



Mycorrhization, root tip vitality and biomass of *Fagus sylvatica*, *Picea abies* and *Pseudotsuga menziesii* in monospecific and mixed combinations under water reduction and nitrogen addition

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Abstract

Key message In mixed combinations, the negative impact of water reduction and N addition is mitigated for *F. sylvatica*, but not for conifers.

Insight into the responses of trees in mixed and monospecific cultivation to water scarcity and nitrogen (N) excess is necessary to recommend suitable tree mixtures for future European forests. Our aim was to investigate the impact of water reduction, N addition or water reduction + N addition in comparison with control (well-watered, no excess N) on mycorrhizal roots and biomass of three temperate forest species (*Fagus sylvatica* Fs, *Picea abies* Pa, and *Pseudotsuga menziesii* Pm) grown in monospecific (FsFs, PaPa, PmPm) and mixed combinations (PaFs, PmFs). We predicted the probability of the responses with a Bayesian approach. Mycorrhizal colonization declined marginally for *P. menziesii* in PmFs under water reduction. Under water reduction and N addition combined, we found a decline in root tip vitality of *F. sylvatica* in PaFs and of *P. abies* in PaPa. *F. sylvatica* shoot biomass declined under water reduction + N addition in the monospecific combination. *P. abies* and *P. menziesii* had lower root biomass in mixture with *F. sylvatica* under water reduction + N addition and under water reduction as single factor, respectively. With water reduction and N addition, *P. menziesii* performed better than *P. abies* in terms of biomass production. *F. sylvatica* had a growth advantage in mixture with conifers instead of conspecifics. These findings suggest that young trees show rapid and interspecific responses to species mixing and water + N availability. The selection of suitable tree species should, therefore, consider their interactive responses to changing abiotic factors.

Keywords Ectomycorrhiza · Forest trees · Plant competition · Abiotic stress · Bayesian statistics · Root traits

Introduction

In many areas of the globe, the occurrence of drought events and nitrogen (N) deposition are expected to increase in the near future (Dai 2013; IPCC 2022; Janssens et al. 2020; Lamarque et al. 2013). Both phenomena can strongly impact soil water and nutrient availability (Galloway et al. 2008; Su et al. 2020; Zhang et al. 2020) as well as microbial processes (Anderegg et al. 2015; Erisman et al. 2013; Janssens et al. 2020; Wu et al. 2019), with negative consequences on tree growth and forest health (IPCC 2022; Krejza et al. 2021). In Central Europe, the last decades saw a higher frequency of drought events, and climate models project a drier and hotter climate (Lindner et al. 2010; Schuldt et al. 2020). Additionally, elevated N deposition cause soil acidification and leaching of base cations, which impact forest tree nutrition and growth (De Vries et al. 2014; Eugster and Haeni 2013) and

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increase their sensitivity to biotic and abiotic stress (Bobbink and Hetteling 2011). However, the responses of trees to drought events combined with high N deposition, a scenario that forests are still experiencing in some areas of Europe, such as Northern Germany (Huang et al. 2016; Schaap et al. 2018; Van der Graaf et al. 2021), are unknown.

The selection of suitable tree species, which combine tolerance to climate-change induced stress with productivity, plays a central role in forestry (Rukh et al. 2020). For example, in Central Europe, the admixture of the dominant native species, *Fagus sylvatica* with the non-native conifer species, *Pseudotsuga menziesii*, is considered a potential solution to deal with challenges imposed by climate change (Thurm and Pretzsch 2016). *P. menziesii*, a highly productive and relatively drought tolerant conifer species (Bréda et al. 2006; Eilmann and Rigling 2012), has been suggested as a valid substitution of the native conifer *Picea abies* in mixture with *F. sylvatica* (Cremer et al. 2016). Since the beginning of the twenty-first century, despite remaining the main timber supply in Central Europe (Brus et al. 2012), *P. abies* has experienced serious declines (Pretzsch et al. 2018). This phenomenon was due to large-scale dieback episodes, following extreme drought, wind throws and bark beetle outbreaks (Bošefa et al. 2021; Mezei et al. 2017), suggesting that *P. abies* is highly susceptible to climate change.

Therefore, attempts are underway to find more suitable tree species and species mixtures that can withstand stressful environmental conditions. Theoretical considerations (stress-gradient hypothesis) suggest that positive interactions prevail under abiotic stress in heterospecific mixtures because complementary effects are stronger when the environmental conditions are unfavourable (Bertness and Callaway 1994; Wang et al. 2013). Trees in mixed forests, typically characterized by coniferous and broadleaf species, might be able to cope with and recover from stress imposed by climate change—in particular drought—better than trees in monospecific forests (Metz et al. 2016; Pardos et al. 2021). For instance, *P. abies* in mixture with *F. sylvatica* shows a higher survival probability under unfavourable climate conditions compared to monocultures (Neuner et al. 2015). *P. menziesii* in mixture with *F. sylvatica* had a shorter drought recovery time compared to pure stands (Thurm et al. 2016).

The effect of species identity of trees interacting in a forest on the belowground system is yet not well understood. For instance, overyielding of root biomass in *F. sylvatica*—*P. menziesii* mixed stands was not found (Lwila et al. 2021, 2023) and mycorrhizal fungal abundances were decreased in *P. menziesii* pure and mixed stands with *F. sylvatica* compared to pure *F. sylvatica* stands (Likulunga et al. 2021). However, these responses are in contrast with those of Wambsganss et al. (2021); they found that trees in mixed stands show a general reduction in fine root biomass linked to morphological and chemical adaptations of fine

root traits and higher mycorrhizal colonization rate, indicating a shift towards a faster foraging strategy. The roots of most temperate forest trees associate with ectomycorrhizal (ECM) fungi, which mediate tree nutrition and water supply (Nehls and Plassard 2018; Lehto and Zwiazek 2011). The colonization of roots by ECM fungi is easily visible, as the symbiotic fungi wrap root tips with a hyphal mantle (Agerer 2001). The relative proportion of mycorrhizal root tips is scored as mycorrhizal colonization rate (Pena et al. 2017), indicating the intensity of the plant association with the fungal partners. The proportion of dry non-vital root tips, together with the mycorrhizal colonization rate, can be scored to gain insight into stress responses of trees (Danielsen and Polle 2014; Pena and Polle 2014; Pena et al. 2017). Tree root mycorrhizal status is likely to be negatively impacted by water scarcity as well as N excess. In temperate forests, water shortage impairs the number of vital mycorrhizae (Nickel et al. 2018) and decreases host tree nutrition (Clausing et al. 2021b). Different tree species adopt different strategies to cope with drought: according to Nikolova et al. (2020), the conifer *P. abies* relies strongly on mycorrhizal symbionts during drought, while the broadleaf *F. sylvatica* increases the production of non-mycorrhizal roots. Moreover, negative effects of N deposition on mycorrhizal associations have been reported for conifer forests (Kuyper and Suz 2023; Lilleskov et al. 2019; Nilsson and Wallander 2003) but not for pure *F. sylvatica* forests (Clausing et al. 2021a; Likulunga et al. 2022). N addition can increase drought sensitivity of trees, impacting both tree growth and ECM colonization (Nilsen 1995); in *F. sylvatica* seedlings, drought treatment combined with N fertilization led to a reduction of root biomass (Dziedek et al. 2016). Drought treatment and N fertilization combined significantly reduced mycorrhizal colonization of *P. abies* (Nilsen et al. 1998). The effect of simulated N deposition combined with artificial precipitation reduction caused a strong reduction of mycorrhizal colonization of different Chinese tree species (Yan et al. 2019). These divergent results suggest that intra- and inter-specific interactions and belowground responses of trees are highly complex.

Additionally, whether the responses of mycorrhizal roots and tree biomass are mediated by the identity of the species interacting in a mixture, and more specifically if there is a difference between the native drought-sensitive *P. abies* and the non-native drought-tolerant *P. menziesii*, is unknown. To address this knowledge gap, we setup a pot experiment with juvenile trees of *F. sylvatica*, *P. abies* and *P. menziesii* with the aim of investigating the changes in mycorrhizal colonization rate, root tip vitality, and tree biomass in monospecific and mixed combinations, experiencing different levels of water and N supply. In detail, we exposed the trees to either water reduction or N addition or both simultaneously, and we compared them with a control group (well-watered trees,

no N excess); we defined the application of water reduction, N addition or both as abiotic stress treatment, and the control conditions as favourable conditions. We then measured mycorrhizal colonization rate, root tip vitality index and tree biomass.

We hypothesised that (I) for all species and species combinations there is a higher probability of negative responses (decline of tree biomass, mycorrhizal colonization rate and root tip vitality index) under the scenario of combined water reduction and N addition in comparison with favourable conditions or application of single stress factors (water reduction or N addition), and that (II) the negative responses under abiotic stress are lower for trees in mixed combinations than in mono-specific combinations.

Materials and methods

Study site, plant materials and experimental design

This study was carried out at the Experimental Botanical Garden of the Georg-August University of Göttingen, Lower Saxony, Germany (51.55615 N, 9.95715 E), from May to September 2021. During this time, the average daily temperature recorded from the climate station at the study site was 14.8 °C, and the average daily relative humidity was 79.7%. We used 3-year-old *F. sylvatica* (Fs), *P. menziesii* (Pm) and 4-year-old *P. abies* (Pa) trees. The trees were purchased from a local nursery (Baumschule Willenbocke GmbH, Walsrode, Germany) in May 2019 and transferred to the study site. At the time of purchase, *F. sylvatica* and *P. menziesii* saplings were 1-year-old, and *P. abies* 2-year-old. The selected saplings of each species were of similar height and collar diameter. The trees were immediately planted into 65L plastic pots (surface area: 0.22 m²). The pots were equipped with a bottom layer (approximately 50 mm depth) of coarse gravel for water drainage, and filled with low-nutrient sand (grain size: 0 to 2 mm). Thin fleece cloths were placed over the gravel to prevent the sand from leaching out from drainage holes at the bottom. In total, we set up 315 pots, equally distributed among species combinations. Each pot contained four saplings, either of the same species (monospecific pots), or the combination of two *F. sylvatica* and two *P. menziesii* or two *F. sylvatica* and two *P. abies* (mixed pots). We planted the trees of the same species diagonally opposite each other, to maximize the interactions between the two species (Fig. S1). In total, we obtained three monospecific combinations (*F. sylvatica*, FsFs; *P. menziesii*, PmPm; *P. abies*, PaPa) and two mixed ones (*P. menziesii* + *F. sylvatica*, PmFs and *P. abies* + *F. sylvatica*, PaFs). The pots were placed under a rain shelter in a randomized order. The shelter was approximately 5 m high and with a transparent

plexiglass roof, under which the photosynthetically active radiation (PAR) was 20–30% lower and the air temperature 2–4 °C higher than outside of it. Prior the start of the experiment, the pots were treated equally with periodical addition of fertilizer (Wuxal© 8.0% N, 8.0% P₂O₅, 6.0% K₂O) and irrigated with an average of 2 to 4 L of tap water per pot per week, depending on the weather conditions. The insecticide Spruzid 20 mL L⁻¹ (Neudorff, Emmerthal, Germany) and the fungicide Ortiva 1 mL L⁻¹ (Syngenta, Frankfurt am Main, Germany) were also applied to ensure plant health. Furthermore, we installed time-domain reflectometry (TDR) probes (Campbell Scientific, Logan, Utah, USA) in a subset of representative pots to monitor volumetric water content in the substrate. In May 2021, we began the treatment application. We used the R package randomizr (Coppock 2022) to apply the treatments to all the pots in a random design. The pots were exposed to four treatments (Fig. 1a): control, mimicking the current scenario in Central Germany, with N fertilization corresponding to 20 kg ha⁻¹ yr⁻¹ (based on current atmospheric N deposition rate in addition to natural N mineralization rate in forest soil; Fleck et al. 2019; Schaap et al. 2018) and an irrigation treatment corresponding to 450 mm of precipitation (typical rainfall pattern from May to September; Schipka et al. 2005); water reduction (Wr), mimicking a scenario with unchanged N deposition rate (20 kg ha⁻¹ yr⁻¹) but reduced precipitation during the growth period, corresponding to 250 mm; N addition (Na), mimicking a scenario with three-fold increased rate of N deposition (60 kg ha⁻¹ yr⁻¹) and unchanged precipitation (450 mm); and water reduction + N addition (WrNa), mimicking a scenario that combines reduced precipitation (250 mm) and increased N deposition (60 kg ha⁻¹ yr⁻¹).

To achieve the treatments, we scaled the amount of water and N to the surface of the pots. We watered each pot assigned to the treatments “control” and “Na” three times a week with 0.66 L day⁻¹, and each pot assigned to the treatments “Wr” and “WrNa” with 0.36 L day⁻¹. We fertilized the pots assigned to the treatments “control” and “Wr” with 1.2 mM NH₄NO₃ every 2 weeks, and the pots assigned to the treatments “Na” and “WrNa” with 3.7 mM NH₄NO₃ with the same frequency. The NH₄NO₃ was added in a 500 mL modified Hoagland solution (Hoagland and Synder 1933) with the following composition: CaCl₂ (1.8 mM), MgSO₄ (0.4 mM), K₂SO₄ (0.3 mM), KH₂PO₄ (0.7 mM), H₃BO₃ (18.7 μM), MnSO₄ (6.6 μM), ZnSO₄ (5.6 μM) and CuSO₄ (0.9 μM). For each treatment and species combination, we set up five to six repetitions (pots), plus additional pots for test measurements. The rest of the pots were exposed to other treatments; however, they are not part of this experiment and will not be mentioned further.

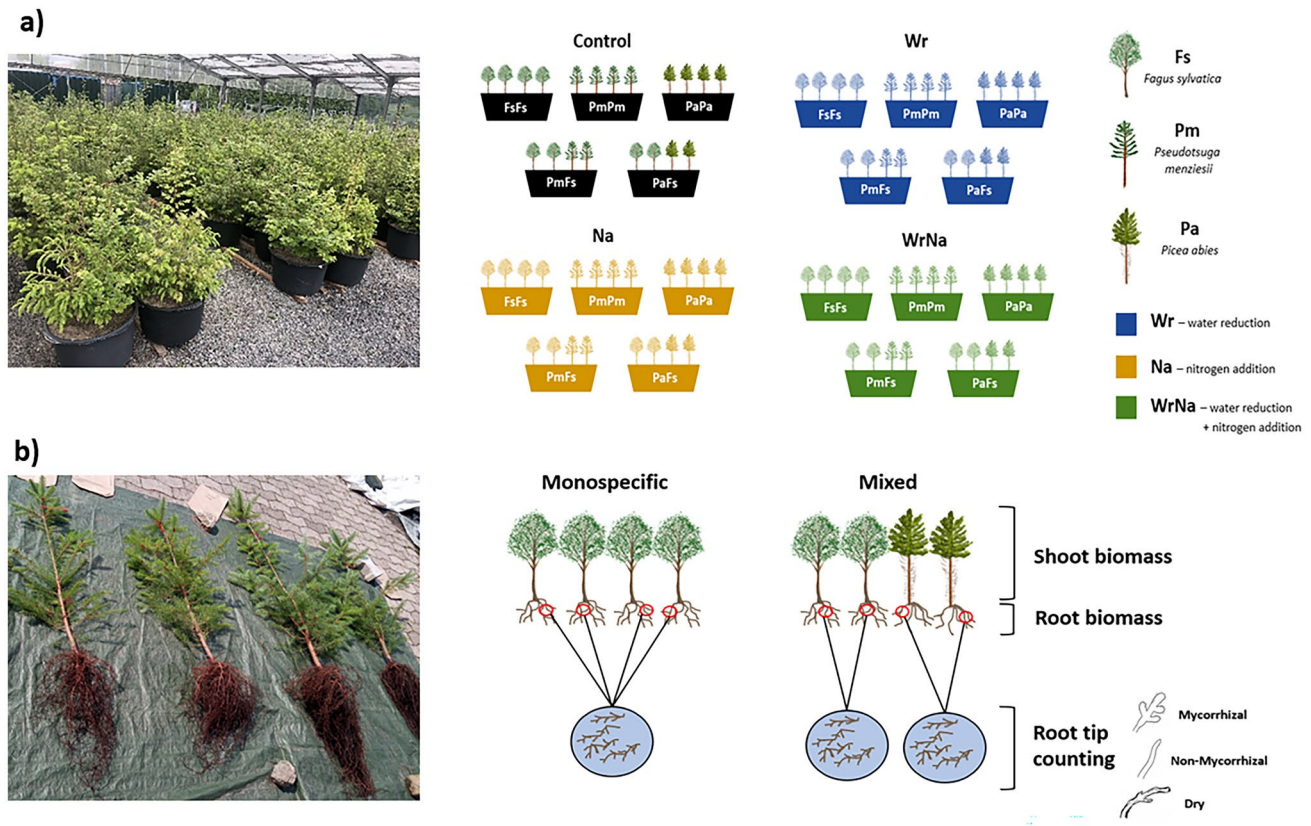


Fig. 1 Photo and scheme of the experiment **a** the three species are *Fagus sylvatica* (Fs), *Pseudotsuga menziesii* (Pm) and *Picea abies* (Pa), here represented in monospecific (FsFs, PaPa, PmPm, PaPa) and mixed pots (PmFs, PaFs). Different colours indicate the different treatments (Wr, water reduction; Na, N addition; WrNa, water reduc-

tion + N addition). Photo and scheme of the sample collection **b** for each pot, the shoot and root biomass were measured for the four saplings; fine root samples were collected and pooled per pot and species, and then observed under the microscope to count mycorrhizal, non-mycorrhizal and dry root tips

Harvest and sample collection

The harvest took place at the end of August 2021 after approximately four months of N and water treatments. For CN analysis, we collected sandy soil samples from three pots per treatment and species combinations. We collected approximately 100 g of the sandy soil from the top layer of each pot and dried it at 70 °C for 72 h.

We harvested 4–6 pots for each treatment and species combination, resulting in a total of 84 pots (Table S2). We extracted the four trees from pots and washed the roots with running tap water (Fig. 1b). We collected three to four root segments (50–100 mm long) per plant. We pooled the root segments of the same pot and same species. We wrapped them in wet paper towels and stored them in plastic bags in a cooling room at 4 °C.

We used 66 pots (2–6 pots per treatment and species combination) to measure tree biomass. For each pot, we recorded the biomass of all four saplings, for a total of 264 trees (Table S3). To measure tree biomass, we cut the trees at the base of the stem, dividing shoot (stem, branches and

leaves) and root (whole root system), placed the two parts separately in paper bags and dried at 70 °C for 72 h. We then weighed them to determine shoot and root biomass.

Determination of C and N in the sandy soil

The dry sandy soil samples were sieved with a metal sieve (mesh size: 2 mm) and homogenized by grinding them in a ball mill (Retsch, Haan, Germany). We weighed about 20 mg of ground dry material into tin capsules and analysed C and N in the samples with a vario MICRO cube elemental analyser (Elementar Analysensysteme GmbH). We used acetanilide (C₈H₉NO, HEKAtsch GmbH, Wegberg, Germany) as the standard.

Determination of mycorrhizal colonization rate and vitality index

We used the stored root segments within 14 days since harvest for the observation of root tips. We cut all the roots into shorter segments (30–50 mm long), randomly selected

at least 10 of them per sample, placed them in a Petri dish and inspected them under a stereo microscope (M205 FA, Leica, Wetzlar, Germany). To determine the proportion of vital mycorrhizal (M), non-mycorrhizal (NM) and dry (D) root tips for each sample, we categorized the first 200 visible root tips of the selected segments as vital mycorrhizal when the hyphal mantle was visible, non-mycorrhizal when the mantle was absent and the root tips were turgid, and dry when the root tips appeared darkened and shrunken (Pena et al. 2017; Valtanen et al. 2014).

Bayesian regression models

Data analysis was carried out with the software *R*, version 4.2.2 (R Core Team 2022). Model estimation was run using the *R* add-on package *brms* (Bürkner 2017, 2018), which builds a statistical modelling environment based on the probabilistic programming language *Stan* (Carpenter et al. 2017).

Because of the time-consuming observation process, the large number of treatments and combinations, and the restricted number of repetitions, we used a Bayesian approach to predict the variation of tree biomass, mycorrhizal colonization rate and root tip vitality as conditional probability to test our hypotheses.

To model the root tip mycorrhizal colonization rate and vitality index, conditional on covariate scenarios, we applied binomial regression models—in a data format grouped for pot ID, denoted by index j , $j = 1, \dots, J$, where J denotes the number of pots—using the logit link-function, separately for each tree species. We calculated the mycorrhizal colonization rate and the root tip vitality index following the equations by Pena et al. (2017). For the mycorrhizal colonization rate, we defined outcome variables $y_{A,j}$ as ‘number of M ’ out of ‘number of M + number of NM ’ trials. To model the vitality index, we defined the variable $y_{B,j}$ as ‘number of M + number of NM ’ out of ‘number of M + number of NM + number of D ’ trials. We set up linear predictors $\eta_{A,j}$ and $\eta_{B,j}$ with treatment (levels control, Wr, Na and WrNa) and species combination (levels FsFs, PaFs and PmFs for the *F. sylvatica* model, levels PaPa and PaFs for the *P. abies* model, and levels PmPm and PmFs for the *P. menziesii* model)—including all pairwise and two-fold interactions—as categorical covariates, x_j , where respective regression coefficient vectors are denoted β_A , and β_B , respectively, to explain the variation in the conditional probabilities, $\text{Prob}(y_{A,j} = M | x_j; y_{A,j} \in \{M, NM\})$, and $\text{Prob}(y_{B,j} \in \{M, NM\} | x_j; y_{B,j} \in \{\{M, NM\}, D\})$. In order to consider potential correlation among measurements taken from the same pots, the variable pot ID was further added to the linear predictors as a grouping term—with respective pot ID specific coefficients γ_j (in frequentist statistical modelling referred to as *random intercepts*) having the conventional ‘coming from a normal distribution with mean 0 and common

standard deviation’ assumption as in frequentist mixed effects regression models i.e. $y_{A,j} \sim N(0, \sigma_{\gamma_A}^2)$, or $y_{B,j} \sim N(0, \sigma_{\gamma_B}^2)$, respectively. With this, and by application of the logit link-function, we model the conditional probabilities by:

$$\begin{aligned} \text{Prob}(y_{A,j} = M | x_j; y_{A,j} \in \{M, NM\}) \\ = \frac{\exp\left(\eta_{A,j} + \sum_{j=1}^J \gamma_{A,j}(\text{potID}_i = j)\right)}{1 + \exp\left(\eta_{A,j} + \sum_{j=1}^J \gamma_{A,j}(\text{potID}_i = j)\right)}, \end{aligned}$$

and

$$\begin{aligned} \text{Prob}(y_{B,j} \in \{M, NM\} | x_j; y_{B,j} \in \{\{M, NM\}, D\}) \\ = \frac{\exp\left(\eta_{B,j} + \sum_{j=1}^J \gamma_{B,j}(\text{potID}_i = j)\right)}{1 + \exp\left(\eta_{B,j} + \sum_{j=1}^J \gamma_{B,j}(\text{potID}_i = j)\right)}. \end{aligned}$$

For intercept parameters, we used a scaled- $t(\nu = 3, \mu = 0, \sigma = 2.5)$ prior, for effects of (interactions of) treatments and species combinations we used a Normal($\mu = 0, \sigma = 2.5$) prior, and for the standard deviation for grouping terms, we again used a scaled- $t(\nu = 3, \mu = 0, \sigma = 2.5)$ prior. The scaled- t priors are *brms* default priors, the normal priors for covariate effects can be considered as ‘weakly informative’ (Lemoine 2019). Prior and posterior predictive checks were performed to check adequacy of the model, and sensitivity of results on choice of priors. For the *Stan* algorithm generating posterior draws, we used 4 independent chains per model, with 2000 iterations per chain, including the first 1000 iterations as warm-up. We verified the convergence of these chains by checking that the potential scale reduction factors (Gelman and Rubin 1992) for all parameters were below a value of 1.01 (Stan Development Team 2023). With this build-up, we obtained the estimated conditional probabilities for y_A (mycorrhizal colonization rate) and y_B (vitality index) under all potential combinations of the predictors (treatments and species combinations) for Fs, Pm and Pa separately. To compare the effect of treatment and species combination, for each tree species and species combination, we first obtained the difference between the estimated probabilities of y_A and y_B in the abiotic stress treatments (Wr, Na and WrNa) and the control treatment, and then plotted the mean, 66% and 95% inner quantile intervals of the posterior distribution to visualize the location and uncertainty of covariate effects. The difference between any abiotic stress treatment and the control was considered statistically significant when the 95% inner quantile interval did not include the zero.

For tree biomass, the total dry weights (g) of roots and shoots—which are strictly positive and continuously scaled outcome variables, i.e. $0 < y_i \in \mathbb{R}$, $i = 1, \dots, n$, where index i denotes a single tree and n the total number of plants investigated (for a specific tree species)—were analysed. For

improved readability, we refrain from denoting each biomass response variable with a separate index as in the above binomial logit analyses: separate, but with identical build-up—models were estimated for each biomass outcome. In order to get to comparable scales for effects across tree species and outcome variables, each response value was divided by the respective empirical mean $\bar{y} = \frac{\sum_{i=1}^n y_i}{n}$, i.e. $\tilde{y}_i = \frac{y_i}{\bar{y}}$. Using covariates treatment and species combination (including their interaction), we applied hierarchical—pot ID as grouping factor—Bayesian regression models, separately for each tree species, with the Gamma distribution as distributional model for the unknown process generating the outcome variables, i.e. $\tilde{y}_i \sim \text{Gamma}(\mu_i, \theta)$, where θ denotes the second distributional parameter. Grouping coefficients γ_j were again estimated using the common normal distributional assumption as introduced above for binomial logit regression models. Applying the logarithmic link-function for the conditional mean $\mu_i = E(\tilde{y}_i | \text{treatment}_{1,i}, \text{combination}_{2,i}, \dots, \text{potID}_i)$, we get:

$$\log(\mu_i) = \eta_i + \sum_{j=1}^J \gamma_j (\text{potID}_i = j),$$

where η_i is a linear predictor for categorically scaled covariates treatment and combination, with including their interaction terms, where respective regression coefficients are denoted β . Our applied priors were $\beta \sim N(0, 2.5^2)$, $\sigma_\gamma \sim \text{scaled-t}(3, 0, 2.5)$, and $\theta \sim \text{Gamma}(0.01, 0.01)$, which are *brms* default (σ_γ, θ), or considered ‘weakly informative’ (Lemoine 2019) for β . Again, prior and posterior predictive checks were performed, and the convergence of posterior sampling was monitored using the potential scale reduction factors. Moreover, we compared the effect of treatment and species combination with the same method used for mycorrhizal colonization rate and vitality index, obtaining the difference in conditional probability between the estimated biomass values in the abiotic stress treatments and the control, and then plotting the mean, 66% and 95% inner quantile intervals of the posterior distribution. Data visualization was done using the *R* add-on package *ggplot2* (Wickham 2016).

Results

During our experiment, the volumetric water content in pots with reduced water input (water reduction and water reduction + N addition) was on average 62.5% lower than in pots under well-watered conditions (control and N addition; Fig. S2). We did not find a significant effect of water and N treatment on the N content in the sandy soil substrate (Table S1; mean \pm standard deviation: 0.18 ± 0.04 mg/g of dry mass). The C content of the sandy soil substrate was also not affected by any treatment (Table S1; mean \pm standard deviation: 0.94 ± 0.15 mg/g of dry biomass).

The mean mycorrhizal colonization rate varied from 95% (72–100%) for *F. sylvatica* (Table S2) to 97% (88–100%) for *P. abies* (Table S3) and to 99% (98–100%) for *P. menziesii* (Table S4). The vitality index spanned from 83% (42–98%) for *F. sylvatica* (Table S2) to 89% (72–100%) for *P. abies* (Table S3), and to 90% (66–99%) for *P. menziesii* (Table S4).

The mean root biomass varied from 46.1 g (10.2 g–121.0 g) for *P. abies* (Table S3) to 77.3 g (12.4 g–179.0 g) for *P. menziesii* (Table S4), and to 89.0 g (20.0 g–181.0 g) for *F. sylvatica* (Table S2). The mean shoot biomass ranged from 64.8 g (7.9–139.0 g) for *F. sylvatica* (Table S2) to 105.0 g (13.4–296.0 g) for *P. abies* (Table S3), and to 149.0 g (11.6 g–349.0 g) for *P. menziesii* (Table S4).

Responses of *F. sylvatica*

The variation of mycorrhizal colonization rates in response to abiotic stress treatments was small. The mycorrhizal colonization rate of *F. sylvatica* (Fig. 2a) showed slight increases (< 1%) under water reduction, and under water reduction + N addition (range of 95% credible intervals: -0.002 to 0.154) compared to the control in the mixed combination with *P. abies*. No relevant changes were found for mycorrhizal colonization rate of *F. sylvatica* growing in monospecific conditions or in mixed combination with *P. menziesii* (Fig. 2a).

The vitality index of root tips (Fig. 2b) declined with a median value of 20% under water reduction + N addition in mixed combination with *P. abies* (-0.438 to -0.038). In combination with *P. menziesii*, the root tip vitality of *F. sylvatica* showed a slight decrease under water reduction, whereas in monospecific combination, no treatment had significant effect on root tip vitality of *F. sylvatica*.

In monospecific combination, the shoot biomass of *F. sylvatica* (Fig. 2c) declined with a median value of 44% under water reduction + N addition compared to control (-0.877 to -0.089). No change of shoot biomass was found under the other treatments, regardless of the tree mixture.

The root biomass of *F. sylvatica* (Fig. 2d) showed a 27% decline in monospecific combination under water reduction + N addition (-0.645 to 0.027) compared to control, although zero and positive values cannot be excluded. No changes were found in the other treatments and species combinations.

Responses of conifers (*P. abies* and *P. menziesii*)

The mycorrhizal colonization rate of *P. abies* (Fig. 3a) showed a small (< 1%) increase under water reduction + N addition compared to control in mixed combination with *F. sylvatica*, although zero and negative values cannot be excluded (range of 95% credible intervals: -0.001 to 0.066).

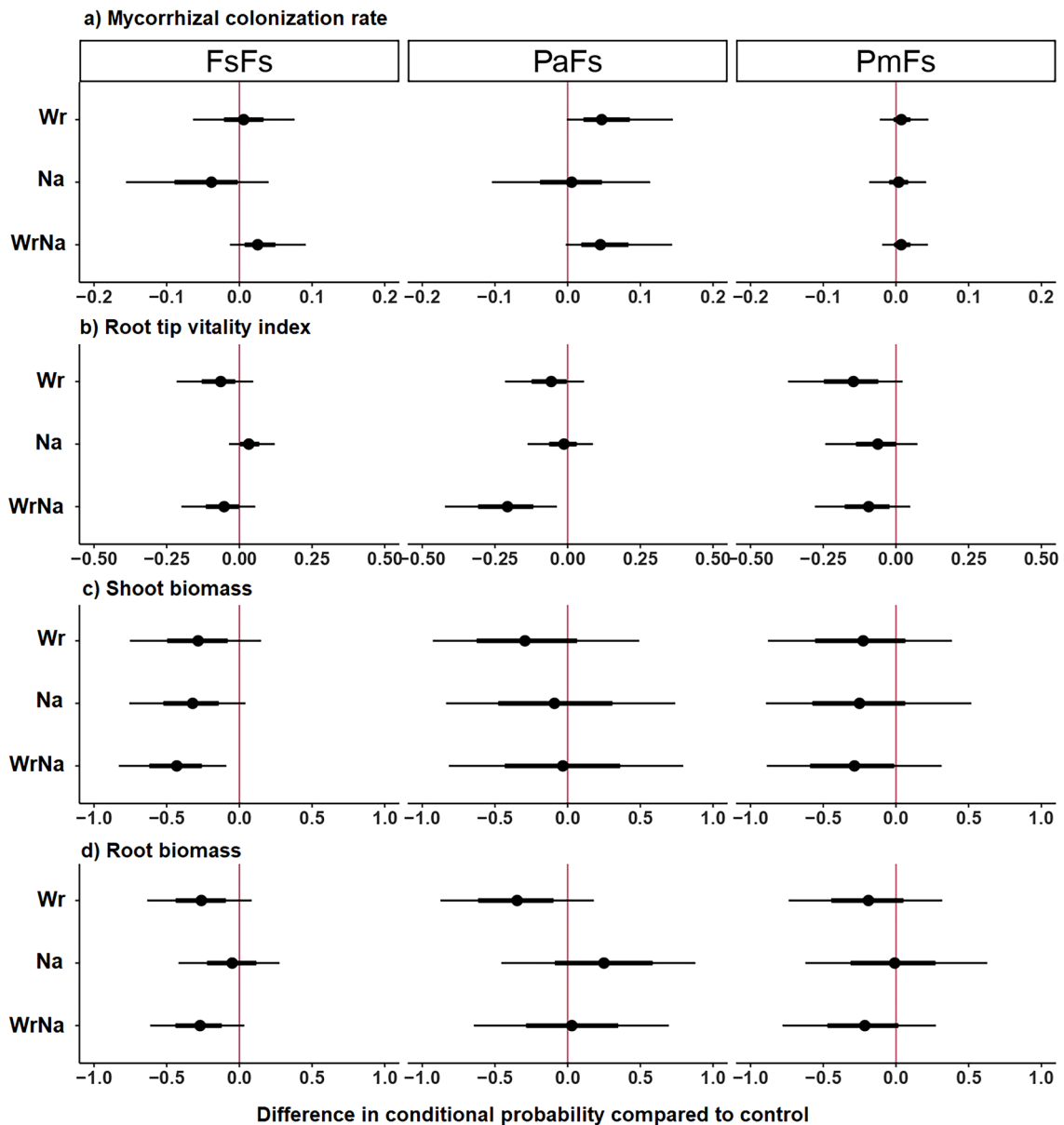


Fig. 2 Variation of **a** mycorrhizal colonization rate, **b** root tip vitality index, **c** normalized shoot biomass and **d** normalized root biomass for *F. sylvatica* in monospecific combination (FsFs), mixed combination with *P. abies* (PaFs) and mixed combination with *P. menziesii* (PmFs). The differences in conditional probability of the measured traits were predicted for water reduction (Wr), N addition (Na) and

water reduction+N addition (WrNa) compared to control. A conditional probability of $-1/+1$ defines a 100% probability of a reduction/increase in the trait due to the treatment. Red line denotes expectation of zero change compared to control. Black bar depicts 66% (thick) and 95% (thin) credible intervals of the estimate; closed circles indicate medians of the posterior distribution

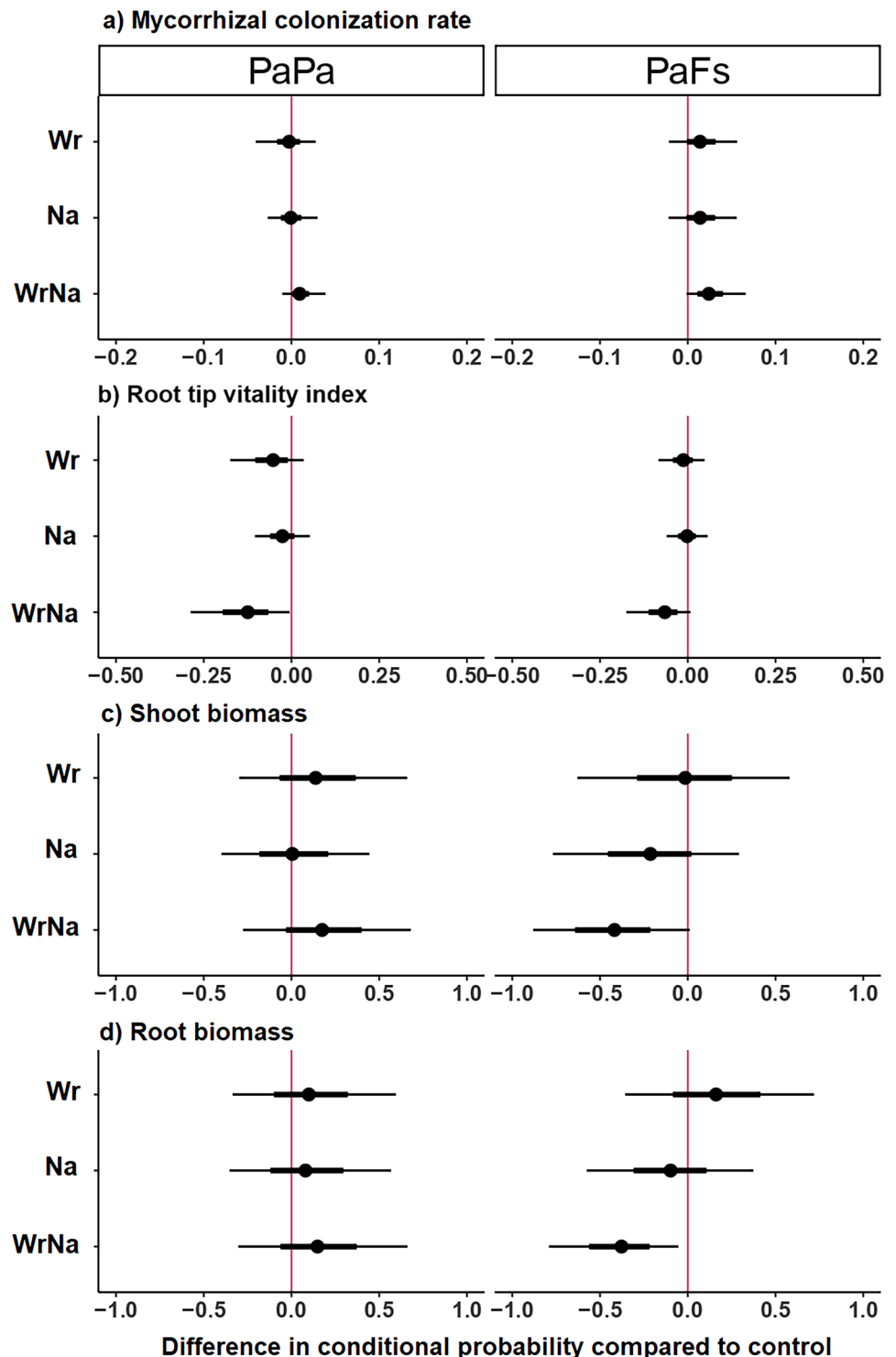
No effects on mycorrhization rates were found in any other treatment.

The vitality index (Fig. 3b) of *P. abies* was 12% lower under water reduction + N addition than that of the controls in monospecific combination (-0.287 to -0.005), and also showed 6% decline in mixed combination with *F. sylvatica* under the same treatment (-0.175 to 0.008), although zero and positive values cannot be excluded. Water reduction or N addition as single factors did not affect root

vitality in monospecific conditions or in combinations with *F. sylvatica*.

In mixture with *F. sylvatica*, *P. abies* showed 40% shoot biomass reduction under water reduction + N addition compared with the control (-0.964 to -0.006) (Fig. 3c). The root biomass (Fig. 3d) was 38% lower in mixed combination under water reduction + N addition than that of the control (-0.809 to -0.063). Under the other treatments, the shoot

Fig. 3 Variation of **a** mycorrhizal colonization rate, **b** Root tip vitality index, **c** Normalized shoot biomass and **d** normalized root biomass for *P. abies* in monospecific combination (PaPa) and mixed combination with *F. sylvatica* (PaFs). The differences in conditional probability of the measured traits were predicted for water reduction (Wr), N addition (Na) and water reduction + N addition (WrNa) compared to control. A conditional probability of $-1/+1$ defines a 100% probability of a reduction/increase in the trait due to the treatment. Red line denotes expectation of zero change compared to control. Black bar depicts 66% (thick) and 95% (thin) credible intervals around the estimate; symbols indicate medians of the posterior distribution



and root biomass of *P. abies* in both monospecific and mixed combinations did not show any relevant variations.

For *P. menziesii*, the mycorrhizal colonization rate (Fig. 4a) was slightly reduced in the mixed combination with *F. sylvatica* under water reduction compared to control (-0.013 to 0) and not in any other treatment or species

combination. No significant variations from control was found for the vitality index for *P. menziesii* (Fig. 4b).

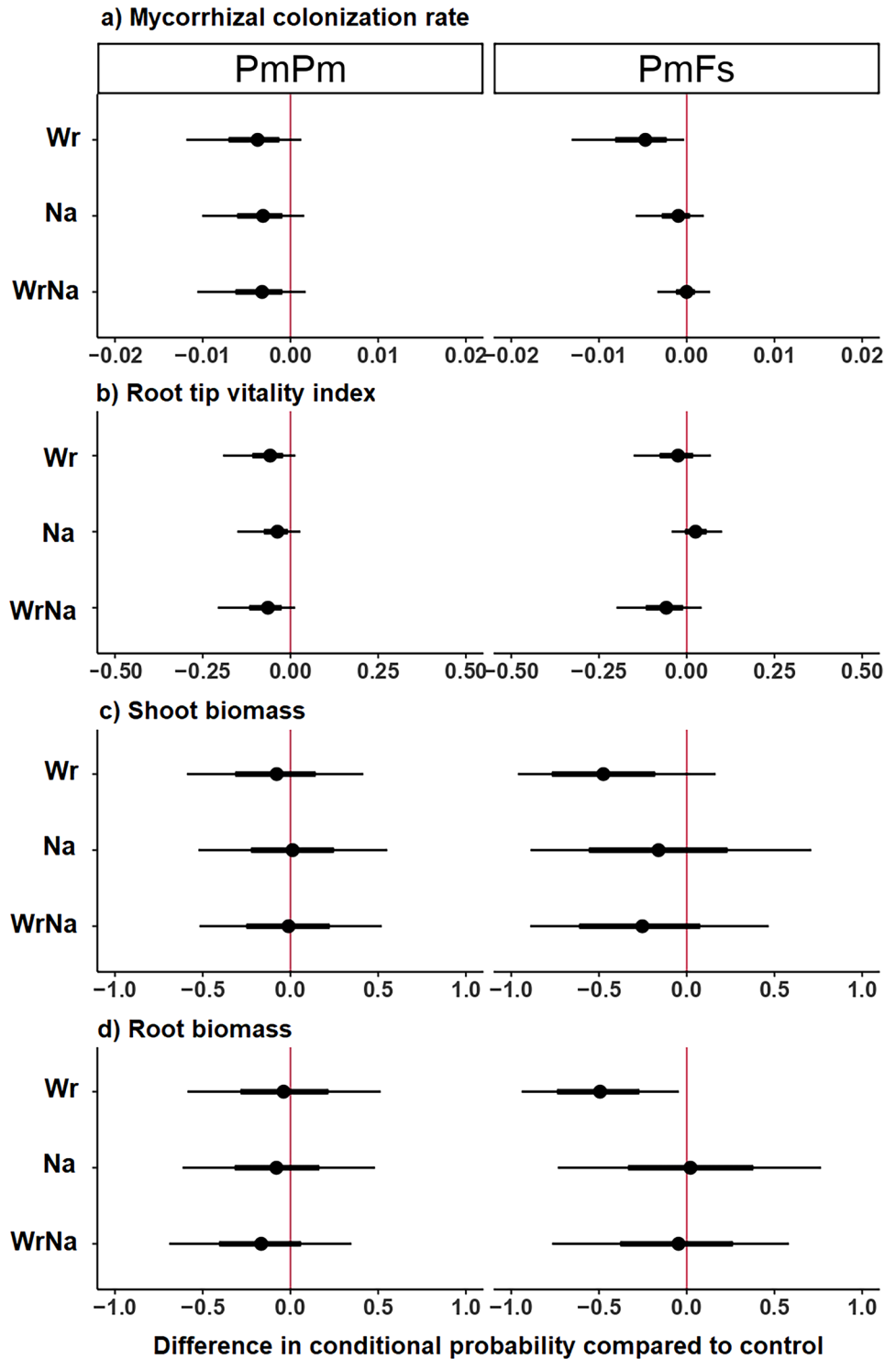
The shoot biomass of *P. menziesii* (Fig. 4c) showed a 56% reduction under water reduction in mixture with *F. sylvatica* compared to control (-1.602 to 0.078), although zero and positive values cannot be excluded. Further under

these conditions, root biomass of *P. menziesii* (Fig. 4d) was 54% lower than that of controls (−1.278 to −0.042). No other changes of shoot and root biomass were found in response to the treatments and species mixtures.

Discussion

In this study, we showed that three economically relevant forest tree species reacted differently to species mixing under the impact of water scarcity and N addition already

Fig. 4 Variation of **a** mycorrhizal colonization rate, **b** root tip vitality index, **c** normalized shoot biomass and **d** normalized root biomass for *P. menziesii* in monospecific combination (PmPm) and mixed combination with *F. sylvatica* (PmFs). The differences in conditional probability of the measured traits were predicted for water reduction (Wr), N addition (Na) and water reduction + N addition (WrNa) compared to control. A conditional probability of $-1/+1$ defines a 100% probability of a reduction/increase in the trait due to the treatment. Red line denotes expectation of zero change compared to control. Black bar depicts 66% (thick) and 95% (thin) credible intervals around the estimate; symbols indicate medians of the posterior distribution



after one growth season.

In line with other studies, the water reduction and N treatments only had a marginal effect on mycorrhizal colonization (Bogeat-Triboulot et al. 2004; Clausing et al. 2021a; Morte et al. 2001), but the combination of both impacted root tip vitality. Studies show that water scarcity can impair plant photosynthesis and, as a consequence, the carbon supply to mycorrhizal symbionts, leading to root tip death; an increase of non-vital root tips was observed in *Quercus pubescens* forests in Slovenia in association with seasonal drought events (Mrak et al. 2021) and for *Pinus edulis* in northern Arizona after prolonged drought (Swaty et al. 2004) as well as in response to girdling when the carbon supply to the roots was interrupted (Clausing et al. 2021b; Pena et al. 2010). In our experiment, water reduction alone did not show a significant impact, but the root tip vitality index declined for *F. sylvatica* in mixed combination with *P. abies* and for monospecific *P. abies* under water reduction + N addition. Root tip vitality declined significantly for trees with the highest root biomass. This observation suggests that under high N availability the trees invested in root biomass production when experiencing root tip mortality to compensate for the loss of active roots, or, alternatively, that a large root system exposed the trees to a greater risk of root tip drying because of increased water demand (Dziedek et al. 2016; Meyer-Grünefeldt et al. 2015). Both options require more studies to be clarified.

An unexpected, novel result was that negative impacts on biomass experienced under abiotic stress (water reduction and N addition) were mitigated in mixed combinations only for *F. sylvatica* but not for conifers. This result does not concur with our initial hypothesis, which states that all studied species—in monospecific and mixed combinations—would show more negative effects under the combination of low water supply + N addition rather than when exposed to individual stress factors. Instead, the results firstly suggest that *F. sylvatica* shows stronger intraspecific competition under stress than conifers. Second, in mixed combinations, competition strengths of the studied species are not equal; field studies (e.g. Schwarz and Bauhus 2019) show greater mixing benefits for *F. sylvatica* under drought compared to the admixed species possibly due to the reduction of the intraspecific competition that *F. sylvatica* experiences in mixture with conifers. Our study partially agrees with this observation, although mixing benefits, in particular on shoot biomass, were observed only when water reduction was combined with N addition.

Our results highlight a species-specific response to environmental stress. *F. sylvatica* shoot biomass and root tip vitality decreased under water reduction combined with N addition; greater stress symptoms were reported by Dziedek

et al. (2016) for *F. sylvatica* seedlings when exposed to drought and N excess. No significant variations were found under the N addition treatment alone: the study of Likulunga et al. (2022) also did not find any variation of root biomass under N fertilization in *F. sylvatica* forests. *P. abies* showed the strongest negative impacts on root biomass and root tip vitality under water reduction + N addition. Lower root biomass might impair water uptake ability, thereby failing to meet the increasing transpiration demand, especially during severe drought (Hammond et al. 2022). Even though we did not measure any root morphological traits, a reduction in fine root diameter could also be responsible for lower biomass, in this case improving water uptake by increasing root–soil interface (Olmo et al. 2014). Negative impacts of drought combined with N addition on root biomass were reported also in a young *P. abies* stand (Palátová 2004). This suggests that the simultaneous occurrence of water reduction and increased N supply represents unfavorable conditions for *F. sylvatica* and *P. abies*, both naturally adapted to a moderate level of soil moisture and N-limited ecosystems (Ellenberg 1988). For *P. menziesii*, mycorrhizal colonization rate, root biomass and marginally shoot biomass were negatively impacted by water reduction as single factor. The observation that, unlike *F. sylvatica* and *P. abies*, *P. menziesii* was not impacted by water reduction combined with N addition, but by water reduction alone, might point to a higher N demand because of its high growth rate (Thomas et al. 2022). We speculate that this demand cannot be met when water limitation most likely reduces N availability and root uptake (McMurtrie and Näsholm 2018). Under low water availability, enhanced N supply might be beneficial. Our result does not fit in the context of the claimed drought tolerance of *P. menziesii* in European forests (Eilmann and Rigling 2012), suggesting that the performance of *P. menziesii* under drought might vary with nutrient availability in the soil.

The three studied species differentially responded to species mixing. First of all, *F. sylvatica* showed a reduction of shoot biomass under water reduction + N addition only in the monospecific combination, while it was not altered in mixed combinations with either *P. abies* or *P. menziesii*. Contrary to our expectations, the responses of *P. abies* and *P. menziesii* to water reduction and N addition were not minor in mixed compared to monospecific combinations; for *P. abies* root biomass declined significantly (38%) under water reduction + N addition when growing with *F. sylvatica*; for *P. menziesii* root biomass showed a 54% decline under water reduction alone in combination with *F. sylvatica*. This suggests that *F. sylvatica* benefits from interactions with conifer neighbors because of the release from intraspecific competition, and that the negative effect of water reduction

+ N addition could be mitigated for this species in mixed combinations. A positive effect of conifer presence on the biomass of *F. sylvatica* was found by Bebre et al. (2021) in a controlled experiment; in detail, *F. sylvatica* seedlings growing with *P. menziesii* reached a higher biomass compared to seedlings growing with conspecifics. However, in this study, no differential water and/or nitrogen treatments were applied, and the positive effect of conifers on *F. sylvatica* was found only when the trees were shaded with 50% light reduction. In mixed forests, the presence of conifers has divergent impacts on *F. sylvatica*: with *P. abies*, either facilitative interaction (Bolte et al. 2013), or a competitive advantage (Schmidt 2002) or disadvantage (Kozovits et al. 2005) was found; the presence of *P. menziesii* favored *F. sylvatica* growth (Reyer et al. 2010) or had no effect (Thurm et al. 2016). Taken together, these results indicate that confounding factors may blur the response of trees to species mixing under field conditions.

Moreover, our results support the notion that the differences between the response of biomass production of *F. sylvatica* and the two conifers, in particular the reduction of shoot biomass of *F. sylvatica* and the reduction of root biomass for conifers, could be due to opposite resource allocation strategies that broadleaved and conifer trees activate during stress (Villar-Salvador et al. 2015).

Facing fast environmental change, even small, divergent responses of tree species to stress factors might have important consequences to be considered in the decision-making process of forest management. Applying Bayesian regression models to our data, we quantified mycorrhizal colonization rate, root tip vitality and tree biomass under different scenarios for all species and species combinations. By estimation of full probability distributions of changes in the conditional expectation of our response variables, the Bayesian approach to statistical inference allowed us to have a direct quantification of uncertainty in these changes. This represents an advantage compared to frequentist probability statistical approaches (i.e., classical ANOVA) that (a) require believing in approximate normality in order to get to statistical inference statements, and (b) only allow for indirect statements based on hypothetical long-run frequentist attributes of the statistical or sampling method.

A limitation of our study is that the controlled pot experiment does not reflect the complexity of natural conditions that trees experience in forest soil. Specifically, we used nutrient poor sand as substrate to be able to assess root tip vitality and mycorrhization. However, in northern Germany, forests of *F. sylvatica*, *P. abies* and *P. menziesii* do occur on sandy soil with low water-holding capacity (BMEL 2012; Foltran et al. 2023) and experience relatively high deposition of N (Schaap et al. 2018). Therefore, our study reflects relevant and current environmental cues for the studied species under controlled conditions.

Conclusion

Our study clearly demonstrates young tree species-specific responses to abiotic stress and neighboring tree species. Under the occurrence of water reduction and N addition, young *P. menziesii* performed better than *P. abies* in terms of biomass production. This could mean that the response of *P. menziesii* depends on nutrient availability, with N addition mitigating the negative effect of water reduction. Additionally, *F. sylvatica* had an advantage when growing with conifers instead of conspecific species. Whether this novel result holds for mature stands, requires further investigation. Based on our results, we recommend that, to withstand the risks imposed by climate change, the selection of suitable tree species should be not only based on their individual responses but also on their interactive responses to changing abiotic factors. On inherently nutrient-rich sites or sites exposed to high N deposition rates, the mixture of *F. sylvatica* with *P. menziesii* might be preferable to *P. abies* or to monospecific *F. sylvatica* stands.

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Author contributions AP, DH, CL conceived the ideas and set up the experiment, MA designed the methodology with the help of AP; MA, IS, KM, and SP collected the data; MA and HS-R analysed the data; MA led the writing of the manuscript; all authors contributed to the drafts. All authors read and approved the final manuscript.

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Data availability The data used for this study are available at <https://doi.org/10.25625/GJ6XUR>.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This manuscript is original and has not been published elsewhere.

Consent to participate Not applicable.

Consent for publication Not applicable.

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References

- Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107–114. <https://doi.org/10.1007/s005720100108>
- Anderegg WR, Schwalm C, Biondi F, Camarero JJ, Koch G, Litvak M et al (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science* 349(6247):528–532. <https://doi.org/10.1126/science.aab1833>
- Bebre I, Riebel H, Annighöfer P (2021) Seedling growth and biomass production under different light availability levels and competition types. *Forests* 12(10):1376. <https://doi.org/10.3390/f12101376>
- Bertness MD, Callaway RM (1994) Positive interactions in communities. *Trends Ecol Evol* 9:191–193. [https://doi.org/10.1016/0169-5347\(94\)90088-4](https://doi.org/10.1016/0169-5347(94)90088-4)
- BMEL (2012) Ergebnisse der Bundeswaldinventur 2012: BMEL Bonn. <https://www.bmel.de/SharedDocs/Downloads/DE/Broschueren/ErgebnisseBW12012.html>. Accessed 15 May 2023
- Bobbink R, Hettelingh JP (2011) Review and revision of empirical critical loads and dose-response relationships. In Coordination Centre for Effects, National Institute for Public Health and the Environment (RIVM), Bilthoven
- Bogeat-Triboulot MB, Bartoli F, Garbaye J et al (2004) Fungal ectomycorrhizal community and drought affect root hydraulic properties and soil adherence to roots of *Pinus pinaster* seedlings. *Plant Soil* 267:213–223. <https://doi.org/10.1007/s11104-005-5349-7>
- Bolte A, Kampf F, Hilbrig L (2013) Space sequestration below ground in old-growth spruce-beech forests—signs for facilitation? *Front Plant Sci* 4:322. <https://doi.org/10.3389/fpls.2013.00322>
- Bošefa M, Tumajer J, Cienciala E, Dobor L, Kulla L, Marčič P et al (2021) Climate warming induced synchronous growth decline in Norway spruce populations across biogeographical gradients since 2000. *Sci Total Environ* 752:141794. <https://doi.org/10.1016/j.scitotenv.2020.141794>
- Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann for Sci* 63(6):625–644. <https://doi.org/10.1051/forest:2006042>
- Brus DJ, Hengeveld GM, Walvoort DJJ, Goedhart PW, Heidema AH, Nabuurs GJ, Gunia K (2012) Statistical mapping of tree species over Europe. *Eur J for Res* 131:145–157. <https://doi.org/10.1007/s10342-011-0513-5>
- Bürkner PC (2017) brms: an R package for Bayesian multilevel models using Stan. *J Stat Softw* 80:1–28
- Bürkner PC (2018) Advanced Bayesian multilevel modeling with the R Package brms. *R J* 10(395):411
- Carpenter B, Gelman A, Hoffman MD, Lee D, Goodrich B, Betancourt M, Brubaker M (2017) Stan: a probabilistic programming language. *J Stat Softw* 76(1):32
- Clausing S, Likulunga LE, Janz D, Feng HY, Schneider D, Daniel R, Krüger J, Lang F, Polle A (2021a) Impact of nitrogen and phosphorus addition on resident soil and root microbiomes in beech forests. *Biol Fertil Soils* 57:1031–1052. <https://doi.org/10.1007/s00374-021-01593-x>
- Clausing S, Pena R, Song B, Müller K, Mayer-Gruner P, Marhan S, Grafe M, Schulz S, Krüger J, Lang F, Schloter M, Kandeler E, Polle A (2021b) Carbohydrate depletion in roots impedes phosphorus nutrition of young forest trees. *New Phytol* 229:2611–2624. <https://doi.org/10.1111/nph.17058>
- Coppock A (2022) Randomizr: Easy-to-use tools for common forms of random assignment and sampling. <https://cran.r-project.org/web/packages/randomizr/randomizr.pdf>. Accessed 15 May 2023
- Cremer M, Kern NV, Prietzel J (2016) Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. *For Ecol Manag* 367:30–40. <https://doi.org/10.1016/j.foreco.2016.02.020>
- Dai A (2013) Increasing drought under global warming in observations and models. *Nat Clim Change* 3(1):52–58. <https://doi.org/10.1038/nclimate1633>
- Danielsen L, Polle A (2014) Poplar nutrition under drought as affected by ectomycorrhizal colonization. *Environ Exp Bot* 108:89–98. <https://doi.org/10.1016/j.envexpbot.2014.01.006>
- De Vries W, Dobbertin MH, Solberg S, van Dobben HF, Schaub M (2014) Impacts of acid deposition, ozone exposure and weather conditions on forest ecosystems in Europe: an overview. *Plant Soil* 380:1–45. <https://doi.org/10.1007/s11104-014-2056-2>
- Dziedek C, Haerdle W, Von Oheimb G, Fichtner A (2016) Nitrogen addition enhances drought sensitivity of young deciduous tree species. *Front Plant Sci* 7:1100. <https://doi.org/10.3389/fpls.2016.01100>
- Eilmann B, Rigling A (2012) Tree-growth analyses to estimate tree species' drought tolerance. *Tree Physiol* 32(2):178–187. <https://doi.org/10.1093/treephys/tps004>
- Ellenberg H (1988) Vegetation ecology of central Europe. Cambridge University Press, Cambridge
- Erisman JW, Galloway JN, Seitzinger S, Bleeker A, Dise NB, Petrescu AR et al (2013) Consequences of human modification of the global nitrogen cycle. *Philos Trans R Soc Lond B Biol Sci* 368(1621):20130116. <https://doi.org/10.1098/rstb.2013.0116>
- Eugster W, Haeni M (2013) Nutrients or pollutants? Nitrogen deposition to European forests. *Dev Environ Sci* 13:37–56. <https://doi.org/10.1016/B978-0-08-098349-3.00003-7>
- Fleck S, Eickenscheidt N, Ahrends B, Evers J, Grüneberg E et al (2019) Nitrogen status and dynamics in German forest soils. In: Wellbrock N, Bolte A (eds) Status and dynamics of forests in Germany: ecological studies, vol 237. Springer, Cham
- Foltran EC, Ammer C, Lamersdorf N (2023) Do admixed conifers change soil nutrient conditions of European beech stands? *Soil Res* 61(7):647–662. <https://doi.org/10.1071/SR22218>
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR et al (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320(5878):889–892. <https://doi.org/10.1126/science.1136674>
- Gelman A, Rubin DB (1992) Inference from iterative simulation using multiple sequences. *Stat Sci* 7(4):457–472. <https://doi.org/10.1214/ss/1177011136>
- Hammond WM, Williams AP, Abatzoglou JT et al (2022) Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. *Nat Commun* 13:1761. <https://doi.org/10.1038/s41467-022-29289-2>
- Hoagland DR, Snyder WC (1933) Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) susceptibility to injury from sodium salts. *Proc Am Soc Horticult Sci* 30:288–294. <https://doi.org/10.5555/19340300363>
- Huang Z, Liu B, Davis M, Sardans J, Peñuelas J, Billings S (2016) Long-term nitrogen deposition linked to reduced water use

- efficiency in forests with low phosphorus availability. *New Phytol* 210(2):431–442. <https://doi.org/10.1111/nph.13785>
- IPCC (2022) Climate Change 2022: impacts, adaptation and vulnerability. contribution of working group ii to the sixth assessment report of the intergovernmental panel on climate Change H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Lösschke, V. Möller, A. Okem, B. Rama Eds. Cambridge University Press. Cambridge, UK and New York, USA. <https://doi.org/10.1017/9781009325844>
- Janssens IA, Dieleman WIJ, Luyssaert S, Subke J-A, Reichstein M et al (2020) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geosci* 3:315–322. <https://doi.org/10.1038/ngeo844>
- Kozovits AR, Matyssek R, Blaschke H, Göttlein A, Grams TE (2005) Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO₂ and/or O₃ concentrations throughout two subsequent growing seasons. *Glob Change Biol* 11(9):1387–1401. <https://doi.org/10.1111/j.1365-2486.2005.00993.x>
- Krejza J, Cienciala E, Světlík J, Bellan M, Noyer E, Horáček P et al (2021) Evidence of climate-induced stress of Norway spruce along elevation gradient preceding the current dieback in Central Europe. *Trees* 35:103–119. <https://doi.org/10.1007/s00468-020-02022-6>
- Kuyper TW, Suz LM (2023) Do ectomycorrhizal trees select ectomycorrhizal fungi that enhance phosphorus uptake under nitrogen enrichment? *Forests* 14(3):467. <https://doi.org/10.3390/f14030467>
- Lamarque J-F, Dentener F, McConnell J, Ro C-U, Shaw M, Vet R, Bergmann D, Cameron-Smith P, Dalsoren S, Doherty R (2013) Multimodel mean nitrogen and sulfur deposition from the atmospheric chemistry and climate model intercomparison project (ACCMIP): evaluation of historical and projected future changes. *Atmos Chem Phys* 13:7997–8018. <https://doi.org/10.5194/acp-13-7997-2013>
- Lehto T, Zwiazek JJ (2011) Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* 21:71–90. <https://doi.org/10.1007/s00572-010-0348-9>
- Lemoine NP (2019) Moving beyond noninformative priors: Why and how to choose weakly informative priors in Bayesian analyses. *Oikos* 128(7):912–928. <https://doi.org/10.1111/oik.05985>
- Likulunga LE, Pérez CAR, Schneider D, Daniel R, Polle A (2021) Tree species composition and soil properties in pure and mixed beech-conifer stands drive soil fungal communities. *For Ecol Manag* 502:119709. <https://doi.org/10.1016/j.foreco.2021.119709>
- Likulunga LE, Clausing S, Krueger J, Lang F, Polle A (2022) Fine root biomass of European beech trees in different soil layers show different responses to season, climate, and soil nutrients. *Front Glob Change* 5:955327. <https://doi.org/10.3389/fgc.2022.955327>
- Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA (2019) Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. *Environ Pollut* 246:148–162. <https://doi.org/10.1016/j.envpol.2018.11.074>
- Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J et al (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *For Ecol Manag* 259:698–709. <https://doi.org/10.1016/j.foreco.2009.09.023>
- Lwila AS, Mund M, Ammer C, Glatthorn J (2021) Site conditions more than species identity drive fine root biomass, morphology and spatial distribution in temperate pure and mixed forests. *For Ecol Manag* 499:119581. <https://doi.org/10.1016/j.foreco.2021.119581>
- Lwila AS, Post-Leon A, Ammer C, Mund M (2023) Site properties, species identity, and species mixture affect fine root production, mortality, and turnover rate in pure and mixed forests of European Beech, Norway spruce, and Douglas-fir. *Ecol Indic* 147:109975. <https://doi.org/10.1016/j.ecolind.2023.109975>
- McMurtrie RE, Näsholm T (2018) Quantifying the contribution of mass flow to nitrogen acquisition by an individual plant root. *New Phytol* 218(1):119–130. <https://doi.org/10.1111/nph.14927>
- Metz J, Annighöfer P, Schall P, Zimmermann J, Kahl T, Schulze ED, Ammer C (2016) Site-adapted admixed tree species reduce drought susceptibility of mature European beech. *Glob Change Biol* 22(2):903–920. <https://doi.org/10.1111/gcb.13113>
- Meyer-Grünefeldt M, Friedrich U, Klotz M, Von Oheimb G, Härdtle W (2015) Nitrogen deposition and drought events have non-additive effects on plant growth—evidence from greenhouse experiments. *Plant Biosyst* 149:424–432. <https://doi.org/10.1080/11263504.2013.853699>
- Mezei P, Jakuš R, Pennerstorfer J, Havašová M, Škvarenina J, Ferenčík J et al (2017) Storms, temperature maxima and the Eurasian spruce bark beetle *Ips typographus*—an infernal trio in Norway spruce forests of the Central European High Tatra Mountains. *Agric for Meteorol* 242:85–95. <https://doi.org/10.1016/j.agrformet.2017.04.004>
- Morte A, Díaz G, Rodríguez P, Alarcón JJ, Sánchez-Blanco MJ (2001) Growth and water relations in mycorrhizal and nonmycorrhizal *Pinus halepensis* plants in response to drought. *Biol Plant* 44:263–267. <https://doi.org/10.1023/A:1010207610974>
- Mrak T, Šibanc N, Brailey-Jones P, Štraus I, Gričar J, Kraigher H (2021) Extramatrical mycelium and ectomycorrhizal community composition of *Quercus pubescens* in a sub-Mediterranean stress-prone environment. *Front for Glob Change* 4:599946. <https://doi.org/10.3389/fgc.2021.599946>
- Nehls U, Plassard C (2018) Nitrogen and phosphate metabolism in ectomycorrhizas. *New Phytol* 220(4):1047–1058. <https://doi.org/10.1111/nph.15257>
- Neuner S, Albrecht A, Cullmann D, Engels F, Griess VC, Hahn WA, Hanewinkel M, Härtl F, Kölling C, Staupendahl K, Knoke T (2015) Survival of Norway spruce remains higher in mixed stands under a dryer and warmer climate. *Glob Change Biol* 21:935–946. <https://doi.org/10.1111/gcb.12751>
- Nickel UT, Weikl F, Kerner R, Schäfer C, Kallenbach C, Munch JC, Pritsch K (2018) Quantitative losses vs. qualitative stability of ectomycorrhizal community responses to 3 years of experimental summer drought in a beech–spruce forest. *Glob Change Biol* 24(2):e560–e576. <https://doi.org/10.1111/gcb.13957>
- Nikolova PS, Bauerle TL, Haeberle KH, Blaschke H, Brunner I, Matyssek R (2020) Fine-root traits reveal contrasting ecological strategies in European beech and Norway spruce during extreme drought. *Front Plant Sci* 11:1211. <https://doi.org/10.3389/fpls.2020.01211>
- Nilsen P (1995) Effect of nitrogen on drought strain and nutrient uptake in Norway spruce *Picea abies* (L.) Karst. trees. *Plant Soil* 172:73–85. <https://doi.org/10.1007/BF00020861>
- Nilsen P, Børja I, Knutsen H, Brean R (1998) Nitrogen and drought effects on ectomycorrhizae of Norway spruce [*Picea abies* L. (Karst.)]. *Plant Soil* 198:179–184. <https://doi.org/10.1023/A:1004399303192>
- Nilsson LO, Wallander H (2003) Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytol* 158:409–416. <https://doi.org/10.1046/j.1469-8137.2003.00728.x>
- Olmo M, Lopez-Iglesias B, Villar R (2014) Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species implications for a drier climate. *Plant Soil* 384:113–129. <https://doi.org/10.1007/s11104-014-2178-6>
- Palátová E (2004) Effect of increased nitrogen depositions and drought stress on the development of young Norway spruce *Picea abies* (L.) Karst. stands. *Dendrobiology* 51:41–45
- Pardos M, Del Río M, Pretzsch H, Jactel H, Bielak K, Bravo F et al (2021) The greater resilience of mixed forests to drought mainly

- depends on their composition: analysis along a climate gradient across Europe. For Ecol Manag 481:118687. <https://doi.org/10.1016/j.foreco.2020.118687>
- Pena R, Polle A (2014) Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. ISME J 8:321–330. <https://doi.org/10.1038/ismej.2013.158>
- Pena R, Offermann C, Simon J, Naumann PS, Geßler A, Holst J et al (2010) Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl Environ Microbiol 76:1831–1841. <https://doi.org/10.1128/AEM.01703-09>
- Pena R, Lang C, Lohaus G, Boch S, Schall P, Schöning I, Ammer C, Fischer M, Polle A (2017) Phylogenetic and functional traits of ectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27:233–245. <https://doi.org/10.1007/s00572-016-0742-z>
- Pretzsch H, Schütze G, Biber P (2018) Drought can favour the growth of small in relation to tall trees in mature stands of Norway spruce and European beech. For Ecosyst 5(1):1–19. <https://doi.org/10.1186/s40663-018-0139-x>
- R Core Team (2022) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna
- Rukh S, Poschenrieder W, Heym M, Pretzsch H (2020) Drought resistance of Norway spruce (*Picea abies* [L.] Karst) and European beech (*Fagus sylvatica* [L.]) in mixed vs. monospecific stands and on dry vs. wet sites. From evidence at the tree level to relevance at the stand level. Forests 11(6):639. <https://doi.org/10.3390/f11060639>
- Schaap M, Hendriks C, Kranenburg R et al (2018) PINETI-3: Modellierung atmosphärischer Stoffeinträge von 2000 bis 2015 zur Bewertung der öko- system-spezifischen Gefährdung von Biodiversität durch Luftschadstoffe in Deutschland. Abschlussbericht. UBA-Texte 79/2018, Dessau-Roßlau. <https://www.umweltbund.esam.de/publikationen/pineti-3-modellierung-atmosphaerischer>. Accessed 14 Feb 2024
- Schipka F, Heimann J, Leuschner C (2005) Regional variation in canopy transpiration of Central European beech forests. Oecologia 143:260–270. <https://doi.org/10.1007/s00442-004-1798-6>
- Schmidt I (2002) The influence of soil type and interspecific competition on the fine root system of Norway spruce and European beech. Basic Appl Ecol 3(4):339–346. <https://doi.org/10.1078/1439-1791-00116>
- Schuldt B, Buras A, Arend M, Vitasse Y, Beierkuhnlein C, Damm A et al (2020) A first assessment of the impact of the extreme 2018 summer drought on Central European forests. Basic Appl Ecol 45:86–103. <https://doi.org/10.1016/j.baae.2020.04.003>
- Schwarz JA, Bausch J (2019) Benefits of mixtures on growth performance of silver fir (*Abies alba*) and European beech (*Fagus sylvatica*) increase with tree size without reducing drought tolerance. Front for Glob Change 2:79. <https://doi.org/10.3389/ffgc.2019.00079>
- Stan Development Team (2023) Runtime warnings and convergence problems. <https://mc-stan.org/misc/warnings.html>. Accessed 8 Mar 2023
- Su X, Su X, Zhou G, Du Z, Yang S, Ni M, Qin H, Huang Z, Zhou X, Deng J (2020) Drought accelerated recalcitrant carbon loss by changing soil aggregation and microbial communities in a subtropical forest. Soil Biol Biochem 148:107898. <https://doi.org/10.1016/j.soilbio.2020.107898>
- Swaty RL, Deckert RJ, Whitham TG, Gehring CA (2004) Ectomycorrhizal abundance and community composition shifts with drought: predictions from tree rings. Ecology 85(4):1072–1084. <https://doi.org/10.1890/03-0224>
- Thomas FM, Rzepecki A, Werner W (2022) Non-native Douglas fir (*Pseudotsuga menziesii*) in Central Europe: ecology, performance and nature conservation. For Ecol Manag 506:119956. <https://doi.org/10.1016/j.foreco.2021.119956>
- Thurm EA, Pretzsch H (2016) Improved productivity and modified tree morphology of mixed versus pure stands of European beech (*Fagus sylvatica*) and Douglas-fir (*Pseudotsuga menziesii*) with increasing precipitation and age. Ann for Sci 73(4):1047–1061. <https://doi.org/10.1007/s13595-016-0588-8>
- Thurm EA, Uhl E, Pretzsch H (2016) Mixture reduces climate sensitivity of Douglas-fir stem growth. For Ecol Manag 376:205–220. <https://doi.org/10.1016/j.foreco.2016.06.020>
- Valtanan K, Eissfeller V, Beyer F, Hertel D, Scheu S, Polle A (2014) Carbon and nitrogen fluxes between beech and their ectomycorrhizal assemblage. Mycorrhiza 24:645–650. <https://doi.org/10.1007/s00572-014-0581-8>
- Van der Graaf SC, Janssen TAJ, Erisman JW, Schaap M (2021) Nitrogen deposition shows no consistent negative nor positive effect on the response of forest productivity to drought across European FLUXNET forest sites. Environ Res Commun 3(12):125003. <https://doi.org/10.1088/2515-7620/ac2b7d>
- Villar-Salvador P, Uscola M, Jacobs DF (2015) The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees. New for 46:813–839. <https://doi.org/10.1007/s11056-015-9499-z>
- Wambsgans J, Freschet GT, Beyer F, Goldmann K, Prada-Salcedo LD, Scherer-Lorenzen M, Bausch J (2021) Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests. Funct Ecol 35(9):1886–1902. <https://doi.org/10.1111/1365-2435.13856>
- Wang J, Zhang CB, Chen T, Li WH (2013) From selection to complementarity: the shift along the abiotic stress gradient in a controlled biodiversity experiment. Oecologia 171:227–235. <https://doi.org/10.1007/s00442-012-2400-2>
- Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York
- Wu J, Liu W, Zhang W, Shao Y, Duan H, Chen B, Wei X, Fan H (2019) Long-term nitrogen addition changes soil microbial community and litter decomposition rate in a subtropical forest. Appl Soil Ecol 142:43–51. <https://doi.org/10.1016/j.apsoil.2019.05.014>
- Yan G, Zhou M, Wang M, Han S, Liu G, Zhang X, Sun W, Huang B, Wang H, Xing Y, Wang Q (2019) Nitrogen deposition and decreased precipitation altered nutrient foraging strategies of three temperate trees by affecting root and mycorrhizal traits. CATENA 181:104094. <https://doi.org/10.1016/j.catena.2019.104094>
- Zhang H, Shi L, Lu H, Shao Y, Liu S, Fu S (2020) Drought promotes soil phosphorus transformation and reduces phosphorus bioavailability in a temperate forest. Sci Total Environ 732:139295. <https://doi.org/10.1016/j.scitotenv.2020.139295>

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