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

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

Abstract

Climate warming can lead to changes in alpine plant species' interactions through the amelioration of environmental conditions. Consistent with the stress-gradient hypothesis, many studies have shown that release from environmental stress can lead to increased competition. However, most of these studies were based on neighbour removal experiments, whereas the response of natural communities has received less attention. We explored the effects of four years of experimental warming with open-top chambers on *Vaccinium myrtillus* stands with different neighbouring shrub species at the Pyrenean treeline. Our aim was to find possible shifts in the interaction between *V. myrtillus* and its neighbours following warming that were demonstrated through changes in *V. myrtillus* performance. We examined the effects of warming on above-ground growth parameters, below-ground biomass and the C and N content and isotope composition of *V. myrtillus* growing in pure stands, in stands mixed with *Vaccinium uliginosum*, and in stands mixed with *Rhododendron ferrugineum*. We also analysed variations in soil N pools and rhizospheric soil C/N ratios, and evaluated the effects of warming on the neighbouring *V. uliginosum* in mixed plots. Our results showed that warming induced positive changes in the above-ground growth of *V. myrtillus*, but not below-ground, while *V. uliginosum* did not respond to warming. *Vaccinium myrtillus* performance did not differ between stand types under increased temperatures, which indicates that warming did not induce any shifts in the interaction between *V. myrtillus* and its neighbours. These findings contrast with many studies in which species interactions changed when environmental conditions were modified, and shows that the interaction between our study species may not be altered with warmer temperatures at the Pyrenean treeline.

Introduction

In the recent decades, climate warming and land-use change (i.e. abandonment of extensive grazing) have led to shrub encroachment processes in the treeline ecotone (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 (Collins et al. 2013) could accelerate these processes. This could have a dramatic impact on Arctic and alpine tundra ecosystems due to shifts in community composition and potential feedbacks to warming, such as decreasing albedo or reducing radiative cooling at night 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 carry out species-specific studies to understand vegetation changes with warming, since co-occurring 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, when studying vegetation responses to temperature increase, it is also important to consider plant–plant interactions, since they are one of the main drivers of community dynamics (Callaway and Walker 1997). The stress-gradient hypothesis (SGH; 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. In fact, 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, amongst others). 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 tundra ecosystems. Amongst them, clonal dwarf shrub species are of great importance in terms of Arctic and alpine vegetation cover, structure and functionality. They present a complex network of subterranean rhizomes bearing fine roots, and producing individual above-ground ramets. Thus, the below-ground system of clonal shrubs is essential for their persistence and vegetative expansion, as well as an important source of soil carbon (Cornelissen et al. 2014). Changes in the below-ground structure of dominant clonal shrubs could translate into major changes in the community and ecosystem structure and composition. Consequently, the study of below-ground responses to warming is an essential part of the complex responses to temperature increase in Arctic and alpine areas. However, the impacts of below-ground sampling and the difficulty encountered when attempting 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 above- and below-ground plant measurements are less common (but see Hollister and Flaherty 2010 and Yang et al. 2015, amongst others).

Global warming can also have strong impacts on N mineralization, with effects on nitrogen availability and, ultimately, plant growth (Bardgett and Wardle 2010). Several studies in cold ecosystems have found an increase in the 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 an increase in the mineralization and decomposition processes at higher temperatures. Since co-occurring species show different N preferences and N-acquisition strategies (Körner et al. 2003, Pornon et al. 2007), shifts in the N pools can lead to changes in their niches that alter their interactions.

Vaccinium myrtillus is a key species forming 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), experiencing low temperatures and short growing seasons. Warmer temperatures could favour the growth and expansion of this species in the treeline ecotone, as has already been reported in a soil warming experiment in the Alps (Dawes et al.

2011, Anadon-Rosell et al. 2014) and in other warming experiments in the Arctic tundra (Rinnan et al. 2009, Taulavuori et al. 2013). On the other hand, in line with the SGH, an amelioration of the environment 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 effects that warming may have on the interaction of this species with its neighbours have not been reported. Moreover, the previously mentioned experiments on *V. myrtillus* have mainly focused on its above-ground responses to warming, whereas below-ground responses have mostly been ignored.

At the treeline in the Central Pyrenees, *V. myrtillus* grows in pure patches (stands hereafter) or in mixed stands together with *Vaccinium uliginosum* ssp. *microphyllum,* or *Rhododendron ferrugineum*. The objective of this study was to investigate the above- (AG) and below-ground (BG) responses of *V. myrtillus* to four years of passive warming and whether warming induced changes in the interaction between *V. myrtillus* and its neighbouring shrub species. For this purpose we assessed *V. myrtillus* phenology, AG and BG biomass, carbon and nitrogen contents and isotopic signatures ($\delta^{13}C$ and $\delta\ ^{15}N$), soil inorganic N pools (ammonium and nitrate) and nitrogen and carbon availability in the rhizosphere in different types of *V. myrtillus* stands subjected to warming treatments. We hypothesized that warming would (i) benefit *V. myrtillus* AG and BG growth, but that it would also (ii) increase the competition with its neighbouring species, which would be manifested as a positive AG and BG growth response of *V. myrtillus* to warming in pure stands but not in mixed stands, and also by a greater increase in the soil inorganic N pool under warming in pure stands than in mixed stands.

Materials and methods

Study area

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), on a Nfacing 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 *Vaccinium myrtillus* L., *Vaccinium uliginosum* subsp. *microphyllum* (Lange) Tolm. and *Rhododendron ferrugineum* L. The mean annual precipitation and mean annual temperature for the study period (2010–2013) were 1223.1 mm and 2.7 °C, respectively. The mean monthly precipitation and the mean temperature for the main months of the growing season (June–August) were 99.1 mm and 10.2 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).

Experimental design

In July 2010 we established 30 plots of 1.1 m^2 combining a temperature and a coexistence treatment. In 15 plots we placed an open-top chamber (OTC) similar to the model used in the International Tundra Experiment (ITEX; Marion et al. 1997), which increased summer air temperature by 1.1 ºC (measured by *ibuttons* placed at ground level); the other 15 plots served as controls for temperature. Within each temperature treatment, five plots were assigned to pure *Vaccinium myrtillus* stands (M stands), five to mixed stands of *V. myrtillus* and *V. uligiosum* subsp. *microphyllum* (hereafter *V. uliginosum*; U stands) and five to mixed stands of *V. myrtillus* and *Rhododendron ferrugineum* (R stands). The distance between two plots ranged from one to a few metres (< 20 m), always ensuring that the studied patches were independent.

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 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 (Supplementary material Appendix 1 Table A1).

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. 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. Soil samples were kept in sealed plastic bags in a cool box until freezing in the lab. Two of these cores were used for BG biomass measurements at the plot scale; two were used for measurements of soil nitrate and ammonia content; and the other two were used for rhizosphere carbon and nitrogen content and isotope composition analyses. The two 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* spp. 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, and dried them at 60 ºC for 48 hours. Leaves and new shoots were weighed for AG biomass measurements and subsequently used for nitrogen and carbon content and isotope composition analyses. Rhizomes and roots were only used for nitrogen and carbon content and isotope composition analyses, since BG biomass was measured at the plot scale on material obtained from the soil cores. Since *Vaccinium myrtillus* is a clonal plant with a long and complex rhizome network, we carried out 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 (≥ 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 content and isotope composition

For the analyses of C and N content 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 *Vaccinium* spp. Then we ground the material and weighed *ca.* 1 mg subsamples in small tin capsules. The nitrogen and carbon contents of samples were determined using an elemental analyzer (EA1108, Series 1; Carbo Erba Instrumentazione, Milan, Italy). The carbon and nitrogen isotope composition of samples were determined 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). The results of carbon 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.

Carbon isotope discrimination ($\Delta^{13}C$) 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.

Nitrogen results were 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:

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

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

All EA-IRMS analyses were performed at the CCiT of the University of Barcelona. The δ^{13}C of CO_2 of the air and the δ^{15}N of the bulk soil were analysed in 2013 and were ca. -10.91‰ and ca. 7.33 ‰, respectively.

Soil inorganic nitrogen pool

Nitrate 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 ammonia 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

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 carbon and nitrogen content and isotope composition following the same procedure as for plant tissues.

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. 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 carbon and nitrogen content and isotope composition of the different AG and BG tissues, BG biomass, soil nitrate and ammonia contents and rhizosphere carbon and nitrogen content and isotope composition we used simple linear model functions. We included ramet age as a covariate in plant analyses when it was significant to account for possible age effects on our growth-related response variables. This included the models for *V. myrtillus* height and AG biomass (except for dry weight per shoot unit), plus the models for number of shoots and dry weight per shoot unit for *V. uliginosum*. We tested for significance with analysis of variance tests and we 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) 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 marginally significant at $0.05 > P < 0.10$ to account for the relatively low replication. When we found significant differences between stand types, we carried out 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. 2008); 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).

Results

Phenology

Warming advanced early-season vegetative phenology through an earlier onset of bud burst and leaf expansion (see Supplementary material Appendix 1 Fig. A1). On the 21st May 2011 (day of year, DOY, 141), *V. myrtillus* ramets in unwarmed plots were at the bud swelling phase, whereas buds in ramets of warmed plots had already started bursting ($F_{1,24}$ = 3.92, P = 0.059). In 2012, *V. myrtillus* ramets in warmed plots were already 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 Supplementary material Appendix 1 Fig. A1 for visit dates). We only found significant differences between stand types (regardless of the warming treatment) in September 2011, when ramets in pure plots were already shedding the 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 pure plots with respect to the other stand types was especially obvious in warmed plots (marginally significant interaction for coexistence x warming, $F_{2,24}$ = 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 ($F_{1,6}$ = 0.07, P = 0.802).

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, Supplementary material Appendix 1 Fig. A2), although 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 ($F_{1,6} = 14.81$, $P = 0.009$).

Vaccinium myrtillus *BG biomass*

There were no effects of warming on *V. myrtillus* BG biomass (Table 2, Fig. 2). We only found differences in rhizome and coarse root biomass between stand types. Plots with *R. ferrugineum* showed lower rhizome biomass per soil volume than in the other two stand types (Table 2, Fig. 2a). Plots with *V. uliginosum* showed marginally significant greater coarse root biomass than pure populations (Table 2, Fig. 2b). Fine root biomass did not differ between stand types (Table 2, Fig. 2c). We did not find any warming x stand type interaction for any of the BG compartments analysed.

Carbon and nitrogen content and isotope composition of AG and BG plant fractions

Carbon concentration in *V. myrtillus* tissues was similar across warming treatments and stand types for leaves, shoots and roots. Rhizomes, however, had greater carbon content under warming than in control plots (Table 3, Fig. 3), which was not related to any rhizome biomass increase under warming (see above). Carbon concentration values of *V. uliginosum* new shoots, rhizomes and roots did not show any response to warming, but there was a marginally significant positive effect of warming on leaf C concentration (Table 4, Fig. 5).

The δ^{13}C of *V. myrtillus* and *V. uliginosum* tissues did not differ between warming treatments (Fig. 3, 5) 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 plots with *R. ferrugineum* than in the other two situations of coexistence for leaves (only marginally significant), shoots and rhizomes. There were no significant differences between stand types for the δ^{13}C composition of roots (Table 3, Fig. 3), or any warming x stand type interaction.

There was no warming effect on the nitrogen content and $\delta^{15}N$ of any of the *V. myrtillus* tissues, and only a very marginally significant interaction between warming and stand type in the N content of *V. myrtillus* rhizomes, which was higher in control plots than in warmed plots in U stands (Table 3, Fig. 4). However, we found significant differences between stand types. Leaf N content was higher in R stands than in U stands, but this was not the case for any of the other plant fractions. Leaf and shoot $\delta^{15}N$ values were higher in M stands than in the other two stand types, which is consistent with a previous study carried out in the area (Anadon-Rosell et al. in prep.). Finally, rhizome $\delta^{15}N$ values were also higher in M stands than in the other two stand types, but only significantly higher than in R stands (Table 3, Fig. 4).

Vaccinium uliginosum shoots showed a significantly lower N content under warming than in control plots, but this was associated with an increase in leaf N content under warming (although the latter was not significant). $\delta^{15}N$ values did not differ significantly between warming treatments (Table 4, Fig. 5).

Soil inorganic N pools and rhizosphere C and N

Soil nitrate content decreased by 36% in warmed plots compared with unwarmed plots ($F_{1,24}$ = 5.87, P = 0.023, Fig. 6a), but the ammonia content remained similar between warming treatments (F = 0.45, P = 0.508, Fig. 6b). As a consequence, the nitrate/ammonia 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, Supplementary material Appendix 1 Fig. A3). Both rhizosphere soil C and N content 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 content 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 δ^{13}C and δ^{15}N values ($P >$ 0.28).

Discussion

This study provides evidence that four years of passive warming did not lead to changes in the interaction between *V. myrtillus* and its neighbours at our treeline study site. *Vaccinium myrtillus* slightly benefitted from increased temperatures regardless of whether or not it was growing with a neighbouring species, or the identity of this neighbour.

According to the stress-gradient hypothesis (Bertness and Callaway 1994), we expected *V. myrtillus* to perform worse in warmed plots with neighbours than in pure stands due to increased competition caused by the amelioration of the environmental conditions with warming. However, our results indicate that, although the applied experimental warming of 1.1 ºC was sufficient to improve *V. myrtillus* growth conditions, it was not sufficient to change the outcome of the interactions between *V. myrtillus* and its neighbouring shrubs. This 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*, but not when the latter was inside an OTC, indicating that warming could affect 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 elevation sites when removing neighbours of target individuals at 11 different mountain sites across the world. In addition, a study in seminatural grasslands across precipitation and temperature gradients in southern Norway found increased competitive interactions with increasing temperature (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 (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 the desired species. In contrast, our approach was based on naturally established populations and species, and the avoidance of any disturbance caused by removing part of this natural assemblage allowed us to assess the natural response of our study species to warming. According to our results, species interactions can be less responsive to warming when studying them in their natural conditions and distribution.

Vaccinium myrtillus, a species that is responsive to temperature change (Rinnan et al. 2009, Taulavuori et al. 2013, Anadon-Rosell et al. 2014, Dawes et al. 2015), showed positive growth responses to warming regardless of the neighbouring species. Its AG biomass was increased under warming, which could be the result of a longer growing period caused by the advancement of its early-vegetative phenology. A previous study on this same species in the Swiss Alps showed that its increase in 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 in OTCs than in plots with warmed soil. However, it could also be that other factors related to warming but not directly linked to a longer growing season influenced *V. myrtillus* growth in our study, such as direct warming effects on photosynthetic rates (Heskel et al. 2013, Fu et al. 2015) or higher N uptake rates with increased temperatures, which would be supported by the lower soil nitrate values found at our study plots under warming. Although there was no increase in the nitrogen content of our study ramets with warming that would support the notion of increased N uptake rates, the N increase could be diluted by the increased growth under warming. In fact, a study in the Swedish Lapland by Hartley et al. (1999) found no response of *V. myrtillus* and *V. uliginosum* leaf N concentrations to warming despite increased mineralization rates, which the authors attributed to an increase in N in their study plants through increased biomass.

In our study, the BG biomass of *V. myrtillus* did not change with warming in any of the stands analysed. Thus, the increase in *V. myrtillus* AG growth did not result in increased BG growth. Moreover, BG interactions between our study species did not change with warming either. Although OTCs mainly increase ground-level and air temperature, 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 a tundra site in Alaska 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, indicating contrasting BG responses to warming depending on the study site and community composition. *Vaccinium myrtillus* can expand its rhizomes several metres below-ground (Flower-Ellis, 1971); therefore our warming treatment might not have reached a large enough area to capture 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.

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 warmer temperatures, as shown by its lower altitudinal range (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 carbon content of *V. uliginosum* increased with warming, but the statistical significance was marginal, and was not accompanied by any other changes in the performance of this shrub. Our study provides evidence that although *V. myrtillus* is more responsive to warming than *V. uliginosum*, when they coexist *V. myrtillus* does not benefit more from warming than when it grows in pure stands.

The slightly lower N content in *V. myrtillus* rhizomes in warmed plots than in unwarmed plots when coexisting with *V. uliginosum* indicates an increase in competition for N with warming. In fact, competition for N in mixed stands of these two species under natural conditions was found in a previous study at the same site (Anadon-Rosell et al. in prep.). The higher rhizosphere C/N ratio in these mixed stands than in the other stand types further supports the idea of the occurrence of natural competition for N without warming, which would be increased with the higher temperatures inside the OTC. A study in the Swiss Alps found a positive response of warming in *V. gaultherioides* (synonym of *V. uliginosum* subsp. *microphyllum*) leaf N content but only a short-term positive response in *V. myrtillus* (Dawes et al. 2011). On the other hand, contrasting effects were found in an experiment in the Swedish Lapland, where *V. myrtillus* showed a positive response in terms of leaf N content to warming whereas *V. uliginosum* responded negatively (Richardson et al. 2002). However, none of these studies tested the effects of warming on these species' interactions and the ultimate effects they would have on their performance. Our study demonstrates that although warming increased the competition for N between *V. myrtillus* and *V. uliginosum*, this ultimately did not outbalance the positive growth response of *V. myrtillus* to warming.

A meta-analysis of experimental warming effects on N pools in terrestrial ecosystems based on 51 studies showed that warming increased N mineralization rates and N pools across different ecosystem types (Bai et al. 2013). However, in our experiment soil nitrate decreased with warming (regardless of the stand type). This could be explained by greater nitrate uptake rates promoted by increased temperatures, since temperature has been proven to be a modulator of plant N assimilation in previous studies (Laine et al. 1994, Volder et al. 2000). The lack of an increase in the N concentration of plant tissues could be due to a dilution effect caused by the greater biomass or to increased nitrate assimilation by other species (especially grasses, due to their abundance), which were not assessed in this study. Another explanation for the reduced nitrate concentrations in the OTCs could be earlier consumption of nitrate through an advanced root phenology promoted by warming (Sullivan and Welker 2005, Nord and Lynch 2009). A study by Rinnan et al. (2009) in a tundra heath dominated by *V. myrtillus*, *V. vitis-idaea* and *Empetrum nigrum* in southwestern Finland found no increase in soil N content with warming either, but there was a decrease in the 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 preferential use of a specific N form at different sites with different community composition, or a greater availability of nitrate than ammonia 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 growth 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.

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Tables

Table 1. Results of ANOVA for effects of warming and stand type on *Vaccinium myrtillus* above-ground (AG) growth and biomass parameters at the ramet scale. Significant ($P > 0.05$) and marginally significant ($0.01 > P > 0.05$) effects 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	-	-	-
	Warming	1, 23	3.85	**0.062**
	Stand type	2, 23	2.59	0.100
	Stand type x warming	2, 23	0.55	0.584

Table 2. Results of ANOVA for effects of warming and stand type on stand below-ground (BG) biomass. Significant (*P* > 0.05) and marginally significant (0.01 > *P* > 0.05) effects 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

Table 3 . Results of ANOVA for effects of warming (W) and stand type (ST) on the C and N concentration and isotope composition (δ^{13}C, δ^{15}N) of *Vaccinium myrtillus* leaves, new shoots, rhizomes and roots. *F*-values and *P*-values (in parentheses) are given. Significant ($P > 0.05$) and marginally significant ($0.01 > P > 0.05$) effects 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 rhizome C and N concentration (22) and root δ^{15}N (23).

Fraction	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)
	δ^{13}C	0.90 (0.352)	2.72 (**0.086**)	0.85 (0.441)
	δ^{15}N	0.08 (0.780)	10.28 (**0.001**)	0.04 (0.960)
New shoots	C concentration	1.68 (0.207)	0.94 (0.404)	0.94 (0.404)
	N concentration	0.07 (0.793)	0.63 (0.540)	0.77 (0.472)
	δ^{13}C	0.07 (0.794)	8.16 (**0.002**)	1.85 (0.179)
	δ^{15}N	0.33 (0.571)	9.39 (**0.001**)	0.00 (1.000)
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**)
	δ^{13}C	0.42 (0.522)	8.78 (**0.001**)	0.03 (0.972)
	δ^{15}N	0.02 (0.884)	6.53 (**0.005**)	0.08 (0.921)
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)
	δ^{13}C	1.59 (0.218)	0.15 (0.860)	2.53 (0.101)
	δ^{15}N	0.21 (0.650)	2.04 (0.153)	0.19 (0.826)

Table 4. 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 ($P > 0.05$) and marginally significant ($0.01 > P > 0.05$) effects are in bold.

Fraction	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 legends

Figure 1. *Vaccinium myrtillus* AG biomass in different stand types (ST) and warming treatments in September 2013 (W; $n = 5$, means + 1 SE are shown). Asterisks (**) show 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 2. BG biomass per soil volume at the plot scale in different stand types (ST) and warming treatments in September 2013 (W; $n = 5$, means + 1 SE are shown). Asterisks show significant differences (* marginally significant differences 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.

Figure 3. Carbon concentration and δ^{13}C of *V. myrtillus* tissues 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). Asterisks show significant differences (* marginally significant differences 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.

Figure 4. Nitrogen concentration and δ^{15}N of *V. myrtillus* tissues 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). For N concentration of rhizomes and roots see the righthand Y-axis scale. Asterisks show significant differences (* marginally significant differences $0.1 > P > 0.05$; *** 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 5. Carbon and nitrogen concentrations and $\delta^{13}C$ and $\delta^{15}N$ of *V. uliginosum* tissues under different warming treatments in September 2013 (W; $n = 4$, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Asterisks (***) show significant differences between warming treatments at $P < 0.01$.

Figure 6. Soil nitrate and ammonia content 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.

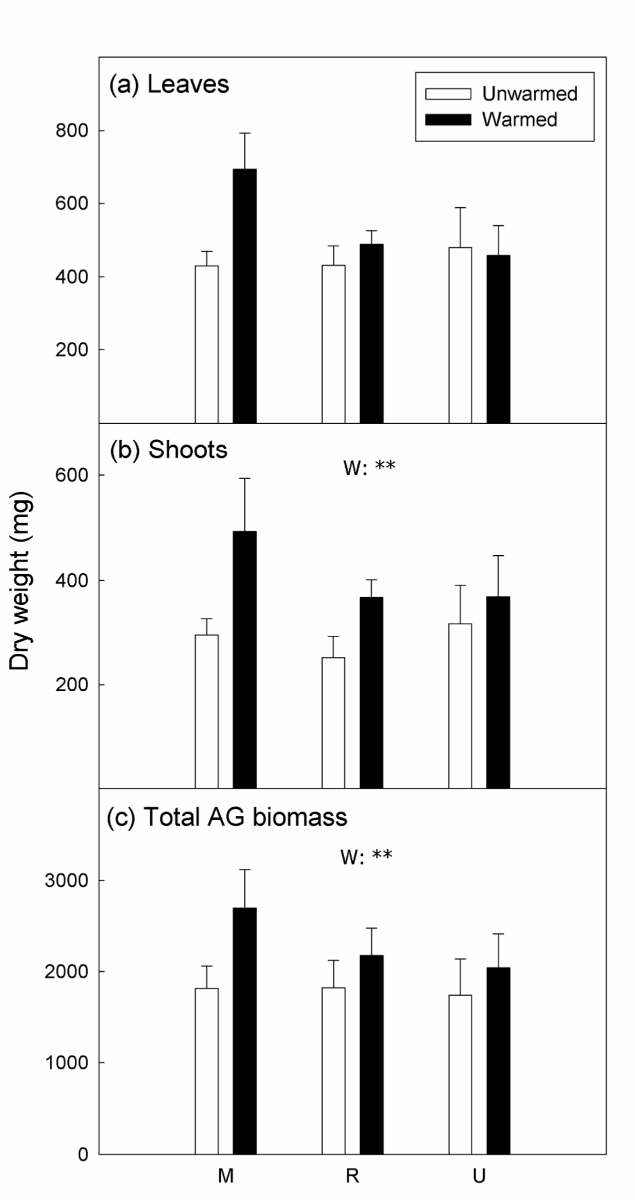

Figure 1. *Vaccinium myrtillus* AG biomass in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means + 1 SE are shown). Asterisks (**) show significant differences at 0.05 > P > 0.01. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
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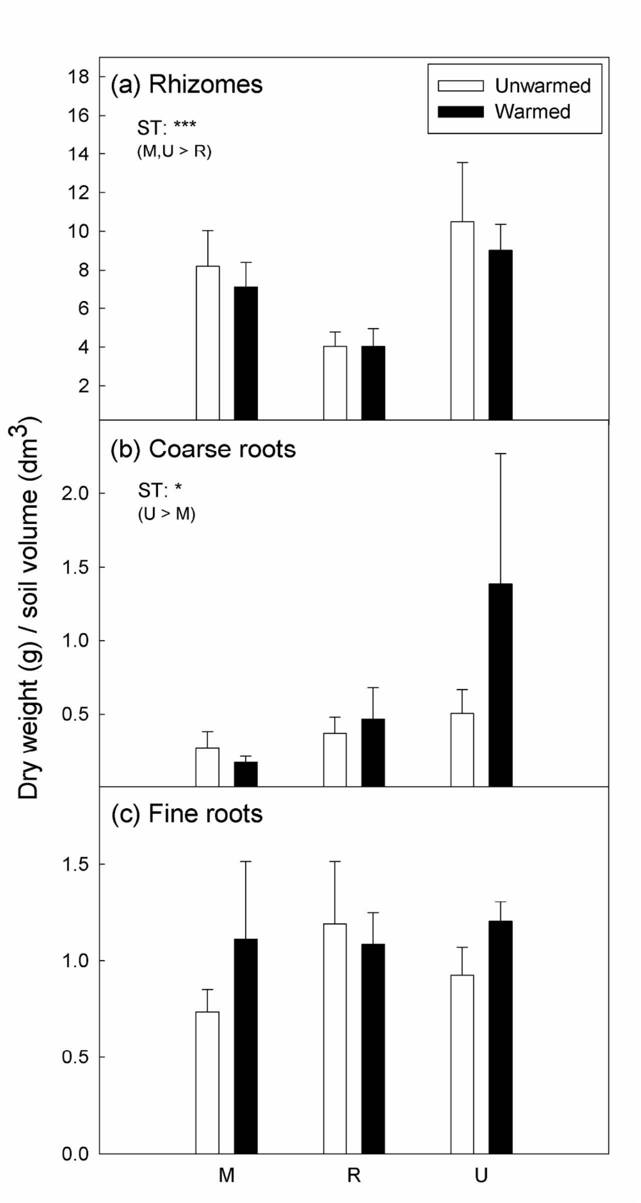

Figure 2. BG biomass per soil volume at the plot scale in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means + 1 SE are shown). Asterisks show significant differences (* marginally significant differences at 0.1 > P > 0.05; ** significant differences at 0.05 > P > 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.
150x281mm (300 x 300 DPI)

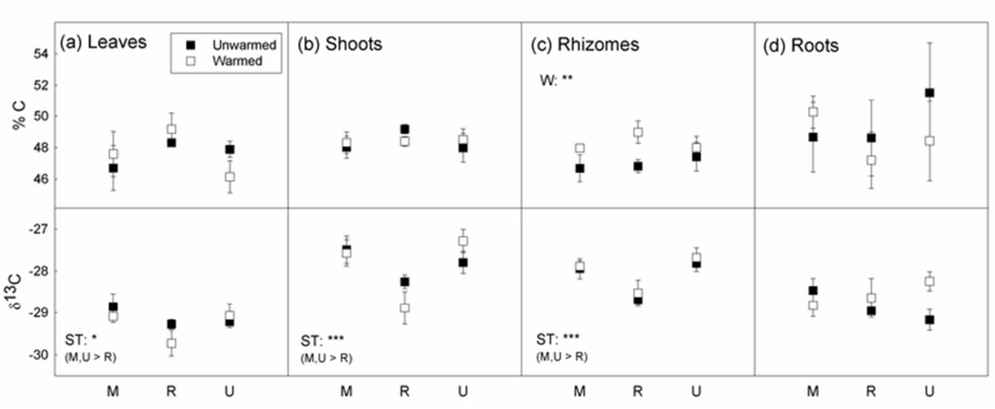

Figure 3. Carbon concentration and δ^{13}C of *V. myrtillus* tissues in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Asterisks show significant differences (* marginally significant differences 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.
67x27mm (300 x 300 DPI)

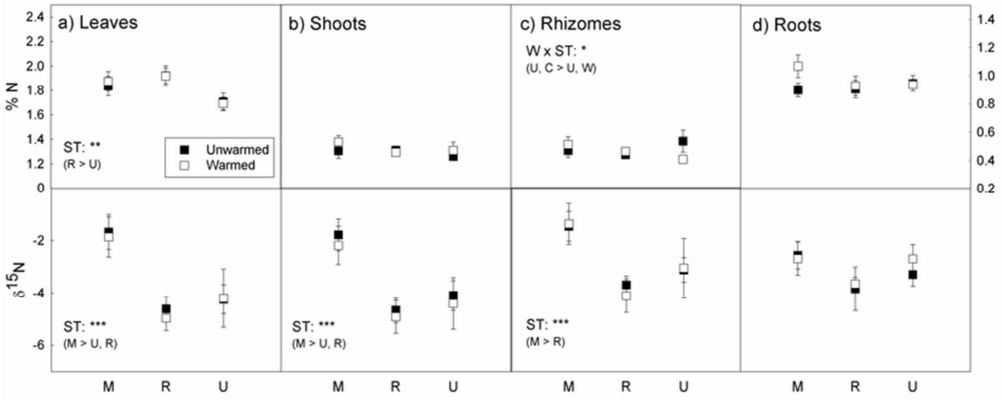

Figure 4. Nitrogen concentration and δ^{15}N of V. myrtillus tissues in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). For N concentration of rhizomes and roots see the righthand Y-axis scale. Asterisks show significant differences (* marginally significant differences 0.1 > P > 0.05; *** 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.
69x29mm (300 x 300 DPI)

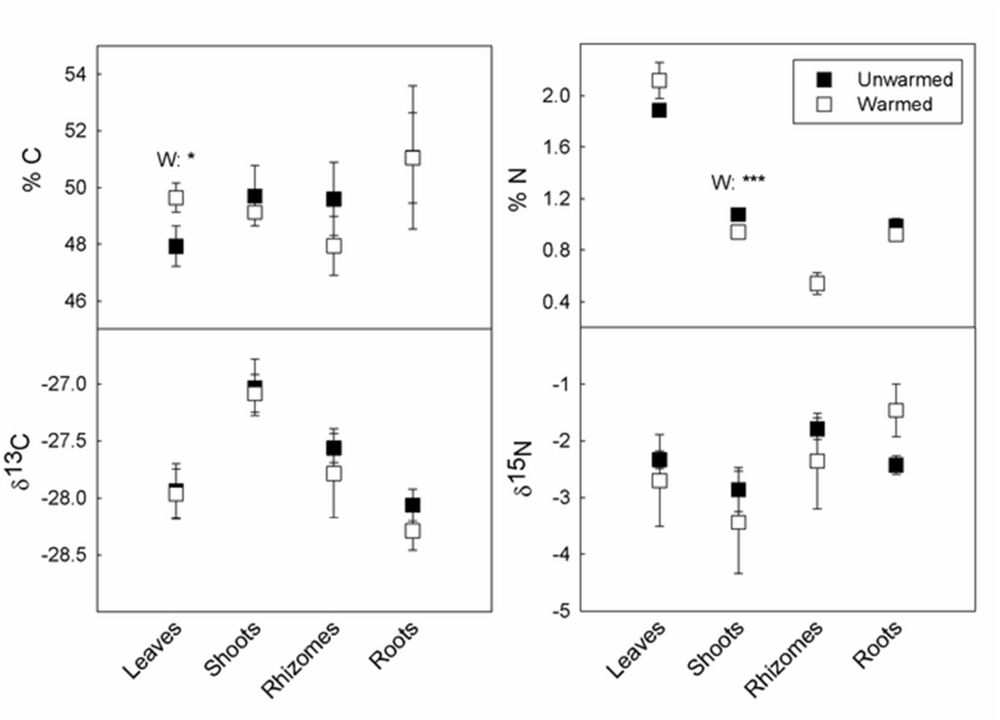

Figure 5. Carbon and nitrogen concentrations and δ^{13}C and δ^{15}N of *V. uliginosum* tissues under different warming treatments in September 2013 (W; *n* = 4, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Asterisks (***) show significant differences between warming treatments at P < 0.01.
57x41mm (300 x 300 DPI)

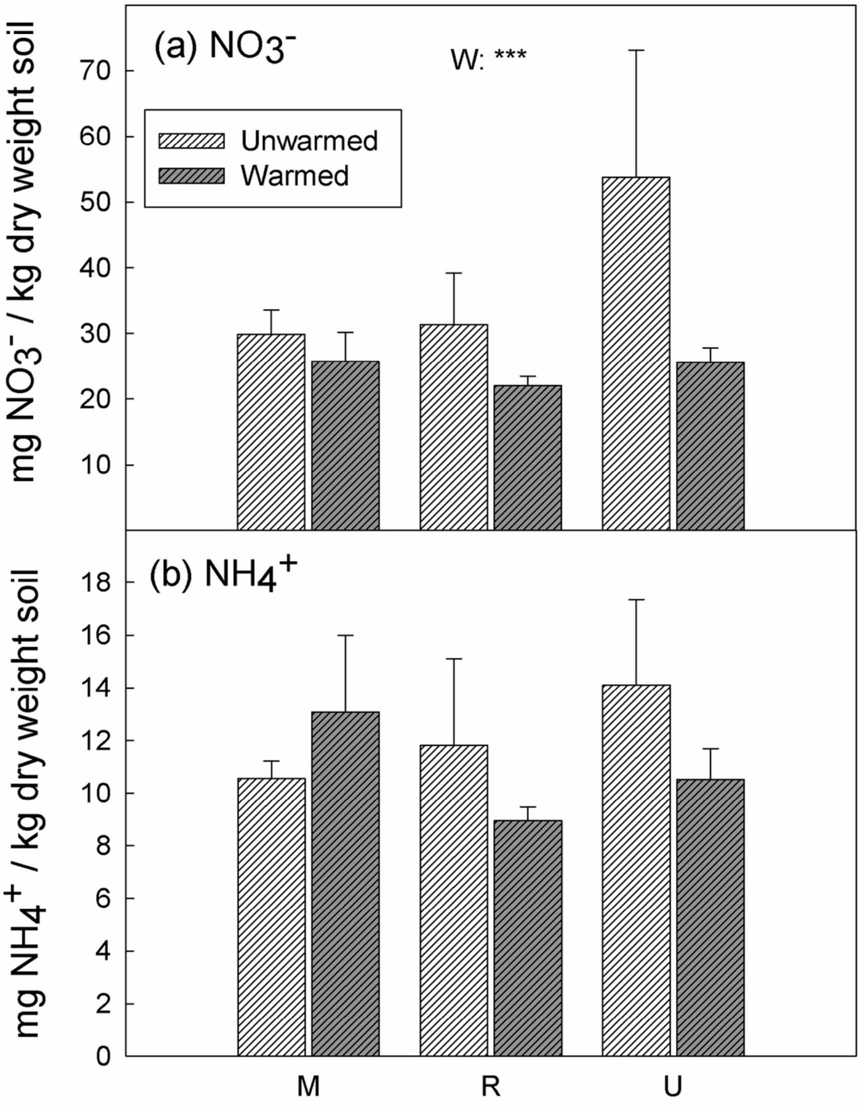

Figure 6. Soil nitrate and ammonia content in our study plots in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means + 1 SE are shown). Asterisks (***) show significant differences at P < 0.01. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
104x136mm (300 x 300 DPI)

Supplementary Material Appendix 1

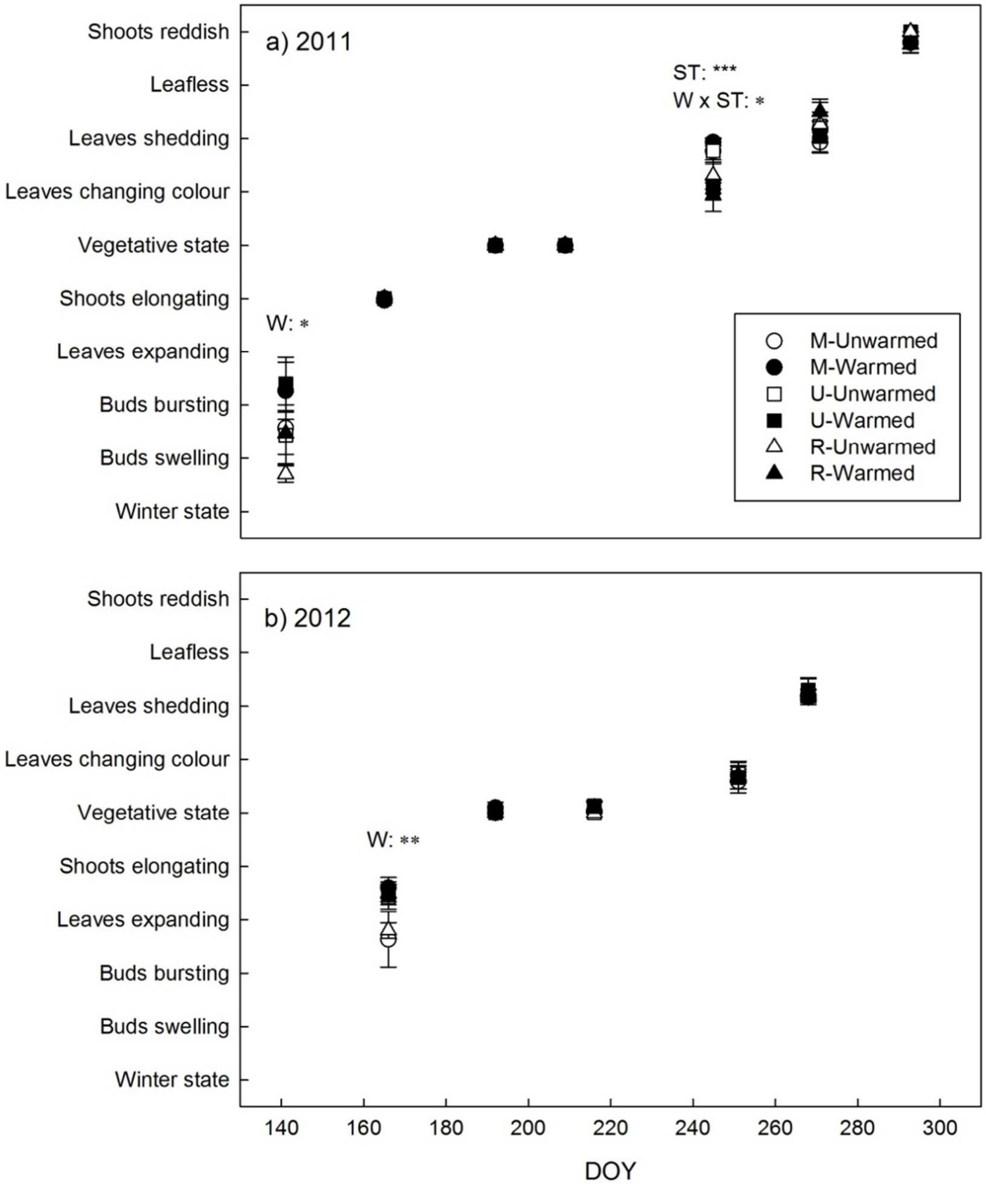

Figure A1. Vegetative phenology (mean phenophase calculated as the average numeric phenophase of six ramets per plot in each visit, day of year: DOY) of *Vaccinium myrtillus* at the study plots during the growing seasons of 2011 and 2012 under different stand types (ST) and warming treatments (W; *n* = 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.

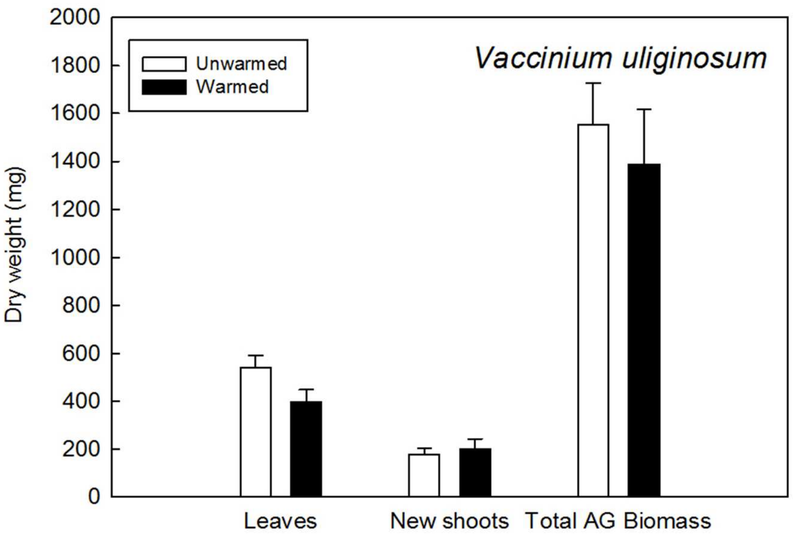

Figure A2. *Vaccinium uliginosum* above-ground (AG) biomass under different warming treatments in September 2013 (*n* = 4, means + 1 SE are shown). There were no significant differences between warming treatments.

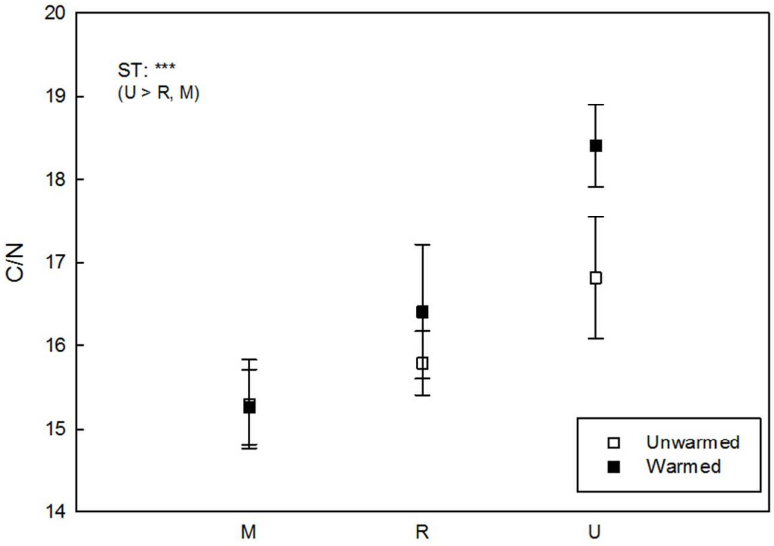

Figure A3. Rhizospheric soil C/N ratio for the different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means ± 1 SE are shown). Asterisks (***) show significant differences at $P < 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

Table A1. 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.

Stand type	Warming treatment	*V. myrtillus*	*V. uliginosum*	*R. ferrugineum*	Grasses	Forbs
M *(V. myrtillus)*	Control	70 69	0 0	0 0	28 20	- 8
	Warmed	68 83	0 0	0 0	25 17	- 8
U *(V. myrtillus + V. uliginosum)*	Control	52 43	48 44	0 2	14 23	- 7
	Warmed	41 34	66 68	0 0	15 13	- 9
R *(V. myrtillus + R. ferrugineum)*	Control	43 44	0 1	73 82	20 17	- 6
	Warmed	40 49	0 0	77 75	12 11	- 6