

ORIGINAL ARTICLE

Combined Effects of Nitrogen Deposition and Climate on Root-to-Shoot Ratios and Root Morphology in Temperate Tree Regeneration

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ABSTRACT

Nitrogen (N) availability is known to interact with the drought tolerance in plants, but its effects on the morphological acclimation of forest trees to low precipitation have rarely been investigated. Based on a Germany-wide field-study design covering different combinations of climatic conditions and atmospheric N deposition, we harvested young trees up to 70 cm in height of *Fagus sylvatica*, *Quercus petraea*, *Pseudotsuga menziesii*, and *Abies alba*. We tested the hypothesis that high N deposition decreases the root/shoot ratio, alters fine root morphology (for example, by reducing specific root length of absorptive roots) and reduces mycorrhizal colonization rate, which are factors that would reduce the capacity for water uptake under drought.

European beech showed reduced root/shoot ratios at high N deposition and reduced specific root length and mycorrhizal colonization of absorptive roots at sites with both high N deposition and low precipitation. These observations were consistent with the optimal portioning theory, as less investment in belowground biomass was required to meet the N demand at high N deposition. Sessile oak showed no effect of N deposition or mean annual precipitation on the parameters studied, indicating that the gradients studied were not large enough to cause changes. Douglas-fir and silver fir expanded their root systems with increasing N deposition, with higher specific root length in Douglas-fir and a trend towards higher root tip abundance in silver fir. Thus, in contrast to European beech, we found no indication that the tree's ability to cope with drought was reduced by high N deposition in sessile oak, Douglas-fir, and silver fir. The combined negative effect of drought and high N deposition on the root system in European beech as Central Europe's most dominant tree species in the natural vegetation emphasizes the need for curtailing N emissions, especially from agriculture.

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Key words: Climate change; Nitrogen availability; Forest regeneration; Temperate forests; Biomass allocation; Specific root length.

HIGHLIGHTS

- Beech invests less biomass belowground with increasing nitrogen availability.
- No reduction in root system plasticity for sessile oak, Douglas fir and silver fir.
- Promotional effect of N on fine roots of Douglas fir and silver fir for water uptake.

INTRODUCTION

Forests are being impacted globally by climate change, as evidenced by drought- and heat-related productivity and vitality reductions and increased tree mortality across all forest biomes (Allen others 2010; Hauck others 2019). Stable and hot summer droughts, a recently evolving characteristic of climate change in Northern Hemisphere mid-latitudes, are a main cause of drought stress in temperate trees (Martinez del Castillo and others 2022; Rubio-Cuadrado and others 2018; Schuldt and others 2020). High N deposition originating from agriculture and fossil fuel combustion (Galloway and others 2008) can be another stress factor for temperate forests, which were originally widely limited by low N availability (Elser and others 2007, 2010). To date, there have also been reports of interactions between the effects of drought and high N loads on the vitality and productivity of forest trees (Dietrich and others 2024; Dulamsuren and Hauck 2021; Sigala and others 2020).

The ability to cope with drought in trees is generated by a variety of morphological, anatomical, and physiological traits, which are either determined by species-specific (or phylogenetic group-specific) genetic adaptations or result from acclimatization within the species' individual plasticity range. Important traits that influence drought tolerance include the sensitivity of stomatal control, the ability to minimize cuticular transpiration and to lower plant water potentials with progressive soil drying, the resistance to xylem embolism, as well as the ability to reduce the transpiring leaf area as a response to acute or chronic drought (Kerstiens 1996; Bartlett and others 2012; Mantova and others 2022; Descals and others 2022). Tolerance to high N concentrations depends largely on

the capacity to maintain high rates of N assimilation and thus to avoid toxic intracellular NH_4^+ concentrations (Cánovas and others 2018; Esteban and others 2016), but also on tolerance to soil acidification (Tian and Niu 2015). Trees along spatial climatic gradients differ in their morphological, anatomical, and physiological acclimation to drought (Leuschner and others 2006; Knutzen and others 2015; Weithmann and others 2023), but whether this acclimation is significantly influenced by N deposition has hardly been scrutinized.

The biomass ratio of roots to above-ground plant organs, fine root morphology, and mycorrhizal colonization are further important parameters with regard to both drought tolerance and high N deposition (Poorter and others 2012). The optimal partitioning theory suggests an increasing investment in root biomass to increase water and nutrient uptake during drought and nutrient deficiency (Bloom and others 1985; Ågren and Franklin 2003; Kobe and others 2010). This has been extensively studied across different plant taxa in analyses focussed on either water or nutrient availability, for example by analysing root/shoot ratios (for example, Brenchley 1916; Chapin 1980; Ingestad and Ågren 1991; McCarthy and Enquist 2007). In trees, this control of biomass allocation by water and nutrition is modified by other environmental or intrinsic factors, like light availability, competition, or tree size (van Hess and others 1997; van Hess and Clercx 2003; Schall and others 2012). Accordingly, a large number of studies have shown increases in the belowground/aboveground biomass ratio with increasing dryness (Markesteijn and Poorter 2009; Olmo and others 2014; Brunner and others 2015; Ledo and others 2018) or with decreasing N availability (Nadelhoffer 2000; McCarthy and Enquist 2007; Li and others 2015; Feng and others 2023). However, biomass studies have also proven the existence of substantial differences in the response to drought and N availability between species, phylogenetic groups, ecosystems, and climate zones. This is especially true for N availability, as some studies have shown no significant response of the root/shoot ratio (Qi and others 2019) or even an increase with increasing N supply (Gao and others 2023), highlighting the need to clarify effects at the species and forest ecosystem level.

Adaptations in root morphology can alter the volume of soil available and the conditions for water and nutrient uptake. Thin and long fine roots with a high specific root length can explore a greater soil volume and also have a higher hydraulic conductivity, because they have a lower

proportion of exodermis, but a higher proportion of xylem in the cross section (Rieger and Litvin 1999; Eissenstat and others 2000; Huang and Eissenstat 2000). Specific root length has been shown to increase with decreasing moisture (Li and others 2023) and increasing N supply (Razaq and others 2017), but also decreases of specific root length due to high N supply have been observed (Ostonen and others 2007). Root tissue density is typically reduced under good water supply, which is consistent with a decrease in strengthening tissues to the advantage of water-conducting cells (Li and others 2023) and reflects the plasticity of tree hydraulic architecture in response to site-dependent and interannual variations in water supply (Chenlemuge and others 2015). Consistent responses of root tissue density to N availability are not known (Noguchi and others 2013; Wang and others 2018; Li and others 2021, 2023).

The amount of ectomycorrhiza on the root surfaces is usually not influenced by water availability, but the species composition of mycorrhizal fungi is (Shi and others 2002). In contrast, high N supply has been repeatedly shown to decrease mycorrhizal colonization rate (Teste and others 2012; Li and others 2015; Wang and others 2017) in addition to reductions in the species richness of ectomycorrhizal fungi (Parrent and Vilgalys 2007). The negative effect of high N supply on mycorrhizal colonization is attributed to a reduced carbohydrate transfer from the tree roots to the fungi, because the tree can meet its N demands without support from the mycorrhiza (Nilsson and Wallander 2003; Li and others 2015).

Our study focussed on European beech (*Fagus sylvatica* L.), which is naturally Central Europe's dominant tree species (Leuschner and Ellenberg 2017), but has again and again shown a high susceptibility to climate change-induced summer droughts (for example, Schuldt and others 2020; Martinez del Castillo and others 2022; Dulamsuren and others 2022). Therefore, we compared beech with three other temperate tree species, which are widely discussed as potentially more drought-tolerant replacement tree species in the context of climate change adaptation of forestry (Bärman and others 2023; Bolte and others 2023; Hauck 2023). These species include the two European tree species sessile oak (*Quercus petraea* (Matt.) Liebl.) and silver fir (*Abies alba* Mill.), and the western North American Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), all of which are being discussed as alternatives with greater tolerance to drought and heat (Dziedek and others 2016a; Dyderski and others 2018; Vitasse and others 2019). In the same

field study design used here, covering a large climatic and atmospheric N deposition gradient across Germany, Dietrich and others (2024) have already shown that shoot length increment of the natural regeneration were differently affected by drought and N deposition in these four tree species, possibly due to different mechanisms driving biomass allocation and root anatomical traits: Shoot length increment in European beech was reduced by high N deposition, that in Douglas-fir was not affected by N, but dry spring climate; shoot length increment in silver fir benefitted from high N deposition combined with drought, and was not influenced by either drought or N deposition in sessile oak.

Existing studies of response of the belowground/aboveground biomass ratio to drought and N availability in European beech did not deliver consistent results. In two related greenhouse experiments with 1–2-year-old European beech plants, Dziedek and others (2016b) found a negative response of the root/shoot ratio to drought, but indifference to N availability, whereas Dziedek and others (2017) found no response to drought, but a negative response to N fertilization. Rose and others (2009) also found a negative response in the root/shoot ratio due to drought in 1-year-old European beech, but Zang and others (2014) observed an increase due to drought in 2-year-old saplings. In oak species, increases in the root/shoot ratio following drought exposure have been shown for both *Quercus petraea* and *Q. robur* (Gieger and Thomas 2002, 2005; Kuster and others 2013; Thomas and Gausling 2000). Moreover, Thomas and Hilker (2000) have shown a decrease in the root/shoot ratio with increasing N availability. Root/shoot ratio in Douglas-fir has been shown to be rather indifferent to drought, but to decrease with increasing N availability (Jacobs and others 2004; Olsthoorn and others 1991). Published results for the species we studied are therefore only partially consistent with the optimal partitioning theory, are scarce for silver fir, and also report variable results for root morphological parameters. In European beech, Taylor and Davies (1990) found reduced specific root length after drought exposure and Meier and others (2018) found increasing root tissue density with increasing mean annual precipitation. By contrast, Hertel and others (2013) observed no effect of mean annual precipitation on these two parameters in a similar study.

Given this partially conflicting information from the literature, we assumed the existence of marked differences in the drought and N response of the root/shoot ratio, root mass fraction, root morphology and mycorrhizal colonization rate among our

study species, which would imply N deposition-induced differences between species in root system water access during drought. Due to the high importance of fine root surface area for water and nutrient uptake in regard to our hypothesis, we focussed on fine root anatomical traits of absorptive roots, following the functional classification approach by McCormack and others (2015). Based on Dzialek and others (2016a, b), we hypothesized (1) that high N deposition decreases the root/shoot ratio, specific root length, root tip frequency, and mycorrhizal colonization in European beech in accordance with the optimal partitioning theory. Furthermore, we expected that adaptations in biomass allocation and root anatomical traits of European beech on drier sites (for example, increased root/shoot ratio, specific root length) will be reduced by increased N deposition (hypothesis 2). Since shoot length increment was shown to be significantly reduced by N deposition in the regeneration of European beech, but not of sessile oak, Douglas-fir, and silver fir (Dietrich and others 2024), we also tested the hypothesis (3) that biomass allocation to the root system and root morphology is less affected by N deposition in the other study species than in European beech.

MATERIALS AND METHODS

Study Area

During the summer of 2021, field work was conducted in managed forests across Germany with sub-oceanic to sub-continental temperate climate. Most forests of Germany would naturally be dominated by European beech, but at many places beech has been widely replaced by Norway spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) as the result of forest management in modern times (Leuschner and Ellenberg 2017). This has resulted in a decline of beech to 15% of the total forest area recently, whereas Norway spruce and Scots pine represent 25% and 22%, of Germany's forest area, respectively (BMEL 2020).

Study Sites

In total, 45 forest stands with natural regeneration of European beech with an average height of 50 cm and adult beech trees in the overstorey were selected along a gradient of atmospheric N deposition ranging from 10 to 32 kg N ha⁻¹ a⁻¹ (Figure 1). The independent climatic gradient ranged from 565 to 1690 mm of annual precipitation (1991–2020, Figure 2). To compare beech with more drought-tolerant species, 25 naturally regenerating stands of

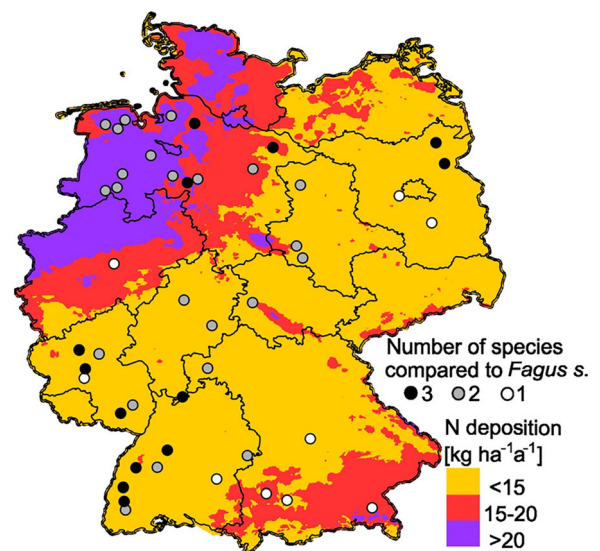


Figure 1. Studied sites ($N = 45$) for *Fagus sylvatica* with at least one of the comparison species across the atmospheric N deposition gradient in Germany (low: $< 15 \text{ kg ha}^{-1} \text{ a}^{-1}$, medium: $15\text{--}20 \text{ kg ha}^{-1} \text{ a}^{-1}$, high: $> 20 \text{ kg ha}^{-1} \text{ a}^{-1}$).

sessile oak, 35 of Douglas-fir, and 34 of silver fir were identified within a maximum distance of 3 km from the beech stands (Figure 2). For each species, 3 plots of 10 m radius were established at least 40 m apart in a forest stand. To avoid pseudo-replication, each cluster of forest stands (study site) containing at least 0.3 ha of beech and one of the comparison species had to be 10 km apart. The forest stands of all the species have been regularly managed according to their needs. Regeneration patches often occurred in areas of recent disturbance within the forest stand. The root/shoot ratio was determined for all harvested trees and sites. Due to the high workload of fine root analysis, absorptive and transport roots were analysed in subsets that covered the extremes of the climatic and N deposition gradients sampled (Figure 2). Therefore, data on absorptive roots were collected at 34 sites for European beech, 24 for sessile oak, 33 for Douglas-fir, and 32 for silver fir; transport roots were analysed at 13 sites for European beech, 15 for sessile oak, 12 for Douglas-fir, and 10 for silver fir (Figure 2).

Quantification of Biomass and Fine Root Morphology

For each species and site, 3 undamaged 5–15-year-old young trees no taller than 50 cm (70 cm for Douglas-fir) were harvested from each of the 3 plots, resulting in 9 harvested trees per site. A total

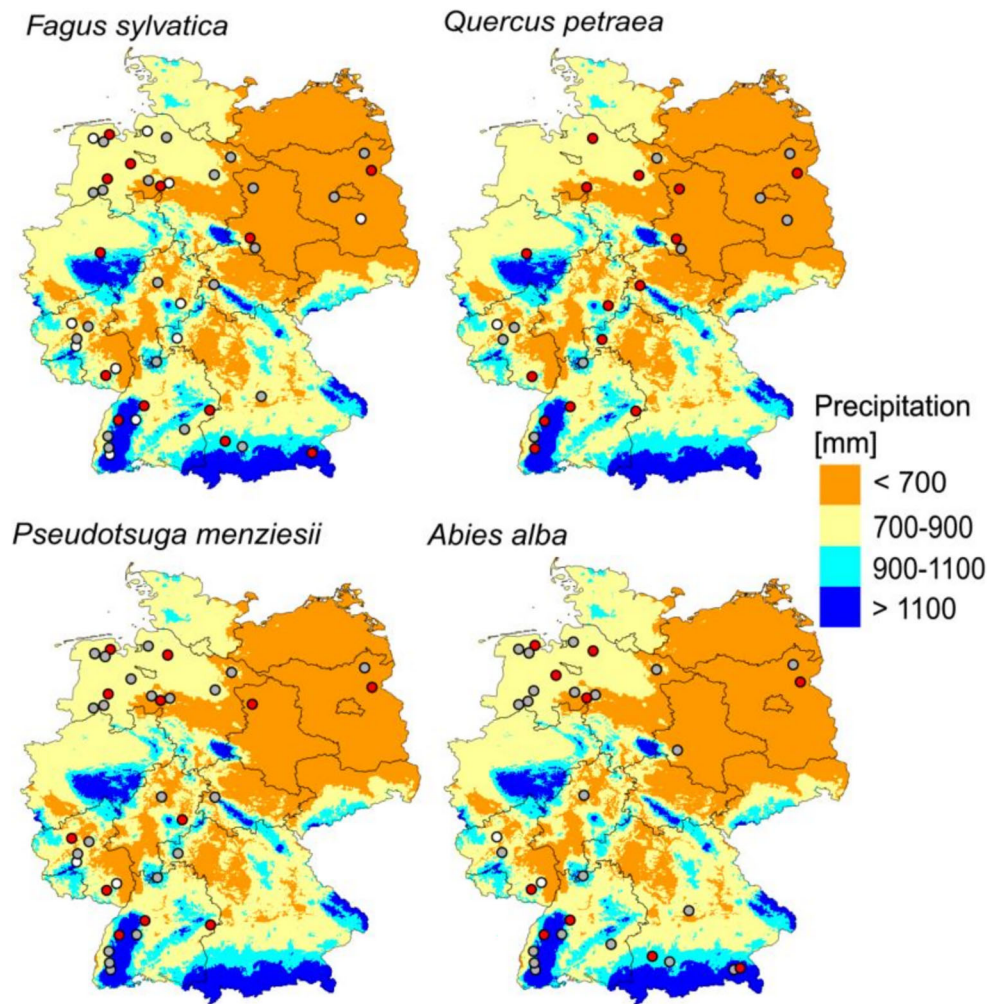


Figure 2. Study sites with tree regeneration of *Fagus sylvatica* ($N = 45$; present at all locations), *Quercus petraea* ($N = 25$), *Pseudotsuga menziesii* ($N = 35$), and *Abies alba* ($N = 34$) in Germany plotted against the annual precipitation gradient [mm] from 1991 to 2020 in Germany grouped in < 700 mm, 700–900 mm, 900–1100 mm and > 1100 mm precipitation per year, filled circles mark sites with fine root analysis (red: including transport roots, grey: only absorptive roots), biomass analysis was done at all sites (including sites marked with open circles).

of 1191 saplings was excavated. To characterize the forest stand, total regeneration density, estimates of canopy cover in per cent, and basal area and height of the adult trees were recorded for each plot. Mixed mineral soil samples were taken from a depth of 0–10 cm to analyse field capacity and, from dried (40°C), sieved and homogenized sub-samples, C and N content (TruSpec Micro, Leco, St. Joseph, Michigan, USA). A garden fork was used to lift a $30 \times 30 \times 30 \text{ cm}^3$ volume of soil around each tree. In case of an already developed tap root and roots that were more than 30 cm deep or wide, the lifted soil volume around the sampled tree was increased. The root system was roughly cleaned manually, before aboveground and belowground biomass was separated at the root collar. While the

aboveground biomass was dried at 70°C for 72 h and weighed, the root system was stored without any additional treatment either in the refrigerator at 4°C (immediate analysis, up to 3 months) or in the freezer at -20°C (delayed analysis, after 3–15 months). Root/shoot ratio is defined as the total belowground to aboveground biomass ratio, with the latter including shoots and foliage; root mass fraction was calculated as the ratio of belowground biomass weight to total seedling weight.

For analysis of fine root morphology, frozen root samples were thawed overnight in the refrigerator. Roots were carefully washed under rinse water to remove larger soil aggregates. Roots were kept underwater throughout the following steps and smaller soil particles were removed with tweezers.

From every tree, 3–5 subsamples of fine roots (≤ 2 mm) were cut off from the coarse roots. Following the functional classification approach (McCormack and others 2015), each sample was divided in absorptive (first three root orders) and transport (higher order) roots. The number of tips and ectomycorrhizal tips per sample was counted manually using a microscope, and a visual comparison between root tips and with existing reference images was made to assess ectomycorrhizal colonization (Wambsganss and others 2021). Roots were scanned with a flatbed scanner using a resolution of 800 dpi and root length and volume were calculated using the WinRhizo software (Regent Instruments, Quebec, Canada). Root volume was recalculated by summing up volumes of different fine root diameter classes following Rose (2017). Each diameter class included a 0.1 mm interval of the total diameter range of 0–2 mm. Both the scanned root samples and the rest of the root system were dried at 70 °C for 72 h and were weighed using a high precision scale (TE1502S, Sartorius, Göttingen, Germany).

All parameters were analysed at the tree level. Thus, fine root parameters were means of the 3–5 subsamples per tree. We determined (1) the ectomycorrhizal colonization rate as percentage fine root tips with visible ectomycorrhiza relative to the total number of tips, (2) specific root length (in cm mg^{-1}), (3) root tip frequency (including abrupt tips; in tips mg^{-1}), and (4) root tissue density (in g cm^{-3}) related to the fine root volume per diameter class. Specific root length and root tissue densities were calculated separately for absorptive and transport fine roots; root tip frequency was related to absorptive root biomass.

Atmospheric N Deposition and Climatic Data

Modelled atmospheric N deposition data (means for 2013 to 2015) describing the deposition from natural and anthropogenic sources were taken from the Pollutant Impact and Ecosystem Impact (PINETI-3) data base of the German Environmental Agency (Schaap and others 2017). Depending on the tree species, we used either the model outputs for the broadleaved forest or coniferous forest. Mean annual precipitation and temperature data from 1991 to 2020 were obtained from the Climate Data Centre of the German Meteorological Service (Deutscher Wetterdienst, Offenbach, Germany). These data sets were provided at a resolution of 1 km $\times$ 1 km. For data analysis, study sites were assigned to three atmospheric N deposition classes

defined as “low” ($< 15 \text{ kg N ha}^{-1} \text{ a}^{-1}$), “medium” ($15\text{--}20 \text{ kg N ha}^{-1} \text{ a}^{-1}$), and “high” ($> 20 \text{ kg N ha}^{-1} \text{ a}^{-1}$) deposition (Figure 1). Furthermore, we distinguished four classes of mean annual precipitation: (1) < 700 mm, (2) 700–900 mm, (3) 900–1100 mm, (4) > 1100 mm (Figure 2).

Statistical Analysis

Throughout the paper, arithmetic means $\pm$ standard errors (SE) are presented and all analysis were carried out with R 4.3.1 software. Normality of the data was tested parametrically using the Shapiro–Wilk test and visually using quantile–quantile (QQ) plots. Non-normally distributed data and count/percentage data of ectomycorrhizal tip frequency were compared using the Wilcoxon rank sum test.

Linear mixed effects modelling using the R package ‘lme4’ 1.1–33 was used to explore relationships between parameters describing root/shoot ratio, root mass fraction, absorptive fine root morphology and N deposition, precipitation, and temperature as continuous variables. For all sites, the selected fixed variables used as means per site have shown no collinearity, as verified by the Spearman rank sum coefficient ($\rho < 0.7$). To account for confounding effects, canopy cover (light availability), field capacity, tree age, and height were included in the models describing the root/shoot ratio and root mass fraction. Because soil pH decreased and organic layer depth increased with increasing N deposition at our study sites, but soil N content was not affected by N deposition (Dietrich and others 2024), only soil N content was included as a confounding effect in all models. Site identity was included as a random effect to account for the variance explained by grouping the data. The variance inflation factor of each final model selection should be a conservative choice of 5 or less (Dormann and others 2013). Interaction effects of N deposition with precipitation, temperature, tree age or size that introduced variance inflation much greater than 5 were therefore excluded from the final model. As the laboratory methods used to determine absorptive fine root parameters are highly prone to measurement errors, these high or low outlier measurements were excluded from modelling when they were rare and randomly distributed across the data set. For each model, the normality of residuals was checked for compliance with model assumptions using the R package DHARMA 0.4.6, and if residuals were not normally distributed, response variables were transformed to the natural logarithm (Dormann, 2017). The

goodness of fit was calculated with the R package MuMin 1.40.4.

RESULTS

Relationship Between Belowground to Aboveground Biomass

In linear mixed-effects modelling, the root/shoot ratio was strongly impacted by site location (included as a random effect) for each species (Table 1). In addition to random site-specific effects, the root/shoot ratio decreased with increasing N deposition in European beech, but was unaffected by N deposition in the other tree species (Table 1, Figure S1). For sessile oak, the only significant effect on root/shoot ratio was an increase caused by increasing soil N content (Table 1, Figure S1). In Douglas-fir, root/shoot ratios increased with increasing mean annual precipitation, and this effect was marginally significantly enhanced by increasing N deposition (Table 1, Figure S1), which is related to the increased aboveground biomass with increasing N deposition (Figure S3). No species showed significant variation of belowground biomass with N deposition (Figure S4). Except for Douglas-fir, no interaction effect of N deposition with mean annual precipitation on the root/shoot ratio was found for the other species (Table 1), which was also true for all species when above-

ground and belowground biomass was considered separately (Figure S5 & S6). The root/shoot ratio decreased with increasing tree age and height, except for sessile oak where age had no effect (shown as an example for silver fir and seedling height in Figure S1). Temperature, canopy cover, field capacity, as well as N deposition in interaction with either tree age or height (results not shown), did not significantly affect the root/shoot ratio in any species.

Selected variables for linear mixed effects modelling described the root mass fraction less well in terms of model quality, significant effects and explained variance than the root/shoot ratio (compare Table 1 and S3). The root mass fraction increased significantly with mean annual precipitation in silver fir, and the interaction effect of increasing mean annual precipitation, enhanced by increasing N deposition, was significant in these models for Douglas-fir (Table S3), while other effects observed for the root/shoot ratio were absent.

Root/shoot ratios were generally higher for the broadleaved species than for the studied conifers (Figure S2), and were higher for sessile oak than for European beech and for silver fir than for Douglas-fir. This pattern corresponded to higher aboveground biomass in Douglas-fir than in silver fir and to slightly but also significantly higher aboveground biomass in sessile oak than in European beech (Figure S3). Belowground, European beech

Table 1. Linear Mixed Effect Models for Root/Shoot Ratio

	<i>Fagus sylvatica</i>	<i>Quercus petraea</i>	<i>Pseudotsuga menziesii</i>	<i>Abies alba</i>
Intercept	1.009 ± 0.005 ***	1.13 ± 0.02 ***	0.766 ± 0.005 ***	0.838 ± 0.007 ***
N deposition ^c	− 0.014 ± 0.006 *	− 0.006 ± 0.019	0.006 ± 0.005	0.0004 ± 0.0082
Precipitation ^d	0.003 ± 0.009	− 0.05 ± 0.03 (*)	0.025 ± 0.009 *	0.02 ± 0.01 (*)
Temperature ^d	0.007 ± 0.007	0.03 ± 0.02	0.005 ± 0.006	− 0.004 ± 0.009
Precipitation × N deposition	− 0.02 ± 0.01	− 0.06 ± 0.04	0.02 ± 0.01 (*)	0.0003 ± 0.0122
Canopy cover	0.005 ± 0.006	− 0.02 ± 0.02	− 0.002 ± 0.005	− 0.009 ± 0.007
Field capacity	− 0.0002 ± 0.0057	0.02 ± 0.02	0.0006 ± 0.0046	0.0002 ± 0.0071
Soil N content	− 0.002 ± 0.007	0.05 ± 0.02 *	0.005 ± 0.006	− 0.003 ± 0.008
Tree age	− 0.008 ± 0.004 *	− 0.009 ± 0.007	− 0.008 ± 0.003 *	− 0.017 ± 0.004 ***
Tree height	− 0.039 ± 0.003 ***	− 0.059 ± 0.008 ***	− 0.032 ± 0.003 ***	− 0.035 ± 0.004 ***
Conditional R ²	47%	62%	42%	49%
Marginal R ²	33%	35%	34%	36%
Random effects σ ²				
Site	0.0009	0.005	0.0003	0.0008
Residual	0.0031	0.007	0.0022	0.0032

Model coefficients (± SE)^a of linear models analysing the effects of N deposition, precipitation, their interaction, and temperature, canopy cover, field capacity, soil N content, and tree age and height^b on root/shoot ratio in *Fagus sylvatica* (N = 393 trees), *Quercus petraea* (N = 190), *Pseudotsuga menziesii* (N = 309), *Abies alba* (N = 295)

^aLevels of significance: (*) P ≤ 0.10, * P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

^bThe interaction between tree age or size with N deposition did not significantly improve model quality

^cModeled N deposition for broadleaved forests was used for *Fagus sylvatica* and *Quercus petraea*, N deposition for coniferous forests was used for *Pseudotsuga menziesii* and *Abies alba*

^dMean annual precipitation and mean annual temperature for 1991–2020.

produced less biomass than the three other species (Figure S4).

Mycorrhizal Colonization Rate in Variation of Climate, N Supply, and Tree Species

European beech was the only species with reduced mycorrhizal colonization on dry sites with high N deposition (Figure 3). This pattern was absent in the other species.

Mycorrhizal colonization rates differed between species (Figure 4) and decreased in the order sessile oak > European beech, silver fir > Douglas-fir. There were no trends for a steady increase or decrease of mycorrhizal colonization with increasing N deposition in any species (Figure 4). Mycorrhizal colonization was reduced at high precipitation in European beech and sessile oak, but increased in Douglas-fir (Figure 4).

Species-Specific Differences in Fine Root Morphology

The two conifer species had significantly lower specific root lengths, root tissue densities, and root tip frequencies than the two broadleaved species (Figure 5). European beech had higher specific root lengths, and lower root tissue densities in absorptive fine roots than sessile oak, but in contrast, the specific root length in transport roots was higher in sessile oak than in European beech (Figure 5a, b). Between Douglas-fir and silver fir, the specific root length of both transport and absorptive fine roots was comparable (Figure 5a, b). Root tissue density in transport fine roots was higher in sessile oak than in European beech and did not differ between Douglas-fir and silver fir (Figure 5b).

Morphology of Absorptive Fine Roots Changing with Climate and N Supply

Linear mixed effect modelling based on specific root length, root tip frequency and root tissue density of absorptive roots per species showed a strong impact of site location (included as random effect) on these parameters (Table 2), along with significant effects of atmospheric N deposition, mean annual precipitation and mean annual temperature that varied among species.

In European beech, specific root length decreased with decreasing precipitation, but only at sites with high N deposition (Table 2, Figure 6). A similar, but only marginally significant ($P \leq 0.10$) effect was found for root tip frequency (Table 2, Figure 6). No effect of mean annual precipitation

(alone or in interaction) on fine root morphology was observed for the other species (Table 2). Root tip frequency in European beech also decreased with increasing mean annual temperature (Table 2). Douglas-fir showed increases in specific root length with increasing N deposition, and decreases in root tissue density with increasing N deposition and soil N content (Table 2). The morphology of absorptive fine roots in sessile oak and silver fir were not influenced by either N deposition or precipitation. Rather, mean annual temperature had an effect, as sessile oak trees at warmer sites had higher mean specific root length (Table 2).

Morphology of Transport Fine Roots Changing with Climate and N Supply

In European beech and sessile oak, specific root length and root tissue density in transport roots were not influenced by mean annual precipitation or N deposition (Figure 7). In contrast, both conifer species showed a trend towards increasing specific root length with decreasing mean annual precipitation, and Douglas-fir also showed a trend towards decreasing root tissue density (Figure 7a). In parallel to the behaviour of absorptive roots (Table 2), specific root length increased with increasing N deposition and root tissue density decreased with increasing N deposition in Douglas-fir (Figure 7b). Silver fir showed a similar response for root tissue density, but not for specific root length (Figure 7b).

DISCUSSION

Response of the Broadleaved Species, European Beech and Sessile Oak, to Low Precipitation and High N Deposition

European beech showed clear negative responses of the root system to high atmospheric N deposition ($P \leq 0.05$; Tables 1 and 2; Figures 3 and 6), which is in agreement with our first hypothesis. Reduced root/shoot ratios with increasing N deposition follow the optimal partitioning theory (Ågren & Franklin 2003) and suggest less biomass investment below ground due to increased N availability, as the theory describes a biomass investment only when resources are scarce. Lower specific absorptive root length, lower fine-root mycorrhizal colonization rates, and the marginally significant trend for lower root tip frequency with increasing N deposition are also in line with the optimal partitioning theory, as both investment in thin, long absorptive roots with many root tips and in the support of mycorrhizal fungi serve to explore a greater soil volume and

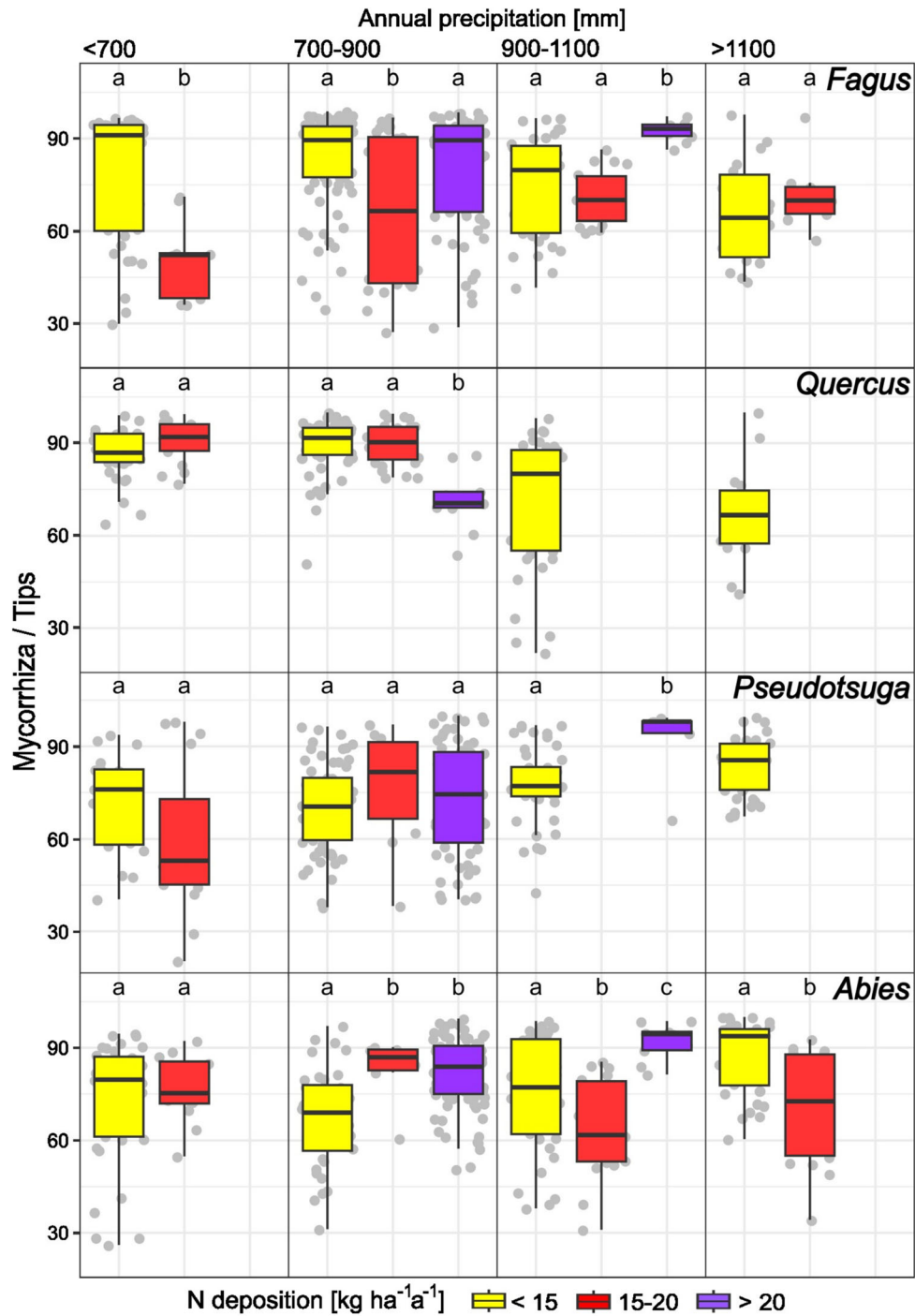


Figure 3. Comparison of ectomycorrhized tip frequency per species and level of mean annual precipitation between sites differing in N deposition. Number of samples: *Fagus sylvatica*, 304 trees; *Quercus petraea*, 190; *Pseudotsuga menziesii*, 260; *Abies alba*, 288. In each subgraph, different letters indicate significant differences, $P \leq 0.05$, Wilcoxon rank-sum test.

thus to foster nutrient uptake (George and Marschner 1996; Kramer-Walter and others 2016; Wang and others 2018). Increased N availability from deposition was most likely due to an increase in the depth of the organic layer, which increased

at the study sites, rather than N accumulation in the mineral soil, which was unaffected at the study sites (Dietrich and others 2024). However, these changes in specific absorptive root length, mycorrhizal colonization, and root tip frequency under

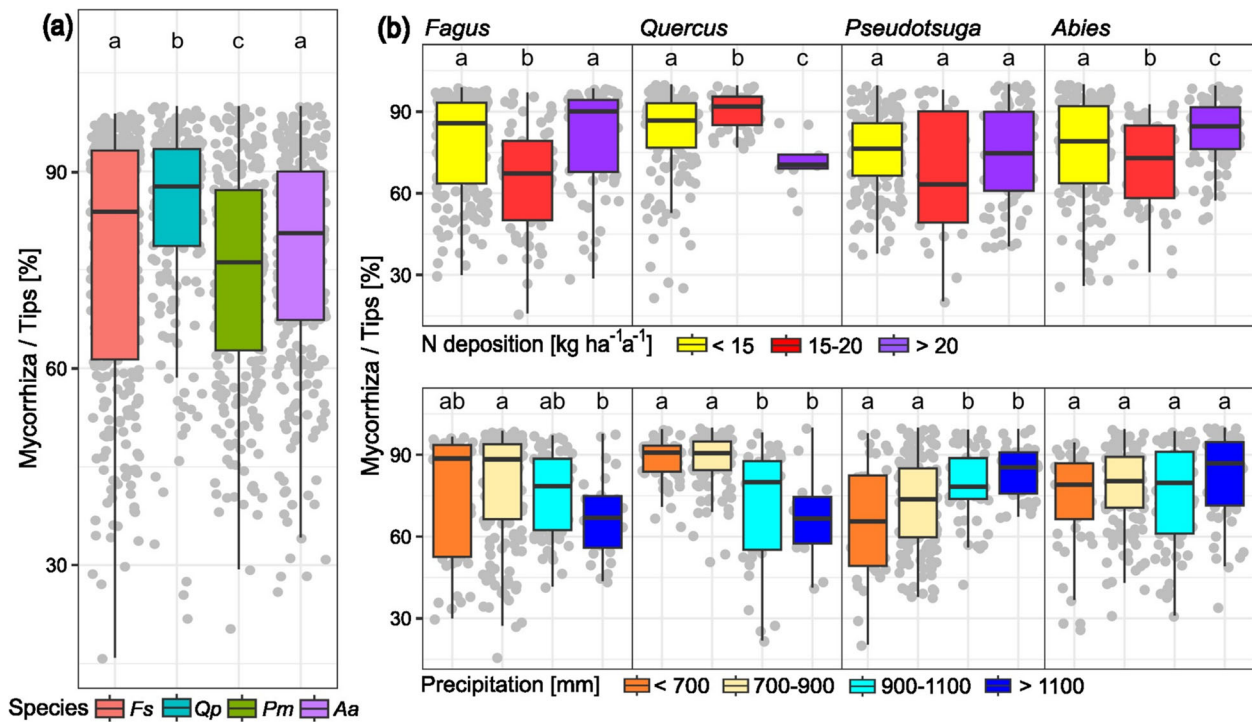


Figure 4. Comparison of ectomycorrhized tip frequency **a** between species and **b** between different levels of N deposition and mean annual precipitation. Number of samples: *Fagus sylvatica* (Fs), 304 trees; *Quercus petraea* (Qp), 190; *Pseudotsuga menziesii* (Pm), 260; *Abies alba* (Aa), 288. In each subgraph per precipitation class or species, different letters indicate significant differences, $P \leq 0.05$, Wilcoxon rank-sum test.

high N deposition in European beech only occurred at sites with low mean annual precipitation, which supports our second hypothesis for fine root anatomical traits. This suggests that European beech reduced carbon allocation to the root system for producing thin, long absorptive roots and for transferring carbohydrates to symbiotic fungi under these conditions because of low carbon assimilation under dry conditions (Pflug and others 2018). An effect of mean annual precipitation alone (without interaction with N deposition) on the root/shoot ratio or root-morphological traits was not found for European beech or any other species, which is not consistent with Dzionek and others (2016a, b), who reported reduced root/shoot ratios from European beech due to drought. Increased fine root mortality due to drought found by Leuschner and others (2001) and Mainiero and Kazda (2006) also shows that the root system of European beech is generally affected by water supply, but the N deposition gradient was apparently more influential than the precipitation gradient in our study. In agreement with our findings, drought alone has not been found in other studies to be sufficient to reduce the mycorrhizal colonization rate in European beech regeneration (Shi and others 2002) or in mature

European beech along a precipitation gradient (Hertel and others 2013). We found even reduced mycorrhizal colonization in European beech at sites with high mean annual precipitation > 1100 mm, which is consistent with reduced investment into the symbiosis by the tree under ample water supply following the optimal partitioning theory.

Our results also match with reports of reduced root/shoot ratios in European beech due high N availability (Dzionek and others 2017), decreasing specific root length with decreasing mean annual precipitation (Meier and Leuschner 2008), and decreased fine root biomass due to high N deposition (Finér and others 2007). The reduced root/shoot biomass at high N deposition together with reduced specific absorptive root length, reduced mycorrhizal colonization and (as marginally significant trend) reduced root tip frequency under combined high N deposition and drought suggest that the known vulnerability of European beech to climate change-induced summer droughts (Dulamsuren and others 2017; Schuldt and others 2020) is aggravated due to high N deposition. Observations of reduced shoot length increment with increasing N deposition (Dietrich and others 2024) in the European beech regeneration on our

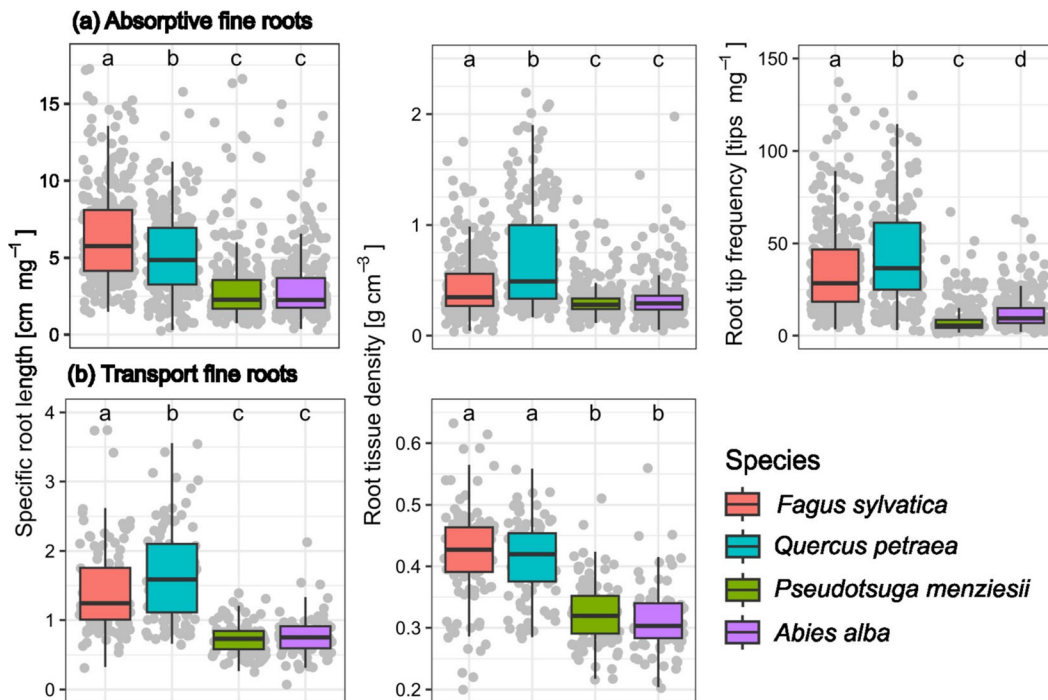


Figure 5. Morphological traits in **a** absorptive fine roots and **b** transport fine roots including specific root length, root tissue density, and root tip frequency. Number of samples (absorptive/transport fine roots): *Fagus sylvatica*, $N = 304/112$ trees; *Quercus petraea*, $N = 190/98$; *Pseudotsuga menziesii*, $N = 260/102$; *Abies alba*, $N = 288/80$. Different letters indicate significant differences, $P \leq 0.05$, Wilcoxon rank-sum test.

sample sites in addition to the reduced root/shoot ratios underline that these concerns are justified. In Germany, the main source of high N deposition is from intense agriculture and from industrial livestock farming in particular (Salomon and others 2016).

Sessile oak was generally less influenced in biomass allocation and fine root morphology by mean annual precipitation and N deposition than European beech, supporting our third hypothesis for this species. The root/shoot ratio was generally higher in sessile oak than in European beech (or any other study species), which indicates that sessile oak relies more on safety than on plasticity for optimal resource partitioning when it comes to water and nutrient uptake. Furthermore, the increasing root/shoot ratio with increasing soil N content rather indicates a N limitation according to the optimal partitioning theory, independent of the amount of N deposition (Dietrich and others 2024). The high root/shoot ratio in sessile oak was achieved by low aboveground biomass in combination with high root biomass, which was as high as in the studied conifers and higher than in European beech. The generally well-developed deep root system of *Quercus petraea* and other oak species (Drexhage and others 1999; Aranda and

others 2000) supports our conclusion. Nevertheless, even in *Q. petraea* (and *Q. robur*) saplings, experimental drought can induce increases in the root/shoot ratios (Thomas 2000; Thomas and Gausling 2000; Gieger and Thomas 2002, 2005), but the threshold for such shift in biomass partitioning was obviously not crossed in the climatic gradient of our study, even though it covered most of Germany. The positive effect of mean annual temperature on specific root length in sessile oak is consistent with observations of Teskey and Hinkelley (1981) that root elongation in the North American *Q. alba* was promoted by increasing soil temperatures. Usually mean annual air temperature has no significant effect on root growth or root morphology (Ostonen and others 2007; Kuster and others 2013).

Douglas-Fir and Silver Fir in Comparison to European Beech and Sessile Oak

In contrast to European beech and sessile oak, Douglas-fir produced thinner and longer fine roots with increasing N deposition, as indicated by higher specific root length and lower root tissue density, and silver fir tended to produce more root tips with increasing N deposition. This means that the roots

Table 2. Linear Mixed Effect Models Describing Fine Root Morphology of Absorptive Fine Roots

	<i>Fagus sylvatica</i>	<i>Quercus petraea</i>	<i>Pseudotsuga menziesii</i>	<i>Abies alba</i>
<i>Root tip frequency</i>				
Intercept	3.41 ± 0.07 ***	3.57 ± 0.09 ***	1.88 ± 0.06 ***	2.33 ± 0.04 ***
N deposition ^b	0.05 ± 0.08	0.06 ± 0.09	− 0.04 ± 0.07	0.09 ± 0.05 (*)
Precipitation ^c	0.15 ± 0.09	0.04 ± 0.16	0.11 ± 0.08	0.03 ± 0.05
Temperature ^c	− 0.17 ± 0.08 *	0.02 ± 0.08	− 0.04 ± 0.08	− 0.009 ± 0.053
Precipitation × N deposition	0.24 ± 0.12 (*)	− 0.02 ± 0.21	-	-
Soil N content	− 0.14 ± 0.08 (*)	− 0.16 ± 0.09 (*)	− 0.09 ± 0.08	− 0.05 ± 0.05
Conditional R ²	40%	18%	19%	5%
Marginal R ²	17%	4%	4%	3%
<i>Random effects σ²</i>				
Site	0.12	0.08	0.07	0.009
Residual	0.29	0.45	0.36	0.35
<i>Specific root length</i>				
Intercept	1.77 ± 0.04 ***	1.48 ± 0.05 ***	0.74 ± 0.03 ***	0.85 ± 0.04 ***
N deposition ^b	0.11 ± 0.05 *	− 0.002 ± 0.060	0.12 ± 0.03 **	0.07 ± 0.04
Precipitation ^c	− 0.04 ± 0.06	0.02 ± 0.07	− 0.06 ± 0.03	− 0.01 ± 0.05
Temperature ^c	− 0.05 ± 0.05	0.12 ± 0.06 *	0.04 ± 0.04	0.03 ± 0.04
Precipitation × N deposition	0.16 ± 0.07 *	-	-	0.002 ± 0.063
Soil N content	0.06 ± 0.05	− 0.07 ± 0.07	0.03 ± 0.03	− 0.06 ± 0.04
Conditional R ²	22%	9%	20%	11%
Marginal R ²	10%	4%	17%	8%
<i>Random effects σ²</i>				
Site	0.03	0.02	0.005	0.006
Residual	0.20	0.42	0.12	0.20
<i>Root tissue density</i>				
Intercept	− 0.97 ± 0.05 ***	− 0.47 ± 0.07 ***	− 1.28 ± 0.02 ***	− 1.26 ± 0.04 ***
N deposition ^b	0.001 ± 0.05	0.02 ± 0.06	− 0.08 ± 0.03 **	− 0.04 ± 0.04
Precipitation ^c	− 0.02 ± 0.06	− 0.03 ± 0.11	0.05 ± 0.03	− 0.02 ± 0.06
Temperature ^c	0.01 ± 0.05	− 0.07 ± 0.06	0.02 ± 0.03	0.03 ± 0.04
Precipitation × N deposition	0.007 ± 0.086	0.21 ± 0.15	-	− 0.04 ± 0.06
Soil N content	0.03 ± 0.05	0.08 ± 0.07	− 0.07 ± 0.03 *	0.04 ± 0.04
Conditional R ²	12%	9%	6%	11%
Marginal R ²	0.3%	6%	6%	2%
<i>Random effects σ²</i>				
Site	0.04	0.01	0	0.01
Residual	0.30	0.46	0.11	0.15

Model coefficients (± SE)^a of linear models analysing effects of N deposition, precipitation, their interaction and temperature on root tip frequency, specific root length and root tissue density of absorptive roots in *Fagus sylvatica* (N = 304 trees), *Quercus petraea* (N = 190), *Pseudotsuga menziesii* (N = 260), *Abies alba* (N = 288)

^aLevels of significance: (*) $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

^bModeled N deposition for broadleaved forests was used for *Fagus sylvatica* and *Quercus petraea*, N deposition for coniferous forests was used for *Pseudotsuga menziesii* and *Abies alba*

^cMean annual precipitation and mean annual temperature for 1991–2020.

of the two conifers were increasingly optimized for high uptake and transport rates for water and nutrients with increasing N deposition (Huang and Eissenstat 2000; Weemstra and others 2020), which also supports our third hypothesis for the conifer species in comparison to European beech. Increased mycorrhizal colonization with increasing mean annual precipitation additionally promoted water and nutrient acquisition in Douglas-fir. Furthermore, decreasing root/shoot ratios, but increasing specific transport root length, with

decreasing precipitation suggest that soil exploration of Douglas-fir and silver fir depends on fine root plasticity rather than increased belowground biomass investment under drier conditions. However, the increased specific root length combined with lower root tissue density of Douglas-fir roots puts them at risk for increased mortality during drought (Fitter 1987; Konôpka and others 2007), as they might be less suberized, increasing their vulnerability to stress compared to more lignified and denser fine roots (Kozłowski and Pallardy 1996).

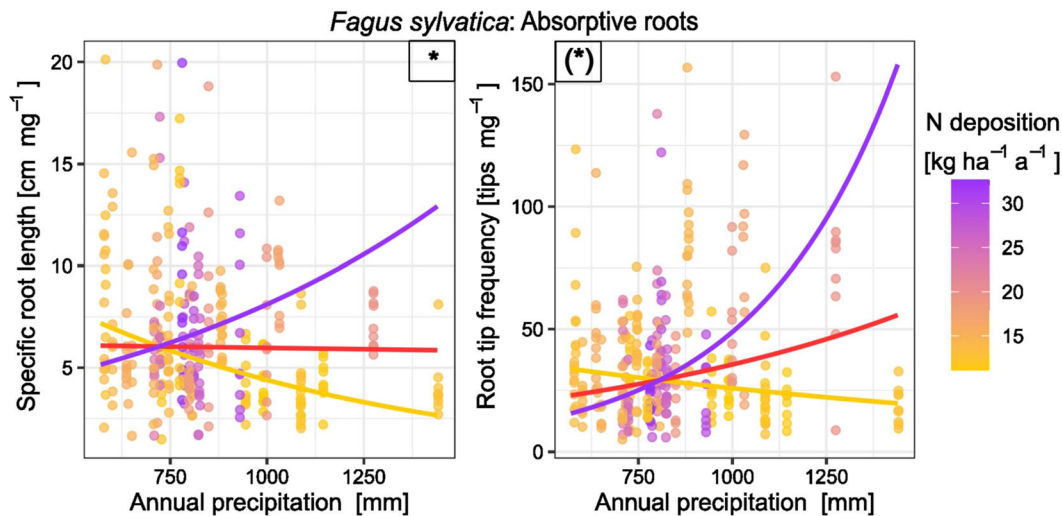


Figure 6. Specific root length and root tip frequency for absorptive fine roots of *Fagus sylvatica* in dependence of mean annual precipitation and N deposition in interaction according to model results in Table 2; presented interaction effect was not significant in the other species (*: $P \leq 0.05$, (*): $P \leq 0.10$; $N = 304$ trees, of which 171 trees at $< 15 \text{ kg N ha}^{-1} \text{ a}^{-1}$), 62 trees at $15\text{--}20 \text{ kg N ha}^{-1} \text{ a}^{-1}$, and 71 trees at $> 20 \text{ kg N ha}^{-1} \text{ a}^{-1}$).

While the root/shoot biomass ratio and the root mass fraction were reduced by mean annual precipitation under high N deposition (Table 1, S3), morphological plasticity in Douglas-fir thus promoted water and nutrient transport at high N deposition levels, explaining the increased above-ground biomass allocation with increased N deposition also observed in this study. Reductions in specific root length in fertilization trials with Douglas-fir, however, showed that also this promotional effect is limited and dose-dependant (Olsthoorn and others 1991; Jacobs and others 2004).

The generally lower root/shoot biomass ratios and specific root lengths in Douglas-fir and silver fir than in European beech and sessile oak reflect the evolutionary trend towards greater quantities (Qi and others 2019; Wang and others 2023) and thinner fine roots in seed plants (Bergmann and others 2020). This trend, which is often found in gymnosperm/angiosperm comparisons (Bauhus and Messier 1999; Wang and others 2019), made plants less dependent on the mycorrhizal symbiosis for water and nutrient uptake in the course of evolution (Bergmann and others 2020; Comas and others 2012), which is, however, not reflected by the mycorrhizal colonization rates in European beech, sessile oak, Douglas-fir, and silver fir in our study.

CONCLUSIONS

Our results show that high N deposition reduces the root surface area for water uptake of European beech by decreasing the root/shoot ratio and (if high deposition occurs combined with low mean annual precipitation) also by reducing specific absorptive root length and mycorrhizal colonization. Effects of high N deposition on the fine root system of sessile oak were not found. Higher specific root length and lower root tissue density in Douglas-fir and a trend towards higher root tip abundance in silver fir at sites with high N deposition even proved a promotional effect on the trees' capacity to take up water and nutrients.

The results show that N deposition is highly relevant to the ability of European beech and other temperate tree species in Central Europe to cope with dry site conditions and presumably also drought spells. So far, interaction of climate change-induced drought with N deposition is rarely discussed in the context of climate change adaptation of forest management in Europe despite the existing large regional variability in N availability due anthropogenic emissions. Although our results are derived from independent N deposition and precipitation gradients and do not include replicate studies of wet and dry periods, they suggest that high N deposition is likely to have a negative impact on the ability of European beech to cope with drought, which is alarming given that European beech is the dominant plant species in the natural vegetation of Central Europe. This should not only

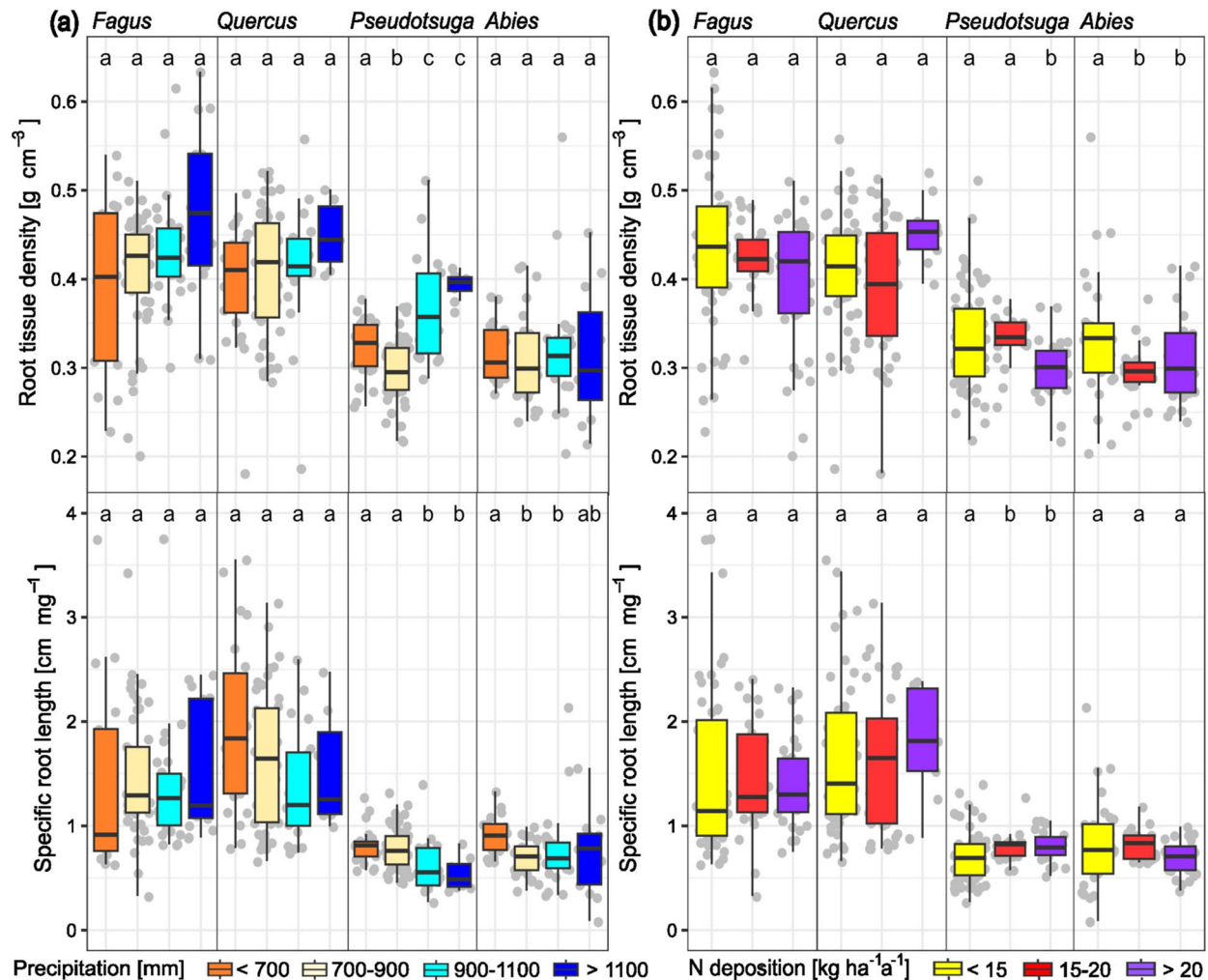


Figure 7. Root tissue density and specific root length of transport fine roots at different levels of **a** mean annual precipitation and **b** N deposition in *Fagus sylvatica* ($N = 112$ trees), *Quercus petraea* ($N = 98$), *Pseudotsuga menziesii* ($N = 107$), *Abies alba* ($N = 80$). Within each species, different letters indicate significant differences, $P \leq 0.05$, Wilcoxon rank-sum test.

be taken as a motivation to give greater consideration to N deposition in silvicultural planning in addition to climate, but also to effectively reduce anthropogenic N emissions, as European beech forests are of outstanding importance for biodiversity conservation in Central Europe.

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DATA AVAILABILITY

Data are available at: [https://urldefense.com/v3/_https://zenodo.org/records/14586609_!!Mak6IKo!LB1bmMM1c-W9wSQdD0sjsT3XUCnvqaT-wtH3NKMRcmclOO9xB9ZtB7PWhtvYmtZ0Gj2NAUqFEIZt_hZcRSdAFwOGsxhtVTozKRsf1bgZPPFGA QAL\\$](https://urldefense.com/v3/_https://zenodo.org/records/14586609_!!Mak6IKo!LB1bmMM1c-W9wSQdD0sjsT3XUCnvqaT-wtH3NKMRcmclOO9xB9ZtB7PWhtvYmtZ0Gj2NAUqFEIZt_hZcRSdAFwOGsxhtVTozKRsf1bgZPPFGA QAL$).

Declarations

Conflict of interest The authors declare no conflict of interest.

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