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REGULAR ARTICLE

Acid and calcareous soils affect nitrogen nutrition and organic nitrogen uptake by beech seedlings (*Fagus sylvatica* L.) under drought, and their ectomycorrhizal community structure

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Abstract
Aims The role of different soil types for beech productivity and drought sensitivity is unknown. The aim of this experimental study was to compare mycorrhizal diversity between acid sandy and calcareous soils and to investigate how this diversity affects tree performance, nitrogen uptake and use efficiency (NUE).
Methods Beech trees were germinated and grown in five different soil types (pH 3.8 to 6.7). One-and-a-half-year-old plants were exposed for 6 weeks to sufficient or low soil humidity. Tree biomass, root tip mycorrhizal colonization and community structure, root tip mortality, leaf area, photosynthesis, nitrogen concentrations, NUE and short-term ^{15}N uptake from glutamine were determined.
Results Soil type did not affect photosynthesis or biomass formation, with one exception in calcareous soil, where root mortality was higher than in the other soil types. Beech in acid soils showed lower mycorrhizal colonization, higher nitrogen tissue concentrations, and lower NUE than those in calcareous soils. Drought had no effect on nitrogen concentrations or NUE but caused reductions in mycorrhizal colonization. Mycorrhizal species richness correlated with nitrogen uptake and NUE. Nitrogen uptake was more sensitive to drought in calcareous soils than in acid soils.
Conclusions Beech may be more drought-susceptible on calcareous sites because of stronger decrease of organic nitrogen uptake than on acid soils.

Keywords Ectomycorrhizal fungi · Organic nitrogen · Glutamine uptake · Drought · N concentration · Beech · *Fagus sylvatica* · Calcareous soil · Acid soil

Abbreviations

EM	ectomycorrhizal
N	nitrogen
NUE	nitrogen use efficiency

Introduction

The productivity of forests is deteriorating when aridity is increasing in a changing climate (Ciais et al. 2005; Williams et al. 2013). Drought limits water uptake,

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suppresses photosynthesis and, thereby, leads to decreases in biomass production as well as diminished belowground carbon allocation. Consequently, energy and carbon-demanding physiological processes such as root formation and nutrient uptake can be hampered (Ruehr et al. 2009; Kreuzwieser and Gessler 2010). Reduced soil humidity further impedes soil mineralization processes and so may also decrease plant nitrogen (N) nutrition resulting in growth depression (Sardans and Penuelas 2012). A long-standing debate concerns the question whether beech (*Fagus sylvatica* L.), a major deciduous species in Central European forests, is particularly endangered by climate change because of restricted water supply or aggravation of N limitation (Gessler et al. 2007; Rennenberg et al. 2009). Beech trees have a number of morphological characteristics that could render the species drought-sensitive (Granier et al. 2007). However, beech is also known for its wide ecological range occurring from moist to moderately dry climatic conditions and on acid as well as on calcareous soils (Leuschner et al. 2006; Bolte et al. 2007; Ellenberg 2009). Field surveys showed that leaf biomass production and N concentrations were relatively unaffected by soil types and variation in climate (Meier and Leuschner 2008, 2014), whereas a comparison of dry-warm and cool-moist beech forests revealed significant reductions in N uptake and radial stem growth in the warmer climate (Gessler et al. 2001, 2005). These results led to divergent conclusions regarding the drought susceptibility of beech. However, potential impacts of different soil types and confounding effects of other environmental variables cannot be excluded under field conditions and therefore experimental studies are required to elucidate whether soil chemistry affects beech N nutrition and performance under drought.

Variations in soil chemistry and drought conditions are also known to affect the structures of ectomycorrhizal (EM) assemblages (Lilleskov et al. 2002; Shi et al. 2002; Kranabetter et al. 2009; Pena et al. 2010; Zhang et al. 2013; Moeller et al. 2014; Walker et al. 2014; Zavišić et al. 2016). EM fungi form symbioses with the root tips of temperate forest trees and play major roles in ecosystem nutrient cycling because of their ability to access N from different soil pools (Talbot and Treseder 2010; Hobbie and Högberg 2012; van der Heijden et al. 2015). Although relationships between soil N and EM community structures have frequently been studied (Talbot and Treseder 2010), little is known on their impact on organic N supply. It is now widely recognized that amino acids are often as abundant in soil solutions of temperate forests as inorganic N forms and can be used by plants as well as by EM fungi (Finlay et al. 1992; Keller 1996; Näsholm and Persson 2001; Persson et al. 2003; Grelet et al. 2009; Näsholm et al. 2009; Stoelken et al. 2010; Talbot and Treseder 2010). ^{15}N labelling with mixtures of inorganic N and amino acids in a beech forest indicated that glutamine is the preferred source for N uptake by EM roots (Dannenmann et al. 2009). When other N sources are lacking, growth of EM plants benefits more strongly from amino acid-derived N than that of non-mycorrhizal plants, suggesting that EM symbionts widen the adaptability of plants to limiting resources (Turnbull et al. 1995; Plassard et al. 2000; Schmidt et al. 2006). One reason may be that composed EM assemblages as well as distinct EM taxa in their natural habitats vary in their abilities to access and utilize different N sources (Clemmensen et al. 2008; Pena et al. 2013a; Pena and Polle 2014; Valtanen et al. 2014; Kranabetter et al. 2015; Leberecht et al. 2015). Whether soils from different climatic areas and geological formations structure the root-associated EM assemblages with implications for beech performance, nitrogen use efficiency (NUE) and organic N uptake under drought is not known.

To close this knowledge gap, we conducted a pot study, in which European beech seedlings were raised in five different soil types that represented acid and calcareous conditions. Beech was chosen because of its relevance as a keystone species in temperate forests, its wide ecological range and its ability to form diverse EM assemblages. We hypothesized that the beech seedlings in different soil types (i) develop different EM communities under common climatic conditions, (ii) exhibit differences in ^{15}N uptake from glutamine and (iii) that N uptake and NUE are more resilient to drought in soils from dry habitats than in soils from moist conditions. To test these hypotheses, biomass, growth parameters, photosynthesis, and root vitality and mycorrhizal colonization were analyzed in relation to NUE and uptake of ^{15}N from glutamine.

Materials and methods

Beech cultivation and drought treatment

Beech (*Fagus sylvatica* L.) nuts (certificate no.: D-03,001 2 0031 09, FSB Oerrel, Oerrel, Germany)

were germinated in humid cloths at 4 °C in darkness. After 6 weeks the seed coat of germinating nuts was removed. The seedlings were incubated in a sterilization solution [5 ml of Proplant (Stähler, Stade, Germany), 5 ml Tween 80 (Roth, Karlsruhe, Germany), 500 mg tetracycline] overnight and subsequently washed with tap water. Three germinating beech nuts were planted together in 2-L pots. The soils were collected immediately before use in March 2011 in five beech forests with closed canopy that differed in climatic and edaphic conditions (Table 1). The soils from Unterluess (UL) and Calvörde (CL, CS) are highly acidic nutrient poor sandy soils originating from Pleistocene fluvio-glacial sands from the penultimate Saalian ice age with up to 30 % sand fraction, classified as moderate to intense podzolic Umbrisols (Müller-Haubold et al. 2013). The mineral soils are covered by a thick organic layer (9-4 cm). The soils in the Tuttlingen forests are characterized as Rendzic Leptosols derived from limestone (Weißjura beta and gamma series) and are shallow with a thin organic layer (Gessler et al. 2005). Top soils of the first 0.2 m were used after sieving (20 × 20 mm mesh width).

The seedlings were grown until May 2011 in a greenhouse and then placed randomized outside in a nursery (Forest Botanical Garden, University of Göttingen, longitude 9°57'E, latitude 51°33'N, 171 m above sea level). The two weakest seedlings were removed, keeping only one seedling per pot. The plants were watered as necessary and kept outside from May 2011 until the end of the experiment in August 2012 (mean annual air temperature 9.9° in 2011 and 9.4 °C in 2012).

In June 2012, the soil humidity was regularly determined by a sensor (ML2X, HH2, Delta-T Devices, Cambridge, UK) in each individual pot, and kept in the range from 0.2 to 0.3 $m^3\ m^{-3}$. Watering was stopped for half of the plants in each soil treatment on 25th June until the soil humidity had dropped to about 0.1 $m^3\ m^{-3}$. Thereafter, the drought-exposed plants were watered

Table 1 Location, annual precipitation, mean annual temperatures, soil nutrients and pH in five beech forests. Mean annual climate data refer to long-term 30-year-averages (1971–2000). Data for CL (Calvörde, clay), CS (Calvörde, sand) and UL (Unterluess, clay) were compiled from Carsjens et al. (2014) and Müller-Haubold et al. (2013) and those for TCN (Tuttlingen, calcareous, northern exposition) and TCS (Tuttlingen, calcareous, south exposition) from Gessler et al. (2001). Because of the higher irradiance on the TCS site, the soil temperature at 10 cm depth is 0.8 °C higher and the soil water potential generally more negative than on the TCN site (Gessler et al. 2005). Total nutrient element concentrations and pH of soils from five sites were determined after soil collection. Data are means (mg g^{-1} dry soil) ($n = 6 \pm$ SE). Different letters in columns indicate significant differences at $P \leq 0.05$. CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, northern exposition, TS = Tuttlingen, calcareous, south exposition

Site parameters	CL	CS	UL	TN	TS
Latitude (N)	52.40396	52.38032	52.83135	47.97865	47.98301
Longitude (E)	11.26102	11.2901	10.3201	8.74758	8.75458
Elevation (m)	105	105	117	740	760
Precipitation (mm)	544	544	766	810	810
Temperature (°C)*	9.1	9.1	8.5	6.6	6.6
Soil nutrients (mg g^{-1})					
N	3.12 ± 0.04a	3.58 ± 0.02b	6.24 ± 0.04c	6.08 ± 0.06c	9.46 ± 0.13d
C	77.35 ± 0.10a	80.41 ± 0.20b	125.60 ± 0.57d	103.78 ± 0.54c	205.05 ± 0.95e
P	0.30 ± 0.01a	0.43 ± 0.00c	0.35 ± 0.01b	0.82 ± 0.02e	0.75 ± 0.01 d
S	0.40 ± 0.08a	0.33 ± 0.02a	0.79 ± 0.02b	0.86 ± 0.07b	1.16 ± 0.01c
K	1.18 ± 0.05b	1.14 ± 0.01ab	0.95 ± 0.02a	7.07 ± 0.12c	8.29 ± 0.08d
Ca	1.15 ± 0.02a	1.14 ± 0.03a	2.90 ± 0.08a	14.24 ± 2.07b	20.52 ± 0.17c
Mg	0.34 ± 0.01a	0.36 ± 0.01a	0.49 ± 0.01a	5.72 ± 0.13c	4.90 ± 0.04b
Mn	0.23 ± 0.01a	0.27 ± 0.01b	0.43 ± 0.01c	1.04 ± 0.02e	0.66 ± 0.01d
Fe	19.86 ± 0.27b	19.58 ± 0.07b	16.47 ± 0.08a	34.14 ± 0.61d	25.97 ± 0.22c
pH	3.82 ± 0.03a	3.78 ± 0.02a	4.39 ± 0.03b	6.40 ± 0.04c	6.93 ± 0.03d

*mean day temperature in the vegetation period 13.9 °C at TN and 14.3 °C at TS

individually to maintain the reduced soil humidity of about 0.08 to 0.1 m^3 m^{-3} until the harvest (10th August) (n = 5 per soil type and water level).

Plant performance

In the week before harvest, four plants per drought and soil treatment were used for measurements of gas exchange. A mean value for an individual plant was obtained by eight measurements on that plant and plant means were used for further data analyses. All measurements were conducted between 9:00 h und 14:00 h with a photosynthesis analyzer (LCpro+, ADC, Hoddesdon, UK) at a photosynthetic light intensity of 435 μmol photons m^{-2} s^{-1}, a mean air temperature of 22.6 ± 0.5 °C and an ambient CO_2 concentration of 385 ± 11 μmol CO_2 mol.

Plant height and stem diameter at soil level were measured, and the total number of leaves per plant was counted the day before harvest.

Labeling with ^{15}N glutamine

The labeling with L-glutamine ($C_5H_{10}{}^{15}N_2O_3$, 98 %, LOT # PR-19,070, Cambridge Isotope Laboratories, Andover, USA) was achieved by soil injection. Each pot received 7.8 mg glutamine dissolved in 30 ml deionized water. The glutamine solution was injected at 15 positions at soil depths of 60, 30, and 0 mm. The plants were harvested 5 h after soil injection. Five plants per soil and drought treatment were used. Non-labelled plants were treated with water in same way.

Harvest

Plants were carefully removed from the pots. Leaves and above-ground woody tissues were separated and weighed. Five leaves per plant (sample leaves) were weighed separately, photographed with a camera KP-C551 (Hitachi, Tokyo, Japan) and used for leaf area determination (ImageJ 1.47v, National Institute of Health, Bethesda, USA). Whole plant leaf area was calculated as leaf area of sample leaves x whole plant leaf mass / mass of sample leaves.

Roots were washed, separated into coarse roots (> 2 mm diameter) and fine roots (< 2 mm diameter) and weighed. Fine roots were kept in humid tissue papers at 4 °C in darkness until analysis of mycorrhizal colonization.

Aliquots of all fresh tissues were dried for 7 days at 60 °C and used to determine the dry biomass.

The soil from each pot was mixed carefully, and 15 g were removed and stored for soil analyses at −20 °C.

Soil analyses

Five g of fresh soil (stored frozen) was suspended in 12.5 ml deionized water, shaken for 4 h at 230 rpm (Shaker 3018, GFL, Burgwedel, Germany) and then used to determine the pH (pH 538, WTW, Weilheim, Germany).

For elemental analyses soil aliquots were extracted in 65 % HNO_3 for 12 h at 170 °C (Heinrichs et al. 1986). After filtering the extracts were analyzed by inductively coupled plasma atomic emission spectroscopy (Spectro Analytic Instruments, Kleve, Germany).

Root tip and mycorrhizal analyses

Fine roots were cut into about 20 mm long pieces and spread under a compound microscope (205 FA, Leica, Wetzlar, Germany). Adhering soil particles were removed. Randomly selected root segments were used for counting root tips. In each sample, a total number of 700 root tips was counted and classified as vital mycorrhizal, vital non-mycorrhizal or dead according to their morphological appearances (Winkler et al. 2010). Vital mycorrhizal root tips were assigned to morphotypes after morphological characters such as color, mantle structures, ramnification, presence/absence of hyphae or rhizomorphs (Agerer 2006). Morphotypes were documented with a digital camera (DFC420 C, Leica, Wetzlar, Germany). Aliquots of different morphotypes were collected and used for ITS sequencing to determine fungal identities as described elsewhere (Lang et al. 2011). The sequences were deposited in Genbank under the accessions numbers KU564080 - KU564088 and KX355262 (Table S1).

Carbon and nitrogen analysis in plant tissues and soil

Dry aliquots of all plant tissues were ground to a fine powder in a ball mill (Retsch, Düsseldorf, Germany). Aliquots of about 1 mg were weighed into tin cartouches (5 × 9 mm, IVA Analysetechnik) and subjected to N and C analyses in a CNS analyzer (EA 1108, Carlo Erba Strumentazione, Rodano, Italy). Dry, milled soil samples of 1.2 mg were used for N and

C analyses in soil. Whole plant N content was determined as N_{leaves}*$mass_{leaves}$ + $N_{(stem+branches)}$*$mass_{(stem+branches)}$ + $N_{coarse\ roots}$ * $mass_{coarse\ roots}$ + $N_{fine\ roots}$*$mass_{fine\ roots}$ with N = N concentration (mg g^{-1} dry mass). Whole plant C content was determined correspondingly. Mean plant N concentration (mg g^{-1}) was determined as whole plant N content (mg) / whole plant biomass (g). Nitrogen use efficiency (NUE) was determined as the annual amount of biomass produced per amount of N taken up. NUE = (biomass at harvest) / (whole plant $N_{content}$ at harvest) / 1.5 years according to Finzi et al. (2007). Because the plants were raised from the same seed lot, the amounts of N and C at the beginning (t = 0) were the same and tiny for all treatments and therefore neglected. We used 1.5 years as the time period because the plants were harvested after about 1.5 full growth periods.

^{15}N and ^{13}C was measured in milled fine roots at the service unit KOSI (Kompetenzzentrum für Stabile Isotope, University Göttingen, Germany) on a Delta Plus mass spectrometer (Finnigan MAT, Bremen, Germany; Interface: Conflo III, Finnigan MAT, Bremen, Germany; elemental analyzer: NA2500, CE Instruments, Rodano, Milano, Italy). Stable isotope ratios of ^{13}C to ^{12}C are expressed with the delta notation ($\delta^{13}C$ ‰) relative to the Vienna Pee Dee Belemnite standard. Stable isotope ratios of ^{15}N to ^{14}N were calculated as APE (^{15}N atom% excess) with ^{15}N APE = atom $\%_{sample}$ − atom $\%_{natural\ abundance}$ with

$$\text{atom } \% = \frac{^{15}N}{^{14}N + {}^{15}N} \times 100$$

Fine root ^{15}N uptake rate (μg $g^{-1} \times h^{-1}$) was determined as (1000 × ^{15}N $APE_{fine\ roots}$*10*$N_{fine\ roots}$)/5 h. ^{15}N recovery (%) in roots was determined as (1000 × ^{15}N $APE_{fine\ roots}$ x $N_{fine\ roots}$ x dry mass of fine whole plant fine roots)/7.8.

Statistical analyses

Data are means of n = 5 biological replicates (± SE) or of n = 4 (± SE) for the gas exchange measurements. Statistical analyses were conducted with the program Statgraphics Centurion XVII (Statpoint Technologies, Inc. Warrenton, Virginia, USA). Data were tested for normal distribution by the Shapiro-Wilk's test and variance homogeneity by the Levine test. When these requirements were not fulfilled, data were log-transformed to achieve normal distribution. Two-way-ANOVA was conducted for the main factors soil type and drought treatment. P values ≤0.05 were considered to indicate significant differences. LSD tests were conducted post hoc to determine the means that differed at $P < 0.05$. Multiple variable regression analyses were based on Pearson product moment correlations and regression coefficients (R); P values <0.05 were considered to indicate significant relationships. Multiple regression analyses were conducted by stepwise addition of variables. To compare EM communities, analyses of similarities (ANOSIM) with the Bay-Curtis indices as similarity measure were conducted with 9999 permutations using the free software package PAST 3.08 (http://folk.uio.no/ohammer/past/, Hammer et al. 2001).

Results

Performance and drought response of beech seedlings in different soil types

There were no significant differences in biomass among the beech seedlings raised in acid (CS, CL, UL) and calcareous soils (TN), except plants in TS soil (Fig. 1a). They exhibited significantly lower biomass than those in the other soils tested here. Reduced water supply for 6 weeks resulted in a decline of the relative soil humidity from a mean of 0.267 ± 0.003 across all control treatments to a mean of 0.066 ± 0.005 $m^3\ m^{-3}$ during the last week of the drought treatments (Fig. 2), but had no significant effect on plant biomass (Fig. 1a). In addition to biomass, we also determined plant height, stem diameter, and leaf area. All performance parameters were strongly correlated among each other (Pearson product moment correlations for all combinations, $P < 0.001$) showing no drought effects, but significantly lower values for beeches in TS than in the other soil types.

Net photosynthesis of control plants showed no significant variations among well-watered plants in different soil types (Fig. 1b). Drought treatment caused significant reductions in photosynthesis, with the exception of the smaller seedlings in TS soil and the UL seedlings (Fig. 1b). Photosynthesis was unrelated to biomass (Pearson product moment, P = 0.778). Other gas exchange parameters (transpiration, stomatal conductance) were significantly correlated with photosynthesis (Pearson product moment correlations for all combinations, $P < 0.001$).

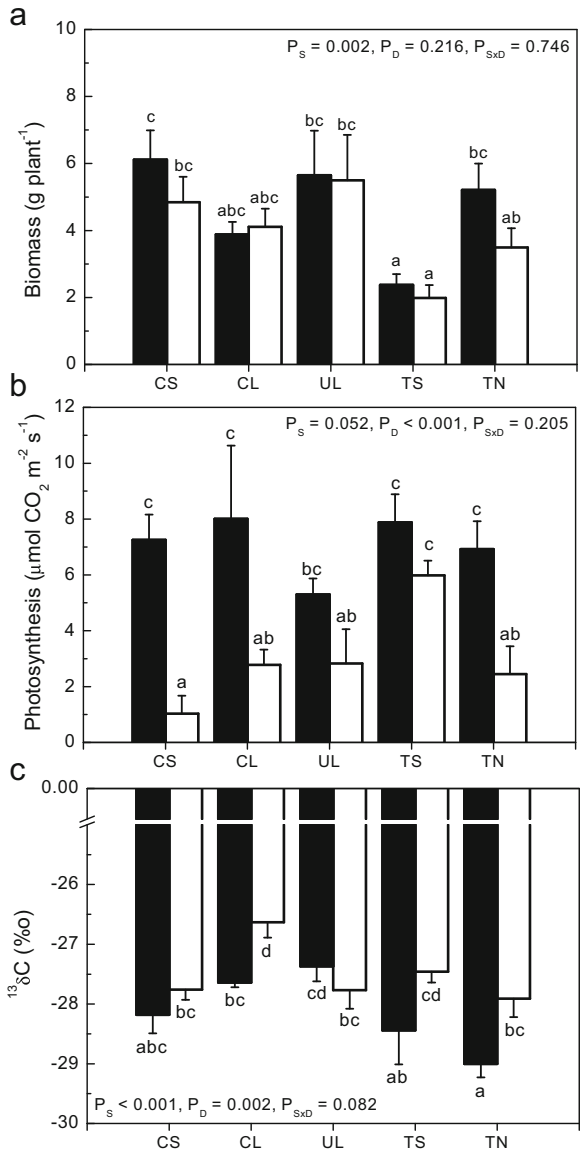


Fig. 1 Biomass (g plant^{-1}) (**a**), net photosynthesis (µmol CO_2 m^{-2} s^{-1}) (**b**) and carbon discrimination δ^{13}C (‰) in fine roots (**c**) of 1.5-year-old beech (*Fagus sylvatica*) trees raised in different soil types and exposed for 6 weeks to diminished water supply. Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, north exposition, TS = Tuttlingen, calcareous, south exposition) as described in detail in Table 1. *Bars* indicate means (±SE) of well irrigated controls (*black*) and drought-treated plants (*white*). Different letters indicate significant differences with $p \leq 0.05$

To characterize the impact of drought, we determined δ^{13}C in fine roots (Fig. 1c). It was notable that the control roots showed differences in ^{13}C discrimination among the soil types, with the strongest discrimination in roots in TN and the lowest in CL and UL soils (Fig. 1c). Drought resulted in significant decreases in ^{13}C discrimination in CL, TS, and TN roots, reaching the lowest values in CL roots (Fig. 1c). On the contrary, the variation of δ^{13}C in response to drought in CS and UL roots was not significant (Fig. 1c).

Mycorrhizal assemblages of beech in different soils in response to drought

An unexpected result was that the root tips of beech plants in the acid soil (CL, CS, UL) were significantly less colonized by mycorrhizas than those in the calcareous soils (TN, TS, Fig. 3a). Drought resulted in significant reductions in mycorrhizal colonization of beech plants in calcareous soils, which were stronger in TS than in TN soil (Fig. 3a).

Beech seedlings in different soil types also developed different EM fungal communities at the root tips (Fig. 3b). Roots of well-watered controls were colonized by 5 to 10 EM species and those of drought-stressed beech plants by 3 to 7 EM taxa per soil origin (Fig 3b). The plants in acid soils exhibited lower EM species richness than those in calcareous soils resulting in significant differences between the acid and the calcareous EM assemblages under control conditions (Fig. 3b, Table 2). While no significant differences were found among EM assemblages of the well-watered controls in acid soils (CS, CL, UL), the communities on roots in calcareous soil from TS were significantly different from those in TN soil (Table 2). Because of EM species loss in response to drought, the significant differences among the communities disappeared among the beech seedlings in different soil types in response to drought (Table 2).

Cenococcum geophilum was the only fungal species that was found in all treatments, but its abundance was generally lower on roots grown in calcareous than on those in acid soils (Fig. 3b). *Sebacina incrustans* was also frequently found and was more abundant on the roots of plants in calcareous than in acid soils (Fig. 3b). A large fraction of the mycorrhizal root tips in acid soil was colonized by an ascomycete morphotype (uncultured Heliotales, Hesp2) whose identity could not be determined unequivocally because in several extraction attempts the samples yielded two ITS sequences (Table S1). It is possible that two species co-colonized the root tips.

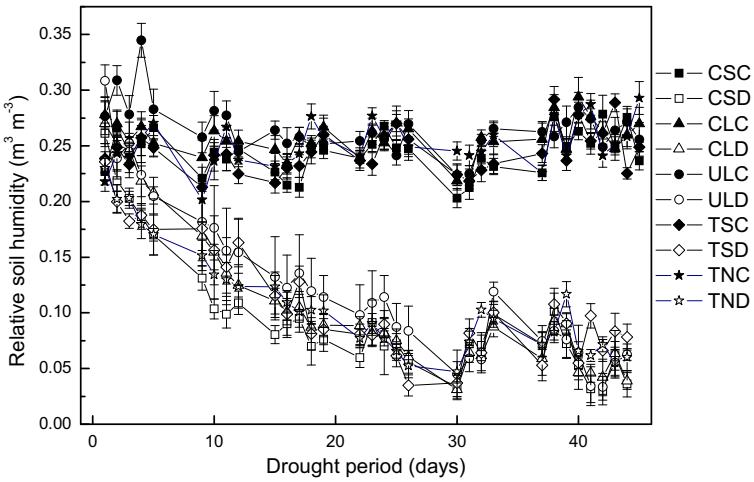


Fig. 2 Time course of the decrease in soil humidity in five different soil types after reduced water supply (*white symbols*) and with sufficient irrigation (*black symbols*). Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, north exposition, TS = Tuttlingen, calcareous, south exposition) and treatments (C = well irrigated controls, D = drought treatment) as described in detail in Table 1. Data indicate means of $n = 5$ (± SE) pots per treatment

We further observed differences in root tip mortality among the beech seedlings in different soil types (Fig. 3c). Under control conditions, the root tips of beech in TS soil showed about 5-fold higher mortality than those from CS, CL, and UL, while those from TN had an intermediate position (Fig. 3c). Drought resulted in strong increases in dead root tips reaching about 65 % mortality in soils from US, TN and TS, whereas the mortality was lower in soils from UL and CL (Fig. 3b).

To find out whether relationships existed between root EM taxon richness, colonization, mortality and performance parameters, we conducted multiple regression analyses (Table 3). Linear regression analysis showed that EM species richness, the degree of root tip colonization and net photosynthesis were strongly correlated (Table 3). Negative relationships were found between EM colonization respective EM species richness and $\delta^{13}C$, as well as between root tip mortality and photosynthesis (Table 3).

Beech N use efficiency and N uptake from ^{15}N-glutamine

Beech seedlings in CS soil contained the highest and those in TS the lowest N contents (Fig. 4a). The effect of drought on beech N content was insignificant (Fig. 4a). Overall, the whole plant N contents were strongly correlated with whole plant biomass ($R = 0.909$, Table 3).

Beech in CS and CL soils exhibited the highest and those in TN and TS the lowest mean plant N concentrations (Fig. 4b). Drought had no effect on the mean plant N concentrations (Fig. 4b). The foliar concentrations ranged from 21.5 to 28.5 mg N g^{-1} dry mass of leaves and showed similar patterns as the mean plant N concentrations (Fig. 4b). In contrast to leaves and whole-plant mean N concentrations, the N concentrations in roots from calcareous soils were significantly lower than those from acid soils and declined in response to drought, while no significant drought-induced decline was apparent in roots from the acid soils (Fig. 4c).

We also determined NUE as the amount of biomass produced per unit of N taken up (Fig. 4d). NUE was inversely related to mean plant N concentrations (Table 3), with beech seedlings in CS and CL exhibiting lower NUE than those in UL, TS, and TN soils (Fig. 4d). NUE, mean plant N concentrations and whole plant N contents were correlated among each other (Table 3), but this could be expected because of partial autocorrelation of the values. Notably, there were also significant, negative relationships between the mean plant N concentration and the degree of mycorrhizal root colonization or EM species richness (Table 3).

To investigate the capacity of the plant for N uptake from an organic source, ^{15}N-glutamine was administered (Fig. 5a). Under control conditions, the ^{15}N uptake rate was significantly lower for roots grown in acid soils than for those in the calcareous soils (Fig. 5a). In response to drought, decreases in ^{15}N uptake were observed in roots from all soil types (Fig. 5a). The decline was stronger in roots of seedlings grown in UL, TN, and TS soils than in CS and CL soils (Fig. 5a).

The uptake rate of ^{15}N was negatively correlated with the relative abundance of non-mycorrhizal roots tips ($P < 0.001$, not shown) and with decreasing carbon discrimination (Table 3). Positive relationships were found between the uptake rate of ^{15}N and EM species

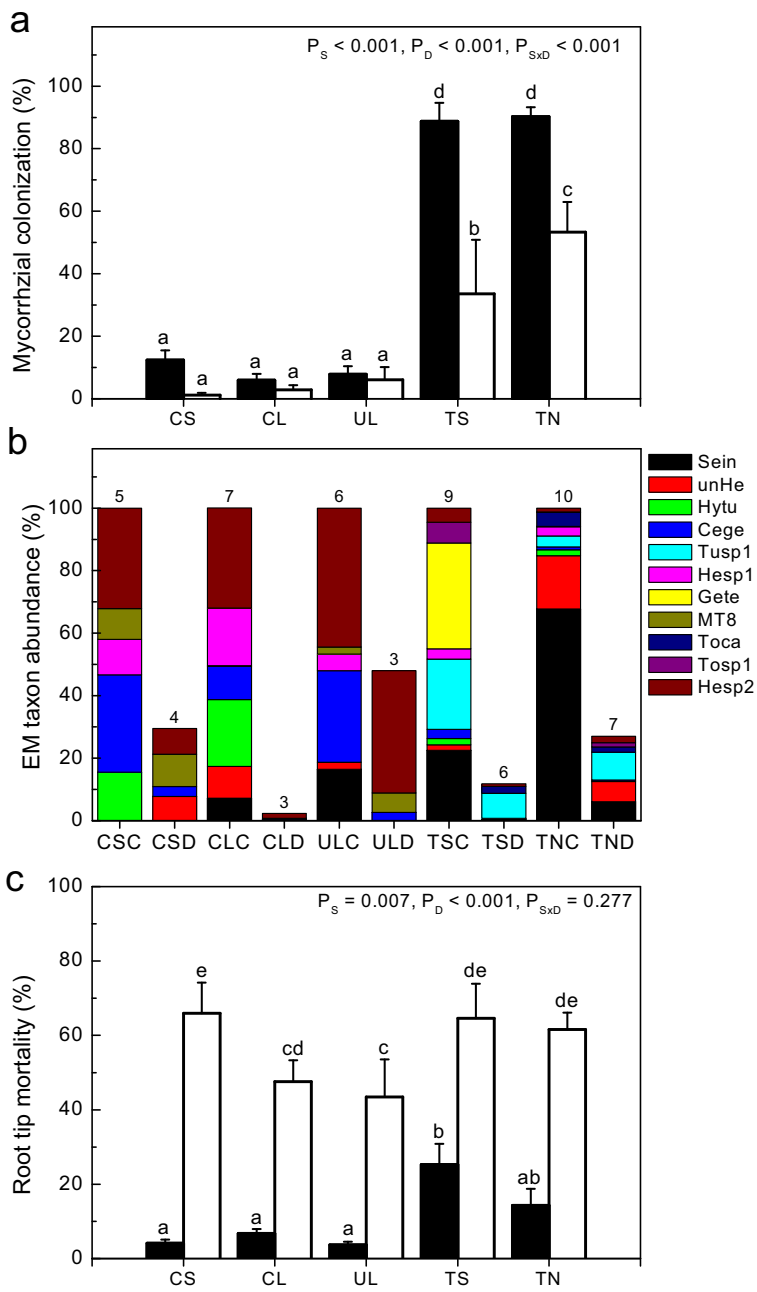


Fig. 3 Ectomycorrhizal colonization of root tips (% of vital root tips) (**a**) ectomycorrhizal species composition (% of mycorrhizal root tips of the controls) (**b**) and root tip mortality (% of all counted root tips) (**c**) of 1.5-year-old beech (*Fagus sylvatica*) trees raised in different soil types and exposed for 6 weeks to diminished water supply. Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, north exposition, TS = Tuttlingen, calcareous, south exposition) as described in detail in Table 1. Ectomycorrhizal species names: Sein: *Sebacina incrustans*, unHe: uncultured *Hebeloma*, Hytu: *Hydnotrya tulasnei*, Cege: *Cenococcum geophilum*, Tusp1: uncultured Tuber, Hesp1: uncultured Heliotales, Gete: *Geopora tenuis*, Toca: *Tomentella castanea* syn. *T. sublilacina*, Tosp1: uncultured Tomentella, Hesp2: Uncultured Helotiales. Further details are shown in supplement Table S1. *Bars* indicate means of $n = 5$ plants per treatment (± SE) of well irrigated controls (*black*) and drought-treated plants (*white*). Different letters indicate significant differences with $p \leq 0.05$

richness, photosynthesis, mycorrhizal colonization and ^{15}N recovery in fine roots (Table 3). Testing the relationship of ^{15}N uptake with EM species richness, photosynthesis, and mycorrhizal colonization by multiple correlation analyses revealed that species richness was the best and among the three variables the only parameter that remained after stepwise testing and explained 52 % of the variation (Fig. 5b).

Discussion

Soil types structure beech EM fungal assemblages

In agreement with our expectation, beech seedlings were colonized by different EM fungal assemblages when raised in different soil types. Many EM taxa detected here were also present on the roots of mature

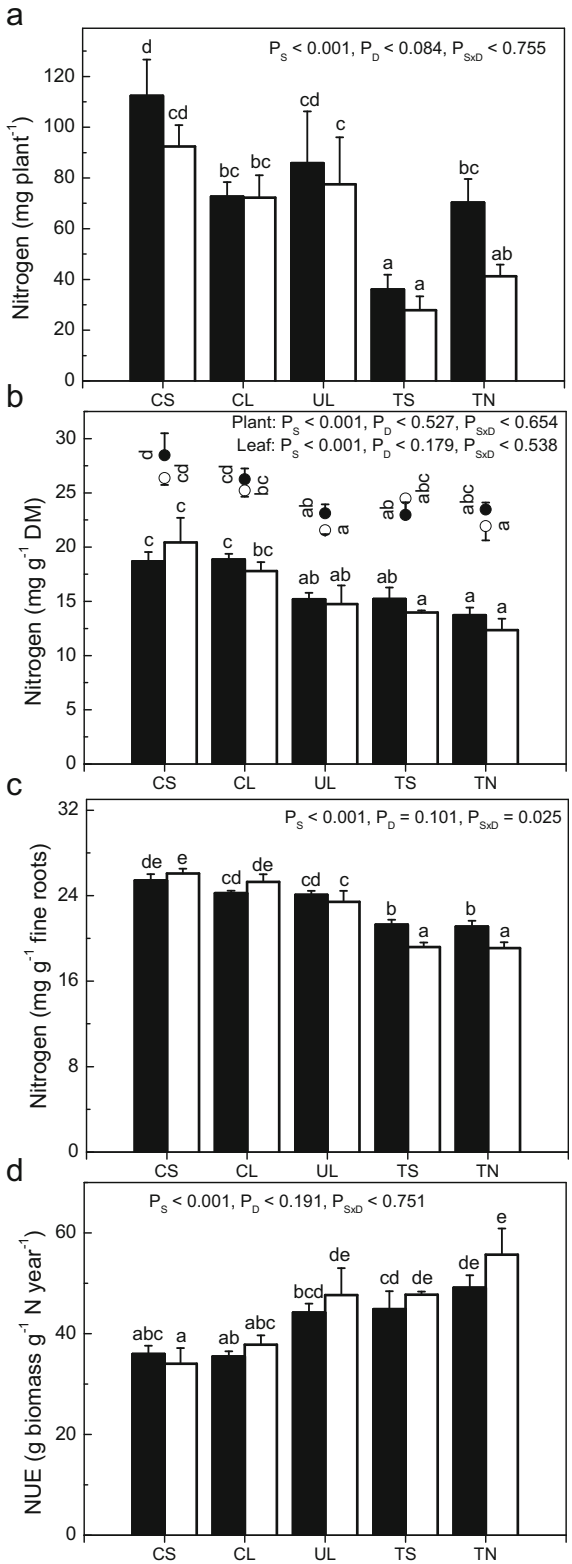
Table 2 Analysis of similarities (ANOSIM) among the ecomycorrhizal fungal communities. ANOSIM was conducted with the Bray Curtis indices as similarity measure of the mycorrhizal communities and 9999 permutations. *P* values were Bonferroni sequentially corrected. Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, north exposition, TS = Tuttlingen south exposition) and treatments (C = control, D = Drought). Bold letters indicate significant *P* values (<0.05)

	CSC	ULC	TSC	TNC	CLD	CSD	ULD	TSD	TND
CLC	0.673	0.384	**0.033**	**0.007**	**0.047**	**0.047**	**0.030**	**0.015**	0.059
CSC		0.278	**0.007**	**0.006**	0.063	0.188	0.128	**0.029**	**0.009**
ULC			**0.009**	**0.008**	**0.015**	**0.040**	**0.024**	**0.015**	**0.010**
TSC				**0.049**	**0.031**	**0.031**	**0.008**	0.068	0.221
TNC					**0.007**	**0.009**	**0.010**	**0.009**	**0.026**
CLD						1.000	0.930	0.624	0.089
CSD							0.921	0.436	0.097
ULD								0.166	**0.008**
TSD									0.319

and young beech trees in the TN and UL forests, for example *C. geophilum*, *Sebacina incrustans*, *Tomentella* sp., *Heliotales*, and *Tuber* sp. (Pena et al. 2010; Leberecht et al. 2015) indicating that the roots were colonized by members of the site-specific EM assemblages. Field studies along geographic and environmental gradients indicated that soil chemistry was an important factor for differences in EM species composition (Lilleskov et al. 2002; Kranabetter et al. 2009; Moeller et al. 2014; Walker et al. 2014; Pretzsch et al. 2014; Zavišić et al. 2016). The present results support this conclusion because potentially confounding effects of temperature and soil humidity were excluded. Other potentially interfering factors such as host species identity (Lang et al. 2011), progeny (Dučić et al. 2009), health and productivity (Druebert et al. 2009; Twieg et al. 2009; Pena et al. 2010; Horton et al. 2013; Pena et al. 2013a) were also excluded here because all plants were raised from the same seed lot and did not differ in photosynthesis, leaf area, growth, and biomass, with one

Table 3 Multiple variable analyses among ectomycorrhizal species richness (S), relative abundance of root tip colonization (EM col), relative abundance of dead root tips (mortality), net Photosynthesis (NP), $^{13}\delta C$ in fine roots (^{13}Cfr), whole plant biomass (WPB), whole plant nitrogen content (WPNC), mean plant N concentration (PN), leaf N concentration (LN), root N concentration (RN), nitrogen use efficiency (NUE), ^{15}N uptake rate (^{15}Nup) and relative ^{15}N recovery ($^{15}Nrec$). Lower diagonal shows the *P* values and upper diagonal Pearson correlation coefficients (R). Bold letters indicate significant R and *P* values (< 0.05)

	S	EMcol	Mortality	NP	^{13}Cfr	WPB	WPNC	PN	LN	RN	NUE	^{15}Nup	$^{15}Nrec$
S		**0.832**	**−0.388**	**0.557**	**0.322**	−0.120	−0.246	**−0.376**	−0.108	**−0.517**	**0.326**	**0.733**	0.559
EMcol	**0.000**		−0.074	**0.422**	**0.369**	−0.191	**−0.373**	**−0.451**	**−0.311**	**−0.643**	**0.460**	**0.722**	**0.412**
Mortality	**0.006**	0.613		**−0.557**	−0.135	**−0.356**	**−0.359**	0.035	−0.174	−0.151	0.060	**−0.393**	**−0.518**
NP	**0.000**	**0.008**	**0.000**		0.232	−0.013	−0.025	−0.038	0.159	−0.240	0.034	**0.555**	**0.458**
^{13}Cfr	**0.024**	**0.009**	0.357	0.155		−0.029	−0.038	0.071	−0.042	−0.012	−0.029	**0.317**	0.189
WPB	0.411	0.190	**0.012**	0.937	0.842		**0.909**	−0.106	0.078	0.181	0.079	−0.041	**0.498**
WPNC	0.089	**0.008**	**0.011**	0.882	0.797	**0.000**		0.274	**0.331**	**0.478**	**−0.312**	−0.149	**0.366**
PN	**0.008**	**0.001**	0.810	0.819	0.630	0.469	0.057		**0.555**	**0.742**	**−0.945**	**−0.295**	−0.238
LN	0.459	**0.030**	0.232	0.334	0.775	0.595	**0.020**	**0.000**		**0.454**	**−0.557**	−0.156	−0.062
RN	**0.000**	**0.000**	0.299	0.141	0.935	0.213	**0.001**	**0.000**	**0.001**		**−0.755**	**−0.308**	−0.141
NUE	**0.023**	**0.001**	0.681	0.839	0.841	0.588	**0.029**	**0.000**	**0.000**	**0.000**		0.249	0.195
^{15}Nup	**0.000**	**0.000**	**0.005**	**0.000**	**0.026**	0.779	0.307	**0.040**	0.285	**0.031**	0.085		**0.673**
$^{15}Nrec$	0.000	**0.003**	**0.000**	**0.003**	0.195	**0.000**	**0.010**	0.099	0.674	0.334	0.181	**0.000**	



◀ **Fig. 4** Nitrogen content (mg N plant^{-1}) (**a**), nitrogen concentrations of whole plant means (*bars*) and of leaves (*circles*) (mg g^{-1} dry mass) (**b**), nitrogen concentrations of roots (mg g^{-1} dry mass) (**c**) and nitrogen use efficiency (NUE, g biomass g^{-1} N year^{-1}) (**d**) of 1.5-year-old beech (*Fagus sylvatica*) trees raised in different soil types and exposed for 6 weeks to diminished water supply. Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, north exposition, TS = Tuttlingen, calcareous, south exposition) as described in detail in Table 1. *Bars* indicate means of $n = 5$ plants per treatment (±SE) of well irrigated controls (*black*) and drought-treated plants (*white*). Different letters indicate significant differences with $p \leq 0.05$

exception (TS). These observations are in agreement with field studies showing that beech is generally able to cope with a large pH gradient and different soil nutrient concentrations, maintaining for example similar leaf production rates across edaphic gradients (Leuschner et al. 2006; Meier et al. 2005).

Experimental manipulation of soil pH and Ca content by liming influenced the root tip EM colonization rate (Børja and Nilsen 2009; Dučić et al. 2009; Monfort-Salvador et al. 2015) and the EM communities, leading for instance to decreases in *C. geophilum* and increases in *Sebacina* sp. (Rineau and Garbaye 2009; Rineau et al. 2010). In our study *C. geophilum* was also less abundant on the roots of beech in calcareous soil than on the roots of trees in acid soil, whereas the opposite behavior was observed for *S. incrustans*. However, in the TN forest *C. geophilum*, a drought tolerant EM species (Herzog et al. 2013), was the most abundant taxon and *S. incrustans* rare. Knowledge on the ecology and physiological requirements of Sebacinales is quite limited (Oberwinkler et al. 2013). Our results indicate that the abundance of these fungi is controlled by soil types and other, yet unknown environmental factors.

Unexpectedly, here the beech seedlings in calcareous soil exhibited higher EM taxon richness and also higher root tip colonization than those in the acid soils. Because regression analyses showed that both root colonization and species richness decreased with decreasing photosynthesis, our results suggest that the colonization of the roots of the young trees is critically depending on recently assimilated carbon.

Host nitrogen use is intertwined with mycorrhizal colonization and soil-born stress

The impact of N on EM community structures has often been demonstrated (Talbot and Treseder 2010). The

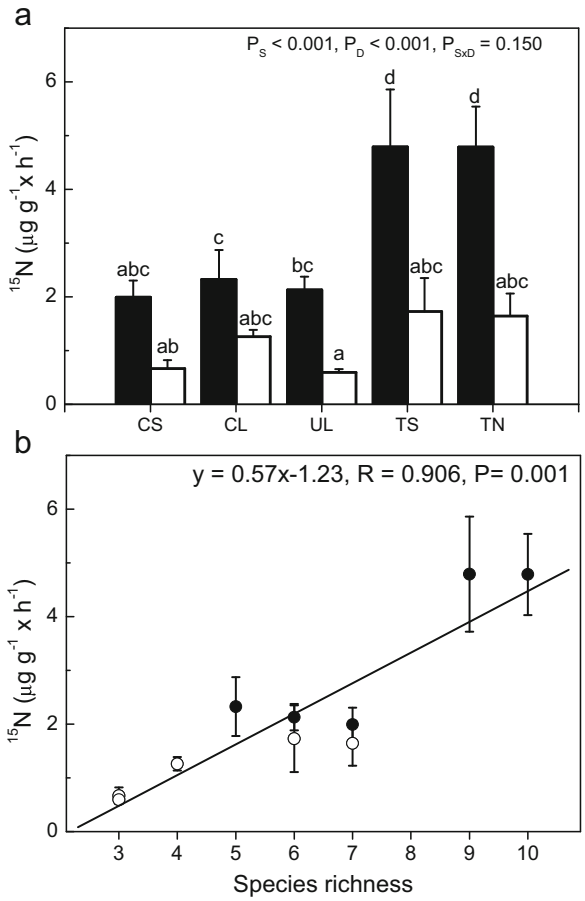


Fig. 5 ^{15}N uptake rate of roots (µg ^{15}N g^{-1} dry mass x h^{-1}) (**a**) and linear regression curve for the relationship between ectomycorrhizal species richness and ^{15}N uptake rate (**b**) of 1.5-year-old beech (*Fagus sylvatica*) trees raised in different soil types and exposed for 6 weeks to diminished water supply. ^{15}N was administered as ^{15}N-glutamine to the soil. Rates were calculated after correction for the natural ^{15}N abundance determined in unlabeled controls. Abbreviations indicate soil types (CL = Calvörde, clay, Calvörde, sand, UL = Unterlüß, clay, TN = Tuttlingen, calcareous, north exposition, TS = Tuttlingen, calcareous, south exposition) as described in detail in Table 1. *Bars* and *circles* indicate means of n = 5 plants per treatment (±SE) of well irrigated controls (*black*) and drought-treated plants (*white*). Different letters indicate significant differences with $p \leq 0.05$

negative relationship between EM species richness respective root colonization and plant N concentrations found here, therefore, suggest that nitrogen also played a major role affecting the establishment of EM host interactions in different soil types. Foliar concentrations of beech plants in the range of 19 to 23 mg g^{-1} dry mass of leaves, detected for beech plants in UL, TN and TS soils, and of >26 mg g^{-1} dry mass observed for the plants in CL and CS soils, indicate sufficient and luxurious N supply, respectively (Mellert and Göttlein 2012). At the first glance the high foliar N concentrations were surprising, but the threshold values have been determined for adult trees, whereas here seedlings were analyzed, which owing to their small size have a lower whole-plant N demand than larger trees. Furthermore, the N availability for beech plants in acid soil might have been higher than in the calcareous soils despite lower total soil N concentrations in the acid soils because the N mineralization potential is lower at high than at acid pH (Leuschner 1999; Andrianarisoa et al. 2009). Low plant N availability may require the aid of EM fungi to compete with soil microbes for soil N, but under certain conditions EM fungi can also withhold N from their host plants (Dučić et al. 2009; Näsholm et al. 2013; Pena et al. 2013b). Both mechanisms may increase NUE. Therefore, it is an open question whether lower N availability in calcareous soil of the TS and TN forests fostered higher root colonization to achieve "normal" N nutrition as indicated by the foliar N concentrations of the plants in calcareous soils or whether there was a trade-off with other edaphic stresses that promoted high EM colonization at the expense of lower plant N transfer. The latter stress theory has some support because we found higher root tip mortality despite similar soil humidity in unstressed plants and higher drought sensitivity of EM root tip colonization in calcareous than in acid soils. This stress was apparently stronger for plants in TS than in TN soil because only under these conditions significantly less biomass was produced and the highest root tip mortality was found in absence of drought stress. It is notable that the radial stem growth of the mature beech trees in the TS forest is lower than in the TN forest (Gessler et al. 2001), suggesting that in addition to climatic stresses and low N availability (Gessler et al. 2005), soil properties may have impinged on tree performance.

Drought sensitivity of beech N uptake correlates with EM taxon richness and root tip colonization, but not with whole plant nitrogen use efficiency

The inverse relationships of EM colonization with plant N concentrations and plant N uptake might appear contradictory at the first glance. In line with other studies, N uptake was strongly suppressed by drought (Gessler et al. 2005; Winkler et al. 2010; Danielsen and Polle 2014; Pena and Polle 2014), but the changes in the

amounts taken up were too small to affect plant N tissue concentrations or NUE. Whole plant N levels are the result of long-term processes reflecting the conservative nutrient and biomass allocation strategy of beech, which is characterized by a strong increment in N in leaves during the early expansion phase and a subsequent decrease until the leaves are fully expanded (Bauer et al. 1997). Since beech usually forms only one flush at the beginning of the season, the N content of the leaves remains quite stable after leaf maturation and is unaffected by precipitation gradients (Meier et al. 2005; Meier and Leuschner 2014). Correspondingly, the N content, NUE and foliar concentrations were not affected by drought in this pot experiment.

Nitrogen uptake processes at the root level are regulated by root physiology and availability of different N sources in the soil (Rennenberg et al. 2009). In mixtures of inorganic (NH_4^+, NO_3^-) and organic N (glutamine, arginine) preferential ^{15}N uptake from glutamine was found for beech roots in the TN forest (Dannenmann et al. 2009). Similarly, non-mycorrhizal beech roots also preferred glutamine and suppressed uptake of inorganic N in mixtures (Kreuzwieser et al. 1997; Stoelken et al. 2010; Winkler et al. 2010). Although ^{15}N was derived from glutamine in those experiments as well as in the present one, the main uptake form of N into the roots is not known. In field studies (Finzi and Berthrong 2005; Dannenmann et al. 2009, the present study), fast turn-over processes in the soil cannot be excluded. Application of ^{13}C dual-labelled glutamine showed that even beech roots in artificial soil solutions did not acquire corresponding $^{13}C/^{15}N$ amounts (Winkler et al. 2010). The reasons could either be rapid degradation of glutamine before root uptake or whole glutamine uptake and respiratory loss of the ^{13}C label (Näsholm et al. 2009).

EM fungi can grow on various organic substances, but glutamine was the only compound efficiently utilized by all fungi tested (Talbot and Treseder 2010). Our results suggest that EM fungi play a major role in ^{15}N acquisition from glutamine and transfer to the host because we observed only a relatively low ^{15}N uptake by roots with low EM colonization and a strong correlation of ^{15}N uptake with EM species richness. The uptake of ^{15}N by diverse EM communities varies with their species composition and environmental constraints (Pena and Polle 2014; Leberecht et al. 2015). The finding that the multiple correlation analyses of ^{15}N uptake with EM taxon richness, mycorrhizal colonization and photosynthesis extracted EM taxon richness as the main explanatory factor further supports that the community structure may have controlled ^{15}N uptake. Apparently, the properties of the EM assemblage were overriding the impact of photosynthesis, which is required for energy supply. Because of the strong co-variation of mycorrhizal colonization and species richness, these effects cannot be separated. The strong drought-induced reductions in N uptake of the plants in calcareous soil at similar levels of soil humidity as in acid soil suggest high lability of N supply of the young plants in these soils. The limitations of N uptake also resulted in small, but significant decreases in root N tissue concentrations under drought in the calcareous soils. The reductions in N uptake were stronger in TS than in TN soil indicating that drought constrained plant N nutrition in this soil type more strongly than in those from the other forests.

In conclusion, we showed that (i) beech roots in different soils were colonized by taxonomically different EM assemblages, (ii) that high EM colonization and species richness correlated with high ^{15}N uptake from glutamine and increased NUE, and that (iii) glutamine-derived ^{15}N uptake was more susceptible to drought in calcareous than in acid soils, whereas NUE was unaffected by drought. It is clear that under field conditions further factors such as high temperatures (Williams et al. 2013), which enhance N mineralization (Gessler et al. 2005) and re-wetting events, which can potentially compensate for N deprivation (He and Dijkstra 2014), affect tree N availability and productivity. Although the results of this relatively short-term pot study cannot be directly applied to explain field observations, our findings enhance our understanding of the mechanisms impinging on beech performance under drought. The interaction of soil type and ecosystem functions of EM communities should be tested in forests along larger geographical gradients because these factors may have important implications for management decisions, when forest ecosystems have to be converted to withstand a future, more arid climate.

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Compliance with ethical standards

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

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