



ELSEVIER

Contents lists available at ScienceDirect

# Environmental and Experimental Botany

journal homepage: [www.elsevier.com/locate/envexpbot](http://www.elsevier.com/locate/envexpbot)

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

Cristina C. Bastias<sup>a,\*</sup>, Fernando Valladares<sup>a,b</sup>, Natalia Ricote M.<sup>c,d</sup>, Raquel Benavides<sup>a</sup><sup>a</sup> Departamento de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales, MNCN-CSIC, Madrid, Spain<sup>b</sup> Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain<sup>c</sup> Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile<sup>d</sup> Centro de Ecología Aplicada y Sustentabilidad (CAPES), Pontificia Universidad Católica de Chile, Santiago 6513677, Chile

### ARTICLE INFO

#### Keywords:

Species richness  
Light treatment  
Nutrient treatment  
Trait variation  
Functional groups  
Phenotypic plasticity

### ABSTRACT

Species diversity seems to favour the environmental heterogeneity and habitat complexity, which in turn favour the selection of greater levels of phenotypic plasticity of plant species. While recent evidence suggested that local species diversity and environmental heterogeneity act as selective pressures over mean traits and plasticity for herbs, which adjust their traits to the identity and species richness of their neighbors, little is known for tree species. Here, we investigated shifts in the phenotypic expression and plasticity of seedlings of two functional groups (broadleaved and conifers) coming from communities with different canopy species richness in two contrasting forest types (a hemiboreal and a Mediterranean forest). We carried out a greenhouse experiment to test the response of seedlings to different light and nutrient availability, measuring different functional traits related to resource acquisition. Our results indicated that seedlings coming from monospecific and more diverse communities had similar phenotypic expression and response capacity to the study treatments. This finding suggests that canopy species richness did not exert a detectable selective pressure on tree phenotypes and plasticity at this early stage. Additionally, we found great differences in the phenotypic expression and plasticity between the two major functional groups (conifers and broadleaved species). Specifically, we found that broadleaves reached higher mean values in key functional traits by a greater plasticity to both light and nutrient treatment than coniferous species, mainly in the Mediterranean forest. This finding suggests that broadleaved species potentially have a higher capacity to respond to future changing environmental conditions than conifers at early stages, conferring an advantage that can be crucial for the species competitive ability for resources and, therefore, for survival.

### 1. Introduction

In forest ecosystems, trees are exposed to multiple environmental factors varying considerably in space and time (Valladares et al., 2007). Phenotypic plasticity is an important and well-known mechanism by which individual plants are able to cope with environmental heterogeneity, adjusting their phenotypes (i.e. functional traits) to shifts in the environment (Pigliucci, 2001). Thus, the ecological range of species may be partly determined by the capacity of their individuals to show plastic responses to the environment (Sultan, 2001; Valladares et al., 2014, 2007). Moreover, individuals rarely present the same plastic response, even within species. Indeed, broad evidence shows differentiation in plasticity patterns at different organization levels such as functional groups, species and populations (Balaguer et al., 2001; Sardans et al., 2006; Vaz et al., 2011; Wyka et al., 2012). Divergence in

the plasticity of traits among individuals may be promoted by the functional environmental heterogeneity (i.e. heterogeneity functionally relevant for a plant; Gomez et al., 2004) (Alpert and Simms, 2002; Gianoli and González-Teuber, 2005). Some theoretical studies suggested that greater levels of functional environmental heterogeneity should select for higher plastic responses (reviewed by Matesanz et al., 2010; Pigliucci, 2001), with empirical evidence for some key ecological factors such as heterogeneity in light (De Kroon et al., 2009, 2005; Pugnaire and Valladares, 1999) or nutrient availability (De Kroon et al., 2009; Wijesinghe and Hutchings, 1999)

A forest attribute that can trigger environmental heterogeneity is species richness. As the number of species increases, the number of interactions among species also raises, leading to complex networks where multiple ecological factors and trophic levels interact (Rzanny and Voigt, 2012). For instance, plant species diversity alters soil

\* Corresponding author.

E-mail addresses: [crbasc@gmail.com](mailto:crbasc@gmail.com), [cristina.crespo@mncn.csic.es](mailto:cristina.crespo@mncn.csic.es) (C.C. Bastias).<https://doi.org/10.1016/j.envexpbot.2018.08.023>

Received 26 April 2018; Received in revised form 14 August 2018; Accepted 17 August 2018

Available online 22 August 2018

0098-8472/ © 2018 Elsevier B.V. All rights reserved.

bacterial community compositions and, subsequently, the nutrient abundance within the rhizosphere (Schlatter et al., 2015). It is based on the species-specific modification of the ecological conditions on its influence area, at both above- and below-ground levels (Rothe and Binkley, 2001; Yankelevich et al., 2006). The creation of heterogeneous conditions reciprocally favours community species diversity by providing different niches to be filled by different species (Stein et al., 2014; Tews et al., 2004). As a result, the relationship between species richness and environmental heterogeneity becomes positively reciprocal. Assuming more environmental heterogeneity in species-rich communities, plasticity of functional traits may be very advantageous for plants. It may be advantageous as adjustments of traits in response to the local biotic diversity may reduce competition among heterospecifics leading to a better use of the available resources and an increase in species complementarity (Aschehoug and Callaway, 2014; Ashton et al., 2010; Berg and Ellers, 2010; Callaway et al., 2003; Levins, 1979). Moreover, if plants reach optimal plant performance and fitness under these heterogeneous environmental conditions thanks to plastic responses, then plasticity may become adaptive, and hence, subject for selection (Matesanz et al., 2010; Pigliucci, 2001). While several studies have reported both local species diversity and environmental heterogeneity as selective pressures over mean traits and plasticity for herbaceous species (Gubsch et al., 2011; Lipowsky et al., 2015; Roscher et al., 2011; Zuppinger-Dingley et al., 2014), little is known for long-lived species such as trees.

In this study, we investigated the phenotypic expression (i.e. trait means in each environment) and phenotypic plasticity patterns of tree seedlings coming from communities with different canopy richness in response to two contrasted light and nutrient availabilities. Specifically, we hypothesized that plants from species-rich communities have evolved in more heterogeneous sites, where increased plasticity would be a target of selection, and therefore, we expect higher plasticity in comparison to plants coming from monospecific communities. Additionally, differential plastic responses among plants from communities differing in species richness could be also reflected in different phenotypic expressions (i.e. trait mean values) in each environment with different resource availability. To test this hypothesis, we collected seeds from tree species belonging to two different functional groups (conifers and broadleaved species) in communities with different species richness levels in the canopy, namely monospecific, mixture of 2–3 species and mixture of 4–5 species, in two contrasting European forests. Then, we carried out a common garden experiment, in which plants, keeping the identification of their origin community, grew in three environments differing in light and nutrient availability. We measured different functional traits associated with light and nutrient acquisition to address the following questions. 1) Do seedlings from communities varying in canopy richness differ in the phenotypic expression (i.e. trait means in each environment) as well as in phenotypic plasticity in response to light and nutrient availability? 2) Do the plastic responses of seedlings have any pattern according to their functional group and type of forest?

## 2. Materials and methods

### 2.1. Background experimental design and seed collection

We collected seeds from dominant tree species growing naturally in two contrasting European forests, a hemiboreal forest in the Białowieża Park, Poland (52.7°N, 23.9°E) and a continental Mediterranean-mixed forest located in the Alto Tajo Natural Park, Spain (40.7°N, 1.9°W) (Table A1 in Supplementary material). The hemiboreal forest encompassed five tree dominant species: Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.) as coniferous species and pedunculate oak (*Quercus robur* L.), silver birch (*Betula pendula*) and hornbeam (*Carpinus betulus* L.) as broadleaved species. And, the Mediterranean forest includes Scots pine (*P. sylvestris*) and European

black pine (*Pinus nigra* Arn.) as conifers, and holm oak (*Quercus ilex* L.) and Portuguese oak (*Quercus faginea* Lam.) as broadleaved species. For this study, we used three out of five tree dominant species (*P. sylvestris*, *P. abies* and *Q. robur*) from the hemiboreal forest and all dominant tree species from the Mediterranean forest.

Seeds from each selected species and forest type were collected from 2 to 6 individuals in different plots, which encompassed three different canopy diversity levels of the dominant tree species in each forest: monocultures (hereafter ‘monospecific canopy’), plots with 2 and 3 species coexisting (hereafter ‘medium canopy diversity’) and plots with 4 and 5 coexisting species (hereafter ‘high canopy diversity’) (Table A2 in Supplementary material). In the Mediterranean forest, seed production of broadleaf species was so poor in high-diverse plots the year of collection, that we only collected seeds of conifers. After collection, we pooled seeds from different individuals collected in a given plot and stored them to 4 °C until the beginning of the experiment.

The plots from where the seeds were sampled are part of a broader network of plots designed within the European project FunDivEUROPE ([www.fundiveurope.eu](http://www.fundiveurope.eu)) to test the role of biodiversity on ecosystem functioning. Particularly, plots were set under two main criteria: i) each study tree 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). More information about the experimental design can be found in Baeten et al., (2013).

A brief description of the averages of the main environmental characteristics for each selected forest, in terms of light, soil properties and others, such as altitude or mean annual precipitation and temperature, can be found in the Table A1 in Supplementary material.

### 2.2. 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. We sowed a total of 1540 seeds of broadleaved species (423 seeds of *Q. ilex*, 666 seeds of *Q. faginea* and 451 seeds of *Q. robur*) in pots of 1.6 L, and 8845 seeds of coniferous species (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) in trays of 32 single-cells (400 cc; 61 × 59 × 200 mm). Pots and trays were 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 and single-cell with the forest type, plot ID (associated with the canopy richness level), species and individual ID. We placed the pots and trays randomly in the greenhouse, regularly moved to avoid microsite effects. Plants were grown under favorable controlled conditions of light and water during two and a half months for broadleaved and three and a half months for coniferous species. The number of seedlings germinated and suitable for the oncoming experiment totalled 754 plants of broadleaved (213 plants of *Q. ilex*, 387 plants of *Q. faginea* and 154 plants of *Q. robur*) and 1991 plants of conifers (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 A2 in Supplementary material for details about sample sizes per forest, species and canopy richness level.

Then, plants were divided into three groups to apply different conditions in light and nutrient supply, two key abiotic factors for plants with high temporal and spatial heterogeneity in natural conditions. Specifically, the conditions exerted on each group were: i) a sun-limited environment with 20% of full sunlight radiation ( $\sim 490 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (‘Shade environment’); ii) a control environment, meaning 50% of full sunlight radiation ( $\sim 1350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and basic nutrient supply provided by the peat moss (0.045 g N/l – 0.03 g P/l – 0.09 g K/l); and iii) a high nutrient-supplied environment, receiving 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

**Table 1**  
Overview of functional traits measured in the study.

	Units	Trait description	Trait functions	Species
<b>Growth-related trait</b>				
Relative growth rate (RGR)	cm/ month	Plant height per unit time: $RGR = (\ln H_2 - \ln H_1) / (t_2 - t_1)$ , where $H_2$ and $H_1$ is plant height at the end and at the beginning of the experiment, respectively, and $t_2 - t_1$ is the duration of the experiment.	Plant growth efficiency related to environmental conditions. Surrogate of 'plant fitness' for long-lived species.	Broadleaved and coniferous
<b>Architecture-related traits</b>				
Height	cm	Elongation from the soil surface to the upper boundary of the main photosynthetic tissue of the plant.		Broadleaved and coniferous
No. of leaves	-	Number of leaves per plant.		Broadleaved
Internode length	cm	Plant height divided by the number of leaves.	Competitive vigour to capture vertical light.	Broadleaved Coniferous
Crown height	cm	Distance above the cotyledons to the node of the most recent fully expanded leaf.		
<b>Morphological traits</b>				
Leaf size (LA)	mm <sup>2</sup>	One-side leaf lamina area.		Broadleaved and coniferous
Specific leaf area (SLA)	mm <sup>2</sup> /mg	Ratio between leaf area and dry mass.	Related to plant ecological strategy: tradeoff between acquisition and conservation of resources.	Broadleaved and coniferous
Leaf dry matter content (LDMC)	mg/g	Ratio between leaf dry mass and the leaf saturated mass.		Broadleaved and coniferous
<b>Physiological traits</b>				
Maximum photochemical efficiency of PSII ( $F_v/F_m$ )	-	Maximum quantum efficiency of PSII electron transport in the light.	Indicator of the physiological status and related to plant growth.	Broadleaved and coniferous
Stomatal conductance	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}^{-1}$	Rate of gas exchange and transpiration through the leaf stomata as determined by the degree of stomatal aperture.	Indicator of the environmental stress tolerance of the plant, mainly to water.	Broadleaved

/l of a Plantacote mix 8/9-month slow-release fertilizer 14-8-15 N-P-K, (Plantacote plus, Projar, Spain) ('High nutrient environment'). Summarizing, we tested the phenotypic expression and plasticity of seedlings in response to two independent treatments: a shading treatment (from 50% vs 20% of full radiation, i.e. from  $\sim 1350 \mu\text{mol m}^{-2} \text{ s}^{-1}$  to  $\sim 490 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and a nutrient treatment (from 0.045 g 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) (Fig. A1 in Supplementary material). Throughout the experiment, all plants were regularly well watered, and also temperature and humidity were recorded every 1 min with temperature and humidity (4–20 mA) sensors (Sistemas electrónicos Progres SA, Lleida, España). The mean day temperature (8 a.m. – 10 p.m.) was  $22.4 \pm 1.7^\circ\text{C}$  and the air humidity of  $49.2 \pm 10.5\%$ .

### 2.3. Functional traits measurements

We measured a set of traits at the end of experiment (after 3.5 months) associated with the plant fitness and distinct aspects of the plant functioning and ecological strategy (physiology, morphology), particularly those that have a strong relationship with the functional significance for resource acquisition (Pérez-Harguindeguy et al., 2013) (Table 1). Specific details on trait measurement protocol can be found in the supplementary material (Appendix B).

### 2.4. Statistical analysis

We assessed the phenotypic expression and plasticity separately for each treatment (light and nutrient treatment) and forest type (hemiboreal and Mediterranean forests) since both forests differed in species and richness levels. We performed linear mixed-effects models (Bates, 2010) for each particular functional trait, with canopy species richness (SR; monospecific -1 species-, medium -2/3 species mixture- and high -4/5 species mixture), functional group (FG; broadleaved vs conifers) and treatment (Tr; control vs. shade or control vs. high nutrient), and all possible double interactions, as fixed factors. Finally, we included 'Plot' and 'Species' as random factors to account for the intrinsic characteristics of each plot and species identity.

Trait  $\sim$  Tr x SR + Tr x FG + FG x SR + (1|Plot) + (1|Species).

We considered a significant treatment effect as evidence for plasticity in the trait (e.g. Matesanz and Milla, 2018). A significant effect of SR would indicate differences in the mean trait values of seedlings coming from different canopy richness (i.e. dissimilarity in the phenotypic expression). A significant Tr x SR interaction would imply different responses of seedlings coming from communities with different canopy richness levels to the experimental treatments (i.e. non-parallel reaction norms for a given trait). A significant Tr x FG interaction would indicate differences in plastic responses to treatments between broadleaved and coniferous species. When double interactions were significant, we ran posthoc analyses to test differences in the slope means between groups.

All statistical analyses were carried out in R v. 3.2.2 (R Development Core Team, 2015) using the packages 'lme4' (Bates et al., 2014) and 'lsmeans' (Lenth, 2016).

## 3. Results

### 3.1. Trait expression and plasticity

Seedlings were unequally affected by treatments, that is, their phenotypic expression was more affected by nutrient than by light availability in both forests (Tables 2 and 3). In general, seedlings under higher nutrient availability were significantly taller with larger crowns and larger leaves with lower LDMC (Table 3; Figs. 1 and 2). Additionally, seedlings from the Mediterranean forest under a high

**Table 2**

Results of the linear mixed models testing the plasticity and phenotypic expression (trait means) of different functional traits to the light availability per forest type (hemiboreal and Mediterranean forest).

	FG df = 1	SR df = 2	Tr df = 1	SR x FG df = 2	Tr x FG df = 1	Tr x SR df = 2
<b>(a) Hemiboreal</b>						
<b>1. Growth-related trait</b>						
RGR	1.89	0.58	2.46	1.29	0.28	0.75
<b>2. Architecture-related traits</b>						
Height	19.21†	1.70	0.01	4.67	<b>6.99**</b>	0.70
Crown height	–	0.58	1.01	–	–	1.47
No. of leaves	–	0.32	1.06	–	–	0.48
Internode length	–	–	–	–	–	–
<b>3. Morphology leaf traits</b>						
Leaf area	11.43	0.37	<b>9.19**</b>	<b>5.80**</b>	<b>21.90***</b>	1.09
SLA	5.47	1.38	<b>14.74***</b>	0.17	1.09	2.55
LDMC	<b>18.57***</b>	0.47	<b>7.02**</b>	0.03	1.84	0.25
<b>4. Physiological traits</b>						
F <sub>v</sub> /F <sub>m</sub>	0.06	1.09	0.88	0.89	<b>5.77*</b>	<b>3.69*</b>
Stom. conduct.	–	0.04	0.00	–	–	0.10
<b>(b) Continental Mediterranean-mixed</b>						
<b>1. Growth-related trait</b>						
RGR	<b>26.55***</b>	0.04	1.39	0.12	<b>44.01***</b>	0.40
<b>2. Architecture-related traits</b>						
Height	1.62	0.92	1.06	0.71	<b>71.07***</b>	0.57
Crown height	–	3.03†	3.15†	–	–	<b>3.13*</b>
No. of leaves	–	0.98	0.44	–	–	0.17
Internode length	–	0.79	<b>54.40***</b>	–	–	1.22
<b>3. Morphology leaf traits</b>						
Leaf area	<b>97.62**</b>	1.65	<b>2.87†</b>	2.12	<b>39.97***</b>	0.30
SLA	0.79	0.83	<b>36.02***</b>	0.21	<b>7.87**</b>	0.49
LDMC	<b>61.75*</b>	0.13	<b>63.75***</b>	0.02	<b>34.66***</b>	0.70
<b>4. Physiological traits</b>						
F <sub>v</sub> /F <sub>m</sub>	<b>21.88***</b>	3.11†	<b>4.99*</b>	2.76	<b>97.90***</b>	0.86
Stom. conduct.	–	0.30	0.07	–	–	0.89

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 (Control-50% of full sun exposition- vs Shade environment -20% of full sun exposition-). 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. We used the symbol (–) when traits were measured only in one functional group and thereby, not included in the model.

nutrient availability also presented larger amount of leaves, higher stomatal conductances and F<sub>v</sub>/F<sub>m</sub> (Table 3; Figs. 1 and 2). Under the light treatment, seedlings from both forests mainly presented morphological changes in leaves (Fig. 2), 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 in both treatments (Tables 2 and 3). Additionally, we found that seedlings from different canopy diversities responded similarly (i.e. parallel slopes in reaction norms) to both treatments (i.e. non-significant Tr. x SR interaction) for most of the traits. As an exception, seedlings from monospecific and medium canopy richness communities in the hemiboreal forest presented higher plasticity in relative growth rate to a high nutrient environment than those coming from high canopy richness; and seedlings from monospecific communities had a higher plastic response to shaded conditions than those that came from mixed communities (Figs. 1 and 2). In the Mediterranean forest, conifers from monospecific communities presented a larger significant plastic response in crown height to both light and nutrient treatments than those from mixed communities (Fig. 1).

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

We observed divergent responses between the two functional groups to both treatments ('Tr x FG' interaction), mainly in the

**Table 3**

Results of the linear mixed models testing the plasticity and phenotypic expression (trait means) of different functional traits to the nutrient availability per forest type (hemiboreal and Mediterranean forest).

	FG df = 1	SR df = 2	Tr df = 1	SR x FG df = 2	Tr x FG df = 1	Tr x SR df = 2
<b>(a) Hemiboreal</b>						
<b>1. Growth-related trait</b>						
RGR	2.37	0.59	0.82	0.66	0.14	<b>3.21*</b>
<b>2. Architecture-related traits</b>						
Height	3.85	4.07†	<b>12.60***</b>	2.20	<b>8.25**</b>	2.10
Crown height	–	2.37	<b>12.15***</b>	–	–	1.42
No. of leaves	–	2.64	1.59	–	–	2.87
Internode length	–	–	–	–	–	–
<b>3. Morphology leaf traits</b>						
Leaf area	6.28	0.21	<b>32.49***</b>	<b>3.93*</b>	<b>7.88**</b>	1.25
SLA	3.02	0.46	<b>3.99*</b>	0.00	0.63	3.12
LDMC	<b>24.80***</b>	0.27	<b>11.10***</b>	0.01	3.57†	0.02
<b>4. Physiological traits</b>						
F <sub>v</sub> /F <sub>m</sub>	0.07	0.07	0.49	0.84	0.06	0.80
Stom. conduct.	–	2.02	1.54	–	–	0.85
<b>(b) Continental Mediterranean-mixed</b>						
<b>1. Growth-related trait</b>						
RGR	<b>12.18*</b>	0.67	2.13	<b>4.20*</b>	<b>34.50***</b>	0.01
<b>2. Architecture-related traits</b>						
Height	1.69	0.05	<b>7.12**</b>	0.17	<b>55.79***</b>	0.32
Crown height	–	<b>4.35*</b>	<b>40.05***</b>	–	–	<b>3.12*</b>
No. of leaves	–	0.37	<b>31.31***</b>	–	–	0.47
Internode length	–	0.01	<b>10.61**</b>	–	–	0.17
<b>3. Morphology leaf traits</b>						
Leaf area	<b>58.75*</b>	1.23	<b>144.40***</b>	1.24	<b>82.78***</b>	1.54
SLA	3.37	0.40	<b>50.74***</b>	1.26	<b>119.03***</b>	0.26
LDMC	<b>62.50**</b>	0.53	<b>4.40*</b>	0.13	0.04	1.44
<b>4. Physiological traits</b>						
F <sub>v</sub> /F <sub>m</sub>	<b>26.18***</b>	0.51	<b>40.87***</b>	0.88	3.12†	0.56
Stom. conduct.	–	0.04	<b>21.79***</b>	–	–	0.94

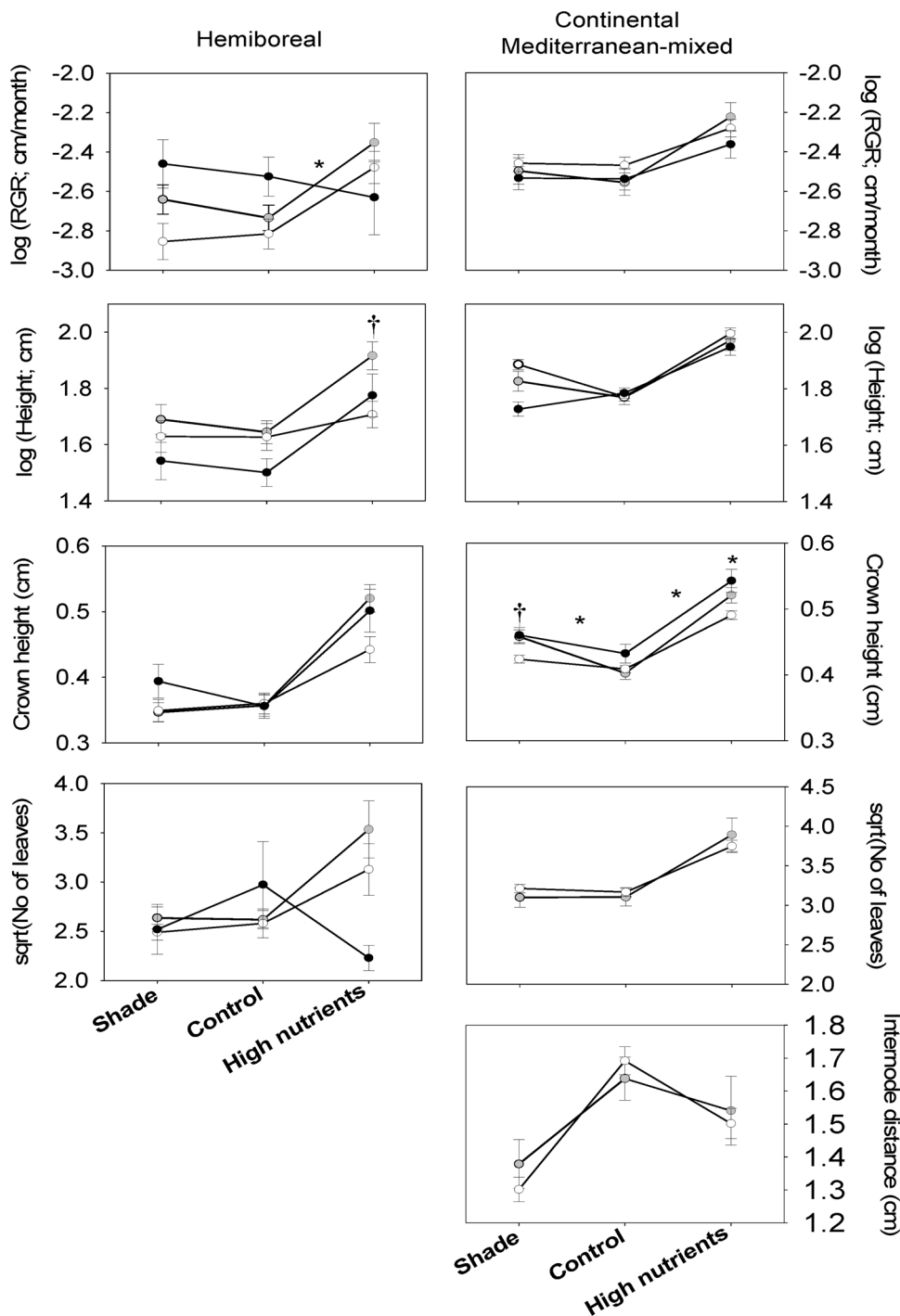
FG: functional group (broadleaved vs conifer species). SR: canopy species richness of communities from where seeds were collected (monospecific, medium and high canopy richness). Tr: nutrient treatment (control vs high nutrient supply). Data shown are the *F* values, the degrees of freedom (df) and the statistical significance level of each model using Type III tests. † *P* < 0.10; \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001. We used the symbol (–) when traits were measured only in a functional group and thereby, these main terms are not included in the model.

Mediterranean forest (Tables 2 and 3). In this forest, broadleaved seedlings showed larger mean trait values and plasticity in RGR and height in response to shaded and high nutrient supply conditions than conifers (Fig. 3; Tables 2 and 3), and higher mean trait values and plasticity of height and F<sub>v</sub>/F<sub>m</sub> than conifers in response to light treatment (Figs. 3 and 4). Moreover, both functional groups increased significantly their LA in response to higher nutrient supply, and increased SLA and reduced LDMC in response to higher light availability. In general, for these morphological traits, the plasticity was greater in conifers than in broadleaved seedlings, except for SLA in response to light (Fig. 4).

## 4. Discussion

Ecological theory predicts a selection of 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. Nevertheless, 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





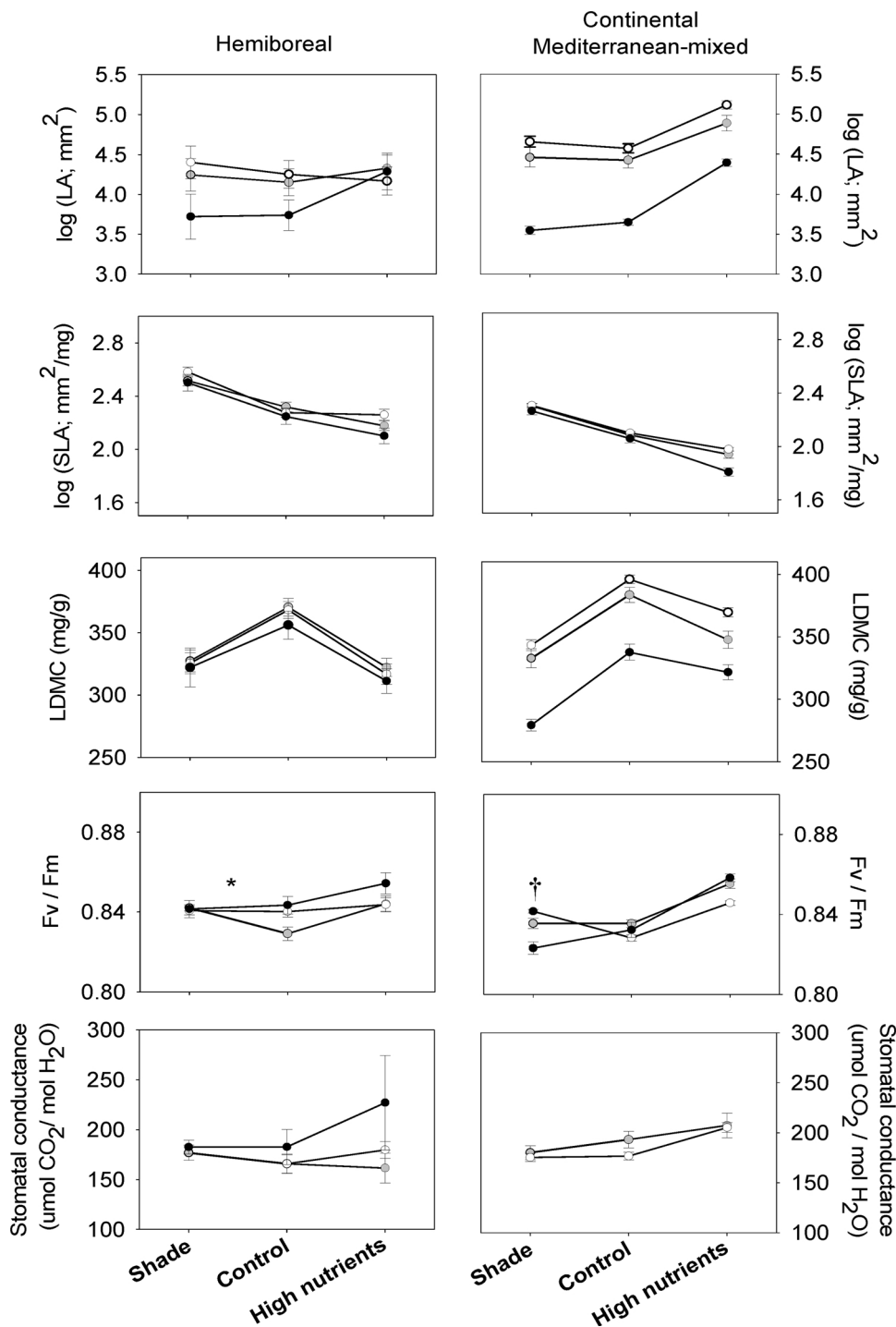
**Fig. 1.** Reaction norms of growth-related and architecture-related traits under light and nutrient treatments of seedlings coming from communities with different canopy richness levels (black circle: high mixture of species richness - 4 and 5 species mixtures; white circle: medium mixture of species richness - 2 and 3 species mixtures; grey circle: mono-specific - 1 species-) and type of forest. Trait means  $\pm$  S.E.M. for the species within each richness level included in the study are shown. A significant SR  $\times$  Treatment, i.e. seedlings from different canopy richness levels differ in their plasticity, is indicated over the reaction norms (lines) with: (†)  $P < 0.10$  and (\*)  $P < 0.05$ . Significant differences in the mean trait value among seedlings coming from different canopy diversities within each treatment are also indicated over the mean values (points) of each treatment with (†):  $P < 0.10$  and (\*)  $P < 0.05$ . Note that the Y axis has a logarithm or square root transformation for some traits.

traits to environmental shifts similarly to plants coming from communities with higher species diversity. Our findings suggest that species richness in the canopy does not exert detectable selective pressure on tree phenotypes and plasticity at this early stage.

#### 4.1. Influence of canopy species richness on trait means and plasticity

Few studies have addressed the diversity-induced differences in plasticity and phenotypic expression, and the existing ones have mainly focused on herbs (Burns and Strauss, 2012; Gubsch et al., 2011; Lipowsky et al., 2015, 2011; Roscher et al., 2011). Some of these works based on the Jena experiment (i.e. a large biodiversity grassland experiment; <http://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  $\delta^{13}\text{C}$  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; Lipowsky et al., 2015; Roscher et al., 2011; Zuppinger-Dingley et al., 2014). These studies suggested that species coexistence is enhanced by trait displacement. In this same line, other studies carried out in another long-term grassland plant experiment (Cedar Creek reserve, Minnesota) found that the spatiotemporal resource partitioning among dominant herb species in the community seemed to be very effective in promoting species coexistence and ecosystem stability (McKane et al., 1990; Tilman et al., 2006). Meanwhile, the effect of diversity on phenotypic expression and plasticity in long-



**Fig. 2.** Reaction norms of morphological leaf traits and physiological traits under light and nutrient treatments of seedlings coming from communities with different canopy richness levels (black circle: high mixture of species richness – 4 and 5 species mixtures-; white circle: medium mixture of species richness – 2 and 3 species mixtures-; grey circle: monospecific -1 species-) and type of forest. Trait means  $\pm$  S.E.M. for the species within each richness level included in the study are shown. A significant SR  $\times$  Treatment, i.e. seedlings from different canopy species richness levels differ in their plasticity, is indicated over the reaction norms (lines) with (\*):  $P < 0.05$ . Significant differences in the mean trait value among seedlings coming from different canopy diversities within each treatment are also indicated over the mean values (points) for each treatment with (†):  $P < 0.10$ . Note that the Y axis has a logarithm transformation for some traits.

lived species has remained almost unexplored and in general poorly understood under real field conditions (but see [Aschehoug and Callaway, 2014](#); [Callaway et al., 2003](#)). In our study, we found that seedlings from tree communities differing in species diversity had similar trait mean values and plasticity ([Figs. 1 and 2](#)). Our findings do not match our expectation that more heterogeneous environments induce higher plasticity ([Alpert and Simms, 2002](#); [Baythavong et al., 2011](#); [Gianoli, 2004](#); [Gianoli and González-Teuber, 2005](#)). In particular, they suggested that more heterogeneous sites in terms of biotic conditions did not exert enough selective pressure on mother trees to trigger shifts in traits heritable by their progeny, including the plasticity to cope with the study environmental changes.

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. low light intensity). 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 ([Mediavilla and Escudero, 2004](#); [Mitchell and Bakker, 2014](#)). No differential response among plants from monocultures and more diverse communities might also be due to an insufficient intensity of the environmental shift along the study gradient, i.e. species richness gradient of the tree canopy and the ensuing environmental heterogeneity associated. Nevertheless, studies conducted in the same study forests have documented perceptible diversity-induced changes of species

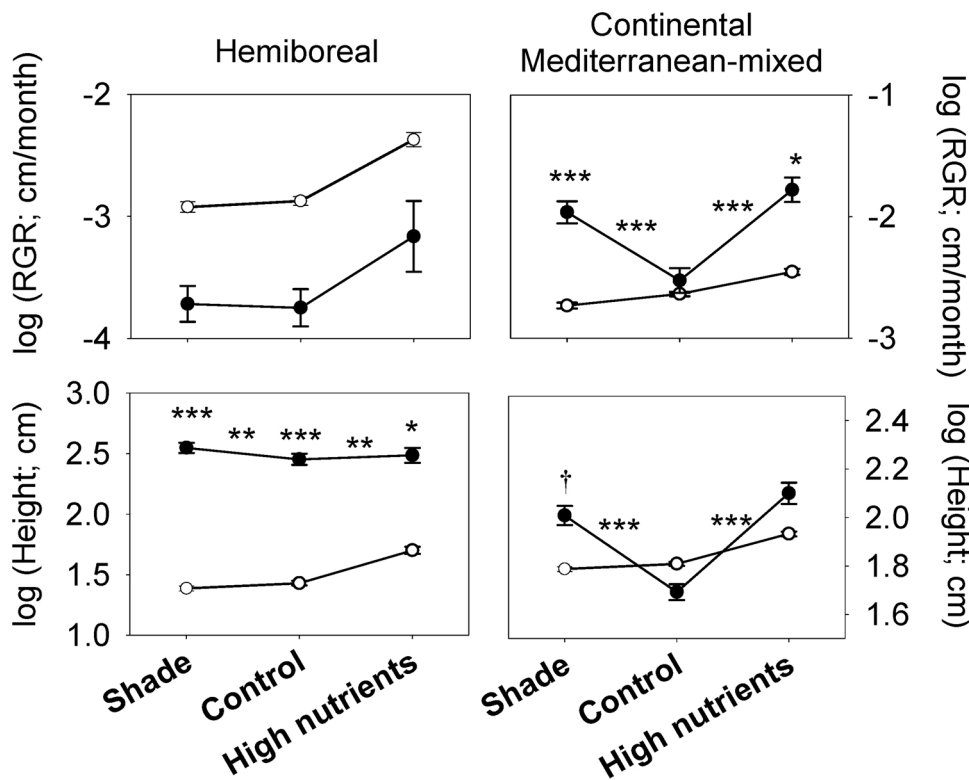


Fig. 3. Reaction norms of relative growth rate and height traits to light and nutrient treatments for conifers and broadleaved seedlings per type of forest. Trait means  $\pm$  S.E.M. per species of each functional group included in the study are shown. A significant  $FG \times Treatment$ , i.e. when broadleaved and conifers differ in their plasticity to the treatment, is indicated with asterisks over the reaction norms (lines) (\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). Differences in the mean trait between functional groups within treatments are indicated with asterisks over the points (†:  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). Note that the Y axis has a logarithm transformation for both traits. Black circles represent broadleaved species, and empty circles represent conifers.

phenotypes of adult trees (Benavides et al., Unpublished results) and ecosystem functions under the same diversity gradient, e.g. the higher above-ground wood production in mixed than in monospecific forest stands by complementary light use strategies among neighbouring trees (Jucker et al., 2014).

Another explanation for the lack of diversity effect may lie in the ontogenetic signal on plant responses, i.e. we did not find differences in traits or plasticity at this early stage, but trees might respond later when increasing competition for resources triggers greater phenotypic differentiation (Mediavilla and Escudero, 2004; Mitchell and Bakker, 2014; Moll and Brown, 2008). It is known that the ability to respond in a plastic way is not stable along developmental stages (Watson et al., 1995), and conditions experienced at some stages may be reflected in their response to the 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 different outcomes from experiments using herbaceous species vs trees reflect the challenge of studying long-lived species, particularly in nature, that entails difficulties not only associated to dealing with large size individuals, but also long temporal scales from human perspective. Populations of tree species typically require much longer time than herbs to express differentiation and local adaptation to the environment (Aitken and Bemmels, 2016), and observers must deal with uncertainties regarding the tracking of past external factors and environmental conditions occurring much earlier than the period of the study. Although species with long lifespan tissues may 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 (Borges, 2009; Sultan, 1987). Clearly, long-living species such as trees deserve more attention to disentangle these complex logistical barriers at the time to study the evolution of plasticity in these woody systems.

#### 4.2. 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 (Tables 2 and 3). We found that broadleaved individuals in the Mediterranean forest exhibited higher plasticity levels, conferring higher relative growth, height, photochemical efficiency and SLA mean values in shaded and nutrient-rich environments than coniferous seedlings (Figs. 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 and Johnsen, 1996) and stomatal conductance favouring their growth rates in productive habitats (Lusk et al., 2003; Lusk and Matus, 2000; but see Becker, 2000).

We also found differential plastic responses of both functional groups, suggesting that broadleaved species (oaks) may have a 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 of leaf traits to changing nutrients (Aerts, 1995) and light availability (Wyka et al., 2012). Similar 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 two conifers (*Pinus pinaster* and *P. sylvestris*) than in 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 pointed to a carbon-acquisition strategy with larger SLA and lower LDMC in response to low light availability (Givnish, 1988; Valladares et al., 2016).

In summary, our results showed large differences between the two functional groups in the response capacity to environmental changes, mainly in the Mediterranean forest, with conifers responding at the leaf level and broadleaved species responding at the whole-plant level. These results can be interpreted as a better potential capacity of

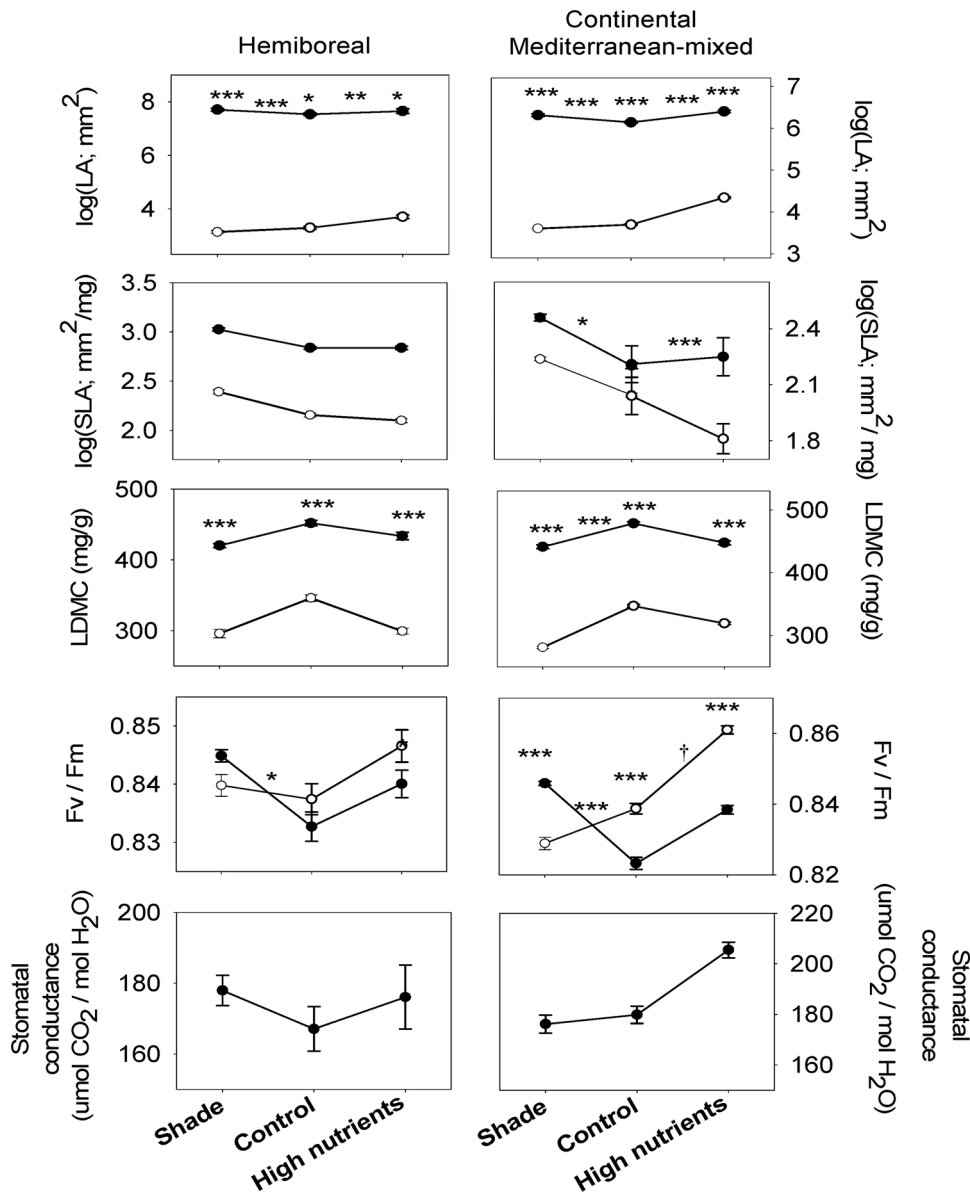


Fig. 4. Reaction norms of morphological leaf traits and physiological traits to light and nutrient treatments for conifers and broadleaved seedlings per type of forest. Trait means  $\pm$  S.E.M. per species of each functional group included in the study are shown. A significant  $\text{FG} \times \text{Treatment}$ , i.e. when broadleaved and conifers differ in their plasticity to the treatment, is indicated with asterisks over the reaction norms (lines) (†  $P < 0.10$ ; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). Differences in the mean trait between functional groups within treatments are indicated with asterisks over the points (\*\*\*  $P < 0.001$ ). Note that the Y axis has a logarithm transformation for LA and SLA. Black circles represent broadleaved species, while empty circles represent conifers.

broadleaved against coniferous seedlings at this early stage in traits related to plant performance and fitness, which can be crucial for the species competitive ability for resources (Latham, 1992) and, therefore, for survival (Becker, 2000; Bond, 1989).

#### 4.3. 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 a 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 and Niinemets, 2008). A greater nutrient availability led to taller seedlings, with higher crowns and more and larger leaves with lower LDMC, and better eco-physiological conditions (higher  $F_v/F_m$  and stomatal conductance). As expected, seedlings in the nutrient-rich environment invested on new productive leaf tissues and woody stems, which improve their performance and competitive ability particularly for light capture (Poorter et al., 2012; Tilman, 1988). 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; Portsmouth and Niinemets, 2007; Schreeg et al., 2005).

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' (Iwasa and Roughgarden, 1984; Poorter et al., 2012; Thornley, 1972). Soil water availability, for instance, is another key environmental factor influencing plasticity and determining the plant phenotype (Gianoli and González-Teuber, 2005; Lázaro-Nogal et al., 2015; Molina-Montenegro et al., 2010). Particularly in Mediterranean climates, plants may exhibit higher potential plasticity in key traits related to water such as  $F_v/F_m$  and SLA since they have to cope with contrasting water variations along seasons. We did not test the effect of water availability in our study but we found that plants from the Mediterranean site presented higher variations in phenotypes than plants from the hemiboreal forest. A plausible explanation may be an advantage in plasticity of certain traits for plants from arid or semiarid



climates, which usually experiment spatial and temporal variations in numerous key environmental factors.

In this context, factorial experiments with both light and nutrient availability gradients, and also including water availability, typically shed more light on the evolutionary and ecological implications of plasticity since plants are almost always challenged by complex, multifactor environmental changes (Niinemets and Valladares, 2006; Portsmouth and Niinemets, 2007).

## 5. Conclusions

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. Our study showed that the species richness in the two forests did not exert any effect, i.e. it did not act as a selective filter, on either the phenotypic expression or the degree of plasticity of seedlings in response to different light and nutrient availability, at least at these early stages. Additionally, our results showed substantial differences in plasticity between functional groups, presenting broadleaf species greater plastic responses to environmental shifts than conifers. 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. Specifically, broadleaf species had a higher capacity to respond to future changing conditions than conifers at this early stage. This study represents the 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, M<sup>a</sup> 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. This work was supported by '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). 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).

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2018.08.023>.

## References

Aerts, R., 1995. The advantages of being evergreen. *Trends Ecol. Evol.* 10, 402–407. [https://doi.org/10.1016/S0169-5347\(00\)89156-9](https://doi.org/10.1016/S0169-5347(00)89156-9).

Aitken, S.N., Bemmels, J.B., 2016. Time to get moving: assisted gene flow of forest trees. *Evol. Appl.* 9, 271–290. <https://doi.org/10.1111/eva.12293>.

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? *Evol. Ecol.* 16, 285–297.

Aschehoug, E.T., Callaway, R.M., 2014. Morphological variability in tree root architecture indirectly affects coexistence among competitors in the understory. *Ecology*

95, 1731–1736.

Ashton, I.W., Miller, A.E., Bowman, W.D., Suding, K.N., 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91, 3252–3260.

Baeten, L., Verheyen, K., Wirth, C., Bruelheide, H., Bussotti, F., Finér, L., Jaroszewicz, B., Selvi, F., Valladares, F., Allan, E., Ampoorter, E., Auge, H., Avacáriei, 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., Castagnayrol, B., Charbonnier, Y., Češko, E., Coomes, D., Coppi, A., Dalmaris, E., Dániilá, G., Dawud, S.M., de Vries, W., De Wandeler, H., Deconchat, M., Domsch, 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. *Perspect. Plant Ecol. Evol. Syst.* 15, 281–291. <https://doi.org/10.1016/j.ppees.2013.07.002>.

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. *Funct. Ecol.* 15, 124–135.

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 Found. Stat. Comput., Vienna.

Baythavong, B.S., Agrawal, A.E.A., McPeck, 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. *Am. Nat.* 178, 75–87. <https://doi.org/10.1086/660281>.

Becker, P., 2000. Competition in the regeneration niche between conifers and angiosperms: bond's slow seedling hypothesis. *Funct. Ecol.* 14, 401–412. <https://doi.org/10.1046/j.1365-2435.2000.00455.x>.

Berg, M.P., Ellers, J., 2010. Trait plasticity in species interactions: a driving force of community dynamics. *Evol. Ecol.* 24, 617–629. <https://doi.org/10.1007/s10682-009-9347-8>.

Bond, W.J., 1989. The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. *Biol. J. Linn. Soc.* 36, 227–249. <https://doi.org/10.1111/j.1095-8312.1989.tb00492.x>.

Borges, R.M., 2009. Phenotypic plasticity and longevity in plants and animals: cause and effect? *J. Biosci.* 34, 605–611.

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

Callaway, R.M., Pennings, S.C., Richards, C.L., 2003. Phenotypic plasticity and interactions among plants. *Ecology* 84, 1115–1128. [https://doi.org/10.1890/0012-9658\(2003\)084\[1115:PPAIAP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2).

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? *J. Veg. Sci.* 14, 311–322. <https://doi.org/10.1111/j.1654-1103.2003.tb02157.x>.

De Kroon, H., Huber, H., Stuefer, J.F., van Groenendael, J.M., 2005. A modular concept of phenotypic plasticity in plants: research review. *New Phytol.* 166, 73–82. <https://doi.org/10.1111/j.1469-8137.2004.01310.x>.

De Kroon, H., Visser, E.J.W., Huber, H., Mommer, L., Hutchings, M.J., 2009. A modular concept of plant foraging behaviour: the interplay between local responses and systemic control. *Plant Cell Environ.* 32, 704–712. <https://doi.org/10.1111/j.1365-3040.2009.01936.x>.

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

Gianoli, E., González-Teuber, M., 2005. Environmental Heterogeneity and Population Differentiation in Plasticity to Drought in *Convolvulus Chilensis* (Convolvulaceae). *Evol. Ecol.* 19, 603–613. <https://doi.org/10.1007/s10682-005-2220-5>.

Givnish, T., 1988. Adaptation to Sun and shade: a whole-plant perspective. *Aust. J. Plant Physiol.* 15 (63). <https://doi.org/10.1071/PP9880063>.

Gomez, J.M., Valladares, F., Puerta-Pinero, C., 2004. Differences between structural and functional environmental heterogeneity caused by seed dispersal. *Funct. Ecol.* 18, 787–792. <https://doi.org/10.1111/j.0269-8463.2004.00912.x>.

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. *Ann. Bot.* 107, 157–169. <https://doi.org/10.1093/aob/mcq220>.

Huber, H., Chen, X., Hendriks, M., Keijsers, D., Voisenek, L.A.C.J., Pierik, R., Poorter, H., Kroon, H., de Visser, E.J.W., 2012. 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 Phytol.* 194, 572–582.

Iwasa, Y., Roughgarden, J., 1984. Shoot / root balance of plants: optimal growth of a system with many vegetative organs. *Theor. Popul. Biol.* 25, 78–105.

Jucker, T., Bouriaud, O., Avacaritei, D., Dániilá, I., Duduman, G., Valladares, F., Coomes, D.A., 2014. Competition for light and water play contrasting roles in driving diversity-productivity relationships in Iberian forests. *J. Ecol.* 102, 1202–1213. <https://doi.org/10.1111/1365-2745.12276>.

Lasky, Jesse R., Bachelot, Bénédicte, Muscarella, Robert, Schwartz, Naomi, Forero-Montaña, Jimena, Nytch, Christopher J., Swenson, Nathan G., Thompson, Jill, Zimmerman, Jess K., Uriarte, María, 2015. Ontogenetic shifts in trait-mediated mechanisms of plant community assembly. *Ecology*. [46](https://doi.org/10.1890/14-</a></p>
</div>
<div data-bbox=)

- 1809.1.
- Latham, R.E., 1992. Co-occurring tree species change rank in seedling performance with resources varied experimentally. *Ecology* 73, 2129–2144. <https://doi.org/10.2307/1941461>.
- 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. *J. Ecol.* 103, 338–350.
- Lenth, R.V., 2016. Least-squares means: the R Package: lsmeans. *J. Stat. Softw.* 69. <https://doi.org/10.18637/jss.v069.i01>.
- Levins, R., 1979. Coexistence in a variable environment. *Am. Nat.* 114, 765–783.
- Lipowsky, A., Schmid, B., Roscher, C., 2011. Selection for monoculture and mixture genotypes in a biodiversity experiment. *Basic Appl. Ecol.* 12, 360–371. <https://doi.org/10.1016/j.baae.2011.03.005>.
- 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. *Perspect. Plant Ecol. Evol. Syst.* 17, 66–77. <https://doi.org/10.1016/j.ppees.2014.11.003>.
- 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. *J. Biogeogr.* 27, 1011–1020. <https://doi.org/10.1046/j.1365-2699.2000.00449.x>.
- Lusk, C.H., Wright, L., Reich, P.B., 2003. Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats. *New Phytol.* 160, 329–336. <https://doi.org/10.1046/j.1469-8137.2003.00879.x>.
- Maire, V., Gross, N., Hill, D., Martin, R., Wirth, C., Wright, L.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. <https://doi.org/10.1371/journal.pone.0077372>.
- 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.
- Matesanz, S., Milla, R., 2018. Differential plasticity to water and nutrients between crops and their wild progenitors. *Environ. Exp. Bot.* 145, 54–63. <https://doi.org/10.1016/j.envexpbot.2017.10.014>.
- Matesanz, S., Gianoli, E., Valladares, F., 2010. Global change and the evolution of phenotypic plasticity in plants. *Ann. N. Y. Acad. Sci.* 1206, 35–55. <https://doi.org/10.1111/j.1749-6632.2010.05704.x>.
- McKane, R.B., Grigal, D.F., Russelle, M.P., 1990. Spatiotemporal differences in 15N uptake and the organization of an old-field plant community. *Ecology* 71, 1126–1132. <https://doi.org/10.2307/1937380>.
- Mediavilla, S., Escudero, A., 2004. Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. *For. Ecol. Manage.* 187, 281–294. <https://doi.org/10.1016/j.foreco.2003.07.006>.
- Mitchell, R.M., Bakker, J.D., 2014. Intraspecific Trait Variation Driven by Plasticity and Ontogeny in *Hypochaeris radicata*. *PLoS One* 9, e109870. <https://doi.org/10.1371/journal.pone.0109870>.
- Molina-Montenegro, M.A., Atala, C., Gianoli, E., 2010. Phenotypic plasticity and performance of *Taraxacum officinale* (dandelion) in habitats of contrasting environmental heterogeneity. *Biol. Invas.* 12, 2277–2284. <https://doi.org/10.1007/s10530-009-9638-6>.
- Moll, J.D., Brown, J.S., 2008. Competition and coexistence with multiple life-history stages. *Am. Nat.* 171, 839–843. <https://doi.org/10.1086/587517>.
- Niinemets, Ü., Valladares, F., 2006. Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecol. Monogr.* 76, 521–547.
- 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. *Aust. J. Bot.* 61, 167–234.
- Pigliucci, M., 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. The Johns Hopkins University Press, Baltimore, MD.
- 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 Phytol.* 193, 30–50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>.
- 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. *Funct. Ecol.* 21, 61–77.
- Pugnaire, F., Valladares, F., 1999. *Handbook of Functional Plant Ecology*. CRC Press.
- R Development Core Team, 2015. R: A Language and Environment for Statistical Computing. [WWW Document]. GBIF.ORG. URL. (Accessed 7 April 2015). <http://www.gbif.org/resource/81287>.
- 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. <https://doi.org/10.1007/s00442-010-1735-9>.
- Rothe, A., Binkley, D., 2001. Nutritional interactions in mixed species forests: a synthesis. *Can. J. For. Res.* 31, 1855–1870. <https://doi.org/10.1139/cjfr-31-11-1855>.
- Rzanny, M., Voigt, W., 2012. Complexity of multitrophic interactions in a grassland ecosystem depends on plant species diversity: plant diversity and multitrophic interactions. *J. Anim. Ecol.* 81, 614–627. <https://doi.org/10.1111/j.1365-2656.2012.01951.x>.
- Sanchez-Gomez, D., Valladares, F., Zavala, M.A., et al., 2006. Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species. *Tree Physiol.* 26, 1425–1433.
- 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. <https://doi.org/10.2980/11195-6860-13-2-258.1>.
- 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. *Can. J. For. Res.* 35, 263–273. <https://doi.org/10.1139/x04-168>.
- Stein, A., Gerstner, K., Kreft, H., 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecol. Lett.* 17, 866–880. <https://doi.org/10.1111/ele.12277>.
- Sultan, S.E., 1987. Evolutionary implications of phenotypic plasticity in plants. In: Hecht, M.K., Wallace, B., Prance, G.T. (Eds.), *Evolutionary Biology*. Springer, US, pp. 127–178. [https://doi.org/10.1007/978-1-4615-6986-2\\_7](https://doi.org/10.1007/978-1-4615-6986-2_7).
- Sultan, S.E., 2000. Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* 5, 537–542. [https://doi.org/10.1016/S1360-1385\(00\)01797-0](https://doi.org/10.1016/S1360-1385(00)01797-0).
- Sultan, S.E., 2001. Phenotypic plasticity for fitness components in polygonum species of contrasting ecological breadth. *Ecology* 82, 328–343. [https://doi.org/10.1890/0012-9658\(2001\)082\[0328:PPFFCI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0328:PPFFCI]2.0.CO;2).
- Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M.C., Schwager, M., Jeltsch, F., 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. *J. Biogeogr.* 31, 79–92.
- Thornley, J.H.M., 1972. A balanced quantitative model for root: shoot ratios in vegetative plants. *Ann. Bot.* 36, 431–441. <https://doi.org/10.1093/oxfordjournals.aob.a084602>.
- Tilman, D., 1988. In: Princeton (Ed.), *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press, Princeton, USA.
- Tilman, D., Reich, P.B., Knops, J.M.H., 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441, 629–632. <https://doi.org/10.1038/nature04742>.
- Valladares, F., Niinemets, Ü., 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Syst.* 39, 237–257. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173506>.
- Valladares, F., Gianoli, E., Gómez, J.M., 2007. Ecological limits to plant phenotypic plasticity. *New Phytol.* 176, 749–763. <https://doi.org/10.1111/j.1469-8137.2007.02275.x>.
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garazón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.* 17, 1351–1364. <https://doi.org/10.1111/ele.12348>.
- Valladares, F., Laanisto, L., Niinemets, Ü., Zavala, M.A., 2016. Shedding light on shade: ecological perspectives of understorey plant life. *Plant Ecol. Divers.* 9, 237–251. <https://doi.org/10.1080/17550874.2016.1210262>.
- 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. *Agrofor. Syst.* 82, 173–181. <https://doi.org/10.1007/s10457-010-9343-6>.
- Watson, M., Geber, M., Jones, 1995. Ontogenetic contingency and the expression of plant plasticity. *Trends Ecol. Evol.* 10, 474–475.
- 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. *J. Ecol.* 87, 860–872. <https://doi.org/10.1046/j.1365-2745.1999.00395.x>.
- 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. <https://doi.org/10.1007/s00442-012-2279-y>.
- 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 Soil* 286, 123–139. <https://doi.org/10.1007/s11104-006-9031-5>.
- Zuppinge-Dingledy, 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. <https://doi.org/10.1038/nature13869>.