

Research

The functional trait space of tree species is influenced by the species richness of the canopy and the type of forest

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Analysing how species modify their trait expression along a diversity gradient brings insight about the role that intraspecific variability plays over species interactions, e.g. competition versus complementarity. Here, we evaluated the functional trait space of nine tree species dominant in three types of European forests (a continental-Mediterranean, a mountainous mixed temperate and a boreal) growing in communities with different species richness in the canopy, including pure stands. We compiled whole-plant and leaf traits in 1719 individuals, and used them to quantify species trait hypervolumes in communities with different tree species richness. We investigated changes along the species richness gradient to disentangle species responses to the neighbouring environment, in terms of hypervolume size (trait variance), shape (trait relative importance) and centroid translation (shifts of mean trait values) using null models. Our main results showed differences in trait variance and shifts of mean values along the tree diversity gradient, with shorter trees but with larger crowns in mixed stands. We found constrained functional spaces (trait convergence) in pure stands, suggesting an important intraspecific competition, and expanded functional spaces (trait divergence) in two-species admixtures, suggesting competition release due to interspecific complementarity. Nevertheless, further responses to increasing species richness were different for each forest type, waning species complementarity in sites with limiting conditions for growth. Our results demonstrate that tree species phenotypes respond to the species richness in the canopy in European forests, boosting species complementarity at low level of canopy diversity and with a site-specific pattern at greater level of species richness. These outcomes evidence the limitation of functional diversity measures based only on traits from pure stands or general trait database values.

Keywords: biodiversity, functional traits, FunDivEUROPE, hypervolume, intraspecific trait variation, phenotypic space, species interactions

Introduction

Approximately 25% of total estimated trait variation in plant communities worldwide is found within species (Albert et al. 2010, Siefert et al. 2015). Hence, the concept of intraspecific variability is changing from being noise to become a fundamental piece of information underlying many ecological processes. Accordingly, increasing evidence highlights the role that trait variability within species have for different ecological questions like species distribution forecasting (Valladares et al. 2014, Cochrane et al. 2015), functioning of plant communities (Crutsinger et al. 2006, Lecerf and Chauvet 2008), mechanisms ruling species interactions and coexistence (Lichstein et al. 2007, Roscher et al. 2015), and community assembly (Jung et al. 2010, Siefert 2012).

Functional traits determine how individuals use resources from their environment (McGill et al. 2006). Therefore, the distribution of trait values within species in a community, defined by the mean, range and variance, is commonly considered a proxy of their realised niche. This approach captures species interactions by showing the degree of similarity among coexisting species in terms of trait distribution overlaps (Violle et al. 2012), which is directly linked to the complementarity and competitive ability of coexisting species (Carroll et al. 2011, Loreau et al. 2012). Together with mean values and intraspecific variance, recent studies advocate the importance of the covariation of traits, i.e. a multivariate approach to functional diversity, as a third ingredient to describe appropriately the functional trait space occupied by species (Cornwell et al. 2006, Violle and Jiang 2009, Laughlin and Messier 2015, Messier et al. 2017, Bittebiere et al. 2018) and the niche differences among species (Kraft et al. 2015, Carscadden et al. 2017, Bittebiere et al. 2018). Covariation of traits involves that phenotypes are coordinated expressions of multiple traits that are jointly influenced (Pigliucci 2003, Bonser 2006) by environmental conditions (Maire et al. 2013). Thus, this covariation reflects tradeoffs among plant functions and processes under given conditions (e.g. growth versus reproduction), which turns into tradeoffs among traits (e.g. plant size versus seed traits) (Westoby 1998, Wright et al. 2004, Chave et al. 2009).

Functional biodiversity research is based on measures of functional diversity often calculated on the bases of selected traits, measured either in monocultures or extracted from trait databases (such as TRY, Kattge et al. 2011). These approaches ignore changes in trait expression along environmental gradients, such as those imposed by the number of coexisting species. Changes in trait expression along diversity gradients can have substantial effects on the interactions among species that drive biodiversity–ecosystem functioning relationships, but remains largely unknown. Here, assuming that variability within species underlies and boosts species complementarity (Ashton et al. 2010, Zuppinge-Dingley et al. 2014, Zhu et al. 2015), we measured functional traits in dominant tree species in European forests, assessed the functional trait hypervolumes (phenotypic space) (Blonder et al. 2014,

2018), and analysed their variation along a gradient of canopy richness. Specifically, we aimed at deciphering the species-specific responses of trees in natural communities to the biotic milieu (species richness in the canopy), in terms of mean trait values (hypervolume centroid), intraspecific variability (hypervolume size) and trait covariation (hypervolume shape). We propose the following alternative cases (Fig. 1):

1. Functional trait space is smaller in mixed stands (convergence of traits), implying space partitioning to avoid overlap with other species based on the limiting similarity theory (MacArthur and Levins 1967).
2. Functional trait space is larger in mixed stands (divergence of traits), implying higher temporal or spatial heterogeneity in the community that provides new opportunities and decreases competition among individuals (Stein et al. 2014).
3. Functional space shifts to release competition, similarly to the character displacement phenomenon described in an evolutionary context (Dayan and Simberloff 2005).

These cases are not necessarily mutually exclusive, and real responses can be a combination of 1) and 3) when limiting similarity triggers both trait convergence and shifts, or a combination of 2) and 3) when divergence occurs at single traits, causing changes in hypervolume shapes and consequently centroid shifts.

Methods

Study sites

This study was located in three mature forests representative of some of the major European forest types: a continental-Mediterranean mixed forest in the Alto Tajo Natural Park (Spain), a mountainous beech forest (hereafter temperate) in Râșca (Carpathian Mountains, Romania) and a boreal forest in North Karelia (Finland) (Table 1). They belong to a network of plots established for the European project FunDivEUROPE (Functional significance of forest biodiversity, <www.fundiveurope.eu>). Within these three sites, 92 plots (30×30 m) were established following a species richness gradient of regional dominant tree species in different combinations (replicated at least twice), ranging the species richness of the canopy from 1 to 3 in Finland, and from 1 to 4 in Romania and Spain. Every dominant species was present in all species richness levels (see Supplementary material Appendix 1 Table A1.1 for a detailed species combination design).

The tree species were *Pinus nigra*, *Pinus sylvestris*, *Quercus faginea* and *Quercus ilex* in the Mediterranean forest with 36 plots in total, *Abies alba*, *Acer pseudoplatanus*, *Fagus sylvatica* and *Picea abies* in the temperate forest, and *Betula pendula*, *Picea abies* and *Pinus sylvestris* in the boreal forest, both with 28 plots. Criteria for plot selection are explained in detail in Baeten et al. (2013) and relied on two main

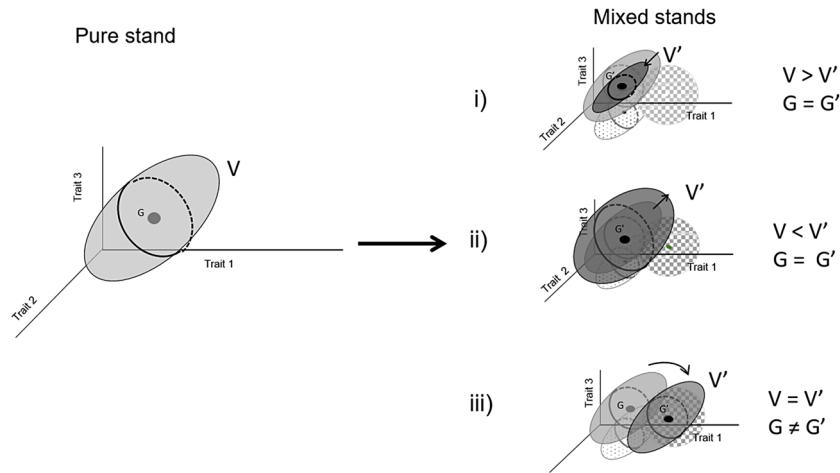


Figure 1. Alternative hypotheses for the relationship between species functional trait space and species richness in a forest community comparing individuals in pure stands (V , light grey) versus mixed stands (V' , dark grey). 1) Functional space decreases with species richness, i.e. variance of traits diminishes around the centroid (G , mean value), or 2) functional space increases with species richness either by variance increment or 3) by trait mean shift and the ensuing increment of the overall phenotypic space at a larger scale. G is the centroid of V and G' centroid of V' . Neighbouring species in mixed stands are represented by dotted and gridded hypervolumes.

principles: 1) mixed plots had high evenness, i.e. species had similar relative abundances, 2) covariation between environmental gradients (e.g. soil, topography) and species richness was reduced by minimizing variation of environmental conditions among plots within a region. In accordance with the design rationale, Baeten et al. (2013) showed that soil and site conditions did not significantly differ among species richness levels, neither did the total basal area along the species richness gradient of each species (Supplementary material Appendix 1 Fig. A1.1).

Study traits, sampling and measuring

We randomly selected ten adult individuals per tree dominant species in each plot to capture as much trait variability

within species as possible, totalling 1719 surveyed trees. From each tree, we compiled seven traits at the whole-plant level and six at the leaf level (Table 2). They represent two of the most critical dimensions in the global plant spectrum (Díaz et al. 2016): plant size which reflects the ability to pre-empt light resources and disperse seeds, and leaf economic spectrum which balances the acquisition and conservation of resources – i.e. construction costs against growth potential (Wright et al. 2004, Díaz et al. 2016).

In the field, tree height (H), diameter at breast height (dbh), crown height (H_c) and crown perpendicular diameters were measured in each selected tree. Then, we calculated crown projection area (CP), using the projection of two perpendicular diameters assuming elliptical areas, the slenderness (SS) as the ratio between height and dbh,

Table 1. Brief description of the study sites, species and environmental conditions.

| | North Karelia, Finland (boreal) | Carpathian mountains, Romania (temperate) | Alto Tajo Natural Park, Spain (continental-Mediterranean) |
|-------------------------------|--|---|--|
| Coordinates | 62.4°N–29.4°E | 47.3°N–25.5°E | 40.7°N–1.9°W |
| Dominant species | <i>Betula pendula</i> <i>Picea abies</i> <i>Pinus sylvestris</i> | <i>Abies alba</i> <i>Acer pseudoplatanus</i> <i>Fagus sylvatica</i> <i>Picea abies</i> | <i>Pinus nigra</i> <i>Pinus sylvestris</i> <i>Quercus faginea</i> <i>Quercus ilex</i> |
| Annual mean T (°C)* | 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) |
| Elevation range (m) | 87–233 | 655–1062 | 960–1404 |
| Soils | Podzols and glacial tills, histosols on peatlands | Tertiary sandstone and clay, loamy sediments | Calcareous, reddish Terra rossa |
| Main forest types (EEA-code)* | Boreal | Carpathian mountainous beech forest | Coniferous and broadleaf Mediterranean forests |
| Species richness gradient | 1–3 | 1–4 | 1–4 |
| Number of plots | 28 | 28 | 36 |
| Plots per richness level | 12/12/4 | 8/10/7/3 | 12/15/6/3 |

*T=temperature, P=precipitation, EEA: European Environmental Agency (source: EEA Technical Report No 9/2006, <<https://eea.europa.eu>>).

Table 2. Overview of functional traits measured in each individual.

| | Trait | Units | Trait description | Trait functions |
|--------------------|---------------------------------|----------------------------------|---|---|
| Whole-plant traits | Height (H) | m | Distance from the soil surface to the top end of the crown | Competitive vigour to capture light, competing either in the vertical or horizontal plane |
| | Diameter at breast height (dbh) | cm | Diameter of the stem at breast height | |
| | Crown height (Hc) | m | Distance between the crown base and the top end of the crown | |
| | Crown projection area (CP) | m ² | Area estimation of the crown projection, assuming elliptical areas and using two perpendicular diameters of the crown | |
| | Slenderness (SS) | unitless | Ratio between height and dbh | Tradeoffs between investment in support and photosynthetic structures |
| | Crown ratio (CPdbh) | m ² cm ⁻¹ | Ratio between crown projection area and dbh | |
| | Crown length ratio (CLR) | unitless | Ratio between crown height and total height | |
| Leaf traits | Leaf area (LA) | mm ² | One-side leaf lamina area | |
| | Specific leaf area (SLA) | mm ² mg ⁻¹ | Ratio between leaf area and dry mass | |
| | Leaf dry matter content (LDMC) | mg g ⁻¹ | Ratio between leaf dry mass and leaf saturated mass | |
| | N content | % | N concentration in leaf | |
| | C content | % | C concentration in leaf | |
| | C:N | unitless | Ratio among C and N contents | |

the crown ratio as the relationships between crown projection and dbh (CPdbh) and crown length ratio (CLR) as the ratio between crown height and total height. Those traits can be considered individual performance measures, doubtfully comparable among cohorts (Garnier et al. 2016). However, we surveyed only adult trees, and their whole-plant traits represent the relative aboveground competitive ability of each individual (Siefert et al. 2015).

In addition, we harvested one branch from the top of the crown of each surveyed tree, and collected five leaves/needles for morphological characterization. We weighed rehydrated leaves, scanned them and measured their area using WinFOLIA and WinSEEDLE for broadleaves and needles, respectively (Regent Instruments, Canada). Then, they were dried up in an oven at 60°C for 72 h and weighed for dry mass. These measurements let the assessment of morphological leaf traits averaged by tree: leaf area (LA), specific leaf area (SLA, the area of an individual fresh leaf divided by its dry mass) and leaf dry matter content (LDMC, the oven-dry mass of an individual leaf divided by its water-saturated fresh mass). Leaf collection, storage, processing and trait measurement followed Garnier et al. (2001) and Pérez-Harguindeguy et al. (2013). Finally, we collected few more leaves from each branch, that were also dried, pooled and grinded in order to estimate the N and C leaf content and the C:N (CN), using a dry combustion method (Elementar Vario EL cube). A summary of sample sizes per trait, species and forest type is shown in Supplementary material Appendix 1 Table A1.2.

Hypervolume calculations

We quantified the size, shape and centroids of the functional trait space of the study species growing in communities with different species richness levels following the approach developed by Blonder et al. (2014), extended in Blonder et al.

(2018), and implemented in the ‘hypervolume’ R package (Blonder and Harris 2017, <www.r-project.org>). This method includes algorithms that infer n-dimensional volumes using a set of observations and assuming a Gaussian kernel density estimation. Compared to previous mathematical approaches (Hutchinson 1957, Nix 1986, Doledec et al. 2000, Cornwell et al. 2006), this hypervolume calculation can describe complex shapes in high dimensionalities with smooth boundaries (density decays smoothly towards the boundaries), it is not sensitive to outliers and allows holes (Blonder 2016) and input weighting. Moreover, metrics like the relevance of each dimension on the final volume building or pairwise overlaps among species hypervolumes at different species richness levels can be easily obtained (detailed in Blonder and Harris 2017).

Before running the hypervolume estimations, we reduced the number of axes (traits) to four, following the recommendations about the ratio between observations and number of dimensions (ca 10) and orthogonality (i.e. the smaller correlations among traits, the better) (Blonder et al. 2014) (Supplementary material Appendix 1, Supplementary material Appendix 1 Table A1.3). Thus, we selected two traits per plant dimension (plant size and leaf traits). Namely, we chose H and CPdbh, which represent the competitive ability for space in two directions (vertically and horizontally); and amongst leaf traits, we chose one chemical trait (C:N) and one morphological trait (LDMC) that were slightly correlated. Moreover, our choice for LDMC (rather than SLA or LA) was also supported because LDMC is considered a morphological trait more reliable for non-broadleaves, like needles in conifers (Wilson et al. 1999).

We normalized the data (z-scores) in order to have axes with comparable units (standard deviations), obtaining output hypervolumes expressed by product of the axis units, i.e. forth power of standard deviations (SD⁴) (Blonder et al. 2014). Then, we performed the calculations of the hypervolumes

setting a quantile threshold of 5%, and a fixed bandwidth of 0.35. The selection of the bandwidth was done following Barros et al. (2016) (detailed in their Supplementary material Appendix 1). We calculated the hypervolumes, their centroids and centroid distances between hypervolumes of each species growing in pure and in mixed stands, both pooling all mixed communities and stratifying per species richness level, totalling 63 hypervolumes (see details in Supplementary material Appendix 2 Table A2.5, Supplementary material Appendix 2). Finally, we also estimated the contribution of each variable (trait) to the total hypervolume with the function ‘hypervolume_variable_importance’, which implements an algorithm that compares the n-dimensional input hypervolume’s volume to all possible n – 1 dimensional hypervolumes where each trait of interest has been deleted (Blonder and Harris 2017). The score reported is the ratio of the n-dimensional hypervolume relative to each of the n – 1 dimensional hypervolumes.

Null models and analyses of the functional space variation along a species richness gradient

We used null models to analyse patterns in the functional trait space occupied by each species along the species richness gradient (Gotelli and Graves 1996). In particular, we tested whether the size of functional space (hypervolumes) enlarges or shrinks, whether their shape changes and whether the functional space shifts in response to the presence of heterospecifics (Fig. 1). We built up hypervolumes based on null communities (null expectations) for each species and species richness level, generating 999 random communities for each case, constraining the species combination and number of individuals. The individual pools included all individuals belonging to the study species no matter the species richness of their original community. In biological terms, these null communities represent communities where the functional space occupied by species is not affected by the species richness of the community, in other words, in the absence of any particular process or mechanism allowing species to adjust their traits and functional space in response to the presence of heterospecifics. Then, we obtained the three metrics from each realisation: the hypervolume size, the centroid coordinates and the relative contribution of each trait. We also calculated pairwise distances among centroids of realisations of different species richness levels.

We added the observed values to the 999 generated in each of the three hypervolume components, i.e. hypervolume size, distance between hypervolume centroids of species richness levels and the relative trait contribution. First, we compared distances among centroids of observed hypervolumes along the species richness gradient to those assessed from null communities to infer shifts of the functional space. We obtained a P-value (Eq. 1), that represents the probability of getting the observed distance or lower, and a statistically significant shift was considered when $(1 - P) < 0.05$. Similarly, analysing diversity-induced shape changes in hypervolumes, the scores

of relative contribution of traits in observed hypervolumes were compared to those found in null communities.

$$P = \frac{\Sigma(\text{null values} < \text{observed value}) + \frac{\Sigma(\text{null values} = \text{observed values})}{2}}{1000} \quad (1)$$

Secondly, we tested whether the observed hypervolumes (size) were significantly different to null communities calculating again the probability P (Eq. 1) that the observed value is equal or lower than expected in a null distribution. We standardised the metric to range from –1 to 1 obtaining the effect size ES (Eq. 2) (Chase et al. 2011, Bernard-Verdier et al. 2012). We considered that the observed hypervolume of a species with a given species richness is statistically significant greater than by chance (trait divergence) when $ES > 0.95$ (at the 0.05 significance level), and smaller than by chance (trait convergence) when $ES < 0.95$.

$$ES = 2 \times (P - 0.5) \quad (2)$$

Data deposition

Data available from the Dryad Digital Repository: <doi:10.5061/dryad.5ck2qs1> (Benavides et al. 2019b).

Results

Species functional trait space

The inferred hypervolumes based on the four standardised traits (H, CPdbh, C:N, LDMC) showed that species occupied different functional spaces with relative low overlap (Fig. 2, Supplementary material Appendix 2 Table A2.6), except for coniferous species with overlaps among themselves close to or exceeding 50% in each forest type. *Betula pendula* in the boreal forest and both *Quercus* species in the Mediterranean forests are the species with most constrained functional trait space (smaller hypervolume sizes) meaning less variable phenotypes; while *Picea abies*, *Fagus sylvatica* and *Pinus sylvestris*, were the species with more variable phenotypes (larger hypervolume size) (Supplementary material Appendix 2 Table A2.5).

Changes in functional trait spaces using null models

At the species level, the effect sizes (ES) showed that variation of hypervolume size of each species along the species richness gradient compared to null expectations was mainly context-dependant (Fig. 3), with a similar pattern in both boreal and Mediterranean forests, and different in the temperate. Most species in boreal and Mediterranean forests presented smaller functional spaces in pure stands (statistically significant for *Betula pendula* in the boreal and

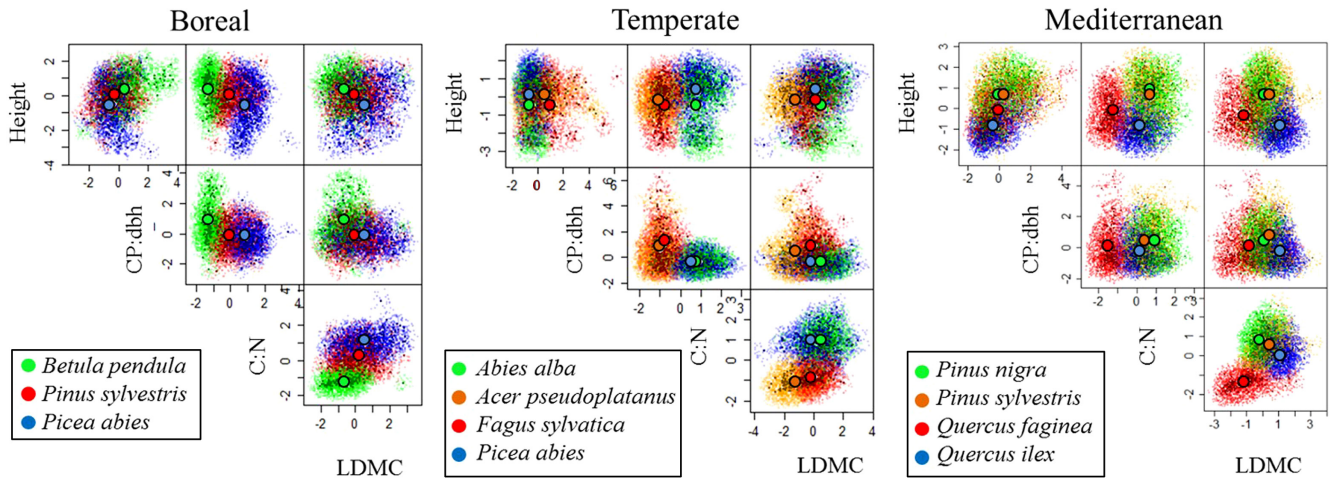


Figure 2. Hypervolumes assessed by a multidimensional kernel density estimation procedure of the study species in the three forest types (a boreal forest, a mountainous beech forest (temperate), and a Mediterranean forest). The hypervolumes are shown as pair plots and are based on four standardised functional traits selected for their low correlation representing four dimensions, i.e. plant height, crown ratio – ratio between crown area projection and diameter at breast height – (CP:dbh), ratio between C and N content in leaves (C:N), and leaf dry matter content (LDMC). Units are standard deviation (SD⁴). Larger, coloured dots are species centroids.

for every species in the Mediterranean forest) and greater in mixed stands than expected by chance (with P ranges between 0.9985 and 0.9995, and ES range between 0.997 and 0.999). However, when data from mixed stands were itemised per richness level, we found that species trait divergence occurred mainly in communities with two species, especially when the two species belonged to different functional groups (conifer versus broadleaved species) (Supplementary material Appendix 2 Fig. A2.2). Species trait variability dropped again when three or more species coexisted, and species functional space appeared more constrained than expected by chance (Fig. 3). We found no contraction in the functional space in the temperate forest, regardless of the species richness of the canopy. Indeed, the functional trait space occupied by the study species was significantly larger in communities with the four species than expected by chance (Fig. 3).

All species in the Mediterranean forests experienced shifts of their phenotypic space from pure to mixed communities. In the other two forest types, centroids shifts occurred only in some species (Table 3). For instance, in the boreal forest, the functional space occupied by *Betula pendula* and *Pinus sylvestris* in pure stands shifted compared to mixed stands, while in *Picea abies* it did not. In the temperate forest, despite the four species showed significant shifts between pure versus mixed stands, only *Abies alba* showed significant centroid distance between that one assessed in pure stands and the rest of species richness levels. For the other three species, shifts were more gradual along the species richness gradient.

Looking at the coordinates of centroids (Table 4, Supplementary material Appendix 2 Table A2.7), conspecific individuals in mixed stands tended to be shorter with larger crowns in the temperate and boreal forests. In the

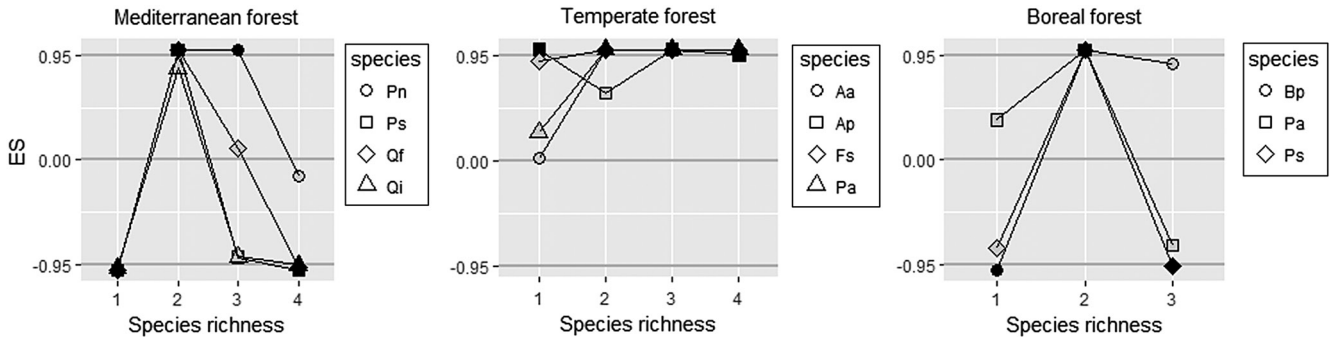


Figure 3. Variation in effect size (ES) of the hypervolumes of each tree species along a species richness gradient in the three study forest types (a boreal forest, a mountainous beech forest (temperate), and a Mediterranean forest). The line showing ES=0 represents the null expectation, and the lines $|ES| > 0.95$ represent the significant levels beyond which it is assumed that species richness affects the functional space of each species (filled symbols). Empty symbols mean no significant difference from the null expectation. (Bp: *Betula pendula*, Pa: *Picea abies*, Ps: *Pinus sylvestris*, Aa: *Abies alba*, Ap: *Acer pseudoplatanus*, Fs: *Fagus sylvatica*, Pn: *Pinus nigra*, Qf: *Quercus faginea*, Qi: *Quercus ilex*.)

Table 3. Analyses of centroid distance between hypervolume pairs assessed for each species at different species richness levels within the three forest types (boreal, mountainous beech forest –temperate–, and Mediterranean forest), including also the comparison between pure stands versus mixed stands that combine all plots with more than one species. A significant p-value means that the centroid distance between two species richness levels is significantly greater than the expected by chance. Significance: • p<0.1, * p<0.05, ** p<0.01, *** p<0.001, ns: non-significant. (Centroids' coordinates can be seen in Supplementary material Appendix 2 Table A2.7).

| Type of forest | Species | Pure versus mixed | 1 versus 2 | 1 versus 3 | 1 versus 4 | 2 versus 3 | 2 versus 4 | 3 versus 4 |
|----------------|----------------------------|-------------------|------------|------------|------------|------------|------------|------------|
| Boreal | <i>Betula pendula</i> | *** | *** | *** | | ns | | |
| | <i>Picea abies</i> | ns | ns | * | | • | | |
| | <i>Pinus sylvestris</i> | • | * | * | | ns | | |
| Temperate | <i>Abies alba</i> | *** | *** | ** | *** | ns | • | ns |
| | <i>Acer pseudoplatanus</i> | ns | ns | ns | ns | • | • | ns |
| | <i>Fagus sylvatica</i> | ** | • | ** | ** | ns | ns | * |
| | <i>Picea abies</i> | ns | ns | ns | * | ns | ** | ns |
| Mediterranean | <i>Pinus nigra</i> | * | * | ** | *** | ns | * | ** |
| | <i>Pinus sylvestris</i> | *** | *** | *** | *** | * | • | ** |
| | <i>Quercus faginea</i> | ** | *** | • | ** | ** | ** | • |
| | <i>Quercus ilex</i> | *** | *** | ** | *** | • | ** | * |

Mediterranean forest, *Pinus* species tended to be shorter, while *Quercus* species were taller in mixed stands. Differences among forest types were also found in leaf traits, in particular, leaves in the boreal and Mediterranean forests had larger LDMC compared to pure stands, while in the temperate one the opposite was found (lower LDMC).

Finally, the relative trait weights shaping species hypervolumes varied depending on the species (Fig. 4, Supplementary material Appendix 2 Table A2.8). For some species, the relative role of each trait barely changed between pure and mixed stands, like both pine species in the Mediterranean forest, or *Acer pseudoplatanus* in the temperate one. In the rest of species, some differences were found along the gradient. For instance, the variability in LDMC contributed greatly to the entire hypervolumes in pure stands, while in mixed stands, architectural traits gained relevance. In particular, conifers showed individuals more variable in height, and broadleaved species more variable in crown size, compared to other traits' variability in more diverse communities. Leaf C:N contribution to the

integrated phenotypic variability was always moderate, but for *Quercus ilex*.

Discussion

We found a significant response of species functional trait space to the species richness in the community, in line with a number of studies based on biodiversity experiments and studies analysing single traits (Ashton et al. 2010, Roscher et al. 2015, Zuppinger-Dingley et al. 2014, Zhu et al. 2015, Benavides et al. 2019a). The functional space occupied by study species expanded and shifted when trees coexist with heterospecific individuals in comparison to pure stands, suggesting an increased complementarity among species. In addition, our results indicated that this effect depends on the environmental context, so species complementarity levels off in favour of interspecific competition earlier at sites with longer drought periods or colder climates.

Trait divergence within species in response to heterospecifics

We found different patterns in trait variability between conspecific individuals growing in pure stands with trait convergence (constrained hypervolume), and mixed stands with trait divergence (expanded hypervolume). Trait convergence is usually seen as a sign of habitat filtering process (Keddy 1992, Weiher and Keddy 1995), mainly in relation to abiotic factors (Cornwell et al. 2006), which would tally with our results for sites with more adverse climatic conditions (Mediterranean and boreal forests). Nevertheless, given that our experimental design involves plots with the very same conditions where trees currently thrive, trait convergence suggests that competition amongst the most similar individuals (i.e. conspecifics) is the underlying process, instead of the habitat filtering (MacArthur and Levins 1967, File et al. 2012). Thus, intraspecific competition seems here strong enough to drive traits towards phenotypic optima

Table 4. Direction of centroid shifts comparing centroids assessed in pure versus mixed stands, distinguishing shifts in each dimension or trait, in the three study sites, a boreal forest, a mountainous mixed forest (temperate), and a Mediterranean forest. Increasing mean trait value from pure to mixed stands is represented by ↑, decreasing mean trait value by ↓, no change in a given trait is represented by ≈, and non-significant centroid shift is shown as ns.

| Type of forest | Species | H | CPdbh | CN | LDMC |
|----------------|----------------------------|----|-------|----|------|
| Boreal | <i>Betula pendula</i> | ↓ | ↑ | ↑ | ↑ |
| | <i>Pinus sylvestris</i> | ↓ | ↑ | ↓ | ↑ |
| | <i>Picea abies</i> | ns | ns | ns | ns |
| Temperate | <i>Abies alba</i> | ↓ | ↑ | ↓ | ↓ |
| | <i>Acer pseudoplatanus</i> | ns | ns | ns | ns |
| | <i>Fagus sylvatica</i> | ↓ | ≈ | ↓ | ↓ |
| | <i>Picea abies</i> | ns | ns | ns | ns |
| Mediterranean | <i>Pinus nigra</i> | ↓ | ↓ | ↓ | ↑ |
| | <i>Pinus sylvestris</i> | ↓ | ↑ | ↑ | ↑ |
| | <i>Quercus faginea</i> | ↑ | ↑ | ≈ | ↑ |
| | <i>Quercus ilex</i> | ↑ | ↑ | ↓ | ↑ |

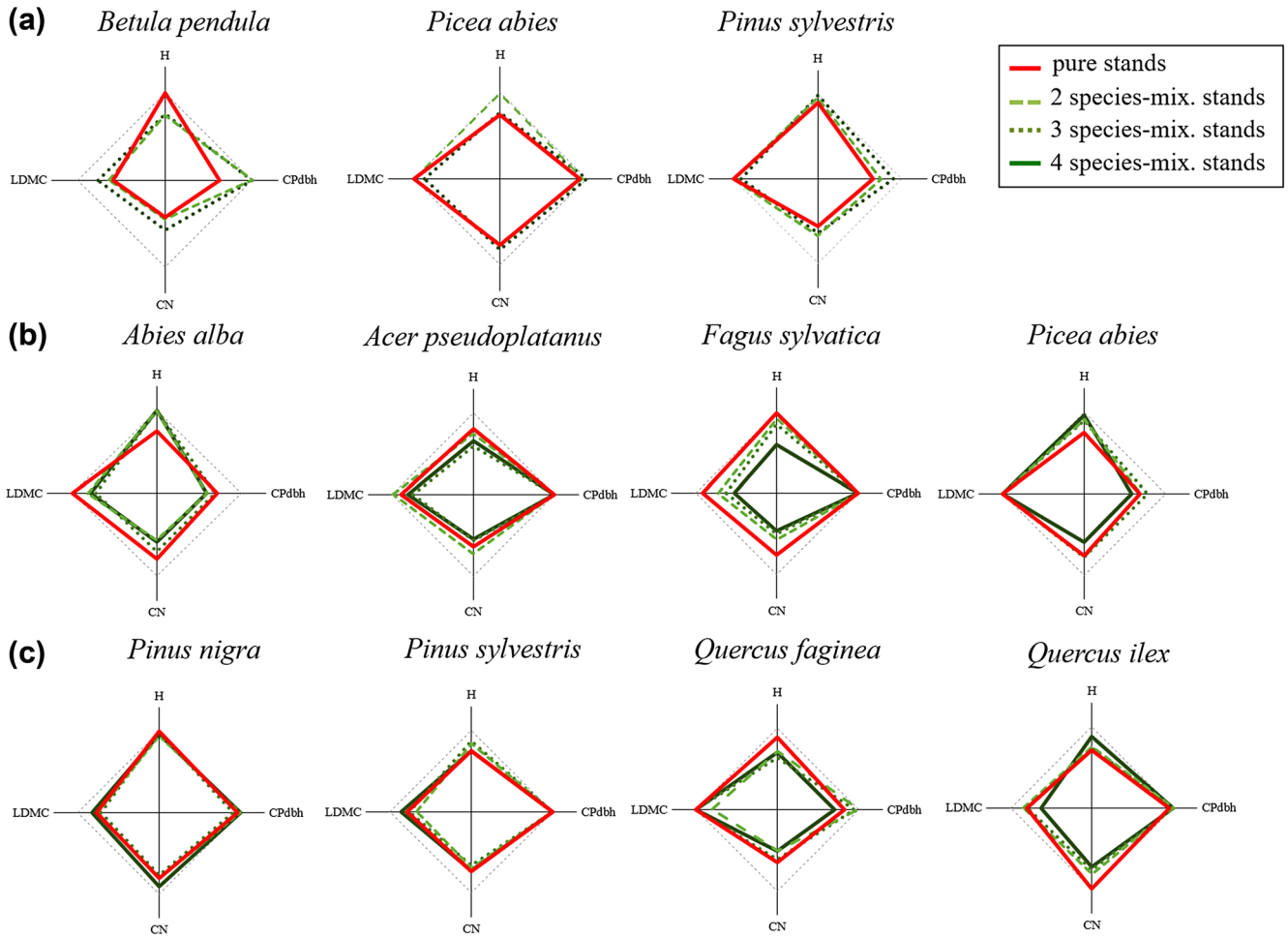


Figure 4. Relative importance of each trait shaping species hypervolumes in pure stands (red line) and mixed stands (green lines), in the three study forest types: (a) a boreal forest, (b) a mountainous mixed forest (temperate), and (c) a Mediterranean forest. For graphical purpose, we re-scaled the scores relative to the largest value (the trait contributing more to the hypervolume), which reached value 1. Each axis represents one trait, and the grey dashed line shows isoline 1, which is the maximum relative importance of observed trait values in each case. H: plant height, CPdbh: crown ratio (ratio between crown area projection and diameter at breast height), C:N: ratio between C and N content in leaves, LDMC: leaf dry matter content. Absolute values and significance of observed data in comparison to relative importance assessed with null models can be seen in Supplementary material Appendix 2 Table A2.8.

(Drenovsky et al. 2012), reducing intraspecific variance and excluding the inferior individuals (Abbott and Stachowicz 2016).

Accordingly, we found that most of the study species occupied larger functional space (trait divergence) in mixed stands than expected by chance. This larger space can be interpreted as a result of heterogeneity or greater resource supply that provided larger niche space available in mixed stands (Forrester 2014, Stein et al. 2014), intraspecific competition reduction (Vandermeer 1989), or complementarity among coexisting species (review in Forrester and Bauhus 2016). Complementarity comprises collectively processes that reduce competition like canopy or root stratification (Aschehoug and Callaway 2014, Schwendenmann et al. 2015) and promote facilitation like symbiotic nitrogen fixation (Forrester 2006) or hydraulic lift (Zapater et al. 2011), which depends highly on the species identity. Our

results support this latter statement when we analysed species responses according to the functional group of the companion species in two-species mixtures (Supplementary material Appendix 2 Fig. A2.2). In particular, we found that the Mediterranean species have convergent traits as in pure stands, when they coexist with species belonging to the same functional group (i.e. conifers and broadleaved species), and divergent traits when coexisting species belonged to the opposite functional group.

Extensive literature encapsulating the ‘stress-gradient hypothesis’ and its nuances evidenced that interactions among plant species (either facilitation, complementarity or competition) strongly depend on the environmental context (Callaway and Walker 1997, Callaway 2007, Maestre et al. 2009). In short, this hypothesis poses that competitive interactions prevails in productive and undisturbed environments, and shifts to facilitation in stressful or disturbed

environments. Accordingly, we found important differences in patterns of species functional trait space among forest types, although our results did not support this hypothesis. In the temperate forest, species phenotypic spaces were larger in mixed stands than expected by chance along the entire species richness gradient, not supporting the resource partitioning expected by strong competition. On the other hand, despite the complementarity found between two dissimilar species, species phenotypic spaces were more constrained than by chance at higher levels of species richness in the boreal and Mediterranean forests, dismissing any facilitation. These results suggest that forests with limiting conditions for growth ‘fill up’ earlier and, therefore, resource partition (Harpole et al. 2016) and exhaustion (Gebauer et al. 2012, Grossiord et al. 2014) occur earlier along the diversity gradient.

Trait shifts in response to heterospecific neighbours

Phenotypic space of most of the study species shifted in response to the species richness reflecting changes in single trait means, with the exceptions of *Picea abies* and *Acer pseudoplatanus*. These shifts, similar to the character displacement phenomenon in an evolutionary context (Dayan and Simberloff 2005), are not new and several studies based on biodiversity experiments with grasslands have already shown diversity-induced shifts (Gubsch et al. 2011, Zupping-Dingley et al. 2014, Roscher et al. 2015, Bittebiere et al. 2018), including a previous single-trait based study with our data (Benavides et al. 2019a). Endorsing the weak trait shifts found in this previous univariate analysis, we found conspecific trees being shorter and with larger crowns in communities with higher species richness, probably in response to a reduction in intraspecific competition. This result agrees also with studies that reported better canopy stratification (niche packing) and a more efficient use of aboveground space in mixed stands compared to pure stands (Jucker et al. 2014, 2015 – who worked on our same plots –, Pretzsch 2014, Swenson and Weiser 2014), especially when species with different shade tolerance are together (Jucker et al. 2014, Pretzsch et al. 2015). *Quercus* individuals in the Mediterranean forests also have larger crowns in mixed stands that would support a better canopy stratification. However, they were taller in mixed than in pure stands. This result may probably reflect their ability to tolerate shade, which would involve relatively consistent growth rates regardless of the identity of their neighbours, associated to the competitive advantage conferred on larger crowns in mixed stands versus the typical stump crowding of pure *Quercus* coppice stands that hinders individual stem growth.

We also found shifts in leaf traits comparing figures in pure and mixed stands, but in this case, the direction varied among forest types, supporting again the single-trait study of Benavides et al. (2019a). In the temperate forest, individuals had lower C:N and LDMC in more diverse communities as typically shown in resource-rich environments (Wright et al. 2004). Increasing nutrient or water availability in soils of

mixed versus monospecific tree stands has often been postulated, based on positive diversity effects on litter decomposition and nutrient cycling, and based on belowground resource use complementarity through niche partitioning and/or facilitation (Rothe and Binkley 2001, Ashton et al. 2010, Richards et al. 2010). On the contrary, individuals in species-rich stands in the boreal and Mediterranean forests had leaves with higher LDMC, suggesting more stressful conditions for individuals in high diverse plots, i.e. under interspecific and intraspecific competition. Previous studies in our boreal and Mediterranean study sites, confirmed that stressful conditions (e.g. droughts) boost competition for belowground resources in more diverse communities (Grossiord et al. 2014, Jucker et al. 2014), especially when trees developed larger crowns in mixtures and require more water to meet their evapotranspiration demands and nutrients to support this biomass.

Finally, together with shifts in mean trait values, phenotypic space shifts emerge also from changes in trait relative importance. Some study species (both pine species in the Mediterranean) turned out to have hypervolumes with quite stiff shapes, i.e. trait variations synchronized in pure and mixed stands, due to either high degree of phenotypic integrations in these species or to an insufficient gradient forcing the variation of a given dimension. However, we chose traits that were weakly correlated representing different plant dimensions, and therefore able to vary independently (Westoby et al. 2002, Díaz et al. 2004). In pure stands, leaf trait variability contributed greatly to shaping species functional space, especially LDMC. This higher relative extend of leaf traits can be explained by both smaller variability of architectural traits in pure stands, together with contrasting local conditions influencing leaf characteristics within a plot, like light, soil fertility and moisture (Wilson et al. 1999, Pérez-Harguindeguy et al. 2013). In mixed stands, architectural traits gained relevance, in particular tree height for conifers and crown size for broad-leaved species. As mentioned before, species dissimilarities allow certain complementarity and favour spatial heterogeneity that yields larger size variability within conspecifics, favoured by the modular nature of plants as variability accumulates according to the local conditions where each module develops (Marks 2007, Herrera 2009).

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Supplementary material (available online as Appendix oik-06348 at <www.oikosjournal.org/appendix/oik-06348>). Appendix 1–2.