Procesos subyacentes a la mortalidad arbórea tras incendio

Carles Arteaga López

http://hdl.handle.net/10803/673858

Universitat de Lleida

TESI DOCTORAL

Procesos subyacentes a la mortalidad arbórea tras incendio

Carles Arteaga López

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida

Programa de Doctorat en Gestió Forestal i Medi Natural

Director/a

Víctor Resco de Dios

Tutor/a

Víctor Resco de Dios

2021

Índice

Resumen

Perturbaciones como el fuego y la sequía son naturales en la cuenca mediterránea, pero el calentamiento global y la ausencia de gestión forestal favorecen que los episodios de sequía sean cada vez más frecuentes y severos. Estos episodios de sequía, acompañados del abandono de las zonas rurales durante el último siglo, han favorecido el desarrollo de una gran cantidad y continuidad de vegetación (combustible desde el punto de vista de los incendios) que está cada vez más y, por tanto, más disponible para las llamas. Esta mayor cantidad de combustible seco aumenta la probabilidad de que sufrir grandes incendios.

Aparte de estos aspectos relacionados con el cambio global, la propia fisiología de las especies forestales puede favorecer las pérdidas de agua a través de la transpiración nocturna o cuticular, por ejemplo, aunque su regulación resulta todavía una gran desconocida a día de hoy. Se ha indicado como estos dos procesos pueden aumentar todavía más la falta de agua, sobretodo en condiciones de estrés, lo que aumentaría el riesgo de incendios y podría dificultar la recuperación de la vegetación tras la perturbación. En este sentido, se ha apuntado que las adaptaciones fisiológicas para resistir a la sequía pueden llegar a ser antagónicas con las adaptaciones para resistir a los incendios

Esta tesis doctoral busca unir los procesos fisiológicos responsables por la resistencia a la sequía con la respuesta de las plantas a los incendios y, en particular, el rebrote. A tal fin se han combinado una serie de estudios bibliográficos y meta-analíticos con estudios de campo. Hemos constatado como adaptaciones a la sequía, tales como resistencia al embolismo pueden estar negativamente relacionadas con la resistencia a los incendios de baja intensidad. Sin embargo, la recuperación del rebrote tras incendios de alta intensidad que eliminan la biomasa arbórea depende del grado de embolismo antes del incendio. Hemos documentado también como la variación en la conductancia cuticular puede, efectivamente, aumentar de forma considerable el estrés hídrico sobretodo en especies perennes y, finalmente, hemos realizado una síntesis global sobre los factores que explican la apertura estomática nocturna.

Resum

Les pertorbacions com el foc i la sequera són naturals a la conca mediterrània, però per la situació actual d'escalfament global i absència de gestió forestal, els episodis de sequera cada cop són més freqüents i severs. Aquests episodis de sequera, acompanyats de l'abandonament de les zones rurals de l'últim mig segle, han afavorit el desenvolupament d'una gran quantitat i continuïtat de vegetació (combustible des del punt de vista dels incendis) més seca i morta (disponible). Aquesta major quantitat de combustible i més sec generen uns incendis de major intensitat.

A part d'aquests aspectes relacionats amb el canvi global, la mateixa fisiologia de les espècies forestals, pot afavorir les pèrdues d'aigua a través de la transpiració nocturna o cuticular, per exemple, malgrat que la seva regulació encara resulta ser una gran desconeguda avui en dia. S'ha indicat com aquests dos processos poden augmentar encara més la falta d'aigua, sobretot en condicions d'estrès, el que augmentaria el risc d'incendis i podria dificultar la recuperació de la vegetació després de la pertorbació. En aquest sentit, s'ha apuntat que les adaptacions fisiològiques per resistir la sequera poden arribar a ser antagòniques amb les adaptacions per resistir els incendis.

Aquesta tesi doctoral busca unir els processos fisiològics responsables per la resistència a la sequera amb la resposta de les plantes als incendis i, en particular, el rebrot. Amb aquesta finalitat s'han combinat una sèrie d'estudis bibliogràfics i meta-analítics amb estudis de camp. Hem constatat com adaptacions a la sequera, com ara la resistència a l'embolisme poden estar negativament relacionades amb la resistència als incendis de baixa intensitat. Però, la recuperació del rebrot després d'incendis d'alta intensitat que eliminen la biomassa arbòria depenen de què el grau d'embolisme hagi estat limitant. Hem documentat com la variació en la conductància cuticular pot, efectivament, augmentar de forma considerable l'estrès hídric sobretot en espècies perennes i, finalment, hem realitzat una síntesi global sobre els factors que expliquen les pèrdues d'aigua nocturnes.

Abstract

Disturbances such as fire and drought are natural in the Mediterranean basin, but global warming and lack of forest management interact to enhance drought episodes that are becoming more frequent and severe. These drought episodes, accompanied by the abandonment of rural areas over the last century, have favoured the development of a large vegetation (fuel) loads that are increasingly drier and, consequently, more available to the fire. This increasing amount of dry fuel is consequently enhancing the probability of suffering a large fire.

In addition to global change factors, some physiological traits may favour water losses through nocturnal or cuticular transpiration, for instance, although their regulation is still largely unknown at present. It has been pointed out how these two processes can further increase water scarcity, especially under stressful conditions, which would increase the risk of fire and could hinder the recovery of vegetation after disturbance. In this sense, it has been suggested that physiological adaptations to resist drought may become antagonistic to adaptations to resist fire.

This PhD thesis seeks to link the physiological processes responsible for drought resistance with the response of plants to fire and, in particular, resprouting. To this end, a series of bibliographical and meta-analytical studies have been combined with field studies. I found that adaptations to drought, such as resistance to embolism, can be negatively related to resistance to low intensity fire. However, resprouting recovery after high-intensity fires that remove aboveground biomass depends on the degree of hydrcaulic conductance before the perturbation. I have documented how variation in cuticular conductance can indeed considerably increase water stress, especially in perennial species and, finally, I have performed a global synthesis on the factors that explain nocturnal stomatal conductance.

Listado de Publicaciones

La presente Tesis doctoral se compone de cuatro capítulos que han dado lugar a cuatro publicaciones:

Capítulo IV: A trade-off betwen embolism resistance and bark thickness in conifers: are drought and fire adaptations antagonistic.

Víctor Resco de Dios, Carles Arteaga, Javier Hedo, Eustaquio Gil Pelegrín, Jordi Voltas.

Publicado en septiembre de 2018 en Plant Ecology & Diversity

Capítulo V: Hydraulic and photosyntetic limitation prevail over root non-structural carbohydrates reserves as drives of resprouting in two Mediterranean oaks: Hydraulics and photosynthesis limit resprouting.

Víctor Resco de Dios, Carles Arteaga, José Javier Peguero-Pina, Domingo Sancho-Knapik, Haiyan Qin, Obey Zveushe, Wei Sun, David Williams, Matthias M.Boer, Jordi Voltas, José Manuel Moreno, David T. Tissue, Eustaquio Gil Pelegrín.

Publicado en mayo de 2020 en Plant Cell and Environment

Capítulo VI: Radiation and drought impact residual leaf conductance in two oak species with implications for water use Models.

Haiyan Qin, Carles Arteaga, Faqrul Islam Chowdhury, Elena Granda, Yinan Yao, Ying Han and Víctor Resco de Dios.

Publicado en noviembre de 2020 en Frontiers in Plant Science

Capitulo VII: Drivers of nocturnal stomatal conductance in C3 and C4 plants.

Faqrul Islam Chowdhury, Carles Arteaga, Mohammed Shafiul Alam, Iftakharul Alam, Víctor Resco de Dios

Enviado en octubre de 2021 a Science of Total Environment

I. Introducción

En los últimos 15 años, más de 3 millones de hectáreas de bosque se han convertido en matorrales y pastizales en los países de europeos de la cuenca mediterránea, principalmente a causa del fuego y la sequía (San-Miguel-Ayanz, et al., 2012). El detonante inicial de este proceso lo encontramos en los cambios socioeconómicos de los siglos XIX y XX, que promovieron el éxodo de las zonas rurales a las zonas urbanas y, en consecuencia, el abandono del aprovechamiento de los bosques y de los campos menos productivos. Este abandono rural, a su vez, ha propiciado aumentos en la carga de combustible a escala de paisaje, en su grado de continuidad y en su homogeneidad (Lloret, et al., 2002; Vega-Garcia, et al., 2010) Estos aumentos de la carga, continuidad y homogeneidad de combustible favorecen una intensificación del régimen de incendios (Rego, 1992).

Durante las últimas décadas hemos observado un aumento en la intensidad y recurrencia de los incendios forestales. Los incendios forestales de la década de los 50 del siglo pasado eran de superficie y no sobrepasaban las 5.000 hectáreas. A partir de la década de los 90 observamos los grandes incendios de copas, caracterizados por una alta intensidad y su capacidad de calcinar entre 10.000 y 20.000 hectáreas (Costa, et al., 2011). En los últimos años se han observado con más frecuencia los incendios piroconvectivos, consecuencia directa del cambio climático, donde una atmosfera muy cálida y bosques estresados por la sequía dan incendios capaces de alterar laestabilidad atmosférica (Castellnou, et al., 2021).

Dichos cambios en el régimen de incendios son el resultado de la interacción entre el cambio climático y el cambio global, esto es, el abandono rural y el consecuente aumento de la carga de combustible junto con el aumento en la temperatura y aridez (Pausas & Fernández-Muñoz, 2011). Así como a principios del s. XX los incendios eran eventos extraños debido a la falta de combustible (vegetación), las acumulaciones de combustible actuales hacen que la presencia y, en cierto grado, la magnitud del incendio dependa de la disponibilidad, o sequía, del combustible (Pausas & Fernández-Muñoz, 2011). Este aumento en la disponibilidad de los bosques ha propiciado la la aparición reciente de mega incendios, que queman superficies sin precedentes en ecosistemas boscosos (Boer, et al., 2020; Nolan, et al., 2020). Estos nuevos incendios, combinados con limitaciones bióticas y abióticas, están produciendo el remplazo a gran escala de bosques por matorrales o pastizales tras el paso del fuego (Karavani, et al., 2018) .

I.1. Estrategias y adaptaciones frente a los incendios

La variación espacial de los regímenes de incendios (pirogeografía) se debe principalmente a la interacción entre la productividad del bioma y su aridez (Boer, et al., 2016). La máxima actividad de incendios se da en los biomas que presentan niveles intermedios de productividad y aridez. En los biomas donde encontramos escasa productividad (muy poca vegetación) y mucha aridez, como los desiertos; o en los que encontramos mucha productividad (mucha vegetación) y poca aridez, como los bosques templados o los bosques tropicales húmedos, es donde menos actividad de incendios existe (Pausas & Bradstock, 2007; Keeley, et al., 2012; Pausas & Ribeiro, 2013).

En respuesta a los incendios, podemos encontrar especies que sucumben tras el incendio, que lo toleran o que regeneran de semilla o rebrote. Los pinos son un género predominante en el hemisferio norte que está marcado, salvo algunas excepciones, por la ausencia de rebrote. En base a la interacción entre la productividad y el gradiente de aridez, (Keeley, 2012) propuso una clasificar las diferentes especies de pino en base a su estrategia para hacer frente al fuego como tolerantes, dependientes o evitadoras del fuego.

En biomas de alta productividad y poca aridez encontramos especies que toleran el paso del fuego. Se trata de ecosistemas donde la elevada humedad limita la intensidad del fuego, por lo que predominan los incendios de baja intensidad. En estos biomas encontramos por tanto pinos cuya corteza gruesa les asegura la supervivencia (p. ej. *Pinus nigra*, *P. ponderosa*, *P. sylvestris*), aunque presentan problemas para regenerar tras incendios de alta severidad. Por el contrario, en biomas con niveles intermedios de productividad y aridez hay una mayor actividad de incendios. Ahí se dan especies cuya regeneración depende de que el fuego abra sus conos serótinos para diseminar las semillas en un suelo sin vegetación (p. ej. *P. attenuata* o *P. halepensis*). Finalmente, en biomas con menor productividad y mayor aridez (o zonas subalpinas), la actividad de incendios es infrecuente y limitada, por lo que dominan especies evitadoras que no presentan ninguna estrategia frente el fuego como *P. aristata* y *P. uncinata*.

En el capítulo IV nos preguntamos si las especies de coníferas con cortezas más gruesas presentaban una menor resistencia a la embolia, para descubrirlo, cruzamos los datos de tolerancia al fuego con los datos de resistencia a la sequía, en concreto los datos de resistencia a la embolia para distintas especies de coníferas.

I.2. El rebrote: una estrategia frente a las perturbaciones

Se considera que el rebrote es la respuesta ancestral al incendio en las angiospermas. Sin embargo, la capacidad de rebrote de las plantas parece estar limitada y disminuyendo notablemente (Karavani, et al., 2018; Resco de Dios, 2020) en diferentes zonas del mundo, principalmente por una mayor severidad de las sequías o la recurrencia de incendios (Batllori, et al., 2018; Collins, 2019; Fairman, et al., 2019; Pratt, et al., 2014). Dicha capacidad para rebrotar es fundamental para el mantenimiento de los ecosistemas ya que proporciona resiliencia frente a perturbaciones (Sánchez-Pinillos, et al., 2016). El paradigma clásico establece que el rebrote está controlado por la disponibilidad de carbohidratos almacenados por la planta (Bowen & Pate, 1993; Pate, et al., 1990). Por lo que es más probable que una planta no rebrote si: i) crece bajo condiciones de sombra (limitaciones de luz); o ii) una sequía prolongada reduce considerablemente sus reservas de carbohidratos. Debemos considerar que el almacenamiento de carbohidratos en la planta sirve como estrategia de supervivencia para reponer la biomasa aérea desaparecida tras una perturbación como el fuego (Wiley & Heliker, 2012).

Sin embargo, otro problema para el rebrote lo puede suponer una embolización en tallos y/o raíces inducida por la sequía que típicamente precede al incendio. Se sabe que, dependiendo del tipo de rebrote, el estrés hídrico puede llegar a ser uno de las mayores limitantes para el rebrote tras incendio (Cruz, et al.,2003; Karavani, et al., 2018), aunque el papel de la pérdida de conductividad hidráulica no se ha documentado todavía. Por tanto, la interacción entre sequía y falta de carbono se conforman como los principales responsables del mecanismo de rebrote y su efectividad, aunque desconocemos los detalles sobre el mecanismo subyacente.

Este es el tema que se aborda en el capítulo V, donde buscamos aclarar la interacción entre la limitación hídrica y la falta de reservas (carbohidratos no estructturales) como agentes que controlan el rebrote en dos especies de robles diferentes, la esclerófila *Quercus ilex* y la caducifolia *Quercus faginea*. Concretamente, las hipótesis a testar son: que las limitaciones hidráulicas controlan el número de plantas que rebrotan, pero que el crecimiento de dicho rebrote estaría limitado por el contenido de NSC (carbohidratos no estructurales por su acrónimo en inglés). Debido a que *Quercus ilex* presenta un mayor grado de esclerofília que *Quercus faginea*, y los costos de construcción de las hojas (gramos de glucosa por gramos de materia seca) son por tanto mayores, esperamos que el contenido de NSC fuera más limitante en *Quercus ilex* que en *Quercus faginea*. La otra hipótesis que planteamos fue que la reducción de luz y agua disponible, reduciría la fotosíntesis como fuente de carbono, lo que también limitaría el rebrote, aunque se esperaba que la fotosíntesis fuera menor en relación con la influencia de las limitaciones del NSC almacenado.

I.3. Las respuestas fisiológicas frente al estrés hídrico

Bajo un escenario de cambio climático, donde aumentan la intensidad y la frecuencia de las sequías, aumenta también el interés por comprender la variación ecológica en la conductancia residual de la hoja (en adelante: g_{res}). Y es que aún después del cierre de los estomas, la hoja sigue perdiendo agua lo que aumenta el estrés hídrico y, en consecuencia, la inflamabilidad de la masa, y puede crear incluso la muerte por sequía. Esto es debido a una mezcla entre la pérdida de agua a través de la cutícula y a un cierre estomático incompleto (conductancia residual; (Blackman, et al., 2016; Martin-Stpaul, et al., 2017).

Se ha descrito que tanto las condiciones de sombra cómo la sequía hacen disminuir la conductancia residual (Boyer, et al., 1997). Sin embargo, se desconoce el efecto que causan ambos factores de estrés a la vez sobre la planta, especialmente se desconoce si su efecto es aditivo o interactivo. Sin embargo, se conocen los efectos de la conductancia residual sobre la mortalidad, y es que el tiempo hasta la desecación se puede doblar si la g_{res} desciende de 4 a 2 mmol m^{-2} s^{-1} (Duursma, et al., 2019).

Aparte de sus efectos sobre el balance hídrico de la planta y, en consecuencia, de su inflamabilidad, entender la variación en conductancia residual es necesario para los modelos de uso del agua (Leuning, 1995; Barnard & Bauerle, 2013; De Kauwe, et al., 2015). La conductancia residual actúa como el "intercept" en los modelos estomáticos usados comúnmente (g_{int}). Los modelos estomáticos más usados en modelos de superficie son los de tipo Ball-Berry, en su forma general $g_{\mathrm{s}} = g_{\mathrm{int}}$+m A/C_{a} f(D), donde g_{s} es la conductancia estomática, A es la fotosíntesis, C_{a} la concentración ambiente de CO_2, D el déficit de presión de vapor y m es el parámetro de la pendiente. Cuando estimamos g_{int} a través del ajuste de la regresión, esta puede ser igual a 0, lo que crea problemas porque entonces la razón entre la concentración de CO_2 intercelular con el ambiente (C_i/C_{a}) no varía con la luz (Collatz, et al., 1991; Leuning, 1995; Duursma, et al., 2019). g_{int} estimada a través de regresión también puede resultar negativa, lo que carece de todo sentido.

En el tercer capítulo evaluamos los efectos de la sombra y la sequía sobre la conductancia residual en las especies de *Quercus* antes comentadas Ambas especies son comunes en los suelos calcáreos de la península ibérica y en general en toda la zona occidental de la cuenca mediterránea. En este tercer capítulo intentamos responder a las siguientes preguntas: i) ¿cómo la interacción entre la sequía y la sombra afectan a la gres?; ii) ¿cuáles son algunos de los posibles mecanismos subyacentes a la variación de gres a través de la sequía y los tratamientos de sombra?; iii) ¿obtenemos distintos valores de gres dependiendo de la técnica de medida?; y iv) ¿cómo incorporamos la conductancia residual en los modelos estomáticos de Ball-Berry y cuáles son las consecuencias en la variación de gres a través de los tratamientos y tipos de mediciones para modelos de intercambios de gases? Estas preguntas son necesarias para poder descifrar hasta qué punto la g_{res} afecta al balance hídrico y, por ende, a la sequía, con su consiguiente efecto sobre el riesgo de incendios.

I.4. La importancia de conductancia estomática nocturna en la pérdida de agua

La transpiración nocturna representa el 12% de la traspiración diurna, y su inclusión en modelos hidrológicos conduce a una reducción de hasta el 50% de la humedad del suelo estimada en ambientes semiáridos (Lombardozzi, et al.,2017). Comprender la conductancia estomática nocturna (g_n) es también esencial para las evaluaciones del balance hídrico desde la hoja hasta la escala de ecosistema (Zeppel, et al., 2014). Esto es porque se trata de una fuente significantiva de pérdidas de agua ecosistémicas en distintos tipos de biomas (Barbeta, et al., 2012; Oishi, et al.,2008; Wallace & McJannet, 2010).

Se conoce la conductancia estomática nocturna es prevalente entre distintas especies C3 y C4, incluidas las herbáceas, los arbustos, árboles y cultivos de distintos de biomas de todo el mundo (Dawson, et al., 2007; Snyder, et al., 2003). Hay diferentes factores que afectan a la g_n, lo que implica que no es simplemente una consecuencia de un cierre deficiente de los estomas (Zeppel et al., 2014), sino que tiene una función ecológica o evolutiva, aunque todavía no se conoce. Algunos estudios indican que los factores que regulan g_n pueden ser diferentes a los que regulan la conductancia estomática diurna (g_d). La g_n es a menudo más alta en condiciones de concentraciones elevadas de CO2 que en concentraciones ambientales de CO_2 (Easlon & Richards, 2009; Zeppel, et al., 2012), lo que es opuesto a la respuesta diurna típica de una reducción de g_d en niveles elevados de CO_2 (Ainsworth & Rogers, 2007). Las distintas respuestas ambientales de g_n vs. g_d complican el modelado, ya que indican que los procesos del modelo diurno no se pueden aplicar para predecir respuestas nocturnas.

También se debe tener en cuenta que no solo los impulsores abióticos influyen en la g_n, los procesos bióticos como los ritmos circadianos conducen a un patrón temporal en g_n (Resco de Dios, et al., 2013). Es por esto que todavía existen muchas incógnitas y poco consenso sobre la regulación de g_n, aunque existe una gran cantidad de estudios sobre g_n en la literatura científica que pueden usarse para sintetizar la información actual sobre los diferentes impulsores de g_n.

La g_n es un proceso fisiológico particularmente relevante para los incendios forestales. Y es que los incendios se suelen apagar por la noche, que es cuando aumenta la humedad de los combustibles muertos. Las plantas suelen "rellenar" los capacitores del tallo por la noche, de forma que la humedad del combustible vivo es también mayor en esa parte del día. Sin embargo, cuando la g_n es elevada, los tallos son incapaces de rellenar los capacitores, lo que disminuye aún más la humedad. Es por elloque debemos entender la influencia de g_n, y los factores que la regulan, como estudio de base para avances posteriores que asesoren su influencia sobre la humedad nocturna y propagación de incendios. Asimismo, g_n puede disminuir la disponibilidad hidráulica, de nuevo repercutiendo negativamente obre la planta, especialmente en condiciones de sequía.

Por todo ello, en este capítulo se hace una revisión de la literatura sobre el efecto de las olas de calor sobre g_n. Dado que nos encontramos con una escasez de datos al respecto, se amplió la búsqueda bibliográfica para obtener resultados más generales sobre los factores que regulan g_n.

I.5. Hipótesis

La hipótesis de partida del capítulo IV es la posible existencia de un compromiso en la asignación del carbono a la protección contra el fuego o contra la sequía. Esto es, que el fotosasimilados se pueden asignar a: i) crear cortezas más gruesas que protejan al cambium y al floema frente al fuego; o ii) crear un xilema más resistente a la embolia como consecuencia de un aumento en la densidad de la madera. Aunque hay muchos más caracteres implicados en la tolerancia al fuego o la resistencia a la sequía, el grosor de corteza y la resistencia a la embolia son vitales.

En el capítulo V examinamos los factores que controlan el rebrote e hipotetizamos que las limitaciones hidráulicas controlarían el número de rebrotes y que el crecimiento de dicho rebrote sí estaría limitado por el contenido de NSC. Asimismo, también hipotetizamos que la importancia de las concentraciones de reserva como agente que controla el rebrote aumentaría con los costes de construcción de las hojas y, por tanto, con la esclerófila.

En el capítulo VI evaluamos los efectos de la sombra y la sequía sobre la conductancia residual en dos especies de robles diferentes. Testamos las hipótesis que la conductancia residual disminuiría tras la aplicación de sombra y estrés hídrico de forma interactiva debido a los efectos sobre los carbohidratos no estructurales de reserva, pero que estas variaciones, aunque significativamente diferentes, jugarían un papel menor en la modelización.

En el capítulo VII testamos la hipótesis general que la regulación estomática responde a los factores ambientales de misma manera tanto de día como de noche. A tal efecto, se estudió la respuesta de g_n a un gran número de parámetros ambientales.

II. Objetivos

La presente tesis doctoral se compone de 3 objetivos principales.

Objetivo 1: Estudiar la relación entre la tolerancia a los incendios y a la sequía en coníferas, centrándonos en la resistencia a la cavitación y el grosor de corteza.

Objetivo 2: Evaluar la capacidad rebrotadora post-incendio en *Quercus ilex L.* y *Q. faginea Lam.* bajo condiciones limitantes de agua (cavitación) y de luz (reservas).

Objetivo 3: Conocer los aspectos que regulan las pérdidas de agua cuticulares y nocturnas por su relevancia sobre la inflamabilidad de las hojas

III. Materiales y métodos

El experimento presentado en los capítulos IV y V se realizó en los campos experimentales de la Escuela Técnica Superior de Agricultura de la Universidad de Lleida (España, coordenadas 41,62N; 0,59E). Construimos una “rain-out shelter” que estaba cubierta por plástico de polietileno transparente. La mitad de la estructura recibía la radiación solar, y la otra mitad estaba cubierta por una malla de sombreo densa, como se puede observar en la figura 1. La malla de sombreo, a diferencia de la cobertura de plástico, no solo cubría el tejado del experimento, sino que cubría también los laterales para evitar la entrada de luz, pero permitiendo la ventilación para evitar la acumulación de calor.

Figura 1: Localización del experimento

Las especies elegidas en este experimento fueron dos especies de robles mediterráneos, *Quercus faginea* Lamk. y *Quercus ilex* L., que obtuvimos plántulas de 2 de savias de viveros locales.

Para el experimento las plantas se trasplantaron de alveolo forestal a macetas de 11 l., el trasplante se realizó en febrero de 2017, y se situaron en el interior de la rain-out shelter 6 meses antes del inicio de los tratamientos del experimento. Las plántulas desarrollaron las hojas nuevas bajo las condiciones de luz experimentales de los tratamientos, pero pudo existir un legado por las condiciones de crecimiento anteriores en vivero que no se pudo cuantificar. Las plantas fueron fertilizadas regularmente y se regaron diariamente a capacidad de campo hasta el inicio de los tratamientos.

Los tratamientos se implementaron a partir de julio de 2017 y duraron dos 2 años. Inducimos el rebrote eliminado la parte aérea de la planta (copa) cortando por encima del cuello de la raíz en julio de 2017 el primer año y en julio de 2018 el segundo año.

Los tratamientos consistieron en dos tratamientos de luz diferentes (sol y sombra), y en tres tratamientos de riego distintos (0%, 50%, 80% de pérdida de conductividad hidráulica, Fig. 2). Con los tratamientos de luz se buscó el agotamiento de las reservas de carbohidratos y consistía de dos niveles: luz ambiente y el tratamiento de sombra (35-45 µmol m^{-2} s^{-1} de PPFD). Los tres tratamientos de riego se establecieron en base al porcentaje de pérdida de conductividad (PLC) de 0%, 50% y 80% (P0, P50 y P80 respectivamente). Estos valores representan diferentes umbrales críticos de estrés hídrico y potencial de recuperación de las angiospermas (Resco de Dios, et al., 2009; Urli, et al., 2013).

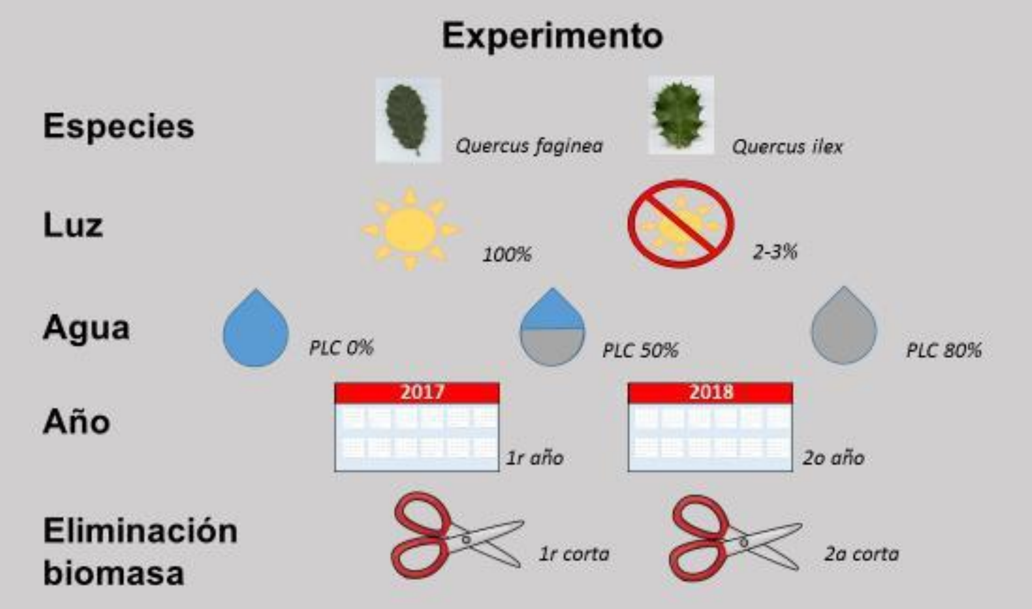


Figura 2: Resumen experimento

Cuando los tratamientos llegaron a los PLC buscados, mantuvimos esas condiciones de estrés hídrico para cada tratamiento durante 2 semanas. Transcurridas esas 2 semanas, realizamos las mediciones de PLC, Ψ_{md} y fotosíntesis y los muestreos para biomasa, área foliar, NSC y costos de construcción. Finalmente, para inducir el rebrote eliminamos la parte área de la planta cortando por encima del cuello de la raíz.

Respecto a los tratamientos de estrés hídrico, no siempre pudimos alcanzar los niveles objetivo pero nuestro enfoque nos permitió establecer un gradiente de PLC que era el objetivo principal. Repetimos los mismos tratamientos, medidas y muestreos durante los 2 años del experimento.

El tamaño de la muestra de nuestro experimento fue de 360 plantas: 30 repeticiones x 2 especies x 3 tratamientos de agua x 2 niveles de luz. Aunque todas las plantas estaban en el mismo rain-out shelter, cada 15 días eran cambiadas de posición para evitar las diferencias microclimáticas del interior del experimento.

Este experimento de campo fue acompañado por un estudio bibliográfico en el que se recabó la información publicada sobre grosores de corteza y resistencia a la cavitación en coníferas, así como toda la información disponible en conductancia nocturna.

IV. A trade-off between embolism resistance and bark thickness in conifers: are drought and fire adaptations antagonistic.

A trade-off between embolism resistance and bark thickness inconifers: are drought and fire adaptations antagonistic?

Víctor Resco de Dios[1,2,*], Carles Arteaga[1], Javier Hedo[1], Eustaquio Gil-Pelegrín[3,4], Jordi Voltas [1]

[1]Departament de Producció Vegetal i Ciència Forestal -AGROTECNIO Center, Universitat de Lleida, E 25198 Lleida, Spain; [2] School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China; [3]Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Gobierno de Aragón, E 50059 Zaragoza, Spain; [4]Instituto Agroalimentario de Aragón - IA2- (CITA-Universidad de Zaragoza), E 50013 Zaragoza, Spain

Abstract

Background**:** Understanding the mechanisms that explain the spatial distribution of conifers across biogeographical gradients is important for anticipating potential range shifts owing to global change. Classical explanations have involved trade-offs between shade and drought tolerances, but more recent studies observed that trade-offs between fire and drought tolerances could also be important.

Aims**:** Here we propose that a contributing mechanism to explain how conifer species are distributed across productivity gradients – with marked variation in the incidence of fire - involves a trade-off between allocation to bark, which serves to protect against fire, or to embolism resistance, which serves to protect against drought.

Methods**:** We compiled information from different datasets and performed regression analyses.

Results**:** We observed a trade-off between bark thickness and embolism resistance in conifer species such that species show either large investments of carbon to the bark or have thinner barks but xylem resistant to embolism; we did not observe conifer species concomitantly showing high fire tolerance and embolism resistance.

Conclusions**:** This study serves as a starting point for a novel framework on how fire and drought adaptations affect conifer biogeography. Additional studies will be necessary to discover the generality of our findings by including other species of conifers, e.g. those in the Southern Hemisphere.

Keywords: bark, cavitation, climate change, conifers, fire, drought, life strategies, trade-off

Introduction

Variation in fire regimes (pyrogeography) across biomes is primarily driven by productivity and aridity gradients (Boer et al. 2016). Maximum global fire activity occurs at intermediate levels of productivity and aridity. Conversely, minimum fire activity is observed at sites with low productivity and high aridity (i.e. deserts, with strong fuel limitation), or at sites with high productivity and low aridity (i.e. wet temperate/tropical forests, where the large amounts of accumulated biomass are seldom dry enough to burn) (Keeley et al. 2012; Pausas and Bradstock 2007; Pausas and Ribeiro 2013).

Considering these interactions between productivity and aridity as drivers of pyrogeography, Keeley (2012) has proposed a division of life strategies across the species of *Pinus,* later expanded by Pausas (2015), and classified species in relation to fire as tolerant, embracer and avoider. Fire-tolerant species occur at the more productive sites, where fire activity is often limited by high moisture that results in low intensity surface fires (Figure 1). Consequently, fire-tolerant species (e.g. *P. nigra*, *P. ponderosa*, *P. sylvestris*) have thick barks that allow the survival of individuals under low intensity fires, but do not regenerate under high intensity crown defoliating fires. Fire-embracer species occur at sites with intermediate productivity, where fire activity is highest, and have a low degree of self-pruning and an overall canopy architecture that enhances crown fires (Figure 1). Their regeneration depends on stand-replacing fires that open their serotinous cones (e.g.: *P. attenuata*, *P. halepensis*). Fire-avoider species occur at dry (or upper montane) environments, where fires are very rare and limited by fuel load (or moisture), and lack adaptations to fire (e.g.: *P. aristata*, *P. uncinata*; Figure 1).

The analyses of trade-offs among traits have been useful to explain species distributions across productivity gradients (Rueda et al. 2016; Valladares et al. 2016).

Here we elaborate on a mechanism that could explain, at least partly, recently reported trade-offs in the ability to cope with stress (drought) and disturbance (fire) in conifer species (Karavani et al. 2018; Rueda et al. 2016). We hypothesised that adaptations to fire and drought incurred a cost and that conifers might not concomitantly survived disturbance and stress. Thus, conifer species may either show large investments of carbon to the bark, which protects the cambium and phloem against fire; or have thinner barks and xylem highly resistant to embolism. Consequently, we hypothesised that conifer species concomitantly showing high bark thickness and embolism resistance may not occur. There are many traits involved in fire tolerance and drought resistance, but bark thickness and embolism resistance are of pivotal importance.

We begin by providing some basic calculations on the carbon costs of constructing bark and xylem resistant to embolism. We then revisit recent work indicating the potential for a trade-off between fire resistance and drought tolerance (Karavani, et al. 2018). Further, we test for the existence of a trade-off in embolism resistance and bark thickness. Finally, we explain the relevance of our findings in a broader context of conifer biogeography.

The carbon cost of constructing bark and xylem

The presence of a trade-off in the allocation of C to either building fire-resistant bark or drought-resistant xylem is conditional on the existence of substantial construction costs. Fernandes et al. (2008) have reported normalised bark thickness (the ratio of bark thickness to stem radius) in western European pines ranging from 7.4% in *P. uncinata* to 24.5% in *P. pinaster*; bark density is ca. 90% that of the xylem in pines (Miles and Smith 2009). Assuming that C concentration is similar across tissues (ca. 50% in both cases (Chave et al. 2009; Hansson et al. 2004)), it thus follows that resources consumed by the bark range from ca. 6.7 % (7.4 × 0.9) to 22.1 % (24.5 × 0.9) those of the xylem, thus representing a substantial construction cost.

In terms of embolism resistance, different adjustments of tracheid anatomy and morphology occur for different conifer families. In species of the Pinaceae and Cupressaceae (which largely dominate our analyses), wood density and tracheid 'thickness-to-span' ratio are strongly correlated with protection from drought-induced embolism (Pittermann et al. 2006). Consequently, mechanical strength is required in these species to avoid tracheid collapse under drought and the correlation between density and resistance to embolism indicates that these trees incur substantial C costs during xylem construction [i.e.: higher wood density leads to higher embolism resistance; (Pittermann, et al. 2006)]. In fact, recent studies have demonstrated that the amount of lignin, which varies between 25 and 36% of wood dry mass in conifers (Fengel and Grosser 1975; Pettersen 1984), is directly related to embolism resistance.

Pereira et al. (2017) have observed a significant correlation between the values of Ψ_{50} (the xylem potential where 50% of the hydraulic conductivity is lost) for different species (Choat et al. 2012) with their respective lignin concentrations (Fengel and Grosser 1975; Pettersen 1984). Based on such correlation, they quantified that an increase of 1% of dry mass allocation to lignin reduced Ψ_{50} by -0.3MPa.

Evidence for trade-offs in embolism resistance and fire tolerance in conifers

The existence of a trade-off between fire tolerance and cavitation resistance in conifer species may be tested with previously published datasets. Data on embolism resistance were obtained from a global database (Choat, et al. 2012). We used Ψ_{50} as this is a commonly used indicator of embolism resistance in conifers (Brodribb and Cochard 2009). We collected data on fire tolerance from the USDA PLANTS database (www.plants.usda.gov, 13 Dec 2016). This database provides a value on "the relative ability to resprout, regrow, or re-establish from residual seed after a fire", and there are four possible levels (none, low, medium, high). After crossing the two datasets, we were able to examine hydraulic resistance and fire tolerance across 41 conifer species (Table S1). No species from those present in the database was able to resprout.

In this analysis we found evidence for a trade-off between Ψ_{50} and fire tolerance (Figure 2a). Conifers with xylem more resistant to embolism (e.g. with $\Psi_{50} \leqslant$ -6 MPa, and down to -12MPa) had either low or no tolerance to fire (levels 1-2 in Figure 1a), whereas conifers with mid or high fire tolerance (levels 3-4 in Figure 2a) always showed smaller embolism resistance (e.g. with $\Psi_{50} >$ -5 MPa) (Figure 2a). We also observed some conifer species with low resistance to cavitation and low tolerance to fire but there were no species showing both high fire tolerance and high Ψ_{50} (Figure 2a).

Despite the scatter in the data, the lack of conifers concomitantly showing high fire tolerance and high embolism resistance points towards a trade-off between these two traits.

As proposed by Grubb (2016), the existence of trade-offs may be formally demonstrated through quantile regression (when fitting an upper quantile the regression becomes significant). Consequently, we examined the significance of the 0.5, 0.75 and 0.95 quantiles and found only the 0.95 quantile regression significant ($P < 0.05$) (using the "quantreg" package (Koenker 2016) within the R software environment (R Core Team 2016)), hence suggesting a trade-off between Ψ_{50} and fire tolerance in conifers (Figure 2a). We note that using different datasets could introduce additional uncertainty around mean trait values. However, this problem affected equally all species and should not lead to any systematic bias.

Evidence for trade-offs in embolism resistance and bark thickness in conifers

The previous analysis is not exempt of criticism because of the ambiguous definition of fire tolerance in the USDA PLANTS database. To overcome this limitation, we conducted further analyses with independent sources of information to test the specific hypothesis that there was a trade-off in the allocation of resources to the bark or to embolism resistance. First, we digitised data on resistances to cambium kill from a previous study on fire resistance across European pines (Fernandes, et al. 2008). This allowed the examination of six species in total (Table S1), a small number, but respresenting a substantial proportion of the pines in Europe (Barbéro et al. 1998; Willis et al. 1998). Cambium kill resistance in the study by Fernandes, et al. (2008) was derived from the percentage of tree radius occupied by bark (at 1.3 m). We observed a negative relationship between resistance to cambium kill and Ψ_{50} (Figure 2b), which is consistent with our hypothesis of a trade-off.

We additionally used an independent dataset on bark thickness at a normalised stem diameter of 10 cm across 20 conifer species (Table S1) from a recent global study (Pellegrini et al. 2017). We observed again that species with a thicker bark were least resistant to embolism and there were no species concomitantly showing thick bark and high embolism resistance (Figure 2c). While more data would be needed to generalise this claim, the trade-off is formally anticipated by the significant (at $P < 0.05$) 0.95 quantile regression.

We also addressed patterns of intra-specific genetic variation underlying a potential trade-off between drought and fire tolerances by digitising graphs with data on bark thickness (Tapias et al. 2004) and on Ψ_{50} (Corcuera et al. 2011) for six different provenances of *P. pinaster* grown under uniform conditions. This Mediterranean pine has a very broad ecological niche (from sea level to 1900 m), which has elicited adaptive divergence among populations arising through localised selection (González-Martínez et al. 2004). The provenances spanned a broad precipitation (348 - 1,257 mm yr^{-1}) and soil fertility gradient (mainly acid soils, but also basic soils and even sandy and

poor soils such as those of Landes, France). From a fire perspective this species is important as a large proportion of all fires in the western Mediterranean basin occur in *P. pinaster* stands. In Spain alone, one-third of all forest fires have been reported from forests dominated by this species (MAGRAMA 2012). This dataset, at intra-specificevel, once more indicated a negative relationship between bark thickness and Ψ_{50} was also significant (Figure 2d).

Conclusions and outlook

Overall, our analyses using independent datasets and across different geographical scales indicate that there are no conifers concomitantly showing high embolism resistance and high tolerance to fire and that this may be owing to an C allocation trade-off between bark and lignin. Moreover, the intra-specific association observed for *Pinus pinaster* under common-garden conditions (Figure 2d) indicates that this trade-off has a genetic basis, although further studies on genetic variation would be required to generalise this claim.

Our hypothesis on a trade-off between embolism resistance and fire tolerance raises the question as to whether adaptations to fire and to drought may, at least to some degree, be antagonistic in conifers.

This hypothesis is still tentative because many additional mechanisms, including stomatal regulation, root: shoot allocation or stem capacitance to name a few, are also involved in drought resistance. However, it could provide a mechanistic explanation, at least partially, to the distribution of the species of *Pinus* and, in general, conifers with different life strategies across productivity gradients (Rueda, et al. 2016).

Further studies should address the role of site fertility as an additional driver of productivity. From the perspective of pyrogeography, productivity gradients have so far been defined as precipitation gradients (Pausas and Bradstock 2007). However, site productivity is also a function of nutrient concentrations and that could be particularly important for species that occurg across a wide range of soil nutrient availabilities (e.g.: *P. pinaster, P. sylvestris, Larix laricina, Taxodium distichum,* etc). This is particularly important because nutrient concentrations tend to decrease xylem vulnerability to embolism (Ewers et al. 2000; Resco de Dios et al. 2013), although less is known on its effects on bark production or fire tolerance, and also because of human-induced nutrient imbalances such as nitrogen deposition (Ochoa-Hueso et al. 2011).

Fire-embracing species, that occupy intermediate productivity sites, would need a high resistance to drought because they are regularly exposed to periods of water scarcity, but they also live in crown-fire environments and, since non-resprouting trees often succumb under high intensity crown defoliating fires, any carbon investment into building structures enhancing fire survival may be futile. Consequently, the mechanism that allows the maintenance of these species in such environments, rather than cambium or crown adaptations to withstand fires, relies on a reproductive strategy that ensures post-fire seed availability via an aerial seed bank (i.e. serotinous cones) (Martín-Sanz et al. 2016). Similarly, fire-avoiding species from arid environments (e.g. *P. edulis, P.monophylla*), which likely show the highest drought tolerance, often lack fire adaptations (Keeley 2012). Additionally, fire-avoiding upper montane treeline-forming species which experience cavitation caused by freeze-thaw cycles notably also lack fire adaptations. Conversely, mesic conifer species need protection from surface fires but are less exposed to periods of intense drought and, consequently, may preferentially allocate more resources into protecting the phloem and cambium than the xylem.

Considering forecasts of increased drought and fire occurrence across parts of the geographical range of temperate conifers (Moritz et al. 2012), understanding conifer strategies for dealing with these factors should be at the forefront of our research efforts. Subsequent work should also address the possibility of recovery from cavitation either by stem refilling or by resprouting.

Acknowledgements

We acknowledge funding form the Spanish Government (RYC-2012-10970, AGL2015-69151-R, AGL2015-68274-C3-3-R).

Notes on contributors

Víctor Resco de Dios is interested in understanding biological processes regulating biosphere-atmosphere interactions.

Carles Arteaga is interested in post-fire regeneration processes.

Javier Hedo is interested in understanding the effects of fire on forest soils.

Eustaquio Gil-Pelegrín is interested in whole-plant ecophysiology of woody plants, especially in the functional responses of trees and shrubs to drought.

Jordi Voltas is interested in understanding adaptive patterns of Mediterranean conifers and their implications for species resilience under global change.

References

Barbéro M, Loisel R, Quézel P, Richardson DM, Romane F. 1998. Pines of the Mediterranean basin. In: Ecology and biogeography of *Pinus* Cambridge: Cambridge University Press. p. 153–170.

Boer MM, Bowman DMJS, Murphy BP, Cary GJ, Cochrane MA, Fensham RJ, Krawchuk MA, Price OF, Resco De Dios V, Williams RJ, et al. 2016. Future changes in climatic water balance determine potential for transformational shifts in Australian fire regimes. Environmental Research Letters 11:065002.

Brodribb TJ, Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiology 149:575-584.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009. Towards a worldwide wood economics spectrum. Ecology Letters 12:351-366.

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, et al. 2012. Global convergence in the vulnerability of forests to drought. Nature 491:752-755.

Corcuera L, Cochard H, Gil-Pelegrin E, Notivol E. 2011. Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P50) under severe drought. Trees 25:1033-1042.

Ewers BE, Oren R, Sperry JS. 2000. Influence of nutrient versus water supply on hydraulic architecture and water balance in Pinus taeda. Plant, Cell and Environment.23:1055-1066.

Fengel D, Grosser D. 1975. Chemische Zusammensetzung von Nadel- und Laubhölzern. Holz als Roh- und Werkstoff. 33:32-34.

Fernandes PM, Vega JA, Jiménez E, Rigolot E. 2008. Fire resistance of European pines. Forest

Ecology and Management. 256:246-255.

González-Martínez S, Mariette S, Ribeiro M, Burban C, Raffin A, Chambel M, Ribeiro C, Aguiar A, Plomion C, Alía R, et al. 2004. Genetic resources in maritime pine (Pinus pinaster Aiton): molecular and quantitative measures of genetic variation and differentiation among maternal lineages. For Ecol Management 197:103-115.

Grubb PJ. 2016. Trade-offs in interspecific comparisons in plant ecology and how plants overcome proposed constraints. Plant Ecology and Diversity 9:3-33.

Hansson K-M, Samuelsson J, Tullin C, Åmand L-E. 2004. Formation of HNCO, HCN, and NH3 from the pyrolysis of bark and nitrogen-containing model compounds. Combustion and Flame 137:265-277.

Karavani A, Boer MM, Baudena M, Colinas C, Díaz-Sierra R, Pemán J, de Luís M, Enríquez-de-Salamanca Á, Resco de Dios V. 2018. Fire-induced deforestation in drought-prone Mediterranean forests: drivers and unknowns from leaves to communities. Ecological Monographs 88:141-169.

Keeley JE. 2012. Ecology and evolution of pine life histories. Ann For Sci. 69:445-453.

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. Fire in Mediterranean ecosystems-ecology, evolution and mangement Cambridge, UK: Cambridge University Press.

Koenker R. 2016. quantreg: Quantile Regression. R package version 5.29. https://CRAN.R-project.org/package=quantreg.

MAGRAMA. 2012. Los incendios forestales en España. Decenio 2001–2010. Madrid.

Martín-Sanz RC, Santos-del-Blanco L, Notivol E, Chambel MR, San-Martín R, Climent J. 2016. Disentangling plasticity of serotiny, a key adaptive trait in a Mediterranean conifer. American Journal of Botany 103:1582-1591.

Miles PD, Smith WB. 2009. Specific gravity and other properties of wood and bark for 156 tree species found in North America. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Moritz MA, Parisien M-A, Batllori E, Krawchuk MA, Van Dorn J, Ganz DJ, Hayhoe K. 2012. Climate change and disruptions to global fire activity. Ecosphere 3:art49.

Ochoa-Hueso R, Allen EB, Branquinho C, Cruz C, Dias T, Fenn ME, Manrique E, Perez-Corona ME, Sheppard LJ, Stock WD. 2011. Nitrogen deposition effects on Mediterranean-type ecosystems: an ecological assessment. Environmental Pollution159:2265-2279.

Pausas J, Bradstock RA. 2007. Plant persistence fire traits along a productivity and disturbance gradient in Mediterranean shrublands of southeastern Australia. Global Ecology and Biogeography 16:330-340.

Pausas JG. 2015. Evolutionary fire ecology: lessons learned from pines. Trends in Ecology &

Evolution. 20:318-324.

Pausas JG, Ribeiro E. 2013. The global fire–productivity relationship. Global Ecology and Biogeography 22:728-736.

Pellegrini AF, Anderegg WR, Paine CE, Hoffmann WA, Kartzinel T, Rabin SS, Sheil D, Franco AC, Pacala SW. 2017. Convergence of bark investment according to fire and climate structures ecosystem vulnerability to future change. Ecology Letters 20:307-316.

Pereira L, Domingues-Junior AP, Jansen S, Choat B, Mazzafera P. 2017. Is embolism resistance in plant xylem associated with quantity and characteristics of lignin? Trees32:349-358.

Pettersen RC. 1984. The chemical composition of wood. In: The chemistry of solid wood. Washington, DC.: American Chemical Society. p. 57-126.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH. 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant, Cell and Environment. 29:1618-1628.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Resco de Dios V, Turnbull MH, Barbour MM, Ontedhu J, Ghannoum O, Tissue DT.2013. Soil phosphorous and endogenous rhythms exert a larger impact than CO_2 or temperature on nocturnal stomatal conductance in *Eucalyptus tereticornis*. TreePhysiology 33:1206-1215.

Rueda M, Godoy O, Hawkins BA. 2016. Spatial and evolutionary parallelism between shade and drought tolerance explains the distributions of conifers in the conterminous United States. Global Ecology and Biogeography. 26:31-42.

Tapias R, Climent J, Pardos JA, Gil L. 2004. Life histories of Mediterranean pines. Plant Ecology. 71:53-68.

Valladares F, Laanisto L, Niinemets Ü, Zavala MA. 2016. Shedding light on shade: ecological perspectives of understorey plant life. Plant Ecology & Diversity. 9:237-251.

Willis KJ, Benneth KD, Birks HJ. 1998. The late Quaternary dynamics of pines in Europe. In: Ecology and biogeography of *Pinus* Cambridge: Cambridge UniversityPress. p. 107-121.

Figure legends

Figure 1: Idealised and simplified representation of how different conifer functional groups are distributed across productivity gradients according to a presumed trade-off between fire tolerance and embolism resistance.

Figure 2: Trade-offs between embolism resistance and bark thickness. (a) Relationship between fire tolerance (1, none; 2, low; 3, medium; 4, high; from PLANTS USGS database) and the xylem potential where 50% (Ψ_{50}) of the hydraulic conductivy is lost (Choat, et al. 2012) across 41 conifer species. (b) Relationships between cambium and kill resistance (in relative units) (Fernandes, et al. 2008), against the xylem water potential where 50% of the hydraulic conductivity is lost (Ψ_{50}) (Choat, et al. 2012) across six European pine species. (c) Relationship between bark thickness for a diameter of 10 cm (Pellegrini, et al. 2017) against the xylem potential where 50% of the hydraulic conductivity is lost (Ψ_{50}) (Choat, et al. 2012) across conifer species. (d) Relationship between bark thickness (Tapias, et al. 2004) and Ψ_{50} (Corcuera, et al. 2011) across six *Pinus pinaster* provenances. The line indicates results of quantile (a, c) or linear (c, d) regression. *P*-values and R^2 are given for quantile and linear regression, respectively. In quantile regressions, we examined the significance of the 0.5, 0.75 and 0.95 quantile regressions for all plots but only the 0.95 quantile regressions were significant at $P < 0.05$, as indicated by the plotted line. X-axis scales may differ between panels. (a) modified from (Karavani, et al. 2018).

Figure 1

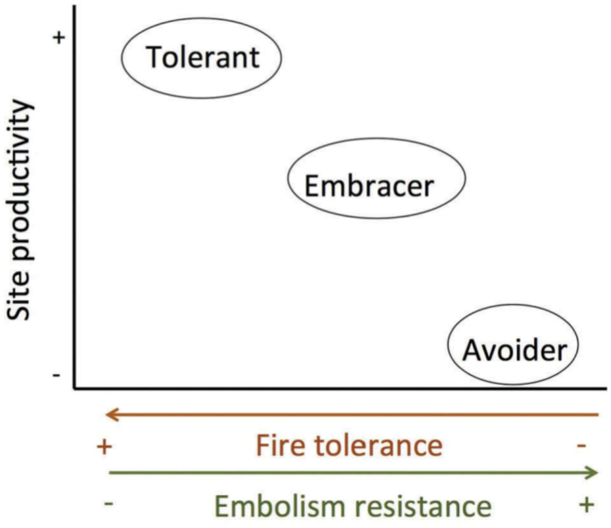
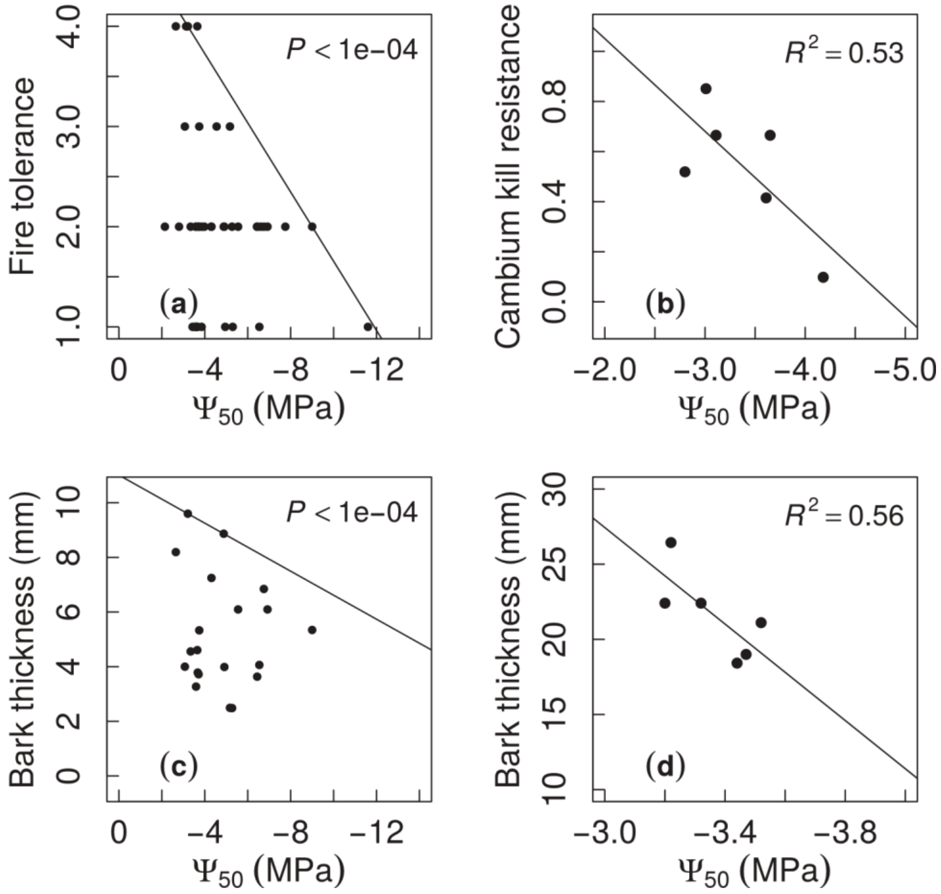


Figure 2


Fire tolerance
1.0
2.0
3.0
4.0
P < 1e−04
(a)
0
−4
−8
−12
Ψ50 (MPa)
Cambium kill resistance
0.0
0.4
0.8
R2 = 0.53
(b)
−2.0
−3.0
−4.0
−5.0
Ψ50 (MPa)
Bark thickness (mm)
0
2
4
6
8
10
P < 1e−04
(c)
0
−4
−8
−12
Ψ50 (MPa)
Bark thickness (mm)
10
15
20
25
30
R2 = 0.56
(d)
−3.0
−3.4
−3.8
Ψ50 (MPa)

V. Hydraulic and photosyntetic limitation prevail over root non-structural carbohydrates reserves as drives of resprouting in two Mediterranean oaks: Hydraulics and photosynthesis limit resprouting

Hydraulic and photosynthetic limitations prevail over root nonstructural carbohydrate reserves as drivers of resprouting in two Mediterranean oaks

Víctor Resco de Dios[1,2], Carles Arteaga[3], José Javier Peguero-Pina[4] , Domingo Sancho-Knapik, Haiyan Qin, Obey K. Zveushe[1], Wei Sun[5], David G. Williams, Matthias M. Boer, Jordi Voltas[2,3], José M. Moreno[8], David T. Tissue[7], Eustaquio Gil-Pelegrín

[1]School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China

[2]Joint Research Unit CTFC-AGROTECNIO, Universitat de Lleida, Lleida, Spain

[3]Department of Crop and Forest Sciences, University of Lleida, Lleida, Spain

[4]Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Gobierno de Aragón, Zaragoza, Spain

[5]Key Laboratory of Vegetation Ecology, Ministry of Education, Institute of Grassland

Science, Northeast Normal University, Changchun, China

[6]Department of Botany, University of Wyoming, Laramie, Wyoming

[7]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia

[8]Department of Environmental Sciences, University of Castilla-La Mancha, Toledo, Spain

Correspondence

Víctor Resco de Dios, School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China.

Funding information

Generalitat de Catalunya, Grant/Award Number: SGR2017-1518; Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain, Grant/Award Number: RTA2015-00054-C02-01; Ministerio de Ciencia e Innovación, Grant/Award Number: AGL2015-69151-R; Natural National Science Foundation of China, Grant/Award number: 31850410483; the talent funds of Southwest University of Science and Technology, Grant/ Award Number: 18ZX7131

Abstract

Resprouting is an ancestral trait in angiosperms that confers resilience after perturbations. As climate change increases stress, resprouting vigor is declining in many forest regions, but the underlying mechanism is poorly understood. Resprouting in woody plants is thought to be primarily limited by the availability of non-structural carbohydrate reserves (NSC), but hydraulic limitations could also be important. We conducted a multifactorial experiment with two levels of light (ambient, 2–3% of ambient) and three levels of water stress (0, 50 and 80 percent losses of hydraulic conductivity, PLC) on two Mediterranean oaks (Quercus ilex and Q. faginea) under a rain-out shelter (n = 360). The proportion of resprouting individuals after canopy clipping declined markedly as PLC increased for both species. NSC concentrations affected the response of Q. ilex, the species with higher leaf construction costs, and its effect depended on the PLC. The growth of resprouting individuals was largely dependent on photosynthetic rates for both species, while stored NSC availability and hydraulic limitations played minor and non-significant roles, respectively. Contrary to conventional wisdom, our results indicate that resprouting in oaks may be primarily driven by complex interactions between hydraulics and carbon sources, whereas stored NSC play a significant but secondary role.

KEYWORDS

construction costs, fire, growth, hydraulics, non-structural carbohydrate concentrations, perturbation, photosynthesis, resilience, resprouting vigor, survival

1 |INTRODUCTION

Understanding the effects of climatic changes in land cover during this 21st century is becoming a major research effort globally (IPCC, 2019). Associated with rapid climate change, a drying atmosphere and more variable precipitation patterns are enhancing water scarcity in many areas of the world. Moreover, as forested ecosystems become drier, the prevalence of mega-fires has accelerated, burning unprecedented fractions of forested ecosystems (Boer, Resco de Dios, & Bradstock, 2020; Nolan et al., 2020). In combination with other biotic and abiotic stresses, large-scale replacements of forests by shrublands or grasslands may become more common (Karavani et al., 2018).

The capacity for resprouting, particularly widespread across angiosperm trees, is a major mechanism providing post-fire and postdrought resilience in Mediterranean ecosystems and other parts of the world (Sánchez-Pinillos, Coll, De Cáceres, & Ameztegui, 2016). However, the capacity for resprouting appears to have limits and is diminishing (Karavani et al., 2018; Resco de Dios, 2020), notably with increased drought severity or fire recurrence (Batllori et al., 2018; Collins, 2019; Fairman, Bennett, & Nitschke, 2019; Pratt et al., 2014).

The mechanisms underlying reductions in the capacity to resprout are still relatively unknown. The classic paradigm is that the availability of stored carbohydrate reserves is the major driver of resprouting (Bowen & Pate, 1993; Pate, Froend, Bowen, Hansen, & Kuo, 1990) and that resprouting exhaustion would be more likely if carbohydrate reserves are reduced, particularly in plants growing under deep shade or that experience protracted drought. There is some debate regarding the impact of protracted drought on carbohydrate reserves because declines in sink activity under drought may preclude the decline of source photo-assimilates (Korner, 2003; Palacio, Hoch, Sala, Körner, & Millard, 2014).

The role of carbohydrate reserves and plant function has been the topic of considerable research (Dietze et al., 2014). Non-structural carbohydrates (NSC) account for, on average, 10% of dry plant biomass (Martínez-Vilalta et al., 2016). Despite the significant investment into NSC, plant growth is seldom limited by carbon, even under extreme droughts, and NSC depletion is a rare phenomenon in angiosperm trees (Adams et al., 2017; Duan et al., 2013; Martínez-Vilalta et al., 2016; Mitchell et al., 2013). Some authors have proposed that the major function of carbohydrate storage is to serve as a bethedging strategy that promotes survival after a perturbation that removes a large part of the aboveground biomass (Wiley & Helliker, 2012).

However, other authors consider that hydraulic limitations developed during drought, either in stems or roots, depending on resprouting type, may impose a higher limitation to post-fire resprouting (Cruz, Pérez, & Moreno, 2003; Karavani et al., 2018). Post-fire resprouting requires new growth, which cannot occur if the capacity for transporting water to meristems is impaired. Additional factors, such as nutrients or bud scarcity, may also limit resprouting (Clarke et al., 2013; Karavani et al., 2018), but the interaction between hydraulics and C starvation (i.e., lack of carbohydrates to support plant function) as drivers of post-fire resprouting is particularly important to resolve under climate change. The frequency of drought-induced defoliation and mortality events are increasing globally (Allen, Breshears, & McDowell, 2015; Carnicer et al., 2011). Subsequently, fire following a protracted drought could catalyze the conversion of forests to shrublands or grasslands through interactive, but largely unknown, effects on plant hydraulics and carbon balance (Karavani et al., 2018; Parra & Moreno, 2018).

Numerous studies have examined the interactive effects of hydraulics and carbohydrate reserve depletion on drought-induced mortality (Adams et al., 2017), and some studies have addressed fire impacts on xylem vulnerability to embolism in surviving branches (Bar, Michaletz, & Mayr, 2019; Hood, Varner, van Mantgem, & Cansler, 2018; Kavanagh, Dickinson, & Bova, 2010; Midgley, Kruger, & Skelton, 2011). However, studies jointly addressing how increasingly larger losses in hydraulic conductance, in addition to increasing levels of carbohydrate reserve depletion, may subsequently limit resprouting are more scarce.

Here, we report the results of an experiment that sought to disentangle the mechanisms affecting resprouting in potted oak seedlings experimentally exposed to three levels of water availability and two levels of light. Treatments were jointly imposed to generate a range in both stored NSC concentrations and losses of hydraulic conductivity.

The oak species (Quercus ilex L. and Quercus faginea Lam.) in this study are the dominant oaks in the drier parts of the oak expansion range in the Western Mediterranean basin. Regeneration of Mediterranean oaks heavily depends on resprouting because of high seed predation (Herrera, 1995). Understanding the limits to resprouting in these two species is thus important to improve estimates on potential species replacements under climate change. Furthermore, these species show contrasting life history strategies (Palacio et al., 2018): Q. ilex is sclerophyllous and evergreen, whereas Q. faginea is deciduous and exhibits hydraulic segmentation (Peguero-Pina et al., 2015).

Overall, we sought to elucidate whether: (a) resprouting capacity (percent individuals resprouting) was controlled by hydraulic limitations, NSC limitations (that is, the depletion of NSC reserves) or their interaction; and (b) the growth of the resprouts exhibited a legacy effect due to previous impacts on hydraulic limitations, reserve availability or concurrent photosynthesis limitations.

We hypothesized that hydraulic limitations would control the percentage of plants that resprout, but subsequent growth of the resprouts would be dependent on NSC content and mobilization to grow the resprouting tissues. Because Q. ilex is more scleromorphic than Q. faginea, and leaf construction costs (in terms of g glucose g^{-1} dry matter) increase with schlerophylly, we expected that resprouting growth would be more dependent on NSC in Q. ilex than in Q. faginea. Finally, we hypothesized that imposed reductions of light and water availability would decrease concurrent photosynthesis as a source of C, which would additionally limit resprouting. However, we expected that this photosynthesis effect would be minor relative to that of sink (stored NSC) limitations.

2 |METHODS

2.1 | Study site

The experiment was performed at the experimental field site at the School of Agrifood and Forestry Science and Engineering at the University of Lleida (Spain; 41.62 N, 0.59 E). We constructed a rain-out shelter that was covered by clear polyethylene plastic, as commonly used in greenhouse building. Half of the structure received natural solar radiation, and the other half was covered by dense shade cloth. Plants under ambient light (i.e., under the polyethylene plastic) received a maximum PPFD of 1,500 μmol m^{-2} s^{-1} and plants under the shade cloth received 2–3% (35–45 μmol m^{-2} s^{-1}) of full sun. The structure had openings on both sides to increase ventilation and minimize heat build-up. The temperature in the rain-out shelter was 3C higher than outside the shelter under ambient light, and 2.5C higher in the shade treatment (Figure S1). The difference in vapour pressure deficit across sun and shade treatments was 0.1 kPa (Figure S1).

For this study, we obtained 2-year-old seedlings from local nurseries. The provenances originated from the mountain range of the Iberian System. Plants were grown in 11 L pots (20 cm × 20 cm × 27.5 cm). The rooting medium used was the commercial potting mix Humin Substrat N6 (Neihaus, Klasman-Deilmann GmbH, Geeste, Germany). Pots were regularly fertilized with a slow release NPK MgO fertilizer (17-09-11-2, Osmocote Universal, KB, Ecully, France) and daily watered to field capacity until treatment implementation.

The plants grew for 6 months (since February 2017) in the rainout shelter before experiment inception. They developed new leaves under the experimental light conditions, but there may have been legacy effects from the previous growing seasons in the nursery, which could not be quantified. The experiment began in the northern hemisphere summer (July) of 2017 and lasted for 2 years. We induced resprouting by removing the canopy by clipping above the root collar in July 2017, and then again in July 2018 (twice in total; details below).

2.2 | Experimental treatments

We induced depletion of carbohydrate reserves and water stress by implementing two different light regimes: sun (ambient) and shade (35–45 µmol m^{-2} s^{-1}) in concert with three different levels of water stress. Light manipulations may alter additional aspects of plant physiology and morphology, but our experimental design was to mimic a comparison between seedlings growing in the understory of a forest or in an open woodland under different levels of water stress.

The three different watering treatments were set to achieve different values of percentage loss of conductivity (PLC); 0, 50 and 80% PLC (P_0, P_{50} and P_{80}, respectively), which represent different critical water stress thresholds and the potential for recovery in angiosperms (Resco et al., 2009; Urli et al., 2013). P_{50} represents moderate water stress in angiosperms and P_{80} represents severe stress, and potential mortality. During treatment implementation, we stopped watering and allowed plants to dehydrate and we measured midday stem water potential (Ψ_{md}) every other day in a subset of plants ($n = 5$). Stem water potential was measured after introducing the cut stem in zip lock bag, and allowing it to equilibrate for 30 min in the dark at room temperature (PMS1000, PMS, Albany, OR). We then calculated the expected PLC based on Ψ_{md} from previously published vulnerability curves for our species and provenances (Esteso-Martínez, Camarero, & Gil-Pelegrín, 2006; Peguero-Pina et al., 2014). Once plants reached the target PLC, we kept soil moisture constant at that level for 2 weeks. This was achieved by weighing a subset of pots ($n = 5$ per each treatment) and adding back the water that had evaporated every day. After this 2 weeks period, we conducted the relevant measurements (PLC, $\Psi_{md,}$ photosynthesis) and sampling protocols (for biomass, leaf area, NSC and construction costs) and then induced resprouting. We could not always reach our target levels, but our approach allowed us to establish a gradient of PLCs which was the major goal (see Table 1, Table S1, Figure S2). We repeated the same measurements over the 2 years of the experiment.

Sample size for this experiment was 360 plants: 30 replicates × 2 species × 3 water treatments × 2 light levels. Although all plants were inside the same shelter, we rotated them every fortnight to minimize the impact of microclimatic differences within the shelter on our experimental plants. We measured the basal diameter and height of all plants at the beginning of the experiment. We used a random number generator to assign plants to treatments. After assigning each plant to a treatment, we ensured there were no significant a priori differences in plant size across treatments (ANOVA, $p > .05$).

2.3 | Resprouting

Plants were clipped above the root collar and the pots were then kept at constant weight (to maintain a constant water level) for two more weeks after clipping and, at that point, all pots were regularly watered to field capacity until the next growing season, when the experiment was repeated. That is, we clipped (and sampled) all the 360 plants twice: once in 2017 and then again in 2018. The only exceptions were: (a) the plants that died during the experiment as a result of our treatments (i.e., a plant that did not resprout in 2017 was not treated again in 2018); and (b) the plants that were destructively harvested for NSC and construction costs analyses, as detailed below.

We monitored resprouting starting after the first re-watering, 2 weeks after clipping, by counting the number of resprouting individuals daily for the first 2 weeks, every 2 days for the next 2 weeks, and weekly for the next 7 months (no resprouting occurred before rewatering). We quantified resprouting growth by measuring, 9 months after clipping, the total length of the re-growing shoots (i.e., the sum of the length of all the shoots within a plant), the basal diameter (above the point of clipping) and the volume (calculated from length and diameter assuming the branches formed a cylinder). Throughout the manuscript, resprouting capacity refers to the percentage of individuals that resprouted and resprouting growth relates to changes in length, diameter and volume of resprouting shoots. There was mortality during the experiment as a result of treatment implementation.

The percentage of resprouting individuals was thus calculated relative to the total number of individuals alive (i.e., with stem and green leaves) per treatment before clipping on a given year.

TABLE 1 Target midday water potential (Ψ_{md}) to reach the desired PLC according to Esteso-Martínez et al. (2006) for Q. faginea and to Peguero-Pina et al. (2014) for Q. ilex, and actual values

Species	Light treatment	Water treatment	Target Ψ_{md} (MPa)	Target PLC (%)	Actual Ψ_{md} (MPa)	Actual PLC (%)
Q. ilex	Sun	P_0	>−4	<20	−1.3 (0.20)	11.9 (2.3)^{c}
		P_{50}	−6	50	−5.4 (0.54)	64.7 (6.87)^{a}
		P_{80}	−8.9	80	−6.4 (0.62)	69.1 (7.56)^{ab}
	Shade	P_0	>−4	<20	−0.8 (0.14)	12.3 (2.84)^{c}
		P_{50}	−6	50	−2.9 (0.77)	41.2 (10.9)^{abc}
		P_{80}	−8.9	80	−4.4 (0.56)	54.3 (10.69)^{ab}
Q. faginea	Sun	P_0	>−2.8	<20	−1.1 (0.22)	15.6 (2.73)^{c}
		P_{50}	−3.9	50	−3.9 (0.28)	53.2 (8.04)^{ab}
		P_{80}	−5.5	80	−4.2 (0.30)	66.1 (9.89)^{a}
	Shade	P_0	>−2.8	<20	−0.7 (0.12)	7.5 (1.35)^{c}
		P_{50}	−3.9	50	−1.1 (0.21)	27.9 (9.86)^{bc}
		P_{80}	−5.5	80	−2.6 (0.51)	39.8 (13.0)^{abc}

Note: Mean (and SE) actual values are presented. The letters in 'Actual PLC' indicate the results of post-hoc analyses (Tukey HSD).

2.4 | PLC measurements

We tested whether the watering treatments had reached the target PLC levels by measuring native PLC in five plants per treatment. We measured PLC in both experimental years, and always immediately before the clipping. That is, we first measured PLC, then we induced clipping and monitored resprouting twice throughout the experiment (once in each year). We used stem PLC as a proxy of root PLC, an assumption that will be addressed in the discussion.

We measured hydraulic conductivity in stems (50 mm long) before and after removing the air embolisms, as in Sperry, Donnelly, and Tyree (1988). We cut the entire shoot, from the root collar, under water in the field. The stem was wrapped in wet paper towels inside zip lock bags in a 4C cooler and transported to the laboratory. The measured stems were then recut under water once in the laboratory. The procedure used for this study was the same that is detailed in Peguero-Pina, MendozaHerrer, Gil-Pelegrín, and Sancho-Knapik (2018). Briefly, we perfused distilled, degassed and filtered water (0.22 mm) with 0.005% (v/v)

Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth. The hydraulic conductivity was measured at a pressure of 0.005 MPa and air embolism was removed at a pressure of 0.15 MPa for 60–90 s. The water flow was measured with a digital mass flowmeter (Liqui-Flow, Bronkhorst High-Tech, Ruurlo, The Netherlands) connected to a computer.

2.5 |Leaf gas exchange measurements

We measured leaf gas exchange with a portable photosynthesis system (LI-6400XT, Li-Cor Inc., Lincoln, NE) during the last round of growth measurements on five plants per treatment. Measurements were conducted between 10:00 hr and 14:00 hr local time, to minimize circadian effects and afternoon stomatal closure. We measured each plant under the two treatment levels of PPFD: 1,500 (full sun) and 40 μmol $m^{-2} s^{-1}$ (shaded) but here we only report values under growth (treatment) conditions. The other conditions in the chamber were set to 25.0C, 400 ppm, and 1.5 kPa for temperature, CO_2 and vapour pressure deficit, respectively.

2.6 |Biomass, leaf area and NSC analyses

Immediately prior to clipping, we randomly selected five individuals per treatment for destructive measurements. Fresh leaves were first scanned and leaf area and number were calculated with ImageJ for all the leaves in each individual (Schneider, Rasband, & Eliceiri, 2012). Samples were microwaved quickly after harvest for 30 s at 700 W, to prevent enzymatic reactions. We then calculated dry biomass by harvesting and weighing the different plant parts (roots, stem, old leaves and new leaves) after drying (48 hr at 105C) and calculated leaf mass area (LMA). Roots were manually extracted and washed from the potting mix, carefully to minimize losses of root biomass.

We extracted soluble sugars and starch in roots following Palacio, Maestro, and Montserrat-Marti (2007). After grinding the dried biomass with a mill (IKA A10, IKA-Werke, Staufen, Denmark), soluble sugars (sugar from now on) and starch were extracted separately. For sugar, we first added 10 ml of ethanol (80% v/v) to 50 mg of sample and waited for 30 min at 60C in a water bath. After this time, we centrifuged (NEYA 8, REMI ELEKTROTECHNIK LTD., Vasai, India) the sample for 10 min at 3,200 rpm. We then added 50 µl of supernate, 450 µl of ethanol (80%), 500 µl of phenol (28%) and 2,500 µl of sulphuric acid (96%), mixed it and let it sit for 30 min. The third step was to read the absorbance at 490 nm with the spectrophotometer (Spectrophotometer UV-1600PC, VWR, Radnor, PA) after removing the supernatant and drying the sample at 70C for 16 hr.

To measure starch content, we added 4 ml of sodium acetate (pH 4.5) to the dry sample and left it for 60 min in a 60C water bath. We then left it to cool and added 1 ml of Amyloglucosidase (0.5% w/v), which was then incubated in the stove (16 hr at 50C). After the incubation, we added 50 µl of supernatant to the sample, 450 µl of sodium acetate (pH 4.5), 500 µl of phenol (28%) and 2,500 µl of sulphuric acid (96%). We then mixed it and let it sit for 30 min, we measured again the absorbance at 490 nm with the spectrophotometer. NSC was calculated as the sum of starch and sugars. Unless otherwise noted, NSC refers to the concentration of stored NSCs in the roots.

2.7 | Construction cost analyses

We estimated the leaf construction costs following previously developed methods (Nagel et al., 2002; Poorter, 1994; Vertregt & Penning de Vries, 1987). We collected leaves from five more individuals per treatment (different to those used in NSC) and we measured ash content after exposing the sample to 400C for 6 hr (Nagel et al., 2002). We analysed organic carbon and nitrogen contents as in Aspinwall et al. (2017) with a combustion elemental analyser (TruSpec Micro, Leco, St Joseph, MI). Construction costs (CC) were then calculated using the formula from Poorter (1994) as:

$$CC = (-1.041 + 5.077C)(1-M) + (5.325N)$$

where M, C and N indicate ash content and organic carbon and nitrogen concentrations, respectively.

2.8 | Data analyses

We first tested whether our implementation of the water treatments had been successful with linear mixed-effects models (LMM), using Ψ_{md} and PLC at the time of inducing resprouting as dependent variables; with light and water treatments as well as species and their interactions as independent variables; and year as a random factor.

We tested for differences in percent resprouting with a generalized linear mixed model (GLMM), using a binomial distribution, where species, light, water treatments and their interactions were fixed factors and year was a random factor. Differences in NSC, construction costs, net assimilation (A_{net}) and growth were analysed with LMM, where species, light, water treatments and their interactions were fixed factors and year was a random factor.

We sought to understand whether resprouting capacity and growth were correlated using GLMM with a binomial distribution, where year was the random factor. In order to understand whether resprouting capacity was limited by hydraulics or by reserves, we used LMM where PLC and NSC and their interactions were the explanatory variables and year was included as a random factor. In order to understand whether growth was driven by hydraulics, NSC reserves or photosynthesis, we additionally used LMM with PLC, NSC and A_{net} and their interactions as fixed factors and year as a random factor.

All statistical analyses were implemented in the R software environment (version 3.5.2; R Development Core Team, 2018) using the ‘lme4’ package for GLMM and LMM (Bates, Maechler, Bolker, & Walker, 2015), the ‘car’ package for Wald tests in GLMM and LMM (Fox & Weisberg, 2019). Additionally, we performed model selection in all of the above cases using automated dredging procedures (Barton, 2018). We also calculated the marginal and conditional R^2 (R^2_m and R^2_c), which refer to the fixed or to the random and fixed components in mixed models, respectively, following Nakagawa and Schielzeth (2013).

3 |RESULTS

3.1 |Water treatment implementation

Our water treatments were successful in that we managed to create different levels of water stress and, importantly, there were no significant differences across species (Table 1, Table S1, Figure S1) or across years. However, we could not always reach the desired PLC levels for the following reasons. First, our seedlings were more vulnerable to cavitation than previously published values for the same species (Esteso-Martínez et al., 2006; Peguero-Pina et al., 2014). The value of PLC for a given value of Ψ_{md} was higher in our seedlings than in published values. Since we measured Ψ_{md} during our experiment to infer PLC from the published literature, this generated a higher experimental PLC under the P_{50}_sun treatment (between 65 and 68%) than our target value for the oaks (Table 1, Table S1, Figure S1). The second issue was that our species were very resistant to cavitation and we could not reach the water potentials necessary to induce PLC over 80%, even after leaving the plants without water for 2 months. Consequently, measured PLC values under the P_{80}_sun treatment (between 69 and 72%) were lower than expected (Table 1, Table S1, Figure S1). The third issue was that evapotranspiration was low in the shade treatment; consequently, PLC was consistently lower than in the full sun treatment. Indeed, PLC ranged between 28 and 41% in the

P_{50}_shade treatment, and between 40 and 54% in the P_{80}_shade treatment (Table 1, Table S1, Figure S2) in both species. Given this problem P_{50}_shade and P_{80}_shade in the second experimental year were merged under a single treatment (P_{50}_shade).

3.2 | Resprouting capacity

Resprouting capacity was significantly affected by our experimental treatments. There were differences across species and water levels, and there were also significant interactions between light and water treatments and between species and light treatments (Table 2, Figure 1).

Taken in isolation, water scarcity had a more important effect on resprouting than light availability: the effect of water was significant ($p < .0001$), but light was not significant ($p > .05$). Indeed, mean resprouting was 68% in the shade and 63% the sun (Figure 1a,b), but resprouting significantly declined with water stress from 88% in P_0, to 57% in P_{50} and to 47% in P_{80} (Figure 1a,c). There was however a

significant interaction between the light and water treatments (Figure 1c). Resprouting in the sun treatment declined from 97% at P_0, to 62% at P_{50} and to 31% at P_{80} (Figure 1c). However, the effect of drought was highly variable in the shade treatment as resprouting varied from 80% in P_0, to 51% in P_{50} and to 79% in P_{80} (Figure 1c).

Regarding the species, Q. faginea showed significantly higher (Table 2, Figure 1a,b) resprouting (77%) than Q. ilex (54%). However, species effects depended on the interactions with light (Figure 1b). Resprouting in Q. faginea was favoured by the shade, as resprouting increased from 61% in the sun to 95% in the shade (Figure 1b). However, shade exerted a negative effect on resprouting in Q. ilex, which declined from 65% in the sun to 45% in the shade (Figure 1b).

TABLE 2 Results of a fitting a generalized linear mixed-effects model (GLMM) on resprouting capacity, a linear mixed-effects model (LMM) on length, diameter, volume, net assimilation (Anet) and non-structural carbohydrate concentrations (NSC), and an ANOVA on leaf construction costs on a mass (CC) and an area (CC_area) basis

	Resprouting capacity	*Length*	*Diameter*	*Volume*	A_{net}	*NSC*	*CC*	*CC_area*
Species	26.6***				3.1·	9.58**	46.3***	1,299***
Light		157***	155***	154.9***	108***	50.1***		165.1***
Water	86***	67.7***	43.8***	43.9***	91.7***			699.5***
Light × water	64.4***	34.2***	52.9***	52.9***	88.7***	0.01**		206.6***
Species × light	67.4***	7.8**			3.2·			67.7***
Species × water								24.7***
Species × water × light								38.6***

Note: The explanatory variables were in all cases species, light, water and their two- and three-way interactions included. We applied dredging techniques for automatic model selection and only the variables selected are included. Year was included as a random factor in GLMM and LMM models. ANOVA was performed for CC and CC_area because only data for 2018 was available. Values indicate either the $\chi 2$ (GLMM and LMM) or the F-values (ANOVA) and stars indicate the significance level. Significance levels: ***<.001; **<.01; *< .05; < .1.

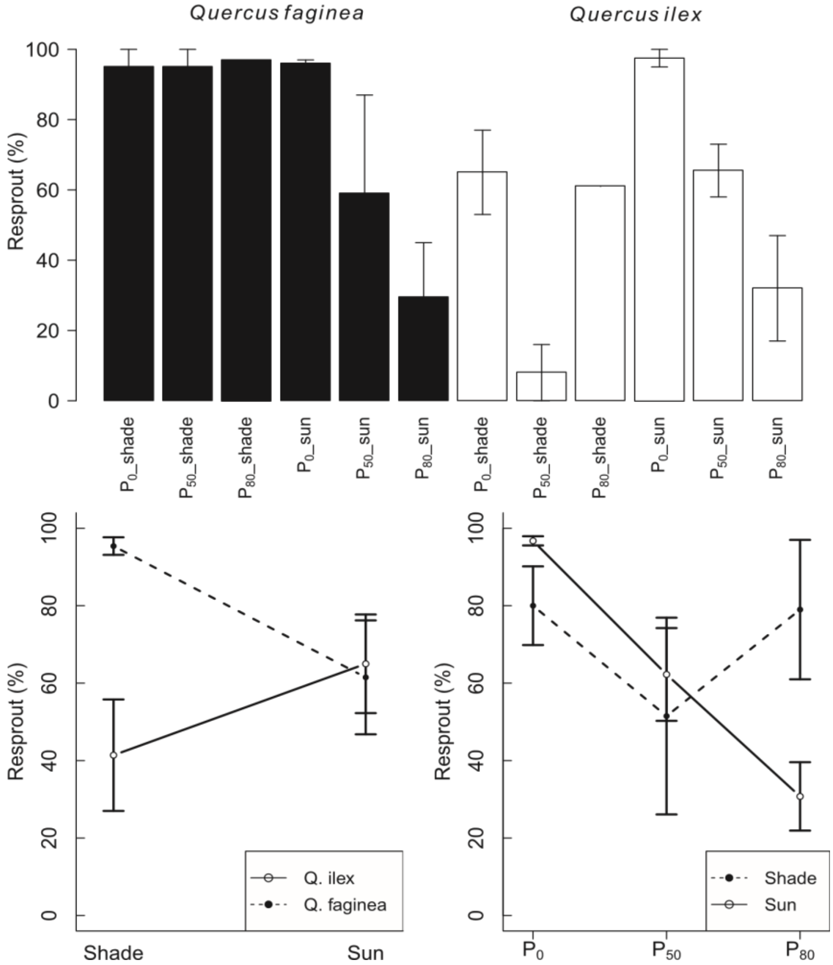


FIGURE 1 Changes in resprouting capacity as a function of species, water and light levels (a) and significant interactions (b, c) The species are *Quercus ilex* (Qi) and *Q. faginea* (Qf). The water levels refer to target levels of PLC (P_0, P_{50} or P_{80}, but see Table 1 for actual values). Sun or shade indicated whether the plants received either ambient, or 2–3% of ambient radiation, respectively. Values indicate mean and standard error

3.3 | Resprouting growth

Resprouting growth was affected by light, water, and their interaction and, in the case of total shoot length, treatment effects depended on the species (Table 2, Figure S3, Figure 2). Total shoot length, basal diameter and shoot volume were always higher in the sun than in the shade treatments (Figure 2, Figure S3). Total length was higher for Q. ilex in the shade, but differences across species vanished at high light (Figure 2a). Total resprouting length in the sun declined from 61 cm to 30 cm to 26 cm as water stress increased from P_0 to P_{50} to P_{80}, respectively (Figure 2b). Total resprout length in the shade showed a less marked declined from 21 cm to 14 cm to 15 cm as water stress increased from P_0 to P_{50} to P_{80}, respectively (Figure 2b). A similar pattern was apparent for basal diameter, which decreased in the sun from 2.9 mm at P_0 to 2.5 mm at P_{50} and to 2.2 mm in P_{80} (Figure 2c). In the shade, however, diameter was higher in P_{80} (1.85 mm) than in P_0 (1.4) or P_{50} (1.5) (Figure 2c). Differences in volume followed the same trend as those in length (Figure 2d).

3.4 | Coordination between resprouting capacity and resprouting growth

We observed that the patterns of correlation between resprouting capacity and growth were highly contrasting across species (Figure 3). On the one hand, percent resprouting in Q. ilex correlated positively with total length ($p < .0001$, $R^2_m = 0.28$) basal diameter ($p < .0001$, $R^2_m = 0.18$) and volume ($p < .0001$, $R^2_m = 0.32$). On the other hand, the correlation between percent resprouting and growth in Q. faginea was not significant for length ($p = .15$) and volume ($p = .5$), and negative in the case of diameter ($p < .0001$), although the power of the negative correlation was very low ($R^2_m = 0.08$).

3.5 | Photosynthesis, carbohydrates and construction costs

Photosynthetic rates measured at ambient light varied across species marginally ($p < .1$), across light, water and their interaction significantly ($p < .05$) and across the interaction between light and species marginally ($p < .1$; Table 2, Figure S4). In particular, A_{net} in P_0_sun treatment for Q. faginea (13 µmol m^{-2} s^{-1}) was significantly higher than in the P_0_sun treatment for Q. ilex (9.5 µmol m^{-2} s^{-1}). Average A_{net} in all other treatments ranged between −0.7 and 2.8 µmol m^{-2} s^{-1} but they were not statistically significant, nor was year.

The concentration of root NSC ranged between 7.6 and 27% in Q. faginea and between 8 and 22% in Q. ilex. Consequently, the minimum value of root NSC concentration reached across species was not different (7.6% vs. 8.0%) indicating that we reached similar levels of reserve depletion across species. However, Q. faginea showed a significantly higher capacity for accumulating NSC on average (18.5% in Q. faginea vs, 14.2% in Q. ilex; Table 2, Figure S5a).

There were also significant differences in NSC across light treatments (Table 2), with higher concentrations in the sun (20%) relative to the shade (13%) treatment (Figure S5a,b). However, the effect of light depended on its interaction with the water treatment (Table 2, Figure S5b). NSC in the shade was highest at the P_{80} treatment (15%) and lower at the P_{50} (10%) and P_0 (12%) treatments, while in the sun treatments, NSC was lower in the P_{80} treatment (18%) compared to the P_{50} (19%) and P_0 (22%) treatments. NSC concentrations reflected storage capacity as starch accounted for 71% of the total NSC pool across species.

Importantly, year was not significant in the model.

We estimated construction costs on a mass and leaf area basis. On a mass basis, the construction cost of a Q. faginea leaf were significantly lower (1.28 g glucose g^{-1} dry matter) than for a Q. ilex leaf (1.44 g glucose g^{-1} dry matter; Table 2, Figure S6), indicating that leaf construction costs for Q. faginea were 11% lower than for Q. ilex. However, the LMA of Q. faginea (8.8 g cm^{-2}) was 39% lower than Q. ilex (14.3 g cm^{-2}). Consequently, the construction costs of Q. faginea on a leaf area basis (0.011 g cm^{-2}) were 45% lower than for Q. ilex (0.020 g cm^{-2}; Figure S6a,b). On a leaf area basis, construction costs were also affected by light, water and their interactions with species (Table 2, Figure S6c–e). Leaf construction costs on an area basis were higher in the sun than in the shade (Figure S6c), and they also increased with drought, particularly in P_{80} (Figure S6d). The interaction between light and water meant that costs in P_{80} were constant regardless of light level (Figure S6e).

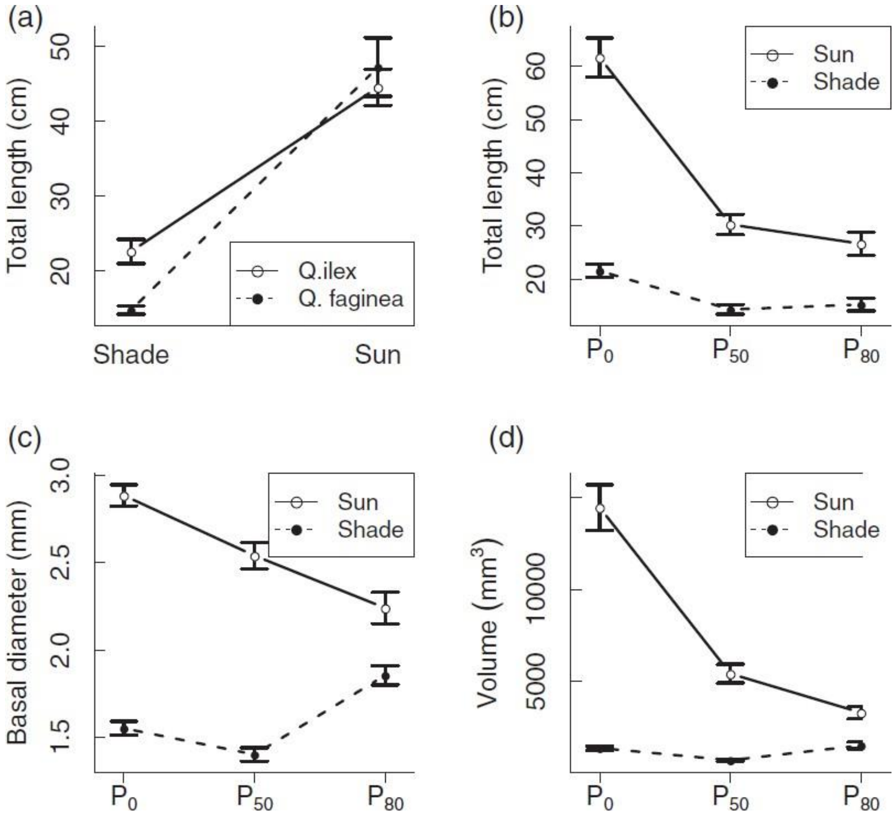


FIGURE 2 Changes in total length (a, b), basal diameter (c) and volume (d) across species, light, water treatments and their interactions. Only significant interactions are included. The water levels refer to target levels of PLC (P0, P50 or P80, but see Table 1 for actual values). Sun or shade indicated whether

the plants received either ambient, or 2–3% of ambient radiation, respectively. Values indicatemean and standard error. PLC, percentage loss of conductivity.

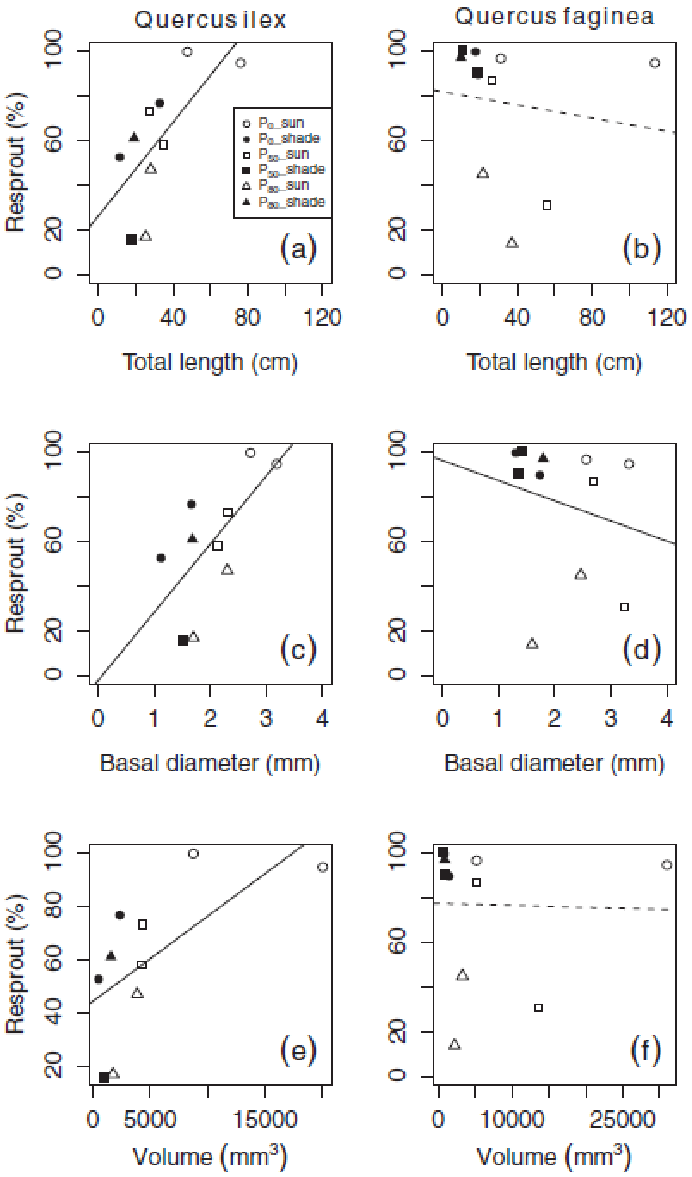


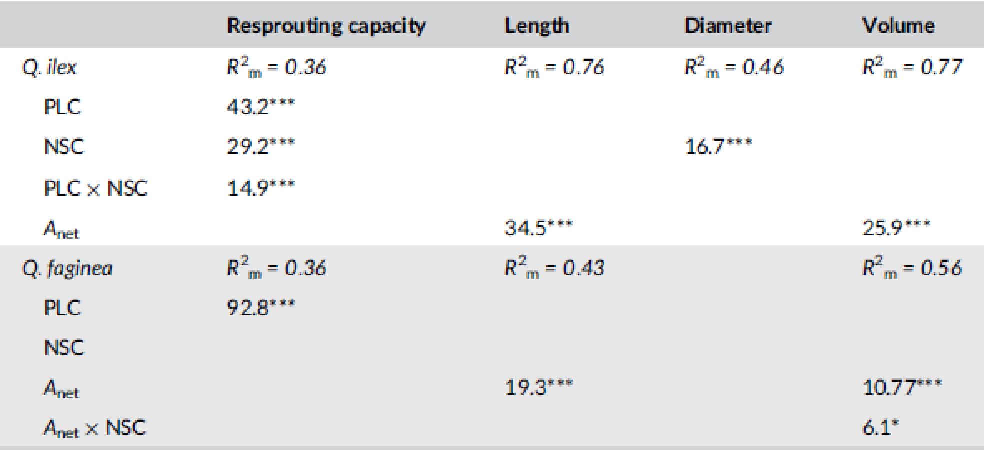
FIGURE 3 Correlations between % resprouting and the three growth variables (total length, basal diameter and volume) in Q. ilex (a, c, e) and Q. faginea (b, d, f). Best-fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant. Each point is a treatment mean.

3.6 |Factors affecting resprouting capacity

We examined the correlation between resprouting capacity and PLC and NSC and we observed different patterns across species (Table 3, Figure 4). Higher PLC had a negative effect on resprouting, which was significant in Q. faginea and Q. ilex ($p < .001$, Table 3). We can examine the point where 50% resprouting occurs, as an analogy with epidemiological studies, which use LD_{50} as an indicator of the median lethal dose (or as an analogy with, P_{50} broadly used in plant hydraulics). In Q. faginea, resprouting was below 50% only when water stress was above P_{50} in all cases (Figure 4b). For Q. ilex, resprouting was below 50% only when water stress was above P_{50} in all cases except for one point (lower left of Figure 4a), indicating that NSC limitations may have played an additional role (Figure 4c).

In Q. ilex, there was a significant ($p > .001$, Table 3, Figure 4c) and positive correlation between NSC and resprouting capacity, and an interaction between PLC and NSC (Table 3). Resprouting in Q. faginea was independent of NSC (Table 3). The R^2_m of our statistical models were 0.36 for both species (Table 3).

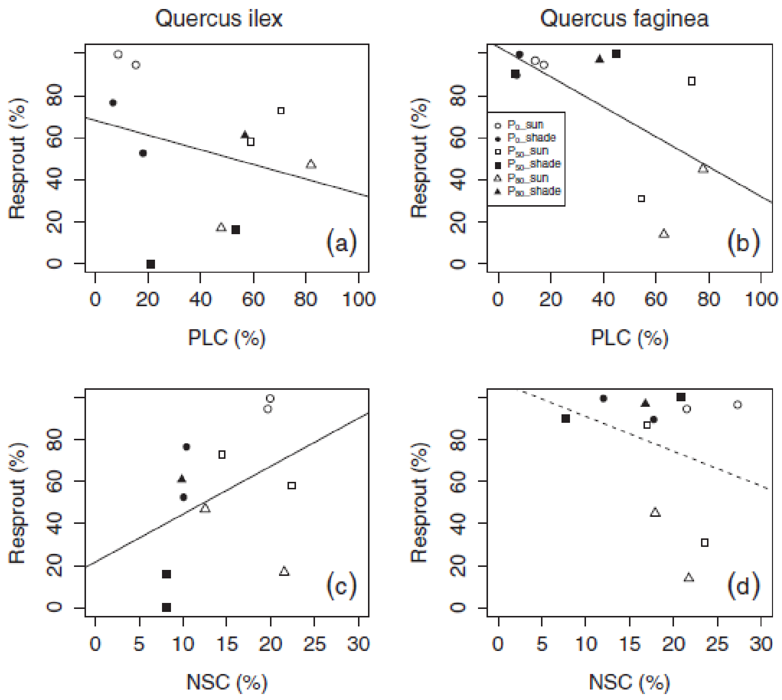
TABLE 3 Effects of PLC additionally of net photosynthesis (A_{net}) over shoot total length, basal diameter and volume and NSC over resprouting capacity and volume.



	Resprouting capacity	Length	Diameter	Volume
Q. ilex	$R^2_m = 0.36$	$R^2_m = 0.76$	$R^2_m = 0.46$	$R^2_m = 0.77$
PLC	43.2***			
NSC	29.2***		16.7***	
PLC × NSC	14.9***			
A_{net}		34.5***		25.9***
Q. faginea	$R^2_m = 0.36$	$R^2_m = 0.43$		$R^2_m = 0.56$
PLC	92.8***			
NSC				
A_{net}		19.3***		10.77***
A_{net} × NSC				6.1*

Note: Resprouting capacity was fitted with generalized linear mixed model (GLMM) and growth with a linear mixed-effects model (LMM). We applied dredging techniques for automatic model selection and only the variables selected in the best model are included. Values indicate the $\chi 2$ and stars indicate the significance level. Values are also provided for the marginal R2 (R2m) for each model. Significance levels: ***<.001; **<.01; *<.05; · < 0.1.

FIGURE 4 Effects of NSC and PLC over resprouting capacity in Q. ilex (a, b) and Q. faginea (c, d). Best-fit lines indicate the results of simple regression rather than the full model and they are included only for visualization purposes. Dashed lines indicate that the full model was not significant. Each point *is a treatment mean. NSC, non-structural carbohydrate; PLC, percentage loss of conductivity.*



3.7 | Factors affecting resprouting growth

Photosynthesis was the most important variable regulating growth of resprouts (Table 3, Figure 5). The best model included A_{net} as the only significant parameter for total shoot length in Q. faginea ($p < .0001$; $R_m^2 = 0.43$) and Q. ilex ($p < .0001$; $^2 = 0.76$) and for volume in Q. ilex ($p < .0001$; $R_m^2 = 0.77$). Photosynthesis alone ($p = .001$) and, to a minor degree, its interaction with NSC ($p = .01$) were significantdrivers of volume in Q. faginea ($R_m^2 = 0.56$). NSC was an important predictor for diameter in Q. ilex ($p < .0001$; $R_m^2 = .46$), but no variable was a significant predictor of diameter in Q. faginea ($p > .05$). It is worth noting that PLC did not show a significant correlation with any of the growth parameters (Table 3).

4 | DISCUSSION

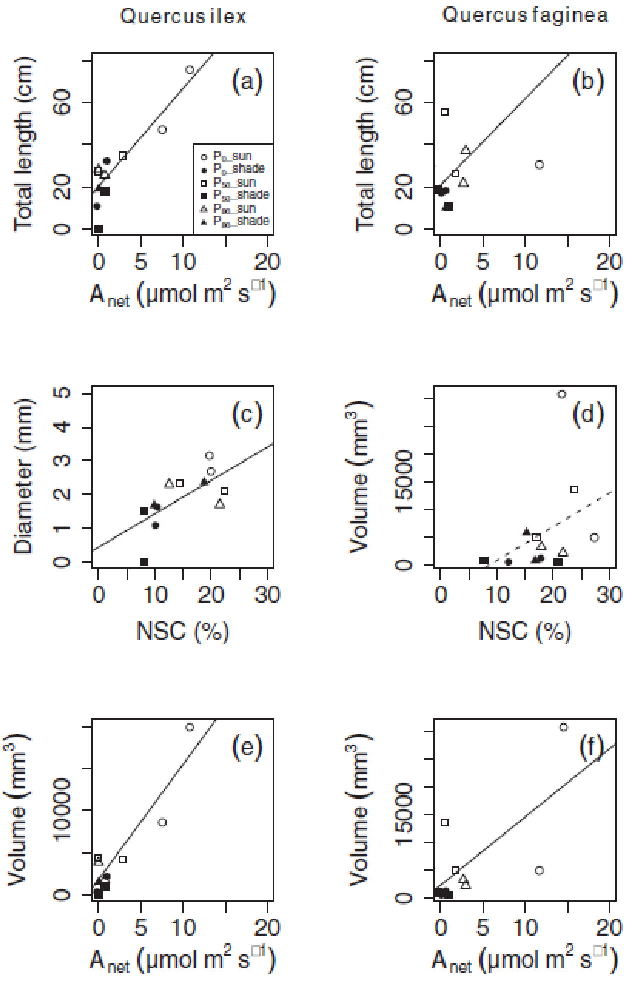
We exposed seedlings of two naturally co-occurring oak species to contrasting light and water conditions for 2 years to understand the drivers of resprouting. We observed that hydraulic limitations on the capacity to resprout were present in both species, but that carbohydrate depletion only affected Q. ilex, which was the species with the highest leaf construction costs. Subsequent growth of resprouts was primarily driven by current photosynthesis, while stored carbon (NSC) played a significant but secondary role. The mechanisms affecting the capacity for resprouting (primarily hydraulics) differed from those affecting the growth of the resprouts (primarily C uptake), although additional differences were observed in two closely related and cooccurring oak species.

4.1 |Factors affecting resprouting capacity

The resprouting literature in woody species has traditionally discussed the role of stored carbohydrates and nutrients as the main drivers of resprouting (Clarke et al., 2013; Pate et al., 1990). Only a handful of studies have documented water limitations, as indicated by predawn water potential (Cruz et al., 2003), as a potential factor affecting resprouting. However, to the best of our knowledge, this is the first study directly demonstrating that the degree of losses in hydraulic conductance before the perturbation that induces resprouting will limit subsequent resprouting capacity in woody species. Hydraulic limitations were more severe in Q. faginea than in Q. ilex, even though Q. faginea shows a higher degree of hydraulicsegmentation (PegueroPina et al., 2015), which is often considered advantageous for recovery after waterstress is relieved because runaway cavitation is isolated (Tyree & Zimmerman, 2002).

Our results were only partly consistent with the hypothesis that NSC limits resprouting capacity. We observed a correlation between resprouting and NSC only in Q. ilex, but the mechanistic reason underlying this relationship remained puzzling after examining differences in leaf construction costs and the amount of available NSC reserves, across species. The lowest NSC concentrations in Q. ilex roots were 8.1% at the P_{50}_shade treatment which, multiplied by total root weight, adds up to 0.67 g of NSC. The construction costs of a Q. ilex leaf at the P_{50}_shade treatment were 0.01 g glucose cm^{-2}.

FIGURE 5Correlates of growth across species. Photosynthesis (A_{net}) correlated with length (a,b) and volume (e,f) in both species. NSC only correlated with diameter in Q. ilex (c) and its interaction with A_{net} in volume of Q. faginea (d, Table 3). Best-fit lines indicate the result of simple regression, not of the full model, and are included for visualization purposes only. Dotted lines indicate marginal relationships. Each point is a treatment mean. NSC, non-structural carbohydrate.



These reserves should thus have been enough to build 67 cm^2 of leaves, which is equivalent to 16 leaves in the P_{50}_shade treatment, and to 37% of total leaf area grown within one season. In addition to building new leaves, basal resprouters also need to rebuild the stem (which has lower construction costs than the leaf, [Poorter, 1994]) and support respiratory costs. However, these numbers indicate that the amount of NSC left would have been enough to resprout and recover canopy photosynthetic capacity.

This apparent contradiction could be partly explained by interactions with hydraulic impairment, which could have limited NSC remobilization (Table 2). Q. ilex often showed low resprouting (<50%) when NSC and PLC were low (<10%) and high (>50%), respectively. The opposite pattern was also true, as we observed a number of cases in the shade treatment with high resprouting rates (>50%) despite NSC being below 10% (Figure 4c), and this coincided with PLC being <50% (Figure 4a).

However, and contrary to this hypothesis, PLC was only 20% in the P_{50}_shade treatment for 2018, when resprouting was 0% and NSC was 8.1% (Figure 4a,c). A plausible, but speculative, explanation for this observation is that the root seedlings were disconnected from the soil in the P_{50}_shade treatment. That is, in conditions of low water and shade, the plant may have encountered disruption in the soil-root continuum such that hydraulic constraints occurred, although they could not be picked up by our PLC sampling scheme which focused on main stems. This could also explain why PLC values in the shade were lower than in the sun, also VPD differences across treatments were only minimal. Further studies on the mechanisms addressing resprouting should thus address the disconnection between roots and soils. To our knowledge, this is the first study indicating this could be a possibility affecting resprouting capacity.

Differences between Q. ilex and Q. faginea in resprouting further supports the hypothesis that NSC may only limit resprouting if accompanied by hydraulic impairment. Resprouting in Q. faginea was not limited by NSC reserves, although it reached lower NSC concentrations than Q. ilex, and this could be because low NSC did not co-occur with high PLC in Q. faginea. That is, the lowest NSC concentrations in Q. faginea were 7.6% in the P_{50}_shade treatment, which multiplied by total root weight, equals 0.24 g of NSC. Construction costs were 0.006 g glucose cm^{-2}, which indicates that reserves were sufficient to build 40 cm^2 of leaves (or 27% of total leaf area after one growing season). The potential to build new material in Q. faginea was thus lower than in Q. ilex (which was of 67 cm^2 of leaves, as stated above) because of the lower NSC content. However, we did not observe NSC limitations in this species, which we attribute to the fact that PLC at low NSC levels was never above 50% in this species. That is, low NSC alone was not enough to induce reserves limitation in Q. faginea. Additional processes, such as bud limitation, could account for some of the explained variation.

4.2 |Factors affecting resprouting growth

We observed that resprouting growth was not driven by the same mechanisms as resprouting capacity. One of the differences was that hydraulic limitations developed before the perturbation did not exert any carry-over effects over the growth of the resprouts. This contrasts with drought responses, where hydraulics have been documented to affect plant growth for some years after the drought (Anderegg et al., 2015; Ogle et al., 2015). This difference is likely driven by the fact that we focused on basal resprouting, where new stem tissue that is free from embolisms is grown. We thus cannot discard a role for hydraulics limiting the growth of epicormic sprouts, particularly for species that maintain their xylem functional for many years. We note that one could have encountered drought legacies if the period between clipping and re-watering had been longer than in this experiment.

Another difference between the drivers of resprouting growth and capacity was that net photosynthesis appeared to correlate best with growth. Photosynthesis was the sole correlate of height in both species and of volume in Q. ilex, indicating that source rather than sink limitations control resprouting in these species. This is perhaps not surprising considering the strong reductions in C uptake imposed by both our shading and drought treatments.

The potential role of NSC as a driver of growth was overall weak. NSC correlated with secondary growth in Q. ilex and, along with photosynthesis, it affected volume in Q. faginea. Such a weak role of NSC may be puzzling, considering the wealth of literature supporting a role of carbohydrates as major drivers of resprouting (Pausas & Keeley, 2014; Zeppel et al., 2015). However, our results are consistent with the drought literature, where overall, NSC concentrations seldom limit plant survival or growth in angiosperms (Adams et al., 2017; Korner, 2003).

Our results indicate that source limitations to growth were the most common under the different stresses, but sink processes also played a role. Following (and simplifying) the model from Huang et al. (2019) and Gessler and Grossiord (2019), under source limitation we should observe that photosynthetic declines lead to a decline in root reserves. This pattern of source limitation is consistent with our results in the shade treatment, where photosynthetic rates and root NSC concentrations were lower than in the P_0_sun. Our temporal design does not allow us to determine the precise moment when NSC concentrations declined, but most likely it occurred during the first few months after treatment implementation because we did not observe differences across years at the moment prior to clipping.

Similarly, there were significant declines in NSC concentration as water stress increased in the sun treatments, further indicating source limitations to growth in the sun across drought treatments. However, we observed that NSC concentrations in P_{80} treatments were higher than in P_0 treatments in the shade, a pattern that is consistent with sink limitations. That is, Huang et al. (2019) and Gessler and Grossiord (2019) propose that when sink limitations occur, a decline in root metabolic activity leads to an increase in metabolite accumulation. This pattern was consistent with the results obtained after imposing the double stress of shade and drought, indicating that multiple stress responses and metabolic limitations co-occur.

The evolutionary role of NSC reserves requires further assessment since NSC constituted significant portions of plant dry mass in the roots and yet exhibited a limited role in regulating resprouting capacity and primary growth.

4.3 | Limitations on the use of stem PLC as a proxy for root PLC

We need to acknowledge that a limitation of this study was the use of stem PLC as a proxy for root PLC. Previous studies have indicated how this proxy can be problematic when there is substantial hydraulic segmentation (Johnson et al., 2016) because roots have sometimes (Tyree & Zimmerman, 2002), but not always (Wu et al., 2020), been documented to be more vulnerable to embolism than stems. Although the existence of hydraulic segmentation was formulated long ago (Zimmermann, 1978), it is still unclear how prevalent it is across species (Wu et al., 2020).

For the two species of concern here, we could not find any study addressing whether hydraulic segmentation occurs in seedlings. Previous studies conducted with adult trees (Peguero-Pina et al., 2015), however, observed that leaves of Q. subpyrenaica (the hybrid between Q. faginea and Q. pubescens) withered prematurely as leaf water potentials dropped below −3 MPa, which indicates hydraulic segmentation between leaves and stems. The same study did not find vulnerability segmentation between leaves and stems in Q. ilex.

For adult trees of Q. ilex Martínez-Vilalta, Prat, Oliveras, & Piñol (2002) documented that roots were more vulnerable to cavitation than stems. The differences in the relationship between applied pressure and PLC across tissues were particularly high as PLC increased above 50%. That is, while there were no statistical differences in the P_{50} of roots and stems, roots became increasingly more vulnerable to cavitation than stems at higher water potentials (Martínez-Vilalta et al., 2002).

We thus cannot discard the possibility that root PLC in the P_{80}_sun treatment for Q. ilex would have been larger than the one measured for stem PLC. A similar response may also have occurred in Q. faginea, if this species also displays vulnerability segmentation between roots and stems. That is, stem PLC may have underestimated root PLC, and the differences in PLC across organs would have been more accentuated at higher PLCs. If this is the case, we can speculate that the slope of the relationship between PLC and resprouting capacity would have been steeper than reported here (Figure 4, Table 3).

We must acknowledge also the possibility that stem PLC in our species is not indicative of root PLC at all. Under this scenario, our results would indicate that stem PLC before clipping, and not root PLC, controls basal resprouting after clipping. However, there is no known mechanism to explain this relationship and we thus consider it unlikely.

4.4 | Quercus resilience

Overall, our results indicated that oak seedlings were more resilient to clipping in the shade than in the sun. That is, light and water limitations (shade and drought) induced a higher level of resprouting than water limitations (drought) under no light limitation (sun). However, there were differences in PLC across treatments, which were substantially higher in the sun than in the shade. Indeed, comparisons on drought responses across different light treatments were affected by the different severities of drought. It should thus be noted that in the field, shady sites may sometimes be drier than sunny sites because of increased plant–plant competition for water (Valladares, Laanisto, Niinemets, & Zavala, 2016). Under these circumstances, when shady environments are drier than sunny sites, we would thus expect a lower resprouting capacity in the shade than in the sun.

Previous studies assessing the recovery from drought in angiosperms indicate that lack of recovery may occur when PLC > 80% (Resco et al., 2009; Urli et al., 2013). Our results on this regard are still tentative, and limited as they are based on stem PLC (and not root PLC), but they allow us to hypothesize that resprouting may become particularly limited when PLC is below 50%. Hence, resprouters are more susceptible to stress and disturbance, than to stress alone. It should be noted that we induced resprouting by clipping, to ensure homogeneity in treatments, but fire exerts an additional thermal stress (Casals & Rios, 2018). It is thus feasible that the hypothesized PLC threshold of 50% for limiting resprouting may be lower in burned plants.

It should also be noted that our results are limited to basal resprouting and further studies will need to address potential limitations in epicormic and other types of resprouting. In particular, the role of hydraulic legacies may be more important in epicormic resprouters. There are an increasing number of studies indicating increased intensity of forest fires under global warming, resulting from higher temperatures and drought intensities (Boer et al., 2016; Jolly et al., 2015; Nolan et al., 2020). Our results offer new mechanistic insights and hypotheses to be tested in further experiments that may be used to improve model predictions. We thus hope this study serves as a starting point for more studies on this area to further clarify outstanding issues.

ACKNOWLEDGEMENTS

This study has been supported by the National Natural Science Foundation of China (No. 31850410483), the talent funds of Southwest University of Science and Technology (No. 18ZX7131), the MICINN

(AGL2015-69151-R), INIA (RTA2015-00054-C02-01) and Generalitat de Catalunya (SGR2017-1518), Spain. We acknowledge technical support from M. J. Pau, M. P. Sopeña, A. Cunill, J. Hedo, B. Lavaquiol and C. Maier. This manuscript benefited from the insightful comments provided by two reviewers and the Associate Editor and from statistical advice by J. G. Alday.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Víctor Resco de Dios conceived the project with input from Carles Arteaga, José Javier Peguero-Pina, Domingo Sancho-Knapik, Wei Sun, David G. Williams, Matthias M. Boer, Jordi Voltas,, José M. Moreno, David T. Tissue and Eustaquio Gil-Pelegrín; Carles Arteaga, Eustaquio Gil-Pelegrín, José Javier Peguero-Pina and Domingo Sancho-Knapik cultivated the plants; Carles Arteaga, Víctor Resco de Dios, Eustaquio Gil-Pelegrín, José Javier Peguero-Pina, Domingo Sancho-Knapik, Obey K. Zveushe, David T. Tissue and Haiyan Qin performed measurements; Carles Arteaga prepared the data files; Víctor Resco de Dios analysed the data and wrote the manuscript and all co-authors contributed to edits to the manuscript.

REFERENCES

Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landhäusser, S. M., Tissue, D. T., … McDowell, N. G. (2017). A multispecies synthesis of physiological mechanisms in drought-induced tree mortality. Nature Ecology & Evolution, 1, 1285–1291.

Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere, 6, 129.

Anderegg, W. R. L., Schwalm, C., Biondi, F., Camarero, J. J., Koch, G., Litvak, M., … Pacala, S. (2015). Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. Science, 349, 528–532.

Aspinwall, M. J., Varhammar, A., Blackman, C. J., Tjoelker, M. G., Ahrens, C., Byrne, M., … Rymer, P. D. (2017). Adaptation and acclimation both influence photosynthetic and respiratory temperature responses in Corymbia calophylla. Tree Physiology, 37, 1095–1112.

Bar, A., Michaletz, S. T., & Mayr, S. (2019). Fire effects on tree physiology. The New Phytologist, 223, 1728–1741.

Barton K. (2018) MuMIn: Multi-model inference. R Package Version 1.42.1. Retrieved from https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixedeffects models using lme4. Journal of Statistical Software, 67, 1–48.

Batllori, E., De Cáceres, M., Brotons, L., Ackerly, D. D., Moritz, M. A., Lloret, F., & Oliveras, I. (2018). Compound fire-drought regimes promote ecosystem transitions in Mediterranean ecosystems. Journal of Ecology, 107, 1187–1198.

Boer, M. M., Bowman, D. M. J. S., Murphy, B. P., Cary, G. J., Cochrane, M. A., Fensham, R. J., … Bradstock, R. A. (2016). Future changes in climatic water balance determine potential for transformational shifts in Australian fire regimes. Environtal Research Letters, 11, 065002.

Boer, M. M., Resco de Dios, V., & Bradstock, R. A. (2020). Unprecedented burn area of Australian mega forest fires. Nature Climate Change, 10, 171–172.

Bowen, B. J., & Pate, J. S. (1993). The significance of root starch in postfire shoot recovery of the resprouter Stirlingia latifolia R. Br. (Proteaceae). Annals of Botany, 72, 7–16.

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., & Peñuelas, J. (2011). Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences, 108, 1474–1478.

Casals, P., & Rios, A. I. (2018). Burning intensity and low light availability reduce resprouting ability and vigor of Buxus sempervirens L. after clearing. Science of the Total Environment, 627, 403–416.

Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., … Knox, K. J. E. (2013). Resprouting as a key functional trait: How buds, protection and resources drive persistence after fire. New Phytologist, 197, 19–35.

Collins, L. (2019). Eucalypt forests dominated by epicormic resprouters are resilient to repeated canopy fires. Journal of Ecology, 108, 310–324.

Cruz, A., Pérez, B., & Moreno, J. M. (2003). Plant stored reserves do not drive resprouting of the lignotuberous shrub Erica australis. New Phytologist, 157, 251–261.

Dietze, M. C., Sala, A., Carbone, M. S., Czimczik, C. I., Mantooth, J. A., Richardson, A. D., & Vargas, R. (2014). Nonstructural carbon in woody plants. Annual Reviews in Plant Biology, 65, 667–687.

Duan, H., Amthor, J. S., Duursma, R. A., O'Grady, A. P., Choat, B., & Tissue, D. T. (2013). Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO_2] and elevated temperature. Tree Physiology, 33, 779–792.

Esteso-Martínez, J., Camarero, J. J., & Gil-Pelegrín, E. (2006). Competitive effects of herbs on Quercus faginea seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, Northeast Spain). Écoscience, 13, 378–387.

Fairman, T. A., Bennett, L. T., & Nitschke, C. R. (2019). Short-interval wildfires increase likelihood of resprouting failure in fire-tolerant trees. Journal of Environmental Management, 231, 59–65.

Fox, J., & Weisber, S. (2019). An R Companion to Applied Regression, 3rd Thousand Oaks, CA: Sage.

Gessler, A., & Grossiord, C. (2019). Coordinating supply and demand: Plant carbon allocation strategy ensuring survival in the long run. New Phytologist, 222, 5–7.

Herrera, J. (1995). Acorn predation and seedling production in a lowdensity population of cork oak (Quercus suber L.). Forest Ecology and Management, 76, 197–201.

Hood, S. M., Varner, J. M., van Mantgem, P., & Cansler, C. A. (2018). Fire and tree death: Understanding and improving modeling of fire-induced tree mortality. Environmental Research Letters, 13, 113004.

Huang, J., Hammerbacher, A., Weinhold, A., Reichelt, M., Gleixner, G., Behrendt, T., … Hartmann, H. (2019). Eyes on the future—Evidence for trade-offs between growth, storage and defense in Norway spruce. New Phytologist, 222, 144–158.

IPCC. (2019). Climate Change and Land: An IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.

Johnson, D. M., Wortemann, R., McCulloh, K. A., Jordan-Meille, L., Ward, E., Warren, J. M., … Domec, J. C. (2016). A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species. Tree Physiology, 36, 983–993.

Jolly, W. M., Cochrane, M. A., Freeborn, P. H., Holden, Z. A., Brown, T. J., Williamson, G. J., & Bowman, D. M. J. S. (2015). Climate-induced variations in global wildfire danger from 1979 to 2013. Nature Communications, 6, 7537.

Karavani, A., Boer, M. M., Baudena, M., Colinas, C., Díaz-Sierra, R., Pemán, J., … Resco de Dios, V. (2018). Fire-induced deforestation in drought-prone Mediterranean forests: Drivers and unknowns from leaves to communities. Ecological Monographs, 88, 141–169.

Kavanagh, K. L., Dickinson, M. B., & Bova, A. S. (2010). A way forward for fire-caused tree mortality prediction: Modeling a physiological consequence of fire. Fire Ecology, 6, 80–94.

Korner, C. (2003). Carbon limitation in trees. Journal of Ecology, 91, 4–17.

Martínez-Vilalta, J., Prat, E., Oliveras, I., & Piñol, J. (2002). Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia, 133, 19–29.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., … Lloret, F. (2016).

Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis. Ecological Monographs, 86, 495–516.

Midgley, J. J., Kruger, L. M., & Skelton, R. (2011). How do fires kill plants? The hydraulic death hypothesis and Cape Proteaceae “fire-resisters”. South African Journal of Botany, 77, 381–386.

Mitchell, P. J.,O'Grady, A. P.,Tissue, D.T., White, D. A., Ottenschlaeger, M. L., & Pinkard, E. A. (2013). Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytologist, 197, 862–872.

Nagel, J. M., Griffin, K. L., Schuster, W. S., Tissue, D. T., Turnbull, M. H., Brown, K. J., & Whitehead, D. (2002). Energy investment in leaves of red maple and co-occurring oaks within a forested watershed. Tree Physiology, 22, 859–867.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution, 4, 133–142.

Nolan, R. H., Boer, M. M., Collins, L., Resco de Dios, V., Clarke, H., Jenkins, M., … Bradstock, R. A. (2020). Causes and consequences of eastern Australia's 2019–20 season of mega-fires. Global Change Biology, 26, 1039–1041.

Ogle, K., Barber, J. J., Barron-Gafford, G. A., Bentley, L. P., Young, J. M., Huxman, T. E., … Tissue, D. T. (2015). Quantifying ecological memory in plant and ecosystem processes. Ecology Letters, 18, 221–235. Palacio, S., Camarero, J. J., Maestro, M., Alla, A. Q., Lahoz, E., & Montserrat-Martí, G. (2018). Are storage and tree growth related? Seasonal nutrient and carbohydrate dynamics in evergreen and deciduous Mediterranean oaks. Trees, 32, 777–790.

Palacio, S., Hoch, G., Sala, A., Körner, C., & Millard, P. (2014). Does carbon storage limit tree growth? New Phytologist, 201, 1096–1100.

Palacio, S., Maestro, M., & Montserrat-Marti, G. (2007). Relationship between shoot-rooting and root-sprouting abilities and the carbohydrate and nitrogen reserves of Mediterranean dwarf shrubs. Annals of Botany, 100, 865–874.

Parra, A., & Moreno, J. M. (2018). Drought differentially affects the postfire dynamics of seeders and resprouters in a Mediterranean shrubland. Science of the Total Environment, 626, 1219–1229.

Pate, J. S., Froend, R. H., Bowen, B. J., Hansen, A., & Kuo, J. (1990). Seedling growth and storage characteristics of seeder and resprouter species of Mediterranean-type ecosystems of S.W. Australia. Annals of Botany, 65, 585–601.

Pausas, J. G., & Keeley, J. E. (2014). Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. New Phytologist, 204, 55–65.

Peguero-Pina, J. J., Mendoza-Herrer, O. S., Gil-Pelegrín, E., & SanchoKnapik, D. (2018). Cavitation limits the recovery of gas exchange after severe drought stress in Holm Oak (Quercus ilex L.). Forests, 9, 443.

Peguero-Pina, J. J., Sancho-Knapik, D., Barron, E., Camarero, J. J., Vilagrosa, A., & Gil-Pelegrin, E. (2014). Morphological and physiological divergences within Quercus ilex support the existence of different ecotypes depending on climatic dryness. Annals of Botany, 114, 301–313.

Peguero-Pina, J. J., Sancho-Knapik, D., Martín, P., Saz, M. A., GeaIzquierdo, G., Cañellas, I., & Gil-Pelegrín, E. (2015). Evidence of vulnerability segmentation in a deciduous Mediterranean oak (Quercus subpyrenaica E. H. del Villar). Trees, 29, 1917–1927.

Poorter, H. (1994). Construction costs and payback time of biomass: A whole plant approach. In J. Roy & J. Garnier (Eds.), A whole plant perspective on carbon-nitrogen interactions (pp. 111–127). Leiden: Backhuys Publishers.

Pratt, R. B., Jacobsen, A. L., Ramirez, A. R., Helms, A. M., Traugh, C. A., Tobin, M. F., … Davis, S. D. (2014). Mortality of resprouting chaparral shrubs after a fire and during a record drought: Physiological mechanisms and demographic consequences. Global Change Biology, 20, 893–907.

Resco, V., Ewers, B. E., Sun, W., Huxman, T. E., Weltzin, J. F., & Williams, D. G. (2009). Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C_3 woody legume, Prosopis velutina. New Phytologist, 181, 672–682.

Resco de Dios, V. (2020). Plant-fire interactions: Applying ecophysiology to wildfire management. Switzerland: Springer Nature.

Sánchez-Pinillos, M., Coll, L., De Cáceres, M., & Ameztegui, A. (2016). Assessing the persistence capacity of communities facing natural disturbances on the basis of species response traits. Ecological Indicators, 66, 76–85.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. Nature Methods, 9, 671–675.

Sperry, J. S., Donnelly, J. R., & Tyree, M. T. (1988). A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment, 11, 35–40.

Tyree, M. T., & Zimmerman, M. H. (2002). Xylem structure and the ascent of sap (2nd ed.). Berlin: Springer.

Urli, M., Porté, A. J., Cochard, H., Guengant, Y., Burlett, R., & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiology, 33, 672–683.

Valladares, F., Laanisto, L., Niinemets, Ü., & Zavala, M. A. (2016). Shedding light on shade: Ecological perspectives of understorey plant life. Plant Ecology & Diversity, 9, 237–251.

Vertregt, N., & Penning de Vries, F. W. T. (1987). A rapid method for determining the efficiency of biosynthesis of plant biomass. Journal of Theoretical Biology, 128, 109–119.

Wiley, E., & Helliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytologist, 195, 285–289.

Wu, M., Zhang, Y., Oya, T., Marcati, C. R., Pereira, L., & Jansen, S. (2020). Root xylem in three woody angiosperm species is not more vulnerable to embolism than stem xylem. Plant and Soil. https://doi.org/10.1007/ s11104-020-04525-0.

Zeppel, M. J. B., Harrison, S. P., Adams, H. D., Kelley, D. I., Li, G., Tissue, D. T., … McDowell, N. G. (2015). Drought and resprouting plants. New Phytologist, 206, 583–589.

Zimmermann, M. H. (1978). Hydraulic architecture of some diffuse-porous trees. Canadian Journal of Botany, 56, 2286–2295.

VI. Radiation and drought impact residual leaf conductance in two oak species with implications for water use models

Radiation and Drought Impact Residual Leaf Conductance in Two Oak Species With Implications for Water Use Models

Haiyan Qin[1], Carles Arteaga[2], Faqrul Islam Chowdhury[3], Elena Granda[4], Yinan Yao[1], Ying Han[1] and Víctor Resco de Dios[1,2,5]*

[1] School of Life Sciences and Engineering, Southwest University of Science and Technology, Mianyang, China, [2] Department of Crop and Forest Sciences, University of Lleida, Lleida, Spain, [3] Institute of Forestry and Environmental Sciences, University of Chittagong, Chattogram, Bangladesh, [4] Department of Life Sciences, University of Alcalá, Alcalá de Henares, Spain, [5] Joint Research Unit CTFC-AGROTECNIO, Universitat de Lleida, Lleida, Spain

ABSTRACT

Stomatal closure is one of the earliest responses to water stress but residual water losses may continue through the cuticle and incomplete stomatal closure. Residual conductance (g_{res}) plays a large role in determining time to mortality but we currently do not understand how do drought and shade interact to alter g_{res} because the underlying drivers are largely unknown. Furthermore, g_{res} may play an important role in models of water use, but the exact form in which g_{res} should be incorporated into modeling schemes is currently being discussed. Here we report the results of a study where two different oak species were experimentally subjected to highly contrasting levels of drought (resulting in 0, 50 and 80% losses of hydraulic conductivity) and radiation (photosynthetic photon flux density at 1,500 µmol m^{-2} s^{-1} or 35–45 µmol m^{-2} s^{-1}). We observed that the effects of radiation and drought were interactive and species-specific and g_{res} correlated positively with concentrations of leaf non-structural carbohydrates and negatively with leaf nitrogen. We observed that different forms of measuring g_{res}, based on either nocturnal conductance under high atmospheric water demand or on the water mass loss of detached leaves, exerted only a small influence on a model of stomatal conductance and also on a coupled leaf gas exchange model. Our results indicate that, while understanding the drivers of g_{res} and the effects of different stressors may be important to better understand mortality, small differences in g_{res} across treatments and measurements exert only a minor impact on stomatal models in two closely related species.

Keywords: cuticular conductance, stomatal conductance, night conductance, dark respiration, drought, shade

INTRODUCTION

Plant transpiration through stomatal pores and leaf cuticles dominates global evapotranspiration (Hetherington and Woodward, 2003). As water stress intensifies under global warming, there is an increasing interest toward understanding ecological variation in residual leaf conductance (g_{res}). After stomatal closure, water loss continues until mortality due to a mixture of cuticular water loss and incomplete stomatal closure (residual conductance; Blackman et al., 2016; Martin-Stpaul et al., 2017).

Studies addressing ecological and physiological variation in the drivers of residual conductance are currently rare (Heredia-Guerrero et al., 2018). According to a recent review on this topic (Duursma et al., 2019), only 10 studies have addressed the effect of drought on g_{res} and, from those, only 4 had been performed on trees. Consequently, multifactorial studies addressing ecological variation in residual conductance are much needed to understand its variation. For instance, while shade and drought are both known to decrease residual conductance (Boyer et al., 1997; Shepherd and Wynne Griffiths, 2006), it is currently unknown whether the effect of both stressors would be additive or interactive. However, the effects of residual conductance on mortality have been documented to be dramatic: time to mortality nearly doubles if g_{res} declines from 4 to 2 mmol m^{-2} s^{-1}(Duursma et al., 2019).

Understanding the physiological and ecological drivers of g_{res} has been the topic of some discussion (Riederer and Müller, 2007; Fernández et al., 2017). Some studies report that variations in the degree of sclerophylly (as indicated by leaf mass area) would increase g_{res} because leaves that are more scleromorphic will show thicker cuticles, but other work has demonstrated that changes in wax composition may compensate for such effect (Bueno et al., 2020). Another alternative, explored to a lesser degree, is that further reductions in g_{res} may be inhibited by changing carbohydrate allocation priorities (Zhang et al., 2020). In other words, as non-structural carbohydrate reserve pools deplete, cuticle production to prevent cuticular water losses may be limited by NSC availability.

Understanding variation in residual conductance is also necessary for models of water use (Leuning, 1995; Barnard and Bauerle, 2013; De Kauwe et al., 2015), where residual conductance acts as the intercept of commonly used stomatal models (g_{int}). The most common stomatal models being used in land surface models are Ball-Berry model types, which have the general form:

$$g_s = g_{int} + m\, A/C_a\, f(D) \qquad (1)$$

Where g_s is stomatal conductance, A, C_a, and D represent photosynthesis, ambient CO_2 concentration and vapor pressure deficit, respectively, and m is the slope parameter. When g_{int} is estimated through regression fitting, it may either be equal to 0, which creates problems because then the ratio of intercellular to ambient CO_2 (C_i/C_a) does not vary with light (Collatz et al., 1991; Leuning, 1995; Duursma et al., 2019), or it may be negative, which is nonsensical.

There are at least two possible definitions of g_{int}: (1) g_0, which represents the lowest conductance reached as photosynthesis tends to 0 because light declines (Leuning, 1995; Barnard and Bauerle, 2013); (2) g_{min}, which refers to the residual conductance after (complete or not) stomatal closure under strong water stress (Duursma et al., 2019). We note that some studies use g_{min} and g_{res} interchangeably but, for clarity, we will differentiate them here as previously defined.

The problem then becomes how to measure g_0 and g_{min}. g_0 could simply be measured as daytime conductance (g_d) under low light in non-droughted plants and, similarly, g_{min} could similarly be measured from g_d in droughted plants (for as long as photosynthesis tends to zero, in both cases; Barnard and Bauerle, 2013; Duarte et al., 2016). Additionally, residual conductance has most often been measured by monitoring the water mass loss in detached leaves (g_{MLD}; Kerstiens, 1996; Schuster et al., 2017). g_0 and g_{min} could thus be measured with this method by comparing g_{MLD} in plants that have grown under strong light limitation or under strong water limitation, respectively. The problem with this approach, however, is that some acclimation responses (particularly in response to low radiation) could alter leaf morphology and it is unclear whether g_0 measured through g_{MLD} after low light acclimation would be representative of that in plants without acclimation to low radiation.

An alternative would be to use nocturnal conductance (g_n; Lombardozzi et al., 2017) in non-droughted and droughted plants. An advantage would be that photosynthesis would always be zero in this case. Duursma et al. (2019), however, proposed that g_n should not be used given the evidence of active regulation of stomatal conductance overnight (Resco De Dios et al., 2019), and that the drivers of nocturnal conductance could differ from those driving daytime conductance (Ogle et al., 2012). Amongst other processes, g_n varies through time due to circadian regulation (Resco De Dios et al., 2015). However, g_n often retains some sensitivity to D such that maximum stomatal closure and, potentially, residual conductance, may be achieved at lower D than during the daytime (Barbour and Buckley, 2007). One could thus hypothesize that measurements of g_n under high D may be indicative of g_{res}.

Regardless of how g_0 and g_{min} are estimated, Duursma et al. (2019) proposed to replace Eq. 1 by:

$$g_s = \max \max g_0, g_{\min}, m\, A/C_a\, f\, (D) \qquad (2)$$

That is, according to Eq. 2, residual conductance would not be added to the right-hand term of Eq. 1. Instead, one would use measured residual conductance (the maximum between g_0 and g_{min}) as an actual minimum (De Kauwe et al., 2015). However, this formulation has not yet been tested against data and, therefore, we do not yet know whether it enhances the predictive power of stomatal models.

Here we evaluate the effects of shade and water stress on g_{res} across two different oak species, the deciduous *Quercus faginea* and the sclerophyll *Q. ilex*. These two species are common in the calcareous soils from Spain and the Western Mediterranean Basin and we expected conductance to be significantly lower in *Q. ilex*, a species with a more conservative water use. More specifically, we sought to test: (1) how do drought and shade interact to affect g_{res}? and (2) what are some of the possible mechanisms underlying variation in g_{res} across drought and shade treatments? Because g_{MLD} is probably the most accepted method to measure residual conductance, here we focused on g_{MLD}. In particular, we addressed whether g_{MLD} would be driven by water stress (as indicated by water potential), NSC, LMA, or nitrogen concentration (N_{mass}, an indicator of photosynthetic capacity), and whether g_{res} could limit respiration. We also sought to understand: (3) whether we obtain different values of g_{res} depending upon whether it is measured from g_{MLD}, g_n, and g_d; and (4) how do we incorporate residual conductance into BallBerry type stomatal models and what are the consequences of variation in g_{res} across treatments and types of measurements for coupled leaf gas exchange models?

MATERIALS AND METHODS

Experimental Design and Growing Conditions

The experiment was performed at the experimental fields from the University of Lleida (Spain; 41.62 N, 0.59 E). We built a rain-out shelter covered by clear polyethylene plastic, which is commonly used in greenhouse building. Half of the structure received solar radiation (sun treatment), with a maximum photosynthetically active radiation (PAR) of 1,500 µmol m^{-2} s^{-1}. The other half was covered by a dense shading cloth (shade treatment) with a maximum PAR of 35–45 µmol m^{-2} s^{-1}, which was near the light compensation point in this species (*data not shown*). The structure had openings on both sides to increase ventilation. Temperature inside the rain out shelter was 3°C higher than outside, but differences between the sun and shade treatment were negligible. Full details on the infrastructure have been provided elsewhere (Resco De Dios et al., 2020).

For this study we sourced 2 year-old seedlings from local nurseries (n = 120). The ecotypes for both species were original from the mountain range of the Iberian System. Plants were grown in 11 L cubic pots (20 cm × 20 cm × 27.5 cm). The substrate used was Humin Substrat Neuhaus N6 [KlasmanDeilmann GmbH, Geeste, Germany], a commercial potting mix. Pots were regularly fertilized with a slow release NPK MgO fertilizer (17-09-11-2, Osmocote Universal, KB, Ecully, France) and daily watered to field capacity until treatment implementation. The position of the pots was randomly shifted every other week.

The plants grew for 4 months into the rainout shelter before experiment inception in July 2017. That is, they developed new leaves under the assigned experimental light conditions. Although we cannot discard legacy effects from the previous growing season in the nursery (Aranda et al., 2001), all plants were treated equally.

We performed a full factorial experiment with the plants experiencing two light treatments crossed with three water stress treatments. Half of the plants grew under the sun treatment and the other half under the shade treatment, as previously described. We implemented three different water stress treatments using three different levels of percent loss of hydraulic conductivity (PLC): (i) P_0, where plants were irrigated at field capacity; (ii) P_{50}, where plants experienced 50% losses in hydraulic conductivity and which represents an important stress; and (iii) P_{80}, where the plant experienced 80% losses in hydraulic conductivity, which represents a major stress and potentially mortality (Resco et al., 2009).

We kept plants at field capacity until treatment implementation. We then stopped watering and allowed plants to dehydrate and we measured midday stem water potential (9_{md}) every other day in a subset of plants (n = 5). The levels of PLC were controlled from the relation between midday shoot water potential (9_{md}) and PLC values reported previously in vulnerability curves from *Quercus faginea Lam.* (Esteso-Martínez et al., 2006) and *Quercus ilex L.* (Peguero-Pina et al., 2014). Shoot 9_{md}

was regularly measured during treatment implementation with a pressure bomb (PMS 1000, PMS Instruments, Albany, Oregon) after clipping the sample and allowing for equilibration in the dark for ~30 min. Once plants reached the target PLC, we kept soil moisture constant at that level for 2 weeks. This was achieved by weighing a subset of pots (n = 5 per each treatment) and adding back the water that had evaporated every day. We also measured native embolism to test the actual levels of PLC that we achieved in every treatment, as previously published (Resco De Dios et al., 2020). It is important to note that we did not always reach the target PLC levels (see **Supplementary Table 1**), but treatment implementation was successful in that we created a gradient in water availability with our treatments. Full details have been provided by Resco De Dios et al. (2020).

Gas Exchange Measurements

Leaf gas exchange was measured with a portable photosynthesis system (LI-6400XT, Li-Cor Inc., Lincoln, NE, United States). We measured 3–5 plants in each treatment at two different periods during the night: between 23:00 h and 01:00 h and between 03:00 h and 05:00 h, and also during the day (10:00–13:00 h). We did not observe significant differences between the stomatal conductance measured over night at the different times (p = 0.79) and measurements were pooled together in subsequent analyses. To understand if measurement errors arising from low flux rates affected our measurements, we also conducted measurements with an empty chamber for 4–5 h, following previously published protocols (Resco De Dios et al., 2013). Results were always one or more orders of magnitude lower or negative. Given these results, we concluded that leaf observations were reliable and that a general correction was not required.

Block temperature was set at 25°C during the night and at 30°C during the day, CO_2 at 400 ppm and relative humidity at ~30%. This meant that D during nighttime measurements was at ~2.2 kPa, which was substantially higher than that naturally occurring during the night (Resco De Dios et al., 2020). We chose this design to induce nocturnal stomatal closure and test whether g_n indicates g_{res}.

During the daytime, we performed measurements at two different levels of PAR: 1,500 µmol m^{-2} s^{-1} and 40 µmol m^{-2} s^{-1}. We first measured under growth PAR (1,500 µmol m^{-2} s^{-1} for plants in the sun treatment and 40 µmol m^{-2} s^{-1} for plants in the shadow treatment) and then at the other PAR level (40 µmol m^{-2} s^{-1} for plants in the sun treatment and 1,500 µmol m^{-2} s^{-1} for plants in the shadow treatment). The leaves were exposed for 10–20 min under the different light intensities until acclimation to the new light level. We only used data measured under growth PAR for analyses, and the rest was reserved for model validation. We note that a sudden exposure to 1,500 µmol m^{-2} s^{-1} for a plant growing in the shade would represent a sunfleck, and this could affect the performance of steady-state stomatal models (Way and Pearcy, 2012). When the leaf did not cover the chamber completely, it was scanned and we corrected measurements for leaf area.

In order to parameterize the photosynthesis component of the coupled leaf gas exchange model, we also measured the response of photosynthesis (A) to different internal CO_2 concentrations (C_i) following the protocols from Long and Bernacchi (2003). Briefly, we started measurements with an ambient CO_2 concentration (C_a) of 400 ppm and, after 5 min of acclimation, we sequentially changed C_a to 300, 250, 200, 150, 100, 50, 0, 400, 500, 650, 800, 1000, 1250, and 1500 ppm. These measurements were performed at saturating light (1,500 µmol m^{-2} s^{-1}), setting block temperature at 30°C and with RH as high as we could achieve, which was ~50%.

Measurements of g_{MLD}

g_{MLD} was measured as the mass loss of detached leaves following Phillips et al. (2010) in five leaves per treatment, weighting the leaves every 5 min during 2 h after collection. We wrapped the petiole with paraffilm so that only water lost through the leaf was measured. We performed the measurements in the laboratory, briefly after collection, where we monitored the temperature and relative humidity. Residual conductance was then calculated as:

$$g_{\mathrm{MLD}} = E_{\mathrm{MLD}} \, P \, D^{-1} \qquad (3)$$

where E_{MLD} is mass loss per projected leaf area (mol m^{-2} s^{-1}), P is atmospheric pressure (kPa) and D is the vapor pressure deficit (kPa). g_0 was defined as g_{MLD} when the leaf originated from the shade treatment at P_0 (P_0_shade) and g_{min} was defined as g_{MLD} at P_{80} in the sun treatment (P_{80}_sun).

Analyses of Non-structural Carbohydrates and Elemental Composition

To better understand the physiological mechanisms explaining variations in g_{res} with treatments, we analyzed the concentrations of non-structural carbohydrates, changes in leaf mass per area (LMA) and nitrogen concentrations (N_{mass}). We collected all the leaves in five plants for each treatment.

Immediately after collection, we scanned the leaves to measure the total area and they were then microwaved for 30 s and 700W to stop further metabolic processes. We then oven dried the samples (48 h in 105°C) and recorded the dry mass. Leaf area and dry weight was used to estimate LMA.

We followed previously developed protocols for extracting the percentage for sugar and starch (Palacio et al., 2007). This method consists of grinding the dried leaves with a mill (IKA A10, IKA-Werke, Staufen, Denmark) and making two extractions: one for extracting soluble sugars (sugars from now on) and a second extraction for starch. The first step of the sugar extraction consisted of adding 10 ml of ethanol (80% v/v) to 50 mg of sample, which we then left for 30 min at 60°C in water bath, and then we centrifuged (NEYA 8, REMI ELEKTROTECHNIK LTD., Vasai, India) the sample for 10 min at 3200 rpm. In the second step we added 50 µl of the supernatant, 450 µl of ethanol (80%), 500 µl of phenol (28%), and 2500 µl of sulfuric acid (96%), we shook the mix and let it stand for 30 min. In the third step we read the absorbance at 490 nm with spectrophotometer (Spectrophotometer UV-1600PC, VWR, Radnor, PA, United States) after removing the supernatant and drying the sample at 70°C during 16 h.

In the starch extraction, we added 4 ml of sodium acetate (pH 4.5) to the dry sample and left it for 60 min in a water bath (60°C). Once the sample cooled down, we added 1 ml of Amyloglucosidase (0.5% w/v) and we incubated the mix in the stove for 16 h at 50°C. We then added sample 50 µl of supernatant, 450 µl of sodium acetate (pH 4.5), 500 µl of phenol (28%), and 2,500 µl of sulfuric acid (96%). We then mixed it and let sit for 30 min, and then we measured the absorbance at 490 nm with the spectrophotometer.

We analyzed nitrogen concentration in an elemental analyzer (Carlo Erba 1110 Elemental Analyzer) at the University of Wyoming following previously published procedures (Hoffman et al., 2019).

Statistical Analyses

We examined statistical differences across treatments in g_{MLD}, g_n, and g_d using an ANOVA (followed by Tukey's HSD test) with species, light and water treatments as explanatory variables. Measurements of g_{MLD}, were conducted on different individuals within a treatment. Consequently, we examined whether values were comparable within a given treatment by examining variation in the mean ± 95% CI in g_{MLD}, g_n, and g_d.

To examine potential drivers of variation in g_{res}, we additionally performed correlation analyses between conductance and NSC, LMA, gas exchange parameters and 9_{md}.

All data was analyzed with R 3.6.3 (R Core Team, 2020) using base packages and, additionally, “corrplot” or plotting the correlation table (Wei and Simko, 2017).

Modeling

In order to examine the effects of the different forms of measuring residual conductance over stomatal predictions and coupled photosynthetic responses, we performed two exercises. First, we examined the effects on stomatal predictions on different implementations of Eqs 1, 2. Second, we examined the effects of the different measured values of g_{res} on a photosynthesis-stomatal conductance coupled model.

For the first exercise, we compared the performance of different versions of the Ball-Berry (BB) model (Ball et al., 1987). First, we examined the version proposed by Duursma et al. (2019, BBD):

$$g_s = \max \max g_0, g_{min}, m \text{ A RH}/C_a \qquad (4)$$

and we used three different forms of the left hand term [max (g_0, g_{min})]. That is, we compared model performance when the left hand term used g_0 and g_{min} estimated from g_{MLD} (BBD$_{MLD}$), g_n (BBD$_n$), and g_d (BBD$_d$). In all cases, g_0 was defined as conductance (g_{MLD}, g_n, or g_d, depending on the case) in the shade treatment without water stress (P_0_shade) and g_{min} as conductance in the sun under strong water stress (P_{80}_sun). We compared these results with the original version of the Ball-Berry model (BB):

$$g_s = g_{int} + m \text{ A RH}/C_a \qquad (5)$$

where g_{int} and m were both estimated through least squares fitting.

Finally, we used an intermediate option where we used Eq. 5 but where g_{int} was replaced by actual g_{MLD} measurements (BB_meas_g$_{MLD}$), instead of being estimated through least squares. We also tried with g_n, in addition to g_{MLD}, but differences were negligible, as will be discussed later in more detail.

Model calibration was performed with data collected under growth PAR (1,500 µmol m^{-2} s^{-1} for sun treatment and 40 µmol m^{-2} s^{-1} for the shade treatment). Model validation was performed with data collected under different PAR levels. That is, with PAR at 40 µmol m^{-2} s^{-1} for the sun treatment and at 1,500 µmol m^{-2} s^{-1} for the shade treatment. Model comparison was performed calculating the Akaike Information Criterion (AIC) and a model was considered more plausible when the AIC was smaller by a difference of 2 or more units (Burnham and Anderson, 2002). We also examined the variation in the slope, intercept and R^2 of the observed *vs* predicted relationship.

For the second exercise, we simulated the effects of the different values of g_{MLD}, g_n and g_d on predictions of C_i with varying PAR and on the effect temperature on leaf evaporation. We used the A/C_i curves to parameterize a coupled photosynthesis model (Duursma, 2015) and we conducted the simulation following previously published protocols (Duursma et al., 2019). We note that differences in mesophyll conductance across species and treatments could affect estimates of photosynthetic parameters (Flexas et al., 2012).

RESULTS

Effects of Shade and Drought on g_{MLD} and g_n

We observed that g_{MLD} varied significantly with species and light and also with light and water (**Table 1** and **Figure 1**). The interactions between species and light resulted in g_{MLD} significantly declining from 6.9 in the sun to 3.4 mmol m^{-2} s^{-1} shadow in *Q. faginea*. However, g_{MLD} in *Q. ilex* did not differ across light levels (5.6 in the sun and 4.4 mmol m^{-2} s^{-1} in the shade). The interaction between light and water was such that g_{MLD} declined with drought in the sun treatment (from 7.4 at P_0 to 5.5 mmol m^{-2} s^{-1} at P_{80}), but g_{MLD} increased with drought in the shade from 3.1 at P_{50} to 5.0 mmol m^{-2} s^{-1} at P_{80}).

Variation in g_n followed a pattern of variation similar to that of g_{MLD} in that it also varied significantly with species and light treatments (**Table 1** and **Figure 2**). g_n was not different between species at the shade treatment (4.5 and 5.6 mmol m^{-2} s^{-1} in *Q. faginea* and *Q. ilex*, respectively), but there was a significant increase in g_n in *Q. faginea* (7.8 mmol m^{-2} s^{-1}) in the sun treatment. Instead, g_n in *Q. ilex* at the sun treatment was similar to that in the shade (4.0 mmol m^{-2} s^{-1}; **Figure 1B**). Differences across water treatments were not significant.

TABLE 1 | ANOVA Table on the effects of species, light treatment, water treatment on residual conductance measured from the mass loss of detached leaves (g_{MLD}), from nocturnal conductance (g_n), and also from daytime conductance (g_d).

Factor	**Df**	***F***	***P*-value**
g_{MLD} **Species**	1	0.12	0.73
Light	1	23.4	<0.0001
Water	2	1.35	0.27
Species × Light	1	5.41	0.02
Species × Water	2	0.75	0.48
Light × Water	2	4.04	0.02
Species × Light × Water	2	0.35	0.71
g_n **Species**	1	0.89	0.35
Light	1	1.27	0.26
Water	2	2.14	0.12
Species × Light	1	4.98	0.03
Species × Water	2	2.53	0.09
Light × Water	2	0.14	0.87
Species × Light × Water	2	0.20	0.82
g_d **Species**	1	5.84	0.02
Light	1	51.21	<0.001
Water	2	138.66	<0.001
Species × Light	1	16.99	<0.001
Species × Water	2	1.08	0.34
Light × Water	2	91.74	<0.001
Species × Light × Water	2	21.24	<0.001

Effects of Shade and Drought on g_d

Of particular relevance for this study is to examine g_d when A_{net} approaches zero (**Figure3B**), so that one can test the potential use of g_d as an indicator of residual conductance. There are different definitions in the literature as to what is meant by photosynthesis approaching zero (Leuning, 1995; Barnard and Bauerle, 2013). Here we used g_d when A_{net} was at, or below, 1 µmol m^{-2} s^{-1}. In *Q. faginea*, this occurred under the shade treatments at all water stress levels, where g_d varied between 14.6 and 29.5 mmol m^{-2} s^{-1} (**Figure 3**).

In *Q. ilex*, A_{net} was always below 1 µmol m^{-2} s^{-1} in the shadow treatments at all water stress levels. However, there was significant variation in g_d as it varied from 58 mmol m^{-2} s^{-1} in P_0 to 14 and 4 mmol m^{-2} s^{-1} in P_{50} and P_{80}, respectively. Within the sun treatments, A_{net} was always below 1 under water stress (at P_{50} and P_{80}) where g_d varied between 4 and 1 mmol m^{-2} s^{-1}, respectively. g_d under water stress (P_{50} and P_{80}) was not different between shadow and sun treatments (**Figures 3A,B**).

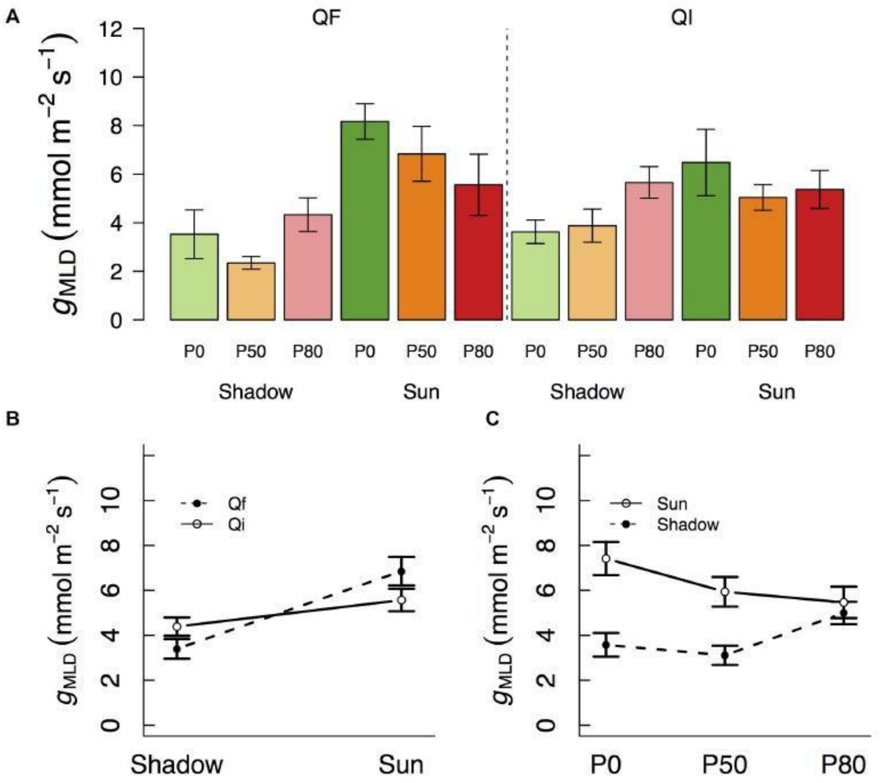


FIGURE 1 | (A) Variation in residual conductance measured from the mass loss of detached leaves (g_{MLD}) across different light treatments (shadow vs sun) and water treatments (P_0, P_{50}, and P_{80}). Significant interactions across treatments are plotted in **(B,C)**. Bars indicate mean values per treatment and error bars indicate SE.

Differences Between g_{MLD}, g_n, and g_d

Within a given treatment, g_n was indistinguishable from g_{MLD}: 95% CI error bars always overlapped (**Figure 3C**). In *Q. faginea*, values of g_{MLD} were usually below those of g_n, but the absolute difference was less than 4 mmol m^{-2} s^{-1}. In *Q. ilex*, the difference between g_n and g_{MLD} was less than 1 mmol m^{-2} s^{-1}.

In contrast, g_d was consistently and significantly above both, g_{MLD} and g_n in *Q. faginea*. It should be noted that, for this comparison, we only used g_d when A_{net} was below 1 µmol m^{-2} s^{-1}. That is, we did not seek to compare values of g_d with g_{MLD} and g_n if A_{net} was above 1 µmol m^{-2} s^{-1} because, in that case, photosynthesis does not tend to zero. The average difference of g_d with g_{MLD} was 17.7 mmol m^{-2} s^{-1} and the average difference of g_d with g_n was 10 mmol m^{-2} s^{-1}. The only case in which g_d was not different from g_{MLD} and g_n was in the sun treatments in *Q. ilex*.

Correlates Explaining Variation in g_{MLD}

Overall, the relationships between the different indicators of g_{MLD} and other physiological parameters were species-specific (**Figure 4A**). The only exceptions were N_{mass} and NSC concentrations which had a negative and a positive correlation, respectively, with g_{MLD} in both species (**Figure 4**). In turn, N_{mass} correlated negatively with NSC concentrations and LMA in both species. NSC also correlated with LMA in both species, albeit positively. In *Q. faginea*, g_{MLD} and g_n also correlated positively with LMA and g_{MLD} also correlated positively. In *Q. ilex*, g_n showed a negative correlation with respiration (*R*) and a positive correlation with 9_{md} and with $A_{net.}$ NSC concentrations were negatively affected by the shade treatment (**Supplementary Figure 1A**).

Modeling g_d: Comparing Different Formulations of the BB Model

We first compared the performance of the model proposed by Duursma et al. (2019) when g_0 and g_{min} had been defined on the basis of g_{MLD} (BBD_{MLD}), of g_n (BBD_n), and of g_d (BBD_d). In all cases, the original g_0 and g_{min} were defined as the level conductance (g_{MLD}, g_n, or g_d, depending on case) in the P_0_shade treatment (low light) and in the P_{80}_sun treatment, respectively, (high water stress).

Model performance was superior when the model was based on g_{MLD} (BBD_{MLD}), but differences with the model based on g_n were minor (*1*AIC = 0.3 for *Q. faginea* and 2.3 for *Q. ilex*). However, the model based on g_d showed consistently a larger AIC, indicating smaller plausibility (**Table 2**).

We compared the performance of these three models against the original Ball- Berry (BB) and we observed that BBD_{MLD} and BBD_n performed better only in *Q. faginea*, where the difference in AIC was bigger than 4. For *Q. ilex*, however, the AIC was similar across models although the intercept of the observed *vs* predicted relationship was significantly different from 0 only in the BB model.

Finally, we compared the performance of the Ball-Berry model but where, instead of fitting g_{int} through least squares, we use actual g_{MLD} measurements (BB_meas_g_{MLD}), which we defined originally as g_{MLD} under water stress (P_{80}_sun). We observed that this was the best model in *Q. ilex* as it had the smallest AIC although the difference was not significant with BBD_{MLD} (*1*AIC = 1.64). In *Q. faginea*, BB_meas_g_{MLD} peformed worst than BBD_{MLD} (*1* AIC = 2.6).

Differences between BB_meas_g_{MLD} were significant with the BB model (AIC = 2 for both species) and it was also more plausible than the BBD_d model in *Q. faginea* (AIC > 2). Differences between BB_meas_g_{int} and the other models were not significant. We tried fitting BB_meas_g_{int} with different values of g_{int} (e.g., using values under shade, or from g_n), but differences were not significant (*data not shown*).

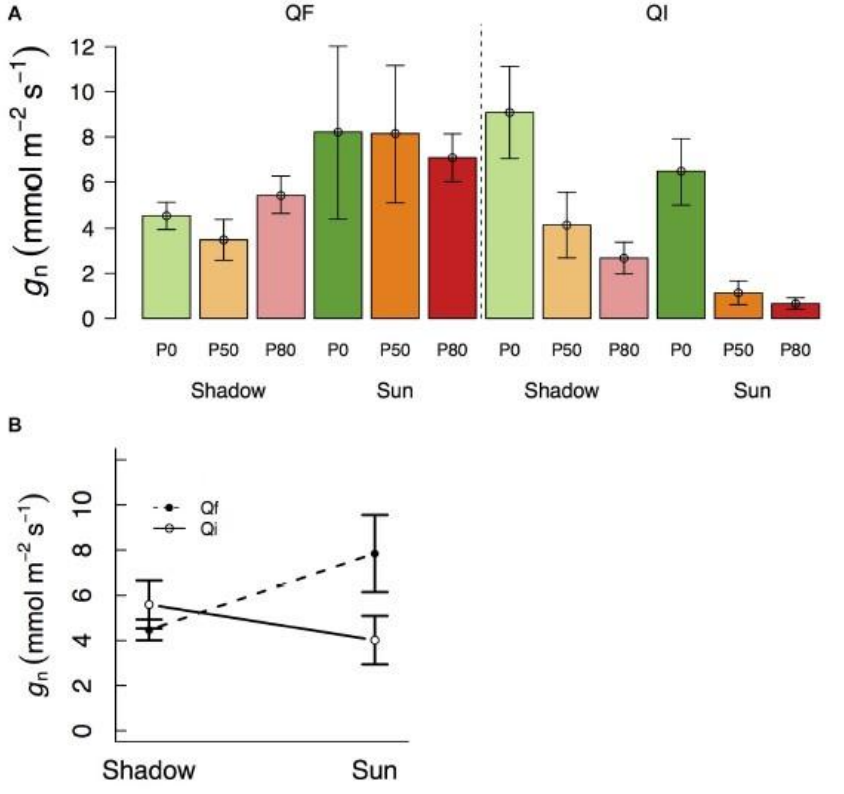


FIGURE 2 | (A) Variation in residual conductance measured from nocturnal conductance under high D (g_n) across different light treatments (shadow vs sun) and water treatments (P_0, P_{50}, and P_{80}). Significant interactions across treatments are plotted in **(B)**. Bars indicate mean values per treatment and error bars indicate SE.

Modeling g_d: Coupled Leaf Gas Exchange Model

Depending on how g_{res} was measured, we found significant differences of simulated gas exchange. In particular, when g_d was used we always observed higher values of C_i at any PAR level and also higher leaf transpiration rates (E_l) as temperatures increased because g_d was often larger than g_{MLD} and g_n (**Figure 5**).

Generally speaking, there was little difference in simulated C_i and E_l regardless of whether g_{MLD} or g_n were used, and whether they were defined from g_0 or from g_{min}. The only exception was that, in *Q.*

ilex, there were some differences in predicted C_i (particularly at low PAR levels) and in predicted leaf transpiration (particularly at peak E_l) when g_{res} was defined from g_n: using g_n from the P_0_shade (g_0) treatment led to higher predicted C_i and E_l than using g_n from the P_{80}_sun treatment (g_{min}). It should be noticed that g_n from the P_0_shade treatment was one order of magnitude larger than g_n from the P_{80}_sun treatment (9.1 vs 0.6 mmol m^{-2} s^{-1}, respectively).

DISCUSSION

We observed that residual conductance varied significantly across light and water treatments in an interactive (nonadditive) fashion and the responses differed across species. There were no significant differences as to whether residual conductance was measured from g_{MLD} or from g_n, but the values were significantly higher when using g_d. g_{MLD} was positively correlated with NSC concentrations, suggesting that further reductions in g_{MLD} under drought may be limited by low NSC availability. From a modeling perspective, the small measured differences between g_{MLD} and g_n generally did not impact model performance. Although residual conductance differed significantly under experimental treatment, such differences in residual conductance showed only a moderate impact on model performance. That is, model performance did not critically depend upon whether residual conductance was measured under strong shade or under strong water stress. There was also little difference in model fit when either g_{MLD} or g_n were used as an absolute minimum in Eq. 4 (BBD_{MLD} or BBD_n), or when they were used as the intercept of the BB equation (BB_meas_g_{MLD}).

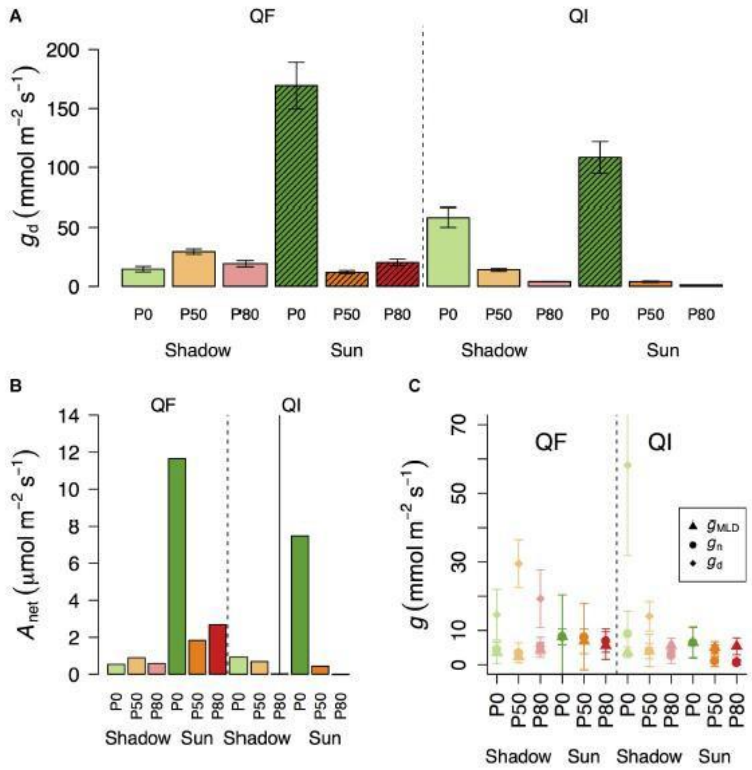


FIGURE 3 | Variation in daytime leaf conductance (**A**, g_d) and net assimilation (**B**, A_{net}) across different light treatments (shadow vs sun) and water treatments (P_0, P_{50}, and P_{80}). Bars indicate mean values per treatment and error bars indicate SE. Hatched bars in **(A)** indicate that A_{net} in that treatment is significantly higher than 1. **(C)** Differences in residual conductance as measured by from the mass loss of detached leaves (g_{MLD}), from nocturnal conductance under high D (g_n), or from g_d when A_{net} was smaller than 1 [note that some values of g_d are missing in **(C)** if A_{net} for that treatment was higher than 1]. Error bars indicate 95% CI.

Shade and Drought Interact as Drivers of g_{MLD} Although Response Are Species-Specific

We observed that g_{MLD} declined under increasing drought in the sun treatment. In the shade treatment, however, g_{MLD} remained low and constant, regardless of the water treatment. This result indicates that drought only affects g_{MLD} under high light because, under shade, light limitations lower g_{MLD} to a minimum that is not affected by water stress. It is worth noting that, at least for some species, full acclimation after changes in the light growth environment may require more than one growing season (Aranda et al., 2001). In other words, the strong limitation imposed by the low light over g_{MLD} may increase even more in subsequent years.

Previous studies had identified how g_{MLD} often decreases under exposure to water stress and light, as a result of changes in wax composition, when each effect is examined in isolation (Shepherd and

Wynne Griffiths, 2006). However, our experiment may be the first to examine g_{MLD} responses in a multifactorial experiment. Interestingly, light and water effects were not additive. That is, we did not observe a lower g_{MLD} under low light and high water stress, as would be expected from an additive effect of both factors.

The response to shade was, however, species-specific. g_{MLD} increased in the sun treatment only in the deciduous Q. *faginea*, whereas the increase in *Q. ilex* g_{MLD} under sun was not significant.

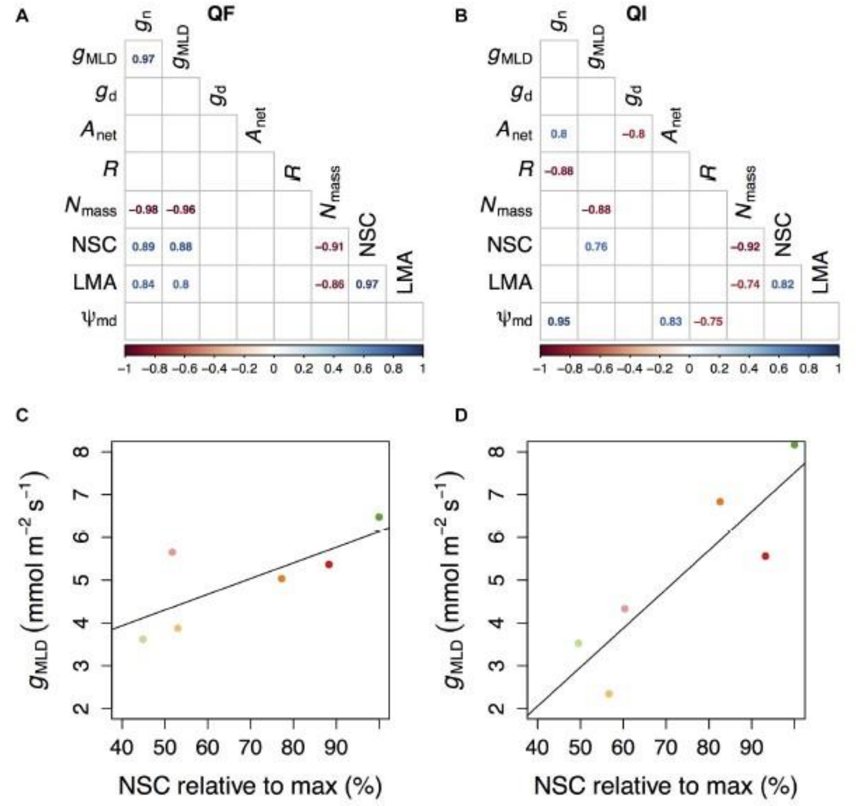


FIGURE 4 | Correlation table between the measured parameters in *Q. faginea* (Qf, **A**) and *Q. ilex* (Qi, **B**). Relationship between conductance measured as mass loss of detached leaves under and non-structural concentrations (NSC) in Qf **(C)** and Qi **(D)**. NSC are provided relative to the maximum values measured in our experiment. Values in **(C,D)** indicate mean per treatment, and each treatment is indicated by a different color following the same convention as in **Figures 1–3**.

TABLE 2 | Model comparison. We compared for *Q. faginea* (QF) and *Q. ilex* (QI) different models based on the Akaike Information Criterion (AIC), the change in AIC relative to the lowest (*1*AIC) and the R^2, slope and intercept of the observed vs predicted relationship. For the slope and intercept we show the mean value (and SE).

	g_{int} (mmol m 2 s 1)	**AIC**	***1* AIC**	**R_2**	**Slope**		**Intercept**	
QF **BBD$_{MLD}$**	5.5	−106.88	0	0.88	1.04	(0.09)	0.001	(0.005)
BBD$_n$	7.1	−106.6	0.28	0.87	1.06	(0.09)	0.0006	(0.005)
BBD$_d$	20.3	−102.32	4.56	0.84	*1.23*	*(0.13)*	*−0.013*	*(0.007)*
BB[1]	8.9	−102.3	4.58	0.85	1.02	(0.09)	−0.01	(0.006)
BB_meas_g_{MLD}	5.5	−104.3	2.58	0.86	0.99	(0.09)	0.002	(0.005)
QI **BBD$_{MLD}$**	5.3	−98.52	1.64	0.97	1.2	(0.05)	−0.0001	(0.003)
BBD$_n$	9.1	−96.22	3.94	0.97	1.2	(0.05)	−0.002	(0.008)
BBD$_d$	58.2	−69.92	30.24	0.85	1.6	(0.17)	0.067	(0.01)
BB[1]	16.8	−98.14	2.02	0.98	1.3	(0.05)	0.018	(0.004)
BB_meas_g_{MLD}	5.3	−100.16	0	0.98	1.2	(0.04)	−0.001	(0.003)

The models compared include the Ball-Berry model (Eq. 5, BB), the BB model where the intercept is measured, rather than estimated (BB_meas_g_{MLD}) and the modification of the Ball-Berry model proposed by Duursma (Eq. 4, BBD) using g_{MLD} (BBD$_{MLD}$), g_n (BBD$_n$), or g_d (BBD$_d$) as g_0 and g_{min}. We also provide the values of the intercept, g_{int} (the maximum between g_0 and g_{min} in the first three models), that were used in each case. Values in bold (or italics) in slope and intercept indicate values significantly (or marginally) different from 1 and 0, respectively.

---

[1] *Note that g_{int} in this case is estimated through least squares, rather than measured as in the other models.*

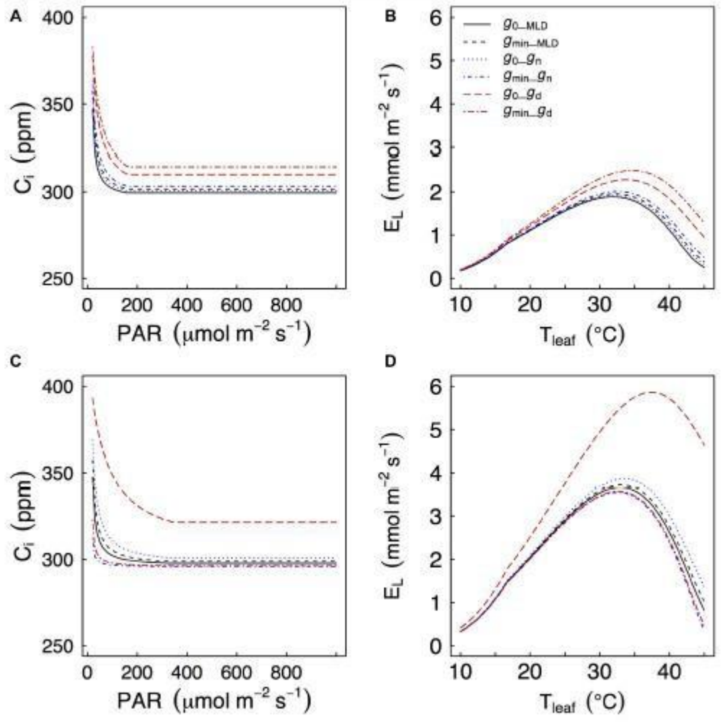


FIGURE 5 | Effects of different values of g_{res} on a coupled photosynthesis model as in Duursma et al. (2019). Models are for *Q. faginea* **(A,B)** and *Q. ilex* **(C,D)**. g_0 and g_{min} represent g_{res} under low light (P_0_shadow treatment) and water stress (P_{80}_sun treatment), respectively. Subscripts $_{MLD}$, $_n$, and $_d$ indicate that g_0 or g_{min} (depending on case) were estimated from mass loss of detached leaves, from nocturnal conductance, or from daytime conductance.

g_{MLD} Correlated With Low NSC Concentrations

We can speculate that the reason why g_{MLD} was not lower under the high water stress and shade treatment (relative to other shade treatments under less water stress) is related to carbohydrate limitations. We observed a significant and positive correlation between NSC and g_{MLD} across species. A synthesis of variation in NSC across species reports that a minimum NSC of 46% is always conserved (Martínez-Vilalta et al., 2016). In our results we also observed a minimum NSC that was close to the 46% of the maximum NSC that we measured (**Figures 4C,D**).

A possible explanation on why g_{MLD} did not decrease further under the joint drought and shade stress is related to a lack of NSC to feed the building of additional wax and/or epidermal layers. That is, once plants have reached the minimum NSC threshold of 46% relative to maximum, they will seek to preserve their NSC for other functions, such as osmoregulation, at the expense of building thicker cuticles or additional wax layers. We note that osmoregulation under shade may be impaired in oaks (Aranda et al., 2005; Rodriguez-Calcerrada et al., 2010).

At any rate, this is the first study, to our knowledge to raise this possibility. This result should thus be interpreted with caution. We acknowledge that the correlation between g_{MLD} and NSC may have been affected by jointly considering plants under different light and water regimes. Subsequent work would thus be needed to confirm this hypothesis.

Residual Conductance in Relation to Respiration, LMA and N_{mass}

Despite stomatal closure, g_{res} did not limit CO_2 diffusion out of the leaf. In fact, there was a negative correlation between nocturnal conductance and respiration in one of our (*Q. ilex*) species, indicating higher CO_2 efflux at lower g_n and, consequently, that reduced g_n levels were far from limiting respiration. This contradicts earlier studies that cytotoxic CO_2 build-up could occur under nocturnal stomatal closure (Fricke, 2019) but it aligns along with the results of modeling, indicating that only under conductances that are orders of magnitude lower to those reported here could a cytotoxic CO_2 build-up occur (Resco DeDios et al., 2019).

g_{MLD} increased in the sun in *Q. faginea.* LMA also increased with light (*data not shown*) and it was significantly correlated with g_n and with g_{MLD} in *Q. faginea*. LMA is an indicator of the degree of sclerophylly, which could serve to decrease residual conductance by increasing cuticle thickness. However, LMA also increased with light in the sclerophyll *Q. ilex*, where LMA did not correlate with g_n or g_{MLD}. This result matches with previous studies in *Quercus* indicating that any effects of LMA in g_n and g_{MLD} may be modified by changes in the cuticle composition (Bueno et al., 2020). We note that this argument is speculative and based only on circumstantial evidence. N_{mass} showed a negative correlation with g_{MLD} in both species, and with g_n in *Q. faginea*. This result indicates that species with a higher photosynthetic investment will decrease the investment in residual conductance. This points toward a potential mechanism underlying the trade-off between investment for C uptake (higher N_{mass}) and preventing catastrophic water losses (reduced g_{MLD}). Further studies will be necessary to test the generality of this hypothesis.

Residual Conductance May Be Measured via g_{MLD} or via g_n Under High D

Measurements of nocturnal conductance under the relatively high D from this experiment were statistically indistinguishable from independent measurements of residual conductance indicating that the latter was driving the former. It had been previously argued that measurements of g_n would not be valid indicators of residual conductance, because g_n is actively regulated (Duursma et al., 2019). Our results suggest that this argument from Duursma et al. (2019) may only be valid when g_n is measured under low D.

Previous studies document that stomata often reach complete closure (or as complete as it can be) under lower D in the night, than in the day (Barbour and Buckley, 2007). This phenomenon would explain why g_n was much lower than g_d although D was comparable, and it is likely explained by the capacity of stomata to sense and open in response to light.

We also show how modeling results were not affected by either using g_{MLD} or g_n. This result, however, needs to be interpreted with caution. We only focused on BB-type stomatal models and other results may be obtained in different model types. For instance, as Duursma et al. (2019) noted, changing minimum conductance from 2 to 4 mmol m^{-2} s^{-1} halved the time to reach mortality in a hydraulics model because it doubled the water losses (Duursma et al., 2019). Although differences were statistically not significant between g_{MLD} and g_n, we still observed differences in mean residual conductance of 4 mmol m^{-2} s^{-1} across measurements, indicating that measurement errors and other sources of uncertainty may play a large for other model types, such as mortality models.

Modeling g_s

We acknowledge our dataset was limited to thoroughly test the best form of the BB model: we sampled under highly contrasting light and water conditions, but only once in time. We would thus need data over more time periods for a more thorough evaluation. However, our dataset allows for the development of some hypotheses, which may be expanded in subsequent studies.

We observed that there were only little differences between Eq. 5, where the original BB function was used but including measured g_{MLD} (BB_meas_g$_{MLD}$), instead of the version proposed by Duursma et al. (2019; Eq. 4). Duursma et al. (2019) note that residual conductance acts as an actual minimum in the function they propose. However, if the goal is to use residual conductance as an actual stomatal minimum, one could consider the following equation instead:

$$g_s = \max \min g_0, g_{\min}, m\, A\, RH/Ca \qquad (6)$$

where the minimum between g_0 and g_{min} is chosen [not the maximum, as proposed by Duursma et al. (2019)].

At any rate, we did not observe major differences in model performance between the BBD model or Eq. 5. This result indicates that it is unlikely that losses in model performance will derive from the adoption of the alternative model formulations, as proposed by previous studies (De Kauwe et al., 2015; Duursma et al., 2019).

Our results also indicate that g_{MLD} and g_n can both be interchangeably, and that the choice between g_0 and g_{min} exerts negligible consequences for model fitting. Earlier studies indicate a major effect of g_{res} (De Kauwe et al., 2015; Duursma et al., 2019). This is because those studies used a wide range of g_{res} values (10–40 mmol m^{-2} s^{-1}, depending on the case), much higher that the variability we reported here when using g_{MLD} and g_n across treatments (**Table 2**). Synthesis studies similarly indicate limited variation in g_{res} within a family (Duursma et al., 2019). After discarding g_d as a reliable indicator of g_{res}, our results indicate a minor effect of different methods and approaches used for measuring g_{res} and for modeling water use, at least in our two closely related species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

VR designed the experiment. CA performed research with the help of FC. VR analyzed the data and wrote the manuscript with important feedback from all co-authors. All authors contributed to the article and approved the submitted version.

FUNDING

We acknowledge funding from the Natural Science Foundation in China (31850410483), the talent proposals from Sichuan Province (2020JDRC0065), the Southwest University of Science and Technology talents fund (18ZX7131), and the Spanish MICINN (AGL2015-69151-R). EG is supported by MCIU/AEI/FEDER, UE [Postdoctoral Contract (IJCI-2017-32511)].

ACKNOWLEDGMENTS

We acknowledge technical assistance from P. Sopeña, M.J. Pau, B. Lavaquiol, A. Cunill, and J. Hedo. We remain grateful to earlier comments from E. Gil-Pelegrín, J.J. Peguero-Pina, D. Sancho-Knapik, D.G. Williams, and D.T. Tissue.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020. 603581/full#supplementary-material

Supplementary Figure 1A | Variation in leaf non-structural carbohydrate concentrations measured across different light treatments (shadow vs sun) and water treatments (P_0, P_{50}, and P_{80}) in *Quercus faginea* (QF) and *Q. ilex* (QI). Bars indicate mean values per treatment and error bars indicate SE.

Supplementary Table 1A | Target midday water potential (9_{md}) to reach the desired PLC according to Esteso-Martínez et al. (2006) for Q. *faginea* and to Peguero-Pina et al. (2014) for *Q. ilex*, and actual values. Mean (and SE) actual values are presented. The letters in “Actual PLC” indicate the results of *post hoc* analyses (Tukey HSD). This is a reproduction (with permission from the publisher) of Table 1 originally published in Resco De Dios et al. (2020).

REFERENCES

Aranda, I., Bergasa, L. F., Gil, L., and Pardos, J. A. (2001). Effects of relative irradiance on the leaf structure of *Fagus sylvatica* L. seedlings planted in the understory of a *Pinus sylvestris* L. stand after thinning. *Ann. For. Sci.* 58, 673–680. doi: 10.1051/forest:2001154

Aranda, I., Castro, L., Pardos, M., Gil, L., and Pardos, J. A. (2005). Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. *For. Ecol. Manag.* 210, 117–129. doi: 10.1016/j.foreco.2005.02.012

Ball, T. J., Woodrow, I. E., and Berry, J. A. (1987). “A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions,” in *Progress in Photosynthesis Research*, ed. J. Biggins (Dordrecht: Martinus Nijhoff), 221–224. doi: 10.1007/978-94-017-0519-6_48

Barbour, M. M., and Buckley, T. N. (2007). The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant Cell Environ.* 30, 711–721. doi: 10.1111/j.1365-3040.2007. 01658.x

Barnard, D. M., and Bauerle, W. L. (2013). The implications of minimum stomatal conductance on modeling water flux in forest canopies. *J. Geophys. Res. Biogeosci.* 118, 1322–1333. doi: 10.1002/jgrg.20112

Blackman, C. J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S. M., and Duursma, R. A. (2016). Toward an index of desiccation time to tree mortality under drought. *Plant Cell Environ.* 39, 2342–2345. doi: 10.1111/pce. 12758

Boyer, J. S., Wong, S. C., and Farquhar, C. D. (1997). CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiol.* 114, 185–191. doi: 10.1104/pp.114.1.185

Bueno, A., Sancho-Knapik, D., Gil-Pelegrin, E., Leide, J., Peguero-Pina, J. J., Burghardt, M., et al. (2020). Cuticular wax coverage and its transpiration barrier properties in *Quercus coccifera* L. leaves: does the environment matter?. *Tree Physiol.* 40, 827–840. doi: 10.1093/treephys/tpz110

Burnham, K. P., and Anderson, D. R. (2002). *Model Selection and Multi/Model Inference: A Practical Information-Theoretic Approach*. New York, NY: Springer-Verlag.

Collatz, G. J., Ball, J. T., Grivet, C., and Berry, J. A. (1991). Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agric. For. Meteorol.* 54, 107–136. doi: 10.1016/0168-1923(91)90002-8

De Kauwe, M. G., Kala, J., Lin, Y. S., Pitman, A. J., Medlyn, B. E., Duursma, R. A., et al. (2015). A test of an optimal stomatal conductance scheme within the CABLE Land Surface Model. *Geosci. Model. Dev.* 8, 431–452. doi: 10.5194/ gmd-8-431-2015

Duarte, A. G., Katata, G., Hoshika, Y., Hossain, M., Kreuzwieser, J., Arneth, A., et al. (2016). Immediate and potential long-term effects of consecutive heat waves on the photosynthetic performance and water balance in Douglas-fir. *J. Plant Physiol.* 205, 57–66. doi: 10.1016/j.jplph.2016.08.012

Duursma, R. A. (2015). Plantecophys - an R package for analysing and modelling leaf gas exchange data. *PLoS One* 10:e0143346. doi: 10.1371/journal.pone. 0143346

Duursma, R. A., Blackman, C. J., Lopez, R., Martin-Stpaul, N. K., Cochard, H., and Medlyn, B. E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytol.* 221, 693–705. doi: 10.1111/nph.15395

Esteso-Martínez, J., Camarero, J. J., and Gil-Pelegrín, E. (2006). Competitive effects of herbs on *Quercus faginea* seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, northeast Spain). *Écoscience* 13, 378–387. doi: 10.2980/i1195-6860-13-3-378.1

Fernández, V., Bahamonde, H. A., Javier Peguero-Pina, J., Gil-Pelegrín, E., SanchoKnapik, D., Gil, L., et al. (2017). Physico-chemical properties of plant cuticles and their functional and ecological significance. *J. Exp. Bot.* 68, 5293–5306. doi: 10.1093/jxb/erx302

Flexas, J., Barbour, M. M., Brendel, O., Cabrera, H. M., Carriqui, M., Diaz-Espejo, A., et al. (2012). Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. *Plant Sci.* 193–194, 70–84. doi: 10.1016/j. plantsci.2012.05.009

Fricke, W. (2019). Night-time transpiration - favouring growth? *Trends Plant Sci.* 24, 311–317. doi: 10.1016/j.tplants.2019.01.007

Heredia-Guerrero, J. A., Guzman-Puyol, S., Benitez, J. J., Athanassiou, A., Heredia, A., and Dominguez, E. (2018). Plant cuticle under global change: biophysical implications. *Glob. Chan. Biol.* 24, 2749–2751. doi: 10.1111/gcb. 14276

Hetherington, A. M., and Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature* 424, 901–908. doi: 10.1038/ nature01843

Hoffman, A. S., Albeke, S. E., Mcmurray, J. A., Evans, R. D., and Williams, D. G. (2019). Nitrogen deposition sources and patterns in the greater yellowstone ecosystem determined from ion exchange resin collectors, lichens, and isotopes. *Sci. Total Environ.* 683, 709–718. doi: 10.1016/j.scitotenv.2019.05.323

Kerstiens, G. (1996). Cuticular water permeability and its physiological significance. *J. Exp. Bot.* 47, 1813–1832. doi: 10.1093/jxb/47.12.1813

Leuning, R. (1995). A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. *Plant Cell Environ.* 18, 339–355. doi: 10.1111/j.1365-3040. 1995.tb00370.x

Lombardozzi, D. L., Zeppel, M. J. B., Fisher, R. A., and Tawfik, A. (2017). Representing nighttime and minimum conductance in CLM4.5: global hydrology and carbon sensitivity analysis using observational constraints. *Geosci. Model. Dev.* 10, 321–331. doi: 10.5194/gmd-10-321-2017

Long, S. P., and Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* 54, 2393–2401. doi: 10.1093/jxb/erg262

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., et al. (2016). Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecol. Monogr.* 86, 495–516. doi: 10.1002/ecm.1231

Martin-Stpaul, N., Delzon, S., and Cochard, H. (2017). Plant resistance to drought depends on timely stomatal closure. *Ecol. Lett.* 20, 1437–1447. doi: 10.1111/ele. 12851

Ogle, K., Lucas, R. W., Bentley, L. P., Cable, J. M., Barron-Gafford, G. A., Griffith, A., et al. (2012). Differential daytime and night-time stomatal behavior in plants from North American deserts. *New Phytol.* 194, 464–476. doi: 10.1111/j.1469- 8137.2012.04068.x

Palacio, S., Maestro, M., and Montserrat-Marti, G. (2007). Relationship between shoot-rooting and root-sprouting abilities and the carbohydrate and nitrogen reserves of Mediterranean dwarf shrubs. *Ann. Botany* 100, 865–874. doi: 10. 1093/aob/mcm185

Peguero-Pina, J. J., Sancho-Knapik, D., Barron, E., Camarero, J. J., Vilagrosa, A., and Gil-Pelegrin, E. (2014). Morphological and physiological divergences within *Quercus ilex* support the existence of different ecotypes depending on climatic dryness. *Ann. Bot.* 114, 301–313. doi: 10.1093/aob/mcu108

Phillips, N. G., Lewis, J. D., Logan, B. A., and Tissue, D. T. (2010). Inter- and intraspecific variation in nocturnal water transport in *Eucalyptus*. *Tree Physiol.* 30, 586–596. doi: 10.1093/treephys/tpq009

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Resco, V., Ewers, B. E., Sun, W., Huxman, T. E., Weltzin, J. F., and Williams, D. G. (2009). Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C3 woody legume, *Prosopis velutina*. *New Phytol.* 181, 672–682. doi: 10.1111/j.1469-8137.2008.02687.x

Resco De Dios, V., Arteaga, C., Peguero-Pina, J. J., Sancho-Knapik, D., Qin, H., Zveushe, O. K., et al. (2020). Hydraulic and photosynthetic limitations prevail over root non-structural carbohydrate reserves as drivers of resprouting in two Mediterranean oaks. *Plant Cell Environ.* 43, 1944–1957. doi: 10.1111/pce.13781

Resco De Dios, V., Chowdhury, F. I., Granda, E., Yao, Y., and Tissue, D. T. (2019). Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. *New Phytol.* 223, 1696–1706. doi: 10.1111/nph.15881

Resco De Dios, V., Diaz-Sierra, R., Goulden, M. L., Barton, C. V., Boer, M. M., Gessler, A., et al. (2013). Woody clockworks: circadian regulation of night-time water use in *Eucalyptus globulus*. *New Phytol.* 200, 743–752. doi: 10.1111/nph. 12382

Resco De Dios, V., Roy, J., Ferrio, J. P., Alday, J. G., Landais, D., Milcu, A., et al. (2015). Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. *Sci. Rep.* 5:10975.

Riederer, M., and Müller, C. (2007). *Biology of the Plant Cuticle*. Hoboken, NJ: Blackwell Publishing.

Rodriguez-Calcerrada, J., Pardos, J. A., and Aranda, I. (2010). Contrasting responses facing peak drought in seedlings of two co-occurring oak species. *Forestry* 83, 369–378. doi: 10.1093/forestry/cpq019

Schuster, A. C., Burghardt, M., and Riederer, M. (2017). The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions? *J. Exp. Bot.* 68, 5271–5279. doi: 10.1093/jxb/ erx321

Shepherd, T., and Wynne Griffiths, D. (2006). The effects of stress on plant cuticular waxes. *New Phytol.* 171, 469–499.

Way, D. A., and Pearcy, R. W. (2012). Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiol.* 32, 1066– 1081.

Wei, T., and Simko, V. (2017). *R Package "Corrplot": Visualization of a Correlation Matrix (Version 0.84)*. Available online at: https://github.com/taiyun/corrplot (accessed September 1, 2020).

Zhang, P., Wen, Y., Wang, L., Zhang, H., Wang, G. G., and Wu, T. (2020). Leaf structural carbohydrate decreased for *Pinus thunbergii* along coast-inland gradients. *Forests* 11:449.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

VII. Drivers of nocturnal stomatal conductance in C3 and C4 plants

Drivers of nocturnal stomatal conductance in C_3 and C_4 plants

Faqrul Islam Chowdhury [a, b, *], Carles Arteaga [c], Mohammed Shafiul Alam [a], Iftakharul Alam [d], Víctor Resco de Dios [c, e, f]

[a] Institute of Forestry and Environmental Sciences University of Chittagong, Chattogram 4331, Bangladesh;

[b] Erasmus Mundus Master Course Mediterranean Forestry and natural Resources Management

[c] Department of Crop and Forest Sciences, University of Lleida, Lleida, Spain

[d] Centre for Tropical Environmental and Sustainability Science, College of Science and Engineering, James Cook University, Cairns, Queensland 4878, Australia

[e] School of Life Science and Engineering, Southwest University of Science and Technology, 621010 Mianyang, China

[f] Joint Research Unit CTFC-AGROTECNIO-CERCA Center, Lleida, Spain

** Corresponding author: Faqrul Islam Chowdhury; e-mail: faqrul@cu.ac.bd; Phone: +8801624369907; Address: Institute of Forestry and Environmental Sciences University of Chittagong, Chattogram 4331, Bangladesh.*

Abstract

Nocturnal water losses were for long considered negligible, but it is now known that incomplete stomatal closure during the night leads to significant water losses at leaf, plant and ecosystem scales. However, only daytime transpiration is currently accounted for in evapotranspiration studies. Important uncertainties on the drivers of nocturnal water fluxes hinder its incorporation within modelling frameworks because some studies indicate that night-time stomatal drivers may differ from day-time responses. Here, we synthesize the studies on nocturnal stomatal conductance (g_n) to determine underlying drivers through a systematic literature review and, whenever possible, meta-analytical techniques. Similar to daytime responses, we found negative effects of vapour pressure deficit, predawn water potential, air temperature, and salinity on g_n across the plant species. However, the most apparent trend was an increase of g_n from the beginning until the end of the night, indicating significant and widespread endogenous regulation by the circadian clock. We further observed how neither elevated CO_2 nor nutrient status affected g_n significantly across species. We also did not find any significant associations between g_n and elevated ozone or increasing plant age. There was a paucity of studies on climatic extremes such heat waves and also few studies connected g_n with anatomical features such as leaf specific area or stomatal density. Further studies are also needed to address the effects of plant sex, abscisic acid concentrations and genotypic variations on g_n. Our findings solve the long-term conundrum on whether stomatal responses to daytime drivers are the same as those that during the nighttime.

Keywords: Nocturnal stomatal conductance, transpiration, evapotranspiration, circadian clock

1. Introduction

Evapotranspiration (ET) is responsible for the return of 60% of annual precipitation to the atmosphere, a proportion that increases up to 95% of precipitation in water-limited ecosystems (Hetherington and Woodward, 2003; Huxman et al., 2005; G. Sun et al., 2010). ET is thus a major component of the global hydrological cycle (Wilcox and Thurow, 2006), and it is currently assumed that transpiration is the dominant contributor of ET (Schlesinger and Jasechko, 2014). Nocturnal transpiration accounts for an additional 12% of daytime transpiration (Forster, 2014), and its inclusion in hydrologic models leads to reductions of up to 50% of available soil moisture worldwide in semi-arid environments (Lombardozzi et al., 2017). Understanding nocturnal stomatal conductance (g_n) is thus essential for assessments of the water balance from leaf to ecosystem scales (Zeppel et al., 2014) because it underlies a substantial fraction of ecosystem water use in different biomes and ecosystems (Barbeta et al., 2012; Oishi et al., 2008; Wallace and McJannet, 2010).

Nocturnal stomatal conductance has been reported for a wide range of C_3 and C_4 species, including grasses, shrubs, trees, crops, and different biomes across the world (Dawson et al., 2007; Resco de Dios et al., 2019; Snyder et al., 2003). Different drivers affect g_n, implying that it is not simply a consequence of "leaky" stomata (Zeppel et al., 2014), which consequently hinders model implementation (Caird et al., 2007). Drivers of g_n such as vapour pressure deficit (VPD), CO_2 concentration, water availability or temperature, to name a few, have been assessed in many studies, but different results have been obtained. For example, higher g_n has been observed under nutrient-limiting conditions (Kupper et al., 2012; Ludwig et al., 2006; Scholz et al., 2007), supporting the notion that g_n is beneficial as it enhances nutrient uptake, but g_n reductions under nutrient limitation have also been reported (Howard and Donovan, 2010, 2007).

Some studies indicate that g_n responses to environmental drivers may be different to daytime responses. For instance, g_n is often higher under elevated CO_2 than under ambient CO_2 concentrations (Easlon and Richards, 2009; Zeppel et al., 2012), which is opposite to the typical daytime response where g_d (daytime stomatal conductance) declines under elevated CO_2 (Ainsworth and Rogers, 2007). Similarly, increases in g_n with VPD, which is contrary to the daytime response, have been observed in some cases (Bucci et al., 2004; Muchow et al., 1980), but not in others (Barbour et al., 2005; Barbour and Buckley, 2007). Different g_n vs g_d environmental responses complicate modelling, as they indicate that daytime model processes cannot be applied for predicting nocturnal responses. The inclusion of g_n within modelling schemes is consequently constrained by a lack of mechanistic understanding on the drivers of g_n.

In addition to abiotic drivers, biotic processes such as circadian rhythms lead to a temporal pattern in g_n resulting higher rates of g_n in late night time when compared with early night time (Easlon and Richards, 2009; Hennessey et al., 1993; Resco de Dios et al., 2019). This regulation of g_n may facilitate plants to uptake more C in the early morning (Mansfield and Heath, 1961; Resco deDios et al., 2019). In fact, although environmental responses of stomata are reasonably fast, stomatal “priming” at predawn may foster responses to morning light, decrease diffusion limitations to photosynthesis and it has sometimes been correlated with enhanced growth (Resco de Dios et al., 2019, 2016a).

Overall, there are still major unknowns and little consensus on the drivers and regulators of g_n. However, there is a wealth of studies on g_n in the scientific literature which can be used to synthesize current information on the different drivers of g_n. Here, we combined these studies through a systematic literature survey to find generality in the environmental drivers and biotic regulators of g_n (Fig. 1A, B). Although our primary goal was to review the literature, we also used meta-analytical analyses to assess general patterns across studies and functional types. That is, when the number of studies on a particular driver was large enough, we used meta-analytical techniques to quantify global trends and separately across biomes and functional types.

Our first objective was to identify which potential drivers and regulators of g_n had been studied, and to which extent. Our second objective was to understand (and quantify whenever possible) the effects of each driver and regulator over $g_{n,}$ based on the currently available literature. Ultimately, we sought to understand to which degree is g_n regulation different from g_d, so we can identify current research gaps to guide future research.

The review is structured such that we first present the responses of g_n to changes in environmental factors (e.g., vapour pressure deficit, temperature, predawn water potential, soil nutrient concentration, atmospheric CO_2, ozone pollution, salinity and radiation dynamics). Second, we address the responses of g_n to biotic factors (e.g., endogenous circadian clock, photosynthesis, genetic variations, leaf specific area, stomatal density, leaf nitrogen, sex and age). Finally, we present the conclusions and implication of this work. For the sake of parsimony, our original predictions were that experiments reporting differential day and night stomatal regulation were the exception and that it would be more common to observe similar daytime and nighttime responses. That is, (a) VPD will have a negative effect on g_n and temperature will have a hump shape response, (b) higher soil nutrient concentration will reduce g_n, (c) elevated CO_2 will reduce g_n, (d) lower predawn water potentials will decrease g_n; (e) elevated O_3 will enhance stomatal closing. Additionally, we expected major influences from biotic processes including: (f) circadian regulation lowering early night stomatal conductance and increasing late night g_n; and (g) photosynthesis increasing g_n on the subsequent night, by providing the osmoticant necessary for opening (Easlon and Richards, 2009). We included a rather large number of potential drivers and regulators, and the full range of hypotheses are available at Fig. 1A, B. We considered bioclimatic factors that are external to plants as drivers and physiological responses are noted as regulators.

2. Methods

We took advantage of the reference list from a recent review on nocturnal conductance (Resco de Dios et al., 2019). This reference list was elaborated after exhaustive data searches and it includes the response for a total of 176 species reported within 85 publications and 339 studies (some publications reported the results of more than one study). Then, we updated the database to include publications from the last couple of years with additional database searches and 7 additional publications were included. Most studies are concentrated in temperate (79 species), Mediterranean (33 species) and desert and xeric (29) species, whereas tropical (12 species) and boreal species (5 species) were less common (Fig. 1C, D). Across plant functional types (PFTs), deciduous angiosperm trees were most common (49 species), followed by C_3 shrubs (35 species), crops (27 species) and evergreen angiosperm trees (21 species). The number of species for gymnosperm trees, C_4 grasses, forbs, C_3 grasses, and tropical trees decreased from 15 to 7.

We examined g_n responses to a rather extensive list of environmental and biotic processes (Fig. 1A, B). Data from each article were obtained from the text and tables or by digitizing graphs using WebPlotDigitizer-3.8-Desktop software. When there were data for more than 5 studies on the relationship between g_n and a given driver, we conducted a semi-quantitative analyses by examining the percent response ratio (RR) of g_n to such driver. This process is commonly practiced in ecological meta-analyses (Curtis and Wang, 1998; Hedges et al., 1999; Resco de Dios et al., 2016b; Wang et al., 2012). CIs for effect-size estimates were calculated by bootstrapping the unweighted data with a resampling of 10000 iterations. An effect was considered significant when the interval captured by the CI did not overlap the zero line. In case of nutrient treatment, different nutrient concentration such as nitrogen (N), phosphorus (P), and nitrogen-phosphorus-potassium (NPK) were combined to get ample data for meta-analysis.

When there were 20 or more studies, we conduct these semi-quantitative analyses separately for biomes and plant functional types (PFTs). In order to avoid publication bias, we calculated Rosenthal's fail-safe number (or the number of non-published studies with non-significant results necessary to affect our conclusions) (Rosenthal, 1979) using the library *metaphor* (Viechtbauer, 2010). We did not observe significant publication bias for any of the results presented. Further details of this method can be found in (Wang et al., 2012). All analyses and plots were performed with R (version 4.0.5) statistical software environment (R Core Team, 2021).

It should be noted that we used meta-analytical techniques in order to complement the results of the literature review. That is, while sample size was certainly enough for meta-analytical techniques in some drivers and regulators, there was a rather low sample size for other factors. Consequently, the use of this technique was simply used as a means to summarize the results available in the literature when there were more than 5 studies.

3. Effects of environmental drivers on nocturnal stomatal conductance

3.1. Vapour pressure deficit

Atmospheric water demand is driven by the gradient in vapour pressure from inside-to-outside the leaf (VPD). It is well known that VPD is one of the major drivers of g_d and the response may be characterised by a negative logarithmic function (Oren et al., 1999). VPD effects over g_n are less clear. VPD typically declines overnight and, during this time course, g_d only sometimes increases (as expected from a the negative effect of VPD over g_d). For instance, Howard and Donovan (2010) document increases in g_n as VPD declines overnight in *Populus angustifolia*, while g_n remained constant in *P. balsamifera*. However, Barbour et al., (2005) report no correlation between g_n and VPD in six temperate tree species, and some studies on different *Eucalyptus* and *Acer* species report a positive effect of VPD on g_n (Dawson et al., 2007; Resco de Dios et al., 2013b; Zeppel et al., 2012). For a given species, different relationships between VPD and g_n have also been documented across studies like in *Acer rubrum* where both positive (Dawson et al., 2007) and neutral (Daley and Phillips, 2006) responses have been documented.

Using the natural covariation between VPD and g_n as the night advances to infer the dependence of the latter over the former is problematic because other factors may have changed during that time that alter that response, including endogenous circadian regulation (Resco de Dios, 2017). Additionally, VPD was altered in some studies by changing temperature, which may introduce additional confounding as the positive response of stomata to VPD could actually be driven by a positive response to temperature. Studies using response curves that solely alter relative humidity are rare and they have not been able to clarify whether the apparent neutral or positive relationships between VPD and g_n are artefactual or real. Response curves in *Ricinus communis* indicate that daytime negative effects of VPD over g_s remain overnight, albeit with a less steep slope (Barbour and

Buckley, 2007), whereas a positive effect of VPD over g_s was observed in ramped response functions in *Eucalyptus globulus* (Resco de Dios et al., 2013a). Unfortunately, studies reporting response functions are rare and it is more common to find studies based on the natural covariation approach.

We calculated the response ratio to changes in VPD across all studies and species available (using either the covariation approach or the response curve) and observed an overall significant reduction of 75% (±38%, 95% CI) (Fig. 2) across 58 studies. Changes in VPD ranged from 1 to 2 kPa on average in the assessed studies. From our review of the literature we can conclude that there is generally a negative effect of VPD over g_n (42 out of 57 species). It is important to note, however, that positive responses occur in 12 species and both positive and negative responses to VPD occurred in 3 species across different studies.

Across biomes, we observed that plants in tropical (-108 ± 85%, n = 20), temperate (-65 ± 55%, n = 26), and Mediterranean (-83 ± 49%, n = 5) biomes showed significant negative responses. Across PFTs, evergreen angiosperm trees (-82 ± 65, n = 8) and crops (-135 ± 105 %, 95% CI; n = 17) and tropical trees (-72 ± 71%; n = 7) showed significant and negative effects of VPD over g_n. Non-significant effects of VPD over g_n were largely restricted to deciduous angiosperm trees (-14 ± 24%, n = 17). We were limited by number of studies in gymnosperm trees (n=2) and C_3 plants (n=4) and did not conduct quantitative analyses (Fig. 3A, B).

In addition to studies on short-term effects of VPD over g_n, the effect of long-term exposures under different growth VPD conditions has been seldom studied. Studies on hybrid aspen (Rohula et al., 2017) and on tomato (Arve and Torre, 2015) both observed that decreasing VPD positively affects g_n (although the effect was only significant at predawn in hybrid aspen). The response could be mediated by abscisic acid (ABA), as significantly lower ABA content was measured in leaves growing under high humidity in tomato (Arve and Torre, 2015).

3.2. Air temperature, soil temperature and heatwaves

Temperature affects stomatal behaviour directly but also indirectly by altering atmospheric water demand, as previously noted. We did not, unfortunately, find studies that separated temperature from VPD effects. Consequently, we acknowledge that temperature induced changes in VPD could affect the analysis and discussion reported here. At the same time, this indicates the need for further studies that decouple temperature from VPD responses in g_n.

Different studies have observed varying responses to temperature. For instance, some studies report that g_n increases with temperature in *Eucalyptus* (Zeppel et al., 2012), that it declines in

Populus (Furukawa et al., 1990) or *Sorghum* (Muchow et al., 1980) and that it exerts no effect also in *Eucalyptus* (Resco de Dios et al., 2013a).

We calculated the response ratio of g_n to changes in air temperature (on average from 17 to 25ºC) across all studies and species available and, similar to VPD responses, we observed an overall significant reduction in g_n (55 ± 30%) (Fig. 2) across 26 studies. From our literature review we may conclude that, generally (25 out of 29 species) temperature exerts a negative effect over g_n. Only 3 of species reported positive responses and 1 species showed a neutral effect across studies.

When examining responses across biomes, there was generally a paucity of data. We observed a significant decline in g_n with temperature in the tropical (-117±72%, n = 7) and temperate (-33±29%, n = 12) biomes. We observed no effect in the few data available from deserts (-15±31%, n = 6) and only 1 study in the boreal biome (Fig. 3C). Across PFTs, we observed more significant declines in crops (-131 ± 65%, n = 7) and in C_3 plants (-39±30%, n = 5), and paucity of data did not allow for examinations across other PFTs (Fig. 3D). The negative response of g_n to temperature may become increasingly important under global warming, as nocturnal air temperatures are expected to increase more rapidly than daytime temperatures (Davy et al., 2017).

In addition to changes in air temperature, only one published study has addressed the effects of different soil temperatures and it was performed on *Vitis vinifera* (Rogiers and Clarke, 2013). The authors reported a 16% increase in $g_{n,}$ from cool (17ºC) to ambient (23ºC) temperatures, but g_n stayed constant after further increases in soil temperature.

We only found two studies addressing how heat waves affect g_n. Duarte et al. (2016) observed that g_n in *Pseudotsuga menziesii* declined by 50% after a 12ºC increase in air temperature and Resco de Dios et al. (2018) similarly reported a 36% decline after a 10ºC increase in air temperature in *Eucalyptus camaldulensis*. We were not able to find additional reports on heat wave effects. A particularly interesting question that yet remains unresolved is whether heat waves could have carry-over effects over g_n, meaning that the decline in g_n after the heat wave continues even after the heat wave has passed. For instance, Duarte et al. (2016) hypothesized (but did not measure) that carry-over effects in *P. menziesii* could occur if changes in anatomical properties, such as stomatal occlusion or changes in epicuticular wax, occurred during the heat wave. The frequency of heat waves will increase under global warming, so addressing the general impacts of heat waves over g_n, along with potential carry-over effects, should be at the forefront of our research efforts.

3.3. Predawn water potential

We expected that decreases in predawn water potential (Ψ_{pd}) would lead to drastic reductions in g_n. That is, one would not expect to have significant stomatal opening under strong water scarcity and any residual conductance should result from cuticular conductance or the inability to close stomata further. We tested this using the available data from 43 studies, with 34 species where Ψ_{pd} changed, on average, from -3 to -0.1 MPa. As expected, we observed a very marked and significant negative response (-102% ± 46%), meaning that to g_n declined as rhizosphere water scarcity increased (Fig. 2). The negative response was consistent across tropical (-56% ± 50%, n = 9), temperate (-163% ±89%, n = 17) and Mediterranean (-134% ±116%, n = 8) biomes. However, the response was not significant in the desert biome (3 ± 25%, n = 7) and we could not analyse the boreal biome (n=2) because of lack of data (Fig. 3E). Across PFTs, the response was significant and negative for C_3 forbs (-75% ±44%, n = 8), crops (-107% ±92%, n = 11) and deciduous angiosperm trees (133 ± 110%, n = 15). For tropical trees (58 ± 59%, n = 6) we observed non-significant responses while data on C_4 grasses (n=2) and gymnosperm trees (n=1) was limited (Fig. 3F).

The negative effect of Ψ_{pd} on g_n occurred in 26 out of 34 species. Surprisingly, lowering Ψ_{pd} had a positive effect in 6 species, namely, *Sporobolus wrightii* (a C_4 grass), *Celtis reticulate, Prosopis velutina* (W. Sun et al., 2010), *Populus tremula* (Rohula et al., 2016), *Helianthus annuus* (Howard and Donovan, 2007) (which are deciduous angiosperm trees), *Tamarix chinensis* (Moore et al., 2008) (C_3 plant). In 2 additional species, *Vitis vinifera* (a Mediterranean crop) (Escalona et al., 2013; Fuentes et al., 2014) and *Qualea grandiflora* (a tropical crop) (Scholz et al., 2007), responses were either negative or positive depending on their biome or PFTs. The positive response of g_n to Ψ_{pd} was unexpected and it deserves further investigation.

3.4. Soil nutrient concentrations

Because nutrient uptake through mass flow and its transport to distal plant parts relies on the transpirational stream, some studies hypothesized that g_n should increase with nutrient scarcity. That is, that nocturnal water losses could indirectly enhance C uptake when they serve to increase nutrient delivery, particularly in nutrient poor soils. The pattern observed after combining different studies is, however, more complex. For instance, g_n increases under nitrogen additions have been reported for *Populus angustifolia* and *P. balsamifera* (Howard and Donovan, 2010) and decreases have been documented for *Populus balsamifera* (Howard and Donovan, 2010), *Ulmus laevis* and *Fraxinus excelsior* (Eller et al., 2017). Regarding phosphorous (P) concentrations, g_n increased under low soil

P in *E. tereticornis* (Resco de Dios et al., 2013b) and in *U. laevis* but it increased in *F. excelsior* (Eller et al., 2017). Scholz et al. (2007) found higher g_n in *Ouratea hexasperma*, *Qualea grandiflora,* and *Blepharocalyx salicifolius* when both N and P were added separately. However, g_n declined when both nutrients were applied together.

In our literature review, we found a total of 27 studies with 15 different species that directly tested the potential effects of g_n in response to increases in nutrient uptake using fertilization experiments. There were 8 studies using N, 4 studies using P, 6 studies using N and P and 9 studies using NPK, which low concentrations were and high concentration were respectively. Given the overall low number of studies for individual nutrients, we pooled all the data together and did not examine separately the changes in response to each nutrient addition. The response to nutrient additions was overall negative but the effect was not significant (-19 ± 21%) (Fig. 2). There were no significant effects in tropical (-24 ± 35%; n=13), temperate (-2 ± 20%; n=7) and Mediterranean (-25 ± 47 n=6) biomes (Fig. 3G). Across PFTs, there were also no significant effect on deciduous angiosperm trees (-9 ± 27%; n=12) and crops (-15 ± 40%; n=11) (Fig. 3H). Overall, our study indicates that responses to nutrient additions are idiosyncratic and species-specific, and no general trend became apparent. An overall lack of data, however, limits the generality of this finding.

3.5. Atmospheric CO_2

Daytime stomatal conductance often declines in response to elevated CO_2. The literature on g_n, however, shows a more complex pattern. Some studies report that CO_2 significantly increases g_n in *Triticum aestivum* (Wheeler et al., 1999), *E. camaldulensis* (Resco de Dios et al., 2016a), *Ipomea batatas* (Wheeler et al., 1999) and *E. sideroxylon* (Zeppel et al., 2012) while other reported negative effect, e.g., *Solanum tuberosum* (Wheeler et al., 1999), *E. tereticornis* (Resco de Dios et al., 2013b), *Ricinus communis* (Cernusak et al., 2004) and *Arabidopsis thaliana* (Costa et al., 2015).

The positive effect of CO_2 on g_n is puzzling because one would need a mechanism to explain stomatal opening under increasing CO_2 concentrations. Easlon and Richards (2009) hypothesized that the mechanism would be related to higher photosynthate production under elevated CO_2 increases g_n by providing the osmoticants necessary for stomatal regulation.

In our literature analyses, we observed a total of 14 studies, with 11 species examining the responses to average changes in CO_2 from 398 to 614 ppm. The effects of CO_2 over g_n were, overall, not significant (-1±33%, Fig. 2). This response is consistent with the notion that CO_2 does not regulate nighttime stomatal conductance and it differs from daytime responses.

3.6. Ozone pollution

Tropospheric ozone (O_3) is a pollutant agent that injures leaves by inducing oxidative stress. Daytime stomatal responses are often negative to O_3 and they could result from both the leaf injury as well a response to diminish O_3 influx (Paoletti, 2005). In our literature review, we were able to obtain data from 8 species across 11 studies where O_3 varied from 4 to 100 ppb. We observed that there was a positive but not-significant response (20% ±26%, Fig. 2). That is, we did not observe any significant effects of O_3 on g_n. This response is different from that in the daytime and again points towards a weaker nighttime than daytime stomatal response.

The lack of nocturnal stomatal closure under elevated O_3 could, however, be problematic. That is, showing no g_n response to O_3, instead of stomatal closure, could enhance O_3-induced injuriesas stomata remain open and O_3 influx may thus continue. In fact, previous studies have documented that plants exposed to O_3 only during the night also showed leaf injuries (Matyssek et al., 1995).

3.7. Salinity

Soil salinity exerts two different types of physiological effects. First, is a decline in the water potential and, second, the induction of ion toxicity through the influx of excess Na^+ and Cl^- (Gao et al., 2021; Munns, 2002; Yao et al., 2021). Daytime conductance often declines in response to salinity. The effects of salinity on g_n have so far been examined in only 3 different plant species across 8 different studies, and a significant negative response (-68% ± 65) was observed (Fig. 2). The response was negative in the non-salt tolerant *Chrysothamnus nauseosus* and in the salt tolerant *Sarcobatus vermiculatus* (Donovan et al., 1999) and in different cultivars of *Distichlis spicata* (Christman et al., 2009). This response is consistent with the previously documented negative effects of Ψ_{PD}.

3.8. Radiation

A number of studies have addressed different aspects associated with g_n responses to radiation and its dynamics, including differences between sun and shade leaves (canopy position), photoperiod, light spectrum and daytime shading.

Regarding differences between sun and shade leaves, Konarska et al. (2016) observed that g_n/g_d in seven tree species within a urban ecosystem was two-fold higher for shaded (23%) leaves than for sunlit leaves (11%). g_n also varied within *Quercus rubra* canopies, and shaded leaves showed

g_n values that were twice those in sunlit leaves (Barbour et al., 2005). These results indicate that morphological changes in shaded leaves lead to higher g_n, and the response could be mediated by the links between specific leaf area and leaf venation (Resco de Dios et al., 2021) .

We found two studies that experimentally shaded leaves during the daytime and they both document increases in g_n. Barbour et al. (2005) observed that shading sunlit *Q. rubra* leaves increased g_n by 4-fold. Similarly, Easlon and Richards (2009) observed that shading *Vicia faba* leaves triggered a 3.7-fold increase in g_n. Changes derived through shading could be mediated by photosynthesis feedbacks, and will be discussed below in the relevant section. Recent studies showed that *Quercus ilex* reduced its g_n when growing under shade while *Quercus faginea* increased g_n, relative to values under ambient radiation (Qin et al., 2020).

The effects of daylength have similarly been examined in a couple of studies: one using examining the response in different rose cultivars Blom-Zandstra et al. (1995) and another one with *Vicia faba* (Easlon and Richards, 2009). In both cases, longer day-lengths led to lower g_n. The decline was almost 50% in some rose cultivars after shifting from 4 to 12 hours of light, and of 25% in *Vicia faba* after increasing from 8 to 16 hours of daylight

The effects of differing radiation spectra have only been assessed in one study using lettuce (Kim et al., 2004). They observed the highest g_n, and also the highest g_n/g_d, in plants growing under green fluorescent lamps (51 μmol m^{-2} s^{-1} and 39%, respectively). However, there was no difference in g_n between plants grown under red and blue light emitting diodes with, or without green fluorescent lamps, or under cool white fluorescent lamps (35-36 mmol m^{-2} s^{-1}). Overall, these results indicate that daytime radiation does exert carry-over effects of g_n in the following night. The mechanisms are, however, more difficult to understand and will be addressed later on when discussing photosynthesis effects.

4. Effects of biotic drivers on nocturnal stomatal conductance

4.1. Endogenous circadian clock

A number of studies have reported that g_n increases through the night. This response could be partly attributed to the decrease in VPD that often occurs as the night advances, as previously discussed. However, the nocturnal change in VPD is often mild and the increase in g_n is usually higher than that which one would expect based VPD alone. We found 81 studies that addressed temporal changes in g_n in 63 species and there was a significant increase in g_n between 20:00h and 03:00 h (average local time for experiments) of 40±7% (Fig. 2). The increase was consistent across tropical (42±19%; n=22), temperate (43±12%; n=32), Mediterranean (33±10%; n=12) and desert (28±23%; n=11) environments, but lack of data prevented the evaluation in boreal environments (n=4) (Fig. 3I). Across PFTs, we also observed significant increases in those with enough sample size, including tropical trees (40 ± 20%; n=5), evergreen angiosperm trees (40±17%; n=17), deciduous angiosperm trees (24 ± 19%; n=16), crops (46±9%; n=30) and C_3 forbs (31 ± 26%; n = 6) (Fig. 3J). This pattern points towards a widespread role of circadian regulation as a driver of g_n.

4.2. Photosynthesis

It has been proposed that photosynthesis could affect g_n by altering the availability of non-structural carbohydrate (NSC) reserves, which provide the raw material for the osmoticants necessary for stomatal regulation (Easlon and Richards 2009). Indeed, a study comparing the response in wildtype *Arabidopsis* against a starch deficient mutant observed much lower values of g_n in the latter (Lascève et al., 1997). That is, conditions promoting low NSC would lower g_n. Some studies have challenged this assumption as, for instance, relationships between assimilation and g_n were not significant in cotton or bean (Resco de Dios et al., 2015).

Here we observed that assimilation (A) showed, generally, a positive and significant association with g_n (26±16%) across 20 studies and 20 species (Fig. 2). Positive associations between A and g_n associations were found in temperate (43 ± 17%; n=6), but not in desert environments (7 ± 22%; n=10) (Fig. 3K). Across PFTs, positive associations were observed in crops (32 ± 17%; n=5) and C_3 plants (55 ± 15%; n=6) but in C_4 grasses (-16 ± 20%; n=6) g_n response to A were not significant (Fig. 3L).

Our results broadly support the notion that daytime photosynthesis could exert a carry-over effect over g_n in the subsequent night. The response, however, seems to be idiosyncratic and dependant on biome and PFT. However, these results are in sharp contrast with previous responses to shading, which increased g_n. Such contrast indicates that the long-term acclimation to shading may induce alternative mechanisms (e.g. changes in leaf morphology) that compensate for the changes in NSC that result from short-term shading.

4.3. Genotypic variation

We observed 8 studies that addressed the role of genotype variation, and all of them documented significant genotypic variation. That is, there was significant genetic variation in the tree *Eucalyptus camaldulensis* (Resco de Dios et al., 2016a), in the crops *Oryza* (Zhang et al., 2021), *Vitis vinifera* (Escalona et al., 2013), *Phaseolus vulgaris* (Salvatori et al., 2013) and *Rose spp.* (Blom-Zandstra et al., 1995), in the shrub *Distichlis spicata* (Christman et al., 2009), and in the forb *Arabidopsis thaliana* (Costa et al., 2015; Leymarie et al., 1998). The range in g_n variation across genotypes was between 158% and 82%. Overall, this data indicates that g_n is a heritable trait and that it has been subject to selection.

This heritability in g_n has led to an increasing number of studies proposing that g_n should be the target of breeding programs (Coupel-Ledru et al., 2016; Schoppach et al., 2020). In particular, those studies have suggested that breeding should select for genotypes with low g_n as a means for reducing water loss and, also, because g_n is decoupled from g_d. In addition to an overall low g_n, those studies also suggest selecting genotypes with a high endogenous predawn rise in g_n, as that serves as a priming that increases productivity (Ramírez et al., 2018; Schoppach et al., 2020).

5. Morphological, anatomical and developmental features

5.1. Specific leaf area and leaf elemental concentrations

There are not many g_n studies where specific leaf area (SLA) was also measured. We only found a study performed with *Helianthus anomalus* (Ludwig et al., 2006), another on the C_3 grass *Bartsia trixago* (Press et al., 1993) and study a comparing 12 oak species (Resco de Dios et al., 2021). In the first two, SLA was positively correlated with g_n but, in the last one, the correlation was negative. Resco de Dios et al. (2021) hypothesized that, in oaks, the negative relationship could be mediated by leaf vein density, which was negative related to SLA. According to this model, increasing leaf vein density in leaves with small SLA would increase water transport redundancy, but at the expense of increasing nocturnal water losses. This hypothesis awaits further testing in other clades.

Very few studies have addressed correlations with leaf elemental concentrations, carbon isotope concentrations and other functional traits (Resco de Dios et al., 2021). Linking g_n with functional properties would lead to better understanding the drivers and also on the function of g_n.

5.2. Stomatal density

Only a handful of studies have addressed the links between g_n and stomatal density (SD), and the responses also vary with each study. Rogiers and Clarke (2013) and Zhang et al. (2021) reported a negative relationship in between g_n and SD in *Vitis vinifera* and 30 *Oryza* genotypes, respectively. No significant relationship between SD and g_n has been reported for Arabidopsis (Lascève et al., 1997), poplars (Ceulemans et al., 1988) and oaks (Resco de Dios et al., 2021). Finally, a positive response between SD and g_n has been documented in a C_4 grass (Chieppa et al., 2021). These conflicting results indicate potentially different roles of g_n in different environments. Chieppa et al. (2021), for instance, argued that the positive link between SD and g_n was driven by necessity for transpirational cooling in *Panicum*, a hot desert grass. In our analyses, we were able to analyse 5 studies addressing how g_n changes with SD and we observed a non-significant positive trend (8±57%) (Fig. 2). Further studies needs to address how links between SD and g_n change across climate gradients and PFTs.

5.3. Age

We could expect that young trees show higher water and nutrient requirements for their development and growth. Consequently, at least on a per leaf area basis, we would expect higher g_n values in younger individuals.

We were able to find 19 studies addressing how g_n changes with plant age and we observed a negative trend, meaning that younger trees showed higher g_n rates but the trend was not-significant (-40±57%) (Fig. 2). There was a negative effect of age in *Helianthus annuus* (Howard and Donovan, 2007), *Rosa spp*. (Madelon and Sonia cultivar) (Blom-Zandstra et al., 1995), *Pinus ponderosa* (Grulke et al., 2004), and *Rubus spectabilis*, *Rubus laciniatus* and *Rubus armeniacus* (McNellis and Howard, 2015). Contrastingly, older individuals of *Glycine max* (Rawson et al., 1978) and *Rubus ursinus* (McNellis and Howard, 2015) showed higher rates of g_n.

It is important to note that the previous studies compared g_n values in leaves of comparable age: only plant age was different. However, leaf age could also affect g_n. In particular, we could expect that new leaves show higher g_n, given their higher growth rates and water needs. This hypothesis has been confirmed in the two studies we compiled on this issue (Phillips et al., 2010; Zeppel et al., 2010). They both worked with different species of Myrtaceae and reported that g_n was 4-5 times larger in in young than in old leaves.

5.4. Sex

Only one study has so far examined differences in g_n with sex, observing values that were 30% higher in female *Salix arctica*, relative to male trees in the Canadian Arctic Archipelago (Dawson and Bliss, 1989).

6. Conclusions and implications

Nocturnal conductance is a widespread phenomenon also in C_3 and C_4 plants. Here we have shown that different processes affect g_n and their effect is likely to differ across temporal scales (Fig. 4). That is, while some short term responses may occur in response to changes in environmental drivers, longer-term effects through acclimation may occur in response to plastic adaptation or to changes in anatomical and structural features. While continued exposure to different environmental factors could also induce long-term responses, most studies conducted to date report only short-term responses. Longer-term studies addressing the response to environmental factors is thus an important research gap.

Our literature review has allowed identifying that previous claims for a different day-time vs night-time regulation of stomatal conductance may occur only in a few species or drivers. The positive effect of VPD, for instance, has only been documented in a few studies and the global average effect was negative. The mechanism explaining why g_n would open in response to VPD in some species, however, remains elusive.

Our results also support the hypothesis that nighttime stomatal control is weaker than during the daytime. This was particularly apparent when examining ozone responses as there was no global effect of ozone on g_n averaged our datasets. Similarly, the lack of CO_2 effects over g_n similarly indicate that stomata do not sense CO_2 concentrations during the night, again pointing towards weakernighttime stomatal regulation.

Perhaps one of the most shocking data gaps was in relation to the paucity of studies jointly addressing g_n and leaf morphological traits such as stomatal density, leaf venation and also functional attributes such as specific leaf area of $\delta^{13}C$. Further work connecting these different data would likely lead to a better of g_n with other leaf and whole plant processes.

Nocturnal conductance has a series of implications that are yet to be assessed. There is the obvious implication over the plant water balance, which has been only assessed in some studies (Dayer et al., 2021) . However, studies from plot to regional and global scales are still rare. To this end, we hope the results of this analysis will be of relevance. The costs of productivity in terms of water lost over night for crops and also for natural ecosystems is thus largely unknown.

Nocturnal conductance could also favour plant damage through different processes. One is continuous O_3 uptake, as already reported. The other is related to serving as pathogenic inputs. Several studies have identified that stomatal openings serve as entry points for different pathogenic microorganisms (Melotto et al., 2008; Zeng et al., 2010). Some of these pathogens have a marked circadian rhythm and they enter preferentially over the night (Panchal et al., 2016; Panchal and

Melotto, 2017). Particularly, those processes enhancing g_n opening, such as circadian regulation or higher photosynthesis, could incur into costs associated with pathogenicity.

7. References

Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising [CO 2]: Mechanisms and environmental interactions. Plant, Cell Environ. 30, 258–270. https://doi.org/10.1111/j.1365-3040.2007.01641.x

Arve, L.E., Torre, S., 2015. Ethylene is involved in high air humidity promoted stomatal opening of tomato (Lycopersicon esculentum) leaves. Funct. Plant Biol. 42, 376–386. https://doi.org/10.1071/FP14247

Barbeta, A., Ogaya, R., Peñuelas, J., 2012. Comparative study of diurnal and nocturnal sap flow of Quercus ilex and Phillyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). Trees - Struct. Funct. 26, 1651–1659. https://doi.org/10.1007/s00468-012-0741-4

Barbour, M.M., Buckley, T.N., 2007. The stomatal response to evaporative demand persists at night in Ricinus communis plants with high nocturnal conductance. Plant, Cell Environ. 30, 711–721. https://doi.org/10.1111/j.1365-3040.2007.01658.x

Barbour, M.M., Cernusak, L.A., Whitehead, D., Griffin, K.L., Turnbull, M.H., Tissue, D.T., Farquhar, G.D., 2005. Nocturnal stomatal conductance and implications for modelling δ 18 O of leaf-respired CO2 in temperate tree species. Funct. Plant Biol. 32, 1107–1121.

Blom-Zandstra, M., Pot, C.S., Maas, F.M., Schapendonk, A.H.C.M., 1995. Effects of different light treatments on the nocturnal transpiration and dynamics of stomatal closure of two Rose cultivars. Sci. Hortic. (Amsterdam). https://doi.org/10.1016/0304-4238(94)00751-Z

Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C., Hinojosa, J.A., Hoffmann, W.A., Franco, A.C., 2004. Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. Tree Physiol. 24, 1119–1127. https://doi.org/10.1093/treephys/24.10.1119

Caird, M.A., Richards, J.H., Donovan, L.A., 2007. Nighttime stomatal conductance and transpiration in C3 and C4 plants. Plant Physiol. 143, 4–10. https://doi.org/10.1104/pp.106.092940

Cernusak, L.A., Farquhar, G.D., Wong, S.C., Stuart-Williams, H., 2004. Measurement and interpretation of the oxygen isotope composition of carbon dioxide respired by leaves in the dark. Plant Physiol. 136, 3350–3363. https://doi.org/10.1104/pp.104.040758

Ceulemans, R., Impens, I., Imler, R., 1988. Stornatal conductance and stornatal behavior in Populus clones and hybrids. Can. J. Bot. 66, 1404–1414.

Chieppa, J., Brown, T., Giresi, P., Juenger, T.E., Resco de Dios, V., Tissue, D.T., Aspinwall, M.J., 2021. Climate and stomatal traits drive covariation in nighttime stomatal conductance and daytime gas exchange rates in a widespread C4 grass, New Phytologist. https://doi.org/10.1111/nph.16987

Christman, M.A., James, J.J., Drenovsky, R.E., Richards, J.H., 2009. Environmental stress and genetics influence night-time leaf conductance in the C4 grass Distichlis spicata. Funct. Plant Biol. 36, 50–55. https://doi.org/10.1071/FP08103

Costa, J.M., Monnet, F., Jannaud, D., Leonhardt, N., Ksas, B., Reiter, I.M., Pantin, F., Genty, B., 2015. Open all night long: The dark side of stomatal control. Plant Physiol. 167, 289–294. https://doi.org/10.1104/pp.114.253369

Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., Doligez, A., Simonneau, T., 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. Proc. Natl. Acad. Sci. U. S. A. 113, 8963–8968. https://doi.org/10.1073/pnas.1600826113

Curtis, P.S., Wang, X., 1998. A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113, 299–313. https://doi.org/10.1007/s004420050381

Daley, M.J., Phillips, N.G., 2006. Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. Tree Physiol. 26, 411–419. https://doi.org/10.1093/treephys/26.4.411

Davy, R., Esau, I., Chernokulsky, A., Outten, S., Zilitinkevich, S., 2017. Diurnal asymmetry to the observed global warming. Int. J. Climatol. 37, 79–93. https://doi.org/10.1002/joc.4688

Dawson, T.E., Bliss, L.C., 1989. Patterns of water use and the tissue water relations in the dioecious shrub, Salix arctica: the physiological basis for habitat partitioning between the sexes. Oecologia 79, 332–343. https://doi.org/10.1007/BF00384312

Dawson, T.E., Burgess, S.S.O., Tu, K.P., Oliveira, R.S., Santiago, L.S., Fisher, J.B., Simonin, K.A., Ambrose, A.R., 2007. Nighttime transpiration in woody plants from contrasting ecosystems.

Tree Physiol. 27, 561–575. https://doi.org/10.1093/treephys/27.4.561

Dayer, S., Herrera, J.C., Dai, Z., Burlett, R., Lamarque, L.J., Delzon, S., Bortolami, G., Cochard, H., Gambetta, G.A., 2021. Nighttime transpiration represents a negligible part of water loss and does not increase the risk of water stress in grapevine. Plant Cell Environ. 44, 387–398. https://doi.org/10.1111/pce.13923

Donovan, L.A., Grisé, D.J., West, J.B., Pappert, R.A., Alder, N.N., Richards, J.H., 1999. Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. Oecologia 120, 209–217. https://doi.org/10.1007/s004420050850

Duarte, A.G., Katata, G., Hoshika, Y., Hossain, M., Kreuzwieser, J., Arneth, A., Ruehr, N.K., 2016. Immediate and potential long-term effects of consecutive heat waves on the photosynthetic performance and water balance in Douglas-fir. J. Plant Physiol. 205, 57–66. https://doi.org/10.1016/j.jplph.2016.08.012

Easlon, H.M., Richards, J.H., 2009. Photosynthesis affects following night leaf conductance in Vicia faba. Plant, Cell Environ. 32, 58–63. https://doi.org/10.1111/j.1365-3040.2008.01895.x

Eller, F., Jensen, K., Reisdorff, C., 2017. Nighttime stomatal conductance differs with nutrient availability in two temperate floodplain tree species. Tree Physiol. 37, 428–440. https://doi.org/10.1093/treephys/tpw113

Escalona, J.M., Fuentes, S., Tomás, M., Martorell, S., Flexas, J., Medrano, H., 2013. Responses of leaf night transpiration to drought stress in Vitis vinifera L. Agric. Water Manag. 118, 50–58. https://doi.org/10.1016/j.agwat.2012.11.018

Forster, M.A., 2014. How significant is nocturnal sap flow? Tree Physiol. 34, 757–765. https://doi.org/10.1093/treephys/tpu051

Fuentes, S., De Bei, R., Collins, M.J., Escalona, J.M., Medrano, H., Tyerman, S., 2014. Night-time responses to water supply in grapevines (Vitis vinifera L.) under deficit irrigation and partial root-zone drying. Agric. Water Manag. 138, 1–9. https://doi.org/10.1016/j.agwat.2014.02.015

Furukawa, A., Park, S., Fujinuma, Y., Division, E.B., Studies, E., Division, E., 1990. Hybrid poplar stomata unresponsive to changes in environmental conditions. Trees 4, 191–197.

Gao, Y., Sun, Y., Ou, Y., Zheng, X., Feng, Q., Zhang, H., Fei, Y., Luo, J., Resco de Dios, V., Yao, Y., 2021. Pretreating poplar cuttings with low nitrogen ameliorates salt stress responses by increasing stored carbohydrates and priming stress signaling pathways. Ecotoxicol. Environ. Saf. 225, 112801. https://doi.org/10.1016/j.ecoenv.2021.112801

Grulke, N.E., Alonso, R., Nguyen, T., Cascio, C., Dobrowolski, W., 2004. Stomata open at night in pole-sized and mature ponderosa pine: Implications for O3 exposure metrics. Tree Physiol. 24, 1001–1010. https://doi.org/10.1093/treephys/24.9.1001

Hedges, L. V., Gurevitch, J., Curtis, P.S., 1999. The Meta-Analysis of Response Ratios in Experimental Ecology. Ecology 80, 1150. https://doi.org/10.2307/177062

Hennessey, T.L., Freeden, A.L., Field, C.B., 1993. Environmental effects on circadian rhythms in photosynthesis and stomatal opening. Planta 189, 369–376. https://doi.org/10.1007/BF00194433

Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901–908. https://doi.org/10.5005/jp/books/10202_34

Howard, A.R., Donovan, L.A., 2010. Soil nitrogen limitation does not impact nighttime water loss in Populus. Tree Physiol. 30, 23–31. https://doi.org/10.1093/treephys/tpp100

Howard, A.R., Donovan, L.A., 2007. Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. Plant Physiol. 143, 145–155. https://doi.org/10.1104/pp.106.089383

Huxman, T.E., Wilcox, B.P., Breshears, D.D., Scott, R.L., Snyder, K.A., Small, E.E., Hultine, K., Pockman, W.T., Robert, B., 2005. Ecohydrological Implications of Woody Plant Encroachment. Ecology 86, 308–319.

Kim, H.H., Goins, G.D., Wheeler, R.M., Sager, J.C., 2004. Stomatal conductance of lettuce grown under or exposed to different light qualities. Ann. Bot. 94, 691–697. https://doi.org/10.1093/aob/mch192

Konarska, J., Uddling, J., Holmer, B., Lutz, M., Lindberg, F., Pleijel, H., Thorsson, S., 2016. Transpiration of urban trees and its cooling effect in a high latitude city. Int. J. Biometeorol. 60, 159–172. https://doi.org/10.1007/s00484-015-1014-x

Kupper, P., Rohula, G., Saksing, L., Sellin, A., Lõhmus, K., Ostonen, I., Helmisaari, H.S., Sõber, A., 2012. Does soil nutrient availability influence night-time water flux of aspen saplings? Environ. Exp. Bot. 82, 37–42. https://doi.org/10.1016/j.envexpbot.2012.03.013

Lascève, G., Leymarie, J., Vavasseur, A., 1997. Alterations in light-induced stomatal opening in a starch-deficient mutant of Arabidopsis thaliana L. deficient in chloroplast phosphoglucomutase activity. Plant, Cell Environ. 20, 350–358. https://doi.org/10.1046/j.1365-3040.1997.d01-71.x

Leymarie, J., Lascève, G., Vavasseur, A., 1998. Interaction of stomatal responses to ABA and CO2 in

Arabidopsis thaliana. Aust. J. Plant Physiol. 25, 785–791. https://doi.org/10.1071/PP98031

Lombardozzi, D.L., Zeppel, M.J.B., Fisher, R.A., Tawfik, A., 2017. Representing nighttime and minimum conductance in CLM4.5: Global hydrology and carbon sensitivity analysis using observational constraints. Geosci. Model Dev. 10, 321–331. https://doi.org/10.5194/gmd-10-321-2017

Ludwig, F., Jewitt, R.A., Donovan, L.A., 2006. Nutrient and water addition effects on day- and night-time conductance and transpiration in a C3 desert annual. Oecologia 148, 219–225. https://doi.org/10.1007/s00442-006-0367-6

Mansfield, T., Heath, O., 1961. Photoperiodic effects on stomatal behaviour in Xanthium pennsylvanicum. Nature 191, 974–975.

Matyssek, R., Gunthardt-Goerg, M.S., Maurer, S., Keller, T., 1995. Nighttime exposure to ozone reduces whole-plant production in Betula pendula. Tree Physiol. 15, 159–165. https://doi.org/10.1093/treephys/15.3.159

McNellis, B., Howard, A.R., 2015. Phylogenetic and ecological patterns in nighttime transpiration among five members of the genus Rubus co-occurring in western Oregon. Ecol. Evol. 5, 3557–3569. https://doi.org/10.1002/ece3.1608

Melotto, M., Underwood, W., He, S.Y., 2008. Role of stomata in plant innate immunity and foliar bacterial diseases. Annu. Rev. Phytopathol. 46, 101–122. https://doi.org/10.1146/annurev.phyto.121107.104959

Moore, G.W., Cleverly, J.R., Owens, M.K., 2008. Nocturnal transpiration in riparian Tamarix thickets authenticated by sap flux, eddy covariance and leaf gas exchange measurements. Tree Physiol. 28, 521–528. https://doi.org/10.1093/treephys/28.4.521

Muchow, R., Fisher, M., Ludlow, M., Myers, R., 1980. Stomatal Behaviour of Kenaf and Sorghum in a Semiarid Tropical Environment. II. During the Day. Funct. Plant Biol. 7, 621. https://doi.org/10.1071/pp9800621

Munns, R., 2002. Comparative physiology of salt and water stress. Plant, Cell Environ. 25, 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x

Oishi, A.C., Oren, R., Stoy, P.C., 2008. Estimating components of forest evapotranspiration: A footprint approach for scaling sap flux measurements. Agric. For. Meteorol. 148, 1719–1732. https://doi.org/10.1016/j.agrformet.2008.06.013

Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N., Schäfer, K.V.R., 1999.

Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant, Cell Environ. 22, 1515–1526. https://doi.org/10.1046/j.1365-3040.1999.00513.x

Panchal, S., Melotto, M., 2017. Stomate-based defense and environmental cues. Plant Signal. Behav. 12, e1362517. https://doi.org/10.1080/15592324.2017.1362517

Panchal, S., Roy, D., Chitrakar, R., Price, L., Breitbach, Z.S., Armstrong, D.W., Melotto, M., 2016. Coronatine facilitates pseudomonas syringae infection of arabidopsis leaves at night. Front. Plant Sci. 7, 1–11. https://doi.org/10.3389/fpls.2016.00880

Paoletti, E., 2005. Ozone slows stomatal response to light and leaf wounding in a Mediterranean evergreen broadleaf, Arbutus unedo. Environ. Pollut. 134, 439–445. https://doi.org/https://doi.org/10.1016/j.envpol.2004.09.011

Phillips, N.G., Lewis, J.D., Logan, B.A., Tissue, D.T., 2010. Inter- and intra-specific variation in nocturnal water transport in Eucalyptus. Tree Physiol. 30, 586–596. https://doi.org/10.1093/treephys/tpq009

Press, M.C., Parsons, A.N., Mackay, A.W., Vincent, C.A., Cochrane, V., Seel, W.E., 1993. Gas exchange characteristics and nitrogen relations of two Mediterranean root hemiparasites:Bartsia trixago and Parentucellia viscosa. Oecologia 95, 145–151. https://doi.org/10.1007/BF00649518

Qin, H., Arteaga, C., Chowdhury, F.I., Granda, E., Yao, Y., Han, Y., Resco de Dios, V., 2020. Radiation and Drought Impact Residual Leaf Conductance in Two Oak Species With Implications for Water Use Models. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.603581

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Ramírez, D.A., Yactayo, W., Rolando, J.L., Quiroz, R., 2018. Preliminary Evidence of Nocturnal Transpiration and Stomatal Conductance in Potato and their Interaction with Drought and Yield. Am. J. Potato Res. 95, 139–143. https://doi.org/10.1007/s12230-017-9618-9

Rawson, H., Turner, N., Begg, J., 1978. Agronomic and Physiological Responses of Soybean and Sorghum Crops to Water Deficits. IV. Photosynthesis, Transpiration and Water Use Efficiency in Leaves. Funct. Plant Biol. 5, 195. https://doi.org/10.1071/pp9780195

Resco de Dios, V., 2017. Circadian regulation and diurnal variation in gas exchange. Plant Physiol. 175, 3–4. https://doi.org/10.1104/pp.17.00984

Resco de Dios, V., Alonso-forn, D., Peguero-pina, J., Sancho-knapik, D., Gil-pelegrín, E., Aspinwall, M.J., Blackman, C., Williams, D.G., Granda, E., 2021. Leaf vein density enhances vascular redundancy instead of carbon uptake at the expense of increasing water leaks in oaks. Environ. Exp. Bot. 188, 104527. https://doi.org/http://dx.doi.org/10.1016/j.envexpbot.2021.104527

Resco de Dios, V., Chowdhury, F.I., Granda, E., Yao, Y., Tissue, D.T., 2019. Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. New Phytol. 223, 1696–1706. https://doi.org/10.1111/nph.15881

Resco de Dios, V., Díaz-Sierra, R., Goulden, M.L., Barton, C.V.M., Boer, M.M., Gessler, A., Ferrio, J.P., Pfautsch, S., Tissue, D.T., 2013a. Woody clockworks: Circadian regulation of night-time water use in Eucalyptus globulus. New Phytol. 200, 743–752. https://doi.org/10.1111/nph.12382

Resco de Dios, V., Loik, M.E., Smith, R., Aspinwall, M.J., Tissue, D.T., 2016a. Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth. Plant Cell Environ. 39, 3–11. https://doi.org/10.1111/pce.12598

Resco de Dios, V., Loik, M.E., Smith, R.A., Tissue, D.T., 2018. Effects of a heatwave on nocturnal stomatal conductance in Eucalyptus camaldulensis. Forests 9, 1–11. https://doi.org/10.3390/f9060319

Resco de Dios, V., Mereed, T.E., Ferrio, J.P., Tissue, D.T., Voltas, J., 2016b. Intraspecific variation in juvenile tree growth under elevated CO2 alone and with O3: A meta-analysis. Tree Physiol. 36, 682–693. https://doi.org/10.1093/treephys/tpw026

Resco de Dios, V., Roy, J., Ferrio, J.P., Alday, J.G., Landais, D., Milcu, A., Gessler, A., 2015. Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. Sci. Rep. 5, 1–8. https://doi.org/10.1038/srep10975

Resco de Dios, V., Turnbull, M.H., Barbour, M.M., Ontedhu, J., Ghannoum, O., Tissue, D.T., 2013b. Soil phosphorous and endogenous rhythms exert a larger impact than CO 2 or temperature on nocturnal stomatal conductance in Eucalyptus tereticornis. Tree Physiol. 33, 1206–1215. https://doi.org/10.1093/treephys/tpt091

Rogiers, S.Y., Clarke, S.J., 2013. Nocturnal and daytime stomatal conductance respond to root-zone temperature in “Shiraz” grapevines. Ann. Bot. 111, 433–444. https://doi.org/10.1093/aob/mcs298

Rohula, G., Tulva, I., Tullus, A., Sõber, A., Kupper, P., 2017. Endogenous regulation of night-time water relations in hybrid aspen grown at ambient and elevated air humidity. Reg. Environ. Chang. 17, 2169–2178. https://doi.org/10.1007/s10113-016-1024-1

Rosenthal, R., 1979. The file drawer problem and tolerance for null results. Psychol. Bull. 86, 638–641. https://doi.org/10.1037/0033-2909.86.3.638

Salvatori, E., Fusaro, L., Mereu, S., Bernardini, A., Puppi, G., Manes, F., 2013. Different O3 response of sensitive and resistant snap bean genotypes (Phaseolus vulgaris L.): The key role of growth stage, stomatal conductance, and PSI activity. Environ. Exp. Bot. 87, 79–91. https://doi.org/10.1016/j.envexpbot.2012.09.008

Schlesinger, W.H., Jasechko, S., 2014. Transpiration in the global water cycle. Agric. For. Meteorol. 189–190, 115–117. https://doi.org/10.1016/j.agrformet.2014.01.011

Scholz, F.G., Bucci, S.J., Goldstein, G., Meinzer, F.C., Franco, A.C., Miralles-Wilhelm, F., 2007. Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. Tree Physiol. 27, 551–559. https://doi.org/10.1093/treephys/27.4.551

Schoppach, R., Sinclair, T.R., Sadok, W., 2020. Sleep tight and wake-up early: Nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment. Funct. Plant Biol. 47, 1117–1127. https://doi.org/10.1071/FP20044

Snyder, K.A., Richards, J.H., Donovan, L.A., 2003. Night-time conductance in C3 and C4 species: Do plants lose water at night? J. Exp. Bot. 54, 861–865. https://doi.org/10.1093/jxb/erg082

Sun, G., Alstad, K., Chen, J., Chen, S., Ford, C.R., Lin, G., Liu, C., Lu, N., McNulty, S.G., Miao, H., Noormets, A., Vose, J.M., Wilske, B., Zeppel, M., Zhang, Y., Zhang, Z., 2010. A general predictive model for estimating monthly ecosystem evapotranspiration. Ecohydrology 130, 245–255. https://doi.org/10.1002/eco

Sun, W., Resco, V., Williams, D.G., 2010. Nocturnal and seasonal patterns of carbon isotope composition of leaf dark-respired carbon dioxide differ among dominant species in a semiarid savanna. Oecologia 164, 297–310. https://doi.org/10.1007/s00442-010-1643-z

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. J. Stat. Softw. 36, 1–48. https://doi.org/10.18637/jss.v036.i03

Wallace, J., McJannet, D., 2010. Processes controlling transpiration in the rainforests of north Queensland, Australia. J. Hydrol. 384, 107–117. https://doi.org/10.1016/j.jhydrol.2010.01.015

Wang, D., Heckathorn, S.A., Wang, X., Philpott, S.M., 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO 2. Oecologia 169, 1–13. https://doi.org/10.1007/s00442-011-2172-0

Wheeler, R.M., Mackowiak, C.L., Yorio, N.C., Sager, J.C., 1999. Effects of CO2 on stomatal conductance: Do stomata open at very high CO2 concentrations? Ann. Bot. 83, 243–251. https://doi.org/10.1006/anbo.1998.0813

Wilcox, B.P., Thurow, T.L., 2006. Emerging issues in rangeland ecohydrology: Vegetation change and the water cycle. Rangel. Ecol. Manag. 59, 220–224. https://doi.org/10.2111/05-090R1.1

Yao, Y., Sun, Y., Feng, Q., Zhang, X., Gao, Y., Ou, Y., Yang, F., Xie, W., Resco de Dios, V., Ma, J., Yousefi, M., 2021. Acclimation to nitrogen × salt stress in Populus bolleana mediated by potassium/sodium balance. Ind. Crops Prod. 170, 113789. https://doi.org/https://doi.org/10.1016/j.indcrop.2021.113789

Zeng, W., Melotto, M., He, S.Y., 2010. Plant stomata: a checkpoint of host immunity and pathogen virulence. Curr. Opin. Biotechnol. 21, 599–603. https://doi.org/10.1016/j.copbio.2010.05.006

Zeppel, M., Tissue, D., Taylor, D., MacInnis-Ng, C., Eamus, D., 2010. Rates of nocturnal transpiration in two evergreen temperate woodland species with differing water-use strategies. Tree Physiol. 30, 988–1000. https://doi.org/10.1093/treephys/tpq053

Zeppel, M.J.B., Lewis, J.D., Chaszar, B., Smith, R.A., Medlyn, B.E., Huxman, T.E., Tissue, D.T., 2012. Nocturnal stomatal conductance responses to rising [CO 2], temperature and drought. New Phytol. 193, 929–938. https://doi.org/10.1111/j.1469-8137.2011.03993.x

Zeppel, M.J.B., Lewis, J.D., Phillips, N.G., Tissue, D.T., 2014. Consequences of nocturnal water loss: A synthesis of regulating factors and implications for capacitance, embolism and use in models. Tree Physiol. 34, 1047–1055. https://doi.org/10.1093/treephys/tpu089

Zhang, Q., Yang, Y., Peng, S., Li, Y., 2021. Nighttime transpirational cooling enabled by circadian regulation of stomatal conductance is related to stomatal anatomy and leaf morphology in rice. Planta 254, 1–14. https://doi.org/10.1007/s00425-021-03661-w

Figure captions

Fig. 1: Hypothesized drivers (green – A) and regulators (blue – B) of g_n and availability of studies across climatic MAT (mean annual temperature) and MAP (mean annual precipitation) spaces in g_n/g_d (C) and g_n (D). Abbreviations indicate Temp as temperature, VPD as vapour pressure deficit, Ψ_{PD} as predawn water potential, Nut as nutrients, SLA as specific leaf area, LVD as leaf vain density, SD as stomatal density.

Fig. 2: Main effects of environmental drivers and biotic regulators over g_n from the meta-analyses (when $n \geqslant 5$). Abbreviations include Temp as temperature, VPD as vapour pressure deficit, Ψ_{PD} as predawn water potential.

Fig. 3: Effect of drivers and regulators (when $n \geqslant 20$) over g_n across biomes (left column) and plant functional types (PFTs, right column) for VPD (vapour pressure deficit, A-B), temperature (C-D), Ψ_{PD} (predawn water potential, E-F), nutrient concentrations (G-H), circadian regulation (I-J) and assimilation (*A*, K-L). Abbreviations for biomes indicate boreal (Bor), desert and xeric (DX), Mediterranean (Med), temperate (Temp), tropical (Trop). Abbreviations for plant functional types indicate deciduous angiosperm tree (Deci Angio tree), evergreen angiosperm tree (Ever angio tree), gymnosperm tree (Gymno tree) and tropical tree (Trop tree).

Fig. 4: The effects of different drivers and regulators over g_n depends on the temporal scale. The results indicate the summary of the responses available from the literature and the time-scale over which they vary.

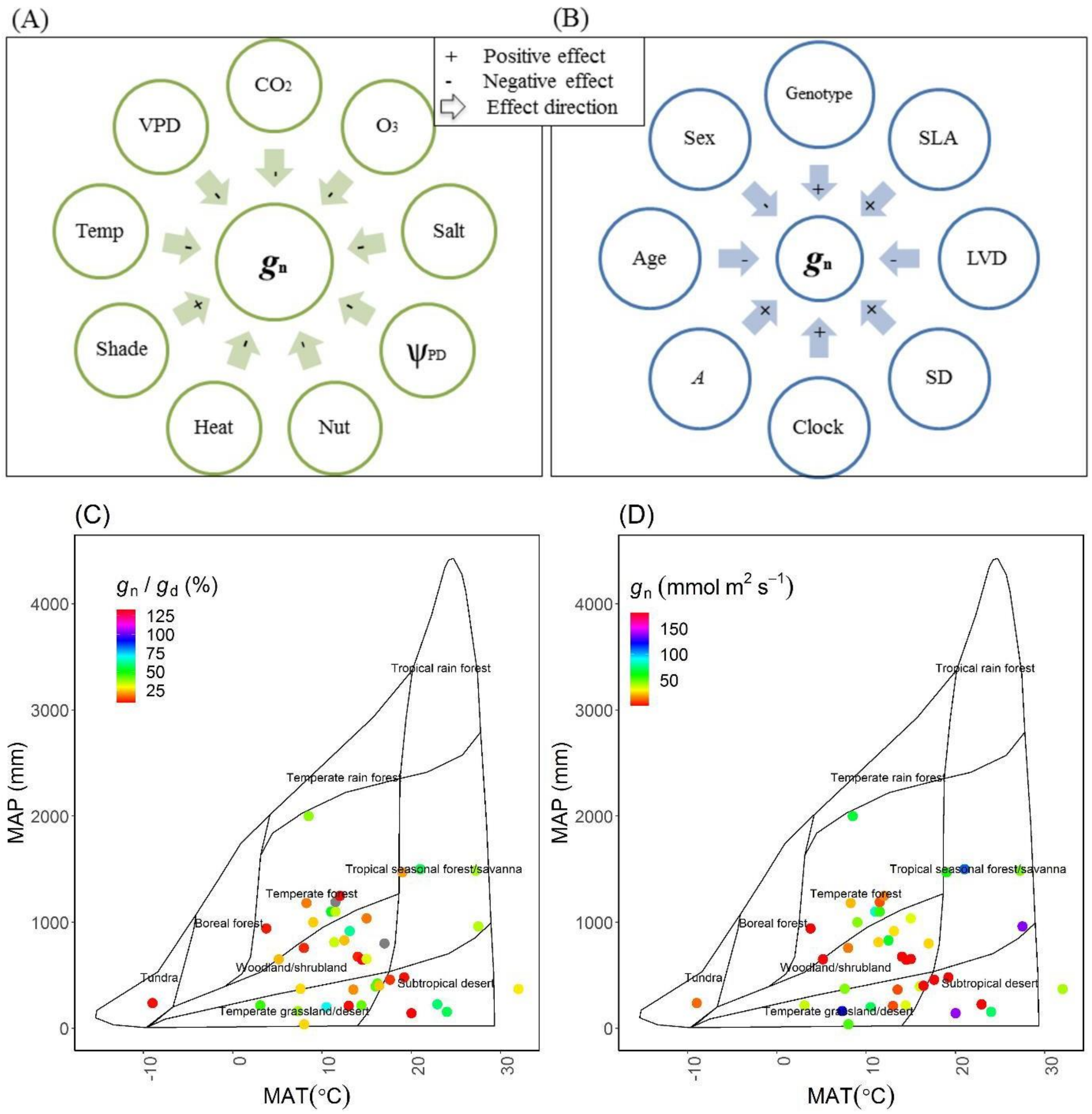

(A)
+ Positive effect
- Negative effect
Effect direction
CO2
VPD
O3
Temp
Salt
gn
Shade
ψPD
Heat
Nut
(B)
Genotype
Sex
SLA
Age
gn
LVD
A
SD
Clock
(C)
gn / gd (%)
125
100
75
50
25
MAP (mm)
4000
3000
2000
1000
0
MAT(°C)
-10
0
10
20
30
Tropical rain forest
Temperate rain forest
Tropical seasonal forest/savanna
Temperate forest
Boreal forest
Woodland/shrubland
Tundra
Subtropical desert
Temperate grassland/desert
(D)
gn (mmol m2 s−1)
150
100
50
MAP (mm)
MAT(°C)

Fig. 1

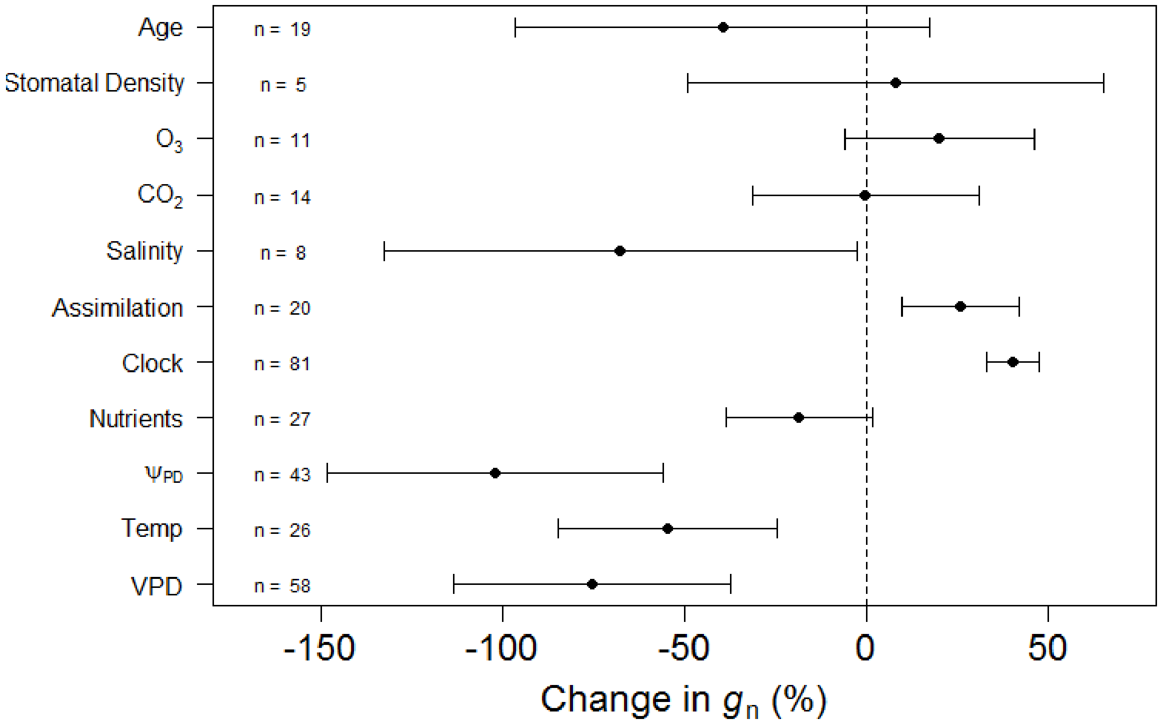

Age
Stomatal Density
O_3
CO_2
Salinity
Assimilation
Clock
Nutrients
Ψ_{PD}
Temp
VPD
n = 19
n = 5
n = 11
n = 14
n = 8
n = 20
n = 81
n = 27
n = 43
n = 26
n = 58
-150
-100
-50
0
50
Change in g_n (%)

Fig. 2

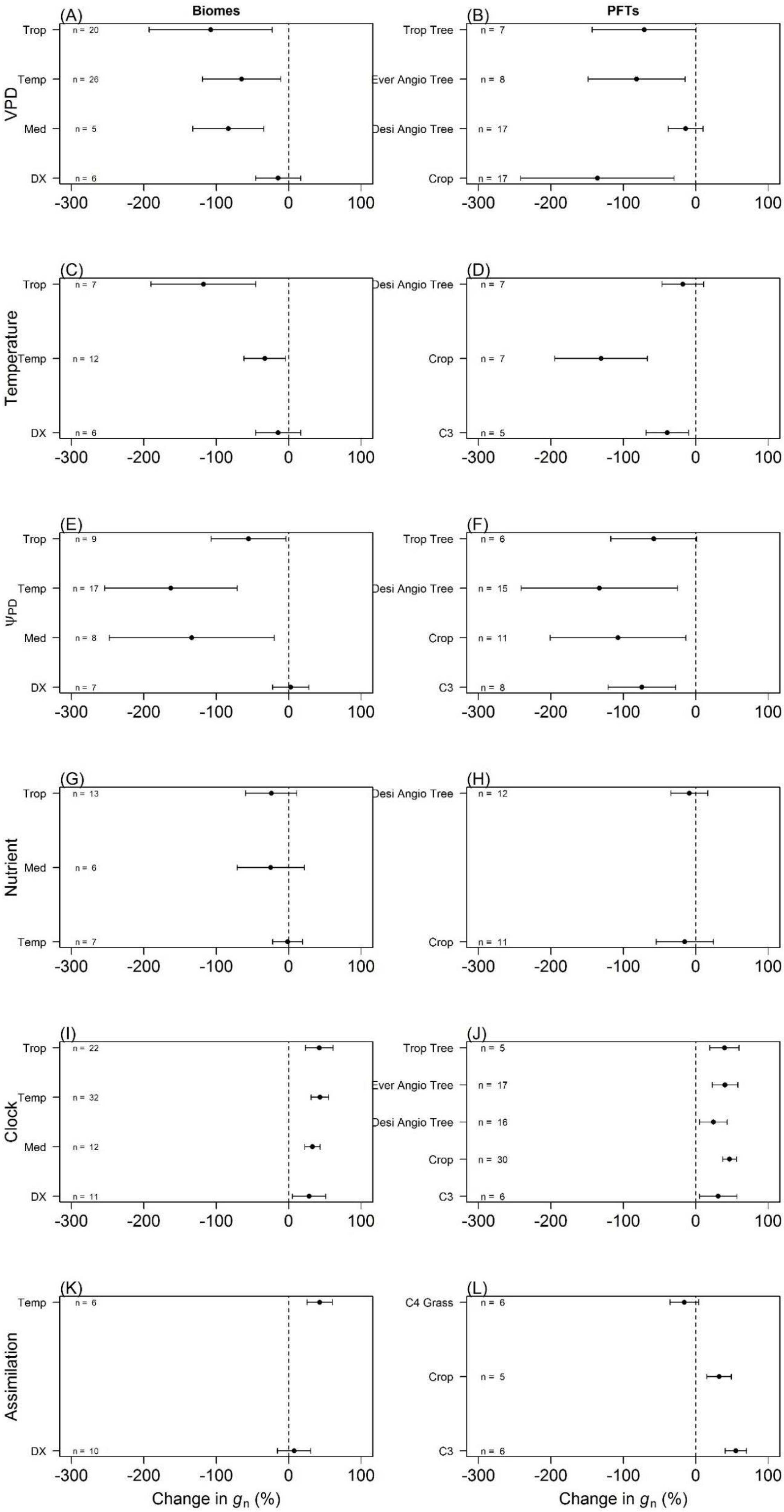

Biomes
PFTs
VPD
(A)
Trop n = 20
Temp n = 26
Med n = 5
DX n = 6
(B)
Trop Tree n = 7
Ever Angio Tree n = 8
Desi Angio Tree n = 17
Crop n = 17
Temperature
(C)
Trop n = 7
Temp n = 12
DX n = 6
(D)
Desi Angio Tree n = 7
Crop n = 7
C3 n = 5
Ψ_{PD}
(E)
Trop n = 9
Temp n = 17
Med n = 8
DX n = 7
(F)
Trop Tree n = 6
Desi Angio Tree n = 15
Crop n = 11
C3 n = 8
Nutrient
(G)
Trop n = 13
Med n = 6
Temp n = 7
(H)
Desi Angio Tree n = 12
Crop n = 11
Clock
(I)
Trop n = 22
Temp n = 32
Med n = 12
DX n = 11
(J)
Trop Tree n = 5
Ever Angio Tree n = 17
Desi Angio Tree n = 16
Crop n = 30
C3 n = 6
Assimilation
(K)
Temp n = 6
DX n = 10
(L)
C4 Grass n = 6
Crop n = 5
C3 n = 6
-300
-200
-100
0
100
Change in g_n (%)

Fig. 3

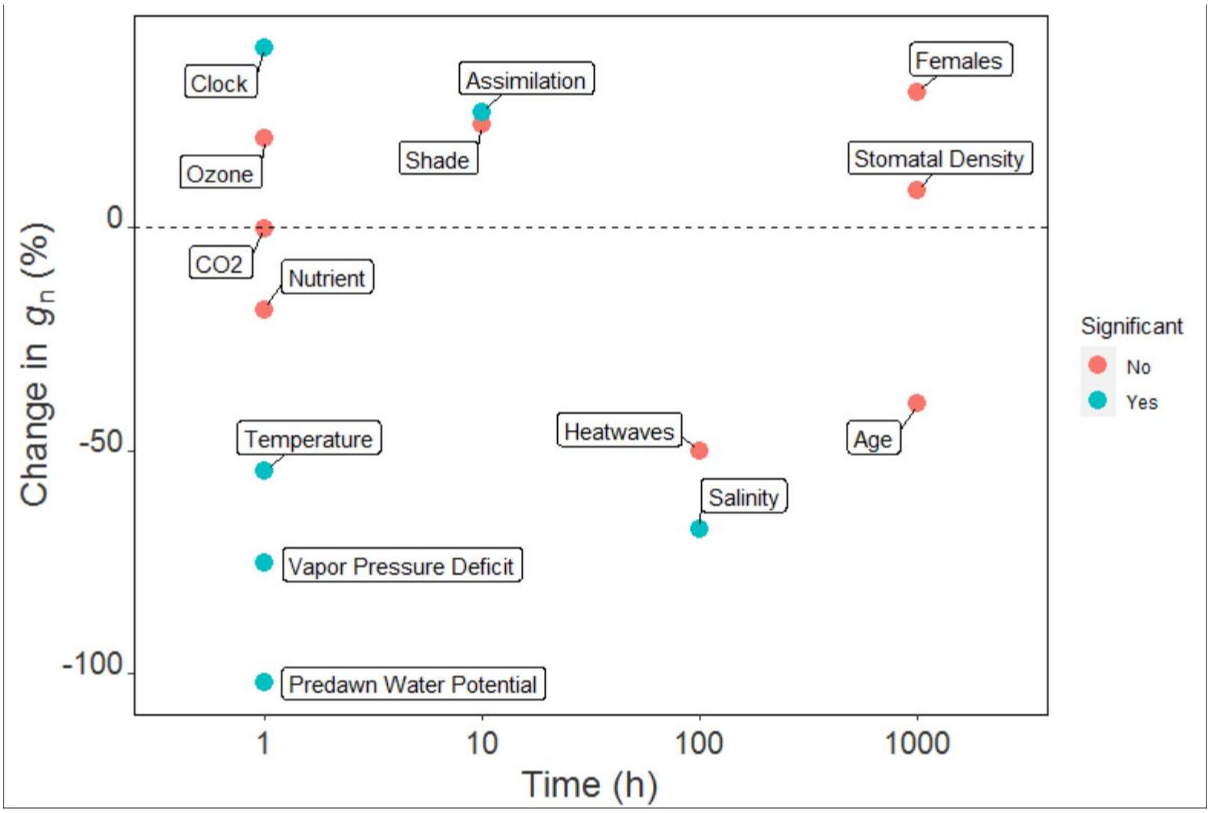

Clock
Assimilation
Females
Ozone
Shade
Stomatal Density
0
CO2
Nutrient
Significant
● No
● Yes
-50
Temperature
Heatwaves
Age
Salinity
Vapor Pressure Deficit
-100
Predawn Water Potential
Change in g_n (%)
1
10
100
1000
Time (h)

Fig. 4

VIII. Discusión general

VIII.1. Capítulo IV

En base al análisis de datos realizados en el capítulo I y con los resultados obtenidos, observamos que las coníferas no presentan simultáneamente alta resistencia a la embolia y alta tolerancia al fuego.Una de las explicaciones puede ser debido a la asignación de C entre corteza y lignina. Además, la asociación intraespecifica observada para *Pinus pinaster* indica que esta compensación tiene una base genética, aunque para poder generalizar esta afirmación se deben realizar más estudios.

Esta observación apunta a que las adaptaciones al fuego y a la sequía son, por lo menos hasta cierto punto, antagónicas en las coníferas. Resulta evidente que semejante afirmación no puede ser categórica, ya que muchos mecanismos y atributos responsables de la resistencia frente al estrés y la perturbación no fueron incluidos en este etudio. A pesar de ello, este estudio nos permite lanzar esta hipótesis para testar hasta qué punto el compromiso en la resistencia a incendios y sequía ha influido en la distribución de especies de Pinus y, en general, a las diferentes estrategias que adoptan las coníferas en función de la productividad del ecosistema (Rueda, et al., 2016).

Las especies que denominamos por su estrategia frente al fuego como dependientes ocupan lugares con una productividad intermedia. Es por ello que necesitan de una alta resistencia a la sequía y también a los fuegos de copas. Cualquier inversión en la mejora de la tolerancia al fuego será de utilidad limitada ya que no hay inversión posible en resistencia que permita la supervivencia a incendios de copas en las no rebortadoras. Por eso su estrategia se basa en la disponibilidad de un banco de semillas aéreo como son los conos serótinos (Martín-Sanz, et al., 2016). Las especies con estrategia evitadora, que habitan ecosistemas áridos (o alpinos), muestran mayor tolerancia a la sequía (o al frío), pero carecen de adaptaciones al fuego (Keeley, 2012). Finalmente, las especies tolerantes, al desarrollarse en ecosistemas poco expuestos a períodos de sequía intensa, pueden asignar preferentemente el carbono a la protección del floema y al cambium que al xilema desarrollando cortezas gruesas.

VIII.2. Capítulo V

En referencia a los factores que afectan a la capacidad de rebrotar de las especies leñosas, tradicionalmente se ha discutido en la literatura el papel de los carbohidratos y de los nutrientes almacenados como los principales limitantes del rebrote (Clarke, et al., 2013; Pate, et al., 1990). Solo unos pocos estudios han documentado las limitaciones hídricas (Cruz, et al., 2003) como un factor potencial que afecte el rebrote. Este es el primer estudio que demuestra directamente que el porcentaje de pérdida de conductividad hidráulica antes de la perturbación limita la capacidad posterior de rebrote en especies leñosas. Encontramos que las limitaciones hidráulicas fueron más graves en *Quercus faginea* que en *Quercus ilex*. Este resultado es hasta cierto punto sorprendente ya que *Quercus faginea* presenta mayor grado de segmentación hidráulica (Peguero-Pina, et al., 2015), algo que muchos autores han considerado ventajoso por aislar el estrés hídrico en compartimentos determinados (Tyree & Zimmerman, 2002).

Los resultados que obtuvimos fueron solo parcialmente consistentes con la hipótesis de que NSC limita la capacidad de rebrote. Observamos que NSC solo actuaba en una de las especies (*Quercus ilex*) y que lo hacía modulando el efecto principal de PLC.

Observamos que los factores que afectan al crecimiento del rebrote difieren de los mecanismos que intervienen en la capacidad de rebrotar. Una de las principales diferencias fue que las limitaciones hidráulicas sufridas antes de la perturbación no ejercieron ningún efecto sobre el crecimiento del rebrote. Hecho que contrasta con respuestas a la sequía, donde se ha documentado que las limitaciones hidráulicas previas afectan algunos años después de la sequía (Anderegg, et al., 2015; Ogle, et al., 2015). Aunque la explicación puede ser que nos centramos en el rebrote basal, donde la planta desarrolla nuevos tallos libres de embolias. Por lo tanto, debemos tener en cuenta que sí puede existir un legado en el crecimiento provocado por la sequía para aquellas especies que mantienen su xilema funcional varios años.

Otra de las diferencias entre los factores que explican la capacidad de rebrote y el crecimiento del rebrote fue que la fotosíntesis neta correlacionaba mejor con el crecimiento. Esto es, solo la fotosíntesis correlacionaba con la altura de ambas especies y con el volumen para *Quercus ilex*, lo que indica que la fuente de recursos controla el rebrote en lugar de las reservas en estas especies.

El contenido de NSC como motor de crecimiento fue en general débil, y solo se correlacionó con el crecimiento secundario en *Quercus ilex*, junto con la fotosíntesis, afectó el volumen de *Quercus faginea*. Los resultados obtenidos son, por lo menos en parte, contrarios a la literatura de incendios donde se otorga un papel predominante a los carbohidratos (Pausas & Keeley, 2014; Zeppel, et al., 2015). Sin embargo, los resultados son consistentes con la literatura de respuestas a la sequía, donde la concentraciones de NSC rara vez limitan la supervivencia o el crecimiento de las plantas angiospermas (Adams, et al., 2017; Korner, 2003).

Estudios previos que evaluaban la recuperación de la sequía en las angiospermas establecían que los límites de la recuperación pueden aparecer entorno un PLC>80% (Resco de Dios, et al., 2009; Urli, et al., 2013). Nuestros resultados nos permiten plantear la hipótesis que las limitaciones en la capacidad de rebrotar están por debajo del PLC 50%. Por lo tanto, las especies con estrategias rebrotadoras son más susceptibles al estrés y perturbaciones que no solo al estrés.

VIII.3. Capítulo VI

La conductancia residual (g_{res}) varió significativa e interactivamente entre los tratamientos de luz y también entre especies. No encontramos diferencias significativas al medir la conductancia residual en base a g_{MLD} o g_{n}, pero los valores fueron significativamente más elevados al usar g_{d}. Sí que pudimos relacionar g_{MLD} con las concentraciones de NSC, lo que nos sugiere que la reducción de g_{MLD} bajo condiciones de estrés hídrico está limitada por la baja disponibilidad de NSC.

En el tratamientos de luz, g_{MLD} disminuyó con el estrés hídrico, mientras que g_{MLD} permaneció baja y constante en la sombra, independientemente de las condiciones de estrés hídrico. Esto nos indica que el estrés hídrico solo afecta a g_{MLD} en condiciones de plena luz, ya que la sombra reduce tanto la g_{MLD} que no se ve afectada por el estrés hídrico.

En la literatura encontramos estudios donde identifican cómo la g_{MLD} a menudo disminuye bajo las condiciones de estrés hídrico y luz, como resultado de cambios en la composición de las ceras que recubren la cutícula, cuando estos efectos se evalúan de forma aislada (Shepherd & Wynne Griffiths, 2006). Nuestro experimento puede ser el primero en examinar las respuestas de g_{MLD} en un contexto de experimento multifactorial. Curiosamente, los efectos de luz y agua no fueron aditivos, es decir, no observamos una g_{MLD} menor bajo las condiciones de estrés hídrico moderado y estrés hídrico elevado, como deberíamos esperar en un efecto aditivo de ambos factores.

Una de las posibles explicaciones por qué g_{MLD} no disminuía más bajo en condiciones de estrés hídrico y sombra, se relaciona con la falta de disponibilidad de NSC para dedicar a la construcción de cera adicional. Esto es, una vez las plantas han alcanzado una concentración de NSC mínimo, la planta buscará preservar su NSC para otras funciones, como la osmoregulación. Aunque reconocemosque la correlación entre g_{MLD} y NSC puede haber sido afectado al considerar conjuntamente las plantas bajo diferentes condiciones de luz y estrés hídrico.

En relación a modelizar la g_{s}, nuestro estudio propone que las variaciones experimentales en la forma como se mide gres ejercen un efecto menor, siempre y cuando no se use g_{n} como indicador.

VIII.4. Capítulo VII

En distintos biomas alrededor del mundo, una parte importante de las pérdidas de agua son debidas a la conductancia estomática nocturna (g_n), y esto no solo es debido a un cierre incompleto de los estomas (Resco de Dios, et al., 2019). Hasta nuestro estudio, no estaba establecido si esto es debido a que son distintas las respuestas de los estomas a la condiciones nocturnas que a las diurnas.

Concluimos que g_n estaba afectada negativamente por varias variables ambientales como el déficit de presión de vapor, el potencial hídrico antes del amanecer, la salinidad y la temperatura, al igual que pasa durante el día. Sin embargo, cambios en la concentración de CO_2 o en la disponibilidad de nutrientes no tenían ningún efecto. Este hecho nos demuestra que los procesos de control estomático son distintos entre el día y la noche.

La mayoría de los modelos de superficie modelizan g_d e ignoran la implicación de g_n (O'Keefe & Nippert, 2018), con los resultados obtenidos hemos visto la importancia de g_n, que debe tenerse en cuenta y modelizar por separado de g_d. Los resultados obtenidos no apoyan la hipótesis que una disponibilidad limitada de nutrientes mejora la g_n. Y por el contrario los ritmos circadianos si tienen un papel importante en la regulación de la g_n. Esto es debido a que los valores máximos de g_n a menudo ocurren antes del amanecer, lo que puede ser una mejora para la fotosíntesis a primeras horas de la mañana.

IX. Conclusiones

- Las coníferas no presentan simultáneamente resistencia a la embolia (sequía) y una alta tolerancia al fuego. Esto puede ser debido al reparto de carbono en la planta, en corteza o en lignina.
- Una intensificación de la sequía puede conllevar un aumento en la intensidad en zonas de montaña actualmente dominadas por especies tolerantes a los incendios, que serán las más afectadas tanto por el aumento en la sequía como por un aumento en intensidad que pueda fomentar el desarrollo de incendios de copas.
- El principal factor que limita la capacidad de rebrotar en las especies de Quercus estudiadas es el estrés hídrico. En particular, la pérdida de la conductividad hidráulica. El efecto de las bajas concentraciones de NSC, cuando lo había, dependía del nivel de conductividad hidráulica.
- El crecimiento del rebrote depende principalmente de la capacidad para fijar carbono (fotosíntesis) y no tanto de las reservas almacenadas. El crecimiento de los rebrotes no se ve afectado por las condiciones de sequía previas a la perturbación (corte) debido a que se trataba de nuevos tallos.
- Las condiciones de sombra y estrés hídrico interaccionan al disminuir la g_{MLD} de forma que el estrés hídrico solo disminuía la g_{MLD} cuando no había limitaciones lumínicas.
- Cuando la planta tiene escasez de NSC bajo condiciones de sombra, no invertirá esos carbohidratos en aumentar la g_{MLD}.
- Una parte muy importante de las pérdidas de agua en las plantas ocurren durante la noche, debido a la conductancia estomática nocturna, la cual no solo es debida a un mal cierre de los estomas.
- Es importante destacar la implicación de los ritmos circadianos en la conductancia estomática nocturna (g_{n}), ya que los valores máximos de g_{n} se dan antes del amanecer.
- El conocimiento del comportamiento de los mecanismos fisiológicos de la planta bajo condiciones de estrés nos ha ayudado a hacernos una idea de cómo va evolucionar una masa después de una perturbación.
- Los efectos de g_{MLD} y g_{n} pueden agravar el problema de los incendios forestales al aumentar la falta de agua y, particularmente g_{n}, cuando no permite que se recarguen los capacitores del tallo sobretodo bajo condiciones de ola de calor.

X. Bibliografia

Adams, H. D., Zeppel, M. J., Anderegg, W. R., Hartmann, H., Landhäusser , S. M., Tissue, D. T., . . . McDowell, N. G. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution, 1*, 1285-1291.

Ainsworth, E. A., & Rogers, A. (2007). The response of photodynthesis and stomatal conductance to rising [CO 2]: Mechanisms and environmental interactions. *Plant Cell and Environment, 30*, 258-270.

Anderegg, W. R., Schwalm, C., Biondi, F., Camarero, J. J., Koch, G., Litvak, M., . . . Pacala, S. (2015). Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science, 349*, 528-532.

Barbeta, A., Ogaya, R., & Peñuelas, J. (2012). Comparative study of diurnaland nocturnal sap flow Quercus ilex and Phyllyrea latifolia in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). *Trees Structure and Function, 26*, 1651-1659.

Barnard, D. M., & Bauerle, W. L. (2013). The implications of minimum stomatal conductance on modeling water flux in forest canopies. *Journal of Geophysical Research-Biogeosciences, 118*, 1322-1333.

Batllori, E., De Cáceres, M., Brotons, L., Ackerly, D. D., Moritz, M. A., Lloret, F., & Oliveras, I. (2018). Compound fire-drought regimes promote ecosystem transitions in Mediterranean ecosystems. *Journal of Ecology, 107*, 1187-1198.

Blackman, C. J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S. M., & Duursma, R. A. (2016). Toward an index of desiccation time to tree mortality under drought. *Plant Cell & Environment, 39*, 2342-2345.

Boer, M. M., Bowman, D., Murphy, B. P., Cary, G. J., Cochrane, M. A., Fenshman, R. J., . . . Bradstock, R. A. (2016). Future changes in climatic water balance determine potential for transformational shifts in Australian fire regimes. *Environmental Research Letters, 11*(065002).

Boer, M. M., Resco de Dios, V., & Bradstock, R. A. (2020). Unprecedented burn area of Australian mega forest fires. *Nature Climate Change, 10*, 171-172.

Bowen, B. J., & Pate, J. (1993). The significance of root starch in postfire shoot recovery of the resprouter Stirlingia latifolia R. Br. (Proteaceae). *Annals of Botany, 72*, 7-16.

Boyer, J. S., Wong, S. C., & Farquhar, C. D. (1997). CO2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology, 114*, 185-191.

Brodribb, T. J., & Cochard, H. (2009). Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology, 149*, 575-584.

Castellnou, M., Miralles, M., Larrañaga, A., Nebot, E., Arilla, E., Castellarnau, X., . . . Pallars, J. (2021). Clasificación de las generaciones de incendios forestales: actualización. *Revista Incendios y Riesgos Naturales*, 70-72.

Choat, J., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., . . . Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature, 491*, 752-755.

Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., . . . Knox, K. J. (2013). Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist, 197*, 19-35.

Collatz, G. J., Ball, J. T., Grivet, C., & Berry, J. A. (1991). Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology, 54*, 107-136.

Collins, L. (2019). Eucalypt forests dominated by epicormic resprouters are resilient to repeated canopy fires. *Journal of Ecology, 108*, 310-324.

Costa, P., Castellnou, M., Larrañaga, A., Miralles, M., & Kraus, D. (2011). *La Prevención de los Grandes Incendios Forestales adaptada al Incendio Tipo.* Cerdanyola del Vallès: Unitat Tècnica del GRAF.

Cruz, A., Pérez, B., & Moreno, J. M. (2003). Plant stored reserves do not drive resprouting of the lignotuberous shrub Erica australis. *New Phytologist, 157*, 251-261.

Dawson, T. E., Burgess, S. S., Tu, K. P., Oliveira, R. S., Santiago, L. S., Fisher, J. B., . . . Ambrose, A. R. (2007). Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology, 27*, 561-575.

De Kauwe, M. G., Kala, J., Lin, Y. S., Pitman, A. J., Medlyn, B. E., Duursma, R. A., . . . Miralles, D. G. (2015). A test of an optimal stomatal conductance scheme within the CABLE land surface model. *Geoscientific Model Development, 8*, 431-452.

Duursma, R. A., Blackman, C. J., Lopez, R., Martin-Stpaul, N. K., Cochard, H., & Medlyn, B. E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist, 221*, 693-705.

Easlon, H. M., & Richards, J. H. (2009). Photosynthesis affects following night leaf conductance in Vicia faba. *Plant Cell and Environment, 32,* 58-63.

Fairman, T. A., Bennet, L. T., & Nitschke, C. R. (2019). Short-interval wildfires increase likelihood of resprouting failure in fire-tolerant trees. *Journal of Environmental Management, 231,* 59-65.

Karavani, A., Boer, M. M., Baudena, M., Colinas, C., Diaz-Sierra, R., Peman, J., . . . Resco de Dios, V. (2018). Fire-induced deforestation in drought-prone Mediterranean forests: drivers and unknowns from leaves to communities. *Ecological Monographs, 88*, 141-169.

Keeley, J. E. (2012). Ecology and evolution of pine life histories. *Annals of Forest Science, 69,* 445-453.

Keeley, J. E., Bond, W. J., Bradstock, R. A., Pausas, J. G., & Rundel, P. W. (2012). *Fire in Mediterranean Ecosystems; Ecology, Evolution and Management.* Cambridge: Cambridge University Press.

Korner, C. (2003). Carbon limitation in trees. *Journal of Ecology, 91*, 4-17.

Leuning, R. (1995). A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. *Plant Cell & Environment, 18,* 339-355.

Lloret, F., Calvo, E., Pons, X., & Díaz-Delgado, R. (2002). Wildfires and landscape patterns in the Eastern Iberian Peninsula. *Landscape Ecology, 17*, 742-759.

Lombardozzi, D. L., Zeppel, M. J., Fisher, R. A., & Tawfik, A. (2017). Representing nighttime and minimum conductance in CLM4.5: global hydrology and carbon sensitivity analysis using observational constraints. *Geoscientific Model Development, 10*, 321-331.

Long, S. P., & Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany, 54*, 2393-2401.

Martín-Sanz, R. C., Santos-del-Blanco, L., Notivol, E., Chambel, M. R., San-Martin, R., & Climent, J. (2016). Disentangling plasticity of serotiny, a key adaptative trait in a Mediterranean conifer. *American Journal of Botany, 103,* 1582-1591.

Martin-Stpaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought depends on timely stomatal closure. *Ecology Letters, 20*, 1437-1447.

Nagel, J. M., Griffin, K. L., Schuster, W. S., Tissue, D. T., Turnbull, M. H., Brown, K. J., & Whitehead, D. (2002). Energy investment in leaves of red maple and co-occurring oaks within a forested watershed. *Tree Physiology, 22*, 859-867.

Nolan, R. H., Boer, M. M., Collins, L., Resco de Dios, V., Clarke, H., Jenkins, M., . . . Bradstock, R. A. (2020). Causes and consequences of eastern Australia's 2019-20 season of mega-fires. *Global Change Biology, 26*, 1039-1041.

Ogle, K., Barber, J. J., Barron-Gafford, G. A., Bentley, L. P., Young, J. M., Huxman, T. E., . . . Tissue, D. T. (2015). Quantifying ecological memory in plant and ecosystem processes. *Ecology Letters, 18*, 221-235.

Oishi, A. C., Oren, R., & Stoy, P. C. (2008). Estimating components of forest evapotranspiration: A footprint approach for scaling sap flux measurements. *Agricultural and Forest Meteorology, 148*, 1719-1732.

O'Keefe, K., & Nippert, J. B. (2018). Drivers of nocturnal water flux in a tallgrass prairie. *Functional Ecology, 32*, 1155-1167.

Palacio, S., Maestro, M., & Montserrat-Marti, G. (2007). Relationship between shoot-rooting and root-sprouting abilities and the carbohydrate and nitrogen reserves of Mediterranean dwarf shrubs. *Annals of Botany, 100*, 865-874.

Pate, J. S., Froend, R. H., Bowen, B. J., Hansen, A., & Kuo, J. (1990). Seedling growth and storage characteristics of seeder and resprouter species of Mediterranean-type ecosystems of S.W. Australia. *Annals of Botany, 65*, 585-601.

Pausas, J. G., & Fernández-Muñoz, S. (2011). Fire regime changes in the Western MediterraneanBasin: from fuel-limited to drought-driven fire regime. *Climatic Change, 110*, 215-226.

Pausas, J. G., & Keeley, J. E. (2014). Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist, 204*, 55-65.

Pausas, J. G., & Ribeiro, E. (2013). The global fire-productivity realtionship. *Global Ecology Biogeography, 20*, 728-736.

Pausas, J., & Bradstock, R. A. (2007). Plant persistence fire traits along a productivity and disturbance gradient in Mediterranean shrublands of southeastern Australia. *Global Ecology Biogeography, 16*, 330-340.

Peguero-Pina, J. J., Sancho-Knapik, D., Martin, P., Saz, M. A., Gea-Izquierdo, G., Cañellas, I., & Gil-Pelegrín, E. (2015). Evidence of vulnerability segmentation in a deciduous Mediterranean oak (Quercus subpyrenaica E. H. del Villar). *Trees, 29*, 1917-1927.

Philips, N. G., Lewis, J. D., Logan, B. A., & Tissue, D. T. (2010). Inter- and intraspecific variation in nocturnal water transport in Eucalyptus. *Tree Physiology, 30*, 586-596.

Poorter, H. (1994). Construction costs and payback time of biomass: A whole plant approach. En E. J. Garnier, *A whole plant perspective on carbon-nitrogen interactions* (págs. 111-127). Leiden: Backhuys Publishers.

Pratt, R. B., Jacobsen, A. L., Ramirez, A. R., Helms, A. M., Traugh, C. A., Tobin, M. F., . . . Davis, S. D. (2014). Mortality of resprouting chaparral shrubs after a fire and during a record drought: physiological mechanisms and demographic consequences. *Global Change Biology, 20,* 893-907.

Rego, F. C. (1992). Land Use Changes and Wildfires. En M. P. Editors. Teller A., *Responses of Forest Ecosystems to Environmental Changes* (págs. 363-376). Springer.

Resco de Dios, V. (2020). *Plant-Fire Interactions.* Switzerland: Springer Nature.

Resco de Dios, V., Chowdhury, F. I., Granda, E., Yao, Y., & Tissue, D. T. (2019). Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. *New Phytologist, 223*, 1696-1706.

Resco de Dios, V., Ewers, B. E., Sun, W., Huxman, T. E., Weltzin, J. F., & Williams, D. G. (2009). Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C3 woody legume, Prosopis velutina. *New Phytologist, 181,* 672-682.

Resco de Dios, V., Turnbull, M. H., Barbour, M. M., Ontedhu, J., Ghannoum, O., & Tissue, D. T. (2013). Soil phosphorous and endogenous rhytms exert a larger impact than CO 2 or temperature on nocturnal stomatal conductance in Eucalyptus tereticornis. *Tree Physiology, 33*, 1206-1215.

Rueda, M., Godoy, O., & Hawkins, B. A. (2016). Spatial and evolutionary parallelism between shade and drought tolerance explains the distributions of conifers in the conterminous United States.

Global Ecology Biogeography, 26, 31-42.

Sánchez-Pinillos, M., Coll, L., De Cáceres, M., & Ameztegui, A. (2016). Assessing the persistence capacity of communities facing natural disturbances on the basis of species response traits. *Ecological Indicators, 66*, 76-85.

San-Miguel-Ayanz, J., Rodrigues, M., Oliveira, S., Kemper Pacheco, C. J., Moreira, F., Duguy, B., & Camia, A. (2012). Land Cover Change and Fire Regime in the European Mediterranean Region. En d. l. Editors: Moreira F, *Post-fire management and restoration of Southern European forests* (págs. 21-43). New York: Springer.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671-675.

Shepherd, T., & Wynne Griffiths, D. (2006). The effects of stress on plant cuticular waxes. *New Phytologist, 171*, 469-499.

Snyder, K. A., Richards, J. H., & Donovan, L. A. (2003). Night-time conductance in C3 and C4 species: Do plants lose water at night? *Journal of Experimental Botany, 54*, 861-865.

Tyree, M. T., & Zimmerman, M. H. (2002). *Xylem structure and the ascent of sap.* Berlin: Springer.

Urli, M., Porté, A. J., Cochard, H., Guengant, Y., Burlett, R., & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology, 33*, 672-683.

Vega-Garcia, C., Tatay, J., Blanco, R., & Chuvieco, E. (2010). Evaluation of the Influence of Local Fuel Homogeneity on Fire Hazard through Landsat-5 TM Texture Measures. *Photogrammetric Engineering and Remote Sensing, 76*, 853–864.

Vertregt, N., & Penning de Vries, F. W. (1987). A rapid method for determining the efficiency of biosynthesis of plant biomass. *Journal of Theoretical Biology, 128*, 109-119.

Wallace, J., & McJannet, D. (2010). Processes controlling transpiration in the reinforests of north Queensland, Australia. *Journal of Hydrology, 384*, 107-117.

Wiley, E., & Heliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist, 195*, 285-289.

Zeppel, M. J., Harrison, S. P., Adams, H. D., Kelley, D. I., Li, G., Tissue, D. T., . . . McDowell, N. G. (2015). Drought and resprouting plants. *New Phytologist, 206*, 583-589.

Zeppel, M. J., Lewis, J. D., Chaszar, B., Smith, R. A., Medlyn, B. E., Huxman, T. E., & Tissue, D. T.

(2012). Nocturnal stomatal conductance responses to rising [CO 2], temperature and drought. *New Phytologist, 193*, 929-938.

Zeppel, M. J., Lewis, J. D., Phillips, N. G., & Tissue, D. T. (2014). Consequences of nocturnal water loss: A synthesis of regulating factors and implications for capacitance, embolism and use in models. *Tree Physiology, 34*, 1047-1055.