

Does excess nitrogen supply increase the drought sensitivity of European beech (*Fagus sylvatica* L.) seedlings?

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Abstract Climate change and atmospheric deposition of nitrogen affect biodiversity patterns and functions of forest ecosystems worldwide. Many studies have quantified tree growth responses to single global change drivers, but less is known about the interaction effects of these drivers at the plant and ecosystem level. In the present study, we conducted a full-factorial greenhouse experiment to analyse single and combined effects of nitrogen fertilization (N treatment) and drought (D treatment) on 16 morphological and chemical response variables (including tissue $\delta^{13}\text{C}$ signatures) of one-year-old *Fagus sylvatica* seedlings originating from eight different seed families from the Cantabrian Mountains (NW Spain).

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Drought exerted the strongest effect on response variables, reflected by decreasing biomass production and increasing tissue $\delta^{13}\text{C}$ signatures. However, D and N treatments interacted for some of the response variables, indicating that N fertilization has the potential to strengthen the negative effects of drought (with both antagonistic and amplifying interactions). For example, combined effects of N and D treatments caused a sevenfold increase of necrotic leaf biomass. We hypothesize that increasing drought sensitivity was mainly attributable to a significant reduction of the root biomass in combined N and D treatments, limiting the plants' capability to satisfy their water demands. Significant seed family effects and interactions of seed family with N and D treatments across response variables suggest a high within-population genetic variability. In conclusion, our findings indicated a high drought sensitivity of Cantabrian beech populations, but also interaction effects of N and D on growth responses of beech seedlings.

Keywords Cantabrian mountains · Global change · Interaction effects · Spain · Tissue $\delta^{13}\text{C}$ signature

Introduction

Climate change and atmospheric deposition of nitrogen (N) are key drivers of biodiversity loss and shifts in ecosystem functioning on regional and global scales

(Vitousek et al. 1997; Sala et al. 2000). The deleterious effects of these drivers are of particular interest in forest ecosystems, since forests provide important ecosystem services, play a crucial role in the global carbon cycle and host a system-specific biodiversity (Peñuelas et al. 2008).

Recent surveys demonstrated that temperate forest ecosystems in Europe were sensitive to changes in the meteorological and chemical climate observed in recent decades, but responses differed with regard to site conditions, stand structure and tree species (Friedrichs et al. 2009; Pretzsch et al. 2014; Ruiz-Benito et al. 2014). Many stands showed accelerated growth rates (e.g. in terms of basal area increment, stand volume growth and carbon accumulation) and still followed general allometric rules, but simultaneously proceeded more rapidly through species-specific growth trajectories (i.e. age-related shifts in allometric relationships; Pretzsch et al. 2014; Ruiz-Benito et al. 2014). More frequent and intense drought events (IPCC 2013), however, are expected to adversely affect carbon and water cycles of forest ecosystems and may impose constraints on growth and competitiveness of tree species that are considered susceptible to drought events (Thomas 2000; Geßler et al. 2007; Grossiord et al. 2014).

Airborne N loads have tripled since 1860 and are expected to further increase in the coming decades (Galloway et al. 2004). In forest ecosystems, N deposition is considered responsible for the increase of productivity which has been observed in recent decades, because growth in forest ecosystems is often limited by the availability of N (Rennenberg et al. 1998; Pretzsch 1999; Nadelhoffer 2000). Moreover, N deposition may mediate biomass allocation in trees and the sequestration of carbon in forest soils (Högberg 2007; Magnani et al. 2007; de Vries et al. 2009). Long-term N loads have been shown to alter soil nutrient cycling and to promote soil acidification and leaching of nitrate and soil cations (Magill et al. 1997; Aber et al. 1998; Rennenberg et al. 1998).

Despite a growing body of literature with a focus on the single effects of the abovementioned global change drivers on forest ecosystems (see recent overviews on the effects of climate change on tree growth and mortality: Allen et al. 2010; effects of N deposition on plant diversity: Bobbink et al. 2010), only few studies have analysed the interactive effects of co-occurring global change drivers (Högberg et al.

1993; Nilsen 1995; Yang et al. 2013). This applies to climate change and N deposition in particular, because little is known about their interaction at the individual and ecosystem level. As a consequence, many recent studies have emphasized the need for multi-factor analyses in order to better understand and predict the possible impacts of co-occurring global change drivers on ecosystem functions (Lindenmayer et al. 2010; Ochoa-Hueso et al. 2014). Yang et al. (2013) analysed single and combined effects of drought and warming on the growth and nutritional status of *Abies fabri*, a fir species typical of the eastern Tibetan Plateau. The authors found that both factors negatively affected seedling growth, but adverse effects were intensified when both factors acted simultaneously (i.e. mutual amplification). In contrast, effects of drought and warming showed no significant interaction in a model ecosystem experiment with different oak provenances (Kuster et al. 2013). Results from process-based ecosystem models identified atmospheric N deposition in combination with rising carbon dioxide levels as the most explanatory factors for the net carbon storage capacity of European forest ecosystems (Churkina et al. 2010). Meyer-Grünefeldt et al. (2015) demonstrated (at the example of the dwarf shrub *Calluna vulgaris*) that N fertilization has the potential to increase the plants' shoot:root ratios and thus increase the risk of severe water shortage during periods of drought. This, in turn, suggests non-additive effects between N deposition and climate change components such as drought events (Norby 1998).

The objective of the present study was to analyse the interaction effects of N fertilization and drought using seedlings of European beech. *Fagus sylvatica* is the most abundant and dominant broad-leaved tree species in Central European forests and thus of particular importance from an ecological and economic point of view (Ellenberg and Leuschner 2010). European beech is considered sensitive to climate shifts such as increasing summer temperatures or drought events (Thomas 2000; Meier and Leuschner 2008; Friedrichs et al. 2009; Scharnweber et al. 2011). Decreasing competitiveness of beech trees resulting from drought has been attributed to mechanisms such as decreasing productivity, shifts in biomass and carbon allocation patterns, pre-senescent leaf shedding, fine root dieback and a deterioration of the trees' nutritional status (Pretzsch 1999; Pretzsch and Dursky 2002; Peuke and Rennenberg 2004; Rose et al. 2009;

Härdtle et al. 2013). Within its European distribution area, *Fagus sylvatica* covers a broad range of site conditions, and hence is characterized by a high genotypic variation and phenotypic plasticity (Peuke and Rennenberg 2004; Rose et al. 2009). Genotypic plasticity and allelic richness are expected to be particularly high in glacial refuges of *Fagus sylvatica*, for example on the Balkan or the Iberian Peninsula (Widmer and Lexer 2001, Magri et al. 2006). Thus, these populations may play an important role in the context of diversity conservation and the selection of proper genotypes for forestry under the prospect of a drier and warmer climate (Hampe and Petit 2005; Jump et al. 2006; Rose et al. 2009; Hampe and Jump 2011). *Fagus sylvatica* populations of the Cantabrian Mountains (NW Spain) belong to the species' southwestern range margin, and the area is considered one of its glacial refugia on the Iberian Peninsula (Magri et al. 2006). It is therefore of interest, how sensitive beech populations of the Cantabrian Mountains (including different seed families as an expression of the within-population's genetic variability) respond to drought. Although several studies have investigated the drought sensitivity of beech provenances along precipitation gradients in Central Europe and the Mediterranean region (see Rose et al. 2009), there is yet no study with a focus on beech populations of the Cantabrian Mountains. Given that both climate change and N deposition will affect forest ecosystems and tree growth in the course of this century (Sala et al. 2000) it is further of interest, whether the drought sensitivity of beech trees might interact with increasing N availability.

To this end, we conducted a greenhouse experiment with beech seedlings originating from eight seed families of a population originating from the Cantabrian Mountain. Our experiments comprised a full-factorial combination of N fertilization and drought treatments, and we measured a total of 16 response variables describing the plants' morphology (such as height, stem diameter, dry weight of leaves, stems and roots), their nutritional status (such as carbon (C) and nitrogen (N) concentrations of leaves and roots) and transpirational demands (using $\delta^{13}\text{C}$ signatures of leaves and roots as a proxy; Kleinebecker et al. 2009; Mölder et al. 2011). We hypothesized that (i) N fertilization and drought would non-additively affect the seedlings' biomass production, and (ii) responses of seedlings would differ for the different seed

families as an expression of their within-population genetic variability.

Materials and methods

Plant material

Fagus sylvatica seeds were collected on north-facing slopes in the Cantabrian Mountains (NW Spain) in autumn 2009 (Fig. 1; site characteristics see Table 1). To relate global change impacts to the within-population genetic variability of beech trees, seeds were collected from eight different mother trees (i.e. eight seed families) growing in near-natural forest stands in an area measuring about 10 km \times 40 km. Since our study did not intend to analyse seed family characteristics in relation to the variability of natural site conditions (resulting from local adaptations), mother trees were selected at sites that were considered as homogeneous as possible with regard to soil morphology and chemistry (soil type: humic cambisols; chemical soil properties based on means of four samples (± 1 SE): litter layer depth: 8.6 cm (1.8), $\text{pH}_{(\text{H}_2\text{O})}$: 4.3 (0.5), C:N ratio: 14.2 (1.0), cation exchange capacity: 9.9 cmol kg^{-1} (2.0); data from Marcos et al. 2010; plant community: Blechno spicanti-Fagetum sensu Rivas-Martinez 1963). The climatic conditions of the forest locations are summarized in Table 1.

Seedling cultivation and treatments

After stratification of the seeds in winter 2009/2010, seeds were planted in small pots (so-called Jiffy Strips, Meyer KG, Rellingen, Germany; two seeds/pot), filled with a germination substrate (TKS 1, Floragard, Oldenburg, Germany) in a greenhouse at the Thünen-Institute (Hamburg, Germany) in spring 2010. Seedlings emerged at the beginning of May 2010 and were then pricked out and transplanted into circular plastic pots (one seedling per pot with 1L of volume) with standard tree cultivation substrate (TKS 2, Floragard, Oldenburg, Germany). A total of 40 pots per seed family were randomly assigned to the four different treatments control, drought, nitrogen and nitrogen plus drought treatment (i.e. 10 pots per treatment; treatments henceforth referred to as C, D, N and ND treatments; total n of pots/seedlings: 8 seed

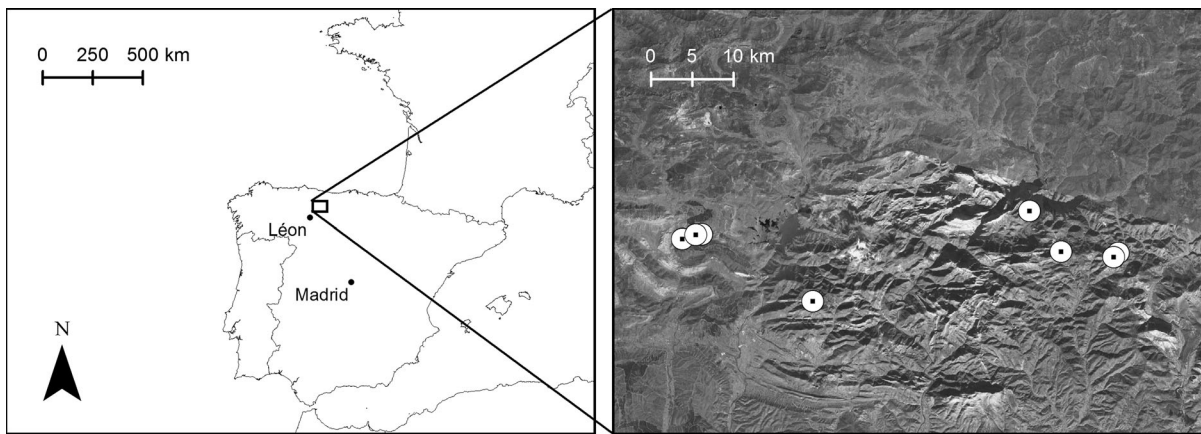


Fig. 1 Locations of the seed origin areas in the Cantabrian Mountains in NW Spain

Table 1 Climatic conditions for the seed family locations

Seed family	Elevation a.s.l. (approximate)	Mean annual temperature	Mean annual precipitation	Mean precipitation in the growing season
1	1375 m	8.8 °C ^b	1221 mm ^b	417 mm ^b
2	1400 m	10.1 °C ^a	920 mm ^a	333 mm ^a
3	1275 m	8.8 °C ^b	1221 mm ^b	417 mm ^b
4	1150 m	9.1 °C ^c	1279 mm ^c	432 mm ^c
5	1375 m	10.1 °C ^a	920 mm ^a	333 mm ^a
6	1300 m	8.8 °C ^b	1221 mm ^b	417 mm ^b
7	1300 m	10.1 °C ^a	920 mm ^a	333 mm ^a
8	1300 m	10.1 °C ^a	920 mm ^a	333 mm ^a

Distances between weather stations and sampling sites ranged between 5 and 11 km; description of soil morphological and soil chemical properties of sampling sites see method section

^a Weather station in Boñar from 1987 to 2006

^b Weather station in Boca de Huergano from 1988 to 2007

^c Weather station in Prioro from 1987 to 2006 (Instituto Nacional de Meteorología, España)

families \times 4 treatments \times 10 replicates = 320). Pots in the C and N treatments were well watered during the experiment to avoid drought effects (40 % soil water content (weight percentage), kept by means of a daily surveillance of the water status of all pots during the experiment). Seedlings in the D and ND treatments were subjected to one severe drought period, during which no watering took place and the soil water content was reduced to 10 % (weight percentage). This drought event lasted for 3 weeks and took place in August 2010 (i.e. in correspondence with the seasonal occurrence of drought events in the natural environment). The two soil moisture levels (40 and 10 %) were roughly equivalent to 20 and 5 vol%, respectively. Soil water reduction in the D and ND

treatments was quantified by daily weighing the pots during the drought event. After the drought, plants were again regularly watered (i.e. 40 % soil water content). The strength of the drought was chosen for two reasons: First, a reduction of the soil water content to 10 % corresponds with soil water losses in upper soil layers after summer drought events in the natural environment (Leuschner 2002). Second, the strength of the drought event in our experiment coincided with experiments with 1-year-old beech seedlings conducted by Rose et al. (2009), which allows for a comparison of experimental outcomes. In the N and ND treatments, N was applied (as NH_4NO_3) in a quantity equivalent to $50 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (as solution in deionized water). This treatment strength

was chosen to simulate the effects of airborne N loads which some areas with beech forest ecosystems in W and NW Europe currently receive (with 50 kg N ha⁻¹ year⁻¹ representing the upper range limit of current deposition rates; Galloway et al. 2004, Bobbink et al. 2010). Nutrient solutions were applied biweekly from July 15 to September 15 (except for the drought period in the D and ND treatment). The mean temperature in the greenhouse was 17.5 °C and the relative humidity was 77 % during the course of the experiment (July 22 to October 6). All pots were randomly relocated every 4 weeks to avoid position effects.

Biomass harvest and measurement of response variables

All seedlings were harvested at the end of the experiment (October 8, 2010) and the following morphological and growth-related variables were measured: stem diameter (measured 5 cm above the root collar in N–S and E–W direction in mm), plant height (measured from the root collar to the top in cm), total number of leaves and number of necrotic leaves and specific leaf area (SLA in m² kg⁻¹; after scanning all leaves of five randomly chosen individuals out of each treatment and seed family). The root biomass was sampled by carefully cleaning roots from adhered soil material (using a sieve) until all soil residues were removed. All biomass samples (shoots, leaves and roots) were dried at 40 °C for 3 days (until weight constancy), subsequently weighed and the following variables determined: leaf biomass (all leaves), aboveground biomass (shoots and leaves), biomass of necrotic leaves, root biomass and shoot:root ratios (aboveground biomass:belowground biomass ratio).

For chemical analyses (biomass C and N concentrations; tissue $\delta^{13}\text{C}$ signatures) all biomass samples were grinded in a centrifugal mill (ZM 200, Retsch, Haan, Germany) and re-dried at 40 °C for 3 days. The values for the C and N concentrations of the samples were corrected for the remaining water content compared to samples dried at 105 °C. C and N concentrations of leaves and roots as well as tissue $\delta^{13}\text{C}$ signatures of leaves and roots were measured using a continuous flow elemental analyzer-isotope mass spectrometer (vario EL cube, Elementar, Hanau, Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK). Biomass element concentrations were given in g kg⁻¹ biomass dry weight

(dw). In addition, we calculated the C:N ratios from the C and N concentrations of leaves and roots. Isotope signatures were presented in the delta (δ) notation (in per mil; ‰) as a relative deviation from an international standard (Pee Dee Belemnite). The relative precision of repeated analyses of IAEA standards (IAEA-CH-3) was ± 0.1 ‰.

Data analysis

Treatment effects on response variables were tested using linear models (LM) with N, D and seed family (henceforth referred to as “Family”) as fixed factors. Each LM included the single factors and the respective interaction terms. Comparisons of means of response variables related to treatments were analysed with an ANOVA combined with a Tukey’s post hoc test (note that analyses of treatment interactions (e.g. N \times D) are shown in Table 2, and combined effects of treatments (ND effects) are shown in Figs. 2, 3). Model residuals were checked for normality (Q–Q plots) and homogeneity of variances (Levene’s test). To meet these prerequisites, the following response variables were log-transformed: stem diameter, height, aboveground and belowground biomass, and shoot:root ratios. The magnitude of treatment effects (MTE) on response variables was calculated as $\text{MTE} = (\bar{x}_t - \bar{x}_c)/\bar{x}_c$, where \bar{x}_t is the average absolute value of a response variable in the treatment N, D or ND and \bar{x}_c is the average absolute value of a response variable in the control (Karban and Huntzinger 2006). All statistical analyses were conducted with SPSS Statistics 22.0 (SPSS Inc.; Chicago, IL, USA). Figures were created with ArcGIS 10.2.1 (Esri Inc.; Bonn, Germany) and R 3.1.2 (R Project for Statistical Computing; <http://www.R-project.org>).

Results

Treatment effects on morphological response variables

The drought event significantly decreased stem diameter, plant height and biomass production (leaf, aboveground, root), and significantly increased the shoot:root ratio of beech seedlings (Table 2; Fig. 2). Single D effects were particularly evident for aboveground and root biomass, with a significant drought-induced decline of 15 and

Table 2 Treatment effects on morphological and chemical response variables of beech seedlings from eight seed families

Morphological variables	Stem diameter		Height		Biomass of leaves (all)		Aboveground		Necrotic leaves		Roots		Shoot:root ratio		SLA	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
N	1.531	0.217	4.127	0.043	0.986	0.322	0.668	0.414	2.852	0.092	2.850	0.092	0.712	0.399	0.527	0.469
D	107.375	<0.001	7.140	0.008	22.687	<0.001	28.412	<0.001	15.813	<0.001	101.987	<0.001	15.954	<0.001	0.292	0.590
Family	11.996	<0.001	6.198	<0.001	17.604	<0.001	13.286	<0.001	5.392	<0.001	19.604	<0.001	13.082	<0.001	2.337	0.028
N × D	5.235	0.023	2.294	0.131	4.118	0.043	3.874	0.050	2.852	0.092	3.006	0.084	0.501	0.480	0.085	0.771
N × Family	1.646	0.122	0.526	0.815	1.456	0.183	1.068	0.384	1.156	0.328	2.016	0.053	0.769	0.614	0.423	0.886
D × Family	1.174	0.317	1.034	0.407	1.041	0.402	0.203	0.985	5.490	<0.001	2.072	0.047	3.111	0.004	1.577	0.148
N × D × Family	1.028	0.412	1.301	0.250	0.256	0.970	0.689	0.682	0.871	0.530	1.049	0.397	1.785	0.090	1.048	0.401
Chemical variables	C concentration of															
	N concentration of				C:N ratio of				$\delta^{13}C$ signature of							
	Leaves		Roots		Leaves		Roots		Leaves		Roots		Leaves		Roots	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
N	6.498	0.011	7.275	0.007	12.111	<0.001	56.723	<0.001	15.742	<0.001	58.194	<0.001	0.194	0.660	3.213	0.074
D	44.770	<0.001	15.085	<0.001	18.164	<0.001	53.768	<0.001	25.160	<0.001	39.489	<0.001	65.281	<0.001	86.112	<0.001
Family	10.927	<0.001	38.463	<0.001	8.788	<0.001	19.786	<0.001	7.614	<0.001	15.854	<0.001	37.483	<0.001	36.017	<0.001
N × D	7.277	0.007	0.073	0.787	15.254	<0.001	0.104	0.747	7.597	0.006	1.041	0.308	0.009	0.926	0.363	0.547
N × Family	2.699	0.010	2.229	0.032	7.692	<0.001	9.915	<0.001	6.638	<0.001	10.475	<0.001	3.526	0.001	6.667	<0.001
D × Family	1.892	0.071	9.244	<0.001	4.619	<0.001	4.199	<0.001	4.363	<0.001	4.339	<0.001	2.046	0.049	3.105	0.004
N × D × Family	1.776	0.092	7.464	<0.001	4.093	<0.001	1.069	0.383	3.365	0.002	0.828	0.565	0.893	0.512	1.479	0.174

N nitrogen fertilization, D drought treatment, Family seed families, SLA specific leaf area

Significant differences ($P < 0.05$) are marked in bold type

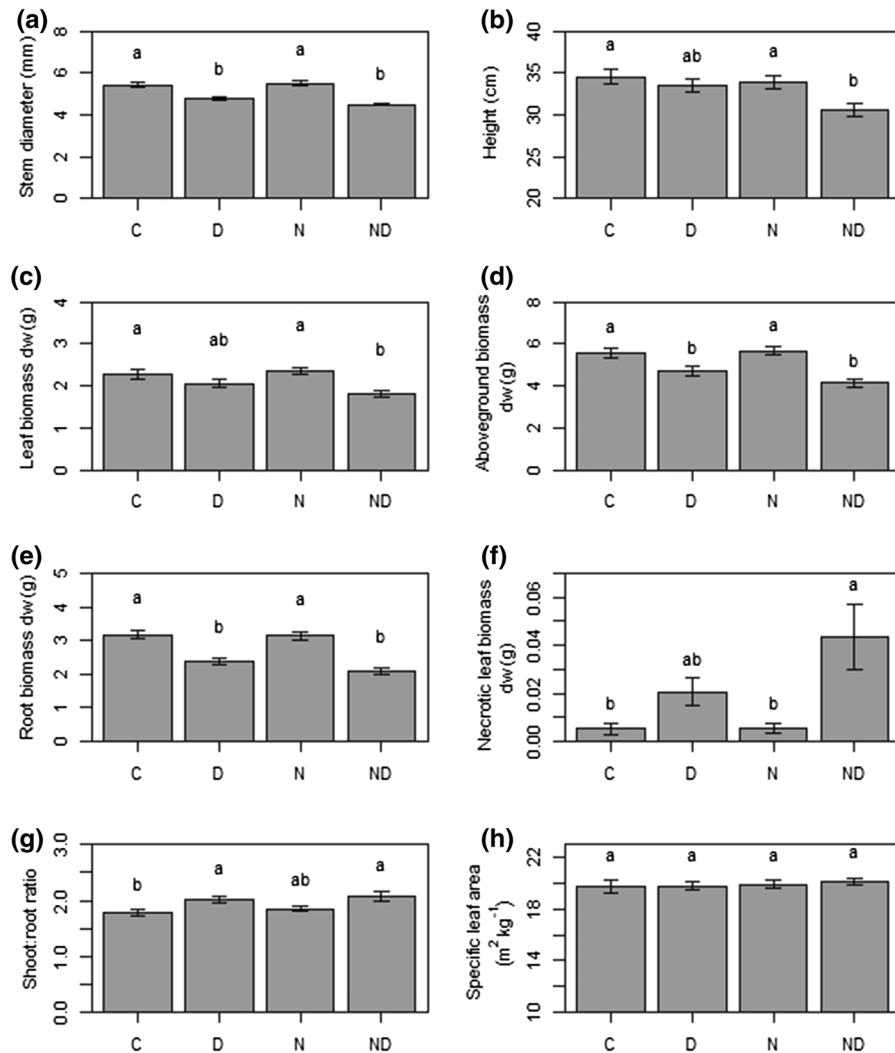


Fig. 2 Treatment effects on morphological response variables of beech seedlings. *Different letters indicate significant differences* ($P < 0.05$); (Tukey's post hoc test; error bars show ± 1 SE)

25 %, respectively, as compared to the control treatment (Appendix Table 3). SLA was not affected by drought. The effect of single N treatments was only significant for plant height (Table 2), resulting in a small reduction (-2 %) of seedling height in comparison to the control.

Although the negative growth responses of plants in the ND treatment were similar as in the D treatment, we found significant $N \times D$ interactions for stem diameter and leaf biomass, and marginally significant $N \times D$ interactions for aboveground, root and necrotic leaf biomass (Table 2). Interaction effects were antagonistic, with the exception of the necrotic leaf biomass, where interactions were amplifying. For example, non-additive effects of N and D reduced root

biomass by about 34 % (Appendix Table 3), and induced a sevenfold increase of the biomass of necrotic leaves. Thus, N fertilization tendentially strengthened the negative effects of drought.

Interestingly, we found significant Family-effects across all the response variables measured, either as main effect, or in interaction with the D treatment (Table 2).

Treatment effects on chemical response variables

In comparison to the control, single D treatments significantly increased leaf C (+2 %) and root N (+15 %) concentrations, and decreased the C:N ratios

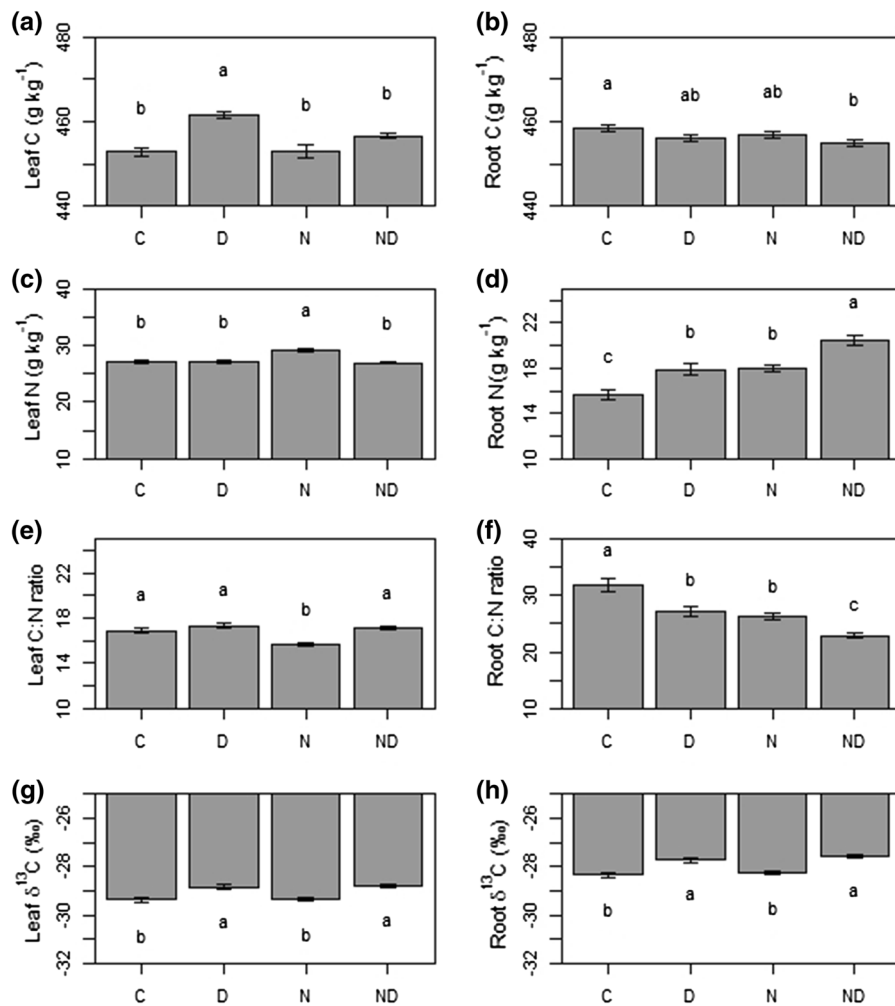


Fig. 3 Treatment effects on chemical response variables of beech seedlings. Different letters indicate significant differences ($P < 0.05$); (Tukey's post hoc test; error bars show ± 1 SE)

of the roots (-15% ; Appendix Table 3). Moreover, we found a significant increase in the leaf and root $\delta^{13}\text{C}$ signatures as a result of D treatments with a mean increase of 0.53 and 0.60 ‰ in leaf and root tissue, respectively (Fig. 3). As expected, N treatments significantly increased tissue N (leaves: $+7\%$, roots: $+15\%$), and thus lowered tissue C:N ratios (leaves: -7% , roots: -17% ; Appendix Table 3) compared to the control.

We found significant $N \times D \times \text{Family}$ interactions for the C concentration of roots and the N concentration and C:N ratio of leaves. Interestingly, root N concentrations (Fig. 3) were highest in the ND treatment (20.5 g kg^{-1}) and lowest in the control (15.6 g kg^{-1}).

Again, we found highly significant Family-effects across all the chemical response variables measured, which interacted with N ($N \times \text{Family}$), with D ($D \times \text{Family}$) or with N and D (see description of $N \times D \times \text{Family}$ interactions above).

Discussion

Drought effects on response variables

The drought event exerted the strongest effect on all response variables indicated by P values < 0.001 ; with

the exception of the SLA. This was reflected in significant reductions of the aboveground and belowground biomass, and increasing tissue $\delta^{13}\text{C}$ signatures. Since the negative effect of drought was stronger for belowground than for aboveground biomass production, we observed an increase in shoot:root ratios with drought. This finding concurs with studies from Meier and Leuschner (2008) and Rose et al. (2009), in which both young and mature beech trees showed stronger belowground than aboveground responses to drought events, with these findings attributed not only to a drought-induced dieback of the trees' fine and coarse roots, but also to a reduced fine root biomass production in dry soils. Although beech provenances from several sites from the Iberian Peninsula showed morphological adaptations to summer drought events (García-Plazaola and Becerril 2000), the population in our study proved to be highly drought sensitive. This finding suggests that the selection of drought resistant ecotypes at a given site is likely related to the precipitation patterns typical of this site (Peuke and Rennenberg 2004). In our study, beech seeds were collected on north-facing slopes in the Cantabrian Mountains and thus at sites with higher summer precipitation (see Table 1). As a consequence, seedling responses to drought in our study were well comparable with the responses found for Central European beech populations (Thomas 2000; Löff et al. 2005; Rose et al. 2009).

N fertilization effects on response variables

With regard to the N treatment, we expected more pronounced positive responses of growth-related variables compared to those observed in our study. The weak effects found for single N treatments might be attributable to a high plant-available N_{inorg} concentration in the germination substrate (as, for example, indicated by relatively low leaf C:N ratios in the control; cf. Figure 3; Anderson (1973), Aranda et al. 2004). The lowered height growth of plants in the N treatment (Table 2) might be attributable to the fact that there was no competition for light between potted seedlings, since seedling height growth, to improve light foraging, is mainly fostered by competition (Beaudet and Messier 1998; Runkle and Yetter 1987). However, fertilization with N caused significant interaction effects with drought (cf. $\text{N} \times \text{D}$ interactions).

Interaction effects between N fertilization and drought

Drought interacted with N fertilization for some of the response variables, indicating that the effects of drought were at least partly mediated by N fertilization. For example, we found antagonistic effects of $\text{N} \times \text{D}$ on the stem diameter and the leaf biomass, and marginally significant effects on the aboveground (antagonistic) and necrotic leaf biomass (amplifying). This supports our first hypothesis, according to which we expected non-additive effects of drought and N fertilization. This indicates that N fertilization has the potential to strengthen the negative effects of drought on the growth of beech seedlings, although the differences of the D and ND effects on response variables were non-significant in the post hoc test. Our result is in agreement with a study by Nilsen (1995), in which N fertilization also interacted with drought events in an experiment with saplings of Norway spruce. Nilsen (1995) found that N fertilization strongly increased the saplings' water consumption, making them more susceptible for stress during drought events. Negative interaction effects of N fertilization and drought were also reported for plants of other life forms, for example for grasses and dwarf shrubs (Friedrich et al. 2012; Meyer-Grünefeldt et al. 2015). In these studies, increasing drought sensitivity was mainly related to an increase of shoot:root ratios of N-fertilized plants, which in turn resulted in higher transpirational demands (Meyer-Grünefeldt et al. 2015). This response is not supported by our data, because N-fertilized plants only showed a slight, but not significant increase of their shoot:root ratios. However, combined effects of N and D reduced the plants' belowground biomass production (effect size: -34.3%), which might have affected their capability to satisfy water demands and thus intensifying the water shortage resulting from the drought event. Although the (non-additive) $\text{N} \times \text{D}$ interaction effect on the belowground biomass proved to be only marginally significant, the responses indicate that joint N fertilization and drought in tendency caused a stronger reduction of the belowground biomass as compared to the sole effect of the D treatment. This finding might be attributable to the fact that N fertilization can increase the biomass of very fine roots, which in turn are more sensitive to drought stress (Meier and Leuschner 2008; Noguchi et al.

2013). Increased fine root mortality thus could have contributed to the lower root biomass in the ND treatment, and this was not compensated for by additional root growth (as indicated by the high root N concentrations in the ND treatment).

Family-effects on response variables

Seed family significantly affected all the response variables analysed. This provides evidence in support of our second hypothesis that beech individuals of different seed families differ with regard to morphological and physiological properties as an expression of their genetic variability. In our study, Family not only proved to be an important single-factor, but also interacted with D and N treatments (i.e. $D \times \text{Family}$, $N \times \text{Family}$, $N \times D \times \text{Family}$). This indicated that tree individuals of the different seed families responded differently to drought and N fertilization. For example, the formation of necrotic leaf tissue as a result of drought differed significantly between seed families, an indication of family-related differences in drought sensitivity. This was also mirrored by significant interactions between D and Family for tissue $\delta^{13}\text{C}$ signatures, indicating that stomatal limitation varied across seed families. The presence of a high genetic variability in European beech stands has also been reported in other studies, and even in Central European populations, within-stand genetic variability was found to be very high (Müller-Starck et al. 1992; Sander et al. 2000; Kriebitzsch and Veste 2012). However, in the present study, we only compared a total of eight seed families, which might be a too restricted number to deduce reliable conclusions on the genetic variation inherent to the population analysed.

Generalizability of findings

With regard to a generalization of our findings it is important to note that the growth behaviour of tree seedlings in the field cannot be inferred directly from greenhouse experiments. Although greenhouse and growth chamber trials allow to keep environmental conditions as constant as possible and thus to exclusively focus on plant responses related to treatments, they often lack the ability to account for a complete suite of biotic interactions such as competition, facilitation, herbivory or symbiosis (Thomas et al.

2002; Baudis et al. 2014). Hence, realistic tests addressing both impacts of abiotic and biotic interactions on seedling growth require additional observations or experiments in natural ecosystems (Thomas et al. 2002; Baudis et al. 2014; Grossiord et al. 2014).

In addition, our experiments focused on one-year-old seedlings, and caution is needed to extrapolate findings to older seedling or sapling stages. Recent studies showed that tree responses to climate change are strongly mediated by the trees' life history stage, because tree growth mostly follows allometric trajectories that are characterized by age-related shifts in biomass allocation patterns (e.g. partitioning in favour of belowground tissue with increasing tree age; Weiner 2004). This coincides with findings by Luo and Chen (2013), according to which tree mortality as a result of climate change was strongly related to stand development processes, and impacts of drought and summer heatwaves decreased with decreasing tree age thus emphasizing the need to investigate tree growth response to climate shifts in relation to different life history stages. This is related to the rejuvenation phase of forests in particular, since young trees are expected to exhibit higher climate sensitivity, but empirical evidence for tree individuals in the rejuvenation phase is still limited (Baudis et al. 2014).

Conclusions

Beech seedlings proved to be highly sensitive to drought, but drought interacted with N fertilization at least for some of the response variables. These interactions were mostly antagonistic, but also amplifying in the case of necrotic leaf biomass. This result indicates that plant responses are difficult to anticipate by means of single-factor approaches, particularly in the face of likely interacting drivers such as altered temperatures or precipitation regimes, N deposition and elevated CO_2 levels.

In our experiments, drought and N fertilization were applied simultaneously. It is, however, conceivable that responses may also depend on the temporal sequence in which global change drivers affect tree growth. For example, effects of N deposition could aggravate the drought sensitivity of seedlings even further if they precede drought events by one or 2 years (i.e. drought takes effect on already fertilized plants). This suggests that further research should

combine global change drivers in full-factorial experiments with simultaneous and time-delayed impacts of drivers to further improve our understanding of the dynamic responses of trees to global change.

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Compliance with ethical standards

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

Appendix

See Appendix Table 3.

Table 3 Effect sizes (%) of treatment effects on morphological and chemical response variables of beech seedlings

Treatment	D	N	ND
Morphological variables			
Stem Diameter	-12.3	1.6	-17.0
Height	-3.1	-1.9	-11.4
Leaf biomass dw	-9.4	3.6	-19.8
Aboveground biomass dw	-15.3	2.2	-25.7
Root biomass dw	-25.4	-0.8	-34.3
Necrotic leaf biomass dw	296.2	0.0	736.5
Shoot:root ratio	13.3	4.0	16.5
Specific leaf area	0.5	0.8	2.3
Chemical variables			
Leaf C	1.9	0.0	0.8
Root C	-0.5	-0.3	-0.8
Leaf N	-0.3	7.1	-0.8
Root N	14.7	15.1	31.2
Leaf C:N	2.4	-7.1	1.1
Root C:N	-14.7	-17.4	-28.0
Leaf $\delta^{13}\text{C}^a$	-1.8	-0.1	-1.9
Root $\delta^{13}\text{C}^a$	-2.1	-0.3	-2.7

For statistical significant differences between the control and treatments see Figs. 2 and 3

^a Note that negative effect size values indicate an increase in $\delta^{13}\text{C}$ signatures

N nitrogen fertilization, D drought treatment, ND nitrogen fertilization and drought treatment

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