UNIVERSIDAD DE CÓRDOBA

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

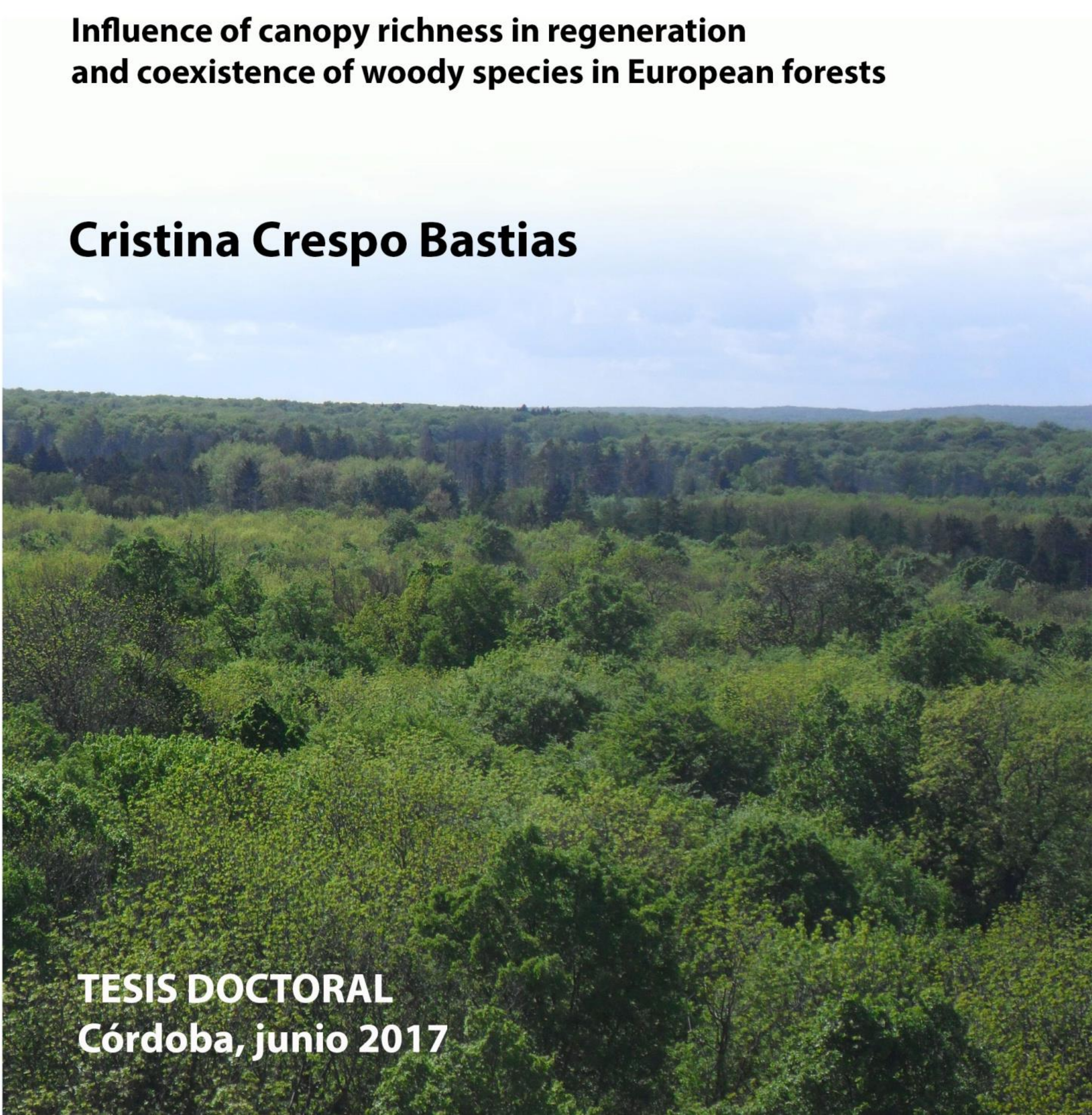
mncn
museonacionaldecienciasnaturales

Influencia de la riqueza del dosel en la regeneración y coexistencia de especies leñosas en bosques europeos

---

Influence of canopy richness in regeneration and coexistence of woody species in European forests

Cristina Crespo Bastias



TESIS DOCTORAL
Córdoba, junio 2017

TITULO: *Influencia de la riqueza del dosel en la regeneración y coexistencia de especies leñosas en bosques europeos*

AUTOR: *Cristina Crespo Bastias*

Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

www.uco.es/publicaciones
publicaciones@uco.es

Diseño de la portada: Isabel Torres Miguel.

Fotografía de la portada: vista por encima del dosel arbóreo del bosque templado (Parque Nacional de Hainich; Alemania). Autora: Cristina Crespo Bastias. **Fotografía de la contraportada:** bosques europeos de estudio. Autora: Cristina Crespo Bastias e Inés Carrascón.

Esta tesis ha sido realizada en el departamento de Biogeografía y Cambio Global del Museo Nacional de Ciencias Naturales-CSIC, gracias a la concesión de una beca predoctoral de Formación de personal Universitario (FPU), AP2010-5600, por parte del Ministerio de Educación, Cultura y Deporte. Los estudios llevados a cabo en esta tesis han sido financiados por el proyecto europeo "Functional Significance of forest biodiversity" (FUNDIV-EUROPE) concedido por séptimo Programa Marco de Investigación y Desarrollo Tecnológico por la Unión Europea (FP7/2007-2013) con el no. 265171 y el proyecto REMEDINAL3eCM (S2013/MAE-2719) concedido por Programa de Actividades de I+D de la Comunidad de Madrid (Consejería de Educación).

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

mncn
museonacionaldecienciasnaturales

UNIVERSIDAD DE CÓRDOBA

TESIS DOCTORAL

“Influencia de la riqueza del dosel en la regeneración y coexistencia de especies leñosas en bosques europeos”

Memoria presentada por la Licenciada Dña. Cristina Crespo Bastias para optar al grado de Doctor en Biología, dentro del programa de doctorado “Recursos Naturales y Sostenibilidad” de la Universidad de Córdoba.

La doctoranda

Cristina Crespo Bastias

Director de la Tesis

Dr: Fernando Valladares
Profesor de Investigación
Museo Nacional de Ciencias Naturales – CSIC

El Tutor de la Tesis

Rafael V

Dr: Rafael Villar Montero
Profesor Titular
Universidad de Córdoba

Codirectora de la Tesis

Raquel Benavides

Dra: Raquel Benavides Calvo
Investigadora científica
Museo Nacional de Ciencias Naturales – CSIC

Córdoba, junio 2017

TÍTULO DE LA TESIS

Influencia de la riqueza del dosel en la regeneración y coexistencia de especies leñosas en bosques europeos

DOCTORANDO/A: CRISTINA CRESPO BASTIAS

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

Esta tesis supervisada por nosotros desde sus inicios ha abordado distintos aspectos sobre la relación entre regeneración forestal, coexistencia de especies y biodiversidad combinando trabajo descriptivo en campo con trabajo experimental en invernadero. Buena parte de los trabajos se han apoyado en el esfuerzo investigador del proyecto europeo FUNDIV en el que tanto la doctoranda como los directores hemos estado implicados activamente. La tesis partió de unas hipótesis generales que se fueron refinando a medida que se iba comprendiendo la naturaleza de las relaciones entre rasgos funcionales, biodiversidad y regeneración, y al final se plantearon objetivos experimentales concretos que se derivaron de estas primeras observaciones en las parcelas de los seis tipos de bosques europeos estudiadas. Los resultados obtenidos son de gran calidad e interés y han dado lugar a publicaciones en revistas especializadas de ecología.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Madrid, 23 de Junio de 2017

Firma del/de los director/es

Raquel Benavide

Fdo.: Fernando Valladares Ros

Fdo.: Raquel Benavides Calvo

TÍTULO DE LA TESIS

Influencia de la riqueza del dosel en la regeneración y coexistencia de especies leñosas en bosques europeos

DOCTORANDO/A: CRISTINA CRESPO BASTIAS

ESCRITO RAZONADO DEL RESPONSABLE DE LA LÍNEA DE INVESTIGACIÓN

Esta tesis aborda varias cuestiones candentes en el área de Ecología como es el papel de la biodiversidad y las funciones y servicios ecosistémicos, la importancia de la variabilidad intraespecífica versus interespecífica, así como el papel de la plasticidad fenotípica. Otro mérito de esta tesis es que el sistema en que se comprueban las hipótesis es sumamente complejo, como son los bosques. Muchos de los trabajos anteriores sobre las relaciones de la biodiversidad y función de los ecosistemas se han realizado en ecosistemas más simples (por ejemplo los pastizales), en el que las especies tienen un ciclo de vida corto y es posible estudiar la dinámica de las poblaciones. Otro aspecto que llama la atención es que las zonas de estudio comprenden un gradiente climático importante desde los bosques boreales a los bosques mediterráneos, con lo que los resultados obtenidos se pueden extrapolar a un gran número de formaciones boscosas del mundo. Además, los resultados de esta tesis han sido publicados en varias revistas de prestigio internacional en el campo de la Ecología. Por último, sus resultados contribuyen a entender mejor la dinámica de los bosques europeos y permite preveer los cambios que se están produciendo en éstos, y que influirán en los bienes y servicios que nos prestan.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 23 de Junio de 2017
Firma del responsable de línea de investigación

Rafael V

Fdo.: Rafael Villar

INFORME SOBRE APORTACIONES DERIVADAS DE LA TESIS DOCTORAL Y FACTOR DE IMPACTO DE LAS REVISTAS CIENTÍFICAS (JOURNAL CITATION REPORT).

Publicaciones en revistas científicas (Capítulos de la tesis doctoral)

Bastias CC, Fortunel C, Valladares F, Baraloto C, Benavides R, Cornwell W, Markesteijn, L, de Oliveira, A. A, Sansevero, JBB, Vaz, MC, Kraft, NJB. (2017) Intraspecific leaf trait variability along a boreal-to-tropical community diversity gradient. PLoS ONE 12(2): e0172495. (Capítulo 4).
Factor de impacto: 3.54, Posición de la revista en relación a su categoría específica (Multidisciplinary sciences) 9/63; primer cuartil (Q1)

Manuscritos enviados a revistas científicas (Capítulos de la tesis doctoral)

Bastias CC, Benavides R, Ricote NM, Valladares F. (2017) Local canopy richness does not influence phenotypic expression and plasticity of tree seedlings exposed to different resource availabilities. Manuscrito enviado a *Journal of Vegetation Sience.* (Capítulo 3).
Factor de impacto: 2.92, Posición de la revista en relación a su categoría específica (Plant Sciences) 42/211; primer quartil (Q1)

Truchado DA†, **Bastias CC†,** Valladares F & De la Cruz M. (2017) Species richness influences the spatial structure of European forests. Manuscrito enviado a *Global Ecology and Biogeography.* (Capítulo 5). † Coautoría compartida.
Factor de impacto: 6.045, Posición de la revista en relación a su categoría específica (Ecology) 10 /153; primer quartil (Q1).

Otras publicaciones en las que he colaborado

Baeten L, Verheyen K, Wirth C. (…), **Bastias CC** et al. (2013) A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. Perspectives in Plant Ecology, Evolution and Systematics, 15, 281–291.
Factor de impacto: 3.12, Posición de la revista en relación a su categoría específica (Ecology) 42/153; primer quartil (Q1)

Valladares F, **Bastias CC**, Godoy O, Granda E, Escudero A. *(2015).* Species coexistence in a changing world. *Frontiers in Plant Science.*
Factor de impacto: 4.29, Posición de la revista en relación a su categoría específica (Plant Sciences) 19/211; primer quartil (Q1)

Van der plas F, Maaning P, Soliveres S, (…), **Bastias CC,** et al. (2016). Jack-of-all-trades effects drive biodiversity–ecosystem multifunctionality relationships in European forests. *Nature communications.*
Factor de impacto: 12.12, Posición de la revista en relación a su categoría específica (Multidisciplinary sciences) 3/63; primer quartil (Q1)

Van der plas F, Maaning P, Soliveres S, (…), **Bastias, CC** et al. (2016) Biotic homogenization can decrease landscape-scale forest multifunctionality. *Proceedings of the National Academy of Sciences (PNAS).*
Factor de impacto: 9.42, Posición de la revista en relación a su categoría específica (Multidisciplinary sciences) 4/63; primer quartil (Q1).

Otros artículos enviados

Ricote NM, **Bastias CC**, Valladares F, Pérez FT, Bozinovic F. (2017). Selfing and drought tolerance in a warming world: 1 balance and future changes for two 2 herbaceous species in the South American Andes. Manuscrito enviado a *Functional Ecology*.
Factor de impacto: 5.63, Posición de la revista en relación a su categoría específica (Ecology) 14/153; primer quartil (Q1).

Ratcliffe S, Wirth C, Jucker T, (…), **Bastias CC** et al (2017). Context dependency of biodiversity and ecosystem functioning relations in European forests: a search for scaling rules. Manuscrito enviado a *Ecology Letters*.
Factor de impacto: 9.41, Posición de la revista en relación a su categoría específica (Ecology) 4/153; primer quartil (Q1).

Van der Plas F, Ratcliffe S, (…),**Bastias CC** et al (2017). Continental mapping of forest ecosystem functions reveals a high but unrealized potential for forest multifunctionality. Manuscrito enviado a *Ecology Letters*.
Factor de impacto: 9.41, Posición de la revista en relación a su categoría específica (Ecology) 4/153; primer quartil (Q1).

Comunicaciones presentadas a congresos

Bastias CC, Benavides R, García-Rabasa S, Godoy A, Sansevero JBB, Carrascón I & Valladares F. Regeneración natural de seis bosques europeos: efecto de la diversidad del dosel e importancia relativa de factores bióticos y abióticos. Congreso Asociación española de Ecología Terrestre (AEET). Tipo de comunicación: póster. 6-10 de mayo de 2013.

Bastias CC, Benavides R, García-Rabasa S, Godoy, A, Sansevero JBB, Carrascón I & Valladares F. Efecto de la diversidad del dosel en la regeneración de diferentes especies arbóreas. Congreso Forestal Español. Tipo de comunicación: póster. 10-1 de Junio de 2013.

Bastias, CC, Fortunel F, Valladares F, Baraloto C, Benavides R, Cornwell W, Markesteijn L, de Oliveira AA, Sansevero JBB, Vaz MC, Kraft NJK. Using leaf traits to explain woody species coexistence along a species richness gradient. Congreso Asociación española de Ecología Terrestre (AEET). Tipo de comunicación: comunicación oral. 16-19 de Junio de 2015.

Bastias CC, Benavides R, García-Rabasa S, Godoy, A, Sansevero JBB, Carrascón I & Valladares F. The influence of canopy diversity together with biotic and abiotic factors on tree speciesregeneration in six contrasting european forests. IUFRO

Landscape Ecology Conference. Tipo de comunicación: comunicación oral. 23-27 de Agosto de 2015.

Bastias CC, Morán-López T, Benavides R & Valladares F. Efecto de la riqueza de árboles en el dosel forestal y factores bióticos y abióticos en la regeneración natural de diferentes especies arbóreas en bosques europeos. REMEDINAL. Tipo de comunicación: comunicación oral. 21 y 22 de Junio de 2016.

Bastias CC, Morán-López T, Benavides R, & Valladares F. Richer tree canopies do not increase recruitment richness nor abundance in five mature European forests. Congreso Asociación española de Ecología Terrestre (AEET). Tipo de comunicación: póster. 31 de Enero al 4 de Febrero de 2017.

A mis padres, por su
apoyo incondicional y por
hacerme tal y como soy.

“Me mudé al bosque porque deseaba vivir deliberadamente, hacerle frente sólo a los hechos esenciales de la vida, probar si podía no aprender lo que tenía para enseñarme, en vez de quedarme esperando la hora de mi muerte para darme cuenta de que no había vivido”

Henry David Thoreau

Índice


capacity
treatment
differences
indicated
evidence
conifers
abundance
biodiversity
between
gradient
recruitment
trees
Mediterranean
groups
environmental
isotopic
showed
response
intraspecific
similarity
overlap
community
results
seed
six
studies
plant
pattern
interactions
interspecific
richness
isotopes
point
expression
role
individual
coming
forests
seedlings
canopy
functional
five
ITV
vs
total
effect
nutrient
structure
boreal
functioning
play
theory
woody
inter
species
relationship
extent
two
three
SLA
sites
tree
traits
light
signals
competition
leaf
trait
variation
heterogeneity
increased
European
communities
plasticity
different
diversity
level
size
variance
spatial
forest
phenotypic
mechanisms
assembly
higher
scales
found
individuals
breadth
plots
variability
differing
regeneration
studied
potential
content
similar
plantplant
important
continental

RESUMEN

Recientes estudios están mostrando una relación positiva entre la riqueza de especies (biodiversidad) y las funciones y servicios ecosistémicos de los bosques. De ahí que para mantener la multifuncionalidad del bosque, parezca necesaria la conservación de la biodiversidad. La conservación de la riqueza de especies, a su vez, pasa por el mantenimiento de la dinámica natural de los bosques, incluyendo procesos clave como la regeneración y la coexistencia de las especies. Sin embargo, hasta la fecha, apenas hay estudios sobre esta relación y/o el papel que juega la riqueza de especies en estos dos aspectos clave. Su comprensión puede ayudar a predecir tendencias futuras en la composición, estructura y funcionamiento de los bosques ante un escenario de cambio global, y por tanto proporcionar a las políticas de gestión forestal un conocimiento adecuado en cuanto a las estrategias a aplicar ante dichos cambios.

En este marco conceptual, el objetivo principal de esta tesis consiste en entender el papel de la riqueza de especies dominantes del dosel de diferentes tipos de bosques europeos en la regeneración y la coexistencia de individuos tanto en la fase juvenil como en la fase adulta. La tesis que incluye un total de 5 capítulos, además de la introducción y la discusión general, se desarrolló utilizando una red de parcelas establecidas para el proyecto europeo FunDivEUROPE. Éstas incluyen un total de 209 parcelas distribuidas en seis tipos de bosques europeos diferentes a lo largo de un gradiente bioclimático, desde un bosque boreal (Finlandia) a un bosque continental Mediterráneo (España). Dentro de cada tipo de bosque, las parcelas varían en nivel de riqueza de las especies arbóreas dominantes de cada bosque, con parcelas monoespecíficas, y parcelas mixtas con combinaciones de 2, 3, 4 y hasta 5 especies diferentes. Cada una de las parcelas fue caracterizada tomando diversas medidas de variables ambientales tanto abióticas como bióticas, que fueron utilizadas de forma común en todos los capítulos de la presente tesis. Además, se tomaron una serie de medidas particulares para el desarrollo de los objetivos planteados en cada uno de los capítulos: medidas de riqueza y abundancia de juveniles (en 5 de los 6 bosques; Capítulo 1), medidas de la diversidad funcional total de la comunidad de juveniles a nivel inter e intraespecífica mediante la medición de una serie de rasgos funcionales (en 3 bosques diferentes; Capítulo 2 y 4), estimación de la plasticidad fenotípica de juveniles mediante un experimento en condiciones controladas (en 2 bosques diferentes; Capítulo 3), y localización espacial de los árboles adultos (en los 6 bosques europeos; Capítulo 5).

Los resultados mostraron que la riqueza del dosel no influyó en la regeneración ni de forma directa, a través de un mayor aporte de semillas sobre la abundancia y más diverso aporte de semillas sobre la riqueza del regenerado, ni de forma indirecta a través de una mayor creación de heterogeneidad ambiental. Es más, encontramos una discordancia entre la composición de especies en adultos vs. juveniles. Una vez establecidos los juveniles, nuestros resultados mostraron que la variabilidad intraespecífica (i.e. variación de rasgos entre individuos de una misma especie) explicó gran parte de la diversidad funcional de la comunidad. Sin embargo, la importancia de ésta dependió del tipo de rasgo medido, siendo el contenido foliar de isótopos ^{13}C y ^{15}N los que más variabilidad presentaron dentro de una especie, mientras que otros rasgos foliares como el SLA (de las siglas en inglés, área foliar específica) y el contenido de nitrógeno en la hoja los que variaron más entre especies, i.e. mayor variabilidad interespecífica y menor intraespecífica. Además, encontramos que la variabilidad intraespecífica de la comunidad de juveniles no estuvo influenciada por la riqueza del dosel ni a escala de gradiente de diversidad europeo (1-5 especies/ha), ni a una escala mayor incluyendo los hiperdiversos bosques tropicales (>250 species/ha). De hecho encontramos que los individuos juveniles presentaron valores de rasgos similares (i.e. individuos funcionalmente equivalentes), solapando en el espacio funcional ocupado independientemente de la riqueza de especies de la comunidad. Dichos resultados apuntaron a una coexistencia en fase juvenil regida por mecanismos estocásticos o por actuación principalmente de mecanismos ecualizadores (*sensu* Chesson 2000). Mientras que en la fase adulta, sí que observamos un patrón espacial más agregado de los individuos con el aumento la riqueza de especies del dosel en los bosques europeos, sugiriendo complementariedad de nicho entre individuos de diferentes especies.

Centrándonos en una de las principales fuentes de variabilidad intraespecífica, la plasticidad fenotípica de los juveniles, con un experimento en jardín común observamos de nuevo que, la riqueza del dosel o la heterogeneidad ambiental de la comunidad de la que procedían no ejercieron efecto apreciable sobre la plasticidad de las plántulas, siendo el grupo funcional al que pertenecían el factor más relevante explicando diferencias tanto en la expresión fenotípica como en su plasticidad frente a cambios en la disponibilidad de luz y nutrientes. Concretamente, en el bosque situado más al sur del gradiente europeo (bosque continental mediterráneo en España), vimos que los individuos de las especies de *Quercus* fueron más plásticas que las de especies de *Pinus*.

Nuestros resultados sugieren que la riqueza del dosel no es un buen predictor de la regeneración natural de los bosques europeos, evidenciando la complejidad de dicho proceso para poder modelizarlo y predecirlo. Además apuntan a un posible cambio en la dominancia de las especies arbóreas de las masas forestales europeas, la cual sería efectiva si los juveniles, con una coexistencia regida por mecanismos (aparentemente) estocásticos en la etapa temprana estudiada en este trabajo, respondieran satisfactoriamente a los cambios ambientales mediante ajuste de sus rasgos (plasticidad fenotípica), superando filtros tanto bióticos como abióticos en etapas posteriores. Un cambio en la composición de especies en las masas forestales europeas podría provocar cambios en el funcionamiento de los bosques, con relevantes implicaciones potenciales sobre los bienes y servicios que éstos nos aportan en el momento actual.

SUMMARY

Recent studies are evidencing a positive relationship between species richness (biodiversity) and forest ecosystem functions and services. Thus, the conservation of biodiversity is key to assure forest multifunctionality. The maintenance of the species richness requires, in turn, successful natural forest dynamics, including key processes such as regeneration and species coexistence. However, up to date, few studies have focused on the role of forest species richness on these two key aspects, being their understanding essential to predict future shifts in the forest composition, structure and functioning under a global change scenario. Furthermore, disentangling this relationship may provide suitable guidelines for forest management policies in order to face these global changes.

In this conceptual framework, the aim of this thesis seeks to understand the role of the species richness of dominant tree species in the canopy on the regeneration and the species coexistence at the juvenile and adult stages in different European forest types. This thesis, which includes a total of 5 chapters together with an introduction and general discussion, was performed using a plot network established within the European project FunDivEUROPE. This plot network comprises a total of 209 plots distributed in six different European forests along a bioclimatic gradient, from a boreal forest in Finland to a continental Mediterranean forest in Spain. Within each forest type, plots were set along a diversity gradient considering the dominant tree species in each forest, including monospecific and mixed plots with different combination of 2, 3, 4 and 5 species. We recorded several abiotic and biotic variables to characterize the environment in each plot, data that were used in all chapters of this thesis. In addition, we performed certain measurements for each chapter according to their specific goals: measures of juvenile abundance and richness (in five out of six forests; Chapter 1), characterization of the functional diversity of juvenile communities at the inter- and intraspecific trait levels using different functional traits (in three different forests; Chapter 2 and 4), estimation of the phenotypic plasticity of seedlings in response to different resource availabilities in a greenhouse experiment (in two different forests; Chapter 3), and finally, the spatial localization of adult trees within each plot (in the six European forests, Chapter 5).

The results showed that canopy species richness did not directly affect natural regeneration through a larger seed supply on juvenile abundance or a richer (more diverse) seed pool on juvenile species richness. Neither had the canopy species richness an indirect effect on regeneration through environmental heterogeneity. Furthermore, we found a mismatch between the species composition of the adults (in the canopy) and the juveniles in the studied European forests. Once juveniles established, we found that a substantial part of their functional diversity was explained at the intraspecific trait variability level (i.e. trait variability among individuals within species). However, the extent of the intraspecific trait variability depended on the functional trait measured, being the leaf ^{13}C y ^{15}N contents the traits with the highest variation at the intraspecific level, while SLA and leaf nitrogen content the traits with the least variation at the intraspecific and largest at the interspecific levels. Moreover, the intraspecific trait variability at the juvenile community level was not influenced by the canopy species richness considering multiple and contrasting forest ecosystems such as boreal (with only 3 dominant species) or hyperdiverse tropical forests (>250 species/ha). In fact, we found that juveniles had similar trait values (i.e. individuals functionally equivalent), overlapping in their functional space regardless of the community species richness. These results pointed at stochastic or equalizing mechanisms as the mechanisms underlying the juvenile coexistence (*sensu* Chesson 2000). In contrast, in the adult stage, we observed that individuals belonging to different woody species were more spatially aggregated as the species richness increased in the studied European forests, suggesting niche complementarity in the resource use among individuals from different species.

Focusing on one of the main source of intraspecific trait variability, we observed that the phenotypic plasticity was similar among individuals coming from tree communities differing in canopy species richness and environmental heterogeneity. In other words, the species richness of the canopy did not exert any effect on the plasticity of seedlings to light and nutrient availability, being the main differences in plasticity and phenotypic expression explained by the functional group they belonged to. Particularly, we found in the southernmost forest (the continental Mediterranean forest) that individuals of *Quercus* were more plastic than individuals of species from genus *Pinus*.

Our results suggested that canopy species richness was not a good predictor of natural regeneration, evidencing the complexity of this process. Moreover, our findings pointed at a potential shift in the dominant tree species in the European forests, which would be effective if juveniles coexisting due to stochastic mechanisms in this early phase were able to surpass environmental changes and filters (both biotic and abiotic) in later developmental phases through adjusting their trait values (phenotypic plasticity). A shift in the species composition of European forest stands might provoke changes in the forest functioning with relevant implications in the goods and services that these forests are currently providing.

INTRODUCCIÓN

ANTECEDENTES

Los bosques europeos y la riqueza del dosel arbóreo.

La superficie forestal total mundial se cifra en torno a unas 4.000 millones de hectáreas, lo que constituye aproximadamente un tercio de la superficie total del planeta (~31%) (EC 2013; FAO 2015). La importancia ecológica de los bosques reside en su carácter multifuncional, ya que proveen a la sociedad de un amplio rango de servicios y bienes vitales para su bienestar (MEA 2005). Los bosques constituyen el hábitat de numerosos animales y plantas, pero además, juegan un importante papel en la mitigación del cambio climático (a través del almacenaje de carbono), ayudan a proteger al suelo de la erosión, mantener los ciclos de nutrientes y mejorar la calidad del agua, nos proveen de numerosas materias primas tales como madera o frutos y tiene un importante papel como uso recreativo.

Sin embargo, la cobertura forestal no se distribuye de forma homogénea en el planeta. Entre las diferentes regiones, Europa representa un 5% de la superficie mundial forestal y aproximadamente un 40% de su superficie está ocupada por masa forestal (FAO 2010; EC 2013). Sin embargo, a pesar de su pequeña representación a nivel mundial, los bosques europeos destacan por abarcar una amplia variedad de condiciones abióticas y bióticas, reflejo de su diversidad climática, edáfica, altitudinal y topográfica. Otra de las características principales de los bosques europeos es, que a pesar de presentar una importante variedad de tipologías de bosque que van desde bosques boreales a bosques mediterráneos, la riqueza arbórea es relativamente baja en comparación con otros bosques en su misma latitud (bosques templados de América o Asia) (Walter 1985). Dicha característica se debe por un lado, a la disposición este-oeste de los principales sistemas montañosos del continente que han actuado de barreras naturales a la migración de especies frente a las numerosas glaciaciones a lo largo de la historia y por otro lado, a la ocupación antrópica desde la antigüedad y a la gestión de los montes que han favorecido principalmente bosques dominados por una sola especie.

Recientemente, numerosos estudios han evaluado el efecto de la riqueza del dosel arbóreo en las diferentes funciones del ecosistema, mostrando un efecto positivo generalizado de la riqueza de especies arbóreas sobre numerosas funciones del ecosistema como por ejemplo resistencia a plagas, producción primaria o en el ciclo de nutrientes (Jactel & Brockerhoff 2007; Zeugin *et al.* 2010; Gamfeldt *et al.* 2013; Jucker *et al.* 2014a; van der Plas *et al.* 2016). Por tanto, preservar y mejorar la biodiversidad ha llegado a ser uno de los objetivos principales en las políticas de gestión de los bosques (McElhinny *et al.* 2005; Kelty 2006), considerando el escenario de pérdida de biodiversidad de las últimas décadas. La reciente puesta en práctica de medidas favoreciendo bosques mixtos frente a los monoespecíficos ha conducido a un incremento considerable en la proporción de los primeros (Knoke *et al.* 2008). Pero, para preservar la biodiversidad y asegurar la continuidad de las funciones ecosistémicas brindadas por los sistemas forestales en el futuro es necesario mantener la dinámica natural de los bosques y por tanto, favorecer procesos clave como la regeneración y la coexistencia de especies (Chesson 2000). Sin embargo, hasta la fecha, la incertidumbre acerca del papel de la riqueza de especies arbóreas de los bosques europeos sobre aspectos clave en la dinámica de comunidades, como el proceso de regeneración y el de interacción de unas especies con otras (coexistencia) desde la etapa juvenil es aún elevada. Dada la relevancia del tema y las implicaciones potenciales en la gestión que aseguren la conservación de los bosques en un escenario de cambio global, la presente tesis se encuadra dentro de este gran marco conceptual.

El efecto de la riqueza de especies en la regeneración y la coexistencia de especies a través de la heterogeneidad ambiental.

Uno de los mecanismos a través del cual la riqueza de especies puede ejercer un efecto sobre ambos procesos (regeneración y coexistencia de especies) es mediante la creación de una mayor heterogeneidad ambiental (Chávez & Macdonald 2010; Pérez-Ramos & Marañón 2012; Granda, Escudero & Valladares 2014). Esta premisa se basa en que cada una de las especies presente en una comunidad tiene unos requerimientos ecológicos particulares y unas estrategias propias para captar los recursos. Esto permite que las especies, por un lado, puedan aprovechar los recursos disponibles a diferentes niveles y, por otro lado, ocupar de manera diferente el espacio de acuerdo a sus requerimientos, dando como resultado una modificación de las características abióticas de su entorno más próximo (Yankelevich *et al.* 2006) y por consiguiente, la creación de diferentes microhábitats con diferente disponibilidad de recursos. A su vez, cuanto más heterogéneo es un ambiente, mayor número de especies diferentes podrán establecerse aprovechando los distintos microhábitats de acuerdo a sus requerimientos del nicho de regeneración (Grubb 1977), coexistiendo en la misma comunidad por complementariedad en el uso de los recursos y minimizando la competencia entre especies (MacArthur & Levins 1967; Pacala & Tilman 1994; Chesson 2000).

La relación entre diversidad de especies y heterogeneidad ambiental es un tema estudiado durante décadas en ecología, y tradicionalmente se ha asumido una única dirección positiva (Ricklefs 1977; Shorrocks & Sevenster 1995; Bell, Lechowicz & Waterway 2000; Stein, Gerstner & Kreft 2014). Sin embargo, nuevos estudios han cuestionado la generalidad de los resultados positivos de esta relación, mostrando una ausencia o incluso una relación negativa entre heterogeneidad ambiental y riqueza de especies (Laanisto *et al.* 2013; Gazol *et al.* 2013; Ampoorter *et al.* 2016). Por un lado, nuevos resultados muestran que una mayor diversidad no necesariamente tiene un efecto aditivo en las condiciones ambientales favoreciendo una mayor heterogeneidad ambiental, sino que es la interacción entre las especies presentes las que dan lugar a nuevas condiciones ambientales diferentes a las esperadas en sus doseles monoespecíficos, y estas nuevas condiciones pueden o no ser más heterogéneas (Ball *et al.* 2008; Ampoorter *et al.* 2014). La relación de la diversidad de especies y la heterogeneidad ambiental es abordada en esta tesis pretendiendo arrojar una mayor claridad al entendimiento de la misma en los bosques europeos.

Regeneración y coexistencia de especies: dos aspectos clave para la composición y la diversidad de los bosques en el futuro.

La composición y la diversidad de especies de una comunidad es el resultado de diversos procesos y factores no excluyentes que actúan a diferentes escalas de una manera jerarquizada; abarcando procesos a escala global como el azar en la distribución inicial de los *phyla* dentro de diferentes zonas bioclimáticas, procesos de especiación, extinción y migración, pasando por procesos de actuación a nivel regional como la dispersión, hasta procesos a escala local tanto abióticos (filtros ambientales impuestos por las condiciones abióticas locales) como bióticos (interacción local entre especies). Estos factores, son conocidos como filtros jerarquizados y constituyen las reglas de ensamblaje de las especies (Götzenberger *et al.* 2012).

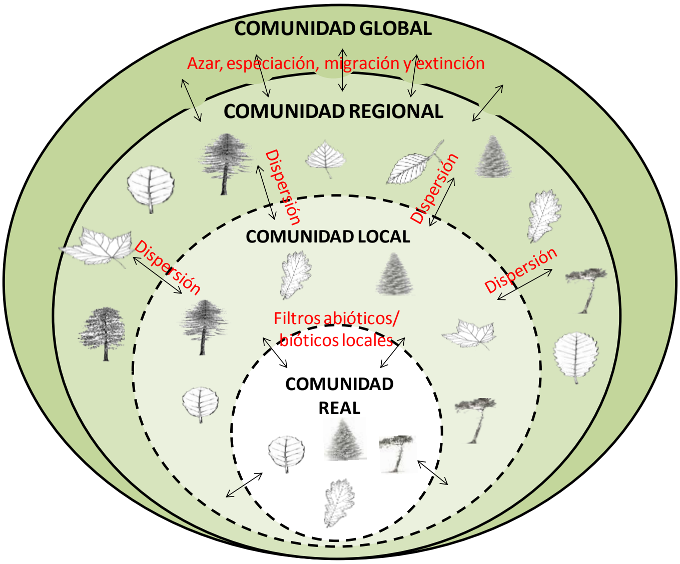


Figura 1. Formación de la composición y riqueza de una comunidad siguiendo las reglas de ensamblaje de las especies (adaptada de Götzenberger *et al.* (2012)). En un

determinado momento existe un conjunto de especies potenciales a nivel global que, a través de procesos de especiación, extinción y migración y azar, dará lugar a un conjunto regional de especies potenciales más reducido. A partir del conjunto regional se constituye otro local potencial formado por aquellas especies que son capaces de dispersase (filtro de dispersión). A escala local, las condiciones abióticas del hábitat podrán una limitación para el establecimiento de las especies (regenerado), una vez establecidas las especies tendrán que hacer frente a la interacción con otras especies (filtros bióticos) para su supervivencia. Al final aquellas especies capaces de sobrepasar todos estos filtros son las que compondrán la comunidad final.

En esta tesis, como avanzábamos en la sección anterior, nos centraremos en estudiar como el factor biótico local de riqueza de especies del dosel afecta a dos aspectos clave en la dinámica de comunidades que ocurren a escala local: la regeneración y la coexistencia de especies en la fase juvenil y adulta. El éxito de ambos procesos vendrá determinado por las especies que componen la comunidad haciendo frente a los filtros abióticos y bióticos mencionados anteriormente y será determinante en la composición y diversidad futura de esas comunidades.

(i) Regeneración natural. Filtros a la dispersión y a la post-dispersión.

Una de las etapas de mayor importancia para una los individuos de una población es su fase de reclutamiento. El éxito del proceso de regeneración natural es indispensable para mantener el equilibrio de las comunidades vegetales y asegurar la actual composición y diversidad del bosque a largo plazo (Harper 1977). A la vez que crítico, el proceso de regeneración es un proceso complejo, en el que numerosos factores tanto estocásticos como determinísticos intervienen en su éxito. La complejidad de este proceso hace que el estudio de la regeneración de una especie sea uno de los mayores retos en la ecología de comunidades (Grubb 1977; Pacala & Tilman 1994).

Retomando las reglas de ensamblaje de especies, los dos filtros principales a los que se enfrenta una especie para un establecimiento exitoso dentro de la comunidad son: (i) una dispersión efectiva; para ello la cantidad de semillas producidas por adultos circundantes deber ser suficiente y su llegada a la comunidad por los agentes dispersantes debe ser efectiva (Muller-Landau *et al.* 2002; Schupp, Jordano & Gómez 2010); y (ii), unas condiciones ambientales locales del hábitat (temperatura, radiación, humedad, etc) deben ser las apropiadas para la regeneración de la especie, i.e. el llamado nicho de regeneración (Grubb 1977; Muller-Landau *et al.* 2002; Clark *et al.* 2007), el cual se define como el conjunto de requerimientos ambientales necesarios para la germinación y establecimiento de una especie (Gurevitch & Fox 2006). En este contexto, la diversidad local de especies arbóreas puede jugar un papel muy importante ayudando 'a priori' a sobrepasar ambos filtros (dispersión y post-dispersión) a través, por un lado, de un aporte de semillas más diverso y, por otro lado, induciendo heterogeneidad ambiental y favoreciendo diferentes microhábitats disponibles para ser ocupados por individuos de diferentes especies. En ambos casos, bosques con doseles más diversos en especies favorecerían 'a priori' la continuidad de bosques mixtos.

Sin embargo, es preciso destacar que la regeneración de las especies puede limitarse por la presencia de sus congéneres de acuerdo a los procesos de denso-dependencia negativa, los cuales han sido ampliamente estudiados en bosques tropicales (Janzen 1970; Connell 1971; Comita *et al.* 2014). Una de las hipótesis más destacadas es la de Janzen-Connell, según la cual los enemigos naturales de una especie, incluyendo patógenos y predadores, son más abundantes y por tanto causan una mayor mortalidad de semillas y plántulas cuánto más cerca se encuentren de adultos conespecíficos (doseles monoespecíficos) (Clark & Clark 1984; Schupp, Milleron & Russo 2002). De acuerdo con esta hipótesis, una dispersión alejada de adultos de la misma especie permite reducir la depredación e infección por patógenos, favoreciéndose así la regeneración a cierta distancia y dejando libre espacio que puede ser aprovechado por plántulas de otras especies. Esta hipótesis ampliamente estudiada sugiere que la composición actual de especies arbóreas del bosque no coincida necesariamente con la composición actual de juveniles de la comunidad, lo que apunta a una composición del bosque dinámica en el tiempo (Granda *et al.* 2012; Galiano *et al.* 2013; Granda *et al.* 2014).

(ii) Interacciones bióticas de las especies. Múltiples teorías para explicar la coexistencia de especies.

Una vez que las especies han logrado superar los filtros de dispersión y los filtros abióticos impuestos por el hábitat para establecerse exitosamente en la comunidad, ahora su supervivencia dentro de ella dependerá de su capacidad de hacer frente a las interacciones con otras especies así como a las fluctuaciones ambientales que puedan ocurrir en el tiempo (Fenner and Thompson, 2005).

En cuanto a las interacciones bióticas, la competencia es el principal proceso estructurador que da forma a la composición y a la diversidad final de las comunidades vegetales (Chesson 2000). De acuerdo con la teoría clásica de coexistencia, cada especie posee un nicho particular dentro de la comunidad, que engloba una serie de factores ambientales específicos para satisfacer sus requerimientos ecológicos y que le permiten coexistir con otras especies, siempre y cuando los requerimientos difieran de unas especies a otras (teoría de nicho; Gause 1934; Hutchinson 1957). Bajo esta premisa, si hay un solapamiento en el uso de los recursos por dos especies dentro de la comunidad, la especie que presente una mayor ventaja competitiva en el uso del recurso será capaz de desplazar a la otra (desplazamiento competitivo) o incluso excluirla de la comunidad (exclusión competitiva). Los individuos con requerimientos ecológicos más similares como por ejemplo individuos de una misma especie (conespecíficos), competirán más fuertemente existiendo un umbral (límite de similitud) por encima del cual no pueden coexistir (MacArthur & Levins 1967; Abrams 1983). Por tanto, de acuerdo con esta teoría de nicho, se podría decir que la riqueza de especies de una comunidad será proporcional al rango de las condiciones ambientales de la comunidad dividido por la amplitud de nicho de cada especie (MacArthur & Levins 1967). Posteriormente ha surgido la teoría neutral (Hubbell 2001) que desafía la tan aceptada teoría de nicho, la cual postula que las especies son funcionalmente equivalentes y que por tanto, poseen la misma capacidad de competir por los recursos. De acuerdo con esta teoría, los eventos estocásticos son los principales motores que modelan la composición final de especies de una comunidad (Hubbell 2001; Chave 2004; Hubbell 2005). Sin embargo, ambas teorías han resultado en muchos casos insuficientes para explicar la coexistencia de especies en comunidades reales, y numerosas discrepancias han sido encontradas entre las predicciones teóricas y los estudios empíricos (Nathan, von Hardenberg & Meron 2013).

Con la necesidad de entender los mecanismos que determinan la coexistencia de especies, y por tanto la composición y diversidad de especies de una comunidad, ciertos autores han reformulado las ideas de competencia en un marco más mecanicista, cuyos principios reconcilian ambas teorías neutral y nicho, hasta entonces vistas como teorías excluyentes (Adler, HilleRisLambers & Levine 2007). Esta nueva formulación para explicar la coexistencia de especies (Teoría Moderna de Coexistencia; Chesson 2000) se basa en dos tipos de mecanismos. Por un lado, los mecanismos estabilizadores, los cuales mantienen la diversidad vía diferenciación de nicho controlando las densidades de poblaciones de las especies que llegan a ser dominantes y evitando la extinción de aquellas especies con densidades menores (Adler *et al.* 2007). Por otro lado, los mecanismos ecualizadores basados en los principios de neutralidad y que se definen por la capacidad similar de competir de las especies (Chesson 2000). En definitiva, Chesson (2000) postula que la coexistencia de especies vendrá determinada por el *trade-off* o solución intermedia considerando la fuerza relativa de ambos mecanismos en cada comunidad.

Cabe añadir que la competencia no es el único factor modulador de la coexistencia de las especies, sino que también existen otros factores, como la facilitación que favorece la diversidad y la estructura de comunidades (Callaway 2007; McIntire & Fajardo 2014), o la heterogeneidad ambiental o la variabilidad intraespecífica (Violle *et al.* 2012) que minimizan la competencia entre las especies ayudando así a la coexistencia. Hay que resaltar que la coexistencia no es, ni mucho menos, un asunto cerrado y la generación de evidencia empírica y la evaluación de estas hipótesis es un tema de actualidad que se abordará en varios capítulos a lo largo de esta tesis.

La diversidad funcional y la variabilidad intraespecífica: un nuevo enfoque funcional para estudiar el ensamblaje y la coexistencia de especies.

Tradicionalmente los cambios en la diversidad de una comunidad se han analizado a través de cambios en la abundancia y la riqueza de especies mediante la utilización de los clásicos índices de diversidad y equidad (Morin *et al.* 2011). Esta clásica aproximación supone una equivalencia ecológica entre las especies, lo que implica que todas tienen la misma probabilidad de sobrevivir, competir, y reproducirse (Chave 2004). Sin embargo, las especies no deben ser consideradas 'a priori' equivalentes, ya que la pérdida o adición de especies en la comunidad puede tener un efecto, mayor o menor, en la estructura de las comunidades y más allá, en los procesos ecosistémicos (Tilman *et al.* 1997). Debido a esta limitante, en los últimos años ha surgido la necesidad de incorporar la diversidad funcional de la comunidad i.e. la variabilidad en los rasgos funcionales de las especies de una comunidad, los cuales se puede definir como cualquier característica medible a nivel individual y que tiene un efecto directo o indirecto a la adecuación de la planta al ambiente y su éxito reproductivo (los llamados *performance* y *fitness*) reflejado en su crecimiento, reproducción y la supervivencia (Violle *et al.* 2007). La diversidad funcional ha sido propuesta como la clave para tener una visión más completa de la relación entre la diversidad, la estructura de las comunidades y el funcionamiento de los ecosistemas (Tilman *et al.* 1997; McGill *et al.* 2006; Adler *et al.* 2013). Esta novedosa aproximación funcional supone un avance importante en la ecología de comunidades que nos permite ir más allá de la mera descripción tradicional de la diversidad con el uso de los índices clásicos, abordando cuestiones relacionadas con las interacciones existentes entre los individuos que componen una comunidad, que a su vez están íntimamente relacionadas con sus estrategias funcionales, como la de éstos con su medio ambiente (Escudero & Valladares 2016).

Desde su inicio, el estudio de ensamblaje de especies y la composición y diversidad de las comunidades vegetales desde un punto de vista funcional se ha centrado en la variación que existe en los rasgos entre las diferentes especies que componen la comunidad (i.e. variabilidad interespecífica) (McGill *et al.* 2006; Kraft, Valencia & Ackerly 2008; Weiher *et al.* 2011). De esta manera, cada especie en la comunidad está representada mediante su valor medio del rasgo ponderado generalmente por la abundancia de la especie en la comunidad (Lavorel *et al.* 2008). Esta aproximación se basa en la asunción de que la variación en un rasgo es mucho mayor entre especies que dentro de una especie (Garnier *et al.* 2001; Kattge *et al.* 2011; Laughlin *et al.* 2011). El enfoque a nivel interespecífico, es decir usando el rasgo medio de la especie, puede ser suficiente para entender aquellas cuestiones ecológicas a mayor escala, relacionadas con la detención de los filtros abióticos impuestos por el hábitat local (Kraft *et al.* 2008; Fortunel *et al.* 2014), permitiéndonos la consideración de todas las especies de la comunidad con un esfuerzo de muestreo reducido. Esto llega a ser especialmente importante en comunidades ricas en especies (bosques tropicales) donde el número de especies por hectárea llega a ser superior a las 200 especies/ha (Baraloto *et al.* 2010).

Sin embargo, en los últimos años numerosos estudios han revelado que la variación de rasgos entre individuos de la misma especie (i.e. variabilidad intraespecífica) puede tener una gran importancia tanto a nivel de comunidad (Messier, McGill & Lechowicz 2010; Jung *et al.* 2010; Violle *et al.* 2012), como a nivel de especie entre los individuos que la forman (Bastias *et al.* 2017). Estudios recientes han demostrado que la variabilidad intraespecífica puede explicar una parte sustancial de la variación de rasgos (diversidad funcional) (Cianciaruso *et al.* 2009; de Bello *et al.* 2011), pudiendo llegar incluso a tener rangos similares a la variación interespecífica (Messier *et al.* 2010). De esta forma, numerosos autores han señalado la importancia de la incorporación de la variación intraespecífica en los estudios de ensamblaje de comunidades para entender el mantenimiento de la coexistencia de especies (Jung *et al.* 2010; Long *et al.* 2011), la riqueza de especies (Cianciaruso *et al.* 2009; Violle *et al.* 2012) y la dinámica de comunidades (Courbaud, Vieilledent & Kunstler 2012). Por ejemplo, la variabilidad intraespecífica explicaría como individuos de una especie con un valor de rasgo diferente al valor medio de su especie podrían sobrepasar ciertos filtros abióticos y bióticos impuestos por el ambiente, que con el valor medio de su especie no podrían (Figura 2). El mecanismo subyacente que permitiría el ajuste del rasgo de algunos de los individuos de una especie para sobrepasar los filtros tanto bióticos como abióticos y mediar con la heterogeneidad ambiental impuesta por el medio sería a través de la plasticidad fenotípica (Sultan 1987; Valladares, Gianoli & Gómez 2007).

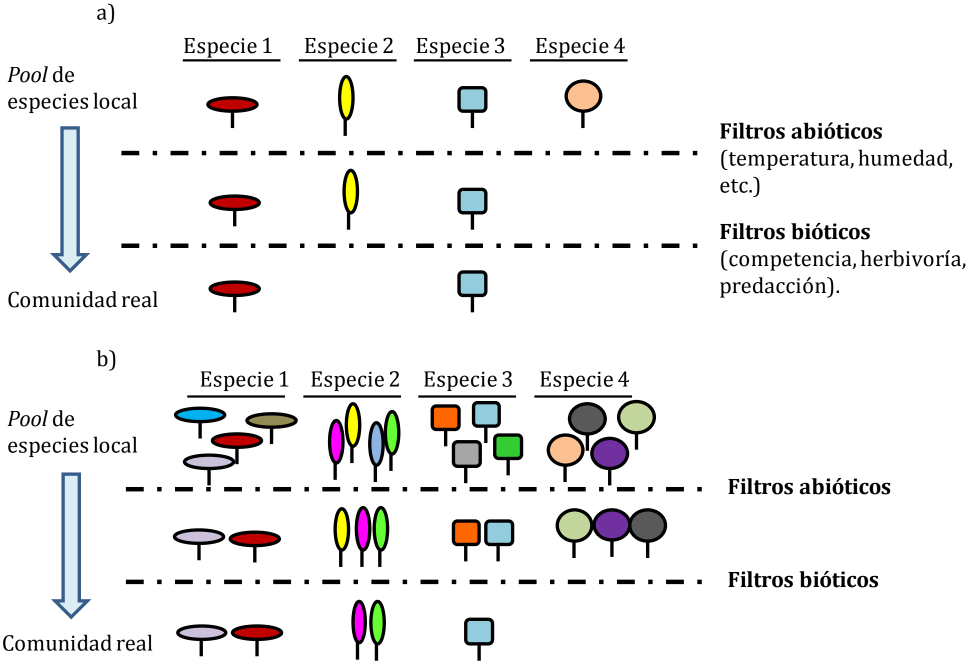


Figura 2. Influencia de la variabilidad intraespecífica en los distintos filtros ambientales a escala local y en la riqueza especies de la comunidad final. (A) Teoría clásica de ensamblaje de especies sin tener en cuenta la variabilidad intraespecífica, las especies

son representadas con su valor medio del rasgo y; (B) teoría de ensamblaje de especies teniendo en cuenta la variabilidad dentro de la especie (variabilidad intraespecífica). En el escenario A, si el valor medio de la especie se adecúa a las condiciones ambientales del hábitat y es suficientemente diferente para no competir en el uso de los recursos con otras especies, entonces en ese caso las especies serán capaces de sobrepasar los filtros y formar parte de la comunidad final. Incorporando la variabilidad intraespecífica, se permite que algunos individuos de una especie con un valor medio de rasgo de su especie no adecuado para prosperar bajo ciertas condiciones tanto abióticas como bióticas impuestas por el ambiente, presenten valores del rasgo que si les permitan sobrepasar los filtros y sean capaces de formar parte de la comunidad final, contribuyendo así a una mayor riqueza de la comunidad. Figura adaptada de Valladares *et al.* (2015).

Por otra parte, aunque la variabilidad de rasgos a nivel intraespecífico favorece la diversidad de comunidad, una mayor riqueza de especies podría significar una menor variabilidad intraespecífica a nivel de comunidad de acuerdo con la clásica teoría de nicho. Según la teoría de nicho, las especies se verían obligadas a disminuir su variabilidad intraespecífica cuando aumenta la diversidad de la comunidad con el fin de evitar la competencia y el solapamiento de su nicho con otras especies (MacArthur & Levins 1967). En este caso, la coexistencia de especies funcionalmente diferentes se vería favorecida, ganando importancia relativa la variabilidad de rasgos a nivel interespecífico frente al intraespecífico cuando la riqueza de especies aumenta (partición de la varianza intra-interespecífica; (Violle *et al.* 2012). Sin embargo, bajo la teoría neutral, las especies podrían coexistir a pesar de ser funcionalmente similares, siendo la única limitación el espacio funcional disponible, y por tanto el patrón de variación intra- *versus* interespecífica con la riqueza de especies se verían desdibujados. Hasta la fecha, pocos estudios han evaluado la relación entre variabilidad intraespecífica y riqueza de especies, debido al enorme esfuerzo de medición requerido a nivel intraespecífico (Baraloto *et al.* 2010), y los pocos estudios existentes han mostrado resultados inconsistentes (Hulshof *et al.* 2013; Le Bagousse-Pinguet *et al.* 2014; Siefert *et al.* 2015).

A pesar del enorme crecimiento de estudios incorporando la variabilidad de rasgos a nivel intraespecífico en los estudios de coexistencia (Jung *et al.* 2010; Fajardo & Piper 2011; Hulshof *et al.* 2013; Siefert *et al.* 2015; Bastias *et al.* 2017), aún quedan numerosas incognitas en torno a la importancia de la variabilidad intraespecífica y cómo le afectan cambios en el medio tanto a nivel biótico de riqueza de especies como a nivel abiótico, con cambios en la disponibilidad de recursos y heterogeneidad ambiental. Una parte importante de la presente tesis pretende mejorar y dar respuesta a algunas de estas cuestiones, focalizando en especies de vida larga como los árboles y en bosques pobres en especies arbóreas como los bosques europeos, donde estas cuestiones resultan aún más desconocidas.

Análisis espacial: una visión más integradora del ensamblaje y coexistencia de las especies.

Actualmente existe un incremento exponencial de estudios que usan la estadística espacial en Ecología para explicar los mecanismos subyacentes de ensamblaje de especies (Seabloom *et al.* 2005; Perry, Miller & Enright 2006; Wiegand *et al.* 2012; Wang *et al.* 2016; Chacón-Labella, de la Cruz & Escudero 2016). Y es que el espacio es una característica fundamental para individuos sésiles como las plantas, y por tanto, la distribución de los individuos en él puede ser el reflejo más fiel de las numerosas interacciones entre los mismos por los recursos disponibles del medio (Stoll & Weiner 2000). Desde distribuciones agregadas mostrando procesos de dispersión limitada o interacciones de facilitación entre individuos (Maestre & Cortina 2002; Escudero *et al.* 2005; Jara-Guerrero *et al.* 2015), pasando por distribuciones regulares de los individuos fruto de la competencia por los recursos, siendo especialmente acentuadas en comunidades monoespecíficas (todos los individuos compiten por los mismos recursos) (Kenkel 1988; Rietkerk & Koppel 2008), hasta las distribuciones más azarosas resultantes de los procesos estocásticos como los procesos estructuradores de las comunidades (Williamson 1975; Kuuluvainen *et al.* 1996).

Además, el espacio se ha puesto aún más de relevancia, sirviendo de base en dos de las tres reglas de la Teoría Unificada de Biodiversidad presentada por McGill (2010), en su intento de unificar y buscar patrones comunes entre las diferentes teorías de biodiversidad existentes. (i) Individuos de una misma especie permanecen agrupados: (ii) individuos de una especie están localizados independientemente de los individuos de otras especies. Dichas reglas espaciales han resultado suficientes para explicar patrones como el recambio de especies, la acumulación de riqueza de especies o el decaimiento de la similitud con la distancia (McGill 2010).

Estos métodos espaciales que utilizan información espacial explícita (coordenadas x, y) son especialmente útiles para inferir la escala espacial a la que ocurren ciertos procesos ecológicos y pueden proporcionar una visión más integradora del ensamblaje actual de especies de una comunidad mediante la diferenciación entre una disposición espacial de los individuos debida a interacciones bióticas por el uso de los recursos (Amarasekare 2003), de aquellas ligadas a la heterogeneidad espacial de los recursos en el medio (fenómeno de 'agregación virtual') (Maestre & Cortina 2002; Schiffers *et al.* 2008). En la presente tesis, dedicaremos un capítulo a estudiar como la riqueza de especies influye en la distribución espacial de los individuos lo cual resulta especialmente novedoso (pero véase Chacón-Labella *et al.* 2016) en base a las reglas de la Teoría Unificada de Biodiversidad (McGill 2010) y las asunciones de la Teoría Moderna de Coexistencia (Chesson 2000).

OBJETIVOS Y ESTRUCTURA DE LA TESIS

El objetivo principal de la tesis es la evaluación del efecto de la riqueza de especies del dosel dominantes en bosques europeos en dos aspectos clave de la dinámica de comunidades vegetales: 1) el proceso de reclutamiento (regeneración) y 2) la coexistencia de individuos tanto en la fase juvenil como en la fase adulta. Para ello, nuestros trabajos se realizaron siguiendo un enfoque multiescalar y contemplando numerosas variables tanto ambientales como funcionales. Esta aproximación nos permite predecir la tendencia futura en composición, estructura y funcionamiento de los bosques europeos de acuerdo a su riqueza actual ante un escenario de cambio global.

La presente Tesis doctoral se estructura en cinco capítulos, además de una introducción, una discusión y unas conclusiones generales. Los objetivos específicos de cada uno de los cinco capítulos experimentales se detallan a continuación.

Capítulo 1

1. Evaluar la hipótesis de que una mayor riqueza de especies del dosel forestal induce una mayor heterogeneidad ambiental.

2. Examinar el efecto de la riqueza de especies del dosel en el proceso de reclutamiento de nuevos individuos, en términos tanto de abundancia como de riqueza.

3. Analizar el efecto de la heterogeneidad lumínica como recurso clave en el establecimiento y supervivencia de los juveniles sobre la abundancia y riqueza del regenerado.

Capítulo 2

1. Analizar la diversidad funcional de la comunidad juvenil a través de la partición de la varianza del rasgo a nivel interespecífico y a nivel intraespecífico.

2. Conocer que variables ambientales pueden estar influyendo en la variación del rasgo a nivel inter e intraespecífico.

Capítulo 3

1. Evaluar la variabilidad fenotípica y la plasticidad fenotípica (motor de la variabilidad intraespecífica) en caracteres relacionados con la disponibilidad de nutrientes y luz. El objetivo se aborda en juveniles de especies de dos grupos funcionales diferentes procedentes de comunidades forestales que varian en el nivel de riqueza del dosel en dos bosques muy contrastados bioclimáticamente mediante un experimento de jardín común.

Capítulo 4

1. Cuantificar y evaluar cambios en la variabilidad intraspecífica en juveniles de diferentes especies leñosas coexistiendo en comunidades que difieren drásticamente en la riqueza de especies del dosel, desde comunidades boreales hasta tropicales.

2. Estudiar si la similaridad en el valor del rasgo (solapamiento de nicho) entre pares de especies leñosas coexistiendo en una misma comunidad disminuye o aumenta dependiendo de la riqueza de especies del dosel forestal bajo el cual coexisten.

Capítulo 5

1. Evaluar el papel de la riqueza de especies del dosel en el patrón espacial de los individuos adultos leñosos a dos escalas diferentes y sus implicaciones en la coexistencia de las especies leñosas.

2. Examinar la influencia de la heterogeneidad ambiental espacialmente explícita en el patrón espacial de los individuos adultos. En este objetivo se pretenden detectar patrones espaciales que puedan derivarse no solo de interacciones bióticas entre las especies foestales sino tambien de la existencia de agregaciones espaciales de recursos que generen una “agregación virtual” de las especies.

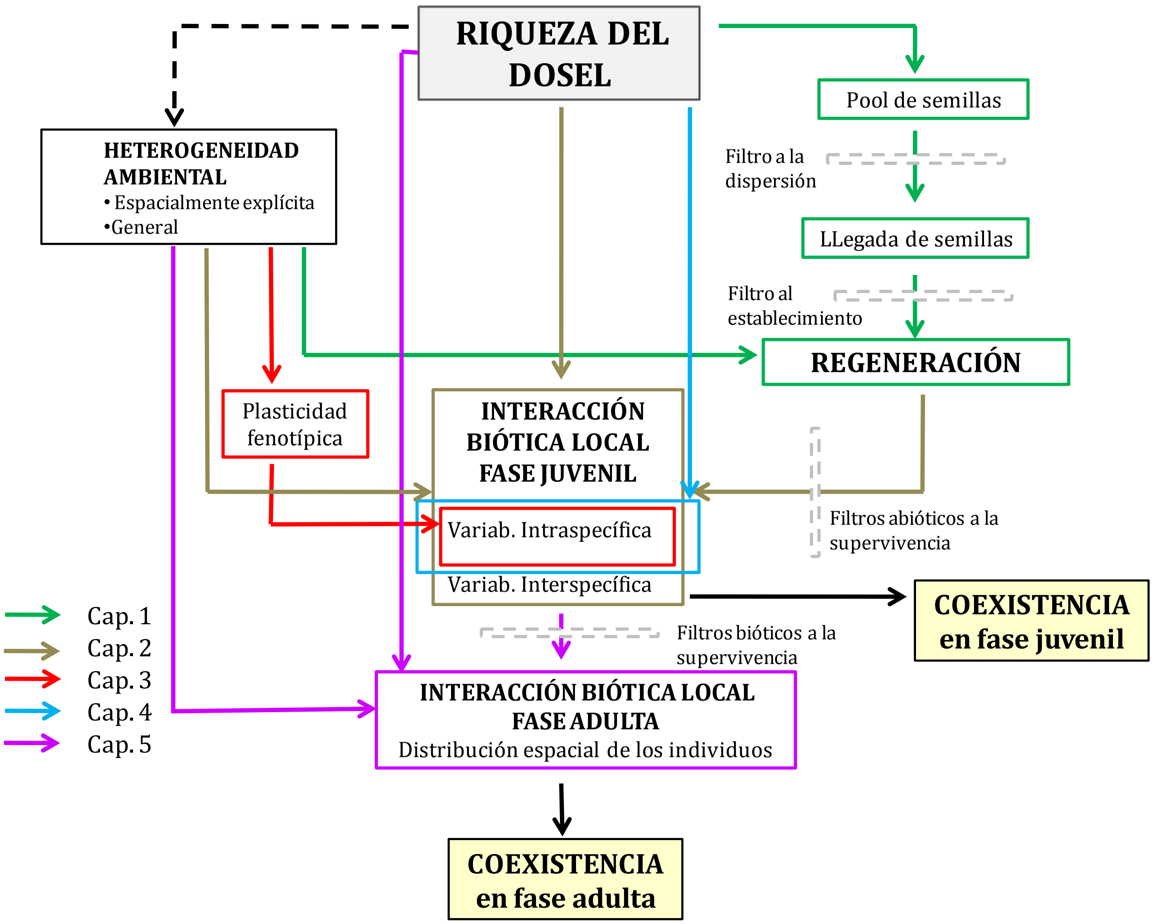


Figura 3. Esquema resumen del contenido de los cinco capítulos que componen la presente tesis doctoral.

METODOLOGÍA GENERAL

Sitios de estudio

Los estudios desarrollados en la presente tesis han sido llevados a cabo fundamentalmente dentro de la plataforma exploratoria diseñada por el proyecto europeo FunDiv-EUROPE (www.fundiveurope.eu), cuyo objetivo es conocer el papel de la biodiversidad en el funcionamiento de los ecosistemas forestales europeos. La plataforma exploratoria consiste en una red de 209 parcelas permanentes repartidas en un total de seis bosques diferentes maduros en Europa. Los bosques seleccionados se encuentran entre los bosques más representativos a escala continental, cubriendo un amplio gradiente bioclimático Europeo: un bosque boreal y hemiboreal en Finlandia y Polonia, un bosque templado-mixto y templado continental en Alemania y Rumania y un bosque mediterráneo templado y continental mediterráneo-mixto en Italia y España (Figura 4). Ver características generales de cada sitio de estudio en la Tabla 1.

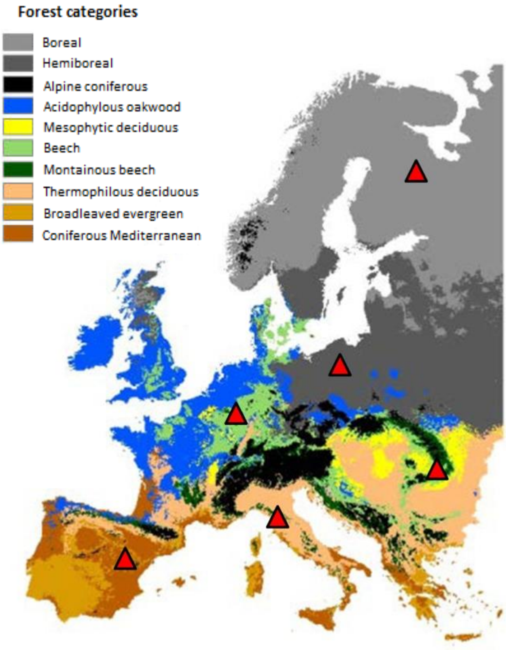


Figura 4. Localización geográfica de los seis sitios de estudio señalados con un triángulo rojo. De Norte a Sur, bosque boreal en Finlandia, bosque hemiboreal en Polonia, bosque templado-mixto en Alemania, bosque continental templado en Rumania, bosque mediterráneo templado en Italia y bosque continental mediterráneo-mixto en España. Cada color sobre el mapa muestra la distribución de los tipos de bosque según la EEA (2007). Figura extraída de Casalegno *et al.* (2011).

Tabla 1. Características generales de cada tipo de bosque de estudio.

	Boreal	**Hemiboreal**	**Templado-mixto**	**Continental templado**	**Mediterráneo templado**	**Continental mediterráneo-mixto**
Localización	Karelia del norte (Finlandia)	Parque Nacional de Bialowieza (Polonia)	Parque Nacional de Hainich (Alemania)	Cárpatos (Rumania)	Toscana (Italia)	Parque Natural del Alto Tajo (España)
Coordenadas (Norte, Este)	62.6º, 29.9º	52.7°, 23.9º	51.5º, 10.2º	47.6º, 25.3º	43.2º; 11.1º	40.7º, -1.9º
Zona de estudio (km x km)	150 x 150	30 x 40	15 x 10	5 x 5	50 x 50	50 x 50
Tra media anual (ºC)	2.1	6.9	7.4	5.5	13	9.7
Pp media anual (mm)	631.8	580.8	689.4	691.1	850	537.4
Rango de altitud	87-233	140-200	273-496	655-1062	254-523	960-1404
Topografía	Plana	Plana	Principalmente plana	Pendientes moderadas a muy empinadas	Pendientes moderadas a muy empinadas	De plano a pendientes moderadas
Geología y suelos	Podzoles, tillitas glaciales e histosoles en turberas.	Arcillas y arenas cuaternarias y sustratos orgánicos sobre arcillas y arenas terciarias.	Piedra caliza triásica y arenisca con depósitos de loess.	Arenisca terciaria y sedimentos franco arcillosos.	Diferentes formaciones de origen sedimentario, metamórfico y magmático.	Suelos calizos que tienen su origen en la era secundaria y principios de la Terciaria.
Tipo de bosque[1]	Bosque boreal	Bosque hemiboreal y bosque nemoral de coníferas y mixto de coníferas y frondosas.	Bosque de roble acidófilo, bosque de roble-abedul. Bosque caduco mesofítico. Bosque de haya.	Bosque de hayas montañoso	Bosque caducifolio termófilo, Bosque de hoja ancha perenne	Bosque mediterráneo de coníferas. Bosque perenne de hoja ancha.
Manejo del bosque en el pasado	Cortas a hecho	Aclareos sucesivos por bsoquetes, cortas selectivas y cortas a hecho	-	Resalveos, cortas selectivas	Resalveo	Corta selectiva
Manejo actual del bosque	Bajo	Bajo	Mínimo	Mínimo	Mínimo	Mínimo
Propiedad actual	Estado y compañías privadas madereras	Estado	Estado	Estado	Estado	Estado

[1] Clasificación de acuerdo con la Agencia medioambiental europea (EEA 2007)

Figura 5. Fotografía de los bosques de estudio: (A) bosque boreal en Finlandia; (B) bosque hemiboreal en Polonia; (C) bosque templado continental en Rumanía; (D) bosque templado-mixto en Alemania; (E) bosque Mediterráneo templado en Italia; y (F) bosque continental Mediterráneo-mixto en España.

Diseño experimental

Dentro de cada tipo de bosque, entre 28 y 43 parcelas de 30 x 30 m fueron delimitadas siguiendo un gradiente de riqueza de las especies arbóreas dominantes en el dosel. Es decir, se establecieron parcelas con distinto nivel de riqueza, desde parcelas monoespecíficas (una sola especie) de cada especie dominante, hasta parcelas mixtas incluyendo todas las especies dominantes de cada bosque, en concreto 3 especies en el caso del bosque boreal en Finlandia, 4 especies en el caso del bosque templado-mixto en Alemania, bosque templado-continental en Rumanía y en el bosque continental mediterráneo-mixto en España, y finalmente, 5 especies en el caso de bosque hemiboreal en Polonia y el bosque mediterráneo templado en Italia. Cada una de las especies dominantes en cada bosque estuvo representada en todos los niveles de riqueza (monoespecífico y mixto de varios niveles de riqueza de especies) y además, presentando una frecuencia similar de las especies presentes en combinaciones mixtas. Cada combinación de riqueza para cada especie fue replicada al menos dos veces.

Las especies arbóreas dominantes incluyen diferentes grupos funcionales y abarcan especies de coníferas (*Pinus sylvestris, Pinus nigra, Picea abies y Abies alba*), y especies frondosas, la mayor parte de ellas especies caducifolias (*Quercus faginea, Quercus robur, Quercus petreae, Quercus cerris, Fagus sylvatica*, *Acer pseudoplatanus, Fraxinus excelsiour, Betula pendula, Castanea sativa*) excepto una perenne, *Quercus ilex* (Tabla 2).

Tabla 2. Diseño experimental para cada tipo de bosque que forman la plataforma exploratoria del proyecto FunDiv-EUROPE.

	Boreal	**Hemiboreal**	**Templado-mixto**	**Continental templado**	**Mediterráneo templado**	**Continental mediterráneo -mixto**
Gradiente de riqueza[1]	1-3	1-5	1-4	1-4	1-5	1-3
No. de parcelas total	28	43	38	28	36	36
No. de parcelas por nivel de riqueza	14/14/3	6/11/13/11/2	6/14/14/4	8/10/8/2	9/10/9/7/1	11/18/4/3
Especies arbóreas dominantes en el dosel	*Pinus sylvestris, Picea abies, Betula spp.*	*Pinus sylvestris, Picea abies, Betula pendula, Quercus robur, Carpinus betulus*	*Fraxinus excelsiour, Acer pseudoplata-nus, Fagus sylvatica, Picea abies, Quercur robur.*	*Fagus sylvatica, Acer pseudoplata-nus, Picea abies, Abies alba.*	*Quercus petreae, Quercus cerris, Quercus ilex, Castanea sativa, Ostrya carpinifolia*	*Quercus ilex, Quercus faginea, Pinus nigra, Pinus sylvestris.*
Especies matorrales y otras especies arbóreas presentes	*Salix caprea, Sorbus aucuparia, populus tremula, Alnus* sp.	*Sorbus aucuparia, Tilia* sp., *Corylus avellana*	*S. caprea, Sorbus torminalis, Tilia cordata, Tilia platyphyllos, Ulmus glabra*	*P. tremula, Ulmus minor, Alnus* sp., *Betula* sp.	*Arbutus unedo, Ilex aquifolium, Tilia cordata, Acer campestre, Corylus avellana, P. tremula,*	*Buxus sempervirens, Crataegus* spp., *Junipeus thurifera, Juniperus communis, Viburnum* sp.

[1] Gradiente de riqueza teniendo en cuenta las especies arbóreas dominantes en el dosel.

Una de las características básicas que cumple el diseño experimental, siendo común en los experimentos de biodiversidad y que permite evaluar el efecto aislado de la riqueza de especies es evitar el 'efecto de dilución', es decir, se evitó que cualquier especie del *pool* total de especies dominantes estuviera presente en todas las parcelas seleccionadas dentro de cada nivel de riqueza en cada bosque con el fin de evitar que el efecto se deba a la dilución de una especie a lo largo del gradiente de riqueza más que a la propia riqueza de especies *per se*.

Las parcelas dentro de cada tipo de bosque fueron a su vez delimitadas con las condiciones ambientales lo más similares posibles (al menos dentro de cada bosque), es decir, similar topografía y/o propiedades del suelo, con el fin de descartar cualquier factor ambiental asociado con el gradiente de riqueza de especies. Además, las parcelas compartían un pasado histórico común dedicado al manejo forestal, teniendo en la actualidad una intervención baja o mínima.

Metodología por capítulos

Durante el desarrollo de la presente Tesis doctoral empleamos una amplia variedad de técnicas y metodologías para abordar cada uno de los objetivos planteados en cada capítulo. Dichas metodologías y técnicas están desarrolladas detalladamente en la sección de material y métodos de cada capítulo, y son resumidas a continuación:

Caracterización ambiental: general (Capítulo 1 y 3) y espacialmente explícita (Capítulo 5).

En cada parcela de 30 x 30 m, se tomaron 17 puntos (16 puntos distribuidos uniformemente separados entre sí 10 metros cada uno, más el centro de la parcela) de todos los bosques de estudio, excepto en el bosque Mediterráneo templado, donde hemos llevado a cabo una caracterización de luz disponible a través de la toma de fotografías hemisféricas. Posteriormente, utilizando el programa Hemiview v.2.1 (Delta-T Devices Ltd,Burwell, UK) fueron analizadas para obtener la luz global total (GSF) y la luz indirecta (ISF). Además, en tres de los seis bosques, los más contrastados y uno intermedio del gradiente bioclimático, boreal (Finlandia), templado continental (Rumanía) y continental mediterráneo-mixto (España), se midió la profundidad del suelo y el porcentaje de cobertura de rocas, matorral, herbáceas y hojarasca.

En los capítulos 1 y 3 calculamos el coeficiente de variación (CV) como una medida de heterogeneidad ambiental para cada variable ambiental y parcela y además, un estadístico de la heterogeneidad ambiental general mediante el promedio de todos los coeficientes de variación de las variables ambientales por parcela. Además de las medidas ambientales tomadas por nosotros, en el capítulo 3 utilizamos datos disponibles de diferentes propiedades del suelo medidas en los primeros 20 cm del suelo en cada una de las parcelas de cada tipo de bosque. Las variables disponibles fueron pH, concentración de C y N y reserva de C y N calculada a través de la densidad aparente (Dawud *et al.* 2017).

En el capítulo 5 llevamos a cabo un análisis del patrón espacial de cada una de las variables ambientales en cada parcela mediante un análisis de índices de distancia (Perry 1998; Perry & Dixon 2002). A continuación, un modelo de regresión de ejes principales estandarizados (Warton *et al.* 2006) para analizar la relación potencial entre el patrón espacial de los individuos y el patrón espacial de las variables ambientales. SMA permite la no asunción previa de una relación causa-efecto de las variables, siendo la relación bidireccional.

Estudio de la regeneración (Capítulo 1)

En este capítulo examinamos el efecto de la riqueza y la composición de especies del dosel en el proceso de reclutamiento de nuevos individuos, en términos de abundancia y riqueza. Para ello, en un subplot de 4 x 4 m situado en el centro de cada parcela calculamos e identificamos todos los juveniles de hasta 1.60 m de altura de las especies dominantes. Por otro lado, estimamos un índice de cantidad de semillas de la parcela a través del área basal total de cada una de

Figura 6. Diseño de la parcela 4 x 4 m situada en el centro de la parcela principal donde se cuantificó e identificó a todos los juveniles por debajo de 1.60m.

especies en cada parcela ponderado por un factor proporcional a la estrategia reproductiva de la especie. La riqueza de semillas fue inferida con la riqueza de especies de la parcela. Los datos fueron analizados globalmente utilizando un modelo linear generalizado mixto (GLMM) para cada una de las variables respuesta (abundancia y riqueza de juveniles) en función de la cantidad y riqueza de semillas, y además añadiendo la heterogeneidad de luz de cada parcela (factor clave en la regeneración y supervivencia de muchas especies). Además, usamos el índice de disimilaridad de Bray-Curtis (Bray & Curtis 1957) para cuantificar la disimilaridad composicional entre el dosel y el regenerado en cada parcela. Finalmente, llevamos a cabo un análisis de potencia estadística *a posteri* (Cohen 1988) para asegurar la fiabilidad de nuestros resultados con nuestro diseño experimental.

Diversidad funcional de la comunidad: partición de la varianza inter- e intraespecífica (Capítulo 2).

En este capítulo cuantificamos la importancia de la variabilidad interespecífica e intraespecífica (diversidad funcional) en comunidades de juveniles de tres bosques diferentes contrastados climáticamente (boreal, continental templado y continental mediterráneo). Para ello, en cada una de las parcelas de los bosques seleccionados, escogimos aleatoriamente 10 juveniles (de hasta 1.60 m de altura) de cada especie arbórea dominante en cada tipo de bosque siempre que fuera posible. En cada individuo medimos un total seis rasgos foliares representativos de la estrategia ecológica de la planta, tanto morfológicos (SLA, de su siglas en inglés Specific Leaf Area y LDMC, de sus siglas en inglés Leaf Dry Matter Content) como químico tales como el contenido de carbono y nitrógeno en la hoja e isótopos δ^{13}C and δ^{15}N.

Una vez procesados, llevamos a cabo la cuantificación de la diversidad funcional meditante el método de la partición de la varianza propuesto por de Bello *et al.* (2011). Concretamente, calculamos la diversidad funcional a nivel interspecifico como la varianza en los valores medios de los rasgos entre las especies de juveniles que coexisten en la misma comunidad, mientras que la diversidad funcional a nivel intraespecífico la calculamos mediante la varianza de los valores de los rasgos entre individuos de cada especie ponderada por el número de individuos muestreados de cada especie (de Bello *et al.* 2011).

Experimento de plasticidad fenotípica en un jardín común (Capítulo 3).

El jardín común consiste en un ensayo que nos permite analizar diferencias en la respuesta fenotípica de individuos, caracterizados por estar expuestos a distintas condiciones ecológicas, cuando éstos son sometidos a un ambiente común (Pigliucci 2001). En el capítulo 4, llevamos a cabo un experimento en jardín común en dos años consecutivos (2013 y 2014) con seis especies arbóreas, pertenecientes a dos grupos funcionales diferentes: coníferas (*Pinus sylvestris, Pinus nigra y Picea abies*) y especies de hoja ancha (*Quercus robur, Quercus ilex y Quercus faginea*). Las especies seleccionadas son especies del dosel dominantes en dos de los seis tipos de bosques europeos, los cuales presentan condiciones ambientales muy contrastadas: el bosque hemiboreal de Polonia y el continental mediterráneo-mixto localizado en España. Recogimos semillas de cada una de las especies de entre 3-6 madres, localizadas en entre 2-5 parcelas con diferente nivel de riqueza de especies en cada tipo de bosque. Las semillas se sembraron y aquellas que germinaron en un tiempo determinado fueron seleccionadas para el experimento. Fueron un total de 2745 plantas de los dos grupos funcionales procedentes de diferentes niveles de riqueza y bosque.

Las plantas seleccionadas fueron sometidas durante 4 meses a tres tipos de condiciones diferentes que fueron atribuidas al azar dentro de cada especie y riqueza de la comunidad de procedencia: (i) sol (50% de la radiación solar total que se recibe en campo abierto; ~1350 μmol m^{-2} s^{-1}) - disponibilidad baja de nutrientes (0.045g N/l – 0.03 g P/l – 0.09 g K/l); (ii) sombra (20% de la radiación solar total en campo abierto; ~490 μmol m^{-2} s^{-1}) - disponibilidad baja de nutrientes (0.045g N/l – 0.03 g P/l – 0.09 g K/l); y (iii) sol (50% radiación solar total; ~1350 μmol m^{-2} s^{-1}) - disponibilidad alta de nutrientes (0.22-0.31 g N/l - 0.12-0.18 g P/l - 0.23-0.34 g K/l). De esta forma conseguimos un experimento no-factorial, que permite analizar independientemente la plasticidad a un tratamiento de disponibilidad de luz (sombra-sol) y a un tratamiento de disponibilidad de nutrientes (baja-alta disponibilidad de nutrientes).

Figura 7. (A) Recolección de semillas con una escalera y una pértiga extensible que tiene en su extremo unas tijeras. Recogida en el bosque continental mediterráneo-mixto en España y (B) experimento de coníferas en condiciones controladas. En primer plano se puede observar las plantas de coníferas en el tratamiento de sol y en un segundo plano las plantas en sombra cubiertas con una malla que reduce la luz solar hasta en un 80% con respecto a la luz solar total exterior.

Durante el desarrollo del experimento se midieron diferentes rasgos funcionales de la planta como la tasa de crecimiento relativa, rasgos de arquitectura como la altura, altura de copa en coníferas, número de hojas y distancia de internodo en las frondosas, rasgos morfológicos de la hoja como el tamaño foliar, área foliar específica y contenido de materia seca de la hoja; y rasgos fisiológicos como la eficiencia fotoquímica del fotosistema II [Fv/Fm] y conductancia estomática. Los tratamientos se analizaron independientemente, y para cada uno de los rasgos funcionales se llevó a cabo un modelo lineal generalizado mixto (GLMMs; Pinheiro et al. (2015)) con el fin de determinar las diferencias en la respuesta fenotípica al tratamiento entre plantas procedentes de comunidades con diferente nivel de riqueza, entre grupos funcionales y entre bosques. Finalmente cuantificamos la plasticidad a ambos tratamientos utilizando el índice de plasticidad creado por Valladares *et al.* (2000b). $IP = [|X(amb_1) - X(amb_2)| / Max(X(amb_1), X(amb_2))]$, donde $X(amb_1)$ y $X(amb_2)$ es el valor del rasgo medio en el ambiente 1 y 2, respectivamente; y es representado matemáticamente como una norma de reacción (Figura 8).

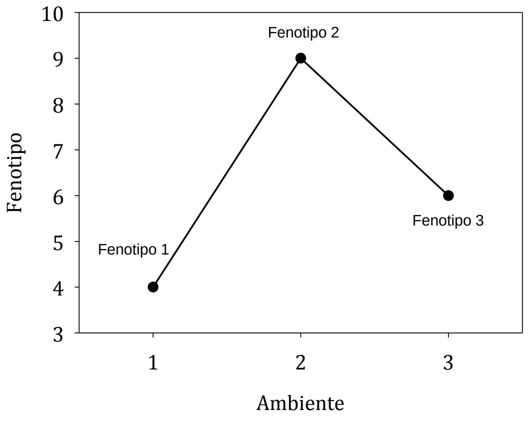


Figura 8. Norma de reacción. Un individuo expresa un fenotipo diferente dependiendo de las condiciones tanto bióticas como abióticas del medio. La plasticidad fenotípica estaría representando como la diferencia entre el valor medio del rasgo entre dos ambientes distintos (o también pendiente de la recta). Figura adaptada de Sultan (1987).

Cuantificación de la variabilidad intraespecífica y la similaridad en los valores de rasgos morfológicos entre especies (Capítulo 4).

Para este capítulo, nos centramos en dos rasgos morfológicos foliares ampliamente utilizados en otros trabajos basados en rasgos funcionales: el tamaño foliar y el área foliar específica (SLA, de sus siglas en inglés Specific Leaf Area). Estos rasgos fueron medidos en un total de 10 juveniles por especie (siempre que estuvieran presente) en cada una de las parcelas de los tres bosques más contrastados bioclimáticamente: boreal, continental templado y continental Mediterráneo-mixto, lo que supuso un total de 746 individuos medidos en 92 parcelas. Estos datos se analizaron junto con datos de tamaño foliar y SLA de las especies más dominantes en otras comunidades forestales con valores de riqueza de especies muy distintos, desde bosques templados en California hasta bosques tropicales en Brasil o Ecuador. En total, recolectamos datos de un total de 3712 individuos pertenecientes a 521 especies en un total de 21 comunidades diferentes. La variabilidad intraespecífica para cada especie fue calculada a través del coeficiente de variación, y diferencias en el tamaño muestral entre individuos fueron resueltas llevando a cabo un análisis de rarefacción previo. A continuación estudiamos la similaridad de ambos rasgos morfológicos (o solapamiento del rasgo) entre pares de especies (de Bello *et al.* 2013) mediante dos métodos diferentes: (i) asumiendo una distribución normal de los rasgos y (ii) mediante el método de estimación de la densidad del Kernel. Posteriormente, se emplearon modelos lineales para relacionar el tamaño de la variabilidad intraespecífica y la similaridad en el rasgo con la riqueza de especies y así testar la hipótesis de que existirá una menor variabilidad intraespecífica al aumentar la riqueza de especies en la comunidad, para minimizar la competencia en el uso de los recursos (disminuir la similaridad o solapamiento en la distribución de rasgos funcionales).

Análisis de patrones de puntos (Capítulo 5)

La posición espacial (coordenadas x,y) de todos los individuos con un diámetro a la altura del pecho (DBH; de sus siglas en inglés Diameter at Breast Height) >7.5cm se midieron en las 209 parcelas, es decir, en los seis tipos de bosque. Con estos datos, evaluamos el efecto de la riqueza de especies del dosel en el patrón espacial de los individuos adultos (agregado, regular o al azar) utilizando tres tipos de funciones diferentes, la función K de Ripley, la función de distribución de distancia al vecino más cercano (función-G) y la función de correlación de par (Función-g) (Wiegand & Moloney 2014). La función K de Ripley mide la densidad de individuos dentro de un círculo de radio *r* alrededor de un individuo elegido del patrón de puntos, mientras que la función-G mide la distancia entre el individuo elegido y el individuo más cercano. A diferencia de la función K de Ripley, la función G permite analizar patrones más precisos a escalas más pequeñas que la función K-Ripley. Ambas funciones, son en cierta forma funciones de distribución acumulada, ya que a cada escala o distancia *r,* todos los pares de puntos separados por una distancia menor que *r* se usan para estimar el valor de la correspondiente función. En cambio la función-g no caracteriza de forma acumulativa y para evaluar el patrón espacial tan solo tiene en cuenta los pares de puntos que se encuentran separados por un distancia exactamente igual a la distancia *r.*

El patrón espacial de los individuos de cada parcela se analizó utilizando las tres funciones a dos escalas diferentes: escala media *r*= 7.5 m y escala fina *r*= 3 m, y a continuación se comparó con un test de aleatoriedad completa (o 'CSR', de Complete Spatial Randomness) para determinar si existe segregación, agrupación o una distribución al azar de los individuos de la parcela. Posteriormente, se aplico un modelo lineal (Rao & Toutenburg 1995) para estudiar la relación entre el patrón espacial de los individuos y la riqueza de especies de la parcela.

ASPECTOS MÁS NOVEDOSOS Y DESTACABLES DE LA TESIS

Los aspectos más relevantes de esta tesis se resumen en los siguientes apartados:

1. Muestreo a gran escala. La mayoría de los estudios en ecología se llevan a cabo a una escala local. Esto hace que los resultados pueden estar sesgados por las características intrínsecas propias de la zona de estudio, y por tanto la extrapolación o generalización de conclusiones ha de ser muy cautelosa. En esta tesis incluimos capítulos donde la misma cuestión es probada hasta en seis bosques con características bioclimáticas diferentes distribuidos a lo largo de un gradiente europeo amplio. Esto nos permite buscar patrones generales o generalizables en torno a una misma cuestión, así como discernir peculiaridades de los diferentes ecosistemas.

2. Incorporación de la variabilidad intraespecífica. La mayoría de los estudios funcionales centrados en comprender los mecanismos que determinan el ensamblaje de especies en una comunidad y la coexistencia de las especies ha utilizado valores de rasgos medios de las especies obviando la variación existente dentro de la especie. Una parte importante de la presente tesis se centra en estudiar la importancia de la variabilidad intraespecífica en los procesos de coexistencia. De ahí que tres de los cinco capítulos incluidos aborden cuestiones actualmente en discusión activa por parte de la comunidad científica relacionadas con la variabilidad intraespecífica.

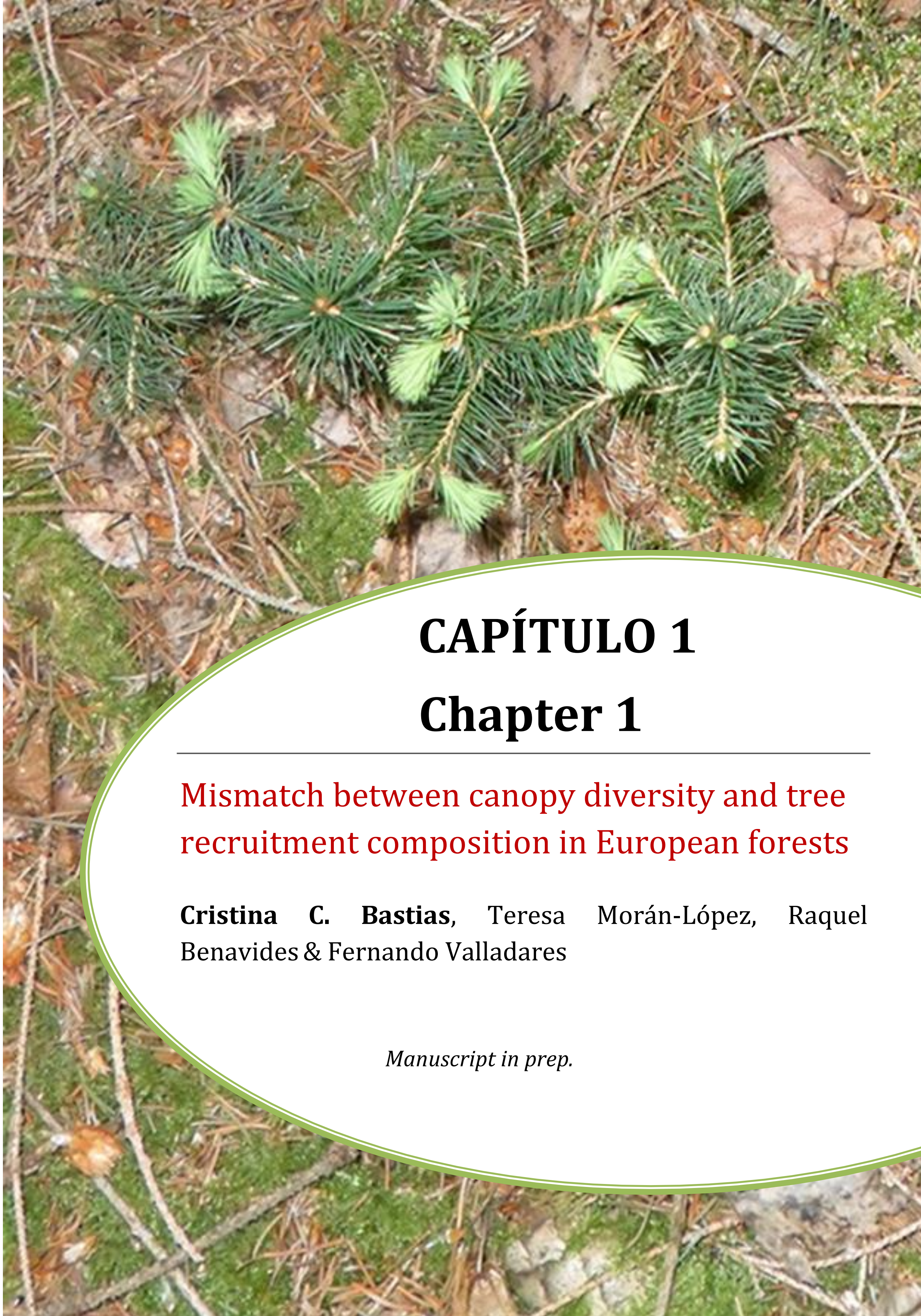
3. Estudio de la variabilidad de los rasgos funcionales en condiciones naturales y en condiciones controladas (experimentos en invernadero). La combinación de ambas aproximaciones permite obtener una visión más completa, por un lado de la variabilidad encontrada en comunidades reales, y por otro lado, la respuesta potencial de la variabilidad de rasgos en individuos ante unas condiciones ambientales cambiantes, y con el componente genético de la variabilidad fenotípica controlado al menos parcialmente.

4. Consideración de una gran cantidad de variables ambientales, así como de múltiples rasgos funcionales en un tamaño muestral grande. El estudio a nivel intraespecífico requiere de un enorme esfuerzo, con un muestreo intensivo y un elevado numero de muestras por especie. Las diferencias en general más sutiles que caben esperarse en los valores del rasgo dentro de una misma especie complican el diseño experimental y explica el hecho de que muchas investigaciones con una aproximación funcional al de diversidad no hayan abordado hasta la fecha con solvencia esta fuente de variabilidad.

5. Utilización de una amplia gama de herramientas estadísticas para el análisis de los datos ecológicos. Con el fin de abordar los objetivos específicos planteados en esta tesis desde diferentes perspectivas (ambiental, funcional y espacial), y la consiguiente variabilidad asociada en los datos encontrados entre bosques, especies, individuos y procesos, hemos empleado una gran variedad de técnicas estadísticas para obtener los resultados más completos y las conclusiones más claras de las cuestiones planteadas.

6. Valoración de la riqueza de especies del dosel como factor principal para dos aspectos clave de la dinámica de comunidades. Hasta la fecha, numerosos estudios han evaluado el efecto de la diversidad de especies arbóreas de los bosques en diferentes funciones del ecosistema (Jactel & Brockerhoff 2007; Zeugin *et al.* 2010; Gamfeldt *et al.* 2013; Jucker *et al.* 2014a; van der Plas *et al.* 2016). Sin embargo, existen muy pocos estudios que hayan cuantificado el papel de la riqueza del dosel como impulsor de la regeneración y de la coexistencia de especies.

CAPÍTULOS



CAPÍTULO 1

Chapter 1

Mismatch between canopy diversity and tree recruitment composition in European forests

Cristina C. Bastias, Teresa Morán-López, Raquel Benavides & Fernando Valladares

Manuscript in prep.

Image from the front page (Chapter 1): juvenile of *Picea abies* in the boreal forest (Finland).

Author: Cristina Crespo Bastias

SUMMARY

Increasing evidence supports that biodiversity favors forest multifunctionality. However, tree canopy richness effects on key forest functions such as regeneration have been overlooked despite the relevance of the natural regeneration for the future forest functioning. Here, through a large-scaled European platform (in five types of European forests, including 173 plots along five levels of canopy species richness), we explored whether plots richer in tree species had higher recruitment abundance and richness by two non-exclusive sources: 1) directly by supplying more abundant and richer seed rain, and 2) indirectly by inducing environmental heterogeneity. Our results showed non-significant direct effects of seed quantity and richness (inferred from canopy composition and richness) on abundance and richness of recruits. Moreover, we also found that increased canopy richness does not necessarily imply increased overall environmental heterogeneity at ground level. Only heterogeneity of understory light increased with canopy richness, but still it did not significantly influence recruitment. Power analyses showed that our experimental design was statistically robust to prove the lack of relationship between seed supply and recruitment richness but it was not enough powerful to prove it for recruitment abundance, which requires a larger amount of forest plots (>100 forest plots) per canopy richness level. Our design was statistically powerful to evidence the absence of effect of canopy richness on overall environmental heterogeneity at ground level. Our findings suggest that canopy richness is not a good predictor of recruitment. Further work is required to assess whether post-dispersal ecological filters and/or seed arrival from regional pools are behind the mismatch between canopy and recruitment composition.

Keywords: canopy species richness, ecosystem functioning, environmental heterogeneity, local seed supply, power analysis, regeneration abundance, regeneration richness.

INTRODUCTION

Natural regeneration of tree species becomes a key function since it anticipates the structure and dynamics of future forest communities (Harper 1977; Nathan & Muller-Landau 2000). Consequently, regeneration is critical to assure and stabilize the provision of multiple forest functions and services in the future. Nevertheless, despite its importance, the relationship between biodiversity in the canopy and the natural regeneration has been rarely investigated (but see Carnevale & Montagnini 2002; van der Plas *et al.* 2016), generating a research gap whose findings seem essential to develop optimal strategies for forest management (Guariguata & Pinard 1998). If recruitment abundance and richness are promoted by local canopy diversity (Carnevale & Montagnini 2002), forest composition may turn out particularly stable over time and resilient to environmental changes. In such scenario, management practices should aim at ensuring diversity maintenance of the canopy layer. In contrast, if diversity of recruits does not rely on canopy diversity, other factors like seed arrival from the regional pool (Myers & Harms 2011) or non-random demographic filters (Wills *et al.* 2006) should be taken into account to maintain high diversity levels in the recruit cohort.

At the local scale, successful natural recruitment overcomes two consecutive ecological 'filters': seed arrival and post-dispersal survival (Muller-Landau *et al.* 2002; Clark *et al.* 2007; Schupp, Jordano & Gómez 2010), which are both influenced directly or indirectly by canopy attributes. In a first step, seed arrival is determined by seed rain (i.e. amount and richness of seeds produced) and seed dispersal quality (i.e. effectiveness in dispersal process, *sensu* Schupp *et al.* 2010), and thereby, seed limitation can arise when either process is compromised. In this stage, canopy composition through species' identities is expected to affect directly to seed rain in terms of quantity and quality (species identity) (Figure 1). Thus, seed rain richness is expected to increase with the number of species in the canopy layer; whereas the number of seeds will be determined by the species' reproduction strategy (i.e. seed production).

Moreover, despite recruitment cannot occur without seed arrival, seed arrival does not guarantee the establishment. Post-dispersal processes such as microsite limitation (John *et al.* 2007; Clark *et al.* 2007) may have strong impacts over the seedling community by limiting the establishment and survival of new recruits (Nathan and Muller-Landau 2000). In this sense, how canopy richness modulates post-dispersal seed survival is key to understand the link between the adult and juvenile cohorts.

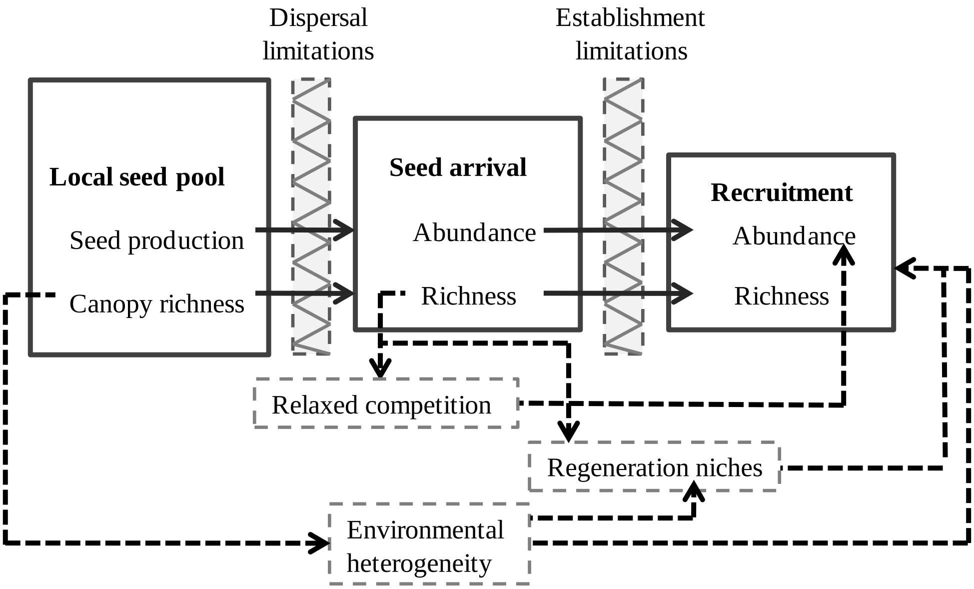


Figure 1. Conceptual model of the expected relationship between forest canopy attributes and seedling recruitment taking into account seed arrival and post-dispersal survival filters. Solid and dashed lines depict direct and indirect relationships respectively.

In the absence of establishment limitation (*sensu* Clark *et al.* 2007), larger seed rain in terms of quantity and richness is expected to enhance directly both the abundance of recruits and the number of species represented in the seedling community. In contrast, assuming post-dispersal mortality, the effects of tree canopy are tightly linked to the interplay between environmental heterogeneity and species regeneration niches (*sensu* Grubb 1977). Higher canopy richness is expected to induce environmental heterogeneity through a mosaic of distinct environmental conditions relying on species' identities combined (Yankelevich *et al.* 2006). Under such scenario, the chances for tree species present in the seed rain pool to find suitable or safe sites increases, and consequently, the expected richness of seedlings rises. Moreover, according to the resource partitioning hypothesis (Hardin 1960; MacArthur & Levins 1967), different species tend to coexist by exploiting different resources. Thus, given a number of seeds, recruitment abundance is expected to be higher as the number of species present in the seed rain increases since intraspecific competition diminishes (Figure 1). All together, high diverse canopy composition is expected to enhance recruits density and richness by supplying larger and more diverse seed rain and/or by providing contrasting microhabitats for the establishment of different species.

Here, we analyze the relationship between canopy richness and natural recruitment, using a large-scale European platform comprising 173 forest plots along a canopy species richness gradient (from monospecific to mixed plots up to 5 tree species) across Europe. We hypothesize that:

(i) Recruitment abundance will increase directly with the amount of seed supply and indirectly with seed rain richness (inferred from canopy richness) by reducing the intraspecific competition.

(ii) Recruitment richness is expected to increase from monospecific to more diverse canopies directly by a more diverse supply of seeds. Moreover, we expect that recruitment richness will increase from monospecific plots with tree species with low seed production, to mixed forest plots with high seed production.

(iii) We expect that high local canopy richness enhances recruitment abundance and richness indirectly by inducing environmental heterogeneity (Ricklefs 1977).

MATERIALS AND METHODS

Study sites

The study was conducted within the framework of the European project FunDivEUROPE (http://www.fundiveurope.eu/), which includes a network of permanent forest plots distributed in five mature European forests. These forests are included among the most representative of European forest types, covering a large European bioclimatic gradient: from the southernmost continental Mediterranean-mixed forest (CM) located in Spain (40.7º N, -1.9º E), through continental mountainous-mixed forest (CB) in Romania (47.6º N, 25.3º E) and temperate-mixed forest (TB) in Germany (51.5º N, 10.2º E), to the northernmost forests as a hemiboreal (H) forest in Poland (52.7° N, 23.9º E) and boreal one (B) located in Finland (62.6º N, 29.9º E). In each forest type, between 28 and 43 plots of 30 x 30 m size were established following a species richness gradient of the local dominant tree species from monospecific to mixed plots with three species (in the case of B forest), four species (in CM, TB and CB forests) and five species in the canopy (in the case of H forest). The dominant species considered in the five sites encompassed conifers (e.g. *Picea abies, Pinus sylvestris*), deciduous broadleaved species (e.g. *Fagus sylvatica, Betula pendula*) and one evergreen broadleaved species (e.g. *Quercus ilex*). Most of species were present only in one forest type, except for few of them (i.e. *P. sylvestris, P.abies* or *Acer pseudoplatanus*) (Table 1). Plots within a forest were chosen following three main criteria to analyze the effect of the species richness in the canopy to different ecosystem functions: (i) similar relative abundance of the dominant species within mixed plots (i.e. high evenness), with each species composition replicated at least twice whenever possible; (ii) minimal signals of human intervention in recent years, and finally (iii), similar environmental characteristics in terms of topography, slope and soil properties, to avoid confounding factors. Further information about experimental design can be found in Baeten *et al.* (2013). In summary, we used five types of forests, 173 plots and 13 target species to test the relationship between recruitment and canopy richness (see Table 1 for more details about the study sites).

Table 1. Description of study sites with mean environment characteristics at plot level (Plot). Standard deviation is presented in brackets. CV: coefficient of variation. GSF: global site factor from hemispherical photographs. ISF: indirect site factor from hemispherical photographs.

		Boreal	Hemiboreal	Continental temperate	Temperate -mixed	Continental Mediterranean-mixed
Region	Location	North Karelia region (Finland)	Bialowieza National Park (Poland)	Carpathian mountains (Romania)	Hainich National Park (Germany)	Alto Tajo Natural Park (Spain)
	Target tree species	*Pinus sylvestris* ^{1}, *Picea abies* ^{1}, *Betula pendula* ^{1}	*Pinus sylvestris* ^{1}, *Picea abies* ^{1}, *Betula spss* ^{1}, *Quercus robur* ^{2}, *Carpinus betulus* ^{3}	*Picea abies* ^{1}, *Fagus sylvatica* ^{2}, *Abies alba* ^{1}, *Acer pseudoplatanus* ^{3}	*Fagus sylvatica* ^{2}, *Fraxinus excelsior* ^{3}, *Acer pseudoplatanus* ^{3}, *Quercus spss* ^{2}, *Picea abies* ^{1}	*Pinus nigra* ^{1}, *Pinus sylvestris* ^{1}, *Quercus ilex* ^{2}, *Quercus faginea* ^{2}
	Canopy richness range	1-3	1-5	1-4	1-4	1-4
	No. plots	28	43	28	38	36
	Sampling month	August-2012	June-2012	August-2012	May-2012	April-2012
	Annual mean T (ºC)	2.1 (0.3)	6.9 (0.1)	5.5 (0.5)	7.4 (0.3)	9.7 (0.6)
	Annual mean P (mm)	631.8 (5.9)	580.8 (3.7)	691.1 (24.9)	689.4 (45.8)	537.4 (27.3)
Plot level	Altitudinal range	87-233	140-200	655-1062	273-496	960-1404
	Mean GSF	0.22 (0.04)	0.11 (0.02)	0.19 (0.03)	0.11 (0.04)	0.50 (0.12)
	CV GSF	0.19 (0.05)	0.23 (0.06)	0.17 (0.06)	0.31 (0.10)	0.23 (0.07)
	Mean ISF	0.28 (0.03)	0.15 (0.02)	0.23 (0.02)	0.14 (0.03)	0.51 (0.10)
	CV ISF	0.12 (0.05)	0.14 (0.07)	0.11 (0.04)	0.23 (0.08)	0.20 (0.07)
	Soil depth (cm)	46.32 (9.73)	-	44.26 (9.54)	-	32.45 (9.45)
	CV Soil depth	0.21 (0.07)	-	0.23 (0.08)	-	0.33 (0.13)
	Mean stoniness	3.28 (5.92)	-	2.10 (3.30)	-	11.09 (10.77)
	CV stoniness	1.58 (1.36)	-	1.68 (1.37)	-	1.07 (0.68)
	Mean shrubs (%)	11.60 (8.79)	-	0.04 (0.18)	-	15.82 (14.81)
	CV shrubs	1.08 (0.72)	-	0.29 (1.08)	-	1.20 (0.49)
	Mean herbs (%)	29.47 (18.12)	-	9.05 (8.64)	-	25.17 (18.58)
	CV Herbs	0.80 (0.31)	-	1.20 (0.49)	-	0.86 (0.36)

Species clasification for BA_{seed}: ^{1} Species with a high seed production (x 3.6; SSS); ^{2} Species with a low seed production (x 1; LSS); ^{3} Species with a medium

Data collection

Regeneration samplings

Regeneration surveys were carried out in 2012, from April to late August, ensuring that all tree species had fully expanded leaves by the sampling date. Samplings were performed in a subplot of 16 m^2 (4 x 4 m) located in the central part of each plot, where we identified and counted all saplings of the dominant tree species from one year old (i.e. avoiding newly emerged seedlings) up to 1.60 m tall, and quantified the species richness. Saplings were considered one year old or older when at least one internode could be counted along its main stem or cotyledons were not visible. We used species richness instead of other diversity indices since it is the simplest indicator of the community diversity, easily to be interpreted and basis of many ecological models (Gotelli & Colwell 2001, 2011).

Seed supply data

Seed supply in terms of quantity and quality (richness) directly depends on stem density and tree species identity. To account for seed quantity, we calculated a proxy for the potential amount of seeds (BA $_{\text{seed}}$) with the following equation:

$$BA_{seed} = \frac{\sum_1^n \varpi ij * BA_i}{\sum_1^n BAi}$$

, where BAi is the basal area of the *i*-th species and ϖij is a weighted factor calculated according to the *j*-th species' reproduction strategy. To obtain ϖij, we sorted out the target species into three different categories according to their reproductive strategy (Table 1). Small-seeded species (SSS): species that produce a high number of small seeds (conifers and *B. pendula*); large-seeded species (LSS): species with low seed production and a greater investment in seed size (e.g. *Quercus spp, F. sylvatica*) and finally, medium-seeded species

(MSS): species with intermediate characteristics (producers of samaras; i.e. *F. excelsior, A. pseudoplatanus*). Then, we assigned a weighting value to each species based on existing literature reporting seed productions (number of seeds per m^2) for species within each previous category (SSS: Béland *et al.* 2000; Calama *et al.* 2015; LSS: Beamonte, 2009; Gómez, 2003; MSS: Shibata, Tanaka & Nakashizuka 1998). Then, we calculated the ratio of change in seed production in relation to the category with the lowest seed production (LSS), resulting in 3.6 for species included in SSS (annual seed production of SSS / annual seed production of LSS); 1.8 for species included in MSS (annual seed production of MSS / annual seed production of LSS) and 1 for species included in LSS. Although, ϖij seems a coarse approximation to species' seed production, it allows considering differences in seed production among tree species co-occurring. BA$_{\text{seed}}$ ranges from 1 when canopy is entirely formed by large-seeded species to 3.6 when it is totally composed by small-seeded species.

As estimates for seed pool richness, we considered the number of different tree species in the canopy (canopy richness) in each plot.

Environmental data

We characterized every single plot (30 x 30 m) recording environmental data in a total of 17 quadrants of 1 m^2 distributed uniformly within each plot (one at the center of the plot and the other 16 located regularly in grid points every 10 meters). We took hemispherical photographs and analyzed them with Hemiview v.2.1 software (Delta-T Devices Ltd,Burwell, UK) to quantified the light availability (Valladares & Guzmán 2006) through the Global Site Factor (GSF) and Indirect Light (ISF). Besides, in three forest types with contrasting climatic conditions (CM, CB and B forests), we measured soil depth till 60 cm (depth enough to ensure a normal root growth of saplings) and we estimated visually the percent of stoniness, shrubs and herbs in each squared meter.

We estimated the heterogeneity of the environmental variables using the coefficient of variation in each plot (CV=(standard deviation/mean) x 100) (e.g. Wiens 2000; Lundholm & Larson 2003; Quilchano *et al.* 2008). We also calculated the overall environmental heterogeneity in the three contrasting forest types (CM, CB and B forests) averaging the coefficients of variation (CV) of all environmental variables in each plot.

Statistical analyses

Environmental heterogeneity and canopy richness

In a first analysis, we fitted a generalized linear mixed model (GLMM, Breslow & Clayton 1993) to test whether an increase in canopy richness increased overall environmental heterogeneity using forest type as a random factor. Different GLMM were also performed separately for each particular environmental variable, using canopy richness as explanatory variable and forest type as a random factor. Since no relationship was found between overall environmental heterogeneity and canopy richness (see results section) and we only had available data for three out of five study forests, we decided to use light heterogeneity (a key factor for regeneration; Nicotra, Chazdon & Iriarte 1999; Benavides *et al.* 2016) rather than overall environmental heterogeneity in the regeneration models.

Regeneration models

We studied the effect of canopy richness on natural recruitment through two different non-exclusives sources: (i) seed supply (quantity and richness) and (ii) environmental heterogeneity in terms of light heterogeneity. Therefore, we performed a full generalized linear mixed model (GLMM) for each recruitment response variable (abundance and richness) with all potential variables affecting recruitment. We used canopy richness (a proxy for seed pool richness), BA_{seed} (a proxy for seed quantity) and light heterogeneity (CV GSF) as explanatory variables and type of forest as a random factor. As the response variables are count data, GLMM's were run assuming a Poisson distribution, except for overdispersed models that entailed negative binomial distributions. For the abundance model, plots with absence of recruitment were discarded to avoid underestimation of recruitment, and four more plots (3 from TB forest and 1 from CM forest) were also discarded as outliers, making a total of sample size = 139. In the case of richness model, only plots able to contribute to species richness of recruitment were included in the analysis (i.e. plots with at least 2 individuals; sample size = 129). Marginal and conditional-R^2 were calculated in all cases following the method outlined by Nakagawa & Schielzeth (2013).As additional analyses, we performed a generalized linear model (GLM) studying the effect of canopy richness on recruitment abundance and richness per each type of forest separately.

Finally, we used the Bray-Curtis dissimilarity index (Bray & Curtis 1957) to quantify the compositional dissimilarity between canopy and recruitment composition in each plot through the percent of representation of individuals present in both cohorts. We did not take into account plots with absence of recruitment since it is meaningless without regeneration. Values of Bray-Curtis dissimilarity ranged from 0 to 1, being 1 completely dissimilar.

Power analyses

We performed statistical post-hoc power analyses in order to evaluate if our experimental design could detect the minimum magnitude of the expected effects among response and explanatory variables (Cohen 1988). First, we needed a theoretical value of the percent of change for each response variable. For this purpose, we searched for studies documenting changes in tree regeneration and environmental heterogeneity in response to different treatments and environmental conditions (e.g. fragmentation, thinning, shelterwood cutting; see Table S1). We calculated for each study the rate of change of our response variables (R_{max}/R_{min}) among treatments discarding those too different to our environmental conditions (e.g. clear cuttings or extreme fragmentation), and averaged them. This value gave us the expected change in abundance and richness of recruits in contrasting conditions in nature ("Minimum theoretical ratio", MTR, hereafter).

Subsequently, we performed a sensitivity analysis to obtain the statistical power of our experimental design. First, we calculated the percentage of significant p-values obtained from simulating 100 times the effect size of the canopy richness on overall heterogeneity considering among- and within-forest variability. Then, we plotted statistical power against effect size to obtain the minimum response that our experimental design could detect with an adequate statistical power (80% chance) (Cohen 1988) ("Minimum detectable effect" hereafter; MDE). This minimum detectable effect was converted to the minimum detectable ratio (MDR) of our response variable following our experimental design. In other words, we calculated the rate of change of our response variable between the minimum and maximum values of our explanatory variables.

Finally, when MDR was greater than MTR we calculated the minimum number of plots per forest and canopy richness level in order to detect the minimum theoretical effect size. For this purpose, we simulated our experimental design varying the number of plots sampled by canopy richness level (and forest type) from 3 to 30. In each experimental design we simulated 100 times the response of the effect size of the minimum theoretical value and calculated the statistical power. We also reported the sample size at which a satisfactorystatistical power was detected. This procedure was performed whenever we found no significant relationship between our response variables (overall environmental heterogeneity, abundance and richness of recruitment) and explanatory variables (canopy richness, light heterogeneity and BA_{seed}).

All statistical analyses were carried out in R v. 3.2.1 (R Development Core Team 2013) using the packages lme4 (Bates *et al.* 2014) and MuMIn (Barton 2015).

RESULTS

Effect of canopy richness on environmental heterogeneity and recruitment

We did not find any relationship between overall environmental heterogeneity and canopy richness (marginal-R^2 = 1.7 %) (Table 2). We neither found a significant effect of canopy richness on environmental heterogeneity when variables were analyzed separately, except for light heterogeneity measurements (CV GSF and CV ISF) (Table 2). Although overall light heterogeneity (CV GSF, used in the regeneration models) was positively and significantly related to canopy richness, the variance explained was very low (Marginal-R^2 = 2.5 %) (Figure 2; Table 2). The relationship between CV ISF and canopy richness was only marginally significant. In general, variance explained by random factor 'forest type' was much larger (conditional-R^2 ranged between 6% for stoniness and 50% for shrubs) than variance explained by canopy richness (fixed effect) for all our environmental heterogeneity models (Table 2).

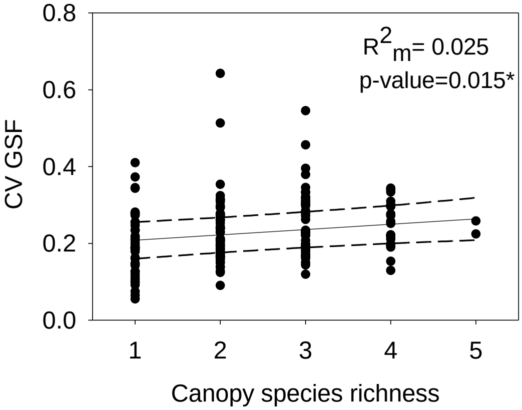


Figure 2. Effect of the canopy species richness on global light heterogeneity measured as coefficient of variation (CV GSF). Solid lines correspond to GLMM predictions and dashed lines to 95% confident intervals.

Table 2. Results from GLMMs examining the effect of tree canopy richness on global environmental heterogeneity and the heterogeneity of each particular environmental variable (measured as coefficient of variation; CV). Type of forest was taken as a random factor. N: sample size used for each variable. Light characterization was performed in all plots from five mature forests included in the study (N= 172) and the rest of environmental variables was carried out in the three most contrasting forests (N= 92). CV: coefficient of variation, GSF: global site factor from hemispherical photographs. ISF: indirect site factor from hemispherical photographs. R^2_m: marginal-R^2; R^2_c: conditional-R^2.

Response	N	Estimate	SD. Error	z-value	p-value	R^2_m	R^2_c
CV global	92	0.038	0.030	1.279	0.201	0.017	0.017
CV GSF	172	0.014	0.005	2.433	**0.015***	0.025	0.359
CV ISF	172	0.010	0.005	1.929	0.053.	0.015	0.400
CV Soil depth	92	0.007	0.012	0.587	0.557	0.003	0.263
CV Stoniness	92	0.143	0.138	1.038	0.299	0.011	0.064
CV Shrubs	92	0.027	0.041	0.659	0.509	0.005	0.506
CV Herbs	92	0.037	0.047	0.777	0.437	0.005	0.205

(***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value <0.1.

Regarding recruitment results, neither canopy richness nor light heterogeneity showed any effect on abundance recruitment, only BA_{seed} had a marginal significant positive effect (Table 3). However, the proportion of variance explained by the random term (forest type) was again much higher than the fixed effects (conditional-R^2 96% and marginal-R^2 13%, respectively). For recruitment richness, none of our explanatory variables showed a significant effect (Table 3). In addition, we observed that the mixed forest plots with the greatest presence of SSS did not necessarily have the highest mean values of recruitment richness, confirming the absence of relationship between BA_{seed} and recruitment richness (Figure S2.1). Again, among forest-type differences absorbed much of our model variance effects (conditional-R^2 16% and marginal-R^2 1%, respectively). Absence of relationship between canopy richness and recruitment was also observed when different forest types were analyzed separately (Figs. S2.2 and S2.3).

Table 3. Summary table of the GLMM for the abundance and richness of recruitment. BA_{seed} is a proxy of the supply of amount of seeds (see calculation in material and methods section); N= sample size, number of plots included into analyses. mar-R^2: marginal-R^2. cond-R^2: conditional-R^2. CV: coefficient of variation; GSF: global site factor from hemispherical photographs.

Response	Exp. variable	Estimate	SD. Error	z-value	p-value	R^2_m	R^2_c
Abundance of recruitment N= 139	Intercept	2.746	0.671	4.092	0.000***	0.125	0.957
	Canopy richness	0.083	0.101	0.817	0.414		
	BA_{seed}	0.279	0.147	1.898	0.058.		
	CV GSF	-2.053	1.593	-1.289	0.197		
Richness of recruitment N= 129	Intercept	0.400	0.352	1.135	0.256	0.010	0.157
	Canopy richness	0.036	0.062	0.582	0.560		
	BA_{seed}	0.064	0.084	0.409	0.445		
	CV GSF	0.307	0.750	0.763	0.683		

(***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value <0.1.

Power analyses

Sensitivity analysis showed that the minimum detectable effect (MDE) of canopy richness on overall environmental heterogeneity was 0.08 (Figure S1.1), resulting in a minimum detectable ratio (MDR) of 1.5. Since MDR was equal to minimum theoretical ratio (MTR), our experimental design was suitable for detecting a potential relationship between both variables.

For the abundance of recruitment analysis, we performed three different post-hoc power analyses for each explanatory variable, since none of them showed a significant effect. In the case of its relationship with canopy richness, the MDR value was greater than the minimum theoretical value (MTR) (Table 4; Figure S1.2) and thereby, our experimental design was not able to detect the minimum magnitude of the expected effect between the abundance of recruitment and canopy richness. In fact, we calculated that a minimum of 12 plots per canopy richness level and forest type was needed to detect an effect size of the minimum theoretical value (Table 4). Our experimental design was also not enough powerful to detect the relationship between recruitment abundance and light heterogeneity, with at least 30 plots required per canopy richness and forest type to detect the MTR. In contrast, our experimental design was statically robust to detect the MTR of BA$_{seed}$ effect on recruitment abundance (Table 4).

Table 4. Summary table of the post-hoc power analyses. Range: simulated effect sizes analyzed during the sensitivity analysis; MDE: size effect with an acceptable power (0.80); MDR: minimum detectable ratio corresponding to our experimental design; MTR: Minimum theoretical ratio; Experimental design: if our design allowed us to detect the theoretical threshold ratio; Sample size: minimum estimated number of plots per richness level and forest type to detect the minimum theoretical ratio. BA $_{seed}$ is a proxy of the supply of amount of seeds (see calculation in material and methods section); GSF: global site factor from hemispherical photographs.

Response	Exp. variable	Range	MDE	MDR	MTR	Experimental design	Sample size
Global heterogeneity	Canopy richness	[0,1]	0.08	1.5	1.5	Reliable	-
Abundance of recruitment	Canopy richness	[0,1]	0.28	3.06	1.85	Unreliable	12
	BA $_{seed}$	[0,2]	0.46	1.51		Reliable	-
	CV GSF	[0,10]	4.27	46.7		Unreliable	>30
Richness of recruitment	Canopy richness	[0,1]	0.18	1.34	1.75	Reliable	-
	BA $_{seed}$	[0,2]	0.20	1.19		Reliable	-
	CV GSF	[0,4]	1.68	4.54		Unreliable	>30

Regarding recruitment richness, we also performed a post-hoc power analysis for each explanatory variable similar to abundance recruitment. From the sensitivity analysis, we obtained a minimum detectable effect (MDE) of 0.18 and 0.20 for canopy richness and BA_{seed}, respectively (Figure S1.3). This meant a MDR below the minimum theoretical value. Therefore, our experimental design was suitable to detect the minimum theoretical effect of canopy richness and BA_{seed} on richness of recruitment (Table 4). However, it was underpowered for light heterogeneity, requiring more than 30 plots per canopy richness level and type of forest to detect the minimum theoretical effect (Table 4).

DISCUSSION

Our results indicated that variations in abundance and richness of recruitment in the European forests studied were not associated to tree species richness in the canopy. This overall finding is derived from the fact that: (i) we did not find an effect of either seed quantity (calculated according to species' reproduction strategy) or richness (inferred from local canopy richness) on abundance and richness of recruitment; and (ii) local canopy richness did not enhance recruitment indirectly by generating heterogeneity in the environment. In addition, we showed that our experimental design was statistically robust to be certain about the lack of relationship between environmental heterogeneity and canopy richness, as well as the lack of relationship between seed supply (in terms of quantity and richness) and recruitment richness. However, for recruitment abundance, its absence of relationship with seed richness (canopy richness) could not be proven, neither for the light heterogeneity-recruitment relationship, being required larger experimental designs to detect the minimum theoretical ratio of change in natural conditions.

Seed rain and recruitment

Contrary to our expectations, our results showed that neither the amount of seeds nor the richness of the seed pool had an effect on tree recruitment patterns. Regarding seed quantity, results from the power analysis showed that such lack of effect was not due to not enough power of the experimental design. We found no differences in recruitment abundance among forest plots with indistinctly large- and small-seeded species, that may be explained by a higher thriving probability of large seeds than small ones (Gómez, Puerta-Piñero & Schupp 2008; Chen *et al.* 2014). Moreover, at least two reasons would support differences in the chance of survival of large *vs* small seeds: (i) the existence of efficient dispersal agents moving large seeds (animals) to safer microsites than small ones, and (ii) seedlings emerged from large seeds usually endure adversity better than those from small ones due to a larger amount of stored resources (Leishman *et al.* 2000). In fact, large-seeded species have shown higher survival rates at the seedling stage than small-seeded species, facing drought events, herbivory, litter coverage, shade and competition interactions (Leishman *et al.* 2000; Westoby *et al.* 2002; Moles & Westoby 2004). Since seed dispersal effectiveness arises from the interaction between the quantity of seeds dispersed and the probability of post-dispersal survival (Schupp *et al.* 2010), an advantage for survival of large-seeded over small-seeded species may compensate their lower fecundity, equalizing differences in recruitment abundance among plots due to contrasting seed production rates. Regarding recruitment richness, we found that mixed plots with high presence of tree species having a high seed production (i.e. high presence of different species following SSS strategy) did not necessarily show high levels of recruitment richness contrary to our expectations, being the mixed plots of LSS and MSS species those with the highest richness in recruitment. This finding suggests that source limitation is not the main driver of recruitment diversity in the European forest studied, and again, low probability of survival of small seeds overriding their higher abundance (dispersal limitation) could explain patterns here found. Our results are in line with previous works, which pointed out that environmental factors determining post-dispersal survival may be as important as seed arrival and have longstanding consequences in plant community composition (Levine & Murrell 2003; Clark *et al.* 2007).

No significant effects of seed richness (inferred from canopy richness) on recruitment abundance or richness was found. However, we can only be certain that local seed richness does not enhance recruitment richness in the studied forests, but it could not be proven for abundance. Lack of concordance between seed pool richness (inferred from canopy composition) and juvenile richness contradicts a previous study showing positive association between the species richness of juveniles and both canopy richness and basal area (Olson & Wagner 2011). Moreover, Carnevale & Montagnini (2002) showed that higher levels of recruitment abundance and richness were reached in mixed-tree species rather than pure-tree species plantations. The uncoupling between community composition of adult trees and juveniles results particularly interesting since it suggests that forest composition is dynamic over time and thereby, changes might be observed in long-term (Granda *et al.* 2012, 2014). Two processes occurring at different recruitment stages could explain this pattern, i.e. similar level of recruitment richness reached in monospecific and mixed forest plots (see Figure S2.4). Firstly, seed arrival from nearby stands might cancel out local canopy richness effects ("regional pool hypothesis"; Eriksson 1993). In this case, species pool at the regional scale would determine local communities' composition rather than local diversity and thereby, landscape connectivity and beta diversity should be a conservation priority. In our study, the median of Bray-Curtis dissimilarity between canopy-recruitment composition was 0.55, suggesting that the composition between adults and recruits is more than 50% dissimilar in average of all plots. High canopy-recruitment dissimilarity was due to either absent recruits of certain dominant tree species present in the canopy or new tree species in the regeneration not present in adjancent adults. Secondly, high post-dispersal mortality favoured by negative density-dependent mechanisms may also explain this mismatch between canopy-recruitment composition (i.e. *'Janzen-Connell effects';* Janzen 1970; Connell 1971; Chesson 2000). The negative density-dependent mechanisms may constraint locally the juvenile abundance in the proximity or in highly dense areas of conspecific adults (monospecific stands), leaving potential sites available to be colonized by heterospecific recruits.

Recruitment and environmental heterogeneity

Forest canopy attributes such as composition or diversity are two of the main factors determining the microhabitats within a forest (Jennings, Brown & Sheil 1999; Augusto, Dupouey & Ranger 2003; Barbier, Gosselin & Balandier 2008). For instance, Chávez & Macdonald (2010) found relevant differences in light, soil moisture, temperature and soil nutrients in small patches within a boreal mixed-wood forest depending on the dominant canopy species (broadleaves, conifers or mixed-codominance). In this context, we expected that increasing the local canopy richness would enhance recruitment abundance and richness indirectly by introducing heterogeneity in the environment and, therefore, offering a wider range of environmental conditions under which different species could establabish. In contrast, our results showed that high local canopy richness did not lead to greater heterogeneity in the environment, considering either individual environmental factor (shrubs, stoniness, herbs and soil depth) or pooled, except for light (Table 2). This lack of relationship was corroborated by the power analysis results (Table 4).

Our finding suggests that monospecific plots may have similar values in the study environmental heterogeneity at ground level to mixed plots, since no relationship between overall environmental heterogeneity and canopy richness was found (Table 2). Then, we can assume that the number of available recruitment niches were similar in both forest stands, as other studies have reported (Barbier *et al.* 2008; Ampoorter *et al.* 2016). In particular, Ampoorter *et al.* (2016) working on understory diversity in the same study sites of this work, showed the lack of support for the environmental heterogeneity hypothesis (Ricklefs 1977) (i.e. the additive effect of the presence of multiple species on environmental conditions) at the stand-level. They proposed the creation of new environmental conditions (called '*novel environment creation*'), which not necessarily imply more heterogeneity in the environment (i.e. non-additive effect of species richness). Nevertheless, we found that heterogeneity in light (GSF and ISF) was the only environmental variable that showed a positive response with canopy richness, although with a very small squared-R. This is consistent with previous works showing that species mixtures increase light quantity and its spatial variability (Kelty 2006; Puerta-Piñero, Gómez & Valladares 2007). The generation of variable light patches has been shown to enhance the opportunities of establishment and survival of multiple species with different light requirements (Ameztegui & Coll 2011; Ligot *et al.* 2013; Carrari *et al.* 2016). However, contrary to our expectations, we obtained that light heterogeneity did not have any effect on abundance or richness of recruits, despite being one of the major factors determining the establishment and survival of juveniles (Nicotra *et al.* 1999; Benavides *et al.* 2016). Consequently, further works assessing light heterogeneity effects on seedling community composition is needed to shed light on these results.

Suitability/Limitation of experimental design

Despite the lack of relationship between canopy richness and recruitment patterns in the studied European forests, we can assure that our experimental design was sufficiently powerful to detect the majority of these potential relationships (Table 4). On the contrary, our results indicated that huge experimental designs are needed to explore some other questions like the relationship between light heterogeneity and recruitment, pointing at designs hardly approachable.

We observed that type of forest (random factor) explained a substantial part of variance in the recruitment-canopy richness relationship in comparison to the predictors considered. This makes sense considering the contrasting types of forests and the idiosyncrasy of the dominant tree species pool in each one. In any case, analyses performed at forest level corroborated the patters found at global scale, namely lack of relationship between recruitment and canopy richness (Figure S2.2 for recruitment abundance and Figure S2.3 for recruitment richness). However, we could not assess which early-stage filters underpin such mismatch. In fact, our data did not allow us to distinguish between seed arrival and establishment limitation, while disentangling these two processes will be key to detect bottlenecks for diversity maintenance (see Carlo & Morales 2016) and establish suitable management guidelines. Thus, although this experiment represent a first step aimed at understanding how canopy composition determines regeneration patterns in European forests, future studies jointly quantifying the real seed supply, also from regional pools and post-dispersal filters are needed to clarify patterns here found.

CONCLUSION

The continuity of the current forest compositions and the provisioning of multiple ecosystem services in the future depend on a successful regeneration process. In this context, we tested whether the canopy richness enhances recruitment in terms of abundance and richness using, for the first time to our knowledge, a large European scale. Our results show that (1) canopy richness does not necessarily enhance environmental heterogeneity, and (2) there is a mismatch between local seed source (inferred from the local canopy species richnes) and forest regeneration. We argue that the relationships between local seed supply, environmental heterogeneity and the attributes of the juvenile community in terms of abundance, richness, composition may not be as straightforward as we expected. This is probably due to the influence of seed supply from regional pools and/or the existence of post-dispersal ecological filters (i.e. biotic interactions), which can be as important as seed arrival (Levine & Murrell 2003; Myers & Harms 2011). However, our study has limitations that must be acknowledged, mainly the coarse approach used to estimate seed availability and richness. Therefore, future studies quantifying the real seed supply, also from regional pools, and the importance of post-dispersal filters are crucial for the understanding of the biological mechanisms underlying the mismatch between canopy richness and recruitment composition and would help the design of adequate management practices.

ACKNOWLEDGMENTS

We are very grateful to all colleagues and field teams that participated in the numerous field campaigns, especially to Inés Carrascón, David López, Alice Godoy, Jerónimo B.B Sansevero and the Belgium field team of FunDivEUROPE project, among others for their valuable help in the field. We thank Sonia Rabasa for her initial comments and ideas on the research. We want also thank Laura Barrios for her statistical support. We are grateful to the site managers for establishing and maintaining of the permanent plots. C.C.B. is beneficiary of a FPU grant funded by the Spanish Government (AP2010-5600). R.B. was funded by a Marie Curie IEF fellowship (FP7-PEOPLE-2011-IEF). This research was supported by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 265171, and also from Spanish-funded project REMEDINAL3eCM (S2013/MAE-2719).

SUPPLEMENTARY MATERIAL

Appendix S1. Additional information to power analyses: MTR and results from sensitivity analyses.

Table S1. References used to determine the "Minimum Theoretical Ratio" (MTR). MTR is the theoretical expected change in abundance and richness of recruits in contrasting conditions in nature. Therefore, it is the minimum effect size of each one of our response variables to be detected by our experimental design.

Response variable	Reference	MTR
Light heterogeneity	(Quilchano *et al.* 2008)	1.5
	(Nicotra *et al.* 1999)	
	(Valladares & Guzmán 2006)	
Abundance of recruitment	(Benítez-Malvido & Martínez-Ramos 2003)	1.85
	(Suffice *et al.* 2015)	
	(Ares, Neill & Puettmann 2010)	
Richness of recruitment	(Suffice *et al.* 2015)	1.75
	(Ares *et al.* 2010)	

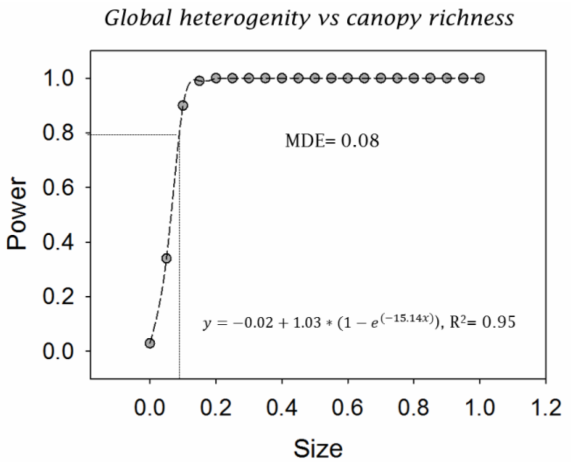


Figure S1.1. Result of sensitivity analysis of size effects (slope) on the predictive power of model on the effect of canopy richness on global environmental heterogeneity. Minimum detectable effect (MDE) depicts size value beyond which prediction power is acceptable (≥80%).

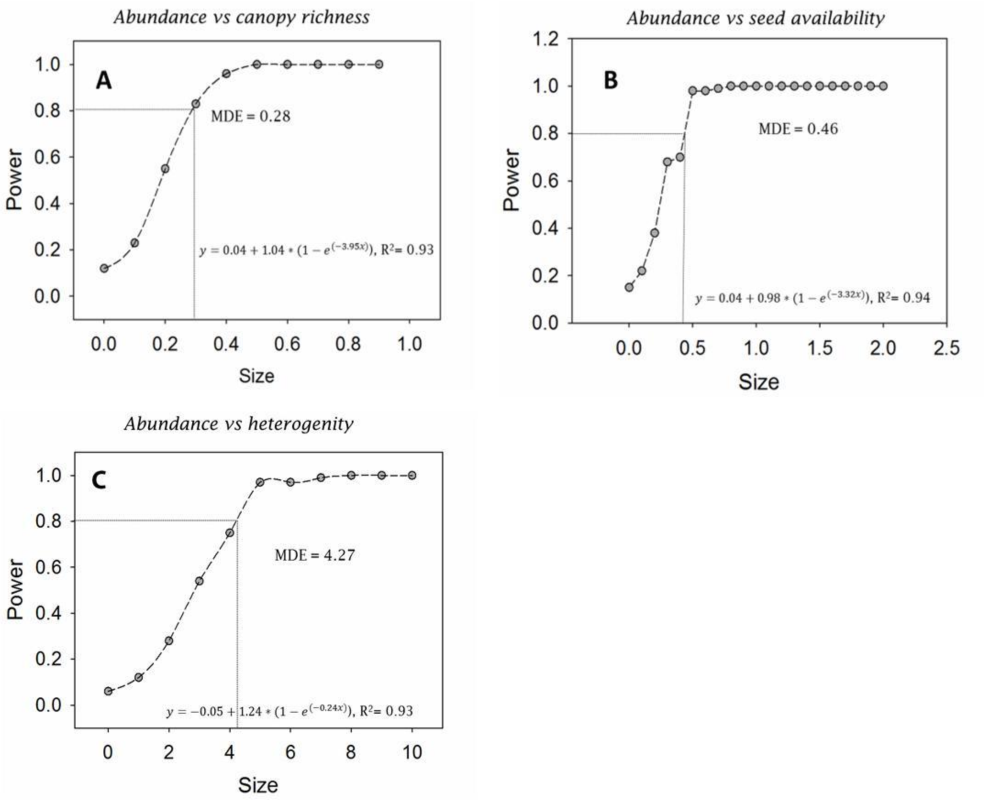


Figure S1.2. Result of sensitivity analysis of size effect (slope) on the predictive power of model on the effect of (A) canopy richness, (B) seed availability and (C) light heterogeneity on recruitment abundance. Minimum detectable effect (MDE) depicts size value beyond which prediction power is acceptable (≥80%).

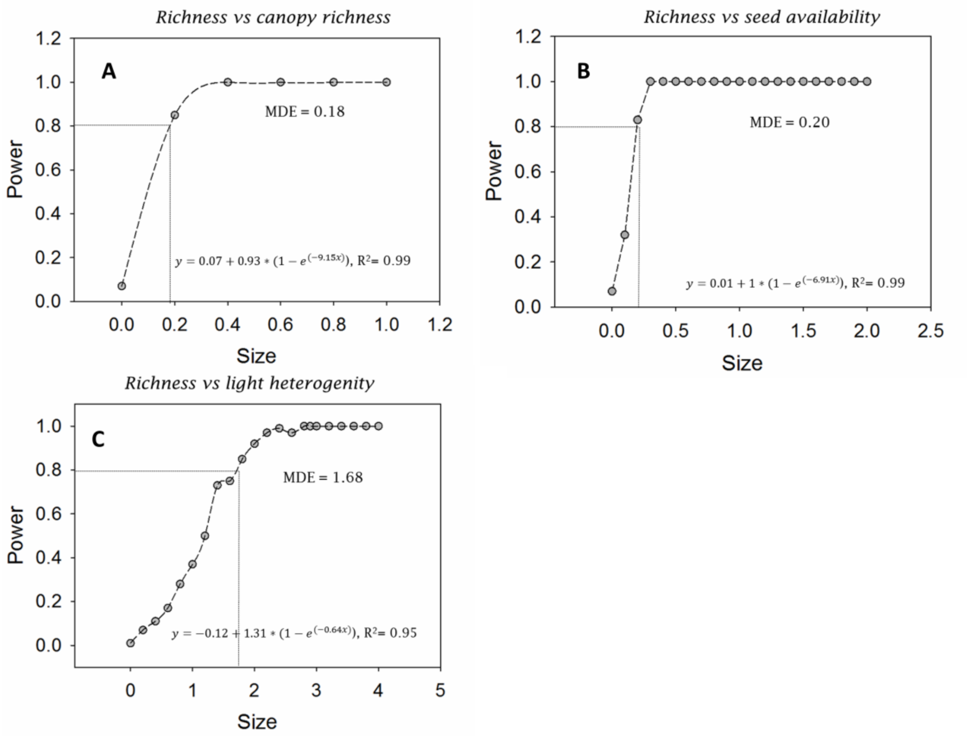


Figure S1.3. Results of sensitivity analysis of size effects (slopes) on the predictive power of models on the effects of (A) canopy richness, (B) seed availability and (C) light heterogeneity on species richness of recruits. Minimum detectable effect (MDE) depicts size value beyond which prediction power is acceptable (≥80%).

Appendix S2. Additional results from regeneration models.

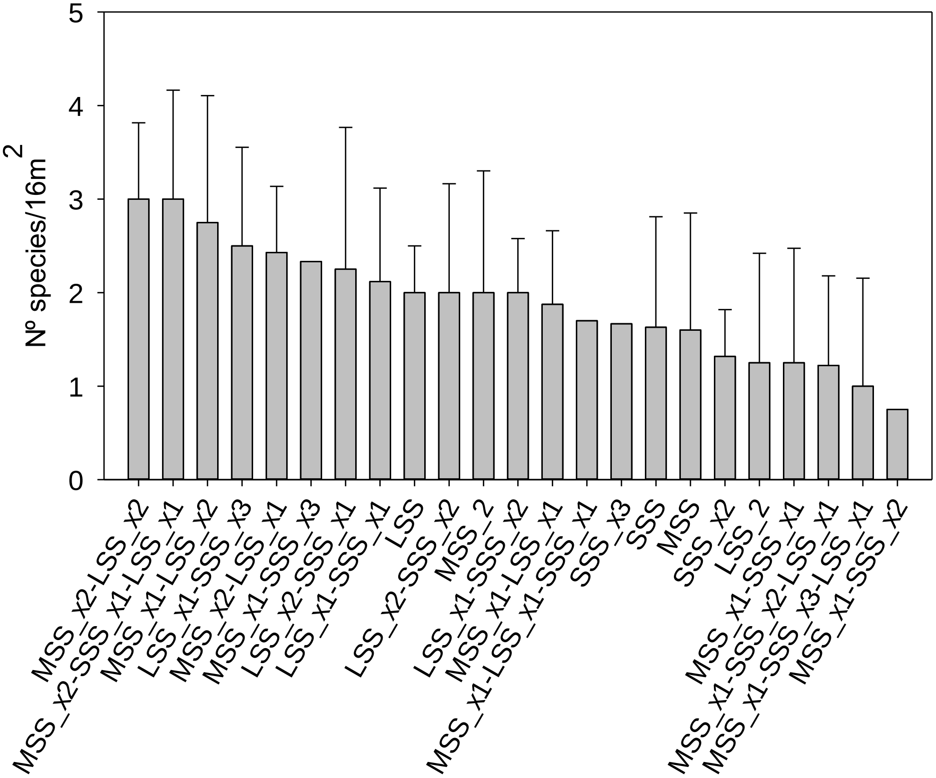


Figure S2.1. Mean recruitment richness across all plot combinations according to species' reproduction strategy. LSS: large-seeded species; SSS: small-seeded species; MSS: intermediate conditions between LSS and SSS (*see* Table 1 in the main text for classification of species). The number accompanying to (x) is the number of species having that particular reproduction strategy. Graph is ordered by mean recruitment richness.

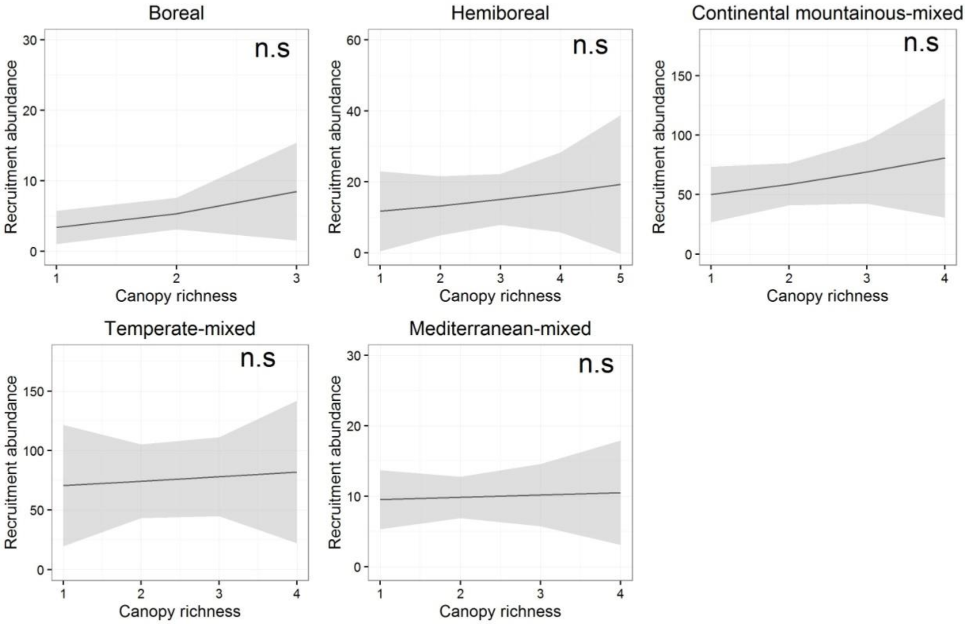


Figure S2.2. Effect of the canopy richness on recruitment abundance for each type of forest. Solid line corresponds to GLMM predictions and the grey area to 95% confident intervals.

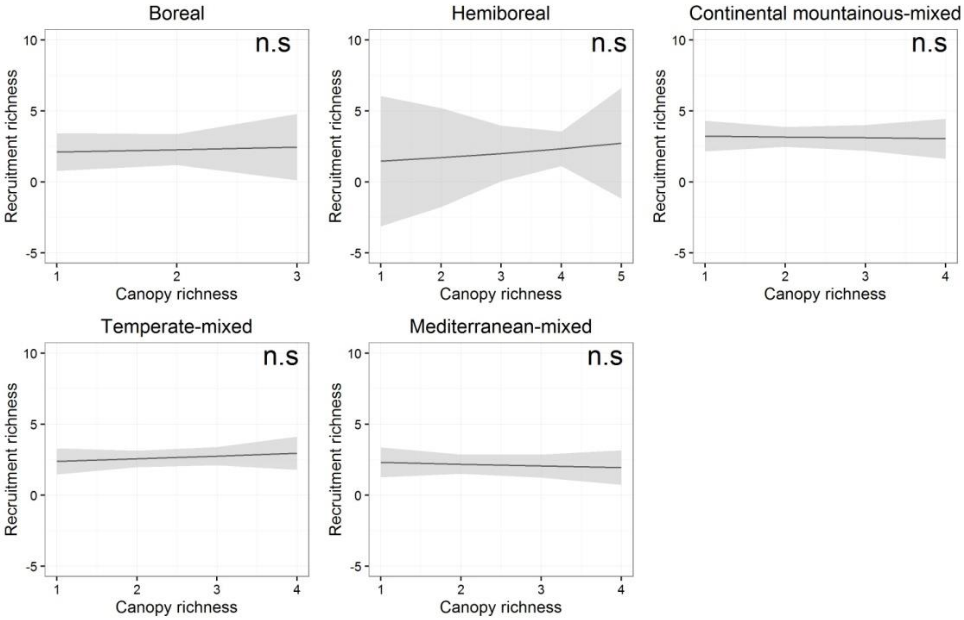


Figure S2.3. Effect of the canopy richness on recruitment richness for each type of forest. Solid line corresponds to GLMM predictions and the grey area to 95% confident intervals.

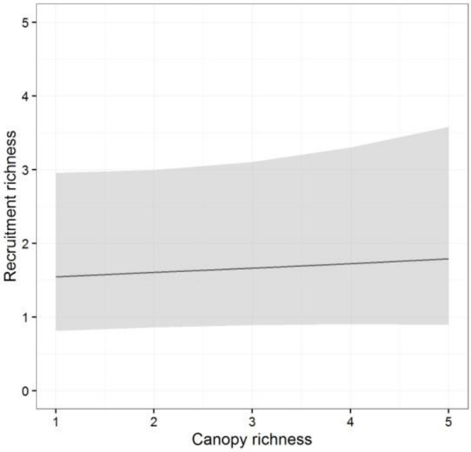
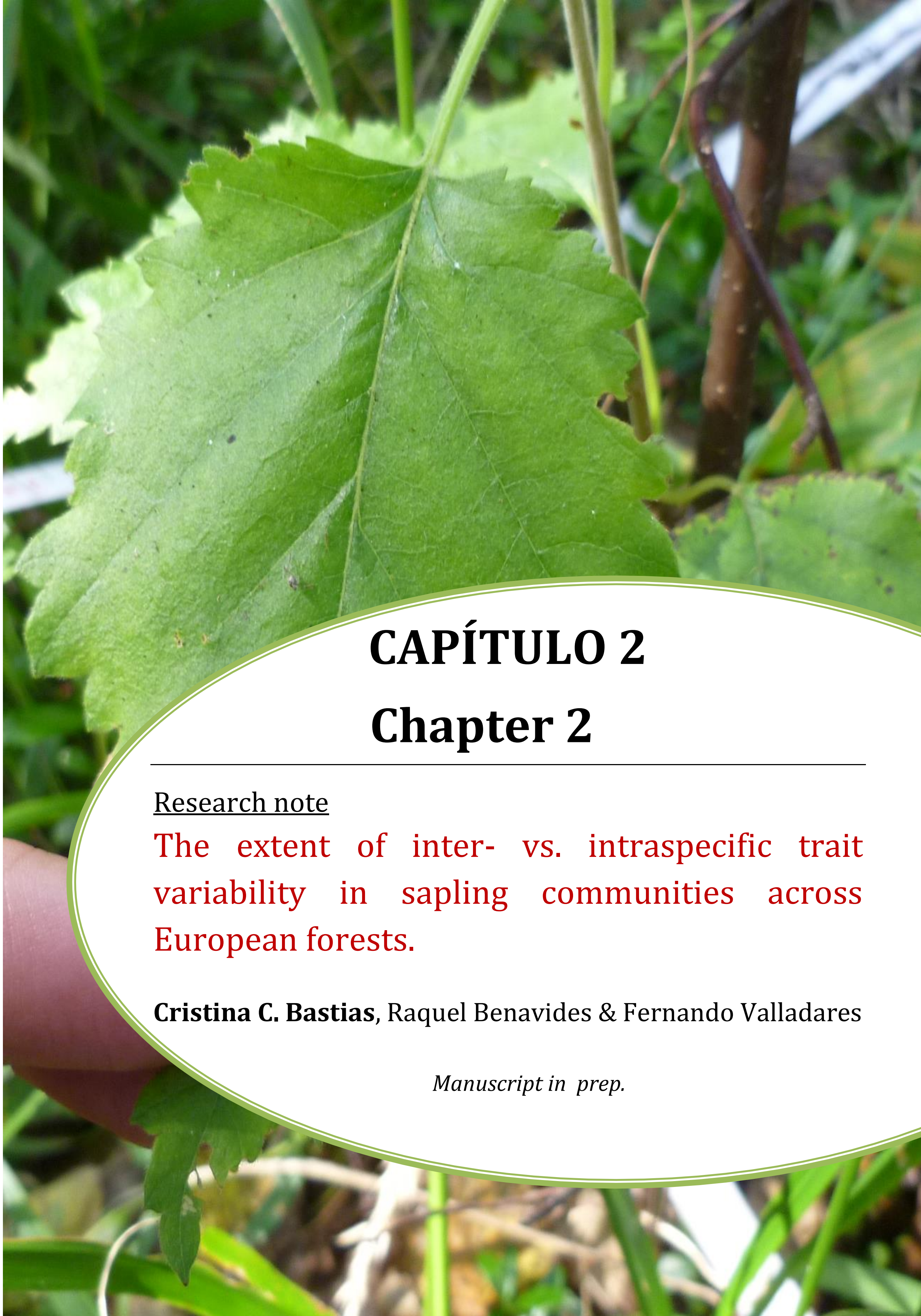


Figure S2.4. Effect of the canopy richness on recruitment richness. Solid line corresponds to GLMM predictions and the grey area to 95% confident interval



CAPÍTULO 2

Chapter 2

Research note

The extent of inter- vs. intraspecific trait variability in sapling communities across European forests.

Cristina C. Bastias, Raquel Benavides & Fernando Valladares

Manuscript in prep.

Image from the front page (Chapter 2): Leaf of *Betula pendula*.

Author: Inés Carrascón.

SUMMARY

Studying the partitioning of functional diversity into inter- and intraspecific trait variability is a crucial step for a mechanistic understanding of species coexistence. The extent of interspecific trait variability is relatively well-known in trait-based studies of community ecology whereas the contribution of intraspecific trait variability to functional diversity and community dynamics remains poorly understood. Here, we partitioned the variance of different leaf morphological (SLA and LDMC) and chemical (Leaf C content, leaf N content, $\delta^{13}C$ and $\delta^{15}N$ isotopes) traits into inter- and intraspecific components 92 sapling communities of up to four species coexisting (5-10 individuals measured per species) distributed in three contrasting European forests: a boreal (Finland), a continental temperate (Romania) and a continental Mediterranean (Spain) forests. We found that intraspecific trait variation was the main trait variation component for leaf $\delta^{13}C$ and $\delta^{15}N$ isotopic signals accounting for 47-75% of the total variance followed by LDMC with 37-70% of the total variance. In contrast, the extent of intraspecific variation was much smaller than interspecific variation for SLA (4-16% vs 84-96%) and leaf nitrogen content (7-23%vs 76-93%). Interestingly, we found a similar extent of inter- and intraspecific variation for the same traits across the three bioclimatically contrasted forests. Our findings indicated that intraspecific trait variability can play very different roles for each trait, with similar patterns across the three European forests. Traits like those related to C and N isotopic signals should receive a larger level of attention at the intraspecific level than that received so far in trait-based studies.

Keywords: leaf morphological traits, leaf chemical traits, functional diversity, community assembly, isotopes, tree species.

INTRODUCTION

Trait-based approaches applied to community ecology, i.e. the use of functional characteristics linked to individual fitness (Violle *et al.* 2007), have led to a considerable advance in understanding key ecological questions related to community assembly and dynamics (Valladares *et al.* 2015; Escudero & Valladares 2016). The mean trait approach has historically dominated the literature of functional ecology (McGill *et al.* 2006; Weiher 2011). This fact is due to the assumption of the differences in trait values are much larger among than within species (Garnier *et al.* 2001; McGill *et al.* 2006). Therefore, it is reasonable that the majority of trait-based community studies has used mean trait values to characterize species, ignoring trait variation within species (Violle *et al.* 2012). However, ignoring intraspecific trait variability depends on its relative contribution to the total functional trait diversity compared to interspecific trait variability. Thus, if intraspecific trait variability becomes substantial, it might have a deep influence on community assembly and thereby it should be taken into account in the study. Some recent works have already found that the relative extent of intraspecific trait variability is large and, in some cases, even larger than interspecific trait variability (Messier *et al.* 2010; Lepš *et al.* 2011). These studies have pointed up to the need of including intraspecific trait variability in trait-based ecological studies in order to avoid biases in the interpretation of the empirical results related to assembly processes and ecosystem functioning (Cianciaruso *et al.* 2009; Jung *et al.* 2010; Albert *et al.* 2011; Lepš *et al.* 2011).

However, the relative importance of intraspecific trait variability has been shown to be highly context-dependent, i.e. it is strongly dependent on the particular study community and on the functional trait used. Thus, an improvement of our understanding about how intraspecific trait variability varies among plant communities and traits is essential for integrating the intraspecific trait variability in trait-based ecological studies (Albert *et al.* 2011). In our study, we decomposed total trait variation of different leaf morphological and chemical traits of sapling communities in three different European forests to quantify the relative importance of trait variability among and within species. We concentrated on leaf morphological (SLA and LDMC) traits because they are directly related to the plant ecological strategy in terms of resource acquisition-conservation attributes and their capacity to adapt to different environmental conditions (Wright *et al.* 2004; Reich 2014), and on leaf chemical traits (Leaf N and C concentrations, $\delta^{13}C$ and $\delta^{15}N$ isotopes) because they are related to metabolic activities and play an important role in controlling nutrient assimilation and primary production (Cornelissen *et al.* 2001; Wright *et al.* 2004; Kerkhoff *et al.* 2006). More importantly, both morphological and chemical leaf traits can be measured extensively and they are widely used in many trait-based ecological studies across different taxa and communities allowing for effective comparisons with other works (e.g. Hulshof & Swenson 2010; Auger & Shipley 2013; Kazakou *et al.* 2014; Siefert *et al.* 2015).

MATERIALS AND METHODS

Study sites

The study was carried out within the network of permanent plots designed by FunDivEUROPE project (http://www.fundiveurope.eu/), particularly in plots located in three European forest types along the European bioclimatic gradient: 28 plots in a boreal forest (62.6º N, 29.9º E; Finland), 28 plots in a continental temperate forest (47.6º N, 25.3º E; Romania) and 36 plots in a continental Mediterranean forest (40.7º N, -1.9º E; Spain). In each forest, plots of 30 x 30 m size were delimited along a species richness gradient of the dominant tree species, ranging from 1 species (monospecific plots) to mixed plots with the complete species pool, i.e. 3 species in the boreal forest and 4 species in the case of the continental temperate and the continental Mediterranean forests. See Table 1 for general characteristics of each forest. Further information about experimental design can be found in Baeten *et al.* (2013).

Table 1. Brief description of study forests with the mean environment characteristics at plot level (Plot). Standard deviation is presented in brackets. GSF: global site factor from hemispherical photographs.

	Boreal	**Continental temperate**	**Continental Mediterranean**
Location	North Karelia region (Finland)	Carpathian mountains (Romania)	Alto Tajo Natural Park (Spain)
Canopy species richness range	1-3	1-4	1-4
Tree species forming part of the diversity gradient	*Pinus sylvestris, Picea abies, Betula spp.*	*Fagus sylvatica, Acer pseudoplatanus, Picea abies, Abies alba*	*Pinus nigra, Pinus sylvestris, Quercus ilex, Quercus faginea*
Total number of plots	28	28	36
Annual mean T (ºC)	2.1 (0.3)	5.5(0.5)	9.7 (0.6)
Annual mean P (mm)	631.8 (5.9)	691.1 (24.9)	537.4 (27.3)
Altitudinal	87-233	655-1062	960- 1404
GSF	0.22(0.04)	0.19(0.03)	0.50 (0.12)
Soil depth (cm)	46.32 (9.73)	44.26(9.54)	32.45 (9.45)
pH	4.01 (0.26)	4.58 (0.66)	6.93 (0.51)
Soil N concentration	1.37 (0.50)	2.81 (0.79)	3.57(1.41)
Soil C concentration	22.92 (9.03)	37.51 (12.88)	68.58(27.42)

Sampling and trait measurements

In each plot, we selected randomly a total of ten juveniles over one year old, which is often considered one of the most critical bottleneck in the recruitment process (Harper 1977), up to 1.60 m tall of each dominant tree species in each forest type. In very few cases (8 from a total of 253 sampling units) all present juveniles of a given species should be selected and measured because they were only 5-9 individuals in total. We harvested one branch with young and fully expanded leaves in every selected individual to assess morphological and chemical leaf traits. The samples were manipulated and processed following (Garnier *et al.* 2001; Pérez-Harguindeguy *et al.* 2013).

Morphological traits. We selected three leaves from each branch that were previously rehydrated for at least 12 hours in darkness. We weighed them after removing the petiole in broadleaves to obtain the leaf saturated mass. Then, we scanned them with a high-resolution scanner (CANON-scan LiDE 25), and the leaf area was determined using Image-Pro Plus software (Image-Pro Plus 4.5, Media Cybernetic Inc. USA). Finally, the leaves were oven-dried at 70 °C for 48 h and then weighed to obtain leaf dry mass. With these data, we estimated the specific leaf area (SLA; mm^2/mg) of an individual as the ratio between leaf area and dry mass, averaging the figures of the three leaves per individual. Likewise, the leaf dry matter content (LDMC, mg/g) was obtained averaging the ratio between the leaf dry mass and the leaf saturated mass of the three leaves considered.

Chemical traits. Those three selected leaves per individual (together with additional needles in conifers taken from the same branch) were used for chemical analysis on five individuals in the boreal and continental temperate forests, and seven out of ten in the continental Mediterranean. After drying, we pooled the leaves of each individual and grinded them with a stainless steel mill obtaining a dry homogenous leaf powder per individual. The leaf carbon and nitrogen concentrations, and $\delta^{13}C$ and $\delta^{15}N$ isotopes contents were then assessed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California, Davis. Further information about the analysis method can be found in http://stableisotopefacility.ucdavis.edu/. The precision for both the $\delta^{13}C$ and $\delta^{15}N$ analyses was approximately 0.2 per mil and 0.3 per mil, respectively.

Quantification of the extent of inter- vs intraspecific functional diversity at community level.

We calculated the partitioning of the total community diversity into interspecific (extent of trait dissimilarity among species) and intraspecific (extent of trait dissimilarity within species) functional diversities for each of the 92 sampled juvenile communities (i.e. plots) in the three forest types. We applied the method of variance partitioning explained in de Bello *et al.* (2011).

The interspecific functional diversity was calculated as the variance of trait mean values among species coexisting in the community while the intraspecific functional diversity was calculated as the variance of trait values within each species being weighed by the number of individuals sampled for each species (see de Bello *et al.* 2011 for details). Calculations were performed for each single trait in R program v. 3.2.1 (R Development Core Team 2013) using the R-function called RaoAdo (de Bello *et al.* 2011).

RESULTS

The extent of the intraspecific functional diversity (or intraspecific trait variance) at community level varied substantially depending on the functional trait considered. However, the extent of the intraspecific vs interspecific trait variation remained similar for all functional traits (except LDMC) across the three contrasting European forest, showing a generalized pattern across Europe (Figure 1).

In general, intraspecific trait variance was negligible in comparison with interspecific variation for SLA and leaf nitrogen concentration (4-16% and 7-23%, respectively), and this was true for the three forests. In contrast, we observed that the extent of the intraspecific trait variance was large for both $\delta^{13}C$ and $\delta^{15}N$ isotopes, accounting for between 47-75% and 57-68%, respectively. The trait variance at the intraspecific level also explained a great part of the total functional diversity for LDMC, especially in boreal forest where it encompassed 70% of the total community variance, in contrast to the 37% found in the Mediterranean forest.

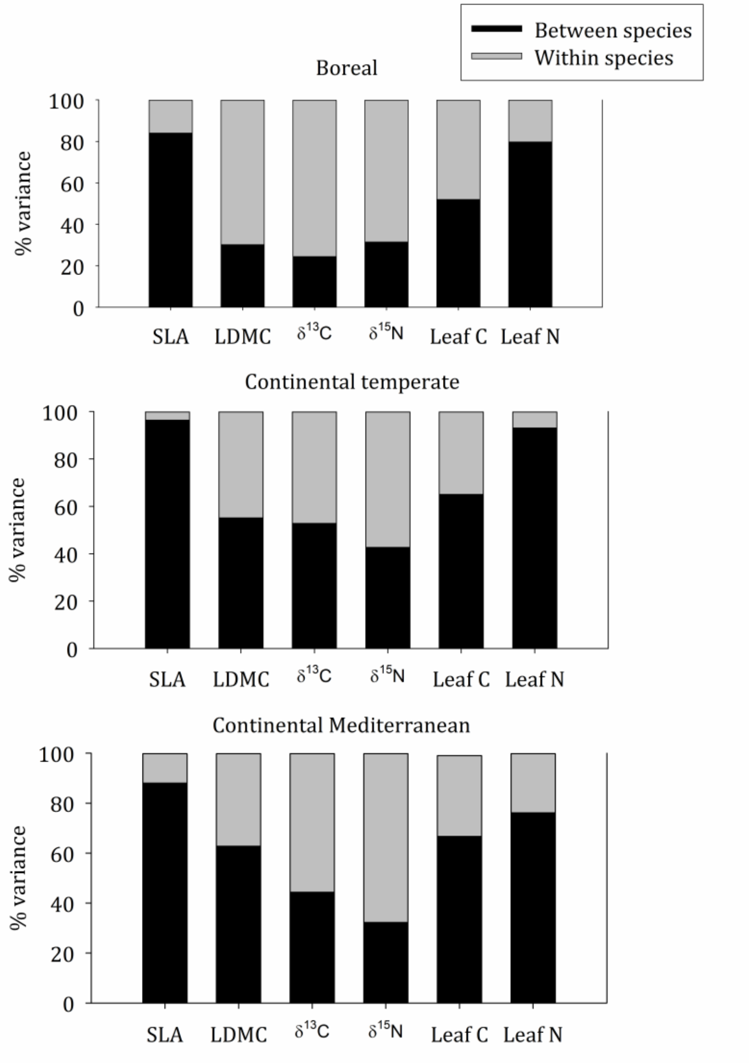


Figure 1. Relative variance decomposition of specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon isotopic signal ($\delta^{13}C$), leaf nitrogen isotopic signal ($\delta^{15}N$), leaf carbon concentration (Leaf C) and leaf nitrogen concentration (Leaf N) at community level (i.e. interspecific –between species- and intraspecific -within species- trait variability at plot level). Bars represent the average of the relative extent of the inter- and intraspecific trait variability for each trait of all communities within each forest except for monospecific ones, which were discarded due to the total functional variation is attributed to intraspecific trait variation. The number of monospecific communities, which were discarded were 9 out of 28 communities in the boreal forest and 6 out of 36 communities in the continental Mediterranean forest).

DISCUSSION

In trait-based community studies, intraspecific trait variability has been systematically ignored because interspecific trait variability has been considered the primary source of variability of functional traits within a community (Garnier *et al.* 2001; McGill *et al.* 2006). However, this premise is not always true since several works have shown that the relative contribution of intraspecific trait variability can be similar and even larger than the interspecific trait variation (Hulshof & Swenson 2010; Messier *et al.* 2010). Here, we found that intraspecific trait variability became a relevant trait variation component of the total functional diversity of juvenile communities for certain functional traits. In particular, we found that the contribution of intraspecific variation for $\delta^{13}C$ and $\delta^{15}N$ isotopic concentrations was much larger, explaining up to 75% and 68% of the total trait variance respectively, in comparison to the functional contribution at the interspecific level in the three European forests. Therefore, it does not seem possible to generalize that interspecific variability is more relevant than intraspecific variability and further quantifications of the variability within species across ecosystems and taxa is necessary to better understand their importance and role in community assembly processes (Jung *et al.* 2010; Siefert 2012; Kichenin *et al.* 2013).

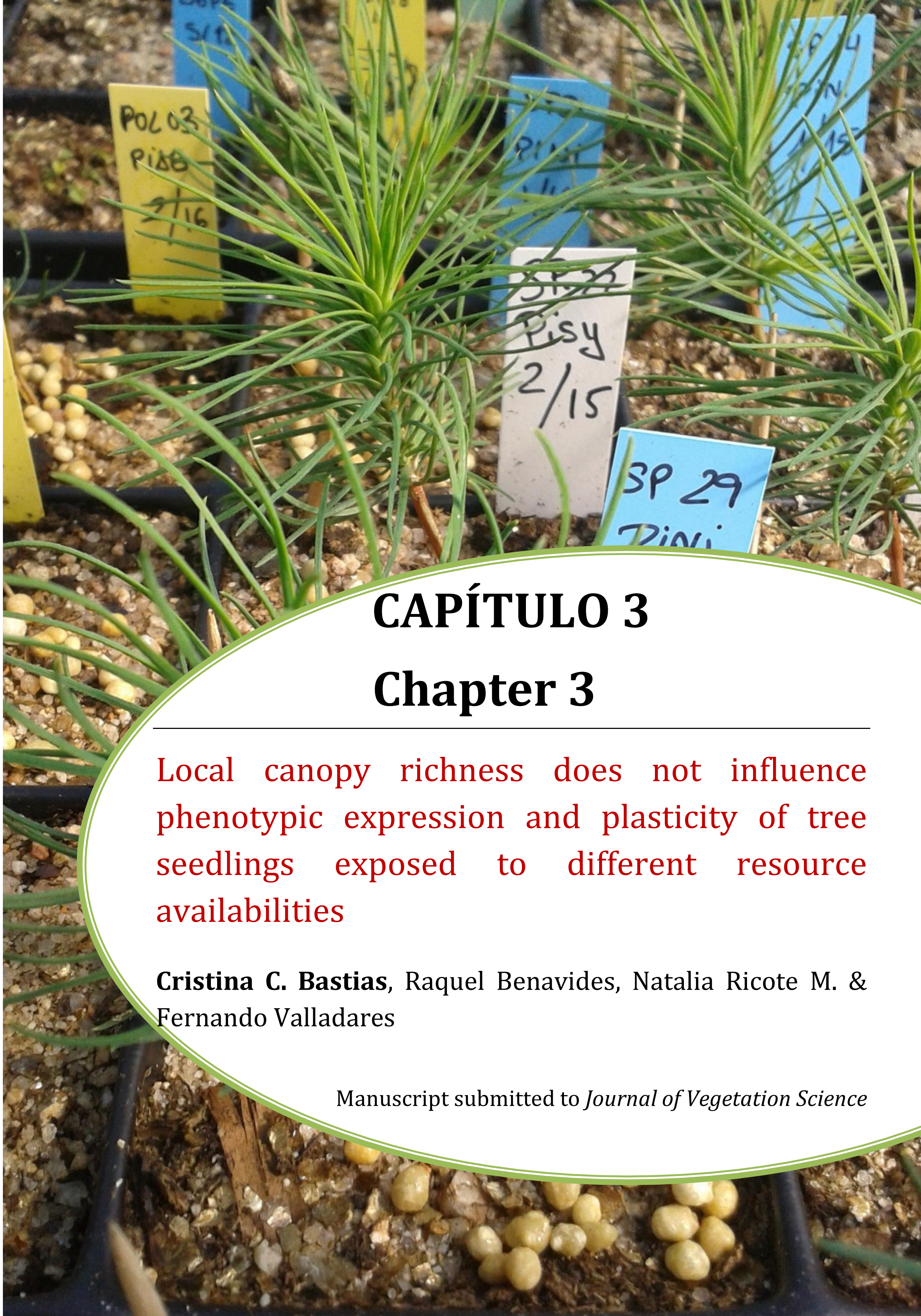
Our findings showing a substantial contribution of the intraspecific trait variability for $\delta^{13}C$ and $\delta^{15}N$ isotopic concentrations support previous studies showing large intraspecific trait variability in leaf chemical traits (Kazakou et al. 2014; Siefert et al. 2015). This variability is likely explained by the labile nature of leaf nutrient concentrations that allows individuals to respond plastically to the resource availability in the environment (Rozendaal, Hurtado & Poorter 2006; Kazakou et al. 2014). This substantial variation in traits at the intraspecific level linked to leaf nutrient contents (Leaf C, N, $\delta^{13}C$ and $\delta^{15}N$ contents) has also been shown by Pescador *et al.* (2015) in another plant system (grasslands). Some authors (e.g. Kazakou et al. 2014; Siefert et al. 2015) have suggested that the importance of intraspecific trait variability in leaf chemical traits is much larger than in morphological traits because the latter tend to be more stable within species in comparison to the formers. This suggestion partly agrees with our results since we found that SLA was the trait showing the least variation at the intraspecific level in the three forests (less than16% of total variation). However, it does not match with the result regarding leaf nitrogen content, which was the second least variable trait at intraspecific level, accounting for the 23% of total trait variation at the most.

The revision of previous studies reveals that the extent of inter- and intraspecific trait variability for the same traits is not consistent. In some cases, the sampling protocol can significantly affect the various sources of trait variability. The relatively small intraspecific variation for traits like SLA could be due, at least in part, to the minimization of the variability of this trait within individuals. Extensive SLA gradients within individuals have been documented in many studies (Valladares & Pearcy 1999; Messier *et al.* 2010) and the systematic sampling of fully developed leaves would therefore reduce the intra-individual source of trait variability. Contrary to our results, Auger & Shipley (2013) found in a temperate forest in Canada that variation in some community-weighted traits such as leaf N content and SLA, were mainly caused by the intraspecific trait variation rather than changes in community-weighted means. Siefert *et al.* (2015) also found large intraspecific variation for leaf N content and a similar trend was also found by Kazakou *et al.* (2014). In contrast, and similar to our results, other studies carried out in other temperate/subtropical systems have also found more than 50% of the variance in SLA is attributed to trait differences at species level (Hulshof & Swenson 2010; Kumordzi *et al.* 2014; Carlucci *et al.* 2015). In summary, the relative extent of inter *vs.* intraspecific trait variability is quite different among study systems and traits (Jung *et al.* 2010; Lepš *et al.* 2011; Auger & Shipley 2013), likely influenced by the goals of the study, scale and sampling protocols (Albert *et al.* 2011).

An outcome that we consider particularly relevant was the similar inter- and intraspecific trait variation patterns were found across the three contrasting European forests. In other words, the relative extent of the intraspecific trait variability in juveniles of tree communities was similar for the same traits measured in three contrasting forests with different species composition, with the only exception of LDMC. The similar pattern observed in the three European forest types suggests a consistent tendency for some traits to respond greatly at the intraspecific level and, therefore, may guide the selection on which traits should be considered and the most efficient sampling strategy to explore sources and implications of trait variation in complex ecosystems.

ACKNOWLEDGMENTS

We are very grateful to David López, Pablo Tabarés, Alice Godoy, Jerónimo B.B Sansevero for their valuable help in the field and in the laboratory. C.C.B. is beneficiary of a FPU grant funded by the Spanish Government (AP2010-5600). R.B. was funded by a Marie Curie IEF fellowship (FP7-PEOPLE-2011-IEF). This research was supported by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 265171, and also from Spanish-funded project REMEDINAL3eCM (S2013/MAE-2719).



CAPÍTULO 3

Chapter 3

Local canopy richness does not influence phenotypic expression and plasticity of tree seedlings exposed to different resource availabilities

Cristina C. Bastias, Raquel Benavides, Natalia Ricote M. & Fernando Valladares

Manuscript submitted to *Journal of Vegetation Science*

Image from the front page (Chapter 3): Conifer experiment in the “sun-high nutrient” environment in the greenhouse.

Author: Cristina Crespo Bastias.

SUMMARY

While recent evidence shows that individual plants adjust their traits to the identity and diversity of their neighbours in herbs, there is a gap in evaluating the diversity-induced plasticity in long-lived species such as trees. It is often hypothesized that species diversity favors environmental heterogeneity and ecological theory predicts, in turn, greater levels of phenotypic plasticity with increasing heterogeneity. Under these two assumptions, we investigated here shifts in phenotypic expression and plasticity in seedlings of two different functional groups (broadleaves and conifers) coming from a hemiboreal and Mediterranean forests differing in canopy species richness. We first characterized the environmental heterogeneity in terms of light and soil properties of the tree communities from which seedlings came from and we then carried out a greenhouse experiment to test plasticity in response to light and nutrient availabilities. Our results indicated a similarity in the phenotypic expression and the capacity of response to treatments of seedlings from monospecific and those coming from more diverse communities and in more heterogeneous environments. Canopy species richness (and the environmental heterogeneity associated to it) did not exert detectable selective pressure on tree phenotypes and plasticity at this early stage. We found great differences in phenotypic expression and plasticity between the two major functional groups mainly in the Mediterranean forest, pointing up to broadleaved species having a higher potential adaptive capacity to respond to future, rapidly changing conditions than conifers.

Keywords: environmental heterogeneity, light treatment, nutrient treatment, species richness, trait variation, intraspecific trait variability.

INTRODUCTION

In forest ecosystems, trees are exposed to multiple environmental factors varying considerably in space and time (Valladares *et al.* 2007). Ecological theory predicts an association between environmental heterogeneity of a given habitat and the level of phenotypic plasticity displayed by plants living in these conditions (i.e. the capacity of an individual to adjust their phenotype in response to spatiotemporal resource fluctuations) (Alpert & Simms 2002; Gianoli 2004; Gianoli & González-Teuber 2005; Baythavong, Agrawal & McPeek 2011). The direction of this relationship would depend on the “amount” and “scale” of spatiotemporal environmental heterogeneity at which plasticity is advantageous for a plant. It is important to distinguish functional from structural heterogeneity, i.e. heterogeneity perceived and not perceived by a given organism (Gomez, Valladares & Puerta-Pinero 2004). When an environmental factor varies on a scale much smaller than the response unit (e.g. whole individual), environmental conditions are perceived as uniform and thereby, plasticity is not expected to have any advantage (i.e. low plasticity exhibited) (Alpert & Simms 2002). However, above this limit, plasticity seems advantageous in heterogeneous ecosystems allowing plants to respond to the range of resource availabilities. Evidence of plants growing under more spatiotemporal heterogeneous environments and showing greater levels of plasticity to different ecological factors has been reported for soil moisture (Gianoli & González-Teuber 2005; Molina-Montenegro, Atala & Gianoli 2010; Lázaro-Nogal *et al.* 2015) or nutrient availability (Wijesinghe & Hutchings 1999). Nevertheless, a large spatio-temporal heterogeneity may exceed the threshold from which a plastic response might not beneficial for plants and might not compensate for its costs (Alpert & Simms 2002).

The “amount” of spatial environmental heterogeneity seems to be positively related to the presence of different species in the forest (i.e. species richness) (Stein *et al.* 2014). This premise agrees well with classical theory suggesting that neighboring individuals have different resource uses (i.e. niche partitioning) for a stable coexistence (Pacala & Tilman 1994; Chesson 2000). In this sense, each individual adjusts its traits according to their requirements avoiding competition, and in turn, modifies the ecological conditions of its influence area, both above and below-ground (Yankelevich *et al.* 2006). Additionally, when species richness is high, the number of interactions among species increases, leading to complex systems where multiple of ecological factors and trophic levels interact. For instance, species diversity has been found to alter soil bacterial community compositions and, subsequently, the nutrient abundance within the rhizosphere (Schlatter *et al.* 2015). Plasticity is likely advantageous in heterogeneous environments. Following this reasoning, individuals living in rich communities (with high species richness) would exhibit different phenotypes (i.e. trait mean shifts) as a result of their greater plastic responses in comparison to those individuals growing in low diverse communities. This differentiation would be due to both a higher environmental heterogeneity and to the complementarity and interactions with other species and trophic levels (Rothe & Binkley 2001; Jucker *et al.* 2014b; Ligot *et al.* 2016).

However, available evidence does not always support this hypothesis, showing neutral or even negative effects of species richness on environmental heterogeneity (Tamme *et al.* 2010; Laanisto *et al.* 2013; Gazol *et al.* 2013; Ampoorter *et al.* 2016). In these cases, similar plasticity levels could be expected in individuals from communities differing in species richness, or even less plasticity in species-rich communities (Debat & David 2001) if local biotic selective pressure penalizes plasticity costs, regardless of the environmental heterogeneity (MacArthur & Levins 1967; Violle *et al.* 2012). There is only few studies evidencing that plant individuals adjust their traits to the identity and diversity of their neighbours (Callaway, Pennings & Richards 2003; Berg & Ellers 2010) and there is an important gap in the evaluation of the effect of species diversity on plasticity of long-lived species such as trees.

Among the key environmental factors affecting plant performance, light and nutrients are the two presenting the largest spatial and temporal heterogeneity. Therefore, they are considered very suitable for studying the potential plasticity of plants, generating numerous studies on these relationships (Valladares *et al.* 2000a; Balaguer *et al.* 2001; Sanchez-Gomez *et al.* 2006; Sardans, Peñuelas & Rodà 2006; Vaz *et al.* 2011; Wyka *et al.* 2012; Santiso & Retuerto 2015). These studies revealed that individuals do not respond in the same way or to the same extent to environmental shifts in these key factors as some authors have previously suggested (Kawecki 2008; Banta *et al.* 2012), but rather differences in plasticity have been observed among individuals at different organization levels, functional groups, species and populations. Studying the capacity of response of individuals provides a better understanding of the functioning of plant species in response to contrasting ecological conditions (Valladares *et al.* 2007, 2015), and of the sensibility and their adaptive capacity of forests to respond to climate change (Lindner *et al.* 2010).

In this study, we investigated the phenotypic expression and phenotypic plasticity patterns of tree seedlings coming from communities with different canopy richness in response to two contrasted light and nutrient availabilities. Firstly, we collected seeds from tree species belonging to two different functional groups (conifers and broadleaves) in communities that varied according to three canopy species richness levels, (monospecific, mixture of 2-3 species and mixture of 4-5 species), in two contrasting European forests: a Mediterranean forest in Spain and a hemiboreal forest in Poland. We then recorded environmental heterogeneity in terms of light availability and soil properties in each community. Finally, we carried out a common garden experiment in order to compare phenotypic expression of seedlings originated in communities with different canopy richness and their plasticity in response to different light and nutrient availabilities. We measured different functional traits associated with light and nutrient availability to address the following questions. 1) Do seedlings from communities differing in canopy richness differ in the phenotypic expression (i.e. trait mean) and/in the magnitude of phenotypic plasticity in response to light and nutrient availability?. 2) Is there convergence in the plastic responses to light and nutrients among seedlings despite that they belong to different functional groups and come from different forests?

MATERIALS AND METHODS

Background experimental design and seed collection

We used plots differing in canopy species richness that belong to a network designed and established by the European project, FunDivEUROPE (www.fundiveurope.eu) aimed at testing the role of biodiversity on ecosystem functioning. In particular, we used those located in two contrasting forests, a hemiboreal forest in the National Park of Bialowieza, Poland (52.7° N, 23.9º E) and a continental Mediterranean-mixed forest located in the Alto Tajo Natural Park, Spain (40.7º N, -1.9º E). In each forest, 43 and 36 plots respectively, were established along a canopy species richness gradient of the dominant tree species, from monospecific stands (only one species) to mixed stands with five in the hemiboreal forest and four species richness in the Mediterranean forest. Plots were set under two main criteria: i) each dominant species was present in all diversity levels and, ii) the relative abundances of the dominant tree species were similar in mixed stands (i.e. maximum evenness). See Table S1 for a description of the environmental characteristics of each type of forest.

For our experiment, we selected six dominant tree species that belonged to two different functional groups (coniferous and broadleaved), growing naturally in both forests. In the hemiboreal forest, we selected Scots pine (*Pinus sylvestris* L.)*,* Norway spruce (*Picea abies* [L.] Karst.) and pedunculate oak (*Quercus robur* L.); and in the Mediterranean forest, Scots pine (*P. sylvestris),* European black pine (*Pinus nigra* Arn.), holm oak (*Quercus ilex* L.) and the Portuguese oak (*Quercus faginea* Lam.).

We collected seeds from three to six mothers for each selected species and forest type at three different canopy species richness levels, i.e. in two monospecific plots (hereafter 'monospecific canopy'), in 2-6 plots with 2 and 3 species coexisting (hereafter 'medium canopy richness') and in one or two plots with 4 and 5 coexisting species (hereafter 'high canopy richness') for hemiboreal and Mediterranean forest, respectively (Table S2). In the Mediterranean forest, we collected seeds from the maximum level of diversity only for conifers because of the negligible seed production of broadleaved species the year of collection. After collection, we pooled seeds from different mothers collected in a given plot and stored them to 4ºC until the beginning of the experiment.

Environmental heterogeneity of surveyed natural communities

Several *in situ* measurements of light and soil properties were taken in all plots (30 x 30 m) where our selected species were present (a total of 75 plots). Global light availability (GSF) was quantified through hemispherical photographs (Valladares & Guzmán 2006) taken in 17 points uniformly distributed (every 10 meters plus one in the center) within each plot. Regarding soil properties, we used data of soil pH, soil C and N concentrations and soil C and N stocks based on bulk density in the first 20 cm of soil. Nine soil cores samples were obtained in every plot at two different soil layers (0-10cm and 10-20cm layers), and pooled per layer for the analyses. Details about soil analyses are reported in Dawud *et al.* (2017). For this study, we used the averaged values for both layers, obtaining one single value for each soil property and plot.

Greenhouse experiment setup and treatments

The experiment was carried out in two consecutive years, from mid November 2012 to mid June 2013 for broadleaved species, and from mid November 2013 to mid July 2014 for coniferous species. The total number of seeds for broadleaved species was 1540 (423 seeds of *Q. ilex*, 666 seeds of *Q. faginea* and 451 seeds of *Q. robur*), which were weighed and sowed in pots of 1.6L filled with a 2:1:1 mixture of peat moss (Kekkilä White 420 F6, Projar, Spain) (autoclaved), vermiculite (0-3 mm grain, Projar, Spain) and washed coarse sand (0-4 mm grain, Leroy Merlin, Spain). We identified each pot with the forest type, plot ID (associated to the canopy richness level), species and number of individual. We placed the pots randomly in the greenhouse, regularly moved to avoid microsite effect. Plants were grown under favorable controlled conditions of light and water during 2 months and half. In mid February 2013, we counted a total of 754 plants germinated (213 plants of *Q. ilex*, 387 plants of *Q. faginea* and 154 plants of *Q. robur*). See table S2 for the total number of plants selected per forest, species and canopy richness level.

Regarding the conifer experiment, a total of 8845 seeds (1782 seeds of *P. sylvestris* and 3125 of *P. abies* from hemiboreal forest; 1977 of *P. sylvestris* and 1961 of *P. nigra* from the Mediterranean forest) were sowed in trays of 32 single-cells (400 cc; 61 x 59 x 200 mm) filled each one with a mixture of 2:1:1 (v:v:v; peat moss [autoclaved]: vermiculite: washed coarse sand). Similar to the experiment with the broadleaved species, we planted the seeds randomly in the trays identifying each single cell with the forest type, plot ID (associated canopy richness level), species and number of individual. We placed the trays in the greenhouse, and we regularly moved them to avoid micro-site effects. Plants were grown in optimal conditions during 3 and half months. We finally had 1991 plants to continue the experiment (473 plants of *P. sylvestris* and 233 plants of *P.abies* from the hemiboreal forest and 571 of *P. sylvestris* and 714 of *P. nigra* from the Mediterranean forest). See table S2 for the total number of plants selected per forest, species and canopy richness level.

In both broadleaf and conifer experiments, plants were divided into three different treatments differing in light and nutrient availability, two key abiotic factors for plants presenting a high temporal and spatial heterogeneity in natural conditions. The treatments were: i) full exposition inside the greenhouse, which means 50% of full sunlight radiation (~1350 μmol m^{-2} s^{-1}) and basic nutrient supply provided by the peat moss (0.045g N/l – 0.03 g P/l – 0.09 g K/l) ('Sun-Low nutrient'), ii) a shaded environment with 20% of full sunlight radiation (~490 μmol m^{-2} s^{-1}) and also basic nutrient supply (0.045g N/l – 0.03 g P/l – 0.09 g K/l) ('Shade-Low nutrient'); and iii) full exposition inside the greenhouse (~1350 μmol m^{-2} s^{-1}) with an additional nutrient supply of one initial input through the experiment of a Plantacote mix 8/9-month slow-release fertilizer 14-8-15 N-P-K, (Plantacote plus, Projar, Spain) ('Sun-High nutrient'). Seedlings received a dose equivalent between 0.22-0.31 g N /l, 0.12-0.18 g P /l and 0.23-0.34 g K /l. Summarizing, we designed a non-factorial experiment aimed at a light treatment (from 50% vs. 20% of full radiation, i.e. from ~1350 μmol m^{-2} s^{-1} to ~490 μmol m^{-2} s^{-1}) with the same nutrient availability, and a nutrient treatment with the same light condition (from 0.045g N/l – 0.03 g P/l – 0.09 g K/l to 0.22-0.31 g N/l - 0.12-0.18 g P/l - 0.23-0.34 g K/l nutrients). In the case of the broadleaf experiment, when treatments started, we removed acorns from germinated seedlings to avoid that seed mass could affect differently the further capacity to response to a treatment. During the whole experiment, temperature and irradiance were recorded every 5 min with a Hobo H08-006-04 data logger (Onset, Pocasset, MA).

Functional traits measurements

We measured a growth trait associated with the plant fitness and different traits in relation to distinct aspects of the plant functioning and ecological strategy (physiology, morphology), particularly those that keep a close relationship with the functional significance for resource acquisition.

Growth-related trait. We measured the relative growth rate (RGR), which is often considered as a surrogate of 'plant fitness' in long-lived species (Gianoli & González-Teuber 2005; Ramírez-Valiente *et al.* 2011). Particularly RGR is a measure of the growth efficiency of the plant related to environmental stress (Pérez-Harguindeguy *et al.* 2013). We calculated RGR for all seedlings as the increase in plant height per unit time (Salgado-Luarte & Gianoli 2010; Lázaro-Nogal *et al.* 2015): $RGR = (\ln H_2 - \ln H_1)/(t_2 - t_1)$, where H_2 and H_1 is plant height (cm) at the beginning and end of the treatment duration (in months), being $(t_2 - t_1)$ equal to 3.5.

Architecture-related traits. We considered plant height since it is related to the ability to compete and obtain resources (both vertical light capture and nutrient uptake). Taller plants with higher number of leaves and smaller internode lengths usually present higher potential light capture (Pérez-

Harguindeguy *et al.* 2013). We measured plant height in cm as the elongation from the soil surface to the upper boundary of the main photosynthetic tissue of the plant at the end of experiment. Moreover, for broadleaved seedlings, we measured the number of leaves and assumed the internode lengths (cm) as the plant height divided by the number of leaves. While for conifers, we measured the stem covered by leaves as the distance above the cotyledons to the node of the most recent fully expanded leaf.

Morphological leaf traits. We measured leaf size (LA, one-side leaf lamina area in mm^2), specific leaf area (SLA, ratio between leaf area and dry mass (mm^2/mg)) and leaf dry matter content (LDMC, ratios of leaf dry mass divided by the leaf saturated mass (mg/g)), because they are closely associated with the trade-off between conservation and acquisition of resources (Westoby 1998; Hodgson *et al.* 2011). Four months after initiating the treatments, we selected in every plant three fully-expanded leaves grown during the treatment to assess the three leaf traits. Once leaves were collected, we kept them in the fridge 24 h at 4ºC, completely hydrated and we weighed them to obtain the saturated leaf mass. Afterwards, we immediately assessed their leaf area using a LI-3000C portable area meter (LI-COR) for broadleaved species, whereas we scanned needles with a high-resolution scanner (CANON-scan LiDE 25) and calculated the area using an image processing software (Image Pro Plus program, Media Cybernetics). Later, all leaves and needles were oven dried (60ºC during 72h) and weighed to obtain the dry leaf mass (Pérez-Harguindeguy *et al.* 2013). Finally, we obtained a mean value per individual averaging the values of the three leaves/needles used.

Physiological traits. We measured the maximum photochemical efficiency of PSII (F_v/F_m), which is a good indicator of the physiological status and it is related to plant growth. We quantified the maximum photochemical efficiency (F_v/F_m) in all broadleaved seedlings and in a subsample of conifers (between 187-281 seedlings per species representative of different diversity levels) using a portable pulse-modulated fluorometer (FMS2, Hansatech, UK). We used one fully-expanded leaf per plant in the case of broadleaved individuals, and a bunch of needles previously dark-adapted for 30 min with leaf clips. Measurements started at 7.00 a.m and finished at 11.30 a.m before seedlings showed signals of heat stress. Also, we measured the stomatal conductance in broadleaved seedlings, which is associated to environmental stress tolerance of the plant (μmol CO_2 mol^{-1} H_2O^{-1}), with a leaf porometer (SC-1, Decagon Devices, Pullman, WA) in one leaf per plant from 12 to 15 p.m. in 7 consecutive and comparable sunny days. These physiological traits were measured after three months and half treatment lengths.

Statistical analysis

We used the coefficient of variation (CV) as a measure of heterogeneity of the light and soil properties in the surveyed natural communities (i.e. the natural conditions, in which mother trees of the study seedlings developed) (Wiens 2000; Lundholm 2009). We calculated CV of GSF at plot level and then we averaged CVs per diversity level (monospecific, medium and high canopy richness) and forest. Regarding soil properties, since we had only one measure for each soil property per plot, we calculated CV using plot values with similar diversity level. To deal with differences in the sample size (number of plots) in each diversity level, we previously run a rarefaction analysis at sample size of n=4 (i.e. minimum sample size) to calculate CV of each soil property for each diversity level and forest at the same sample size (Gotelli & Chao 2013), except for high canopy richness level in the Mediterranean forest whose CV was calculated using n=3. Later, we carried out ANOVA models to test differences in the mean and in the heterogeneity (measured as CV) of light availability and several soil properties among the three diversity levels considered (monospecific, medium and high canopy richness), followed by post-hoc Tukey tests performed on diversity level means.

For testing phenotypic plasticity, we performed linear mixed-effects models (Bates 2010) for each particular functional trait to assess the plasticity to both treatments (Tr) independently: ‘light treatment’ (Sun vs Shade) and ‘nutrient treatment’ (Low *vs* High nutrient supply):

$$\text{Trait} \sim \text{Tr} \times \text{FG} \times \text{SR} + \text{FT} \times \text{Tr} + (1|\text{FT/plot})$$

, where FG is the functional group (broadleaved or coniferous species), SR is canopy richness (monospecific -1 species-, medium -2/3 species mixture- and high -4/5 species mixture), FT is forest type (hemiboreal or continental Mediterranean). Finally, we included ‘plot’ nested in forest type as a random factor to account for the intrinsic conditions of plots that might lead to higher correlations among individuals within a plot. We introduce the term FT x Tr assuming different responses between species from different forest types. The triple interaction was removed from the full model when it turned out non-significant, while we kept double interactions in order to account for the weight of the main interactions responding to our key questions. A significant effect of the SR would indicate differences in the mean trait values of seedlings from different canopy diversities (i.e. dissimilarity in the phenotypic expression). This could suggest local adaptation of the seedlings to the level of species diversity coexisting in natural conditions. A significant Tr x SR interaction would imply plausible differences in plastic responses of seedlings from different canopy richness levels to the experimental treatments (i.e. non-parallel reaction norms). In other words, seedlings might respond differently to treatments depending on whether seedlings come from monospecific stands, medium-mixture stands or from a high-mixture of tree species in the stands.

When FT and/or FT x Tr terms were significant, we additionally run the models for each type of forest separately, keeping ‘plot’ as the only random factor, for the sake of an easy and straightforward model interpretation within each forest. Similarly, when FG x Tr or SR x Tr interactions were significant for forest-specific analyses, individual linear mixed-effects analyses were performed to evaluate the (fixed) effect of FG and SR and its interaction to each treatment.

We also quantified the magnitude of plasticity as the percentage of change in the mean trait value from one environment to other using the unbiased plasticity index (PI) (Valladares *et al.* 2000b).

$$IP = [|\mathrm{Mean}(env_1) - \mathrm{Mean}(env_2)| / \mathrm{Max}(\mathrm{Mean}(env_1), \mathrm{Mean}(env_2))],$$

where Mean(env_1) and Mean(env_2) is the mean trait value in the environment 1 and 2, respectively, and it is represented mathematically as a reaction norm. We standardized the index from 0 (no plasticity) to 1 (maximum plasticity) divided by (Max(Mean(env_1), Mean(env_2)), which is the maximum mean trait value found in both environments. We calculated IP for each trait in response to light (Sun vs Shade) and nutrient (Low vs High supply) treatments, differentiating per diversity level, functional group and forest.

All statistical analyses were carried out in R v. 3.2.2 (R Development Core Team 2015) using the package lme4 (Bates *et al.* 2014).

RESULTS

Environmental heterogeneity in natural communities with different canopy richness

Results from the ANOVA analyses showed similar heterogeneity values of both light and soil properties across canopy richness levels in the hemiboreal forest (Figure S1 and S2), except for N concentration, which were significantly higher in plots with higher tree diversity than in the monospecific plots (Figure S2). In the case of the Mediterranean forest, we found a significantly higher heterogeneity of both light and soil properties in plots with high canopy species richness in comparison to monospecific and medium canopy richness plots (Figure S1 and S2). We did not find differences either in mean values of GSF (*F*-value: 0.83; *P*-value: 0.44 for the hemiboreal forest; *F*-value: 0.26; *P*-value: 0.77

for the Mediterranean forest) or in soil characteristics across plots with different diversity levels, except for soil pH in the hemiboreal forest (Figure S3).

Trait expression and plasticity

Seedlings were unequally affected by the treatments, being their phenotypic expression more affected by nutrient than by light availability (Table 1 and 2; Figure S4). As expected, seedlings under higher nutrient availability were significantly taller with larger crowns and more and larger leaves, which had lower LDMC (Table 1, 2, S3 and S4; Figure 1, 2 and S4). Additionally, seedlings from the Mediterranean forest presented also larger stomatal conductance and F_v/F_m (Table S4). Under the light treatment, seedlings mainly presented morphological changes in leaves (Figure S4), with large SLA and reduced LDMC when they grew in shaded conditions.

We did not find differences among mean trait values of seedlings coming from communities differing in canopy species richness ('SR' effect) for any trait, neither at the overall scale, i.e. pooling all data, nor at the forest scale (Table 1, 2, S3 and S4; Figure 1 and 2). Additionally, we found that seedlings from different canopy diversities responded similarly to both treatments (i.e. non-significant Tr. x SR interaction), except for four traits (number of leaves, crown height, leaf size and RGR) in response to nutrients. In the case of RGR, the plastic responses of seedlings from different canopy diversities to both treatments differed between functional groups (significant triple interaction; Table 1 and 2).

At the forest level, phenotypic plasticity remained similar among seedlings from different canopy diversities. However, some marginal differences in plasticity were observed in the hemiboreal forest, particularly in traits such as crown height and F_v/F_m in response to light, and RGR, height, number of leaves and SLA in response to nutrients (Figure 1 and 2; Table S3), with seedlings from communities with intermediate diversity level being the least plastic, except for RGR.

Table 1. Linear mixed models testing the plasticity of different functional traits to the light availability. FG: functional group (broadleaved vs. conifer species). SR: canopy species richness of communities from where seeds were collected (monospecific, medium and high canopy richness). Tr: light treatment (Sun-50% of full sun exposition- *vs* Shade environments-20% of full sun exposition-). FT: type of forest (Continental Mediterranean forest and hemiboreal forest). Data shown are the *F* values, the degrees of freedom (df) and the statistical significance level of each model using Type III tests. † $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.i: triple interaction removed from the full model when it was not significant. We used the symbol (-) when traits were measured only in a functional group and thereby, these main terms are not included in the model.

	FG df=1	**SR** df=2	**Tr.** df=1	**SR x FG** df= 2	**Tr. x FG** df= 1	**Tr. x SR** df= 2	**FG x SR x Tr.** df=2	**FT** df=1	**FTx Tr.** df=1
1. Growth-related trait									
RGR	1.13	0.09	2.09	3.67*	0.43	1.48	7.28***	25.98***	1.28
2. Architecture-related traits									
Height	63.42***	0.06	0.16	7.50**	63.20***	0.29	n.i	0.41	1.77
Crown height	-	0.97	8.10**	-	-	1.41	-	12.45**	8.45**
No. of leaves	-	0.06	0.68	-	-	0.41	-	28.60***	0.22
Internode length	-	0.01	52.53***	-	-	1.18	-	-	-
3. Morphology leaf traits									
Leaf area	557.94***	0.46	4.39*	12.36***	25.50***	0.48	n.i	0.12	0.00
SLA	62.75***	0.24	29.97***	6.42**	1.37	0.64	n.i	30.38***	2.37
LDMC	55.24***	0.30	56.76***	0.66	25.53***	0.83	n.i	1.11	4.04*
4. Physiological traits									
F_v/F_m	1.00	0.56	6.13*	0.38	83.59***	0.38	n.i	1.78	1.39
Stom. conduct.	-	0.11	0.32	-	-	0.46	-	0.01	2.88 †

Abbreviations: RGR: relative growth rate; F_v/F_m: maximum photochemical efficiency of FSII. SLA: specific leaf area; LDMC: leaf dry matter content. Stom. conduct: stomatal conductance.

Table 2. Linear mixed models testing the plasticity of different functional traits to the nutrient availability. FG: functional group (broadleaved vs. conifer species). SR: canopy species richness of communities from where seeds were collected (monoespecific, medium and high canopy richness). Tr: nutrient treatment (Low vs High nutrient supply). FT: type of forest (Continental Mediterranean forest and hemiboreal forest). Data shown are the F values, the degrees of freedom (df) and the statistical significance level of each model using Type III tests. † P < 0.10; * P < 0.05, ** P < 0.01, *** P < 0.001. n.i: triple interaction removed from the full model when it was not significant. We used the symbol (-) when traits were measured only in a functional group and thereby, these main terms are not included in the model.

	FG df=1	**SR** df=2	**Tr.** df=1	**SR x FG** df= 2	**Tr. x FG** df= 1	**Tr. x SR** df= 2	**FG x SR x Tr.** df=2	**FT** df=1	**FTx Tr.** df=1
1. Growth-related trait									
RGR	8.57**	0.38	0.71	7.58***	1.01	1.54	4.03***	4.25*	12.81***
2. Architecture-related traits									
Height	28.48***	0.14	14.17***	5.55**	14.79***	2.27	n.i	0.08	0.00
Crown height	-	1.60	36.77***	-	-	4.94**	-	2.24	0.93
No. of leaves	-	3.19*	1.39	-	-	3.30*	-	5.57*	0.03
Internode length	-	0.24	10.31**	-	-	0.18	-	-	-
3. Morphology leaf traits									
Leaf area	267.01***	0.46	102.14***	9.76***	16.70***	3.31*	n.i	1.01	28.69***
SLA	87.55***	0.59	52.12***	7.28**	50.50***	2.13	n.i	53.64***	22.74***
LDMC	41.94***	0.05	9.09**	0.14	0.82	0.70	n.i	13.42***	5.63*
4. Physiological traits									
F_v/F_m	6.80**	0.42	40.62***	0.63	1.58	1.34	n.i	4.85*	19.86***
Stom. conduct.	-	1.32	2.09	-	-	1.11	-	6.39*	1.28

Abbreviations: RGR: relative growth rate; F_v/F_m: maximum photochemical efficiency of FSII. SLA: specific leaf area; LDMC: leaf dry matter content. Stom. conduct: stomatal conductance.

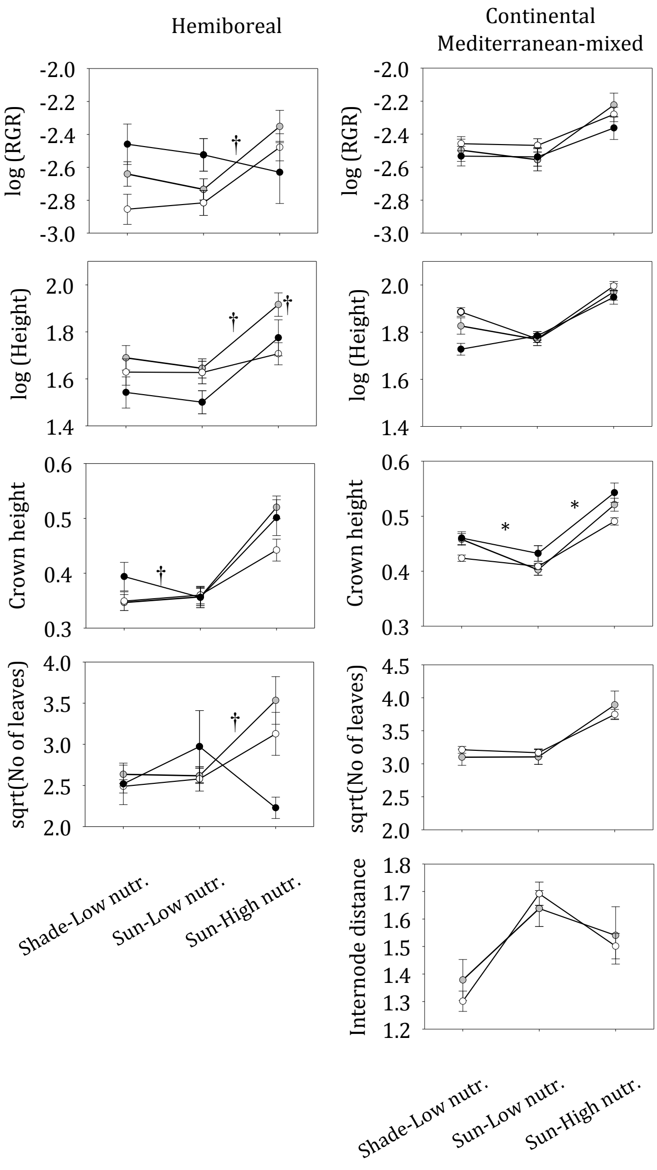


Figure 1. Norms of reaction of growth-related and architecture-related traits under light and nutrient treatments of seedlings coming from communities with different canopy richness levels (Mono: monospecific –1 species-, Medium: 2 and 3 species mixtures, and High: 4 and 5 species mixtures) and type of forest. Trait means ± S.E.M. averaged for the species within each diversity level included in the study are shown. A significant SR × Treatment, i.e. seedlings from different canopy richness levels differ in their plasticity, is indicated with asterisks on the lines († *P* < 0.10; * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001). Significant differences in the mean trait value among seedlings coming from different

canopy diversities within each treatment are also indicated with asterisks on the points of each treatment. Note that the Y axis has a logarithm transformation for some traits. Black circle: high mixture of species richness; Empty circle: Medium mixture of species richness; Grey circle: monospecific.

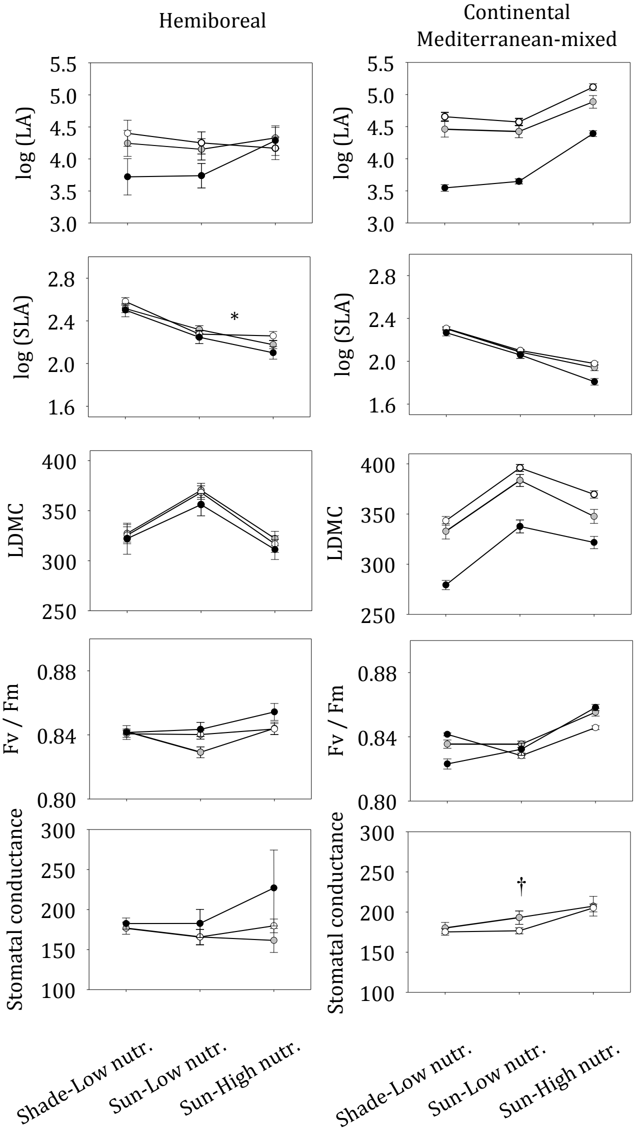


Figure 2. Norms of reaction of morphological leaf traits and physiological traits under light and nutrient treatments of seedlings coming from communities with different

canopy richness levels (Mono: monospecific –1 species-, Medium: 2 and 3 species mixtures, and High: 4 and 5 species mixtures) and type of forest. Trait means ± S.E.M. averaged for the species within each diversity level included in the study are shown. A significant SR × Treatment, i.e. seedlings from different canopy species richness levels differ in their plasticity, is indicated with asterisks on the lines († $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Significant differences in the mean trait value among seedlings coming from different canopy diversities within each treatment are also indicated with asterisks on the points of each treatment. Note that the Y axis has a logarithm transformation for some traits. Black circle: high mixture of species richness; Empty circle: Medium mixture of species richness; Grey circle: monospecific.

The influence of the functional group on trait expression and plasticity.

We observed divergent responses between the two functional groups to the different environmental treatments ('Tr x FG' interaction), especially in terms of RGR, height, F_v/F_m and morphological leaf traits (Table 1 and 2), and mainly due to the results derived from the Mediterranean forest (Table S4).In the latter forest, broadleaved seedlings showed larger mean trait values and plasticity in RGR and height in response to nutrients than conifers (Figure 3; Table 1, S3 and S4), and higher mean trait values and plasticity of height and F_v/F_m than conifers in response to light treatment (Figure 3, 4 and 5; Table S4). Moreover, both functional groups increased significantly their LA in response to nutrients, and increased SLA and reduced LDMC in response to light, being the plasticity in general greater in conifers than in broadleaved seedlings, except for SLA in response to light (Figure 4).

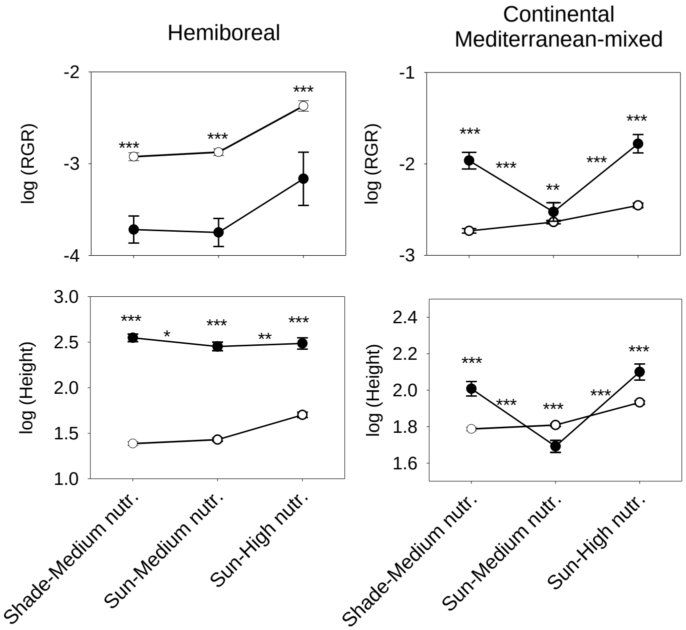


Figure 3. Norms of reaction of relative growth rate and height traits to light and nutrient treatments for conifers and broadleaved seedlings per type of forest. Trait means ± S.E.M. averaged per species of each functional group included in the study are shown. A significant SR × Treatment, i.e. when broadleaved and conifers differ in their plasticity to the treatment, is indicated with asterisks on the lines. Differences in the mean trait between functional groups within treatments are indicated with asterisks († $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) Note that the Y axis has a logarithm or root squared transformation for some traits. Black circles: broadleaved species, empty circles: conifers.

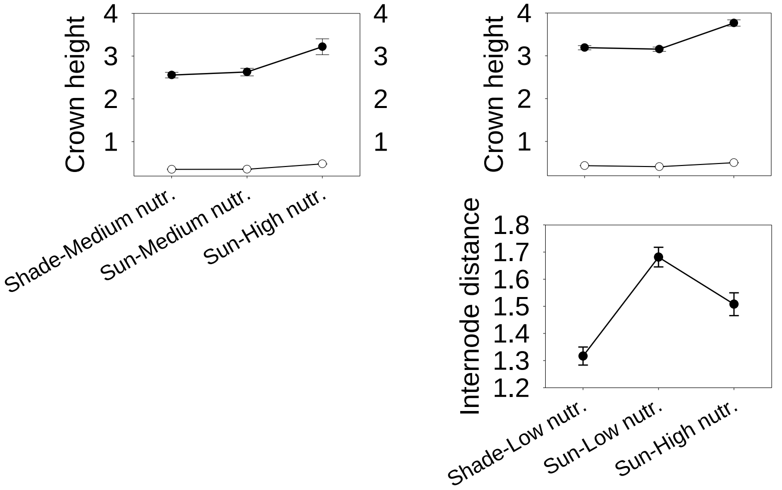


Figure 4. Norms of reaction of architecture-related traits to light and nutrient treatments for conifers and broadleaved seedlings per type of forest. Trait means ± S.E.M. averaged per species of each functional group included in the study are shown. A significant SR × Treatment, i.e. when broadleaved and conifers differ in their plasticity to the treatment, is indicated with asterisks on the lines. Differences in the mean trait between functional groups within treatments are indicated with asterisks († *P* < 0.10; * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001) Note that the Y axis has a logarithm or root squared transformation for some traits. Black circles: broadleaved species, empty circles: conifers.

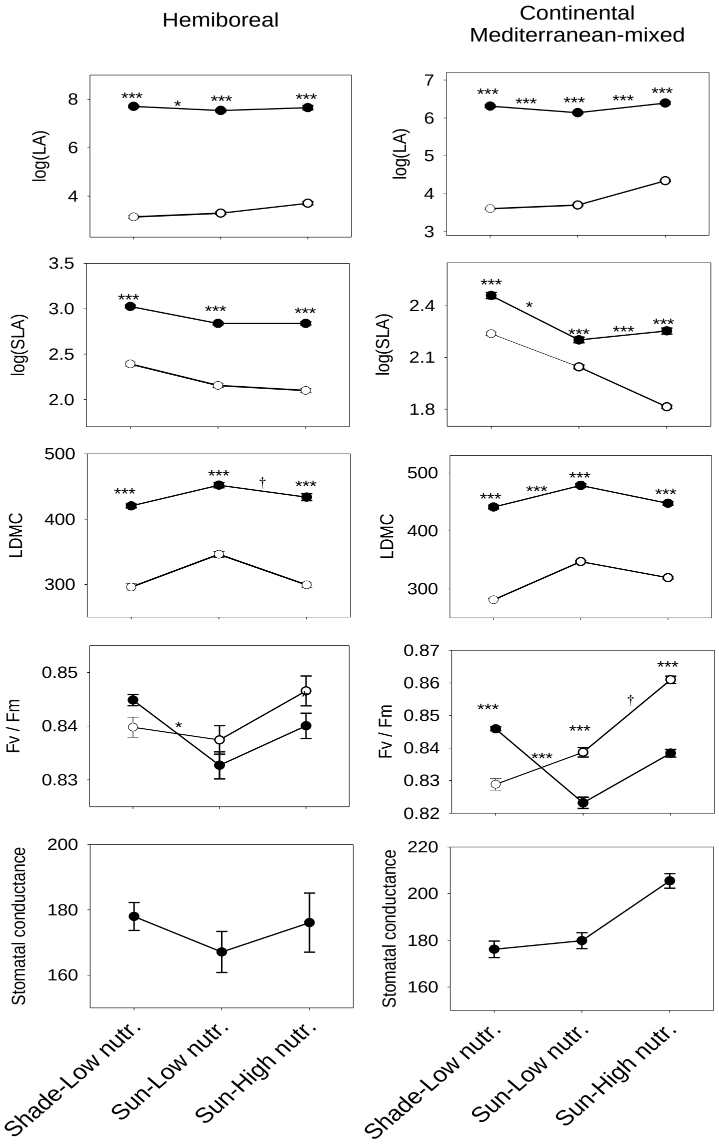


Figure 5. Norms of reaction of growth-related and architecture-related traits to light and nutrient treatments for conifers and broadleaved seedlings per type of forest. Trait means ± S.E.M. averaged per species of each functional group included in the study are shown. A significant SR × Treatment, i.e. when broadleaved and conifers differ in their plasticity to the treatment, is indicated with asterisks on the lines. Differences in the mean trait between functional groups within treatments are indicated with asterisks († $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) Note that the Y axis has a logarithm or root squared transformation for some traits. Black circles: broadleaved species, empty circles: conifers.

DISCUSSION

Ecological theory predicts greater levels of phenotypic plasticity with increasing environmental heterogeneity, which in turn is often hypothesized to be induced by canopy species richness in forest ecosystems. In our study sites, we found that heterogeneity in light and soil properties did not increase with canopy species richness in the hemiboreal forest, but it did in the Mediterranean forest. Nevertheless, despite an increasing environmental heterogeneity in the Mediterranean forest with increasing canopy species richness, we found similar mean trait values and levels of phenotypic plasticity in seedlings coming from either monospecific and mixed communities with different levels of canopy diversity. In other words, seedlings from monospecific communities adjusted their functional features to environmental shifts equally to plants living in more diverse communities and in more heterogeneous environments in our Mediterranean study case. Our findings suggest that species richness in the canopy (and its environmental heterogeneity associated) does not exert detectable selective pressure on tree phenotypes and plasticity at this early stage.

Influence on canopy species richness on trait means and plasticity

The few existing studies addressing diversity-induced plasticity and differences in the phenotypic expression have focused on herbs (e.g. Gubsch *et al.* 2011; Roscher *et al.* 2011a; Lipowsky, Schmid & Roscher 2011; Burns & Strauss 2012; Lipowsky *et al.* 2015). Most of these works were based on the Jena experiment (i.e. a large biodiversity grassland experiment; www.the-jena-experiment.de*)* have reported variations in the trait means of plant height and mainly in foliar traits such as leaf length, SLA, foliar ^{13}C isotope in individuals growing in monospecific communities in comparison to individuals growing in experimental mixed communities with different level of species richness (Gubsch *et al.* 2011; Roscher *et al.* 2011; Zuppinger-Dingley *et al.* 2014;

Lipowsky *et al.* 2015). These studies suggested that species coexistence is enhanced by trait displacement. However, the effect of diversity on phenotypic expression and plasticity in long-lived species has remained almost unexplored and in general poorly understood under real field conditions. In our study, we found that seedlings from tree communities differing in species diversity had similar trait mean values and plasticities (Figure 1 and 2). Our findings do not match our expectation that more heterogeneous environments induce higher plasticity (Alpert & Simms 2002; Gianoli 2004; Gianoli & González-Teuber 2005; Baythavong *et al.* 2011). In particular, they suggest that canopy richness and its increased environmental heterogeneity exerts a negligible selective pressure on mother trees, at least not enough to trigger shifts in the traits heritable by their progeny, including the plasticity to cope with environmental changes. In other words, plasticity does not seem advantageous under these conditions (see discussion in Alpert & Simms 2002). The fact that plants from monospecific canopies exhibited similar phenotypes and responded similarly to plants from more diverse communities might then result from the convergence in abiotic filters (i.e. similar mean abiotic conditions). If the communities studied are mainly shaped by abiotic filtering processes, seedlings would tend to converge toward similar trait values that are optimum to cope with local abiotic conditions, which explaining our results and match previous studies (e.g. Mediavilla & Escudero 2004; Mitchell & Bakker 2014). While species diversity as a biotic force shaping functional features and plasticity of tree communities remains uncertain, there are many studies demonstrating that the availability of light and nutrient in forests do determine the phenotypic expression of individuals living there (Sack, Grubb & Marañón 2003; Domínguez *et al.* 2012; Santiso & Retuerto 2015), and further, it influences tree plasticity particularly under limiting conditions (Van Kleunen & Fischer 2005).

Another explanation for the lack of a diversity effect may lie in the ontogenetic signal in plant responses: we did not find differences in traits or plasticites at this early stage, but trees might respond in more advanced stages when competition might trigger more extensive phenotypic differentiation (Moll & Brown 2008; Lasky *et al.* 2015). It is known that the degree of plasticity is not stable along developmental stages (Watson, Geber & Jones 1995), and conditions experienced at some stages may be reflected in their response to environment in later developmental phases (Sultan 2000). In this sense, ontogeny plays an important role in changing trait expression and plasticity of tree individuals along *their* lifetime as some works have already reported (Cornelissen *et al.* 2003; Huber *et al.* 2012; Lasky et al. 2015).

The contrasting outcomes of herbaceous vs. trees reflect the challenge of approaching communities of long-lived species particularly under real field conditions. This is true not only for the practical difficulties associated to the study of large individuals, but also for the challenge imposed by a long temporal scale. Populations of tree species typically require much longer time than herbs to express differentiation and local adaptation to the environment (Aitken & Bemmels 2016), and observers must deal with uncertainties regarding the tracking of past external factors i.e. of environmental conditions taking place much earlier than the time of the study. Although species with long lifespan tissues may need to limit the plasticity due to the high construction costs of these tissues (Maire *et al.* 2013), long-lived individuals face highly variable conditions during their entire lives and their acclimation frequently requires larger ontogenetic variation that bestows higher phenotypic variation display compared to short-lived individuals (Sultan 1987; Borges 2009). Clearly, long living species such as trees deserve more attention regarding these evolutionary complex processes.

The influence of functional groups on trait means and plasticity

Large differences in plasticity to both treatments were found between the two functional groups in the Mediterranean forest for most of the traits, being less significant in the hemiboreal forest (Table S3). We found that broadleaved individuals in the Mediterranean forest exhibited higher plasticity levels, leading them to have higher relative growth, height, photochemical efficiency and SLA mean values in shaded and nutrient-rich environments than coniferous seedlings (Figure 1 and 2). A competitive advantage of angiosperms over conifers has been reported in other studies, which showed higher photosynthetic rates related to the stability of photosystem II (Major & Johnsen 1996) and stomatal conductances favoring their growth rates in productive habitats (Lusk & Matus 2000; Lusk, Wright & Reich 2003; but see Becker 2000). We found differential plastic responses of both functional groups, suggesting that broadleaved species (oaks) may have higher adaptive capacity to respond to future changing conditions than conifers (pines) in the Mediterranean areas. Conifers only showed substantial plasticity in morphological leaf traits, in contrast to results showing low responsiveness in leaf traits to changes in both nutrients (Aerts 1995) and light (Wyka *et al.* 2012). Similarly to our outcomes, Sanchez-Gomez *et al.* (2006) studying plasticity in response to light in seedlings of four Iberian forest tree species, found higher plasticity in morphological and physiological traits in the two conifers (*Pinus pinaster* and *P. sylvestris*) than in the two study oaks (*Q. robur* and *Quercus pyrenaica*). Nevertheless, Sanchez-Gomez *et al.* (2006) suggested that this plasticity was an avoidance mechanism of the shade intolerant *Pinus* species rather than a persistence mechanism. In contrast, our result points to a carbon acquisition strategy with larger SLA and lower LDMC at least in response to low light availability.

In summary, our results showed large differences between the two functional groups in the capacity to respond to environmental changes mainly in the Mediterranean forests, with conifers responding by morphological variations at the leaf level and broadleaved species responding at the whole-plant level. These results may be interpreted as a better potential adaptive capacity of broadleaved against coniferous seedlings at this early stage in traits related to plant performance, which can be crucial for the species competitive ability for resources (Latham 1992) and, therefore, for survival (Bond 1989; Becker 2000). In fact, some works have already reported evidence of recent colonization of oaks (the broadleaved species of our study) in stands currently dominated by conifers (Lookingbill & Zavala 2000; Urbieta *et al.* 2011), which might be pointing to significant species composition shifts in the future.

Overall trait variations in response to light and nutrient availabilities

Nutrient availability affected most of the functional traits studied, while light availability only affected leaf morphology. Seedlings in shaded environment had leaves with greater specific leaf areas and lower leaf dry matter contents in comparison with seedlings under full exposition. These morphological variations are associated with the optimization of light interception and they have also been found in other plant life forms (Givnish 1988; Valladares & Niinemets 2008). A greater nutrient availability led to taller seedlings, with higher crowns and more and larger leaves with lower LDMC, and better ecophysiological conditions (higher F_v/F_m and stomatal conductances). As expected, seedlings in the nutrient-rich environment invested on new productive leaf tissues and woody stems, which may improve their performance and competitive ability particularly for light capture (Tilman 1988; Poorter *et al.* 2012). Surprisingly, none of the two treatments had an effect on the relative growth rate, in contrast with other studies reporting effects of light and nutrient availabilities on mean plant growth and plasticity (Latham 1992; Schreeg, Kobe & Walters 2005; Portsmuth & Niinemets 2007).

Nevertheless, we missed part of the whole picture as we have approached the study of two environmental factors independently. The acquisition of a single resource is dependent on the availability of others, and plants allocate proportionally more resources to organs in charge of capturing the most limiting resource to achieve a 'functional equilibrium' (Thornley 1972; Iwasa & Roughgarden 1984; Poorter *et al.* 2012). Therefore, factorial experiments with both light and nutrient availability gradients typically shed more light on the evolutionary and ecological implications of plasticity since plants are almost always challenged by complex, multifactor environmental changes (Niinemets & Valladares 2006; Portsmuth & Niinemets 2007).

CONCLUSION

Recent efforts to understand the effects of diversity on plant functional traits and their plasticity aim to decipher the mechanisms underlying community assembly and the maintenance of diversity. To our knowledge, this is the first study analyzing diversity-induced plasticity in tree species, taking into account the heterogeneity in the environmental conditions of their natural habitats. Our study showed that the number of species of two contrasting European forests and their associated local heterogeneity did not exert any effect on either the phenotypic expression or the degree of plasticity of seedlings in response to different light and nutrient availabilities. We suggest that the lack of relationship may reflect the slight or absent increment in the environmental heterogeneity in rich communities in comparison to monospecific communities. Hence, the environmental heterogeneity may not act as a selective filter either favoring or limiting phenotypic plasticity, at least at these early stages. These findings on the differences between the two functional groups entail important implications regarding regeneration patterns, plant-plant interactions and species coexistence under changing environmental conditions. This study represents a first step to understand the effect of species diversity on phenotypic plasticity in forests, advocating the need for more studies on the footprint of species diversity on ecological and evolutionary processes.

ACKNOWLEDGMENTS

We are very grateful to Bogdan Jaroszewicz, Ewa Chécko and Kuba for the collection of seeds from Poland site. We thank to Inés Carrascón, David López for their valuable help in collecting seeds from the Mediterranean forest, and together with Borja Ginés, Mª Carmen Bastias, José Crespo, José Ángel Peñas, Alicia Forner, Dulce Flores helping in the setting up of the common garden and during the greenhouse experiment. We also thank to Silvia Matesanz for her valuable help improving the clarity of the concepts and the manuscript in general. C.C.B. is beneficiary of a FPU grant funded by the Spanish Government

(AP2010-5600). R.B. was funded by a Marie Curie IEF fellowship ‘DIVERFOR’ (FP7-PEOPLE-2011-IEF. No. 302445). This research was funded through the ‘FunDivEUROPE’ research project as part of the European Union Seventh Framework Programme (FP7 2007–2013) under grant agreement no. 265171, the Spanish-funded project REMEDINAL3eCM (S2013/MAE-2719), and also the Ecometas excellence network (CGL2014-53840-REDT).

SUPPLEMENTARY MATERIAL

Table S1. Description of two study forests with the average of their main environment characteristics in term of light and soil properties. Standard deviation is presented in brackets. GSF: global site factor (global light availability). ISF: indirect site factor (i.e. indirect light availability). Both obtained from hemispherical photographs. Nconc: soil N concentration in mg/g. Cconc: soil C concentration in mg/g. Nstock: N stock based on bulk density in mg/ha. Cstock: C stock based on bulk density in mg/ha. Soil properties are calculated on the average of the first 20 cm of soil.

	Hemiboreal	**Mediterranean-mixed**
Location	Bialowieza National Park (Poland)	Alto Tajo Natural Park (Spain)
Canopy species richness range	1-5	1-4
Tree species forming part of the diversity gradient	*Pinus sylvestris, Picea abies, Betula spss, Quercus robur, Carpinus betulus*	*Pinus nigra, Pinus sylvestris, Quercus ilex, Quercus faginea*
Total number of plots	43	36
No. of plots per canopy richness level	6/11/13/11/2	11/18/4/3
Annual mean T (ºC)	6.9 (0.1)	9.7 (0.6)
Annual mean P (mm)	580.8 (3.7)	537.4 (27.3)
Altitudinal range(m.a.s.l)	140- 200	960- 1404
GSF	0.11 (0.02)	0.50 (0.12)
ISF	0.15 (0.02)	0.51 (0.10)
Forest Floor Thickness	1.97(0.65)	1.38(1.17)
pH	3.83 (0.33)	6.93 (0.51)
Nconc	1.89(0.49)	3.57(1.41)
Cconc	32.43(10.14)	68.58(27.42)
Nstock	1.80(0.41)	3.09(1.03)
Cstock	30.58(7.71)	59.27(19.23)

Table S2. Total number of selected plants per species, treatment and canopy richness level (SR) of the communities from where mother trees grow. Number of plots per each canopy level where seeds were collected.

		SR	No. plots in each diversity level	Number of plants selected per treatment			TOTAL
Hemiboreal forest				Shade-Low nutrient	Sun-Low nutrient	Sun-High nutrient	
Conifers	*Pinus sylvestris*	Mono	2	67	73	62	473
		Medium	2	61	68	56	
		High	1	27	34	25	
	Picea abies	Mono	2	30	47	27	233
		Medium	2	25	37	41	
		High	1	8	13	5	
Broadleaved species	*Quercus robur*	Mono	2	28	27	14	154
		Medium	2	29	28	15	
		High	1	5	5	3	
Continental Mediterranean-mixed forest							
Conifers	*Pinus sylvestris*	Mono	2	55	57	49	571
		Medium	5	114	124	103	
		High	2	25	25	19	
	Pinus nigra	Mono	2	35	40	35	714
		Medium	6	165	172	155	
		High	2	39	39	34	
Broadleaved species	*Quercus ilex*	Mono	2	15	15	7	213
		Medium	3	60	60	56	
		High	-	-	-	-	
	Quercus faginea	Mono	2	26	26	23	387
		Medium	6	105	105	102	
		High	-	-	-	-	

Table S3. Results from linear mixed-effects model of different functional traits under a light (A) and nutrient (B) treatments, measured on saplings from a hemiboreal forest. FG: functional group (two levels: broadleaf species and conifers). SR: species richness level of plot from where seeds were collected. Tr.: nutrient treatment (low *vs* high nutrient supply environments). The values shown are the *F* values, and degrees of freedom (df). The statistical significance of each factor was assessed with Type III tests. † $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.i: triple interaction removed from the full model when it was not significant. We used the symbol (-) when traits were measured only in a functional group and thereby, these main terms are not included in the model.

	FG df=1	**SR** df=2	**Tr.** df=1	**SR x FG** df= 2	**Tr. x FG** df= 1	**Tr. x SR** df= 2	**FG x SR x Tr.** df=2
(A) Light Treatment							
1. Growth-related trait							
RGR	**4.66***	0.46	2.15	1.18	0.21	0.71	n.i
2. Architecture-related traits							
Height	**67.09*****	0.37	0.00	4.72	**5.97***	0.58	n.i
Crown height	-	0.47	3.32†	-	-	2.77†	-
No. of leaves	-	0.32	1.06	-	-	0.48	-
Internode length	-	-	-	-	-	-	-
3. Morphological traits							
Leaf size	**381.67*****	0.23	3.28†	2.19	**6.42***	0.28	n.i
SLA	**39.08*****	0.00	**13.34*****	0.04	0.89	1.95	n.i
LDMC	**25.55*****	0.34	**7.24****	0.04	1.99	0.25	n.i
4. Physiological traits							
F_v/F_m	0.03	0.02	0.36	0.55	**4.37***	2.87†	n.i
Stom. conduct.	-	0.04	0.00	-	-	0.10	-
(B) Nutrient Treatment							
1. Growth-related trait							
RGR	**8.46****	0.09	1.36	0.43	0.15	2.70†	n.i
2. Architecture-related traits							
Height	**12.21*****	3.23	**13.98*****	2.76†	**7.12****	2.82†	n.i
Crown height	-	0.19	**13.62*****	-	-	2.15	-
No. of leaves	-	2.64	1.59	-	-	2.87†	-
Internode length	-	-	-	-	-	-	-
3. Morphological leaf traits							
Leaf size	**153.71*****	0.30	**14.54*****	2.50	1.52	1.78	n.i
SLA	**44.88*****	0.32	**4.87***	0.22	0.28	**3.97***	n.i
LDMC	**27.58*****	0.30	**10.73****	0.02	3.57†	0.03	n.i
4. Physiological traits							
F_v/F_m	**4.15***	0.15	2.08	1.47	0.01	1.08	n.i
Stom. conduct.	-	2.02	1.54	-	-	0.85	n.i

Abbreviations: RGR, relative growth rate; F_v/F_m: maximum photochemical efficiency of FSII. SLA, specific leaf area; LDMC, leaf dry matter content. Stom. conduct: stomatal conductance.

Table S4. Results from linear mixed-effects model of different functional traits under a light (A) and nutrient (B) treatments, measured on saplings from a continental Mediterranean-mixed forest. FG: functional group (two levels: broadleaf species and conifers). SR: species richness level of plot from where seeds were collected. Tr.: nutrient treatment (low *vs* high nutrient supply environments). The values shown are the *F* values, and degrees of freedom (df). The statistical significance of each factor was assessed with Type III tests. † $P < 0.10$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.i: triple interaction removed from the full model when it was not significant. We used the symbol (-) when the traits were measured only in a functional group and thereby, these main terms are not included in the model.

	FG df=1	**SR** df=2	**Tr.** df=1	**SR x FG** df= 2	**Tr. x FG** df= 1	**Tr. x SR** df= 2	**FG x SR x Tr.** df=2
(A) Light Treatment							
1. Growth-related trait							
RGR	**11.00****	0.40	1.40	2.20	0.29	0.49	**8.01****
2. Architecture-related traits							
Height	2.50	0.60	1.05	0.08	**69.84*****	0.59	n.i
Crown height	-	1.60	**4.28***	-	-	**6.85***	-
No. of leaves	-	1.07	0.43	-	-	0.17	-
Internode length	-	0.01	**52.52*****	-	-	1.18	-
3. Morphological leaf traits							
Leaf size	**475.53*****	0.32	2.39	**0.29**	**34.93*****	0.33	n.i
SLA	**6.73***	0.10	**28.06*****	**0.23**	**5.77***	0.38	n.i
LDMC	**385.52*****	0.04	**60.01*****	0.53	**33.41*****	0.68	n.i
4. Physiological traits							
F_v/F_m	**23.08*****	3.09 †	**5.00***	2.69	**97.84*****	0.86	n.i
Stom. conduct.	-	0.30	0.07	-	-	0.89	-
(B) Nutrient Treatment							
1. Growth-related trait							
RGR	**41.83*****	0.64	1.99	2.92†	**32.42*****	0.01	n.i
2. Architecture-related traits							
Height	3.27†	0.08	**7.00****	0.45	**52.74*****	0.32	n.i
Crown height	-	1.51	**34.79*****	-	-	**3.01***	-
No. of leaves	-	0.37	**31.32*****	-	-	0.48	-
Internode length	-	0.24	**10.31****	-	-	0.18	-
3. Morphological leaf traits							
Leaf size	**244.34*****	0.03	**128.09*****	0.00	**69.71*****	1.44	n.i
SLA	**29.27*****	0.33	**40.20*****	0.00	**96.33*****	0.28	n.i
LDMC	**223.57*****	0.39	**4.13***	0.64	0.09	0.51	n.i
4. Physiological traits							
F_v/F_m	**28.81*****	0.46	**41.27*****	0.85	3.10†	0.59	n.i
Stom. conduct.	-	0.04	**21.77*****	-	-	0.95	n.i

Abbreviations: RGR, relative growth rate; F_v/F_m: maximum photochemical efficiency of FSII. SLA, specific leaf area; LDMC, leaf dry matter content. Stom. conduct: stomatal conductance.

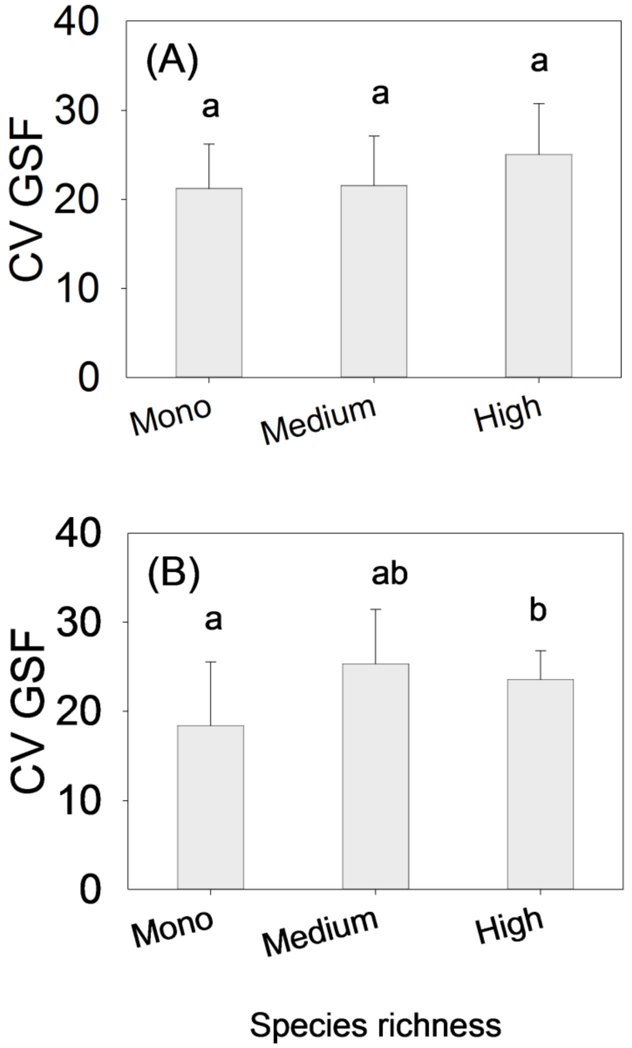


Figure S1. Means and standard errors of the coefficient of variation of light availability (Global Site Factor, CV GSF) assessed in plots with different species richness category in the hemiboreal forest (A) and in the continental Mediterranean-mixed forest (B).

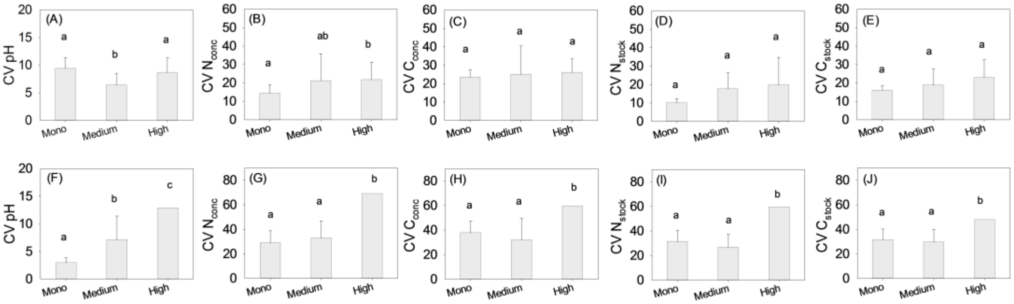


Figure S2. Means and standard errors of coefficient of variation (CV) of different soil properties measured at 0-20 cm of soil depth for communities with different tree species richness categories (Mono: monospecific -1 species-; Medium: stands with 2 and 3 species mixture; High: stands with 4 and 5 species mixture). A rarefaction analysis was performed in order to standardize by the minimum sample size, i.e. minimum number of plots included in each category (n=4). Upper row (graphs from A to E) belongs to a hemiboreal forest in Poland and lower row (graphs from F to J) to a continental Mediterranean-mixed forest in Spain.

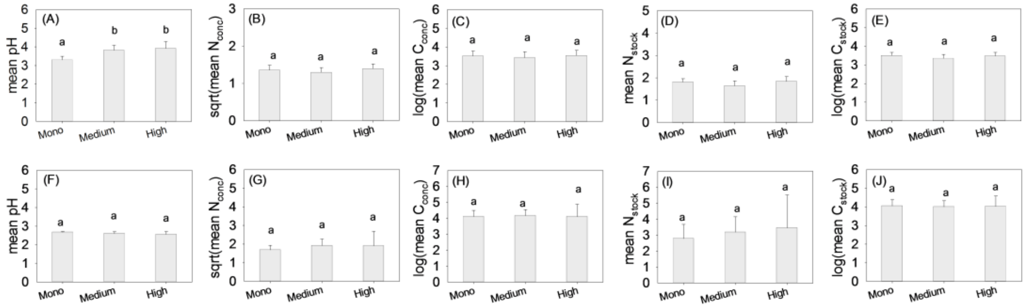


Figure S3. Means and standard errors of different soil properties measured at 0-20 cm of soil depth for communities with different tree species richness categories (Mono: monospecific -1 species-; Medium: stands with 2 and 3 species mixture; High: stands with 4 and 5 species mixture). A rarefaction analysis was performed in order to standardize by the minimum sample size, i.e. minimum number of plots included in each category (n=4). Upper row (graphs from A to E) belongs to a hemiboreal forest in Poland and lower row (graphs from F to J) to a continental Mediterranean-mixed forest in Spain.

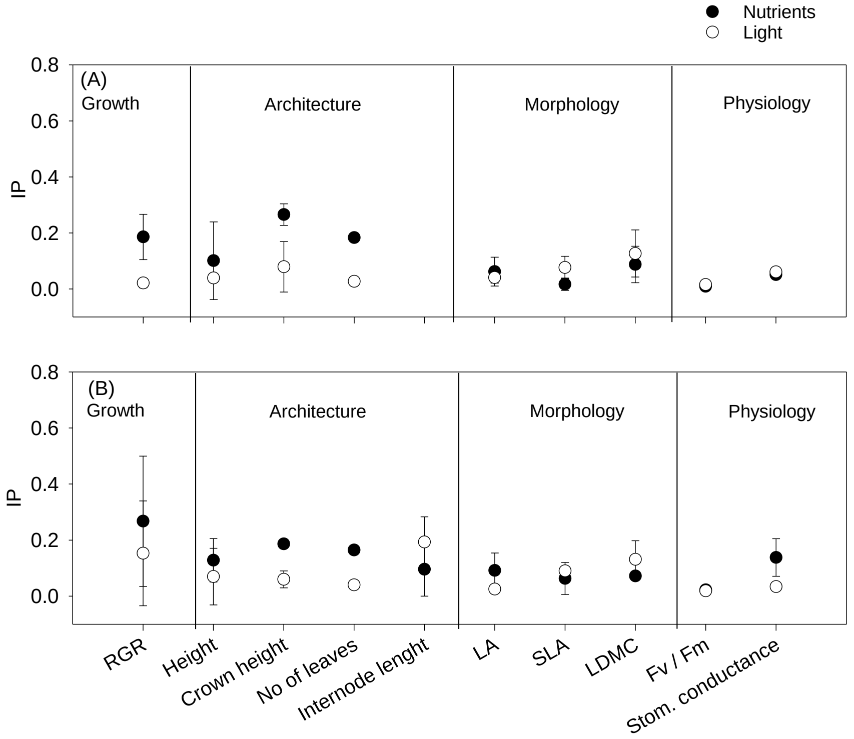
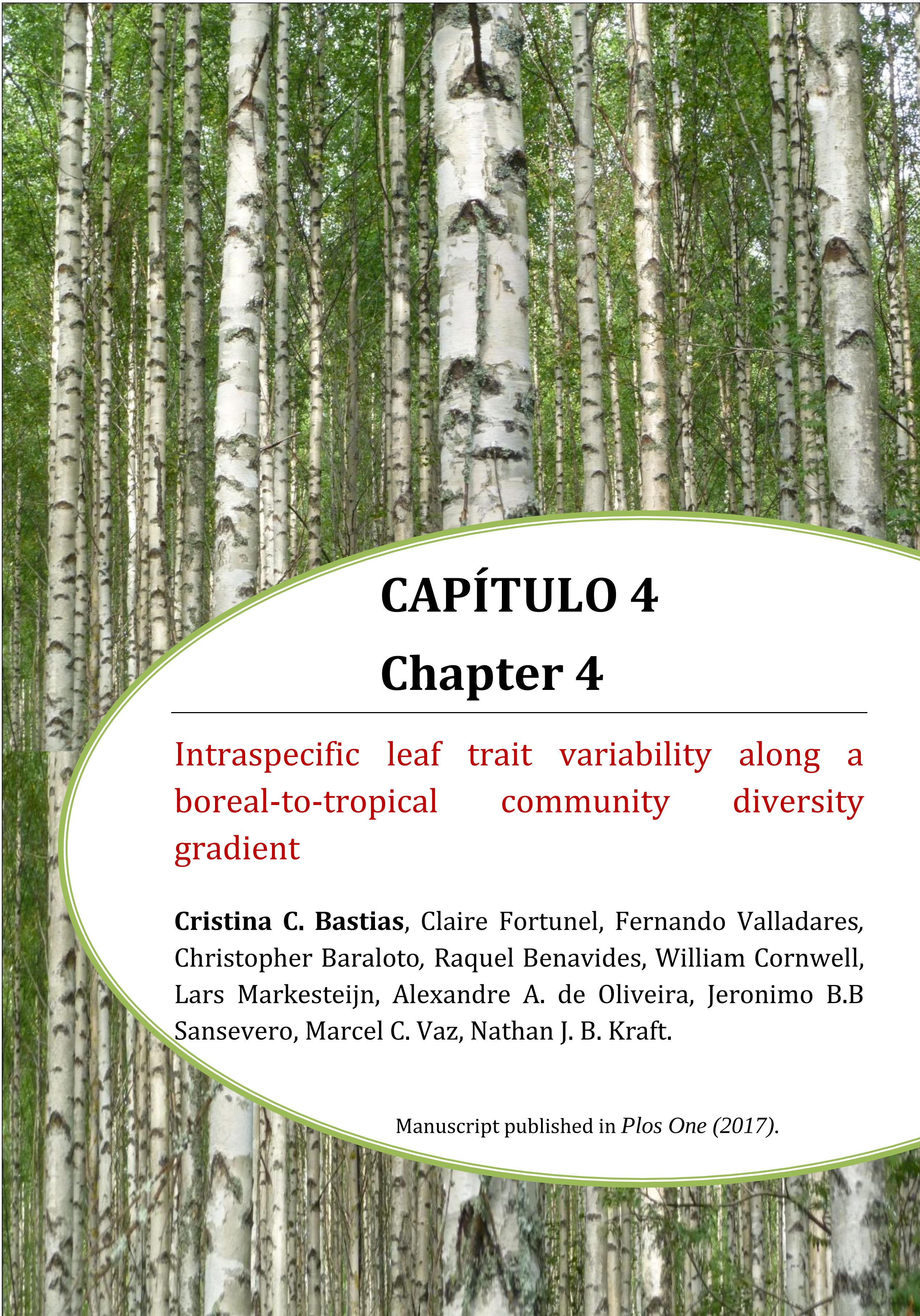


Figure S4. Mean phenotypic plasticity index (IP) ± Standard deviation (corresponding to species in each forest) of each single trait in response to 'light treatment' (Sun vs Shade) and to 'nutrient treatment' (Low vs High) in both forest types (A) hemiboreal forest and (B) continental Mediterranean-mixed forest. Light treatment is represented with white circles and nutrients treatment with black circles.



CAPÍTULO 4

Chapter 4

Intraspecific leaf trait variability along a boreal-to-tropical community diversity gradient

Cristina C. Bastias, Claire Fortunel, Fernando Valladares, Christopher Baraloto, Raquel Benavides, William Cornwell, Lars Markesteijn, Alexandre A. de Oliveira, Jeronimo B.B Sansevero, Marcel C. Vaz, Nathan J. B. Kraft.

Manuscript published in *Plos One (2017)*.

Image from the front page (Chapter 4): monospecific forest of *Betula pendula* in North of Karelia (Finland).

Author: Inés Carrascón.

SUMMARY

Disentangling the mechanisms that shape community assembly across diversity gradients is a central matter in ecology. While many studies have explored community assembly through species average trait values, there is a growing understanding that intraspecific trait variation (ITV) can also play a critical role in species coexistence. Classic biodiversity theory hypothesizes that higher diversity at species-rich sites can arise from narrower niches relative to species-poor sites, which would be reflected in reduced ITV as species richness increases. To explore how ITV in woody plant communities changes with species richness, we compiled leaf trait data (leaf size and specific leaf area) in a total of 521 woody plant species from 21 forest communities that differed dramatically in species richness, ranging from boreal to tropical rainforests. At each forest, we assessed ITV as an estimate of species niche breadth and we quantified the degree of trait overlap among co-occurring species as a measure of species functional similarity. We found ITV was relatively invariant across the species richness gradient. In addition, we found that species functional similarity increased with diversity. Contrary to the expectation from classic biodiversity theory, our results rather suggest that neutral processes or equalizing mechanisms can be acting as potential drivers shaping community assembly in hyperdiverse forests.

Keywords: species richness; trait breadth; trait overlap; SLA; leaf size; species assembly; trait differences; trait similarity.

INTRODUCTION

The relative importance of ecological factors in shaping plant communities across species diversity gradients is the subject of longstanding debate in ecology (Stevens 1989; Platnick 1991; Hill & Hill 2001; Myers *et al.* 2013) that has been recently invigorated by the lens of functional trait diversity (Ackerly & Cornwell 2007; Adler *et al*. 2013). The use of traits in a community ecology context hinges on the hypothesis that there is a link between traits and the breadth and position of species' realized niches (McGill *et al*. 2006; Weiher *et al.* 2011). Trait-based studies have often used a trait mean approach (i.e. assigning all conspecific individuals a species average trait value) to examine community assembly mechanisms (Kraft *et al.* 2008; Fortunel *et al.* 2014). The implicit assumption in many of these studies is that interspecific trait differences are much larger than intraspecific trait differences (Garnier *et al.* 2001; Laughlin *et al.* 2011). However, there is increasing evidence that community assembly at local scales depends critically on the extent of intraspecific trait variation (ITV) (Albert *et al.* 2010; Paine *et al.* 2011; Kraft *et al.* 2014; Siefert *et al.* 2015). Recently, the scientific community has reconsidered the importance of ITV (Lake & Ostling 2009; Bolnick *et al.* 2011; Violle *et al.* 2012) and its non-negligible contribution to the total trait variability, being sometimes as important as interspecific trait variation (Messier *et al.* 2010; Ainley, Vergés & Bishop 2016). Even when interspecific trait differences are larger, incorporating ITV can improve the answer to key questions about the assembly and functioning of plant communities (Jung *et al.* 2010; Kraft *et al.* 2014).

The extent of ITV among species in a community is expected to vary depending on community attributes such as the number of co-occurring species or the community trait diversity (Albert *et al.* 2010; Violle *et al.* 2012). Previous studies have suggested that ITV should be greater in species-poor than species-rich communities (Whitham *et al.* 2006; Crutsinger *et al.* 2006). If the biotic pressure via competitive interactions is lower in species-poor than in species-rich communities, conspecific individuals in species-poor communities could occupy a greater extension of available trait space (i.e. substantial extent of ITV) without increasing interspecific interactions (Figure 1A and 1A’). As the number of co-occurring species increases under the assumption of all co-occurring species with equal fitness (i.e. flat fitness landscape), species’ trait breadths are expected to be reduced (i.e. decline of ITV) to accommodate more species without increasing the potential for interspecific competition by resource use, consistent with classical niche theory (MacArthur & Levins 1967; Violle *et al.* 2012) (Figure 1B). However, if the extent of ITV does not change from species-poor to species-rich communities or even increases (for example, see ‘individual variation’ theory by Clark 2010) and the trait range within a community in turn does not increase when species richness increases (Figure 1C), species’ trait overlaps would be expected to increase in more diverse communities (Figure 1C’). If traits map to resource use (and stabilizing niche differences, *sensu* Chesson 2000), species can coexist more readily by being functionally distinct, thereby promoting trait dissimilarity among species for coexistence (MacArthur & Levins 1967). However, some have argued that the lack of interspecific dissimilarity could lead to neutral dynamics (i.e. all individuals are considered functionally equivalent), reducing or removing the role of niche differences in shaping community assembly outcomes (Hubbell 2001; Hubbell 2005), or alternatively, communities structured primarily by the acting of equalizing mechanisms in a non-neutral model (Adler *et al.* 2007). On the other hand, under an alternative niche differentiation model, specifically if species are differentiating along a landscape represented by combinations of peaks (high-fitness) and deep valleys (low-fitness) (i.e. multi-peak fitness landscape), a decrease of ITV is not predicted with species richness (Svensson, Eroukhmanoff & Friberg 2006) despite a limiting similarity principle playing a role to select species on each peak.

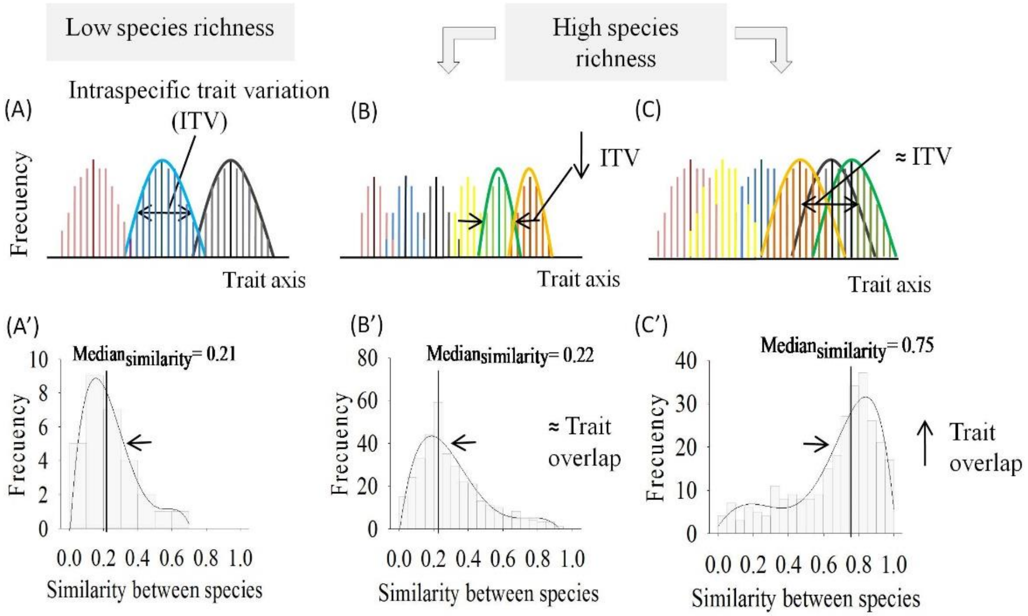


Figure 1. A schematic of possible changes in the extent of ITV and the degree of trait overlap (measured as the similarity between species; (de Bello *et al.* 2013)) when species richness increases assuming a flat fitness landscape. (A) A substantial extent of ITV is expected in species-poor communities without increasing interspecific interactions since the biotic pressure is low. Therefore, low values of trait overlap are expected in species-poor communities leading to a right-skewed distribution (A'). (B) The extent of ITV is reduced, remaining trait overlap fairly constant to accommodate more species. It translates into a right-skewed distribution to low trait overlap values in species-rich forests (B'). (C) Trait overlap is increased without a change in the extent of ITV, resulting in greater degree of functional similarity among individuals. A left-skewed distribution of trait overlap values would be expected in species-rich forests (C').

While the relationships between species richness, ITV and trait overlap have important implications for community assembly and the maintenance of species richness (Lamanna *et al.* 2014), they have been poorly studied due to the substantial effort required to measure ITV, especially in species-rich systems (Baraloto *et al.* 2010). Moreover, the few studies testing these relationships so far have shown contrasting results. For instance, some studies have found a decrease in the extent of ITV in specific leaf area (SLA) accompanied by declining SLA overlap with increased species richness (Hulshof *et al.* 2013; Kumordzi *et al.* 2015); whereas others have found no change in ITV in SLA or an increase of ITV and overlap using multiple approach (included SLA) with species richness (Le Bagousse-Pinguet *et al.* 2014). These contradictory results show evidence that there is a need to improve our understanding of these relationships between species richness, ITV and trait overlap, exploring all possible approaches for a given trait in combination with the attributes of the study system in order to infer community assembly mechanisms across diversity gradients.

Here, we explore how the extent of ITV and trait overlap changes across a broad species richness gradient. We compile leaf trait data for 521 woody plant species found in 21 forest communities that varied in species richness from 1 to 284 species per hectare (from boreal and temperate to tropical forests). In accordance with the classic niche theory under a flat fitness landscape scenario, we hypothesize that the extent of ITV will decrease when more species are added to the community (Figure 1B) (MacArthur & Levins 1967; Chesson 2000; Adler *et al.* 2007). By extension, this hypothesis would suggest a reduction of the contribution of local intraspecific variation to the total trait diversity of the community with increasing species richness. Alternatively, if ITV does not decline and the overall length of the trait gradient does not increase in diverse communities, we expect a higher overlap in trait distributions among species with increasing species richness (Figure 1C). This would translate into a left-skewed distribution of trait overlap values, i.e. higher median values of trait overlap as well as a higher proportion of pairs of species with high trait overlap in species-rich than species-poor forests (Figure 1C’). By extension, ITV could be expected to contribute to the total trait diversity similarly or even more than interspecific trait differences in rich communities.

MATERIALS AND METHODS

Data Collection

We used two leaf traits to quantify ITV: leaf size refers to individual leaf area (cm^2) and specific leaf area (SLA), defined as the ratio of leaf area to dry mass ($cm^2 \cdot g^{-1}$) (Pérez-Harguindeguy *et al.* 2013). We focused on these traits, firstly, because different empirical studies of woody plants from a wide range of environments have shown that SLA and leaf size are weakly or not correlated across species or at species level (Fonseca *et al.* 2000; Ackerly *et al.* 2002). Secondly, these key leaf traits have been widely used in species distribution studies across gradients to predict future shifts in individual species distributions or even in species-realized niches due to their strong response to abiotic and biotic changes (Ackerly *et al.* 2002; Cornwell & Ackerly 2009). Moreover, SLA and leaf size are traits with an important ecological significance in relation to plant economics and plant resource acquisition: plants investing in greater SLA values increase light-capture efficiency, but are more vulnerable to high temperatures, drought, nutrient-limitation and herbivory (Westoby 1998). On the other hand, leaf size is more related to the thermal conductance of the leaf boundary layer. Smaller leaf size helps to keep optimal leaf temperature and a higher water balance efficiency, especially under high solar radiation and low water availability conditions (Miller & Stoner 1979). Finally, these traits can easily be measured on a large number of individuals.

We measured leaf traits in 3712 individuals of 521 woody plant species in 21 forest communities with contrasting species richness, ranging from the species-poor boreal and temperate European forests (1-4 species/ha) to hyperdiverse rainforests in Ecuador and Brazil (>200 species/ha) (Table 1). Species richness was calculated for each forest as the number of species with a diameter at breast height (DBH) ≥ 10 cm as it is customary (Avery, Thomas Eugene & Burkhart, Harold 2002; Markesteijn, Poorter & Bongers 2007; Ricklefs & He 2016; Yeboah, Chen & Kingston 2016). We also obtained climatic variables (the mean annual temperature and annual precipitation) per each forest community from the Worldclim global climate models (Hijmans *et al.* 2005). We included leaf trait data for species from a given community using the following criteria: (i) at least five individuals per species were measured in each forest in order to estimate ITV (see Albert *et al.* 2011), but it was higher whenever was possible (Table 1). (ii) To minimize the influence of ontogenetic variation on ITV, we restricted our sampling to understory individuals (saplings and small-stature trees), whose DBH is smaller than 20 cm and height less than 10 m. To restrict the sampling to understory individuals may imply either an underestimation or overestimation in the number of sampled species compared to the species richness of the community (calculated taking into account individuals with a DBH >10cm). An underestimation may occur since we are sub-sampling woody species from a forest layer, but also an overestimation since the arrival of propagules from neighbor canopies can lead to new established individuals in the understory of the canopy of the focal community. (iii) Due to the considerable effort required in sampling at ITV level in hyperdiverse forests, we only conducted trait measurements on a subset of the common species in tropical forests (those species that we found at least 5 individuals) (see Table 1 for the number of sampled species in each forest). This restriction in tropical forests may result in an underestimation of the extent of ITV and trait overlap among species in these forests since we do not have trait data available for the whole range of naturally occurring trait variation (i.e. we do not consider those rare species that occupy unique trait space compared to common species (Mouillot *et al.* 2013; Umaña *et al.* 2015). Besides, common species could show less trait variation, which further contributes to underestimating actual ITV. (iv) Also, it is important to mention that trait data used here were previously collected for other specific goals (see Table 1 for original references), but in general, individuals and leaves were chosen under standardized abiotic conditions (i.e. recently matured and fully expanded leaves) (Pérez-Harguindeguy *et al.* 2013). As a consequence, we may underestimate the actual ITV since ITV measured here is controlled for two main sources of variation: phenotypic plasticity in response to local abiotic conditions (i.e. we biased ITV towards natural standardized conditions) and ontogenetic variations (i.e. we biased ITV at a single ontogenetic stage: understory individuals). Overall, all criteria were consistently in direction of the underestimation of actual ITV.

Sampling permissions were granted by Delegación Provincial de la Conserjería de Agricultura y Medio Ambiente (Guadalajara-Castilla La Mancha) for the Mediterranean mixed forest in Alto Tajo Natural Park (Spain), by Instituto Boliviano de Investigación Forestal (IBIF) and the logging companies INPA Parket Ltd. and Planet La Chonta Investment Ltda for the tropical lowland dry deciduous forest in Bolivia, by Ministerio de Ambiente de Panama (MiAmbiente) for the tropical lowland semi-deciduous seasonal moist forest in Panama, by ICMBio/SISBIO- license number 23191-1 for the lowland tropical Atlantic Forest (Poço das Antas Biological Reserve – Southeastern Brazil), by Ministerio del Ambiente of Ecuador for the evergreen lowland tropical rainforest in Yasuní National Park (Ecuador) and by ICMBio/SISBIO- license number 18757-1 for the lowland tropical rainforest in Central Amazon (Brasil). For the rest of forests were not required specific permissions. The authorities responsible of these areas were informed and they expressed their consent to this sampling. Moreover, sampling did not involve endangered or protected species.

Table 1. Description of each forest community included in the study. Pp: annual precipitation. SR: number of species with a DBH ≥10cm /ha.

Type of forest	Location	Latitude	Longitude	Mean annual Tra (ºC)	Pp (mm)	SR	Representative families of sampled species	No. species measured with ≥ 5 individuals	Individuals measured per species [Min, Max]	Original references
Boreal forest	Joensuu, North Karelia (Finland)	62.616	29.89	2.1	628	1	Pinaceae, Betulaceae	3	[19, 55]	(Bastias et al. unpublished data)
Boreal forest	Joensuu, North Karelia (Finland)	62.504	29.76	2.1	628	2	Pinaceae, Betulaceae	2	[5, 64]	(Bastias et al. unpublished data)
Boreal forest	Joensuu, North Karelia (Finland)	62.558	30.16	2.1	628	3	Pinaceae, Betulaceae	2	[7, 8]	(Bastias et al. unpublished data)
Mountainous beech forest	Carpathian mountains (Romania)	47.295	26.05	5.6	689	1	Pinaceae, Fagaceae,Sapindaceae	4	[11, 54]	(Bastias et al. unpublished data)
Mountainous beech forest	Carpathian mountains (Romania)	47.294	26.05	5.6	689	2	Pinaceae, Fagaceae,Sapindaceae	4	[8, 60]	(Bastias et al. unpublished data)
Mountainous beech forest	Carpathian mountains (Romania)	47.292	26.05	5.6	689	3	Pinaceae, Fagaceae,Sapindaceae	3	[10, 37]	(Bastias et al. unpublished data)
Mountainous beech forest	Carpathian mountains (Romania)	47.291	26.05	5.6	689	4	Pinaceae, Fagaceae,Sapindaceae	2	[5, 20]	(Bastias et al. unpublished data)
Mediterranean mixed forest	Alto Tajo Natural Park (Spain)	40.731	-2.25	9.9	533	1	Pinaceae, Fagaceae	4	[6, 57]	(Bastias et al. unpublished data)
Mediterranean mixed forest	Alto Tajo Natural Park (Spain)	40.713	-2.19	9.9	533	2	Pinaceae, Fagaceae	4	[21, 70]	(Bastias et al. unpublished data)
Mediterranean mixed forest	Alto Tajo Natural Park (Spain)	40.698	-2.13	9.9	533	3	Pinaceae, Fagaceae	2	[5, 10]	(Bastias et al. unpublished data)
Tropical lowland dry deciduous forest	Inpa, Concepcion, Santa Cruz (Bolivia)	-16.117	-61.72	23.5	1124	34	Fabaceae, Flacourtiaceae, Euphorbiaceae	52	[5, 10]	(Markesteijn et al. 2007)

Type of forest	Location	Latitude	Longitude	Mean annual Tra (ºC)	Pp (mm)	SR	Representative families of sampled species	No. species measured with ≥ 5 individuals	Individuals measured per species [Min, Max]	Original references
Riparian, chaparral, broadleaf evergreen forest	Jasper Ridge Biological Preserve (California, USA)	37.4	-122.25	13.8	598	54	Fagaceae, Rosaceae, Rhamnaceae	43	[5, 42]	(Cornwell & Ackerly 2009)
Tropical lowland semi-deciduous seasonal moist forest	Soberania National Park (Panama)	9.162	-79.75	26	2553	131	Fabaceae, Piperaceae, Rubiaceae	16	[5, 6]	(Markesteijn, unpublished data)
Lowland tropical rainforest	Acarouany (French Guiana)	5.544	-53.81	26.5	2237	148	Annonaceae, Burseraceae, Lecythidaceae	11	[3, 22]	(Baraloto et al. 2012; Fortunel et al. 2014)
Lowland tropical rainforest	Paracou (French Guiana)	5.272	-52.93	25.8	2821	150	Euphorbiaceae, Fabaceae, Lecythidaceae	35	[3, 39]	(Baraloto et al. 2012; Fortunel et al. 2014)
Lowland tropical rainforest	BAFOG (French Guiana)	5.494	-53.99	26.4	2460	156	Annonaceae, Burseraceae, Lecythidaceae	11	[3, 25]	(Baraloto et al. 2012; Fortunel et al. 2014)
Lowland tropical rainforest	Nouragues (French Guiana)	4.087	-52.67	24.8	3337	197	Lecythidaceae, Malvaceae, Sapotaceae	24	[3, 25]	(Baraloto et al. 2012; Fortunel et al. 2014)
Lowland tropical rainforest	Montagne Tortue (French	4.219	-52.41	24.6	3591	213	Sapotaceae	14	[3, 11]	(Baraloto et al. 2012; Fortunel et al. 2014)
Lowland tropical rainforest	Saut Lavilette (French Guiana)	4.151	-52.2	25.7	3590	224	Annonaceae, Sapotaceae	10	[3, 28]	(Baraloto et al. 2012; Fortunel et al. 2014)
Evergreen lowland tropical rainforest	Yasuní National Park (Ecuador)	0.683	-76.4	25	3129	251	Euphorbiaceae, Annonaceae, Fabaceae,	59	[3, 21]	(Kraft et al. 2008)
Lowland tropical rainforest	Biological Dynamics of Forest Fragments	-2.433	-59.83	27	2410	284	Fabaceae, Lecythidaceae, Sapotaceae	16	[3, 7]	(Vaz, 2011), Oliveira, unpublished data

Statistical analyses

We used the coefficient of variation (CV; 100 * standard deviation / mean) as an estimate of ITV. Because ITV may be influenced by the number of individuals sampled, we performed a rarefaction analysis in order to account for differences in sample size among species within and among forest communities (Gotelli & Colwell 2011). This rarefaction analysis generated an expected trait value for each species in each forest by randomly drawing five individuals from the total pool of individuals of each species. We repeated this re-sampling process 1000 times for each species in each forest community. We then calculated the CV for each species from the average of the expected trait values generated by 1000 randomizations. To be sure of unbiased statistics estimated from rarefaction analysis, we checked both the community' rarefaction curves did not cross (Figure S1) and also, species ranks in ITV were the same across sample sizes (Figure S2 and FigureS3) (Gotelli & Colwell 2011). Moreover, we confirm that species with smaller sample sizes did not have systematically lower ITV values (Figure S4 for leaf size and Figure S5 for SLA). We performed a generalized linear mixed model (GLMM; Breslow & Clayton 1993) using ITV as response variable, species richness as an explanatory variable together with the mean annual temperature and annual precipitation as explanatory covariates in order to account for climate differences among forests. Type of forest was included as a random factor to control for other intrinsic characteristics of each community.

We also assessed the degree of trait overlap among co-occurring species and its relationship with species richness. Trait overlap is defined as the overlapping area between two trait distribution curves (de Bello *et al.* 2013) and calculated by (1) assuming that trait values of a species are normally distributed around the mean (MacArthur & Levins 1967; Leps *et al.* 2006) or (2) using kernel density estimators, which do not assume any particular shape of the trait distribution (Mouillot *et al.* 2005). Using a normal distribution rather than kernel density tends to overestimate trait overlap, but at the same time it is considered more robust to small sample size (i.e. in our case, species with 5 individuals) than kernel distribution (Mouillot *et al.* 2005). Because of these concerns, we estimated trait overlap with both normal and kernel density approaches using the R function Trova (de Bello *et al.* 2013). For analyses with normal distributions, a mean and standard deviation of the traits is required for each species in each forest community. Given the differences among species in sampling intensity, we first ran a rarefaction analysis for each species by randomly re-sampling 5 individuals per species per site, repeated 1000 times. We then calculated the mean and standard deviation from the average of expected trait values from 1000 randomizations for each species and forest community. Trait overlap figures for both methods range from 0 to 1, where values close to 1 indicate a high overlap between species or a high trait similarity. We calculated the median and the proportion of values obtained with low (less than 0.25 out of 1) and high trait overlap (higher than 0.75 out of 1) as a categorical description of the distribution of trait overlap values for each community from both methods. Finally, we applied linear regression models using categorical parameter description of the distribution of trait overlap values as response variables and species richness as explanatory variable. Categorical parameters were square root transformed to improve normality.

All statistical analyses were carried out in R v. 3.2.1 (R Development Core Team 2013) using the packages lme4 (Bates *et al.* 2014) and MuMIn (Barton 2015).

RESULTS

Intraspecific trait variability and species richness

We found considerable ITV for both leaf traits among species co-occurring in all forest communities (Figure 2A, B). Accordingly, ITV for leaf size and SLA did not vary consistently with species richness (Figure 2A, B; Table 2). None significant effects were also observed for climatic covariates (mean annual

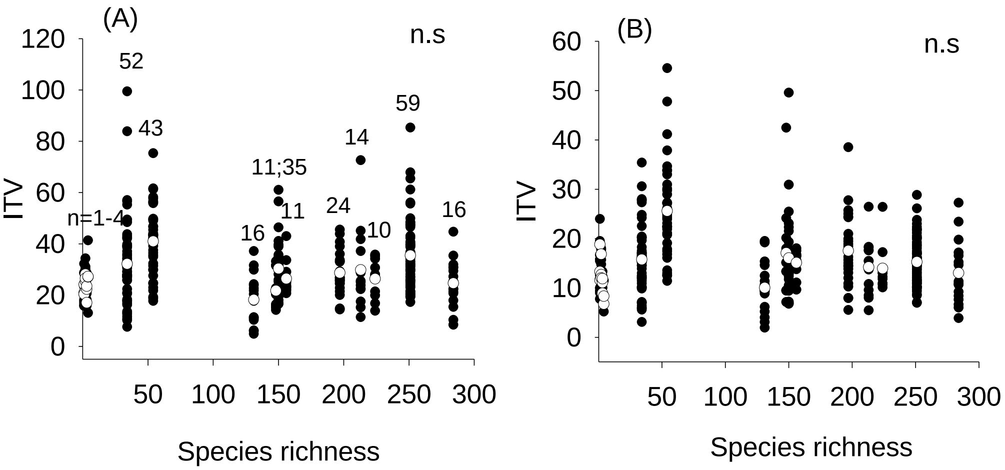


Figure 2. Effect of species richness on the extent of ITV (estimated as coefficient of variation, CV) for (A) leaf size and (B) SLA. n= number of species measured for each forest community with ≥ 5 individuals measured. Empty circles indicate the average of ITV values for each forest community and richness level. The number of sampled species (n) may be both lower than the species richness of the community since we sub-sampled a forest layer, but also, potentially larger than the species richness of that community since the arrival of seeds from neighbor canopies may result in the establishment of new individuals that previously were not part of the main canopy of the focal community.

Table 2. Summary table of the GLMM testing the change in the extent of ITV with species richness (SR), mean annual temperature (Mean. Ann. T^{ra}) and annual precipitation (Pp) for both leaf size and SLA. n= number of species included in the analyses; R^2_m: marginal-R^2; R^2_c: conditional-R^2.

Response	Exp. variable	Estimate	SD. Error	t-value	p-value	R^2_m	R^2_c
Leaf size n=321	Intercept	5.098	0.379	13.436	0.000***	0.010	0.213
	SR	0.027	0.004	0.706	0.480		
	Mean. Ann. T^{ra}	0.003	0.031	0.119	0.905		
	Pp	-0.000	0.000	-0.577	0.564		
SLA n=315	Intercept	3.726	0.302	12.338	0.000***	0.009	0.243
	SR	0.001	0.003	0.469	0.639		
	Mean. Ann. T^{ra}	0.013	0.025	0.512	0.459		
	Pp	-0.000	0.000	-0.740	0.609		

(***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value <0.1.

Trait overlap and species richness

For both leaf traits, median trait overlaps between species for each forest were significantly lower when assuming normal trait distribution than using kernel density estimators (Wilcoxon-Signed Rank test: n = 21; Z = 3.7; p-value < 0.001 for leaf size; n = 21, Z = 3.8, p-value < 0.001 for SLA). We found increasing trait overlap with species richness for both leaf traits using normal trait distribution (Figure 3A, B). We obtained similar results using kernel density estimators ($r^2 = 0.64$, p-value < 0.001 for leaf size, Figure S6A; and $r^2 = 0.44$, p-value < 0.001 for SLA, Figure S6B). We found that the proportion of species pairs with very low trait overlap (< 0.25) decreased significantly with species richness for both traits assuming normal trait distribution (Figure 3C, D) and kernel density approximation ($r^2 = 0.72$, p-value < 0.001 for leaf size, Figure S6C and $r^2 = 0.27$, p-value < 0.001 for SLA, Figure S6D). Moreover, we found that the proportion of species pairs with high trait overlap (> 0.75) increased significantly with species richness for both traits assuming normal distribution (Figure 3E, F). However, under kernel approach, the relationship between the proportion of species pairs with high trait overlap with species richness was only marginally significant for leaf size ($r^2 = 0.14$, p-value = 0.07 for leaf size, Figure S6E) and none relationship was observed for SLA (Figure S6F).

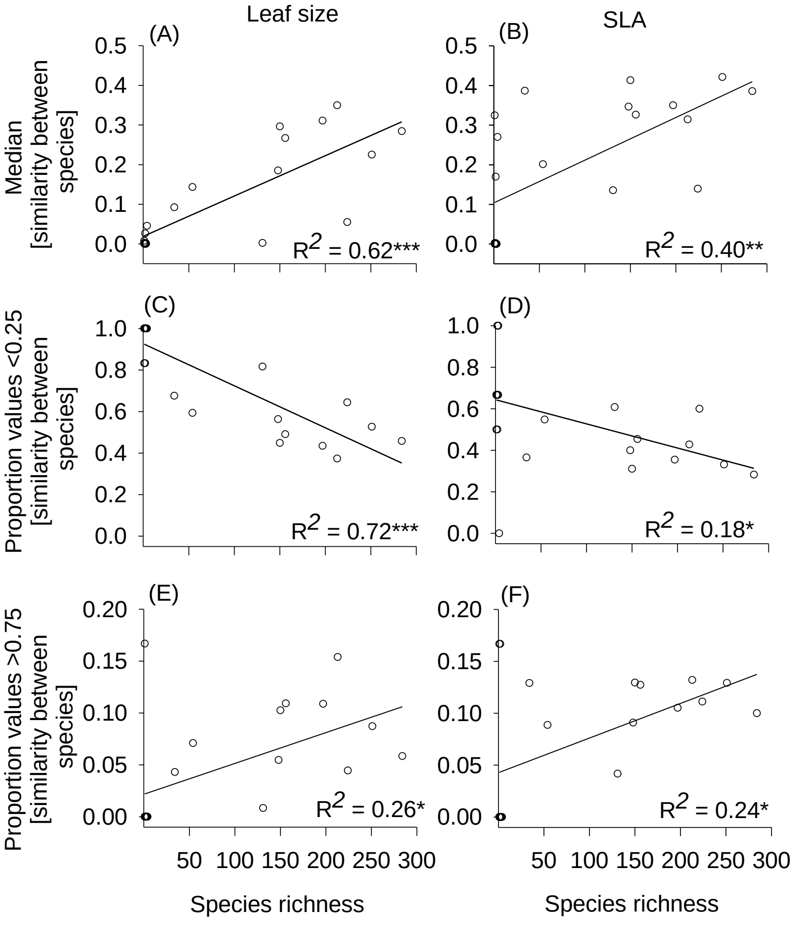


Figure 3. Linear regression models of the median values of trait overlap (panel A, B) and the proportion of low (less than 0.25; panel C, D) and high (> 0.75; panel E, F) values of degree of trait overlap between species for each forest community against species richness for both leaf size (left) and SLA (right). Trait similarity was calculated by assuming normal trait distribution of species with ≥ 5 individuals measured. (***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (.): p-value <0.1.

DISCUSSION

A largely unanswered question in biodiversity theory is whether ITV actually varies with species richness as predicted by classical niche theory (MacArthur & Levins 1967; Violle *et al.* 2012) assuming all co-occurring species with equal fitness, that postulates that species would show narrower trait breadths (i.e. decrease of ITV) with increased species richness in order to avoid competition. Contrary to niche theory, our results showed a lack of relationship between ITV and species richness for leaf size and SLA, suggesting that the species' niches did not exhibit tight packing of the trait space in species-rich forests. In addition, we found greater trait overlap in species-rich communities for both traits, reflected here by an increase of the median values in trait overlap, with decreasing proportion of species pairs with low trait overlap and thus increasing proportion of species pairs with high trait overlap as species richness increased. Our results did not support the predictions from the principle of limiting similarity, which predicts a higher spread of trait values (i.e. trait dissimilarity among species) at the community level (Cornwell & Ackerly 2009; de Bello *et al.* 2009) since co-occurring individuals with high similarity in ecological requirements are more likely to face competitive exclusion (MacArthur & Levins 1967).

The few studies testing the species richness-ITV-trait overlap relationships have so far focused on a single study system, plant growth form or functional trait, and found inconsistent results (Hulshof *et al.* 2013; Le Bagousse-Pinguet *et al.* 2014; Kumordzi *et al.* 2015). Some studies have shown declines in ITV and reduction of trait overlap with increasing species richness. For example, Hulshof *et al.* (2013) found that the ratio between intraspecific and interspecific variability (a good proxy of trait overlap among species (Violle *et al.* 2012; de Bello *et al.* 2013) in SLA in woody plant communities decreased with increasing species richness. A similar pattern was also found by Kumordzi *et al.* (2015) when studying variation in SLA of understory vegetation across different boreal forest communities differing in species diversity. Felten *et al.* (2009) found a decrease of niche breadth and niche overlap in temperate grasslands with increasing species richness, indicating complementarity of soil N use from different soil depth. In contrast, others have shown increasing ITV and trait overlap with increasing species richness, in concordance with our findings. For example, using a multi-trait approach (including SLA) in limestone grasslands, Le Bagousse-Pinguet *et al.* (2014) found increases in both ITV and the ratio between intraspecific and interspecific variability with increasing species richness, with no effect of environment on ITV in agreement with our results. They suggest asymmetric light competition among competing species as a potential explanation to this pattern. It may merely be due to the increased probability of having individual plants from a shade-intolerant species being slightly more tolerant than an individuals of a supposed shade-tolerant species; this situation would induce ITV and would minimize the differences in plant fitness and competitive ability among co-occurring species. Finally, a recent global meta-analysis Siefert *et al.* (2015) reports that the relative extent of ITV to the total community trait variance decreases with increasing species richness, but this pattern is mainly due to an increase in interspecific variance and consequently, in the total community variance, whereas the absolute extent of ITV remains fairly constant with species richness. While the analysis from Siefert *et al.* (2015) supports partly our results, it includes different plant growth forms (i.e. both herbaceous and woody plants) from multiple community types (from grasslands to forests). Differences in ITV between plant forms can be expected since long-lived woody plants may present limited plasticity (i.e. less ITV) due to higher investment in longer lifespan tissues over their lifetimes compared to short-lived herbaceous species. Unlike, our study is based on a single growth form (freestanding woody plants in forest communities) on which debates about community assembly processes across the latitudinal gradient have been especially focused in recent years (e.g. Hubbell 2001, 2005; HilleRisLambers, Clark & Beckage 2002; Kraft *et al.* 2011; Myers *et al.* 2013; Lamanna *et al.* 2014). It is important to note that a different way to calculate the ITV is used in our study in comparison with these aforementioned studies. Whereas our study measures ITV as the coefficient of variation at individual species level (i.e. absolute ITV at species level), others measure the mean intraspecific trait variance at community level (including ITV of all coexisting species in relation to total community trait variance). This difference in ITV measurement could explain in somehow differences in patterns found with respect to our results (Hulshof *et al.* 2013; Siefert *et al.* 2015).

Our findings showing an increased functional similarity in hyperdiverse forests suggest, as Hubbell and Chave have argued (e.g. Hubbell 2001, 2005; Chave 2004), that it is more likely that individuals in high diversity forests are more functionally similar to each other than individuals in lower diversity forests. On the one hand, this can lead to a greater chance for neutral or nearly neutral dynamics in these more diverse communities (Hubbell 2001). On the other hand, if a higher degree of functional similarity between species pairs in diverse forests translates into smaller fitness differences between species (*sensu* Chesson 2000), only modest stabilizing niche differences between species (e.g. resource partitioning, density-dependent effects or population density fluctuations) would be required to drive community dynamics in a non-neutral fashion (Adler *et al.* 2007). Unfortunately, these questions cannot be resolved without deeper understanding of how trait differences in woody plant communities relate to fitness and stabilizing niche differences. While recent experimental works have made these links for algal (Narwani *et al.* 2013) and annual plant communities (Kraft, Godoy & Levine 2015), considerable logistical barriers remain in long-lived plants as woody communities.

We suggest that our findings contradict niche theory and the principle of limiting similarity (promoting trait dissimilarity among species), but only based on previous assumptions of a flat fitness landscape (i.e. all species with equal fitness). However, an alternative conclusion could be reached assuming a multi-peak fitness landscapes (Gavrilets 2004). Under multi-peak fitness landscapes, species on each peak may have been selected by the interplay of different processes among which competitive limit to similarity. In this scenario, species can reach equal fitness and maintain similar ITV (Svensson *et al.* 2006) despite species richness increases (i.e. no decrease of ITV with species richness is expected).

In this study we explored shifts in the extent of ITV and trait overlap along a broad species richness gradient, but it is important to mention that our results may be in part limited since we did not carry out trait measurements on all individuals or species that were part of the whole community, particularly in species-rich communities. This likely may translates into an underestimation of the extent of ITV and trait overlap between species in diverse forests. Nevertheless, the high degree of species rarity in our tropical forests [70] makes it difficult to reach a complete range for ITV. Moreover, it is important to take into account that we are using two dimensions of the plant ecological strategy (SLA and leaf size) and they may not be good proxies of plant species' realized niches in species-rich forests. In other words, they may not be capturing niche differences among species, being the competitive ability for limiting resource use determined by other key traits that we did not take into account. Further analyses incorporating other traits representative of different plant strategy axes, such as architecture traits and woody density, and even a multi-trait approach could improve our understanding about the traits that best relate to fitness and, thus, drive niche differentiation (Spasojevic & Suding 2012). Furthermore, local biotic and abiotic factors also have effects on the extent of ITV by selecting a particular subset of trait values according to the local environment (Diaz, Cabido & Casanoves 1998). Future studies integrating other local environmental factors, such as crowding, light availability or water availability (Whitmore 1996; Ackerly 2004; Le Bagousse-Pinguet *et al.* 2015), as well as the environmental heterogeneity (Galloway 1995) would improve our understanding of the factors driving the relationship between ITV and species richness.

CONCLUSION

To conclude, our study highlights the key role that trait variability within species can play in understanding community assembly along biodiversity gradients, and emphasizes the value of estimating intraspecific variability in studies exploring trait diversity at the community level. We found an increase of functional similarity among co-occurring species in more diverse communities, to the widely recognized classical niche theory. Our study points to neutral processes or equalizing mechanisms to explain that species with similar ecological requirements can be present in the same community at the same time.

ACKNOWLEDGMENTS

We would like to thank all colleagues and technicians that assisted with field and laboratory work. Leaf data used to come to this manuscript resulted from many different studies involving different funding sources: European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 265171; Spanish-funded project REMEDINAL3eCM (S2013/MAE-2719); Ecometas excellence network (CGL2014-53840-REDT); a FPU grant by the Spanish Government (AP2010-5600); a Marie Curie IEF fellowship (FP7-PEOPLE-2011-IEF. No. 302445); a Investissement d'Avenir' grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-0025); a collaborative NSF DEB-0743103/0743800 and Institut National de la Recherche Agronomique (INRA); Instituto Interamericano para Pesquisas em Mudanças Globais (IAI); CAPES; Wageningen graduate school, Production Ecology and Resource Conservation (PE&RC); Center for Tropical Forest Science and the University of California, Berkeley, Department of Integrative, Biology; a NSF graduate fellowship and a DDIG. This is the study 709 of the Technical Series of the Biological Dynamics of Forest Fragments Project.

SUPPLEMENTARY MATERIAL

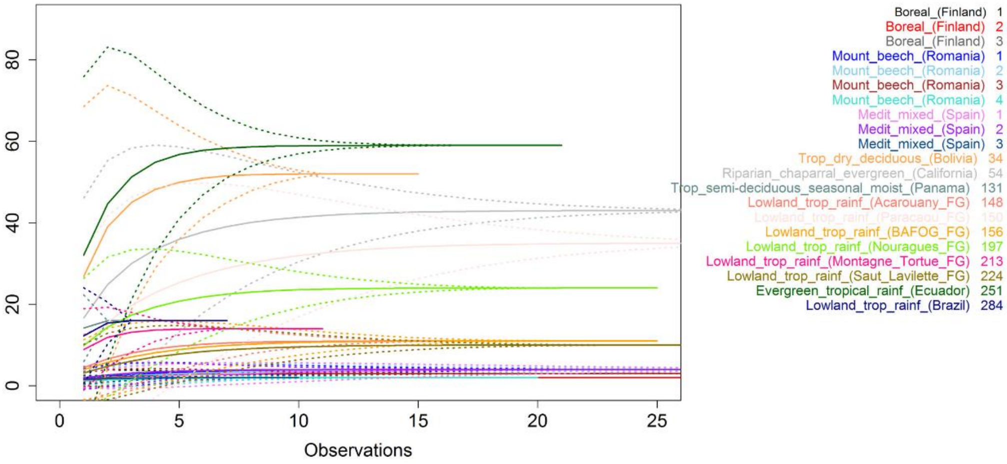


Figure S1. Community accumulation curves at sample size of 5 individuals. Dashed lines are 95% confident.

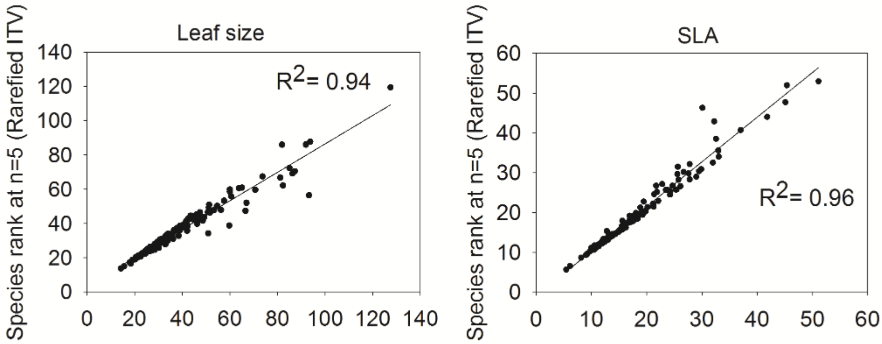


Figure S2. Species ranks at sample size of 10 individuals per species (ITV) *vs* species ranks at sample size of 5 individuals (rarefied ITV) in order to detect bias in the ITV estimate by a small sample size. R^2 close to 1 means no bias (i.e. similar ITV values obtained for a species using 10 individuals and using 5 individuals).

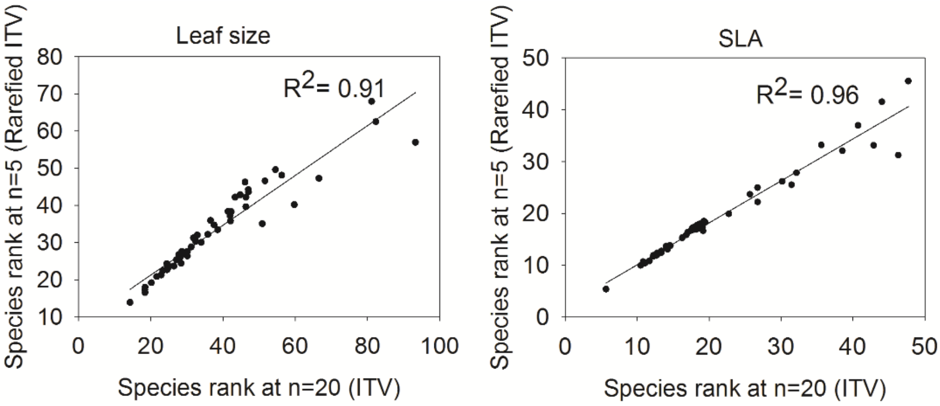


Figure S3. Species ranks at sample size of 20 individuals per species (ITV) *vs* species ranks at sample size of 5 individuals (rarefied ITV) in order to detect bias in the ITV estimate by a small sample size. R^2 close to 1 means no bias (i.e. similar ITV values obtained for a species using 20 individuals and using 5 individuals).

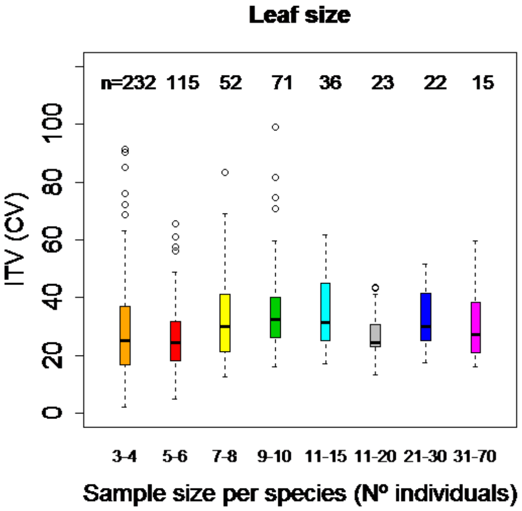


Figure S4. Boxplot of the ITV values for leaf size grouped in 7 categories of sample size per species (No. of individuals). n: number of observations in each category.

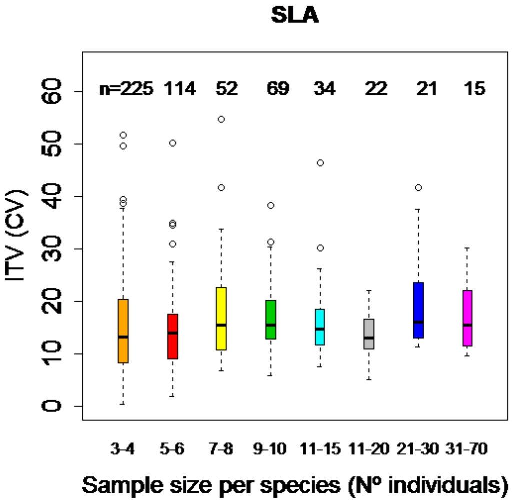


Figure S5. Boxplot of the ITV values for SLA grouped in 7 categories of sample size per species (No. of individuals). n: number of observations in each category.

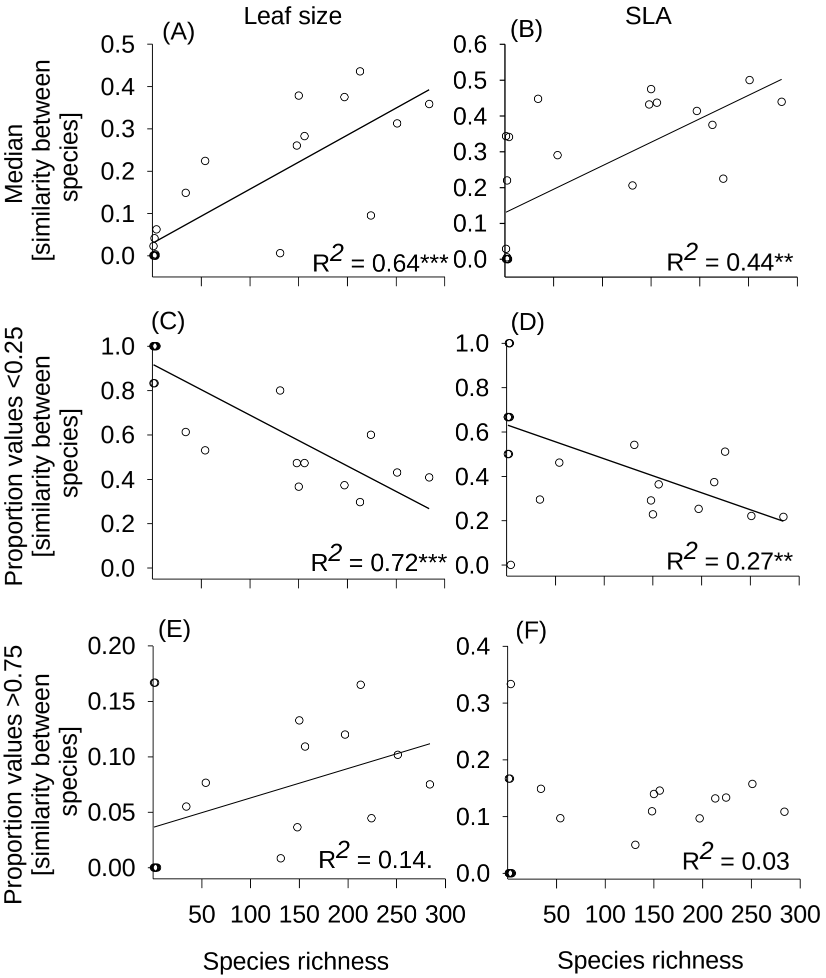
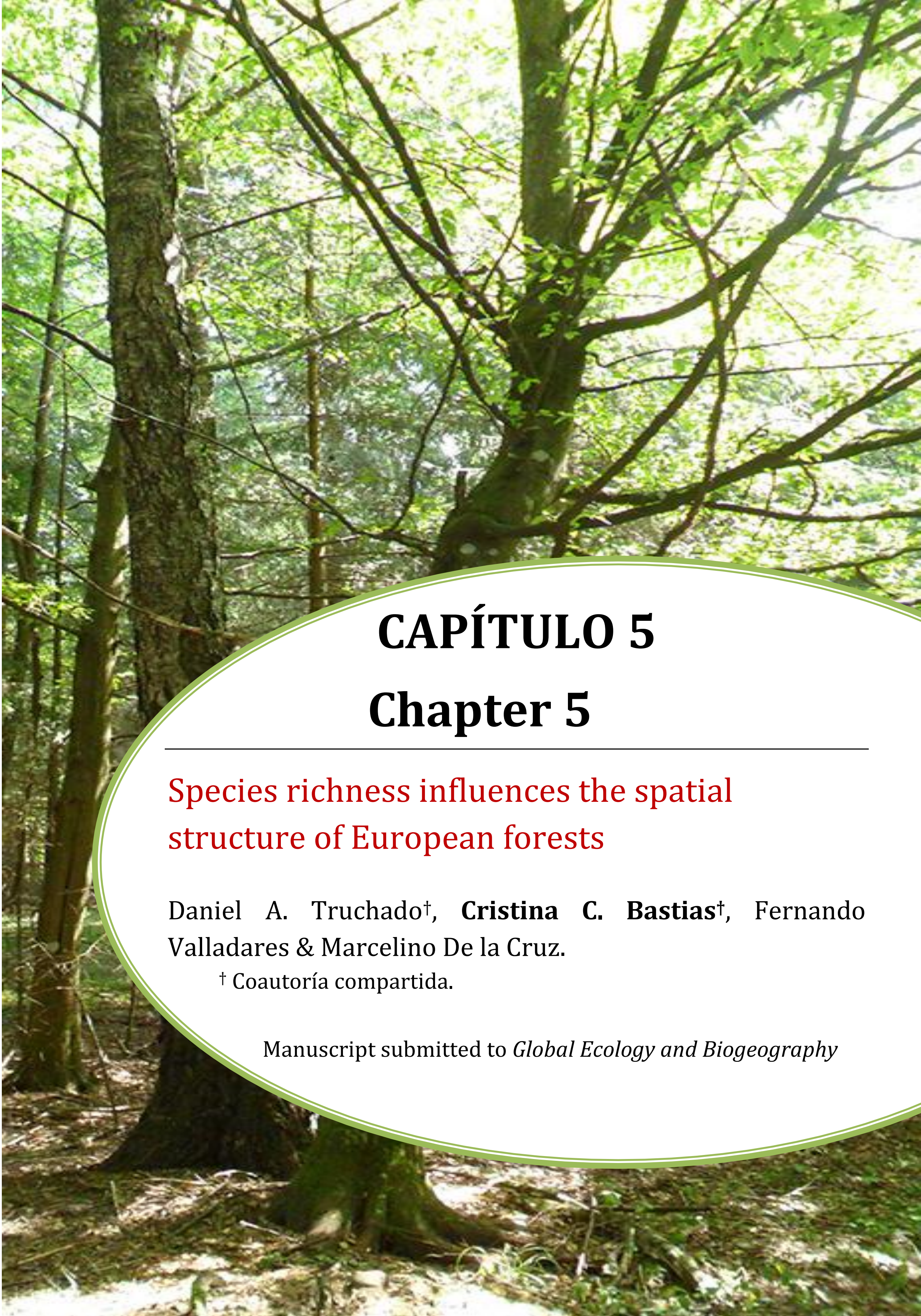


Figure S6. Linear regression models of the median values of trait overlap (panel A, B) and the proportion of low (less than 0.25; panel C, D) and high (> 0.75; panel E, F) values of degree of trait overlap between species for each forest community against species richness for both leaf size (left) and SLA (right). Trait similarity was calculated by kernel density approach using species with ≥ 5 individuals measured. (***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (.): p-value <0.1.



CAPÍTULO 5

Chapter 5

Species richness influences the spatial structure of European forests

Daniel A. Truchado†, **Cristina C. Bastias†**, Fernando Valladares & Marcelino De la Cruz.

† Coautoría compartida.

Manuscript submitted to *Global Ecology and Biogeography*

Image from the front page (Chapter 5): Adults of *Picea abies* and *Carpinus betulus* coexisting in the Bialowieza National Park (Poland).

Author: Cristina Crespo Bastias.

SUMMARY

Spatial structure of individuals and species richness are two important characteristics in the functioning of plant communities whose relation has been little studied in scientific literature. As an increase of species richness in a community have been recently demostrated to affect the balance of intra- vs. inter-specific competition, the potential effect of species richness on the final spatial structure of the plant community remain unknown. In this paper, we evaluate the effect of species richness on the spatial arrangement of the woody individuals (canopy and understory trees and shrubs, irrespective of species) in 209 different forest stands differing in species richness (from 1 up to 10 different species) located in six different forests along a European latitudinal gradient. We used complete mapped plots to characterize the spatial structure of individuals in each forest stand with different point pattern techniques at medium and fine scales. Our results showed a significant and positive relationship between clustering of individuals and species richness at both scales in five of the six study forests. The magnitude of this relationship was independent of the type of forest and could be considered a neglected macroecological pattern. These results show that species richness plays an important role in plant community assembly by controling the frequency of interspecific vs. intraspecific plant-plant interactions experimented by an individual in the studied forests.

Keywords: spatial point pattern; spatial heterogeneity; SADIE; intraspecific competition; plant-plant interactions; stochastic geometry of biodiversity

INTRODUCTION

In plant communities, the available space is a key feature for their individuals since plants, as sessile organisms, will mainly interact with their nearest neighbours, being spatial pattern a reflection of these interactions (Watt 1947; Stoll & Weiner 2000). This fact makes spatial pattern analysis (i.e. the study of changes of type and intensity of spatial patterns) a useful tool to infer mechanisms explaining species assembly and the maintenance of species diversity, as well as community structure and functioning (Bergelson 1990; Tirado & Pugnaire 2003; Maestre *et al.* 2005). Spatial point pattern analysis of plant communities (Wiegand & Moloney 2014), for example, has demonstrated how facilitative interactions result in clumped or aggregated patterns (Escudero *et al.* 2005) whereas competition produces regular distributions (Kenkel 1988).

The relevance of space as a key component of community assembly results evident by its role at the core of the Stochastic Geometry of Biodiversity theory (SGB) proposed by McGill (2010). Synthesizing several unconnected "unified" theories of biodiversity, the SGB theory summarizes three basic rules common to all of them, with two of these rules closely related to the spatial distribution of individuals in the community (McGill 2010). In particular, (1) individuals of the same species are clumped together; and (2) individuals of the same species are placed without regard to individuals of other species (also known as the "independence assertion"). Although this latter rule could appear contrary to the foundations of community ecology, e.g., Chesson's (2000), Modern Coexistence Theory (MCT), McGill (2010) explains that the independence assertion is a consequence of statistical (i.e., geometric) constraints (e.g. in rich forest, the number of possible pairwise interactions in the community increases but the likelihood of two species encountering each other decreases, thereby leading to independence by chance); and thereby, he does not discard the importance of species interactions such as competition or facilitation for community assembly. From the point of view of the SGB, the intra-specific clustered patterns resulting from dispersal limitation and/or microhabitat preferences are assumed to be a critical requirement for species coexistence, as they avoid competitive exclusion (Stoll & Prati 2001). In fact, intra-specific clumping might promote inter-specific segregation (Granda *et al.* 2012) or at least inter-specific independence leading to what the second rule assumes (Hubbell & Foster 1986; McGill 2010) and, as an indirect result, to a decrease of spatial inter-specific interactions as richness increases in the community (Chacón-Labella *et al.* 2016). Based on those simple spatial rules, the SGB has been able to explain macroecological patterns such as species turnover and distance decay or the accumulation of species richness (McGill 2010). Although several studies have addressed the assumptions of the SGB, e.g. by testing the prevalence of clustered patterns (e.g. Hubbell & Foster 1986; Seidler & Plotkin 2006; Jara-Guerrero *et al.* 2015) or the absence of inter-specific interactions in species-rich communities (Perry *et al.* 2009; Wiegand *et al.* 2012; Perry *et al.* 2014, 2016; Wang *et al.* 2016), no study has tested the role of species richness on the spatial assembly of communities (but see Chacón-Labella *et al.* 2016).

In this paper, under the stochastic rules of the SHG, we address the assumptions of Modern Coexistence Theory (MCT) (Chesson 2000) about the niche differences of coexisting species in a community. In fact, MCT assumes “stabilizing niche differences” among coexisting species as those differences that cause intra-specific interactions to be more limiting than inter-specific interactions. Therefore, an increase of species richness, which by simple geometrical reasons will decrease the probability of intra-specific interactions, would affect the balance of intra- vs. inter-specific competition (Barabás, J. Michalska-Smith & Allesina 2016; Saavedra *et al.* 2017) and consequently, it could modify the final spatial structure of the community. Based on this, we hypothesize the existence of a relationship between species richness and the spatial pattern of individuals in a community. Specifically, we hypothesize that increasing species richness would result in stronger spatial clustering of the individuals (irrespective of the species) in the community (or, in other words, a less regular spatial pattern). This derives from the dilution of intra-specific interactions as species richness increases. This dilution would reduce the strength of the competition experienced by every individual as the available space will be partitioned among the individuals of different species and thereby, coexistence instead of thinning would be favoured (Figure 1) (Kenkel 1988).

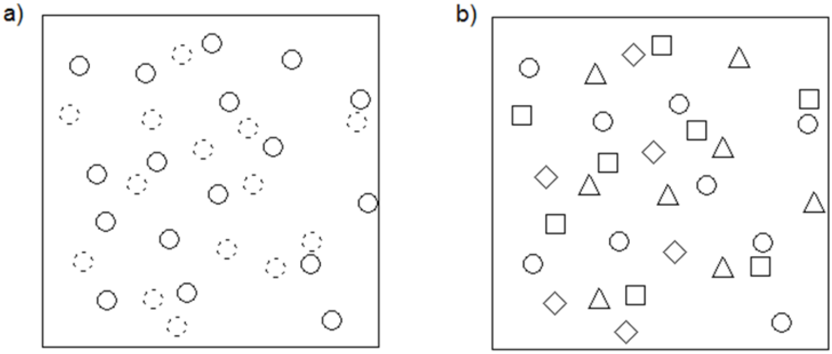


Figure 1. Comparison of spatial pattern between two hypothetical plant communities differing in species richness: a monospecific stand (a) and a mixed stand of five different species (b). Both communities share the same initial spatial pattern but over time, individuals close to others start to dead by a strong intraspecific competition by the similarity in the resource use in monospecific stands, leading to regular pattern (a). In species-rich communities, individuals tend to show random to clumped patterns by using different resources since the probability that the nearest neighbour is conspecific is low. Each species is represented by a different figure. Solid circles represent live individuals and *dashed circles* represent dead individuals.

To test the hypothesis about changes in spatial structure of individuals promoted by increasing species richness, we analyse the spatial arrangement of all woody individuals (canopy trees and understory trees and shrubs, irrespective of species) in 209 different forest stands differing in species richness (ranging from 1 species up to 10 different species) distributed in six different forests along a European latitudinal gradient: from Mediterranean to boreal forests. As the scale of the study may influence the spatial patterns found (Levin 1992; Chase 2014), we propose analysing these completely mapped communities with point pattern analysis techniques (Wiegand & Moloney 2014) which allow a detailed assessment of the scale or range of spatial scales where biotic interactions occur. Furthermore, we control the individual spatial clustering by patchy habitat variation (Plotkin *et al.* 2000; Getzin *et al.* 2008; Perry *et al.* 2008; Jara-Guerrero *et al.* 2015) (i.e. spatial structure of the environmental factors in the habitat leading to the density of individuals increased in resource-rich spots, it is known as “virtual aggregation”) (Schiffers *et al.* 2008) since it could hinders the signal of biotic interactions from the observed spatial patterns.

MATERIALS AND METHODS

Study design

The spatial analysis was assesed in a network of permanent plots, which was designed by FunDivEUROPE project to explore the effect of tree diversity on different ecosystem functions in mature European forests (http:/*/www.fundiveurope.eu*). The plot network consists of a total of 209 square plots of 30 × 30 m distributed in six different mature forests, which span a wide bioclimatic European range: from boreal forest situated in Finland and hemiboreal forest in Poland, through temperate forest in Germany and continental temperate forest in Romania, to temperate Mediterranean forest in Italy and continental Mediterranean-mixed forest located in Spain. In each forest type, plots were set up following a species richness gradient, where all woody species (i.e. canopy trees and understory trees and shrubs) with a diameter at breast height (DBH) > 7.5 cm were considered. In this way, plots ranged from monospecific stands to mixed stands of up to 10 different species, relying on the regional species pool (Table S1). Mixed stands were selected taking care that dominant species share similar relative abundances (i.e. high evenness) and the presence of nontarget species was minimal (preferably < 5% of the total basal area). Further information about experimental design can be found in Baeten *et al.* (2013)

Data collection

In each forest stand, all woody individuals were identified, and their spatial coordinates (x, y) were recorded with 1 cm precision. Individuals with multiple stems were counted as a single individual (Condit *et al.* 2000).

Moreover, we recorded several abiotic and biotic variables in order to characterize the spatial strucuture of these environmental factors in each forest stand of the three most constrasting forest types, i.e., boreal, continental temperate and continental Mediterranean forests. In each stand, a total of 17 quadrants of 1 m^2 were delimited (one at the centre of the forest stand and the other 16 located regularly in grid points every 10 meters). In each quadrant, we recorded soil depth at a random point with a tubular soil sampler (till 60 cm), we visually estimated the percent of cover of stoniness, litter, shrubs and herbs and we quantify the total light availability (GSF) through a hemispheric photograph (Valladares & Guzmán 2006). Photographs were processed with Hemiview v. 2.1 software (Delta-T Devices Ltd. Burwell, UK). This set of factors is among the major environmental factors influencing plant establishment and competitive interactions among species, which could have influenced in turn plant spatial patterns (Nicotra *et al.* 1999).

Species richness and spatial structure of woody individuals

To characterize the spatial pattern of the whole community (i.e. canopy trees and understory trees and shrubs, irrespective of species) of each forest stand, we employed three different spatial point pattern analysis functions, i.e. Ripley's *K*-function, nearest neighbor distance distribution function and pair-correlation function. Ripley's K-function $K(r)$ determines the expected number of points (i.e. individuals), weighted by the intensity (or density), occurring within a circle of radius r around a random point of the spatial pattern (Wiegand & Moloney 2014). The nearest-neighbour function $G(r)$ represents the cumulative distribution of r distances from each point to its nearest neighbour point in the pattern. The G function characterizes the small-scale properties from the spatial pattern that the K function does not reflect (e.g. the mean distance between the typical individual and its nearest neighbour; Wiegand & Moloney 2014), thereby represents a useful complement to the commonly used Ripleys's K–function. Finally, pair-correlation function $g(r)$ calculates the expected number of points within a ring of radius r around a typical individual of the pattern. Due to its non-accumulative properties, $g(r)$ has the advantage that one can isolate specific distance classes (Wiegand & Moloney 2014).

These three functions were estimated for r varying from r = 0 m to r = 7.5 m with intervals of 0.15 m. To control for edge effects, we applied Ripley's isotropic correction for K and g function and 'reduced-sample' correction for G-function. In a first exploratory analysis, we tested whether the observed spatial pattern deviated significantly from randomness, comparing the observed function (K, G and g) with the expectations under a null model of complete spatial randomness (CSR). Simulation envelopes were obtained through 99 Monte Carlo simulations of a CSR pattern for each plot. Departures from null model expectations were tested using a goodness-of-fit (*Go*F) test (Loosmore & Ford 2006). Observed functions remaining within the envelopes indicate not significant difference from CSR. Observed values smaller or larger than the envelopes at some spatial scale r were interpreted respectively as evidence of regular or aggregated pattern at that scale (Figure S1).

To measure clustering strength, we defined the statistic DR_F (i.e., deviation from randomness for the function F) to summarize the magnitude of spatial structure in each stand as follows:

$$DR_F = \sum_{r=1}^{r\,max}(F_{obs}(r) - F_{CSR}(r)),$$

where $F_{obs}(r)$ and $F_{CSR}(r)$ are respectively the observed and the expected value under CSR. Even if the CSR test is non significant, larger values of DR_F indicate stronger clustering. For each forest stand, we computed this deviation for two different ranges of spatial scales: from r = 0 to r_{max} = 7.5 m (hereinafter called “medium scale”) and from r = 0 to r_{max} = 3 m (hereinafter referred to “fine scale”). We chose those scales since they are within the distance range (10 m -or even further-) at which interactions among individuals are expected to occur in temperate forests (Getzin *et al.* 2008).

A DR_F statistic was calculated for each one of the spatial functions (i.e., we computed DR_K, DR_G and DR_g). Values of the DR_F statistic close to 0 indicate CSR compatibility whereas the higher the value of DR_F is, the greater the aggregation (and conversely, the more negative the value of DR_F is, the greater the regularity).

We explored the effect of species richness on DR_F fitting linear models (Rao & Toutenburg 1995), one for each statistic (DR_K, DR_G and DR_g) and spatial scale (medium and fine scales). To do so, we used DR_F as response variable and species richness as a predictor. We included forest type (and its interaction with species richness) to control by the potential local processes affecting the relationship between species richness and the spatial structure of individuals in each type of forest. When the interaction between forest type and species richness turned out significant, *post-hoc* tests were performed for comparisons of the relationship of spatial structure with species richness among forests.

Spatial heterogeneity and spatial structure of woody individuals.

We performed a spatial analysis by distance indices (SADIE; Perry 1998; Perry & Dixon 2002; Conrad & Perry 2008) to characterize the spatial pattern of each environmental variable in each forest stand. For those variables estimated visually (i.e. cover of rock, litter, shrub and herb), SADIE was performed when at least 4 out of 17 sample quadrants within a forest stand had values ≥ 20%. As result of SADIE analysis, we obtained an index of aggregation, I_a, which quantifies how far form spatially uniform is the distribution of values of the variable (Perry 1998) (i.e. it measures the "distance to regularity"). Then, we used standardized major axis regression models (SMA) (Warton *et al.* 2006) to test the relationship between the spatial pattern of each environmental variable

(I_a) and the spatial pattern of woody individuals (DR_F) in each forest stand. Unlike simple linear regression models, standardized major axis regression models give information about bidirectional relationship between two variables without assumption of a cause-effect link (Warton *et al.* 2006). As the sampling scheme for environmental variables had a coarse spatial grain, we used the DR_K statistic because the K function it is the most sensitive to coarse scale structures among the three functions employed.

All statistical analyses were carried out in R 3.1.2 (R Development Core Team 2013) using the packages *spatstat* (Baddeley & Turner 2005) for spatial analyses and *lmodel2* (Legendre 2015) for standardized major axis regression models. For SADIE analysis, we used SADIEshell program (Conrad & Perry 2008).

RESULTS

With either of the functions employed (i.e., Ripley's K, G and g), we found that randomness was the dominant spatial pattern in all forest types, except for the boreal forest where the regular pattern predominated (> 80% of forest stands) (Table S2). At fine scale, results showed a significant and positive effect of species richness on the spatial structure of woody individuals using DR_K ($F = 7.35$; p-value = 0.007) and DR_G ($F = 6.92$; p-value = 0.009) (Table 1; Figure 2) and a marginally positive one for DR_g ($F = 3.13$; p-value = 0.079). At medium scale, we also found a significant, positive relationship between species richness and spatial structure of woody individuals for DR_G ($F = 5.07$; p-value = 0.026) and a marginally significant one over DR_K. The interaction between species richness and forest type turned out only significant for DR_G at both fine scale ($F = 2.34$; p-value = 0.043) and medium scale ($F = 2.38$; p-value = 0.040) (Table 1, Figure 2). *Post-hoc* analyses comparing the slopes of the model fitted for different forests using G-function showed that the temperate forest presented a negative slope for the relationship between species richness and spatial structure of individuals in comparison to the positive ones for the rest of forests (Table S3). In fact, when the model was fitted without temperate forest, the interaction species richness × forest type for DR_G stopped being significant (Table S4).

Additionally, all environmental variables showed the random structure as the dominant spatial pattern in the three study forests (Table S5). None of the indexes of aggregation (I_a) of environmental variables (a proxy of the spatial heterogeneity) had an effect on clustering strenght of individuals in any forest (Table S6).

Table 1. Summary table of fitted linear models. Effect of species richness (SR) and forest type on clustering intensity of individuals for DR_K (*Ripley's K-Function*); DR_G (*G* function) and DR_g (*g* function). Data shown are the degrees of freedom (df), the *F* values and the statistical significance level of model using Type III tests and model coefficients (Coef.). (***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value < 0.1; (ns): not significant. The species richness: Forest type term means the interaction between species richness and forest type in order to control by the potential local processes affecting the relationship between species richness and the spatial structure of individuals in each type of forest.

			RESPONSE					
			DR_K		DR_G		DR_g	
Scale	Exp. variable	df	F	Coef.	F	Coef.	F	Coef.
Medium scale	SR	1	3.34 †	12.91	5.07**	2.23	1.67 ns	5.13
	Forest type	5	20.93***		29.62***		21.15***	
	SR: Forest type	5	1.54 ns		2.38*		0.82 ns	
	R^2		0.34		0.45		0.34	
Fine scale	SR	1	7.35**	7.26	6.92**	2.69	3.13 †	6.14
	Forest type	5	27.27***		39.65 ***		22.29***	
	SR: Forest type	5	1.05 ns		2.34 ***		0.53 ns	
	R^2		0.41		0.50		0.36	

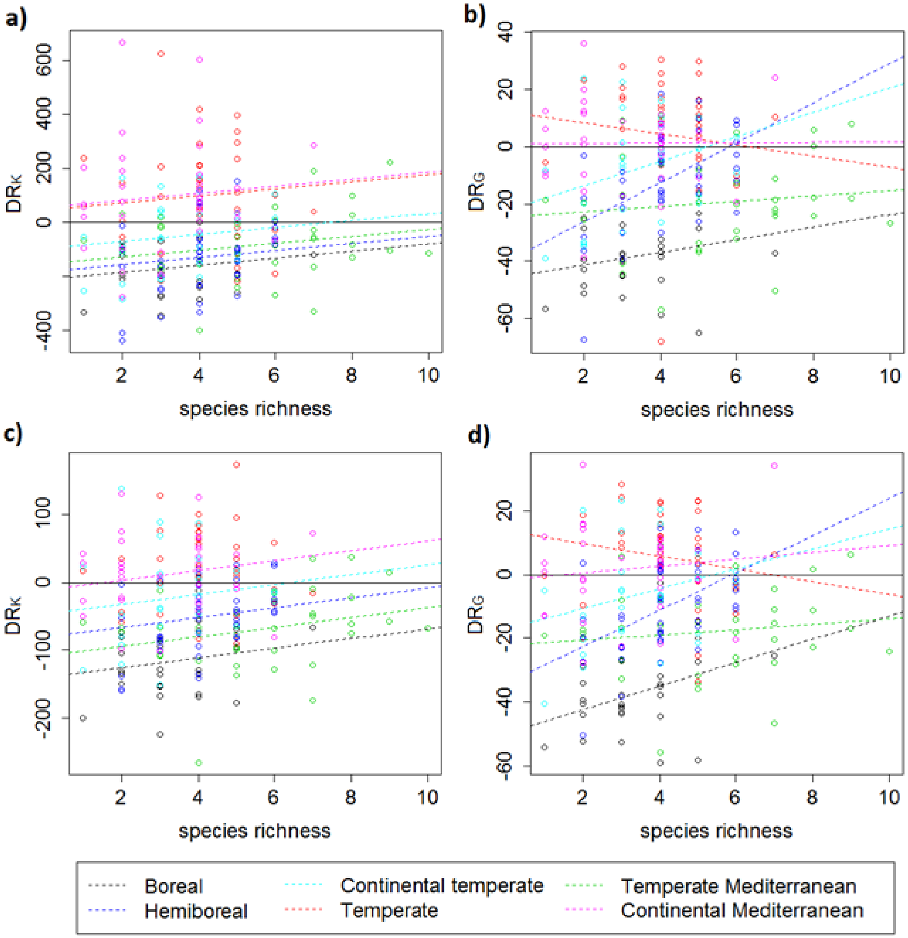


Figure 2. Relationship between species richness and DR_K and DR_G (b) for each type of forest at two different scale: at medium scale (a and b) and at fine scale (c and d). Values of DR_F above 0 imply clustering, whereas negative values mean regular patterns. Values close to 0 indicate random pattern.

DISCUSSION

From the pioneering work of Watt (1947), the study of spatial patterns has been employed as a surrogate to disentangle the ecological processes and biotic interactions involved in the assembly of plant communities (McIntire & Fajardo 2009). Although this approach has provided an impressive record of worthwhile insights (Tirado & Pugnaire 2003; Maestre *et al.* 2005; Wiegand & Moloney 2014; Pommerening 2016), there still remain highly relevant ecological questions to answer. Some of these questions require advanced analysis techniques (Velázquez *et al.* 2016), while others, like the questions posed in this paper, could be answered with simple tools but require an appropriate data set. In fact, no study up to date has evaluated the role of species richness on the spatial patterns of the community, probably because of the logistics complications involved in the observational design.

Our study, employing completely mapped forest stands differing in the total number of species and deployed along a climatic gradient throughout the European continent showed that, in all but one of the studied forests, the greater the species richness, the less regular the spatial distribution of woody individuals was, especially at fine scale. Moreover, our findings indicated the lack of effect of environmental heterogeneity on the clustering of woody individuals, what supports our main result about individual's arrangement is due to a direct effect of biotic interactions. Some times (e.g. Maestre & Cortina 2002) it has been found that the patchiness of environmental factors in the habitat was significantly correlated with the aggregation of the plants in a community. However, in our study, although SADIE analysis detected a clumped distribution in some of the measured environmental variables, they were not related to the strength of clustering of individuals at stand level, thereby ruling out the occurrence of virtual aggregation.

Our result showing a decrease in the intensity of clustering as species richness decrease (or the other way round, less regular patterns when species richness increases), was in agreement with our initial hypothesis. It is usually assumed, especially in forest communities, that the spatial structure of the stand changes throughout the ontogeny, with early recruits showing clumped distributions (consequence of limited dispersa, in line with the first assertion of SGB; McGill (2010)) which, owing to negative density dependent (Getzin *et al.* 2008; Chacón-Labella *et al.* 2014) or competitive effects (Kenkel 1988; Moeur 1993; Getzin *et al.* 2006) evolve to random or, even, regular spatial structures as some individuals grow and others die. We hypothesized that as species richness increased in the community, the probability of intra-specific encounters would decrease and in the same way would decrease the strength of intra-specific competition, hindering or delaying the apparition of regular patterns. Even though most observed patters are indistinguishable from random in the CSR tests, our DR_F statistics showed that there were differences in the strength of clustering and that these differences were significantly related to species richness in the plot. The fact that the effect of species richness on clustering of individual was stronger at fine scale reinforces the hypothesis that the observed patterns are related to plant-plant interactions (Stoll & Weiner 2000). This finding suggests that the "independence assertion" of SGB (McGill 2010) may not be relevant at the spatial scale of our study and that the degree of niche complementarity between coexisting species could affect the final spatial structure of the community. Further studies analyzing the relationship between the spatial association and functional similarity among coexisting species (e.g. Chacón-Labella *et al.* (2016)) could shed light on these aspects.

As far as we know, this is the first time that such a relationship has been documented. In one of few studies that have (superficially) examined the relationships between spatial patterns and species richness, Perry *et al.* (2008) found a negative relationship between species richness and the occurrence of clustered patterns in Mediterranean-like shrubland communities in Australia. However, in this case, this relationship was due to the prevalent environmental restrictions of a stressful and resource-limited habitat. Thus, environmental filtering allowed the coexistence of only a few tolerant species whose individuals concentrated in the least environmentally unfavourable patches, probably via facilitation processes (Pugnaire, Haase & Puigdefábregas 1996; Callaway & Walker 1997) or even by virtual aggregation (Schiffers *et al.* 2008). So, unlike in our studied forests, environmental factors played a key role in the generation of clumped structures in the Australian shrubland communities. In other unrelated study, (Kikvidze *et al.* 2005) found that both regular and aggregated patterns could appear linked to high species richness in alpine vegetation, However, in this case they considered just one, fixed, spatial scale in their study, so the "patchy" vegetation typical from this environment appeared as "regular" despite showing great clustering at smaller scales (at patch scale). Unlike the study by (Kikvidze *et al.* 2005), our research carried out with a wide range of scales allows us to check that in a forest environment, where competition is the predominant interaction among individuals, the increase of species richness leaves a mark on spatial structuring of individuals, leading to more aggregated patterns. Also quite recently, (Pretzsch & Biber 2016) have shown that mixed stands of European forest species (different combinations of pairs of species) could attain higher densities than mono-specific ones. Our study goes far beyond this result because our point pattern statistics ($K(r)$, $g(r)$ and $G(r)$ functions) control for differences of density among stands and measure the degree of clustering, i.e., they show that irrespective of stand density, trees are more clustered as species richness increases.

In the last two decades, several studies have analysed the effects of species richness on different ecosystem and community attributes such as functioning and productivity (Vilà *et al.* 2007; Paquette & Messier 2011; Morin *et al.* 2011; Zhang, Chen & Reich 2012) or individual growth rates (Chamagne *et al.* 2017) and have found a positive relationship. For example, (Morin *et al.* 2011), in a study of European temperate forests across a large climatic gradient similar to ours, found that the increase of productivity with species richness was promoted through functional complementarity between species, with higher richness generating higher variation of shade tolerance and growing strategies, which resulted in faster responses (i.e. colonization) to small-scale mortality events. Other studies have pointed also complementarity in light use strategies (Jucker *et al.* 2014b) or root architectures (Brassard *et al.* 2013) linked to an increase of species richness in forests. Complementarity is the base for resource partitioning (Paquette & Messier 2011; Ruiz-Benito *et al.* 2014), i.e., for the stabilizing niche differences that promote coexistence in ecological communities (Chesson 2000; Saavedra *et al.* 2017) so, in addition to increasing productivity (Zhang *et al.* 2012; Chamagne *et al.* 2017), it could render inter-specific competition less intense that intra-specific competition and would explain our findings.

In the case of DR_G, most forests showed also a positive relationship with species richness, except the temperate forest. It is important remembering that for an effect of species richness to occur on the balance of interactions, there must exists clear functional differences between species (Levine & HilleRisLambers 2009; Kraft *et al.* 2015; Saavedra *et al.* 2017). The temperate forest is the most productive along the European climatic gradient (Ratcliffe *et al.* 2016) and, in these conditions, competition between species may result in community trait convergence (Mayfield & Levine 2010; Kraft *et al.* 2015). If this were the case, increasing richness would not result in an increase of stabilizing niche differences, and the "dilution effect" would not occur. It is also possible that in this forest, the effects of species diversity should have been measured throughout species evenness instead of species richness (Zhang *et al.* 2012). In fact, it has been proposed that the species survival probability (i.e. the smaller effects of competitive exclusion) is maximized with the combination of a high evenness and intermediate levels of productivity (Rohr *et al.* 2016).

It is worth noticing that with the only exception of DR_G, in the temperate forest, the positive relationship between species richness and the strength of spatial clustering was prevalent, irrespective of the type of forest (Table 1, Figure 2), throughout the entire European climatic gradient. In other words, species richness has a similar effect on the spatial structure of individuals in from the boreal to the Mediterranean forests. This points out to a neglected macroecological pattern that has remained elusive to ecological research up to date. In this paper, we have seen in this paper the important role that species richness plays shaping the spatial structure of plant communities, probably modifying the intensity of the interactions among individuals.

CONCLUSIONS

Our study has presented empirical evidence that the spatial pattern of woody individuals in European forests was affected by community species richness. Our results point out that the spatial pattern of woody individuals tends to be less regular with increased species richness. Moreover, these observed patterns were largely caused by plant-plant interactions rather than by a spatial heterogeneity in the abiotic environment. Further studies taking into account species identity of the closest neighbours could help to a better understanding of mechanisms behind species assembly in European forests. Our study highlights the relative importance of species richness as a key community attribute affecting in the community spatial structure and functioning.

ACKNOWLEDGMENTS

We greatly acknowledge all colleagues and technicians that participated in the establishment of plots and the collection of spatial data. We thank to site managers for maintaining of the plots and logistical help during field data collection. CCB is beneficiary of a FPU grant funded by the Spanish Government (AP2010-5600). This research was supported by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 265171, and also from Spanish-funded project REMEDINAL3eCM (S2013/MAE-2719).

SUPPLENTARY MATERIAL

Table S1. Description of the six different forests of study, including their location, predominant woody species, species richness range and the number of plots selected in each forest type.

Type of forest	Location	Coordenates	Woody species (canopy and understory trees and shrubs; DBH ≥ 7.5 cm)	Dominant canopy trees (>90% plot basal area)	Species richness range	No. of plots
Boreal	North Karelia region (Finland)	(62.6º N, 29.9º E)	*Alnus* sp., *Betula pendula, Betula pubescens, Picea abies, Pinus sylvestris, Populus tremula, Salix caprea, Sorbus aucuparia*	*Betula pendula, Betula pubescens, Picea abies, Pinus sylvestris,*	1-7	28
Hemiboreal	Bialowieza National Park (Poland)	(52.7° N, 23.9º E)	*Acer pseudoplatanus, B. pendula, Carpinus betulus, Corylus avellana, Fagus sylvatica, Fraxinus excelsior, P. abies, P. sylvestris, P. tremula, Quercus petraea, Quercus robur, Sorbus aucuparia, Tilia* sp.	*B. pendula, Carpinus betulus, P. abies, P. sylvestris, Quercus robur*	2-6	43
Continental temperate	Carpathian Mountains (Romania)	(47.6º N, 25.3º E)	*Abies alba, A. pseudoplatanus, Alnus* sp., *Betula* sp., *C. betulus, F. sylvatica, F. excelsior, P. abies, P. tremula, Ulmus minor*	*Abies alba, A. pseudoplatanus, F. sylvatica, P. abies*	1-6	28
Temperate	Hainich National Park (Germany)	(51.5º N, 10.2º E)	*Acer campestre, Acer platanoides, A. pseudoplatanus, Alnus glutinosa, Alnus incana, B. pendula, C. betulus, Crataegus* sp., *F. sylvatica, F. excelsior, P. abies, P. sylvestris, Prunus avium, Q-. petraea, Q. robur, Quercus rubra, S. caprea, Sorbus torminalis, Tilia cordata, Tilia platyphyllos, Ulmus glabra*	*A. pseudoplatanus, F. sylvatica, F. excelsior, P. abies, Q-. petraea*	1-7	38
Temperate Mediterranean	Southern Central Tuscany (Italy)	(43° N, 11° E)	*A. campestre, Acer monspessulanum, Arbutus unedo, C. betulus, Castanea sativa, Chamaecyparis lawsoniana, Corylus avellana, F. sylvatica, Fraxinus ornus, Ilex aquifolium, Ostrya carpinifolia, P. tremula, P. avium, Pseudotsuga menziesii, Quercus cerris, Quercus crenata, Quercus ilex, Q. petraea, Quercus pubescens, Sorbus domestica, S. torminalis, T. cordata*	*Castanea sativa, Ostrya carpinifolia, Quercus cerris, Quercus ilex, Q. petraea*	1-10	36
Continental Mediterranean	Alto Tajo Natural Park (Spain)	(40.7º N, -1.9º E)	*Buxus sempervirens, Crataegus* sp., *Juniperus communis, Juniperus oxycedrus, Juniperus phoenicia, Juniperus* sp., *Junipeus thurifera, Pinus nigra, P. sylvestris, Quercus faginea, Q. ilex, Viburnum* sp.	*Pinus nigra, P. sylvestris, Quercus faginea, Q. ilex*	1-7	36

Table S2. Percentage of forest stands (i.e. plots) in each forest type that showed an aggregated (Agg), random (Ran) or regular (Reg) spatial pattern using Ripley's *K*-function (*K(r)*), the nearest-neighbour function (*G(r)*) and pair-correlation function (*g(r)*).

	K(r)			***G(r)***			***g(r)***		
Forest type	Agg	Ran	Reg	Agg	Ran	Reg	Agg	Ran	Reg
Boreal	0	14	86	0	18	82	0	18	82
Hemiboreal	0	74	26	0	95	5	0	93	7
Continental temperate	0	75	25	11	87	3	8	89	3
Temperate	21	74	5	0	89	11	0	93	7
Temperate Mediterranean	6	64	31	0	67	33	3	86	11
Continental Mediterranean	31	61	8	11	86	3	8	86	6

Table S3. Results from post-hoc analyses. Comparisons were carried out using the 'relevel' R-function on the linear models changing the 'forest type' taken as reference. Values of model coefficients for the fitted linear model of *G* function are shown at fine and medium scale.

	Coefficient	**Standard error**	**t-value**	**p-value**
Medium scale				
Boreal	2.20	2.49	0.885	0.377
Hemiboreal	6.94	2.06	3.365	<0.001
Continental temperate	4.28	2.32	1.846	0.066
Temperate	-1.96	2.12	-0.922	0.357
Temperate Mediterranean	0.95	1.24	0.763	0.446
Continental Mediterranean	0.06	1.82	0.035	0.972
Fine scale				
Boreal	3.68	2.14	1.72	0.086
Hemiboreal	5.83	1.77	3.30	0.001
Continental temperate	3.12	1.99	1.57	0.119
Temperate	-1.99	1.82	-1.09	0.276
Temperate Mediterranean	0.87	1.06	0.82	0.416
Continental Mediterranean	1.07	1.56	0.69	0.493

Table S4. Summary table of fitted linear models excluding temperate forest. Effect of species richness (SR) and forest type on clustering intensity of individuals for DR_G (G function). Data shown are the degrees of freedom (df), the F values and the statistical significance level of model using Type III tests and model coefficients (Coef.). (***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value < 0.1; (ns): not significant. The species richness: Forest type term means the interaction between species richness and forest type in order to control by the potential local processes affecting the relationship between species richness and the spatial structure of individuals in each type of forest.

		DR_G		
Scale	Exp. variable	df	F	Coef.
Medium scale	SR	1	6.24**	0.11
	Forest type	4	33.17***	
	SR: Forest type	4	2.12 †	
	R^2		0.47	
Fine scale	SR	1	4.79*	3.68
	Forest type	4	33.80 ***	
	SR: Forest type	4	2.14†	
	R^2		0.52	

Table S5. Results from SADIE analyses studying the spatial structure of environmental factors in each forest stand for the three most contrasting forests (Boreal, continental temperate and continental Mediterranean). In the table is shown for each forest type, the percentage of forest stands that presented an aggregated (Agg), random (Ran) or regular (Reg) spatial distribution for each environmental factor. Sign (–): SADIE analysis was not calculated for this variable (see *material and methods section* for criteria).

		Boreal	**Continental temperate**	**Continental Mediterranean**
GSF	Agg	6	4	11
	Ran	94	92	82
	Reg	0	4	7
Soil depth	Agg	18	7	17

	Ran	79	79	83
	Reg	3	14	0
Stoniness	Agg	-	-	29
	Ran	-	-	66
	Reg	-	-	5
Litter	Agg	7	11	17
	Ran	86	86	83
	Reg	7	3	0
Shrubs	Agg	15	-	0
	Ran	77	-	96
	Reg	8	-	4
Herbs	Agg	26	17	18
	Ran	74	83	82
	Reg	0	0	0

Table S6. Results from standardized major axis regression models testing the relationship between the I_a of each particular environmental variable and clustering intensity of individuals (DR_K). The sign of model coefficients shows positive or negative correlation. Sign (–): SADIE analysis was not calculated for this variable (see *material and methods section* for criteria). (***): p-value <0.001; (**): p-value <0.01; (*): p-value < 0.05; (†): p-value < 0.1; (ns): not significant.

Predictor variable	**Boreal**			**Continental temperate**			**Continental Mediterranean**		
	R^2	**p-value**	**Coef.**	**R^2**	**p-value**	**Coef.**	**R^2**	**p-value**	**Coef.**
GSF	0.00	0.743	-0.004	0.04	0.339	0.002	0.04	0.229	-0.001
Soil depth	0.01	0.594	0.002	0.02	0.505	-0.001	**0.12**	**0.043***	**0.001**
Cover of Stoniness	-	-	-	-	-	-	0.01	0.670	0.001
Cover of litter	0.02	0.502	-0.002	0.02	0.535	0.002	0.00	0.989	0.000
Cover of shrub	0.03	0.560	-0.002	-	-	-	0.01	0.578	0.000
Cover of herb	0.01	0.772	-0.002	0.00	0.961	0.000	0.00	0.802	0.000

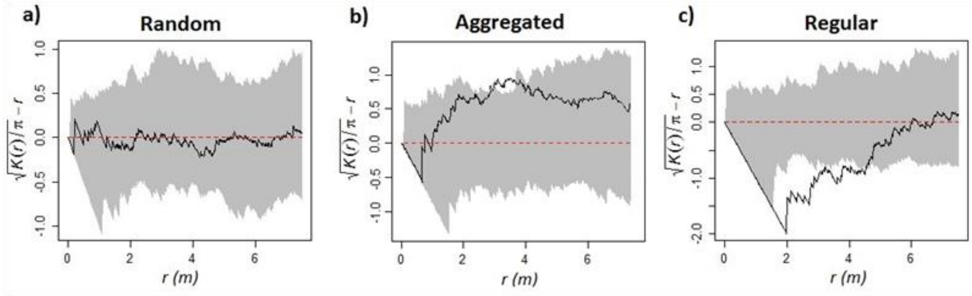


Figure S1. Examples of the three types of spatial pattern found in our study forests. Spatial pattern is calculated using K function. For an easier interpretation of the K function, we employed the linearization proposed by Besag (1977) and modified as $L(r) = \sqrt[2]{K(r)/\pi} - r$

The grey zone represents the confidence interval between the envelopes obtained from 99 CSR pattern simulations. We considered random pattern when the observed function lay within the envelopes for all distances (a); aggregated when the observed function lay outside the upper part of the envelopes (b) and regular when it lay outside the lower part of the envelopes (c).

DISCUSIÓN GENERAL

Los bosques europeos se caracterizan por un número bajo de especies arbóreas dominantes en comparación con otros sistemas forestales en su misma latitud (Walter 1985). A pesar de la baja riqueza de especies de los bosques europeos, diversos estudios han señalado su importancia sobre el número de funciones y servicios que prestan a la sociedad (multifuncionalidad del bosque) (Jactel & Brockerhoff 2007; Zeugin *et al.* 2010; Gamfeldt *et al.* 2013; Jucker *et al.* 2014a; van der Plas *et al.* 2016). Pero, para conservar y mantener no sólo la diversidad sino también las funciones ecosistémicas que proporcionan, determinados procesos que mantienen la dinámica natural de los bosques como la regeneración y la coexistencia de los individuos desde la fase juvenil deben ser exitosos.

Hasta la actualidad, el papel de la riqueza de especies del dosel en la regeneración y en los mecanismos que promueven la coexistencia de especies ha permanecido prácticamente desconocido (pero véase Olson & Wagner 2011). Sin embargo, a través del desarrollo de cada uno de los capítulos de la presente tesis (con muestreos llevados a cabo en comunidades naturales y experimentos controlados) y de la presente discusión de los resultados principales, contribuimos a un mejor entendimiento de la relación entre la riqueza de especies del dosel y la regeneración y la coexistencia de especies en fase juvenil y adulta en los bosques europeos. Además, con el desarrollo de las cuestiones aquí planteadas y estudiadas en hasta seis tipos de bosques europeos diferentes, podemos conocer la generalidad de nuestros resultados y tener una visión más amplia de la posible evolución futura de la diversidad y la composición de estas masas forestales europeas. Entender los procesos que permiten el mantenimiento de la diversidad en los bosques europeos es un reto que se debe abordar para desarrollar planes de manejo y estrategias de conservación efectivas que contribuyan a mitigar los posibles efectos negativos del cambio global, tales como la pérdida de funciones ecosistémicas, la fragmentación o la pérdida de diversidad de especies tanto animales como plantas (FAO 2013).

La riqueza del dosel no influye en la heterogeneidad ambiental de los bosques europeos.

Uno de los resultados más importantes y consistentes obtenidos en esta tesis doctoral es que el número de especies que conforman el dosel arbóreo no favoreció la creación de una mayor heterogeneidad ambiental tanto de factores bióticos como abióticos (Capítulo1). Además, encontramos que la disposición de los mismos en el espacio a la escala medida (cada 10 m) estuvo lejos de mostrar un patrón espacial definido (agregado o regular) ya que en la mayoría de las parcelas (>80% de éstas), independientemente de su nivel de riqueza, la distribución espacial encontrada de las variables ambientales medidas no fue diferente de lo que cabría esperar por azar (Capítulo5). Una excepción fue la magnitud de la heterogeneidad lumínica que se incrementó significativamente con la presencia de un mayor número de especies en el dosel pero con un R^2 muy bajo (marginal) (Nakagawa & Schielzeth 2013) (Capítulo1), siendo la mayor parte de la varianza en esta relación explicada por la diferencia al comparar los distintos tipos de bosques entre sí (R^2 condicional alto) (Capítulo1). Una prueba de la diferencia en la heterogeneidad lumínica entre bosques en su relación con la riqueza la encontramos en el Capítulo3 donde la heterogeneidad lumínica sí se incrementó significativamente con la riqueza de especies en el bosque continental mediterráneo (España), mientras que encontramos en el bosque hemiboreal (Polonia) valores similares de heterogeneidad lumínica tanto en doseles monoespecíficos como en doseles mixtos. Diferencias similares en los resultados sobre la relación riqueza de especies-heterogeneidad ambiental fue observada entre tipos bosques para las propiedades del suelo (Capítulo3).

Nuestros resultados sugirieron que los bosques monoespecíficos podrían tener valores similares de **heterogeneidad ambiental** que los bosques con doseles mixtos. Estos resultados apoyarían la hipótesis de que las especies en doseles mixtos interactúan entre sí dando lugar a unas condiciones ambientales nuevas, más que tener un efecto aditivo en las condiciones ambientales con respecto a sus doseles monoespecíficos (Ball *et al.* 2008;

Ampoorter *et al.* 2016). Y en este caso particular, estas nuevas condiciones no fueron diferentes en términos de heterogeneidad ambiental con respecto a sus parcelas monoespecíficas. De hecho, Ampoorter *et al.* (2016) encontró evidencia de la creación de dicho “nuevo ambiente” en las mismas parcelas del bosque hemiboreal y del bosque continental mediterráneo que utilizamos para el desarrollo de esta tesis, ya que la riqueza de especies acompañantes del sotobosque (arbustivas) que se encontraron en masas mixtas fue completamente distinta a la riqueza encontrada en las masas monoespecíficas. Sin embargo, Stein *et al.* (2014), con un meta-análisis incluyendo 1148 puntos de todo el mundo, encontró que la heterogeneidad ambiental estuvo en promedio positivamente relacionada con la riqueza de especies. Esta relación no se cumple en nuestros bosques de estudio en un gradiente europeo y podría atribuirse bien a la falta de poder estadístico (tamaño muestral pequeño), a la escala espacial utilizada que podría ser demasiado gruesa para captar la heterogeneidad ambiental a nivel de parcela, o bien a que las variables ambientales seleccionadas no fueron las idóneas para captar la heterogeneidad ambiental en el hábitat debida a la riqueza de especies. Mientras que la primera de ellas la descartamos en base a los resultados que obtuvimos del análisis de poder estadístico (Capítulo1), las dos últimas no podemos estar seguros de ellos. De hecho, ciertos estudios han mostrado la importancia de la escala y de la heterogeneidad de una variable para detectar tanto la magnitud (fuerza) como la forma (tipo) de relación entre la heterogeneidad y la riqueza de especies (e.g. Costanza, Moody & Peet 2011).

La regeneración de bosques con diferente riqueza en el dosel.

Un mayor número de microhábitats disponibles (mayor heterogeneidad) supondría mayores oportunidades para el establecimiento de juveniles, tanto de una misma especie (aumentando la abundancia) como de diferentes especies (aumentando la riqueza de especies) (Ricklefs 1977; Masaki *et al.* 2007; Pérez-Ramos & Marañón 2012; Stein *et al.* 2014). El hecho de que nuestros resultados apunten a una heterogeneidad ambiental similar entre masas con distinto nivel de riqueza de especies tiene importantes implicaciones, ya que indicaría un número similar de microhábitats diferentes disponibles para el establecimiento de nuevos individuos comparando masas monoespecíficas con masas mixtas. Esto parece corroborarse con los resultados obtenidos ya que no encontramos diferencias en abundancia ni en riqueza del regenerado entre bosques con diferente grado de riqueza de especies (Capítulo1). Sólo la heterogeneidad lumínica aumentó con la riqueza de especies, aunque con un R^2 bastante bajo. Aun así, esta hereogeneidad lumínica tampoco tuvo un efecto sobre la abundancia o la riqueza de juveniles (Capítulo1). Este resultado en un principio resultó sorprendente ya que la luz es uno de los principales factores que influye en el establecimiento y supervivencia de los juveniles, especialmente en las primeras etapas de vida (Nicotra *et al.* 1999; Benavides *et al.* 2016), y tanto su magnitud como su heterogeneidad espacialmente explícita favorecerían el establecimiento de individuos con diferentes requerimientos de luz o lo que es lo mismo, de tolerancia a la sombra (Valladares & Niinemets 2008). Una de las posibles explicaciones a la falta de relación entre riqueza de especies del dosel, heterogeneidad lumínica y la riqueza y abundancia del regenerado, y que nosotros además observamos en el Capítulo3 de la presente tesis, sería que individuos en etapas tempranas fuesen capaces de ajustar sus características funcionales en función de la disponibilidad de los recursos de cada parcela para contribuir al cómputo del regenerado total. Es decir, nosotros, en el Capítulo3, observamos que plántulas procedentes de comunidades con diferente nivel de riqueza de especies, e incluso con diferente heterogeneidad ambiental, exhibieron valores similares de plasticidad tanto a la disponibilidad de luz como de nutrientes. Lo que podría llevar a pensar que los individuos en etapas tempranas serían capaces de aclimatarse a las diferentes condiciones ambientales entre parcelas mediante el ajuste de sus rasgos funcionales, de manera similar independientemente de la heterogeneidad ambiental y riqueza de especies de la comunidad de origen (donde están los árboles madre), pudiendo contribuir con igualdad de condiciones al regenerado de la parcela siempre que la dispersión garantice su presencia.

El resultado obtenido por esta falta de relación entre la riqueza del dosel y la riqueza de los juveniles fue la discordancia en la composición de especies entre ambas cohortes (Capitulo 1). Es decir, que los individuos no regeneran necesariamente mejor en los bosques dominados por adultos de su misma especie (conespecíficos). Este patrón puede ser clave para explicar un cambio potencial de dominancia de las especies en estos bosques europeos a medio plazo, y puede deberse, a las interacciones planta-planta y otros factores asociados a patrones de denso-dependencia negativa relacionados con la depredación e infección por patógenos específicos de una especie (Janzen 1970; Connell 1971). Aunque en la presente tesis no se analiza la depredación de semillas e infección por patógenos como posibles factores que puedan contribuir a esta disparidad entre comunidades adulto-juvenil, otros autores han encontrado evidencia de mecanismos de denso-dependencia negativa en bosques templados y mediterráneos (Packer & Clay 2000; Pérez-Ramos & Marañón 2012; Comita *et al.* 2014), incluido el estudio de Granda *et al.* (2014) llevado a cabo en el mismo bosque continental mediterráneo de este trabajo. En particular, Granda *et al.* (2014) encontró mayor mortalidad de semillas de *Quercus ilex* debajo de doseles conespecíficos, señalando de esta manera potenciales cambios en la composición de este bosque, al favorecerse la regeneración de individuos lejos de sus congéneres adultos. Este cambio potencial de composición de estos bosques se vería favorecido si existe una gran efectividad en el proceso de dispersión de semillas desde masas cercanas (contribución de semillas desde escala regional; Eriksson 1993), así como por una capacidad de respuesta similar (plasticidad) de juveniles de las distintas especies para adaptarse a cualquier nuevo ambiente (Capítulo3).

Por tanto, los resultados obtenidos en esta tesis parecen apuntar a un cambio en la dominancia de las especies en el futuro, siempre y cuando a través de las diferentes etapas del desarrollo de los individuos, éstos sean capaces de hacer frente a los distintos filtros impuestos por el ambiente. Este cambio en las masas podría en principio ocurrir a una escala general europea, pues en todos los tipos de bosques se observó dicho desacoplamiento entre ambas cohortes. Además, en principio, dicho desacoplamiento no vendría determinado porque las especies que conformen la masa forestal tengan una limitada producción de semillas ya que encontramos valores similares de abundancia y riqueza de juveniles en parcelas compuestas por especies de frondosas, a pesar de ser especies de baja producción de semillas en comparación con parcelas con coníferas, especies de alta producción (Capítulo1). Esta capacidad similar de prosperar a pesar de la baja producción de semillas de la mayoría de las especies de frondosas en estudio, se debe a la compensación producida por un tamaño y calidad mayor de las semillas (Leishman *et al.* 2000; Westoby *et al.* 2002; Moles & Westoby 2004). De hecho, en el bosque continental mediterráneo, encontramos evidencia de que dicho desacoplamiento parece estar encaminado a favorecer las frondosas (*Quercus*) frente a las coníferas (*Pinus*), ya que encontramos una capacidad potencial mayor de respuesta (plasticidad) frente a cambios ambientales en rasgos funcionales clave para el desarrollo de los individuos (e.g. crecimiento, SLA, altura) de plántulas del género *Quercus* frente individiduos de especies pertenecientes al género *Pinus* (Capítulo3). Este fenómeno, sin embargo, no es sorprendente a la vista de otros trabajos que ya han detectado un reemplazamiento con especies de *Quercus* en masas actualmente dominadas por coníferas en otros bosques del ámbito mediterráneo (Lookingbill & Zavala 2000; Urbieta *et al.* 2011; Galiano *et al.* 2013).

La importancia de la variabilidad intraespecifica en el ensamblaje de comunidades.

Una vez las plántulas se establecen, los individuos tienen que hacer frente no sólo a las condiciones abióticas ambientales, sino también a las interacciones con otros individuos de su misma o diferentes especies presentes en la comunidad. Por tanto, conocer los mecanismos que explican estas interacciones bióticas y que dan lugar a los patrones de ensamblaje y la coexistencia de especies se convierte en algo fundamental para entender la dinámica, la composición y la riqueza futura de las masas forestales (Chesson 2000).

Desde que en los trabajos de comunidades se empezara a utilizar el enfoque funcional para estudiar las interacciones bióticas entre las especies y así poder comprender mejor los mecanismos por el cual las especies coexisten, se ha dado una gran importancia al estudio de la variabilidad de rasgos a nivel interespecífico (entre especies) (Kraft *et al.* 2008; Fortunel *et al.* 2014), y se ha considerado que la variabilidad entre individuos de una misma especie (a nivel intraespecífico) es despreciable en comparación con la variabilidad entre las diferentes especies que componen una comunidad (Garnier *et al.* 2001; Laughlin *et al.* 2011). Sin embargo, recientemente, numerosos trabajos han señalado la importancia de considerar la variabilidad intraespecífica en los estudios de comunidades basados en rasgos para inferir los mecanismos que mantienen la coexistencia y la diversidad de especies (Jung *et al.* 2010; Bolnick *et al.* 2011; Violle *et al.* 2012) y además, se han presentado ciertas pautas de cuando ésta puede o debe incluirse en los estudios de comunidades dependiendo del objetivo del trabajo, sistema de estudio, y tipo de rasgos medidos entre otros (Albert *et al.* 2011).

En esta tesis, uno de los resultados principales que mostramos es que la magnitud de la variabilidad los rasgos funcionales exhibida por los juveniles dentro de una especie **(variabilidad intraespecífica)** llegó a ser tan importante como la variabilidad entre juveniles de las diferentes especies que formaban la comunidad **(variabilidad interespecífica)** (Capítulo 2). En otras palabras, que la variabilidad intraespecífica explicó una gran parte de la diversidad funcional total de los bosques europeos, al menos en la fase de juvenil y para ciertos rasgos. Más aún, ampliando la escala y utilizando datos de otras partes del mundo, incluidos algunos bosques tropicales, encontramos que la magnitud de la variabilidad intraespecífica absoluta mostrada por individuos del sotobosque (i.e. juveniles y árboles acompañantes de baja estatura) coexistiendo en estas comunidades ricas en especies (>250 especies/ha) fue similar o incluso mayor a la alcanzada por las especies en los bosques europeos (Capítulo 4). Esta gran variabilidad intraespecífica encontrada también en los bosques tropicales hizo que al estudiar la relación entre la riqueza de especies y la variabilidad intraespecífica obtuviésemos una falta de relación entre ambas (Capítulo 4). Este hallazgo en parte sorprende ya que de acuerdo con la teoría de nicho (MacArthur & Levins 1967), la variabilidad entre especies (variabilidad interespecífica) ganaría importancia con respecto a la variabilidad intraespecífica en bosques ricos en especies, ya que el espacio total funcional se repartiría entre las diferentes especies, las cuales para evitar la competencia y poder coexistir de forma estable con otras especies tendrían que reducir su variabilidad intraespecífica en torno a su valor medio óptimo de rasgos. Aunque la reducción de la variabilidad intraespecífica con la riqueza de especies ha sido observada por ciertos autores en concordancia con lo predicho por la teoría de nicho (Felten *et al.* 2009; Hulshof *et al.* 2013; Kumordzi *et al.* 2015), dicha relación no está demostrada pues otros trabajos (e.g. Le Bagousse-Pinguet *et al.* 2014) junto con el nuestro (Bastias *et al.* 2017) muestran una falta de relación entre ambas. Además, en un meta-análisis global analizando dicha relación (Siefert *et al.* 2015) encontraron que aunque la importancia relativa de la variabilidad intraespecífica disminuyó con la riqueza de especies, la variabilidad intraespecífica absoluta no se vio afectada. La inconsistencia de los resultados de estos trabajos pone de manifiesto que el debate sobre la riqueza de especies y la variabilidad intraespecífica sigue aún abierto y más estudios en dicha dirección son necesarios. A pesar de esto, nuestros resultados ponen de manifiesto la importancia de la variabilidad intraespecífica tanto en bosques europeos como en bosques tropicales, y que su consideración en los estudios de ensamblaje de especies es fundamental para entender mejor los procesos implicados en la coexistencia, dinámica y funciones del ecosistema (Leps *et al.* 2006; Cianciaruso *et al.* 2009; de Bello *et al.* 2011).

Hay que mencionar que la relevancia de la variabilidad intraespecífica dependió en gran medida del rasgo funcional medido. Concretamente observamos que el SLA y el contenido de nitrógeno en la hoja son los que mostraron una menor variación dentro de la especie (i.e. mayor variabilidad a nivel interespecífico) y rasgos como el LDMC o el contenido de isótopos 13C y 15N los que presentaron un porcentaje de variación intraespecifíco superior al 75% (Capítulo2). Esto demuestra que la importancia de la variabilidad intraespecífica está influida considerablemente por el tipo de rasgo funcional medido (Lepš *et al.* 2011). Aun considerando las diferencias entre rasgos, hubo un patrón similar en cuanto a la importancia intraespecífica para los mismos rasgos en los tres tipos de bosques europeos estudiados, contrastados bioclimáticamente (boreal, continental templado y continental mediterráneo) (Capítulo2). Esto nos permite generalizar nuestros resultados sobre qué rasgos responderían más a nivel intraespecífico en comparación con otros. En otras palabras, que rasgos son más variables a nivel de individuo y por tanto pueden ser considerados más interesantes para estudios donde la variabilidad intraespecífica sea relevante (Albert *et al.* 2011).

Mecanismos de coexistencia. Fase juvenil vs fase adulta.

Entre los resultados obtenidos en la presente tesis se muestran indicios que sugieren que los mecanismos que explican las interacciones planta-planta cambian a lo largo de las etapas de desarrollo de los árboles (ontogenia) (Moll & Brown 2008; Barabás, Meszéna & Ostling 2014; Lasky *et al.* 2015). Y es que durante la fase del desarrollo de un individuo desde su etapa de juvenil hasta adulta, especialmente en especies de vida larga como los árboles, los individuos están sometidos a una multitud de cambios ambientales en su entorno tanto a nivel biótico como abiótico, así como en sus interacciones con otros niveles tróficos (e.g. herbivoría) (Niinemets 2010). Dichos cambios generan una respuesta por parte del individuo a nivel tanto fisiológico, morfológico como estructural (Niinemets & Kull 1995; Sack & Grubb 2001; Niinemets 2002) que puede hacer que los mecanismos que promueven la coexistencia de individuos en cierto nivel de desarrollo puedan cambiar a lo largo de diferentes fases del desarrollo (Moll & Brown 2008; Niinemets 2010).

Con nuestros resultados observamos que en una fase temprana, juveniles y otros individuos del sotobosque llegaron a presentar valores de rasgos similares (i.e. individuos funcionalmente similares) solapando así en el espacio funcional disponible. Además, dicha similaridad funcional se hizo más grande cuanto mayor fue la riqueza de especies de la comunidad desde bosques boreales a tropicales (Capítulo 4). Una comunidad donde coexisten especies funcionalmente similares (i.e. contrario al principio de similaridad; Hutchinson 1957; MacArthur & Levins 1967) se concebiría bajo la teoría neutral (Hubbell 2001; Chave 2004; Hubbell 2005), donde la comunidad estaría regida por procesos y mecanismos estocásticos y donde el ensamblaje de las especies fuera producto del azar (Hubbell 2001; Chave 2004; Hubbell 2005). Otra posible explicación aplicando la nueva versión mecanicista de la teoría de la coexistencia (Chesson 2000), se basaría en que la coexistencia de los individuos en esta fase temprana estaría principalmente regida por mecanismos ecualizadores (i.e. especies con una capacidad similar para competir), y con una menor intervención de los mecanismos estabilizadores (aquellos que regulan las densidades de las poblaciones que coexisten basándose en las diferencias en sus requerimientos) (Adler *et al.* 2007).

En cambio, esta tesis sí muestra evidencias de la actuación de mecanismos de diferenciación de nicho (mecanismos estabilizadores *sensu* Chesson 2000) en la fase adulta frente a los mecanismos ecualizadores dominando la coexistencia en la fase juvenil. En la fase adulta, concretamente encontramos a través del análisis del patrón espacial que los individuos presentaron patrones menos regulares a medida que incrementó la riqueza de especies, derivado de una dilución de la competencia intraespecífica en bosques mixtos en comparación con los bosques monoespecíficos; donde en éstos últimos el patrón regular se esperaría ser el dominante por similaridad entre los individuos conespecíficos en el uso de los recursos (Kenkel 1988; Rietkerk & Koppel 2008) (Capítulo 5). Este resultado apuntando a patrones más agregados de los individuos adultos cuanto mayor fue la riqueza sugirió una coexistencia de especies basada en la complementariedad en el uso de los recursos, lo que les permitiría coexistir de manera más estable muy próximos en el espacio (Pacala & Tilman 1994; Cardinale, Nelson & Palmer 2000). Esto apoya los resultados obtenidos con nuevos estudios, como por ejemplo el estudio llevado a cabo por Pretzsch & Biber (2016), quienes observaron una mayor densidad de individuos en masas mixtas que en masas monoespecíficas y lo basaron en un uso más eficiente de los recursos. Si bien, basándonos en nuestros trabajos (Capítulo 4 y

5), no podemos saber si el cambio de mecanismos subyacentes que permiten la coexistencia entre la fase juvenil-adulto es debido a un cambio en la ontogenia de los individuos interaccionando con su ambiente (abiótico y biótico) en fases intermedias a las dos fases estudiadas aquí o si se debe a la diferencia *per se* en la composición de especies en las dos cohortes estudiadas. Aun así, nuestros resultados van en la misma línea de numerosos trabajos que han mostrado cambios en las interacciones bióticas a lo largo de la ontogenia señalando que los individuos pasan por fases de diferenciación de nicho y solapamiento con otras especies a lo largo de su ciclo de vida (Cornelissen *et al.* 2003; Moll & Brown 2008; Barabás *et al.* 2014). Concretamente y en concordancia con nuestros resultados, Lasky *et al.* (2015) encontró que rasgos asociados con la partición de nicho fueron más relevantes a nivel de adulto que a nivel de juvenil. Una parte importante de los cambios en las interacciones bióticas se debe a cambios en la variabilidad intraespecífica a lo largo de la ontogenia incluida la plasticidad fenotípica (Werner & Gilliam 1984; Watson, Geber & Jones 1995; Sultan 2000; Huber *et al.* 2012), afectando consecuentemente al balance final intra-interespecífica (Barabás *et al.* 2016; Saavedra *et al.* 2017).

En resumen, nuestros resultados ponen de manifiesto la complejidad del proceso de regeneración, y sugieren que la riqueza del dosel no es un buen predictor de la misma. La actuación de diferentes mecanismos como los de denso-dependencia, así como una dispersión efectiva desde masas cercanas, unido a una capacidad similar de respuesta (plasticidad) de los juveniles independientemente de la riqueza y la heterogeneidad ambiental de su comunidad de origen, favorecen la falta de concordancia entre la composición de especies en la comunidad de adultos y juveniles. Nuestros resultados apuntan a un posible cambio a medio plazo de las especies dominantes de las masas actuales forestales europeas, favoreciéndose las especies de frondosas frente a las coníferas. Este cambio en la composición de especies de las masas forestales europeas podría llegar a ser real si las plántulas teniendo una coexistencia regida por mecanismos estocásticos, como nuestros resultados sugieren, son capaces de hacer frente a las condiciones abióticas y a las interacciones bióticas, ajustando sus rasgos en fases más tardías para complementarse con otros individuos en el uso de los recursos, gracias a la variabilidad intraespecífica (incluyendo la plasticidad fenotípica). Un cambio en la composición de especies de las masas europeas trae consigo un posible riesgo de cambiar o incluso perder el carácter multifuncional de los bosques, perdiendo parte de los bienes y servicios que éstos nos están proporcionando actualmente.

CONCLUSIONES

Integrando los cinco capítulos experimentales de la presente tesis se pueden extraer las siguientes conclusiones:

1. La riqueza de especies del dosel no tuvo un efecto directo sobre la abundancia y riqueza de juveniles en bosques europeos dado que la cantidad de semillas disponibles (medido a través del índice BA_{seed}) y la riqueza de semillas (inferido a través de la riqueza de especies del dosel) no tuvieron ningún efecto en la abundancia y la riqueza de juveniles, respectivamente, ni a nivel general ni a nivel de bosque.

2. La riqueza del dosel tampoco tuvo un efecto indirecto en la regeneración natural de estos bosques en términos de abundancia y riqueza de especies a través de la heterogeneidad ambiental. Esta conclusión se basa (i) en un efecto no significativo de la riqueza del dosel en la abundancia y riqueza de juveniles, (ii) en una falta de relación entre la heterogeneidad de la luz y la abundancia y riqueza de juveniles y, (iii) en la falta de relación entre riqueza del dosel y la heterogeneidad ambiental apuntando a un número similar de microhábitats disponibles en bosques monoespecíficos y mixtos.

3. El diseño experimental mostró tener una potencia estadística suficiente para tener certeza de la ausencia de relación entre la heterogeneidad ambiental general y la riqueza de juveniles con la riqueza de especies del dosel. También resultó ser fiable para corroborar la ausencia de relación entre la cantidad de semillas y la abundancia de juveniles. Sin embargo, más de 30 parcelas por nivel de riqueza en cada tipo de bosque serían necesarias para estar seguros de la falta de relación entre la heterogeneidad lumínica y el regenerado en términos de abundancia y riqueza, diseño experimental no abordable en esta tesis.

4. La composición de especies del dosel no coincidió con la composición de especies del regenerado, indicando un posible cambio potencial en la composición de especies dominantes de los bosques europeos en el futuro. Este resultado junto con la falta de relación de la riqueza del dosel con la riqueza del regenerado podrían apuntar a la actuación de mecanismos de denso-dependencia negativa en este tipo de bosques.

5. La variabilidad intraespecífica representó una parte importante de la diversidad funcional total de la comunidad de juveniles en bosques europeos, llegando incluso a ser más importante que la variabilidad interespecífica en algunos rasgos (ver punto 6).

6. La importancia de la variabilidad intraespecífica relativa a la interespecífica estuvo determinada por el tipo de rasgo funcional estudiado, siendo el SLA y el contenido de nitrógeno los que mostraron menor variación a nivel intraespecífico (mayor importancia interespecífica), mientras que los dos rasgos de contenido foliar de isótopos ^{13}C y ^{15}N fueron los que más variaron a nivel intraespecífico de forma relativa a la variabilidad interespecífica. Además, esta conclusión es generalizada observándose en tres tipos de bosques diferentes.

7. La expresión fenotípica y la plasticidad de rasgos tanto morfológicos, fisiológicos o aquellos relacionados con el crecimiento y la arquitectura de la planta en respuesta a una diferente disponibilidad de luz y nutrientes fue similar para plántulas provenientes de masas forestales con distinto nivel de riqueza de especies y diferente heterogeneidad ambiental. Este resultado apoyaría en parte la disparidad en composición y riqueza entre adultos y

juveniles, ya que la plasticidad permitiría aclimatarse a cualquier individuo a las condiciones ambientales del nuevo ambiente donde ha sido dispersado y contribuir al cómputo del regenerado total.

8. Existieron importantes diferencias en la expresión fenotípica para todos los rasgos morfológicos, fisiológicos, y aquellos relacionados con el crecimiento y la arquitectura de la planta entre grupos funcionales. Además, la plasticidad fenotípica estuvo fuertemente determinada por el grupo funcional de las especies, principalmente en el bosque continental mediterráneo (ver punto 9).

9. En el bosque continental mediterráneo, los individuos de especies frondosas (*Quercus*) mostraron una mayor capacidad potencial de respuesta a cambios en la disponibilidad de luz y nutrientes que los individuos de especies de pino (*Pinus)*. Además, esta mayor capacidad de respuesta se tradujo en valores más altos en rasgos importantes relacionados con la capacidad de competir por los recursos disponibles como crecimiento, altura o SLA. Este resultado junto con la discordancia encontrada entre la composición del dosel y de los juveniles, podría apuntar a un reemplazamiento potencial de especies, favoreciéndose el regenerado de las especies de *Quercus* por encima de los pinos.

10. La magnitud absoluta de la variabilidad del rasgo a nivel intraespecífico en especies del sotobosque se mantuvo en valores similares desde comunidades boreales a tropicales a pesar de aumentar mucho la riqueza de especies. Este resultado contrario a la teoría clásica de nicho, sugirió que la variabilidad intraespecífica puede llegar a jugar un papel clave en los

procesos de ensamblaje de especies independientemente del tipo de comunidad.

11. El solapamiento de rasgos entre especies del sotobosque en una misma comunidad aumentó a medida que se incrementaba la riqueza de especies de la misma (desde bosques boreales a bosques tropicales). Este resultado sugirió que las especies son funcionalmente más similares a medida que aumenta la riqueza de especies, contrario a la teoría clásica de nicho, sugiriendo así que son los procesos estocásticos y/o la actuación de los mecanismos ecualizadores (*sensu* Chesson 2000) los que modelan el ensamblaje de especies en estas etapas tempranas.

12. En fase adulta, los individuos se mostraron más agregados en el espacio cuando aumentó la riqueza de especies de la comunidad. Además, dicho patrón espacial no estuvo asociado a una distribución espacial de los recursos en el hábitat (i.e. se descartó la 'agregación virtual'). Nuestros resultados sugieren una complementariedad en el uso de los recursos por parte de los diferentes individuos de las distintas especies para mantener una coexistencia estable.

13. Nuestros resultados sugieren un cambio en los mecanismos de coexistencia a lo largo de las diferentes etapas del desarrollo de los individuos (ontogenia).

CONCLUSIONS

We obtain the following conclusions from the five experimental chapters:

1. Canopy species richness did not directly affect either the regeneration abundance or richness in European forests since BA_{seed} (a proxy for seed quantity) and seed pool richness (inferred from canopy species richness) did not have any effect on juvenile abundance and richness, respectively, neither at the overall nor at the forest scales.

2. Canopy species richness neither had an indirect effect on natural regeneration of European forests in terms of juvenile abundance and richness through environmental heterogeneity. This conclusion is based on (i) the non-significant relationship between canopy species richness and the abundance and richness of juveniles; (ii) the lack of relationship between light heterogeneity and both juvenile abundance and richness and (iii) the absence of relationship between canopy species richness and environmental heterogeneity.

3. Our experimental design was statistically powerful enough to be certain about the absence of relationships between overall environmental heterogeneity and regeneration abundance and richness with canopy species richness. Moreover, our experimental design was suitable to corroborate the lack of relationship between seed quantity and juvenile abundance. However, we would need more than 30 plots per richness level and forest type in order

to be sure about the absence of relationship between light heterogeneity and natural regeneration in terms of abundance and richness.

4. There was a mismatch in the species composition between the tree canopy and the recruitment, indicating a potential shift in the composition of dominant tree species of European forests in the future. This result, together with the lack of relationship between canopy species richness and regeneration richness, suggested the presence of negative density-dependence mechanisms in these forests.

5. The intraspecific trait variability explained a substantial part of the total functional diversity at the juvenile community level in European forests, becoming even more relevant than interspecific trait variability for some traits (see point 6).

6. The relative extent of intraspecific trait variability was influenced by the functional trait. SLA and leaf nitrogen content were the two traits that showed the least variation at the intraspecific level (i.e. the largest interspecific variability), while leaf content of ^{13}C y ^{15}N isotopes exhibited a substantial relative extent of the intraspecific variation to the interspecific variability. Moreover, these patterns were generalized across three contrasting European forests.

7. The phenotypic expression and plasticity of morphological, physiological traits and those related to growth and plant architecture in response to a different light and nutrient availabilities were similar among seedlings coming

from tree communities differing in species richness and environmental heterogeneity. This result would partly support the mismatch in species composition between juveniles and adults, since any juvenile would have a similar capacity to adjust its traits to any new environmental conditions where they arrived at (dispersed) and therefore, to contribute to the regeneration.

8. There were relevant differences in the phenotypic expression for all leaf morphological, architectural physiological and growth-related traits between functional groups. Moreover, phenotypic plasticity was strongly affected by the functional group, mainly in the continental Mediterranean forest (see point 9).

9. In the continental Mediterranean forest, broadleaved species (*Quercus*) exhibited a higher potential capacity of response to shifts in the light and nutrient availabilities than conifers (*Pinus*), translating into higher values of growth, height and SLA (key traits related to the competitive capacity). This result, together with a mismatch in the composition between juveniles and adults, might point to a potential replacement of species of genus *Pinus* by *Quercus* species.

10. The absolute extent of the intraspecific trait variability in juveniles and small trees remained similar from boreal to tropical forests despite the increasing community species richness. This result, contrary to classical niche theory, suggested that intraspecific trait variability may play a key role in species assembly regardless of the forest type.

11. The trait overlap between species within a community increased with the species richness from boreal to tropical forests. This result suggested that species were functionally more similar as species richness increased, contrary to the classical niche theory, pointing at stochastic and/or equalizing mechanisms (*sensu* Chesson 2000) as responsible for shaping species assemblage in these early stages.

12. At the adult stage, individuals were more aggregate spatially as the community species richness increased. We discarded that this spatial pattern was derived by a specific spatial ordination of resources within the habitats –plots- (i.e. 'virtual aggregation' discarded). Our result suggested a complementarity in the resource use by individuals from different species contributing to a stable coexistence.

13. In summary, our results showed shifts in the mechanisms underlying the species coexistence along different stages of developmental of individual (ontogeny).

REFERENCIAS

Abrams, P. (1983) The Theory of Limiting Similarity. *Annual Review of Ecology and Systematics*, **14**, 359–376.

Ackerly, D. (2004) Functional Strategies of Chaparral Shrubs in Relation to Seasonal Water Deficit and Disturbance. *Ecological Monographs*, **74**, 25–44.

Ackerly, D.D. & Cornwell, W.K. (2007) A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecology Letters*, **10**, 135–145.

Ackerly, D., Knight, C., Weiss, S., Barton, K. & Starmer, K. (2002) Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. *Oecologia*, **130**, 449–457.

Adler, P.B., Fajardo, A., Kleinhesselink, A.R. & Kraft, N.J.B. (2013) Trait-based tests of coexistence mechanisms (ed C Scherber). *Ecology Letters*, **16**, 1294–1306.

Adler, P.B., HilleRisLambers, J. & Levine, J.M. (2007) A niche for neutrality. *Ecology Letters*, **10**, 95–104.

Aerts, R. (1995) The advantages of being evergreen. *Trends in Ecology & Evolution*, **10**, 402–407.

Ainley, L.B., Vergés, A. & Bishop, M.J. (2016) Congruence of intraspecific variability in leaf traits for two co-occurring estuarine angiosperms. *Oecologia*.

Aitken, S.N. & Bemmels, J.B. (2016) Time to get moving: assisted gene flow of forest trees. *Evolutionary Applications*, **9**, 271–290.

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G. & Violle, C. (2011) When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics*, **13**, 217–225.

Albert, C.H., Thuiller, W., Yoccoz, N.G., Soudant, A., Boucher, F., Saccone, P. & Lavorel, S. (2010) Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology*, **98**, 604–613.

Alpert, P. & Simms, E.L. (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology*, **16**, 285–297.

Amarasekare, P. (2003) Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters*, **6**, 1109–1122.

Ameztegui, A. & Coll, L. (2011) Tree dynamics and co-existence in the montane-sub-alpine ecotone: the role of different light-induced strategies: Forest dynamics in the montane-sub-alpine ecotone. *Journal of Vegetation Science*, **22**, 1049–1061.

Ampoorter, E., Baeten, L., Koricheva, J., Vanhellemont, M. & Verheyen, K. (2014) Do diverse overstoreys induce diverse understoreys? Lessons learnt from an experimental–observational platform in Finland. *Forest Ecology and Management*, **318**, 206–215.

Ampoorter, E., Selvi, F., Auge, H., Baeten, L., Berger, S., Carrari, E., Coppi, A., Fotelli, M., Radoglou, K., Setiawan Nuri, N., Vanhellemont, M. & Verheyen, K. (2016) Driving mechanisms of overstorey–understorey diversity relationships in European forests. *Perspectives in Plant Ecology, Evolution and Systematics*, **19**, 21–29.

Ares, A., Neill, A.R. & Puettmann, K.J. (2010) Understory abundance, species diversity and functional attribute response to thinning in coniferous stands. *Forest Ecology and Management*, **260**, 1104–1113.

Auger, S. & Shipley, B. (2013) Inter-specific and intra-specific trait variation along short environmental gradients in an old-growth temperate forest. *Journal of Vegetation Science*, **24**, 419–428.

Augusto, L., Dupouey, J.-L. & Ranger, J. (2003) Effects of tree species on understory vegetation and environmental conditions in temperate forests. *Annals of Forest Science*, **60**, 823–831.

Avery, Thomas Eugene & Burkhart, Harold. (2002) Forest Measurements. URL http://www.mheducation.com/highered/product.0073661767.html [accessed 29 June 2016]

Baddeley, A. & Turner, R. (2005) spatstat: An R Package for Analyzing Spatial Point Patterns. *J Stat Softw*, **12**, 1–42.

Baeten, L., Verheyen, K., Wirth, C., Bruelheide, H., Bussotti, F., Finér, L., Jaroszewicz, B., Selvi, F., Valladares, F., Allan, E., Ampoorter, E., Auge, H., Avăcăriei, D., Barbaro, L., Bărnoaiea, I., Bastias, C.C., Bauhus, J., Beinhoff, C., Benavides, R., Benneter, A., Berger, S., Berthold, F., Boberg, J., Bonal, D., Brüggemann, W., Carnol, M., Castagneyrol, B., Charbonnier, Y., Chećko, E., Coomes, D., Coppi, A., Dalmaris, E., Dănilă, G., Dawud, S.M., de Vries, W., De Wandeler, H., Deconchat, M., Domisch, T., Duduman, G., Fischer, M., Fotelli, M., Gessler, A., Gimeno, T.E., Granier, A., Grossiord, C., Guyot, V., Hantsch, L., Hättenschwiler, S., Hector, A., Hermy, M., Holland, V., Jactel, H., Joly, F.-X., Jucker, T., Kolb, S., Koricheva, J., Lexer, M.J., Liebergesell, M., Milligan, H., Müller, S., Muys, B., Nguyen, D., Nichiforel, L., Pollastrini, M., Proulx, R., Rabasa, S., Radoglou, K., Ratcliffe, S., Raulund-Rasmussen, K., Seiferling, I., Stenlid, J., Vesterdal, L., von Wilpert, K., Zavala, M.A., Zielinski, D. & Scherer-Lorenzen, M. (2013) A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 281–291.

Balaguer, L., Martínez-Ferri, E., Valladares, F., Pérez-Corona, M.E., Baquedano, F.J., Castillo, F.J. & Manrique, E. (2001) Population divergence in the plasticity of the response of Quercus coccifera to the light environment. *Functional Ecology*, **15**, 124–135.

Ball, B.A., Hunter, M.D., Kominoski, J.S., Swan, C.M. & Bradford, M.A. (2008) Consequences of non-random species loss for decomposition dynamics: experimental evidence for additive and non-additive effects. *Journal of Ecology*, **96**, 303–313.

Banta, J.A., Ehrenreich, I.M., Gerard, S., Chou, L., Wilczek, A., Schmitt, J., Kover, P.X. & Purugganan, M.D. (2012) Climate envelope modelling reveals intraspecific relationships among flowering phenology, niche breadth and potential range size in Arabidopsis thaliana. *Ecology Letters*, **15**, 769–777.

Barabás, G., J. Michalska-Smith, M. & Allesina, S. (2016) The Effect of Intra- and Interspecific Competition on Coexistence in Multispecies Communities. *The American Naturalist*, **188**, E1–E12.

Barabás, G., Meszéna, G. & Ostling, A. (2014) Fixed point sensitivity analysis of interacting structured populations. *Theoretical Population Biology*, **92**, 97–106.

Baraloto, C., Timothy Paine, C.E., Patiño, S., Bonal, D., Hérault, B. & Chave, J. (2010) Functional trait variation and sampling strategies in species-rich plant communities. *Functional Ecology*, **24**, 208–216.

Barbier, S., Gosselin, F. & Balandier, P. (2008) Influence of tree species on understory vegetation diversity and mechanisms involved—A critical review for temperate and boreal forests. *Forest Ecology and Management*, **254**, 1–15.

Barton, K. (2015) MuMIn: Multi-model inference. R package version 1.15.6. Available on - line at http://CRAN.R-project.org/package=MuMIn.

Bastias, C.C., Fortunel, C., Valladares, F., Baraloto, C., Benavides, R., Cornwell, W., Markesteijn, L., Oliveira, A.A. de, Sansevero, J.B.B., Vaz, M.C. & Kraft, N.J.B. (2017) Intraspecific leaf trait variability along a boreal-to-tropical community diversity gradient. *PLOS ONE*, **12**, e0172495.

Bates, D.M. (2010) *lme4: Mixed-Effects Modeling with R*. Springer New York.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Eigen, C. & Rcpp, L. (2014) Package “lme4.” *R Foundation for Statistical Computing, Vienna*.

Baythavong, B.S., Agrawal, A.E.A. & McPeek, E.M.A. (2011) Linking the Spatial Scale of Environmental Variation and the Evolution of Phenotypic Plasticity: Selection Favors Adaptive Plasticity in Fine-Grained Environments. *The American Naturalist*, **178**, 75–87.

Beamonte, E. (2009) *Variabilidad Espacial Y Temporal En El Tamaño de Semilla de La Encina Quercus Ilex Subsp. Ballota : Repetibilidad Y Compromisos.* MdC Thesis, Complutense University, Madrid, Spain.

Becker, P. (2000) Competition in the regeneration niche between conifers and angiosperms: Bond’s slow seedling hypothesis. *Functional Ecology*, **14**, 401–412.

Béland, M., Agestam, E., Ekö, P.M., Gemmel, P. & Nilsson, U. (2000) Scarification and Seedfall affects Natural Regeneration of Scots Pine Under Two Shelterwood Densities and a Clear-cut in Southern Sweden. *Scandinavian Journal of Forest Research*, **15**, 247–255.

Bell, G., Lechowicz, M.J. & Waterway, M.J. (2000) Environmental heterogeneity and species diversity of forest sedges. *Journal of Ecology*, **88**, 67–87.

de Bello, F., Carmona, C.P., Mason, N.W.H., Sebastià, M.-T. & Lepš, J. (2013) Which trait dissimilarity for functional diversity: trait means or trait overlap? (ed M Zobel). *Journal of Vegetation Science*, **24**, 807–819.

de Bello, F., Lavorel, S., Albert, C.H., Thuiller, W., Grigulis, K., Dolezal, J., Janeček, Š. & Lepš, J. (2011) Quantifying the relevance of intraspecific trait variability for functional diversity: Intraspecific variability in functional diversity. *Methods in Ecology and Evolution*, **2**, 163–174.

de Bello, F., Thuiller, W., Lepš, J., Choler, P., Clément, J.-C., Macek, P., Sebastià, M.-T. & Lavorel, S. (2009) Partitioning of functional diversity reveals the scale and extent of trait convergence and divergence. *Journal of Vegetation Science*, **20**, 475–486.

Benavides, R., Escudero, A., Coll, L., Ferrandis, P., Ogaya, R., Gouriveau, F., Peñuelas, J. & Valladares, F. (2016) Recruitment patterns of four tree species along elevation gradients in Mediterranean mountains: Not only climate matters. *Forest Ecology and Management*, **360**, 287–296.

Benítez-Malvido, J. & Martínez-Ramos, M. (2003) Impact of Forest Fragmentation on Understory Plant Species Richness in Amazonia. *Conservation Biology*, **17**, 389–400.

Berg, M.P. & Ellers, J. (2010) Trait plasticity in species interactions: a driving force of community dynamics. *Evolutionary Ecology*, **24**, 617–629.

Bergelson, J. (1990) Life After Death: Site Pre-Emption by the Remains of Poa Annua. *Ecology*, **71**, 2157–2165.

Besag, J. (1977) Contribution to the discussion of Dr Ripley's paper. *Journal of the Royal Statistical Society*, **39**.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183–192.

Bond, W.J. (1989) The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. *Biological Journal of the Linnean Society*, **36**, 227–249.

Borges, R.M. (2009) Phenotypic plasticity and longevity in plants and animals: cause and effect? *Journal of Biosciences*, **34**, 605–611.

Brassard, B.W., Chen, H.Y.H., Cavard, X., Laganière, J., Reich, P.B., Bergeron, Y., Paré, D. & Yuan, Z. (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *Journal of Ecology*, **101**, 210–219.

Bray, J.R. & Curtis, J.T. (1957) An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs*, **27**, 325–349.

Breslow, N.E. & Clayton, D.G. (1993) Approximate Inference in Generalized Linear Mixed Models. *Journal of the American Statistical Association*, **88**, 9–25.

Burns, J.H. & Strauss, S.Y. (2012) Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. *Ecology*, **93**.

Calama, R., Manso, R., Barbeito, I., Madrigal, G., Garriga, E., Gordo, J., Montero, G., Cañellas, I. & Pardos, M. (2015) Do inter-specific differences in seed size determine natural regeneration traits in Pinus pinea and Pinus sylvestris? *Applied Ecology and Environmental Research*, **13**.

Callaway, R.M. (2007) *Positive Interactions and Interdependence in Plant | Ragan M. Callaway | Springer*. NewYork:Springer-Verlag.

Callaway, R.M., Pennings, S.C. & Richards, C.L. (2003) Phenotypic Plasticity and Interactions Among Plants. *Ecology*, **84**, 1115–1128.

Callaway, R.M. & Walker, L.R. (1997) Competition and Facilitation: A Synthetic Approach to Interactions in Plant Communities. *Ecology*, **78**, 1958–1965.

Cardinale, B.J., Nelson, K. & Palmer, M.A. (2000) Linking species diversity to the functioning of ecosystems: on the importance of environmental context. *Oikos*, **91**, 175–183.

Carlo, T.A. & Morales, J.M. (2016) Generalist birds promote tropical forest regeneration and increase plant diversity via rare-biased seed dispersal. *Ecology*.

Carlucci, M.B., Debastiani, V.J., Pillar, V.D. & Duarte, L.D.S. (2015) Between- and within-species trait variability and the assembly of sapling communities in forest patches. *Journal of Vegetation Science*, **26**, 21–31.

Carnevale, N.J. & Montagnini, F. (2002) Facilitating regeneration of secondary forests with the use of mixed and pure plantations of indigenous tree species. *Forest ecology and management*, **163**, 217–227.

Carrari, E., Ampoorter, E., Verheyen, K., Coppi, A. & Selvi, F. (2016) Former charcoal kiln platforms as microhabitats affecting understorey vegetation in Mediterranean forests. *Applied Vegetation Science*, **19**, 486–497.

Casalegno, S., Amatulli, G., Bastrup-Birk, A., Houston Durrant, T. & Pekkarinen, A. (2011) Modelling and mapping the suitability of European forest formations at 1-km resolution. , **130**, 971–981.

Chacón-Labella, J., de la Cruz, M. & Escudero, A. (2016) Evidence for a stochastic geometry of biodiversity: the effects of species abundance, richness and intraspecific clustering. *Journal of Ecology*.

Chacón-Labella, J., De la Cruz, M., Vicuña, R., Tapia, K. & Escudero, A. (2014) Negative density dependence and environmental heterogeneity effects on tree ferns across succession in a tropical montane forest. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 52–63.

Chamagne, J., Tanadini, M., Frank, D., Matula, R., Paine, C.E.T., Philipson, C.D., Svátek, M., Turnbull, L.A., Volařík, D. & Hector, A. (2017) Forest diversity promotes individual tree growth in central European forest stands. *Journal of Applied Ecology*, **54**, 71–79.

Chase, J.M. (2014) Spatial scale resolves the niche versus neutral theory debate. *Journal of Vegetation Science*, **25**, 319–322.

Chave, J. (2004) Neutral theory and community ecology: Neutral theory and community ecology. *Ecology Letters*, **7**, 241–253.

Chávez, V. & Macdonald, S.E. (2010) The influence of canopy patch mosaics on understory plant community composition in boreal mixedwood forest. *Forest Ecology and Management*, **259**, 1067–1075.

Chen, L., Wang, L., Baiketuerhan, Y., Zhang, C., Zhao, X. & von Gadow, K. (2014) Seed dispersal and seedling recruitment of trees at different successional stages in a temperate forest in northeastern China. *Journal of Plant Ecology*, **7**, 337–346.

Chesson, P. (2000) Mechanisms of Maintenance of Species Diversity. *Annual Review of Ecology and Systematics*, **31**, 343–366.

Cianciaruso, M.V., Batalha, M.A., Gaston, K.J. & Petchey, O.L. (2009) Including Intraspecific Variability in Functional Diversity. *Ecology*, **90**, 81–89.

Clark, J.S. (2010) Individuals and the Variation Needed for High Species Diversity in Forest Trees. *Science*, **327**, 1129–1132.

Clark, D.A. & Clark, D.B. (1984) Spacing Dynamics of a Tropical Rain Forest Tree: Evaluation of the Janzen-Connell Model. *The American Naturalist*, **124**, 769–788.

Clark, C.J., Poulsen, J.R., Levey, D.J. & Osenberg, C.W. (2007) Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *The American Naturalist*, **170**, 128–142.

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*, 2 edition. Routledge, Hillsdale, N.J.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., Beckman, N. & Zhu, Y. (2014) Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival (ed L Gómez-Aparicio). *Journal of Ecology*, **102**, 845–856.

Condit, R., Ashton, P.S., Baker, P., Bunyavejchewin, S., Gunatilleke, S., Gunatilleke, N., Hubbell, S.P., Foster, R.B., Itoh, A., LaFrankie, J.V., Lee, H.S., Losos, E., Manokaran, N., Sukumar, R. & Yamakura, T. (2000) Spatial Patterns in the Distribution of Tropical Tree Species. *Science*, **288**, 1414–1418.

Connell, J. (1971) *On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forest Trees*. In: Den Boer PJ, Gradwell G (eds) Dynamics of populations. PUDOC, Wageningen.

Conrad, K.F. & Perry, J.N. (2008) The SADIEShell Manual: SADIE Red-Blue Analysis for 32-bit Windows Operating Systems. , 1–37.

Cornelissen, J., Aerts, R., Cerabolini, B., Werger, M. & Heijden, M. van der. (2001) Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*, **129**, 611–619.

Cornelissen, J. h. c., Cerabolini, B., Castro-Díez, P., Villar-Salvador, P., Montserrat-Martí, G., Puyravaud, J. p., Maestro, M., Werger, M. j. a. & Aerts, R. (2003) Functional traits of woody plants: correspondence of species rankings between field adults and laboratory-grown seedlings? *Journal of Vegetation Science*, **14**, 311–322.

Cornwell, W.K. & Ackerly, D.D. (2009) Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs*, **79**, 109–126.

Costanza, J.K., Moody, A. & Peet, R.K. (2011) Multi-scale environmental heterogeneity as a predictor of plant species richness. *Landscape Ecology*, **26**, 851–864.

Courbaud, B., Vieilledent, G. & Kunstler, G. (2012) Intra-specific variability and the competition-colonisation trade-off: coexistence, abundance and stability patterns. *Theoretical Ecology*, **5**, 61–71.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006) Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process. *Science*, **313**, 966–968.

Dawud, S.M., Raulund-Rasmussen, K., Ratcliffe, S., Domisch, T., Finér, L., Joly, F.-X., Hättenschwiler, S. & Vesterdal, L. (2017) Tree species functional group is a more important driver of soil properties than tree species diversity across major European forest types (ed R Ostertag). *Functional Ecology*.

Debat, V. & David, P. (2001) Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology & Evolution*, **16**, 555–561.

Diaz, S., Cabido, M. & Casanoves, F. (1998) Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, **9**, 113–122.

Domínguez, M.T., Aponte, C., Pérez-Ramos, I.M., García, L.V., Villar, R. & Marañón, T. (2012) Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant and Soil*, **357**, 407–424.

EC, (European Commision). (2013) *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions*. A new EU Forest Strategy: for forests and the forest-based sector, Brussels.

EEA. (2007) *European Forest Types. Categories and Types for Sustainable Forest Management Reporting and Policy,* 2nd ed. European Environment Agency,. Copenhagen, EEA Technical Report 09/2006.

Eriksson, O. (1993) The Species-Pool Hypothesis and Plant Community Diversity. *Oikos*, **68**, 371–374.

Escudero, A., Romão, R.L., Cruz, M. & Maestre, F.T. (2005) Spatial pattern and neighbour effects on Helianthemum squamatum seedlings in a Mediterranean gypsum community. *Journal of Vegetation Science*, **16**, 383–390.

Escudero, A. & Valladares, F. (2016) Trait-based plant ecology: moving towards a unifying species coexistence theory: Features of the Special Section. *Oecologia*, **180**, 919–922.

Fajardo, A. & Piper, F.I. (2011) Intraspecific trait variation and covariation in a widespread tree species (Nothofagus pumilio) in southern Chile. *New Phytologist*, **189**, 259–271.

FAO. (2010) Global Forest Resources Assessment 2010.

FAO. (2013) Climate change guidelines for forest managers.

FAO. (2015) Evaluación de los recursos forestales mundiales.

Felten, S. von, Hector, A., Buchmann, N., Niklaus, P.A., Schmid, B. & Scherer-Lorenzen, M. (2009) Belowground Nitrogenc Partitioning in Experimental Grassland Plant Communities of Varying Species Richness. *Ecology*, **90**, 1389–1399.

Fenner, M. & Thompson, K. (2005) *The Ecology of Seeds*. Cambridge University Press.

Fonseca, C.R., Overton, J.M., Collins, B. & Westoby, M. (2000) Shifts in trait-combinations along rainfall and phosphorus gradients. *Journal of Ecology*, **88**, 964–977.

Fortunel, C., Paine, C.E.T., Fine, P.V.A., Kraft, N.J.B. & Baraloto, C. (2014) Environmental factors predict community functional composition in Amazonian forests. *Journal of Ecology*, **102**, 145–155.

Galiano, L., Martínez-Vilalta, J., Eugenio, M., Granzow-de la Cerda, Í. & Lloret, F. (2013) Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy. *Journal of Vegetation Science*, **24**, 580–588.

Galloway, L.F. (1995) Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in Mimulus guttatus. *Evolution*, **49**, 1095–1107.

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., Ruiz-Jaen, M.C., Fröberg, M., Stendahl, J., Philipson, C.D., Mikusiński, G., Andersson, E., Westerlund, B., Andrén, H., Moberg, F., Moen, J. & Bengtsson, J. (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. *Nature Communications*, **4**, 1340.

Garnier, E., Laurent, G., Bellmann, A., Debain, S., Berthelier, P., Ducout, B., Roumet, C. & Navas, M.-L. (2001) Consistency of species ranking based on functional leaf traits. *New Phytologist*, **152**, 69–83.

Gause, G.F. (1934) *The Struggle for Existence.* The Williams and Williams Company, Baltimore.

Gavrilets, S. (2004) *Fitness Landscapes and the Origin of Species*. Princeton Univ. Press, Princeton, NJ.

Gazol, A., Tamme, R., Price, J.N., Hiiesalu, I., Laanisto, L. & Pärtel, M. (2013) A negative heterogeneity–diversity relationship found in experimental grassland communities. *Oecologia*, **173**, 545–555.

Getzin, S., Dean, C., He, F., A Trofymow, J., Wiegand, K. & Wiegand, T. (2006) Spatial patterns and competition of tree species in a Douglas-fir chronosequence on Vancouver Island. *Ecography*, **29**, 671–682.

Getzin, S., Wiegand, T., Wiegand, K. & He, F. (2008) Heterogeneity influences spatial patterns and demographics in forest stands. *Journal of Ecology*, **96**, 807–820.

Gianoli, E. (2004) Plasticity of traits and correlations in two populations of Convolvulus arvensis (Convolvulaceae) differing in environmental heterogeneity. *International Journal of Plant Sciences*, **165**, 825–832.

Gianoli, E. & González-Teuber, M. (2005) Environmental Heterogeneity and Population Differentiation in Plasticity to Drought in Convolvulus Chilensis (Convolvulaceae). *Evolutionary Ecology*, **19**, 603–613.

Givnish, T. (1988) Adaptation to Sun and Shade: a Whole-Plant Perspective. *Australian Journal of Plant Physiology*, **15**, 63.

Gómez, J. (2003) Impact of vertebrate acorn- and seedling-predators on a Mediterranean Quercus pyrenaica forest. *Forest Ecology and Management*, **180**, 125–134.

Gómez, J.M., Puerta-Piñero, C. & Schupp, E.W. (2008) Effectiveness of rodents as local seed dispersers of Holm oaks. *Oecologia*, **155**, 529–537.

Gomez, J.M., Valladares, F. & Puerta-Pinero, C. (2004) Differences between structural and functional environmental heterogeneity caused by seed dispersal. *Functional Ecology*, **18**, 787–792.

Gotelli, N.J. & Chao, A. (2013) Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data. *Encyclopedia of Biodiversity*, pp. 195–211. Elsevier.

Gotelli, N.J. & Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Gotelli, N.J. & Colwell, R.K. (2011) Estimating species richness. *Biological diversity: frontiers in measurement and assessment*, **12**, 39–54.

Götzenberger, L., de Bello, F., Bråthen, K.A., Davison, J., Dubuis, A., Guisan, A., Lepš, J., Lindborg, R., Moora, M., Pärtel, M., Pellissier, L., Pottier, J., Vittoz, P., Zobel, K. & Zobel, M. (2012) Ecological assembly rules in plant communities—approaches, patterns and prospects. *Biological Reviews*, **87**, 111–127.

Granda, E., Escudero, A., de la Cruz, M. & Valladares, F. (2012) Juvenile-adult tree associations in a continental Mediterranean ecosystem: no evidence for sustained and general facilitation at increased aridity (ed V Pillar). *Journal of Vegetation Science*, **23**, 164–175.

Granda, E., Escudero, A. & Valladares, F. (2014) More than just drought: complexity of recruitment patterns in Mediterranean forests. *Oecologia*.

Grubb, P.J. (1977) The Maintenance of Species-Richness in Plant Communities: The Importance of the Regeneration Niche. *Biological Reviews*, **52**, 107–145.

Guariguata, M.R. & Pinard, M.A. (1998) Ecological knowledge of regeneration from seed in neotropical forest trees: Implications for natural forest management. *Forest Ecology and Management*, **112**, 87–99.

Gubsch, M., Buchmann, N., Schmid, B., Schulze, E.-D., Lipowsky, A. & Roscher, C. (2011) Differential effects of plant diversity on functional trait variation of grass species. *Annals of Botany*, **107**, 157–169.

Gurevitch, J. & Fox, G.A. (2006) *The Ecology of Plants, Second Edition*, 2 edition. Sinauer Associates, Sunderland, Mass.

Hardin, G. (1960) The competitive exclusion principle. , **131**, 1292–1297.

Harper, J.L. (1977) *Population Biology of Plants*. Academic Press.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hill, J.L. & Hill, R.A. (2001) Why are tropical rain forests so species rich? Classifying, reviewing and evaluating theories. *Progress in Physical Geography*, **25**, 326–354.

HilleRisLambers, J.H.R., Clark, J.S. & Beckage, B. (2002) Density-dependent mortality and the latitudinal gradient in species diversity. *Nature*, **417**, 732–735.

Hodgson, J.G., Montserrat-Martí, G., Charles, M., Jones, G., Wilson, P., Shipley, B., Sharafi, M., Cerabolini, B.E.L., Cornelissen, J.H.C., Band, S.R., Bogard, A., Castro-Díez, P., Guerrero-Campo, J., Palmer, C., Pérez-Rontomé, M.C., Carter, G., Hynd, A., Romo-Díez, A., de Torres Espuny, L. & Royo Pla, F. (2011) Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany*, **108**, 1337–1345.

Hubbell, S.P. (2001) *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ.

Hubbell, S.P. (2005) Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology*, **19**, 166–172.

Hubbell, S. & Foster, R. (1986) Biology, chance, and history and the structure of tropical rain forest tree communities. *Community Ecology*, eds: Diamond J., Case T.J.). Harper and Row Publishers, pp. 314–329. Harper & Row, New York.

Huber, H., Chen, X., Hendriks, M., Keijsers, D., Voesenek, L.A.C.J., Pierik, R., Poorter, H., de Kroon, H. & Visser, E.J.W. (2012a) Plasticity as a plastic response: how submergence-induced leaf elongation in Rumex palustris depends on light and nutrient availability in its early life stage. *New Phytologist*, **194**, 572–582.

Huber, H., Chen, X., Hendriks, M., Keijsers, D., Voesenek, L.A.C.J., Pierik, R., Poorter, H., Kroon, H. de & Visser, E.J.W. (2012b) Plasticity as a plastic response: how submergence-induced leaf elongation in Rumex palustris depends on light and nutrient availability in its early life stage. *The New Phytologist*, **194**, 572–582.

Hulshof, C.M. & Swenson, N.G. (2010) Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. *Functional Ecology*, **24**, 217–223.

Hulshof, C.M., Violle, C., Spasojevic, M.J., McGill, B., Damschen, E., Harrison, S. & Enquist, B.J. (2013) Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of Vegetation Science*, **24**, 921–931.

Hutchinson, G.E. (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

Iwasa, Y. & Roughgarden, J. (1984) Shoot / root balance of plants: optimal growth of a system with many vegetative organs. *Theoretical Population Biology*, **25**, 78–105.

Jactel, H. & Brockerhoff, E.G. (2007) Tree diversity reduces herbivory by forest insects. *Ecology Letters*, **10**, 835–848.

Janzen, D. (1970) Herbivores and number of tree species in tropical forests. , **104**, 501–528.

Jara-Guerrero, A., De la Cruz, M., Espinosa, C.I., Méndez, M. & Escudero, A. (2015) Does spatial heterogeneity blur the signature of dispersal syndromes on spatial patterns of woody species? A test in a tropical dry forest. *Oikos*, **124**, 1360–1366.

Jennings, S.., Brown, N.. & Sheil, D. (1999) Assessing forest canopies and understorey illumination: canopy closure, canopy cover and other measures. , **72**, 61–73.

John, R., Dalling, J.W., Harms, K.E., Yavitt, J.B., Stallard, R.F., Mirabello, M., Hubbell, S.P., Valencia, R., Navarrete, H., Vallejo, M. & Foster, R.B. (2007)

Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences*, **104**, 864–869.

Jucker, T., Bouriaud, O., Avacaritei, D. & Coomes, D.A. (2014a) Stabilizing effects of diversity on aboveground wood production in forest ecosystems: linking patterns and processes (ed J Knops). *Ecology Letters*, n/a-n/a.

Jucker, T., Bouriaud, O., Avacaritei, D., Dănilă, I., Duduman, G., Valladares, F. & Coomes, D.A. (2014b) Competition for light and water play contrasting roles in driving diversity-productivity relationships in Iberian forests (ed M Turnbull). *Journal of Ecology*, **102**, 1202–1213.

Jung, V., Violle, C., Mondy, C., Hoffmann, L. & Muller, S. (2010) Intraspecific variability and trait-based community assembly. *Journal of Ecology*, **98**, 1134–1140.

Kattge, J., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P.B., Wright, I.J., Cornelissen, J.H.C., Violle, C., Harrison, S.P., Van BODEGOM, P.M., Reichstein, M., Enquist, B.J., Soudzilovskaia, N.A., Ackerly, D.D., Anand, M., Atkin, O., Bahn, M., Baker, T.R., Baldocchi, D., Bekker, R., Blanco, C.C., Blonder, B., Bond, W.J., Bradstock, R., Bunker, D.E., Casanoves, F., Cavender-Bares, J., Chambers, J.Q., Chapin Iii, F.S., Chave, J., Coomes, D., Cornwell, W.K., Craine, J.M., Dobrin, B.H., Duarte, L., Durka, W., Elser, J., Esser, G., Estiarte, M., Fagan, W.F., Fang, J., Fernández-Méndez, F., Fidelis, A., Finegan, B., Flores, O., Ford, H., Frank, D., Freschet, G.T., Fyllas, N.M., Gallagher, R.V., Green, W.A., Gutierrez, A.G., Hickler, T., Higgins, S.I., Hodgson, J.G., Jalili, A., Jansen, S., Joly, C.A., Kerkhoff, A.J., Kirkup, D., Kitajima, K., Kleyer, M., Klotz, S., Knops, J.M.H., Kramer, K., Kühn, I., Kurokawa, H., Laughlin, D., Lee, T.D., Leishman, M., Lens, F., Lenz, T., Lewis, S.L., Lloyd, J., Llusià, J., Louault, F., Ma, S., Mahecha, M.D., Manning, P., Massad, T., Medlyn, B.E., Messier, J., Moles, A.T., Müller, S.C., Nadrowski, K., Naeem, S., Niinemets, Ü., Nöllert, S., Nüske, A., Ogaya, R., Oleksyn, J., Onipchenko, V.G., Onoda, Y., Ordoñez, J., Overbeck, G., Ozinga, W.A., Patiño, S., Paula, S., Pausas, J.G., Peñuelas, J., Phillips, O.L., Pillar, V., Poorter, H., Poorter, L., Poschlod, P., Prinzing, A., Proulx, R., Rammig, A., Reinsch, S., Reu, B., Sack, L., Salgado-Negret, B., Sardans, J., Shiodera, S., Shipley, B., Siefert, A., Sosinski, E., Soussana, J.-F., Swaine, E., Swenson, N., Thompson, K., Thornton, P., Waldram, M., Weiher, E., White, M., White, S., Wright, S.J., Yguel, B., Zaehle, S., Zanne, A.E. & Wirth, C. (2011) TRY – a global database of plant traits. *Global Change Biology*, **17**, 2905–2935.

Kawecki, T.J. (2008) Adaptation to Marginal Habitats. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 321–342.

Kazakou, E., Violle, C., Roumet, C., Navas, M.-L., Vile, D., Kattge, J. & Garnier, E. (2014) Are trait-based species rankings consistent across data sets and spatial scales? (ed V Pillar). *Journal of Vegetation Science*, **25**, 235–247.

Kelty, M.J. (2006) The role of species mixtures in plantation forestry. *Forest Ecology and Management*, **233**, 195–204.

Kenkel, N.C. (1988) Pattern of Self-Thinning in Jack Pine: Testing the Random Mortality Hypothesis. *Ecology*, **69**, 1017–1024.

Kerkhoff, A.J., Fagan, W.F., Elser, J.J., Enquist, B.J., Grover, A.E.J.P. & Losos, E.J.B. (2006) Phylogenetic and Growth Form Variation in the Scaling of Nitrogen and Phosphorus in the Seed Plants. *The American Naturalist*, **168**, E103–E122.

Kichenin, E., Wardle, D.A., Peltzer, D.A., Morse, C.W. & Freschet, G.T. (2013) Contrasting effects of plant inter- and intraspecific variation on community-level trait measures along an environmental gradient. *Functional Ecology*, **27**, 1254–1261.

Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. & Callaway, R.M. (2005) Linking patterns and processes in alpine plant communities: a global study. *Ecology*, **86**, 1395–1400.

Knoke, T., Ammer, C., Stimm, B. & Mosandl, R. (2008) Admixing broadleaved to coniferous tree species: a review on yield, ecological stability and economics. *European Journal of Forest Research*, **127**, 89–101.

Kraft, N.J.B., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M., Boyle, B., Anderson, M.J., Cornell, H.V., Davies, K.F., Freestone, A.L., Inouye, B.D., Harrison, S.P. & Myers, J.A. (2011) Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients. *Science*, **333**, 1755–1758.

Kraft, N.J.B., Crutsinger, G.M., Forrestel, E.J. & Emery, N.C. (2014) Functional trait differences and the outcome of community assembly: an experimental test with vernal pool annual plants. *Oikos*, **123**, 1391–1399.

Kraft, N.J.B., Godoy, O. & Levine, J.M. (2015) Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences*, **112**, 797–802.

Kraft, N.J., Valencia, R. & Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, **322**, 580–582.

Kumordzi, B.B., de Bello, F., Freschet, G.T., Le Bagousse-Pinguet, Y., Lepš, J. & Wardle, D.A. (2015) Linkage of plant trait space to successional age and species richness in boreal forest understorey vegetation (ed D Gibson). *Journal of Ecology*, **103**, 1610–1620.

Kumordzi, B.B., Nilsson, M.-C., Gundale, M.J. & Wardle, D.A. (2014) Changes in local-scale intraspecific trait variability of dominant species across contrasting island ecosystems. *Ecosphere*, **5**, 1–17.

Kuuluvainen, T., Penttinen, A., Leinonen, K. & Nygren, M. (1996) Statistical opportunities for comparing stand structural heterogeneity in managed and primeval forests: an example from boreal spruce forest in southern Finland.

Laanisto, L., Tamme, R., Hiiesalu, I., Szava-Kovats, R., Gazol, A. & Pärtel, M. (2013) Microfragmentation concept explains non-positive environmental heterogeneity-diversity relationships. *Oecologia*, **171**, 217–226.

Lake, J.K. & Ostling, A. (2009) Comment on "Functional traits and niche-based tree community assembly in an Amazonian forest." *Science*, **324**, 1015–1015.

Lamanna, C., Blonder, B., Violle, C., Kraft, N.J.B., Sandel, B., imova, I., Donoghue, J.C., Svenning, J.-C., McGill, B.J., Boyle, B., Buzzard, V., Dolins, S., Jorgensen, P.M., Marcuse-Kubitza, A., Morueta-Holme, N., Peet, R.K., Piel, W.H., Regetz, J., Schildhauer, M., Spencer, N., Thiers, B., Wiser, S.K. & Enquist, B.J. (2014) Functional trait space and the latitudinal diversity gradient. *Proceedings of the National Academy of Sciences*, **111**, 13745–13750.

Lasky, J.R., Bachelot, B., Muscarella, R., Schwartz, N.B., Forero-Montaña, J., Nytch, C.J., Swenson, N.G., Thompson, J., Zimmerman, J.K. & Uriarte, M. (2015) Ontogenetic shifts in trait-mediated mechanisms of plant community assembly. *Ecology*.

Latham, R.E. (1992) Co-Occurring Tree Species Change Rank in Seedling Performance with Resources Varied Experimentally. *Ecology*, **73**, 2129–2144.

Laughlin, D.C., Fulé, P.Z., Huffman, D.W., Crouse, J. & Laliberté, E. (2011) Climatic constraints on trait-based forest assembly. *Journal of Ecology*, **99**, 1489–1499.

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N.S.G., Garden, D., Dorrough, J., Berman, S., Quétier, F., Thébault, A. & Bonis, A. (2008) Assessing functional diversity in the field – methodology matters! *Functional Ecology*, **22**, 134–147.

Lázaro-Nogal, A., Matesanz, S., Godoy, A., Pérez-Trautman, F., Gianoli, E. & Valladares, F. (2015) Environmental heterogeneity leads to higher plasticity in dry-edge populations of a semi-arid Chilean shrub: insights into climate change responses. *Journal of Ecology*, **103**, 338–350.

Le Bagousse-Pinguet, Y., de Bello, F., Vandewalle, M., Leps, J. & Sykes, M.T. (2014) Species richness of limestone grasslands increases with trait overlap: evidence from within- and between-species functional diversity partitioning. *Journal of Ecology*, **102**, 466–474.

Le Bagousse-Pinguet, Y., Börger, L., Quero, J.L., García-Gómez, M., Soriano, S., Maestre, F.T. & Gross, N. (2015) Traits of neighbouring plants and space limitation determine intraspecific trait variability in semi-arid shrublands. *Journal of Ecology*, **103**, 1647–1657.

Legendre, P. (2015) lmodel2: Model II Regression. R package version 1.7-2.

Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000) *The Evolutionary Ecology of Seed Size*. In: Fenner M. (ed.), Seeds: the ecology of regeneration in plant communities, 2nd Edition. CAB International,.

Lepš, J., de Bello, F., Šmilauer, P. & Doležal, J. (2011) Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography*, **34**, 856–863.

Leps, J., De Bello, F., Lavorel, S. & Berman, S. (2006) Quantifying and interpreting functional diversity of natural communities: practical considerations matter. *Preslia*, **78**, 481–501.

Levin, S.A. (1992) The problem of pattern and scale in ecology. *Ecology*, **73**, 1943–1967.

Levine, J.M. & HilleRisLambers, J. (2009) The importance of niches for the maintenance of species diversity. *Nature*, **461**, 254–257.

Levine, J.M. & Murrell, D.J. (2003) The Community-Level Consequences of Seed Dispersal Patterns. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 549–574.

Ligot, G., Ameztegui, A., Courbaud, B., Coll, L. & Kneeshaw, D. (2016) Tree light capture and spatial variability of understory light increase with species mixing and tree size heterogeneity. *Canadian Journal of Forest Research*, **46**, 968–977.

Ligot, G., Balandier, P., Fayolle, A., Lejeune, P. & Claessens, H. (2013) Height competition between Quercus petraea and Fagus sylvatica natural regeneration in mixed and uneven-aged stands. *Forest Ecology and Management*, **304**, 391–398.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M.J. & Marchetti, M. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management*, **259**, 698–709.

Lipowsky, A., Roscher, C., Schumacher, J., Michalski, S.G., Gubsch, M., Buchmann, N., Schulze, E.-D. & Schmid, B. (2015) Plasticity of functional traits of forb species in response to biodiversity. *Perspectives in Plant Ecology, Evolution and Systematics*, **17**, 66–77.

Lipowsky, A., Schmid, B. & Roscher, C. (2011) Selection for monoculture and mixture genotypes in a biodiversity experiment. *Basic and Applied Ecology*, **12**, 360–371.

Long, W., Zang, R., Schamp, B.S. & Ding, Y. (2011) Within- and among-species variation in specific leaf area drive community assembly in a tropical cloud forest. *Oecologia*, **167**, 1103–1113.

Lookingbill, T.R. & Zavala, M.A. (2000) Spatial pattern of *Quercus ilex* and *Quercus pubescens* recruitment in *Pinus halepensis* dominated woodlands. *Journal of Vegetation Science*, **11**, 607–612.

Loosmore, N.B. & Ford, E.D. (2006) Statistical Inference Using the G or K Point Pattern Spatial Statistics. *Ecology*, **87**, 1925–1931.

Lundholm, J.T. (2009) Plant species diversity and environmental heterogeneity: spatial scale and competing hypotheses. *Journal of Vegetation Science*, **20**, 377–391.

Lundholm, J.T. & Larson, D.W. (2003) Relationships between spatial environmental heterogeneity and plant species diversity on a limestone pavement. *Ecography*, **26**, 715–722.

Lusk, C.H. & Matus, F. (2000) Juvenile tree growth rates and species sorting on fine-scale soil fertility gradients in a Chilean temperate rain forest. *Journal of Biogeography*, **27**, 1011–1020.

Lusk, C.H., Wright, I. & Reich, P.B. (2003) Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats. *New Phytologist*, **160**, 329–336.

MacArthur, R. & Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *American naturalist*, 377–385.

Maestre, F.T. & Cortina, J. (2002) Spatial patterns of surface soil properties and vegetation in a Mediterranean semi-arid steppe. *Plant and Soil*, **241**, 279–291.

Maestre, F.T., Escudero, A., Martinez, I., Guerrero, C. & Rubio, A. (2005) Does spatial pattern matter to ecosystem functioning? Insights from biological soil crusts. *Functional Ecology*, **19**, 566–573.

Maire, V., Gross, N., Hill, D., Martin, R., Wirth, C., Wright, I.J. & Soussana, J.-F. (2013) Disentangling Coordination among Functional Traits Using an Individual-Centred Model: Impact on Plant Performance at Intra- and Inter-Specific Levels. *PLOS ONE*, **8**, e77372.

Major, J.E. & Johnsen, K.H.; (1996) *Family Variation in Photosynthesis of 22-Year-Old Black Spruce: A Test of Two Models of Physiological Response to Water Stress*.

Markesteijn, L., Poorter, L. & Bongers, F. (2007) Light-dependent leaf trait variation in 43 tropical dry forest tree species. *American Journal of Botany*, **94**, 515–525.

Masaki, T., Osumi, K., Takahashi, K., Hoshizaki, K., Matsune, K. & Suzuki, W. (2007) Effects of microenvironmental heterogeneity on the seed-to-seedling process and tree coexistence in a riparian forest. *Ecological Research*, **22**, 724–734.

Mayfield, M.M. & Levine, J.M. (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters*, **13**, 1085–1093.

McElhinny, C., Gibbons, P., Brack, C. & Bauhus, J. (2005) Forest and woodland stand structural complexity: Its definition and measurement. *Forest Ecology and Management*, **218**, 1–24.

McGill, B.J. (2010) Towards a unification of unified theories of biodiversity: Towards a unified unified theory. *Ecology Letters*, **13**, 627–642.

McGill, B., Enquist, B., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178–185.

McIntire, E.J.B. & Fajardo, A. (2009) Beyond description: the active and effective way to infer processes from spatial patterns. *Ecology*, **90**, 46–56.

McIntire, E.J.B. & Fajardo, A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, **201**, 403–416.

MEA, (Millennium Ecosystem Assessment). (2005) Ecosystems and Human Well -being: Synthesis.

Mediavilla, S. & Escudero, A. (2004) Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. *Forest Ecology and Management*, **187**, 281–294.

Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838–848.

Miller, P.C. & Stoner, W.A. (1979) Canopy Structure and Environmental Interactions. *Topics in Plant Population Biology* (eds O.T. Solbrig, S. Jain, G.B. Johnson & P.H. Raven), pp. 428–458. Macmillan Education UK.

Mitchell, R.M. & Bakker, J.D. (2014) Intraspecific Trait Variation Driven by Plasticity and Ontogeny in Hypochaeris radicata. *PLoS ONE*, **9**, e109870.

Moeur, M. (1993) Characterizing Spatial Patterns of Trees Using Stem-Mapped Data. *Forest Science*, **39**, 756–775.

Moles, A.T. & Westoby, M. (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology*, **92**, 372–383.

Molina-Montenegro, M.A., Atala, C. & Gianoli, E. (2010) Phenotypic plasticity and performance of Taraxacum officinale (dandelion) in habitats of contrasting environmental heterogeneity. *Biological Invasions*, **12**, 2277–2284.

Moll, J.D. & Brown, J.S. (2008) Competition and coexistence with multiple life-history stages. *The American Naturalist*, **171**, 839–843.

Morin, X., Fahse, L., Scherer-Lorenzen, M. & Bugmann, H. (2011) Tree species richness promotes productivity in temperate forests through strong complementarity between species: Species richness promotes forest productivity. *Ecology Letters*, **14**, 1211–1219.

Mouillot, D., Bellwood, D.R., Baraloto, C., Chave, J., Galzin, R., Harmelin-Vivien, M., Kulbicki, M., Lavergne, S., Lavorel, S., Mouquet, N., Paine, C.E.T., Renaud, J. & Thuiller, W. (2013) Rare Species Support Vulnerable Functions in High-Diversity Ecosystems (ed GM Mace). *PLoS Biology*, **11**, e1001569.

Mouillot, D., Stubbs, W., Faure, M., Dumay, O., Tomasini, J.A., Wilson, J.B. & Chi, T.D. (2005) Niche overlap estimates based on quantitative functional traits: a new family of non-parametric indices. *Oecologia*, **145**, 345–353.

Muller-Landau, H.C., Wright, S.J., Calderón, O., Condit, R. & Hubbell, S.P. (2002) Assessing recruitment limitation: Concepts, methods and case-studies from a tropical forest., p. 35–53. In D. J. Levey, W. Silva, and M. Galetti (Eds). Seed dispersal and frugivory: Ecology, evolution, and conservation, CABI Publishing, Wallingford, UK.

Myers, J.A., Chase, J.M., Jiménez, I., Jørgensen, P.M., Araujo-Murakami, A., Paniagua-Zambrana, N. & Seidel, R. (2013) Beta-diversity in temperate and tropical forests reflects dissimilar mechanisms of community assembly. *Ecology Letters*, **16**, 151–157.

Myers, J.A. & Harms, K.E. (2011) Seed arrival and ecological filters interact to assemble high-diversity plant communities. *Ecology*, **92**, 676–686.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models (ed RB O'Hara). *Methods in Ecology and Evolution*, **4**, 133–142.

Narwani, A., Alexandrou, M.A., Oakley, T.H., Carroll, I.T. & Cardinale, B.J. (2013) Experimental evidence that evolutionary relatedness does not affect the ecological mechanisms of coexistence in freshwater green algae (ed L De Meester). *Ecology Letters*, **16**, 1373–1381.

Nathan, J., von Hardenberg, J. & Meron, E. (2013) Spatial instabilities untie the exclusion-principle constraint on species coexistence. *Journal of Theoretical Biology*, **335**, 198–204.

Nathan, R. & Muller-Landau, H.C. (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. , **15**, 278–285.

Nicotra, A.B., Chazdon, R.L. & Iriarte, S.V.B. (1999) Spatial Heterogeneity of Light and Woody Seedling Regeneration in Tropical Wet Forests. *Ecology*, **80**, 1908–1926.

Niinemets, Ü. (2002) Stomatal conductance alone does not explain the decline in foliar photosynthetic rates with increasing tree age and size in Picea abies and Pinus sylvestris. *Tree Physiology*, **22**, 515–535.

Niinemets, Ü. (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management*, **260**, 1623–1639.

Niinemets, U. & Kull, O. (1995) Effects of light availability and tree size on the architecture of assimilative surface in the canopy of Picea abies: variation in needle morphology. *Tree Physiology*, **15**, 307–315.

Niinemets, Ü. & Valladares, F. (2006) Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological monographs*, **76**, 521–547.

Olson, M. g. & Wagner, R. g. (2011) Factors affecting species richness of tree regeneration in mixed-wood stands of central Maine. *Journal of Vegetation Science*, **22**, 303–311.

Pacala, S.W. & Tilman, D. (1994) Limiting Similarity in Mechanistic and Spatial Models of Plant Competition in Heterogeneous Environments. *The American Naturalist*, **143**, 222–257.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404**, 278–281.

Paine, C.E.T., Baraloto, C., Chave, J. & Hérault, B. (2011) Functional traits of individual trees reveal ecological constraints on community assembly in tropical rain forests. *Oikos*, **120**, 720–727.

Paquette, A. & Messier, C. (2011) The effect of biodiversity on tree productivity: from temperate to boreal forests: The effect of biodiversity on the productivity. *Global Ecology and Biogeography*, **20**, 170–180.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E. & others. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.

Pérez-Ramos, I.M. & Marañón, T. (2012) Community-level seedling dynamics in Mediterranean forests: uncoupling between the canopy and the seedling layers. *Journal of Vegetation Science*, **23**, 526–540.

Perry, J.N. (1998) Measures of Spatial Pattern for Counts. *Ecology*, **79**, 1008–1017.

Perry, J.N. & Dixon, P.M. (2002) A new method to measure spatial association for ecological count data. *Écoscience*, **9**, 133–141.

Perry, G.L.W., Enright, N.J., Miller, B.P. & Lamont, B.B. (2008) Spatial patterns in species-rich sclerophyll shrublands of southwestern Australia. *Journal of Vegetation Science*, **19**, 705–716.

Perry, G., Enright, N.J., Miller, B.P. & Lamont, B.B. (2009) Nearest-neighbour interactions in species-rich shrublands: the roles of abundance, spatial patterns and resources.

Perry, G.L.W., Miller, B.P. & Enright, N.J. (2006) A comparison of methods for the statistical analysis of spatial point patterns in plant ecology. *Plant Ecology*, **187**, 59–82.

Perry, G.L.W., Miller, B.P., Enright, N.J. & Lamont, B.B. (2014) Stochastic geometry best explains spatial associations among species pairs and plant functional types in species-rich shrublands. *Oikos*, **123**, 99–110.

Perry, G.L.W., Miller, B.P., Lamont, B.B. & Enright, N.J. (2016) Community-level spatial structure supports a model of stochastic geometry in species-rich shrublands. *Oikos*, n/a-n/a.

Pescador, D.S., de Bello, F., Valladares, F. & Escudero, A. (2015) Plant Trait Variation along an Altitudinal Gradient in Mediterranean High Mountain Grasslands: Controlling the Species Turnover Effect. *PLoS ONE*, **10**, e0118876.

Pigliucci, M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore, MD: The Johns Hopkins University Press.

Pinheiro, J., Bates, D., DebRoy, S, Sarkar, D & R Core Team. (2015) nlme: Linear and nonlinear mixed effects models. *R package version 3.1-120*.

van der Plas, F., Manning, P., Allan, E., Scherer-Lorenzen, M., Verheyen, K., Wirth, C., Zavala, M.A., Hector, A., Ampoorter, E., Baeten, L., Barbaro, L., Bauhus, J., Benavides, R., Benneter, A., Berthold, F., Bonal, D., Bouriaud, O., Bruelheide, H., Bussotti, F., Carnol, M., Castagneyrol, B., Charbonnier, Y., Coomes, D., Coppi, A., Bastias, C.C., Muhie Dawud, S., De Wandeler, H., Domisch, T., Finér, L., Gessler, A., Granier, A., Grossiord, C., Guyot, V., Hättenschwiler, S., Jactel, H., Jaroszewicz, B., Joly, F.-X., Jucker, T., Koricheva, J., Milligan, H., Müller, S., Muys, B., Nguyen, D., Pollastrini, M., Raulund-Rasmussen, K., Selvi, F., Stenlid, J., Valladares, F., Vesterdal, L., Zielínski, D. & Fischer, M. (2016) Jack-of-all-trades effects drive biodiversity–ecosystem multifunctionality relationships in European forests. *Nature Communications*, **7**, 11109.

Platnick, N.I. (1991) Patterns of biodiversity: tropical vs temperate. *Journal of Natural History*, **25**, 1083–1088.

Plotkin, J.B., Potts, M.D., leslie, N., Manokaran, N., LaFrankie, J. & Ashton, P. (2000) Species-area Curves, Spatial Aggregation, and Habitat Specialization in Tropical Forests. *Journal of Theoretical Biology*, **207**, 81–99.

Pommerening, A. (2016) Spatial Analysis. A Guide for Ecologists. A1 - M. R. T. Dale and A1 - M.-J. Fortin (2014). 2nd edition. Cambridge, UK: PB - Cambridge University Press . 438 pages, ISBN: 9780521143509. *Biometrical Journal*, **58**, 242–243.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P. & Mommer, L. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control: Tansley review. *New Phytologist*, **193**, 30–50.

Portsmuth, A. & Niinemets, Ü. (2007) Structural and physiological plasticity in response to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. *Functional ecology*, **21**, 61–77.

Pretzsch, H. & Biber, P. (2016) Tree species mixing can increase maximum stand density [1]. *Canadian Journal of Forest Research*, **46**, 1179–1193.

Puerta-Piñero, C., Gómez, J.M. & Valladares, F. (2007) Irradiance and oak seedling survival and growth in a heterogeneous environment. *Forest Ecology and Management*, **242**, 462–469.

Pugnaire, F., Haase, P. & Puigdefábregas, J. (1996) Facilitation between higher plant species in a semiarid environment. *Ecology*, **77**, 1420–1426.

Quilchano, C., Marañón, T., Pérez-Ramos, I.M., Noejovich, L., Valladares, F. & Zavala, M.A. (2008) Patterns and ecological consequences of abiotic heterogeneity in managed cork oak forests of Southern Spain. *Ecological Research*, **23**, 127–139.

R Development Core Team. (2013) *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing.

R Development Core Team. (2015) R: a language and environment for statistical computing. Text. URL http://www.gbif.org/resource/81287 [accessed 7 April 2015]

Ramírez-Valiente, J.A., Valladares, F., Delgado Huertas, A., Granados, S. & Aranda, I. (2011) Factors affecting cork oak growth under dry conditions: local adaptation and contrasting additive genetic variance within populations. *Tree Genetics & Genomes*, **7**, 285–295.

Rao, C.R. & Toutenburg, H. (1995) *Linear Models: Least Squares and Alternatives.* Springer-Verlag, New York, Inc.

Ratcliffe, S., Liebergesell, M., Ruiz-Benito, P., Madrigal González, J., Muñoz Castañeda, J.M., Kändler, G., Lehtonen, A., Dahlgren, J., Kattge, J., Peñuelas, J., Zavala, M.A. & Wirth, C. (2016) Modes of functional biodiversity control on tree productivity across the European continent: Functional biodiversity control on tree growth. *Global Ecology and Biogeography*, **25**, 251–262.

Reich, P.B. (2014) The world-wide "fast–slow" plant economics spectrum: a traits manifesto. *Journal of Ecology*, **102**, 275–301.

Ricklefs, R.E. (1977) Environmental heterogeneity and plant species diversity: a hypothesis. *The American Naturalist*, **111**, 376–381.

Ricklefs, R.E. & He, F. (2016) Region effects influence local tree species diversity. *Proceedings of the National Academy of Sciences*, **113**, 674–679.

Rietkerk, M. & Koppel, J. van de. (2008) Regular pattern formation in real ecosystems. *Trends in Ecology & Evolution*, **23**, 169–175.

Rohr, R.P., Saavedra, S., Peralta, G., Frost, C.M., Bersier, L.-F., Bascompte, J. & Tylianakis, J.M. (2016) Persist or produce: a community trade-off tuned by species evenness.

Roscher, C., Schmid, B., Buchmann, N., Weigelt, A. & Schulze, E.-D. (2011) Legume species differ in the responses of their functional traits to plant diversity. *Oecologia*, **165**, 437–452.

Rothe, A. & Binkley, D. (2001) Nutritional interactions in mixed species forests: a synthesis. *Canadian Journal of Forest Research*, **31**, 1855–1870.

Rozendaal, D.M.A., Hurtado, V.H. & Poorter, L. (2006) Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology*, **20**, 207–216.

Ruiz-Benito, P., Madrigal-González, J., Ratcliffe, S., Coomes, D.A., Kändler, G., Lehtonen, A., Wirth, C. & Zavala, M.A. (2014) Stand Structure and Recent Climate Change Constrain Stand Basal Area Change in European Forests: A Comparison Across Boreal, Temperate, and Mediterranean Biomes. *Ecosystems*, **17**, 1439–1454.

Saavedra, S., Rohr, R.P., Bascompte, J., Godoy, O., Kraft, N.J.B. & Levine, J.M. (2017) A structural approach for understanding multispecies coexistence. *Ecological Monographs*.

Sack, L. & Grubb, P.J. (2001) Why do species of woody seedlings change rank in relative growth rate between low and high irradiance? *Functional Ecology*, **15**, 145–154.

Sack, L., Grubb, P.J. & Marañón, T. (2003) The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecology*, **168**, 139–163.

Salgado-Luarte, C. & Gianoli, E. (2010) Herbivory on Temperate Rainforest Seedlings in Sun and Shade: Resistance, Tolerance and Habitat Distribution (ed J Chave). *PLoS ONE*, **5**, e11460.

Sanchez-Gomez, D., Valladares, F., Zavala, M.A. & others. (2006) Functional traits and plasticity in response to light in seedlings of four lberian forest tree species. *TREE PHYSIOLOGY-VICTORIA-*, **26**, 1425.

Santiso, X. & Retuerto, R. (2015) Low among-provenance differences in structural and functional plasticity in response to nutrients in saplings of the circum-Mediterranean tree *Arbutus unedo* L. (ed J Pereira). *Tree Physiology*, **35**, 1118–1128.

Sardans, J., Peñuelas, J. & Rodà, F. (2006) Plasticity of leaf morphological traits, leaf nutrient content, and water capture in the Mediterranean evergreen oak Quercus ilex subsp. ballota in response to fertilization and changes in competitive conditions. *Ecoscience*, **13**, 258–270.

Schiffers, K., Schurr, F.M., Tielbörger, K., Urbach, C., Moloney, K. & Jeltsch, F. (2008) Dealing with virtual aggregation - a new index for analysing heterogeneous point patterns. *Ecography*, **31**, 545–555.

Schlatter, D.C., Bakker, M.G., Bradeen, J.M. & Kinkel, L.L. (2015) Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*, **96**, 134–142.

Schreeg, L.A., Kobe, R.K. & Walters, M.B. (2005) Tree seedling growth, survival, and morphology in response to landscape-level variation in soil resource availability in northern Michigan. *Canadian Journal of Forest Research*, **35**, 263–273.

Schupp, E.W., Jordano, P. & Gómez, J.M. (2010) Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist*, **188**, 333–353.

Schupp, E.W., Milleron, T. & Russo, S.E. (2002) Dissemination Limitation and the Origin and Maintenance of Species-Rich Tropical Forests. In book: Seed Dispersal and Frugivory: Ecology, Evolution and Conservation, Publisher: CABI Publishing. *ResearchGate*, 19–33.

Seabloom, E.W., Bjørnstad, O.N., Bolker, B.M. & Reichman, O.J. (2005) Spatial Signature of Environmental Heterogeneity, Dispersal, and Competition in Successional Grasslands. *Ecological Monographs*, **75**, 199–214.

Seidler, T.G. & Plotkin, J.B. (2006) Seed Dispersal and Spatial Pattern in Tropical Trees (ed R Nathan). *PLoS Biology*, **4**, e344.

Shibata, M., Tanaka, H. & Nakashizuka, T. (1998) Causes and Consequences of Mast Seed Production of Four Co-Occurring Carpinus Species in Japan. *Ecology*, **79**, 54.

Shorrocks, B. & Sevenster, J.G. (1995) Explaining Local Species Diversity. *Proceedings: Biological Sciences*, **260**, 305–309.

Siefert, A. (2012) Incorporating intraspecific variation in tests of trait-based community assembly. *Oecologia*, **170**, 767–775.

Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., Aarssen, L.W., Baraloto, C., Carlucci, M.B., Cianciaruso, M.V., de L. Dantas, V., de Bello, F., Duarte, L.D.S., Fonseca, C.R., Freschet, G.T., Gaucherand, S., Gross, N., Hikosaka, K., Jackson, B., Jung, V., Kamiyama, C., Katabuchi, M., Kembel, S.W., Kichenin, E., Kraft, N.J.B., Lagerström, A., Bagousse-Pinguet, Y.L., Li, Y., Mason, N., Messier, J., Nakashizuka, T., Overton, J.M., Peltzer, D.A., Pérez-Ramos, I.M., Pillar, V.D., Prentice, H.C., Richardson, S., Sasaki, T., Schamp, B.S., Schöb, C., Shipley, B., Sundqvist, M., Sykes, M.T., Vandewalle, M. & Wardle, D.A. (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities (ed J Chase). *Ecology Letters*, **18**, 1406–1419.

Spasojevic, M.J. & Suding, K.N. (2012) Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes: *Functional diversity along gradients*. *Journal of Ecology*, **100**, 652–661.

Stein, A., Gerstner, K. & Kreft, H. (2014) Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales (ed H Arita). *Ecology Letters*, **17**, 866–880.

Stevens, G.C. (1989) The Latitudinal Gradient in Geographical Range: How so Many Species Coexist in the Tropics. *The American Naturalist*, **133**, 240–256.

Stoll, P. & Prati, D. (2001) Intraspecific Aggregation Alters Competitive Interactions in Experimental Plant Communities. *Ecology*, **82**, 319.

Stoll, P. & Weiner, J. (2000) A neighborhood view of interactions among individual plants. *The geometry of ecological interactions: Simplifying spatial complexity*, 11–27.

Suffice, P., Joanisse, G., Imbeau, L., Mazerolle, M.J. & Lessard, G. (2015) Short-term effects of irregular shelterwood cutting on yellow birch regeneration and habitat use by snowshoe hare. *Forest Ecology and Management*, **354**, 160–169.

Sultan, S.E. (1987) Evolutionary Implications of Phenotypic Plasticity in Plants. *Evolutionary Biology* (eds M.K. Hecht, B. Wallace & G.T. Prance), pp. 127–178. Springer US.

Sultan, S.E. (2000) Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, **5**, 537–542.

Svensson, E.I., Eroukhmanoff, F. & Friberg, M. (2006) Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution*, **60**, 1242–1253.

Tamme, R., Hiiesalu, I., Laanisto, L., Szava-Kovats, R. & Pärtel, M. (2010) Environmental heterogeneity, species diversity and co-existence at different spatial scales. *Journal of Vegetation Science*.

Thornley, J.H.M. (1972) A Balanced Quantitative Model for Root: Shoot Ratios in Vegetative Plants. *Annals of Botany*, **36**, 431–441.

Tilman, D. (1988) *Plant Strategies and the Dynamics and Structure of Plant Communities*, Princeton. Princeton University Press, Princeton, USA.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. (1997) The Influence of Functional Diversity and Composition on Ecosystem Processes. *Science*, **277**, 1300–1302.

Tirado, R. & Pugnaire, F.I. (2003) Shrub spatial aggregation and consequences for reproductive success. *Oecologia*, **136**, 296–301.

Umaña, M.N., Zhang, C., Cao, M., Lin, L. & Swenson, N.G. (2015) Commonness, rarity, and intraspecific variation in traits and performance in tropical tree seedlings (ed K Suding). *Ecology Letters*, **18**, 1329–1337.

Urbieta, I.R., García, L.V., Zavala, M.A. & Marañón, T. (2011) Mediterranean pine and oak distribution in southern Spain: Is there a mismatch between regeneration and adult distribution? *Journal of Vegetation Science*, **22**, 18–31.

Valladares, F., Bastias, C.C., Godoy, O., Granda, E. & Escudero, A. (2015) Species coexistence in a changing world. *Frontiers in Plant Science*, **6**.

Valladares, F., Gianoli, E. & Gómez, J.M. (2007) Ecological limits to plant phenotypic plasticity. *New Phytologist*, **176**, 749–763.

Valladares, F. & Guzmán, B. (2006) Canopy structure and spatial heterogeneity of understory light in an abandoned Holm oak woodland. *Annals of Forest Science*, **63**, 749–761.

Valladares, F., Martinez-Ferri, E., Balaguer, L., Pérez-Corona, E. & Manrique, E. (2000a) Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytologist*, **148**, 79–91.

Valladares, F. & Niinemets, Ü. (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 237–257.

Valladares, F. & Pearcy, R.W. (1999) The geometry of light interception by shoots of Heteromeles arbutifolia: morphological and physiological consequences for individual leaves. *Oecologia*, **121**, 171–182.

Valladares, F., Wright, S.J., Lasso, E., Kitajima, K. & Pearcy, R.W. (2000b) Plastic Phenotypic Response to Light of 16 Congeneric Shrubs from a Panamanian Rainforest. *Ecology*, **81**, 1925.

Van Kleunen, M. & Fischer, M. (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist*, **166**, 49–60.

Vaz, M., Maroco, J., Ribeiro, N., Gazarini, L.C., Pereira, J.S. & Chaves, M.M. (2011) Leaf-level responses to light in two co-occurring Quercus (Quercus ilex and Quercus suber): leaf structure, chemical composition and photosynthesis. *Agroforestry Systems*, **82**, 173–181.

Velázquez, E., Martínez, I., Getzin, S., Moloney, K.A. & Wiegand, T. (2016) An evaluation of the state of spatial point pattern analysis in ecology. *Ecography*, **39**, 1042–1055.

Vilà, M., Vayreda, J., Comas, L., Ibáñez, J.J., Mata, T. & Obón, B. (2007) Species richness and wood production: a positive association in Mediterranean forests. *Ecology Letters*, **10**, 241–250.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution*, **27**, 244–252.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007) Let the concept of trait be functional! *Oikos*, **116**, 882–892.

Walter, H. (1985) *Vegetation of the Earth*. Springer-Verlag, Berlin, Germany.

Wang, X., Wiegand, T., Kraft, N.J., Swenson, N.G., Davies, S.J., Hao, Z., Howe, R., Lin, Y., Ma, K., Mi, X. & others. (2016) Stochastic dilution effects weaken deterministic effects of niche-based processes in species rich forests. *Ecology*, **97**, 347–360.

Warton, D.I., Wright, I.J., Falster, D.S. & Westoby, M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews*, **81**, 259–291.

Watson, M., Geber, M. & Jones. (1995) Ontogenetic contingency and the expression of plant plasticity. *Trends in Ecology & Evolution*, **10**, 474–475.

Watt, A.S. (1947) Pattern and process in the plant community. *Journal of ecology*, **35**, 1–22.

Weiher, E. (2011) A primer of trait and functional diversity. *Biological diversity: frontiers in measurement and assessment*, p. 175–193.

Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. & Bentivenga, S. (2011) Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **366**, 2403–2413.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, **199**, 213–227.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: Some Leading Dimensions of Variation Between Species. *Annual Review of Ecology and Systematics*, **33**, 125–159.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J., Lonsdorf, E.V., Allan, G.J., DiFazio, S.P., Potts, B.M., Fischer, D.G., Gehring, C.A., Lindroth, R.L., Marks, J.C., Hart, S.C., Wimp, G.M. & Wooley, S.C. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics*, **7**, 510–523.

Whitmore, T.C. (1996) *A Review of Some Aspects of Tropical Rain Forest Seedling Ecology with Suggestions for Further Enquiry*. UNESCO, Paris, France, and also by Parthenon Publishing Group Inc., New York, USA.

Wiegand, T., Huth, A., Getzin, S., Wang, X., Hao, Z., Gunatilleke, C.V.S. & Gunatilleke, I.A.U.N. (2012) Testing the independent species' arrangement assertion made by theories of stochastic geometry of biodiversity. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 3312–3320.

Wiegand, T. & Moloney, K. (2014) *Handbook of Spatial Point-Pattern Analysis in Ecology*, Boca Raton. CRC Press.

Wiens, J. (2000) *Ecological Heterogeneity: An Ontogeny of Concepts and Approaches*. Stewart AJA, John EA, Hutchings MJ (eds) The ecological consequences of environmental heterogeneity. Blackwell, Oxford.

Wijesinghe, D.K. & Hutchings, M.J. (1999) The effects of environmental heterogeneity on the performance of Glechoma hederacea: the interactions between patch contrast and patch scale. *Journal of Ecology*, **87**, 860–872.

Williamson, G.B. (1975) Pattern and Seral Composition in an Old-growth Beech-Maple Forest. *Ecology*, **56**, 727–731.

Wills, C., Harms, K.E., Condit, R., King, D., Thompson, J., He, F., Muller-Landau, H.C., Ashton, P., Losos, E., Comita, L., Hubbell, S., Lafrankie, J., Bunyavejchewin, S., Dattaraja, H.S., Davies, S., Esufali, S., Foster, R., Gunatilleke, N., Gunatilleke, S., Hall, P., Itoh, A., John, R., Kiratiprayoon, S., de Lao, S.L., Massa, M., Nath, C., Noor, M.N.S., Kassim, A.R., Sukumar, R., Suresh, H.S., Sun, I.-F., Tan, S., Yamakura, T. & Zimmerman, J. (2006) Nonrandom processes maintain diversity in tropical forests. *Science (New York, N.Y.)*, **311**, 527–531.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.

Wyka, T.P., Oleksyn, J., Żytkowiak, R., Karolewski, P., Jagodziński, A.M. & Reich, P.B. (2012) Responses of leaf structure and photosynthetic properties to intra-canopy light gradients: a common garden test with four broadleaf deciduous angiosperm and seven evergreen conifer tree species. *Oecologia*, **170**, 11–24.

Yankelevich, S.N., Fragoso, C., Newton, A.C., Russell, G. & Heal, O.W. (2006) Spatial Patchiness of Litter, Nutrients and Macroinvertebrates during Secondary Succession in a Tropical Montane Cloud Forest in Mexico. *Plant and Soil*, **286**, 123–139.

Yeboah, D., Chen, H.Y.H. & Kingston, S. (2016) Tree species richness decreases while species evenness increases with disturbance frequency in a natural boreal forest landscape. *Ecology and Evolution*, **6**, 842–850.

Zeugin, F., Potvin, C., Jansa, J. & Scherer-Lorenzen, M. (2010) Is tree diversity an important driver for phosphorus and nitrogen acquisition of a young tropical plantation? *Forest Ecology and Management*, **260**, 1424–1433.

Zhang, Y., Chen, H.Y.H. & Reich, P.B. (2012) Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *Journal of Ecology*, **100**, 742–749.

Zuppinger-Dingley, D., Schmid, B., Petermann, J.S., Yadav, V., De Deyn, G.B. & Flynn, D.F.B. (2014) Selection for niche differentiation in plant communities increases biodiversity effects. *Nature*, **505**, 108–111.

AFILIACIÓN DE COAUTORES

Christopher Baraloto
INRA, UMR "Ecologie des Forets de Guyane", Kourou Cedex 97387, French Guiana.

Cristina C. Bastias
Departamento de Biogeografía y Cambio Global. Museo Nacional de Ciencias Naturales- CSIC, Madrid, Spain.

Raquel Benavides
Departamento de Biogeografía y Cambio Global. Museo Nacional de Ciencias Naturales- CSIC, Madrid, Spain.

William Cornwell
Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia.

Marcelino de la Cruz
Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain.

Alexandre A. de Oliveira
Departamento de Ecologia, Universidade de São Paulo, São Paulo, SP, Brasil.

Claire Fortunel
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, United States.

Nathan J. B. Kraft
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, United States.

Lars Markesteijn
Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá.

Department of Zoology, University of Oxford, South Parks Road, Oxford, United Kingdom.

School of Environment, Natural Resources and Geography, Bangor University, Bangor LL57 2DG, United Kingdom.

Teresa Morán-López
Laboratorio Ecotono, INIBIOMA-CONICET, Universidad Nacional del Comahue, Bariloche, Argentina.

Natalia Ricote M.
Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

Jerónimo B.B Sansevero
Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Jardim Botânico, Rio de Janeiro, Brazil.

Universidade Federal Rural do Rio de Janeiro-UFRRJ. Departamento de Ciencias Ambientais-DCA. Instituto de Florestas-, Seropédica, Rio de Janeiro, Brazil

Daniel A. Truchado
Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain.

Fernando Valladares
Departamento de Biogeografía y Cambio Global. Museo Nacional de Ciencias Naturales- CSIC, Madrid, Spain

Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain

Marcel C. Vaz
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, United States

AGRADECIMIENTOS

Toca escribir las últimas líneas de esta tesis pero más que una Tesis doctoral, esto ha sido una etapa de vida, en realidad mi vida durante los últimos cinco años y medio. Y aquí, echada en la cama, intento hacer recuento y pienso en todos los momentos que durante estos años he vivido, cuanta gente distinta he conocido, cuantos lugares he visitado por muestreos o por congresos, cuantas risas, cuantos lloros o cuantos enfados, y es que esto es una montaña rusa a veces tocas el cielo y otras veces piensas qué hago yo en este mundo pero desde luego sea cual sea el sentimiento, lo que está claro que esto ha sido una experiencia tan inmesa e intensa que no la cambiaría por nada.

Y esto no hubiese sido posible sin la confianza que depositaron en mí tres personas, las cuales han ido marcando mi rumbo en esta etapa. Fernando Valladares, mi director de tesis, muchas gracias por esta oportunidad y por hacer la tesis doctoral que comencé hace cinco años una realidad. Y es que, te aseguro que esto me lo dicen a mí cuando estaba llorando en tu despacho a la primera semana de llegar y no me lo creo. Pero sí, ¡aquí está!. Muchas gracias por sacar lo mejor de mí, por toda la caña, por iniciarme en el camino de la investigación y por hacerme ver que todavía queda mucho camino y que hay que luchar hasta el final y que si te caes, te levantas. Raquel Benavides, mi codirectora, muchas gracias a ti también por tu confianza desde el primer momento que hablé contigo por teléfono en esa entrevista y por darme la oportunidad de hoy poder escribir estas líneas. Eres un ejemplo para mí de perseverancia, de trabajo, eres una luchadora que demuestra que pasito a pasito las cosas se consiguen y encima con esa risa siempre tan escandalosa jaja. Gracias por tu ánimo en los momentos de bajón, por hacerme seguir adelante, por transmitirme tu conocimiento y experiencia de vida y sobre todo por estar siempre al pie del cañón. Sonia Rabasa, muchas gracias a ti también por tu confianza depositada en mí en esa fase inicial, por impulsarme en esa fase y contribuir a que iniciara mi camino en la investigación.

Y a la planta 8 llegué como dice mi amigo David en una etapa de transición en el laboratorio, entre gente que también había llegado nueva como yo en su nuevo postdoc y por tanto con muchísima más experiencia, entre gente que prácticamente acababa la tesis y que se iría fuera de postdoc, gente que había empezado la tesis meses antes que yo, pero desde luego yo era la última que empezaba a hacer su tesis de esa generación hasta que un par de años más adelante empezará de nuevo el flujo de predoctorales. Y eso en verdad, me hace sólo estar agradecida de la oportunidad de haber conocido a tanta gente buena como persona y como científicos. Me gustaría agradercer esta tesis a mis dos compañeros y amigos que sin ellos nada de esto hubiese sido posible, David López e Inés Carrascón. David, millones de gracias por tu dedicación a que esto saliese adelante, hubiese sido imposible sin tu ayuda y esfuerzo. Pero no sólo en el trabajo, sino también te doy las gracias por tus enseñanzas de vida, sigo la historia corta que un día saliendo me contaste del queso y unos ratones jeje. Que sepas que me llevo lo mejor de tí y de todos los momentos que hemos vivido muy buenos en el Alto Tajo con el coche cuando nos quedamos atrapados en el río, en Rumanía cuando estábamos hasta las narices uno del otro o en Finlandia haciendo kayak y mil más que podría contar. Simplemente aunque haya pasado y pasará mucha gente por el laboratorio, espero que recuerdes que ninguna se despertará más tarde que tú jajaja. Inésss, me encanta tu forma de ver la vida y de hacer las cosas, te estoy enormemente agradecida por tu esfuerzo y dedicación y que sin tí tampoco hubiese sido posible esta tesis. Recuerdo cuando nos perdíamos por Alemania en nuestro primer viaje con otros grupos y me decías que era muy pipiola cuando no tenia ni idea de que hacer jaja, hay mil aventuras que hemos vivido y eso estará guardado eternamente en mi corazón. Sólo decirte que esta tesis se debe principalmente a tí. ¡Mil gracias!.

Pero en la planta 8 hay muchísima más gente que debo agradecer y que esta etapa no hubiese tenido gracia ni hubiese sido lo mismo sin ellos. Silvia Matesanz, ¿has visto como he crecido? Tú que cuando llegue me decias que sólo tenía 12 años jajaja, muchas gracias por servirme de inspiración porque con tu referencia uno confía que con esfuerzo y trabajo al final se llega muy lejos. Elena Granda otro gran referente de buena científica y siempre con esa gran sonrisa que llena de vida aquel sitio donde va. Teresa Gimeno, aunque casi no coindicimos trabajando, siempre me quedaré con tu simpatía y buena educación para todo y con ese día en el campo en el que me mostraste que hay que ser un buen jabalí jaja. A Elena Concepción, por transmitirme tranquilidad y fuerza ante todo. A Anita, por su vitalidad y entusiasmo para hacer cualquier cosa y un gran ejemplo de lucha para defender tus ideas. A Dulce mi referente para saber como funcionaban las cosas y estar dispuesta siempre a ayudarme, ¡mil gracias!. A Tere Morán por todos los momentos divertidos que me has hecho pasar, todas las risas que me has sacado, esas charlas en el despacho, por escucharme y por estar ahí para aconsejarme. A Ali, ejemplo de fuerza y perseverancia, te doy las gracias por estar ahí siempre que te he necesitado, por cuidar de nosotros y hacer que las cosas sean mucho más fáciles, también por escucharme y porque aparte de compañeras siempre seremos amigas. A Curi, por hacerme ver que científico y sencillez van de la mano, se echará de menos tu acento de Usera chungo jajaj ¡que me encanta!. A Ana Rey, porque eres un ejemplo para mí de cómo hay que transmitir todo lo que se hace con estusiamo y viviéndolo al 100%. A Xandra y Jorge porque son únicos, porque son unas de las mejores personas que he conocido y soy muy afortunada de tenerlos como amigos, os quiero mucho y mil gracias por todo vuestro apoyo, consejos, ánimo y por estar siempre ahí. A Sandra por ser un ejemplo para mí de mujer luchadora y por encima de todo por sus ganas de querer mejorar el mundo. A Dani porque me lo paso genial hablando contigo de cualquier cosa y hace mucho más ameno mis horas de trabajo. A Ana Heres por su sencillez y su sabiduría que hace que fácil mantener una conversación de cualquier tema. A Bárbara porque aunque la conozco de hace sólo un año y medio ya ha ocupado un lugar muy grande en mi vida y sabes que será para siempre, gracias por estar ahí siempre que te necesito. A Pablo por su creatividad, por su entusiasmo para hacer cualquier cosa y siempre por estar pendiente de mi y transmitirme toda la energía positiva. A Omar, por tratar siempre las cosas con mucha amabilidad y tranquilidad, y por supuesto también por corregirme siempre que hablo mal jeje. A Mario por esas clases de identificación de pájaros durante los viajes que siempre quise aprender y que nunca pude. A David Montero por ayudarme a analizar las miles de fotos hemisféricas y por hacer más fácil todo el tema de papeles con la administración. A Esteban por brindarnos con sus conocimientos todos los miércoles en el lunch meeting. A Paula por su simpatía y siempre tan amable ante cualquier situación.

También quiero agredecer su enorme contribución aunque haya sido en un corto periodo de tiempo a Naty por ser siempre tan positiva, por todos los buenos momentos que vivimos en Madrid, ¡te echo mucho de menos!. A Jero y Alice por toda la ayuda en el campo y todas las horas empleadas para que saliese adelante el trabajo, sois una pareja genial. Guido por todas las risas, todos los buenos momentos y sufrir codo con codo el final de la tesis. También quería agradecer a Borja, Irene, Margot, Javi, Matheus, Sara, Senna, Ciro, Juan Mentruyt, María Almagro por haber aportado su granito de arena para que esta tesis saliese adelante.

Una experiencia como científica que jamás olvidaré fueron mis días de estancia en EEUU en el laboratorio de Nathan Kraft, me hicieron sentir como en casa, como parte de su familia y me ayudaron en todo lo psible humanamente además de conocer gente con una calidad científica exquisita. Les doy las gracias a Nathan y a Claire Fortunel por dedicarme parte de su tiempo a mejorar con científica y a colaborar y abrir vínculos con otros investigadores. También tengo que agradecer a Marcel Vaz, Gaurav Kandlikar e Ian McFadden por acogerme y hacerme parte de su grupo, incluso en días importantes como el Día de Acción de Gracias.

Y durante mis años en Madrid he tenido la suerte de compartir piso con gente genial y que ya después de tantos años se han convertido en verdaderas amigas. Les tengo que agradecer a ellas, Lucía, Isa, Pastora, Joana y Laura, por escuchar mis historietas cada vez que llego del trabajo, por animarme cuando estoy de bajón y ayudarme a seguir adelante, por aconsejarme cuando he tenido dudas y en definitiva por compartir y disfrutar de todos los ratitos que hemos pasado juntas.

Pero, la otra mitad de mi corazón ha estado siempre en Córdoba donde tengo mi familia y mis amigos de toda la vida, donde están mis raíces. Quería dedicar esta tesis especialmente a José porque desde un primer momento confiaste en mí y hoy día último de la tesis puedo decir lo mismo, sólo tengo palabras de agradecimiento para tí, has estado ahí pasase lo que pasase para animarme, para apoyarme, para hacerme ver que hay un final, para hacer cualquier plan y despejarme en los días malos, para ayudarme en el invernadero levantándote a las tantas de la madrugada o para acompañarme a EEUU. Mil gracias por todo, sabes que siempre siempre siempre estaré aquí. Quería especialmente agradecer a Mª Jesús, sabes que te quiero muchísimo y eres una de las personas más importantes de mi vida, gracias por estar ahí siempre apoyándome en todo, escuchándome y animándome en cualquier situación. Sabes sacar una sonrisa en cualquier circunstancia. A Lucía y Mª Luisa mis dos grandes mejores amigas, sois geniales, sin vuestro apoyo y ánimo esto hubiese sido mucho más complicado. Gracias por escuchar mis bajones y mis aventuras. Gracias por hacerme desconectar de todos los momentos malos con miles de planes e historias, con miles de risas y aventuras. Os quiero muchísimo. También quería agradecer a Tomás porque sabe perfectamente lo que es compartir la montaña rusa de lo que es hacer una tesis jeje, muchas gracias por esas conversaciones. A Fran, Lourdes, Rafa, Cristina, Pablo, Irene, por todos esos buenos ratos que pasamos que hacen especial cada momento. También quería agradecer a mis compis de uni Alex y Sandra por apoyarme siempre, por estar siempre ahí que os necesito aunque estemos lejos y no podamos compartir todos los ratos que solíamos, pero la buena amistad perdura en el tiempo. También quería dar las gracias a profesores de la Universidad de Córdoba como Rafael Villar, Diego Jordano y Joaquín que han hecho posible que pueda empezar en el mundo de la docencia, que me han aconsejado y que me han ayudado a mejorar en mi carrera profesional.

Y finalmente, el mayor apoyo para empezar, continuar y terminar esta tesis han sido mis padres. Mamá y Papá sabéis que yo no soy muy de mostrar a menudo mis sentimientos pero aquí quería haceros saber que esta tesis y que yo como persona os lo debo a vosotros. Quería daros las gracias por vuestra dedicación durante estos 30 años, por haberme dado lo mejor dentro de vuestras posibilidades, de haber renunciado a cosas solo para que yo pudiera estar un poco mejor, de haberme apoyado hasta el final en cualquier decisión y hacerme pensar en aquellas otras que no eran las correctas. Gracias por vuestra paciencia, cariño y amor en esta etapa y por animarme en todos los momentos y sobre todo CREER EN MÍ. Mamá y hermano gracias por dedicar muchas horas de vuestro tiempo en ayudarme a que yo estuviera menos agobiada con el trabajo, os agradezco muchísimo vuestra ayuad en el invernadero rellenando macetas, pesando hojas, clasificando muestras o preparando las muestras para los análisis en el laboratorio. También Mamá a tí por tu dedicación haciéndome miles de comidas para que no tuviera que cocinar durante todo este tiempo, aunque eso nos ha costado más de una pelea, te doy las gracias por tu eterna dedicación a mí para que estuviera un poco mejor aquí. ¡OS QUIERO!. También quería dedicar por último esta tesis a mi abuelo Clemente que aunque hace ya unos años que no está con nosotros yo siento que me acompaña cada día, por su confianza plena en mí desde pequeña y por quererme tanto.

Por último, quería hacer una mención especial para agradecer también aquellos que haciéndome esta etapa muy difícil y dura me han hecho darme cuenta de la fuerza interior que tengo para seguir adelante y cumplir mi sueño.

“Son muchas las manos y los corazones que contribuyen al éxito de una persona”
- Walt Disney-.

Hay muchas veces en que el hecho de encontrar
una respuesta es menos importante que
el de haber sido capaz de vivir a fondo la pregunta,
de avanzar ansiosamene por las pistas
que tiende a abrir en nosotros

Julio Cortázar