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2 **Effects of drought on nitrogen uptake and carbon dynamics** 3 **in trees**

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18 **Summary**

19 Research on drought impact on tree functioning is focused primarily on water and
20 carbon (C) dynamics. Changes in nutrient uptake might also affect tree performance
21 under drought and there is a need to explore underlying mechanisms.

22 We investigated effects of drought on a) *in-situ* nitrogen (N)-uptake accounting for
23 both, N availability to fine-roots in soil and actual N-uptake, b) physiological N-uptake
24 capacity of roots, and c) the availability of new assimilates to fine roots influencing the
25 N-uptake capacity using ¹⁵N and ¹³C labelling. We assessed saplings of six different tree
26 species (*Acer pseudoplatanus*, *Fagus sylvatica*, *Quercus petraea*, *Abies alba*, *Picea*
27 *abies*, *Pinus sylvestris*).

Drought resulted in significant reduction of *in-situ* soil N-uptake in deciduous trees accompanied by reduced carbon allocation to roots and by a reduction in root biomass available for N-uptake. While physiological root N-uptake capacity was not affected by drought in deciduous saplings, reduced maximum ammonium but not nitrate uptake was observed for *A.alba* and *P.abies*.

Our results indicate that drought has species-specific effects on N-uptake. Even water limitations of only 5 weeks as assessed here can decrease whole plant inorganic N-uptake independent of whether the physiological N-uptake capacity is affected or not.

Key words

Carbon; Climate change; Coniferous; Deciduous; Drought; Forest ecosystem; Nitrogen; Nutrient allocation; Plant-soil continuum; Stable isotopes

Introduction

Forest ecosystems are known to be at present strong terrestrial carbon (C) sinks (Lal, 2005). They thus can play a significant role in mitigating the effects of global warming by absorbing atmospheric CO₂ and retaining it in the plant-soil continuum for long. Climate change has, however, the potential to turn forests during drought periods from C sinks into sources on large spatial scales (Ciais et al., 2005; Graf Pannatier et al., 2012; Sun et al., 2006; Unger et al., 2010). On the long-term, drought-induced forest mortality that is reported to increase worldwide (Allen et al., 2010) might continuously decrease C-sequestration in forests (Anderegg et al., 2016; Breshears and Allen, 2002). The major mechanisms discussed to be involved in drought-induced loss of tree functioning and finally mortality are hydraulic failure (mainly due to a disruption of water transport by xylem cavitation) and C starvation (Mcdowell et al., 2008; McDowell, 2011; Sevanto *et al.*, 2014) together with damages by biotic attacks (Manion, 1981).

In addition to the adverse impacts on C assimilation and tree hydraulics, drought might lead also to reduced nutrient uptake resulting in nutrient imbalances in trees and ecosystems (Kreuzwieser and Gessler, 2010). Especially impaired nitrogen (N) uptake is assumed to

deteriorate general metabolic function and cell structural integrity and to cause also negative feedbacks on the C balance (Gessler et al., 2017). Under drought, the depletion of free amino acids in tree leaves resulted in degradation of Rubisco in beech and oak saplings (Fotelli et al., 2002; Hu et al., 2013), indicating that amino acids needed for basic cellular functions were synthesized at the expense of photosynthetic potential. At the same time, reduced N supply increases the sensitivity of stomata to leaf water potential leading to stomatal closure and thus reduces C assimilation (Ghashghaie and Saugier, 1989). As a consequence, N uptake and its reduction by reduced water availability is also an important parameter affecting tree performance under drought.

While impaired N uptake by trees during drought events lasting from weeks to a growing season might be a result of lower ion mobility, reduced microbial nutrient delivery (i.e. soil related processes), plant physiological processes might be also directly involved (Fotelli et al., 2002). Moreover, it has been shown for adult beech and spruce that transpiration driven transport of nutrients in the xylem is related to nutrient net uptake with lower transpiration rates resulting in lower uptake (Gessler *et al.*, 2002). In addition, reduced carbon supply to the roots as a result of drought induced photosynthetic or transport restriction (Sala et al., 2010) leads to a lower availability of recent assimilates and thus energy for active N uptake and assimilation as well as to restricted root growth and delivery of carbon to rhizosphere microorganisms (Gessler et al., 2017). The impairment of carbon allocation to the roots might, however, depend on drought intensity: Moderate drought has been shown to increase root growth and belowground C allocation in beech and oak (Leuschner et al., 2001, adult beech and oak; Hommel et al. 2016, beech saplings), whereas intensive and longer-lasting drought events reduce the availability of recent assimilates to the roots (Hagedorn et al., 2016, beech saplings).

Under field conditions, a long-term reduction of soil water availability over years together with increased air temperature had a strong impact on N uptake kinetics leading to lower maximum uptake rates in adult beech (Gessler *et al.*, 2005). Under the same soil water reduction and high light availability, whole plant uptake of inorganic N was also reduced in beech saplings (Fotelli et al. 2004). For both, adult trees and saplings, it is, however, not clear if physiological impairment of root functioning or reduced overall growth rates that might have reduced nutrient demand lead to reduced uptake rates.

Amongst soil mineral nutrients, N is an abundantly required macro-nutrient for tree growth and maintenance and is also most likely the limiting nutrient for tree growth under future high tropospheric CO₂ concentrations in natural ecosystems not strongly affected by anthropogenic N deposition (Lukac et al., 2010; Reich et al., 2006). N plays a central role in photosynthesis, is as key component of proteins involved in all metabolic processes in plant cells and also in regulating stomatal conductance (Sun et al., 2016). In the soil, N is available to tree roots as inorganic nitrate and ammonium and also in its organic form as amino acids. Even though direct absorption of organic N by the trees roots has been reported especially in temperate and boreal forests, inorganic sources of N constitute the major part of the N taken up by temperate tree species (Liu *et al.* 2017; Dion et al., 2018). Trees are able to store significant amounts of N (Millard and Grelet, 2010), which allows them to temporally uncouple N demand and external N supply. This is mainly important in spring, when growth of foliage and roots cannot be met by recently assimilated N (Millard & Proe, 1992; Gessler *et al.*, 1998). In later spring and summer, however, N demand is mainly met by root uptake from the soil (Millard and Proe, 1992) and thus any reduction of N uptake during drought periods might negatively affect tree functioning thus potentially impair the resistance and resilience capacity of forest ecosystems (Gessler et al., 2017).

With the present work, we aimed at exploring the effects of moderate and severe drought on the *in situ* uptake of inorganic N by different deciduous (*Acer pseudoplatanus*, *Fagus sylvatica*, *Quercus petraea*) and coniferous (*Picea abies*, *Abies alba*, and *Pinus sylvestris*) tree species taking into account the combined effect N availability to the fine roots in the soil and actual N uptake. We have chosen the two lifeform types (deciduous, coniferous) because of their differences in N storage and transport (Gessler et al. 1998). Within life-forms, drought susceptibility is assumed to differ among species with *Q. petraea* being the least sensitive among the deciduous (Scherer et al. 2011) and *A. alba* among the coniferous species (Bouriaud & Popa, 2008). Moreover, we studied drought effects on the N net uptake kinetics and thus the direct effects of drought on the physiological root uptake capacity. For *in situ* uptake we determined ¹⁵N incorporation into the tissues of the saplings growing in soil where the ammonium and nitrate pools had been ¹⁵N labelled. For assessing uptake kinetics, roots of the six species still attached to the tree were incubated in solutions with different

concentrations of ^{15}N nitrate or ammonium to determine the concentration-dependent net uptake rates (Gessler et al., 2004).

To assess the influence of the availability of recent assimilates on root N uptake capacity, we linked ^{15}N uptake to carbon allocation belowground by tracking the fate of ^{13}C labelled assimilates in drought exposed as well as in girdled saplings.

We tested the following hypothesis:

1) N uptake by tree roots is reduced under severe drought due to both reduced physiological uptake capacity and reduced ion mobility and thus N availability to the roots.

2) The availability of new assimilates to fuel the active transport and to provide carbon for N assimilation influences the N uptake capacity. Under moderate drought, an increase in belowground C allocation might occur, resulting in an increased N uptake capacity.

Materials and methods

Experimental design – drought and girdling treatments

In total three experiments were performed in greenhouse or climate chamber settings. One experiment (*experiment i*) was conducted to characterize the influence of drought stress on soil processes (e.g. mobility and thus availability of N for root uptake) and actual ^{15}N uptake by the trees. In *experiment ii*) ammonium and nitrate net uptake kinetics and thus the direct effects on the physiological root uptake capacity was examined. In *experiment i*) the relationship between N uptake and the allocation of recent ^{13}C labelled assimilates within the plant was additionally determined. In an additional *experiment iii*), we aimed at analyzing if N uptake capacity can get restricted as a result of reduced carbon supply (without any additional impacts of drought on root functioning) to the roots by performing a girdling experiment. On overview of the designs of the three experiments is given in Fig. 1.

In *experiments i*) and *ii*) two-year-old saplings of *A. pseudoplatanus*, *F. sylvatica*, *Q. petraea*, *P. abies*, *A. alba*, and *P. sylvestris* were used. Plants were obtained from the nursery of the experimental garden of the Swiss Federal Research Institute WSL and later transferred to 5L pots filled with commercial soil (Ökohum GmbH; see Table S1) with a pH value of 5.5 and grown in the greenhouse. Average air temperature and relative air humidity (RH) during the

experiment were $22.7 \pm 4.5^{\circ}\text{C}$ (SD) and $67\% \pm 16\%$, respectively (Fig. S1). Supplement light provided a constant 16h light period and average daytime photosynthetic photon flux density was $69.62 \pm 61 \mu\text{mol m}^{-2} \text{s}^{-1}$.

After leaves of all deciduous species had been fully developed, trees in both experiments were exposed to two drought levels (severe and moderate drought) for approximately 5 weeks while in the control treatment plants were watered to field capacity. Volumetric water content (VWC) was determined continuously with soil moisture sensors (Decagon EC 5, California, USA) and irrigation was adjusted so that the moderate drought treatment reduced soil water content on average to $11.0 \pm 3.3\%$ and severe drought to $5.5 \pm 3.8\%$. In the well-watered controls VWC was on average $15.7 \pm 2.7\%$ (Fig. S2, Table S2). Soil specific pF curves (obtained from Hyprop (DIN EN ISO 11274; Fig. S3) by UMS GmbH) allowed to convert VWC to soil water potential (Kirste and Durner, 2018). The severe drought resulted in a soil water potential range between -0.6 and -1.2 MPa and thus close to the permanent wilting point (-1.5 MPa) (Farrick et al., 2019) and the moderate drought ranged between -0.2 to -0.6 MPa. Stem diameter and height measurements were done before and during drought stress periodically for monitoring tree growth. ^{15}N uptake. In *experiment i*) the transport of recently ^{13}C labelled assimilates was assessed as described in the section “Experimental design – N uptake and carbon supply”.

For *experiment iii*) we used two-year-old saplings of *F. sylvatica* and *P. sylvestris* grown in a climate chamber. Air temperature and RH were set to 20°C and 65%, respectively. Illumination was provided by daylight fluorescent lamps (Osram Fluora L58W/77, Osram GmbH, Munich, Germany) plus incandescent light bulbs (Osram Krypton 60 W). Photosynthetic photon flux density (PPFD) at the top of the trees was $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. Half of the trees was girdled (i.e. the bark with the phloem was removed around the stem to prevent basipetal phloem transport) at a stem height of 3 cm above the soil surface. These trees were grown under well-watered conditions for 5 weeks as were the controls. ^{13}C allocation to the roots as well as N uptake were assessed as described in the section “Experimental design – N uptake and carbon supply”.

In all experiments (i)-iii)) pre-dawn leaf water potential (Ψ_{PD} , MPa) and leaf-level gas exchange (light saturated photosynthesis (A_{sat} , $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g_s , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and transpiration (E , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were measured once per week during the drought or girdling treatments. Leaf water potential (LWP) was determined with a Scholander bomb (Model 600, PMS Instrument Company, Oregon, USA). For gas exchange measurements carried out with a GFS 3000 system (Heinz WALZ GmbH, Effeltrich, Germany) temperature and air humidity were set to 20°C and 65%, respectively. CO_2 concentration was 400 ppm and photosynthetically active photon flux density was 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Experimental design – N uptake and carbon supply

Experiment i): To characterize the combined effects of soil and root uptake processes we added ^{15}N labelled ammonium nitrate (99% $^{15}\text{NH}_4^{15}\text{NO}_3$) in solution directly to the soil as described in detail by Gockele et al. (2014) with five replicates per species and treatment. We added 35 mg/kg soil of 99% $^{15}\text{NH}_4^{15}\text{NO}_3$ to make sure not to increase the plant available inorganic N concentration by more than 3% to avoid fertilization effects (Total KCl-extractable (Gessler et al. 2005) inorganic N (i.e. NO_3^- and NH_4^+) amounted to approx. 15 mmol N/kg soil). The $^{15}\text{NH}_4^{15}\text{NO}_3$ was applied by the end of 3rd week of the drought period (Fig. 1) via holes predrilled to a depth of 5 cm in an equidistance (3 cm) grid over the pot surface. 50 ml of 40 millimolar $^{15}\text{NH}_4^{15}\text{NO}_3$ solution per pot was injected into the holes through a four-side port needle allowing optimal vertical distribution of the label connected to a dispenser (see Gockele *et al.*, 2014). This procedure was applied to avoid adding high amounts of water and at the same time allowed optimal distribution of the tracer. Five days after ^{15}N labelling, we applied a $^{13}\text{CO}_2$ pulse label to trace the new assimilates through the plants. For ^{13}C labelling we constructed a temporary gas tight transparent plastic chamber to expose the plant with ^{13}C enriched CO_2 generated by mixing 1M $\text{Na}_2\text{-}^{13}\text{CO}_3$ (98%, ^{13}C) and 1M $\text{Na}_2\text{-}^{12}\text{CO}_3$ (98%, ^{12}C) at a 50:50 ratio with 1M H_2SO_4 inside a conical flask kept outside the chamber. The $^{13}\text{C}\text{-CO}_2$ was then directed into the chamber via a pump (N5KP18, M&C TechGroup, Ratingen, Germany) for 3 hours and fans inside the chamber ensured proper mixing. The $^{13}\text{CO}_2$ concentration was continuously measured using an Off-Axis Integrated cavity output spectrometer (LGR -CCIA 36d, LosGatos research, CA, USA) (Fig. S4). Ten days after the ^{13}C pulse (i.e. after five weeks of drought), plants were harvested and separated into fine roots, coarse roots, stems and leaves/needles for analysis of biomass, C and N content, and ^{15}N and

¹³C isotopic composition. All tissues were oven-dried (65°C) and ¹⁵N and ¹³C uptake was based on dry mass.

Experiment ii): To assess N uptake kinetics, we incubated roots in nutrient solutions (Gessler *et al.*, 2005). For these experiments three replicates per species, treatment and nitrate or ammonium concentration were used. After 5 weeks of drought (see Fig. 1), roots were carefully excavated from the soil and while they were still attached to the tree, incubated in solutions with different concentrations with either 99% ¹⁵N nitrate or 99% ¹⁵N ammonium added. Five concentration levels were chosen according to Gessler *et al.*, (2005) and the range for ammonium was 1 to 200 $\mu\text{mol L}^{-1}$ and for nitrate 1 to 500 $\mu\text{mol L}^{-1}$. A nutrient solution containing K⁺ (769 mmol/L), Na⁺ (431 mmol/L), Ca²⁺ (662 mmol/L), HPO₄⁻ (319 mmol/L), SO₄²⁻ (320 mmol/L), Mg²⁺ (190 mmol/L), Cl⁻ (201 mmol/L), and Zn²⁺ (14.6 mmol/L) was also added at a concentration of (0.1 ml/L) to the incubation solution to reach concentrations values of natural forest soil solutions (Gessler *et al.*, 1998). After incubating roots for 2 hours, fine roots (incubated into the solutions and a short section outside the solution; see Gessler *et al.* (2004)) were harvested, washed with demineralized water, weighted, oven dried at 65°C and ground for analysis of C and N content, and ¹⁵N isotopic composition. By fitting uptake rates (based on the fine root fresh weight to allow comparison with previous literature (e.g. Gessler *et al.*, 2005)) versus concentrations applying a Michaelis-Menten model, the kinetic parameter V_{max} (maximum net uptake capacity, based on length and biomass of the incubated roots) and k_m (nitrate or ammonium concentration where 50% of the maximum net uptake is observed) were determined as described in detail by Gessler *et al.* (2005). We acknowledge that our approach is only an approximation as the Michaelis–Menten model can be—*sensu stricto*—only applied to unidirectional reactions, while N net uptake consists of an influx and an efflux term.

Experiment iii): 25 days after the start of the girdling treatment a ¹³CO₂ pulse (see Fig. 1) was applied as described for *experiment i*) with 5 replicates. After ten days, we excavated roots and incubated the fine roots for 2 hours in nutrient solutions with 500 $\mu\text{mol L}^{-1}$ ¹⁵N nitrate as described for *experiment ii*). Then plants were harvested (and root were washed with demineralised water) and separated into different organs and tissues (fine roots, coarse roots, stem, leaves/needles). After drying at 65°C, ¹³C isotope composition was determined

in all tissues and ^{15}N was analysed in the incubated fine roots. ^{15}N and ^{13}C incorporation was based on dry weight.

^{15}N and ^{13}C analysis and uptake calculations

To determine N and C content and ^{15}N and ^{13}C abundance, aliquots of 1 mg of oven dried and homogenized plant parts were transferred into tin capsules. and introduced into an elemental analyzer (Flash, ThermoFisher, Bremen, Germany) coupled with a ConFlo II- Interface (ThermoFisher, Bremen, Germany) to an isotope ratio mass spectrometer (Delta plus^{XP}, ThermoFisher, Bremen, Germany).

The amount of ^{15}N or ^{13}C incorporated as a consequence of labelling into plant parts per gram dry weight (molar excess: $^{nn}\text{X}_{in}$ (μmol (^{13}C or ^{15}N) g^{-1}) was calculated based on the following equation.

$$^{nn}\text{X}_{in} = \frac{(^{nn}\text{X}_t - ^{nn}\text{X}_c) * [\text{X}] * 10^4}{\text{MM}} \quad \text{eqn. 1}$$

Where $^{nn}\text{X}_t$ and $^{nn}\text{X}_c$ are the abundance of ^{15}N or ^{13}C (atom %) in labelled and unlabelled control trees, respectively, [X] is the total N or C concentration (% N or C g^{-1} dry weight), and MM is the molar mass of ^{15}N or ^{13}C (g mol^{-1}). The ^{15}N or ^{13}C incorporated into a particular organ (e.g. leaves) is calculated from $^{nn}\text{X}_{in}$ and the respective organ biomass and summed up over all organs for whole plant uptake. To calculate the relative allocation (i.e. distribution) to different plant organs, the amount of ^{15}N or ^{13}C incorporated into a given organ (leaves, stem, fine roots, coarse roots) was divided by the whole plant incorporation.

Statistical analysis

The effect of treatment on various plant physiological processes like photosynthesis, transpiration, stomatal conductance, ^{15}N assimilation and concentration, and ^{13}C assimilation and concentration, and stress indicators like Leaf water and soil water contents were examined using mixed-model analysis of variance (ANOVA). Separate ANOVAs were conducted for each and species, and Tukey-Kramer, and Sidaks multiple comparison tests were used to isolate specific differences among treatments. Regression models are used to show the correlation between N uptake, fresh carbon allocation to fine roots, root biomass, and transpiration. A Michaelis-Menten model was fitted iteratively for assessing V_{max} and k_m

values using Origin 2016 (OriginLab, Northampton, USA) and a one-way ANOVA procedure combined with a Tukey HSD post hoc test was applied to assess treatment effects. Statistical analyses were performed using the statistical program R version 3.1.2 (R Core Team, 2018) and GraphPad prism software (Prism version 7.00 for Mac OS X, GraphPad Software, La Jolla California USA).

Results

Effects of drought on leaf physiology and growth.

The LWP decreased over time in the drought treatments of experiments i) and ii) (Fig. S5) and drought significantly reduced LWP in all species at the end of the drought period (Table 1, Table S3). Similarly, the drought treatments reduced photosynthesis (A_{sat}), transpiration (E), and stomatal conductance (g_s) across the different levels of drought for all species (Table 1, Figs. S6-8, Tables S4-6). Stem biomass development was assessed using stem height and diameter measurements. Height growth was already completed before the start of the treatments in all species and no further increment was observed during the course of the experiment. In contrast, stem diameter increased during the experiment irrespective of the treatment in all species (Fig. S9). Drought reduced the diameter of *A. pseudoplatanus*, *F. sylvatica*, *A. alba* and *P. sylvestris* and total biomass in *A. pseudoplatanus*, *F. sylvatica*, *Q. petraea* and *P. sylvestris* (Table 2) compared to well-watered controls. A significant reduction in fine root biomass was observed in saplings of all species under intense drought (Fig. S10).

Effects of drought on combined soil and plant processes affecting plant N uptake.

In *experiment i)* $^{15}\text{NH}_4^{15}\text{NO}_3$ was injected into the soil and ^{15}N uptake and incorporation into the plant biomass was determined to address effects of drought on the complete soil-plant continuum. ^{15}N incorporation into plant biomass was found significantly reduced as a consequence of drought in all deciduous tree species while this was only a trend for the conifers (Fig. 2 for deciduous, Fig. 3 for conifers, detailed statistics in Table S7). The highest ^{15}N incorporation in total biomass in the control treatments was observed with *A. pseudoplatanus* and the lowest with *P. abies* (Figs. 2, 3). Severe drought reduced ^{15}N incorporation in deciduous trees by between 47% (*A. pseudoplatanus*) and 79% (*F. sylvatica*). There were some common patterns for most species of drought induced changes in relative

¹⁵N allocation to different plant compartments. In all species except *Q. petraea*, less of the ¹⁵N taken up was allocated to the leaves under extreme drought and instead relatively more ¹⁵N remained in the fine roots (*A. pseudoplatanus*, *F. sylvatica*), in the coarse roots (*P. sylvestris*, *P. abies*) and/or in the stem (*A. pseudoplatanus*, *A. alba*) (Figs. 2, 3). Drought also significantly reduced ¹³C incorporation into plant biomass with the exception of *F. sylvatica* and severe drought led to reductions of between 70% (*A. pseudoplatanus*) and 92% (*P. abies*) (Fig. 2 for deciduous, Fig. 3 for conifers, detailed statistics in Table S8). In the three deciduous species severe drought and in *F. sylvatica* also intermediate drought led to a clear reduction of the relative ¹³C allocation to belowground tissues (Fig. 2). Such pattern was also observed in *A. alba* but *P. abies* showed higher relative ¹³C allocation to belowground tissues under drought (Fig. 3). Changes in total uptake and in allocation patterns affected ¹⁵N concentrations in leaves (where newly assimilated N is needed to sustain photosynthesis) and ¹³C concentrations in roots (where new C assimilates are needed to support nutrient uptake and assimilation) of the six species (Fig. 4, detailed statistics in Table S9). In *F. sylvatica*, *Q. petraea* and *A. alba* drought significantly reduced ¹⁵N concentrations compared to controls and in *A. pseudoplatanus*, *F. sylvatica*, and *P. sylvestris* fine root ¹³C concentrations were reduced. In order to examine if the changes in C allocation under drought affected N uptake, we assessed the linkage between the total ¹⁵N taken up by the plants and parameters representing changes in ¹³C allocation to the fine roots (Fig. 5, Table S10). There was a significant correlation ($R^2 = 0.76$, $p = <0.0001$) between the ¹⁵N incorporated into whole plant biomass and the total amount of ¹³C transported to the fine root across all species and drought treatments (Fig. 5a). Comparable relationships were observed when the relative amount of ¹³C allocated to the roots ($R^2 = 0.77$, $p = <0.0001$; Fig. 5b) or the ¹³C concentration in the fine roots ($R^2 = 0.76$, $p = <0.0001$; Fig. 5c) were considered. There was also a similar relationship between total ¹⁵N incorporation and fine root biomass across all treatments ($R^2 = 0.75$, $p = <0.0001$, Fig. 6, Table S11) indicating that not only short but also longer-term C allocation belowground affects N uptake. Total N uptake per g root biomass was comparable between control and moderate drought (over all species: $102 \pm 66 \mu\text{mol } ^{15}\text{N g}^{-1} \text{ root dry weight}$ and $102 \pm 65 \mu\text{mol } ^{15}\text{N g}^{-1}$, respectively) and slightly lower ($78 \pm 54 \mu\text{mol } ^{15}\text{N g}^{-1}$) for the severe drought treatment. The potential effects of drought induced changes in transpiration on N uptake is shown in Fig. 5d. A low positive linear correlation between ¹⁵N

incorporated into plant biomass and leaf level transpiration ($R^2 = 0.30$, $p = <0.0181$) was observed.

Effects of drought and C transport on nitrate and ammonium uptake systems.

In *experiment ii*) concentration dependent root net uptake capacities as affected by drought were assessed. In contrast to total plant incorporation of ^{15}N from the soil as assessed in *experiment i*) deciduous trees were not significantly affected in their concentration dependent nitrate or ammonium uptake rates by drought (Fig. 7). Even though ^{15}N incorporation from the soil (i.e. in *experiment i*) was not affected in conifers, severe drought reduced maximum ammonium but not nitrate uptake rates ($V_{\max}$) in *A. alba* and *P. abies* (Fig. 8). K_m values for nitrate and ammonium uptake were not affected by drought for neither of the species (Fig. 7 for deciduous trees, Fig. 8 for conifers).

To independently test if reduced C allocation to the roots does or does not affect the root nitrate uptake systems, we applied a girdling treatment in two tree species in *experiment iii*). Girdling did not affect LWP of *F. sylvatica* throughout the course of experiment but caused a significant decrease in LWP for *P. sylvestris* towards the end of the experiment (Table.1, Fig. S11, Table S12). In addition, gas exchange measurement data showed that girdling resulted in a significant reduction in A, E, and g_s in both *F. sylvatica* and *P. sylvestris* (Table 1, Figs S12-13, Tables S13-15). Similarly, girdling reduced ^{13}C uptake by 60.6 % ($p = 0.5901$) and 41.1 % ($p = 0.0032$) in *F. sylvatica* and *P. sylvestris*, respectively (Fig. 9b, Table S16). Moreover, relative to total ^{13}C uptake, significantly lower proportions of ^{13}C were allocated belowground (*F. sylvatica*: Control: 35.5 ± 18.0 %, Girdling: 18.4 ± 13.7 %; $p = 0.0004$; *P. sylvestris*: Control 9.8 ± 8.7 %, Girdling: 1.5 ± 1.4 %; $p = 0.12$; Table S17). This reduction in the transport of newly assimilated C belowground did, however, not significantly affect nitrate uptake capacity from nutrient solutions (Fig. 9a, Table S17).

Discussion

N absorption by trees from the soil mainly depends on three factors: a) Nutrient availability at the root surface, b) the absorbing root surface area and c) the efficiency of the plant uptake and assimilation systems, and all these factors might be severely affected by drought (Kreuzwieser and Gessler, 2010).

With our approaches we were able to distinguish c) from the other factors. Still we need to acknowledge that our study did not account for drought effects on mycorrhizal associations and rhizosphere microorganisms in general, which play an important role in the drought response of plants (e.g., Santander et al., 2017). Moreover, we assessed saplings and the responses of adult trees might be different as they e.g., have larger C storage pools to buffer the reduced C availability under drought. Finally, we only addressed inorganic N uptake even though it is known that the uptake of organic N can be important in forest trees and ecosystems (Näsholm et al., 2009).

Total inorganic N uptake from the soil under drought is impaired by reduced root biomass and lower belowground assimilate allocation

In *experiment i)*, we aimed at assessing the combined effect of soil and plant processes on N uptake. We observed a significant reduction of N uptake under severe drought stress for the three deciduous species and a comparable (though not significant) trend for the conifers. In *experiment ii)* we observed that ammonium and nitrate transporter characteristics were not significantly influenced in deciduous species by drought but that $V_{\max}$ for ammonium uptake was reduced in *A. alba* and *P. abies*. On the one hand, any drought effect on whole plant N uptake from the soil as observed in the deciduous species must thus be attributed to root nutrient availability and/or root biomass or surface area. On the other hand, the reduced $V_{\max}$ ammonium in the two-conifer species did not lead to effects on whole plant N uptake.

A reduction in soil nutrient availability during drought may occur for several reasons, including lower microbial nutrient mineralization, and altered plant – microbe interactions (Fierer and Schimel, 2002; Hawkins and Crawford, 2018). As we added similar amounts of ^{15}N labelled ammonium nitrate to the soil in the different treatment we did not account for such re-supply effects. Reduced soil water content can cause lower diffusion through the soil matrix and also lower nutrient transport by water mass flow influenced by plant transpiration to the root surface (Schoonover and Crim, 2015). As the same soil was used, and water content was similar in the drought treatments across species the differences between deciduous and coniferous species in terms of drought impact on N uptake might not be primarily related to diffusion. In addition, N uptake might also be influenced by changes in root hydraulic conductivity and drought stress is considered to cause a reduction in root hydraulic conductivity due to increased suberization of roots (Steudle, 2000). The positive (though not

very strong) relationship between transpiration and whole plant N uptake, therefore, points to an effect of water mass flow in the soil-plant-atmosphere continuum.

In our drought experiments with saplings, extreme drought reduced fine root biomass (and thus active surface area for nutrient uptake), and in 5 out of 6 species also the amount of newly assimilated ^{13}C carbon. These results of the ^{13}C pulse labelling approach agree with the assessments of assimilation on the leaf level. Drought not only impaired C assimilation but also reduced allocation of new assimilates to roots in most of the species tested. Reduced allocation to roots might be a result of increasing phloem sap viscosity under drought that leads to reduced sieve-tube conductivity (Dannoura et al. 2019).

It has already been shown that fine root biomass is crucial for tree- and stand-based nutrient uptake under drought as is the exploitation of the soil volume by root foraging (de Kroon and Mommer, 2006) to compensate for the reduction of the mobility of ions under water restriction. Our results are in agreement with previous findings showing that severe drought can strongly impede root growth (Eghball and Maranville, 1993). However, in contrast to our hypothesis 2 we could neither observe a stimulation of root growth nor of allocation of new assimilates belowground (cf., Leuschner et al., 2001; Hommel et al. 2016) with moderate drought. Such differences to previous publications might be due to differences in drought intensity, duration of the drought events and species-specific differences. Over all treatments, fine root biomass significantly influenced N uptake, associating 75% of variation in N uptake to root biomass, and a comparably high relationship was observed with allocation of new assimilates belowground.

We conclude that drought mainly negatively affected C allocation to root biomass and that the lower fine root biomass growth reduced the nutrient foraging capacity. Reduced allocation of new assimilates belowground during drought is known to also reduce the exudation of carbon to the rhizosphere and the supply of rhizo-dependent soil microbes (e.g., Rein et al., 2016). Dannenmann et al., (2009) showed that such a reduction of belowground C allocation and exudation might lead to a shift in the abilities between roots of adult trees and free-living microbes to compete for N. The authors observed an increase in nitrate uptake by roots of beech trees and at the same time a reduction of microbial biomass and N immobilisation and argued that trees might be able to “starve-out” the micro-organisms. Such an effect was, however, not observed with beech saplings (Winkler et al., 2010) being in agreement with our findings for six different coniferous and deciduous species. Simon et al.,

(2017) argued that the amount of C transferred to the soil via exudation by saplings is generally too low to introduce a visible change in microbial activity on an ecosystem scale.

Ammonium uptake capacity is reduced by drought in two coniferous species, but such physiological impairment does not significantly restrict total inorganic N uptake

Our data demonstrated that root uptake capacity for inorganic N forms was not affected in deciduous saplings by drought events, even though total *in situ* N uptake was reduced significantly in the three deciduous species. In contrast while $V_{\max}$ for ammonium was reduced in *A. alba* and *P. abies* under severe drought their total N uptake from the soil was not significantly reduced. The reduction of the ammonium uptake capacity in the conifer species is in contrast to findings with *Populus simmonii* saplings (Zhang et al. 2018) and *Malus prunifolia* (Huang et al., 2018) seedlings. The further authors observed an increase in ammonium transporter transcript abundance and ammonium uptake and the latter an increase in ammonium at the expense of nitrate uptake. In coffee trees, $V_{\max}$ and K_m for nitrate uptake were significantly reduced upon drought stress (Martinez et al. 2020). These differences might be due to species-specific responses and due to different drought intensities applied.

The ability of tree roots to absorb N available at the root surface depends upon the characteristics of the N transporters (i.e. their abundance and their activity) involved in the active transport across the cell membrane. The capability of a plant root to efficiently take up the nutrients available in the rhizosphere are defined by the kinetic characteristics of the nutrient transporters (i.e. transporter affinity and maximum uptake capacity) (Glass et al., 1992). As root N uptake and assimilation are important energy demanding processes in roots (Pfautsch et al., 2009), and a large proportion of energy demanding processes in the roots depend on the belowground transport of new assimilates (Högberg et al. 2001), we hypothesised that the amount of recently assimilated carbon transported to the fine roots would modify the nitrate and ammonium uptake capacity. It is without doubt that sugars play a central role in the regulation of N uptake and assimilation (Gessler et al., 2004) supported by observations, that a decrease in nitrate uptake at night was reversed by feeding sucrose to the roots, which affected nitrate transporters on the transcriptional level (Lejay et al., 1999). The close coupling between N and C metabolism can also be explained by the demand for C skeletons during N assimilation; indeed up to 55% of the assimilated C can be committed

to N assimilation and metabolism (Huppe and Turpin, 1994). The findings that root uptake capacity remained unaltered during the drought period in four of six species and was in these species not affected by drought-induced reduction of C transport to the roots lead us, however, to reject our initial hypothesis. This conclusion is also supported by the findings of *experiment i*) that mainly the drought-induced reduction of root biomass is a factor for the reduction of total plant N uptake. Our results from the drought treatment, were corroborated by the girdling experiment that effectively prevented allocation of recent assimilates belowground and did not influence the root N uptake capacity in beech and pine.

We conclude that the reduction of the allocation of newly assimilated carbon to the fine roots during short term drought periods did not induced a general energy or C-skeleton limitation for the active uptake of N by fine roots. One mechanism that could compensate the reduction of allocation of new assimilates to fine roots is the remobilisation of stored carbon. Galiano *et al.*, (2017) showed that 10 weeks of drought, did not deplete but rather increase the starch and sugar pools in *P. sylvestris* and *Tilia platyphyllos* and at the same time root biomass decreased during drought. Lower fine root biomass and thus reduced nutrient absorbing area thus can come with an increase in sugar and starch concentration. We might thus conclude that specific uptake rates (per g root biomass) were not affected by drought in the three deciduous species and in *P. sylvestris* as sufficient carbohydrates (per g root biomass) were still available despite reduced C allocation. We might speculate that the carbohydrate reserves were lower in the two species (*A. alba*, *P. abies*) with the drought induced reduction of ammonium uptake. However, nitrate uptake capacity was not affected in these species even though additional energy is needed for reducing nitrate before being incorporated into amino acids. The reduction in ammonium uptake capacity in response to drought in the conifers did not affect total N extraction from the soil; rather the opposite was the case as the deciduous tree species (even though their uptake kinetics was not affected) and not the conifers showed a significant reduction of N uptake in *experiment i*). We need to conclude that the reduced ammonium uptake capacity was not restricting total N uptake in the soil in *A. alba* and *P. abies*.

Conclusion

Currently, the interaction between carbon starvation and hydraulic failure is in the focus of the scientific debates concerning the effects of drought on the functioning of trees. It is important to address the fact that drought might also impair the functioning of trees via restrictions in the nutrient supply but a clear mechanistic basis that allows to inform biogeochemical models is lacking. By linking N uptake with the carbon and water balance, our study shows that drought events resulted in a) reduction of actual soil nutrient uptake mainly in deciduous trees accompanied by b) reduced carbon allocation to the roots and c) reduction in root biomass and thus surface area available for N uptake. Reduced C allocation belowground did not generally impose any energy or carbon restriction on the functioning of the root N uptake system during short drought periods and several species might compensate a lack of new assimilates by using storage reserves. On the longer term this compensation might not be possible any longer; however, Aaltonen et al. (2017), observed that prolonged drought stress of 15 months led to acclimation and even an increase in N uptake.

Conflict of Interest

None declared

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Author Contribution

A.G., and J.J. designed the experiment. L.B., K.S., N.B., J.L., and J.J collected the samples. J.J. analysed the data. J.J., and A.B. performed the statistics. J.J. and A.G. wrote the manuscript. L.B., K.S., N.B., A.B., J.L. commented on and edited the manuscript.

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Figure Legends

Figure 1: Scheme of the experimental designs of experiment i)-iii). In *experiments i)* and *ii)* 6 tree species were subjected to 5 weeks of drought treatments (moderate drought, severe drought, control). In *experiment i)* $^{15}\text{NH}_4^{15}\text{NO}_3$ was applied to the soil 3 weeks after the start of the drought and 5 days later $^{13}\text{CO}_2$ labelling was applied. At the end of the experiments, the plants were harvested and ^{13}C and ^{15}N incorporation was assessed in the different plant organs. In *experiment ii)* nitrate and ammonium uptake kinetics were determined with excavated roots after 5 weeks of treatment. In *experiment iii)* plants were subjected to girdling (vs. ungirdled controls) for 5 weeks. 25 days after the onset of the treatment plants were labelled with $^{13}\text{CO}_2$. At the end of the treatment nitrate uptake was assessed with excavated roots and plants were harvested to assess label incorporation.

Figure 2: ^{15}N , and ^{13}C distribution across different plant organs in broad leaf species in response to drought in experiment i). The figure shows the relative distribution of the ^{15}N and ^{13}C incorporated in total plant biomass as a consequence of labelling to the different plant organs in *Acer pseudoplatanus* (a, b), *Fagus sylvatica* (c, d) and *Quercus petraea* (e, f), respectively. On top of each column the absolute amount of ^{15}N ($\mu\text{mol}^{15}\text{N} \pm \text{SD}$) and ^{13}C ($\mu\text{mol}^{13}\text{C} \pm \text{SD}$) incorporated in the total plant biomass as a consequence of labelling is given. (Signif. codes: ****: $p < 0.0001$, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$).

Figure 3: ^{15}N , and ^{13}C distribution across different plant organs in conifers in response to drought in experiment i). The figure shows the relative distribution of the ^{15}N and ^{13}C incorporated in total plant biomass as a consequence of labelling to the different plant organs in *Picea abies* (a, b), *Abies alba* (c, d) and *Pinus sylvestris* (e, f), respectively. On top of each column the absolute amount of ^{15}N ($\mu\text{mol}^{15}\text{N} \pm \text{SD}$) and ^{13}C ($\mu\text{mol}^{13}\text{C} \pm \text{SD}$) incorporated in the total plant biomass as a consequence of labelling is given. (Signif. codes: ***: $p < 0.001$, *: $p < 0.05$).

Figure 4: ^{15}N and ^{13}C concentration in leaf and fine root biomass in response to drought. ^{15}N concentration in leaf biomass and ^{13}C concentration in fine root biomass for deciduous (a, c) and coniferous (b, d) tree species. Concentrations are based in dry weight (dw). Signif. codes: **: $p < 0.01$, *: $p < 0.05$.

Figure 5: Correlation between ^{15}N incorporated in total plant biomass and ^{13}C in fine root biomass or plant transpiration. a) ^{15}N incorporated in total plant biomass versus ^{13}C incorporated in total fine root biomass, b) ^{15}N incorporated in total plant biomass and percentage of ^{13}C allocation to fine root biomass, c) ^{15}N incorporated in total plant biomass and to concentration of ^{13}C per gram of fine root biomass, and d) ^{15}N incorporated in total plant biomass versus transpiration measured the day before harvesting. All data shown are based on dry biomass. Each data points shows mean with standard deviation for one tree species.

Figure 6: Correlation between ^{15}N incorporated into total plant biomass in relation to total fine root dry biomass. Black dashed lines denote linear regression, dashed red lines shows

95% confidence interval. Each data points shows mean with standard deviation for one tree species.

Figure 7: Net nitrogen uptake kinetics in broadleaf species. Concentration dependent ammonium (a, c, e) and nitrate (b, d, f) net uptake kinetics of root tips (n=3) in experiment ii). a,b) shows the ^{15}N uptake kinetics of *Acer pseudoplatanus*, c, d) represents *Fagus sylvatica*, e,f) denotes *Quercus petraea*. Letters a, b indicates significant differences ($p < 0.5$) of $V_{\max}$ or k_m between drought treatments. Uptake rates are based on root fresh weight.

Figure 8: Net nitrogen uptake kinetics in conifers. Concentration dependent ammonium (a, c, e) and nitrate (b, d, f) net uptake kinetics of root tips (n=3) in experiment ii). a, b) shows the ^{15}N uptake kinetics of *Abies alba*, c, d) represents *Picea abies*, e, f) denotes *Pinus sylvestris*. Letters a, b indicates significant differences ($p < 0.5$) of $V_{\max}$ or k_m between drought treatments. Uptake rates are based on root fresh weight

Figure 9: ^{15}N in fine root biomass and ^{13}C in total plant biomass in response to girdling. ^{15}N concentration in fine root dry biomass (a), and ^{13}C total incorporated in total plant dry biomass (b) after the girdling in *experiment iii*).

Experiment i) and ii): 5 weeks of treatment: control, moderate, severe drought

A. pseudoplatanus, F. sylvatica, Q. petraea, P. abies, A. alba, P. sylvestris

Experiment ii): 5 weeks of treatment: control, girdling

F. sylvatica, P. sylvestris

Leaves Stem Coarse roots Fine roots

A. pseudoplatanus

F. sylvatica

Q. petraea

Drought treatment

Drought treatment

Leaves
 Stem
 Coarse roots
 Fine roots

P. abies

A. alba

P. sylvestris

Drought treatment

Drought treatment

Broadleaf

Coniferous

Leaves

Fine roots

Table 1: Summary of leaf water potential (LWP), and gas exchange parameters during experiments i) – iii). Data (mean + SD) shown for leaf water potential (LWP), photosynthesis (A_{sat}), stomatal conductance (g_s), and transpiration (E) are average values of measurements made during the respective treatments. Treatments in exp. i) and ii) were a moderate and a severe drought treatment including a well-watered control. In exp. iii) girdling and control treatments were compared. Statistical significance is denoted as different letters ($P>0.05$) among the treatments based on 2-way ANOVA followed by either Tukey's or Dunnett's multiple comparison test.

	Exp i - ii)			Exp iii)	
Tree Species	Control	Moderate	Severe	Control	Girdling
<i>A. pseudoplatanus</i>	-0.23 ± 0.11 ^a	-0.45 ± 0.19 ^b	-1.29 ± 0.75 ^c	-0.74 ± 0.48 ^a	-0.82 ± 0.45 ^a
<i>F. sylvatica</i>	-1.36 ± 0.61 ^a	-1.9 ± 0.81 ^b	-2.34 ± 1.23 ^b		
<i>Q. petraea</i>	-0.25 ± 0.12 ^a	-1.02 ± 0.58 ^b	-1.35 ± 1.10 ^b		
<i>P. abies</i>	-0.46 ± 0.12 ^a	-0.61 ± 0.20 ^a	-1.41 ± 0.76 ^b		
<i>A. alba</i>	-0.30 ± 0.10 ^a	-0.82 ± 0.35 ^b	-1.38 ± 0.78 ^c		
<i>P. sylvestris</i>	-0.33 ± 0.12 ^a	-0.52 ± 0.13 ^a	-1.23 ± 1.10 ^b		
	Photosynthesis A _{sat} (μmol m ⁻² s ⁻¹)				
	Control	Moderate	Severe	Control	Girdling
<i>A. pseudoplatanus</i>	5.97 ± 1.18 ^a	4.48 ± 2.35 ^b	3.18 ± 2.82 ^c	3.14 ± 0.71 ^a	2.03 ± 1.55 ^b
<i>F. sylvatica</i>	6.73 ± 2.31 ^a	3.99 ± 3.33 ^b	3.71 ± 2.74 ^b		
<i>Q. petraea</i>	8.88 ± 1.78 ^a	5.22 ± 3.64 ^b	4.08 ± 2.90 ^b		
<i>P. abies</i>	7.44 ± 2.69 ^a	5.69 ± 3.28 ^b	3.44 ± 2.83 ^c		
<i>A. alba</i>	5.06 ± 1.24 ^a	3.61 ± 2.36 ^b	3.02 ± 2.36 ^b		
<i>P. sylvestris</i>	6.38 ± 3.49 ^a	7.47 ± 4.72 ^a	5.50 ± 5.42 ^b		
	Stomatal conductance G _s (μmol m ⁻² s ⁻¹)				
	Control	Moderate	Severe	Control	Girdling
<i>A. pseudoplatanus</i>	111.3 ± 25.82 ^a	84.04 ± 41.19 ^b	47.91 ± 53.69 ^c	178 ± 58 ^a	159 ± 46 ^b
<i>F. sylvatica</i>	158 ± 39.71 ^a	73.19 ± 85.68 ^b	48.34 ± 39.73 ^c		
<i>Q. petraea</i>	122.4 ± 42.23 ^a	56.99 ± 36.32 ^b	38.52 ± 27.06 ^c		
<i>P. abies</i>	70.76 ± 43 ^a	55.7 ± 31.37 ^a	26.18 ± 21.21 ^b		
<i>A. alba</i>	55.17 ± 21.68 ^a	37.75 ± 24.67 ^b	29.75 ± 24.81 ^b		
<i>P. sylvestris</i>	92.46 ±50.66 ^{aa}	77.68 ± 39.22 ^{ab}	58.87 ± 57.15 ^{bc}		
	Transpiration E (mmol m ⁻² s ⁻¹)				
	Control	Moderate	Severe	Control	Girdling
<i>A. pseudoplatanus</i>	1.10 ± 0.37 ^a	0.95 ± 0.55 ^a	0.63 ± 0.69 ^b	2.4 ± 1.1 ^a	1.8 ± 0.79 ^b
<i>F. sylvatica</i>	1.70 ± 0.30 ^a	0.86 ± 1.0 ^b	0.59 ± 0.49 ^b		
<i>Q. petraea</i>	1.3 ± 0.41 ^a	0.7 ± 0.45 ^b	0.49 ± 0.32 ^c		
<i>P. abies</i>	0.96 ± 0.46 ^a	0.78 ± 0.38 ^a	0.4 ± 0.29 ^b		
<i>A. alba</i>	0.68 ± 0.23 ^a	0.48 ± 0.29 ^b	0.38 ± 0.3 ^b		
<i>P. sylvestris</i>	1.2 ± 0.72 ^{aa}	0.99 ± 0.61 ^{ab}	0.83 ± 0.93 ^{bc}		
	13.16 ± 2.45 ^a				
	11.4 ± 2.915 ^b				

Table.2: Summary of growth parameters during experiments i) and ii). Data (mean + SD) shown for stem height, and stem diameter are average values of measurements made during

last two weeks of respective treatments. Total biomass data have been determined on the day before pulse label application. Statistical significance is denoted as different letters ($P>0.05$) among the treatments based on 1-way ANOVA followed by either Tukey's or Dunnett's multiple comparison test.

	Control	Moderate	Severe
Tree Species	Stem Height (cm)		
<i>A. pseudoplatanus</i>	79.51 ± 7.04 ^a	69.86 ± 3.07 ^b	73.14 ± 2.93 ^b
<i>F. sylvatica</i>	47.72 ± 2.68 ^{aa}	58.66 ± 6.37 ^{ab}	48.19 ± 4.38 ^{aa}
<i>Q. petraea</i>	71.58 ± 9.99 ^a	69.97 ± 9.05 ^a	65.91 ± 10.43 ^a
<i>P. abies</i>	48.23 ± 1.54 ^a	47.39 ± 3.75 ^a	46.19 ± 1.72 ^a
<i>A. alba</i>	39.43 ± 1.46 ^a	42.34 ± 3.49 ^a	42.34 ± 2.84 ^a
<i>P. sylvestris</i>	55.47 ± 5.22 ^a	50.79 ± 6.01 ^a	48.83 ± 6.14 ^a
	Stem Diameter (mm)		
<i>A. pseudoplatanus</i>	10.62 ± 0.76 ^{aa}	9.64 ± 0.59 ^{bc}	9.95 ± 0.84 ^{ac}
<i>F. sylvatica</i>	10.39 ± 0.50 ^a	10.71 ± 0.65 ^a	9.23 ± 0.32 ^b
<i>Q. petraea</i>	9.307 ± 0.86 ^a	8.927 ± 0.31 ^a	9.35 ± 0.44 ^a
<i>P. abies</i>	13.54 ± 0.58 ^a	14.28 ± 0.87 ^a	14.16 ± 0.46 ^a
<i>A. alba</i>	12.2 ± 0.63 ^a	12.34 ± 0.44 ^a	11.33 ± 0.27 ^b
<i>P. sylvestris</i>	13.25 ± 0.65 ^a	10.79 ± 0.57 ^b	9.97 ± 0.19 ^c
	Total Biomass (g)		
<i>A. pseudoplatanus</i>	85.29 ± 21.28 ^a	51.32 ± 12.49 ^b	41.66 ± 5.76 ^b
<i>F. sylvatica</i>	42.24 ± 3.95 ^a	41.09 ± 3.20 ^a	25.73 ± 3.45 ^b
<i>Q. petraea</i>	42.97 ± 12.70 ^{aa}	33.45 ± 4.16 ^{ab}	21.58 ± 2.68 ^{bc}
<i>P. abies</i>	83.47 ± 56.88 ^a	79.85 ± 49.06 ^a	51.63 ± 19.86 ^a
<i>A. alba</i>	100.31 ± 38.56 ^a	94.98 ± 22.94 ^a	70.42 ± 11.31 ^a
<i>P. sylvestris</i>	65.10 ± 17.34 ^a	31.04 ± 6.11 ^b	34.78 ± 17.88 ^b