)	3.74 (**0.038**)	0.23 (0.798)
	C concentration	1.68 (0.207)	0.94 (0.404)	0.94 (0.404)
New shoots	N concentration	0.07 (0.793)	0.63 (0.540)	0.77 (0.472)
	$\delta^{13}C$	0.07 (0.794)	8.16 (**0.002**)	1.85 (0.179)
	$\delta^{15}N$	0.33 (0.571)	9.39 (**0.001**)	0.00 (1.000)
	C/N	0.70 (0.410)	0.84 (0.444)	0.23 (0.794)
Rhizomes	C concentration	5.71 (**0.026**)	0.33 (0.723)	0.7 (0.509)
	N concentration	0.05 (0.829)	0.46 (0.637)	2.57 (**0.099**)
	$\delta^{13}C$	0.42 (0.522)	8.78 (**0.001**)	0.03 (0.972)
	$\delta^{15}N$	0.02 (0.884)	6.53 (**0.005**)	0.08 (0.921)
	C/N	2.71 (0.114)	0.26 (0.775)	2.70 (**0.091**)
Roots	C concentration	0.21 (0.653)	0.43 (0.656)	0.56 (0.578)
	N concentration	1.69 (0.207)	0.62 (0.545)	1.19 (0.323)
	$\delta^{13}C$	1.59 (0.218)	0.15 (0.860)	2.53 (0.101)
	$\delta^{15}N$	0.21 (0.650)	2.04 (0.153)	0.19 (0.826)
	C/N	1.58 (0.222)	0.31 (0.739)	0.38 (0.688)

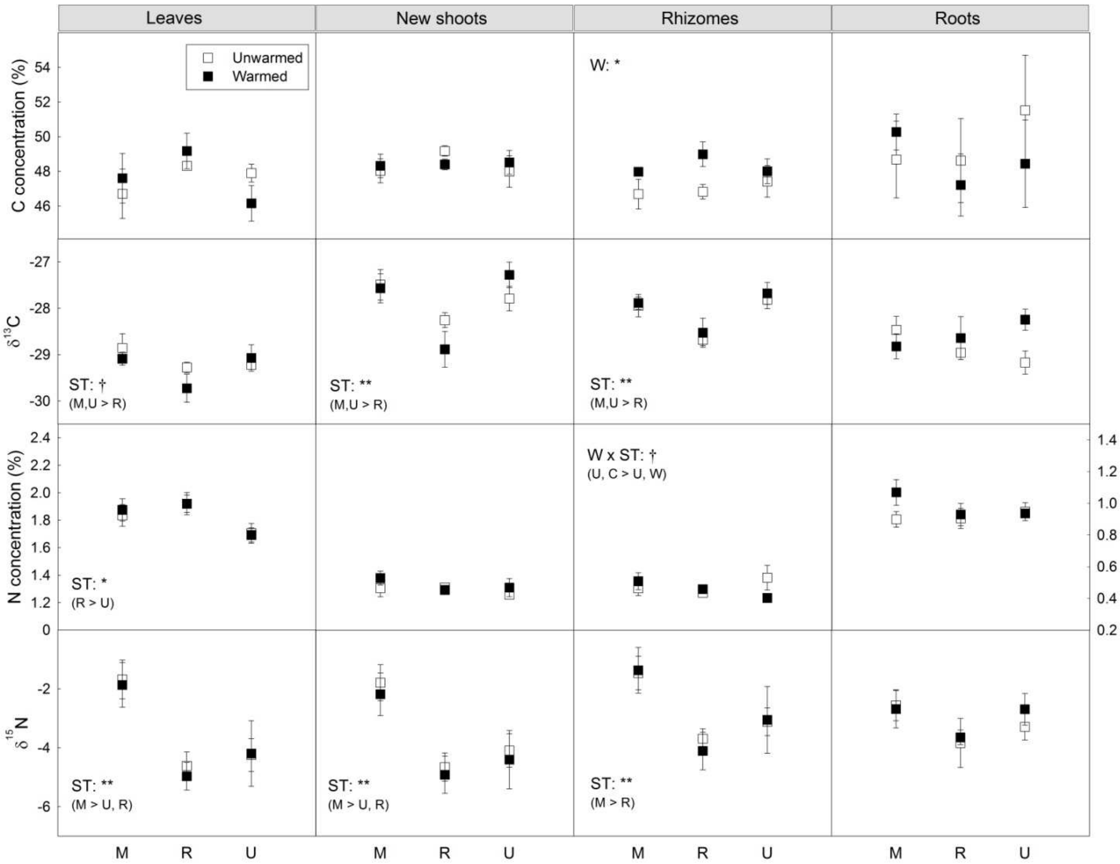

Figure 2. Carbon and N concentrations and isotope compositions (δ^{13}C and δ^{15}N) of *V. myrtillus* organs in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Symbols show significant differences († trend towards significance at 0.1 > *P* > 0.05; * significant differences at 0.05 > *P* > 0.01; ** significant differences at *P* < 0.01). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

Table 3. Results of ANOVA or Wilcoxon tests for the effects of warming on the C and N concentration and isotope composition ($\delta^{13}C$, $\delta^{15}N$) of *Vaccinium uliginosum* leaves, new shoots, rhizomes and roots. Significant effects ($P < 0.05$) and effects trending towards significance ($0.1 > P > 0.05$) are in bold.

Organ	Parameter	df	*F*/*W*	*P*
Leaves	C concentration	1, 6	3.89	**0.096**
	N concentration	1, 6	2.57	0.160
	$\delta^{13}C$	1, 6	0.01	0.934
	$\delta^{15}N$	-	*W* = 6	0.686
New shoots	C concentration	-	*W* = 6	0.686
	N concentration	1, 6	13.91	**0.010**
	$\delta^{13}C$	1, 6	0.03	0.871
	$\delta^{15}N$	1, 6	0.35	0.575
Rhizomes	C concentration	1, 6	0.99	0.357
	N concentration	-	*W* = 8	1.000
	$\delta^{13}C$	-	*W* = 8	1.000
	$\delta^{15}N$	-	*W* = 8	1.000
Roots	C concentration	1, 6	0.00	0.997
	N concentration	1, 6	0.93	0.373
	$\delta^{13}C$	1, 6	1.08	0.339
	$\delta^{15}N$	1, 6	3.86	0.097

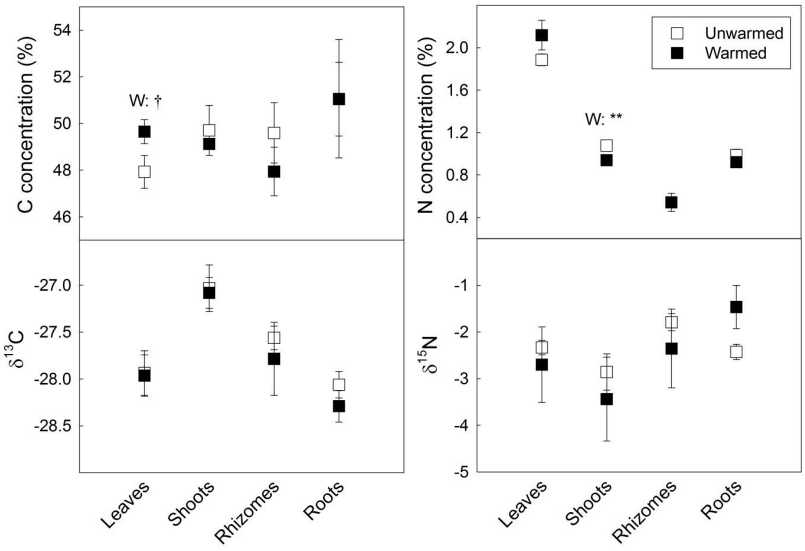

Figure 3. Carbon and nitrogen concentrations and $\delta^{13}C$ and $\delta^{15}N$ of *V. uliginosum* organs under different warming treatments in September 2013 (W; *n* = 4, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Symbols show significant differences († trend towards significance at $0.1 > P > 0.05$; ** significant differences at $P < 0.01$).

Soil inorganic N concentrations, rhizosphere C and N and functionality of the soil microbial community

Soil NO_3^- concentration decreased by 36% in warmed plots compared with unwarmed plots ($F_{1,24}$ = 5.87, P = 0.023, Fig. 4a), but the NH_4^+ concentration remained similar between warming treatments (F = 0.45, P = 0.508, Fig. 4b). As a consequence, the NO_3/NH_4^+ ratio decreased by 27% under warming with respect to control conditions. There was no difference between stand types or any interaction between warming and stand type for any of the two N forms analysed.

The rhizosphere C/N ratio did not differ between warming treatments. However, it differed between stand types, as it was higher in U stands than in the other two ($F_{2,24}$ = 7.99, P = 0.002, Fig. S4). Both rhizosphere soil C and N concentration were significantly higher in U stands than in R and M stands ($F_{2,24}$ = 5.81, P = 0.009 and $F_{2,24}$ = 3.64, P = 0.042, respectively), but the difference in the C concentration was greater than the difference in N (data not shown). There was no significant warming x stand type interaction on the rhizosphere C/N ratio ($F_{2,24}$ = 0.89, P = 0.422), but the high dispersion in the data could have masked possible differences between warming treatments in U stands. Neither warming nor stand type or their interaction had any effects on rhizospheric soil $\delta^{13}C$ and $\delta^{15}N$ values ($P > 0.28$).

The warming treatment did not change the ability of the microbial communities of *V. myrtillus* M stands to degrade the different sources of C present in the Ecoplates (Fig. S5 and S6), indicating that the exposure to 1.1 ºC warmer temperatures during four years was not enough to result in significant changes in the soil C cycling by microbia.

Discussion

Vaccinium myrtillus slightly benefitted from increased temperatures regardless of neighbourhood composition. Thus, our results evidence that four years of passive warming did not lead to changes in the interaction between *V. myrtillus* and its neighbour species at our study site. Although coexistence theory predicts that intraspecific competition should be stronger than interspecific competition (Chesson 2000), we did not find evidence of stronger competition in pure than in mixed stands

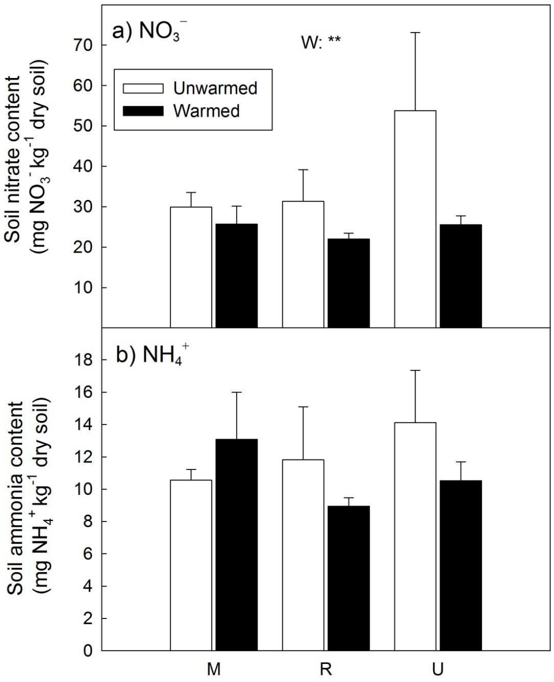

Figure 4. Soil nitrate (NO_3^-) and ammonia (NH_4^+) concentration in our study plots in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means + 1 SE are shown). Asterisks (**) show significant differences at $P < 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

nor that this changed with warming, even when the percentage cover of *V. myrtillus* ramets in pure stands was higher than in mixed stands (and also ramet density, which was measured in 2010 in similar plots at the same study site; Table S1). A previous study at the same area also found that the performance of *V. myrtillus* was similar across stand types, indicating that ramets did not perform worse in pure stands than in mixtures (Anadon-Rosell et al. 2016). Furthermore, other studies have reported that intraspecific facilitation can be more important than competition at moderate to high levels of stress (Chu et al. 2008; Fajardo and McIntire 2011).

The lack of warming effects on species interactions in our study contrasts with previous studies in which the interaction between plant species shifted when temperatures changed (both naturally and experimentally). Dormann et al. (2004) found that the interaction between the rush *Luzula confusa* and the deciduous shrub *Salix polaris* changed with warming in favour of *S. polaris* in Svalbard. In a removal experiment in Finse, Norway, Klanderud and Totland (2005) found that the removal of the neighbour species negatively affected *Thalictrum alpinum* in unwarmed plots, but not inside OTCs, indicating that warming affected the interaction between these species. Callaway et al. (2002) also reported evidence of a shift from facilitation at higher elevation sites to competition at lower elevations when removing neighbours of target individuals at different mountain sites across the world. In addition, a study in seminatural grasslands in southern Norway found increased competitive interactions with increasing temperature across precipitation and temperature gradients (Olsen et al. 2016). Most of these studies consisted of removal experiments, which provide very important ecological and functional information about the community and species studied, and allow to test the effect of species composition on the community response to the environment (see review by Díaz et al. 2003). However, despite their numerous advantages and outcomes, removal experiments cannot avoid the disturbance caused by the extraction of selected species. In contrast, our approach was based on naturally established populations and species and allowed us to assess their natural responses to warming. According to our results, species interactions seem less responsive to warming when studied under natural conditions and within the natural distributions of the study species. While the removal experiments mentioned above captured changes in species interactions after shorter periods of warming than our experiment, four years of passive warming were not sufficient to cause changes in species interactions at our study site.

The AG biomass increase of *V. myrtillus* inside the OTCs could be the result of a longer growing period caused by the advancement of its early-vegetative phenology. A previous study on the same species in the Swiss Alps showed that its increased growth after six years of soil warming with heating cables was not related to a longer growing period (Anadon-Rosell et al. 2014). The above-ground phenology of ramets could be more affected by warming through OTCs than by soil warming, since air temperature at canopy level may be higher inside the OTC. However, other factors related to warming but not directly linked to a longer growing season could have also influenced *V. myrtillus* growth in our study, such as direct warming effects on photosynthetic rates (Heskel et al. 2013; Fu et al. 2015) or through the stimulation of N uptake rates, which would be supported by the lower soil NO_3^- values in warmed plots at our study (see below). In addition, although OTCs are a robust tool for the study of warming effects on plant species (Hollister and Webber 2000; see review by Bokhorst et al. 2013), they might potentially cause confounding effects on microclimate variables that could have influenced the response of our study species to the warming treatment, such as modifications in wind patterns around the study plants (DeBoek et al. 2012) and changes in soil moisture and vapour pressure deficit (Marion et al. 1997; Piiki et al. 2008).

In our study, the BG biomass of *V. myrtillus* did not change with warming, and this was consistent across stand types. Thus, the AG growth stimulation under warming did not result in increased BG growth, indicating an uncoupling between AG and BG responses to warming. This could potentially be related to different phenological responses to increased temperatures. In fact, an uncoupling between AG and BG phenology has been reported along an Arctic elevation gradient (Blume-Berry et al. 2016). Another explanation could be that OTCs mainly increase ground-level and air temperature. However, they have been found to slightly increase soil temperature at 5 cm depth (Hollister et al. 2006) and even at 10 cm in steppe ecosystems in Northern Mongolia (Sharkhuu et al. 2013). Hollister and Flaherty (2010) found a BG biomass increase in *Salix rotundifolia* at the Alaskan tundra after 3–4 years of warming with OTCs, but Shaver et al. (1998) found no BG biomass increase after 6–9 years of passive warming in another Alaskan wet sedge tundra site, indicating contrasting BG responses to warming depending on the study site and community composition. *Vaccinium myrtillus* can expand its rhizomes laterally several metres below-ground (Flower-Ellis, 1971); therefore our warming treatment might have not captured the potential response of a whole functional unit to warming, or a possible transfer of assimilates from AG parts might have been diluted by the complex BG network of this species. The lack of differences between warming treatments in the rhizosphere C/N ratio and in the soil microbial C source use suggests that the degree of warming applied in our study was not enough to induce significant BG changes. Moreover, the similar substrate utilization by the microbial community between warming treatments indicates that the soil C pools were not altered by the increased temperature (Rinnan et al. 2009). Numerous studies on the effect of temperature on the composition and functioning of soil microbial communities have led to contrasting results (see review by Classen et al. 2015). Temperature shifts of as much as 10 ºC did not significantly alter the physiological functioning of the microbiota of humic soils (Pettersson and Bååth 2003). However, a temperature increase of 5 ºC in temperate forests resulted in a significant alteration of soil microbial communities (DeAngelis et al. 2015), and an increase of 4 ºC in a warming experiment at the Swiss treeline led to changes in the microbial substrate use (Streit et al. 2014). All these contrasting results indicate that warming effects on microbial metabolism and BG processes may be mediated through other biotic and abiotic factors (see also Christiansen et al. 2017), and certainly deserve further study.

Vaccinium uliginosum has been shown to be less plastic in response to warming than *V. myrtillus* (Richardson et al. 2002; Kudo and Suzuki 2003; Anadon-Rosell et al. 2014). This can be attributed to the better adaptation of *V. myrtillus* to higher temperatures, which is evidenced by the fact that it grows at lower altitudes than *V. uliginosum* (Bolòs et al. 2005). Although the dry weight of new individual shoots of *V. uliginosum* increased with warming, the number of shoots decreased, probably as a trade-off, which led to an overall lack of AG biomass response to warming in this species. In fact, only the leaf C concentration of *V. uliginosum* increased slightly with warming, and was not accompanied by any other changes in the shrub performance. Our study provides evidence that although *V. myrtillus* is more responsive to warming than *V. uliginosum*, it does not benefit more from warming when it grows in mixtures than when it grows in pure stands.

The slightly lower N concentration in *V. myrtillus* rhizomes in warmed plots than in unwarmed plots when coexisting with *V. uliginosum* suggests that there could be an increase in competition for N with warming. In fact, competition for N was found in mixed stands of the two *Vaccinium* species under natural conditions in a previous study at the same site (Anadon-Rosell et al. 2016), and is supported by the higher rhizosphere and leaf C/N ratios in mixed stands of these two species than in pure stands found in this study. Moreover, the higher $\delta^{15}N$ values in pure stands than in mixed stands, which might be explained by lower N uptake through mycorrhiza or larger ecosystem losses of ^{15}N-depleted N (leaving an enriched remaining pool), are indicative of larger N availability (Craine et al. 2009). A study in the Swiss Alps found a positive response to warming in *V. uliginosum* leaf N concentration but only a short-term positive response in *V. myrtillus* (Dawes et al. 2011). On the other hand, in the Swedish Lapland, *V. myrtillus* increased leaf N concentration in response to warming whereas the opposite was found for *V. uliginosum* (Richardson et al. 2002). However, these studies did not test warming effects on interspecific interactions. Our study suggests that although warming may increase the competition for N between *V. myrtillus* and *V. uliginosum*, this does not outweigh the positive growth response of *V. myrtillus* to warming.

A meta-analysis of experimental warming effects on N pools in terrestrial ecosystems showed that warming increased N mineralization rates and N pools across different ecosystem types (Bai et al. 2013). However, in our experiment soil NO_3^- decreased with warming (regardless of the stand type). This could be due to greater NO_3^- uptake rates promoted by warming, since temperature is a modulator of plant N assimilation (Laine et al. 1994; Volder et al. 2000). The lack of an increase in the N concentration of *V. myrtillus* tissues could be due to a dilution effect caused by the greater biomass, which is supported by the higher total N pool found in new shoots under warming. Additionally, other species (especially grasses, due to their abundance) could have increased their NO_3^- assimilation under warming, which was not assessed in this study. Another explanation for the reduced soil NO_3^- concentrations in the OTCs may be earlier consumption of NO_3^- through an advanced root phenology promoted by warming (Sullivan and Welker 2005; Nord and Lynch 2009). In the Finnish tundra, Rinnan et al. (2009) detected no increase in soil N concentration with warming either, but there was a decrease in soil NH_4^+ concentration inside the OTCs. The authors argued that this reduction could reflect the increased efficiency of N uptake with warming. The differing responses in the N form between that study and ours might reflect the differential use of specific N forms at different sites with different community composition, or a greater availability of NO_3^- than NH_4^+ at our study site.

In conclusion, four years of experimental warming had no effect on the interaction between *V. myrtillus* and *V. uliginosum* or *R. ferrugineum*. *Vaccinium myrtillus* showed a positive AG biomass response to warming regardless of the neighbouring species, but no BG responses were found. Although warming seemed to increase the competition for N between the two *Vaccinium* species, their overall performance was not affected. This study shows that species interactions are not altered by warming at this treeline site and, thus, the performance of these populations will probably not change due to mild warming in the near future.

Acknowledgements

We thank Clara Borrull, Noelia Seguer, Estela Illa, Victoria Lafuente, Elena Lahoz and Santiago Pérez for their field and laboratory assistance. We are grateful to CCiT of the University of Barcelona for the use of their facilities and their technical assistance. This work was supported by Conselh Generau d'Aran and the project ARBALMONT/786-2012 (Organismo Autónomo Parques Nacionales, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain). AAR was funded by an FPU grant (Ministerio de Educación, Cultura y Deporte, Spain) and SP was funded by a Ramón y Cajal fellowship (RYC-2013-14164, Ministerio de Economía y Competitividad, Spain).

Conflict of interest: All authors declare that they have no conflict of interest.

References

Anadon-Rosell A, Rixen C, Cherubini P, Wipf S, Hagedorn F, Dawes M.A (2014) Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. PLoS ONE 9(6): e100577. doi:10.1371/journal.pone.0100577

Anadon-Rosell A, Palacio S, Nogués S, Ninot JM (2016) *Vaccinium myrtillus* stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline. Plant Ecol 217: 115-1128. doi: 10.1007/s11258-016-0637-2

Bai E, Li S, Xu W, Li W, Dai W, Jiang P (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytol 199: 441–451. doi:10.1111/nph.12252

Bardgett RD, Wardle DA (2010) Aboveground-belowground linkages. Biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford.

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends Ecol Evol 9: 187–191.

Blume-Werry G, Wilson SD, Kreyling J, Milbau A (2016) The hidden season: growing season is 50% longer below than above ground along an arctic elevation gradient. New Phytol 209: 978-986. doi: 10.1111/nph.13655

Bokhorst S, Huiskes A, Aerts R, Convey P, Cooper EJ, Dalen L, Erschbamer B, Gudmundsson J, Hofgaard A, Hollister RD, Johnstone J, Jónsdóttir IS, Lebouvier M, Van de Vijver B, Wahren C-H, Dorrepaal E (2013) Variable temperature effects of Open Top Chambers at polar and alpine sites explained by irradiance and snow depth. Glob Change Biol 19:64–74. doi: 10.1111/gcb.12028

Bolòs O, Vigo J, Masalles RM, Ninot JM (2005) Flora Manual dels Països Catalans. 3rd ed. rev. and ext. Ed. Pòrtic SA, Barcelona.

Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78: 1958–1965. doi: 10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ (2002) Positive interactions among alpine plants increase with stress. Nature 417: 844–848. doi:10.1038/nature00812

Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of Arctic tundra to experimental and observed changes in climate. Ecology 76:694–711.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366. doi: 10.1146/annurev.ecolsys.31.1.343

Choler P, Michalet R, Callaway RM (2001) Facilitation and competition on gradients in alpine plant communities. Ecology 82: 3295–3308. doi: 10.1890/0012-9658(2001)082[3295:FACOGI]2.0.CO;2

Christiansen CT, Haugwitz MS, Priemé A, Nielsen CS, Elberling B, Michelsen A, Grogan P, Blok D (2017) Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra. Global Change Biology 23: 406–420. doi: 10.1111/gcb.13362

Chu C-J, Maestre FT, Xiao S, Weiner J, Wang Y-S, Duan Z-H, Wang G (2008) Balance between facilitation and resource competition determines biomass–density relationships in plant populations. Ecology Letters 11: 1189–1197. doi: 10.1111/j.1461-0248.2008.01228.x

Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, Moorhead LC, Patterson CM (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6(8): 130. doi: 10.1890/ES15-00217.1

Collins M, Knutti R, Arblaster J, Dufresne J-L, Fichefet T, Friedlingstein P, Gao X, Gutowski WJ, Johns T, Krinner G, Shongwe, M, Tebaldi C, Weaver AJ, Wehner W (2013) Long-term climate change: projections, commitments and irreversibility. In: Stocker TF, Quin D, Plattner G-K, Tignor M, Allen SK, Bosching J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate Change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 1029-1136.

Cornelissen JHC, Song Y-B, Yu F-H, Dong M (2014) Plant traits and ecosystem effects of clonality: a new research agenda. Ann Bot doi:10.1093/aob/mcu113

Cornelissen, JHC, Van Bodegom PM, Aerts R, Callaghan TV, Van Logtestijn RSP, Alatalo J, Stuart Chapin F, Gerdol R, Gudmundsson J, Gwynn-Jones D, Hartley AE, Hik DS, Hofgaard A, Jónsdóttir IS, Karlsson S, Klein JA, Laundre J, Magnusson B, Michelsen A, Molau U, Onipchenko VG, Quested HM, Sandvik SM, Schmidt IK, Shaver GR, Solheim B, Soudzilovskaia NA, Stenström A, Tolvanen A, Totland Ø, Wada N, Welker JM, Zhao X, Brancaleoni L, Brancaleoni L, De Beus MAH, Cooper EJ, Dalen L, Harte J, Hobbie SE, Hoefsloot G, Jägerbrand A, Jonasson S, Lee JA, Lindblad K, Melillo JM, Neill C, Press MC, Rozema J, Zielke M (2007) Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. Ecol Lett 10: 619–627. doi:10.1111/j.1461-0248.2007.01051.x

Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, MacK MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol 183:980–992. doi: 10.1111/j.1469-8137.2009.02917.x

Dawes MA, Hagedorn F, Zumbrunn T, Handa IT, Hättenschwiler S, Wipf S, Rixen C (2011) Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytol 191: 806–818. doi: 10.1111/j.1469-8137.2011.03722.x

DeAngelis KM, Pold G, Topcuoglu BD, van Diepen LTA, Varney RM, Blanchard JL, Melillo J, Frey SD (2015) Long-term forest soil warming alters microbial communities in temperate forest soils. Front Microbiol 6: 104. doi: 10.3389/fmicb.2015.00104

De Boeck HJ, De Groote T, Nijs I (2012) Leaf temperatures in glasshouses and open-top chambers. New Phytol 194:1155–1164. doi: 10.1111/j.1469-8137.2012.04117.x

Díaz S, Symstad AJ, Chapin III FS, Wardle DA, Huenneke LF (2003) Functional diversity revealed by removal experiments. Trends Ecol Evol 18: 140–146. doi:10.1016/S0169-5347(03)00007-7

Dijkstra FA, Blumenthal D, Morgan JA, Pendall E, Carrillo Y, Follett RF (2010) Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. New Phytol 187:426–437. doi: 10.1111/j.1469-8137.2010.03293.x

D’Odorico P, He Y, Collins S, De Wekker SFJ, Engel V, Fuentes JD (2013) Vegetation-microclimate feedbacks in woodland-grassland ecotones. Glob Ecol Biogeogr 22: 364–379. doi:10.1111/geb.12000

Dormann CF, Van Der Wal R, Woodin SJ (2004) Neighbour identity modifies effects of elevated temperature on plant performance in the High Arctic. Glob Change Biol 10, 1587–1598. doi:10.1111/j.1365-2486.2004.00830.x

Dullinger S, Dirnböck T, Grabherr G (2003) Patterns of shrub invasion into high mountain grasslands of the Northern Calcareous Alps, Austria. Arct Antarct Alp Res 35: 434–441. doi: 10.1657/1523-0430(2003)035[0434:POSIIH]2.0.CO;2

Fajardo A, McIntire EJB (2011) Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. J Ecol 99: 642–650. doi: 10.1111/j.1365-2745.2010.01771.x

Flower-Ellis JGK (1971) Age, structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.) Department of Forest Ecology and Forest Soils. Research Note 9. Royal College of Forestry, Stockholm, Sweden.

Fu G, Shen Z-X, Sun W, Zhong Z-M, Zhang X-Z, Zhou Y-T (2015) A meta-analysis of the effects of experimental warming on plant physiology and growth on the Tibetan Plateau. J Plant Growth Regul 34: 57–65. doi:10.1007/s00344-014-9442-0

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57: 2351-2359.

Hartley AE, Neill C, Melillo JM, Crabtree R, Bowles FP (1999) Plant performance and soil nitrogen mineralization in response to simulated climate change in subarctic dwarf shrub heath. Oikos 86: 331–343. doi: 10.2307/3546450

Heskel M, Greaves H, Kornfeld A, Gough L, Atkin OK, Turnbull MH, Shaver G, Griffin KL (2013) Differential physiological responses to environmental change promote woody shrub expansion. Ecol Evol 3: 1149–1162. doi: 10.1002/ece3.525

Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol Monogr 66: 503–522. doi: 10.2307/2963492

Hollister RD, Webber PJ (2000) Biotic validation of small open-top chambers in a tundra ecosystem. Glob Change Biol 6:835–842. doi: 10.1046/j.1365-2486.2000.00363.x

Hollister RD, Webber PJ, Nelson FE, Tweedie CE (2006) Soil thaw and temperature response to air warming varies by plant community: Results from an open-top chamber experiment in northern Alaska. Arct Antarct Alp Res 38: 206–215. doi:10.1657/1523-0430(2006)38[206:STATRT]2.0.CO;2

Hollister RD, Flaherty KJ (2010) Above- and below-ground plant biomass response to experimental warming in northern Alaska. Appl Veg Sci 13: 378–387. doi:10.1111/j.1654-109X.2010.01079.x

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50: 346–363. doi: 10.1002/bimj.200810425

Insam H (1997) A new set of substrates proposed for community characterization in environmental samples. In: Insam H, Rangger A (eds) Microbial Communities. pp 259-260.

Kaneko S, Inagaki M, Morishita T (2010) A simple method for the determination of nitrate in potassium chloride extracts from forest soils. In: Gilkes RJ, Prakongkep N (eds) Proceedings of the 19th World Congress of Soil Science: Soil solutions for a changing world. pp 4-7.

Kempers AJ, Kok CJ (1989) Re-examination of the determination of ammonium as the indophenol blue complex using salicylate. Anal Chim Acta 221: 147-155. doi: 10.1016/S0003-2670(00)81948-0

Klanderud K (2005) Climate change effects on species interactions in an alpine plant community. J Ecol 93: 127–137. doi:10.1111/J.1365-2745.2004.00944.X

Klanderud K, Totland Ø (2005) The relative importance of neighbours and abiotic environmental conditions for population dynamic parameters of two alpine plant species. J Ecol 93: 493–501. doi:10.1111/j.1365-2745.2005.01000.x

Klanderud K (2008) Species-specific responses of an alpine plant community under simulated environmental change. J Veg Sci 19: 363–372. doi: 10.3170/2008-8-18376

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. 2n ed. Springer, Berlin.

Kudo G, Suzuki S (2003) Warming effects on growth, production, and vegetation structure of alpine shrubs: A five-year experiment in northern Japan. Oecologia 135: 280–287. doi: 10.1007/s00442-003-1179-6

Laine P, Bigot J, Ourry A, Boucaud J (1994) Effects of low temperature on nitrate uptake, and xylem and phloem flows of nitrogen, in *Secale cereale* L. and *Brassica napus* L. New Phytol 127: 675–683. doi: 10.1111/j.1469-8137.1994.tb02970.x

Little CJ, Jägerbrand AK, Molau U, Alatalo JM (2015) Community and species-specific responses to simulated global change in two subarctic-alpine plant communities. Ecosphere 6. doi:10.1890/ES14-00427.1

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Lévesque E, Molau U, Mølgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Glob Change Biol 3: 20–32. doi: 10.1111/j.1365-2486.1997.gcb136.x

de Mendiburu F (2010) agricolae: Statistical procedures for agricultural research. R package version 1.0-9. URL http://CRAN.R-project.org/package=agricolae.

Myers-Smith IH, Forbes BC, Wilmking M, Hallinger M, Lantz T, Blok D, Tape KD, MacIas-Fauria M, Sass-Klaassen U, Lévesque E, Boudreau S, Ropars P, Hermanutz L, Trant A, Collier LS, Weijers S, Rozema J, Rayback SA, Schmidt NM, Schaepman-Strub G, Wipf S, Rixen C, Ménard CB, Venn S, Goetz S, Andreu-Hayles L, Elmendorf S, Ravolainen V, Welker J, Grogan P, Epstein HE, Hik DS (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. Environ Res Lett 6. doi: 10.1088/1748-9326/6/4/045509

Muñiz S, Lacarta J, Pata MP, Jiménez JJ, Navarro E (2014) Analysis of the diversity of substrate utilisation of soil bacteria exposed to Cd and earthworm activity using generalised additive models. PLoS ONE 9(1): e85057. doi: 10.1371/journal.pone.0085057

Nogués-Bravo D, Araújo MB, Errea MP, Martínez-Rica JP (2007) Exposure of global mountain systems to climate warming during the 21st Century. Global Environmental Change 17: 420 – 428. doi: 10.1016/j.gloenvcha.2006.11.007

Nord EA, Lynch JP (2009). Plant phenology: a critical controller of soil resource acquisition. J Exp Bot 60: 1927–1937. doi:10.1093/jxb/erp018

Olsen SL, Töpper JP, Skarpaas O, Vandvik V, Klanderud K (2016) From facilitation to competition: temperature-driven shift in dominant plant interactions affects population dynamics in seminatural grasslands. Glob Change Biol 22: 1915–1926. doi:10.1111/gcb.13241

Pettersson M, Bååth E (2003) Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiol Ecol 45: 13-21. doi: 10.1016/S0168-6496(03)00106-5

Piikki K, Temmerman LD, Högy P, Pleijel H (2008) The open-top chamber impact on vapour pressure deficit and its consequences for stomatal ozone uptake. Atmos Environ 42: 6513 – 6522. doi: http://dx.doi.org/10.1016/j.atmosenv.2008.04.014

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2016) *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.

Pohland B, Owen B (2009) Biolog EcoPlates Standard Methods. TAS Technical Bulletin. Biolog: Hayward, CA, USA. pp 1-3.

Pornon A, Escaravage N, Lamaze T (2007) Complementarity in mineral nitrogen use among dominant plant species in a subalpine community. Am J Bot 94: 1778–1785. doi: 10.3732/ajb.94.11.1778

Pugnaire FI, Zhang L, Li R, Luo T (2015) No evidence of facilitation collapse in the Tibetan plateau. J Veg Sci 26: 233–242. doi:10.1111/jvs.12233

Rangwala I, Sinsky E, Miller JR (2013) Amplified warming projections for high altitude regions of the northern hemisphere mid-latitudes from CMIP5 models. Environmental Research Letters 8: 024040. doi: 10.1088/1748-9326/8/2/024040

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Richardson SJ, Press MC, Parsons AN, Hartley SE (2002) How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. J Ecol 90: 544–556. doi:10.1046/j.1365-2745.2002.00681.x

Rinnan R, Stark S, Tolvanen A (2009) Responses of vegetation and soil microbial communities to warming and simulated herbivory in a subarctic heath. J Ecol 97: 788–800. doi:10.1111/j.1365-2745.2009.01506.x

Ritz C and Streibig JC (2005) Bioassay analysis using R. J Stat Softw 12: 1-22. doi: 10.18637/jss.v012.i05

Ropars P, Boudreau S (2012) Shrub expansion at the forest-tundra ecotone: Spatial heterogeneity linked to local topography. Environ. Res. Lett. 7. doi:10.1088/1748-9326/7/1/015501

Rundqvist S, Hedenås H, Sandström A, Emanuelsson U, Eriksson H, Jonasson C, Callaghan TV (2011) Tree and shrub expansion over the past 34 years at the tree-line near Abisko, Sweden. Ambio 40: 683–692.

Sarkar D (2008) Lattice: Multivariate data visualization with R. Springer, New York.

Schimel J, Schaeffer S (2012) Microbial control over carbon cycling in soil. Frontiers in Microbiology 3: 348. doi: 10.3389/fmicb.2012.00348

Sharkhuu A, Plante AF, Enkhmandal O, Casper BB, Helliker BR, Boldgiv B, Petraitis PS (2013) Effects of open-top passive warming chambers on soil respiration in the semi-arid steppe to taiga forest transition zone in Northern Mongolia. Biogeochemistry 115: 333–348. doi:10.1007/s10533-013-9839-z

Shaver GR, Johnson LC, Cades DH, Murray G, Laundre JA, Rastetter EB, Nadelhoffer KJ, Giblin AE (1998) Biomass and CO_2 flux in wet sedge tundras: Responses to nutrients, temperature, and light. Ecol Monogr 68: 75–97. doi: 10.1890/0012-9615(1998)068[0075:BACFIW]2.0.CO;2

Shevtsova A, Haukioja E, Ojala A (1997) Growth response of subarctic dwarf shrubs, *Empetrum nigrum* and *Vaccinium vitis-idaea*, to manipulated environmental conditions and species removal. Oikos 78: 440–458. doi: 10.2307/3545606

Streit K, Hagedorn F, Hiltbrunner D, Portmann M, Saurer M, Buchmann N, Wild B, Richter A, Wipf S, Siegwolf RTW (2014) Soil warming alters microbial substrate use in alpine soils. Glob Change Biol 20: 1327–1338. doi: 10.1111/gcb.12396

Sullivan PF, Welker JM (2005) Warming chambers stimulate early season growth of an arctic sedge: Results of a minirhizotron field study. Oecologia 142: 616–626. doi: 10.1007/s00442-004-1764-3

Taulavuori K, Laine K, Taulavuori E (2013) Experimental studies on *Vaccinium myrtillus* and *Vaccinium vitis-idaea* in relation to air pollution and global change at northern high latitudes: A review. Environ Exp Bot 87: 191–196. doi:10.1016/j.envexpbot.2012.10.002

Tilman D, Lehman C (2001) Human-caused environmental change: Impacts on plant diversity and evolution. Proc Natl Acad Sci 98:5433–5440. doi: 10.1073/pnas.091093198

Volder A, Bliss LC, Lambers H (2000) The influence of temperature and nitrogen source on growth and nitrogen uptake of two polar-desert species, *Saxifraga caespitosa* and *Cerastium alpinum*. Plant Soil 227: 139–148. doi:10.1023/A:1026528830228

Wheeler JA, Schnider F, Sedlacek J, Cortés AJ, Wipf S, Hoch G, Rixen C (2015) With a little help from my friends: Community facilitation increases performance in the dwarf shrub *Salix herbacea*. Basic Appl Ecol 16: 202–209. doi:10.1016/j.baae.2015.02.004

Yang Y, Wang G, Klanderud K, Wang J, Liu G (2015) Plant community responses to five years of simulated climate warming in an alpine fen of the Qinghai–Tibetan Plateau. Plant Ecol Divers 8: 211–218. doi:10.1080/17550874.2013.871654

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York.

Supporting information

Table S1. Percent cover of the three main shrubs and grasses for the different stand types and warming treatments (n = 5, means are shown) in 2011 (left) and 2013 (right). Forbs were only recorded in 2013 and their percent cover was always < 10. In the right side of the table, *V. myrtillus* ramet density recorded in 2010 at the same study area, in similar stand type plots (n = 5). M: *V. myrtillus* pure stands; U: *V. myrtillus* and *V. uliginosum* mixed stands; R: *V. myrtillus* and *R. ferrugineum* mixed stands.

Stand type	Warming treatment	*V. myrtillus*	*V. uliginosum*	*R. ferrugineum*	Grasses	*V. myrtillus* density (n°ramets dm^{-2})
M	Control	70 69	0 0	0 0	28 20	19 ± 1
	Warmed	68 83	0 0	0 0	25 17	
U	Control	52 43	48 44	0 2	14 23	10 ± 2
	Warmed	41 34	66 68	0 0	15 13	
R	Control	43 44	0 1	73 82	20 17	5 ± 1
	Warmed	40 49	0 0	77 75	12 11	

Table S2. Soil properties for the different stand types and warming treatments. Means and SE are shown for organic matter (OM) and organic C (Corg) concentrations and pH. Values obtained for soil colour type according to Munsell for each treatment combination are given. Results from simple linear models on the effects of stand type and warming treatment on soil OM, Corg and pH are at the right side of the table. M: *V. myrtillus* pure stands; U: *V. myrtillus* and *V. uliginosum* mixed stands; R: *V. myrtillus* and *R. ferrugineum* mixed stands.

Soil properties	Stand type	Warming treatment	Mean	SE	Fixed effect	df	*F*	*P*
OM (%)	M	Control	13.71	1.72	Stand type	2, 24	1.10	0.349
		Warmed	14.22	1.36	Warming	1, 24	2.09	0.161
	U	Control	13.15	1.26				
		Warmed	14.44	1.13				
	R	Control	14.04	2.26				
		Warmed	17.58	0.92				
Corg (%)	M	Control	7.95	1.00	Stand type	2, 24	1.10	0.349
		Warmed	8.25	0.79	Warming	1, 24	2.08	0.162
	U	Control	7.63	0.73				
		Warmed	8.37	0.66				
	R	Control	8.14	1.31				
		Warmed	10.20	0.54				
pH	M	Control	4.63	0.16	Stand type	2, 24	0.38	0.691
		Warmed	4.46	0.15	Warming	1, 24	1.91	0.180
	U	Control	4.55	0.17				
		Warmed	4.32	0.08				
	R	Control	4.49	0.16				
		Warmed	4.40	0.11				

			Munsell value
Colour (dry)	M	Control	10YR3/1, 10YR3/2, 10YR4/2
		Warmed	10YR3/1, 10YR3/2, 10YR4/2
	U	Control	10YR3/1, 10YR3/2, 10YR4/2
		Warmed	10YR3/1, 10YR3/2, 10YR4/2
	R	Control	10YR3/1, 10YR3/2, 10YR4/2
		Warmed	10YR3/2, 10YR4/2
Colour	M	Control	10YR2/1, 10YR3/1, 10YR3/2
		Warmed	10YR2/1, 10YR3/1, 10YR3/2
	U	Control	10YR2/1, 10YR3/1, 10YR3/2
		Warmed	10YR2/1, 10YR3/1, 10YR3/2
	R	Control	10YR3/1, 10YR3/2
		Warmed	10YR3/1, 10YR3/2

The hue component (specific colour) of Munsell system was 10YR (yellow-red towards red) in all plots. The value component (lightness to darkness) ranged between 2 and 4 (dark), and the chroma component (intensity) was 1 or 2 (light).

Table S3. Results of ANOVA for effects of warming and stand type on stand below-ground (BG) biomass. Significant effects ($P < 0.05$) and effects trending towards significance ($0.1 > P > 0.05$) are in bold.

BG Variable	Factor	df	*F*	*P*
Rhizome biomass	Warming	1, 24	2.98	0.100
	Stand type	2, 24	6.93	**0.004**
	Stand type x warming	2, 24	0.03	0.970
Coarse roots biomass	Warming	1, 19	0.91	0.352
	Stand type	2, 19	3.04	**0.071**
	Stand type x warming	2, 19	0.30	0.745
Fine roots biomass	Warming	1, 24	0.88	0.358
	Stand type	2, 24	0.41	0.667
	Stand type x warming	2, 24	0.57	0.575

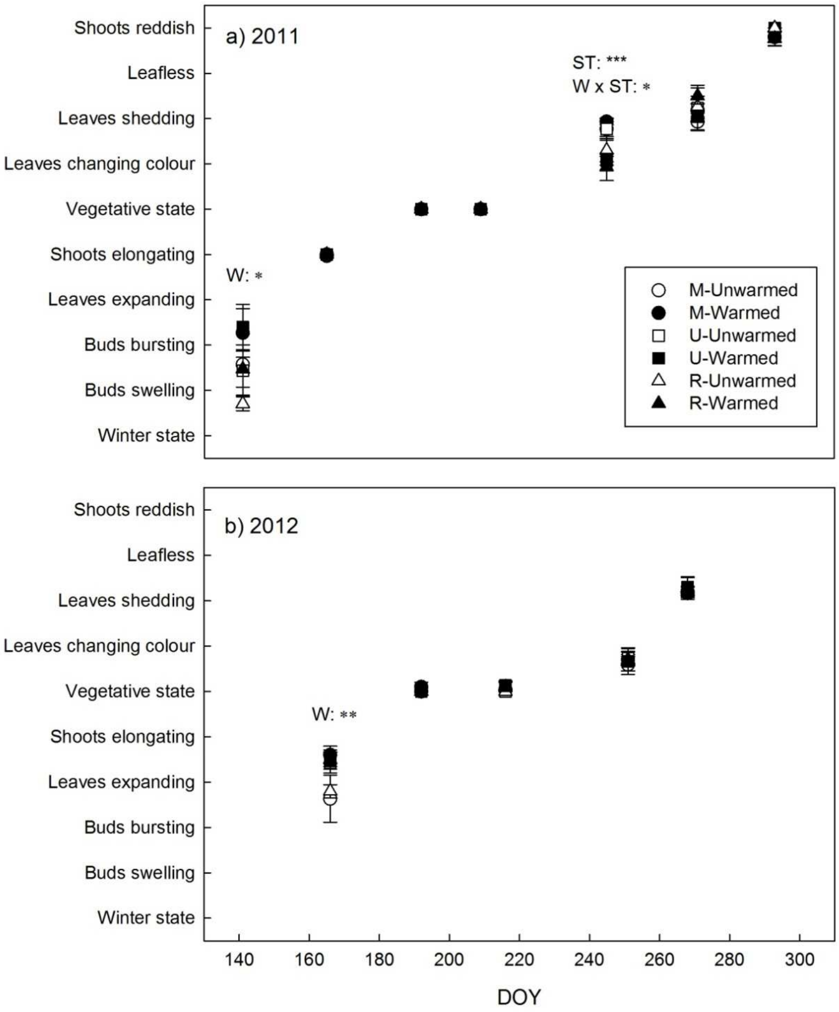

Figure S1. Vegetative phenology (mean phenophase calculated as the average numeric phenophase of six ramets per plot in each visit, day of year: DOY) of *Vaccinium myrtillus* at the study plots during the growing seasons of 2011 and 2012 under different stand types (ST) and warming treatments (W; *n* = 5, means +1SE are shown). Asterisks show differences between treatments (* marginally significant differences 0.1 > *P* > 0.05; ** significant differences at 0.05 > *P* > 0.01; *** significant differences at *P* < 0.01). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

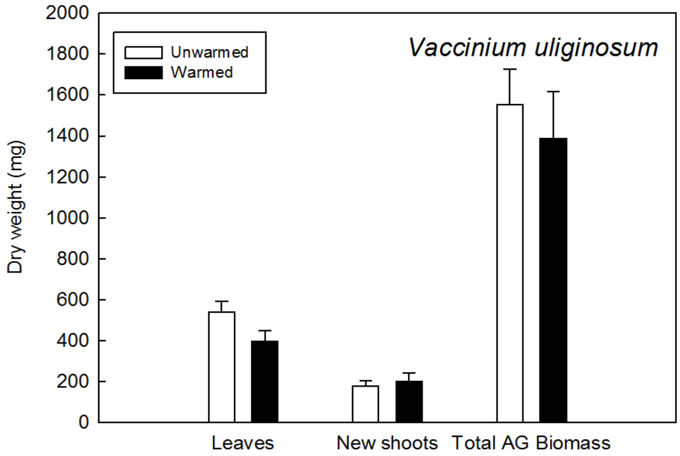

Figure S2. *Vaccinium uliginosum* above-ground (AG) biomass under different warming treatments in September 2013 (*n* = 4, means + 1 SE are shown). There were no significant differences between warming treatments.

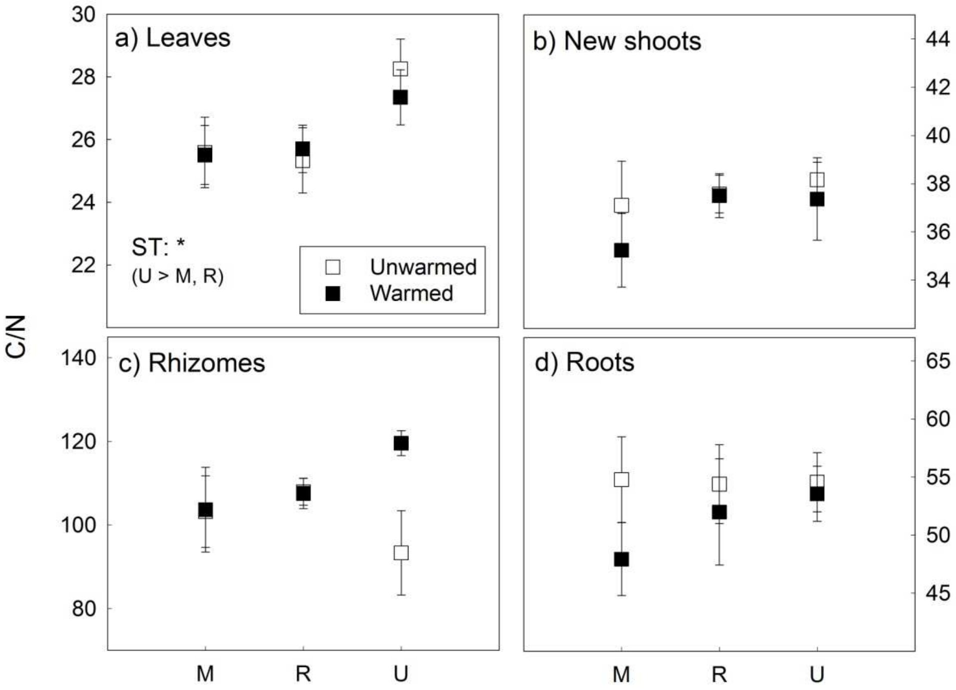

Figure S3. C/N ratios for *Vaccinium myrtillus* tissues for the different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means ± 1 SE are shown). Symbols show significant differences († trend towards significance at $0.1 > P > 0.05$; * significant differences at $0.05 > P > 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

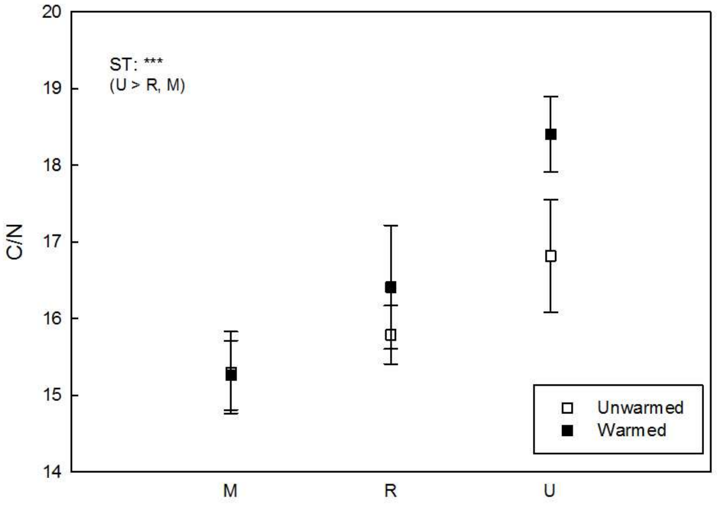

Figure S4. Rhizospheric soil C/N ratio for the different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means ± 1 SE are shown). Asterisks (***) show significant differences at *P* < 0.01. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

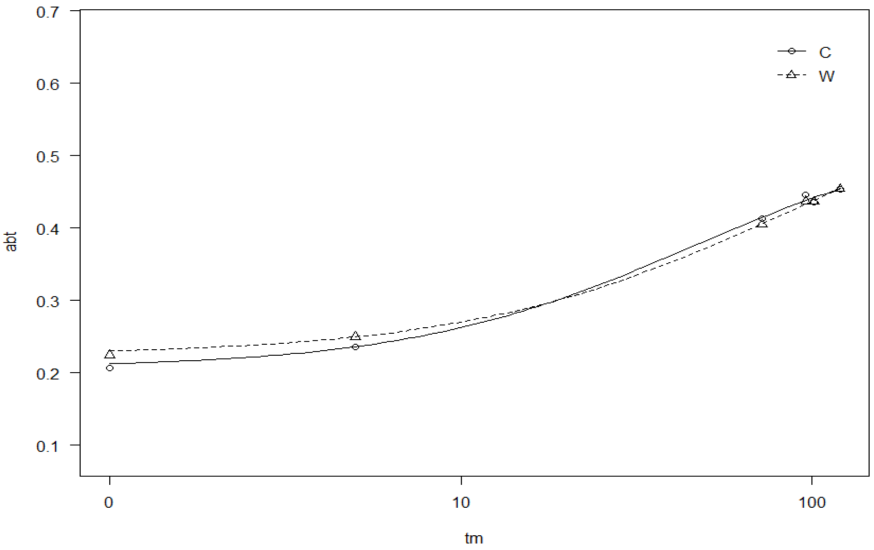

Figure S5. Effects of the warming treatment on the soil microbial community by “Community-level physiological profiling” with Biolog EcoPlate^{TM} (*n* = 3). The X axis *–tm–* represents the time at which optical density (OD_{590}) has been measured, the Y axis *–abt–* represents the OD_{590}. No differences in any of the parameters of these two curves were detected.

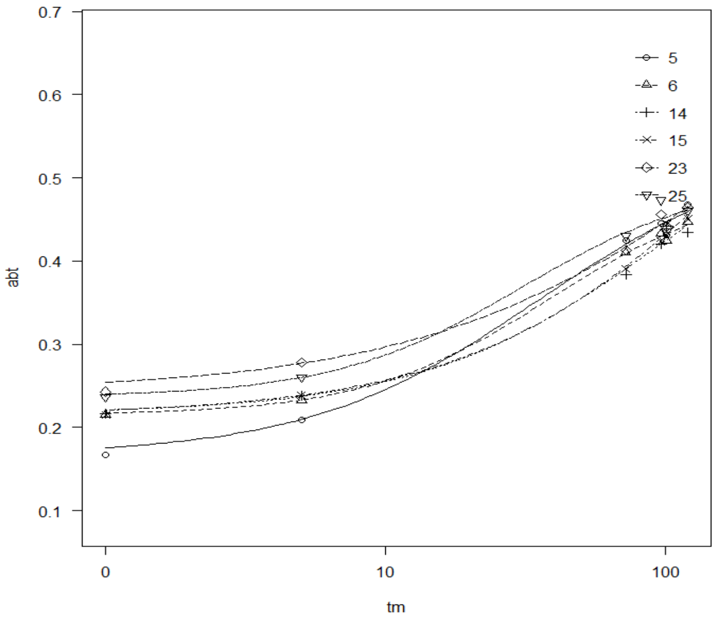

Figure S6. AWCD curves for each plot (unwarmed plots: 5, 14 and 25; warmed plots: 6, 15 and 23). The only plot that significantly differed from the rest was plot 5. The minimum value of OD_{590} at time 0 for curve 5 was significantly lower than the others ($P < 0.01$). This initial lower value may be due to a lower load of colored dissolved substances. After 120 hours all replicates converged and no significant differences were detected at any curve parameter.

Capítol 3 / Chapter 3

Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline

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PLoS ONE (2014) 9: e100577

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Citation: Anadon-Rosell A., Rixen C., Cherubini P., Wipf S., Hagedorn F., Dawes M.A. (2014) Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. *PLoS ONE* 9: e100577

Resum

L'escalfament global pot tenir impactes substancials en els patrons fenològics i de creixement d'espècies alpines i àrtiques, cosa que podria comportar canvis en la composició de les comunitats vegetals i la dinàmica de l'ecosistema. Vam avaluar els efectes d'un tractament d'escalfament del sòl durant sis anys (+ 4 °C, 2007-2012) en la fenologia i creixement de tres espècie subarbustives codominants sota la capçada de les espècies arbòries *Larix decidua* i *Pinus uncinata* al límit supraforestal als Alps Suïssos. Vam fer un seguiment de la fenologia vegetativa i reproductiva de *Vaccinium myrtillus*, *Vaccinium gaultherioides* i *Empetrum hermaphroditum* durant la primera part de l'estació de creixement del 2012 i al pic de l'estació de creixement vam recol·lectar i mesurar la biomassa de les fraccions aèries dels ramets. Per cadascun dels sis anys d'escalfament vam mesurar l'elongació anual de les tiges en les tres espècies i vam analitzar l'edat i l'amplada dels anells de creixement de *V. myrtillus*. Els nostres resultats mostren que la fenologia de les tres espècies estava més influenciada per la data de la fosa de la neu, i també per l'arbre present en la parcel·la (*Larix* o *Pinus*) en el cas de *V. myrtillus*, que per l'escalfament del sòl. Tanmateix, l'escalfament va comportar l'augment de la biomassa aèria total dels ramets de *V. myrtillus* (+ 36%, el 2012), especialment la de les tiges noves (+ 63%, el 2012), així com un increment de la llargada de les tiges noves i de l'amplada dels anells de creixement (+ 22% i + 41%, respectivament; mitjana pels anys 2007-2012). Aquests resultats indiquen una estimulació general del creixement de *V. myrtillus* amb l'escalfament del sòl, la qual va ser sostinguda al llarg dels sis anys d'estudi, i que no va ser causada per un avançament del període de creixement a principis d'estiu. Per contra, *E. hermaphroditum* només va presentar una resposta positiva en el creixement de les tiges noves el 2011 (+ 21%) i *V. gaultherioides* no va mostrar cap resposta significativa. Els nostres resultats indiquen que, amb el futur escalfament global, *V. myrtillus* podria tenir un avantatge competitiu sobre els subarbusts veïns.

Abstract

Global warming can have substantial impacts on the phenological and growth patterns of alpine and Arctic species, resulting in shifts in plant community composition and ecosystem dynamics. We evaluated the effects of a six-year experimental soil warming treatment (+4°C, 2007-2012) on the phenology and growth of three co-dominant dwarf shrub species growing in the understory of *Larix decidua* and *Pinus uncinata* at treeline in the Swiss Alps. We monitored vegetative and reproductive phenology of *Vaccinium myrtillus*, *Vaccinium gaultherioides* and *Empetrum hermaphroditum* throughout the early growing season of 2012 and, following a major harvest at peak season, we measured the biomass of above-ground ramet fractions. For all six years of soil warming we measured annual shoot growth of the three species and analyzed ramet age and xylem ring width of *V. myrtillus*. Our results show that phenology of the three species was more influenced by snowmelt timing, and also by plot tree species (*Larix* or *Pinus*) in the case of *V. myrtillus*, than by soil warming. However, the warming treatment led to increased *V. myrtillus* total above-ground ramet biomass (+36% in 2012), especially new shoot biomass (+63% in 2012), as well as increased new shoot increment length and xylem ring width (+22% and +41%, respectively; average for 2007-2012). These results indicate enhanced overall growth of *V. myrtillus* under soil warming that was sustained over six years and was not caused by an extended growing period in early summer. In contrast, *E. hermaphroditum* only showed a positive shoot growth response to warming in 2011 (+21%), and *V. gaultherioides* showed no significant growth response. Our results indicate that *V. myrtillus* might have a competitive advantage over the less responsive co-occurring dwarf shrub species under future global warming.

Keywords: above-ground biomass; European Alps; phenology; shoot increment length; snowmelt date; treeline; xylem growth ring; *Empetrum hermaphroditum*; *Vaccinium gaultherioides; Vaccinium myrtillus*

Introduction

Alpine and Arctic ecosystems are predicted to be especially vulnerable to global warming [1,2] because plant growth and performance in these environments are strongly constrained by low temperature, short growing seasons and frequent freezing events during the snow-free period. Projected increases of 1.8-4ºC in the global mean surface air temperature by the end of the 21st century [3] can cause dramatic changes in community composition since co-occurring species respond differently to climate variations [4-6]. Community changes are of particular importance in alpine and Arctic ecosystems due to their potential effects on climate feedbacks through shifts in plant community composition and plant species cover [7,8].

Experimental studies have shown that responses of plants growing at high latitude and high elevation to warming are species-specific [9-11], demonstrating that understanding how plant community dynamics might change with higher temperatures can only be achieved through assessing responses of individual species. For some species and study sites, positive growth responses to warming were transient, stopping some years after the experiment started [12,13]. In contrast, other studies found a lag in plant growth response to experimental warming or inconsistent responses over time [6, 14]. Therefore, species-specific studies lasting several years are crucial for understanding community responses to global warming.

Phenological timing is an important factor for plant fitness and abundance, and it can be highly responsive to temperature ([15] and references therein). For instance, advanced leaf phenology in response to warming could lead to an extension of the photosynthetically active season, which, in turn, could lead to greater carbon gain and ultimately growth for plants [16,17]. Advanced phenology of high-latitude and high-elevation species has occurred with experimentally increased growing season temperatures in numerous studies where snowmelt date was not modified [10, 18-22; but see 23]. In a meta-analysis of alpine and Arctic studies, Arft et al. [12] suggested a relationship between advanced leaf bud burst under warming and increased vegetative growth in alpine and Arctic species. However, most studies have focused on phenology and growth separately, whereas the relationship between phenology and growth in response to warming at an individual/ramet level has rarely been reported.

Shrubs are an essential component of alpine and Arctic ecosystems, and several studies have reported an increase in their cover due to climate warming [6,14,24-27]. This “shrubification” strongly impacts ecosystem dynamics because shrubs modify patterns of snow accumulation, decrease albedo and modify carbon storage and nutrient cycling by greater biomass accumulation and more recalcitrant litter (see review by Myers-Smith et al. [8]).

Shrub growth responses to global warming in alpine and Arctic ecosystems have been assessed in many studies through long-term observations [5,28], experimental manipulations [6] and/or studies using gradients across elevation or latitude [29,30]. Most previous warming experiments have measured above-ground primary production by means of new shoot growth or standing biomass [11,21,31-33]. In contrast, relatively few studies have focused on shrub secondary growth [13,34,35] since acquiring these data requires destructive techniques. Therefore, it remains unclear if the reported increases in biomass production or in shoot increment length are caused by shifts in biomass allocation or if greater overall biomass production occurs.

The Stillberg treeline research area in the Swiss Central Alps hosted a six-year soil warming experiment (2007-2012). A short-term investigation of the dominant ericaceous dwarf shrub species growing in the understory of treeline trees after three years of warming showed that *Vaccinium myrtillus* responded to higher temperatures with increased shoot increment length but that *Vaccinium gaultherioides* and *Empetrum hermaphroditum* did not show a growth response [11]. Moreover, there were only few indications for changes in early-season vegetative phenology in these species under warming [36,37]. In the study presented here, we conducted a detailed investigation of dwarf shrub phenological and growth responses during the summer of 2012, the final (sixth) year of the soil warming experiment. A final destructive harvest conducted at the peak of the vegetation period allowed us to investigate warming effects on secondary growth and biomass allocation for the first time. Our aims were (i) to determine whether a positive growth response in *Vaccinium myrtillus* was maintained after six years of soil warming, and whether *Vaccinium gaultherioides* and *Empetrum hermaphroditum* showed any delayed increases in growth after this full experimental period. By tracking the vegetative and flowering phenology of these dwarf shrubs, we also aimed (ii) to relate possible lagged changes in their phenology to changes in growth. Finally, we analyzed the widths of *V. myrtillus* growth rings with the aim of (iii) assessing whether the increases in shoot increment length under soil warming previously reported for this species represented an increase in the entire above-ground biomass of the ramets or if there was simply a shift in biomass allocation.

Material and methods

Study site

The study site was located within the Stillberg treeline site in the Central Alps (Davos, Switzerland, 9° 52'E, 46° 46' N). Stillberg is a 5-ha long-term afforestation research area where tree seedlings were planted into the intact dwarf shrub community in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). Climate data measured by a WSL meteorological station located within the research area (2090 m a.s.l.) indicated a mean annual precipitation of 1155 mm and mean annual air temperature of 2.1°C from 1975 to 2012. For the same period, the main growing season months (June-August) had a mean precipitation of 444 mm and a mean air temperature of 9.2°C. See Table S1 for details on climate data over the study years (2007-2012).

No specific permits were required for this location and activities and the field studies did not involve endangered or protected species.

Experimental design

The experiment consisted of 40 hexagonal 1.1 m^2 plots, 20 with one *Pinus mugo* ssp. *uncinata* (DC.) Domin individual in the center and 20 with one *Larix decidua* Mill. individual in the center. The plots were situated within an area of 2500 m^2 on a NE-exposed 25-30° steep slope at 2180 m a.s.l. at or slightly above the current treeline in the region [38,39]. The trees were sparsely distributed without forming a closed canopy; therefore, each plot contained a single tree surrounded by a dense cover of understory vegetation consisting mainly of the co-dominant dwarf shrub species *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* agg.) and *Empetrum nigrum* subsp. *hermaphroditum* (Hagerup) Böcher (referred to hereafter as *Empetrum hermaphroditum*). Further details about understory species composition were reported by Martin et al. [36] and Dawes et al. [11].

A free air CO_2 enrichment (FACE) experiment was started after snowmelt in early June 2001 and applied during each snow-free season for nine years (ending in 2009). The 40 plots were assigned to 10 groups of four neighboring plots (two *Pinus* and two *Larix* trees per group) and half of these groups were randomly assigned to an elevated CO_2 treatment while the other half served as controls (see a detailed description of the setup and performance of the CO_2 enrichment facility in Hättenschwiler et al. [38] and in Dawes et al. [40]). Dwarf shrub responses to the CO_2 enrichment have been reported in detail by Dawes et al. [11,41]. A soil warming treatment was added to the experiment in spring 2007 and was applied during each snow-free season until early August 2012. Within each of the 10 CO_2 treatment groups, one plot of each tree species was randomly selected and assigned a soil warming treatment, either control or warmed, and the second plot was assigned the other treatment, yielding a balanced split-split-plot design with a replication of five individual plots for each combination of CO_2 level, soil warming treatment and tree species. Therefore, from 2007 to 2009 the experiment included both CO_2 enrichment and soil warming, whereas from 2010 to 2012 the treatment consisted of soil warming only. The soil warming treatment was applied using 420 W-heating cables laid on the ground surface underneath the dwarf shrub layer, with a 5 cm distance between neighboring cables [42]. Each year, the heating was turned on immediately after snowmelt and turned off just before the site was covered in snow for the winter to avoid an interaction between soil temperature and snow cover duration. The warming treatment increased the growing season mean soil temperatures at 5 cm depth by 3.1 to 4.4°C over the 6 seasons of heating, with a difference of +3.5°C in 2012 (HOBO U23 Pro v2 dataloggers, Onset Computer Corporation, Bourne, MA, USA). Air temperature was warmed within the dwarf shrub canopy (0.9°C at 20 cm above ground [42]). The warming treatment had a slight drying effect on the soil organic layer (details in Hagedorn et al. [42] and Dawes et al. [11]). However, the soil matric water potential at 5 cm depth was always above -300 hPa in all plots, indicating overall very moist soil conditions [11,43].

Snowmelt date of each plot from 2007 to 2011 was defined as the date in spring when soil temperatures at 5 cm depth rose sharply from values near 0ºC, supported by visual estimations in the field and photographs. In 2012, snowmelt date was determined by visual estimations as the date when 50% of the plot was snow free and the ramets selected for detailed investigation were uncovered. This way, the estimation was more accurate for the study of the specific ramets selected.

Phenology

At the time of snowmelt in 2012, we marked five ramets of *V. myrtillus*, *V. gaultherioides* and *E. hermaphroditum* in each plot, excluding the area within 10 cm of plot borders to avoid potential edge effects. All plant measurements were made on these ramets. *Vaccinium myrtillus* was present and abundant in every plot, *V. gaultherioides* was present in 35 of the 40 plots, and *E. hermaphroditum* was present in 26 of the 40 plots, which was sufficient replication to assess treatment effects.

We monitored the phenology of the three study species between the start of snowmelt (day of year 142) and peak growing season (day of year 212). We visited all plots and monitored all ramets every 2 to 4 days at early growing season and every 4 to 6 days after flowering. For each marked ramet, we recorded the date when it entered the following phenophases (some of which occurred at the same time): (1) burst of first vegetative bud, (2) first leaf starting to unfold, (3) first leaf fully expanded, (4) start of shoot elongation, (5) burst of first flower bud, (6) anthesis, and (7) first flower withered. In early August, we harvested all vegetation from the experimental plots. Previous phenological data in the same plots from an entire snow-free season [44] confirm our observation that dwarf shrub vegetative development was completed at the time of the harvest, whereas fruits were not mature and leaf senescence had not started.

Shoot increment length and above-ground biomass

To track shoot growth responses during the whole soil warming experimental period, shoot increment length of the three species was measured from 2005 to 2011 on the longest branch of five to seven ramets in the field (2005-2009 data presented in Dawes et al. [11]). During early August 2012, we harvested all the marked ramets in every plot, clipping them at ground level. For *E. hermaphroditum*, we measured the new shoot increment length on the longest branch for every ramet before detaching and drying them for biomass measures. For all three species, we separated new shoots and leaves from the rest of the ramet while they were still fresh and dried them at 60°C for 48h. For the two deciduous *Vaccinium* species, we weighed new shoots and leaves separately and measured the length of three new shoots per ramet, whereas for *E. hermaphroditum*, we weighed the small needle-like leaves and new shoots together. For *V. myrtillus* and *V. gaultherioides*, we counted the number of new shoots per ramet and calculated the average mass per individual shoot. For *E. hermaphroditum*, we counted the number of new shoots from a subsample of ramets and used the mass-count relationship to estimate the number of branches for the remaining ramets in each plot. For all three species, we dried the remaining ramet material (designated as “main stems”, >1 year old) at 60°C for 24h and weighed it.

Growth rings of Vaccinium myrtillus

We made cross-sections of 20 µm thickness from the basal 1.5 cm of dried *V. myrtillus* stems using a sledge microtome (WSL-Lab-microtome). We stained sections with a mixture of Safranin and Astrablue to emphasize the growth ring structure. For dehydrating the sections for preservation, we rinsed them with increasingly concentrated ethanol solutions (75%, 96%, 100%), immersed them in Xylol, imbedded them in Canada-Balsam and dried them at 60°C for 24 hours [45] before photographing them at x20-x200 magnification through a microscope with a digital camera (Canon EOS 650D on Olympus BX41 microscope; Fig. S1). We used the images to visually count rings and to measure xylem ring widths in four radii per section with the program WinCELL [46]. We excluded 23 out of 200 ramets from the statistical analyses because wood was damaged or broken or rings were not distinguishable. Ramets were not old enough for rings to be analyzed statistically using specialized dendrochronological software, so we visually cross-dated the samples to find possible missing rings. Latewood had not formed in the 2012 growth rings, indicating that secondary growth was not finished when we harvested the ramets.

Statistical analysis

We applied linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML) to assess treatment effects on phenology and growth parameters. We used likelihood ratio tests to determine whether the previous CO_2 treatment and interactions between CO_2 and the other treatments contributed significantly to the model fits as fixed effects. Previous investigations already reported a lack of soil warming x CO_2 interactive effects [11,41] and we did not find significant persistent effects of the CO_2 enrichment; therefore, we pooled across CO_2 treatments for all final analyses. Statistical models for phenology and biomass of the study species included warming treatment, plot tree species and their interaction as fixed effects. We included snowmelt date as a covariate when it contributed significantly to the model fits, i.e. in tests of the effect of soil warming and plot tree species on phenology.

Shoot increment length and growth ring width were analyzed as repeated measures and included treatment year (categorical variable) and all interactions between year, warming and plot tree species as additional fixed effects (after Dawes et al. [11]). Measurements averaged over 2005 and 2006 were included as a covariate to account for differences in shoot increment length or ring width before the warming treatment started. When a significant interaction between soil warming and year was found, we additionally tested the effect of soil warming with separate analyses for each individual year. We accounted for violation of independence of residuals from different treatment years by implementing the residual autocorrelation structure corAR1 [47]. As growth rings were not completely finished in 2012, we excluded this year from the repeated measures analysis of growth ring widths. We tested for Pearson's correlations between shoot increment length and above-ground biomass, as well as between shoot increment length and xylem ring width in 2012. We did not extend this analysis to previous years because ramets harvested in 2012 were not the same as those randomly selected for shoot increment measurements in previous years.

For all statistical analyses, the random effects structure reflected the hierarchy of the split-split-plot experimental design, with measurements made on ramets in 40 individual plots, nested within 20 soil warming treatment groups, nested within 10 CO_2 treatment groups. For all analyses, we visually checked assumptions of normality and homoscedasticity of the residuals. We log-transformed response variables when necessary to reach these assumptions. Effects were considered significant at $P < 0.05$ and, because of the relatively low replication, we considered $P \geq 0.05$ but < 0.10 as marginally significant. We performed all the analyses with R version 2.15.2 [48] using the nlme package [47].

Results

Abiotic conditions

In 2012, snow melted from the experimental plots between 21 and 31 May (days of year 142 and 152). There were no differences in the date of snowmelt between soil warming treatments ($F_{1,9}$ = 0.64, P = 0.447) or plot tree species ($F_{1,18}$ = 1.04, P = 0.321). From 21 May to 3 August, when plants were harvested, the mean air temperature at the meteorological station was 9.7°C and temperatures ranged from -0.4 on 12 June to 21.9°C on 30 June.

Phenology

The soil warming treatment did not significantly affect the vegetative phenology of the three dwarf shrub species (Fig. 1). However, we found marginally significant warming effects on their flowering phenology. *Vaccinium myrtillus* and *V. gaultherioides* showed a slightly earlier flower anthesis (by 1 and 2 days, respectively) in warmed plots than in unwarmed plots (*V. myrtillus*: $F_{1,9}$ = 4.3, P = 0.067, Fig.1A; *V. gaultherioides*: $F_{1,7}$ = 4.41, P = 0.074, Fig. 1B). *Vaccinium myrtillus* flowers also withered slightly earlier with increased soil temperatures (by 2 days, $F_{1,9}$ = 4.03, P = 0.076).

The tree species present in the plot had a greater effect than soil warming on *V. myrtillus* vegetative and reproductive phenology (Fig. 1A). The start of leaf unfolding, start of shoot elongation, flower bud break and flower anthesis took place 1 day earlier under larch than under pine (P < 0.011), and leaf full expansion occurred 3 days earlier ($F_{1,17}$ = 29.88, P < 0.001). There was a marginally significant effect of the tree species x warming interaction on the start of shoot elongation for *V. myrtillus,* which was slightly earlier (1 day) in warmed plots under larch than in unwarmed plots under pine ($F_{1,17}$ = 3.7, P = 0.071). The phenology of *V. gaultherioides* and *E. hermaphroditum* did not show significant differences between plots with the two different tree species (Fig. 1B,C).

Most of the phenological phases for *V. myrtillus* and *V. gaultherioides* occurred earlier in plots with an earlier snowmelt date (P < 0.07). However, *V. myrtillus* anthesis and *V. gaultherioides* flower bud break and withering did not show a relationship with snowmelt timing. *Empetrum hermaphroditum* flowering phenophases and start of shoot elongation were also related to snowmelt date ($P < 0.04$), but vegetative bud break was not.

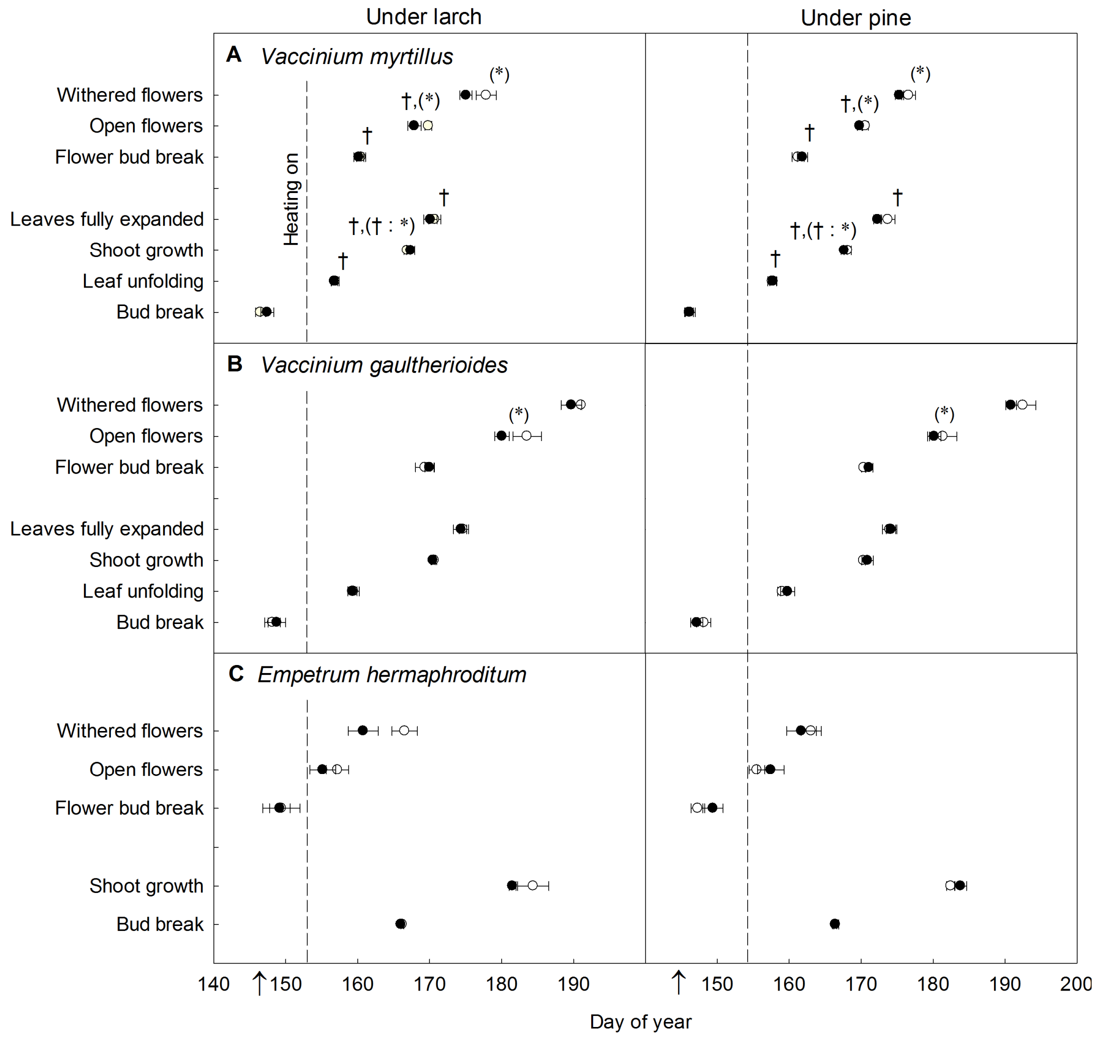

Figure 1. Vegetative and flowering phenology for the three dwarf shrub species studied.
Vegetative and flowering phenology for the three dwarf shrub species studied. Circles represent the mean day of year (±1 SE) of each phenophase for the four different soil warming (open circles, unwarmed; closed circles, warmed) and plot tree species (larch or pine) combinations (n = 10). Crosses show significant differences between plot tree species ($P < 0.05$) and marginally significant differences ($0.05 \leq P < 0.10$) between soil warming treatments are given by asterisks in parentheses. Colons show treatment interactions. Note that in some cases symbols for different soil warming treatments overlap completely. Dotted lines show the date when the warming treatment started in 2012 and arrows on the x-axes show the mean snowmelt date for all plots with the same tree species.

Shoot increment length

Soil warming had a significant positive effect on the annual shoot increment length of *V. myrtillus* (mean increase of 22% over 2007-2012, $F_{1,9}$ = 41.38, P < 0.001, Fig. 2A). Treatment year also had a significant effect ($F_{5,180}$ = 3.67, P = 0.004) but the warming x year effect was not significant ($F_{5,180}$ = 1.89, P = 0.100). Nevertheless, the response was larger in the two last study years (shoot increment length 34% greater in warmed plots than unwarmed plots for 2011-2012) than in the previous years (18% greater in warmed plots than unwarmed plots, averaged over 2008-2010).

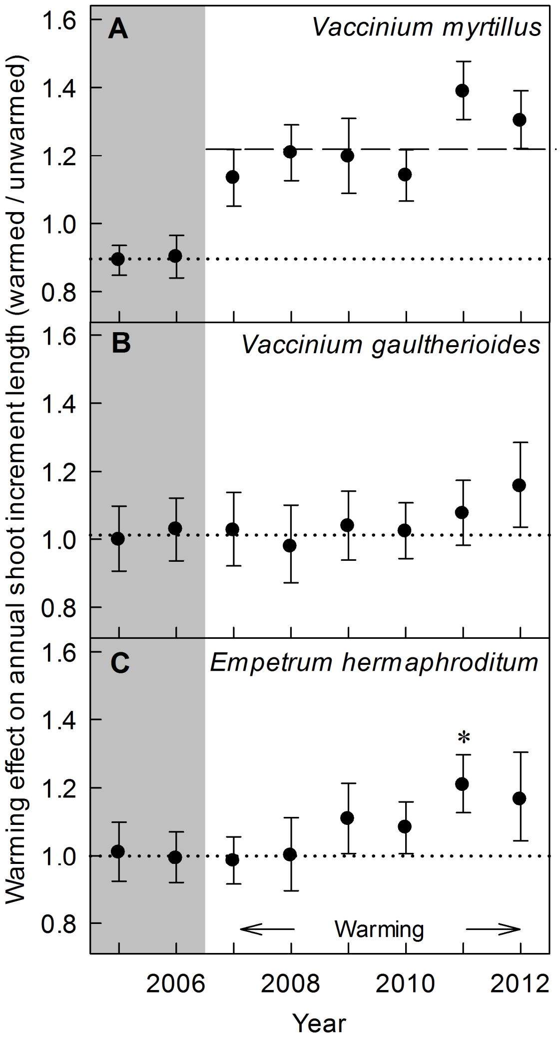

Figure 2. Soil warming effect on dwarf shrub annual shoot increment length. Soil warming effect on dwarf shrub annual shoot increment length from 2007 until 2012, the entire duration of the soil warming experiment. Data through 2009 were presented in Dawes et al. (2011a). The warming effect was calculated as the ratio of the mean shoot increment length of all warmed plots to the mean of all unwarmed plots, pooled across plots containing a larch or pine tree. Error bars represent ±1 SE of the ratio. The dashed line shows the significant warming effect on *V. myrtillus* averaged for 2007-2012. The asterisk shows significant differences between temperature treatments (P < 0.05). Pre-warming ratios are shown in the shaded region (2005-2006) and the dotted line is drawn through the average of these two points, which indicates the mean warmed to unwarmed ratio before treatment began.

The annual shoot increment length of *V. gaultherioides* was not significantly affected by soil warming ($F_{1,9}$ = 0.99, P = 0.346, Fig. 2B) although there was a trend of increase with warming in the last year. Treatment year had a significant effect on *V. gaultherioides* shoot increment length ($F_{5,171}$ = 6.93, P < 0.001) but the warming x year interaction was not significant ($F_{5,171}$ = 0.5, P = 0.776). *Empetrum hermaphroditum* annual shoot increment length showed a significant effect of treatment year ($F_{5,128}$ = 25.97, P < 0.001, Fig. 2C) and the plot tree species x year interaction ($F_{5,128}$ = 2.55, P = 0.031), and also a marginally significant effect of warming ($F_{1,9}$ = 4.83, P = 0.056) and the warming x year interaction ($F_{5,128}$ = 2.21, P = 0.057). Analyses of individual years showed that *E. hermaphroditum* only had a significant (positive) shoot growth response to soil warming in 2011 (+21% increase in warmed plots compared to unwarmed plots, $F_{1,7}$ = 10.01, P = 0.016) and that the shoot increment length of this species was greater under pine than under larch in 2008 (marginally significant, $F_{1,11}$ = 4.04, P = 0.070) and 2009 ($F_{1,10}$ = 5.07, P = 0.048).

Shoot increment length averaged over 2005 and 2006 (pre-treatment covariate) positively influenced the length of new *V. gaultheroides* shoots during the 2007-2012 warming period ($F_{1,17}$ = 18.61, P < 0.001), and to a lesser extent that of *V. myrtillus* and *E. hermaphroditum* (both marginally significant, $F_{1,17}$ = 3.28, P = 0.088 and $F_{1,13}$ = 4.58, P = 0.052, respectively). In 2012, new shoot increment length showed a positive correlation with new shoot biomass for each of the three species (*V. myrtillus* R^2 = 0.53, P < 0.001; *V. gaultherioides* R^2 = 0.39, P< 0.001; *E. hermaphroditum* R^2 = 0.27, P < 0.001).

Above-ground biomass

At the time of harvest in August 2012, *V. myrtillus* ramets showed a 54% greater total leaf biomass ($F_{1,9}$ = 15.52, P = 0.003), a 63% greater total new shoot biomass ($F_{1,9}$ = 14.15, P = 0.005) and a 26% greater main stem (stems >1 year old) biomass ($F_{1,9}$ = 7.82, P = 0.021) in warmed plots than in unwarmed plots (Fig. 3A). Moreover, the main stem biomass of *V. myrtillus* was 35% greater under pine than under larch ($F_{1,18}$ = 5.15, P = 0.036). Although differences in ramet above-ground biomass between warming treatments were, on average, larger in plots with larch than in plots with pine, there were no significant tree species x warming interactions for any of the plant parts (P > 0.77). *Vaccinium gaultherioides* and *E. hermaphroditum* did not show significant differences among treatments in the above-ground biomass for any of the plant parts analyzed (Fig. 3B,C). The three dwarf shrub species showed a positive correlation between new shoot biomass and total ramet biomass (*V. myrtillus* $R^2 = 0.77$, $P < 0.001$; *V. gaultherioides* $R^2 = 0.71$, $P < 0.001$; *E. hermaphroditum* $R^2 = 0.52$, $P < 0.001$).

The number of new shoots per ramet in *V. myrtillus* was significantly greater in warmed plots than in unwarmed plots ($F_{1,9} = 6.466$, $P = 0.032$) and slightly greater under pine than under larch (marginally significant, $F_{1,18} = 3.51$, $P = 0.077$). In addition, the mass per individual shoot was larger in warmed plots than in unwarmed plots ($F_{1,9} = 9.79$, $P = 0.012$) and slightly larger under larch than under pine (marginally significant, $F_{1,18} = 4.14$, $P = 0.060$). Therefore, *V. myrtillus* not only produced more shoots in warmed plots, but these individual shoots were longer and heavier. *Vaccinium gaultherioides* produced slightly more new shoots in warmed plots than in unwarmed plots (marginally significant, $F_{1,9} = 3.74$, $P = 0.085$) and under pine than under larch ($F_{1,13} = 5.91$, $P = 0.030$), but the mass per individual shoot did not differ between warming treatments or plot tree species.

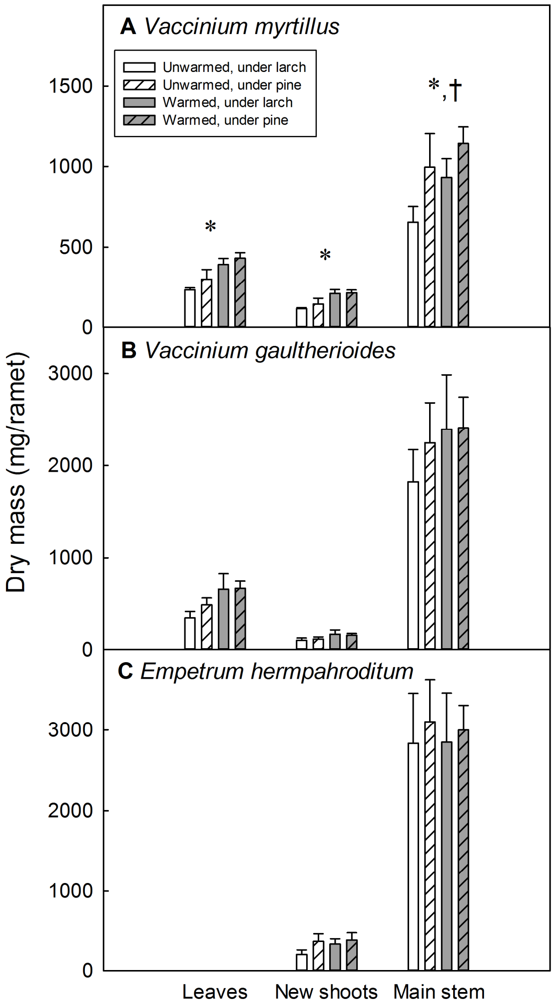

Figure 3. Above-ground biomass partitioning of the three dwarf shrub species studied.

Above-ground biomass partitioning of the study species for each soil warming and plot trees species combination (mean values +1 SE, n = 10). Asterisks show significant differences between soil warming treatments and crosses show significant differences between plot tree species ($P < 0.05$). For *Empetrum hermaphroditum* only, leaves and new shoots are both included in "New shoots". The y-axis scale varies across species to emphasize differences between treatments.

Growth rings of Vaccinium myrtillus

We did not find significant differences in ramet age between warming treatments (8.4 ± 0.5 years old for unwarmed and 7.9 ± 0.3 for warmed plots; $F_{1,9} = 0.58$, $P = 0.467$; Fig. S2). However, ramets were younger under larch (7.4 ± 0.4 years old) than under pine (9.0 ± 0.4; $F_{1,18} = 10.13$, $P = 0.005$). There was a marginally significant warming x tree species interaction ($F_{1,18} = 3.28$, $P = 0.087$): on average, ramets were older in unwarmed plots with pine than in both warmed and unwarmed plots with larch.

Repeated measures analyses showed that, averaged across all treatment years, *V. myrtillus* ring width was 41% greater in warmed plots than in unwarmed plots ($F_{1,9} = 16.45$, $P = 0.003$, Fig. 4). Treatment year and the warming x year interaction also had significant effects ($F_{4,297} = 4.67$, $P = 0.001$ and $F_{4,297} = 3.32$, $P = 0.011$, respectively), as well as the pre-treatment ring width covariate ($F_{1,44} = 17.26$, $P < 0.001$). Plot tree species and interactions between tree species and the other fixed effects did not significantly influence xylem ring width ($P > 0.17$). Analyses of individual years showed that warming had a significant effect on ring width in all years ($P < 0.024$) except for the first year of treatment (Fig. 4).

Although xylem latewood formation was not completed when we collected the samples in 2012, we found a significant correlation between shoot increment length and ring width in that year ($R^2 = 0.157$, $P < 0.001$), as well as between ring width and biomass of new shoots ($R^2 = 0.088$, P < 0.001) and biomass of leaves ($R^2 = 0.068$, P < 0.001). We tested for an age-biomass correlation and results corroborated that older ramets were heavier ($R^2 = 0.24$, $P < 0.001$).

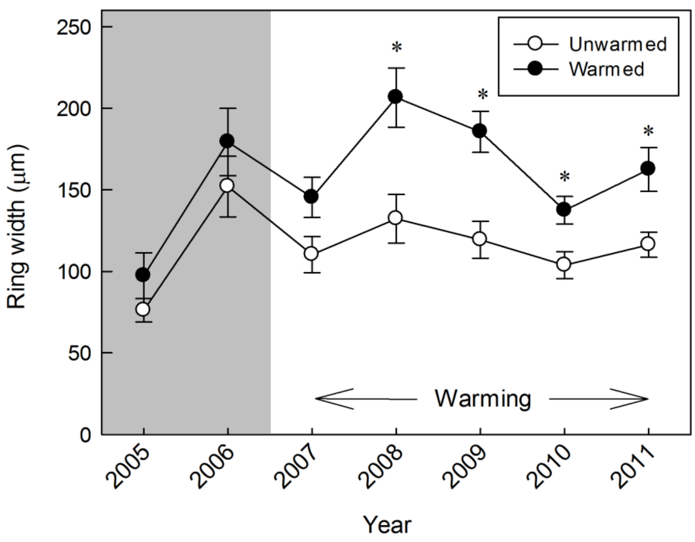

Figure 4. Xylem ring widths of *Vaccinium myrtillus*.
Xylem ring widths of *Vaccinium myrtillus* from 2007 to 2011 for warmed and unwarmed plots, pooled across plots containing a larch or pine tree (mean values ±1 SE, n = 20). Asterisks show significant differences between soil warming treatments ($P < 0.05$). Pre-warming values are shown in the shaded region (2005-2006).

Discussion

Increased above-ground growth after six years of soil warming

Six years of soil warming led to a sustained growth stimulation of *V. myrtillus*, and the greatest response of new shoot increments occurred in the final two years. This increasing response over time contrasts with studies where positive responses of *V. myrtillus* and other alpine and Arctic plant species to warming were transient and stopped after four or five years [12,13,49]. In addition, our findings indicate that other factors that can potentially limit growth, such as nutrient or light availability, did not constrain the warming response of *V. myrtillus* in later years of the experiment [12].

Earlier studies at the same experimental site found more severe freezing damage of *V. myrtillus* under warming than in control plots [36,37], but our results show that these freezing effects were not large enough to substantially offset the enhanced growth over six years. The other two dwarf shrub species showed at least a slight trend of increased shoot increment length with soil warming in the last years of the study, suggesting that the response of these two species to climate warming may be considerably delayed.

The species-specific warming effects on above-ground biomass production in our experiment contrast with a two-year study with open-top polythene tents in subarctic Sweden, where all three study species in common with our study showed a greater above-ground biomass under warming [50]. A five-year study at the same site in the subarctic by Press et al. [51] similarly showed that higher temperatures increased *V. uliginosum* (comparable with *V. gaultherioides*) biomass. Moreover, *Empetrum nigrum* shoot production and shoot increment length increased under two years [31] and five years [32] of warming with open-top chambers in alpine heath in Japan. The different methodologies applied (air warming by open-top chambers *vs*. soil warming by heating cables) might explain some of the differences between studies. In addition, different plant community composition and dynamics might play an important role in explaining the different results across study sites.

Vaccinium myrtillus has a wider elevational distribution (colline to alpine) than *V. gaultherioides* and *E. hermaphroditum* (subalpine to alpine) [52]. Its presence in lower elevational areas indicates that *V. myrtillus* might be adapted to higher temperatures than *V. gaultherioides* and *E. hermaphroditum* [11] and thus may be a stronger competitor under increased temperatures. In fact, a 22-year experiment with open-top greenhouses in the Swedish tundra reported no effects on *E. hermaphroditum* growth under 4ºC of air warming [13], whereas in the same experiment, Graglia et al. [53] found positive effects on this species after six years of 2.5ºC warming but not 4ºC warming. The mean daily air temperature increase with open-top chambers is around 1-2ºC [54] whereas the soil temperature increase by our heating cables is around 4ºC, which is similar to the air temperature increase reached by Campioli et al. [13]. As suggested in their study, *E. hermpahroditum* might suffer from heat stress under high temperatures, responding positively only to smaller increases.

Phenology: effects of growing season temperature, snowmelt and plot tree species

The lack of vegetative phenological responses to soil warming of the three dwarf shrub species studied and the strong effect of snowmelt date on early-season phenology in 2012 are consistent with results from the first three years of the experiment [37]. Some plant species have been shown not to respond to spring warming experiments [55] because they either do not respond to climate warming [55,56], because they are more sensitive to changes in late winter temperatures [55] or because they primarily respond to other cues such as photoperiod [57]. In our experiment, the lack of warming effects on the vegetative phenophases might be partially due to the fact that vegetative development, especially for the two *Vaccinium* species, started directly after snowmelt, which coincided with the start of the warming treatment. Phenophases occurring later in the season (i.e. flowering phenophases in *Vaccinium* spp.) did not depend on the snowmelt date and were slightly influenced by the warming treatment, which is consistent with patterns found for tundra dwarf shrub species in subarctic Alaska [58].

Our study might have underestimated phenological responses to future climate warming because only growing season temperatures were altered [55,59], whereas late winter temperatures, which can be key determinants of plant phenology [15,55], were not experimentally manipulated. Nevertheless, other studies that only altered growing season temperatures found that the same species as in our study showed phenological advances under air warming [18] or that other alpine species showed a lagged response after multiple years of warming [10]. The distinct methodologies applied in these experiments (e.g. soil *vs.* air warming), microclimate conditions, different plant community types and genetic variations between populations might explain these differences [11,60,61].

The tree species present in the plot had a greater effect on *V. myrtillus* phenology than soil warming. The phenological advancement under larch could be due to a greater light incidence under this species at the beginning of the growing season, which could act as a phenological cue [62].

Increased growth in V. myrtillus decoupled from phenology

The increased growth and biomass production found in *V. myrtillus* with soil warming did not correspond to a phenological shift or to the date of snowmelt, which indicates that *V. myrtillus* can produce more biomass under higher growing season temperatures even without a springtime extension of the active season. The peak-season harvest meant that we could not check if *V. myrtillus* phenology at senescence time was delayed by the warming treatment, an effect that has been reported for tundra plants in experiments with open-top chambers [63,64]. Delayed leaf senescence could have influenced *V. myrtillus* growth in the late season (e.g. secondary growth) or in the following year. However, monitoring of leaf senescence in 2008-2009 showed no differences between warming treatments in this species (Dawes, unpublished) and late-season phenophases of alpine plants are generally considered to be more controlled by photoperiod than by temperature [65].

As advanced phenology could not explain the enhanced growth of *V. myrtillus* under soil warming, potential mechanisms for this response include increased rates of photosynthesis [66] and tissue formation [65] directly caused by higher temperatures. Another mechanism could be accelerated decomposition and mineralization of soil organic matter under warmer soil, which can improve nutrient availability for plants in alpine and Arctic environments where low temperatures tend to constrain these processes [49,66-68]. Soil inorganic nitrogen content increased during the first three years of our soil warming experiment [11], suggesting that indirect effects of soil warming via an enhanced nutrient cycling played a role in the *V. myrtillus* growth response.

Above-ground biomass increase under soil warming

To our knowledge, this is a pioneer study on assessing the effects of experimental soil warming on the age and xylem ring width of *V. myrtillus*. Our results show that rings were wider in warmed plots since the second year of treatment, a response that was maintained throughout the six experimental years. In addition, the significant positive correlation between shoot increment length and early xylem growth (xylem latewood formation was not completed) indicates that vessel size in the early xylem growing season is tightly related to the elongation of new shoots, which receive water from newly-formed vessels [69].

Moreover, the positive correlation between shoot increment length and both early xylem growth and annual shoot biomass production, together with the greater biomass in *V. myrtillus* in warmed plots, provide evidence that the previously reported increases in the shoot increment length of *V. myrtillus* with warming were not merely a result of a shift in biomass allocation but an overall increase in above-ground biomass production.

However, below-ground biomass was not measured in this study and thus, it is possible that soil warming led to shifts in biomass allocation between below-ground and above-ground compartments [70].

Although warmer temperatures led to an increased growth of *V. myrtillus*, the age structure of this species was not affected by soil warming. However, there was a lower ramet turnover under pine (older ramets) than under larch, and ramet main stem biomass was also higher under pine. A possible explanation for these differences is that pine provides greater protection against freezing conditions at the beginning of the season before needles are developed on deciduous larch. This effect would be similar to the facilitation exerted by shrubs on young trees [71,72], leading to lower mortality rates. Moreover, Dawes et al. [11] found less canopy shading under pine than under larch, thus the lower main stem biomass under larch may be a consequence of lower light availability.

Concluding remarks

In summary, we found increased growth of *V. myrtillus* under soil warming, a response that was sustained, and even became stronger in the case of shoot increment length, over six years of warming. The application of dendrochronological techniques showed that this increased growth reflected an overall increase in above-ground biomass production. Moreover, the lack of an advanced phenology of *V. myrtillus* under soil warming indicated that an extended growing period was not necessary for the observed growth response. Our results suggest that *V. myrtillus* will experience a larger and more rapid growth benefit from a warming climate than *V. gaultherioides* or *E. hermaphroditum* and could therefore become increasingly dominant in high-elevation treeline environments.

Acknowledgements

We thank many colleagues at the SLF, WSL and other research institutes who contributed to the Stillberg CO_2 enrichment and soil warming experiment. We are especially grateful to Stephan Hättenschwiler and I. Tanya Handa for planning and initiating this treeline study. We thank Yves Bötsch and Michael Liu for their help with field work and Jodi Axelson, Loïc Schneider, Fritz Schweingruber, Agata Buchwal and

Patrick Fonti for their help, support and comments in the growth rings methodology and analysis. We are grateful to Sara Palacio for her comments on an early version of the manuscript and to Josep Maria Ninot for his academic support.

References

1. Chapin III FS, Mcguire AD, Randerson J et al. (2000) Arctic and boreal ecosystems of western North America as components of the climate system. Global Change Biol 6: 211–223.
2. IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland, 73 p.
3. Meehl GA, Stocker TF, Collins WD, et al. (2007) Global climate projections. In: Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change (eds Solomon S, Qin D, Manning M, et al.). Cambridge University Press, Cambridge, UK. pp. 747-845.
4. Cornelissen JHC, Callaghan TV, Alatalo JM, et al. (2001) Global change and arctic ecosystems: Is lichen decline a function of increases in vascular plant biomass? J Ecol 89: 984–994.
5. Hudson JMG, Henry GHR (2009) Increased plant biomass in a high arctic heath community from 1981 to 2008. Ecology 90: 2657–2663.
6. Elmendorf SC, Henry GHR, Hollister RD, et al. (2012a) Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. Ecol Lett 15: 164–175.
7. Wookey PA, Aerts R, Bardgett RD et al. (2009) Ecosystem feedbacks and cascade processes: Understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Global Change Biol 15: 1153–1172.
8. Myers-Smith IH, Forbes BC, Wilmking M, et al. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. Environ Res Lett 6: 10.1088/1748-9326/6/4/045509.
9. Klanderud K (2008) Species-specific responses of an alpine plant community under simulated environmental change. J Veg Sci 19: 363–372.

10. Hoffmann AA, Camac JS, Williams RJ, Papst W, Jarrad FC, Wahren C-H (2010) Phenological changes in six Australian subalpine plants in response to experimental warming and year-to-year variation. J Ecol 98: 927–937.
11. Dawes MA, Hagedorn F, Zumbrunn T, Handa IT, Hättenschwiler S, Wipf S, Rixen C (2011a) Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytol 191: 806–818.
12. Arft AM, Walker MD, Gurevitch J, et al. (1999) Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. Ecol Monogr 69: 491–511.
13. Campioli M, Leblans N, Michelsen A (2012) Twenty-two years of warming, fertilisation and shading of subarctic heath shrubs promote secondary growth and plasticity but not primary growth. PLoS ONE 7, 10.1371/journal.pone.0034842.
14. Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of Arctic tundra to experimental and observed changes in climate. Ecology 76: 694–711.
15. Walther GR (2003) Plants in a warmer world. Perspect Plant Ecol 6: 169-185.
16. Goulden ML, Munger JW, Fan S-M, Daube BC, Wofsy SC (1996) Exchange of carbon dioxide by a deciduous forest: Response to interannual climate variability. Science 271: 1576–1578.
17. Myneni RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the northern high latitudes from 1981 to 1991. Nature 386: 698–702.

18. Suzuki S, Kudo G (2000) Responses of alpine shrubs to simulated environmental change during three years in the mid-latitude mountain, northern Japan. Ecography 23: 553–564.
19. Dunne JA, Harte J, Taylor KJ (2003) Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. Ecol Monogr 73: 69–86.
20. Aerts R, Cornelissen JHC, Dorrepaal E, Van Logtestijn RSP, Callaghan TV (2004) Effects of experimentally imposed climate scenarios on flowering phenology and flower production of subarctic bog species. Global Change Biol 10: 1599–

1609.

21. Xu Z-F, Hu T-X, Wang K-Y, Zhang Y-B, Xian J-R (2009) Short-term responses of phenology, shoot growth and leaf traits of four alpine shrubs in a timberline ecotone to simulated global warming, Eastern Tibetan Plateau, China. Plant Spec Biol 24: 27–34.
22. Smith J, Sconiers W, Spasojevic M, Ashton I, Suding K (2012) Phenological changes in alpine plants in response to increased snowpack, temperature, and nitrogen. Arct Antarct Alp Res 44: 135–142.
23. Totland Ø, Alatalo JM (2002) Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. Oecologia 133: 168–175.
24. Tape K, Sturm M, Racine C (2006) The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. Global Change Biol 12: 686–702.
25. Rundqvist S, Hedenås H, Sandström A, Emanuelsson U, Eriksson H, Jonasson C, Callaghan TV (2011) Tree and shrub expansion over the past 34 years at the tree-line near Abisko, Sweden. Ambio 40: 683–692.
26. Ropars P, Boudreau S (2012) Shrub expansion at the forest-tundra ecotone: Spatial heterogeneity linked to local topography. Environ Res Lett 7: 10.1088/1748-9326/7/1/015501.
27. Elmendorf SC, Henry GHR, Hollister RD et al. (2012b) Plot-scale evidence of tundra vegetation change and links to recent summer warming. Nature Climate Change 2: 453–457.
28. Hill GB, Henry GHR (2011) Responses of High Arctic wet sedge tundra to climate warming since 1980. Global Change Biol 17: 276–287.
29. Rixen C, Schwoerer C, Wipf S (2010) Winter climate change at different temporal scales in *Vaccinium myrtillus*, an Arctic and alpine dwarf shrub. Polar Res 29: 85–94.
30. Pato J, Ramón Obeso J (2012) Growth and reproductive performance in bilberry (*Vaccinium myrtillus*) along an elevation gradient. Ecoscience 19: 59–68.
31. Wada N, Shimono M, Miyamoto M, Kojima S (2002) Warming effects on shoot developmental growth and biomass production in sympatric evergreen alpine dwarf shrubs *Empetrum nigrum* and *Loiseleuria procumbens*. Ecol Res 17: 125–

132.

32. Kudo G, Suzuki S (2003) Warming effects on growth, production, and vegetation structure of alpine shrubs: A five-year experiment in northern Japan. Oecologia 135: 280–287.
33. Van Wijk MT, Clemmensen KE, Shaver GR, et al. (2004) Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: Generalizations and differences in ecosystem and plant type responses to global change. Global Change Biol 10: 105–123.
34. Bret-Harte MS, Shaver GR, Chapin III FS (2002) Primary and secondary stem growth in arctic shrubs: Implications for community response to environmental change. J Ecol 90: 251–267.
35. Campioli M, Schmidt NM, Albert KR, Leblans N, Ro-Poulsen H, Michelsen A (2013) Does warming affect growth rate and biomass production of shrubs in the High Arctic? Plant Ecol 214: 1049–1058.
36. Martin MA, Gavazov K, Körner C, Hättenschwiler S, Rixen C (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Global Change Biol 16: 1057–1070.
37. Rixen C, Dawes MA, Wipf S, Hagedorn F (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO_2 enrichment and soil warming. Oikos 121: 1532–1543.
38. Hättenschwiler S, Handa IT, Egli L, Asshoff R, Ammann W, Körner C (2002) Atmospheric CO_2 enrichment of alpine treeline conifers. New Phytol 156: 363–375.
39. Barbeito I, Dawes MA, Rixen C, Senn J, Bebi P (2012) Factors driving mortality and growth at treeline: a 30-year experiment of 92 000 conifers. Ecology 93: 389–401.
40. Dawes MA, Hättenschwiler S, Bebi P, Hagedorn F, Handa IT, Körner C, Rixen C (2011b) Species-specific tree growth responses to 9 years of CO_2 enrichment at the alpine treeline. J Ecol 99: 383–394.
41. Dawes MA, Hagedorn F, Handa IT et al. (2013) An alpine treeline in a carbon dioxide-rich world: Synthesis of a nine-year free-air carbon dioxide enrichment study. Oecologia 171: 623–637.

42. Hagedorn F, Martin MA, Rixen C, et al. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. Biogeochemistry 97: 7–19.
43. Dawes MA, Zweifel R, Dawes N, Rixen C, Hagedorn F (2014) CO_2 enrichment alters diurnal stem radius fluctuations of 36-yr-old *Larix decidua* growing at the alpine tree line. New Phytol: 10.1111/nph.12742.
44. Zumbrunn T (2004) Alpine dwarf shrubs in a CO_2 enriched world. Unpublished Diploma thesis, University of Basel, Basel, Switzerland.
45. Schweingruber FH (1990) Microscopic Wood Anatomy. 3rd ed. Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, 226 p.
46. Régent Instruments, Inc. (2001) WinCELL pro 2001: image analysis software [computer program]. Québec.
47. Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2008) NLME: linear and nonlinear mixed effects models. R package version 3.1-89.
48. R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/
49. Hartley AE, Neill C, Melillo JM, Crabtree R, Bowles FP (1999) Plant performance and soil nitrogen mineralization in response to simulated climate change in subarctic dwarf shrub heath. Oikos 86: 331–343.
50. Parsons AN, Welker JM, Wookey PA, Press MC, Callaghan TV, Lee JA (1994) Growth responses of four sub-Arctic dwarf shrubs to simulated environmental change. J Ecol 82: 307–318.
51. Press MC, Potter JA, Burke MJW, Callaghan TV, Lee JA (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. J Ecol 86: 315–327.
52. Lauber K, Wagner G, Gygax A (2012) Flora Helvetica. Bern: Haupt. 1656 pp.
53. Graglia E, Jonasson S, Michelsen A, Schmidt IK (1997) Effects of shading, nutrient application and warming on leaf growth and shoot densities of dwarf shrubs in two arctic-alpine plant communities. Ecoscience 4: 191–198.
54. Marion GM, Henry GHR, Freckman DW, et al. (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biol

3: 20–32.

55. Cook BI, Wolkovich EM, Parmesan C (2012) Divergent responses to spring and winter warming drive community level flowering trends. P Natl Acad Sci USA 109: 9000–9005.
56. McEwan RW, Brecha RJ, Geiger DR, John GP (2011) Flowering phenology change and climate warming in southwestern Ohio. Plant Ecol 212: 55–61.
57. *Körner C, Basler* D (*2010*) *Phenology under* global warming. Science, 327: 1461–1462.
58. Wipf S (2010) Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. Plant Ecol 207: 53–66.
59. Wolkovich EM, Cook BI, Allen JM, et al. (2012) Warming experiments underpredict plant phenological responses to climate change. Nature 485: 494–497.
60. Oberbauer SF, Elmendorf SC, Troxler TG et al. (2013) Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. Philos T Roy Soc B 368: 20120481.
61. Schwartz MD, Hanes JM, Liang L (2013) Separating temperature from other factors in phenological measurements. Int J Biometeorol, 10.1007/s00484-013-0723-2.
62. Pau S, Wolkovich EM, Cook BI et al. (2011) Predicting phenology by integrating ecology, evolution and climate science. Global Change Biol 17: 3633–3643.
63. Suzuki S, Kudo G (1997) Short-term effects of simulated environmental change on phenology, leaf traits, and shoot growth of alpine plants on a temperate mountain, northern Japan. Global Change Biol 3: 108-115.
64. Natali SM, Schuur EAG, Rubin RL (2012) Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. J Ecol 100: 488–498.
65. Körner C (2003) Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. 2nd ed. Springer, Berlin, 344 p.
66. Rustad LE, Campbell JL, Marion GM, et al. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126: 543–562.
67. Aerts R, Cornelissen JHC, Dorrepaal E (2006) Plant performance in a warmer

world: General responses of plants from cold, northern biomes and the importance of winter and spring events. Plant Ecol 182: 65–77.

68. Streit K, Hagedorn F, Hiltbrunner D, Portmann M, Saurer M, Buchmann N, Wild B, Richter A, Wipf, S, Siegwolf RTW (2014) Soil warming alters microbial substrate use in alpine soils. Global Change Biol 20: 1327–1338.
69. Esau K (1977) Anatomy of seed plants. 2nd ed. John Wiley & Sons, New York, 550 p.
70. Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. J Exp Bot 57: 267-281.
71. Gómez-Aparicio L, Zamora R, Castro J, Hódar JA (2008) Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores? J Veg Sci 19: 161–172.
72. Grau O, Ninot JM, Blanco-Moreno JM, van Logtestijn RSP, Cornelissen JHC, Callaghan TV (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. Oikos 121: 1680–1690.

Supporting information

Table S1. Climate conditions in the experimental plots during all study years (2007-2012). Mean air temperature and total precipitation at a climate station located *c*. 100 m below the experimental site are given for the main vegetation period, June-August.

Year	Snowmelt date	Air temperature (°C) (mean JJA)	Precipitation (mm) (sum JJA)
2007	07 May	9.5	469
2008	29 May	9.7	470
2009	23 May	10	463
2010	29 May	9.7	539
2011	12 May	9.3	472
2012	28 May	10.7	645

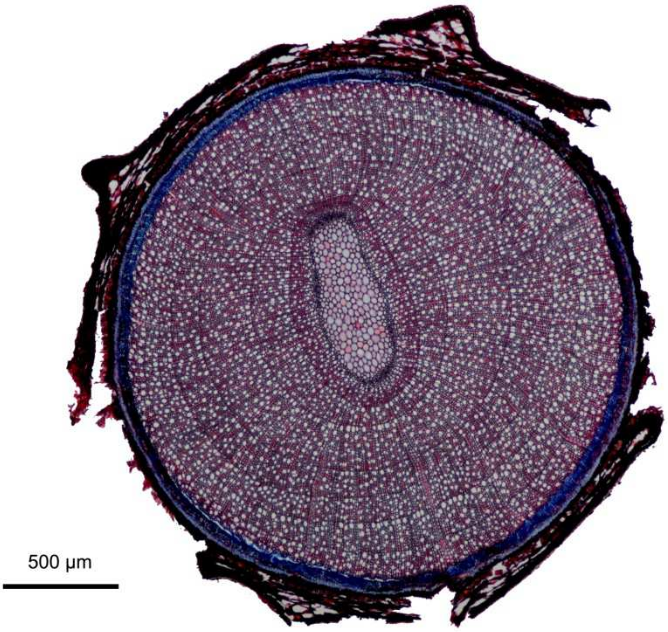

Figure S1. Cross-section of a *Vaccinium myrtillus* ramet.

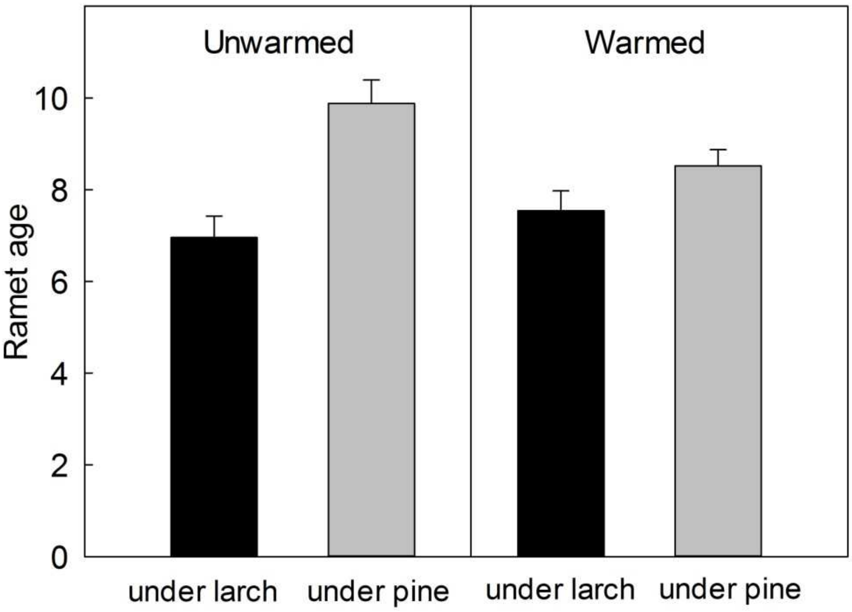

Figure S2. Age of *Vaccinium myrtillus* for each soil warming treatment and plot tree species combination.

Capítol 4 / Chapter 4

Linking above-ground growth and functional xylem anatomy in the dwarf shrub *Vaccinium myrtillus* in a CO_2 enrichment and soil warming experiment at treeline

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Resum

Les respostes de les plantes a les condicions ambientals poden tenir una base estructural en ajustaments en l'anatomia del xilema. Vam investigar el creixement aeri i l'anatomia del xilema del subarbust *Vaccinium myrtillus* sota la capçada de *Larix decidua* i *Pinus uncinata* en una zona experimental al límit supraforestal als Alps Suïssos després de 9 anys de fumigació amb CO_2 a l'aire lliure (+ 200 ppm) i 6 anys d'escalfament del sòl (+ 4 ºC). El nostre objectiu era analitzar la relació entre el creixement i l'anatomia del xilema i determinar si aquesta relació es mantenia amb els tractaments. Vam mesurar paràmetres de creixement i vam realitzar fotografies d'alta resolució de microseccions de la base de la tija principal de *V. myrtillus* per tal de quantificar característiques anatòmiques funcionalment rellevants de vasos conductors i parènquima radial, els quals tenen funcions de transport d'aigua i d'emmagatzematge de carboni, respectivament. Vam trobar fortes correlacions entre la conductivitat hidràulica acumulada (Kh) i paràmetres de creixement per a tots els tractaments en conjunt. Tanmateix, l'escalfament va modificar la relació creixement-anatomia, ja que va estimular l'elongació de la tija i l'amplada i l'àrea dels anells de creixement però va reduir l'eficiència hidràulica a través de la reducció de la mida dels vasos (Dh), la conductivitat hidràulica específica (Ks) i la conductivitat hidràulica acumulada (Kh). L'augment de CO_2 va tenir menys efectes en el creixement i l'anatomia, però va augmentar l'àrea basal de la tija, la densitat absoluta dels radis i, de forma retardada, Dh i Ks. Aquests resultats suggereixen que hi ha un lligam entre creixement i conductivitat hidràulica, però que les respostes de creixement a l'escalfament i l'augment de CO_2 només estan parcialment lligades a canvis en l'anatomia del xilema. Algunes modificacions de la relació creixement-anatomia podrien ser resultat d'una aclimatació al clima fred del límit supraforestal, cosa que podria ser especialment beneficiosa per espècies poc resistents a les gelades com *V. myrtillus*.

Abstract

Plant growth responses to specific environmental conditions may have an underlying mechanistic basis in adjustments of the xylem anatomy. We investigated the above-ground growth and xylem anatomy of the dwarf shrub *Vaccinium myrtillus* growing in the understory of *Larix decidua* and *Pinus uncinata* at a treeline experimental site in the Swiss Alps after 9 years of free-air CO_2 enrichment (+ 200 ppm) and 6 years of soil warming (+ 4 ºC). Our aims were to analyze relationships between growth and xylem anatomy and to determine whether these relationships would be maintained under the two treatments. We measured growth parameters and captured high-resolution images of xylem anatomical microsections from the stem base and quantified functionally relevant anatomical characteristics of both vessels and ray parenchyma, which perform water transport and carbon storage functions, respectively. We found strong correlations between accumulated hydraulic conductivity (Kh) and growth parameters across all treatments. However, warming modified the relationship between growth and anatomy, as it increased shoot elongation and xylem ring width (RW) and area (RA) but reduced hydraulic efficiency by means of reduced vessel size (Dh), xylem-specific hydraulic conductivity (Ks) and accumulated hydraulic conductivity (Kh). Elevated CO_2 caused fewer growth and anatomical responses. It increased stem basal area and absolute ray density and led to lagged increased Dh and Ks. These results suggest that there is a link between growth and hydraulic conductivity, but that growth responses to warming and CO_2 are only partly accompanied by xylem anatomical changes. Some modifications of the growth-anatomy relationship may show acclimation to the cold climate at treeline, which would be particularly advantageous for a frost sensitive species such as *V. myrtillus*.

Keywords: Alps, CO_2 enrichment, dwarf shrub, soil warming, treeline, *V. myrtillus*, xylem anatomy

Introduction

Global atmospheric CO_2 concentrations and air temperature are predicted to increase in the next decades. Climate models forecast a terrestrial air temperature increase of 1.2-4.8 ºC by the end of the 21st century (IPCC 2014), especially at high-elevation and high-latitude ecosystems (Collins et al. 2013; Rangwala et al. 2013; Mountain Research Initiative EDW Working Group 2015). On the one hand, these ecosystems are particularly sensitive to global warming because they experience low temperatures during the growing season, with frequent freezing events, and long snow cover durations. On the other hand, high-elevation ecosystems may be particularly responsive to increasing CO_2 concentrations due to the lower CO_2 partial pressure associated with the reduction of the atmospheric pressure with altitude (Körner 2003). Indeed, some studies at high-elevation sites have shown evidence of plant growth stimulation under CO_2 enrichment (Dawes et al. 2011a, 2015), although other studies have not found such positive growth responses (Körner et al. 1997; Hättenschwiler and Körner 1998; Inauen et al. 2012).

Dwarf shrubs are an important component of many alpine and Arctic ecosystems, where they cover extensive areas. At the alpine treeline, warmer temperatures and the abandonment of extensive grazing and agricultural practices have led to a re-colonization of pastures by shrub species (Rundqvist et al. 2011; Ropars and Bordreau 2012). This shrub expansion can cause drastic changes in the ecosystem C balance, with potential feedbacks to climate warming (Myers-Smith et al 2011; D'Odorico et al 2013). Therefore, an in-depth understanding of the ecology and physiology of alpine shrubs and how these species respond to global change is essential for predicting future community and ecosystem changes.

Plant physiological responses and structural adjustments are key to cope with short and long-term environmental variability. One of the most important xylem physiological processes is axial water transport which, in angiosperms, is carried out by a network of dead lignified tubular cells, i.e. vessels. Plants optimize water transport efficiency and safety by regulating the structural characteristics of the water conducting system (Hacke and Sperry 2001). According to the Hagen-Poiseuille law, the hydraulic efficiency increases with vessel lumen diameter to the fourth power (Tyree and Ewers 1991). However, the wider the vessels, the higher the risk of water transport failures due to frost-induced cavitation (Hacke et al. 2006). This phenomenon takes place during freeze-thaw events when air bubbles are gassed out as xylem sap freezes, interrupting water transport in the concerned vessels. In this situation, a high connectivity or grouping among vessels might provide alternative pathways to bypass cavitated vessels (Tyree et al. 1994, von Arx et al. 2013), or debatably enhance the risk of cavitation spreading from one vessel to the next by the aspiration of air through the pit pores (Sperry & Tyree 1988; Loepfe et al. 2007). Other important xylem functions, i.e. storage and radial transport of water, nutrients and carbon, are performed by ray parenchyma tissue (Plavcová and Jansen 2015). In fact, ray and axial parenchyma represent the greatest stores of non-structural carbohydrates (NSC) in wood. Therefore, variations in ray parenchyma tissues might reflect variations in the amount of NSC in the plant (von Arx et al. 2012). Despite its important physiological function, ray parenchyma has been underrepresented in studies on the relationship between wood anatomy and the environment (but see Lev-Yadun 1998; Olano et al. 2013; Fonti et al. 2015; von Arx et al. 2015). Quantitative assessments of the structures responsible for all these functions may provide knowledge on plant conductive and storage capacity, and allow retrospectively reconstructing plant structural and functional responses to environmental variability (Fonti et al. 2010; Fonti and Jansen 2012; Schweingruber et al. 2013; von Arx et al. 2016).

Many studies have focused on responses of alpine and Arctic shrubs to warming (mainly above-ground growth) and have shown different results depending on the study site and species (e.g., Kudo and Suzuki 2003; Klanderud 2008; Campioli et al. 2013). Contrarily, CO_2 enrichment experiments in high-elevation and high-latitude ecosystems are scarce (Tissue and Oechel 1987; Gwynn-Jones et al. 1997; Körner et al. 1997; Inauen et al. 2012; Dawes et al. 2015). Few of these works have used quantitative wood anatomy approaches (but see Gorsuch and Oberbauer 2002). In fact, most of the existing warming and CO_2 enrichment studies on xylem anatomy are based on trees (mostly tree seedlings or saplings; but see Gorsuch and Oberbauer 2002 and Rico et al. 2013). Some of these studies showed that warming induced positive responses on the size of water conducting cells (Maherali and DeLucia 2000; Gorsuch and Oberbauer 2002; Petit et al. 2011; McCulloh et al. 2016). However, other studies showed the opposite response (Thomas et al. 2004; Kilpeläinen et al. 2007). Regarding CO_2

enrichment, some studies showed that the increase in the atmospheric CO_2 concentrations induced wider vessel lumina (Atkinson and Taylor 1996; Kilpeläinen et al. 2007; Kostiainen et al. 2014), while others showed no response (Maherali and DeLucia 2000; Rico et al. 2013; Watanabe et al. 2016) or even a negative response (Overdieck et al. 2007). Of all these studies, only those by Overdieck et al. (2007) and Kilpeläinen et al. (2007) focused on ray parenchyma: the former found no response of ray parenchyma to the CO_2 enrichment, whereas the latter found a decrease in the number of rays. However, some FACE experiments have found an increase in NSC leaf content under enhanced CO_2 (Handa et al. 2005; Bader et al. 2013), which may increase the demand for more storage tissue. Many questions clearly remain unanswered regarding plant anatomical responses to warming and CO_2 enrichment, especially for tundra shrubs. Furthermore, the scarcity of studies combining both CO_2 and warming treatments highlights the wide area still remaining for exploration (but see Maherali and DeLucia 2000; Ziche and Overdieck 2004; Kilpeläinen et al. 2007; Dieleman et al. 2012).

Here, we studied wood anatomical responses to a combined 9-year-long CO_2 enrichment and 6-year-long soil warming experiment at the Stillberg treeline research site in the Central Swiss Alps. Previous studies found that the dwarf shrub *Vaccinium myrtillus* benefited from both treatments, showing increased shoot elongation, above-ground biomass and xylem growth-ring widths (RW) (Dawes et al. 2011a, 2015; Anadon-Rosell et al. 2014). In this study, we aimed at unraveling the underlying mechanism of these responses by investigating the adjustments at the wood anatomical level. From a mechanistic point of view, such a link should be expected because xylem anatomical structures define key functions for plant survival and performance (Hacke and Sperry 2001; Plavcová and Jansen 2015) and small changes in xylem anatomical characteristics (e.g. in the lumen diameter of vessels) can considerably improve xylem functioning, providing a better plant performance and a potential advantage over competitors. Specifically, we hypothesized that (i) growth and xylem anatomical parameters would be strongly correlated and that the enhanced growth was induced by (ii) an improved hydraulic system and (iii) an increased storage capacity under the release of limiting conditions.

Materials and Methods

Study site and experimental design

This study makes use of plant material collected at the end of a long-term CO_2-enrichment (2001-2009) and soil warming experiment (2007-2012) (Dawes et al. 2015). The experimental site was located at Stillberg, Davos, in the Central Alps, Switzerland (9º 52' E, 46º 46' N). The site lies within a 5-ha long-term afforestation area where tree seedlings were planted into the intact dwarf shrub community in 1975 (Barbeito et al. 2012). The CO_2 enrichment and soil warming experiment was located on a NE-exposed 25-30º slope at 2180 m a.s.l., slightly above the present treeline, and covered an area of 2500 m^2. From 1975 to 2012, the mean air temperature and mean precipitation during the main growing season months (June-August) were 9.2 ºC and 444 mm, respectively (data from a nearby WSL climate station located at 2090 m a.s.l.). See detailed climate information for the study period 2001-2012 in Table S1. Soil types are sandy Ranker and Podzols (Lithic Cryumbrepts and Typic Cryorthods), derived from siliceous paragneis parent material and have a 5–20-cm-thick organic layer (Hagedorn et al. 2010).

In 2001, a free-air CO_2 enrichment (FACE) experiment was started (Hättenschwiler et al. 2002). A total of 40 hexagonal 1.1 m^2 plots were created, 20 with one *Pinus mugo* ssp. *uncinata* (DC.) Domin individual in the center and 20 with one *Larix decidua* Mill. individual in the center. The trees were sparsely distributed, not forming a closed canopy, and therefore each plot consisted of a single tree surrounded by a dense cover of understory vegetation dominated by the dwarf shrub species *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* agg.) and *Empetrum nigrum* L. subsp. *hermaphroditum* (Hagerup) Böcher. The plots were assigned to 10 groups of four neighboring plots, half of which were randomly assigned to an elevated CO_2 treatment (+ 200 ppm) and half of which did not receive additional CO_2. The system released pure CO_2 gas through laser-punched drip irrigation tubes hung vertically around a hexagonal frame surrounding each plot. Enrichment was supplied during daytime hours throughout the snow-free seasons from 2001 to 2009. For more details on the FACE setup and performance see Hättenschwiler et al. (2002) and Dawes et al. (2011b).

In spring 2007, a soil warming treatment was added to the experiment, and it was applied during each snow-free season until August 2012. Within each of the 10 CO_2 treatment groups, one plot of each tree species was randomly assigned to a soil warming treatment, yielding a balanced split-split plot design with a replication of five individual plots for each combination of CO_2 concentration, soil warming and plot tree species. The soil warming treatment was applied using 420-W heating cables laid on the ground surface with a distance of 5 cm between neighboring cables (see Hagedorn et al. 2010 for more details). Soil warming increased the growing season mean soil temperature at 5 cm depth by 3.1 to 4.4 ºC over the six seasons of heating (Dawes et al. 2015). Air temperature within the dwarf shrub canopy was also increased by 0.9 ºC at 20 cm above ground (Hagedorn et al. 2010). The warming treatment had a slight drying effect on the soil organic layer during the first three years of treatment but the soil matric water potential at 5 cm depth was always above -300 hPa in all plots, indicating high soil moisture throughout all the study years (Dawes et al. 2015).

Plant sampling and sample preparation

During early August 2012, we harvested five *V. myrtillus* ramets per plot, clipping them at ground level. We made cross-sections of 20 µm thickness from the basal 1.5 cm of each ramet using a sledge microtome (WSL Lab-microtome) and stained the sections with a mixture of safranin and astrablue. We dehydrated the sections for preservation by rinsing them with ethanol solutions of increasing concentration (75%, 96%, 100%), then the sections were immersed in xylol, imbedded in Canada-Balsam and dried at 60 ºC for 24 hours (Gärtner & Schweingruber 2013). More details on plant harvesting, above-ground biomass and shoot elongation measurements, and sample preparation are given in Anadon-Rosell et al. (2014). For the quantification of wood anatomical parameters, we selected the oldest available ramet per plot and followed the protocol detailed in von Arx et al. (2016). In short, we photographed the sections at ×40 magnification through a microscope with a digital camera (Canon EOS 650D mounted on an Olympus BX41 microscope featuring distortion-free lenses), took several overlapping photographs per cross-section and stitched them with the software PTGui (New House Internet Services BV, Rotterdam, NL) to obtain high-resolution images (0.94 pixels/µm) of the entire cross-sections (Fig. 1). We used the software ROXAS (von Arx et al. 2013; Wegner et al. 2013) to quantify the vessel lumen area and spatial arrangement, as well as ring area and width. Only vessel lumina of > 50 µm^2 were considered, to avoid confounding the smallest vessels with fiber cells. The borders of the xylem growth rings were manually drawn, accounting for the missing rings based on a cross-dating procedure previously performed on the same samples (Anadon-Rosell et al. 2014).

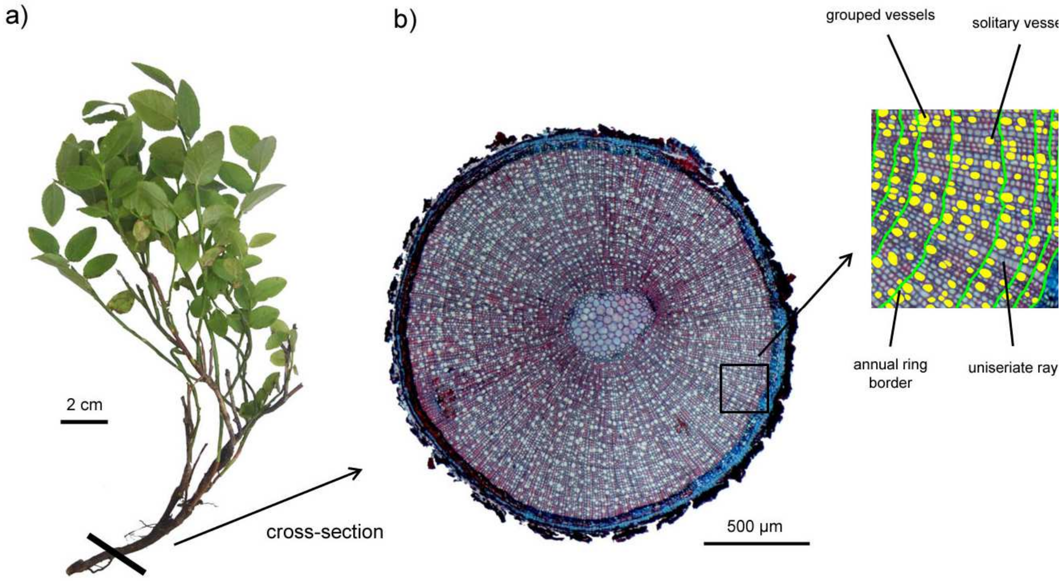

Figure 1. (a) *Vaccinium myrtillus* ramet; (b) cross-section cut at ground level and stained with safranin and astrablue. Details on wood anatomical parameters are shown at the right side.

Quantification of anatomical traits

For each annual ring, we quantified xylem anatomical parameters related to hydraulic efficiency, spatial vessel distribution, storage and radial transport of water, nutrients and carbon, and growth (Table 1). As proxies for hydraulic efficiency, we considered mean hydraulic diameter (Dh; Kolb and Sperry 1999), vessel lumen size at the 95th percentile (CA 95%, i.e. the widest and thus most conductive vessels), accumulated hydraulic conductivity (Kh) and xylem-specific hydraulic conductivity (Ks). The spatial vessel distribution was expressed by vessel density (CD), vessel grouping index (V_G), mean group size of grouped cells (V_M) and vessel solitary fraction (V_S) (von Arx et al. 2013). Storage and radial transport capacity were assessed by ray density and ray seriality, i.e. the number of parallel cell lines per ray (Carlquist 2001). We hypothesized multiseriate rays to be relatively more important for storage than uniseriate rays. Both ray metrics were assessed in the outmost ring (formed in 2012). Ray density was quantified as the number of rays per millimeter of circumference in the stem cross-section. Ray seriality was obtained by counting the number of parallel cell lines forming each ray. Finally, we used the number of vessels (CNo), mean ring width (RW) and ring area (RA) as parameters related to growth.

Table 1. Variables included in the study with their explanation and units.

Abbreviation	Units	Explanation
CA95%	µm^2	Vessel lumen size at 95th percentile
CD	no./mm^2	Vessel density
CNo		Number of vessels
Dh	µm	Mean hydraulic diameter
Kh *	kg MPa^{-1} s^{-1}	Accumulated hydraulic conductivity
Ks *	kg m^{-2} MPa^{-1} s^{-1}	Xylem-specific hydraulic conductivity (Kh/RA)
RW	µm	Ring width
RA	mm^2	Ring area
V_G		Vessel grouping index
V_M		Mean group size of grouped vessels
V_S		Vessel solitary fraction

* based on Hagen-Poiseuille law (Tyree & Ewers 1991)

Statistical analysis

We assessed the relationship among the wood anatomical parameters and checked the coherence within groups of parameters with similar functions using Pearson's correlations. We also tested for correlations between these parameters and biomass and shoot elongation measurements made in 2012 (see Anadon-Rosell et al. 2014). We further explored the relationship between Kh and above-ground growth to determine how many rings were involved in the hydraulic conductivity of *V. myrtillus*, i.e. the sapwood area.

To evaluate the effects of the treatments on the wood anatomical parameters we used linear mixed-effects models fitted with the restricted maximum likelihood estimation method (REML). We fitted two separate models for the two key periods of the experiment, one for the CO_2-treatment period (2001-2009) and one for the warming-treatment period (2007-2012), which also included the three years when the two treatments overlapped. The CO_2-treatment period model included CO_2, plot tree species (larch or pine), study year and their interactions as fixed effects. The warming-treatment period additionally included warming and their interactions as fixed effects. Both models included cambial age as a covariate when it was significant. For the analysis of the treatment effects on ray density, we only included CO_2, warming and plot tree species as fixed factors. We tested 3- and 4-way interactions with likelihood ratio tests and excluded the terms when they did not contribute significantly to the model fit. The random effects structure reflected the hierarchy of the split-split plot design, with plot (i.e. one ramet) nested within soil warming treatment group, nested within CO_2 treatment group. We applied a residual auto-correlation structure, corAR1 (Pinheiro et al. 2016), to account for violation of independence of residuals from measurements made on the same ramet in different years. The year 2012 was excluded from the analyses of xylem ring widths, ring area and number of vessels because ring growth was not completely finished at the sampling time. For all the analyses, we visually checked the assumptions of normality and homoscedasticity of residuals and we log- or square root-transformed response variables when necessary to meet these assumptions. In addition, we used the varIdent structure (Zuur et al. 2009) when the residual variance differed between levels of treatments. Finally, we analyzed the effects of CO_2, warming and plot tree species on ray seriality with the non-parametric Kolmogorov-Smirnov test. We considered effects significant at $P < 0.05$ and marginally significant at $0.05 \leq P < 0.1$ to account for the relatively low replication of the study. We performed all the analyses with R 3.1.2 (R Core Team, 2015). For linear mixed effects models we used the nlme package (Pinheiro et al. 2016).

Results

Correlations among anatomical parameters and their link to growth

Results of the correlation analyses confirmed our grouping of anatomical parameters with similar functions (Fig. S1). Amongst parameters related to hydraulic efficiency, Dh and CA95% were strongly positively correlated (r = 0.99), which is why we excluded CA95% from subsequent analyses. The link between Kh and Ks was comparably weak (r = 0.43), showing a certain independence between area-based (Ks) and absolute (Kh) conductivity. The correlations between parameters describing spatial vessel distribution ranged between |*r*| = 0.45 and 0.96, with CD showing a comparably weak association with the three grouping variables (|*r*| ≤ 0.52). Growth-related parameters (CNo, RW, RA) were correlated between each other with *r* ≥ 0.74. Across functional groups, the hydraulic-efficiency parameters were positively linked to vessel grouping (V_G, *r* ≥0.30) and negatively to the proportion of solitary vessels (V_S, *r* ≤ -0.62).

We did not find a correlation between the great majority of the wood anatomical parameters (measured at the outermost ring) and above-ground biomass or shoot elongation, except for Kh (values accumulated until ring 12, see next paragraph), which was significantly correlated with all the biomass parameters and with shoot elongation (Fig. 2a, Table S2). Moreover, shoot elongation was positively correlated with RA, RW and CNo. All the biomass parameters and shoot elongation were positively correlated

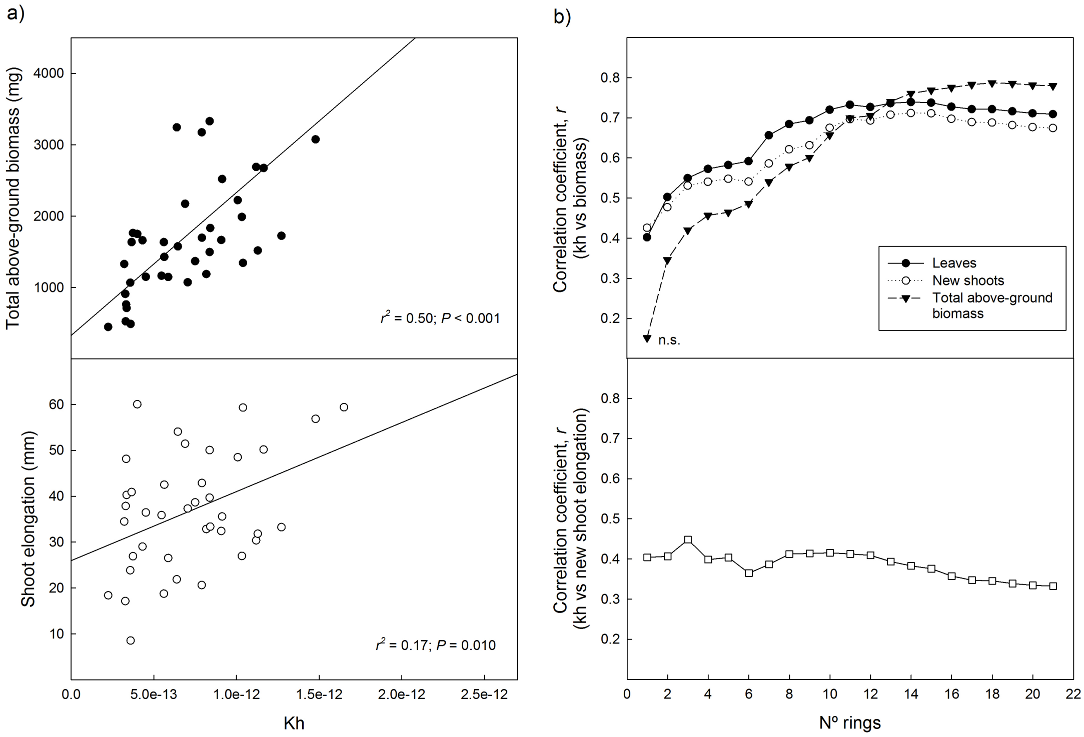

Figure 2. (a) Relationship between total above-ground biomass and Kh (top), and shoot elongation and Kh (bottom) in 2012. Coefficients of determination (r^2) and error probabilities of the regression slope (*P* values) are shown. (b) Variation in the correlation coefficient (*r*) between Kh and above-ground biomass (top) and shoot elongation (bottom) with increasing number of rings considered in Kh measurements, from the outermost ring (left, formed in 2012) to the innermost (right). All the correlations between biomass and shoot elongation and Kh were significant at $P < 0.05$ except for the above-ground biomass in the first ring, indicated by n.s.

with the cross-section area (accumulated RA) and diameter (accumulated RW) (Table S2).

Our analyses on the relationship between Kh and above-ground growth showed increasing values of the correlation coefficient between Kh and biomass until *c.* ring 12 (counted from the outermost ring inwards), when the correlations stopped increasing, indicating that rings closer to the pith than the 12 outermost rings did not additionally contribute to the conductivity (Fig. 2b). Correlations with shoot elongation were more variable, and the highest *r* values were found when considering the three youngest rings only, probably indicating that shoot elongation relies on the outermost part of the xylem.

Xylem anatomical and growth responses to CO_2 enrichment

Responses of the considered functional xylem anatomical traits to CO_2 enrichment were either rather small, lagged, or in interaction with other factors. Within the group of hydraulic efficiency traits, Dh responded positively to CO_2 enrichment during the last years of CO_2-treatment period (2001-2009), especially when *V. myrtillus* was growing under pine (significant CO_2 × tree × year interaction), and marginally significantly during the warming-treatment period (2007-2012; Table 2, Fig. 3. See raw means in Table S3). Ks showed a similar pattern as Dh, with 12% higher values under elevated than under ambient CO_2 in the last years of the CO_2 experiment (2006-2009; significant CO_2 × year interaction), especially under pine (significant CO_2 × tree × year interaction; Table 2, 3). Kh did not respond to CO_2 enrichment. Parameters characterizing the spatial vessel distribution did not show any response to CO_2 enrichment either, except for a marginally significantly increased V_G during the warming-treatment period (Table 2, 3). The analyses of storage and radial transport capacity showed that ray absolute density was higher under enhanced CO_2 (207.8 ± 10.3 rays/cross-section) than under ambient CO_2 (170.4 ± 11.3 rays/cross-section) ($F_{1,8} = 7.10$, $P = 0.029$). However, these differences were linked to stem diameter, which was also higher under enhanced CO_2 (7375.86 ± 300.01 µm *vs.* 6129.14 ± 419.95 µm; $F_{1,8} = 10.08$, $P = 0.013$). Thus, ray density did not differ between treatments ($F_{1,8} = 0.07$, $P = 0.805$). Ray seriality was also unaffected by CO_2 enrichment (Fig. 3). Among the growth-related parameters, CNo showed slightly higher values under enriched than under ambient CO_2 in both study periods (marginally significant differences between CO_2 treatments), whereas RW and RA did not differ significantly between CO_2 treatments for any of the treatment periods (Table 2, 3, Fig. S3). We did not find significant differences in new shoot elongation, nor in leaf, new shoot or total above-ground biomass (measured in 2012) between CO_2 treatments.

Xylem anatomical and growth responses to soil warming

Soil warming had generally larger effects on the anatomical traits than CO_2 enrichment. Among the traits related to hydraulic efficiency, Dh decreased with warming, especially in the first years of the experiment (2007-2009) when Dh of ramets in warmed plots was, on average, 14% smaller than that of ramets in unwarmed plots (significant warming and warming × year effects) (Table 2, Fig. 3. See raw means in Table S3). Similarly, Ks decreased under warming in the first years (significant warming × year interaction). This warming effect was stronger in CO_2 enriched plots, where Ks decreased by 38% during the period 2007-2009, than under ambient CO_2 conditions, where it decreased only by 15% during the same period (significant warming × CO2 × year interaction; Table 2, 3). Kh showed a significant warming × year interaction with an initial decrease and subsequent increase compared to unwarmed conditions. As was the case for CO_2 enrichment, parameters related to the spatial distribution of vessels were less responsive to warming, with only V_G decreasing and V_S increasing in the first treatment years (significant warming × year interaction; Table 2, 3). Regarding storage and radial transport traits, warming did not influence absolute ($F_{1,8}$ = 0.12, P = 0.743) or per unit distance ($F_{1,8}$ = 0.12, P = 0.740) ray density, but reduced the proportion of biseriate rays (3.8% *vs.* 4.7% of the total rays) (D = 0.45, P = 0.035; Fig. 4). The growth-related parameters all responded similarly to warming, with a decrease in the first and a relative increase in the last treatment years compared to unwarmed conditions (significant warming × year interaction; Table 2). At the end of the experiment (2012), there was a significant effect of warming on *V. myrtillus* shoot elongation across tree species and CO_2 treatments. Shoots were 22% longer in warmed plots than in control plots ($F_{1,9}$ = 5.53, P = 0.043, Fig. S2). In contrast, we did not find significant differences in leaf, new shoot or total above-ground biomass between warming treatments.

Cambial age, calendar year and influence of tree species on anatomical traits

The functional anatomical traits showed, with few exceptions, very strong significant trends with cambial age, i.e. ring number counted from the pith (Table 2). Traits related to hydraulic efficiency and growth were also often influenced by conditions in specific years (Table 2). However, the relatively small number of study years did not allow a more detailed analysis of potential effects of climate variability in specific years.

The tree species present in the plot played an important role on *V. myrtillus* anatomy and its response to the treatments. Dh was 7% and Ks 22% larger in *V. myrtillus* ramets growing under pine than under larch when pooled across CO_2 treatments and years. We found a similar pattern for the warming-treatment years, with a 9% (Dh) and 29% (Ks) increase (Table 2, Fig. 3. See raw means in Table S3). In contrast, secondary growth (RW and RA) was marginally significantly reduced under pine compared to larch (Table 2, 3, Fig. S3). Tree species did not have a significant effect on ray absolute density ($F_{1,15} = 1.51$, $P = 0.237$), ray density per unit distance ($F_{1,15} = 1.72$, $P = 0.209$) or ray seriality ($P > 0.832$), and it did not affect spatial vessel distribution parameters either (Table 2, 3).

Table 2. Results of the ANOVA test of the effects of CO_2 enrichment, soil warming, plot tree species and treatment year on the studied wood anatomical parameters of *V. myrtillus*. Three- and four-way interactions only shown when included in the model.

	Dh			**Kh**			**Ks**			**CD**			**V_G**		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
a) CO_2 years															
Cambial age	1, 201	110.42	<**0.001**	1, 209	41.93	<**0.001**	1, 195	161.90	<**0.001**	1, 209	5.80	0.017	1, 208	108.90	<**0.001**
CO_2	1, 8	1.82	0.215	1, 8	1.40	0.270	1, 8	2.89	0.128	1, 8	0.08	0.789	1, 8	2.50	0.153
Tree	1, 28	7.74	**0.010**	1, 28	1.32	0.261	1, 27	12.64	**0.001**	1, 28	1.07	0.311	1, 28	0.76	0.392
Year	8, 201	3.06	**0.003**	8, 209	5.96	<**0.001**	8, 195	2.15	**0.033**	8, 209	1.91	0.061	8, 208	1.10	0.368
CO_2:Tree	1, 28	0.42	0.523	1, 28	0.05	0.823	1, 27	2.53	0.124	1, 28	1.01	0.323	1, 28	0.00	0.990
CO_2:Year	8, 201	1.21	0.296	8, 209	0.54	0.825	8, 195	2.12	**0.036**	8, 209	0.82	0.588	8, 208	0.93	0.497
Tree:Year	8, 201	0.72	0.673	8, 209	2.02	**0.046**	8, 195	0.60	0.774	8, 209	0.46	0.883	8, 208	0.23	0.986
CO2:Tree:Year	8, 201	1.89	**0.064**	-	-	-	8, 195	2.80	**0.006**	-	-	-	-	-	-
b) Warming years															
Cambial age	1, 167	30.82	<**0.001**	1, 167	4.81	**0.030**	1, 152	59.82	<**0.001**	-	-	-	1, 166	40.01	<**0.001**
CO2	1, 8	5.17	**0.053**	1, 8	1.84	0.212	1, 8	2.94	0.125	1, 8	0.15	0.712	1, 8	3.51	**0.098**
Warming	1, 8	9.56	**0.015**	1, 8	0.01	0.906	1, 8	2.35	0.164	1, 8	0.86	0.380	1, 8	0.93	0.363
Tree	1, 17	11.19	**0.004**	1, 17	0.59	0.453	1, 15	11.51	**0.004**	1, 17	0.55	0.467	1, 17	2.01	0.175
Year	5, 167	7.56	<**0.001**	5, 167	4.93	**0.000**	5, 152	2.39	**0.041**	5, 168	4.02	**0.002**	5, 166	0.60	0.700
CO2:Warming	1, 8	0.00	0.960	1, 8	0.41	0.541	1, 8	0.01	0.940	1, 8	0.90	0.371	1, 8	1.03	0.341
CO2:Tree	1, 17	1.08	0.313	1, 17	0.91	0.353	1, 15	2.68	0.122	1, 17	1.56	0.229	1, 17	0.48	0.498
CO2:Year	5, 167	0.52	0.759	5, 167	0.67	0.646	5, 152	0.76	0.581	5, 168	0.32	0.901	5, 166	0.51	0.769
Warming:Tree	1, 17	1.64	0.218	1, 17	0.83	0.376	1, 15	0.38	0.549	1, 17	2.28	0.149	1, 17	0.02	0.900
Warming:Year	5, 167	3.91	**0.002**	5, 167	4.12	**0.002**	5, 152	2.51	**0.032**	5, 168	1.57	0.172	5, 166	2.91	**0.015**
Tree:Year	5, 167	0.83	0.532	5, 167	0.53	0.751	5, 152	0.53	0.753	5, 168	0.92	0.467	5, 166	0.56	0.729
CO2:Warming:Year	-	-	-	-	-	-	5, 152	2.33	**0.045**	-	-	-	-	-	-
CO2:Warming:Tree	-	-	-	-	-	-	1, 15	0.41	0.533	-	-	-	-	-	-
CO2:Tree:Year	-	-	-	-	-	-	5, 152	0.64	0.673	-	-	-	-	-	-
Warming:Tree:Year	-	-	-	-	-	-	5, 152	0.25	0.939	-	-	-	-	-	-

	V_S			**V_M**			**CNo**			**RW**			**RA**		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
a) CO2 years															
Cambial age	1, 208	102.74	**<0.001**	1, 209	56.839	**<0.001**	-	-	-	1, 211	13.73	**0.000**	1, 183	5.14	**0.025**
CO2	1, 8	2.31	0.167	1, 8	0.87	0.378	1, 8	3.66	**0.092**	1, 8	0.07	0.802	1, 8	0.50	0.499
Tree	1, 28	0.90	0.352	1, 28	0.07	0.799	1, 28	0.01	0.914	1, 28	1.99	0.170	1, 28	2.11	0.157
Year	8, 208	1.23	0.284	8, 209	0.47	0.874	8, 182	5.38	**<0.001**	8, 211	5.97	**<0.001**	8, 183	5.18	**<0.001**
CO2:Tree	1, 28	0.00	0.970	1, 28	0.01	0.921	1, 28	0.16	0.689	1, 28	0.02	0.886	1, 28	0.00	0.944
CO2:Year	8, 208	0.94	0.488	8, 209	1.07	0.389	8, 182	1.00	0.440	8, 211	0.69	0.698	8, 183	1.03	0.413
Tree:Year	8, 208	0.25	0.980	8, 209	0.64	0.746	8, 182	0.77	0.630	8, 211	1.97	**0.052**	8, 183	0.79	0.611
CO2:Tree:Year	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b) Warming years															
Cambial age	1, 166	27.6	**<0.001**	1, 167	35.35	**<0.001**	-	-	-	1, 125	6.18	**0.014**	-	-	-
CO2	1, 8	3.28	0.108	1, 8	2.47	0.155	1, 8	4.32	**0.071**	1, 8	0.06	0.817	1, 8	0.35	0.569
Warming	1, 8	1.32	0.284	1, 8	0.05	0.837	1, 8	0.40	0.543	1, 8	1.09	0.328	1, 8	0.03	0.874
Tree	1, 17	2.19	0.157	1, 17	0.68	0.422	1, 17	0.23	0.637	1, 16	4.50	**0.050**	1, 17	3.06	**0.098**
Year	5, 166	0.65	0.663	5, 167	0.63	0.678	4, 134	3.23	**0.015**	4, 125	3.53	**0.009**	4, 138	5.26	**0.001**
CO2:Warming	1, 8	1.09	0.328	1, 8	0.91	0.368	1, 8	1.75	0.222	1, 8	1.18	0.310	1, 8	0.56	0.475
CO2:Tree	1, 17	0.64	0.434	1, 17	0.29	0.597	1, 17	0.00	0.958	1, 16	0.16	0.695	1, 17	0.13	0.728
CO2:Year	5, 166	0.4	0.846	5, 167	0.69	0.635	4, 134	0.49	0.741	4, 125	0.39	0.813	4, 138	0.34	0.852
Warming:Tree	1, 17	0.05	0.820	1, 17	0	0.948	1, 17	0.23	0.640	1, 16	0.36	0.559	1, 17	0.94	0.346
Warming:Year	5, 166	2.52	**0.032**	5, 167	1.66	0.146	4, 134	3.19	**0.015**	4, 125	3.27	**0.014**	4, 138	3.19	**0.015**
Tree:Year	5, 166	0.66	0.658	5, 167	0.34	0.885	4, 134	0.39	0.818	4, 125	0.35	0.845	4, 138	0.27	0.897
CO2:Warming:Year	-	-	-	-	-	-	-	-	-	4, 125	1.29	0.276	-	-	-
CO2:Warming:Tree	-	-	-	-	-	-	-	-	-	1, 16	0.12	0.733	-	-	-
CO2:Tree:Year	-	-	-	-	-	-	-	-	-	4, 125	1.29	0.279	-	-	-
Warming:Tree:Year	-	-	-	-	-	-	-	-	-	4, 125	1.62	0.173	-	-	-

Table 2 (cont.)

Table 3. Mean values (± 1 SE) of the wood anatomical parameters studied for the CO_2-treatment years 2001-2009 (a) and the warming-treatment years 2007-2012 (b). Mean values of original data are given for each combination of CO_2 treatment (ambient or enhanced) and plot tree species (larch or pine) across the study years during the CO_2-treatment years (n = 10) and for each combination of CO_2 treatment, soil warming treatment and plot tree species across the study years during the warming-treatment years (n = 5). For Dh, more detailed data is provided in Fig. 3 and Table S3.

a) CO_2-treatment years

CO_2 treatment	Tree species	Kh (kg MPa^{-1} s^{-1})		Ks (kg m^{-2} MPa^{-1} s^{-1})		CD (no./mm^2)		V_G		V_S		V_M		CNo		RW (µm)		RA (mm^2)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ambient	Larch	4.51E-14	5.35E-15	1.17E-07	3.92E-09	1009.90	21.54	1.35	0.02	56.71	1.24	2.37	0.02	374.22	35.25	112.02	9.96	0.55	0.05
	Pine	5.57E-14	6.96E-15	1.27E-07	4.53E-09	1009.89	18.92	1.35	0.02	56.50	1.36	2.37	0.02	405.12	40.78	98.85	9.88	0.47	0.06
Enhanced	Larch	5.95E-14	7.39E-15	1.11E-07	5.51E-09	991.56	25.15	1.37	0.02	55.03	1.51	2.39	0.03	541.67	55.87	128.77	13.11	0.57	0.06
	Pine	7.34E-14	6.20E-15	1.54E-07	4.82E-09	1060.10	17.86	1.41	0.01	51.95	1.03	2.42	0.02	496.55	36.22	95.86	7.02	0.50	0.05

b) Warming-treatment years

Warming treatment	CO_2 treatment	Tree species	Kh (kg MPa^{-1} s^{-1})		Ks (kg m^{-2} MPa^{-1} s^{-1})		CD (no./mm^2)		V_G		V_S		V_M		CNo		RW (µm)		RA (mm^2)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Unwarmed	Ambient	Larch	5.46E-14	5.92E-15	1.35E-07	6.83E-09	1046.20	38.10	1.42	0.03	51.04	1.79	2.44	0.04	410.96	33.91	101.80	10.29	0.61	0.06
		Pine	8.54E-14	1.37E-14	1.65E-07	6.00E-09	984.46	32.42	1.46	0.02	47.96	1.30	2.49	0.04	484.52	70.36	100.60	21.29	0.66	0.12
	Enhanced	Larch	6.48E-14	9.18E-15	1.42E-07	5.49E-09	1021.94	32.98	1.44	0.02	49.40	1.45	2.48	0.04	459.19	52.99	86.04	9.48	0.57	0.07
		Pine	9.51E-14	8.58E-15	2.04E-07	6.35E-09	1024.86	23.70	1.50	0.02	45.55	1.06	2.56	0.03	485.48	42.76	68.96	6.59	0.54	0.04
Warmed																				
	Ambient	Larch	8.34E-14	1.55E-14	1.31E-07	4.96E-09	1042.38	29.93	1.38	0.02	54.32	1.45	2.45	0.03	573.54	98.37	115.96	15.27	0.91	0.13
		Pine	5.74E-14	1.02E-14	1.36E-07	5.72E-09	1070.38	26.74	1.36	0.02	55.71	1.76	2.40	0.03	370.00	52.51	78.29	8.03	0.47	0.06
	Enhanced	Larch	8.06E-14	1.19E-14	1.15E-07	6.20E-09	962.46	31.73	1.36	0.02	55.86	1.89	2.39	0.03	710.29	98.25	164.33	21.39	0.76	0.10
		Pine	1.03E-13	1.08E-14	1.70E-07	6.41E-09	1090.39	20.30	1.50	0.02	45.71	1.22	2.54	0.03	701.00	88.53	101.16	11.41	0.69	0.08

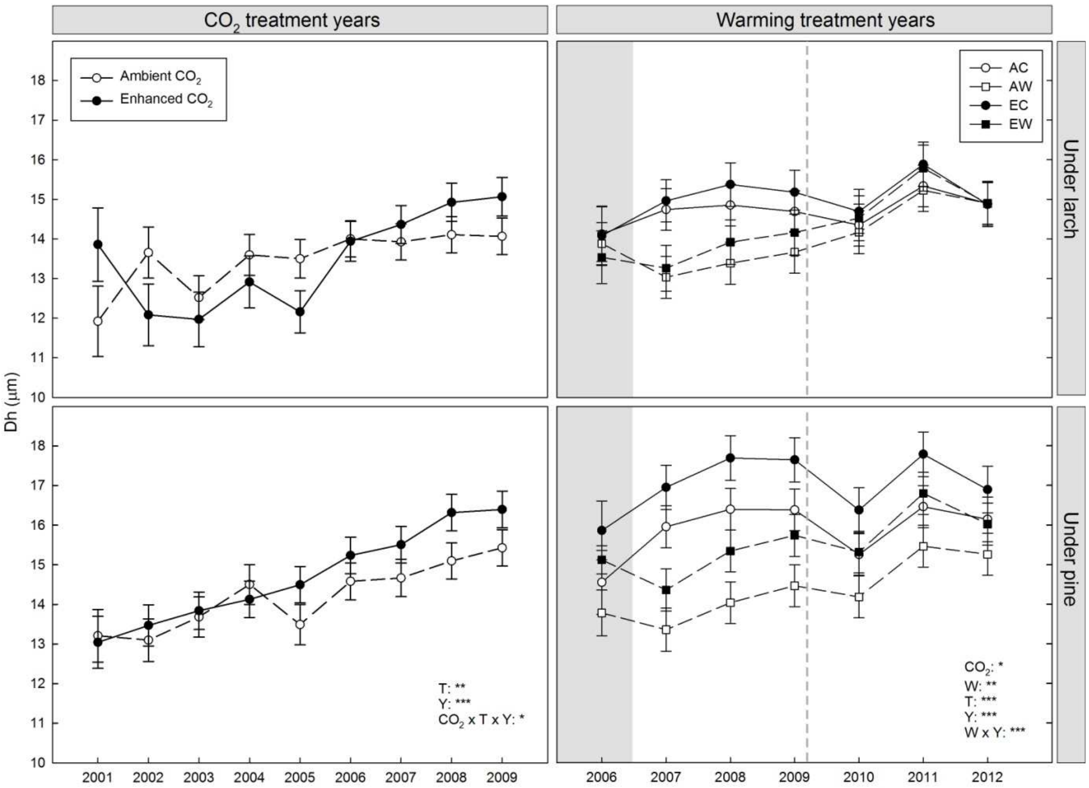

Figure 3. Variation of mean hydraulic diameter (Dh) through time during the CO_2-treatment years 2001-2009 (left panels) and during the warming-treatment years 2007-2012 (right panels) for each plot tree species (larch in top panels, pine in bottom panels). Mean values ± 1 SE are shown, estimated from statistical models for a ramet with an average cambial age, and thus reflect statistical analyses. For the CO_2-treatment years, the combination of each CO_2 treatment (Ambient and Enhanced) and plot tree species are shown (n = 10). For the warming-treatment years, each combination of CO_2 treatment (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species are shown (n = 5). Vertical dashed lines indicate the end of the CO_2 treatment. The shaded area indicates pre-warming values from 2006. Significant effects included CO_2 treatment (CO_2), warming treatment (W), plot tree species (T) and study year (Y) at $0.05 < P < 0.10$ (m.s., *), $0.01 < P < 0.05$ (**) and $P \leq 0.01$ (***), and are shown at the right bottom corner of the lower panels. See Table 3 for detailed statistical results.

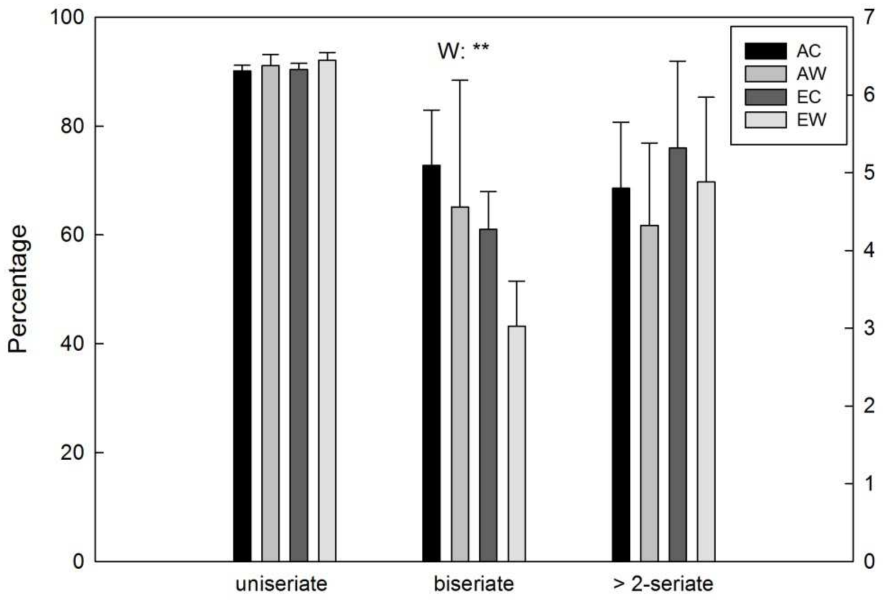

Figure 4. Percentage of uniseriate (lefthand y-axis) and biseriate and > 2-seriate (righthand y-axis) rays for the combination of CO_2 levels (A = ambient, E = enhanced) and soil warming treatments (C = unwarmed, W = warmed). Mean values ± 1 SE are shown. Measurements were taken in the last ring (year 2012). Asterisks (**) indicate significant effects between warming treatments (W) in the percentage of biseriate rays at $0.01 < P < 0.05$.

Discussion

The aim of this study was to investigate the relationship between growth and the xylem anatomical structure in the dwarf shrub *V. myrtillus* under soil warming and CO_2 enrichment, and to find out whether the stimulation of growth found in previous studies at our same experimental site under these treatments (Dawes et al. 2011a; Anadon-Rosell et al. 2014) were related to adjustments of the underlying xylem anatomical structure. The subset of *V. myrtillus* plants in the present study partly supported previous growth findings. Specifically, we found an increased shoot elongation and ring width and area under warming. We did not find ring width and area increases under CO_2 enrichment, but stem basal area increased, which is in line with the previously reported growth stimulation in this species under this treatment (Dawes et al. 2011a). New shoot elongation and biomass measurements in 2012 may not reflect real responses to the CO_2 enrichment, as this treatment was over three years before plant sampling.

Growth links to wood anatomy

Independent of the treatments, we observed a mechanistic link of anatomical features with plant growth through the strong correlation between the plant-level conductance (Kh) and aboveground growth. Kh was the only one from the several hydraulic-efficiency-related parameters to show this effect, which could be explained by the fact that the above-ground performance investigated here is a cumulative trait, particularly for biomass. The consideration of an increasing number of growth rings in the calculation of Kh significantly improved its relationship with above-ground growth, probably because it was closer to the true number of conducting sapwood rings. On the other hand, the relationship between the elongation of new shoots and Kh did not change when including more sapwood rings other than the outmost three, indicating that new shoots are mostly supplied with water by the outmost sapwood rings only. This would imply a more effective strategy for water transport, since crossing ring boundaries with smaller latewood vessels increases the pathway length resistance (Petit et al. 2016).

CO_2 enrichment has small effects on anatomy, growth and storage

An increase of atmospheric CO_2 concentration has been hypothesized to improve carbon assimilation, especially at high elevations where the CO_2 partial pressure is lower (Körner 2003). A stimulation of carbon assimilation does not necessarily translate into a growth enhancement due to a limited carbon sink (Handa et al. 2005), but evidence of plant growth increases under CO_2 enrichment have been reported (e.g. Dawes et al. 2011a, 2015). For this, a more efficient water transport may be required to supply the larger demand for water and nutrients, and a larger storage capacity for carbohydrates. Indeed, some studies have found positive effects of CO_2 enrichment on vessel size. Atkinson and Taylor (1996) reported an increase in vessel size of *Quercus robur* seedlings with higher CO_2 concentrations, and an experiment on aspen and birch seedlings found that radial growth and vessel size of aspen were increased with higher CO_2 concentrations throughout the experimental years (Kostiainen et al. 2014).

Conversely, an improved CO_2 supply may also reduce water loss during photosynthesis (Bunce 2004, Leuzinger and Körner 2007), which may allow the plants to invest more in hydraulic safety by forming narrower vessels. We only found a small, lagged positive response of anatomical features to the 9-year long CO_2 enrichment and the carbon storage capacity did not respond to elevated CO_2. However, we found an increase in basal stem diameter under CO_2 enrichment, indicative of growth stimulation. The lack of changes in *V. myrtillus* hydraulic system at the beginning of the experiment, in conjunction with the higher shoot increments seen under CO_2 in this species compared to ambient plots during the whole CO_2-treatment period (Dawes et al. 2011a), strongly suggest that there was no demand for a more efficient hydraulic system. One possible explanation would be an increased water use efficiency under higher CO_2 concentrations, as *V. myrtillus* could assimilate more carbon with lower water losses in relative terms. Another reason for the small CO_2-effect could be the high water availability at this treeline site, where frequent rainfalls and a thick organic layer with a large water storage capacity may reduce the need to alter xylem anatomical features to support growth enhancements. The lack of CO_2 effects on the ray parenchyma could be explained by the fact that cross-sections were made at the stem base. Belowground structures (i.e. roots and rhizomes) store large amounts of carbohydrates, especially in clonal tundra plant species (Billings and Mooney 1968), and we cannot exclude that there were ray responses in belowground tissues of *V. myrtillus*. In fact, Handa et al. (2008) showed increased starch contents in tree roots under enriched CO_2 at the same experimental site. In addition, some studies have shown that ray parenchyma may be unresponsive to environmental conditions (Overdieck et al. 2007; Fonti et al. 2015). In any case, CO_2 enrichment did not promote storage capacity in *V. myrtillus*.

Hydraulic efficiency vs. *hydraulic safety under warming*

In our experiment, *V. myrtillus* showed a reduced vessel size and Ks under warming, which contrasts with previous studies in cold regions that have reported positive effects of warming on vessel size of woody species. In a study near Toolik Lake, Alaska, Gorsuch and Oberbauer (2002) showed that the shrub *Salix pulchra* had larger vessel diameters and densities under an increase of 5 ºC inside growth chambers. Other warming experiments on tree species have also reported increases in conduit sizes with warming (Maherali and DeLucia 2000; Petit et al. 2011; McCulloh et al. 2016). Nevertheless, like in our study, Kilpeläinen et al. (2007) found narrower lumina in earlywood tracheids of Scots Pine (*Pinus sylvestris*) under warming, and argued that higher temperatures could have increased growth and cell division rates, which could explain the smaller lumen sizes. Thomas et al. (2004) reported similar effects of temperature on the vessels of *Eucalyptus camaldulensis* in a warming experiment in growth chambers. Moreover, they related their findings to a decreased viscosity of water with warming (Roderick and Berry 2001) and suggested that equal amounts of water could be transported by smaller vessel lumina. A plausible explanation for our results is a faster cell differentiation through an increase in auxin levels induced by warming (Franklin et al. 2011) that resulted in narrower vessels due to a reduced cell expansion phase (Aloni and Zimmermann 1983). Moreover, in a high-elevation site such as Stillberg, where early-season frosts are frequent (Rixen et al. 2012), decreasing vessel sizes would be advantageous, as it would reduce the risk of cavitation induced by freeze-thaw events (Tyree and Sperry 1989). This would be further advantageous considering that *V. myrtillus* has a low freezing resistance (Martin et al. 2010; Wheeler et al. 2014), and a faster phenological development under warming may enhance the risk of severe damage in leaf tissue (Martin et al. 2010). In line with this explanation, in the first warming year (2007), snow melted earlier in warmed plots and left plants without the insulating snow cover when a freezing event occurred (Rixen et al. 2012), which might have induced the formation of smaller vessels. The negative effects of warming, however, decreased with time, and were less pronounced after the first three years of warming. On the one hand, this could reflect a progressive recovery from the 2007 frost. On the other hand, since the increase in the above-ground growth did not stop after three years of warming (Anadon-Rosell et al. 2014), the counteracting need for supplying an increasing biomass might have explained the weakening negative response of *V. myrtillus* vessel size. Thus, our results might demonstrate that hydraulic safety is prioritized once a sufficiently efficient hydraulic system is built. The decrease in multiseriate rays with warming (although only significant for biseriate rays) could indicate that the release from limiting conditions promoted above-ground growth at the expense of storage (von Arx et al. 2012).

Effects of CO_2 enrichment and soil warming are mostly independent

We did not find significant CO_2 × warming interactions for most of our parameters, indicating that responses to CO_2 enrichment and soil warming were not influenced by each other. In addition, as the effects of the two treatments on *V. myrtillus* hydraulics were opposite, they might have cancelled each other out in the combined treatment. In a review on plant and soil responses to experimental combinations of CO_2 enrichment and warming, Dieleman et al. (2012) showed that additive and synergistic effects of the two treatments have rarely been reported, as antagonistic effects are more common. However, plants growing under enriched CO_2 showed a stronger warming effect in Ks than plants at ambient CO_2 conditions. Although warming resulted in similarly decreased Ks values for both ambient and enriched plots, the higher Ks under ambient temperature in CO_2 enriched plots than in ambient plots indicates that the warming effect was stronger under elevated CO_2. This evidences that the negative effects of warming clearly surpassed the positive effects of CO_2 enrichment and that warming effects were strong regardless of the original vessel size. The stronger effect of warming than CO_2 enrichment in our study suggests that changes in temperature will likely have stronger consequences on this species physiology and growth than rising CO_2 atmospheric concentrations.

Importance of overstory tree species on xylem anatomy and growth

The xylem anatomy of *V. myrtillus* showed strong differences between plots with different tree species regardless of the treatment applied, and its response to the treatments also differed depending on the tree species. Although the larger vessel sizes and the larger response to CO_2 under pine than under larch could be due to ontogenetic effects (ramets under pine were older), Dh-age relationships showed that for the same cambial age, vessel size was also larger under pine (Fig. S4). In a previous study in the same experiment, *V. myrtillus* phenology was advanced under larch and above-ground biomass was larger under pine (Anadon-Rosell et al. 2014). Thus, if the larger biomass was associated with taller ramets (which was not measured), the larger vessel sizes under pine could also just reflect conduit widening (Carrer et al. 2015). In any case, growing conditions under pine seem more favorable, probably related to the later snowmelt in spring under its perennial canopy, and thus greater protection to frost damage and freezing-induced cavitation during early-season freezing events. This could also explain that under CO_2 enrichment, ramets under pine showed greater increases in Dh and Kh than under larch. Alternatively, the greater growth of *V. myrtillus* under pine could result from a lower nitrogen availability under pine trees, as indicated by smaller nitrogen concentrations and $\delta^{15}N$ values in *V. myrtillus* leaves (Dawes et al. 2017), which may lead to a competitive advantage over more N-demanding graminoids. Surprisingly, RW was the only parameter to show higher values in *V. myrtillus* ramets growing under larch than under pine. Longer growing seasons experienced under larch could have induced an earlier onset of secondary growth despite being more unfavorable for *V. myrtillus* AG growth.

Conclusions

To conclude, our results show that whereas growth and anatomy are linked in terms of accumulated hydraulic conductivity in *V. myrtillus*, they did not respond in the same way to the soil warming and CO_2 enrichment treatments. We did not find evidence that the increased growth found in *V. myrtillus* under these two treatments was linked to immediately accompanying changes in the xylem anatomical structure. Warming had stronger effects on *V. myrtillus* growth and xylem anatomy than CO_2 enrichment. The negative effects of the soil warming treatment on vessel size and xylem-specific hydraulic conductivity of *V. myrtillus* might have improved hydraulic safety without compromising water transport. Altogether, these results suggest that the hydraulic system of *V. myrtillus* had to fulfill growth requirements at some stage, probably when the hydraulic system was not efficient enough for bigger ramets. Together with a lack of investment in above-ground storage tissues, these results provide evidence that growth is the most responsive parameter to CO_2 enrichment and, especially, soil warming in *V. myrtillus* at treeline and that this growth response is partly independent of concomitant adjustments in the xylem anatomical structures.

Acknowledgements

We acknowledge all the colleagues at SLF and WSL who were involved in the Stillberg CO_2 enrichment and soil warming experiment. We are particularly grateful to Stephan Hättenschwiler (CEFE-CNRS) for initiating the Stillberg CO_2 enrichment and soil warming experiment and to Loïc Schneider (WSL) for his help during sample preparation and image capturing. Major funding sources for the experiment included: the Velux foundation from 2007-2012 (grant 371 to FH); the Swiss National Science Foundation from 2007-2010 (grant 315200-116861 to CR); and the WSL from 2012-2014 (grant to CR and MD). AAR was funded by an FPU grant (Ministerio de Educación, Cultura y Deporte, Spain). GvA was supported by a grant from the Swiss State Secretariat for Education, Research and Innovation SERI (SBFI C14.0104).

References

Aloni R and Zimmermann M.H. 1983. The control of vessel size and density along the plant axis. Differentiation 24:203-208

Anadon-Rosell A., Rixen C., Cherubini P., Wipf S., Hagedorn F., Dawes M.A. (2014) Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. PLoS ONE **9**: e100577. doi: 10.1371/journal.pone.0100577

Atkinson C.J., Taylor J.M. (1996) Effects of elevated CO_2 on stem growth, vessel area and hydraulic conductivity of oak and cherry seedlings. New Phytologist **133**:617–626.

Bader M.K.-F., Leuzinger S., Keel S.G., Siegwolf R.T.W., Hagedorn F., Schleppi P., Körner C. (2013) Central european hardwood trees in a high-CO_2 future: Synthesis of an 8-year forest canopy CO_2 enrichment project. Journal of Ecology **101**:1509–1519.

Barbeito I., Dawes M.A., Rixen C., Senn J., Bebi P. (2011) Factors driving mortality and growth at treeline: a 30-year experiment of 92 000 conifers. Ecology **93**:389–401.

Billings W.D., Mooney H.A. (1968) The ecology of Arctic and Alpine plants. Biological Reviews 43:481–529. doi: 10.1111/j.1469-185X.1968.tb00968.x

Bunce J.A. (2004) Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. Oecologia **140**: 1–10. doi: 10.1007/s00442-003-1401-6.

Campioli M., Schmidt NM., Albert KR., Leblans N., Ro-Poulsen H., Michelsen A. (2013) Does warming affect growth rate and biomass production of shrubs in the High Arctic? Plant Ecol 214: 1049-1058.

Carlquist, S. 2001. Comparative wood anatomy: systematic, ecological, and evolutionary aspects of dicotyledon wood. 2nd edition. Springer, Berlin.

Collins M, Knutti R, Arblaster J, Dufresne J-L, Fichefet T, Friedlingstein P, Gao X, Gutowski WJ, Johns T, Krinner G, Shongwe, M, Tebaldi C, Weaver AJ, Wehner W (2013) Long-term climate change: projections, commitments and irreversibility. In: Stocker TF, Quin D, Plattner G-K, Tignor M, Allen SK, Bosching J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate Change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 1029-1136.

D'Odorico P., He Y., Collins S., De Wekker S.F.J., Engel V., Fuentes J.D. (2013) Vegetation-microclimate feedbacks in woodland-grassland ecotones. Global Ecology and Biogeography **22**:364–379.

Dawes M.A., Hagedorn F., Zumbrunn T., Handa I.T., Hättenschwiler S., Wipf S., Rixen C. (2011a) Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytologist **191**:806–818.

Dawes M.A., Hättenschwiler S., Bebi P., Hagedorn F., Handa I.T., Körner C., Rixen C. (2011b) Species-specific tree growth responses to 9 years of CO_2 enrichment at the alpine treeline. Journal of Ecology **99**:383–394.

Dawes MA., Hagedorn F., Handa IT., Streit K., Ekblad A., Rixen C., Körner C., Hättenschwiler S. (2013) An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. Oecologia171: 623-637.

Dawes M.A., Philipson C.D., Fonti P., Bebi P., Hättenschwiler S., Hagedorn F., Rixen C. (2015) Soil warming and CO_2 enrichment induce biomass shifts in alpine tree line vegetation. Global Change Biology **21**:2005–2021.

Dawes M.A., Schleppi P., Hättenschwiler S., Rixen C., Hagedorn F. (2017) Soil warming opens the nitrogen cycle at the alpine treeline. Global Change Biology 23: 421-434. doi:10.1111/gcb.13365

Dieleman W.I.J., Vicca S., Dijkstra F.A., Hagedorn F., Hovenden M.J., Larsen K.S., Morgan J.A., Volder A., Beier C., Dukes J.S., King J., Leuzinger S., Linder S., Luo Y., Oren R., De Angelis P., Tingey D., Hoosbeek M.R., Janssens I.A. (2012) Simple additive effects are rare: A quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. Global Change Biology **18**:2681–2693.

Fonti P., von Arx G., García-González I., Eilmann B., Sass-Klaassen U., Gärtner H., Eckstein D. (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. New Phytologist **185**:42–53.

Fonti P., Jansen S. (2012) Xylem plasticity in response to climate. New Phytologist **195:** 734-736.

Fonti P., Tabakova M.A., Kirdyanov A.V., Bryukhanova M.V., von Arx G. (2015) Variability of ray anatomy of *Larix gmelinii* along a forest productivity gradient in Siberia. Trees - Structure and Function **29**:1165–1175.

Franklin K.A., Lee S.H., Patel D., Kumar S.V., Spartz A.K., Gu C., Ye S., Yu P., Breen G., Cohen J.D., Wigge P.A., Gray W.M. (2011) Phytochrome-Interacting Factor 4 (PIF4) regulates auxin biosynthesis at high temperature. Proceedings of the National Academy of Sciences of the United States of America **108**:20231–20235.

Gärtner H. and Schweingruber F.H. (2013). Microscopic preparation techniques for plant stem analysis. Kessel Publishing House, Remagen.

Gorsuch D.M., Oberbauer S.F. (2002) Effects of mid-season frost and elevated growing season temperature on stomatal conductance and specific xylem conductivity of the arctic shrub, *Salix pulchra*. Tree Physiology **22**:1027–1034.

Gwynn-Jones D., Lee J.A., Callaghan T.V. (1997) Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-arctic forest heath ecosystem. Plant Ecology **128**:243–249.

Hacke U. G. and Sperry J. S. (2001). Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics 4: 97-115.

Hacke U.G., Sperry J.S., Wheeler J.K., Castro L. (2006). Scaling of angiosperm xylem structure with safety and efficiency. Tree Physiology 26: 689-701.

Hagedorn F., Martin M., Rixen C., Rusch S., Bebi P., Zürcher A., Siegwolf R.T.W., Wipf S., Escape C., Roy J., Hättenschwiler S. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. Biogeochemistry **97**:7–19.

Handa I.T., Körner C., Hättenschwiler S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO_2 enrichment and defoliation. Ecology **86**:1288–1300.

Handa I.T., Hagedorn F., Hättenschwiler S. (2008) No stimulation in root production in response to 4 years of in situ CO_2 enrichment at the Swiss treeline. Functional Ecology 22: 348-358.

Hättenschwiler S., Körner C. (1998) Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. Oecologia 113:104–114. doi: 10.1007/s004420050358.

Hättenschwiler S., Handa I.T., Egli L., Asshoff R., Ammann W., Körner C. (2002) Atmospheric CO_2 enrichment of alpine treeline conifers. New Phytologist **156**:363–375.

Inauen N., Körner C., Hiltbrunner E. (2012) No growth stimulation by CO_2 enrichment in alpine glacier forefield plants. Global Change Biology **18**:985–999.

Kilpeläinen A., Gerendiain A.Z., Luostarinen K., Peltola H., Kellomäki S. (2007) Elevated temperature and CO_2 concentration effects on xylem anatomy of Scots pine. Tree Physiology **27**:1329–1338.

Klanderud K. (2008) Species-specific responses of an alpine plant community under simulated environmental change. Journal of Vegetation Science **19**:363–372.

Kolb KJ, Sperry JS. 1999. Transport constraints on water use by the Great Basin shrub, Artemisia tridentata. Plant, Cell and Environment 22: 925–935.

Körner C., Diemer M., Schappi B., Niklaus P., Arnone III J. (1997) The responses of alpine grassland to four seasons of CO_2 enrichment: A synthesis. Acta Oecologica **18**:165–175.

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. 2n ed. Springer, Berlin.

Kostiainen K., Saranpää P., Lundqvist S., Kubiske M.E., Vapaavuori E. (2014) Wood properties of *Populus* and *Betula* in long-term exposure to elevated CO_2 and O_3. Plant, Cell & Environment **37**:1452–1463.

Kudo G., Suzuki S. (2003) Warming effects on growth, production, and vegetation structure of alpine shrubs: a five-year experiment in northern Japan. Oecologia 135: 280-287.

Leuzinger S., Körner C. (2007) Water savings in mature deciduous forest trees under elevated CO_2. Global Change Biology 13: 2498-2508.

Lev-Yadun S. (1998) The relationship between growth-ring width and ray density and ray height in cell number in the earlywood of *Pinus halepensis* and *Pinus pinea*. IAWA Journal **19**:131–139.

Loepfe L, Martinez-Vilalta J, Pinol J & Mencuccini M. 2007. The relevance of xylem network structure for plant hydraulic efficiency and safety. J. Theor. Biol. 247: 788–803.

Maherali H., DeLucia E.H. (2000) Interactive effects of elevated CO_2 and temperature on water transport in ponderosa pine. American Journal of Botany **87**:243–249.

Martin M., Gavazov K., Körner C., Hättenschwiler S., Rixen C. (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Global Change Biology **16**:1057–1070.

McCulloh K.A., Petitmermet J., Stefanski A., Rice K.E., Rich R.L., Montgomery R.A., Reich P.B. (2016) Is it getting hot in here? Adjustment of hydraulic parameters in six boreal and temperate tree species after 5 years of warming. Global Change Biology **22**:4124–4133. doi: 10.1111/gcb.13323

Mountain Research Initiative EDW Working Group (2015) Elevation-dependent warming in mountain regions of the world. Nature Climate Change *5: 424-430.*

Myers-Smith I.H., Forbes B.C., Wilmking M., Hallinger M., Lantz T., Blok D., Tape K.D., MacIas-Fauria M., Sass-Klaassen U., Lévesque E., Boudreau S., Ropars P., Hermanutz L., Trant A., Collier L.S., Weijers S., Rozema J., Rayback S.A., Schmidt N.M., Schaepman-Strub G., Wipf S., Rixen C., Ménard C.B., Venn S., Goetz S., Andreu-Hayles L., Elmendorf S., Ravolainen V., Welker J., Grogan P., Epstein H.E., Hik D.S. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. Environmental Research Letters **6:** 045509. doi: 10.1088/1748-9326/6/4/045509.

Olano J.M., Arzac A., García-Cervigón A.I., von Arx G., Rozas V. (2013) New star on the stage: Amount of ray parenchyma in tree rings shows a link to climate. New Phytologist **198**:486–495.

Overdieck D., Ziche D., Böttcher-Jungclaus K. (2007) Temperature responses of growth and wood anatomy in European beech saplings grown in different carbon dioxide concentrations. Tree Physiology **27**:261–268.

Petit G., Anfodillo T., Carraro V., Grani F., Carrer M. (2011) Hydraulic constraints limit height growth in trees at high altitude. New Phytologist **189**:241–252.

Petit G., Savi T., Consolini M., Anfodillo T., Nardini A. (2016) Interplay of growth rate and xylem plasticity for optimal coordination of carbon and hydraulic economies in *Fraxinus ornus* tres. Tree Physiology **36**:1310-1319.

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2016) *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.

Plavcová L., Jansen S. (2015) The role of xylem parenchyma in the storage and utilization of nonstructural carbohydrates. In: Hacke (ed.) Functional and ecological xylem anatomy. Springer. 281 pp.

Rangwala I., Sinsky E., Miller JR (2013) Amplified warming projections for high altitude regions of the northern hemisphere mid-latitudes from CMIP5 models. Env Res Lett 8: 024040. doi:10.1088/1748-9326/8/2/024040

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Rico C., Pittermann J., Polley H.W., Aspinwall M.J., Fay P.A. (2013) The effect of subambient to elevated atmospheric CO_2 concentration on vascular function in *Helianthus annuus*: Implications for plant response to climate change. New Phytologist **199**:956–965.

Rixen C., Dawes M.A., Wipf S., Hagedorn F. (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO_2 enrichment and soil warming. Oikos **121**:1532–1543.

Roderick M.L., Berry S.L. (2001) Linking wood density with tree growth and environment: A theoretical analysis based on the motion of water. New Phytologist **149**:473–485.

Ropars P., Boudreau S. (2012) Shrub expansion at the forest-tundra ecotone: Spatial heterogeneity linked to local topography. Environmental Research Letters **7:** 015501. doi: 10.1088/1748-9326/7/1/015501

Rundqvist S., Hedenås H., Sandström A., Emanuelsson U., Eriksson H., Jonasson C., Callaghan T.V. (2011) Tree and shrub expansion over the past 34 years at the tree-line near Abisko, Sweden. Ambio **40**:683–692.

Schweingruber F.H., Hellmann, L., Tegel, W., Braun S., Nievergelt D., Büntgen U. (2013). Evaluating the wood anatomical and dendroecological potential of arctic dwarf shrub communities. IAWA Journal 34: 485-497.

Sperry JS & Tyree MT. 1988. Mechanism of water stress-induced xylem embolism. Plant Physiol. 88: 581–587.

Thomas D.S., Montagu K.D., Conroy J.P. (2004) Changes in wood density of *Eucalyptus camaldulensis* due to temperature - The physiological link between water viscosity and wood anatomy. Forest Ecology and Management **193**:157–165.

Tissue D.T., Oechel W.C. (1987) Response of *Eriophorum vaginatum* to elevated CO_2 and temperature in the Alaskan tussock tundra. Ecology **68**:401–410.

Tyree M.T., Ewers F.W. 1991. The hydraulic architecture of trees and other woody plants. New Phytol 119: 345-360

Tyree MT, Davis SD & Cochard H. 1994. Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWA J. 15: 335–360.

Tyree M.T., Sperry J.S. 1989. Vulnerability of xylem to cavitation and embolism. Annu. Rev. Plant Phys. Mol. Bio. 40:19-38

von Arx G, Archer SA, Hughes MK. 2012. Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. Annals of Botany 109: 1091-1100

von Arx G, Kueffer C., Fonti P. 2013 Quantifying plasticity in vessel grouping –added value from the image analysis tool ROXAS IAWA Journal 34: 433-445 von Arx G., Arzac A., Olano J.M., Fonti P. (2015) Assessing conifer ray parenchyma for ecological studies: Pitfalls and guidelines. Frontiers in Plant Science **6**:1016. doi: 10.3389/fpls.2015.01016.

von Arx G., Crivellaro A., Prendin A.L., Čufar K., Carrer M. (2016) Quantitative wood anatomy—practical guidelines. Frontiers in Plant Science **7:**781.

Watanabe Y., Wakabayashi K., Kitaoka S., Satomura T., Eguchi N., Watanabe M., Nakaba S., Takagi K., Sano Y., Funada R., Koike T. (2016) Response of tree growth and wood structure of *Larix kaempferi*, *Kalopanax septemlobus* and *Betula platyphylla* saplings to elevated CO_2 concentration for 5 years exposure in a FACE system. Trees - Structure and Function:1–11.

Wegner L., von Arx G., Sass-Klaassen U., Eilmann B. 2013. ROXAS – an efficient and accurate tool to detect vessels in diffuse-porous species. IAWA Journal 34:425-432.

Wheeler J.A., Hoch G., Cortés A.J., Sedlacek J., Wipf S., Rixen C. (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. Oecologia **175**: 219–229. doi: 10.1007/s00442-013-2872-8.

Ziche D., and Overdieck P. (2004). CO_2 and temperature effects on growth, biomass production and stem wood anatomy of juvenile Scots Pine (*Pinus sylvestris* L.). Journal of Applied Botany 78: 120-132.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York.

Supporting information

Table S1. Climate and snowmelt data for the experimental plots during all the experimental years (2001-2012). Mean annual air temperature, annual precipitation and mean air temperature and precipitation for the main growing season months (June-August) are given. Data obtained from a climate station located *ca.* 100 m below the experimental site.

Year	Snowmelt date	Air T (°C) (annual mean)	Precipitation (mm) (annual)	Air T (°C) (mean JJA)	Precipitation (mm) (sum JJA)
2001	24 May	2.2	1520	9.5	681
2002	21 May	3.0	1436	10.1	452
2003	12 May	3.1	1035	12.7	375
2004	06 Jun	2.1	1088	9.2	466
2005	27 May	1.5	1044	9.1	386
2006	26 May	2.9	1038	9.7	393
2007	07 May	2.7	1235	9.5	469
2008	29 May	2.3	1266	9.7	470
2009	23 May	2.2	1156	10.0	463
2010	29 May	1.1	1268	9.7	539
2011	12 May	3.4	1345	9.3	472
2012	28 May	2.3	1622	10.7	645

Table S2. Correlation coefficients (*r*) and significance values (*P*) between the wood anatomical parameters from the ring formed in 2012 and biomass and shoot elongation of *V. myrtillus* across treatments and sample years.

Wood anatomical parameter	Leaves		New shoots		Total above-ground biomass		Shoot elongation	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Dh	0.22	0.196	0.18	0.280	0.14	0.400	0.12	0.486
Ks	0.08	0.661	0.04	0.805	0.14	0.413	0.14	0.413
Kh*	0.73	**<0.001**	0.69	**<0.001**	0.71	**<0.001**	0.41	**0.010**
CNo	0.31	0.069	0.38	**0.022**	0.06	0.733	0.41	**0.014**
CD	-0.29	0.090	-0.29	0.083	-0.12	0.495	-0.06	0.727
V_G	-0.06	0.719	0.03	0.879	0.00	0.999	0.28	0.097
V_S	0.06	0.747	-0.04	0.825	0.04	0.836	-0.27	0.117
V_M	0.08	0.632	0.07	0.699	0.34	**0.044**	0.17	0.326
RA	0.36	**0.027**	0.43	**0.007**	0.10	0.545	0.46	**0.004**
RA total**	0.67	**<0.001**	0.64	**<0.001**	0.73	**<0.001**	0.31	**0.050**
RW	0.04	0.821	0.16	0.352	-0.19	0.255	0.34	**0.037**
RW total**	0.70	**<0.001**	0.64	**<0.001**	0.74	**<0.001**	0.32	**0.047**

* Kh values for rings 2001-2012, i.e. the sapwood area

** RA total and RW total are accumulated values for the whole cross-section

Table S3. Dh values (mean ± 1 SE) for the CO_2-treatment years 2001-2009 (a) and the warming-treatment years 2007-2012 (b). For each combination of CO_2 treatment (ambient and enhanced), plot tree species (larch or pine) and treatment year (2001-2009) during the CO_2-treatment years (*n* = 10) and CO_2 treatment , soil warming treatment (C = unwarmed, W = warmed), plot tree species and treatment year (2007-2012) during the warming-treatment years raw means are given (*n* = 5), which incorporate variation due to ontogenetic differences (and, thus differ from estimates from statistical models shown in Fig. 3, which accounts for these differences).

a) CO_2-treatment years

Plot tree species	Year	Ambient	SE	Enhanced	SE
Larch	2001	13.05	0.65	14.25	1.25
	2002	14.25	0.98	12.77	0.35
	2003	12.60	0.73	12.53	0.64
	2004	13.61	0.46	13.75	0.62
	2005	13.66	0.40	12.07	0.86
	2006	14.00	0.41	13.88	0.51
	2007	13.96	0.45	14.21	0.51
	2008	14.18	0.42	14.32	0.61
	2009	14.14	0.44	14.49	0.52
Pine	2001	13.13	0.73	13.15	0.82
	2002	12.76	0.73	13.46	0.82
	2003	13.53	0.71	14.02	0.41
	2004	14.40	0.71	14.06	0.58
	2005	13.64	0.85	14.61	0.44
	2006	14.12	0.47	15.49	0.41
	2007	14.74	0.52	15.80	0.59
	2008	15.18	0.63	16.65	0.54
	2009	15.48	0.64	16.73	0.49

b) Warming-treatment years

		Ambient CO_2				Enhanced CO_2			
Plot tree species	Year	Unwarmed	SE	Warmed	SE	Unwarmed	SE	Warmed	SE
Larch	2007	14.82	0.55	13.10	0.49	15.06	0.52	13.15	0.68
	2008	14.34	0.78	14.02	0.40	15.38	0.51	13.48	0.88
	2009	13.98	0.71	14.30	0.61	15.53	0.46	13.66	0.67
	2010	14.48	0.65	13.68	0.57	14.60	0.75	14.46	0.52
	2011	14.88	0.71	15.48	0.47	16.35	0.71	15.08	0.62
	2012	15.06	0.97	14.98	0.66	14.98	0.90	14.30	0.77
Pine	2007	15.68	0.55	13.58	0.56	17.28	0.43	14.32	0.54
	2008	16.76	0.62	13.60	0.36	18.00	0.43	15.30	0.46
	2009	16.94	0.62	14.02	0.63	17.70	0.69	15.76	0.39
	2010	15.46	0.70	14.16	0.48	16.30	0.55	15.40	0.62
	2011	16.64	0.82	15.56	0.83	17.88	1.00	16.62	0.59
	2012	16.17	0.79	14.98	0.60	16.80	0.65	16.56	0.62

	Dh	CA95%	Ks	Kh	CD	V_G	V_S	V_M	CNo	RW	RA
Dh	1 ***	0.99 ***	0.87 ***	0.62 ***	-0.05 n.s.	0.58 ***	-0.63 ***	0.40 ***	0.33 ***	0.10 **	0.33 ***
CA95%		1 ***	0.86 ***	0.62 ***	-0.08 n.s.	0.57 ***	-0.62 ***	0.40 ***	0.32 ***	0.10 *	0.32 ***
Ks			1 ***	0.43 ***	0.36 ***	0.75 ***	-0.76 ***	0.58 ***	0.13 **	-0.22 ***	0.04 n.s.
Kh				1 ***	-0.23 ***	0.30 ***	-0.36 ***	0.17 ***	0.91 ***	0.65 ***	0.90 ***
CD					1 ***	0.52 ***	-0.51 ***	0.45 ***	-0.28 ***	-0.52 ***	-0.42 ***
V_G						1 ***	-0.96 ***	0.81 ***	0.10 *	-0.21 ***	0.03 n.s.
V_S							1 ***	-0.69 ***	-0.17 **	0.13 **	-0.09 *
V_M								1 ***	0.01 n.s.	-0.28 ***	-0.02 n.s.
CNo									1 ***	0.74 ***	0.88 ***
RW										1 ***	0.78 ***
RA											1 ***

Figure S1. Pearson's correlation matrix between the considered wood anatomical parameters. Values for the CO_2 treatments, warming treatments, plot tree species and study years are plotted together. Positive correlations are green and negative correlations are red. Correlation coefficients (*r*) are shown and significant correlations are indicated by asterisks at $0.05 < P < 0.10$ (m.s., *), $0.01 < P < 0.05$ (**) and $P \leq 0.01$ (***). Non-significant correlations indicated by n.s.

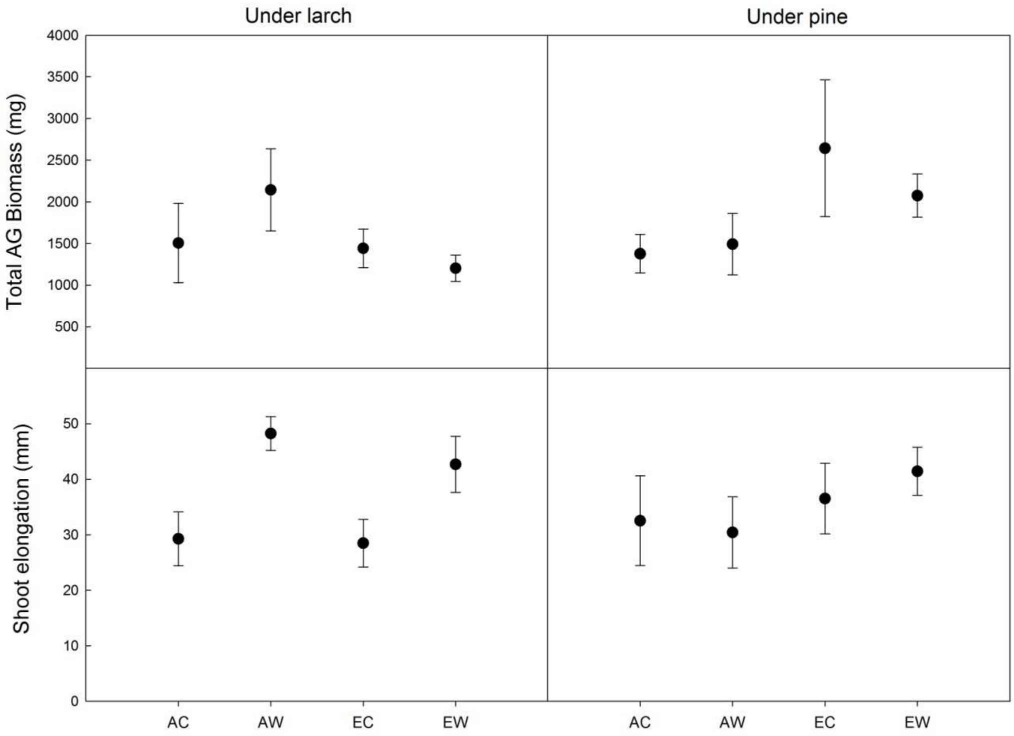

Figure S2. Total above-ground biomass (top) and shoot elongation (bottom) of *V. myrtillus* ramets harvested in 2012. Mean values ± 1 SE for each combination of CO_2 treatment (A = ambient, E = enhanced), warming treatment (C = unwarmed, W = warmed) and plot tree species (larch or pine) are shown (n = 5). There was only a significant effect of warming in *V. myrtillus* shoot elongation across tree species and CO_2 treatments ($P < 0.05$).

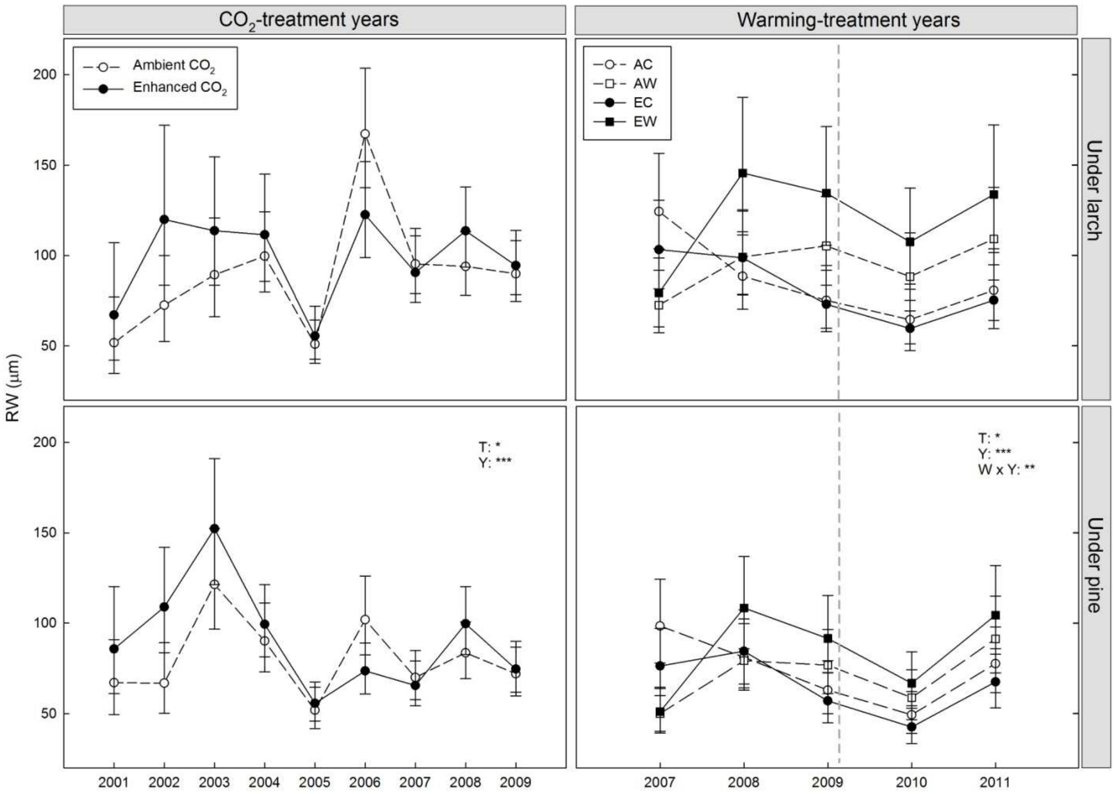

Figure S3. RW variation through time during the CO_2-treatment years 2001-2009 (left panels) and during the warming-treatment years 2007-2012 (right panels) for each plot tree species (larch in top panels, pine in bottom panels). Mean values ± 1 SE are shown, estimated from statistical models for a ramet with an average cambial age, and thus reflect statistical analyses. For the CO_2-treatment years, the combination of each CO_2 treatment (Ambient and Enhanced) and plot tree species are shown (n = 10). For the warming-treatment years, each combination of CO_2 treatment (A = ambient, E = elevated), soil warming treatment (C = unwarmed, W = warmed) and plot tree species are shown (n = 5). Vertical dashed lines indicate the end of the CO_2 treatment. Significant effects included warming treatment (W), plot tree species (T) and study year (Y) at $0.05 < P < 0.10$ (m.s., *), $0.01 < P < 0.05$ (**), $P \leq 0.01$ (***), and are shown at the right top corner of the lower panels. See Table 3 for detailed statistical results.

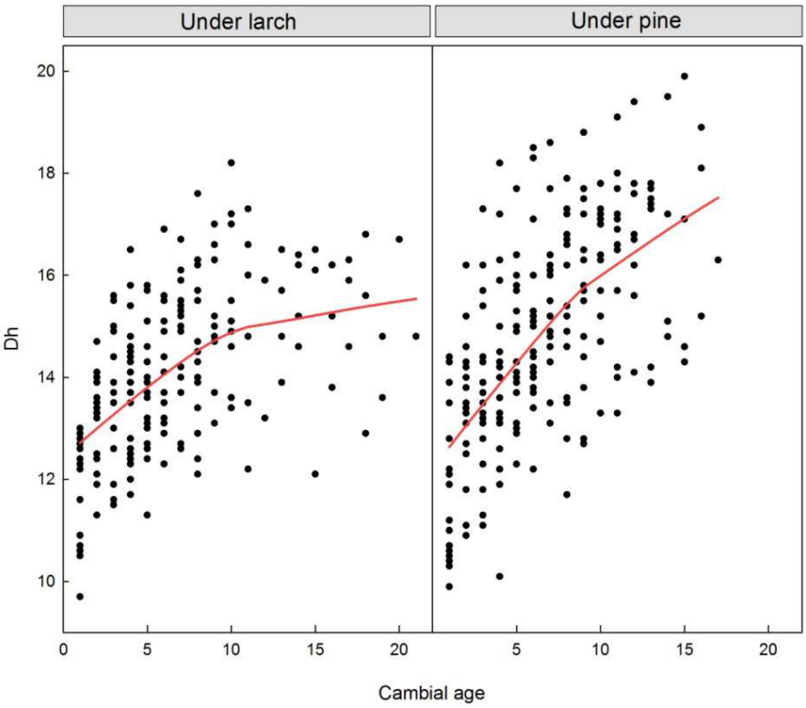

Figure S4. Relationship between cambial age and Dh for *V. myrtillus* ramets growing under larch or under pine trees. The red line was fitted with LOESS smoothing.

Capítol 5 / Chapter 5

Short-term carbon allocation dynamics in subalpine dwarf shrubs and their response to experimental summer drought

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Resum

La distribució de carboni és un procés clau en el funcionament de les plantes i dels ecosistemes, però les seves respostes a les condicions ambientals i al canvi global encara no es coneixen prou. Això és particularment aplicable als subarbusts, que són un component important de la vegetació d'alta muntanya. Vam estudiar la dinàmica de la distribució del C recentment assimilat en dues espècies subarbustives dominants, *Vaccinium myrtillus* i *Vaccinium uliginosum,* en dues zones subalpines, al Pirineu i als Alps, i vam dur a terme una exclusió de pluja experimental per simular una sequera estival a la zona d'estudi dels Alps. Vam comparar la taxa d'assimilació neta (A_{max}), la conductància estomàtica (g_s) i els potencials hídrics de les tiges (Ψ_S) entre les dues espècies i vam realitzar un experiment de marcatge amb ^{13}C per traçar la dinàmica de la seva distribució de C, tant en condicions naturals com en l'experiment de sequera. En condicions naturals, *V. uliginosum* va presentar A_{max} i g_s més elevades, i va utilitzar els assimilats recents com a substrat respiratori més lentament que *V. myrtillus*. La dinàmica de la distribució de C en tiges noves era significativament diferent entre espècies, mostrant una disminució progressiva del ^{13}C en les tiges no fotosintètiques de *V. uliginosum* i un augment progressiu en les tiges fotosintètiques de *V. myrtillus*. En l'experiment de sequera, una reducció significativa de la humitat del sòl va reduir els Ψ_S abans de l'alba fins a -1,2 MPa en totes dues espècies, valor considerat molt proper al punt de pèrdua de turgència. Tanmateix, tant la conductància estomàtica com la velocitat de transferència del C recentment assimilat cap a òrgans subterranis només es van veure reduïdes en *V. uliginosum.* Tot i que totes dues espècies d'estudi són força resistents al dèficit hídric, els nostres resultats indiquen que *V. uliginosum* és més sensible a la sequera, cosa que podria tenir implicacions per la composició de la comunitat i el cicle del C de l'ecosistema en el futur.

Abstract

Carbon allocation is a key determinant of plant functioning and ecosystem processes. Its dynamic responses to environmental conditions and global changes are still poorly understood. This holds particularly for dwarf shrubs, which are an important component of high mountain vegetation. We studied the short-term C allocation dynamics in two dominant dwarf shrub species, *Vaccinium myrtillus* and *Vaccinium uliginosum*, at two subalpine sites, in the Pyrenees and in the Alps, and additionally performed an experimental rain exclusion to simulate summer drought at the site in the Alps. We compared net assimilation rates (A_{max}), stomatal conductance (g_s) and shoot water potentials (Ψ_S) between the two species and carried out a ^{13}C pulse labelling experiment to trace their C allocation dynamics, both under natural conditions and in the drought experiment. Under ambient conditions, *V. uliginosum* showed higher A_{max} and g_s and used recent assimilates as a respiratory substrate more slowly than *V. myrtillus*. C allocation dynamics in current-year shoots differed significantly between the species, showing a progressive decrease in tracer concentrations after the pulse labelling in *V. uliginosum* non-photosynthetic shoots but a progressive increase in *V. myrtillus* photosynthetic shoots. In the drought experiment, a significant reduction of soil moisture caused predawn Ψ_S below -1.2 MPa in both species, which is considered close to the turgor loss point. However, both the stomatal conductance and the speed of transfer of newly assimilated C to belowground organs decreased in *V. uliginosum* only. Thus, although both species were largely resistant to the drought imposed, our results indicate a higher drought sensitivity of *V. uliginosum* compared to *V. myrtillus*, with potential implications for community composition and ecosystem C cycling in the future.

Keywords: Alps, carbon allocation, ^{13}C pulse labelling, drought, dwarf shrub, Pyrenees, subalpine

Introduction

Carbon (C) allocation from photosynthetically active plant parts to other plant compartments is a prerequisite for plant growth and metabolic maintenance, and has important implications for ecosystem C cycling (Högberg and Read 2006; Brüggemann et al. 2011). The study of plant C allocation patterns and their dynamics provides essential information on the transfer, use or storage of assimilates, and how these functions might change directly or indirectly due to environmental changes both at the longer (Litton et al. 2007, Poorter et al. 2012) and at the shorter timescales (Epron et al. 2012). Recent methodological advances have permitted the study of C allocation dynamics *in situ* with isotopic pulse-chase labelling, permitting an understanding of the fate of assimilated C and its responses to environmental changes. While the short-term C allocation dynamics have been studied in grasslands (e.g. Bahn et al. 2009; Burri et al. 2014; Fuchslueger et al. 2014; Hasibeder et al. 2015) and, regarding woody species, mostly in trees (see review by Epron et al. 2012, and more recent studies by Streit et al. 2013; Krepkowski et al. 2013; Blessing et al. 2015; Hartmann et al. 2015 and Desalme et al. 2017), the understanding of the environmental controls and responses to climatic changes is still limited. To date, very little is known on the short-term C allocation dynamics in dwarf shrubs (see Karlsson 1985).

Dwarf shrubs are an important component of many subalpine plant communities (Körner 2003; Ninot et al. 2008) and play a crucial role in treeline processes (Batllori et al. 2009; Grau et al. 2012, 2013; Liang et al. 2016). Climate and land-use changes have recently led to an expansion of dwarf shrub species in many mountain areas, where they colonize abandoned pastures and herbaceous-dominated alpine sites (Tasser and Tappeiner 2002; Dullinger et al. 2003; Sanz-Elorza et al. 2003). Shrub encroachment has profound impacts on ecosystem structure and functioning (Eldridge et al. 2011; Prévosto et al. 2011; Ropars and Bordreau 2012; Bragazza et al. 2013; Maestre et al. 2016). The genus *Vaccinium* contains ecologically important and widespread species across cold regions. *Vaccinium myrtillus* L. and *Vaccinium uliginosum* ssp. *microphyllum* (Lange) Tolm. or *Vaccinium gaultherioides* (hereafter *V. uliginosum*) are clonal dwarf shrub species of similar dimensions, deciduous leaf habit and with a complex and extensive network of subterranean rhizomes. Despite their similar leaf-habit and size, *V. myrtillus* bears photosynthetic shoots that remain green for some years after their formation, whereas *V. uliginosum* does not. Thus, green shoots of *V. myrtillus* represent a significant part of its total photosynthetic area, and remain functional for a longer period than the deciduous leaves, with so far unknown implications for C allocation dynamics.

Several studies have reported re-fixation by branches and leaves of CO_2 respired by other organs and transported by the xylem sap (Stringer and Kimmerer 1993; McGuire et al. 2009; Bloemen et al. 2013a,b). Moreover, large amounts of C have been found to be allocated in xylem for their transportation through the transpiration stream (Heizmann et al. 2001). Therefore, species differing in the photosynthetic behaviour of their shoots might show differences in their C allocation dynamics due to differences in C uptake and transport, and CO_2 re-assimilation processes.

Global change projections for the end of the century point to changing precipitation patterns with an increase of extreme events such as severe summer droughts (Dai et al. 2013; IPCC 2013). Limited water supply alters photosynthesis and respiration and thereby drives major changes in the ecosystem C balance (Ciais et al. 2005; Zhao and Running 2010; Reichstein et al. 2013; Frank et al. 2015; Yuan et al. 2016). Moreover, frequent and intense drought events may decelerate the expansion of forests and shrublands driven by land-use change and warming at high elevations (Barros et al. 2016). When facing water stress, most plants respond with stomatal closure, which reduces water loss at the expense of decreasing C uptake by photosynthesis (Chaves et al. 2002, Flexas et al. 2006). Some studies based on isotopic labelling experiments found that drought slowed down the transfer of recently assimilated C to belowground compartments (Ruehr et al. 2009; Barthel et al. 2011; Fuchslueger et al. 2014; Hasibeder et al. 2015), but other studies did not found such effects (Burri et al. 2014; Blessing et al. 2015; Hommel et al. 2015). Moreover, drought may reduce phloem transport through a reduction of water potential and NSC availability, causing dysfunctions in the transfer of assimilates throughout the plant (Hartmann and Trumbore 2016).

In our study, we aimed to determine the differences in the short-term C allocation between *V. myrtillus* and *V. uliginosum* at two subalpine sites differing in their background climate conditions, one in the Alps and one in the Pyrenees. Moreover, we studied the effects of an experimental summer drought on the short-term C allocation dynamics of both species. We hypothesized that (i) due to the photosynthetic capacity of its shoots, *V. myrtillus* would show higher shoot C uptake and faster turnover transport to belowground organs; that (ii) the dynamics of recently assimilated C would be slower and less pronounced for xylem (carrying re-fixed respired CO_2) than phloem; and that (iii) drought would reduce C uptake and slow down C allocation to rhizomes, with stronger reduction in xylem tracer dynamics than in phloem.

Materials and methods

Study sites and experimental design

The study aimed at comparing natural C allocation dynamics between *V. myrtillus* and *V. uliginosum*. They both grow in acidic substrates and often coexist, although their elevation distribution differs slightly. *Vaccinium myrtillus* can be frequently found from the lower montane until the upper subalpine belt (although it also reaches alpine sites), whereas *V. uliginosum* grows at slightly higher altitudes than *V. myrtillus*, from the subalpine to the alpine belt (Aeschimann et al. 2004; Bolòs et al. 2005). *Vaccinium uliginosum* is more heliophilous, and can resist strong winds and the lack of snow cover (Jacquemart 1996), contrarily to *V. myrtillus*, which needs snow protection in winter (Ritchie 1956) and has a lower freezing resistance (Martin et al. 2010; Wheeler et al. 2014).

We performed two separate pulse labelling experiments at two subalpine sites, in the Alps and in the Pyrenees. Despite their similar annual precipitation, rainfall during the summer months is distinctly higher at the site in the Alps than in the Pyrenees (see below). In the Alps, we also aimed at exploring the effects of experimental drought on C allocation patterns of both species. A summary of the measurements taken at each study site is provided in Table S1.

Stubai valley, Alps

The study site is located at a subalpine grassland colonized by dwarf shrub patches (1820 m a.s.l.) in the Stubai valley, Austrian Central Alps (47º 7' 45'' N, 11º 18' 20'' E). It is immediately adjacent to a site where previous pulse labelling and drought experiments have been performed on herbaceous vegetation (e.g. Bahn et al. 2009;

Seeber et al. 2012; Fuchslueger et al. 2014, 2016; Hasibeder et al. 2015). Vegetation includes the dominant dwarf shrub species *V. myrtillus* and *V. uliginosum*, plus other dwarf shrub species (*Calluna vulgaris* and *Vaccinium vitis-idaea*) and numerous grasses and forbs (*Agrostis capillaris*, *Festuca ovina*, *Deschampsia flexuosa*, *Briza media*, *Luzula multiflora*, *Campanula scheuzeri*, *Chaerophyllum hirsutum*, *Crepis conyzifolia*, *Hypericum maculatum* and *Potentilla erecta*, amongst others). Mean annual temperature is 3 ºC and mean annual precipitation is 1100 mm. Summer precipitation (June-August) is 548 mm.

A drought experiment was set up on the 3 July 2014 and consisted of three blocks, each one with a drought plot and the corresponding control plot, all of them placed on mixed patches of the clonal dwarf shrubs *V. myrtillus* and *V. uliginosum*. Drought was simulated with a rain-out shelter of 3 × 3.5 m basal area and 2.5 m height covered with transparent and UV-B transmissive plastic foil (UV B Window; Folitec, Westerburg, Germany; light transmittance *ca*. 95%, see Hasibeder et al. 2015). Inner ventilation was achieved through openings at the bottom and the top of the face sides of the rain-out shelter. At the centre of each rain-out shelter, an area of 1×1 m was established on which a 15-cm-high plastic frame was installed by inserting it *ca*. 3 cm into the soil. Plastic frames were also installed on the corresponding control plots. The frames served to place the ecosystem chamber airtight for measurements of ecosystem CO_2 fluxes and for pulse labelling. On the hillslope facing side of each rain-out shelter the soil was trenched down to *ca*. 20 cm, and the trench was filled with waterproof plastic foil to avoid runoff water getting inside drought plots.

We installed two sets of sensors for light, air temperature and air moisture (HMP155 with radiation and precipitation shield DTR500; Vaisala, Helsinki, Finland), one outside the rain-out shelters and one inside one of the rain-out shelters. In addition, we installed one soil temperature sensor (S-TMB, Onset Computer Corporation, MA, USA) and one soil moisture sensor (EC-10; Decagon Devices, WA, USA) per plot. Data were recorded by dataloggers (HOBO micro station, Onset Computer Corporation, MA, USA).

Filià valley, Pyrenees

The study site is located at a low shrub heath in Filià valley, in Pallars Jussà, Catalonia, Central Pyrenees, in a 28º steep N-facing slope at 1900 m a.s.l. (42° 27' 41" N, 0° 57' 40" E). The dominant shrub species are *Vaccinium myrtillus*, *Vaccinium uliginosum*, *Rhododendron ferugineum* and *Calluna vulgaris*. Other abundant forbs and grasses are *Festuca nigrescens*, *Deschampsia flexuosa*, *Anthoxantum odoratum*, *Nardus stricta*, *Luzula multiflora*, *Alchemilla* gr. *alpina*, *Galium verum*, *Trifolium alpinum* and *Achillea millefolium*, amongst others. Mean annual temperature is 3.7 ºC and mean annual precipitation 1297 mm (for the period 1951-2002; data from a nearby climate station at Estany Gento, 2120 m a.s.l., provided by Meteorological Service of Catalonia and Spanish National Institute of Meteorology). Summer precipitation is 376 mm (based on datasets gathered between 2007 and 2015). In July 2013 we established four plots of 1 m^2, each one with high cover of *V. myrtillus* and *V. uliginosum*.

Gas exchange measurements

At Stubai, stomatal conductance was measured during the second half of the drought experiment on three sunny days (1, 8 and 18 August 2014) around noon with a leaf porometer (model SC-1, Decagon Devices, WA, USA). We marked 5 leaves in different ramets of each study species (*V. myrtillus* and *V. uliginosum*) with colour straws and took the measurements on the same leaves each sampling day. If leaves were damaged, measurements were taken on another leaf of the same twig. We also took measurements of the net ecosystem exchange (NEE) of CO_2 on two sunny days (8 and 18 August 2014), as described by Schmitt et al. (2010). Light saturated rates of gross primary productivity (GPP) were estimated by subtracting dark respiration rates (obtained with a shading foil reducing photosynthetically active radiation, PAR, to zero) from the net ecosystem exchange (NEE) of CO_2 at photosynthetic photon flux density (PPFD) of 1400-2000 μmol $m^{-2}s^{-1}$ (measured with a PAR quantum sensor, PQS 1; Kipp & Zonen, Delft, the Nederlands). CO_2 concentrations were measured with a CO_2 probe (GMP343, Vaisala, Helsinki, Finland) inserted into the upper part of a transparent Plexiglas chamber of 1 x 1 x 0.7 m^3 fixed on top of the plot. The probe was connected to a hand-held meter (HM70, Vaisala, Helsinki, Finland), which recorded CO_2 concentrations during 1 minute at 5-s intervals. The chamber had a rubber gasket at the bottom to ensure gas tightness once fixed on the plastic frame, and an opening on top, which was closed only once the chamber was fixed on the frame to avoid pressurization of the interior (Schmitt et al. 2010). We maintained air circulation inside the chamber with fans fixed to one side of the chamber. For statistical analyses, we only compared g_s and NEE data from control and drought plots obtained during the period of peak drought. For consistency purposes, we also used this last day measurements only when comparing the two species under ambient conditions.

At Filià, on the 26 June 2015 we measured photosynthetic rates (A_{max}) and stomatal conductance (g_s) in leaves of two different ramets of *V. myrtillus* and *V. uliginosum* in each plot with an infrared gas analyser (LI-6400 system, LI-COR Inc., Lincoln, NB, USA). Measurements were made at a PPFD of 1200 μmol m^{-2} s^{-1} and at a temperature of 24 ºC. We kept all leaves wrapped in moist cellulose paper until arriving at the laboratory, where we scanned them to obtain leaf area to correct leaf A_{max} and g_s values obtained in the field.

$^{13}CO_2$ *Pulse labelling*

At Stubai, we carried out a $^{13}CO_2$ pulse labelling 7.5 weeks after installing the rain-out shelters, as described by Bahn et al. (2009) and Hasibeder et al. (2015). The three blocks were labelled in three consecutive campaigns (25, 28 and 29 August 2014), one for each block (including a drought and the corresponding control plot, which were labelled consecutively). Pulse labellings were always carried out on sunny days, between 10 am and 2 pm. We used the same chamber as for CO_2 concentration measurements. We ensured temperature stabilization by mounting ice packs in the N-facing part of the chamber. We monitored air temperature, CO_2 concentration and irradiance inside the chamber during labelling with a thermocouple, an IRGA (EGM-4, PP Systems, Hitchin, UK), and a PAR quantum sensor, respectively. We estimated the amount of $^{13}CO_2$ from the values obtained with the IRGA. Once we closed the chamber to start the labelling, we waited until the CO_2 concentrations inside the chamber dropped to *ca*. 250 ppm, and then added highly enriched $^{13}CO_2$ (99.9 atom%) at flow rates of 150 ml/min, maintaining a $^{13}CO_2$ concentration inside the chamber of 600-800 ppm. We labelled every plot for 70 minutes.

At Filià, in July 2015 we selected three of the four plots and installed aluminium frames of 0.7 × 0.7 m in the centre of each plot to fix the chambers during labelling. We inserted each frame *ca*. 3 cm into the soil and we sealed the spaces left between the frame and the soil due to terrain heterogeneity with plastic foil. We labelled plots with $^{13}CO_2$ following the same procedure as in Stubai, but we used a smaller labelling chamber, 0.74 m × 0.74 m × 0.70 m. We labelled one plot per day in three consecutive campaigns (27, 28 and 29 July 2015).

Plant sampling

We harvested *V. myrtillus* and *V. uliginosum* ramets immediately before (0 h) and five times after the pulse labelling started, at 2 h, 24 h, 2, 4 and 8 days at both study sites. At Filià, we additionally collected plant samples after 16 days and 2 months. At each sampling time we harvested one ramet of each species, consisting of the aboveground leaves, shoots and main stem, and part of the rhizome, which we dug out down to *ca*. 10-20 cm. We separated a subsample of leaves and rhizomes for respiration measurements (only in Filià, see below) and placed the rest of the ramet in the microwave for 3 minutes at maximum power (800 watts) to stop all enzymatic activity (Popp et al. 1996). Once in the lab, we separated leaves and rhizomes from the main stem, dried them at 60 °C for 48 h and weighed them. In Filià, we also separated and analysed current-year shoots to compare the C allocation dynamics between *V. myrtillus* photosynthetic shoots and *V. uliginosum* non-photosynthetic shoots. In a subsample of each rhizome fraction obtained in Stubai, we carefully separated xylem and phloem with a razor blade under a magnification lens for isotope composition analyses.

Respiration measurements

At Filià, immediately after sampling we washed rhizomes and removed the attached roots (which were only present in some samples). Leaves were kept 10 min in the dark before starting the incubation. We incubated plant material (*ca*. 25-35 mg) in 50 ml Erlenmeyer flasks (sealed with rubber stoppers) in a waterbath placed in a dark, thermally isolated box at constant temperature of 15 °C (Hasibeder et al. 2015). Before starting the incubations and after placing the plant material inside the incubation flask, we flushed the flask with CO_2-free air for 5 minutes. Air was pushed by an air bomb through a soda lime column, a tube at the end of the column collected the air and released it inside the flask through an opening in the stopper (and then air was released by another opening). After 5 minutes, once the air inside the chamber reached *ca.* 0 ppm CO_2 (flushing time established after performing measurements prior to the labelling experiment with an IRGA EGM-4), we sealed the chamber and let the material incubate for 30 minutes. After this time, we extracted 20 ml air sample with a 50 ml syringe (SGE, Ringwood, Australia) and injected the air sample into a 10 ml plastic tube (BD vacutainer, Plymouth).

Water potential measurements

At the end of the drought experiment in Stubai (8 September), we measured shoot water potential (Ψ_S) of the two study species. We collected three current-year shoots (formed in 2014) of different ramets per species and per plot before sunrise and at midday, respectively. Shoots were cut with a sharp razor blade, tightly wrapped in plastic bags and transported to the laboratory. Once in the lab, we measured Ψ_S with a pressure chamber (model 1000 pressure chamber; PMS Instrument, Corvallis, OR, USA).

Isotope composition of plant material

We ground the oven-dried leaves, current-year shoots, rhizomes, xylem and phloem of each study species and weighed *ca.* 1 mg subsamples in small tin capsules for the analyses of C content and isotopic composition. The C content was determined by an Elemental Analyzer Flash 1112 (Carbo Erba, Milan). The C isotope composition was determined with the Elemental Analyser coupled to an IRMS Delta C isotope ratio mass spectrometer through a Conflo III Interface (Thermo-Finnigan, Germany). The results of C isotope analyses are reported in per mil (‰) on the relative δ-scale as $\delta^{13}C$, and refer to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}}\right) - 1$$

(Eq. 1)

where *R* is the $^{13}C/^{12}C$ ratio.

For both experiments, we used pre-labelling samples to calculate the ^{13}C excess. To express the amount of ^{13}C label above the natural isotopic background, we calculated the ^{13}C atom% excess of each plant fraction, which corresponds to the increase in ^{13}C atoms after the pulse labelling, expressed as the percentage of total C atoms present in the sample. First, we expressed the $\delta^{13}C$ in atom%:

$$\text{atom\%} = 100 * \frac{0.0111802 * (\frac{\delta}{1000} + 1)}{1 + 0.0111802 * (\frac{\delta}{1000} + 1)}$$

(Eq. 2)

To calculate the ^{13}C atom% excess in each compartment we used the equation:

$$\text{atom\% excess}^{13}\text{C} = \text{atom\%}_s - \text{atom\%}_b$$

(Eq. 3)

where $\text{atom\%}_s$ is the atom% of the sample and $\text{atom\%}_b$ is the atom% of the natural background (measured in unlabelled samples).

The CO_2 concentrations and isotopic compositions of the leaf and rhizome respired CO_2 were measured by gas–chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS). All EA-IRMS and GC-C-IRMS analyses were performed at the Scientific and Technological Centres of the University of Barcelona (CCiT).

Statistical analyses

In this study, we followed two different approaches pursuing the two main objectives. First, we compared the ^{13}C dynamics between the two species *V. myrtillus* and *V. uliginosum* under natural environmental conditions at both study sites. For this, we used all samples from Filià and samples from control plots in Stubai. Second, we compared the ^{13}C dynamics of both dwarf shrub species between drought and control plots in Stubai.

We used simple linear models to assess the differences in the ^{13}C dynamics of bulk plant material between *V. myrtillus* and *V. uliginosum* in Stubai and in Filià, and in the dynamics of respired ^{13}C in Filià. Models included plot, species identity and their interaction as fixed factors, and sampling time as a covariate. This way, models accounted for the repeated measures through time within plots. Plot was not treated as a random factor because it only had three levels (Zuur et al. 2007). We applied the same models to assess the effect of drought on the ^{13}C dynamics of *V. myrtillus* and *V. uliginosum* in Stubai. In this case, block, drought treatment and their interaction were included as fixed effects, and sampling time as a covariate. We tested for species identity effects and drought effects on pre- and post-labelling measurements (i.e. GPP, A_{max}, g_s and midday Ψ_S) using the same models. We visually tested for normality and homoscedasticity of residuals. When these assumptions were not met, data were log-transformed. To analyse species and drought treatment effects on pre-dawn Ψ_S we used generalized linear models with a gamma distribution. To test differences in the ^{13}C excess between dwarf shrub species and between drought treatment levels on each single date within the time series, we used exact permutation tests (see R script in Supplementary information). To estimate mean residence times (MRT) of the ^{13}C excess in leaves, we fitted the following exponential decay function:

$$N_{(t)} = N_0 \mathrm{e}^{(-\lambda t)}$$

(Eq. 4)

Where t is the time in hours after labelling; N_0 is the initial amount of ^{13}C excess at time $t = 0$ (^{13}C peak); λ is the decay constant and $N_{(t)}$ is the amount of ^{13}C after time t. The MRT was calculated as 1/λ. Only two replicates per species and drought treatment could be fitted due to the nature of the data.

We considered significant effects at $P < 0.05$ and marginally significant effects at $0.05 < P < 0.1$. We performed all the analyses with R 3.1.2 (R Core Team, 2015) and used the *nls* function to fit the exponential decay models by nonlinear least squares.

Results

Allocation of newly assimilated C in **V. myrtillus** *and* **V. uliginosum**

Physiological measurements

At the Stubai site, stomatal conductance (g_s) was higher ($F_{1,24}$ = 31.21, $P < 0.001$) in *V. uliginosum* than in *V. myrtillus* (Table 1, control plots). Ψ_S was zero in most of the samples of both species before sunrise, but some *V. myrtillus* ramets showed slightly negative values ($F_{1,12}$ = 6.12, P = 0.029; Table 1, control plots). Midday Ψ_S reached -1.81 ± 0.14 MPa in *V. uliginosum* and -1.57 ± 0.10 MPa in *V. myrtillus*, but they did not differ significantly ($F_{1,12}$ = 3.12, P = 0.103; Table 1, control plots). At Filià, leaf net assimilation (A_{max}) and g_s were also higher ($F_{1,24}$ = 7.45, P = 0.012; and $F_{1,24}$ = 7.31, P = 0.012, respectively) in *V. uliginosum* (15.37 ±1.2 µmol m^{-2} s^{-1} and 280 ± 30 mmol m^{-2} s^{-1}) than in *V. myrtillus* (11.6 ±1.0 µmols m^{-2} s^{-1} and 190 ± 20 mmols m^{-2} s^{-1}).

Table 1. Gross Primary Productivity (GPP; at plot scale), stomatal conductance (g_s) and water potential (Ψ) in drought and control plots (mean ± SE; n = 3) at Stubai. GPP was measured at plot scale, g_s and Ψ were measured on 5 and 3 ramets per plot, respectively. Results after the ANOVA tests for differences between treatments are shown in the last column. Significant differences (P < 0.05) and marginally significant differences (0.05 < P < 0.1) are in bold.

Parameter	Species	Control	Drought	*P*
GPP (µmol m^{-2} s^{-1})	-	1.68 ± 0.06	1.48 ± 0.03	**0.039**
g_s (mmol m^{-2} s^{-1})	*V. myrtillus*	179 ± 20	238 ± 26	**0.059**
	V. uliginosum	362 ± 61	172 ± 33	**<0.001**
ψ (MPa)	*V. myrtillus*			
Pre-dawn		-0.14 ± 0.14	-1.21 ± 0.19	**<0.001**
Midday		-1.57 ± 0.10	-1.68 ± 0.02	0.376
	V. uliginosum			
Pre-dawn		0.00 ± 0.00	-1.34 ± 0.19	**<0.001**
Midday		-1.81 ± 0.14	-2.04 ± 0.14	0.130

GPP and g_s measured on the 18 August 2014; Ψ measured on the 8 September 2014.

C dynamics in bulk plant material

At Stubai, we did not find significant differences between *V. myrtillus* and *V. uliginosum* in the time series of bulk ^{13}C excess in leaves ($F_{1,23}$ = 0.33, P = 0.572), bulk rhizome ($F_{1,22}$ = 0.19, P = 0.670) or rhizome phloem ($F_{1,22}$ = 0.11, P = 0.740) (Fig. 1a, b, c). The time series of the ^{13}C signal in the rhizome xylem did not differ between species either ($F_{1,23} = 0.90$, $P = 0.353$), but *V. uliginosum* showed higher ^{13}C values two and eight days after labelling ($P = 0.07$ and $P = 0.03$, respectively; Fig. 1d). Leaf MRT of ^{13}C in *V. myrtillus* was larger than in *V. uliginosum* (Table 2), but the statistical significance could not be tested because only one replicate could be fitted for *V. uliginosum*.

At Filià, the overall time series of ^{13}C did not differ between the two study species in leaves and rhizomes ($F_{1,33} = 0.22$, $P = 0.644$ and $F_{1,33} = 0.07$, $P = 0.795$, respectively; Fig. 2a, c). Moreover, the MRT of ^{13}C in leaves did not differ significantly between the two species either ($P = 0.25$, tested with exact permutation, Table 2). However, we found some indication that the decrease in ^{13}C values in *V. myrtillus* leaves was faster than in *V. uliginosum* (^{13}C values were slightly lower in the former at 48 h than in the latter, marginally significant, $P = 0.087$), reflecting a trend towards lower MRT. In rhizomes, we could not identify clear trends in the time series, as the inter-ramet variability was high. In contrast, we found clear differences between the two species in the overall time series of current-year shoots (Fig 2b). *Vaccinium myrtillus* showed higher ^{13}C excess values than *V. uliginosum* across the whole time series ($F_{1,33} = 25.05$, $P < 0.001$). At 2 h, both species exhibited a peak in ^{13}C, but *V. myrtillus* shoots showed slightly higher tracer concentrations than *V. uliginosum* (marginally significant, $P = 0.08$). After this peak, the signal decreased in *V. uliginosum* shoots and increased in *V. myrtillus* until the last sample was taken, two months after labelling.

Tracer dynamics in respired CO_2

Vaccinium myrtillus and *V. uliginosum* showed some differences in the pattern of ^{13}C dynamics of CO_2 respired by leaves and rhizomes (measured at Filià only). *Vaccinium myrtillus* leaves respired the newly assimilated C faster than *V. uliginosum*, as the peak in the respired ^{13}C in the former was at 2 h, whereas the peak in *V. uliginosum* appeared after 24 h. This lag was maintained throughout the whole time series, and 16 days after the labelling no more ^{13}C was respired by any of the *Vaccinium* spp. However, the overall time series of respired ^{13}C by leaves did not differ significantly between the two species ($F_{1,21} = 0.62$, $P = 0.440$; Fig. 3a). Respiration patterns in rhizomes also differed between the two species, although we did not find significant effects on the overall time series either ($F_{1,21}$ = 0.21, P = 0.655; Fig. 3b). Again, we found a lag between the two species, as the ^{13}C excess in respired C peaked earlier in *V. myrtillus* rhizomes (after 4 days) than in *V. uliginosum* (after 8 days) (P = 0.03 at 8 days). Similar as for leaves, respired C showed almost no signal of the tracer16 days after the labelling, only little ^{13}C excess remained in *V. uliginosum* rhizomes.

Table 2. Mean residence time (MRT) of ^{13}C and coefficient of determination (R^2) from the exponential decay function (Eq. 4) of ^{13}C atom% excess in *V. myrtillus* and *V. uliginosum* leaves during the 8-day period after pulse labeling in control and in drought plots at Stubai and during the 16-day period after pulse labeling at Filià. The number of time points used in the functions are indicated in the table (*n*).

		n	peak	MRT (h)	R^2	MRT(h) mean (± SE)
Stubai						
V. myrtillus						
	Control	5	2h	91.4	0.97	90.76 (0.65)
		4	2h	90.1	0.99	
	Drought	4	2h	39.3	0.95	61.78 (22.47)
		3	2h	84.2	0.42	
V. uliginosum						
	Control	5	2h	46.1	0.97	46.1 (-)
		-	-	-	-	
	Drought	4	2h	65.8	0.94	67.51 (1.74)
		4	2h	69.3	0.78	
Filià						
V. myrtillus		6	2h	11	0.95	18.5 (7.4)
		5	2h	25.7	0.98	
V. uliginosum		5	24h	53.6	0.94	33.3 (20.4)
		5	2h	12.9	0.97	

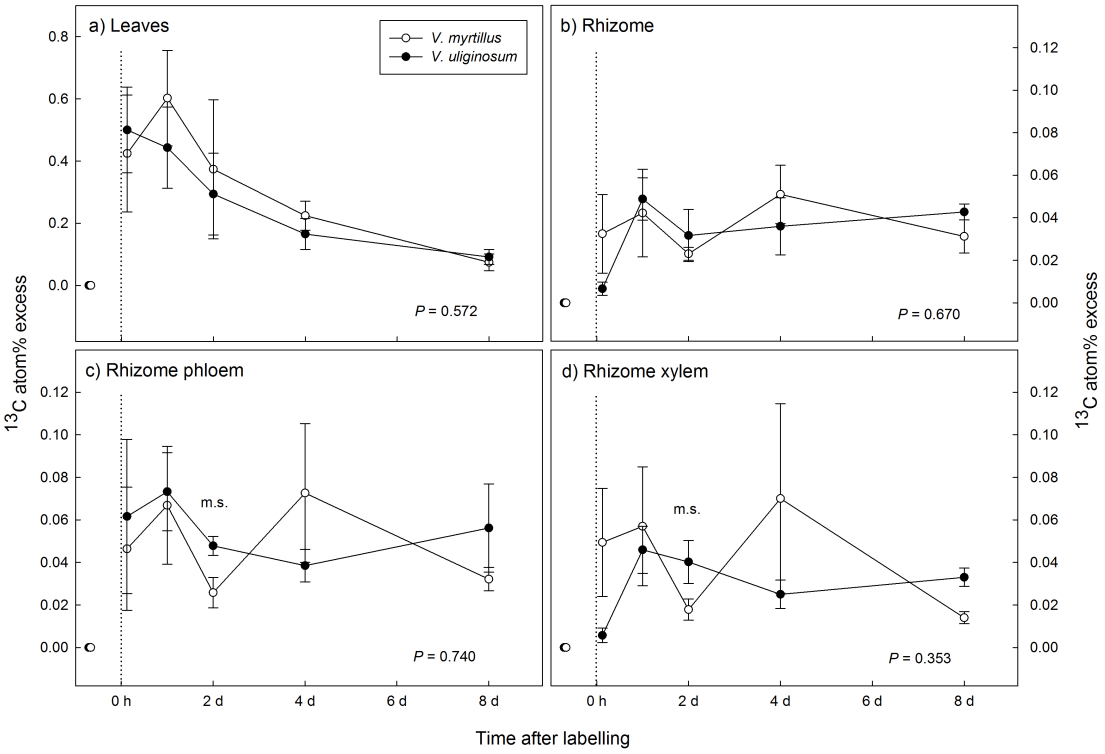

Figure 1. Dynamics of ^{13}C excess (atom%) after pulse labeling in leaves (a), bulk rhizomes (b) and rhizome phloem (c) and rhizome xylem (d) of *V. myrtillus* and *V. uliginosum* under ambient weather conditions at Stubai. The dotted vertical line indicates the labeling time. Asterisks indicate significant differences between *V. myrtillus* and *V. uliginosum* ($P < 0.05$) and m.s. indicates marginally significant differences ($0.05 < P < 0.1$) at the specific sampling time. Mean ±SE.

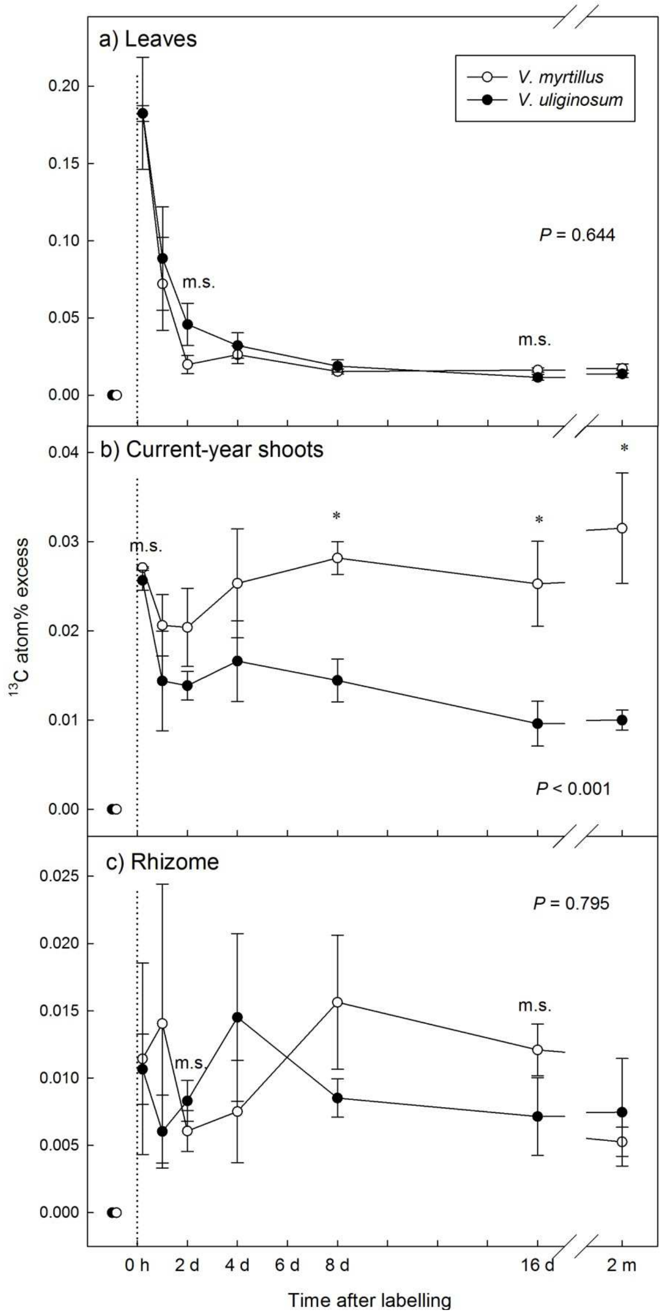

Figure 2. Dynamics of ^{13}C excess (atom%) after pulse labeling in leaves (a), current-year shoots (b) and rhizome (c) of *V. myrtillus* and *V. uliginosum* at Filià. The dotted vertical lines indicate the labeling time. Asterisks indicate significant differences between *V. myrtillus* and *V. uliginosum* ($P < 0.05$) and m.s. indicates marginally significant differences ($0.05 < P < 0.1$) at the specific sampling time.

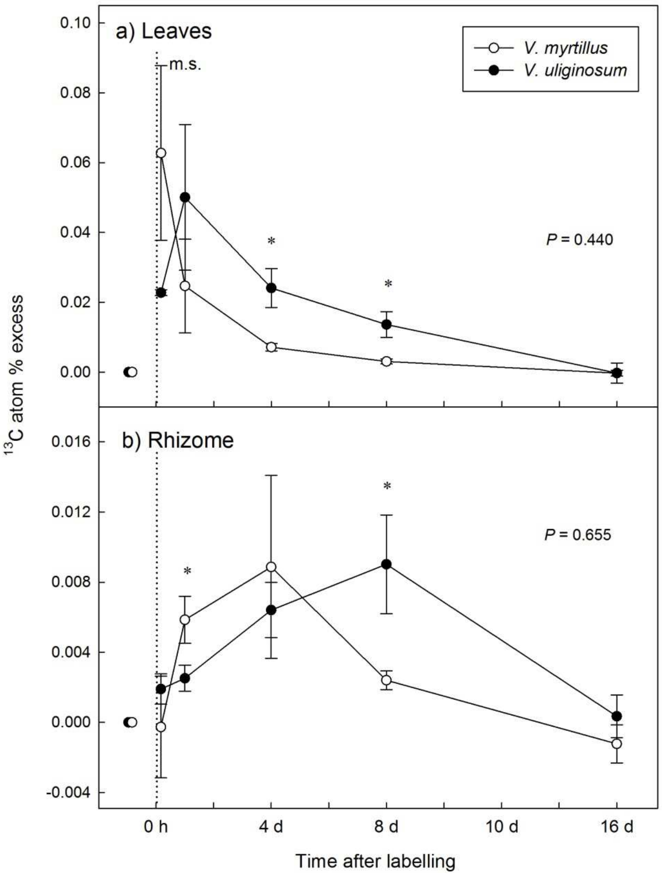

Figure 3. Dynamics of ^{13}C excess (atom%) in respired CO_2 after pulse labeling in leaves (a) and rhizome (b) of *V. myrtillus* and *V. uliginosum* at Filià. The dotted vertical lines indicate the labeling time. Asterisks indicate significant differences between *V. myrtillus* and *V. uliginosum* ($P < 0.05$) and m.s. indicates marginally significant differences ($0.05 < P < 0.1$) at the specific sampling time.

Effects of drought on the C allocation in **V. myrtillus** *and* **V. uliginosum**

Microclimate, gas exchange and water potential

Soil water content at 5 cm depth decreased to *ca.* 16% in drought plots by the end of the experiment, whereas in control plots it remained between 50-67% throughout the experiment ($P < 0.001$, Fig. 4). During the pulse-chase period, soil temperature was 1.2 °C higher in drought plots than in control plots during daytime ($P < 0.001$) and 0.4 ºC higher at night ($P < 0.001$; Fig. 4). Air temperature at *ca*. 150 cm height increased by 1.5 ºC inside the rain-out shelters over the whole experimental period ($P < 0.001$; Fig. 4). For data on RH and PAR see Fig. S1.

GPP in plots under rain-out shelters was reduced by 12% relative to controls (Table 1). g_s decreased by 52% under rain exclusion in *V. uliginosum*, but *V. myrtillus* showed the opposite, as g_s was slightly higher under rain exclusion than in controls (Table 1). Pre-dawn Ψ_S was significantly lower under rain exclusion than in control plots in both

Vaccinium species, and this was more pronounced for *V. uliginosum* (Table 1). At midday, we did not find significant differences between treatments in any species (Table 1).

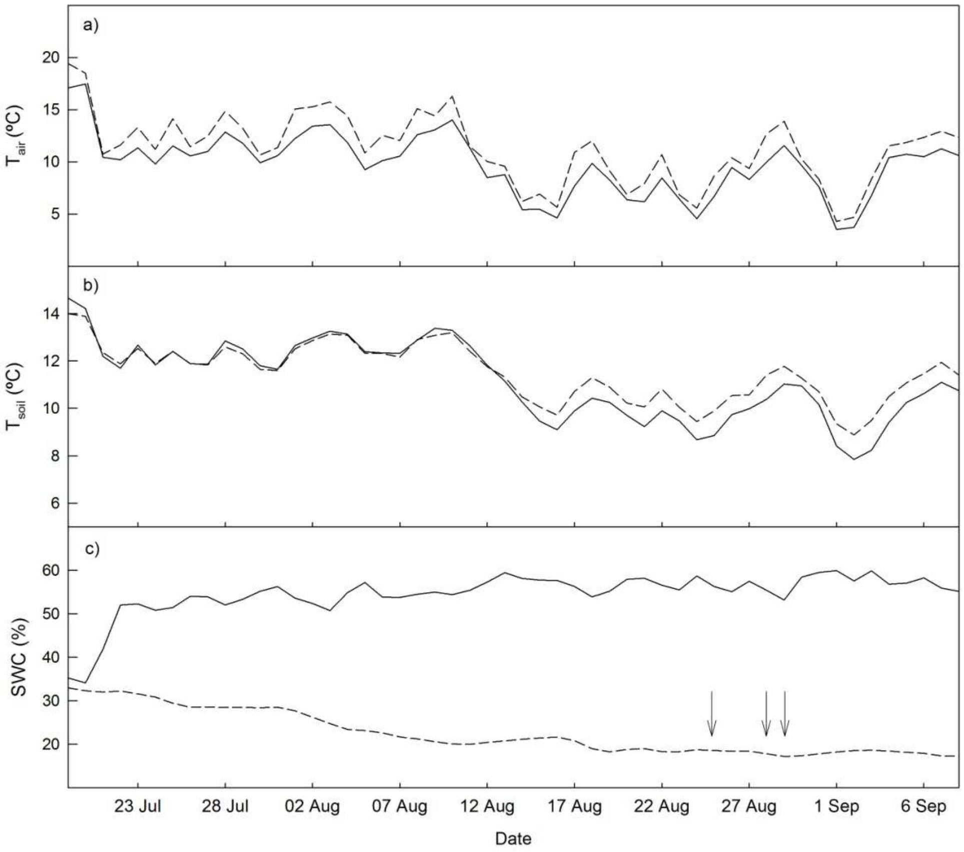

Figure 4. Microclimate during the drought experiment at Stubai: (a) air temperature inside and outside the rainout shelters (*n* = 1); (b) soil temperature and (c) soil volumetric water content (SWC) at 5 cm depth (mean values, *n* = 3). Graphs show daily means in control (solid lines) and drought (dotted lines) plots. Rainout shelters were installed on 3 July and removed on the 8 September 2014, after the last samples were harvested. The arrows indicate the three labeling campaigns (25, 28, 29 August 2014).

Tracer dynamics in bulk plant material

Drought did not have a strong impact on the ^{13}C dynamics of both species (Fig. 5). *Vaccinium myrtillus* did not show differences between drought and control plots in the overall time series of ^{13}C excess dynamics in leaves ($F_{1,23}$ = 0.30, P = 0.592, Fig. 5a), bulk rhizome ($F_{1,21}$ = 0.33, P = 0.573, Fig. 5c), rhizome phloem ($F_{1,22}$ = 0.31, P = 0.586, Fig. 5e) and rhizome xylem ($F_{1,23}$ = 0.15, P = 0.707, Fig. 5g). However, leaf MRT of ^{13}C was slightly higher in control plots than under drought (marginally significant, P = 0.08, tested with exact permutation). In contrast, *V. uliginosum* showed some differences between rain exclusion and control plots. Leaves showed higher ^{13}C excess values under drought throughout the time series (marginally significant, $F_{1,23}$ = 3.82, P = 0.063, Fig. 5b), indicating a slightly higher assimilation under drought than in control plots. ^{13}C MRT was greater in leaves under drought than in control plots, but this was only based in one replicate and statistics could not be applied. In the same line, the ^{13}C transfer to BG organs in *V. uliginosum* was slowed down under drought, as we found lower ^{13}C values in *V. uliginosum* rhizomes under drought than in control plots at 2 h after labelling (P = 0.017, Fig. 5d). From 24 h onwards, the trend was reverted, and rhizome ^{13}C values were higher under drought than in controls. The overall time series of ^{13}C in *V. uliginosum* rhizome phloem and xylem differed significantly between the drought treatments. ^{13}C values were higher under drought than in controls for both rhizome phloem ($F_{1,23}$ = 3.21, P = 0.087, Fig. 5f) and rhizome xylem ($F_{1,23}$ = 9.38, P = 0.006, Fig. 5h).

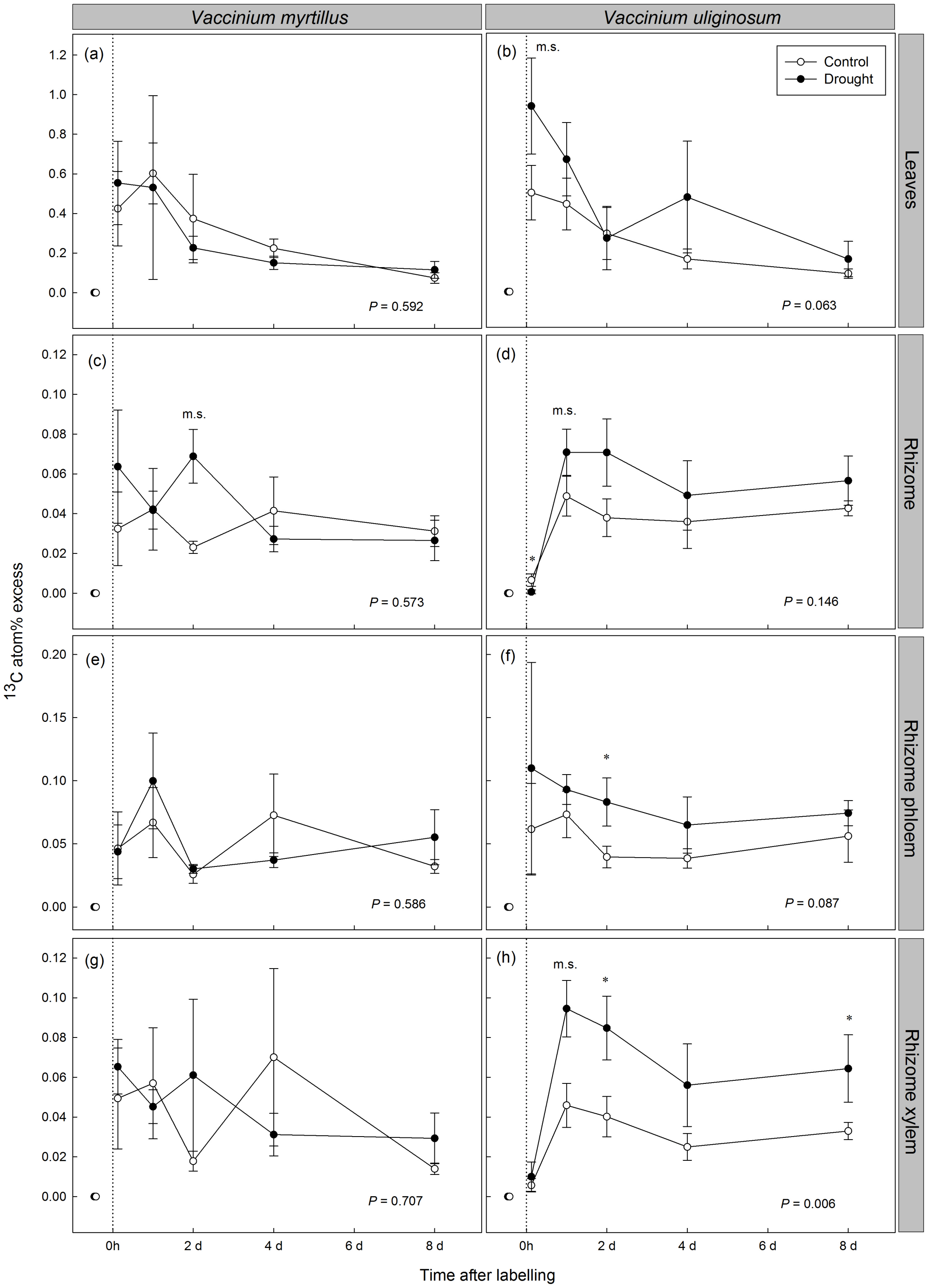

Figure 5. Dynamics of ^{13}C excess (atom %) after pulse labeling in leaves (a,b), bulk rhizome (c,d) and rhizome phloem (e,f) and rhizome xylem (g,h) of *V. myrtillus* and *V. uliginosum* in control and drought plots at Stubai. The dotted vertical lines indicate the labeling time. Asterisks indicate significant differences between control and drought plots ($P < 0.05$) and m.s. indicates marginally significant differences ($0.05 < P < 0.1$) at the specific sampling time.

Discussion

Physiology and C cycling of V. myrtillus *and* V. uliginosum

Our results were overall consistent across the two study sites and showed that intraspecific variability in the study traits was low. The geographical distance, differences in climate and different plant community composition and land-use history did not imply large differences in the physiology and the C cycling of the two *Vaccinium* species. *Vaccinium uliginosum* showed higher uptake of ^{13}C than *V. myrtillus* at both study sites, corresponding to higher A_{max} and higher g_s. (Table 1) A study in northern Sweden found that the photosynthetic rate throughout summer months was also lower in *V. myrtillus* than in *V. uliginosum* (Karlsson 1989), and another study on the same species reported that *V. uliginosum* leaves showed higher levels of photosynthetic light saturation (Karlsson 1987). All these results might be explained by a greater adaptation of *V. uliginosum* to higher irradiations than *V. myrtillus* (Jacquemart 1996; Ellenberg et al. 2001), reflecting the species preferences for more (*V. uliginosum*) versus less open vegetation (Jacquemart 1996).

Studies on hydraulic properties suggest that 50% xylem embolism is reached at a Ψ_s of *ca*. -2.1 MPa in *V. myrtillus* while at -2.7 MPa in *V. uliginosum* (Ganthaler and Mayr 2015a, b). In these studies, the turgor loss point was reached at similar Ψ_s (-1.4 MPa) in both species. This suggests that the minimum Ψ_s values observed in the present study at ambient conditions at midday (lower than -1.5 MPa) were below the turgor loss point previously reported, but not critical regarding embolism formation. The lack of significant differences in Ψ_s between our study species indicates they have a similar hydraulic status under ambient conditions at our study site. Before dawn, however, some *V. myrtillus* ramets showed negative Ψ_s, which could indicate that they were experiencing particularly dry soil conditions.

Vaccinium myrtillus and *V. uliginosum* have a contrasting shoot functionality, since only *V. myrtillus* young shoots have photosynthetic capacity. We hypothesised that this characteristic would lead to higher tracer concentrations in *V. myrtillus* shoots after labelling due to the C uptake by these organs. Indeed, ^{13}C dynamics in current-year shoots differed notably between the two species (Fig. 2b). Immediately after labelling, *V. myrtillus* showed a higher tracer concentration in current-year shoots than *V. uliginosum*, likely because *V. myrtillus* shoots assimilated ^{13}C during the labelling.

However, these differences were less pronounced than expected, possibly due to an overall low photosynthetic capacity of *V. myrtillus* shoots and/or a faster transfer of assimilates from leaves to current-year shoots in *V. uliginosum*. In the following weeks, the dynamics of ^{13}C clearly diverged between the two species, with a decrease of ^{13}C signal in *V. uliginosum* and an increase in *V. myrtillus* shoots. The progressive increase of ^{13}C in *V. myrtillus* current-year shoots might be due to the re-assimilation by photosynthetic shoots of C respired by the same shoots, old stems, rhizomes and roots, and transported through the transpiration stream, a process that has been reported in previous studies (Stringer and Kimmerer 1993; McGuire et al. 2009; Bloemen et al. 2013a,b). Remarkably, ^{13}C tracer concentrations in current-year shoots were not only maintained through time but even increased, with the largest ^{13}C values 2 months after labelling. McGuire et al. (2009) suggested that the importance of the C recycling function could change seasonally depending on conditions affecting leaf photosynthesis; thus, our results might be explained by an increased re-fixation function by photosynthetic shoots when leaf senescence starts. The C recycling by *V. myrtillus* shoots may be especially advantageous in periods of water stress, when stomata are closed and gas exchange is reduced (Aschan and Pfanz 2003).

At Stubai ^{13}C dynamics in rhizome phloem and xylem did not differ. Phloem is the major tissue for sugar transport, and non-structural carbohydrate (NSC) concentrations have been found to be higher in phloem than in xylem of tree species (Landhäusser and Lieffers 2003; Giovannelli et al. 2011; Gruber et al. 2013). Surprisingly, we did not find significant differences in the tracer signal between xylem and phloem either (data not shown), which might be related to the fact that xylem represents the major amount of tissue in woody organs and that xylem ray parenchyma serves as a storage tissue, and thus, can be a sink for new assimilates (Bell and Ojeda 1999; Verdaguer and Ojeda 2002; Plavcová and Jansen 2015). As reported in several studies on herbaceous and tree species (e.g. Bahn et al. 2013; Burri et al. 2014; Blessing et al. 2015), we found fast transport velocities of recent assimilated C to belowground organs, since ^{13}C signal in rhizomes appeared in less than 1 day after labelling.

Vaccinium myrtillus used newly assimilated C as a respiratory substrate faster than *V. uliginosum*, both in leaves and rhizomes (Fig. 3). The lag found in respiration did not correspond to any lags in the bulk material ^{13}C signal, therefore our results point towards a faster metabolism of *V. myrtillus* than *V. uliginosum* concerning the use of recent assimilates as respiratory substrate. Carbone and Trumbore (2007) studied shrub species in California and showed that aboveground and belowground ^{13}C respired peaked within less than 1 day after labelling, which is consistent with our results for leaf respiration. The later peak of ^{13}C in the rhizome respiration of our study species indicates they both reflect a slower use of recent assimilates for respiration in belowground organs. In fact, these results would be closer to those found by Carbone et al. (2007), who reported that the maximum peak of label in leaves and roots of *Picea mariana* in a boreal forest in Canada appeared 1 and 4 days after labelling, respectively. Nevertheless, *V. uliginosum* seems to present a particularly slow belowground respiratory metabolism of recent assimilates, as the peak in rhizomes took 4 more days than in *V. myrtillus* to appear.

Drought effects on the C allocation of two coexisting dwarf shrub species

Physiological measurements carried out before and after labelling suggest that plants under drought were only mildly stressed. Drought effects were more pronounced in *V. uliginosum* than in *V. myrtillus*, as reflected by the stronger decrease in Ψ_S and g_s under drought (Table 1). Our study species are woody, with a complex structure of belowground rhizomes and deeper rooting, which makes them less dependent on the water content in upper soil layers than herbaceous species (Báez et al. 2013; Hoover et al. 2016). This explains why they were less responsive to drought in terms of C uptake and C allocation dynamics than herbaceous species growing close to our study site (Hasibeder et al. 2015). Ganthaler and Mayr (2015a, b) reported that *V. myrtillus* and *V. uliginosum* follow risky hydraulic strategies when facing drought, such as incomplete stomatal closure until shoots reach low Ψ or a low resistance to drought-induced embolism. Such low hydraulic safety is compensated by recovery mechanisms including the refilling of embolized vessels (Ganthaler and Mayr 2015a,b). We also found relatively high g_s despite Ψ_S corresponding to 20-30 % xylem embolism were reached, especially in *V. myrtillus*, which surprisingly showed even higher g_s under drought than in control conditions. A study by Correia et al. (1999) showed that *Lupinus albus* stomatal conductance increased with warming both in well-watered and moderately water-stressed plants. Thus, the slight increase in the stomatal conductance of *V. myrtillus* in our study could be related to the temperature increase inside the rain-out shelters, even if at the shrub canopy height temperature was not largely increased. In addition, an experimental drought in a Walsh heather moorland (UK) showed that leaf gas exchange of *V. myrtillus* was not significantly affected by drought (Llorens et al. 2002). Altogether, these results support the idea that *V. myrtillus* follows a more risky hydraulic strategy than *V. uliginosum*, and suggests it has a relatively high resistance to (moderate) water stress. Interestingly, although *V. uliginosum* showed a clear reduction in g_s under drought, it assimilated more ^{13}C during labelling (Fig. 5b). One explanation could be that in control plots a great amount of $^{13}CO_2$ was assimilated by grasses and forbs during labelling, as they have a more active metabolism than dwarf shrubs (Körner 2003). Contrastingly, in drought plots grasses and forbs were probably more severely drought-stressed (see Hasibeder et al. 2015) and thus, not assimilating much. Higher photosynthetic rates of *V. uliginosum* than *V. myrtillus* would explain that the former was more responsive to this effect and assimilated more C during labelling. The higher ^{13}C assimilation under drought explains that ^{13}C excess values in the other plant parts were higher under rain exclusion than in controls (Fig 5 d, f, h).

We found evidence of a slower C allocation to belowground organs under drought in *V. uliginosum*, as the tracer showed up earlier in rhizomes from control plots than in those subjected to drought (and, although based on a single replicate, this would be supported by the higher MRT in *V. uliginosum* leaves under drought) (Fig 5d, Table 2). These findings are consistent with other drought experiments. Ruehr et al. (2009) also found that the rate of loss in ^{13}C excess from the leaves of one-year-old beech trees (*Fagus sylvatica* L.) was reduced under drought in a glasshouse experiment. Moreover, they found a reduction in phloem transport velocity under drought. Another experiment in a mountain meadow close to our study site also reported a reduction in the speed of C transfer from shoots to roots in drought plots (Hasibeder et al. 2015). We did not find lower tracer concentrations in the rhizome phloem under drought (Fig. 5f), thus we hypothesize that the reduction in the speed of C transfer to belowground organs in *V. uliginosum* could be due to an alteration at the phloem unloading stage rather than during uploading or transport of assimilates. Our findings also indicate that C allocation in *V. myrtillus* was not affected by the simulated drought which, together with Ψ_S and g_s data, suggest that *V. myrtillus* may be more resistant to drought episodes than *V.*

uliginosum. It remains unknown whether *V. uliginosum* would be more negatively affected under severe drought episodes in the long term, which could, in turn, have large impacts on community composition, structure and function.

Concluding remarks

Our study showed differences in physiological traits and C cycling of the two co-occurring subalpine dwarf shrubs *V. myrtillus* and *V. uliginosum*, as well as their response to an experimental summer drought. Intraspecific variability between study sites in traits related to C assimilation and allocation was low. The photosynthetic behaviour of *V. myrtillus* shoots explained the largest differences between the two species in their C allocation dynamics, suggesting an important potential of green shoots for C re-assimilation. Both species were quite resistant to drought, although *V. uliginosum* showed some evidence of suffering from mild stress, and its short-term C allocation dynamics were slightly affected. At our study site in the Alps, drought seems to have more negative effects on the C uptake and allocation dynamics of herbaceous species than on shrubs (Hasibeder et al. 2015). Since drought events affect co-occurring dwarf shrub species and plant functional types differently, shifts on community composition, structure and function might occur in the long term under a more extreme climate.

Acknowldegements

We are thankful to all the people who collaborated during, before and after the labelling experiments in Stubai and Filià. We especially want to thank Irene Febrer, Alexander König, Marc Fradera, Nil Escolà, Isabel Llorca, Zoë Feder, Elias Cabrera, Pere Anadón, Empar Carrillo, Marc Talavera and Oriol Grau for their help in the field. We also want to thank Pilar Rubio and Pilar Teixidor from the CCiT of the University of Barcelona for their technical assistance and support, Biel Obrador and Serveis de Camps Experimentals of the University of Barcelona for lending us the IRGAs used in this study and Eudald Pujol and Luis Cayuela for their statistical advice. This work was supported by the project ARBALMONT /786-2012 (Organismo Autónomo Parques Nacionales, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain). AAR was funded by a STSM grant provided by the COST Action STReESS (FP1106) and an

FPU grant (Ministerio de Educación, Cultura y Deporte, Spain). SP was funded by a Ramón y Cajal fellowship (RYC-2013-14164, Ministerio de Economía y Competitividad, Spain).

References

References

Aeschimann D., Lauber, K., Moser D.M., Theurillat, J.-P. (2004) Flora alpina. Vol 1. Haupt, Bern, Stuttgart, Wien.

Aschan G., Pfanz H. (2003) Non-foliar photosynthesis – a strategy of additional carbon acquisition. Flora - Morphology, Distribution, Functional Ecology of Plants **198**:81 – 97.

Báez S., Collins S.L., Pockman W.T., Johnson J.E., Small E.E. (2013) Effects of experimental rainfall manipulations on Chihuahuan Desert grassland and shrubland plant communities. Oecologia **172**:1117–1127.

Bahn M., Schmitt M., Siegwolf R., Richter A., Brüggemann N. (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale? New Phytologist **182:** 451-460. doi: 10.1111/j.1469-8137.2008.02755.x

Bahn M., Lattanzi F.A., Hasibeder R., Wild B., Koranda M., Danese V., Brüggemann N., Schmitt M., Siegwolf R., Richter A. (2013) Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. New Phytologist **198**:116–126.

Barros C., Guéguen M., Douzet R., Carboni M., Boulangeat I., Zimmermann N.E., Münkemüller T., Thuiller W. (2016) Extreme climate events counteract the effects of climate and land-use changes in Alpine tree lines. Journal of Applied Ecology. doi: 10.1111/1365-2664.12742

Barthel M., Cieraad E., Zakharova A., Hunt J.E. (2014) Sudden cold temperature delays plant carbon transport and shifts allocation from growth to respiratory demand. Biogeosciences **11**:1425–1433.

Barthel M., Hammerle A., Sturm P., Baur T., Gentsch L., Knohl A. (2011) The diel imprint of leaf metabolism on the $\delta^{13}C$ signal of soil respiration under control and drought conditions. New Phytologist **192**:925–938.

Batllori E., Camarero J.J., Ninot J.M., Gutiérrez E. (2009) Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* tree line ecotones. Implications and potential responses to climate warming. Global Ecology and Biogeography **18**:460–472.

Bell T.L., Ojeda F. (1999) Underground starch storage in *Erica* species of the Cape Floristic Region - Differences between seeders and resprouters. New Phytologist **144**:143–152.

Blessing C.H., Werner R.A., Siegwolf R., Buchmann N. (2015) Allocation dynamics of recently fixed carbon in beech saplings in response to increased temperatures and drought. Tree Physiology **35**:585–598.

Bloemen J., McGuire M. A., Aubrey D.P., Teskey R.O., Steppe K. (2013a) Internal recycling of respired CO_2 may be important for plant functioning under changing climate regimes. Plant Signaling & Behavior **8**:e27530.

Bloemen J., McGuire M.A., Aubrey D.P., Teskey R.O., Steppe K. (2013b) Transport of root-respired CO_2 via the transpiration stream affects aboveground carbon assimilation and CO_2 efflux in trees. New Phytologist **197**:555–565.

Bolòs O, Vigo J, Masalles RM, Ninot JM (2005) Flora Manual dels Països Catalans. 3rd ed. rev. and ext. Ed. Pòrtic SA, Barcelona.

Bragazza L. Parisod J., Buttler A., Bardgett R.D. (2013) Biogeochemical plant-soil microbe feedback in response to climate warming in peatlands. Nature Climate Change 3: 273-277. doi: 10.1038/nclimate1781

Brüggemann N., Gessler A., Kayler Z., Keel S.G., Badeck F., Barthel M., Boeckx P., Buchmann N., Brugnoli E., Esperschütz J., Gavrichkova O., Ghashghaie J., Gomez-Casanovas N., Keitel C., Knohl A., Kuptz D., Palacio S., Salmon Y., Uchida Y., Bahn M. (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences **8**: 3457–3489.

Burri S., Sturm P., Prechsl U.E., Knohl A., Buchmann N. (2014) The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO_2 efflux in a temperate grassland. Biogeosciences **11**:961–975.

Carbone M.S., Czimczik C.I., McDuffee K.E., Trumbore S.E. (2007) Allocation and residence time of photosynthetic products in a boreal forest using a low-level ^{14}C pulse-chase labeling technique. Global Change Biology **13**:466–477.

Carbone M.S., Trumbore S.E. (2007) Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. New Phytologist **176**:124–135.

Chaves M.M., Pereira J.S., Maroco J., Rodrigues M.L., Ricardo C.P.P., Osório M.L., Carvalho I., Faria T., Pinheiro C. (2002) How plants cope with water stress in the field. Photosynthesis and growth. Annals of Botany **89**:907–916.

Ciais P., Reichstein M., Viovy N., Granier A., Ogée J., Allard V., Aubinet M., Buchmann N., Bernhofer C., Carrara A., Chevallier F., De Noblet N., Friend A.D., Friedlingstein P., Grünwald T., Heinesch B., Keronen P., Knohl A., Krinner G., Loustau D., Manca G., Matteucci G., Miglietta F., Ourcival J.M., Papale D., Pilegaard K., Rambal S., Seufert G., Soussana J.F., Sanz M.J., Schulze E.D., Vesala T., Valentini R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature **437**:529–533.

Correia M.J., Rodrigues M.L., Ósorio M.L., Chaves M.M. (1999) Effects of growth temperature on the response of lupin stomata to drought and abscisic acid. Functional Plant Biology **26**:549–559.

Dai A. (2013) Increasing drought under global warming in observations and models. Nature Climate Change **3**:52–58.

Desalme D., Priault P., Gérant D., Dannoura M., Maillard P., Plain C., Epron D. (2017) Seasonal variations drive short-term dynamics and partitioning of recently assimilated carbon in the foliage of adult beech and pine. New Phytologist **213**:140–153.

Dullinger S., Dirnböck T., Grabherr G. (2003) Patterns of shrub invasion into high mountain grasslands of the Northern Calcareous Alps, Austria. Arctic, Antarctic, and Alpine Research **35**:434–441.

Eldridge D.J., Bowker M.A., Maestre F.T., Roger E., Reynolds J.F., Whitford W.G. (2011) Impacts of shrub encroachment on ecosystem structure and functioning: Towards a global synthesis. Ecology Letters **14**:709–722.

Ellenberg H., Weber H.E. Düll R., Wirth V., Werner W. (2001) Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica 18. 3rd ed. Göttingen.

Epron D., Bahn M., Derrien D., Lattanzi F.A., Pumpanen J., Gessler A., Högberg P., Maillard P., Dannoura M., Gérant D., Buchmann N. (2012) Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. Tree Physiology **32**:776–798.

Ernst M.D. (2004) Permutation Methods: A Basis for Exact Inference. Statist Sci **19:** 676–685. doi:10.1214/088342304000000396

Flexas J., Bota J., Galmés J., Medrano H., Ribas-Carbó M. (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiologia Plantarum **127**:343–352.

Frank D., Reichstein M., Bahn M., Thonicke K., Frank D., Mahecha M.D., Smith P., van der Velde M., Vicca S., Babst F., Beer C., Buchmann N., Canadell J.G., Ciais P., Cramer W., Ibrom A., Miglietta F., Poulter B., Rammig A., Seneviratne S.I., Walz A., Wattenbach M., Zavala M.A., Zscheischler J. (2015) Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. Global Change Biology **21**: 2861–2880. doi: 10.1111/gcb.12916

Fuchslueger L., Bahn M., Fritz K., Hasibeder R., Richter A. (2014) Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytologist **201**:916–927.

Ganthaler A., Mayr S. (2015a) Dwarf shrub hydraulics: two *Vaccinium* species (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*) of the European Alps compared. Physiologia Plantarum **155**:424–434.

Ganthaler A., Mayr S. (2015b) *Vaccinium gaultherioides*: another insight into water relations of alpine dwarf shrubs. Journal of Plant Hydraulics **2**: e-004. doi:http://dx.doi.org/10.20870/jph.2015.e004.

Giovannelli A., Emiliani G., Traversi M.L., Deslauriers A., Rossi S. (2011) Sampling cambial region and mature xylem for non structural carbohydrates and starch analyses. Dendrochronologia **29**:177 – 182.

Grau O., Ninot J.M., Blanco-Moreno J.M., van Logtestijn R.S.P., Cornelissen J.H.C., Callaghan T.V. (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. Oikos **121**:1680–1690.

Grau O., Ninot J.M., Cornelissen J.C., Callaghan T.V. (2013) Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. Plant Ecology and Diversity **6**:329–342.

Gruber A., Pirkebner D., Oberhuber W. (2013) Seasonal dynamics of mobile carbohydrate pools in phloem and xylem of two alpine timberline conifers. Tree Physiology **33**:1076–1083.

Hartmann H., McDowell N.G., Trumbore S. (2015) Allocation to carbon storage pools in Norway spruce saplings under drought and low CO_2. Tree Physiology **35**:243–252.

Hartmann H., Trumbore S. (2016) Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. New Phytologist **211**: 386–403. doi: 10.1111/nph.13955

Hasibeder R., Fuchslueger L., Richter A., Bahn M. (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phytologist **205**:1117–1127.

Heizmann U., Kreuzwieser J., Schnitzler J.-P., Brüggemann N., Rennenberg H. (2001) Assimilate transport in the xylem sap of pedunculate oak (*Quercus robur*) saplings. Plant Biology **3**:132–138.

Högberg P., Read D.J. (2006) Towards a more plant physiological perspective on soil ecology. Trends in Ecology & Evolution **21**:548 – 554.

Hommel R., Siegwolf R., Zavadlav S., Arend M., Schaub M., Galiano L., Haeni M., Kayler Z.E., Gessler A., Adams W. (2016) Impact of interspecific competition and drought on the allocation of new assimilates in trees. Plant Biology **18**: 785–796. doi: 10.1111/plb.12461

Hoover D.L., Duniway M.C., Belnap J. (2016) Testing the apparent resistance of three dominant plants to chronic drought on the Colorado Plateau. Journal of Ecology. doi: 10.1111/1365-2745.12647

IPCC (2013) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, New York.

Jacquemart A.-L. (1996) *Vaccinium uliginosum* L. Journal of Ecology **84**:771–785.

Karlsson P.S. (1985) Patterns of carbon allocation above ground in a deciduous (*Vaccinium uliginosum*) and an evergreen (*Vaccinium vitis-idaea*) dwarf shrub. Physiologia Plantarum **63**:1-7.

Karlsson P.S. (1987) Micro-site performance of evergreen and deciduous dwarf shrubs in a subarctic heath in relation to nitrogen status. Ecography **10**:114–119.

Karlsson P.S. (1989) In situ photosynthetic performance of four coexisting dwarf shrubs in relation to light in a subarctic woodland. Functional Ecology **3**:481–487.

Körner C. (2003) Alpine plant life. Functional plant ecology of high mountain ecosystems. 2nd edn. Springer, Berlin.

Krepkowski J., Gebrekirstos A., Shibistova O., Bräuning A. (2013) Stable carbon isotope labeling reveals different carry-over effects between functional types of tropical trees in an Ethiopian mountain forest. New Phytologist **199**:441–451.

Kuzyakov Y., Gavrichkova O. (2010) Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology **16**: 3386–3406.

Landhäusser S.M., Lieffers V.J. (2003) Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees **17**:471–476.

Liang E., Wang Y., Piao S., Lu X., Camarero J.J., Zhu H., Zhu L., Ellison A.M., Ciais P., Peñuelas J. (2016) Species interactions slow warming-induced upward shifts of treelines on the Tibetan Plateau. Proceedings of the National Academy of Sciences of the United States of America **113**:4380–4385.

Litton C.M., Raich J.W., Ryan M.G. (2007) Carbon allocation in forest ecosystems. Global Change Biology 13:2089–2109.

Llorens L., Peñuelas J., Emmett B. (2002) Developmental instability and gas exchange responses of a heathland shrub to experimental drought and warming. International Journal of Plant Sciences **163**:959–967.

Maestre F.T., Eldridge D.J., Soliveres S. (2016) A multifaceted view on the impacts of shrub encroachment. Applied Vegetation Science **19**:369–370.

Martin M., Gavazov K., Körner C., Hättenschwiler S., Rixen C. (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Global Change Biology **16**:1057–1070.

McGuire M.A., Marshall J.D., Teskey R.O. (2009) Assimilation of xylem-transported ^{13}C-labelled CO_2 in leaves and branches of sycamore (*Platanus occidentalis* L.). Journal of Experimental Botany **60**:3809–3817.

Ninot J.M., Batllori E., Carrillo E., Carreras J., Ferré A., Gutiérrez E. (2008) Timberline structure and limited tree recruitment in the Catalan Pyrenees. Plant Ecology & Diversity **1**: 47–57. doi: 10.1080/17550870802260764

Plavcová L., Jansen S. (2015) The role of xylem parenchyma in the storage and utilization of nonstructural carbohydrates. In: Hacke (ed.) Functional and ecological xylem anatomy. Springer. 281 pp.

Poorter H., Niklas K.J., Reich P.B., Oleksyn J., Poot P., Mommer L. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193:30–50

Popp M, Lied W, Meyer AJ, Richter A, Schiller P, Schwitte H. (1996) Sample preservation for determination of organic compounds: microwave versus freeze-drying. Journal of Experimental Botany **47**: 1469-1473.

Prévosto B., Kuiters L., Bernhardt-Römermann M., Dölle M., Schmidt W., Hoffmann M., Van Uytvanck J., Bohner A., Kreiner D., Stadler J., Klotz S., Brandl R. (2011) Impacts of land abandonment on vegetation: successional pathways in European habitats. Folia Geobotanica **46**:303–325.

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Ritchie JC (1956) *Vaccinium myrtillus* L. Journal of Ecology **44**:291-299.

Reichstein M., Bahn M., Ciais P., Frank D., Mahecha M.D., Seneviratne S.I., Zscheischler J., Beer C., Buchmann N., Frank D.C., Papale D., Rammig A., Smith P., Thonicke K., van der Velde M., Vicca S., Walz A., Wattenbach M. (2013) Climate extremes and the carbon cycle. Nature **500**: 287–295. doi: 10.1038/nature12350

Ritter W., Andersen C.P., Matyssek R., Grams T.E.E. (2011) Carbon flux to woody tissues in a beech/spruce forest during summer and in response to chronic O_3 exposure. Biogeosciences **8**: 3127–3138. doi: 10.5194/bg-8-3127-2011

Ropars P., Boudreau S. (2012) Shrub expansion at the forest-tundra ecotone: Spatial heterogeneity linked to local topography. Environmental Research Letters **7**. doi:10.1088/1748-9326/7/1/015501

Ruehr N.K., Offermann C.A., Gessler A., Winkler J.B., Ferrio J.P., Buchmann N., Barnard R.L. (2009) Drought effects on allocation of recent carbon: From beech leaves to soil CO_2 efflux. New Phytologist **184**:950–961.

Sanz-Elorza M., Dana E.D., González A., Sobrino E. (2003) Changes in the high-mountain vegetation of the Central Iberian Peninsula as a probable sign of global warming. Annals of Botany **92**: 273–280. doi: 10.1093/aob/mcg130

Schmitt M., Bahn M., Wohlfahrt G., Tappeiner U., Cernusca A. (2010) Land use affects the net ecosystem CO_2 exchange and its components in mountain grasslands. Biogeosciences **7**:2297–2309. doi: 10.5194/bg-7-2297-2010

Streit K., Rinne K.T., Hagedorn F., Dawes M.A., Saurer M., Hoch G., Werner R.A., Buchmann N., Siegwolf R.T.W. (2013) Tracing fresh assimilates through *Larix decidua* exposed to elevated CO_2 and soil warming at the alpine treeline using compound-specific stable isotope analysis. New Phytologist **197**:838–849.

Stringer J.W., Kimmerer T.W. (1993) Refixation of xylem sap CO_2 in *Populus deltoides*. Physiologia Plantarum **89**:243–251.

Tasser E., Tappeiner U. (2002) Impact of land use changes on mountain vegetation. Applied Vegetation Science **5**: 173–184. doi: 10.1111/j.1654-109X.2002.tb00547.x

Verdaguer D., Ojeda F. (2002) Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape Erica (*Ericaceae*) species. American Journal of Botany **89**:1189–1196.

Wheeler J.A., Hoch G., Cortés A.J., Sedlacek J., Wipf S., Rixen C. (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. Oecologia **175**: 219–229. doi: 10.1007/s00442-013-2872-8

Yuan W., Cai W., Chen Y., Liu S., Dong W., Zhang H., Yu G., Chen Z., He H., Guo W., Liu D., Liu S., Xiang W., Xie Z., Zhao Z., Zhou G. (2016) Severe summer heatwave and drought strongly reduced carbon uptake in Southern China. Scientific Reports **6**:18813. doi: 10.1038/srep18813

Zhao M., Running S.W. (2010) Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. Science **329**:940–943.

Zuur A.F., Ieno E. N., Smith G. M. (2007) Analyzing ecological data. Springer, New York, USA.

Supporting information

Table S1. Summary of the measurements taken at each study site. In Stubai, measurements were taken at ambient conditions and under rain exclusion.

Parameter	Stubai	Filià
A_{max}		x
g_s	X	x
Ψ_S	X	
GPP	X	
C allocation dynamics		
Leaves	X	x
Current-year shoots		x
Rhizome	X	x
Rhizome phloem	X	
Rhizome xylem	X	
Leaf respiration		x
Rhizome respiration		x

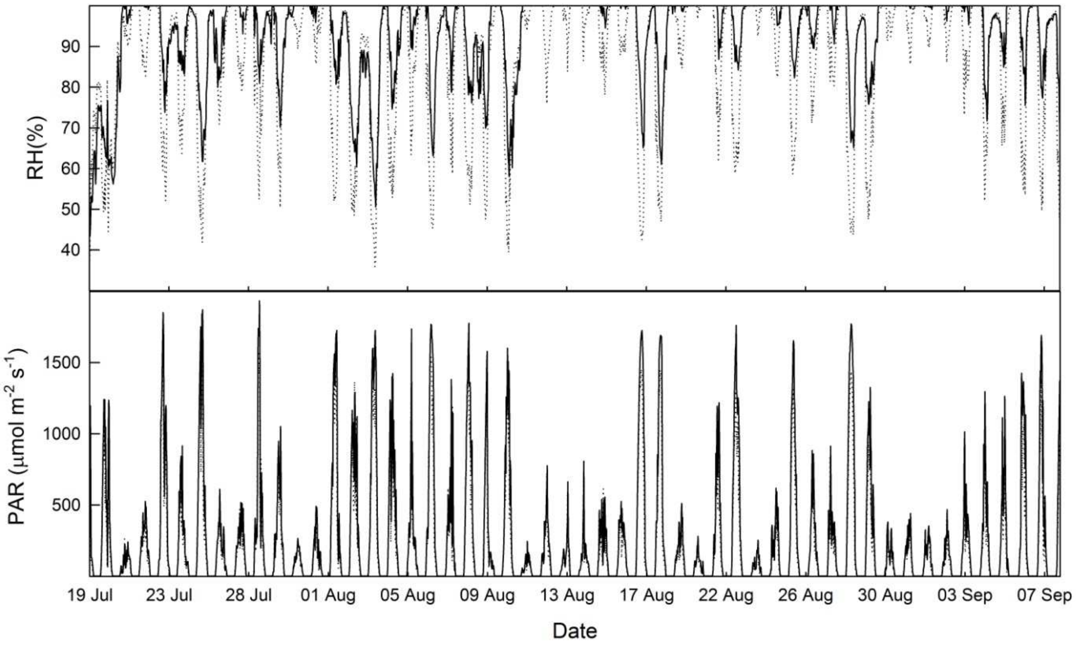

Figure S1. Dynamics of relative air humidity (top) and photosynthetically active radiation (PAR, bottom) during the experiment at Stubai in control (solid lines) and drought plots (dotted lines). Plotted values were recorded every half hour.

R Code Permutation tests

```
##### Permutation tests #########

# The following function was build to create simulations for paired data at single sampling dates. The example here may be used when control (C) values are smaller than drought (D), but it can be modified for those cases where C > D. Thus, here dataC corresponds to the three replicates for control at the specific sampling date, and dataD corresponds to the three drought replicates. It may also be implemented for the comparison between species, in which case C and D should be substituted by the two species V. myrtillus (M) and V. uliginosum (U).

# Function exact permutation for paired data (C < D):
exactpairs <- function (dataC, dataD, sims=999) {
  #dataC <- Vac2$C
  #dataD <- Vac2$D
  #sims <- 999
  #
  sims <- sims
  diffsreal <- dataC - dataD
  df <- matrix(ncol = length(dataC),nrow = sims+1)
  df[1,] <- diffsreal
  #df
  for(i in 1:sims) {
    joint <- c(dataC,dataD)
    jointrandom <- sample(joint)
    #joint
    #jointrandom
    dataCsim <- jointrandom[1:length(dataC)]
    #dataCsim
    ini <- length(dataC)+1
    dataDsim <- jointrandom[ini:length(jointrandom)]
    #dataDsim
    diffsims <- dataCsim - dataDsim
    df[i+1,] <- diffsims
  }
  df}
###

# Once the exactpairs function is run, the following code may be used for the exact permutation tests.
simus <- 9999
labelling <- exactpairs(dataC = Vac2$C, dataD = Vac2$D, sims = simus)
labelling <- as.data.frame(labelling)
labelling$mean <- apply(X = labelling, MARGIN = 1, FUN = mean) #We have organised our data in a dataframe and we have added a new column with the means

hist(labelling$mean, breaks=40, col="grey") #little exploration of our data
abline(v=labelling$mean[1], col="red")

labelling$mean[1] #Let's obtain the p-value
labelling$mean[2:simus+1]

SO <- sort(labelling$mean, decreasing = F)
n <- rank(labeling$mean)[1]
mean(pvals)
```

Discussió general

Les comunitats arbustives poden cobrir notables extensions a l'alta muntanya. Com a conseqüència dels canvis en l'ús del sòl (com ara l'abandonament de la ramaderia extensiva i les pràctiques agrícoles tradicionals) i l'escalfament global (Theurillat i Guissan 2001; Beniston 2003; Dullinger et al. 2003; Roura-Pascual et al. 2005; Komac et al. 2013), aquestes comunitats s'estan expandint, colonitzant pastures i prats. Això implica que el coneixement del seu funcionament i dinàmica sigui de gran importància per entendre i predir el futur d'aquestes comunitats i de la dinàmica dels ecosistemes davant de la societat i el clima canviants. Els subarbusts són un component clau de moltes d'aquestes comunitats (Körner 2003; Ninot et al. 2008; Illa et al. in review) i, com a conseqüència, el seu estudi i l'estudi de les seves respostes al canvi global tenen un interès cabdal.

En aquesta Tesi Doctoral, es tracten nombrosos aspectes relacionats amb els efectes del canvi climàtic en subarbusts d'alta muntanya (vegeu Fig. 8 de la Introducció). Les anàlisis de les respostes fenològiques, fisiològiques, anatòmiques i de creixement en experiments *in situ* d'escalfament, d'augment de CO_2 i de sequera estival han proporcionat una informació molt interessant sobre el comportament dels subarbusts i les seves interaccions amb espècies veïnes sota condicions ambientals predites per al futur. A més, també es tracten diversos aspectes del funcionament de *V. myrtillus* i de la seva interacció amb les espècies veïnes en condicions ambientals naturals, que han contribuït a comprendre millor les respostes d'aquestes espècies als canvis en les condicions ambientals. En la Taula 1 hi ha un resum dels principals efectes dels tractaments aplicats en cadascuna de les espècies estudiades. Tot i que aquesta Tesi se centra principalment en les espècies *V. myrtillus* i *V. uliginosum*, els resultats obtinguts tenen implicacions que abasten tota la comunitat i l'ecosistema.

Taula 1. Resum dels principals resultats obtinguts en aquesta Tesi Doctoral.

Espècie	**Tractament**	**Paràmetre**	**Efecte**	**Capítol**	**Observacions**
V. myrtillus	Escalfament de l'aire	Biomassa aèria i alçada	Augment	2	
		Biomassa subterrània	Cap efecte	2	
		Fenologia vegetativa primerenca	Avançament	2	
		Interaccions planta-planta	Cap efecte	2	
		Concentració de N del sòl	Augment concentració NO_3^- del sòl	2	
	Escalfament del sòl	Biomassa aèria i elongació tija	Augment	3	
		Fenologia vegetativa primerenca	Cap efecte	3	Sí relacionada amb la fosa de la neu
		Fenologia vegetativa tardana	Cap efecte	3	Sí relacionada amb la fosa de la neu
		Floració	Avançament	3	
		Edat	Cap efecte		
		Mida del lumen dels vasos i conductivitat hidràulica	Reducció, sobretot els primers anys	4	
		Distribució espacial dels vasos	Reducció de l'agrupament de vasos	4	
		Teixits de reserva (parènquima radial)	Major proporció de radis biseriats	4	
	Augment de CO_2	Amplada anells de creixement	Cap efecte	4	
		Mida del lumen dels vasos i conductivitat hidràulica	Augment els últims anys	4	
		Distribució espacial dels vasos	Cap efecte	4	
		Teixits de reserva (parènquima radial)	Cap efecte	4	
	Sequera estival	Dinàmica assignació de C	Cap efecte	5	
		Conductància estomàtica	Lleuger augment	5	

Taula 1. (cont.)

		Potencial hídric (tija)	Reducció (alba); Cap efecte (migdia)	5	
	Espècie arbòria	Biomassa aèria i elongació tija	Més gran sota pi	3	
		Amplada anells de creixement	Més gran a larix	4	
		Fenologia vegetativa primerenca	Avançament sota làrix	3	
		Floració	Avançament sota làrix	3	
		Edat	Més joves sota làrix	3	
		Mida del lumen dels vasos i conductivitat hidràulica	Més gran sota pi	4	
		Distribució espacial dels vasos	Cap efecte	4	
	Coexistència (tipus de clap)	Biomassa aèria i alçada	No hi ha diferències entre claps	1	
		Àrea fotosintètica tiges	Cap efecte	1	
		Concentració N plantes	Menor en claps mixtos	1	
		EUA	Menor en claps amb *R. ferrugineum*	1	
		Estructura d'edats	No hi ha diferències entre claps	1	
V. uliginosum	Escalfament de l'aire	Biomassa aèria i alçada	Cap efecte	2	
	Escalfament del sòl	Biomassa aèria i elongació tija	Cap efecte	3	
		Fenologia vegetativa primerenca	Cap efecte	3	Sí relacionada amb la fosa de la neu
		Floració	Avançament	3	
	Sequera estival	Dinàmica assignació de C	Reducció velocitat transferència de C a òrgans subterranis	5	
		Conductància estomàtica	Cap efecte	5	
		Potencial hídric (tija)	Reducció (alba); Cap efecte (migdia)	5	

Taula 1. (cont.)

	Espècie arbòria	Biomassa aèria i elongació tija	Cap efecte	3	
		Fenologia vegetativa primerenca	Cap efecte	3	
		Fenologia vegetativa tardana	Cap efecte	3	
		Floració	Cap efecte	3	
E. hermaphroditum	Escalfament del sòl	Biomassa aèria i elongació tija	Cap efecte	3	Excepte augment elongació el 2011
		Fenologia vegetativa tardana i floració	Cap efecte	3	Sí relacionades amb la fosa d ela neu
	Espècie arbòria	Biomassa aèria i elongació tija	Cap efecte	3	Excepte 2008-09 (més gran sota pi)
		Fenologia vegetativa primerenca	Cap efecte	3	
		Floració	Cap efecte	3	Sí relacionada amb la fosa de la neu

Abreviatures: eficiència en l'ús de l'aigua (EUA)

Aproximacions experimentals per l'estudi dels efectes del canvi climàtic en espècies vegetals

Els estudis experimentals permeten testar l'efecte de factors ambientals concrets en paràmetres diana. Com que en aquests estudis es controlen els factors ambientals desitjats, proporcionen aproximacions mecanístiques i de causa-efecte per l'anàlisi de l'efecte dels canvis ambientals en les espècies vegetals (Dunne et al. 2004). Tanmateix, cal prendre precaucions a l'hora d'interpretar els resultats dels estudis experimentals, ja que no solen englobar tots els processos que potencialment tindrien lloc en una comunitat o ecosistema concrets davant el canvi climàtic (Morison i Morecroft 2006). A més, l'escala temporal d'aquests estudis difereix de la realitat, i processos que necessiten uns quants anys per produir-se, normalment s'intenten simular en curts períodes de temps (a excepció dels episodis extrems). Les alternatives a aquests experiments, però, també tenen desavantatges. Els estudis observacionals a llarg termini no sempre estan disponibles per períodes de temps prou llargs, i sovint manquen dades de sèries temporals històriques. A més, en aquests estudis pot ser difícil establir les relacions causa-efecte, i la variabilitat interanual pot comportar errors a l'hora d'interpretar patrons de resposta (Elmendorf et al. 2015). Finalment, substitucions espai-per-temps (*space-for-time*) permeten extrapolar sèries temporals a partir de patrons espacials al llarg de gradients ambientals. No obstant, infereixen efectes del canvi climàtic actual a partir de composicions d'espècies que són producte d'una llarga cadena de processos. Per tant, aquests estudis no són capaços d'incloure els nombrosos factors que expliquen les disposicions espacials de les espècies a banda del clima (Dunne et al. 2004; Elmendorf et al. 2015). Així, avui en dia no hi ha una manera perfecta d'estudiar els efectes del canvi climàtic en les espècies vegetals. Amb tot, un estudi fet per Elmendorf et al. (2015) va demostrar que mentre les substitucions espai-per-temps comportaven sobreestimacions en la magnitud del canvi, els resultats obtinguts a través de l'escalfament experimental i del seguiment a llarg termini sota temperatura ambient arribaven a conclusions similars en termes de la magnitud de les respostes de les plantes a l'escalfament global.

Estudis realitzats per Mikkelsen et al. (2008) i Dieleman et al. (2012) han posat de manifest la necessitat d'avaluar els efectes del canvi climàtic a través de combinacions experimentals múltiples, incloent diferents factors ambientals i, així, fent simulacions el més properes a la realitat possible. Malauradament, realitzar aquest tipus d'estudis no sempre és possible, principalment per la manca de recursos humans i econòmics. Per una banda, els estudis d'escalfament en ecosistemes alpins i àrtics són nombrosos. Les OTC s'han utilitzat sovint en experiments d'escalfament *in situ* gràcies al seu baix cost i fàcil manteniment. Però per altra banda, els experiments de fumigació de CO_2 a l'aire lliure en ecosistemes d'elevades altituds i latituds són escassos (Tissue i Oechel 1987; Gwynn-Jones et al. 1997; Körner et al. 1997; Dawes et al. 2011; Inauen et al. 2012). Altres estudis s'han centrat en els efectes de l'augment de la concentració d'ozó troposfèric o del nivell de radiacions ultraviolades en les plantes (Taulavuori et al. 1998, 2012; Semerdjieva et al. 2003a, b; Bassin et al. 2009; Boesgaard et al. 2012; Gwynn-Jones et al. 2012, entre altres), i a l'alta muntanya s'han dut a terme nombrosos experiments de sequera (Burri et al. 2014; Hasibeder et al. 2015; Fuchslueger et al. 2016; Sierra-Almeida et al. 2016). Tot i això, experiments *in situ* incloent més de dos tractaments i les seves interaccions són molt escassos (vegeu, però, Zavaleta et al. 2003; Mikkelsen et al. 2008; Kardol et al. 2010; i altres estudis duts a terme a les mateixes zones experimentals). Tot i que, idealment, s'haurien d'incloure diferents tractaments en els estudis experimentals de les respostes de les espècies vegetals als canvis ambientals, els experiments d'un sol tractament també són molt importants, perquè igualment permeten aprofundir en els efectes d'aquell sol factor en les plantes. Els experiments duts a terme en aquesta Tesi han permès capturar els efectes de l'escalfament, l'augment de CO_2 i la sequera en les espècies diana, i fins i tot la combinació de dos d'aquests factors (escalfament i augment de CO_2) en l'anatomia del xilema de *V. myrtillus*. Així, la Tesi aporta una visió de les respostes d'espècies subarbustives davant cadascun d'aquests factors en particular, i ens acosta a la comprensió de la importància de cadascun d'ells en el funcionament de les espècies d'estudi. A més, l'ús de diferents tècniques ens apropa a diferents visions d'aquestes respostes. Gràcies a la combinació de mesures observacionals i força directes (com la fenologia i mesures de biomassa) amb mesures isotòpiques i anatòmiques, i gràcies a l'aproximació a diferents escales (des de comunitat fins a òrgans i teixits; Fig. 8), podem obtenir una visió més profunda del comportament dels subarbusts davant el canvi global a l'alta muntanya.

Coexistència entre espècies subarbustives a l'ecotò del límit supraforestal

Vaccinium myrtillus s'ha descrit com una espècie plàstica, que presenta plasticitat morfològica en boscos i hàbitats oberts (Tolvanen 1995). Tanmateix, els capítols 1 i 2 mostren que la seva estructura i funcionament eren molt similars en claps amb diferents espècies a l'ecotò del límit supraforestal al Pirineu. Tot i que vam trobar indicis d'un comportament més plàstic en claps amb *R. ferrugineum* (els ramets presentaven una tendència cap a volums més elevats i més variabilitat), la majoria dels paràmetres analitzats eren força similars entre claps. El fet que les parcel·les d'estudi es trobessin al límit altitudinal superior de la distribució de *V. myrtillus* podria haver dificultat la seva habilitat per respondre d'una manera més plàstica a les diferències ambientals trobades entre claps a causa de la presència d'espècies diferents. A Eth Corrau des Machos (al voltant dels 2.300 m d'altitud), els ramets creixen menys que a altituds més baixes i que en comunitats forestals, ja que hi troben temperatures més baixes i menys protecció a les ventades. El seu comportament, doncs, sembla força fix davant diferents situacions de coexistència a elevades altituds. De fet, les comunitats dominades per *V. myrtillus* són rares, ja que l'espècie més freqüentment creix en claps barrejats amb altres subarbusts o en claps purs segregats, tal com s'ha mostrat al llarg d'aquesta Tesi. Això suggereix que aquests claps purs podrien ser inestables en el context de la successió vegetal.

No vam trobar evidència que l'escalfament provoqués canvis en la interacció entre *V. myrtillus* i les dues espècies veïnes *V. uliginosum* i *R. ferrugineum*, contràriament als resultats de força estudis en altres comunitats que sí que n'han trobat (Dormann et al. 2004; Pugnaire et al. 2015; Wheeler et al. 2015; Olsen et al. 2016). Tot i que aquesta Tesi no se centra particularment en l'estudi d'interaccions de competència, i en cap cas en la competència intraespecífica, val la pena comentar que no vam trobar evidència d'una major competència intraespecífica que interespecífica, contràriament a allò que prediu la teoria de la coexistència (Chesson 2000). Fins i tot a densitats més elevades de ramets de *V. myrtillus* en claps purs que en claps mixtos, el seu desenvolupament no era pitjor en els primers (vegeu Informació suplementària del capítol 2).

Respostes específiques al canvi climàtic

Aquesta Tesi demostra que *V. myrtillus* pot ser força sensible a determinats canvis ambientals a altituds elevades. Alguns estudis han demostrat que *V. myrtillus* respon positivament a l'escalfament (Rinnan et al. 2009; Taulavuori et al. 2013; Dawes et al. 2011, 2015), incloent aquesta Tesi. Tant l'escalfament del sòl (+ 4 °C) com l'escalfament de l'aire (+ 1,1 °C) durant l'estació de creixement van tenir efectes positius en el creixement de *V. myrtillus*, cosa que indica una limitació de la temperatura per aquesta espècie a les nostres àrees d'estudi i suggereix un possible efecte positiu de l'escalfament global en ella. Tot i que aquesta Tesi no presenta evidències que l'escalfament tingués efectes positius en òrgans subterranis, és plausible que condicions climàtiques futures que comportin un major creixement aeri acabin tenint efectes positius en les parts subterrànies. Aquests efectes podrien tenir lloc a través d'una major transferència d'assimilats (i el conseqüent increment en l'exsudació de les arrels, que comportaria un increment de la biomassa bacteriana del sòl) o a través d'efectes directes de l'escalfament sobre l'activitat microbiana i les taxes de descomposició o mineralització (Bardgett i Wardle 2010; Hollister i Flaherty 2010; Schindlbacher et al. 2011; Bai et al. 2013).

Contrastant amb la sensibilitat de *V. myrtillus*, espècies que hi conviuen a elevades altituds com *V. uliginosum* o *E. hermaphroditum* responen menys a l'escalfament. Això es podria explicar, parcialment, per la diferència en els seus rangs de distribució altitudinal. Quan coexisteixen a elevades altituds, doncs, l'escalfament podria ser positiu per *V. myrtillus* però no per les espècies veïnes, cosa que podria comportar potencials canvis estructurals i de composició de la comunitat. Quatre anys d'escalfament passiu al Pirineu no van estimular més el creixement de *V. myrtillus* quan creixia amb *V. uliginosum* que en claps purs. Així, tot i ser més afavorit per l'escalfament que *V. uliginosum*, *V. myrtillus* no se'n beneficiava més a costa d'aquest. Roman desconegut, però, si *V. uliginosum* es va veure afectat negativament per la coexistència amb *V. myrtillus* en el tractament d'escalfament. A més, *V. myrtillus* podria guanyar espai aeri amb l'escalfament a llarg termini, cosa que podria augmentar les interaccions competitives amb les espècies veïnes, la qual cosa podria ser, finalment, perjudicial per aquestes. Com s'ha esmentat anteriorment, caldria estudiar la capacitat competitiva de les espècies veïnes per tal de preveure possibles canvis en la composició i estructura de la comunitat amb l'escalfament.

Tot i que l'escalfament sembla ser beneficiós per *V. myrtillus*, alguns processos associats a l'escalfament podrien tenir efectes contraris, com una fosa de la neu avançada, que podria desprotegir *V. myrtillus* en un període en què tenen lloc gelades tardanes. Hi ha estudis que han demostrat que *V. myrtillus* presenta una baixa resistència a aquestes gelades (Taschler i Neuner 2004; Martin et al. 2010; Wheeler et al. 2014). A més, estudis previs han demostrat que *V. myrtillus* era més sensible a les gelades sota concentracions elevades de CO_2 i escalfament del sòl a Stillberg (Martin et al. 2010; Rixen et al. 2012). Així, davant l'escalfament global, tot i que les temperatures estivals més altes puguin ser beneficioses per *V. myrtillus*, la fosa avançada de la neu associada podria contrarestar aquests efectes. No obstant, *V. myrtillus* també ha mostrat una elevada capacitat de recuperar-se vegetativament dels danys per gelada en un experiment d'extracció de neu (Gerdol et al. 2013).

Quan vam estudiar els efectes dels tractaments de CO_2 i escalfament del sòl en l'anatomia del xilema de *V. myrtillus*, vam trobar indicis que el seu sistema hidràulic era més sensible a l'escalfament que no pas a l'augment de CO_2, evidenciant la major limitació per baixes temperatures que no pas per pressions parcials més baixes de CO_2 en *V. myrtillus* a l'alta muntanya. No vam trobar pràcticament cap interacció entre aquests dos tractaments en l'anatomia hidràulica o en el creixement de *V. myrtillus*. De la mateixa manera, Dieleman et al. (2012) van mostrar que en estudis que combinaven aquests dos tractaments, era rar observar-ne efectes additius, mentre que les respostes antagonistes eren més freqüents. Els nostres resultats semblen exemplificar-ho, ja que la mida del lumen dels vasos conductors es va reduir amb l'escalfament, però va respondre positivament (tot i que només en els últims anys) a l'augment de CO_2. L'estimulació del creixement de *V. myrtillus* amb l'escalfament del sòl i l'augment de CO_2 trobats en un estudi anterior (Dawes et al. 2011) i en el capítol 3 d'aquesta Tesi no estaven directament acompanyats per ajustaments anatòmics del xilema. Només en els últims anys d'ambdós tractaments la direcció de la resposta anatòmica tenia una tendència més propera a la del creixement. Això es podria explicar per la manca de necessitat d'una millora en l'anatomia hidràulica dels ramets fins a un punt en què l'estimulació del creixement va requerir una millora en l'estructura hidràulica. És interessant l'efecte negatiu observat en la mida dels vasos conductors amb l'escalfament del sòl, especialment durant els primers anys del tractament. Això, que podria estar relacionat amb una gelada tardana que va tenir lloc el 2007 (Rixen et al. 2012), es podria interpretar com un avantatge davant de les gelades, ja que el risc d'embolisme induït per aquestes decreix amb la reducció de la mida dels vasos conductors (Tyre i Sperry 1989). Com que l'augment de la temperatura redueix la viscositat de l'aigua (Roderick and Berry 2001), la reducció dels vasos conductors no tindria per què haver perjudicat el transport d'aigua dins la planta.

L'experiment de sequera també mostra respostes específiques, tot i que cap de les dues espècies de *Vaccinium* van mostrar grans evidències d'estrès davant la sequera aplicada. Les dues espècies es van mostrar força resistents, només *V. uliginosum* va mostrar senyals d'un lleuger estrès, amb una reducció de la conductància estomàtica i del potencial hídric, i algunes alteracions en la dinàmica de la distribució del C. La sequera, doncs, va tenir efectes diferents en les dues espècies, cosa que podria provocar, a llarg termini, canvis en la composició, l'estructura i el funcionament de la comunitat. A més, alteracions en el cicle del C podrien comportar, en última instància, impactes en el balanç de C de l'ecosistema (Reichstein et al. 2013) a través de canvis en l'acoblament entre les parts aèries i subterrànies de les plantes i, com a conseqüència, de canvis en la comunitat microbiana i l'emissió de CO_2 del sòl (Ruehr et al. 2009; Burri et al. 2014; Fuchslueger et al. 2014). Llorens et al. (2002) van dur a terme un experiment de sequera en una landa torbosa al nord de Gal·les (Regne Unit), i van trobar que aquesta no tenia efectes en l'intercanvi de gasos de *V. myrtillus*, en línia amb els nostres resultats. Tot això, juntament amb l'estudi de Ganthaler i Mayr (2015) que mostrava que *V. myrtillus* segueix una estratègia hidràulica de risc, sembla indicar que aquesta espècie és força resistent a la sequera. No obstant, també s'han trobat indicis que el dèficit hídric té efectes negatius en *V. myrtillus*. En l'experiment del nord de Gal·les, Llorens et al. (2002) van trobar que la sequera tenia efectes negatius en l'estabilitat del desenvolupament foliar en *V. myrtillus*. A més, en un altre experiment, Taulavuori et al. (2010) van trobar que les fulles de *V. myrtillus* eren sensibles a l'estrès hídric, especialment en un estadi madur, en què els efectes negatius eren més pronunciats (p. ex. la reducció de les concentracions de clorofil·la, una major degradació d'aquesta o un increment en l'oxidació de les proteïnes) i la senescència de la fulla es veia accelerada.

Així, mentre que l'intercanvi de gasos i el cicle del C en *V. myrtillus* semblen força resistents a la sequera, la sensibilitat a aquesta es podria reflectir en altres paràmetres. A més, és molt probable que la severitat de la sequera influeixi el nivell d'estrès de la planta i, per tant, el seu comportament (Farooq et al. 2009; Nandintsetseg i Shinoda 2013).

En resum, les baixes temperatures són una clara limitació per al creixement de *V. myrtillus* a elevades altituds. L'augment de les concentracions de CO_2 no sembla tenir grans efectes en el seu creixement i funcionament hidràulic, tot i que vam trobar una lleugera estimulació del creixement i efectes retardats en l'estructura anatòmica. Això podria indicar que fins que l'estimulació del creixement no va assolir un cert nivell, no va caldre una millora de l'estructura hidràulica. Tot i que *V. myrtillus* i *V. uliginosum* semblen força resistents a la sequera, indicis d'un lleuger estrès en *V. uliginosum* suggereixen que aquesta espècie és més sensible al dèficit hídric i que, per tant, podria patir un desavantatge competitiu al costat de l'espècie veïna *V. myrtillus* davant episodis de sequera més intensos. A més, els canvis en els patrons de distribució del C en *V. uliginosum* podrien tenir efectes a la llarga en processos subterranis com les taxes d'emissió de CO_2 del sòl o canvis en la comunitat microbiana (Ruehr et al. 2009; Burri et al. 2014; Fuchslueger et al. 2014), amb les consegüents alteracions del balanç de C de la comunitat i l'ecosistema.

Efectes de l'espècie arbòria en el creixement, funcionament i resposta als tractaments de *V. myrtillus* a l'ecotò del límit supraforestal

Els experiments compresos en aquesta Tesi Doctoral s'han dut a terme a la part superior de l'estatge subalpí, en la majoria de casos en l'ecotò del límit supraforestal. Mentre que els experiments corresponents als capítols 1, 2 i 5 s'han desenvolupat en prats colonitzats per subarbusts, que són hàbitats més o menys oberts, els experiments dels capítols 3 i 4 es van dur a terme a l'àrea experimental de Stillberg, que és una plantació al límit supraforestal. Així, els experiments a Stillberg ens han permès avaluar paràmetres relacionats amb el creixement, la fenologia i l'anatomia de *V. myrtillus* al sotabosc de dues espècies arbòries: *Pinus mugo* subsp. *uncinata*, de fulla perenne, i *Larix decidua*, de fulla caduca. Aquestes espècies poden ser dominants en zones extenses del Pirineu i dels Alps (*P. mugo* subsp. *uncinata* al Pirineu i *L. decidua* als

Alps interiors; Ozenda 1985; Ninot et al. 2008), cosa que reforça la importància d'estudiar les espècies arbustives que creixen sota les seves capçades. La identitat de l'espècie arbòria de la parcel·la (només hi havia un arbre per parcel·la) tenia grans efectes sobre la majoria dels paràmetres estudiats en *V. myrtillus* (Taula 1), cosa que evidencia la seva rellevància per al funcionament d'aquest arbust i la importància de tenir-la en compte a l'hora d'estudiar els subarbusts en ecosistemes forestals, en aquest cas en el límit supraforestal.

En aquesta Tesi no s'han avaluat els factors relacionats amb la identitat de l'espècie arbòria que tenien una influència directa sobre *V. myrtillus* i la manera com responia als tractaments. Tanmateix, la hipòtesi que la durada de la fulla de l'arbre podria tenir una gran influència sobre el subarbust és més que plausible. Una capçada caduca (la de *Larix decidua*) permet que una major quantitat de llum arribi al sotabosc al principi de l'estació de creixement. Així, la neu podria fondre's abans, deixant *V. myrtillus* descobert i, per tant, incrementant el risc que aquest patís gelades tardanes. Tal com he descrit a la secció anterior, *V. myrtillus* té una baixa resistència a les gelades, per tant episodis de gelada sense la protecció de la neu podrien comprometre'n el creixement i funcionament. Altres possibles raons per les grans diferències entre parcel·les amb diferents espècies arbòries podrien estar relacionades amb la menor disponibilitat de N al sòl de les parcel·les amb pi en aquest experiment (Dawes et al. 2017), que podria comportar un avantatge competitiu en *V. myrtillus* sobre gramínies amb més requeriments de N.

Hem vist, doncs, que l'espècie arbòria té una influència en la comunitat del sotabosc; però les espècies arbustives també són factors causants de modificacions en les comunitats forestals. La reforestació espontània a elevades altituds ve sovint impulsada per les comunitats arbustives emmatadores, i els arbusts poden tenir un paper clau en la facilitació de la supervivència i el reclutament dels plançons d'arbres (Batllori et al. 2009; Grau et al. 2010, 2012). Contràriament, també poden tenir efectes al·lelopàtics en els arbres, tal com la fullaraca de *V. myrtillus* en la germinació de *P. uncinata* Ninot et al. (2016).

Comunitats arbustives d'alta muntanya davant el canvi global

Tot i que els canvis abruptes en el clima són intrínsecs als ecosistemes alpins, canvis en els règims de temperatura i de precipitació i, com a conseqüència, en els patrons de la neu, tindran conseqüències en aquests ecosistemes (Körner 2003). Les espècies vegetals coexistents presenten adaptacions concretes que expliquen que responguin de manera diferent als estímuls ambientals. Davant de temperatures més elevades, majors concentracions de CO_2 atmosfèric, un augment dels episodis de sequera o una fosa avançada de la neu, les espècies presenten comportaments diferents, tal com s'ha mostrat en aquesta Tesi. Com a conseqüència, podem esperar que amb el canvi climàtic es produeixin canvis en la composició de les comunitats vegetals i que, per tant, la seva estructura i el seu funcionament també canviïn (Sundqvist et al. 2008; Kreyling et al. 2012; Pardo et al. 2013). Amb tot, no podem oblidar un dels principals factors causants dels canvis en els ecosistemes, que és l'activitat humana. Les futures activitats antropogèniques no només tindran impactes indirectes en aquests ecosistemes a través del canvi climàtic, sinó també impactes més directes, com els que tenen lloc en l'actualitat a través de les transformacions socials que han comportat canvis en l'ús del sòl en zones de muntanya (Prévosto et al. 2011; Ameztegui et al. 2016).

L'emmatament de prats subalpins i alpins, i d'altres ecosistemes de tundra, s'ha observat globalment, i s'han demostrat els seus impactes negatius en la biodiversitat i en el funcionament i estructura de l'ecosistema (Wilson i Nilsson 2009; Eldridge et al. 2011; Myers-Smith et al. 2011; Pajunen et al. 2011; Brandt et al. 2013; Koch et al. 2015; entre altres). Tanmateix, un estudi dut a terme per Barros et al. (2016) suggereix que episodis de sequera intensa podrien contrarestar aquest emmatament. Aquests autors van trobar que l'expansió de boscos i matollars es veia accelerada amb la sequera els primers anys d'estudi, però que en anys posteriors, davant sequeres més freqüents i més intenses, l'expansió es desaccelerava. També van trobar que en el límit supraforestal, els canvis en la biodiversitat estaven regulats pel canvi climàtic gradual i els canvis en l'ús del sòl, mentre que la sequera influenciava la taxa d'aquests canvis. Resultats d'un experiment previ en un prat al costat de la nostra àrea d'estudi a Stubai (Alps austríacs) van mostrar que els efectes de la sequera eren més severs en espècies herbàcies (Hasibeder et al. 2015) que no pas en els arbustos estudiats en aquesta Tesi. Tot i això, altres paràmetres relacionats amb la resiliència a la sequera (incloent la recuperació, les taxes de creixement o la supervivència), que podrien ser especialment importants per les espècies no llenyoses (Ingrisch et al. in prep.) i que podrien determinar el comportament de la comunitat davant episodis de sequera, no s'han avaluat aquí.

La diversitat d'espècies en una comunitat vegetal i la gran varietat de processos que hi tenen lloc fa que sigui molt difícil predir com es comportaran aquestes comunitats en un futur, especialment si les prediccions climàtiques difereixen entre models (depenent del factor ambiental poden diferir més o menys). En aquesta Tesi Doctoral es demostra que les respostes de les espècies vegetals a factors climàtics concrets és específica, però fer una extrapolació d'aquests resultats per a la dinàmica de tota la comunitat seria incorrecte. Amb tot, la informació obtinguda a partir de tots els estudis aquí inclosos proporciona una millor comprensió del comportament dels subarbusts (i, com a conseqüència, de les comunitats que formen), i és també una base per a estudis sobre aquestes comunitats, que podrien aportar un coneixement més profund i generalitzat.

Futures línies de recerca

Dur a terme tots els experiments compresos en aquesta Tesi Doctoral ha sigut personalment i científicament molt estimulant, i ens ha permès respondre moltes preguntes relacionades amb el comportament dels subarbusts d'alta muntanya davant el canvi climàtic. Tanmateix, durant aquests anys, també han anat sorgint noves preguntes, i seria molt interessant poder dedicar el temps necessari per respondre-les.

Un dels camps que ha restat inexplorat és l'efecte de la clonalitat a l'hora d'explicar el comportament dels subarbusts davant el canvi climàtic. Tots els estudis d'aquesta Tesi es basen en ramets a causa de la dificultat que suposaria identificar individus genètics al camp (connectats per rizomes o no), especialment en estudis al llarg del temps. Per això, seria molt interessant poder establir una metodologia per poder estudiar en detall com es comporta un clon i com els ramets d'un mateix veget interactuen entre ells davant condicions ambientals canviants.

Una altra línia de recerca molt prometedora seria l'estudi de la capacitat colonitzadora d'aquests subarbusts i de com aquesta capacitat actua davant els canvis en les condicions climàtiques. A més, un seguiment a llarg termini incloent tractaments relacionats tant amb el canvi en l'ús del sòl com amb factors ambientals permetria separar-ne els efectes, cosa que aportaria dades molt interessants.

Una altra línia que ha quedat sense explorar és el mecanisme a través del qual les espècies arbòries afecten els subarbusts que hi creixen a sota. Un experiment que controlés factors com la llum al principi de l'estació de creixement i durant aquesta, la fosa de la neu, el pH del sòl i de la fullaraca, associacions micoríziques específiques i la comunitat microbiana del sòl, entre d'altres, podria aportar moltes respostes sobre aquest tema.

Finalment, respecte els episodis de sequera, seria molt interessant dur a terme un experiment amb dos tractaments de dèficit hídric (més intens i menys intens) que durés uns quants anys. D'aquesta manera, podríem obtenir més informació sobre l'estrès causat per diferents intensitats de sequera i per la seva recurrència. En aquesta línia, l'anàlisi de compostos específics (com els carbohidrats no estructurals) podria aportar més informació sobre els efectes en les espècies d'estudi. Si, a més, es mostregessin grans longituds de rizoma, es podria estudiar com de lluny pot arribar el senyal isotòpic en un període de temps concret. A més, l'estudi de la fase de recuperació després de la sequera podria, sense dubte, aportar nous coneixements sobre els efectes d'aquesta en global.

Conclusions

El creixement i el funcionament de *V. myrtillus* a l'ecotò del límit supraforestal al Pirineu no semblen afectats per les espècies veïnes. Els ramets de claps purs i els de claps mixtos a Eth Corrau des Machos presentaven un creixement i una estructura d'edats similars, cosa que suggereix que la dinàmica d'aquesta espècie a la part superior de l'estatge subalpí no està modificada per la presència d'altres espècies arbustives.

L'escalfament del sòl i de l'aire van afavorir el creixement de *Vaccinium myrtillus* a l'ecotò del límit supraforestal. Experiments al Pirineu i als Alps van demostrar que la biomassa aèria, l'alçada, l'elongació de la tija i el creixement secundari dels ramets s'incrementaven amb l'escalfament, i que aquesta estimulació es mantenia al llarg del temps que van durar els experiments. Vam trobar un desacoblament entre les respostes aèries i subterrànies a l'escalfament al Pirineu, cosa que es podria explicar, parcialment, pel fet que l'escalfament s'havia simulat amb OTC. En qualsevol cas, no es va produir una major transferència d'assimilats cap a òrgans subterranis amb l'augment de la temperatura o, si es va produir, es va diluir en la complexa xarxa de rizomes. L'absència d'efectes de l'escalfament en la diversitat de la comunitat microbiana del sòl aniria associada a una absència d'efectes en el reservori de C del sòl. Per altra banda, es va produir una reducció en la concentració de NO_3^- amb l'escalfament, probablement associada a un augment de la taxa d'assimilació d'aquest per la comunitat vegetal. El creixement de *V. myrtillus* no estava necessàriament relacionat amb una estació de creixement més llarga causada per una fosa de la neu o una brotació i expansió de la fulla avançades (tot i que en l'experiment al Pirineu podria estar-ho). Efectes directes de la temperatura en la fotosíntesi, formació de teixits i processos del sòl que comportessin més disponibilitat de nutrients (p. ex. descomposició i mineralització) podrien explicar l'estimulació del creixement, fins i tot encara que l'estació de creixement no fos més llarga.

L'escalfament de l'aire (1,1 °C) amb tècniques passives no va comportar canvis en la interacció entre *V. myrtillus* i les espècies arbustives *V. uliginosum* i *R. ferrugineum*. Aquests resultats suggereixen que les interaccions entre espècies podrien ser menys sensibles a l'escalfament en comunitats en el seu estat natural que en experiments d'extracció (*removal experiments*), i que l'escalfament global podria no comportar canvis en les interaccions entre espècies a curt termini. Tot i que els efectes de l'escalfament en les espècies coexistents *V. myrtillus* i *V. uliginosum* eren diferents (positius en la primera, nuls en la segona), *V. myrtillus* no es va beneficiar més de l'augment de la temperatura en detriment de *V. uliginosum*. Així, tot i que el creixement de *V. myrtillus* (i probablement, en última instància el seu recobriment) podrien incrementar-se amb l'escalfament global, això no necessàriament implicaria un efecte negatiu directe en *V. uliginosum* en les àrees on coexisteixen.

L'estimulació del creixement de *V. myrtillus* amb l'augment de CO_2 i l'escalfament del sòl al límit supraforestal no va anar acompanyada per canvis immediats en l'anatomia del xilema. La mida del lumen dels vasos conductors de *V. myrtillus* es va veure reduïda per l'escalfament, i només es va incrementar lleugerament amb l'augment de CO_2 al final d'aquest tractament. La reducció de la mida del lumen dels vasos conductors podria no haver tingut implicacions negatives en el creixement de *V. myrtillus* perquè la temperatura redueix la viscositat de l'aigua (i, per tant, el transport de l'aigua pot ser més eficient amb una mateixa mida dels vasos si la temperatura augmenta). A més, vasos conductors més petits podrien suposar un avantatge, ja que comporten un risc menor d'embolisme. Els teixits de reserva de *V. myrtillus* eren poc sensibles als canvis de temperatura i de la concentració de CO_2. Així, l'augment de CO_2 i l'escalfament del sòl van tenir efectes en el creixement i l'anatomia hidràulica més que no pas en la funció de reserva de *V. myrtillus*. L'escalfament del sòl va tenir efectes més grans que no pas l'augment de CO_2 en l'anatomia de la fusta de *V. myrtillus*, posant de manifest la major limitació per baixes temperatures en aquesta espècie al límit supraforestal.

La identitat de l'espècie arbòria té una influència en el creixement i el funcionament de *V. myrtillus* al límit supraforestal. Les dues espècies arbòries incloses en els experiments a Stillberg (*Pinus mugo* subsp. *uncinata* i *Larix decidua*) van tenir efectes diferents en la fenologia, el creixement, la taxa de renovació i l'eficiència hidràulica dels ramets de *V. myrtillus*. Això podria estar relacionat amb les diferents condicions microclimàtiques creades sota la capçada d'aquestes dues espècies arbòries. El pi, de fulla perenne, podria conferir una major protecció que el làrix (de fulla caduca) a *V. myrtillus* al principi de l'estació de creixement davant gelades tardanes. A més, una major incidència de la llum al sotabosc d'una capçada decídua quan la neu comença a fondre's podria accelerar la fosa i, per tant, deixar els ramets descoberts quan aquestes gelades tardanes tenen lloc. Per altra banda, diferències en la disponibilitat de nutrients entre parcel·les amb espècies arbòries diferents també podrien haver influenciat el creixement i funcionament dels subarbusts del sotabosc.

***Vaccinium myrtillus* i *V. uliginosum* presenten estratègies diferents en relació a la dinàmica de distribució del C**, parcialment explicades pel funcionament diferenciat de les seves tiges. Les tiges joves fotosintètiques de *V. myrtillus* podrien reassimilar C respirat per altres òrgans, mentre que les tiges no fotosintètiques de *V. uliginosum* no presentarien aquesta funció. Per altra banda, les fulles de *V. uliginosum* presentaven una major fixació activa de C que les de *V. myrtillus*.

***Vaccinium myrtillus* i *V. uliginosum* són força resistents a episodis de sequera, especialment el primer**. La sequera va causar un lleuger estrès en *V. uliginosum*, la dinàmica del C del qual va canviar lleugerament cap a una transferència més lenta de C a òrgans subterranis. Les comunitats dominades per aquests subarbusts podrien ser força resistents a episodis de sequera, però com que aquests episodis semblen tenir efectes diferents en les dues espècies coexistents, es podrien donar canvis en la composició de la comunitat i en la seva estructura i funcionament, així com en el balanç de C de l'ecosistema. La diferència en les respostes a la sequera i en les estratègies de recuperació entre tipus funcionals dins una comunitat podria exacerbar aquests canvis.

El canvi climàtic té efectes més grans sobre algunes espècies que sobre d'altres que hi conviuen, cosa que reforça la necessitat d'estudiar les respostes particulars de cada espècie als canvis en les condicions ambientals. Els ecosistemes d'alta muntanya són especialment vulnerables als canvis de temperatura, amb els seus efectes directes i indirectes i, molt probablement, les comunitats vegetals patiran canvis de composició, estructura i funcionament en el futur. Juntament amb l'efecte de l'acció humana, l'estudi de l'efecte dels canvis ambientals en el creixement i funcionament de les plantes és bàsic per predir i modelitzar la dinàmica i direcció de les comunitats i els ecosistemes en el futur.

General discussion

Shrub communities may cover large areas in high mountain ecosystems. Due to land-use change (i.e. abandonment of extensive grazing, agricultural practices and tree logging) and global warming (Theurillat and Guissan 2001; Beniston 2003; Dullinger et al. 2003; Roura-Pascual et al. 2005; Komac et al. 2013) they are expanding through the colonization of grassland communities. Thus, the study of the functioning and dynamics of shrub communities is of great importance to understand and predict future community and ecosystem directions under our changing climate and society. Dwarf shrubs are an important component of these communities (Körner 2003; Ninot et al. 2008; Illa et al. in review). Consequently, their study and the study of their responses to global change are of especial interest.

In this Thesis, several aspects of climate change effects on high mountain dwarf shrubs have been tackled (see Fig. 8 in Introduction). The analyses of growth, phenological, physiological and anatomical responses to *in situ* experimental warming, CO_2 enrichment and summer drought have provided interesting information on the behaviour of coexisting dwarf shrub species under current and forecasted changes in environmental conditions. Moreover, several aspects of *V. myrtillus* performance and its interaction with neighbour species at current environmental conditions have been addressed, and these results have contributed to a better understanding of their functioning and their responses to changes in the environment. In Table 1, the main effects of the treatments applied on each species in this Thesis are summarised. Although this Thesis mainly focuses on the species *V. myrtillus* and *V. uliginosum*, results obtained here have implications for the whole community and the ecosystem.

Table 1. Summary of the main results found in this Thesis.

Species	**Treatment**	**Parameter**	**Effect**	**Chapter**	**Observations**
V. myrtillus	Air warming	AG biomass and height	Increased	2	
		BG biomass	No effect	2	
		Early-season vegetative phenology	Advanced	2	
		Plant-plant interactions	No effect	2	
		Soil N concentration	Increased soil NO_3^- concentration	2	At plot scale in different stand types
	Soil warming	AG biomass and shoot elongation	Increased	3	
		Early-season vegetative phenology	No effect	3	Related with snowmelt timing
		Late-season vegetative phenology	No effect	3	Related with snowmelt timing
		Flowering phenology	Advanced	3	
		Age	No effect		
		Vessel lumen size and hydraulic conductivity	Decreased, especially in the first years	4	
		Spatial vessel distribution	Decreased vessel grouping	4	
		Storage tissues (ray parenchyma)	Larger proportion of biseriate rays	4	
	CO_2 enrichment	Xylem ring widths	No effects	4	
		Vessel lumen size and hydraulic conductivity	Increased in the last years	4	
		Spatial vessel distribution	No effects	4	
		Storage tissues (ray parenchyma)	No effects	4	
	Summer drought	C allocation dynamics	No effects	5	
		Stomatal conductance	Slightly increased	5	
		Shoot water potential	Decreased before dawn; No effect at midday	5	

Table 1. (cont.)

	Overstorey tree species	AG biomass and shoot elongation	Larger under pine	3	
		Xylem ring widths	Larger under larch	4	
		Early-season vegetative phenology	Advanced under larch	3	
		Flowering phenology	Advanced under larch	3	
		Age	Younger under larch	3	
		Vessel lumen size and hydraulic conductivity	Larger under pine	4	
		Spatial vessel distribution	No effect	4	
	Coexistence (stand type)	AG biomass and height	No differences between stand types	1	
		Shoot photosynthetic area	No effect	1	
		N concentration in plants	Lower when growing in mixed stands	1	
		WUE	Lower when growing with *R. ferrugineum*	1	
		Age structure	No significant differences between stand types	1	
V. uliginosum	Air warming	AG biomass and height	No effect	2	
	Soil warming	AG biomass and shoot elongation	No effect	3	
		Early-season vegetative phenology	No effect	3	Related with snowmelt timing
		Flowering phenology	Advanced	3	
	Summer drought	C allocation dynamics	Reduced speed of C transfer BG	5	
		Stomatal conductance	Decreased	5	
		Shoot water potential	Decreased before dawn; No effect at midday	5	
	Overstorey tree species	AG biomass and shoot elongation	No effect	3	
		Early-season vegetative phenology	No effect	3	

Table 1. (cont)

		Late-season vegetative phenology	No effect	3	
		Flowering phenology	No effect	3	
E. hermaphroditum	Soil warming	AG biomass and shoot elongation	No effect	3	Except 2011 (elong. increased)
		Late-season vegetative phenology and flowering phenology	No effect	3	Both related with snowmelt timing
	Overstorey tree species	AG biomass and shoot elongation	No effect	3	Except 2008-09 (larger under pine)
		Early-season vegetative phenology	No effect	3	
		Flowering phenology	No effect	3	Related with snowmelt timing

Abbreviations: aboveground (AG), belowground (BG), water-use efficiency (WUE)

Experimental approaches to the study of climate change effects on plants

Experimental studies allow testing the effects of specific environmental factors on target parameters. In these studies, individual environmental factors may be under control; thus, they provide mechanistic and cause-effect approaches to the analysis of environmental change effects on plant species (Dunne et al. 2004). However, caution needs to be taken when interpreting results of experimental studies, as they usually do not encompass all the processes that would potentially take place in a specific community or ecosystem with environmental change (Morison and Morecroft 2006). Moreover, the temporal scale also differs from reality, and processes that need a long time to show up in natural ecosystems are usually simulated in shorter periods of time (except for sudden events). Alternatives to experimental studies, however, also have numerous downsides. Long-term observational studies are not always available for long-enough periods of time, and historical data are often lacking. Moreover, in these studies it may be difficult to establish cause-effect relationships and inter-annual variability may lead to confusion when interpreting response patterns (Elmendorf et al. 2015). Finally, space-for-time substitutions allow extrapolating temporal trends from spatial patterns across environmental gradients, but they infer current climate change effects from species compositions that have followed a long chain of processes explaining their current assembly; thus, they fail to include the large number of causalities explaining spatial arrangements other than climate (Dunne et al. 2004; Elmendorf et al. 2015). Therefore, at present there is no perfect way of dealing with the study of climate change effects on plant species. Nevertheless, a study by Elmendorf et al. (2015) demonstrated that while space-for-time substitutions led to overestimations of the magnitude of change, results obtained with experimental warming and long-term monitoring under natural temperature change led to similar conclusions in terms of the magnitude of plant responses to global warming.

Studies by Mikkelsen et al. (2008) and Dieleman et al. (2012) emphasized the need for evaluating climate change effects through the combination of multiple treatments involving different environmental factors and thus, simulating reality as close as possible. Unfortunately, this is not always feasible, mainly due to human and economic resources. On the one hand, warming studies in alpine and Arctic ecosystems are numerous. Open-top chambers (OTCs) have been widely used in the study of *in situ* warming effects on vegetation due to their low cost and maintenance. On the other hand, experimental studies involving *in situ* CO_2 enrichment in high-elevation and high-latitude ecosystems are scarce (Tissue and Oechel 1987; Gwynn-Jones et al. 1997; Körner et al. 1997; Dawes et al. 2011; Inauen et al. 2012). Other studies have focused on the effects of increasing tropospheric ozone concentrations or UV-B levels on plant species (Taulavuori et al. 1998, 2005, 2012; Semerdjieva et al. 2003a, b; Bassin et al. 2009; Boesgaard et al. 2012; Gwynn-Jones et al. 2012, amongst others), and several drought experiments have been carried out in high mountain ecosystems (Burri et al. 2014; Hasibeder et al. 2015; Fuchslueger et al. 2016; Sierra-Almeida et al. 2016). Nevertheless, experiments *in situ* across the globe involving more than two factors and their interactions are scarce (but see Zavaleta et al. 2003; Mikkelsen et al. 2008; Kardol et al. 2010; and other studies carried out at the same experimental sites). Although, ideally, several environmental factors should be included in experimental studies on plant responses to environmental changes, single-factor experiments are still very important, because they also allow for the understanding of plant responses to individual environmental factors. The experiments performed in this Thesis undoubtedly let us capture the effects of warming, CO_2 enrichment and drought on the target species, and even the effects of the combination of two of these factors (warming and CO_2 enrichment) on *V. myrtillus* xylem anatomy. Thus, this Thesis provides an overview of the response of dwarf shrub species to each factor in particular, leading to a good understanding of the importance of each of these climate factors on the performance of our study species. In addition, the use of different techniques has provided different insights into the responses of dwarf shrubs to environmental change. Thanks to the combination of observational (i.e. phenology) and relatively straight-forward measurements (i.e. biomass) with isotopic and anatomical approaches, and the approximation at different scales (from community scale to plant organs and tissues; Fig. 8), a deep insight into dwarf shrubs behaviour in front of environmental changes has been made possible.

Coexistence between dwarf shrub species at the treeline ecotone

Vaccinium myrtillus has been described as a plastic species, showing morphological plasticity when growing in forests and in open habitats (Tolvanen 1995). Nevertheless, chapters 1 and 2 show that its structure and functioning was very similar when growing with different neighbour species at the subalpine belt of the Pyrenees. Although we found some indication of a greater plastic performance when growing with *R. ferrugineum* (ramets showed a trend towards greater volumes and higher dispersion), most of the parameters analysed were very similar between stand types. The fact that our study plots were at the highest limit of *V. myrtillus* elevation distribution might have hampered its ability to respond in a more plastic way to different conditions encountered in the presence of other species. At Eth Corrau des Machos (*ca*. 2300 m a.s.l.), ramets grow smaller than at lower elevations and forest communities, as they find lower temperatures and less protection to strong winds. Thus, its performance seems rather fixed in front of the coexistence with different neighbouring species at high elevations. In fact, communities dominated by *V. myrtillus* are rare, as this species grows more commonly in mixtures with other shrubs or in patchy pure stands, as shown in this Thesis. This suggests that these pure stands may be unstable in the context of plant succession.

We did not find evidence that the interaction with its neighbour species changed with warming, contrary to many studies that have reported shifts in other species assemblies (Dormann et al. 2004; Pugnaire et al. 2015; Wheeler et al. 2015; Olsen et al. 2016). Although this Thesis does not focus specifically on competitive interactions, and even less on intraspecific competition, it would be worth mentioning that we did not find evidence that intraspecific competition was stronger than interspecific competition in our study plots, contrary to what the coexistence theory would predict (Chesson 2000). Even when the density of *V. myrtillus* ramets was larger in pure stands than in mixed stands, it did not perform worse in the former (see Supporting information in chapter 2).

Species-specific responses to environmental change

This Thesis shows that *V. myrtillus* may be highly responsive to specific environmental changes at high elevations. *Vaccinium myrtillus* has been found to positively respond to warming with increased growth in numerous studies (Rinnan et al. 2009; Taulavuori et al. 2013; Dawes et al. 2011, 2015), including this one. Both soil warming (+ 4 °C) and air warming (+ 1.1 °C) during the growing season months had positive effects on *V. myrtillus* growth, indicating a temperature limitation for this species at the study sites, and suggesting a positive effect of global warming on its performance. Although this Thesis does not provide evidence that warming has positive effects belowground, it is plausible that future climate conditions that lead to aboveground growth stimulation eventually have positive effects belowground. Such effects may take place through greater transfer of assimilates (with subsequent increased root exudation leading to greater soil microbial biomass), or through direct warming effects on decomposition and mineralization rates and soil microbial activity (Bardgett and Wardle 2010; Hollister and Flaherty 2010; Schindlbacher et al. 2011; Bai et al. 2013).

Contrasting with the responsiveness of *V. myrtillus*, species that coexist with it in European high elevations such as *V. uliginosum* and *E. hermaphroditum* are much less responsive to warming. Differences in their elevation distributional range might partially explain their different response. Thus, when they coexist at high elevations, climate warming might be positive for *V. myrtillus* but not for its neighbour species, which could lead to shifts in community composition and structure. Four years of passive warming in the Pyrenees did not lead to a better performance of *V. myrtillus* when growing with *V. uliginosum* than in pure stands. Thus, *V. myrtillus* did not benefit more from warming at the detriment of *V. uliginosum*. It remains unknown, however, if the performance of *V. uliginosum* was still negatively affected by the coexistence with *V. myrtillus* under higher temperatures. In addition, *V. myrtillus* could gain aerial space with warming in the long term, leading to an increased competitive interaction with its neighbours that could ultimately be detrimental for them. As aforementioned, the competitive ability of its neighbours should be assessed to find possible community composition and structure changes with warming.

Although warming may be beneficial for *V. myrtillus*, processes associated to warming might have contrary effects, such as an advanced snowmelt, which could lead to a lack of protection of *V. myrtillus* at a time when spring frosts occur. Some studies have shown that *V. myrtillus* has a low freezing resistance and is highly sensitive to spring frost (Taschler and Neuner 2004; Martin et al. 2010; Wheeler et al. 2014). Moreover, *V. myrtillus* showed a larger freezing sensitivity under soil warming and CO_2 enrichment in Stillberg (Martin et al. 2010; Rixen et al. 2012). Thus, although higher summer temperatures might be beneficial for *V. myrtillus* in a warmer climate, an associated earlier snowmelt could counterbalance these positive effects. Nevertheless, despite its sensitivity to frost, *V. myrtillus* has also shown the capacity to recover vegetatively from frost injuries in a snow removal experiment in the Northern Apennines, Italy (Gerdol et al. 2013).

When both CO_2 enrichment and soil warming effects on *V. myrtillus* xylem anatomy were studied, we found indication that its growth and hydraulic system were more responsive to warming than to CO_2 enrichment. This evidences the stronger constraint by temperature than by the lower partial pressures of CO_2 on *V. myrtillus* performance at high elevations. We found almost no interactions between these two treatments in *V. myrtillus* hydraulic function or growth. Likewise, Dieleman et al. (2012) showed that in studies combining warming and CO_2 experiments, additive effects did not often occur, whereas antagonistic responses were more common. Our results seem to exemplify this, as vessel lumen diameter decreased with warming but responded positively (although only in the later years) to CO_2 enrichment. The growth stimulation of *V. myrtillus* under soil warming and CO_2 enrichment found in a previous work (Dawes et al. 2011) and in chapter 3 in this Thesis was not accompanied by immediate xylem anatomical adjustments. It seems that the hydraulic anatomy did not need to be adjusted until a certain point, when growth stimulation may have required an improved hydraulic architecture. Interestingly, vessel size decreased under warming, especially in the first years. This could be related to a freezing event in 2007 (Rixen et al. 2012). In line with the preceding paragraph, it may be interpreted as an advantage when facing freezing events, as the risk of freezing-induced embolism decreases with vessel size (Tyre and Sperry 1989). Since temperature decreases water viscosity (Roderick and Berry 2001), reduction in vessel size might have not been detrimental for water conduction.

The drought experiment also revealed species-specific responses, although none of the *Vaccinium* species showed large signs of stress under the drought applied. Only *V. uliginosum* showed indication of mild stress under water shortage with decreased stomatal conductance and water potential, and some changes in its C allocation dynamics. Therefore, drought had different effects in these two species, which may imply shifts in community composition, structure and functioning at the long term. Moreover, alterations of the C cycle may have impacts on the ecosystem C balance (Reichstein et al. 2013) through changes in the coupling between aboveground and belowground organs, and its consequences on soil microbial community and CO_2 efflux (Ruehr et al. 2009; Burri et al. 2014, Fuchslueger et al. 2014). Llorens et al. (2002) performed an experimental drought in a heather moorland in northern Wales (UK) and found that *V. myrtillus* leaf gas exchange was not significantly affected by drought, which is in line with our results. Together with the study by Ganthaler and Mayr (2015) showing that *V. myrtillus* follows a risky hydraulic strategy, these results seem to indicate that *V. myrtillus* is quite resistant to drought. However, there is also evidence that water shortage has negative effects on *V. myrtillus*. In the Welsh drought experiment, Llorens et al. (2002) found that drought had negative effects on *V. myrtillus* stability during leaf development. Moreover, in another experiment, Taulavuori et al. (2010) found that *V. myrtillus* leaves were sensitive to drought stress, especially at a mature stage, when negative effects were more pronounced (e.g. decrease in chlorophyll concentrations, enhanced chlorophyll degradation and increase in protein oxidation) and leaf senescence was accelerated. Thus, whereas leaf gas exchange and the C cycle seem quite resistant to drought in *V. myrtillus*, sensitivity to drought stress might be reflected in other parameters. Moreover, drought severity will certainly influence the stress level of a plant and thus, its performance (Farooq et al. 2009; Nandintsetseg and Shinoda 2013).

In summary, temperature is a clear constraint for *V. myrtillus* growth at high elevations. The increase in CO_2 concentrations does not seem to have major effects on its growth and hydraulic function, although we found a slight growth stimulation and some lagged effects on its xylem anatomy. This might indicate that adjustments of the xylem anatomical structure were not needed until growth stimulation reached a certain point. Although *V. myrtillus* and *V. uliginosum* were quite resistant to drought, the indication of a mild stress in *V. uliginosum* suggests that it is more sensitive to drought and thus, it could have a competitive disadvantage respect to *V. myrtillus* under summer drought episodes. Moreover, C allocation shifts found in *V. uliginosum* could have effects on belowground processes, including shifts in soil microbial communities and soil CO_2 efflux rates (Ruehr et al. 2009; Burri et al. 2014; Fuchslueger et al. 2014), with alterations on the community and ecosystem C balance.

Effects of the overstorey tree species on *V. myrtillus* growth, functioning and its response to the treatments

The experiments comprised in this Thesis have been performed at the upper subalpine belt, the great majority within the treeline ecotone, i.e. between the subalpine and the alpine belts. While chapters 1, 2 and 5 were performed in grasslands colonized by shrubs, which are quite open habitats, chapters 3 and 4 were carried out at the Stillberg experimental site, which is a tree plantation at the treeline. Thus, experiments at Stillberg allowed for the analyses of different growth, phenological and anatomical parameters of *V. myrtillus* when growing in the understorey of two different tree species: the evergreen *Pinus mugo* subsp. *uncinata* and the deciduous *Larix decidua*. These tree species may be dominant in extensive areas in the Pyrenees and in the Alps (*P. mugo* subsp. *uncinata* in the Pyrenees and *L. decidua* in the inner Alps; Ozenda 1985; Ninot et al. 2008), which underlines the importance of studying shrub species growing in their understorey. The overstorey tree species identity had large effects on *V. myrtillus* phenology, growth, age and wood anatomy (Table 1), which evidences its relevance for shrub performance, and the importance of considering tree species identity when studying dwarf shrubs in forest ecosystems, more specifically at the treeline.

Factors related to the tree species identity that directly influenced *V. myrtillus* performance and its responses to soil warming or CO_2 enrichment have not been evaluated here. However, the hypothesis that the different leaf habit of the tree species has a strong influence on *V. myrtillus* is more than plausible. A deciduous canopy (i.e. *Larix decidua*) allows more light to pass through and reach the understorey at the early growing season. Thus, snow would melt earlier and leave *V. myrtillus* uncovered, increasing the risk of experiencing early season frosts. As mentioned in the section above, *V. myrtillus* has a low freezing resistance and frost events without the snow protection might compromise its growth. Other possible reasons for the strong differences found in *V. myrtillus* between plot tree species may be related with the lower N availability under pine trees in this experiment (Dawes et al. 2017), which may lead to a competitive advantage of *V. myrtillus* over more N-demanding graminoids.

Thus, tree species influence the understorey community, but shrub species also play an important role on modulating forest ecosystems. Spontaneous reforestation at high elevations is frequently led by encroaching shrub communities, and shrubs can have a key role in facilitating tree seedling survival and recruitment (Batllori et al. 2009; Grau et al. 2010, 2012). Contrarily, they may have allelopathic effects on the tree species, such as those of *V. myrtillus* litter on *P. uncinata* seed germination reported by Ninot et al. (2016).

High mountain shrub communities facing global change

Although abrupt climate changes are intrinsic to alpine ecosystems, changes in temperature and precipitation trends and, as a consequence, snow patterns will certainly have large impacts on these ecosystems (Körner 2003). Co-occurring plant species show specific adaptations that explain their different responses to environmental cues. In front of warmer temperatures, higher CO_2 concentrations, increasing drought events or earlier snowmelt, they will respond differently, as has been shown in this Thesis. Therefore, we might expect changes in plant community assemblages with changing climate conditions and thus, shifts in their structure and functioning (Sundqvist et al. 2008; Kreyling et al. 2012; Pardo et al. 2013). Nevertheless, we must not forget one of the main drivers explaining changes in worldwide ecosystems, which is human activity. Future anthropogenic activity will not only have indirect impacts on these ecosystems through climate change, but more direct impacts, such as those occurring at present through the social transformations that have led to land-use change in mountain areas (Prévosto et al. 2011; Ameztegui et al. 2016).

The encroachment of subalpine and alpine grasslands, as well as other tundra ecosystems has been extensively reported, together with the negative impacts that these processes have on biodiversity and ecosystem structure and function (Wilson and Nilsson 2009; Eldridge et al. 2011; Myers-Smith et al. 2011; Pajunen et al. 2011; Brandt et al. 2013; Koch et al. 2015; amongst others). However, a study by Barros et al. (2016)

suggests that intense drought events can counteract this woody encroachment driven by climate and land-use changes. They found that forest and shrubland expansion was first accelerated with drought in early study years, but in later years, with more frequent and severe drought the expansion decelerated. The authors also reported that at treeline, changes in biodiversity were mostly driven by gradual climate change and land-use change, whereas drought influenced the rate at which these changes occur. Results from a previous experiment in a grassland next to our study site in the Stubai valley (Austrian Alps) showed that drought effects were more severe in herbaceous species (Hasibeder et al. 2015) than the effects found on shrubs in this Thesis. However, other parameters related to resilience to drought (including recovery, growth rates or survival) that could be especially important for non-woody species (Ingrisch et al. in prep.) and which could determine the future behaviour of ecosystems when facing drought episodes have not been assessed here.

The diversity of species forming plant communities and the wide range of processes that these communities encompass makes it extremely difficult to predict how they will behave in the future, especially when climate projections differ between each other (at a higher or lower extent depending on the climate factor). In this Thesis, evidence for species-specific responses to particular climate factor changes has been provided, but extrapolating our results to the whole community would be inaccurate and misleading. Nevertheless, knowledge gained here not only provides a better understanding of the performance of dwarf shrubs (and, as a consequence, also of the communities they form), but it is also a baseline for further studies on these shrub communities that may lead to a wider and more general knowledge.

Future research

Performing all the experiments comprised in this Thesis was scientifically and personally stimulating and provided answers to many questions related to the performance of high mountain dwarf shrubs in front of climate change. However, during these years of research many other questions have arisen, and they certainly deserve time for being studied.

One of the fields that remained unexplored is how clonality may help connected ramets to cope with climate change. All our studies were based on ramets because of the difficulties of identifying genetic individuals (connected with rhizomes or not) in these dwarf shrub species, especially in studies spanning several years. However, it would be very interesting to go into detail about the effects of clonality on the response of these species to environmental changes.

It would be indeed a promising line to study the encroachment ability of these dwarf shrubs, and how this ability acts in front of changing climate conditions. Moreover, a long-term monitoring involving both land-use change and environmental change treatments enabling the discrimination of the effects from each other would definitely be challenging.

Another line that remains open is the mechanism by which overstorey tree species may affect understorey dwarf shrubs. An experiment controlling for light at the early season and during the growing season, snow melt timing, tree litter and soil pH, tree specific mychorrizal associations and the soil microbial community, amongst others, would certainly give us more answers on this topic.

Finally, regarding drought events, it would definitely be interesting to perform a larger experiment in which two different levels of drought (less intense and extremely intense) were applied during several years. In this way, we could have a better understanding of the stress caused by different intensities and also the effects of recurrence. In this line, further analyses of specific C-compounds would provide more information on the effects of drought on the study species. It would be even better if large amounts of rhizomes could be sampled, to find out how far the tracer could be transported in a certain amount of time. Moreover, studying the recovery phase would provide a deeper insight into the overall effects of drought.

Conclusions

***Vaccinium myrtillus* growth and performance at the treeline ecotone in the Pyrenees may not be affected by its neighbouring species.** Ramets growing in pure stands and in mixed stands at Eth Corrau des Machos showed similar growth, functioning and age, suggesting that the dynamics of this species at the upper subalpine belt would not be modified by the presence of its neighbour species.

Air and soil warming clearly favour *Vaccinium myrtillus* growth at the treeline ecotone. Experiments in the Pyrenees and the Alps showed that ramet aboveground biomass, height, shoot elongation and secondary growth increased with warming, and that these positive growth responses were sustained over the experimental years. There was an uncoupling between aboveground and belowground responses to air warming, which may be partially due to the fact that warming was applied with OTC aboveground. In any case, there was either no increased transfer of assimilates to belowground organs after an increase in aboveground biomass under warming, or the transfer was diluted by the extensive network of rhizomes. The lack of warming effects on the functional diversity of the microbial community was associated to a lack of warming effects on soil C pools. Nevertheless, soil N concentrations changed with warming towards a decrease in NO_3^-, most probably associated with an increase in NO_3^- uptake by the plant community. *Vaccinium myrtillus* growth increases were not necessarily related (although in the Pyrenees they may have been) to a longer growing season caused by earlier snowmelt or an earlier leaf bud break and expansion. Direct effects of temperature on photosynthesis, tissue formation and soil processes leading to a greater nutrient availability (i.e. decomposition and mineralization) might explain the increased growth when the growing season was not advanced.

Passive air warming of 1.1 °C did not lead to changes in the interaction between *V. myrtillus* and its neighbour shrubs *V. uliginosum* and *R. ferrugineum*. These results suggest that species interactions could be less responsive to warming when studied in their natural occurrence than in removal experiments, and that future warming might not lead to changes in these species interactions in the short-term. Although warming effects on the coexisting *V. myrtillus* and *V. uliginosum* were contrasting (positive in the former, null in the latter), *V. myrtillus* did not benefit more from warmer temperatures in detriment of *V. uliginosum*. Thus, although *V. myrtillus* growth (and maybe ultimately cover) might increase with warming in the future, this would not necessarily imply a direct negative effect for *V. uliginosum* performance in areas where they coexist.

***Vaccinium myrtillus* growth stimulation under CO_2 enrichment and soil warming at the treeline was not accompanied by immediate changes in xylem anatomy.** Vessel lumen size of *V. myrtillus* decreased under warming and only increased slightly with CO_2 enrichment at the end of the experiment. Reduced vessel lumen sizes may have not had negative implications on *V. myrtillus* growth because temperature increase reduces water viscosity (thus, water transport is more efficient at the same vessel size). Moreover, smaller vessels might imply a benefit as they are associated with a reduced risk of cavitation caused by freeze-thaw events. *Vaccinium myrtillus* storage tissues were quite unresponsive to changes in temperature and CO_2 concentrations. Thus, CO_2 enrichment and soil warming affected *V. myrtillus* growth and hydraulic function rather than storage. Soil warming had larger effects than CO_2 enrichment on *V. myrtillus* growth and wood anatomy, pointing to the stronger limitation by temperature on this species performance at treeline.

The identity of the overstorey tree species has an influence on *V. myrtillus* growth and performance at the treeline. The tree species included in the experiments (*Pinus mugo* subsp. *uncinata* and *Larix decidua*) had different effects on the phenology, growth, turnover and hydraulic efficiency of *V. myrtillus*. This could be related to the different early-season microclimate conditions created under the two tree species. The evergreen pine might have conferred a greater protection to *V. myrtillus* against freezing than the deciduous larch. Moreover, a greater light incidence under a deciduous canopy when snow starts melting may accelerate the melting process and thus leave ramets uncovered when early season frosts are likely to occur. Differences in soil nutrient availability between plots with different tree species might also influence growth and performance of the understorey shrubs.

***Vaccinium myrtillus* and *V. uliginosum* show different strategies with relation to their C allocation dynamics**, partially due to their contrasting shoot functionality. *Vaccinium myrtillus* photosynthetic shoots seemed to re-assimilate C respired by other organs. On the other hand, *V. uliginosum* leaves had a more active C fixation than those of *V. myrtillus*.

***Vaccinium myrtillus* and *V. uliginosum* were quite resistant to a drought event, although the latter showed signs of mild stress**. *Vaccinium uliginosum* C allocation dynamics slightly changed towards a slower C transfer belowground with drought, which could have implications for the ecosystem C balance at the long term. Communities dominated by these dwarf shrubs may be relatively resistant to drought events, but since drought episodes seem to have different effects on these coexisting shrubs, shifts on plant community composition may occur. Different responses to drought and different recovery strategies between coexisting plant functional types could exacerbate these shifts.

Climate change has larger impacts on some species than on other co-occurring species, which underlines the necessity of studying species-specific responses to environmental conditions. High mountain ecosystems are especially vulnerable to changes in temperature with its direct and indirect effects, and plant communities will undergo compositional, structural and functional changes in the future, with large implications for the ecosystem. Together with the role of human action, understanding the role of environmental shifts on plant species growth and performance is basic for forecasting and modelling community and ecosystem future directions.

Referències

References

Referències/References

Aeschimann D., Lauber K., Moser D.M., Theurillat J.-P. (2004) *Flora alpina*, *vol. 1*. Haupt, Bern, Switzerland, 1159 pp.

Ainsworth E.A., Long S.P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165:351–372.

Albert K.R., Ro-Poulsen H., Mikkelsen T.N., Michelsen A., Van Der Linden L., Beier C. (2011) Interactive effects of elevated CO_2, warming, and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem. *Journal of Experimental Botany* 62:4253–4266.

Ameztegui A., Coll L., Brotons L., Ninot J.M. (2016) Land-use legacies rather than climate change are driving the recent upward shift of the mountain tree line in the Pyrenees. *Global Ecology and Biogeography* 25:263–273.

Anthelme F., Villaret J.-C., Brun J.-J. (2007) Shrub encroachment in the Alps gives rise to the convergence of sub-alpine communities on a regional scale. *Journal of Vegetation Science* 18:355–362.

Araki K., Shimatani K., Ohara M. (2009) Dynamics of distribution and performance of ramets constructing genets: a demographic–genetic study in a clonal plant, *Convallaria keiskei*. *Annals of Botany* 104:71-79.

Arft A.M., Walker M.D., Gurevitch J., Alatalo J.M., Bret-Harte M.S., Dale M., Diemer M., Gugerli F., Henry G.H.R., Jones M.H., Hollister R.D., Jónsdóttir I.S., Laine K., Lévesque E., Marion G.M., Molau U., MØlgaard P., Nordenhäll U., Raszhivin V., Robinson C.H., Starr G., Stenström A., Stenström M., Totland Ø., Turner P.L., Walker L.J., Webber P.J., Welker J.M., Wookey P.A. (1999) Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* 69:491–511.

Armas C., Schöb C., Gutiérrez J.R. (2013) Modulating effects of ontogeny on the outcome of plant–plant interactions along stress gradients. *New Phytologist* 200:7–9.

Bader M.K.-F., Leuzinger S., Keel S.G., Siegwolf R.T.W., Hagedorn F., Schleppi P., Körner C. (2013) Central European hardwood trees in a high-CO_2 future: Synthesis of an 8-year forest canopy CO_2 enrichment project. *Journal of Ecology* 101:1509–1519.

Bai E., Li S., Xu W., Li W., Dai W., Jiang P. (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist* 199:441–451.

Bardgett R.D., Wardle D.A. (2010) *Aboveground-belowground linkages. Biotic interactions, ecosystem processes, and global change*. Oxford University Press, Oxford, UK, 301 pp.

Barros C., Guéguen M., Douzet R., Carboni M., Boulangeat I., Zimmermann N.E., Münkemüller T., Thuiller W. (2016) Extreme climate events counteract the effects of climate and land-use changes in Alpine tree lines. *Journal of Applied Ecology*. doi: 10.1111/1365-2664.12742

Bassin S., Werner R.A., Sörgel K., Volk M., Buchmann N., Fuhrer J. (2009) Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. *Oecologia* 158:747–756.

Batllori E., Camarero J.J., Ninot J.M., Gutiérrez E. (2009) Seedling recruitment, survival and facilitation in alpine Pinus uncinata tree line ecotones. Implications and potential responses to climate warming. *Global Ecology and Biogeography* 18:460–472.

Beniston M. (2003) Climatic change in mountain regions: A review of possible impacts. *Climatic Change* 59:5–31.

Bertness M.D., Callaway R. (1994) Positive interactions in communities. *Trends in Ecology and Evolution* 9:187–191.

Boesgaard K.S., Albert K.R., Ro-Poulsen H., Michelsen A., Mikkelsen T.N., Schmidt N.M. (2012) Long-term structural canopy changes sustain net photosynthesis per ground area in high arctic *Vaccinium uliginosum* exposed to changes in near-ambient UV-B levels. *Physiologia Plantarum* 145:540–550.

Bokhorst S., Bjerke J.W., Street L.E., Callaghan T.V., Phoenix G.K. (2011) Impacts of multiple extreme winter warming events on sub-Arctic heathland: Phenology, reproduction, growth, and CO_2 flux responses. *Global Change Biology* 17:2817–2830.

Bolòs O., Vigo J. (1995) *Flora dels Països Catalans*, *vol. 3*. Editorial Barcino, Barcelona, Spain, 1230 pp.

Bolòs O., Vigo J., Masalles R.M., Ninot J.M. (2005) *Flora manual dels Països Catalans*, 3rd ed. Pòrtic, Barcelona, Spain, 1310 pp.

Brandt J.S., Haynes M.A., Kuemmerle T., Waller D.M., Radeloff V.C. (2013) Regime shift on the roof of the world: Alpine meadows converting to shrublands in the southern Himalayas. *Biological Conservation* 158:116 – 127.

Bret-Harte M.S., Shaver G.R., Chapin III F.S. (2002) Primary and secondary stem growth in arctic shrubs: Implications for community response to environmental change. *Journal of Ecology* 90:251–267.

Brooker R.W. (2006) Plant-plant interactions and environmental change: Tansley review. *New Phytologist* 171:271–284.

Brüggemann N., Gessler A., Kayler Z., Keel S.G., Badeck F., Barthel M., Boeckx P., Buchmann N., Brugnoli E., Esperschütz J., Gavrichkova O., Ghashghaie J., Gomez-Casanovas N., Keitel C., Knohl A., Kuptz D., Palacio S., Salmon Y., Uchida Y., Bahn M. (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. *Biogeosciences* 8:3457–3489.

Burri S., Sturm P., Prechsl U.E., Knohl A., Buchmann N. (2014) The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO_2 efflux in a temperate grassland. *Biogeosciences* 11:961–975.

Callaway R.M., Walker L.R. (1997) Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* 78:1958–1965.

Campioli M., Leblans N., Michelsen A. (2012) Twenty-two years of warming, fertilisation and shading of subarctic heath shrubs promote secondary growth and plasticity but not primary growth. *PLoS ONE* 7: e34842.

Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31:343–366.

Choler P., Michalet R., Callaway R.M. (2001) Facilitation and competition on gradients in alpine plant communities. *Ecology* 82:3295–3308.

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General discussion

Chu C.-J., Maestre F.T., Xiao S., Weiner J., Wang Y.-S., Duan Z.-H., Wang G. (2008) Balance between facilitation and resource competition determines biomass–density relationships in plant populations. *Ecology Letters* 11:1189–1197.

Collins M., Knutti R., Arblaster J., Dufresne J.-L., Fichefet T., Friedlingstein P., Gao X., Gutowski W.J., Johns T., Krinner G., Shongwe M., Tebaldi C., Weaver A.J., Wehner W. (2013) Long-term climate change: projections, commitments and irreversibility. In: Stocker T.F., Quin D., Plattner G.-K., Tignor M., Allen S.K., Bosching J., Nauels A., Xia Y., Bex V., Midgley P.M. (eds.) *Climate Change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, pp. 1029-1136.

Cornelissen J.H.C., Van Bodegom P.M., Aerts R., Callaghan T.V., Van Logtestijn R.S.P., Alatalo J., Stuart Chapin F., Gerdol R., Gudmundsson J., Gwynn-Jones D., Hartley A.E., Hik D.S., Hofgaard A., Jónsdóttir I.S., Karlsson S., Klein J.A., Laundre J., Magnusson B., Michelsen A., Molau U., Onipchenko V.G., Quested H.M., Sandvik S.M., Schmidt I.K., Shaver G.R., Solheim B., Soudzilovskaia N.A., Stenström A., Tolvanen A., Totland Ø., Wada N., Welker J.M., Zhao X., Brancaleoni L., Brancaleoni L., De Beus M.A.H., Cooper E.J., Dalen L., Harte J., Hobbie S.E., Hoefsloot G., Jägerbrand A., Jonasson S., Lee J.A., Lindblad K., Melillo J.M., Neill C., Press M.C., Rozema J., Zielke M. (2007) Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. *Ecology Letters* 10:619–627.

D’Odorico P., He Y., Collins S., De Wekker S.F.J., Engel V., Fuentes J.D. (2013) Vegetation-microclimate feedbacks in woodland-grassland ecotones. *Global Ecology and Biogeography* 22:364–379.

Dai A. (2013) Increasing drought under global warming in observations and models. *Nature Climate Change* 3:52–58.

Dawes M.A., Hagedorn F., Zumbrunn T., Handa I.T., Hättenschwiler S., Wipf S., Rixen C. (2011) Growth and community responses of alpine dwarf shrubs to *in situ* CO_2 enrichment and soil warming. *New Phytologist* 191:806–818.

Dawes M.A., Philipson C.D., Fonti P., Bebi P., Hättenschwiler S., Hagedorn F., Rixen C. (2015) Soil warming and CO_2 enrichment induce biomass shifts in alpine tree line vegetation. *Global Change Biology* 21:2005–2021.

Dawes M.A., Schleppi P., Hättenschwiler S., Rixen C., Hagedorn F. (2017) Soil warming opens the nitrogen cycle at the alpine treeline. *Global Change Biology* 23:421–434.

Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H., Tu K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* 33:507–559.

De Boeck H.J., Bassin S., Verlinden M., Zeiter M., Hiltbrunner E. (2016) Simulated heat waves affected alpine grassland only in combination with drought. *New Phytologist* 209:531–541.

Dieleman W.I.J., Vicca S., Dijkstra F.A., Hagedorn F., Hovenden M.J., Larsen K.S., Morgan J.A., Volder A., Beier C., Dukes J.S., King J., Leuzinger S., Linder S., Luo Y., Oren R., De Angelis P., Tingey D., Hoosbeek M.R., Janssens I.A. (2012) Simple additive effects are rare: A quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. *Global Change Biology* 18:2681–2693.

Dormann C.F., Van Der Wal R., Woodin S.J. (2004) Neighbour identity modifies effects of elevated temperature on plant performance in the High Arctic. *Global Change Biology* 10:1587–1598.

Dullinger S., Dirnböck T., Grabherr G. (2003) Patterns of shrub invasion into high mountain grasslands of the northern Calcareous Alps, Austria. *Arctic, Antarctic, and Alpine Research* 35:434–441.

Dunne J.A., Saleska S.R., Fischer M.L., Harte J. (2004) Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85:904–916.

Eldridge D.J., Bowker M.A., Maestre F.T., Roger E., Reynolds J.F., Whitford W.G. (2011) Impacts of shrub encroachment on ecosystem structure and functioning: Towards a global synthesis. *Ecology Letters* 14:709–722.

Eldridge D.J., Soliveres S. (2014) Are shrubs really a sign of declining ecosystem function? Disentangling the myths and truths of woody encroachment in Australia. *Australian Journal of Botany* 62:594–608.

Elmendorf S.C., Henry G.H.R., Hollister R.D., Fosaa A.M., Gould W.A., Hermanutz L., Hofgaard A., Jónsdóttir I.S., Jorgenson J.C., Lévesque E., Magnusson B., Molau U., Myers-Smith I.H., Oberbauer S.F., Rixen C., Tweedie C.E., Walker M.D. (2015) Experiment, monitoring, and gradient methods used to infer climate change effects on plant communities yield consistent patterns. *Proceedings of the National Academy of Sciences* 112:448–452.

Emmerton K.S., Callaghan T.V., Jones H.E., Leake J.R., Michelsen A., Read D.J. (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. *New Phytologist* 151:503–511.

Emmett B.A., Beier C., Estiarte M., Tietema A., Kristensen H.L., Williams D., Peñuelas J., Schmidt I., Sowerby A. (2004) The response of soil processes to climate change: results from manipulation studies of shrublands across an environmental gradient. *Ecosystems* 7:625–637.

Engler R., Randin C.F., Thuiller W., Dullinger S., Zimmermann N.E., Araújo M.B., Pearman P.B., Le Lay G., Piedallu C., Albert C.H., Choler P., Coldea G., De Lamo X., Dirnböck T., Gégout J.-C., Gómez-García D., Grytnes J.-A., Heegaard E., Høistad F., Nogués-Bravo D., Normand S., Puşcaş M., Sebastià M.-T., Stanisci A., Theurillat J.-P., Trivedi M.R., Vittoz P., Guisan A. (2011) 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology* 17:2330–2341.

Epron D., Bahn M., Derrien D., Lattanzi F.A., Pumpanen J., Gessler A., Högberg P., Maillard P., Dannoura M., Gérant D., Buchmann N. (2012) Pulse-labelling trees to study carbon allocation dynamics: A review of methods, current knowledge and future prospects. *Tree Physiology* 32:776–798.

Evans R.D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science* 6:121–126.

Fajardo A., McIntire E.J.B. (2011) Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. *Journal of Ecology* 99:642–650.

Farooq M., Wahid, A., Kobayashi, N. (2009) Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* 29:185–212.

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–537.

Flower-Ellis J.G.K. (1971) Age, structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). *Department of Forest Ecology and Forest Soils, Research Note 9*. Royal College of Forestry, Stockholm, Sweden, 108 pp.

Fonti P., Jansen S. (2012) Xylem plasticity in response to climate. *New Phytologist* 195:734–736.

Fonti P., Von Arx G., García-González I., Eilmann B., Sass-Klaassen U., Gärtner H., Eckstein D. (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* 185:42–53.

Frank D., Reichstein M., Bahn M., Thonicke K., Frank D., Mahecha M.D., Smith P., van der Velde M., Vicca S., Babst F., Beer C., Buchmann N., Canadell J.G., Ciais P., Cramer W., Ibrom A., Miglietta F., Poulter B., Rammig A., Seneviratne S.I., Walz A., Wattenbach M., Zavala M.A., Zscheischler J. (2015) Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. *Global Change Biology* 21:2861–2880.

Fuchslueger L., Bahn M., Fritz K., Hasibeder R., Richter A. (2014) Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. *New Phytologist* 201:916–927.

Fuchslueger L., Bahn M., Hasibeder R., Kienzl S., Fritz K., Schmitt M., Watzka M., Richter A., Austin A. (2016) Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. *Journal of Ecology* 104:1453–1465.

Ganthaler A., Mayr S. (2015) Dwarf shrub hydraulics: Two *Vaccinium* species (*Vaccinium myrtillus, Vaccinium vitis-idaea*) of the European Alps compared. *Physiologia Plantarum* 155:424–434.

Gärtner H., Schweingruber F.H. (2013) *Microscopic preparation techniques for plant stem analysis*. Kessel Publishing House, Remagen, Germany, 78 pp.

Gerdol R., Siffi C., Iacumin P., Gualmini M., Tomaselli M. (2013) Advanced snowmelt affects vegetative growth and sexual reproduction of *Vaccinium myrtillus* in a sub-alpine heath. *Journal of Vegetation Science* 24:569–579.

Ghashghaie J., Tcherkez G. (2013) Chapter Eight - Isotope Ratio Mass Spectrometry Technique to Follow Plant Metabolism: Principles and Applications of $^{12}C/^{13}C$ Isotopes. In: Rolin D. (ed) *Metabolomics Coming of Age with its Technological Diversity*. Academic Press, pp 377 – 405.

Grau O. (2014) *Interaccions planta-planta en gradients d'estrès en ecosistemes freds*. Tesi Doctoral, Universitat de Barcelona, Barcelona, Spain, 171 pp.

Grau O., Ninot J.M., Blanco-Moreno J.M., van Logtestijn R.S.P., Cornelissen J.H.C., Callaghan T.V. (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. *Oikos* 121:1680–1690.

Grau O., Rautio P., Heikkinen J., Saravesi K., Kozlov M.V., Markkola A. (2010) An ericoid shrub plays a dual role in recruiting both pines and their fungal symbionts along primary succession gradients. *Oikos* 119:1727–1734.

Grissino-Mayer H.D. (2001) Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-ring Research* 57: 205-221.

Gwynn-Jones D., Jones A.G., Waterhouse A., Winters A., Comont D., Scullion J., Gardias R., Graee B.J., Lee J.A., Callaghan T.V. (2012) Enhanced UV-B and elevated CO_2 impacts sub-arctic shrub berry abundance, quality and seed germination. *Ambio* 41:256–268.

Gwynn-Jones D., Lee J.A., Callaghan T.V. (1997) Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-arctic forest heath ecosystem. *Plant Ecology* 128:242–249.

Hagedorn F., Martin M., Rixen C., Rusch S., Bebi P., Zürcher A., Siegwolf R.T.W., Wipf S., Escape C., Roy J., Hättenschwiler S. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. *Biogeochemistry* 97:7–19.

Handa I.T., Körner C., Hättenschwiler S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO_2 enrichment and defoliation. *Ecology* 86:1288–1300.

Hasibeder R., Fuchslueger L., Richter A., Bahn M. (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytologist* 205:1117–1127.

Hättenschwiler S., Körner C. (1996) System-level adjustments to elevated CO_2 in model spruce ecosystems. *Global Change Biology* 2:377–387.

Hättenschwiler S., Körner C. (1998) Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. *Oecologia* 113:104–114.

Hobbie E.A., Macko S.A., Williams M. (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate-plant-mycorrhizal interactions. *Oecologia* 122:273–283.

Hoffmann A.A., Camac J.S., Williams R.J., Papst W., Jarrad F.C., Wahren C.-H. (2010) Phenological changes in six Australian subalpine plants in response to experimental warming and year-to-year variation. *Journal of Ecology* 98:927–937.

Hollister R.D., Flaherty K.J. (2010) Above- and below-ground plant biomass response to experimental warming in northern Alaska. *Applied Vegetation Science* 13:378–387.

Holsinger K.E. (2000) Reproductive systems and evolution in vascular plants. *Proceedings of the National Academy of Sciences* 97:7037–7042.

Hutchings M.J., Wijesinghe D.K. (1997) Patchy habitats, division of labour and growth dividends in clonal plants. *Trends in Ecology and Evolution* 12:390–394.

Illa E., Ninot J.M., Anadon-Rosell A., Oliva F. (in review at *Folia Geobotanica*) The role of topography, bedrock and dominant species traits on functional structure and processes of alpine sub-shrub communities.

Inauen N., Körner C., Hiltbrunner E. (2012) No growth stimulation by CO_2 enrichment in alpine glacier forefield plants. *Global Change Biology* 18:985–999.

Ingrisch J., Karlowsky S., Hasibeder R. Anadon-Rosell A., König A., Augusti A., Gleixner G., Bahn M. (in prep.) Land use alters the drought response of productivity and CO_2 fluxes in mountain grassland.

IPCC (2014) *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on*

Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jacquemart A.-L. (1996) *Vaccinium uliginosum* L. *Journal of Ecology* 84:771–785.

Jump A.S., Peñuelas J. (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* 8:1010–1020.

Kaennel M., Schweingruber F.H. (1995) *Multilingual glossary of dendrochronology*. Haupt, Bern, Switzerland, 467 pp.

Kardol P., Campany C.E., Souza L., Norby R.J., Weltzin J.F., Classen A.T. (2010) Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Global Change Biology* 16:2676–2687.

Klanderud K. (2005) Climate change effects on species interactions in an alpine plant community. *Journal of Ecology* 93:127–137.

Klanderud K. (2008) Species-specific responses of an alpine plant community under simulated environmental change. *Journal of Vegetation Science* 19:363–372.

Koch B., Edwards P.J., Blanckenhorn W.U., Walter T., Hofer G. (2015) Shrub encroachment affects the diversity of plants, butterflies, and grasshoppers on two Swiss subalpine pastures. *Arctic, Antarctic, and Alpine Research* 47:345–357.

Komac B., Esteban P., Trapero L., Caritg R. (2016) Modelization of the current and future habitat suitability of *Rhododendron ferrugineum* using potential snow accumulation. *PLoS ONE* 11:1–18.

Komac B., Kefi S., Nuche P., Escós J., Alados C.L. (2013) Modeling shrub encroachment in subalpine grasslands under different environmental and management scenarios. *Journal of Environmental Management* 121:160–169.

Körner C. (2003) *Alpine plant life: functional plant ecology of high mountain ecosystems*. 2n ed. Springer, Berlin, Germany, 344 pp.

Körner C., Diemer M., Schappi B., Niklaus P., Arnone III J. (1997) The responses of alpine grassland to four seasons of CO_2 enrichment: A synthesis. *Acta Oecologica* 18:165–175.

Kreyling J., Haei M., Laudon H. (2012) Absence of snow cover reduces understory plant cover and alters plant community composition in boreal forests. *Oecologia* 168:577–587.

Kudo G., Suzuki S. (2003) Warming effects on growth, production, and vegetation structure of alpine shrubs: A five-year experiment in northern Japan. *Oecologia* 135:280–287.

Lambers H., Chapin III F.S., Pons T.L. (2008) *Plant Physiological Ecology*. 2nd ed. Springer, New York, USA, 604 pp.

Lambert A.M., Miller-Rushing A.J., Inouye D.W. (2010) Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae). *American Journal of Botany* 97:1431–1437.

Le Roux P.C., Shaw J.D., Chown S.L. (2013) Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist* 200:241–250.

Leitinger G., Ruggenthaler R., Hammerle A., Lavorel S., Schirpke U., Clement J., Lamarque P., Obojes N., Tappeiner U. (2015) Impact of droughts on water provision in managed alpine grasslands in two climatically different regions of the Alps. *Ecohydrology* 8:1600–1613.

Lembicz M., Piszczałka P., Grzybowski T., Woźniak M., Jarmołowski A., Borkowska L., Falińska K. (2011) Microsatellite identification of ramet genotypes in a clonal plant with phalanx growth: The case of *Cirsium rivulare* (Asteraceae). *Flora - Morphology, Distribution, Functional Ecology of Plants* 206:792 – 798.

Li H., Shen H., Chen L., Liu T., Hu H., Zhao X., Zhou L., Zhang P., Fang J. (2016) Effects of shrub encroachment on soil organic carbon in global grasslands. *Scientific Reports* 6:28974.

Little C.J., Jägerbrand A.K., Molau U., Alatalo J.M. (2015) Community and species-specific responses to simulated global change in two subarctic-alpine plant communities. *Ecosphere* 6:227.

Livensperger C., Steltzer H., Darrouzet-Nardi A., Sullivan P.F., Wallenstein M., Weintraub M.N. (2016) Earlier snowmelt and warming lead to earlier but not necessarily more plant growth. *AoB Plants* 8: plw021.

Llorens L., Peñuelas J., Beier C., Emmett B., Estiarte M., Tietema A. (2004) Effects of an experimental increase of temperature and drought on the photosynthetic performance of two ericaceous shrub species along a north-south European gradient. *Ecosystems* 7:613–624.

Llorens L., Peñuelas J., Emmett B. (2002) Developmental instability and gas exchange responses of a heathland shrub to experimental drought and warming. *International Journal of Plant Sciences* 163:959–967.

López-Moreno J.I., Beniston M. (2009) Daily precipitation intensity projected for the 21st century: Seasonal changes over the Pyrenees. *Theoretical and Applied Climatology* 95:375–384.

MacDonald D., Crabtree J.R., Wiesinger G., Dax T., Stamou N., Fleury P., Lazpita J.G., Gibon A. (2000) Agricultural abandonment in mountain areas of Europe: Environmental consequences and policy response. *Journal of Environmental Management* 59:47 – 69.

Maestre F.T., Callaway R.M., Valladares F., Lortie C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97:199–205.

Maestre F.T., Cortina J. (2004) Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proceedings of the Royal Society B: Biological Sciences* 271:S331–S333.

Martin M., Gavazov K., Körner C., Hättenschwiler S., Rixen C. (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. *Global Change Biology* 16:1057–1070.

Michelsen A., Quarmby C., Sleep D., Jonasson S. (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia* 115:406–418.

Mikkelsen T.N., Beier C., Jonasson S., Holmstrup M., Schmidt I.K., Ambus P., Pilegaard K., Michelsen A., Albert K., Andresen L.C., Arndal M.F., Bruun N., Christensen S., Danbæk S., Gundersen P., Jørgensen P., Linden L.G., Kongstad J., Maraldo K., Priemé A., Riis-Nielsen T., Ro-Poulsen H., Stevnbak K., Selsted M.B., Sørensen P., Larsen K.S., Carter M.S., Ibrom A., Martinussen T., Miglietta F., Sverdrup H. (2008) Experimental design of multifactor climate change experiments with elevated CO_2, warming and drought: The CLIMAITE project. *Functional Ecology* 22:185–195.

Miriti M.N. (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* 94:973–979.

Mizuki I., Tani N., Ishida K., Tsumura Y. (2005) Development and characterization of microsatellite markers in a clonal plant, *Dioscorea japonica* Thunb. *Molecular Ecology Notes* 5:721–723.

Montané F., Romanyà J., Rovira P., Casals P. (2010) Aboveground litter quality changes may drive soil organic carbon increase after shrub encroachment into mountain grasslands. *Plant and Soil* 337:151–165.

Morison J.I.L., Morecroft M.D. (2006) *Plant growth and climate change*. Blackwell Publishing Ltd, Oxford , UK, 232 pp.

Myers-Smith I.H., Elmendorf S.C., Beck P.S.A., Wilmking M., Hallinger M., Blok D., Tape K.D., Rayback S.A., Macias-Fauria M., Forbes B.C., Speed J.D.M., Boulanger-Lapointe N., Rixen C., Lévesque E., Schmidt N.M., Baittinger C., Trant A.J., Hermanutz L., Collier L.S., Dawes M.A., Lantz T.C., Weijers S., JØrgensen R.H., Buchwal A., Buras A., Naito A.T., Ravolainen V., Schaepman-Strub G., Wheeler J.A., Wipf S., Guay K.C., Hik D.S., Vellend M. (2015) Climate sensitivity of shrub growth across the tundra biome. *Nature Climate Change* 5:887–891.

Myers-Smith I.H., Forbes B.C., Wilmking M., Hallinger M., Lantz T., Blok D., Tape K.D., MacIas-Fauria M., Sass-Klaassen U., Lévesque E., Boudreau S., Ropars P., Hermanutz L., Trant A., Collier L.S., Weijers S., Rozema J., Rayback S.A., Schmidt N.M., Schaepman-Strub G., Wipf S., Rixen C., Ménard C.B., Venn S., Goetz S., Andreu-Hayles L., Elmendorf S., Ravolainen V., Welker J., Grogan P., Epstein H.E., Hik D.S. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. *Environmental Research Letters* 6:045509.

Myers-Smith I.H., Hik D.S. (2013) Shrub canopies influence soil temperatures but not nutrient dynamics: An experimental test of tundra snow–shrub interactions. *Ecology and Evolution* 3:3683–3700.

Nandintsetseg B., Shinoda M. (2013) Assessment of drought frequency, duration, and severity and its impact on pasture production in Mongolia. *Natural Hazards* 66:995–1008.

Neuner G., Ambach D., Aichner K. (1999) Impact of snow cover on photoinhibition and winter desiccation in evergreen *Rhododendron ferrugineum* leaves during subalpine winter. *Tree Physiology* 19:725–732.

Ninot J.M., Batllori E., Carrillo E., Carreras J., Ferré A., Gutiérrez E. (2008) Timberline structure and limited tree recruitment in the Catalan Pyrenees. *Plant Ecology & Diversity* 1:47–57.

Ninot, J.M.; Anadon-Rosell, A.; Carrillo, E.; Grau, O.; Illa, E.; Lloret, F.; Talavera, M. (2016) Els arbusts d'alta muntanya en el context dels canvis de vegetació. In: *La investigació al Parc Nacional d'Aigüestortes i Estany de Sant Maurici. X Jornades sobre Recerca al Parc Nacional d'Aigüestortes i Estany de Sant Maurici*. Parc Nacional d'Aigüestortes i Estany de Sant Maurici i Generalitat de Catalunya. pp. 115-130.

Niu S., Wan S. (2008) Warming changes plant competitive hierarchy in a temperate steppe in northern China. *Journal of Plant Ecology* 1:103–110.

Nogués-Bravo D., Araújo M.B., Errea M.P., Martínez-Rica J.P. (2007) Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change* 17:420 – 428.

Norby R.J., Zak D.R. (2011) Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments. *Annual Review of Ecology, Evolution, and Systematics* 42:181–203.

O’Connor T.G., Puttick J.R., Hoffman M.T. (2014) Bush encroachment in southern Africa: Changes and causes. *African Journal of Range and Forage Science* 31:67–88.

Olsen S.L., Töpper J.P., Skarpaas O., Vandvik V., Klanderud K. (2016) From facilitation to competition: temperature-driven shift in dominant plant interactions affects population dynamics in seminatural grasslands. *Global Change Biology* 22:1915–1926.

Osca J., Romero R., Alonso S. (2013) Precipitation projections for Spain by means of a weather typing statistical method. *Global and Planetary Change* 109:46–63.

Ozenda P. (1985) La végétation de la chaîne alpine dans l'espace montagnarde européen. Masson, Paris. 344 pp.

Pajunen A.M., Oksanen J., Virtanen R. (2011) Impact of shrub canopies on understorey vegetation in western Eurasian tundra. *Journal of Vegetation Science* 22:837–846.

Pardo I., Camarero J.J., Gutiérrez E., García M.B. (2013) Uncoupled changes in tree cover and field layer vegetation at two Pyrenean treeline ecotones over 11 years. *Plant Ecology & Diversity* 6:355–364.

Peñuelas J., Gordon C., Llorens L., Nielsen T., Tietema A., Beier C., Bruna P., Emmett B., Estiarte M., Gorissen A. (2004) Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north–south European gradient. *Ecosystems* 7:598–612.

Popova T.N. (1972) *Vaccinium*. In: Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (eds.). *Flora Europaea, vol. 3*. Cambridge Univ. Press. Cambridge, UK, pp. 12-13.

Prentice I.C., Farquhar G.D., Fasham M.J.R., Goulden M.L., Heimann M., Jaramillo V.J., Kheshgi H.S., Le Quéré C., Scholes R.J., Wallace D.W.R. (2001) The carbon cycle and atmospheric carbon dioxide. In: Houghton J.T., Ding Y., Griggs D.J., Noguer M., van der Linden P.J., Dai X., Maskell K., Johnson C.A. (eds.). *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK and New York, USA, pp. 183–237.

Prévosto B., Kuiters L., Bernhardt-Römermann M., Dölle M., Schmidt W., Hoffmann M., Van Uytvanck J., Bohner A., Kreiner D., Stadler J., Klotz S., Brandl R. (2011) Impacts of land abandonment on vegetation: Successional pathways in European habitats. *Folia Geobotanica* 46:303–325.

Pugnaire F.I., Zhang L., Li R., Luo T. (2015) No evidence of facilitation collapse in the Tibetan plateau. *Journal of Vegetation Science* 26:233–242.

Rangwala I., Sinsky E., Miller J.R. (2013) Amplified warming projections for high altitude regions of the northern hemisphere mid-latitudes from CMIP5 models. *Environmental Research Letters* 8:024040.

Ratajczak Z., Nippert J.B., Collins S.L. (2012) Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology* 93:697–703.

Reichstein M., Bahn M., Ciais P., Frank D., Mahecha M.D., Seneviratne S.I., Zscheischler J., Beer C., Buchmann N., Frank D.C., Papale D., Rammig A., Smith P., Thonicke K., van der Velde M., Vicca S., Walz A., Wattenbach M. (2013) Climate extremes and the carbon cycle. *Nature* 500:287–295.

Rinnan R., Stark S., Tolvanen A. (2009) Responses of vegetation and soil microbial communities to warming and simulated herbivory in a subarctic heath. *Journal of Ecology* 97:788–800.

Ritchie J.C. (1956) *Vaccinium myrtillus* L. *Journal of Ecology* 44: 291-299.

Rixen C., Dawes M.A., Wipf S., Hagedorn F. (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO_2 enrichment and soil warming. *Oikos* 121:1532–1543.

Roderick M.L., Berry S.L. (2001) Linking wood density with tree growth and environment: A theoretical analysis based on the motion of water. *New Phytologist* 149:473–485.

Rogers A., Ellsworth D.S. (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant, Cell and Environment* 25:851–858.

Roudier P., Andersson J.C.M., Donnelly C., Feyen L., Greuell W., Ludwig F. (2016) Projections of future floods and hydrological droughts in Europe under a +2°C global warming. *Climatic Change* 135:341–355.

Roura-Pascual N., Pons P., Etienne M., Lambert B. (2005) Transformation of a rural landscape in the Eastern Pyrenees between 1953 and 2000. *Mountain Research and Development* 25:252–261.

Ruehr N.K., Offermann C.A., Gessler A., Winkler J.B., Ferrio J.P., Buchmann N., Barnard R.L. (2009) Drought effects on allocation of recent carbon: From beech leaves to soil CO_2 efflux. *New Phytologist* 184:950–961.

Rundqvist S., Hedenås H., Sandström A., Emanuelsson U., Eriksson H., Jonasson C., Callaghan T.V. (2011) Tree and shrub expansion over the past 34 years at the tree-line near Abisko, Sweden. *Ambio* 40:683–692.

Schindlbacher A., Rodler A., Kuffner M., Kitzler B., Sessitsch A., Zechmeister-Boltenstern S. (2011) Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology and Biochemistry* 43:1417–1425.

Schweingruber F.H., Hellmann L., Tegel W., Braun S., Nievergelt D., Büntgen U. (2013) Evaluating the wood anatomical and dendroecological potential of Arctic dwarf shrub communities. *IAWA Journal* 34:485–497.

Semenchuk P.R., Gillespie M.A.K., Rumpf S.B., Baggesen N., Elberling B., Cooper E.J. (2016) High Arctic plant phenology is determined by snowmelt patterns but duration of phenological periods is fixed: an example of periodicity. *Environmental Research Letters* 11:125006.

Semerdjieva S.I., Phoenix G.K., Hares D., Gwynn-Jones D., Callaghan T.V., Sheffield E. (2003a) Surface morphology, leaf and cuticle thickness of four dwarf shrubs from a sub-Arctic heath following long-term exposure to enhanced levels of UV-B. *Physiologia Plantarum* 117:289–294.

Semerdjieva S.I., Sheffield E., Phoenix G.K., Gwynn-Jones D., Callaghan T.V., Johnson G.N. (2003b) Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. *Plant, Cell and Environment* 26:957–964.

Sierra-Almeida A., Reyes-Bahamonde C., Cavieres L.A. (2016) Drought increases the freezing resistance of high-elevation plants of the Central Chilean Andes. *Oecologia* 181:1011–1023.

Soliveres S., Maestre F.T. (2014) Plant-plant interactions, environmental gradients and plant diversity: A global synthesis of community-level studies. *Perspectives in Plant Ecology, Evolution and Systematics* 16:154–163.

Soliveres S., Maestre F.T., Eldridge D.J., Delgado-Baquerizo M., Quero J.L., Bowker M.A., Gallardo A. (2014) Plant diversity and ecosystem multifunctionality peak at intermediate levels of woody cover in global drylands. *Global Ecology and Biogeography* 23:1408–1416.

Song Y.-B., Yu F.-H., Keser L.H., Dawson W., Fischer M., Dong M., van Kleunen M. (2013) United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171:317–327.

Sparks T.H., Menzel A. (2002) Observed changes in seasons: An overview. *International Journal of Climatology* 22:1715–1725.

Steffen W., Sanderson A., Tyson P.D., Jäger J., Matson P., Moore III B., Oldfield F., Richardson K., Schellnhuber H.J., Turner B.L.II, Wasson R.J. (2004) *Global change and the earth system: A planet under pressure.* The IGBP global change series. Springer-Verlag, Berlin, Germany, 336 pp.

Stevens C.J., Dise N.B., Mountford J.O., Gowing D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.

Sturm M., Douglas T., Racine C., Liston G.E. (2005a) Changing snow and shrub conditions affect albedo with global implications. *Journal of Geophysical Research: Biogeosciences* 110: G01004.

Sturm M., Schimel J., Michaelson G., Welker J.M., Oberbauer S.F., Liston G.E., Fahnestock J., Romanovsky V.E. (2005b) Winter biological processes could help convert arctic tundra to shrubland. *BioScience* 55:17–26.

Sundqvist M.K., Björk R.G., Molau U. (2008) Establishment of boreal forest species in alpine dwarf-shrub heath in subarctic Sweden. *Plant Ecology & Diversity* 1:67–75.

Suter D., Frehner M., Fischer B.U., Nösberger J., Lüscher A. (2002) Elevated CO_2 increases carbon allocation to the roots of *Lolium perenne* under free-air CO_2 enrichment but not in a controlled environment. *New Phytologist* 154:65–75.

Taschler D., Neuner G. (2004) Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell and Environment* 27:737–746.

Taulavuori E., Bäckman M., Taulavuori K., Gwynn-Jones D., Johanson U., Laine K., Callaghan T., Sonesson M., Björn L.O. (1998) Long-term exposure to enhanced ultraviolet-B radiation in the sub-arctic does not cause oxidative stress in *Vaccinium myrtillus*. *New Phytologist* 140:691–697.

Taulavuori E., Tahkokorpi M., Laine K., Taulavuori K. (2010) Drought tolerance of juvenile and mature leaves of a deciduous dwarf shrub *Vaccinium myrtillus* L. in a boreal environment. *Protoplasma* 241:19–27.

Taulavuori K., Keränen J., Suokanerva H., Lakkala K., Huttunen S., Laine K., Taulavuori E. (2012) Decreased frost hardiness of *Vaccinium vitis-idaea* in reponse to UV-A radiation. *Physiologia Plantarum* 145:516–526.

Taulavuori K., Laine K., Taulavuori E. (2013) Experimental studies on *Vaccinium myrtillus* and *Vaccinium vitis-idaea* in relation to air pollution and global change at northern high latitudes: A review. *Environmental and Experimental Botany* 87:191–196.

Taulavuori K., Taulavuori E., Laine K. (2005) Ultraviolet radiation and plant frost hardiness in the subarctic. *Arctic, Antarctic, and Alpine Research* 37:11–15.

Theurillat J.-P., Guisan A. (2001) Potential impact of climate change on vegetation in the European Alps: A review. *Climatic Change* 50:77–109.

Tielbörger K., Kadmon R. (2000) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81:1544–1553.

Tissue D.T., Oechel W.C. (1987) Response of *Eriophorum vaginatum* to elevated CO_2 and temperature in the Alaskan tussock tundra. *Ecology* 68:401–410.

Tolvanen A. (1995) Aboveground growth habits of two *Vaccinium* species in relation to habitat. *Canadian Journal of Botany* 73:465–473.

Tylianakis J.M., Didham R.K., Bascompte J., Wardle D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11:1351–1363.

Tyree M.T., Sperry J.S. 1989. Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:19-38.

Van Auken O.W. (2000) Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics* 31:197–215.

Vigo J. (2008) *L'alta muntanya catalana. Flora i vegetació*. 2nd ed. Centre Excursionista de Catalunya and Institut d'Estudis Catalans, Barcelona, Spain, 443 pp.

von Arx G, Kueffer C., Fonti P. 2013 Quantifying plasticity in vessel grouping –added value from the image analysis tool ROXAS. *IAWA Journal* 34: 433-445

von Arx G., Crivellaro A., Prendin A.L., Čufar K., Carrer M. (2016) Quantitative wood anatomy—practical guidelines. *Frontiers in Plant Science* 7:781. doi: 10.3389/fpls.2016.00781.

Walker M.D., Wahren C.H., Hollister R.D., Henry G.H.R., Ahlquist L.E., Alatalo J.M., Bret-Harte M.S., Calef M.P., Callaghan T.V., Carroll A.B., Epstein H.E., Jónsdóttir I.S., Klein J.A., Magnússon B., Molau U., Oberbauer S.F., Rewa S.P., Robinson C.H., Shaver G.R., Suding K.N., Thompson C.C., Tolvanen A., Totland Ø., Turner P.L., Tweedie C.E., Webber P.J., Wookey P.A. (2006) Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences* 103:1342–1346.

Walther G.-R. (2003) Plants in a warmer world. *Perspectives in Plant Ecology, Evolution and Systematics* 6:169–185.

Wegner L., von Arx G., Sass-Klaassen U., Eilmann B. 2013. ROXAS – an efficient and accurate tool to detect vessels in diffuse-porous species. *IAWA Journal* 34:425-432.

Wheeler J.A., Hoch G., Cortés A.J., Sedlacek J., Wipf S., Rixen C. (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. *Oecologia* 175:219–229.

Wheeler J.A., Schnider F., Sedlacek J., Cortés A.J., Wipf S., Hoch G., Rixen C. (2015) With a little help from my friends: Community facilitation increases performance in the dwarf shrub *Salix herbacea*. *Basic and Applied Ecology* 16:202–209.

Wilson S.D., Nilsson C. (2009) Arctic alpine vegetation change over 20 years. *Global Change Biology* 15:1676–1684.

Wipf S., Stoeckli V., Bebi P. (2009) Winter climate change in alpine tundra: Plant responses to changes in snow depth and snowmelt timing. *Climatic Change* 94:105–121.

Wookey P.A., Aerts R., Bardgett R.D., Baptist F., Bråthen K., Cornelissen J.H.C., Gough L., Hartley I.P., Hopkins D.W., Lavorel S., Shaver G.R. (2009) Ecosystem feedbacks and cascade processes: Understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Global Change Biology* 15:1153–1172.

Yang Y., Wang G., Klanderud K., Wang J., Liu G. (2015) Plant community responses to five years of simulated climate warming in an alpine fen of the Qinghai–Tibetan Plateau. *Plant Ecology & Diversity* 8:211–218.

Yuan W., Cai W., Chen Y., Liu S., Dong W., Zhang H., Yu G., Chen Z., He H., Guo W., Liu D., Liu S., Xiang W., Xie Z., Zhao Z., Zhou G. (2016) Severe summer heatwave and drought strongly reduced carbon uptake in Southern China. *Scientific Reports* 6:18813.

Zavaleta E.S., Shaw M.R., Chiariello N.R., Thomas B.D., Cleland E.E., Field C.B., Mooney H.A. (2003) Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. *Ecological Monographs* 73:585–604.

Apèndix/Appendix

Plant Ecol (2016) 217:1115–1128
DOI 10.1007/s11258-016-0637-2

Vaccinium myrtillus stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline

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Received: 22 November 2015 / Accepted: 18 July 2016 / Published online: 23 July 2016

Abstract Plant–plant interactions are key drivers of treeline dynamics. At the Pyrenean treeline, the dwarf shrub *Vaccinium myrtillus* grows in pure stands or in mixed stands with *Vaccinium uliginosum* or *Rhododendron ferrugineum*. They form sparse shrub patches that colonize subalpine grasslands, having dramatic impacts on their structure and functioning. We investigated the role of the two co-occurring shrubs as possible modulators of the structure and performance of *V. myrtillus* in the Central Pyrenees. We analysed biomass, growth, functional parameters, age distribution, N and C concentrations and isotope compositions (δ^{15}N and δ^{13}C) of *V. myrtillus* ramets in pure stands, and stands mixed with *V. uliginosum* or *R. ferrugineum*. Volume, above-ground biomass and age of the ramets did not differ between stand types. We found lower δ^{13}C values, indicative of lower water-use efficiency (WUE), in leaves and shoots of *V. myrtillus* in stands with *R. ferrugineum* than in pure stands. The N content and δ^{15}N of *V. myrtillus* leaves and shoots in pure stands were higher than in mixed stands, pointing to a competition for N in mixed stands. Our results indicate that *V. myrtillus* competes for nutrients with its neighbours, but neither this competition nor the lower WUE affect its above-ground performance. Therefore, the interaction with co-occurring shrubs does not have a major effect on *V. myrtillus* structure and functioning at treeline and, consequently, should not be considered as a key driver on the dynamics of this species in the encroachment of subalpine grasslands.

Keywords Biomass partition · Competition · Dwarf shrubs · Stable isotope composition · Treeline · Xylem growth rings

Abbreviations

IRMS	Isotope ratio mass spectrometry
SPA	Shoot photosynthetic area
TOM	Total organic matter
δ^{13}C	Carbon isotope composition
δ^{15}N	Nitrogen isotope composition

Communicated by Kun-Fang Cao.

Electronic supplementary material The online version of this article (doi:10.1007/s11258-016-0637-2) contains supplementary material, which is available to authorized users.

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Introduction

Plant–plant interactions represent one of the major selective forces driving population and community dynamics (Callaway and Walker 1997). At the treeline ecotone, shrubs have been identified as modulators of ecosystem dynamics (Batllori et al. 2009b; Grau et al. 2012). However, the role of plant–plant interactions in shrub communities has often been disregarded in favour of climate modulators (especially temperature; see the numerous warming experiments carried out at treeline areas, e.g. Michelsen et al. 1996; Xu et al. 2009; Kaarlejärvi et al. 2012; Anadon-Rosell et al. 2014). Shrubs can facilitate tree seedling survival and recruitment because they can exert a nurse effect when tree species are in their most vulnerable life stage (Gómez-Aparicio et al. 2008; Batllori et al. 2009b; Grau et al. 2012). Moreover, interactions between woody species can have a key role in the encroachment processes that have been reported during the last decades in alpine and Arctic tundra ecosystems (Eldridge et al. 2011; Myers-Smith et al. 2011).

In the last decades, the increase in temperature and the progressive abandonment of traditional land use practices (Tappeiner and Cernusca 1993; Gellrich et al. 2008; IPCC 2013) have caused a densification of woody plants at European alpine treelines (Stöcklin and Körner 1999; Camarero and Gutierrez 2004; Kullman 2005; Batllori and Gutiérrez 2008) and the re-colonization of abandoned pastures by shrubs (Didier 2001; Vicente-Serrano et al. 2004; Albert et al. 2008). This shrub encroachment, which is predicted to increase in response to the higher temperatures forecasted for this century (Walker et al. 2006; Meehl et al. 2007; IPCC 2013), plays an important role in the first stadia of tree re-colonization at treeline (Tasser and Tappeiner 2002; Targetti et al. 2010; Ninot et al. 2011). Therefore, studies focusing on shrub species interactions at treeline can provide essential understanding of the functioning of treeline ecosystem dynamics (Smith et al. 2003; Batllori et al. 2009b; Grau et al. 2012; Llambí et al. 2013).

In the subalpine belt of the Pyrenees, *Vaccinium myrtillus* has a substantial role in the transitional ecotone between *Pinus uncinata* forests and alpine grasslands (see Batllori et al. 2009a). In these areas, it grows close to the highest altitudinal limit of its distribution (Bolòs et al. 2005), where stressful conditions related to low temperatures, wind abrasion, early-season frost and short growing seasons could lead to a prevalence of facilitative plant–plant interactions (Batllori et al. 2009b; Fajardo and McIntire 2011). Some studies in alpine and Arctic ecosystems have reported facilitative interactions between *Vaccinium myrtillus* and co-occurring species (Maillette 1988; Shevtsova et al. 1995), although allelopathic effects of *V. myrtillus* on some conifer species have also been reported (Mallik and Pellissier 2000; Talavera & Ninot, unpublished). At treeline in the Central Pyrenees, *Vaccinium myrtillus* forms sparse patches together with the similarly sized shrub *Vaccinium uliginosum* ssp. *microphyllum* and the taller shrub *Rhododendron ferrugineum*. These shrub communities have an important role on treeline dynamics due to their potential for colonizing subalpine grasslands. However, the role of *V. uliginosum* and *R. ferrugineum* as modulators of *V. myrtillus* population structure and performance and, ultimately, the effects that these interactions can have on treeline dynamics are poorly understood. Furthermore, studies on shrub interactions involving *Vaccinium* species have usually considered neighbour shrubs of similar dimensions (Maillette 1988; Shevtsova et al. 1995, 1997; Gerdol et al. 2000; Brancaleoni and Gerdol 2006), whereas the effect of taller shrubs remains mostly unexplored (but see Pornon et al. 2007).

Carbon (C) and nitrogen (N) isotope compositions (i.e. δ^{13}C and δ^{15}N) of plant tissues can be used to determine plant nutrient and/or water status. Natural abundance δ^{13}C values have been used to assess plant water-use efficiency (Farquhar et al. 1982), and δ^{15}N values may provide insight into the use of different sources of N (Ghashghaie et al. 2003; Tcherkez and Hodges 2008), the niche partitioning for N-acquisition (Pornon et al. 2007; Gundale et al. 2012) and differential isotopic fractionation of N in response to different mycorrhizal N uptake mechanisms (Emmerton et al. 2001). Moreover, C and N mass-based concentrations can be used as a measure of nutrient availability and dynamics (Aerts 1996; Killingbeck 1996; Palacio et al. 2007; Baptist et al. 2009; 2013; Muller et al. 2011; Vergutz et al. 2012). Previous studies have shown differences in the N-acquisition and N-use strategies between *V. myrtillus* and coexisting species such as *R. ferrugineum* or *Vaccinium vitis-idaea* (Pornon et al. 2007; Gundale et al. 2012).

Nevertheless, although studies on congeneric species have often shown that trait divergence and niche separation are strategies for coexistence (Maillette 1988; Vander Kloet and Hill 2000; Beltrán et al. 2012; Gundale et al. 2012), this has not been assessed for the co-occurring *V. myrtillus* and *V. uliginosum*.

We aimed to determine whether structure and functioning of *V. myrtillus* stands at the treeline ecotone are affected by the coexistence with *Vaccinium uliginosum* ssp. *microphyllum* (*V. uliginosum* hereafter) and *Rhododendron ferrugineum* and to find out the primary plant–plant interactions driving the performance of *V. myrtillus* in the area. To do so, we measured functional, growth and demographical parameters using growth measures, isotopic analyses and dendroecological techniques. We specifically aimed to answer the following questions: (i) does *V. myrtillus* compete for light with the taller shrub *R. ferrugineum* and, thus, presents greater spread (vertically and horizontally) when co-occurring with it?; (ii) does *R. ferrugineum* exert a facilitative effect on *V. myrtillus*, protecting it from environmental damage (e.g. wind damage and early-spring frost), translating into older, more vigorous *V. myrtillus* ramets in these mixed stands?; (iii) are growth and survival of *V. myrtillus* ramets from stands mixed with *V. uliginosum* comparable to those from monospecific stands due to the structural similarity between these two species?; and finally, (iv) does *V. myrtillus* compete for N with the other two shrubs despite the putative different N-acquisition strategy from *R. ferrugineum* and the suggested niche separation from *V. uliginosum*?

Materials and methods

Study area and species

The study site was located at Eth Corrau des Machos, in the buffer zone of the Aigüestortes and Estany de Sant Maurici National Park (Central Pyrenees, Catalonia, 31 N 329, 472). Sampled plots were located on a N-facing 10–25° steep slope at 2250 m a.s.l., slightly above the current treeline in the area, but with some trees scattered above. Climatic data from a nearby location (La Bonaigua, located 6.3 km away from the study site and at similar altitude) for the period 2007–2012 are a mean annual precipitation of 1154.9 mm and a mean annual temperature of 2.7 °C (Meteorological Service of Catalonia, see detailed climatic data for the study period 2010–2012 in Fig. S1).

At this site, vegetation was composed of *Festuca eskia* Ramond ex DC. and *Nardus stricta* L. grasslands (Selino-Festucetum eskiae) mixed with patches of dwarf shrub heath dominated by *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. and *Rhododendron ferrugineum* L. These communities are considered as serial stages in the succession to mountain pine (*Pinus uncinata* Ramond ex DC.) forests (Rhododendro-Pinetum uncinatae, i.e. grassland-shrubland-woodland-forest succession). These forests were formerly extensive in the area but are reduced at present due to anthropogenic deforestation.

Vaccinium myrtillus and *V. uliginosum* are both deciduous dwarf shrubs that reach ca. 15–35 cm height at our study site, whereas *R. ferrugineum* is a taller evergreen shrub that reaches 40–50 cm height.

Sampling design

In mid-September 2010, we evaluated the performance of *V. myrtillus* in three different stand types: pure stands (M stands), stands with *V. myrtillus* co-occurring with *V. uliginosum* (U stands) and stands with *V. myrtillus* co-occurring with *R. ferrugineum* (R stands). The percentage cover of each shrub species in the different stand types is shown in Table S1. For each stand type, we established five 20 cm × 20 cm plots, accounting for the small size but high density of the ramets. The distance between two plots ranged from one to a few metres (<20 m) but always ensuring that different plots belonged to different patches. We carried out this study on a ramet basis due to the clonal structure of *V. myrtillus*, which consists of a horizontal network of subterranean rhizomes from which aerial shoots arise (Ritchie 1956; Flower-Ellis 1971). We measured and clipped at ground level all *V. myrtillus* ramets grounded within each plot boundaries, and we subsequently stored them in sealed plastic bags kept in a portable cooler until we arrived at the laboratory.

Stand structure, above-ground growth and plant performance

To have an estimate of the ability of plants to compete for space at a stand scale, we calculated the volume of all *V. myrtillus* ramets present in each plot. To do so, we measured their height and their canopy diameter (i.e. mean between the maximum and the minimum canopy diameters) and estimated the theoretical volume occupied for each ramet as a canopy cylinder ($V = \pi r^2 h$, Johnson et al. 1988).

To study above-ground biomass partition of individual ramets, we selected 12 ramets per plot when possible (except for one M plot, which only had 11 ramets, and two R plots, which only had 8 and 10 ramets), representative of the wide range of ramet sizes in the plots. We used the last three cohorts of shoots as the sampling unit for biomass allocation comparisons between stand types. Ramets younger than 3-year old were not used for biomass allocation measurements. The following fractions were detached and counted: leaves, new shoots (formed in 2010, Sh1), one-year-old shoots (formed in 2009, Sh2), two-year-old shoots (formed in 2008, Sh3) and main stem (>3-year old). We identified the different cohorts of shoots by the scars left by bud scales after shoot elongation. Before drying, we cut a 1.5 cm segment at the base of the stem of each ramet for counting xylem growth rings to determine ramet age (see below). We dried all the fractions at 70 °C for 72 h and weighed them to the nearest 0.001 g (with a Mettler Toledo PB303 Delta Range scale). We corrected the weight of the main stems by adding the weight of segments of similar dimensions to the part previously cut. For each ramet, we calculated the dry mass of Sh1, Sh2 and Sh3 relative to the two-year-old shoot unit, and we also calculated the total above-ground biomass. Moreover, for each ramet (except for ramets younger than 3 years), we measured the main stem diameter as the mean between the maximum and minimum diameters measured at the base of the stem.

We randomly selected one of the 12 ramets described above in three plots of each stand type and measured length and width of all shoots from the different cohorts to calculate shoot photosynthetic area (SPA). For this, we assumed shoot surface area as that of a tetrahedron, because young shoots of *V. myrtillus* show a tetrahedral shape. The greenness of the shoots gradually decreases as ramets age, but the three last shoot cohorts still show a clear green photosynthetic colour. We carried out linear regressions between photosynthetic area and shoot dry mass separately for each cohort and found positive significant correlations (Sh1: $R^2 = 0.96$, $P < 0.001$; Sh2: $R^2 = 0.91$, $P < 0.001$; Sh3: $R^2 = 0.85$, $P < 0.001$, Fig. S2). Consequently, we used the obtained regression equations to estimate the photosynthetic area of each shoot cohort per two-year-old shoot unit for the rest of ramets.

Ramet age distribution

The structure and growth of *V. myrtillus* ramets depend on age (Flower-Ellis 1971). Thus, it is important to know and consider ramet age when performing comparative studies between *V. myrtillus* stands. To estimate the age of ramets and to identify differences in the above-ground age structure of different stands, we made cross sections of 20–25 μm thickness from the basal segments of *V. myrtillus* stems using a sledge microtome. Sections were stained with Safranin to emphasize the growth ring structure, subsequently rinsed with ethanol (75 %) for dehydration (Schweingruber and Poschlod 2005, modified) and mounted in DPX. We counted xylem growth rings under the microscope (Olympus CH2) to assess ramet age.

C and N concentration and isotope composition (δ^{13}C and δ^{15}N)

At the time of harvest, leaves were already senescent, and therefore we established another sampling period in August 2012. Then, we collected *V. myrtillus* leaf and new shoot samples in fifteen plots similar to those established in 2010, i.e. five plots for each stand type, and analysed their C and N concentrations and isotopic compositions. We sampled leaves and new shoots of five ramets per plot and pooled together the material of these ramets for each plot separately, both for leaves and new shoots. Thus, we had five replicates for each stand type and plant fraction. Samples were dried at 60 °C for 72 h, and subsamples were weighed in small tin capsules.

Samples were analysed to determine the C and N isotope compositions using a Flash 1112 Elemental Analyzer (Carbo Erba, Milan) coupled to an IRMS Delta C isotope ratio mass spectrometer through a Conflo III Interface (Thermo-Finnigan, Germany). Results of C isotope analyses are reported in per thousand (‰) on the relative δ-scale as δ^{13}C and refer to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1, \quad (1)$$

where R is the $^{13}\mathrm{C}/^{12}\mathrm{C}$ ratio.

C isotope discrimination ($\Delta^{13}\mathrm{C}$) of shoot TOM (total organic matter) was calculated from δ_a and δ_p (Farquhar et al. 1989) as

$$\Delta^{13}\mathrm{C} = \frac{\delta_a - \delta_p}{\delta_p + 1}, \quad (2)$$

where a and p refer to air and plant, respectively.

N results are also expressed in δ notation ($\delta^{15}\mathrm{N}$) using international secondary standards of known $^{15}\mathrm{N}/^{14}\mathrm{N}$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) relative to N_2 in air:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1, \quad (3)$$

where R is the $^{15}\mathrm{N}/^{14}\mathrm{N}$ ratio.

N and C contents were determined using an elemental analyser (EA1108, Series 1; Carbo Erba Instrumentazione, Milan, Italy).

In August 2013, we collected air samples with a 50 ml syringe (SGE, Ringwood, Australia) and kept them in 10 ml vacutainers (BD vacutainer, Plymouth, UK) to analyse the $^{13}\mathrm{CO}_2$ isotopic composition at the study site by gas–chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS) according to Nogués et al. (2004). The $\delta^{13}\mathrm{C}$ of CO_2 of the air at the site in the Pyrenees was ca. −10.91 ‰. We also collected soil samples to determine soil N isotope composition at the study site and analysed them following the same technique as mentioned above for plant material. Soil $\delta^{15}\mathrm{N}$ was ca. 7.33 ‰.

All the EA-IRMS and GC-C-IRMS analyses were performed at the CCiT of the University of Barcelona.

Statistical analyses

We used linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML) to test the effects of the different scenarios of coexistence on *V. myrtillus* volume, canopy height and width, biomass partitioning, total above-ground biomass, SPA of each cohort of shoots, ramet age and stem diameter. We included stand type as a fixed factor and plot as a random factor. To account for the possible age effects on response variables, we included ramet age as a covariate in the analyses of the effects of stand type on individual ramet traits when it was significant, that is for total above-ground biomass, stem diameter and SPA in Sh3. For the N and C concentrations and isotope compositions, we evaluated differences between stand type, plant fraction (leaves and shoots) and their interaction with Type III analysis of variance using the linear model function. We tested for Pearson's correlations between ramet age, stem diameter and total above-ground biomass.

For all statistical analyses, we visually evaluated the assumptions of normality and homoscedasticity of residuals (Zuur et al. 2009) and transformed response variables when necessary to reach these assumptions. When homoscedasticity of residuals was not met, we used the varIdent structure (Zuur et al. 2009) to account for the heterogeneity of variances among factor levels. Effects were considered significant at $P < 0.05$ and marginally significant at $0.05 < P < 0.10$ to account for the relatively low replication. When there were significant differences between stand types, we performed Tukey HSD post hoc tests to determine which factor level means differed significantly. All analyses were performed with R 2.15.2 (R Core Team 2012), using the nlme package (Pinheiro et al. 2008) for linear mixed effects models, the lattice package (Sarkar 2008) for graphical evaluation of the assumptions of the models, the multcomp package (Hothorn et al. 2008) for multiple comparisons on linear mixed effects models and the agricolae package (de Mendiburu 2010) for multiple comparisons on simple linear models.

Results

Stand structure, above-ground growth and performance

Ramet volume (calculated as an elliptical cylinder) did not differ between stand types (Tables 1, 2). Despite the average values for R stands were much higher than for the other two stand types, the great data dispersion in these R stands masked possible significant differences. The average ramet height and ramet canopy width did not differ between the three stand types either (Tables 1, 2), although the trend of higher values but higher dispersion in R stands was also evident.

Table 1 Mean (and standard deviation) for the parameters studied at plot scale (all the ramets in the plot)

Stand type	Ramet volume (cm^3)	Ramet height (cm)	Canopy width (cm)
M	223.04 (111.62)	14.46 (2.71)	3.24 (0.71)
R	1074.93 (1691.77)	15.57 (5.76)	5.89 (4.16)
U	137.07 (66.16)	12.21 (1.41)	3.02 (0.66)

M, *V. myrtillus* pure stands; R, *V. myrtillus* mixed with *R. ferrugineum* stands; U, *V. myrtillus* mixed with *V. uliginosum* stands

Table 2 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* volume, height and canopy width studied in all ramets within each plot

Variable	df	*F*	*P*
Ramet volume	2, 12	2.07	0.17
Ramet height	2, 12	1.05	0.38
Ramet canopy width	2, 12	2.38	0.14

No significant differences were found

Similarly, total above-ground ramet biomass did not differ significantly between the three stand types. For ramets in M stands was 868.7 ± 195.9 mg (mean ± SD), in R stands 761.9 ± 635.1 mg and in U stands 507.1 ± 123.6 mg (Table 3). However, we found significant differences in the biomass allocation patterns. *Vaccinium myrtillus* ramets growing with *R. ferrugineum* showed a lower biomass allocation to Sh3

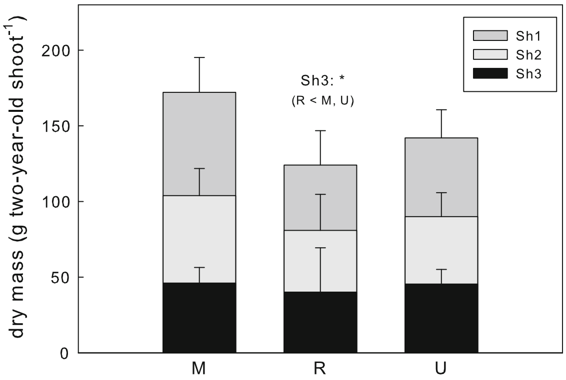

Fig. 1 *Vaccinium myrtillus* biomass allocation to the last three shoot cohorts in the three stand types. The asterisk shows significant differences between stand types ($0.01 < P < 0.05$), referred to Sh3 between *R* stands and *M* and *U*. Data are means + SD, $n = 5$. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus mixed* with *V. uliginosum* stands. *Sh1* new shoots, *Sh2* one-year-old shoots and *Sh3* two-year-old shoots

Table 3 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* biomass partitioning, total above-ground (AG) biomass, shoot photosynthetic area (SPA), age and stem diameter in selected ramets per plot

Variable	Factor/covariable	Df	*F*	*P*
Biomass partition				
Sh1	Stand type	2, 12	2.39	0.13
Sh2	Stand type	2, 12	1.86	0.20
Sh3	Stand type	2, 12	3.96	**0.04**
Total AG biomass	Age covariable	1, 152	100.08	**<0.01**
	Stand type	2, 12	1.92	0.19
SPA				
Sh1	Stand type	2, 12	2.31	0.14
Sh2	Stand type	2, 12	2.72	0.11
Sh3	Age covariable	1, 139	5.26	**0.02**
	Stand type	2, 12	4.53	**0.03**
Age	Stand type	2, 12	0.20	0.82
Stem diameter	Age covariable	1, 123	57.91	**<0.01**
	Stand type	2, 12	0.89	0.44

Significant differences ($P < 0.05$) in bold

Sh1 new shoots, *Sh2* one-year-old shoots, *Sh3* two-year-old shoots than ramets in the other stand types (Table 3; Fig. 1). Although the biomass allocation to Sh1 and Sh2 tended to be lower too, differences between stand types were not significant for these two cohorts. Biomass allocation to Sh1 was 14 % higher than to Sh2, which was 9 % higher than Sh3 averaged across all stand types. Stem diameter did not differ between stand types either (Table 3).

SPA is related to the biomass allocation to each fraction. Therefore, it showed a similar pattern than biomass allocation: it was greater in Sh1, followed by Sh2 and Sh3 for the three stand types (Fig. 2). Moreover, SPA of Sh3 was higher in M and U stands than in R stands (Table 3; Fig. 2).

Ramet age distribution

Ramet age did not differ between stand types (Table 3; Fig. 3). The oldest ramet was 12-year old (U stand), but the majority of the ramets of each stand type were younger than 7-year old (70 % in M stands, 68 % in U stands and 61 % in R stands). Ramet age was positively correlated with stem diameter ($R^2 = 0.55$, $P < 0.001$) and total above-ground biomass ($R^2 = 0.53$, $P < 0.001$).

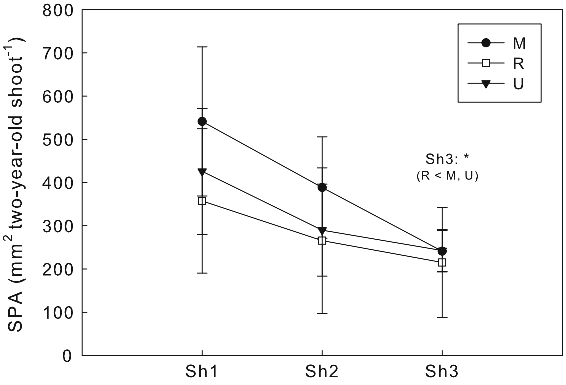

Fig. 2 Shoot photosynthetic area (SPA) of the last three shoot cohorts of *V. myrtillus* ramets in the three stand types. The asterisk shows significant differences between stand types ($0.01 < P < 0.05$), referred to *Sh3* between *R* stands and *M* and *U*. Data are means + SD, $n = 5$. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus mixed* with *V. uliginosum* stands. *Sh1* new shoots, *Sh2* one-year-old shoots and *Sh3* two-year-old shoots

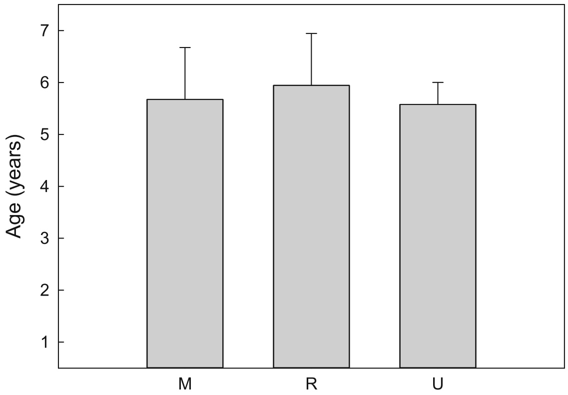

Fig. 3 Ramet age in the three stand types. No significant differences were found. Data are means + SD, $n = 5$. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus mixed* with *V. uliginosum* stands

C and N concentrations and isotope compositions ($\delta\ ^{13}$C and δ^{15}N) of leaves and new shoots

We found significant differences in the C and N isotopic composition and N concentration of leaves and new shoots of *V. myrtillus* between stand types. δ^{13}C values of leaves and new shoots of *V. myrtillus* in pure stands and U stands were higher than those of ramets from R stands (marginally significant, Table 4; Fig. 4a; see Table S2 for ^{13}C discrimination values, Δ^{13}C), and δ^{13}C values of new shoots were higher than values for leaves. The C content of leaves and new shoots did not differ between stand types, and it was higher in new shoots than in leaves (Table 4; Fig. 4b). The N content and δ^{15}N of leaves and new shoots of pure stands were higher than those of mixed stands (Table 4; Fig. 4c, d), and the N content was higher in leaves than in new shoots. The interaction between stand type and plant fraction did not have a significant effect on any of the parameters analysed.

Discussion

Above-ground growth and performance

We did not find major differences in *V. myrtillus* volume, biomass or SPA between the different stand types, which indicates that *V. myrtillus* above-ground growth and performance is similar when it grows in pure and in mixed stands.

Table 4 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) and plant fraction (leaves and new shoots) on *V. myrtillus* C and N isotope compositions ($\delta^{13}C$, $\delta^{15}N$) and concentrations

Variable	Factor	Df	*F*	*P*
$\delta^{13}C$	Stand type	2, 24	2.93	**(0.07)**
	Fraction	1, 24	26.39	**<0.01**
	Stand type × fraction	2, 24	0.20	0.82
$\delta^{15}N$	Stand type	2, 24	39.09	**<0.01**
	Fraction	1, 24	1.30	0.27
	Stand type × fraction	2, 24	0.01	0.99
C concentration	Stand type	2, 24	0.86	0.44
	Fraction	1, 24	45.48	**<0.01**
	Stand type × fraction	2, 24	1.58	0.23
N concentration	Stand type	2, 24	6.67	**<0.01**
	Fraction	1, 24	35.78	**<0.01**
	Stand type × fraction	2, 24	0.19	0.83

Significant differences ($P < 0.05$) in bold and marginally significant differences ($0.10 > P < 0.05$) in bold in parentheses

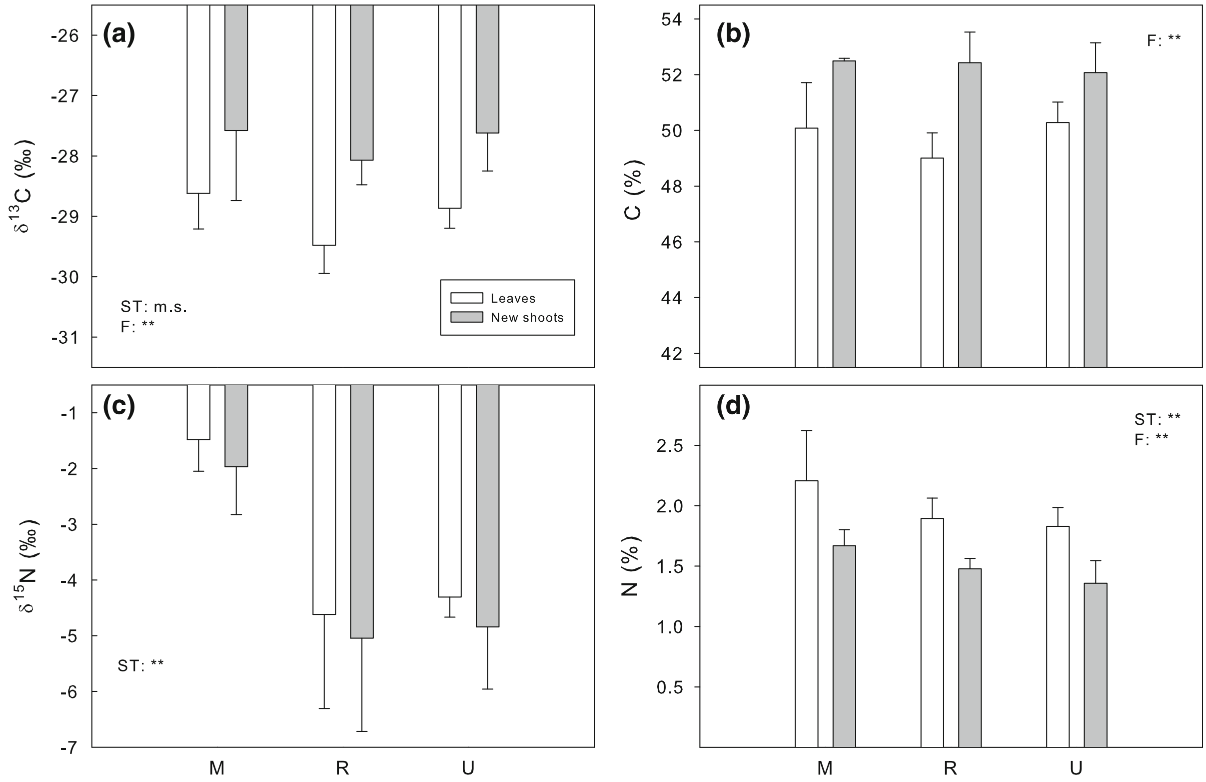

Fig. 4 C and N isotopic compositions (**a**, **c**) and C and N concentrations (**b**, **d**) of leaves and new shoots of *V. myrtillus* in the three stand types. Data are means + (**b**, **d**) or − (**a**, **c**) SD. *Asterisks* (**) show significant differences between situations of coexistence ($P < 0.01$), and marginally significant differences are indicated by m.s. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus mixed* with *V. uliginosum* stands; *ST*, stand type; *F*, plant fraction. For detailed explanation on the differences between factor level means, see the main text

Nevertheless, the higher values and greater spread of *V. myrtillus* volumes when growing with *R. ferrugineum* as compared to ramets growing with *V. uliginosum* or in pure stands suggest that *V. myrtillus* finds more heterogeneous conditions when growing with *R. ferrugineum*. *Vaccinium myrtillus* might face some competition for light when growing with the taller shrub *R. ferrugineum*, which is the reason why *V. myrtillus* would spread its shoots both laterally and upwards to occupy more space, a plasticity that would allow it to favourably compete to intercept more light (van Kleunen and Fisher 2001; Mourelle et al. 2001; Callaway et al. 2003; Lepik et al. 2005).

Although *V. myrtillus* ramets tended to show a reduced biomass allocation to the three last shoot cohorts in plots with *R. ferrugineum*, differences were only significant in two-year-old shoots, and they were not maintained in the next two shoot cohorts. These results could indicate that *V. myrtillus* experienced less favourable conditions in stands with *R. ferrugineum* in 2008 that caused a lower investment in shoot growth that year. Despite these differences, results for the younger shoot cohorts and total above-ground biomass suggest that the coexistence with the other shrub species does not have major effects on the above-ground growth of *V. myrtillus* in the study area.

The photosynthetic potential of *V. myrtillus* shoots is especially important during the cold season, when ramets are leafless, because the green shoots of this species remain partly active in autumn and spring, and can photosynthesize even under the snow cover (Körner 2003). SPA increased with shoot biomass, and therefore, shoots produced in 2008 also had a lower photosynthetic area in ramets growing with *R. ferrugineum*. However, apart from two-year-old shoots, *V. myrtillus* SPA was similar across stand types, indicating a similar productive potential irrespective of the coexistence with *V. uliginosum* and *R. ferrugineum*.

The lack of major significant differences in these functional and growth parameters, however, needs to be carefully interpreted. Although important differences between stand types were not occurring, some differences could be masked by the high dispersion in some of the studied parameters and the relatively low replication.

Ramet age distribution

The lack of differences between the three stand types in ramet age, together with the above-ground biomass results, indicates that this species does not encounter a different environment for its ramets growth or turnover when coexisting with the other two shrubs than when growing in pure stands. We did not expect to find differences in the mean ramet age between *V. myrtillus* pure stands and stands with *V. uliginosum,* because the effects of the intraspecific interaction between *V. myrtillus* ramets would be functionally and structurally similar to the effects from the interspecific interaction between ramets of the two *Vaccinium* species (i.e. we did not expect *V. uliginosum* to exert any protection to *V. myrtillus*). However, our results indicate that we cannot assume that the taller *R. ferrugineum* exerts any facilitative effect on *V. myrtillus* ramets development at our study site either. Grau et al. (2013) found that *R. ferrugineum* reduced winter damage to tree seedlings at treeline sites in the Pyrenees. Also in the Pyrenees, we observed that *R. ferrugineum* had some kind of nurse effect on *V. myrtillus* in subalpine grasslands colonized by shrubs and in open shrublands, since the density of ramets growing next to *R. ferrugineum* was higher than far from it (unpublished data). Consequently, we expected to find older *V. myrtillus* ramets in plots with *R. ferrugineum*, but such a nurse effect was not detected at our study site.

All the stands analysed were formed by relatively young ramets, the oldest ones being only 12-year old. This indicates that we are dealing with patches where *V. myrtillus* has recently established or patches where a high above-ground turnover by environmental constrictions or disturbance maintains ramets at a young stage. The below-ground network of *V. myrtillus* rhizomes can spread and expand clonally and thus, be much older than the above-ground ramets (Flower-Ellis 1971). Therefore, only a long-term monitoring or a below-ground dendrochronological survey could corroborate whether we are dealing with a recent encroaching area where *V. myrtillus* could expand in the near future, or with older populations with a high above-ground turnover.

C and N concentration and isotope composition

Our results on C and N concentrations and isotope compositions showed differences between stand types, which evidence that competition occurred between *V. myrtillus* and its neighbours.

First, our results suggest that the WUE of *V. myrtillus* was reduced in the presence of *R. ferrugineum*. Several studies have reported lower values of δ^{13}C in leaves under shaded conditions than under full light (Gebauer and Schulze 1991; Berry et al. 1997; Le Roux et al. 2001; Duursma and Marshall 2006; Kranabetter et al. 2010). However, stomatal conductance can be reduced under shade (Gross et al. 1996; Forseth et al. 2001), leading to a lower discrimination against ^{13}C and higher δ^{13}C values in shaded plants. The observed lower δ^{13}C values in ramets coexisting with *R. ferrugineum* are probably explained by a greater difference in the assimilation rates between open and shady stands than the difference in stomatal conductance between these two situations (Carelli et al. 1999; Forseth et al. 2001). When growing with *R. ferrugineum*, *V. myrtillus* might have encountered more shade, which probably reduced its photosynthetic rate to a greater extent than the stomatal conductance (i.e. leading to lower WUE values). A study in the semiarid Colorado Plateau showed that shading by neighbour shrub species reduced the performance of the herbaceous perennial *Cryptantha flava* through a reduction of its photosynthetic rate and a non-proportional reduction of its transpiration and stomatal conductance, thus reducing its WUE (Forseth et al. 2001). In our study, we also observed a reduction of the WUE of *V. myrtillus* growing with *R. ferrugineum*, but its performance was not affected. As suggested by Forseth et al. (2001), temperature, vapour pressure deficit and transpiration rates may have also been lower under the shade of *R. ferrugineum*, which would explain why *V. myrtillus* performance was not altered. As expected, the C content was higher in new shoots than in leaves, since the former are permanent structures, whereas leaves are shed every year in this species.

The lower N content and the lower δ^{15}N values of leaves and new shoots in mixed stands than in pure stands suggest that *V. myrtillus* encountered a deficit in available N when growing with *R. ferrugineum* and *V. uliginosum* compared to pure stands (Olsrud et al. 2004; Craine et al. 2009). Wang and Schjoerring (2012) found a positive correlation between δ^{15}N and N concentration in leaves of ryegrass from intensively managed fields in Scotland, and Craine et al. (2009) also found evidence that foliar δ^{15}N increased with increasing N supply at a local and regional scale after reviewing data from 11,000 plants worldwide. These studies showed that foliar δ^{15}N values and foliar N concentrations correlate and that they can both give information on the N availability.

The lower N content values in leaves and new shoots of *V. myrtillus* in mixed plots are probably explained by the competition for N with its neighbours, which might have limited the amount of N that *V. myrtillus* ramets could invest in their tissues. *Vaccinium myrtillus* and *R. ferrugineum* have shown different N-acquisition strategies in an experiment in a subalpine community in the French Pyrenees (Pornon et al. 2007), where *V. myrtillus* took up N early in the growing season, whereas *R. ferrugineum* showed a slower N uptake rate, which was maintained over the growing season. Moreover, a study on *V. myrtillus* and *V. vitis-idaea* in a boreal shrub community (Gundale et al. 2012) showed that the removal of one shrub species did not affect the isotopic signal of the other coexisting shrub, demonstrating that the niches of these species were not affected by the presence of their neighbours. Differences in the study species, community composition and soil N pools (both content and dynamics) between these studies and ours might explain the contrasting results. A N shortage can have dramatic impacts on plant performance, since the majority of the leaf N is allocated to the photosynthetic apparatus, and leaf N content and photosynthetic capacity are well correlated (Evans 1989). Thus, competition for N can have negative impacts on plant growth, as it has been reported in previous studies (Wilson and Tilman 1991; Li et al. 2015). However, despite the evidence of an existing competition for N in our study, *V. myrtillus* could clearly counteract the effects of this competition and grow as vigorously as in pure stands. These results suggest that competition did not lead to a sufficient N shortage that could negatively affect *V. myrtillus* growth. Further studies should be carried out to determine whether the lower δ^{15}N values in leaves and shoots of *V. myrtillus* from mixed populations were due to higher ^{15}N fractionation by mycorrhiza (Emmerton et al. 2001), to an increased proportion of N obtained by mycorrhiza (Hobbie et al. 2000), or to the use of different N sources (Michelsen et al. 1998) as a result of the competition with the other shrub species.

Concluding remarks

We did not find evidence that the performance of *V. myrtillus* at our study site was affected by the presence of *R. ferrugineum* or *V. uliginosum*. Although *V. myrtillus* seems to compete for N with both species, it can counterbalance these competition effects without compromising its growth. This is especially important to understand and predict landscape dynamics at treeline: the possible expansion of *V. myrtillus* at this treeline site would not be tightly related to the presence of *V. uliginosum* and *R. ferrugineum*, but mostly driven by the own population dynamics, mediated by other biotic interactions and environmental factors.

Acknowledgments We are especially grateful to Empar Carrillo, Oriol Grau, Marc Talavera, Claudia Arias and Elena Sánchez for their field and laboratory assistance, and to CCiT of the University of Barcelona for the use of their facilities and their technical assistance. We also thank Jonathan Mitchley for his comments and corrections in an early version of this work, Emilia Gutiérrez for the use of the sledge microtome and Fritz Schweingruber for his support in the research of bibliographical resources. AAR was funded by a grant provided by the Fundació Agustí Pedro i Pons and an FPU grant (Ministerio de Educación, Cultura y Deporte, Spain), and SP was funded by a Ramón y Cajal contract (RYC-2013-14164, Ministerio de Economía y Competitividad, Spain). This work has been partly funded by Conselh Generau d'Aran, the European Research Project OPTIMA and the projects CGL2011-26654 (Ministerio de Ciencia e Innovación, Spain) and ARBALMONT/786-2012 (Organismo Autónomo de Parques Nacionales, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain).

References

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? J Ecol 84:597–608. doi:10.2307/2261481

Albert C, Thuiller W, Lavorel S, Davies ID, Garbolino E (2008) Land use change and sub-alpine tree dynamics: colonisation of *Larix decidua* in French sub-alpine grasslands. J Appl Ecol 45:659–669. doi:10.1111/j.1365-2664.2007.01416.x

Anadon-Rosell A, Rixen C, Cherubini P, Wipf S, Hagedorn F, Dawes MA (2014) Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. PLoS One. doi:10.1371/journal.pone.0100577

Baptist F, Tcherkez G, Aubert S, Pontailler J-Y, Choler P, Nogués S (2009) ^{13}C and ^{15}N allocations of two alpine species from early and late snowmelt locations reflect their different growth strategies. J Exp Bot 60:2725–2735. doi:10.1093/jxb/erp128

Baptist F, Secher-Fromell H, Viard-Cretat F, Aranjuelo I, Creme A, Desclos M, Laine P, Nogués S, Lavorel S (2013) Carbohydrate and nitrogen stores in *Festuca paniculata* under mowing explain dominance in subalpine grasslands. Plant Biol 15:395–404. doi:10.1111/j.1438-8677.2012.00652.x

Batllori E, Gutiérrez E (2008) Regional treeline dynamics in response to global change in the Pyrenees. J Ecol 96:1275–1288. doi:10.1111/j.1365-2745.2008.01429.x

Batllori E, Blanco-Moreno JM, Ninot JM, Gutiérrez E, Carrillo E (2009a) Vegetation patterns at the alpine treeline ecotone: the influence of tree cover on abrupt change in species composition of alpine communities. J Veg Sci 20:814–825. doi:10.1111/j.1654-1103.2009.01085.x

Batllori E, Camarero JJ, Ninot JM, Gutiérrez E (2009b) Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* treelines. Implications and potential responses to climate warming. Glob Ecol Biogeogr 18:460–472. doi:10.1111/j.1466-8238.2009.00464.x

Beltrán E, Valiente-Banuet A, Verdú M (2012) Trait divergence and indirect interactions allow facilitation of congeneric species. Ann Bot 110:1369–1376. doi:10.1093/aob/mcs089

Berry SC, Varney GT, Flanagan LB (1997) Leaf δ^{13}C in *Pinus resinosa* trees and understory plants: variation associated with light and CO_2 gradients. Oecologia 109:499–506. doi:10.1007/s004420050110

Bolòs O, Vigo J, Masalles RM, Ninot JM (2005) Flora manual dels Països Catalans. 3ª ed. rev. i amp. Ed. Pòrtic SA, Barcelona

Brancaleoni L, Gerdol R (2006) Recovery of subalpine dwarf shrub heath after neighbour removal and fertilization. Plant Ecol 183:227–235. doi:10.1007/s11258-005-9020-4

Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958–1965. doi:10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Callaway RM, Pennings SC, Richards CL (2003) Phenotypic plasticity and interactions among plants. Ecology 84:1115–1128. doi:10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2

Camarero JJ, Gutierrez E (2004) Pace and pattern of recent treeline dynamics: response of ecotones to climatic variability in the Spanish Pyrenees. Clim Change 63:181–200. doi:10.1023/B:CLIM.0000018507.71343.46

Carelli MLC, Fahl JI, Trivelin PCO, Queiroz-Voltan RB (1999) Carbon isotope discrimination and gas exchange in *Coffea* species grown under different irradiance regimes. Rev Bras Fisiol Veg 11:63–68

Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, MacK MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol 183:980–992. doi:10.1111/j.1469-8137.2009.02917.x

de Mendiburu F (2010) agricolae: Statistical Procedures for Agricultural Research. R package version 1.0-9. http://CRAN.R-project.org/package=agricolae

Didier L (2001) Invasion patterns of European larch and Swiss stone pine in subalpine pastures in the French Alps. For Ecol Manag 145:67–77. doi:10.1016/S0378-1127(00)00575-2

Duursma RA, Marshall JD (2006) Vertical canopy gradients in δ^{13}C correspond with leaf nitrogen content in a mixed-species conifer forest. Trees 20:496–506. doi:10.1007/s00468-006-0065-3

Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JR, Whitford WG (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. Ecol Lett 14:709–722. doi:10.1111/j.1461-0248.2011.01630.x

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. New Phytol 151:513–524. doi:10.1046/j.1469-8137.2001.00179.x

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9–19. doi: 10.1007/BF00377192

Fajardo A, McIntire EJB (2011) Under strong niche overlap conspecifics do not compete but help each other to survive: facilitation at the intraspecific level. J Ecol 99:642–650. doi:10.1111/j.1365-2745.2010.01771.x

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137. doi:10.1071/PP9820121

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Phys 40:503–537. doi:10.1146/annurev.pp.40.060189.002443

Flower-Ellis JGK (1971) Age, structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). Department of Forest Ecology and Forest Soils, Research Note 9. Royal College of Forestry, Stockholm

Forseth IN, Wait DA, Casper BB (2001) Shading by shrubs in a desert system reduces the physiological and demographic performance of an associated herbaceous perennial. J Ecol 89:670–680. doi:10.1046/j.0022-0477.2001.00574.x

Gebauer G, Schulze ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. Oecologia 87:198–207. doi:10.1007/BF00325257

Gellrich M, Baur P, Robinson BH, Bebi P (2008) Combining classification tree analyses with interviews to study why sub-alpine grasslands sometimes revert to forest: a case study from the Swiss Alps. Agric Syst 96:124–138. doi:10.1016/j.agsy.2007.07.002

Gerdol R, Brancaleoni L, Menghini M, Marchesini R (2000) Response of dwarf shrubs to neighbour removal and nutrient addition and their influence on community structure in a subalpine heath. J Ecol 88:256–266. doi:10.1046/j.1365-2745.2000.00445.x

Ghashghaie J, Badeck F-W, Lanigan G, Nogués S, Tcherkez G, Deléens E, Cornic G, Griffiths H (2003) Carbon isotope fractionation during dark respiration and photorespiration in C_3 plants. Phytochem Rev 2:145–161. doi:10.1023/B:PHYT.0000004326.00711.ca

Gómez-Aparicio L, Zamora R, Castro J, Hódar JA (2008) Facilitation of tree saplings by nurse plants: microhabitat amelioration or protection against herbivores? J Veg Sci 19:161–172. doi:10.3170/2008-8-18347

Grau O, Ninot JM, Blanco-Moreno JM, van Logtestijn RSP, Cornelissen JHC, Callaghan TV (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. Oikos 121:1680–1690. doi:10.1111/j.1600-0706.2011.20032.x

Grau O, Ninot JM, Cornelissen JC, Callaghan TV (2013) Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. Plant Ecol Divers 6:329–342. doi:10.1080/17550874.2013.810311

Gross K, Homlicher A, Weinreich A, Wagner E (1996) Effect of shade on stomatal conductance, net photosynthesis, photochemical efficiency and growth of oak saplings. Ann For Sci 53:279–290. doi:10.1051/forest:19960211

Gundale MJ, Hyodo F, Nilsson M-C, Wardle DA (2012) Nitrogen niches revealed through species and functional group removal in a boreal shrub community. Ecology 93:1695–1706. doi:10.1890/11-1877.1

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar δ^{15}N and nitrogen concentrations may indicate-plant-mycorrhizal interactions. Oecologia 122:273–283. doi:10.1007/PL00008856

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363. doi:10.1002/bimj.200810425

IPCC (2013) Climate change 2013: the physical science basis. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Contribution of Working Group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Johnson PS, Johnson CL, West NE (1988) Estimation of phytomass for ungrazed crested wheatgrass plants using allometric equations. J Range Manag 41:421–425

Kaarlejärvi E, Baxter R, Hofgaard A, Hytteborn H, Khitun O, Molau U, Sjögersten S, Wookey P, Olofsson J (2012) Effects of warming on shrub abundance and chemistry drive ecosystem-level changes in a forest-tundra ecotone. Ecosystems 15:1219–1233. doi:10.1007/s10021-012-9580-9

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727. doi:10.2307/2265777

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems, 2nd edn. Springer, Berlin

Kranabetter JM, Simard SW, Guy RD, Coates KD (2010) Species patterns in foliar nitrogen concentration, nitrogen content and ^{13}C abundance for understory saplings across light gradients. Plant Soil 327:389–401. doi:10.1007/s11104-009-0062-6

Kullman L (2005) Pine (*Pinus sylvestris*) treeline dynamics during the past millennium: a population study in west-central Sweden. Ann Bot Fenn 42:95–106

Le Roux X, Bariac T, Sinoquet H, Genty B, Piel C, Mariotti A, Girardin C, Richard P (2001) Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant Cell Environ 24:1021–1032. doi:10.1046/j.0016-8025.2001.00756.x

Lepik M, Liira J, Zobel K (2005) High shoot plasticity favours plant coexistence in herbaceous vegetation. Oecologia 145:465–474. doi:10.1007/s00442-005-0142-0

Li X, Rennenberg H, Simon J (2015) Competition for nitrogen between *Fagus sylvatica* and *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. Front Plant Sci 6:302. doi:10.3389/fpls.2015.00302

Llambí LD, Puentes Aguilar J, García-Núñez C (2013) Spatial relations and population structure of a dominant tree along a treeline ecotone in the Tropical Andes: interactions at gradient and plant neighbourhood scales. Plant Ecol Divers 6:343–353. doi:10.1080/17550874.2013.810312

Maillette L (1988) Apparent commensalism among three *Vaccinium* species on a climatic gradient. J Ecol 76:877–888. doi:10.2307/2260579

Mallik AU, Pellissier F (2000) Effects of *Vaccinium myrtillus* on Spruce regeneration: testing the notion of coevolutionary significance of allelopathy. J Chem Ecol 26:2197–2209. doi:10.1023/A:1005528701927

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Michelsen A, Jonasson S, Sleep D, Havström M, Callaghan TV (1996) Shoot biomass, $\delta^{13}C$, nitrogen and chlorophyll responses of two arctic dwarf shrubs to in situ shading, nutrient application and warming simulating climatic change. Oecologia 105:1–12. doi:10.1007/BF00328785

Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115:406–418. doi:10.1007/s004420050535

Mourelle C, Kellman M, Kwon L (2001) Light occlusion at forest edges: an analysis of tree architectural characteristics. For Ecol Manag 154:179–192. doi:10.1016/S0378-1127(00)00624-1

Muller O, Hirose T, Werger MJA, Hikosaka K (2011) Optimal use of leaf nitrogen explains seasonal changes in leaf nitrogen content of an understory evergreen shrub. Ann Bot 108:529–536. doi:10.1093/aob/mcr167

Myers-Smith IH, Forbes BC, Wilmking M et al (2011) Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environ Res Lett. doi:10.1088/1748-9326/6/4/045509

Ninot JM, Batllori E, Carrillo E, Carreras J, Casals P, Casas C, Grau O, Gutiérrez E, Montané F, Puente A (2011) Reforestación natural en el dominio de *Pinus uncinata*. In: Ramirez L, Asensio B (eds) Proyectos de investigación en Parques Nacionales: 2007–2010. Organismo Autónomo de Parques Nacionales, Madrid, pp 139–158

Nogués S, Tcherkez G, Cornic G, Ghashghaie J (2004) Respiratory carbon metabolism following illumination in intact French bean leaves using $^{12}C/^{13}C$ labelling. Plant Physiol 136:3245–3254. doi:10.1104/pp.104.048470

Olsrud M, Melillo JM, Christensen TR, Michelsen A, Wallander H, Olsson PA (2004) Response of ericoid mycorrhizal colonization and functioning to global change factors. New Phytol 162:459–469. doi:10.1111/j.1469-8137.2004.01049.x

Palacio S, Millard P, Maestro M, Montserrat-Martí G (2007) Non-structural carbohydrates and nitrogen dynamics in Mediterranean sub-shrubs: an analysis of the functional role of overwintering leaves. Plant Biology 9:49–58. doi:10.1055/s-2006-924224

Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2008) NLME: linear and nonlinear mixed effects models. R package version 3.1-89

Pornon A, Escaravage N, Lamaze T (2007) Complementarity in mineral nitrogen use among dominant plant species in a subalpine community. Am J Bot 94:1778–1785. doi:10.3732/ajb.94.11.1778

Ritchie JC (1956) *Vaccinium myrtillus* L. J Ecol 44:291–299

Sarkar D (2008) Lattice: multivariate data visualization with R. Springer, New York

Schweingruber FH, Poschlod P (2005) Growth rings in herbs and shrubs: life span, age determination and stem anatomy. For Snow Landsc Res 79:195–415

Shevtsova A, Ojala A, Neuvonen S, Vieno M, Haukioja E (1995) Growth and reproduction of dwarf shrubs in a subarctic plant community: annual variation and above-ground interactions with neighbours. J Ecol 83:263–275. doi:10.2307/2261565

Shevtsova A, Haukioja E, Ojala A (1997) Growth response of subarctic dwarf shrubs, *Empetrum nigrum* and *Vaccinium vitis-idaea*, to manipulated environmental conditions and species. Oikos 78:440–458. doi:10.2307/3545606

Smith WK, Germino MJ, Hancock TE, Johnson DM (2003) Another perspective on altitudinal limits of alpine timberlines. Tree Physiol 23:1101–1112. doi:10.1093/treephys/23.16.1101

Stöcklin J, Körner C (1999) Recruitment and mortality of *Pinus sylvestris* near the arctic treeline: the role of climatic change and herbivory. Ecol Bull 47:168–177

Tappeiner U, Cernusca A (1993) Alpine meadows and pastures after abandonment. Pirineos 141–142:97–118. doi:10.3989/pirineos.1993.v141-142.166

Targetti S, Staglianò N, Messeri A, Argenti G (2010) A state-and-transition approach to alpine grasslands under abandonment. iForest 3:44–51. doi:10.3832/ifor0525-003

Tasser E, Tappeiner U (2002) The impact of land-use changes in time and space on vegetation distribution in mountain areas. Appl Veg Sci 5:173–184. doi:10.1111/j.1654-109X.2002.tb00547.x

Tcherkez G, Hodges M (2008) How stable isotopes may help to elucidate primary nitrogen metabolism and its interaction with (photo)respiration in C_3 leaves. J Exp Bot 59:1685–1693. doi:10.1093/jxb/erm115

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org/

van Kleunen M, Fisher M (2001) Adaptive evolution of plastic foraging responses in a clonal Plant. Ecology 82:3309–3319. doi:10.2307/2680154

Vander Kloet SP, Hill NM (2000) Bacca quo vadis: regeneration niche differences among seven sympatric *Vaccinium* species on headlands of Newfoundland. Seed Sci Res 10:89–97. doi:10.1017/S0960258500000106

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Monogr 82:205–220. doi:10.1890/11-0416.1

Vicente-Serrano SM, Lasanta T, Romo A (2004) Analysis of spatial and temporal evolution of vegetation cover in the Spanish Central Pyrenees: role of human management. Environ Manag 34:802–818. doi:10.1007/s00267-003-0022-5

Walker MD, Wahren CH, Hollister RD, Henry GHR, Ahlquist LE, Alatalo JM, Bret-Harte MS, Calef MP, Callaghan TV, Carroll AB, Epstein HE, Jónsdóttir IS, Klein JA, Magnússon B, Molau U, Oberbauer SF, Rewa SP, Robinson CH, Shaver GR, Suding KN, Thompson CC, Tolvanen A, Totland Ø, Turner PL, Tweedie CE, Webber PJ, Wookey PA (2006) Plant community responses to experimental warming across the tundra biome. P Natl Acad Sci USA 103:1342–1346. doi:10.1073/pnas.0503198103

Wang L, Schjoerring JK (2012) Seasonal variation in nitrogen pools and $^{15}N/^{13}C$ natural abundances in different tissues of grassland plants. Biogeosciences 9:1583–1595. doi:10.5194/bg-9-1583-2012

Wilson S, Tilman D (1991) Components of plant competition along an experimental gradient of nitrogen availability. Ecology 72:1050–1065. doi:10.2307/1940605

Xu Z-F, Hu T-X, Wang K-Y, Zhang Y-B, Xian J-R (2009) Short-term responses of phenology, shoot growth and leaf traits of four alpine shrubs in a timberline ecotone to simulated global warming, Eastern Tibetan Plateau, China. Plant Species Biol 24:27–34. doi:10.1111/j.1442-1984.2009.00229.x

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York

Growth and Phenology of Three Dwarf Shrub Species in a Six-Year Soil Warming Experiment at the Alpine Treeline

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Abstract

Global warming can have substantial impacts on the phenological and growth patterns of alpine and Arctic species, resulting in shifts in plant community composition and ecosystem dynamics. We evaluated the effects of a six-year experimental soil warming treatment (+4°C, 2007–2012) on the phenology and growth of three co-dominant dwarf shrub species growing in the understory of *Larix decidua* and *Pinus uncinata* at treeline in the Swiss Alps. We monitored vegetative and reproductive phenology of *Vaccinium myrtillus*, *Vaccinium gaultherioides* and *Empetrum hermaphroditum* throughout the early growing season of 2012 and, following a major harvest at peak season, we measured the biomass of above-ground ramet fractions. For all six years of soil warming we measured annual shoot growth of the three species and analyzed ramet age and xylem ring width of *V. myrtillus*. Our results show that phenology of the three species was more influenced by snowmelt timing, and also by plot tree species (*Larix* or *Pinus*) in the case of *V. myrtillus*, than by soil warming. However, the warming treatment led to increased *V. myrtillus* total above-ground ramet biomass (+36% in 2012), especially new shoot biomass (+63% in 2012), as well as increased new shoot increment length and xylem ring width (+22% and +41%, respectively; average for 2007–2012). These results indicate enhanced overall growth of *V. myrtillus* under soil warming that was sustained over six years and was not caused by an extended growing period in early summer. In contrast, *E. hermaphroditum* only showed a positive shoot growth response to warming in 2011 (+21%), and *V. gaultherioides* showed no significant growth response. Our results indicate that *V. myrtillus* might have a competitive advantage over the less responsive co-occurring dwarf shrub species under future global warming.

Citation: Anadon-Rosell A, Rixen C, Cherubini P, Wipf S, Hagedorn F, et al. (2014) Growth and Phenology of Three Dwarf Shrub Species in a Six-Year Soil Warming Experiment at the Alpine Treeline. PLoS ONE 9(6): e100577. doi:10.1371/journal.pone.0100577

Editor: Bazartseren Boldgiv, National University of Mongolia, Mongolia

Received March 11, 2014; **Accepted** May 26, 2014; **Published** June 23, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript and the supporting information file Dataset S1.

Funding: Funding came from University of Barcelona (collaborative grant to AAR); Spanish Ministry of Education (FPU grant to AAR); WSL (funding for MAD and AAR); Swiss National Science Foundation from 2007 to 2009 (grant 315200-116861 to CR); Velux Foundation (funding for SW and FH). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Alpine and Arctic ecosystems are predicted to be especially vulnerable to global warming [1,2] because plant growth and performance in these environments are strongly constrained by low temperature, short growing seasons and frequent freezing events during the snow-free period. Projected increases of 1.8–4°C in the global mean surface air temperature by the end of the 21st century [3] can cause dramatic changes in community composition since co-occurring species respond differently to climate variations [4–6]. Community changes are of particular importance in alpine and Arctic ecosystems due to their potential effects on climate feedbacks through shifts in plant community composition and plant species cover [7,8].

Experimental studies have shown that responses of plants growing at high latitude and high elevation to warming are species-specific [9–11], demonstrating that understanding how plant community dynamics might change with higher temperatures can only be achieved through assessing responses of individual species. For some species and study sites, positive growth responses to warming were transient, stopping some years after the experiment started [12,13]. In contrast, other studies found a lag in plant growth response to experimental warming or inconsistent responses over time [6,14]. Therefore, species-specific studies lasting several years are crucial for understanding community responses to global warming.

Phenological timing is an important factor for plant fitness and abundance, and it can be highly responsive to temperature ([15] and references therein). For instance, advanced leaf phenology in response to warming could lead to an extension of the photosynthetically active season, which, in turn, could lead to greater carbon gain and ultimately growth for plants [16,17]. Advanced phenology of high-latitude and high-elevation species has occurred with experimentally increased growing season temperatures in numerous studies where snowmelt date was not modified [10,18–22] (but see [23]). In a meta-analysis of alpine and Arctic studies, Arft et al. [12] suggested a relationship between advanced leaf bud burst under warming and increased vegetative growth in alpine and Arctic species. However, most studies have focused on phenology and growth separately, whereas the relationship between phenology and growth in response to warming at an individual/ramet level has rarely been reported.

Shrubs are an essential component of alpine and Arctic ecosystems, and several studies have reported an increase in their cover due to climate warming [6,14,24–27]. This "shrubification" strongly impacts ecosystem dynamics because shrubs modify patterns of snow accumulation, decrease albedo and modify carbon storage and nutrient cycling by greater biomass accumulation and more recalcitrant litter (see review by Myers-Smith et al. [8]).

Shrub growth responses to global warming in alpine and Arctic ecosystems have been assessed in many studies through long-term observations [5,28], experimental manipulations [6] and/or studies using gradients across elevation or latitude [29,30]. Most previous warming experiments have measured above-ground primary production by means of new shoot growth or standing biomass [11,21,31–33]. In contrast, relatively few studies have focused on shrub secondary growth [13,34,35] since acquiring these data requires destructive techniques. Therefore, it remains unclear if the reported increases in biomass production or in shoot increment length are caused by shifts in biomass allocation or if greater overall biomass production occurs.

The Stillberg treeline research area in the Swiss Central Alps hosted a six-year soil warming experiment (2007–2012). A short-term investigation of the dominant ericaceous dwarf shrub species growing in the understory of treeline trees after three years of warming showed that *Vaccinium myrtillus* responded to higher temperatures with increased shoot increment length but that *Vaccinium gaultherioides* and *Empetrum hermaphroditum* did not show a growth response [11]. Moreover, there were only few indications for changes in early-season vegetative phenology in these species under warming [36,37]. In the study presented here, we conducted a detailed investigation of dwarf shrub phenological and growth responses during the summer of 2012, the final (sixth) year of the soil warming experiment. A final destructive harvest conducted at the peak of the vegetation period allowed us to investigate warming effects on secondary growth and biomass allocation for the first time. Our aims were (i) to determine whether a positive growth response in *Vaccinium myrtillus* was maintained after six years of soil warming, and whether *Vaccinium gaultherioides* and *Empetrum hermaphroditum* showed any delayed increases in growth after this full experimental period. By tracking the vegetative and flowering phenology of these dwarf shrubs, we also aimed (ii) to relate possible lagged changes in their phenology to changes in growth. Finally, we analyzed the widths of *V. myrtillus* growth rings with the aim of (iii) assessing whether the increases in shoot increment length under soil warming previously reported for this species represented an increase in the entire above-ground biomass of the ramets or if there was simply a shift in biomass allocation.

Material and Methods

Study site

The study site was located within the Stillberg treeline site in the Central Alps (Davos, Switzerland, 9° 52′E, 46° 46′ N). Stillberg is a 5-ha long-term afforestation research area where tree seedlings were planted into the intact dwarf shrub community in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). Climate data measured by a WSL meteorological station located within the research area (2090 m a.s.l.) indicated a mean annual precipitation of 1155 mm and mean annual air temperature of 2.1°C from 1975 to 2012. For the same period, the main growing season months (June-August) had a mean precipitation of 444 mm and a mean air temperature of 9.2°C. See Table S1 for details on climate data over the study years (2007–2012).

No specific permits were required for this location and activities and the field studies did not involve endangered or protected species.

Experimental design

The experiment consisted of 40 hexagonal 1.1 m^2 plots, 20 with one *Pinus mugo* ssp. *uncinata* (DC.) Domin individual in the center and 20 with one *Larix decidua* Mill. individual in the center. The plots were situated within an area of 2500 m^2 on a NE-exposed 25–30° steep slope at 2180 m a.s.l. at or slightly above the current treeline in the region [38,39]. The trees were sparsely distributed without forming a closed canopy; therefore, each plot contained a single tree surrounded by a dense cover of understory vegetation consisting mainly of the co-dominant dwarf shrub species *Vaccinium myrtillus* L., *Vaccinium gaultherioides* Bigelow (group *V. uliginosum* agg.) and *Empetrum nigrum* subsp. *hermaphroditum* (Hagerup) Böcher (referred to hereafter as *Empetrum hermaphroditum*). Further details about understory species composition were reported by Martin et al. [36] and Dawes et al. [11].

A free air CO_2 enrichment (FACE) experiment was started after snowmelt in early June 2001 and applied during each snow-free season for nine years (ending in 2009). The 40 plots were assigned to 10 groups of four neighboring plots (two *Pinus* and two *Larix* trees per group) and half of these groups were randomly assigned to an elevated CO_2 treatment while the other half served as controls (see a detailed description of the setup and performance of the CO_2 enrichment facility in Hättenschwiler et al. [38] and in Dawes et al. [40]). Dwarf shrub responses to the CO_2 enrichment have been reported in detail by Dawes et al. [11,41]. A soil warming treatment was added to the experiment in spring 2007 and was applied during each snow-free season until early August 2012. Within each of the 10 CO_2 treatment groups, one plot of each tree species was randomly selected and assigned a soil warming treatment, either control or warmed, and the second plot was assigned the other treatment, yielding a balanced split-split-plot design with a replication of five individual plots for each combination of CO_2 level, soil warming treatment and tree species. Therefore, from 2007 to 2009 the experiment included both CO_2 enrichment and soil warming, whereas from 2010 to 2012 the treatment consisted of soil warming only. The soil warming treatment was applied using 420 W-heating cables laid on the ground surface underneath the dwarf shrub layer, with a 5 cm distance between neighboring cables [42]. Each year, the heating was turned on immediately after snowmelt and turned off just before the site was covered in snow for the winter to avoid an interaction between soil temperature and snow cover duration. The warming treatment increased the growing season mean soil temperatures at 5 cm depth by 3.1 to 4.4°C over the 6 seasons of heating, with a difference of +3.5°C in 2012 (HOBO U23 Pro v2 dataloggers, Onset Computer Corporation, Bourne, MA, USA). Air temperature was warmed within the dwarf shrub canopy (0.9°C at 20 cm above ground [42]). The warming treatment had a slight drying effect on the soil organic layer (details in Hagedorn et al. [42] and Dawes et al. [11]). However, the soil matric water potential at 5 cm depth was always above -300 hPa in all plots, indicating overall very moist soil conditions [11,43].

Snowmelt date of each plot from 2007 to 2011 was defined as the date in spring when soil temperatures at 5 cm depth rose sharply from values near 0°C, supported by visual estimations in the field and photographs. In 2012, snowmelt date was determined by visual estimations as the date when 50% of the plot was snow free and the ramets selected for detailed investigation were uncovered. This way, the estimation was more accurate for the study of the specific ramets selected.

Phenology

At the time of snowmelt in 2012, we marked five ramets of *V. myrtillus*, *V. gaultherioides* and *E. hermaphroditum* in each plot, excluding the area within 10 cm of plot borders to avoid potential edge effects. All plant measurements were made on these ramets. *Vaccinium myrtillus* was present and abundant in every plot, *V. gaultherioides* was present in 35 of the 40 plots, and *E. hermaphroditum* was present in 26 of the 40 plots, which was sufficient replication to assess treatment effects.

We monitored the phenology of the three study species between the start of snowmelt (day of year 142) and peak growing season (day of year 212). We visited all plots and monitored all ramets every 2 to 4 days at early growing season and every 4 to 6 days after flowering. For each marked ramet, we recorded the date when it entered the following phenophases (some of which occurred at the same time): (1) burst of first vegetative bud, (2) first leaf starting to unfold, (3) first leaf fully expanded, (4) start of shoot elongation, (5) burst of first flower bud, (6) anthesis, and (7) first flower withered. In early August, we harvested all vegetation from the experimental plots. Previous phenological data in the same plots from an entire snow-free season [44] confirm our observation that dwarf shrub vegetative development was completed at the time of the harvest, whereas fruits were not mature and leaf senescence had not started.

Shoot increment length and above-ground biomass

To track shoot growth responses during the whole soil warming experimental period, shoot increment length of the three species was measured from 2005 to 2011 on the longest branch of five to seven ramets in the field (2005–2009 data presented in Dawes et al. [11]). During early August 2012, we harvested all the marked ramets in every plot, clipping them at ground level. For *E. hermaphroditum*, we measured the new shoot increment length on the longest branch for every ramet before detaching and drying them for biomass measures. For all three species, we separated new shoots and leaves from the rest of the ramet while they were still fresh and dried them at 60°C for 48 h. For the two deciduous *Vaccinium* species, we weighed new shoots and leaves separately and measured the length of three new shoots per ramet, whereas for *E. hermaphroditum*, we weighed the small needle-like leaves and new shoots together. For *V. myrtillus* and *V. gaultherioides*, we counted the number of new shoots per ramet and calculated the average mass per individual shoot. For *E. hermaphroditum*, we counted the number of new shoots from a subsample of ramets and used the mass-count relationship to estimate the number of branches for the remaining ramets in each plot. For all three species, we dried the remaining ramet material (designated as "main stems", >1 year old) at 60°C for 24 h and weighed it.

Growth rings of Vaccinium myrtillus

We made cross-sections of 20 μm thickness from the basal 1.5 cm of dried *V. myrtillus* stems using a sledge microtome (WSL-Lab-microtome). We stained sections with a mixture of Safranin and Astrablue to emphasize the growth ring structure. For dehydrating the sections for preservation, we rinsed them with increasingly concentrated ethanol solutions (75%, 96%, 100%), immersed them in Xylol, imbedded them in Canada-Balsam and dried them at 60°C for 24 hours [45] before photographing them at x20–x200 magnification through a microscope with a digital camera (Canon EOS 650D on Olympus BX41 microscope; Fig. S1). We used the images to visually count rings and to measure xylem ring widths in four radii per section with the program WinCELL [46]. We excluded 23 out of 200 ramets from the statistical analyses because wood was damaged or broken or rings were not distinguishable. Ramets were not old enough for rings to be analyzed statistically using specialized dendrochronological software, so we visually cross-dated the samples to find possible missing rings. Latewood had not formed in the 2012 growth rings, indicating that secondary growth was not finished when we harvested the ramets.

Statistical analysis

We applied linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML) to assess treatment effects on phenology and growth parameters. We used likelihood ratio tests to determine whether the previous CO_2 treatment and interactions between CO_2 and the other treatments contributed significantly to the model fits as fixed effects. Previous investigations already reported a lack of soil warming x CO_2 interactive effects [11,41] and we did not find significant persistent effects of the CO_2 enrichment; therefore, we pooled across CO_2 treatments for all final analyses. Statistical models for phenology and biomass of the study species included warming treatment, plot tree species and their interaction as fixed effects. We included snowmelt date as a covariate when it contributed significantly to the model fits, i.e. in tests of the effect of soil warming and plot tree species on phenology.

Shoot increment length and growth ring width were analyzed as repeated measures and included treatment year (categorical variable) and all interactions between year, warming and plot tree species as additional fixed effects (after Dawes et al. [11]). Measurements averaged over 2005 and 2006 were included as a covariate to account for differences in shoot increment length or ring width before the warming treatment started. When a significant interaction between soil warming and year was found, we additionally tested the effect of soil warming with separate analyses for each individual year. We accounted for violation of independence of residuals from different treatment years by implementing the residual autocorrelation structure corAR1 [47]. As growth rings were not completely finished in 2012, we excluded this year from the repeated measures analysis of growth ring widths. We tested for Pearson's correlations between shoot increment length and above-ground biomass, as well as between shoot increment length and xylem ring width in 2012. We did not extend this analysis to previous years because ramets harvested in 2012 were not the same as those randomly selected for shoot increment measurements in previous years.

For all statistical analyses, the random effects structure reflected the hierarchy of the split-split-plot experimental design, with measurements made on ramets in 40 individual plots, nested within 20 soil warming treatment groups, nested within 10 CO_2 treatment groups. For all analyses, we visually checked assumptions of normality and homoscedasticity of the residuals. We log-transformed response variables when necessary to reach these assumptions. Effects were considered significant at $P<0.05$ and, because of the relatively low replication, we considered $P\geq0.05$ but <0.10 as marginally significant. We performed all the analyses with R version 2.15.2 [48] using the nlme package [47].

Results

Abiotic conditions

In 2012, snow melted from the experimental plots between 21 and 31 May (days of year 142 and 152). There were no differences in the date of snowmelt between soil warming treatments ($F_{1,9} = 0.64$, $P = 0.447$) or plot tree species ($F_{1,18} = 1.04$, $P = 0.321$). From 21 May to 3 August, when plants were harvested, the mean air temperature at the meteorological station was 9.7°C and temperatures ranged from -0.4 on 12 June to 21.9°C on 30 June.

Phenology

The soil warming treatment did not significantly affect the vegetative phenology of the three dwarf shrub species (Fig. 1). However, we found marginally significant warming effects on their flowering phenology. *Vaccinium myrtillus* and *V. gaultherioides* showed a slightly earlier flower anthesis (by 1 and 2 days, respectively) in warmed plots than in unwarmed plots (*V. myrtillus*: $F_{1,9} = 4.3$, $P = 0.067$, Fig.1A; *V. gaultherioides*: $F_{1,7} = 4.41$, $P = 0.074$, Fig. 1B). *Vaccinium myrtillus* flowers also withered slightly earlier with increased soil temperatures (by 2 days, $F_{1,9} = 4.03$, $P = 0.076$).

The tree species present in the plot had a greater effect than soil warming on *V. myrtillus* vegetative and reproductive phenology (Fig. 1A). The start of leaf unfolding, start of shoot elongation, flower bud break and flower anthesis took place 1 day earlier under larch than under pine ($P < 0.011$), and leaf full expansion occurred 3 days earlier ($F_{1,17} = 29.88$, $P < 0.001$). There was a marginally significant effect of the tree species x warming interaction on the start of shoot elongation for *V. myrtillus*, which was slightly earlier (1 day) in warmed plots under larch than in unwarmed plots under pine ($F_{1,17} = 3.7$, $P = 0.071$). The phenology of *V. gaultherioides* and *E. hermaphroditum* did not show significant differences between plots with the two different tree species (Fig. 1B,C).

Most of the phenological phases for *V. myrtillus* and *V. gaultherioides* occurred earlier in plots with an earlier snowmelt date ($P < 0.07$). However, *V. myrtillus* anthesis and *V. gaultherioides* flower bud break and withering did not show a relationship with snowmelt timing. *Empetrum hermaphroditum* flowering phenophases and start of shoot elongation were also related to snowmelt date ($P < 0.04$), but vegetative bud break was not.

Shoot increment length

Soil warming had a significant positive effect on the annual shoot increment length of *V. myrtillus* (mean increase of 22% over 2007–2012, $F_{1,9} = 41.38$, $P < 0.001$, Fig. 2A). Treatment year also had a significant effect ($F_{5,180} = 3.67$, $P = 0.004$) but the warming x year effect was not significant ($F_{5,180} = 1.89$, $P = 0.100$). Nevertheless, the response was larger in the two last study years (shoot increment length 34% greater in warmed plots than unwarmed plots for 2011–2012) than in the previous years (18% greater in warmed plots than unwarmed plots, averaged over 2008–2010).

The annual shoot increment length of *V. gaultherioides* was not significantly affected by soil warming ($F_{1,9} = 0.99$, $P = 0.346$, Fig. 2B) although there was a trend of increase with warming in the last year. Treatment year had a significant effect on *V. gaultherioides* shoot increment length ($F_{5,171} = 6.93$, $P < 0.001$) but the warming x year interaction was not significant ($F_{5,171} = 0.5$, $P = 0.776$). *Empetrum hermaphroditum* annual shoot increment length showed a significant effect of treatment year ($F_{5,128} = 25.97$, $P < 0.001$, Fig. 2C) and the plot tree species x year interaction ($F_{5,128} = 2.55$, $P = 0.031$), and also a marginally significant effect of warming ($F_{1,9} = 4.83$, $P = 0.056$) and the warming x year interaction ($F_{5,128} = 2.21$, $P = 0.057$). Analyses of individual years showed that *E. hermaphroditum* only had a significant (positive) shoot growth response to soil warming in 2011 (+21% increase in warmed plots compared to unwarmed plots, $F_{1,7} = 10.01$, $P = 0.016$) and that the shoot increment length of this species was greater under pine than under larch in 2008 (marginally significant, $F_{1,11} = 4.04$, $P = 0.070$) and 2009 ($F_{1,10} = 5.07$, $P = 0.048$).

Shoot increment length averaged over 2005 and 2006 (pre-treatment covariate) positively influenced the length of new *V. gaultheroides* shoots during the 2007–2012 warming period ($F_{1,17} = 18.61$, $P < 0.001$), and to a lesser extent that of *V. myrtillus* and *E. hermaphroditum* (both marginally significant, $F_{1,17} = 3.28$, $P = 0.088$ and $F_{1,13} = 4.58$, $P = 0.052$, respectively). In 2012, new shoot increment length showed a positive correlation with new shoot biomass for each of the three species (*V. myrtillus* $R^2 = 0.53$, $P < 0.001$; *V. gaultherioides* $R^2 = 0.39$, $P < 0.001$; *E. hermaphroditum* $R^2 = 0.27$, $P < 0.001$).

Above-ground biomass

At the time of harvest in August 2012, *V. myrtillus* ramets showed a 54% greater total leaf biomass ($F_{1,9} = 15.52$, $P = 0.003$), a 63% greater total new shoot biomass ($F_{1,9} = 14.15$, $P = 0.005$) and a 26% greater main stem (stems >1 year old) biomass ($F_{1,9} = 7.82$, $P = 0.021$) in warmed plots than in unwarmed plots (Fig. 3A). Moreover, the main stem biomass of *V. myrtillus* was 35% greater under pine than under larch ($F_{1,18} = 5.15$, $P = 0.036$). Although differences in ramet above-ground biomass between warming treatments were, on average, larger in plots with larch than in plots with pine, there were no significant tree species x warming interactions for any of the plant parts ($P > 0.77$). *Vaccinium gaultherioides* and *E. hermaphroditum* did not show significant differences among treatments in the above-ground biomass for any of the plant parts analyzed (Fig. 3B,C). The three dwarf shrub species showed a positive correlation between new shoot biomass and total ramet biomass (*V. myrtillus* $R^2 = 0.77$, $P < 0.001$; *V. gaultherioides* $R^2 = 0.71$, $P < 0.001$; *E. hermaphroditum* $R^2 = 0.52$, $P < 0.001$).

The number of new shoots per ramet in *V. myrtillus* was significantly greater in warmed plots than in unwarmed plots ($F_{1,9} = 6.466$, $P = 0.032$) and slightly greater under pine than under larch (marginally significant, $F_{1,18} = 3.51$, $P = 0.077$). In addition, the mass per individual shoot was larger in warmed plots than in unwarmed plots ($F_{1,9} = 9.79$, $P = 0.012$) and slightly larger under larch than under pine (marginally significant, $F_{1,18} = 4.14$, $P = 0.060$). Therefore, *V. myrtillus* not only produced more shoots in warmed plots, but these individual shoots were longer and heavier. *Vaccinium gaultherioides* produced slightly more new shoots in warmed plots than in unwarmed plots (marginally significant, $F_{1,9} = 3.74$, $P = 0.085$) and under pine than under larch ($F_{1,13} = 5.91$, $P = 0.030$), but the mass per individual shoot did not differ between warming treatments or plot tree species.

Growth rings of Vaccinium myrtillus

We did not find significant differences in ramet age between warming treatments (8.4±0.5 years old for unwarmed and 7.9±0.3 for warmed plots; $F_{1,9} = 0.58$, $P = 0.467$; Fig. S2). However, ramets were younger under larch (7.4±0.4 years old) than under pine (9.0±0.4; $F_{1,18} = 10.13$, $P = 0.005$). There was a marginally significant warming x tree species interaction ($F_{1,18} = 3.28$, $P = 0.087$): on average, ramets were older in unwarmed plots with pine than in both warmed and unwarmed plots with larch.

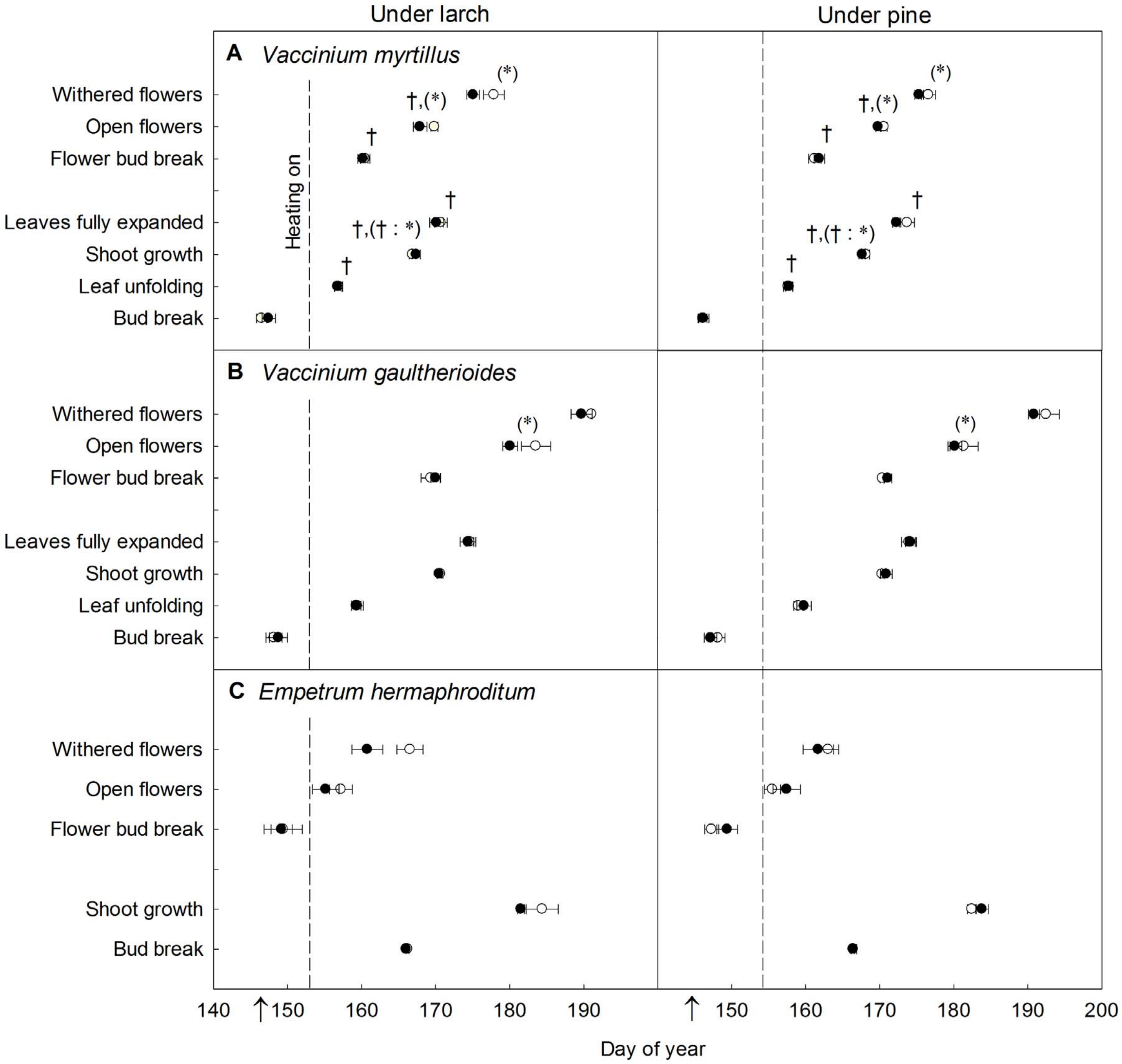

Figure 1. Vegetative and flowering phenology for the three dwarf shrub species studied. Vegetative and flowering phenology for the three dwarf shrub species studied. Circles represent the mean day of year (±1 SE) of each phenophase for the four different soil warming (open circles, unwarmed; closed circles, warmed) and plot tree species (larch or pine) combinations (n = 10). Crosses show significant differences between plot tree species ($P<0.05$) and marginally significant differences ($0.05 \leq P < 0.10$) between soil warming treatments are given by asterisks in parentheses. Colons show treatment interactions. Note that in some cases symbols for different soil warming treatments overlap completely. Dotted lines show the date when the warming treatment started in 2012 and arrows on the x-axes show the mean snowmelt date for all plots with the same tree species.
doi:10.1371/journal.pone.0100577.g001

Repeated measures analyses showed that, averaged across all treatment years, *V. myrtillus* ring width was 41% greater in warmed plots than in unwarmed plots ($F_{1,9} = 16.45$, $P = 0.003$, Fig. 4). Treatment year and the warming x year interaction also had significant effects ($F_{4,297} = 4.67$, $P = 0.001$ and $F_{4,297} = 3.32$, $P = 0.011$, respectively), as well as the pre-treatment ring width covariate ($F_{1,44} = 17.26$, $P<0.001$). Plot tree species and interactions between tree species and the other fixed effects did not significantly influence xylem ring width ($P>0.17$). Analyses of individual years showed that warming had a significant effect on ring width in all years ($P<0.024$) except for the first year of treatment (Fig. 4).

Although xylem latewood formation was not completed when we collected the samples in 2012, we found a significant correlation between shoot increment length and ring width in that year ($R^2 = 0.157$, $P<0.001$), as well as between ring width and biomass of new shoots ($R^2 = 0.088$, $P<0.001$) and biomass of leaves ($R^2 = 0.068$, $P<0.001$). We tested for an age-biomass correlation and results corroborated that older ramets were heavier ($R^2 = 0.24$, $P<0.001$).

Discussion

Increased above-ground growth after six years of soil warming

Six years of soil warming led to a sustained growth stimulation of *V. myrtillus*, and the greatest response of new shoot increments occurred in the final two years. This increasing response over time contrasts with studies where positive responses of *V. myrtillus* and other alpine and Arctic plant species to warming were transient and stopped after four or five years [12,13,49]. In addition, our findings indicate that other factors that can potentially limit

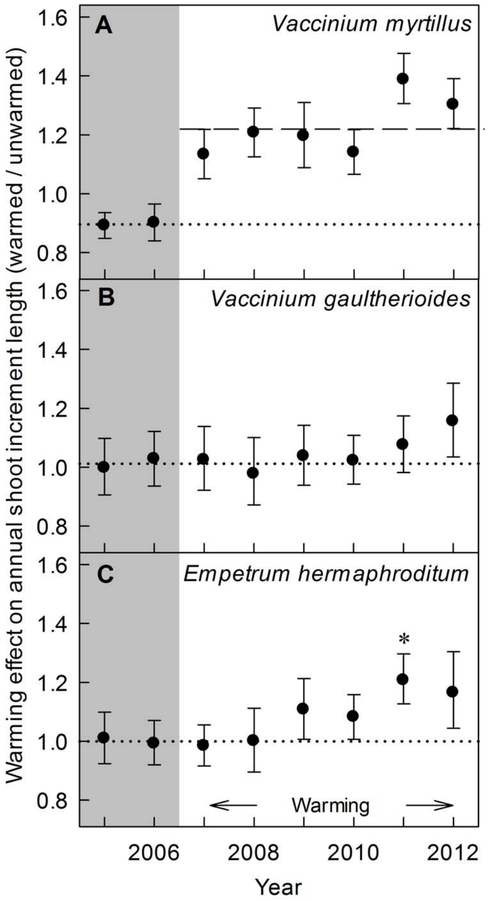

Figure 2. Soil warming effect on dwarf shrub annual shoot increment length. Soil warming effect on dwarf shrub annual shoot increment length from 2007 until 2012, the entire duration of the soil warming experiment. Data through 2009 were presented in Dawes et al. (2011a). The warming effect was calculated as the ratio of the mean shoot increment length of all warmed plots to the mean of all unwarmed plots, pooled across plots containing a larch or pine tree. Error bars represent ±1 SE of the ratio. The dashed line shows the significant warming effect on *V. myrtillus* averaged for 2007–2012. The asterisk shows significant differences between temperature treatments ($P<0.05$). Pre-warming ratios are shown in the shaded region (2005–2006) and the dotted line is drawn through the average of these two points, which indicates the mean warmed to unwarmed ratio before treatment began.
doi:10.1371/journal.pone.0100577.g002

growth, such as nutrient or light availability, did not constrain the warming response of *V. myrtillus* in later years of the experiment [12]. Earlier studies at the same experimental site found more severe freezing damage of *V. myrtillus* under warming than in control plots [36,37], but our results show that these freezing effects were not large enough to substantially offset the enhanced growth over six years. The other two dwarf shrub species showed at least a slight trend of increased shoot increment length with soil warming in the last years of the study, suggesting that the response of these two species to climate warming may be considerably delayed.

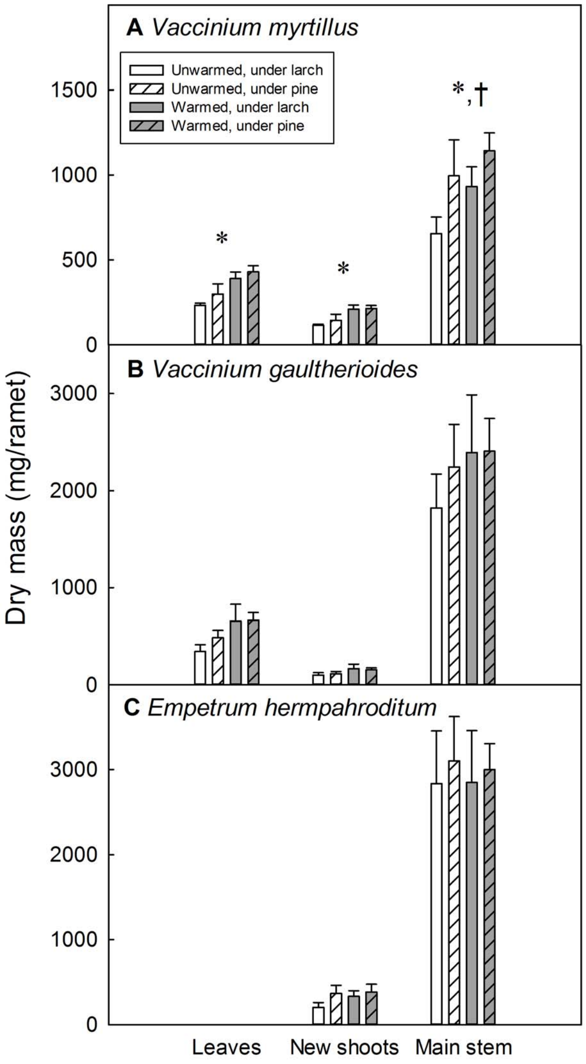

Figure 3. Above-ground biomass partitioning of the three dwarf shrub species studied. Above-ground biomass partitioning of the study species for each soil warming and plot trees species combination (mean values +1 SE, n = 10). Asterisks show significant differences between soil warming treatments and crosses show significant differences between plot tree species ($P<0.05$). For *Empetrum hermaphroditum* only, leaves and new shoots are both included in "New shoots". The y-axis scale varies across species to emphasize differences between treatments.
doi:10.1371/journal.pone.0100577.g003

The species-specific warming effects on above-ground biomass production in our experiment contrast with a two-year study with open-top polythene tents in subarctic Sweden, where all three study species in common with our study showed a greater above-ground biomass under warming [50]. A five-year study at the same site in the subarctic by Press et al. [51] similarly showed that higher temperatures increased *V. uliginosum* (comparable with *V. gaultherioides*) biomass. Moreover, *Empetrum nigrum* shoot production and shoot increment length increased under two years [31] and five years [32] of warming with open-top chambers in alpine heath in Japan. The different methodologies applied (air warming by open-top chambers *vs.* soil warming by heating cables) might explain some of the differences between studies. In addition, different plant community composition and dynamics might play an important role in explaining the different results across study sites.

Vaccinium myrtillus has a wider elevational distribution (colline to alpine) than *V. gaultherioides* and *E. hermaphroditum* (subalpine to alpine) [52]. Its presence in lower elevational areas indicates that *V. myrtillus* might be adapted to higher temperatures than *V.*

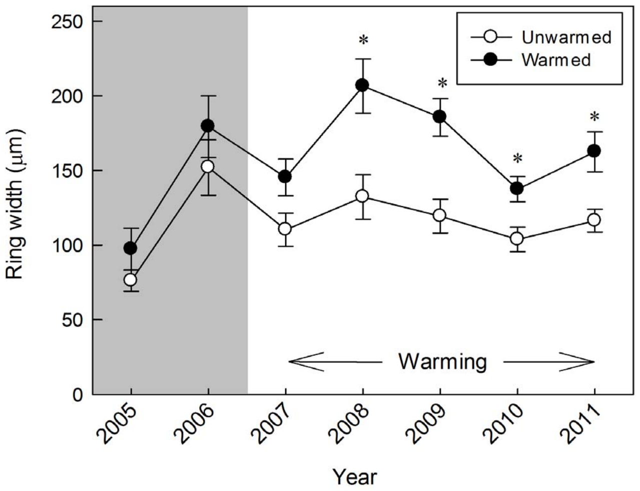

Figure 4. Xylem ring widths of *Vaccinium myrtillus*. Xylem ring widths of *Vaccinium myrtillus* from 2007 to 2011 for warmed and unwarmed plots, pooled across plots containing a larch or pine tree (mean values ±1 SE, n = 20). Asterisks show significant differences between soil warming treatments ($P<0.05$). Pre-warming values are shown in the shaded region (2005–2006).
doi:10.1371/journal.pone.0100577.g004

gaultherioides and *E. hermaphroditum* [11] and thus may be a stronger competitor under increased temperatures. In fact, a 22-year experiment with open-top greenhouses in the Swedish tundra reported no effects on *E. hermaphroditum* growth under 4°C of air warming [13], whereas in the same experiment, Graglia et al. [53] found positive effects on this species after six years of 2.5°C warming but not 4°C warming. The mean daily air temperature increase with open-top chambers is around 1-2°C [54] whereas the soil temperature increase by our heating cables is around 4°C, which is similar to the air temperature increase reached by Campioli et al. [13]. As suggested in their study, *E. hermpahroditum* might suffer from heat stress under high temperatures, responding positively only to smaller increases.

Phenology: effects of growing season temperature, snowmelt and plot tree species

The lack of vegetative phenological responses to soil warming of the three dwarf shrub species studied and the strong effect of snowmelt date on early-season phenology in 2012 are consistent with results from the first three years of the experiment [37]. Some plant species have been shown not to respond to spring warming experiments [55] because they either do not respond to climate warming [55,56], because they are more sensitive to changes in late winter temperatures [55] or because they primarily respond to other cues such as photoperiod [57]. In our experiment, the lack of warming effects on the vegetative phenophases might be partially due to the fact that vegetative development, especially for the two *Vaccinium* species, started directly after snowmelt, which coincided with the start of the warming treatment. Phenophases occurring later in the season (i.e. flowering phenophases in *Vaccinium* spp.) did not depend on the snowmelt date and were slightly influenced by the warming treatment, which is consistent with patterns found for tundra dwarf shrub species in subarctic Alaska [58].

Our study might have underestimated phenological responses to future climate warming because only growing season temperatures were altered [55,59], whereas late winter temperatures, which can be key determinants of plant phenology [15,55], were not experimentally manipulated. Nevertheless, other studies that only altered growing season temperatures found that the same species as in our study showed phenological advances under air warming [18] or that other alpine species showed a lagged response after multiple years of warming [10]. The distinct methodologies applied in these experiments (e.g. soil *vs.* air warming), microclimate conditions, different plant community types and genetic variations between populations might explain these differences [11,60,61].

The tree species present in the plot had a greater effect on *V. myrtillus* phenology than soil warming. The phenological advancement under larch could be due to a greater light incidence under this species at the beginning of the growing season, which could act as a phenological cue [62].

Increased growth in *V. myrtillus* decoupled from phenology

The increased growth and biomass production found in *V. myrtillus* with soil warming did not correspond to a phenological shift or to the date of snowmelt, which indicates that *V. myrtillus* can produce more biomass under higher growing season temperatures even without a springtime extension of the active season. The peak-season harvest meant that we could not check if *V. myrtillus* phenology at senescence time was delayed by the warming treatment, an effect that has been reported for tundra plants in experiments with open-top chambers [63,64]. Delayed leaf senescence could have influenced *V. myrtillus* growth in the late season (e.g. secondary growth) or in the following year. However, monitoring of leaf senescence in 2008–2009 showed no differences between warming treatments in this species (Dawes, unpublished) and late-season phenophases of alpine plants are generally considered to be more controlled by photoperiod than by temperature [65].

As advanced phenology could not explain the enhanced growth of *V. myrtillus* under soil warming, potential mechanisms for this response include increased rates of photosynthesis [66] and tissue formation [65] directly caused by higher temperatures. Another mechanism could be accelerated decomposition and mineralization of soil organic matter under warmer soil, which can improve nutrient availability for plants in alpine and Arctic environments where low temperatures tend to constrain these processes [49,66–68]. Soil inorganic nitrogen content increased during the first three years of our soil warming experiment [11], suggesting that indirect effects of soil warming via an enhanced nutrient cycling played a role in the *V. myrtillus* growth response.

Above-ground biomass increase under soil warming

To our knowledge, this is a pioneer study on assessing the effects of experimental soil warming on the age and xylem ring width of *V. myrtillus*. Our results show that rings were wider in warmed plots since the second year of treatment, a response that was maintained throughout the six experimental years. In addition, the significant positive correlation between shoot increment length and early xylem growth (xylem latewood formation was not completed) indicates that vessel size in the early xylem growing season is tightly related to the elongation of new shoots, which receive water from newly-formed vessels [69].

Moreover, the positive correlation between shoot increment length and both early xylem growth and annual shoot biomass production, together with the greater biomass in *V. myrtillus* in warmed plots, provide evidence that the previously reported increases in the shoot increment length of *V. myrtillus* with warming were not merely a result of a shift in biomass allocation but an overall increase in above-ground biomass production. However, below-ground biomass was not measured in this study and thus, it is possible that soil warming led to shifts in biomass allocation between below-ground and above-ground compartments [70].

Although warmer temperatures led to an increased growth of *V. myrtillus*, the age structure of this species was not affected by soil warming. However, there was a lower ramet turnover under pine (older ramets) than under larch, and ramet main stem biomass was also higher under pine. A possible explanation for these differences is that pine provides greater protection against freezing conditions at the beginning of the season before needles are developed on deciduous larch. This effect would be similar to the facilitation exerted by shrubs on young trees [71,72], leading to lower mortality rates. Moreover, Dawes et al. [11] found less canopy shading under pine than under larch, thus the lower main stem biomass under larch may be a consequence of lower light availability.

Concluding remarks

In summary, we found increased growth of *V. myrtillus* under soil warming, a response that was sustained, and even became stronger in the case of shoot increment length, over six years of warming. The application of dendrochronological techniques showed that this increased growth reflected an overall increase in above-ground biomass production. Moreover, the lack of an advanced phenology of *V. myrtillus* under soil warming indicated that an extended growing period was not necessary for the observed growth response. Our results suggest that *V. myrtillus* will experience a larger and more rapid growth benefit from a warming climate than *V. gaultherioides* or *E. hermaphroditum* and could therefore become increasingly dominant in high-elevation treeline environments.

Supporting Information

Figure S1 Cross-section of a *Vaccinium myrtillus* ramet.
(PDF)

Figure S2 Age of *Vaccinium myrtillus* for each soil warming treatment and plot tree species combination.
(PDF)

Table S1 Climate conditions in the experimental plots during all study years (2007–2012).
(PDF)

Dataset S1 Phenology, above-ground biomass, shoot elongation and xylem growth rings measurements.
(XLSX)

Acknowledgments

We thank many colleagues at the SLF, WSL and other research institutes who contributed to the Stillberg CO_2 enrichment and soil warming experiment. We are especially grateful to Stephan Hättenschwiler and I. Tanya Handa for planning and initiating this treeline study. We thank Yves Bötsch and Michael Liu for their help with field work and Jodi Axelson, Loïc Schneider, Fritz Schweingruber, Agata Buchwal and Patrick Fonti for their help, support and comments in the growth rings methodology and analysis. We are grateful to Sara Palacio for her comments on an early version of the manuscript and to Josep Maria Ninot for his academic support.

Author Contributions

Conceived and designed the experiments: AAR CR PC SW FH MAD. Performed the experiments: AAR MAD. Analyzed the data: AAR MAD. Contributed reagents/materials/analysis tools: SW FH MAD. Wrote the paper: AAR CR PC SW FH MAD.

References

1. Chapin III FS, Mcguire AD, Randerson J, Pielke R, Baldocchi D, et al. (2000) Arctic and boreal ecosystems of western North America as components of the climate system. Global Change Biol 6: 211–223.
2. IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland, 73 p.
3. Meehl GA, Stocker TF, Collins WD, Friedlingstein AT, Gaye JM, et al. (2007) Global climate projections. In: Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change (eds Solomon S, Qin D, Manning M, et al.). Cambridge University Press, Cambridge, UK. pp. 747–845.
4. Cornelissen JHC, Callaghan TV, Alatalo JM, Michelsen A, Graglia E, et al. (2001) Global change and arctic ecosystems: Is lichen decline a function of increases in vascular plant biomass? J Ecol 89: 984–994.
5. Hudson JMG, Henry GHR (2009) Increased plant biomass in a high arctic heath community from 1981 to 2008. Ecology 90: 2657–2663.
6. Elmendorf SC, Henry GHR, Hollister RD, Björk RG, Bjorkman AD, et al. (2012a) Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. Ecol Lett 15: 164–175.
7. Wookey PA, Aerts R, Bardgett RD, Baptist F, Bråthen KA, et al. (2009) Ecosystem feedbacks and cascade processes: Understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Global Change Biol 15: 1153–1172.
8. Myers-Smith IH, Forbes BC, Wilmking M, Hallinger M, Lantz T, et al. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. Environ Res Lett 6: 10.1088/1748-9326/6/4/045509.
9. Klanderud K (2008) Species-specific responses of an alpine plant community under simulated environmental change. J Veg Sci 19: 363–372.
10. Hoffmann AA, Camac JS, Williams RJ, Papst W, Jarrad FC et al. (2010) Phenological changes in six Australian subalpine plants in response to experimental warming and year-to-year variation. J Ecol 98: 927–937.
11. Dawes MA, Hagedorn F, Zumbrunn T, Handa IT, Hättenschwiler S, et al. (2011a) Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytol 191: 806–818.
12. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999) Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. Ecol Monogr 69: 491–511.
13. Campioli M, Leblans N, Michelsen A (2012) Twenty-two years of warming, fertilisation and shading of subarctic heath shrubs promote secondary growth and plasticity but not primary growth. PLoS ONE 7, 10.1371/journal.pone.0034842.
14. Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of Arctic tundra to experimental and observed changes in climate. Ecology 76: 694–711.
15. Walther GR (2003) Plants in a warmer world. Perspect Plant Ecol 6: 169–185.
16. Goulden ML, Munger JW, Fan S-M, Daube BC, Wofsy SC (1996) Exchange of carbon dioxide by a deciduous forest: Response to interannual climate variability. Science 271: 1576–1578.
17. Myneni RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the northern high latitudes from 1981 to 1991. Nature 386: 698–702.
18. Suzuki S, Kudo G (2000) Responses of alpine shrubs to simulated environmental change during three years in the mid-latitude mountain, northern Japan. Ecography 23: 553–564.
19. Dunne JA, Harte J, Taylor KJ (2003) Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. Ecol Monogr 73: 69–86.
20. Aerts R, Cornelissen JHC, Dorrepaal E, Van Logtestijn RSP, Callaghan TV (2004) Effects of experimentally imposed climate scenarios on flowering phenology and flower production of subarctic bog species. Global Change Biol 10: 1599–1609.
21. Xu Z-F, Hu T-X, Wang K-Y, Zhang Y-B, Xian J-R (2009) Short-term responses of phenology, shoot growth and leaf traits of four alpine shrubs in a timberline ecotone to simulated global warming, Eastern Tibetan Plateau, China. Plant Spec Biol 24: 27–34.
22. Smith J, Sconiers W, Spasojevic M, Ashton I, Suding K (2012) Phenological changes in alpine plants in response to increased snowpack, temperature, and nitrogen. Arct Antarct Alp Res 44: 135–142.
23. Totland Ø, Alatalo JM (2002) Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. Oecologia 133: 168–175.
24. Tape K, Sturm M, Racine C (2006) The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. Global Change Biol 12: 686–702.

25. Rundqvist S, Hedenås H, Sandström A, Emanuelsson U, Eriksson H, et al. (2011) Tree and shrub expansion over the past 34 years at the tree-line near Abisko, Sweden. Ambio 40: 683–692.
26. Ropars P, Boudreau S (2012) Shrub expansion at the forest-tundra ecotone: Spatial heterogeneity linked to local topography. Environ Res Lett 7: 10.1088/1748-9326/7/1/015501.
27. Elmendorf SC, Henry GHR, Hollister RD, Björk RG, Boulanger-Lapointe N, et al. (2012b) Plot-scale evidence of tundra vegetation change and links to recent summer warming. Nature Climate Change 2: 453–457.
28. Hill GB, Henry GHR (2011) Responses of High Arctic wet sedge tundra to climate warming since 1980. Global Change Biol 17: 276–287.
29. Rixen C, Schwoerer C, Wipf S (2010) Winter climate change at different temporal scales in *Vaccinium myrtillus*, an Arctic and alpine dwarf shrub. Polar Res 29: 85–94.
30. Pato J, Ramón Obeso J (2012) Growth and reproductive performance in bilberry (*Vaccinium myrtillus*) along an elevation gradient. Ecoscience 19: 59–68.
31. Wada N, Shimono M, Miyamoto M, Kojima S (2002) Warming effects on shoot developmental growth and biomass production in sympatric evergreen alpine dwarf shrubs *Empetrum nigrum* and *Loiseleuria procumbens*. Ecol Res 17: 125–132.
32. Kudo G, Suzuki S (2003) Warming effects on growth, production, and vegetation structure of alpine shrubs: A five-year experiment in northern Japan. Oecologia 135: 280–287.
33. Van Wijk MT, Clemmensen KE, Shaver GR, Williams M, Callaghan TV, et al. (2004) Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: Generalizations and differences in ecosystem and plant type responses to global change. Global Change Biol 10: 105–123.
34. Bret-Harte MS, Shaver GR, Chapin III FS (2002) Primary and secondary stem growth in arctic shrubs: Implications for community response to environmental change. J Ecol 90: 251–267.
35. Campioli M, Schmidt NM, Albert KR, Leblans N, Ro-Poulsen H, et al. (2013) Does warming affect growth rate and biomass production of shrubs in the High Arctic? Plant Ecol 214: 1049–1058.
36. Martin MA, Gavazov K, Körner C, Hättenschwiler S, Rixen C (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO_2. Global Change Biol 16: 1057–1070.
37. Rixen C, Dawes MA, Wipf S, Hagedorn F (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO_2 enrichment and soil warming. Oikos 121: 1532–1543.
38. Hättenschwiler S, Handa IT, Egli L, Asshoff R, Ammann W, et al. (2002) Atmospheric CO_2 enrichment of alpine treeline conifers. New Phytol 156: 363–375.
39. Barbeito I, Dawes MA, Rixen C, Senn J, Bebi P (2012) Factors driving mortality and growth at treeline: a 30-year experiment of 92 000 conifers. Ecology 93: 389–401.
40. Dawes MA, Hättenschwiler S, Bebi P, Hagedorn F, Handa IT, et al. (2011b) Species-specific tree growth responses to 9 years of CO_2 enrichment at the alpine treeline. J Ecol 99: 383–394.
41. Dawes MA, Hagedorn F, Handa IT, Streit K, Ekblad A, et al. (2013) An alpine treeline in a carbon dioxide-rich world: Synthesis of a nine-year free-air carbon dioxide enrichment study. Oecologia 171: 623–637.
42. Hagedorn F, Martin MA, Rixen C, Rusch S, Bebi P, et al. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. Biogeochemistry 97: 7–19.
43. Dawes MA, Zweifel R, Dawes N, Rixen C, Hagedorn F (2014) CO_2 enrichment alters diurnal stem radius fluctuations of 36-yr-old *Larix decidua* growing at the alpine tree line. New Phytol: 10.1111/nph.12742.
44. Zumbrunn T (2004) Alpine dwarf shrubs in a CO_2 enriched world. Unpublished Diploma thesis, University of Basel, Basel, Switzerland.
45. Schweingruber FH (1990) Microscopic Wood Anatomy. 3rd ed. Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, 226 p.
46. Régent Instruments, Inc. (2001) WinCELL pro 2001: image analysis software [computer program]. Québec.
47. Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2008) NLME: linear and nonlinear mixed effects models. R package version 3.1–89.
48. R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/
49. Hartley AE, Neill C, Melillo JM, Crabtree R, Bowles FP (1999) Plant performance and soil nitrogen mineralization in response to simulated climate change in subarctic dwarf shrub heath. Oikos 86: 331–343.
50. Parsons AN, Welker JM, Wookey PA, Press MC, Callaghan TV, et al. (1994) Growth responses of four sub-Arctic dwarf shrubs to simulated environmental change. J Ecol 82: 307–318.
51. Press MC, Potter JA, Burke MJW, Callaghan TV, Lee JA (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. J Ecol 86: 315–327.
52. Lauber K, Wagner G, Gygax A (2012) Flora Helvetica. Bern: Haupt. 1656 pp.
53. Graglia E, Jonasson S, Michelsen A, Schmidt IK (1997) Effects of shading, nutrient application and warming on leaf growth and shoot densities of dwarf shrubs in two arctic-alpine plant communities. Ecoscience 4: 191–198.
54. Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, et al. (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biol 3: 20–32.
55. Cook BI, Wolkovich EM, Parmesan C (2012) Divergent responses to spring and winter warming drive community level flowering trends. P Natl Acad Sci USA 109: 9000–9005.
56. McEwan RW, Brecha RJ, Geiger DR, John GP (2011) Flowering phenology change and climate warming in southwestern Ohio. Plant Ecol 212: 55–61.
57. Körner C, Basler D (2010) Phenology under global warming. Science, 327: 1461–1462.
58. Wipf S (2010) Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. Plant Ecol 207: 53–66.
59. Wolkovich EM, Cook BI, Allen JM, Crimmins TM, Betancourt JL, et al. (2012) Warming experiments underpredict plant phenological responses to climate change. Nature 485: 494–497.
60. Oberbauer SF, Elmendorf SC, Troxler TG, Hollister RD, Rocha AV, et al. (2013) Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. Philos T Roy Soc B 368: 20120481.
61. Schwartz MD, Hanes JM, Liang L (2013) Separating temperature from other factors in phenological measurements. Int J Biometeorol, 10.1007/s00484-013-0723-2.
62. Pau S, Wolkovich EM, Cook BI, Davies TJ, Kraft NJB, et al. (2011) Predicting phenology by integrating ecology, evolution and climate science. Global Change Biol 17: 3633–3643.
63. Suzuki S, Kudo G (1997) Short-term effects of simulated environmental change on phenology, leaf traits, and shoot growth of alpine plants on a temperate mountain, northern Japan. Global Change Biol 3: 108–115.
64. Natali SM, Schuur EAG, Rubin RL (2012) Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. J Ecol 100: 488–498.
65. Körner C (2003) Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. 2nd ed. Springer, Berlin, 344 p.
66. Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, et al. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126: 543–562.
67. Aerts R, Cornelissen JHC, Dorrepaal E (2006) Plant performance in a warmer world: General responses of plants from cold, northern biomes and the importance of winter and spring events. Plant Ecol 182: 65–77.
68. Streit K, Hagedorn F, Hiltbrunner D, Portmann M, Saurer M, et al. (2014) Soil warming alters microbial substrate use in alpine soils. Global Change Biol 20: 1327–1338.
69. Esau K (1977) Anatomy of seed plants. 2nd ed. John Wiley & Sons, New York, 550 p.
70. Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. J Exp Bot 57: 267–281.
71. Gómez-Aparicio L, Zamora R, Castro J, Hódar JA (2008) Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores? J Veg Sci 19: 161–172.
72. Grau O, Ninot JM, Blanco-Moreno JM, van Logtestijn RSP, Cornelissen JHC, et al. (2012) Shrub-tree interactions and environmental changes drive treeline dynamics in the Subarctic. Oikos 121: 1680–1690.