

Functional Ecology

Interactive effects of tree species mixture and climate on foliar and woody trait variation in a widely distributed deciduous tree.

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Conflict of interest

Authors declare no conflict of interest.

Authors' contributions

MD and CG conceived the ideas and designed the methodology; XM selected the field sites; MD, EV, and CG collected the data; MD, CB, and CG analyzed the data; PV supervised the morpho-anatomical analyses; MD, CB, and CG led the writing of the manuscript. All authors contributed critically to the draft and gave final approval for publication.

Data availability

Data used in this manuscript are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.gtht76hn1> (Didion-Gency, 2021).

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Abstract

- Despite increasing reports of severe drought and heat impacts on forest ecosystems, community-level processes, which could potentially modulate tree responses to climatic stress, are rarely accounted for. While numerous studies indicate a positive effect of species diversity on a wide range of ecosystem functions and services, little is known about how species interactions influence tree responses to climatic variability.
- We quantified the intraspecific variation in 16 leaf and wood physiological, morphological, and anatomical traits in mature beech trees (*Fagus sylvatica* L.) at six sites located along a climatic gradient in the French Alps. At each site, we studied pure beech and mixed stands with silver fir (*Abies alba* Mill.) or downy oak (*Quercus pubescens* Willd.). We tested how functional traits differed between the two species mixtures (pure vs. mixed stands) within each site and along the climatic gradient.

- We found significant changes in many traits along the climatic gradient as conditions progressively got drier and warmer. Independent of the mixture, reduced leaf-level CO₂ assimilation, stomatal size, and thicker leaf cuticles, consistent with a more conservative resource use strategy, were found. At the drier sites, higher foliar stable carbon isotopic composition ($\delta^{13}\text{C}$), thicker mesophyll tissues, and lower specific leaf area (SLA) in pure stands suggests that beech had more acquisitive traits there compared to mixed stands. At the wetter sites, trees in beech-silver fir mixtures had higher chlorophyll concentration, lower $\delta^{13}\text{C}$, larger xylem vessels, and higher SLA, suggesting a more acquisitive resource use strategy in mixed stands than in pure stands.
- Our work revealed that species interactions are significant modulators of functional traits, and that they can be just as important drivers of intraspecific trait variation as climatic conditions. We show that downy oak mixtures lead to an adaptive drought response by common beech in dry environments. In contrast, in milder climates, interactions with silver fir seem to increase beech' resource acquisition and productivity. These findings highlight a strong context-dependency and imply that incorporating local interspecific interactions in research on climate impacts could improve our understanding and predictions of forest dynamics.

Keywords: anatomy, climatic stress, functional traits, morphology, phenotypic plasticity, species composition.

Introduction

Tree growth and survival largely depend on their capacity to exploit available resources in the ecosystem, which is ultimately controlled by the interactions and dynamics of the whole community. Species interactions can be either detrimental (i.e., competition) (e.g., Connoily & Wayne, 1996), neutral, or beneficial (i.e., complementarity and facilitation) (Hooper, 1998). For instance, trees can partition water resources in the soil when they have different rooting systems (e.g., beech and oak trees), leading potentially to reduced soil water competition and more stable productivity during drought events (e.g., Zapater et al., 2011). Similarly, evidence suggests that interaction among trees with distinct crown architectures (e.g., conifers and broadleaves) result in a more efficient occupation of the aboveground space, leading to improved productivity (e.g., Jucker et al., 2015).

However, species interactions are not fixed and can be modified by local environmental conditions. In dry regions, mixed stands have been found to reduce water use, growth, and the overall tolerance of trees to drought (e.g., Grossiord, 2019) or enhance it (e.g., Lubbe et al., 2017). In contrast, in moister regions, studies tend to find positive effects of species interactions and diversity on growth (e.g., Liang et al., 2016), suggesting higher water, carbon and nutrient use in mixed-species stands. Species interaction effects are also highly dependent on the identity of the species present with interspecific interactions being potentially beneficial for some species but detrimental to others (e.g., Forrester et al., 2016). Hence, species interaction effects are highly context-dependent, with positive interactions that enhance function potentially turning into negative interactions under different abiotic and biotic conditions (Ratcliffe et al., 2017). Yet, few studies have looked at how species interactions alter tree functional strategies along broad environmental conditions (Paquette et al., 2018), and no study to our knowledge has tried to disentangle the impact of species mixture *vs.* climatic conditions on leaf and wood functional traits.

Tree physiological, morphological, and anatomical traits provide fundamental insights into trees' functional strategies, including, for instance, how trees respond to environmental constraints and the underlying processes driving tolerance to extreme events (Violle et al., 2007). Moreover, how trees adjust their traits in responses to a changing climate will affect their long-term persistence (Allen et al., 2015; Nicotra et al., 2010). Long-lived species that occur over broad

environmental conditions, such as trees, can show substantial genetic adaptation and phenotypic plasticity in their traits. Populations living in drier climates tend, for instance, to have smaller and thicker leaves with denser but smaller stomates (e.g., de Cárcer et al., 2017), which provide higher foliar hydraulic safety (i.e., a conservative resource use strategy; Reich, 2014). Inversely, populations from wetter regions tend to have larger and thinner leaves with bigger but fewer stomates (e.g., Abrams et al., 1994) to increase their maximum photosynthetic rates (i.e., an acquisitive resource use strategy). Thus, if interspecific interactions increase access to resources because of complementarity and facilitation processes, one may expect trees in mixed stands to shift their foliar and woody traits towards a more acquisitive resource use strategy (thinner and larger leaves, larger xylem vessels and denser wood), independently of the background climate. Inversely, if competition is reducing access to resources in mixed stands, trees may develop more conservative traits compared to their counterparts in pure stands.

To compare how tree functional strategies are influenced by climatic conditions and species interactions, we investigated the intraspecific variability of 16 physiological, morphological, and anatomical leaf and woody traits of common beech in pure and mixed stands along a climatic gradient in the French Alps. We determined how these two species mixtures, i.e., pure vs. mixed stands with silver fir or downy oak, influence beech traits along the climatic gradient. We expected (1) beech trees in drier sites to have traits associated with a conservative resource use strategy (e.g., lower maximum assimilation, higher water-use efficiency, lower stomatal size but higher density, and smaller xylem vessel area) compared to wetter sites where trees will show traits associated with an acquisitive resource use strategy (e.g., higher hydraulic diameter, maximum assimilation rates, and thinner and larger leaves), and (2) trees to have a less conservative and more acquisitive resource use strategy in mixed stands due to complementarity and/or facilitation between species (both when mixed with silver fir and downy oak), leading to enhanced resource availability, independently of the climatic conditions (i.e., all along the climatic gradient).

Material and methods

Study sites

The study was conducted at six sites along a climatic gradient in the French Alps (Fig. 1; Jourdan et al., 2020b). All sites are characterized by limestone bedrock, with a North to West orientation (Table S1). Minimum and maximum temperature and precipitation were recorded by meteorological stations at each site, and the gaps were filled by E-OBS gridded dataset (2005-2019) (<https://www.ecad.eu/download/ensembles/download.php>).

In the three Northern sites (BG, VC, VT), the forest is dominated by beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) trees, and in the three Southern sites (LA, LU, SB), the dominant trees species are beech and downy oak (*Quercus pubescens* Willd.) (Table 1). Other tree species growing at the sites are *Acer pseudoplatanus* L., *Acer opalus* Mill., *Picea abies* L., *Sorbus aucuparia* L., *Ilex aquifolium* L., but they represent a negligible part of the total basal area of each stand. To limit confounding effects, stands were selected within sites without management for at least one decade. Forest structure was homogenous among stands, except in LU which has coppice stands for more than 50 years, and SB which has old-growth forest and secondary stands. A stand was delimited by a 17.5 m radius circle, including a central area of 10 m radius and a 7.5 m buffer zone (total surface of 1000 m²). Stands contained between 14 and 46 trees in total (Table 1). Multiple characteristics were measured on all trees (i.e., species identity, location of the trees, height, and diameter at breast height (DBH)).

Stands were separated into two groups based on their species mixture, i.e., beech pure stands, for assessing the impact of intraspecific interactions, and mixed stands, for impacts of interspecific interactions (beech-silver fir and beech-downy oak mixed stands in the three northern and southern sites, respectively, Fig. 1). Stands were determined as pure when at least 90% of the total basal area was represented by beech, while mixed stands were dominated by a mixture of the two species, with the relative basal area of each species representing at least 40% of the total basal area. In each site, four stands were selected with two subgroups at two different elevations, with one pure and one mixed stand at each elevation (Table 1), leading to a total of 24 stands. Elevation differences between the two subgroups were ranging from 50m to 250m among sites (Table 1). The stands were selected as subsets of a large experiment (GMAP plot network, <https://oreme.org/observation/foret/gmap/>). In each stand, five dominant beech trees were

randomly selected, leading to a total of 120 trees. Beech was present over the entire gradient allowing us to study the effect of climatic conditions (variation between sites), species interactions (variation between pure and mixed stands), and the interactive effect of climate and species interactions. However, as different interaction partners are present in mixed stands in the three northern and southern sites, the experimental design does not allow us to disentangle between climatic and forest type effects (mixtures with downy oak vs. silver fir).

Sixteen physiological, morphological, and anatomical traits related to water and carbon transport, use and uptake (Table 2) were measured on all selected trees between mid-July and mid-August 2019. The sampling started in the southern sites, proceeding northwards to account for differences in the growing season length and start (shorter in the northern sites) and ensure that measurements were conducted in the middle of the growing season at all sites. Samples were harvested in non-rainy conditions by tree climbers, except LU where the sampling was performed using a telescopic pole pruner.

Wood anatomical traits

We cut a 20 cm-long twig sample (70 cm from the apex, diameter between 2-3 cm) from the top of the canopy of each tree to determine wood properties. The samples were stored in plastic vials with a 50% ethanol solution. Transversal sections of each sample (12 μm thick) were made with a Leica rotation microtome and stained with Safranin-O and astra blue (1% and 0.5% in distilled water, respectively). The sections were mounted with Canada balsam on glass slides with a cover slip (von Arx et al., 2016). Microscopic digital images were captured at 40x magnification with a compound microscope (BX51, Olympus, Germany) interfaced with a Canon camera (Canon EOS 1200D, Switzerland). Digital images were taken on a radial path (Fig. S1a). Image analyses were performed with the ImageJ software. The total number of vessels and the lumen area (A_{Lumen} , μm^2) were measured on the last two growth rings, as these vessels are responsible for the majority of water transport (Domec & Gartner, 2002). Vessel density (VD, vessels cm^{-2}) was calculated by dividing the number of vessels by the area measured. The hydraulically weighted diameter (D_{H} , μm) was calculated on the last two growth rings, following the Hagen-Poiseuille law as described by Tyree et al. (1983):

$$Dh = 2 \left(\frac{\sum r^5}{\sum r^4} \right)$$

where r is the vessel radius. Wood density (WD, mg cm^{-3}) was measured as the ratio of xylem dry mass to xylem wet volume (Wright et al., 2010).

Leaf physiological traits

We measured predawn leaf water potential (Ψ_{PD} , MPa) on one leaf per tree by sampling one twig from the top of the canopy before sunrise. Measurements were conducted in the field with a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA).

Maximum CO_2 assimilation (A_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) were measured on sunlit leaves of all trees using a LI-COR 6400 infrared gas analyzer system (LI-COR, Lincoln, USA). We sampled 1.5m-long branches from the top of the canopy, placed them in a water bucket and recut them twice to remove potential cavitation (Bachofen et al., 2020). The measurements were carried out between 10am and 1pm when the highest gas exchange rates is expected. Measurements were conducted with the following setting: 400 ppm of reference CO_2 concentration, 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light-saturating photosynthetic photon flux density, block temperature at 20°C or 30°C to match ambient air temperature at each site (i.e., BG, VC, VT = 20°C, LA, LU, SB = 30°C), and relative air humidity set at 50% to reflect ambient conditions. Leaf temperature was estimated using the instrument energy balance calculation in the system software. Measurements were recorded after steady-state gas exchange rates had been maintained for at least 5 min. Intrinsic water-use efficiency (WUE_i , μmol^{-1}) was calculated by dividing A_{max} by g_s (Fischer & Turner, 1978). Chlorophyll concentration (Chl, $\mu\text{mol m}^{-2}$) was measured on the same leaves with a chlorophyll concentration meter (MC-100, Apogee Instruments, Inc., USA).

Foliar carbon isotopic composition ($\delta^{13}\text{C}$, ‰) was measured on sunlit leaves taken from the same branches used for the gas exchange measurements. Leaves were stored in paper bags with a dry environment until further drying at 65°C for 48h in the laboratory, and dry samples were ground to powder. About 1 mg of the powdered material was placed into tin capsules (Säntis, Teufen, Switzerland). $\delta^{13}\text{C}$ measurements were conducted with an elemental analyzer interfaced to a

DeltaPlusXP isotope ratio mass spectrometer (EA-IRMS; Thermo EA 1100 Deltaplus XL; 0.01‰ precision) at the stable isotope research center (SIRC) of WSL in Birmensdorf. $\delta^{13}\text{C}$ values are reported in the standard delta notation relative to the Vienna Pee Dee Belemnite standard (VPDB).

Leaf morphological traits

Leaf morphological traits were assessed by sampling ten leaves from long terminal shoots on one branch from the top canopy (Cornelissen et al., 2003). The mean leaf surface (LS, cm^2) was determined using scanned fresh leaves (EPSON Perfection V800 Photo, EPSON, Amsterdam) and the Silver Fast 8 software (Laser soft imagine AG, Germany). After drying for 48h at 65°C , the leaves were weighed, and the specific leaf area calculated (SLA , $\text{m}^2 \text{kg}^{-1}$).

Leaf anatomical traits

Leaf anatomical traits were measured using leaf material sampled next to the samples collected for morphological assessments (de Cárcer et al., 2017). Stomatal density (SD , mm^{-2}) and size (A_{stomates} , μm^2) were determined using nail varnish imprints realized at mid-leaf location apart from the leaf central vein, on two leaves. Stomata were observed and imaged in diascopic light using the 20x objective of a Leica Leitz DMRB microscope interfaced with the INFINITY 2 ANALYZE camera. The camera software was used to assess selected stomata count and size (Lumenera Corporation, Canada, Fig. S1b). The thickness of the leaf mesophyll (M , μm) and the lower cuticle (LC , μm) (Fig. S1c, d) were measured using 1 cm leaf disks excised at middle leaf position, just after harvesting the branch, and immediately fixed using 2.5% glutaraldehyde buffered at pH 7.0 using 0.067 M Soerensen phosphate buffer. In the laboratory, the samples were further infiltrated by evacuation and the solution renewed before storing at 4°C until further processing. Leaf sections (50 μm thick) were trimmed from the central disk part using a custom-made hand microtome and mounted in 80% glycerin either directly or after 25 min staining in 0.5% Alcian blue (Arend et al., 2008) for assessing cuticles. Mesophyll tissues and cuticles were observed, imaged, and measured using the aforementioned DMRB microscope, 20x and 100x objectives, the Infinity 2 camera and its software's measurement module.

Data analysis

A climatic index considering the long-term variation in mean annual temperature and precipitation along the gradient was calculated by dividing the precipitation by the potential evapotranspiration (P/PET). PET at each site was calculated using the Thornthwaite equation (Kolka, 1998), which takes into account the average daily temperature, the number of days per year (i.e., 365), the average day length, and a heat index estimated using monthly mean temperature. P/PET ranged between 3.31 and 1.39 over the last 20 years (from 2005 to 2019) and between 0.61 and 0.08 over the measurement period (from July to August 2019) for the wettest and the driest site, respectively (Table 1).

We determined the effect of P/PET (average from 2005 to 2019), species mixture (pure vs. mixed stands), and the interactive effect of P/PET and species mixture all along the gradient on each functional trait using linear mixed models (*lme4* and *lmerTest* function). P/PET and species mixture were fixed effects, and the subgroup of stands (high vs. low elevation) were treated as a random effect nested in each site to account for differences in elevation within each site. This is particularly important knowing the potential impact of elevation on some functional traits (e.g., $\delta^{13}\text{C}$).

We evaluated the effect of the forest type (three northern sites where beech and silver fir co-occur vs. three southern sites where beech and downy oak co-occur) and the interaction between the forest type and species mixture on each functional trait in a separate linear mixed model. P/PET was not included in this test to better capture the isolated effect of the forest type and species mixture. Forest type and species mixture were included as fixed effects, and the subgroups of stands nested in each site were treated as random effects. Tukey type post hoc tests were used to reveal significant differences between forest type and species mixture groups (*multcomp* function).

All analyses were performed using the R v.3.6.1 statistical platform (R Development Core Team, 2019).

Results

Impact of the climatic gradient on functional traits

Climatic conditions significantly impacted four leaf traits and only one woody trait. Maximum CO₂ assimilation (A_{\max}), predawn leaf water potential (Ψ_{PD}), chlorophyll concentration (Chl), and stomatal area (A_{stomates}) were significantly decreasing in response to lower P/PET (Table 3). Inversely, leaf cuticles (LC) were increasing in response to lower P/PET (Table 3). No effect of P/PET was found on stomatal conductance (g_s), leaf carbon isotopic composition ($\delta^{13}\text{C}$), intrinsic water-use efficiency (WUE_i), lumen area (A_{Lumen}), vessel density (VD), theoretical hydraulic diameter (D_H), wood density (WD), leaf surface (LS), specific leaf area (SLA), stomatal density (SD), and mesophyll thickness (M) (Table 3).

Interactive impacts of species mixture and climate on functional traits

Species mixture (i.e., pure vs. mixed stands) significantly modified Ψ_{PD} , Chl, $\delta^{13}\text{C}$, A_{Lumen} , SLA and M and this effect depended on P/PET except for M, which was consistently smaller in mixed stands (Figs. 2, 3 & 4, Table 3). Mixed stands had more negative Ψ_{PD} , less negative $\delta^{13}\text{C}$, smaller SLA and higher M compared to pure stands as conditions progressively got drier and warmer (Figs. 2, 3 & 4, Table 3), consistent with a more conservative resource use strategy. In contrast, mixed stands had less negative Ψ_{PD} , more negative $\delta^{13}\text{C}$, higher Chl, A_{Lumen} , SLA, and lower M thickness compared to pure stands as conditions got wetter and colder (Figs. 2, 3 & 4, Table 3), suggesting a more acquisitive resource use strategy. No impact of species mixture or of the interaction between species mixture and P/PET was found for the other traits (Table 3).

Impact of species mixture on functional traits within each forest type

Species mixture significantly modified A_{\max} , Ψ_{PD} , Chl, and A_{Lumen} within both forest types (i.e., beech mixed with downy oak or beech mixed with silver fir) (Fig. 2, 3 & 4, Table 3) but the effects were opposite. Mixed stands had more negative Ψ_{PD} in the sites where beech is mixed with downy oak compared to pure stands (Fig. 2, Table 3). On the contrary, mixed stands had less negative Ψ_{PD} , higher Chl, and A_{Lumen} compared to pure stands in the sites where beech is

mixed with silver fir (Figs. 2 & 3, Table 3). We found no impact of species mixture within the two forest types on all remaining traits (Figs. 2, 3 & 4, Table 3).

Discussion

Impact of the climatic gradient on functional traits

As we initially expected, our study demonstrates that beech has more conservative foliar traits (i.e., lower maximum photosynthesis, leaf water potential, chlorophyll concentration, stomatal area, and thicker cuticle; Figs. 2 & 4) as conditions get warmer and drier along the climatic gradient. This result agrees with previous work reporting strong variation in beech foliar traits in response to climate (Bussotti, 1995; de Cárcer et al., 2017; Martin-Blangy et al., 2021). Although our number of replicates could have impacted these findings, it is important pointing out that many leaf anatomical traits showed low variability along the gradient (e.g., leaf surface, SLA, stomatal density, mesophyll thickness). These results contrast work conducted on other species (McLean et al., 2013; Warren et al., 2005 but see Binks et al., 2016) and suggest that beech responds to reduction in soil water by adjusting its physiology rather than by producing leaves with more xeromorphic characteristics (e.g., smaller leaves, higher stomatal density, thinner mesophyll tissues). Similarly, wood morphological and anatomical traits also showed a homeostatic response to the climatic gradient (Fig. 3). These results contradict previous studies that showed a strong intraspecific variation in beech woody traits along environmental gradients, with often a reduction of hydraulic conductivity linked to smaller and denser vessels as conditions get drier (e.g., Schuldt et al., 2016; Tognetti et al., 1995). A limitation in our work is that we bundled the effects of different climatic drivers (temperature, VPD and soil drought) into one index, P/PET, which does not allow us to associate trait shifts to specific environmental constraints. Yet, because of potential local changes in soil characteristics, the relative importance of atmospheric vs. soil moisture stress can vary substantially under a similar P/PET. Using a similar approach, Martínez-Vilalta et al. (2009) also reported a lack of woody trait variability along an even larger environmental gradient for *Pinus sylvestris*. To better understand the underlying drivers of intraspecific trait variability, future work should disentangle the effects of individual climatic variables (temperature and VPD vs. soil moisture), particularly as their trajectories could differ under future climate. Moreover, apart from abiotic conditions, genetic-based assessments demonstrated that the trees studied here had a gradual genetic differentiation along the gradient, but that they still belonged to the same population (Capblancq et al., 2020), suggesting that plasticity to changing conditions could explain the observed trait variability.

Nevertheless, independently of the underlying processes leading to changes in traits, our findings could suggest that while beech is able to adjust to drought and heat by shifting to more conservative physiological leaf traits, the absence of leaf anatomical and woody trait plasticity points towards high vulnerability during upcoming extreme events. These findings are supported by the high drought-induced mortality rates found for beech throughout central Europe in recent years, including in our study region (e.g., Archaubeau et al., 2020; Etzold et al., 2019).

Interactive impacts of species mixture and climate on functional traits

Our results indicate that species interactions exacerbate competition for water in the driest conditions as trees had more conservative strategies in mixed stands with downy oak (i.e., more negative leaf water potential, thicker M, lower SLA, and more positive $\delta^{13}\text{C}$; Figs. 2 & 4). These responses contradict previous studies suggesting improved water availability and water use efficiency of beech during drought in mixed stands (de Andrés et al., 2017; Pretzsch et al., 2013). Oak and beech exhibit important physiological and structural differences that should lead to complementarity and facilitation mechanisms. For instance, beech has a rather extended horizontal rooting system compared to oak that have a deeper pivoting rooting system allowing them to maintain higher transpiration rates during drought (Bréda et al., 1993; Čermák & Fér, 2007). Beech is also more isohydric compared to oak, by closing its stomata at less negative water potential (Pretzsch et al., 2013; Roman et al., 2015), thereby limiting the competition for soil moisture for oak during dry conditions. However, previous work has shown that while deep-rooted oak trees benefit from interspecific interactions during drought, species with shallower roots, like beech, have an exacerbated response to soil moisture stress in mixtures (Grossiord et al., 2014b, 2015). We could therefore hypothesize that in our mixed stands, downy oak roots outcompete beech roots in the deeper and wetter soil horizons, leading to higher drought stress for beech compared to pure stands. These results are supported by findings of Jourdan et al. (2020) who showed lower productivity of beech when mixed with downy oak compared to pure stands in the same sites.

In contrast, at moister sites, we found that beech had a more acquisitive strategy in mixtures compared to pure stands (i.e., higher chlorophyll concentration, more negative carbon isotopic composition, larger xylem vessels, higher SLA and thinner mesophyll; Figs. 2, 3 & 4). In these

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sites, beech is competing with silver fir. These two species also exhibit fundamental differences, such as a different crown architecture (height, shape, and shade tolerance; Mauri et al., 2016) and the foliar type and habit (broadleaved *vs.* conifer, deciduous *vs.* evergreen). Interaction between species with these different structures could support complementarity processes such as a better utilization of the aboveground space because of a more diversified vertical structure (e.g., Jucker et al., 2015) and/or facilitation processes such as the enrichment of litter by more diverse soil microbial communities (Zak et al., 2003). Furthermore, these findings confirm previous work conducted in the same sites, where beech was found to be more productive in mixed stands with silver fir than in pure stands (Jourdan et al., 2020; Toïgo et al., 2021). Future work would be needed to identify the exact underlying physical and biological mechanisms driving these differential interaction effects along the gradient, including assessing the rooting depth of species, the soil nutritional status, and its microbial composition.

Overall, our work revealed that species interactions are significant modulators of leaf and woody traits. Moreover, the same number of functional traits were altered by climate and species interactions (Table 3), with some traits showing larger variation in response to species interactions than to climate (e.g., Fig. 3). This finding suggests that species interactions are just as important drivers of intraspecific trait variation as climatic conditions. This result is especially striking for woody traits for which no variability was found along the gradient but where clear differences emerged between mixed and pure stands in the moister sites (Fig. 3). These findings are the first to demonstrate the high plasticity of beech' hydraulic strategies and the adjustments of the efficiency to transport water in response to complementarity, facilitation and competition between species. However, our results need to be interpreted with care considering the few interactions included (interactions only with downy oak and silver fir). Future work should consider broader gradients where the same tree species are present along the entire gradient, which, however, might prove difficult due to the different climatic niches of different species. Nevertheless, these findings still highlight the importance of species interactions on the functional strategies of trees and further accentuates the need to include species interaction and identity in trait and climate-vegetation research.

Conclusions

Our study highlights that adjustments of *Fagus sylvatica* L. to a broad range of climatic conditions occurs mainly in the leaf, with trees developing more conservative traits as conditions get drier and warmer. No change in woody hydraulic traits was found, which could point towards a high hydraulic vulnerability with the projected decline in moisture within beech's distribution range. Our work further revealed that intraspecific trait variation heavily depends on species interactions, and that these interactions can have stronger impacts than climatic conditions. However, these effects varied with the climate and the forest type. While the mixture with a more drought resistant species in drier and hotter regions seem to increase moisture constrains, beech seem to benefit from the mixture with a conifer in moister and cooler climates. Which underlying processes are driving these differences remains an open question for future work. Disentangling the impacts of atmospheric and soil drivers on trait variation would deserve further studies to fully decipher the significance of species interactions under a changing climate.

References

- Abrams, M. D., Kubiske, M. E., & Mostoller, S. A. (1994). Relating Wet and Dry Year Ecophysiology to Leaf Structure in Contrasting Temperate Tree Species. *Ecology*, 75(1), 123–133. <https://doi.org/10.2307/1939389>
- Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere*, 6(8), art129. <https://doi.org/10.1890/ES15-00203.1>
- Archambeau, J., Ruiz-Benito, P., Ratcliffe, S., Fréjaville, T., Changenet, A., Muñoz Castañeda, J. M., Lehtonen, A., Dahlgren, J., Zavala, M. A., & Benito Garzón, M. (2020). Similar patterns of background mortality across Europe are mostly driven by drought in European beech and a combination of drought and competition in Scots pine. *Agricultural and Forest Meteorology*, 280, 107772. <https://doi.org/10.1016/j.agrformet.2019.107772>
- Arend, M., Muninger, M., & Fromm, J. (2008). Unique occurrence of pectin-like fibrillar cell wall deposits in xylem fibres of poplar. *Plant Biology*, 8.
- Bachofen, C., D’Odorico, P., & Buchmann, N. (2020). Light and VPD gradients drive foliar nitrogen partitioning and photosynthesis in the canopy of European beech and silver fir. *Oecologia*, 192(2), 323–339. <https://doi.org/10.1007/s00442-019-04583-x>
- Binks, O., Meir, P., Rowland, L., da Costa, A. C. L., Vasconcelos, S. S., de Oliveira, A. A. R., Ferreira, L., & Mencuccini, M. (2016). Limited acclimation in leaf anatomy to experimental drought in tropical rainforest trees. *Tree Physiology*, 36(12), 1550–1561. <https://doi.org/10.1093/treephys/tpw078>
- Bréda, N., Cochard, H., Dreyer, E., & Granier, A. (1993). Field comparison of transpiration, stomatal conductance and vulnerability to cavitation of *Quercus petraea* and *Quercus robur* under water stress. *Annales Des Sciences Forestières*, 50(6), 571–582. <https://doi.org/10.1051/forest:19930606>
- Bussotti, F. (1995). Morpho-anatomical alterations in leaves collected from beech trees (*Fagus sylvatica* L.) in conditions of natural water stress. *Environmental and Experimental Botany*, 35(2), 201–213. [https://doi.org/10.1016/0098-8472\(94\)00040-C](https://doi.org/10.1016/0098-8472(94)00040-C)
- Capblancq, T., Morin, X., Gueguen, M., Renaud, J., Lobreaux, S., & Bazin, E. (2020). Climate-associated genetic variation in *Fagus sylvatica* and potential responses to climate change

in the French Alps. *Journal of Evolutionary Biology*, 33(6), 783–796.

<https://doi.org/10.1111/jeb.13610>

- Čermák, P., & Fér, F. (2007). Root systems of forest tree species and their soil-conservation functions on the Krušné hory Mts. Slopes disturbed by mining. *JOURNAL OF FOREST SCIENCE*, 53, 561–566.
- Connoily, J., & Wayne, P. (1996). Asymmetric competition between plant species. *Oecologia*, 10.
- Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., Reich, P. B., Steege, H. ter, Morgan, H. D., Heijden, M. G. A. van der, Pausas, J. G., & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), 335.
<https://doi.org/10.1071/BT02124>
- Dai, A. (2011). Drought under global warming: A review: Drought under global warming. *Wiley Interdisciplinary Reviews: Climate Change*, 2(1), 45–65. <https://doi.org/10.1002/wcc.81>
- de Cárcer, P. S., Signarbieux, C., Schlaepfer, R., Buttler, A., & Vollenweider, P. (2017). Responses of antinomic foliar traits to experimental climate forcing in beech and spruce saplings. *Environmental and Experimental Botany*, 13.
- Didion-Gency, M. (2021), Interactive effects of tree species mixture and climate on foliar and woody trait variation in in a widely distributed deciduous tree - dataset, Dryad, Dataset, <https://doi.org/10.5061/dryad.gtht76hn1>
- Domec, J. C., & Gartner, B. L. (2002). Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: Inferring the design criteria for Douglas-fir wood structure. *Tree Physiology*, 22(2–3), 91–104. <https://doi.org/10.1093/treephys/22.2-3.91>
- Etzold, S., Ziemińska, K., Rohner, B., Bottero, A., Bose, A. K., Ruehr, N. K., Zingg, A., & Rigling, A. (2019). One Century of Forest Monitoring Data in Switzerland Reveals Species- and Site-Specific Trends of Climate-Induced Tree Mortality. *Frontiers in Plant Science*, 10, 307. <https://doi.org/10.3389/fpls.2019.00307>
- Farquhar, G. D., & Sharkey, T. D. (1982). Stomatal Conductance and Photosynthesis. *Annual Review of Plant Physiology*, 33(1), 317–345.
<https://doi.org/10.1146/annurev.pp.33.060182.001533>

- Accepted Article
- Farquhar, G., & Richards, R. (1984). Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. *Functional Plant Biology*, *11*(6), 539. <https://doi.org/10.1071/PP9840539>
- Fernandez, J. E., Giron, I. F., & Blazquez, O. M. (1997). Stomatal control of water use in olive tree leaves. *Plant and Soil*, *190*, 179–192.
- Fischer, R. A., & Turner, N. C. (1978). Plant Productivity in the Arid and Semiarid Zones. *Annual Review of Plant Physiology*, *29*(1), 277–317. <https://doi.org/10.1146/annurev.pp.29.060178.001425>
- Forrester, D. I., Bonal, D., Dawud, S., Gessler, A., Granier, A., Pollastrini, M., & Grossiord, C. (2016). Drought responses by individual tree species are not often correlated with tree species diversity in European forests. *Journal of Applied Ecology*, *53*(6), 1725–1734. <https://doi.org/10.1111/1365-2664.12745>
- González de Andrés, E., Seely, B., Blanco, J. A., Imbert, J. B., Lo, Y.-H., & Castillo, F. J. (2017). Increased complementarity in water-limited environments in Scots pine and European beech mixtures under climate change: Climate change increases complementarity in pine/beech mixedwoods. *Ecohydrology*, *10*(2), e1810. <https://doi.org/10.1002/eco.1810>
- Grossiord, C. (2019). Having the right neighbors: How tree species diversity modulates drought impacts on forests. *New Phytologist*. <https://doi.org/10.1111/nph.15667>
- Grossiord, C., Forner, A., Gessler, A., Granier, A., Pollastrini, M., Valladares, F., & Bonal, D. (2015). Influence of species interactions on transpiration of Mediterranean tree species during a summer drought. *European Journal of Forest Research*, *134*(2), 365–376. <https://doi.org/10.1007/s10342-014-0857-8>
- Grossiord, C., Gessler, A., Granier, A., Pollastrini, M., Bussotti, F., & Bonal, D. (2014). Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. *Forest Ecology and Management*, *318*, 54–61. <https://doi.org/10.1016/j.foreco.2014.01.004>
- Henry, C., John, G. P., Pan, R., Bartlett, M. K., Fletcher, L. R., Scoffoni, C., & Sack, L. (2019). A stomatal safety-efficiency trade-off constrains responses to leaf dehydration. *Nature Communications*, *10*(1), 3398. <https://doi.org/10.1038/s41467-019-11006-1>

- Accepted Article
- Hooper, D. U. (1998). The role of the complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology*, *79*(2), 704–719. [https://doi.org/10.1890/0012-9658\(1998\)079\[0704:TROCAC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[0704:TROCAC]2.0.CO;2)
- Jourdan, M., Kunstler, G., & Morin, X. (2020). How neighbourhood interactions control the temporal stability and resilience to drought of trees in mountain forests. *Journal of Ecology*, *108*(2), 666–677. <https://doi.org/10.1111/1365-2745.13294>
- Jourdan, M., Piedallu, C., Baudry, J., & Morin, X. (2020). Tree diversity and the temporal stability of mountain forest productivity: Testing the effect of species composition, through asynchrony and overyielding. *European Journal of Forest Research*. <https://doi.org/10.1101/2020.01.20.912964>
- Jucker, T., Bouriaud, O., & Coomes, D. A. (2015). Crown plasticity enables trees to optimize canopy packing in mixed-species forests. *Functional Ecology*, *29*(8), 1078–1086. <https://doi.org/10.1111/1365-2435.12428>
- Kolka, R. K. (1998). Estimating Actual Evapotranspiration for Forested Sites: Modifications to the Thornthwaite Model. *Research Note SRS-6*, 8.
- Liang, J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E.-D., McGuire, A. D., Bozzato, F., Pretzsch, H., de-Miguel, S., Paquette, A., Herault, B., Scherer-Lorenzen, M., Barrett, C. B., Glick, H. B., Hengeveld, G. M., Nabuurs, G.-J., Pfautsch, S., ... Reich, P. B. (2016). Positive biodiversity-productivity relationship predominant in global forests. *Science*, *354*(6309), aaf8957–aaf8957. <https://doi.org/10.1126/science.aaf8957>
- Lübbe, T., Schuldt, B., & Leuschner, C. (2017). Acclimation of leaf water status and stem hydraulics to drought and tree neighbourhood: Alternative strategies among the saplings of five temperate deciduous tree species. *Tree Physiology*, *37*(4), 456–468. <https://doi.org/10.1093/treephys/tpw095>
- Martin-Blangy, S., Charru, M., Gérard, S., Jactel, H., Jourdan, M., Morin, X., & Bonal, D. (2021). Mixing beech with fir or pubescent oak does not help mitigate drought exposure at the limit of its climatic range. *Forest Ecology and Management*, *482*, 118840. <https://doi.org/10.1016/j.foreco.2020.118840>
- Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J. F. J., Llorens, P., Nikinmaa, E., Nolé, A., Poyatos, R., Ripullone, F., Sass-Klaassen, U., &

- Zweifel, R. (2009). Hydraulic adjustment of Scots pine across Europe. *New Phytologist*, 184(2), 353–364. <https://doi.org/10.1111/j.1469-8137.2009.02954.x>
- Mauri, A., de Rigo, D., & Caudullo, G. (2016). *Abies alba* in Europe: Distribution, habitat, usage and threats. *European Atlas of Forest Tree Species*, 48–49.
- McCulloh, K., Sperry, J. S., Lachenbruch, B., Meinzer, F. C., Reich, P. B., & Voelker, S. (2010). Moving water well: Comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. *New Phytologist*, 186(2), 439–450. <https://doi.org/10.1111/j.1469-8137.2010.03181.x>
- McLean, E. H., Prober, S. M., Stock, W. D., Steane, D. A., Potts, B. M., Vaillancourt, R. E., & Byrne, M. (2013). Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant, Cell & Environment*, 27, 1440–1451.
- Nardini, A., Öunapuu-Pikas, E., & Savi, T. (2014). When smaller is better: Leaf hydraulic conductance and drought vulnerability correlate to leaf size and venation density across four *Coffea arabica* genotypes. *Functional Plant Biology*, 41(9), 972. <https://doi.org/10.1071/FP13302>
- Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., Poot, P., Purugganan, M. D., Richards, C. L., Valladares, F., & van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, 15(12), 684–692. <https://doi.org/10.1016/j.tplants.2010.09.008>
- Paquette, A., Vayreda, J., Coll, L., Messier, C., & Retana, J. (2018). Climate Change Could Negate Positive Tree Diversity Effects on Forest Productivity: A Study Across Five Climate Types in Spain and Canada. *Ecosystems*, 21, 960–970. <https://doi.org/10.1007/s10021-017-0196-y>
- Poorter, L., & Rozendaal, D. M. A. (2008). Leaf size and leaf display of thirty-eight tropical tree species. *Oecologia*, 158(1), 35–46. <https://doi.org/10.1007/s00442-008-1131-x>
- Pretzsch, H., Schütze, G., & Uhl, E. (2013). Resistance of European tree species to drought stress in mixed *versus* pure forests: Evidence of stress release by inter-specific facilitation: Drought stress release by inter-specific facilitation. *Plant Biology*, 15(3), 483–495. <https://doi.org/10.1111/j.1438-8677.2012.00670.x>
- Ratcliffe, S., Wirth, C., Jucker, T., van der Plas, F., Scherer-Lorenzen, M., Verheyen, K., Allan, E., Benavides, R., Bruelheide, H., Ohse, B., Paquette, A., Ampoorter, E., Bastias, C. C.,

Bauhus, J., Bonal, D., Bouriaud, O., Bussotti, F., Carnol, M., Castagneyrol, B., ...

Baeten, L. (2017). Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecology Letters*, 20(11), 1414–1426.

<https://doi.org/10.1111/ele.12849>

Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: A traits manifesto.

Journal of Ecology, 102(2), 275–301. <https://doi.org/10.1111/1365-2745.12211>

Roman, D. T., Novick, K. A., Brzostek, E. R., Dragoni, D., Rahman, F., & Phillips, R. P. (2015).

The role of isohydric and anisohydric species in determining ecosystem-scale response to severe drought. *Oecologia*, 179(3), 641–654. <https://doi.org/10.1007/s00442-015-3380-9>

Santiago, L. S., Goldstein, G., Meinzer, F. C., Fisher, J. B., Machado, K., Woodruff, D., & Jones,

T. (2004). Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia*, 140(4), 543–550.

<https://doi.org/10.1007/s00442-004-1624-1>

Schuldt, B., Knutzen, F., Delzon, S., Jansen, S., Müller-Haubold, H., Burrell, R., Clough, Y., &

Leuschner, C. (2016). How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? *New Phytologist*, 210(2), 443–

458. <https://doi.org/10.1111/nph.13798>

Sperry, J. S., Hacke, U. G., & Pittermann, J. (2006). Size and function in conifer tracheids and

angiosperm vessels. *American Journal of Botany*, 93(10), 1490–1500.

<https://doi.org/10.3732/ajb.93.10.1490>

Tognetti, R., Johnson, JonD., & Michelozzi, M. (1995). The response of European beech (*Fagus*

sylvatica L.) seedlings from two Italian populations to drought and recovery. *Trees*, 9(6).

<https://doi.org/10.1007/BF00202499>

Toïgo, M., Ledoux, G., Martin-Blangy, S., Coste, L., & Morin, X. (2021). Mixture effect on

radial stem and shoot growth differs and varies with temperature. *Forest Ecology and*

Management, 488, 119046. <https://doi.org/10.1016/j.foreco.2021.119046>

Turrell, F. M. (1936). The Area of the Internal Exposed Surface of Dicotyledon Leaves.

American Journal of Botany, 23(4), 255–26411.

Tyree, M. T., & Zimmermann, M. H. (2002). *Xylem Structure and the Ascent of Sap*. Springer

Berlin Heidelberg. <https://doi.org/10.1007/978-3-662-04931-0>

- Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*(5), 882–892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- von Arx, G., Crivellaro, A., Prendin, A. L., Čufar, K., & Carrer, M. (2016). Quantitative Wood Anatomy—Practical Guidelines. *Frontiers in Plant Science*, *7*. <https://doi.org/10.3389/fpls.2016.00781>
- Warren, C. R., Tausz, M., & Adams, M. A. (2005). Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (*Eucalyptus sideroxylon* subsp. *Tricarpa*) grown in a common garden? *Tree Physiology*, *25*, 10.
- Williams, L. E., & Araujo, F. J. (2002). Correlations among Predawn Leaf, Midday Leaf, and Midday Stem Water Potential and their Correlations with other Measures of Soil and Plant Water Status in *Vitis vinifera*. *Journal of the American Society for Horticultural Science*, *127*(3), 448–454. <https://doi.org/10.21273/JASHS.127.3.448>
- Wright, S. J., Kitajima, K., Kraft, N. J. B., Reich, P. B., Wright, I. J., Bunker, D. E., Condit, R., Dalling, J. W., Davies, S. J., Díaz, S., Engelbrecht, B. M. J., Harms, K. E., Hubbell, S. P., Marks, C. O., Ruiz-Jaen, M. C., Salvador, C. M., & Zanne, A. E. (2010). Functional traits and the growth–mortality trade-off in tropical trees. *Ecology*, *91*(12), 3664–3674. <https://doi.org/10.1890/09-2335.1>
- Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., & Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: Are there any links? *Ecology*, *84*(8), 2042–2050. <https://doi.org/10.1890/02-0433>
- Zapater, M., Hossann, C., Bréda, N., Bréchet, C., Bonal, D., & Granier, A. (2011). Evidence of hydraulic lift in a young beech and oak mixed forest using ¹⁸O soil water labelling. *Trees*, *25*(5), 885–894. <https://doi.org/10.1007/s00468-011-0563-9>

Table 1: Characteristics of the study sites.

Location	Site code	Forest type	Mean tree height (m)	Mean basal area (cm²)	Mean tree number	Latitude (°N)	Longitude (°E)	High elevation (m a.s.l)	Low elevation (m a.s.l)	MAP* (mm)	MAT* (°C)	P/PET* 2005 – 2019	P/PET* July-August 2019
Vercors	VC	beech – silver fir	13.8	2.81	25	44.90	5.33	1403	1164	888	9.6	3.31	0.61
Bauges	BG	beech – silver fir	20.9	7.59	17	45.71	6.21	1222	1009	1001	9.3	2.64	0.51
Mont Ventoux	VT	beech – silver fir	13.8	3.13	33	44.19	5.24	1350	1201	647	13.6	2.20	0.18
Sainte-Baume	SB	beech – downy oak	19.6	5.66	29	43.33	5.76	772	752	629	14.4	1.76	0.11
Lagarde d’Apt	LA	beech – downy oak	11.6	1.70	27	43.97	5.48	1116	1080	622	13.4	1.41	0.10
Grand Luberon	LU	beech – downy oak	9.3	1.36	38	43.82	5.53	974	925	589	13.1	1.39	0.08

* MAP = mean annual precipitation, MAT = mean annual temperature, P/PET = precipitation divided by potential evapotranspiration

Table 2: List of leaf and wood traits measured.

Trait	Symbol	Unit	Organ	Type	Function
Lumen area	A_{Lumen}	μm^2	Wood	Anatomical	Water transport (Sperry et al., 2006)
Theoretical hydraulic diameter	D_{H}	μm	Wood	Anatomical	Water transport (Tyree & Zimmermann, 2002)
Vessels density	VD	vessels cm^{-2}	Wood	Anatomical	Water transport (Nardini et al., 2014)
Wood density	WD	mg cm^{-3}	Wood	Morphological	Water transport (Santiago et al., 2004)
Maximum CO₂ assimilation	A_{max}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Leaf	Physiological	Carbon uptake (Farquhar & Sharkey, 1982)
Stomatal conductance	g_{s}	$\text{mol m}^{-2} \text{s}^{-1}$	Leaf	Physiological	Water loss and carbon capture (Farquhar & Sharkey, 1982)
Leaf water potential	Ψ_{PD}	MPa	Leaf	Physiological	Water status (Williams & Araujo, 2002)
Chlorophyll concentration	Chl	$\mu\text{mol m}^{-2}$	Leaf	Physiological	Carbon uptake (Tognetti et al., 1995)
Carbon isotopic composition	$\delta^{13}\text{C}$	‰	Leaf	Physiological	Water-use efficiency (Farquhar & Richards, 1984)
Intrinsic water-use efficiency	WUE_i	μmol^{-1}	Leaf	Physiological	Water-use efficiency (Fischer & Turner, 1978)
Leaf surface	LS	cm^2	Leaf	Morphological	Water loss and carbon capture (McCulloh et al., 2010)
Specific leaf area	SLA	$\text{m}^2 \text{kg}^{-1}$	Leaf	Morphological	Water loss and carbon capture (Poorter & Rozendaal, 2008)
Stomatal density	SD	stomata mm^{-2}	Leaf	Anatomical	Water loss and carbon capture (Henry et al., 2019)
Stomatal area	A_{stomates}	μm^2	Leaf	Anatomical	Water loss and carbon capture (Henry et al., 2019)
Mesophyll thickness	M	%	Leaf	Anatomical	Carbon uptake and transport (Turrell, 1936)
Lower cuticle thickness	LC	%	Leaf	Anatomical	Water loss (Fernandez et al., 1997)

Table 3: Summary of the two linear mixed models (F- and p-value) on all functional traits, i.e., maximum CO₂ assimilation (A_{\max}), stomatal conductance (g_s), predawn leaf water potential (Ψ_{PD}), chlorophyll concentration (Chl), stable carbon isotopic composition ($\delta^{13}C$), intrinsic water-use efficiency (WUE_i), lumen area (A_{Lumen}), hydraulic diameter (D_H), vessels density (VD), wood density (WD), leaf surface (LS), specific leaf area (SLA), stomatal density (SD), stomatal area ($A_{stomates}$), mesophyll thickness (M), and lower cuticle thickness (LC). The first model evaluates the effects of P/PET, species mixture (pure vs. mixed stands), and their interaction. The second model evaluates the effects of species mixture, forest type (beech mixed with fir vs. oak), and their interaction. Significant effects ($p \leq 0.05$) are highlighted in bold.

Sources of variation	P/PET	Sp. mixture	P/PET * sp. mixture	Sp. mixture	Forest type	Sp. mixture * forest type
A_{\max}	6.05 (0.034)	0.18 (0.675)	1.09 (0.299)	3.37 (0.069)	14.09 (0.004)	5.51 (0.021)
g_s	3.27 (0.101)	0.29 (0.594)	1.12 (0.293)	2.23 (0.138)	5.19 (0.046)	2.47 (0.119)
Ψ_{PD}	8.61 (0.015)	9.24 (0.003)	9.29 (0.003)	0.24 (0.625)	27.48 (< 0.001)	11.51 (< 0.001)
Chl	13.90 (0.004)	2.03 (0.144)	5.07 (0.023)	5.32 (0.022)	12.53 (0.005)	7.28 (0.007)
$\delta^{13}C$	2.91 (0.119)	9.87 (0.002)	7.92 (0.006)	2.21 (0.140)	2.55 (0.141)	2.81 (0.097)
WUE _i	1.36 (0.270)	0.00 (0.971)	0.23 (0.635)	2.59 (0.110)	0.61 (0.452)	0.57 (0.451)
A_{Lumen}	0.00 (0.968)	1.92 (0.169)	6.92 (0.010)	12.71 (< 0.001)	0.32 (0.582)	6.73 (0.011)
VD	0.69 (0.409)	0.00 (0.989)	0.56 (0.454)	5.46 (0.021)	0.06 (0.810)	0.47 (0.494)
D_H	0.04 (0.850)	0.34 (0.561)	2.12 (0.148)	6.60 (0.012)	0.06 (0.803)	1.10 (0.296)
WD	0.41 (0.538)	1.23 (0.269)	0.59 (0.443)	1.51 (0.222)	0.14 (0.717)	0.39 (0.532)
LS	0.29 (0.599)	0.18 (0.673)	0.38 (0.541)	0.26 (0.611)	0.03 (0.865)	0.61 (0.438)
SLA	1.15 (0.308)	7.15 (0.009)	5.70 (0.019)	1.67 (0.199)	0.59 (0.461)	1.37 (0.244)
SD	3.17 (0.106)	0.91 (0.342)	0.64 (0.424)	0.39(0.533)	1.89 (0.199)	0.15 (0.696)
$A_{stomates}$	5.22 (0.024)	1.04 (0.310)	0.59 (0.443)	0.90 (0.346)	3.77 (0.081)	1.71 (0.194)
M	0.17 (0.693)	5.35 (0.023)	3.75 (0.056)	2.28 (0.134)	0.01 (0.942)	0.31 (0.580)
LC	13.36 (0.005)	0.44 (0.508)	0.43 (0.513)	0.01 (0.904)	17.63 (0.002)	0.01 (0.920)

Note: full model:	$lmer(\text{functional traits} \sim P/PET + \text{sp. mixture} + P/PET * \text{sp. mixture} + (1 \text{Site:subgroups}))$	$lmer(\text{functional traits} \sim \text{sp. mixture} + \text{forest type} + \text{sp. mixture} * \text{forest type} + (1 \text{Site:subgroups}))$
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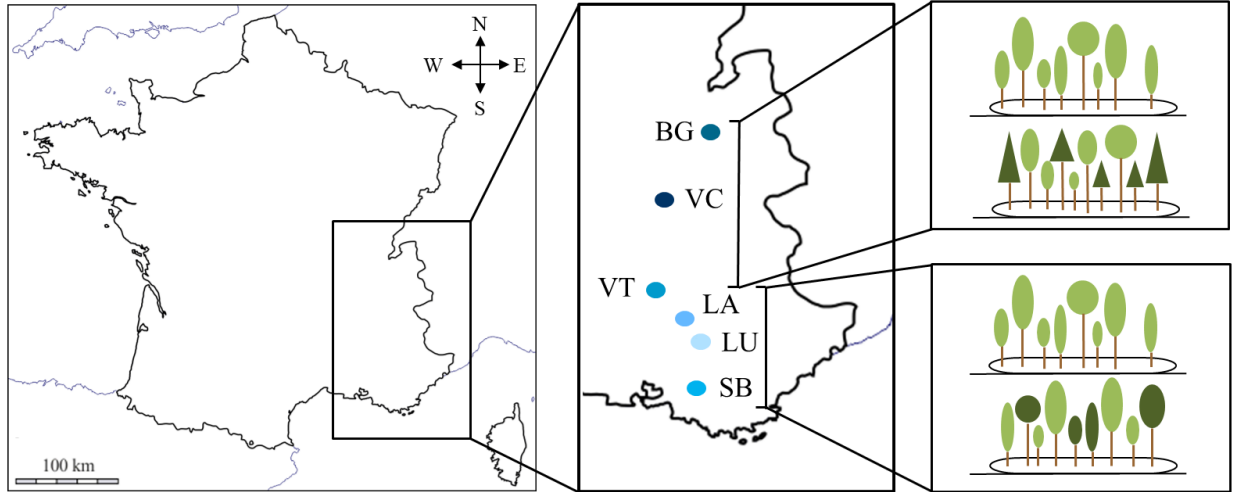


Figure 1: Geographical locations of the study sites. All three northern sites (BG, VC, VT) are composed of beech pure stands and mixed stands where beech is co-occurring with silver fir. The southern sites (LA, LU, SB) are also composed of beech pure stands and mixed stands where beech is co-occurring with downy oak. The color gradient corresponds to the climatic gradient where the dark and light-blue represents the higher and lower P/PET, respectively.

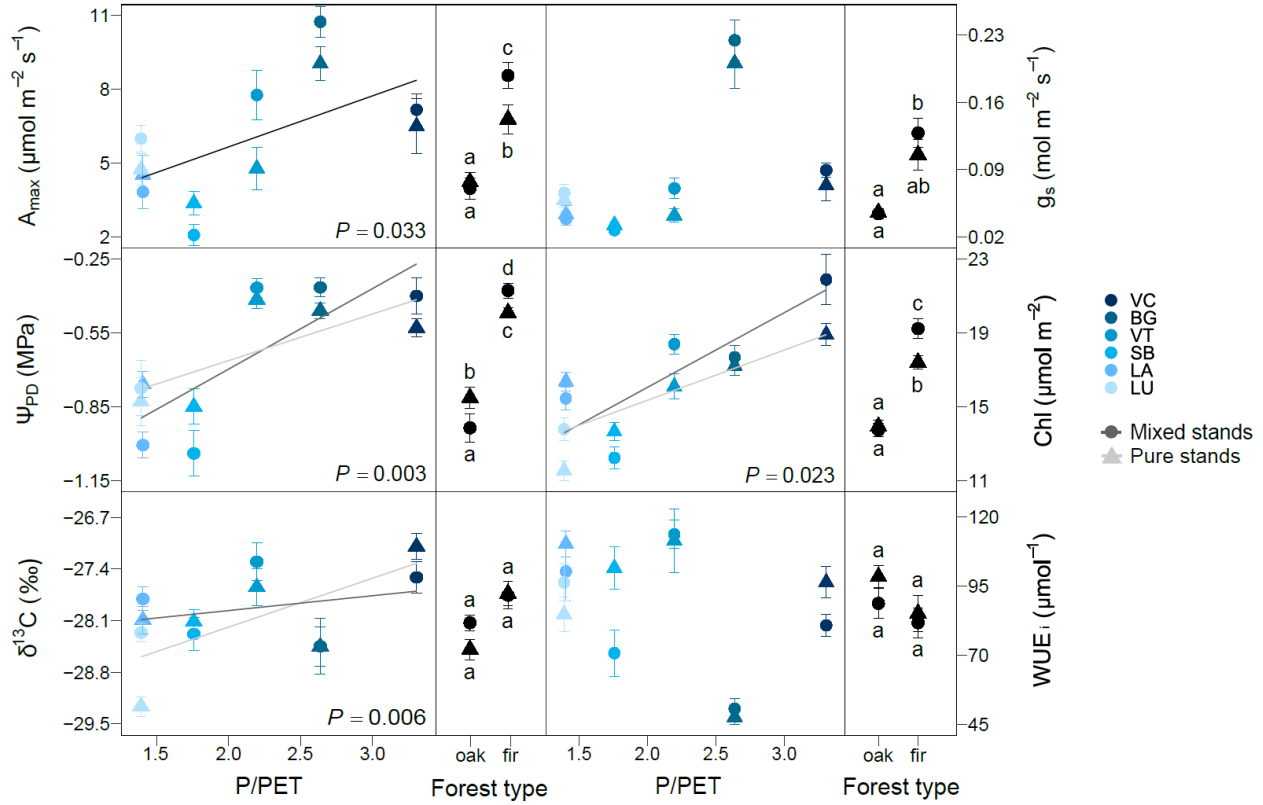


Figure 2: Maximum CO₂ assimilation (A_{\max}), stomatal conductance (g_s), predawn leaf water potential (Ψ_{PD}), chlorophyll concentration (Chl), stable carbon isotopic composition ($\delta^{13}C$), and intrinsic water-use efficiency (WUE_i) as a function of P/PET (mean \pm SE, $n=10$ trees) and forest type (three northern sites where beech and silver fir co-occurs vs. three southern sites where beech and downy oak co-occurs, mean \pm SE, $n=30$ trees). The color gradient corresponds to the climatic gradient where the dark and light-blue represents the higher and lower P/PET, respectively. The triangles and circles stand for the pure and mixed stands, respectively. Shown are the regression lines for the overall model (in black) or for each species mixture (light and dark grey for pure and mixed stands, respectively) when significant. P-values are given in the lower right corner when significant.

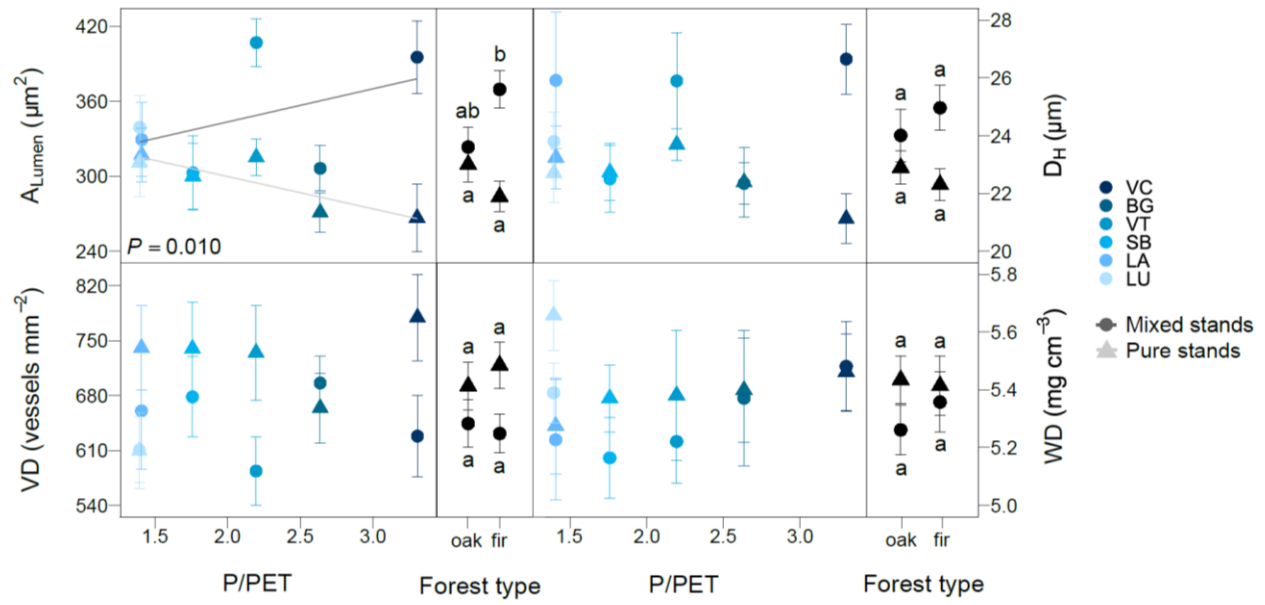


Figure 3: Lumen area (A_{Lumen}), hydraulic diameter (D_H), vessels density (VD), and wood density (WD) as a function of P/PET (mean \pm SE, $n=10$ trees) and forest type (three northern sites where beech and silver fir co-occurs *vs.* three southern sites where beech and downy oak co-occurs, mean \pm SE, $n=30$ trees). The color gradient corresponds to the climatic gradient where the dark and light-blue represents the higher and lower P/PET, respectively. The triangles and circles stand for the pure and mixed stands, respectively. Shown are the regression lines for the overall model (in black) or for each species mixture (light and dark grey for pure and mixed stands, respectively) when significant. P-values are given in the lower left corner when significant.

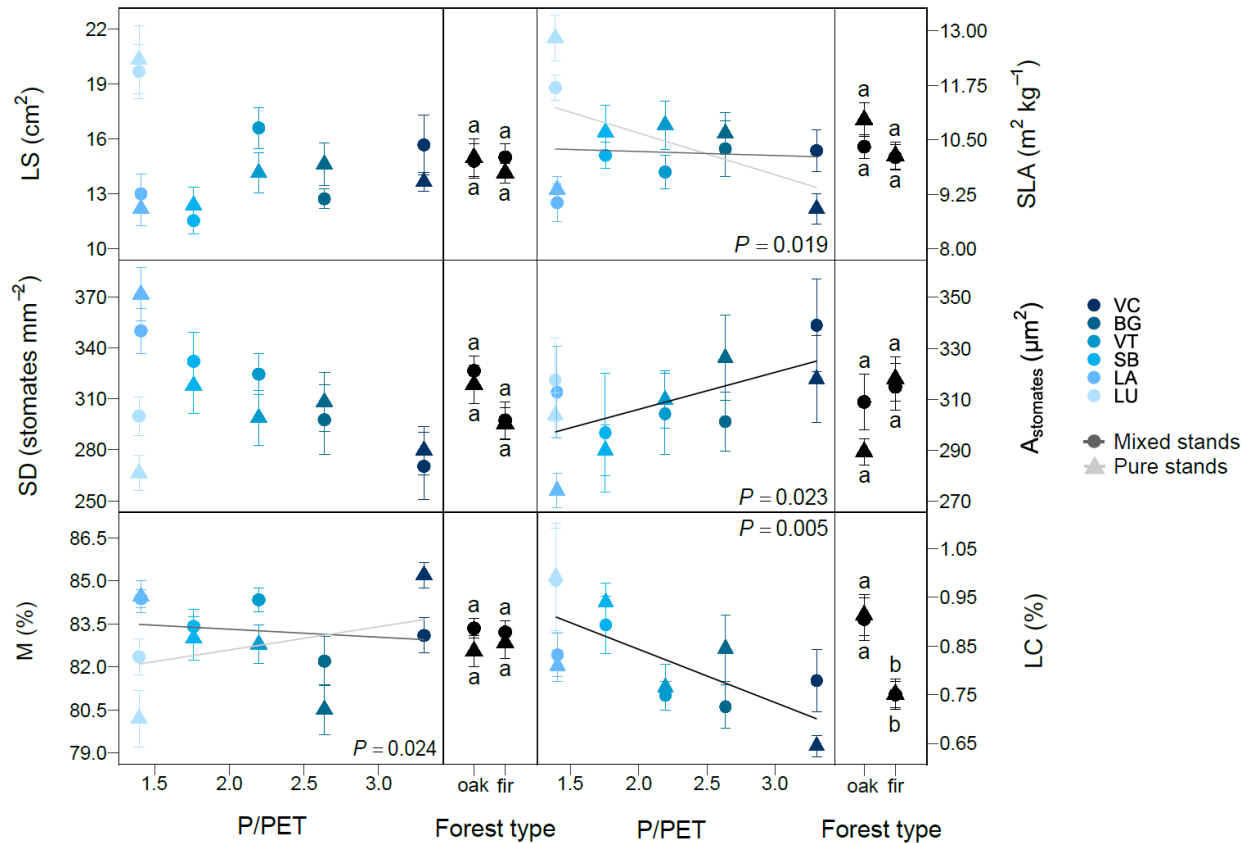


Figure 4: Leaf surface (LS), specific leaf area (SLA), stomatal density (SD), stomatal area (A_{stomates}), mesophyll thickness (M), and lower cuticle thickness (LC) as a function of P/PET (mean \pm SE, $n=10$ trees) and forest type (three northern sites where beech and silver fir co-occurs vs. three southern sites where beech and downy oak co-occurs, mean \pm SE, $n=30$ trees). The color gradient corresponds to the climatic gradient where the dark and light-blue represents the higher and lower P/PET, respectively. The triangles and circles stand for the pure and mixed stands, respectively. Shown are the regression lines for the overall model (in black) or for each species mixture (light and dark grey for pure and mixed stands, respectively) when significant. P-values are given in the right corner when significant.

Supporting information

Table S1: Shallow soil characteristics of the studied sites from 0 to 15 cm depth.

Figure S1: Picture of measurements made on the woody and leaf tissues.