Global Change Impacts on Beech (*Fagus sylvatica* L.) Forest Ecosystems: the Role and Interaction of Nitrogen and Drought

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1 Intention of the Thesis and Research Objectives

It is well known and widely accepted that human-induced global environmental change is a serious threat for the earth's biological system and its species diversity (Butchart et al. 2010, Vitousek et al. 1997). In order to understand the impact of global change on ecosystem function and services, extensive research efforts are needed. Amongst the various effects human influences exert on ecosystems, climate change (e.g. altered precipitation regimes with increased summer drought periods) and nitrogen deposition are major drivers of global change ('global change drivers'; Settele et al. 2014) with severe consequences for ecosystem functioning and species performance (Greaver et al. 2016, Sala et al. 2000). It is without doubt that global change drivers operate simultaneously but our knowledge and understanding of the complex ecosystem responses to co-occurring drivers remains limited (IPCC 2014, Leuzinger 2010, Zavaleta et al. 2003).

Due to the long life-span of trees and concomitant adaptation processes, forests are particularly vulnerable to global environmental change (Pretzsch et al. 2014, Müller-Haubold et al. 2013). As a result, one aim of current forest conservation strategies in Europe is to assist forests in their adaptation to future climatic and atmospheric changes (BMEL 2011). As an ecologically and economically valuable, indigenous forest tree species, European beech (*Fagus sylvatica* L.) plays a leading role in these strategies. *Fagus sylvatica* is a highly competitive, shade-tolerant tree species, which is able to efficiently use photosynthetically active radiation via shade adapted leaf differentiation and high crown plasticity in the forest canopy layer (Ellenberg & Leuschner 2010). The distribution pattern of the heart-shaped root system is flexible and shows superior asymmetric competition in species mixtures, with a tendency to exploit nutrient rich (but drought susceptible) upper soil layers (Bolte & Villanueva 2005, Leuschner et al. 2001).

Regarding soil water requirements, however, beech trees are known to be particularly drought sensitive (Ellenberg & Leuschner 2010, Friedrichs et al. 2009, Geßler et al. 2004). In the face of the high conservation value of European beech, there is thus a need to assess its future performance under the predicted climatic and atmospheric changes.

Within this context, beech populations from the south-western distribution range (NW Spain) may be of particular interest, due to possible adaptation mechanisms to a warmer and drier climate (Robson et al. 2013). Moreover, the Iberian Peninsula is considered to be one of the glacial refugia of European beech. Populations from these refuge regions are expected to exhibit a high allelic richness and high regional genetic diversity (Magri et al. 2006, Hampe & Petit 2005, Widmer & Lexer 2001) and play an important role in conservation of European beech under future environmental conditions (Robson et al. 2013, Hampe & Petit 2005). It is, therefore, crucial to understand the underlying mechanisms of how co-occurring global change drivers affect central- and marginal beech populations, allowing for an assessment of their impact and the resulting implications for forest management and conservation on a European scale.

To this end, I conducted two greenhouse experiments (one- and two-year study, research papers I and II, respectively) to investigate the single and combined effects of nitrogen fertilization and drought on the morphological and physiological response of beech seedlings of a population taken from the Cantabrian Mountains in Spain. Information on the drought sensitivity of these beech populations is limited and to date no study has assessed the sensitivity of these populations to the simultaneously acting global change drivers, nitrogen and drought. In order to investigate the effect of the seedlings ontogenetic stage on the capability of withstanding averse environmental change, I additionally compared the results of the two-year greenhouse experiment with the results of the one-year study (research paper II). Such greenhouse experiments provide the opportunity of controlling environmental conditions thereby gaining an undisturbed insight of the modified factor’s impact on the response variable of interest. On the downside, the absence of biotic and abiotic interactions that would naturally occur in the field gives need for additional experiments that verify the results in natural communities (Gibson et al. 1999). In order to test the combined effects of nitrogen and drought on juvenile beech trees in a natural setting I, therefore, extended the experimental setting and implemented a 4-year field experiment in a privately owned forest in the district of Lüneburg, Lower Saxony (research paper III). In this experiment juvenile beech trees were planted in monoculture as well as in combination with Sessile oak (*Quercus petraea* (Matt.) Liebl.) and Douglas fir (*Pseudotsuga mensziesii* (Mirb.) Franco) and subjected to simulated nitrogen fertilization or drought as well as the combination of these two factors. Thus, I am able to provide insights into the underlying mechanisms of the combined effects of major global change drivers on European beech and discuss the implications for conservation and future research needs.

2 General Introduction

2.1 Beech Forest Ecosystems and Global Change

About one third of the global land area is dominated by natural or managed forest ecosystems (30.7%, FAO 2016). Forests play an important role for global biodiversity, habitat protection, climate regulation and carbon sequestration (Settele et al. 2014). From a human perspective they deliver vital ecosystem services such as the provision of wood (used for construction or energy/heat production), the regulation of the hydrological cycle (water purification and retention) as well as cultural opportunities such as the provision of rest and recreation areas for local communities.

Over the last decades, both nitrogen deposition and climate change have been the focus of intensive research, as their influence on forest ecosystem functioning is not only strong but predicted to increase in the future (Settele et al. 2014). Excess nitrogen deposition has been critically affecting European forest ecosystems for several decades (with consequences for carbon and nitrogen cycling as well as species composition; Reich & Frelich 2002, Aber et al. 1998). Similarly, the impact of human-induced climate change (e.g. higher frequency and severity of drought periods, aggravated by increasing temperatures; Allen et al. 2015) on forests has already led to damage (e.g. tree-mortality; Allen et al. 2010, Breda et al. 2006) and is predicted to further increase over the course of the century with wide ranging consequences for ecosystem functions and services (Settele et al. 2014).

Large areas of Europe's temperate deciduous forests are naturally dominated by European beech (Ellenberg & Leuschner 2010). This tree species prefers a temperate climate, with mild winters and moist summers, without long lasting summer drought periods (Bolte et al. 2007). As European beech covers a broad range of site conditions within its distribution range, it exhibits a high genotypic variability (higher within than amongst populations) and phenotypic plasticity (e.g. Müller & Finkeldey 2016, Sander et al. 2000). Phenotypic plasticity describes the capability of a species to respond to changing environmental conditions within the scope of its genotypic variation in adaptive traits. Environmental stress itself can increase genotypic variability due to a higher frequency of genetic mutations (Debat & David 2002). This process can be crucial for future forest management and conservation, as several studies reveal that populations from drier habitats are better adapted to climate induced stressors (Weber et al. 2013, Robson et al. 2013, Rose et al. 2009, Peuke et al. 2002, Garcia-Plazaola & Becerril 2000).

Beside phenotypic plasticity, epigenetic modification of functional traits is another important mechanism in the context of the species' adaptation to environmental change (Müller & Gailing 2019). A functional trait is defined as a 'measureable property of organisms' that 'strongly influences organismal performance' (McGill et al. 2006). Hrivnák et al. (2017) found evidence for epigenetic effects in European beech in relation to climatic variables, which emphasizes the ability of the species to cope with future environmental stressors.

Species adaptation (genetically and epigenetically) towards environmental stress is indubitably an important mechanism in the context of global environmental change. However, interactions between plants and mycorrhizal fungi modulate the adaptation towards environmental stress as well (Lehto & Zwiazek 2011, Beiler et al. 2010, Shi et al. 2002). In this context, the importance of plant-soil interactions and mycorrhizal networks in forest ecosystems became increasingly important to researchers in the recent decades. The symbiotic relationship with mycorrhiza species and associated bacteria enhance the ability of forest trees to acquire nutrients and water from the forest soil, while the fungal symbionts are able to cover their carbon demand provided by the host plants (Simard et al. 2012). It was shown that mycorrhiza species are linked to mycorrhizal networks in forest ecosystems, which serve as interplant-transfer systems for nutrients, water, and even carbon between the trees associated with the network (Klein et al. 2016, Bingham & Simard 2011, Bonfante & Genre 2010). The finding that forest trees are able to transfer compounds via mycorrhizal networks opens new perspectives in the understanding of ecosystem functioning, tree health, and recovery under a stressful environmental, since stress-sensitive species may benefit from such facilitative interactions (Klein et al. 2016).

The functionality of such networks depends on various biotic and abiotic factors such as soil fertility and humidity, host plant diversity and environmental stress level, the diversity of the fungal community and their interactions (Purahong et al. 2018, Simard et al. 2012), or even the occurrence of non-native invasive plant species (Ruckli et al. 2016). In a Douglas-fir forest in Canada, Beiler et al. (2010) found a widespread symbiotic assemblage of mycorrhizal fungi and trees from all age-classes connected to a mycorrhizal network. Among others, particularly seedlings and young trees were able to benefit from improved nutrient and water supply during the critical establishment phase of forest rejuvenation. The network was reported to be robust against perturbations but susceptible against the removal of mature hub trees (e.g. harvesting or pest calamities). However, the impact of anthropogenic disturbances and land-use history (e.g. due to their impact on the development of belowground networks and microbial communities) also proved to be important in the context of forest responses to environmental stressors (Mausolf et al. 2018, Fichtner et al. 2014, von Oheimb et al. 2014).

Plant species diversity in forest ecosystem is a key factor for the resistance and resilience of ecosystem functions under global change (Tilman et al. 2014, Balvanera et al. 2006). Functional diversity 'is the component of diversity that influences ecosystem dynamics, stability, productivity, nutrient balance, and other aspects of ecosystem functioning' (Tilman 2001). Biodiversity studies suggest that the effects of species richness increase with an increasing number of ecosystem processes observed and that biodiversity effects on ecosystem productivity will increase over time, mainly due to complementarity effects typical of species rich mixtures (Cardinale et al. 2007, Hector & Bagchi 2007). The underlying mechanisms of such 'insurance and stability effects' of biodiversity, however, are complex and still key questions of ecological research (Cardinale et al. 2007). The insurance hypothesis suggests that biodiversity increases the stability of ecosystems, due to differences in species responses to environmental fluctuations or perturbations and the probability of diverse communities to ensure functional redundancy regarding important ecosystem processes (McCann 2000, Yachi & Loreau 1999, Naeem & Li 1997). However, it is still challenging whether and to what extent species richness or functional traits *per se* contribute to the stability of ecosystem functions (Grossiord et al. 2014, Polley et al. 2013). Moreover, the influence of local site conditions on biodiversity-stability relationships in forest ecosystems cannot be generalized, as some forest types did not show positive biodiversity feedbacks in Europe's forests (Grossiord et al. 2014).

2.2 Anthropogenic Climate Change

The debate about climate change and its impact on ecosystems at a regional and global scale is ongoing. The IPCC (2014) defines climate change as a change in the state of the climate, identified by changes of the mean or variability of its properties, which persist for an extended period and are due to natural or anthropogenic processes and impacts. All ecosystems are already impacted by human-induced climate change and are in a state of transition and adaption (IPCC 2014, Sala et al. 2000, Vitousek et al. 1997). The carbon dioxide (CO_2) concentration of the atmosphere has increased by more than 30% compared to pre-industrial levels and is the main cause of the observed change in the mean annual surface temperature over the last decades (IPCC 2014). These climate change related shifts in the global energy system will lead to a higher frequency of extreme weather events such as heat waves, summer drought periods or heavy rain events (IPCC 2014). Many studies have investigated the interaction between climate and non-climate related global change drivers (e.g. global warming, land use change, aerosol emission or nitrogen deposition) and their impact on ecosystem functions or socioeconomic services, with partially contrasting results with regards to the observed biomes, drivers or scales (reviewed in: Settele et al. 2014). In order to identify the future impact of global change drivers on ecosystems, however, it is essential to clarify the underlying processes using multifactorial approaches (e.g. Settele et al. 2014, Leuzinger 2010).

2.2.1 General Drought Response of Forests

The growth and productivity of forests is strongly controlled by soil water availability. Amongst others, it affects photosynthetic capacity, which, in turn, affects carbon and nutrient fluxes in the trees' metabolic system (Breda et al. 2006). Soil water depletion affects the growth as well as the competitiveness of trees and may alter the species composition of forest ecosystems (Geßler et al. 2007). The exacerbation of drought periods due to a concomitant temperature increase has been identified as a growing threat for forest ecosystems (Allen et al. 2015). In order to counteract the negative effects in such situations, plants have evolved several morphological and molecular strategies that maintain a constant water status and avoid cellular damage (Farooq et al. 2012). These strategies differ amongst species and can be separated into drought avoidance (e.g. higher biomass allocation to the root system, decreasing leaf area or rapid stomatal closure) and drought tolerance (e.g. osmotic adjustment) mechanisms (Brunner et al. 2015, Sanders & Arndt 2012). Drought stress occurs whenever soil water content decreases below a species specific threshold with restrictions to growth and transpiration (Breda et al. 2006). A common response of forest trees to drought induced water depletion is a reduction in leaf area and the closure of stomata, which leads to reduced transpiration and CO_2 assimilation. Consequently, drought affects the primary production and carbon sequestration of forest ecosystems (Ruiz-Benito et al. 2014, Breda et al. 2006). Furthermore, it influences the nitrogen balance of forest trees directly via lower soil microbial activity or ion mobility and indirectly due to several metabolic constraints (Gessler et al. 2017, Dannenmann et al. 2016). Severe drought periods in consecutive years lead to tree dieback and tree mortality in interaction with other abiotic and biotic related stress factors (Allen et al. 2010, Breda et al. 2006).

2.2.2 Drought Sensitivity of European Beech

European beech reaches its competitive superiority in an oceanic to sub-continental temperate climate and prefers the relatively humid and mild climate of Central and Western Europe (Bolte et al. 2007). At the southern distribution range the main selective pressure for its occurrence is water limitation, which mitigates the competitive ability of beech trees (Kramer et al. 2010, Gutiérrez 1988). Several studies show an upward shift of beech trees towards higher more temperate altitudinal zones in Spain, even if these are partly caused by temperature (Jump et al. 2006, Penuelas & Boada 2003).

It is important to note, however, that not only marginal populations of beech are affected by current climate change. For Central Europe, drought related impacts on the vitality and competitive performance of beech trees have been reported in several studies. For example, Friedrichs et al. (2009) conducted a dendroecological study at two forest sites with contrasting water supply in Central and West Germany. They investigated the growth response of three tree species (*Q. petraea*, *P. sylvestris*, *F. sylvatica*) in relation to climate variables during the twentieth century. Whilst all three species showed increasing drought sensitivity over the course of the century, of all species, beech was most prone to drought. The authors concluded that even minor changes in the precipitation patterns of drier sites within the distribution range of beech will lead to a shift in species composition towards species that are better adapted to drought. Similar conclusions were drawn by Gessler et al. (2007) with regards to the competitive ability of beech on soils with low water storage capacity.

For withstanding periods of low water availability the root system of a plant is a crucial trait (Brunner et al. 2015). For German forest sites it has been shown that mature beech trees allocate proportionally more biomass to the root system along a precipitation gradient (820-540 mm yr^{-1}), which revealed the high allocation plasticity of the species (Hertel et al. 2013). This is in accordance with the ‘optimal resource partitioning theory’, to which trees enhance biomass allocation to the root system on drier sites compared to more humid sites in order to maintain a balance between water as well as nutrient supply and evaporative demands (Bloom et al. 1985). In general, mature beech trees seem to follow these adaptive trajectories on drier sites when water deficits are moderate and long lasting but suffer from increasing root mortality when water shortage and the frequency of drought periods increase. Consequently, beech trees aim to compensate increasing root mortality under severe drought stress by increasing the production of fine roots. This, however, results in much greater carbon costs under drought (Meier & Leuschner 2008, Leuschner et al. 2001).

Due to their frequently low root to shoot ratios, seedlings are particularly sensitive to drought stress (McDowell et al. 2008). In a common garden experiment with beech seedlings (in mixture with *Rubus fruticosus*, a competitor of beech seedlings in the forest understorey), water shortage revealed distinct negative effects on the growth and biomass allocation patterns of beech seedlings (Fotelli et al. 2001). Even under moderate drought conditions the authors observed a two-fold reduction in aboveground biomass production and a significant decrease in the root to shoot ratio. In contrast, neither water shortage nor competition affected the root to shoot ratio of *Rubus* seedlings. Moreover, beech seedlings seem to be severely affected by a drought induced decreasing nitrogen availability. Such drought related nitrogen limitation of the beech seedlings has been detected up to one year after the application of drought with distinct constraints for growth and the competitive ability of the seedlings (Fotelli et al. 2001). This aspect may be particularly crucial for beech performance under future climate conditions as it affects the rejuvenation phase of beech (Dannenmann et al. 2016).

2.3 Nitrogen Deposition – The Critical Pollutant

Nitrogen, in its reactive forms, is one of the major pollutants that impact ecosystem processes and ranges under the top five global change drivers which critically affect ecosystem functioning (Sala et al. 2000). It is the most abundant element within the Earth's atmosphere but paradoxically the main limiting nutrient in most terrestrial ecosystems with distinct constraints for plant productivity (Aerts & Chapin III 1999). This is because atmospheric nitrogen (i.e. in the nonreactive form N_2) is not directly biologically available for plants. The only biological process to convert nitrogen into the reactive form of ammonium (NH_4^+) is via microbial nitrogen fixation (free living and symbiotic bacteria as well as cyanobacteria; Galloway et al. 2003). The consequence of a limited soil ammonium pool is an almost closed nitrogen cycle, in which plants, heterotrophic microbes, and nitrifiers compete for available nitrogen (Aber et al. 1992).

With the beginning of the industrial era, human activities began to considerably affect global nitrogen cycling mainly due to the combustion of fossil fuels (NO_X emissions) and mineral fertilizer production (as NH_3). Since then, the total amount of reactive nitrogen released into the environment as a result of human activities has increased 10-fold from around 15 million tons per year in the 19th century to approximately 156 million tons per year at the end of the 20th century and is expected to further increase (Galloway et al. 2004). While the process of denitrification returns a proportion of the reactive nitrogen back to the atmosphere as non-reactive N_2, the remaining nitrogen will continue to affect ecosystem processes on a large spatial scale (e.g. reviewed in Galloway et al. 2003, Galloway et al. 2004). If current nitrogen deposition rates remain unchanged in the future, then the impact of excess nitrogen on ecosystem functioning will intensify as ecosystems that reach a state of nitrogen saturation lose their nitrogen retention capacity which, amongst others, will increase nitrate leaching to the groundwater and gaseous emissions of reactive nitrogen forms to the atmosphere (Aber et al. 1998).

2.3.1 Forests and Nitrogen Deposition

The growth and productivity of temperate forest ecosystems is often limited by nitrogen availability (LeBauer & Treseder 2008). Under these conditions forest trees react to enhanced nitrogen availability by increasing foliar nitrogen concentrations, mainly via an increase in the translation of photosynthetic active proteins (Högberg 2007, Evans 1989). This is turn, leads to higher net photosynthesis rates enabling an increase in aboveground biomass production (Pregitzer et al. 2008, Högberg 2007). Furthermore, a shift in carbon allocation patterns towards aboveground biomass, i.e. a lower allocation to roots and symbiotic mycorrhizal fungi, has been observed (Pregitzer et al. 2008, Högberg 2007, Aber et al. 1998). It has been shown, that the belowground to aboveground biomass ratio (i.e. root to shoot ratio) decreases along nitrogen availability gradients (Bobbink et al. 2010, Xia and Wan 2008, Kozlowski & Pallardy 2002, Nadelhoffer 2000). The chronic effect of increased nitrogen deposition on the belowground biomass dynamics of forest trees is, however, not fully understood. It has been postulated that nitrogen deposition leads to an increase in fine-root production but also fine-root turnover rates. Consequently, the total carbon input to the fine-root system increases but, in comparison to aboveground biomass, less is invested in growth leading to a decrease in root to shoot ratios (Hendricks et al. 2006, Nadelhoffer et al. 1985).

Long-term nitrogen deposition ultimately results in nitrogen saturation of former nitrogen limited ecosystems, as has been explicitly described for northern temperate forest ecosystems (nitrogen saturation hypothesis; Aber et al. 1998, Aber et al. 1992). An ecosystems' state of transition towards nitrogen saturation is complex and may depend on stand- and site-characteristics, land-use history as well as nitrogen deposition rates (Högberg et al. 2006, Aber et al. 1998). It has been shown that chronically high levels of nitrogen deposition adversely affect carbon sequestration by altering species composition in the soil (shift from fungal to bacterial dominated microbial communities; Tietema 1998) and trigger nitrate and cation leaching as well as soil acidification (Rennenberg & Gessler 1999, Aber et al. 1998). As a consequence, these processes will lead to nutrient imbalances and cause other elements (e.g. phosphorous, potassium) to become limited with subsequent constraints on tree growth and vitality (Braun et al. 2017, Xia & Wan 2008; Flückinger & Braun 1999, Magill et al. 1997).

2.4 The Interaction of Global Change Drivers

There is a growing awareness about the complexity behind the mechanisms underlying the interactions between major global change drivers and their impact on ecosystem functioning and services. It is without doubt that global change drivers operate simultaneously (Shaw et al. 2002) and it has be known for at least several decades that their impact on ecosystem functioning involves interactive effects. The pressing question is to what extent they influence each other.

In a review article on the causes of tree mortality, Franklin et al. (1987) assumed that the death of a tree is ultimately the result of complex interactions and not just the effect of a single stressor. As mentioned above for nitrogen and drought, many studies have investigated the impact of single global change drivers on various physiological and morphological processes in plants and trees, in particular. Multifactorial studies, which try to address complex feedback mechanisms are scarce and are currently a subject of increased scientific effort. Accordingly, our knowledge and integrated understanding of species' and ecosystems' responses to simultaneously acting stressors remains limited (Drewniak & Gonzalez-Meler 2017, Greaver et al. 2016, Leuzinger 2010).

There are multiple ways in which drivers of global change can interact in their impact on ecosystem functioning. On a general scale, at least three ways have so far been identified in which species and ecosystems respond to co-occurring environmental stressors (Zavaleta et al. 2003):

- Additive (no interaction effect): the process response is equal to the sum of the single responses.
- Synergistic interaction: simultaneously acting drivers amplify each other in their impact on species and ecosystem functioning.
- Antagonistic interaction: This interaction type results in a cancelling or dampening response compared to single impacts of the drivers.

The type of interaction and the direction of the effect can vary between different physiological and morphological processes (Gessler et al. 2017) and cannot be easily assigned from a species to an ecosystem level (Leuzinger et al. 2011). To distinguish between additive and interaction effects, full-factorial design experiments encompassing all single as well as all combinations of treatments are required. These differ from observational studies along environmental gradients in which interactions are quantifiable in absolute terms but from which the contribution of each factor cannot be derived.

2.4.1 Nitrogen and Drought Interaction in Forests

Studies investigating nitrogen and drought interactions in forest ecosystems are scarce. In a review on the combined impact of nitrogen and drought, Drewniak & Gonzalez-Meler (2017) listed only eleven studies that have assessed the interaction of these important drivers in the past, with only four of these relating to trees or forests. A recent forest study within the United States suggests that the response of forest trees to a combination of nitrogen deposition and drought may depend on tree age. In this study, mature trees benefited from nitrogen deposition, in terms of growth response despite extreme climatic conditions (Ibanez et al. 2018). The authors attributed their findings to the ontogenetic stage of the observed trees, as mature trees are able to access deeper soil horizons due to their extensive root system and are thus less affected by concurrent or subsequent drought than younger trees. Within the same study, high nitrogen inputs constantly led to increased growth at drier sites compared to ambient nitrogen levels. In contrast, for seedlings and saplings it has been shown, that the negative effect of drought on growth is enhanced by nitrogen availability (Nagakura et al. 2008, Nilsen 1995).

There is evidence that the interactive effects of global change drivers on forest ecosystems not only depend on the developmental stage of a tree but also on the predisposition to, as well as the timing of the stressors relative to each other (Gessler et al. 2017). It is likely that nitrogen limited forests react differently to concurrent nitrogen deposition and drought than nitrogen saturated forests (s. lat. Aber et al. 1998). High nitrogen availability predisposes plants to increase drought susceptibility by over proportionally increasing aboveground growth rates and thus reducing the root to shoot ratio (Kozlowski & Pallardy 2002, Marschner et al. 1996). As a result, the evaporative demand increases, which, in turn, increases the drought susceptibility. Paradoxically, after periods of water limitation, nutrient, and in particular nitrogen availability, is crucial for tree recovery, as it allows for tissue regrowth and rebalancing of the nutrient stoichiometry (Gessler et al. 2017). In low nitrogen environments, however, drought may enhance nitrogen limitation, due to reduced nitrogen uptake and thus availability, which can result in carbon starvation (Gessler et al. 2017). In this case, background nitrogen deposition may then in fact reduce the drought impact (Dannenmann et al. 2016).

2.4.2 Evidence of Simultaneous Global Change Driver Impact on Beech Forests

There is growing evidence that the drought susceptibility of European beech is enhanced by nitrogen deposition (Dannenmann et al. 2016) and that the effects between these drivers are both additive and interrelated (synergistic and antagonistic), and at least to some extent, depended on the ontogenetic stage (Hess et al. 2018), or forest history (Mausolf et al. 2019).

Braun et al. (2017) were able to show that an observed growth rate decline of beech trees over a 30-year period (1984 – 2014) at Swiss sites was, in large parts, attributable to an interaction effect of nitrogen deposition and drought. Moreover, potassium (K) deficiency (reflected in high N:K ratios resulting from increased nitrogen deposition) is a further contributor to the drought sensitivity of European beech, with a drought and foliar N:K ratio interaction contributing additively to that of nitrogen and drought.

In conclusion, the drought sensitivity of beech trees seems to be reinforced by nitrogen deposition and concomitant nutrient imbalances which suggests that, under current future projections, global change will have a wide-scale effect on the vitality of European beech forests and, therefore, also on their associated biodiversity and ecosystem services.

3 Thesis Outline

To improve our knowledge of global change impacts on ecosystems, and beech forests in particular, multifactorial studies are needed to reduce the uncertainties regarding the impact of simultaneously acting global change drivers and their often complex interactions. With the studies presented in this thesis, I am able to disentangle the single and combined effects of nitrogen addition and drought on the growth of beech trees and contribute to a better understanding of the underlying ecological processes in the interplay of beech forests and abiotic changes.

This thesis comprised three peer-reviewed research papers, which discussed the morphological and physiological response of beech trees to single and combined effects of nitrogen deposition and drought, each with specific research hypotheses and experimental designs. The research papers and their corresponding reference list were embedded at the end of this section in their original publication format.

The following sections give an overview of the partial research aspect of each experiment and respective research paper, its methodological approach and the results as well as the conclusions that can be drawn from these in the context of current knowledge.

Research paper I: Dziedek, C., von Oheimb, G., Calvo, L., Fichtner, A., Kriebitzsch, W.-U., Marcos, E., Pitz, W.T. & Härdtle, W. (2016) Does excess nitrogen supply increase the drought sensitivity of European beech (*Fagus sylvatica* L.) seedlings? Plant Ecology, 217, 393-405. (https://doi.org/10.1007/s11258-016-0581-1).

Research paper II: Dziedek, C., Fichtner, A., Calvo, L., Marcos, E., Jansen, K., Kunz, M., Walmsley, D., von Oheimb, G. & Härdtle, W. (2017) Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought. Forests, 8, 91. (https://doi.org/10.3390/f8030091).

Research paper III: Dziedek, C., Härdtle, W., von Oheimb, G. & Fichtner, A. (2016) Nitrogen addition enhances drought sensitivity of young deciduous tree species. Frontiers in Plant Science, 7, 1100. (https://doi.org/10.3389/fpls.2016.01100).

3.1 Research Paper I (Summary)

The study presented in this paper is based on a cooperation with colleagues from the University of León in Spain. I investigated the single and combined effects of nitrogen (N) fertilization and drought (D) on morphological and physiological response variables of beech seedlings from eight different mother trees ('seed families'). These originated from the Cantabrian Mountains (NW Spain; Figure 1), a region which is considered to be one of the glacial refugia of European beech. Beech populations from such areas play an important role in the face of global environmental change, as they are expected to conserve a high allelic richness and regional genetic diversity, and as such have a large adaptation potential (Robson et al. 2013, Hampe & Petit 2005, Widmer & Lexer 2001). Despite this, our knowledge of the sensitivity of these refuge Spanish populations to simultaneously acting global change drivers is limited.

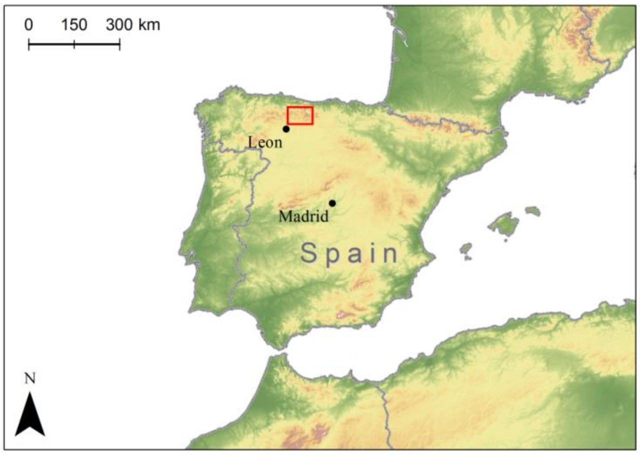

Figure 1 Location of the forest area in the Cantabrian Mountains (North Spain), in which seeds of beech trees were sampled (rectangle; Figure 1 originally published in research paper II).

Therefore beech seeds from eight different seed families were collected on north-facing slopes within the Cantabrian Mountains in autumn 2009, stratified, and planted in plastic pots in a full-factorial greenhouse experiment at the Thünen-Institute in Hamburg, Germany. A total number of 320 seedlings were exposed to treatments (control (CT), nitrogen (NT), drought (DT), combined nitrogen and drought (NDT); 8 seed families x 4 treatments x 10 replicates for each treatment). All seedlings were watered well during the season to exclude unintended drought effects. Seedlings of the DT and NDT were subjected to one severe three-week drought period in August 2010, in which the soil water content within the pots dropped to 5 vol%. Nitrogen was applied (as NH_4NO_3 solution in deionized water) equivalent to a quantity of 50 kg N ha^{-1} yr^{-1} biweekly to the NT and NDT treatments with an intermittence phase during the three weeks of the drought period. In total, 16 morphological and physiological response variables were measured. Single and interaction effects were tested using linear models (LM) and an ANOVA followed by a Tukey's post hoc test.

I hypothesized that (i) combined nitrogen fertilization and drought non-additively affect seedlings biomass production and (ii) seedlings' responses differ between seed families due to their within-population genetic variability.

All beech seedlings proved to be highly drought sensitive as drought exerted the strongest effect on all response variables and throughout all seed families. Nevertheless, some seed families were more drought sensitive than others, indicated by a DT x Family interaction in the LM. In general, seed family affected all response variables in the study, both as a single factor and in interaction with nitrogen and drought for at least some of the response variables. For example, the $\delta^{13}C$ signatures of the leaves differed significantly between the seed families in the CT, the NT as well as the DT. Leaf $\delta^{13}C$ signatures were used as a proxy for the seedlings' transpirational demands (Mölder et al. 2011, Farquhar et al. 1989) with family-related effects again contributing to the seedlings' drought sensitivity. These findings supported my second hypotheses (ii) that the seedlings' response differ between seed families due to their within-population genetic variability.

Solely adding nitrogen (NT) had a comparable weak effect on seedling growth that may, in part, have been due to the relatively high levels of plant-available N_{inorg} in the potting substrate. Importantly, the interaction of nitrogen and drought, observed for some of the response variables analyzed, confirmed my first hypotheses (i). For instance, antagonistic (N x D) effects were found for the stem diameter, the leaf biomass *($P < 0.05$)* as well as for above- and belowground biomass *($P < 0.1$)*. In contrast, synergistic (N x D) effects were found for the extent of necrotic seedling tissue, which can be seen as direct evidence for severe stress and reduced seedling vitality. Furthermore, in combination with nitrogen fertilization, drought led to a significantly larger reduction in belowground biomass production than when applied in isolation (effect size: -34.3 and -25.4, respectively). It is most probable that this contributed to an additional sensitivity of the seedlings towards water stress.

Although other beech populations from the Iberian Peninsula have evolved suitable adaptation mechanisms to deal with drought stress (Robson et al. 2013, Garcia-Plazaola 2000), the beech population used in my study proved to be highly drought sensitive. This might reflect the comparably high precipitation patterns at the collection sites and hence missing or limited adaptation mechanisms of the seedlings to intense drought periods. Accordingly, adaptive traits of beech populations to global change drivers are related to specific site characteristics at the place of origin. This conclusion is supported by the distinct family effect observed in my study, which revealed that the extent to which beech seedlings are sensitive to drought differs between seed families. Even minor changes in the precipitation patterns across the collecting sites might have contributed to the drought sensitivity and related adaptation mechanisms. However, I was not able to evaluate the relationship between the drought sensitivity, the adaptive traits and the mother trees' site characteristics on a seed family level.

In conclusion, nitrogen addition enhanced the seedlings sensitivity to water stress in my study. Thus, future global change research should ascribe importance to the interaction of nitrogen and drought when assessing future climate risks for European beech forests.

3.2 Research Paper II (Summary)

The experimental approach was very similar to the approach of study I. In a parallel approach, beech seeds from the same batch (i.e. collected from the same Cantabrian Mountain beech population in autumn 2009) were grown in the same greenhouse. In contrast to study I, however, the experiment lasted for two years with a simulated drought occurring only in the second season (2011).

In spring 2010, a total number of 336 seedlings were assigned to two treatments, where all plants were watered regularly, but in which half the seedlings (168) received nitrogen fertilization equivalent to 50 kg N ha^{-1} yr^{-1} (applied as NH_4NO_3 solution in deionized water) with the other received no nitrogen. In the second year, each treatment was split again and the pots without fertilization in the first year were equally assigned to a control treatment (CT) in which plants were solely watered and a drought treatment (DT) in which plants were subjected to two drought periods in June and August 2011, with a soil water content reduced to approximately 5 vol%. At the same time, the pots that received nitrogen in the first year were assigned to a nitrogen treatment (NT) in which plants received identical N fertilization as in year one and a nitrogen-drought combination treatment (NDT) in which plants received identical N fertilization as in year one and were subjected to the same drought period as plants of the DT. Thus, each treatment was replicated 84 times. With the exception of the drought period for DT and NDT, all pots were well watered to avoid unintended drought effects. Consequently, in this experimental design, the drought in year two (2011) also affects already fertilized but well-watered saplings (NDT) which is typical of natural conditions as nitrogen deposition affects ecosystems for decades while the effects of changed precipitation regimes and drought periods are a comparably recent phenomenon (Greaver et al. 2016).

At the end of the first growing season in October 2010, the stem diameter, plant height, and total leaf biomass (inferred data) were determined. After harvest, in September 2011, the following response variables were measured: stem diameter, plant height, number of dead branches, number of necrotic leaves, aboveground biomass (shoots, leaves), and belowground biomass (as a subsample, due to the time consuming and laborious procedure). In addition, the root to shoot ratio of the subsample and annual stem-, height-, and leaf biomass increment were calculated. The morphological data were complemented by physiological measurements of the leaf carbon (C) and N concentrations, and $\delta^{13}C$ signatures. Accordingly, I calculated the C:N ratio of the leaves. The effect of sapling age on the root to shoot ratio and the leaf $\delta^{13}C$ signatures was determined by comparing the data with that from the one-year seedling study (research paper I). Treatment effects on response variables were evaluated using linear mixed-effects models (LMMs). Age-related effects of the nitrogen and drought treatments and their interaction effects were assessed by LMMs and generalized mixed-effects models (GLMMs) for non-normal distributed data (age-related effects for root to shoot ratio and $\delta^{13}C$ signatures; combined data with study I). Model selection was based on the Akaike information criterion (AIC).

I hypothesized that (i) nitrogen fertilized saplings exhibit higher drought sensitivity than unfertilized saplings, and (ii) one-year old plants are more sensitive to drought than two-year old plants.

In summary, nitrogen fertilization resulted in an increase of the saplings' aboveground biomass. This was to be expected and is in agreement with other studies, because nitrogen addition potentially changes carbon allocation patterns towards aboveground allocation leading to a decrease in plants' root to shoot ratios (see section 2.3.1.). The NDT did not alleviate the positive growth response of nitrogen fertilization. The combination of nitrogen and drought did however, caused a significant biomass dieback (i.e. number of dead branches), which supported my first hypotheses, that nitrogen fertilized saplings exhibit a higher drought sensitivity compared to unfertilized plants.

Sapling age significantly influenced the response of beech saplings to combined global change drivers. Leaf $\delta^{13}C$ signatures of one year-old plants increased significantly in the DT and NDT whereas the difference in two-year old plants was not significant (D x Age interaction; $P < 0.001$). Moreover, the root to shoot ratio of one-year old plants was significantly lower and increased with sapling age ($P < 0.001$), which explained the higher drought sensitivity of one-year old beech seedlings in the study. Nitrogen treatments, however, enhanced the increase in $\delta^{13}C$ signatures for both, one- and two-year old plants, indicated by a D x N interaction ($P < 0.05$).

The higher drought sensitivity of one-year old seedlings was mainly attributable to lower root to shoot ratios compared to the two-year old saplings. This finding also explained the observed increase in the leaf $\delta^{13}C$ signatures of the seedlings in the DT and NDT. Biomass allocation is an important trait that plants are able to adjust in order to react to changes in water availability (Brunner et al. 2015). Decreasing root to shoot ratios enhance the plants' transpirational demands and thus affect the plants' capability to satisfy their water requirements during periods of drought. Age-related shifts in biomass allocation patterns, due to increasing belowground investments in later life stages can mitigate the plants' susceptibility to water stress (Weiner 2004, Weiner 2001).

The study clearly showed that nitrogen enhanced the drought sensitivity of beech saplings, as reflected by the highest biomass dieback in the NDT and a significant N x D interaction regarding leaf $\delta^{13}C$ signatures. Furthermore, the plants' responses to the treatments were determined by their phenotypic plasticity (shift in biomass allocation patterns), which, in turn, was a function of sapling age and nitrogen fertilization.

3.3 Research Paper III (Summary)

In natural communities, species' responses to changing environmental conditions are related to numerous inter- and intraspecific, biotic and abiotic mechanisms, which may influence the extent to which species' are affected by global change drivers. There is a current debate on how species richness and biodiversity may mitigate the negative impacts of global change on ecosystem functioning and species performance. The related underlying mechanisms are complex and not fully understood (Scherer-Lorenzen 2014, Cardinale et al. 2007). In general, species mixtures are expected to be less sensitive to global change than related monocultures (‘insurance hypotheses’; Yachi & Loreau 1999, Naeem & Li 1997). To date, no consistent mechanism with which biodiversity acts on reducing drought susceptibility has been identified and it remains unclear, whether species’ or functional trait effects are the major drivers behind a better performance of diverse communities (Grossiord et al. 2014, Polley et al. 2013). Forest productivity, as an important ecosystem function, appears to be positively related to increasing species richness levels (Jucker et al. 2014, Morin et al. 2014). The positive biodiversity effect on forest productivity can be ascribed to mechanisms such as complementarity (i.e. niche differentiation or facilitation, resulting in an increased resource use), selection, or sampling effects (a higher probability that mixtures contain species with certain traits, i.e. highly productive species; Loreau & Hector 2001, Yachi & Loreau 1999).

Against this background, I investigated, whether species richness and species composition are able to mitigate the impact of the simultaneously acting drivers, nitrogen and drought, on the growth and productivity of beech on a tree, as well as on a community level. I tested, how single and combined nitrogen fertilization and drought mediate, (i) tree-level growth in relation to species identity, (ii) stand-level growth in relation to species combination and richness, and (iii) complementarity and selection effects and thus net biodiversity effects of tree communities.

In April 2010, a 4-year field study with juvenile trees was established with the aim of investigating the effects of both solitary and joint exposure to nitrogen fertilization and drought on the growth response of three tree species in monoculture and in mixture, respectively. Three ecological and economic important tree species with divergent functional traits regarding productivity (e.g. root system, shade tolerance or leaf longevity; European beech (*Fagus sylvatica* L.), Sessile oak (*Quercus petraea* (Matt.) Liebl.), and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) – henceforth referred to as beech, oak and fir) were selected. The trees were planted under canopy gaps in a forest (district of Lüneburg, Lower Saxony) in a randomized block design with seven replicate blocks. Each block consisted of six plots encompassing all monocultures and three species mixtures containing beech (beech-fir, beech-oak, and beech-oak-fir mixtures). The plots themselves were divided into 4 subplots and each randomly assigned to one of the following 4 treatments: a control (CT), a nitrogen treatment (NT), a drought treatment (DT), and a nitrogen and drought combination treatment (NDT). A schematic overview of the experimental design is shown in Figure 2. In order to minimize planting effects nitrogen fertilization started in 2012 (two years after planting) and was equivalent to 50 kg N ha^{-1} yr^{-1} (applied as NH_4NO_3 solution in deionized water). Trees in the DT and NDT were exposed to drought periods in the years 2012 and 2013 by using UV transparent rain-out shelters. The soil water content was measured in 4 representative blocks by means of permanently installed soil water content sensors. These showed that the soil water content in the drought treatments was reduced by about 20% (volumetric losses compared to field capacity in the last week of the drought periods).

For the assessment of the growth response to treatments and species combinations, tree height and aboveground biomass (stem biomass and leaf or needle biomass) were determined for all target trees. The tree height was measured at the beginning of treatment application in 2012 and at the end of the experiment in September 2013. Additionally, the relative growth rate (RGR) of the tree height was calculated for each target tree individual. In order to evaluate the tree size, treatment, and species richness effect on the RGR, linear mixed models with initial tree height (H), treatment (T), species combination (C), and their interaction as fixed factors as well as block, plot, and treatment as nested random factors were applied. Best fitting models were selected based on the Akaike Information Criterion, maximum likelihood estimations and Akaike weights.

As the aim of the study was to investigate the influence of species richness on the effect of two important global change drivers (N and D) on growth at both the tree- and stand-level, two different methods for determining treatment effect sizes were applied. At the tree level the magnitude of treatment effect (MTE) was calculated to determine the strength of the treatments on the RGR for each species. Values for this standardized effect range from -1 to +1, indicating maximum negative and positive treatment effects, respectively. At the stand level the net biodiversity effect (NE) was calculated to assess the influence of the treatments and species combinations on productivity (total aboveground biomass). As mentioned above, two effects (complementarity and selection effects) may contribute to the NE of mixtures. Therefore, the NE was further partitioned into the complementarity (CE) and selection effect (SE; according to Loreau & Hector 2001). For each species combination linear mixed effect models were used with treatment as fixed and block as random factor.

The treatment effect on the growth response of beech trees was highly dependent on initial tree height (H x T; $P < 0.01$). Furthermore, species combination affected the relationship between RGR and initial height of beech trees significantly (H x C; $P < 0.001$) with a greater increase in the beech-fir mixture compared to other mixtures. The mode of growth response to the treatments (NT, DT, and NDT; as indicated by the MTE), however, was species specific and again depended on the initial tree height ($P < 0.05$; Tukey test). The combination of nitrogen fertilization and drought negatively affected all species, but was highest for large beech trees. Interestingly, in terms of growth response the N x D interaction was antagonistic when only trees that were initially small were considered and synergistic for trees that were large at the beginning of the experiment. In contrast, the responses of oak and fir were additive and independent of initial tree height. Beech and oak were identified as most susceptible to the two simultaneously acting global change drivers assessed in my study (N and D). Finally, I found no influence of species combination on growth rates, indicated by a non-significant treatment and species interaction (T x C) in the linear-mixed models.

Thus it can be hypothesized that the growth response of the investigated tree species (on a tree level) to the global change treatments can be mainly ascribed to species identity (and species specific trait characteristics) rather than species combination (or species diversity). However, with regards to the size of the treatment effect, I was able to demonstrate that larger beech trees suffered most from drought also in the combination of nitrogen fertilization and drought, as deduced from the synergistic N x D interaction in the NDT treatment where the decline in growth rates was a sevenfold larger than compared to the smaller individuals. While initially larger individuals benefited from additional nitrogen fertilization (in contrast to smaller beech trees), the combination of nitrogen and drought changed the direction of the growth response. The growth increase of these individuals after nitrogen fertilization apparently resulted in an unfavorable biomass allocation with consequences for subsequent drought periods.

The NE on stand productivity strongly depended on species combination and species-specific responses to the treatments. In contrast to the mixtures in which fir was included, the beech-oak mixture showed a significant underyielding in the NDT, due to a lack of complementarity, attributable to the negative response of both species to simultaneous acting drivers at the tree level. Moreover, the lack of biodiversity effects in the NT was attributable to a negative SE, indicating that the distinct negative response of oak trees to nitrogen fertilization caused the loss of NE under nitrogen fertilization in this mixture. However, species mixtures with fir showed positive NE on productivity across all treatments. In the beech-fir mixture, overyielding was mainly attributable to fir, reflected in a greater SE than CE, especially in the DT treatment. Apparently, the lower sensitivity of fir to drought, also in the nitrogen and drought combination, more than compensated for the negative treatment effect of beech in the beech-fir mixture. Similar patterns were observed for the three-species mixture, where the presence of fir reduced the negative effects of nitrogen and drought on the beech-oak components of the mixture, as indicated by a distinct CE, i.e. increased productivity of all species in the mixture compared to that in their monocultures.

In summary, my results highlight the importance of multifactorial species diversity experiments in predicting future risks of forests under global environmental change, as species specific responses to nitrogen and drought were interrelated, at least in the case of beech. It is likely that diversity has the potential to mitigate at least some of the negative effects associated with current global change. It is impossible to generalize, however, as the magnitude and direction of such effects may depend on species identity (and inherent species specific trait characteristics) rather than solely on species richness levels.

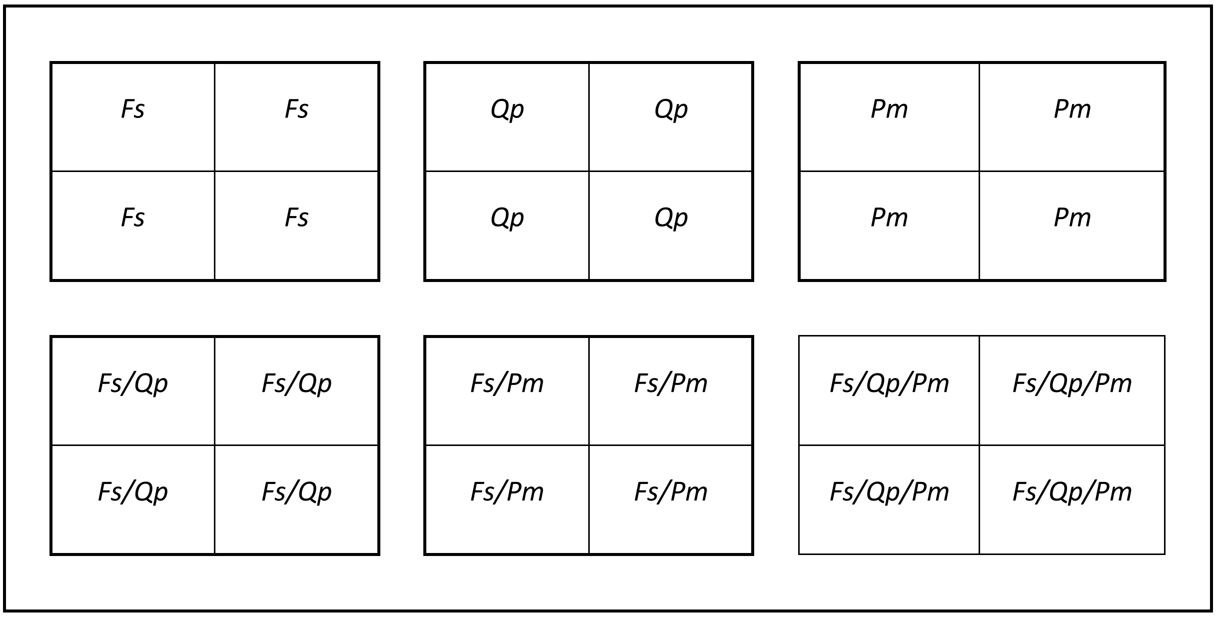

Figure 2 Schematic design of one experimental block with 6 plots (monoculture, 2-species-, 3-species mixture; 100 trees each), and 4 subplots for the different treatments (control, nitrogen, drought, and nitrogen combined with drought; 25 trees each). *Fs* = *Fagus sylvatica*, *Qp* = *Quercus petraea*, *Pm* = *Pseudotsuga menziesii*.

4 Conclusion

The results of this thesis provide experimental evidence that nitrogen is able to enhance the drought sensitivity of European beech. The responses of beech seedlings, saplings, and juvenile trees on the combination of nitrogen and drought were non-additive for some but not all of the measured response variables, both in the greenhouse studies as well as in the field experiment.

Both drivers changed biomass allocation patterns of the seedlings and saplings, by affecting the plants' root to shoot ratio leading to non-additive effects on biomass dieback processes or leaf $\delta^{13}C$ signatures. The negative effect was partly mitigated by the saplings' ontogenetic stage, as biomass allocation patterns were determined by environmental conditions (e.g. nitrogen availability) as well as plants' life history stages, with increasing belowground investments in later stages. Consequently, one-year old seedlings subjected to nitrogen fertilization showed the highest drought sensitivity in my studies. This finding is important, because early life stages are crucial for rejuvenation of European beech in forest ecosystems. Therefore, when predicting the future impacts of global change drivers on beech trees and forests, plants' ontogenetic stages should be taken into consideration.

Although we found interaction effects of nitrogen and drought treatments on growth of beech saplings, differences in response patterns of the seed families (study I) also revealed insights in the potential of European beech to adapt to environmental shifts, as some families proved to be more sensitive to co-occurring drivers than others.

The results of the field experiment supported the findings on the susceptibility of beech trees to interacting global change drivers, which were found in the greenhouse experiments. In contrast to oak and fir trees, the simultaneous effect of nitrogen and drought was non-additive in the case of beech, with stronger effects observed for initially larger trees. Beside beech, oak showed the highest sensitivity to simultaneously acting global change drivers whilst fir was less affected, independent of the initial tree-size. These species-specific response patterns to global change drivers were reflected on a community level. Accordingly, the net biodiversity effect of the beech-oak mixture declined in the combined nitrogen and drought treatment and produced a significant underyielding, while beech-fir and beech-oak-fir mixtures showed overyielding across all treatments. Apparently, the low sensitivity of fir to the levels of nitrogen and drought applied in this study more than compensated the negative impact on beech and oak trees grown in these mixtures. Therefore, in my study, increased biodiversity does not alleviate global change impacts *per se*. Rather it seems that the combination of specific trait characteristics might improve the resistance of diverse forest communities to global environmental change.

My results showed that European beech responded to at least two simultaneously acting major global change drivers. One possible way to counteract conceivable (non-additive) impacts of global change drivers on growth of beech is to consider the possibility of cultivating more drought-adapted populations to strengthen the genetic variability and resistance of central populations in light of current global change scenarios.

However, the acceptance of natural forest dynamics and selection to allow for intra- and interspecific complementarity, adaptation, and natural rejuvenation might be the most expedient strategy to sustain healthy natural forest ecosystems. Additional studies should combine full factorial long term biodiversity experiments with simultaneously acting global change drivers to understand the temporal influence of biodiversity on mitigating global change impacts in forest ecosystems in Europe.

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5 Appendix

5.1 Declaration of Authorship

Table 1 Publication status of the respective article, quality of the journal, and weighted contribution.

Artikel	Zeitschrift	Impact-Faktor (2016)	Status	Anteil des eigenen Beitrags	Gewichtungsfaktor
Does excess nitrogen supply increase the drought sensitivity of European beech (*Fagus sylvatica* L.) seedlings?	Plant Ecology	1.615	Publiziert	Mit-Autorenschaft Überwiegender Anteil	1,0
Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought.	Forests	1.951	Publiziert	Mit-Autorenschaft Überwiegender Anteil	1,0
Nitrogen addition enhances drought sensitivity of young deciduous tree species.	Frontiers in Plant Science	4.298	Publiziert	Mit-Autorenschaft Überwiegender Anteil	1,0

Table 2 Specific author contributions to the respective articles. Abbreviations: Christoph Dziedek (CD), Werner Härdtle (WH), Goddert von Oheimb (GvO), Andreas Fichtner (AF), Leonor Calvo (LC), Elena Marcos (EM), Witja Till Pitz (WT), Wolf-Ulrich Kriebitzsch (WUK), Kirstin Jansen (KJ), David Walmsley (DW), and Matthias Kunz (MK).

Artikel	Konzeption des Forschungsansatzes	Entwicklung von Forschungsmethoden	Erhebung und Aufbereitung der Daten	Analyse und Interpretation der Daten	Schreiben und Überarbeiten des Manuskripts
Does excess nitrogen supply increase the drought sensitivity of European beech (*Fagus sylvatica* L.) seedlings?	CD (20%) WH (20%) LC (15%) EM (15%) GvO (15%) WUK (15%)	CD (40%) WH (20%) LC (10%) EM (10%) GvO (15%) WUK (5%)	CD (80%) WH (10%) WT (10%)	CD (45%) WH (30%) GvO (10%) AF (5%) WT (10%)	CD (35%) WH (35%) LC (5%) EM (5%) GvO (5%) AF (10%) WT (5%)
Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought.	CD (20%) WH (20%) LC (20%) EM (20%) GvO (20%)	CD (50%) WH (20%) LC (10%) EM (10%) GvO (10%)	CD (80%) WH (5%) LC (5%) EM (5%) GvO (5%)	CD (20%) WH (25%) LC (5%) EM (5%) GvO (5%) KJ (5%) MK (5%) DW (5%) AF (25%)	CD (10%) WH (50%) LC (5%) EM (5%) GvO (5%) KJ (10%) MK (5%) DW (5%) AF (5%)
Nitrogen addition enhances drought sensitivity of young deciduous tree species.	WH (50%) GvO (50%)	WH (30%) CD (70%)	CD (90%) AF (10%)	WH (20%) CD (20%) AF (60%)	WH (40%) GvO (10%) CD (10%) AF (40%)

5.2 Declaration of Authenticity

„Ich versichere, dass ich die eingereichte Dissertation „Global Change Impacts on Beech (*Fagus sylvatica* L.) Forest Ecosystems: the Role and Interaction of Nitrogen and Drought“ selbständig und ohne unerlaubte Hilfsmittel verfasst habe. Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient. Alle wörtlich oder sinngemäß anderen Schriften entnommene Stellen habe ich kenntlich gemacht. Zudem versichere ich, dass alle in diesem Anhang gemachten Angaben jeweils einzeln und insgesamt vollständig der Wahrheit entsprechen“

5.3 Original Published Research Articles

See the articles and the supplemental material of articles II and III.

Christoph Dziedek

Research paper I: Dziedek, C., von Oheimb, G., Calvo, L. et al. Plant Ecol (2016) 217: 393.
https://doi.org/10.1007/s11258-016-0581-1

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

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 analyze single and combined effects of nitrogen fertilization (N treatment) and drought (D treatment) on 16 morphological and chemical response variables (including tissue $\delta^{13}C$ signatures) of one-year-old *Fagus sylvatica* seedlings originating from eight different seed families from the Cantabrian Mountains (NW Spain). Drought exerted the strongest effect on response variables, reflected by decreasing biomass production and increasing tissue $\delta^{13}C$ 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 $\delta^{13}C$ signature

Research paper I: Dziedek, C., von Oheimb, G., Calvo, L. et al. Plant Ecol (2016) 217: 393.
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1. 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 above-mentioned 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 analyzed the interactive effects of cooccurring 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) analyzed 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 analyze 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' south-western 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 δ^{13}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.

2. Materials and Methods

2.1. 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 × 40 km. Since our study did not intend to analyze 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 (±1SE): litter layer depth: 8.6cm (1.8), $pH_{(H2O)}$: 4.3 (0.5), C:N ratio: 14.2 (1.0), cation exchange capacity: 9.9cmol 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.

2.2. 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).

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 578 between 5 and 11km; description of soil morphological and soil chemical properties of 579 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).

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 families × 4 treatments × 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 three 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 one-year-old beech seedling 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 kg N ha^{-1} yr^{-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} yr^{-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 four weeks to avoid position effects.

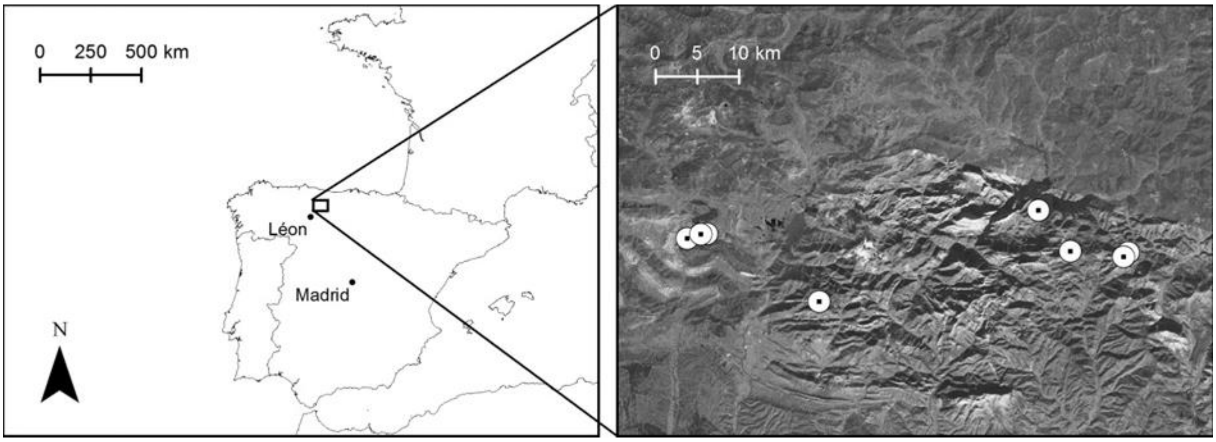

Fig. 1 Locations of the seed origin areas in the Cantabrian Mountains in NW Spain

2.3. 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 5cm 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 m^2 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 (PeeDee Belemnite). The relative precision of repeated analyses of IAEA standards (IAEA-CH-3) was ± 0.1 ‰.

2.4. 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 analyzed with an ANOVA combined with a Tukey's post hoc test (note that analyses of treatment interactions (e.g. N × D) were shown in Table 2, and combined effects of treatments (ND effects) were shown in Figs. 2 and 3). Model residuals were checked for normality (Q-Q-plots) and homogeneity of variances (Levene test). To meet these prerequisites, the following response variables were log-transformed: stem diameter, height, aboveground biomass and shoot:root ratios. The magnitude of treatment effects (MTE) on response variables was calculated as 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).

3. Results

3.1. 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 25%, respectively, as compared to the control treatment (Table 1 in Appendix). 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 × D interactions for stem diameter and leaf biomass, and marginally significant N × 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 1), 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).

3.2. 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 of the roots (-15%; Table 1 in Appendix 1). Moreover, we found a significant increase in the leaf and root $\delta^{13}C$ 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%; Table 1 in Appendix 1) compared to the control.

We found significant N × D × 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 × Family), with D (D × Family), or with N and D (see description of N × D × Family interactions above).

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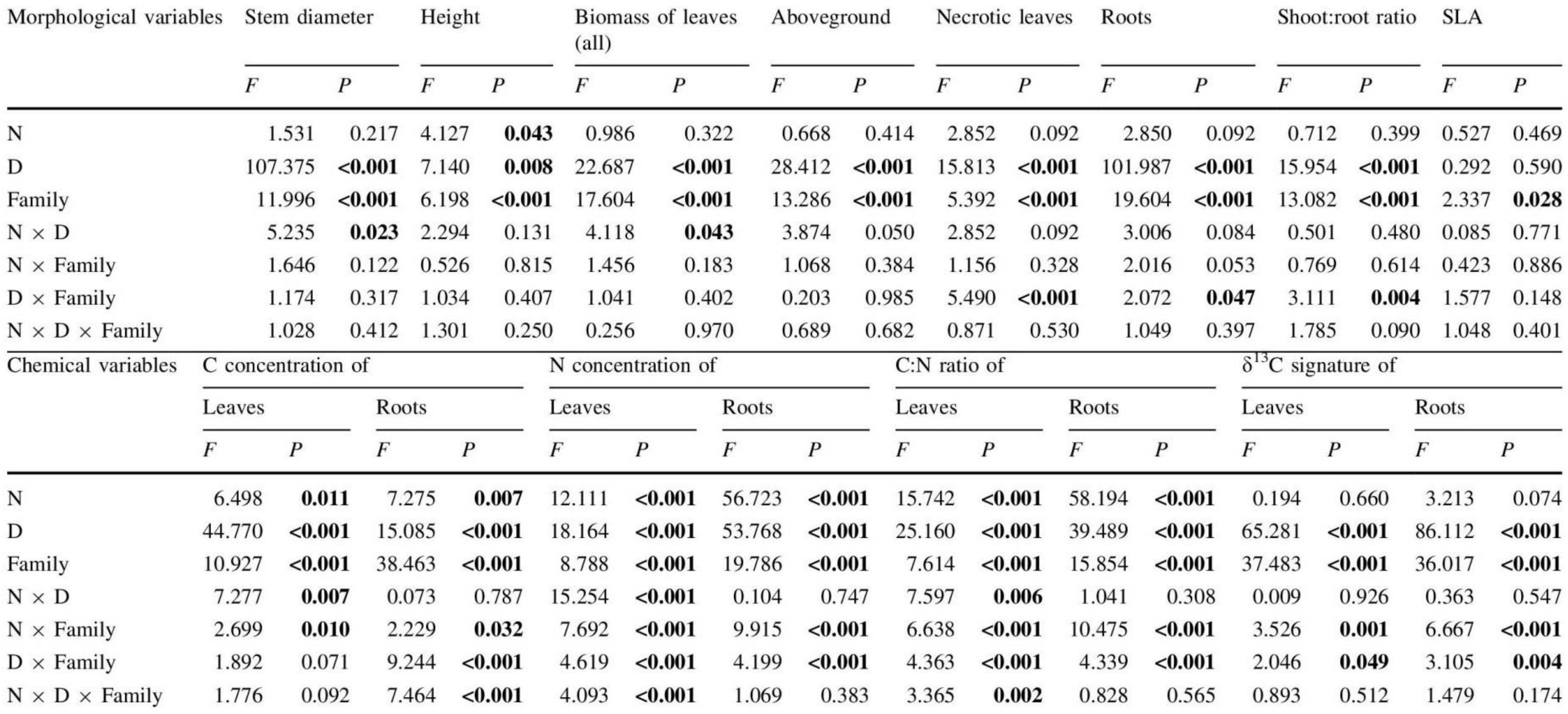
Table 2 Treatment effects on morphological and chemical response variables of beech seedlings from eight seed families. Abbreviations: N: nitrogen fertilization, D: drought treatment, Family: seed families, SLA: specific leaf area. Significant differences ($P<0.05$) are marked in bold type



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				δ^{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

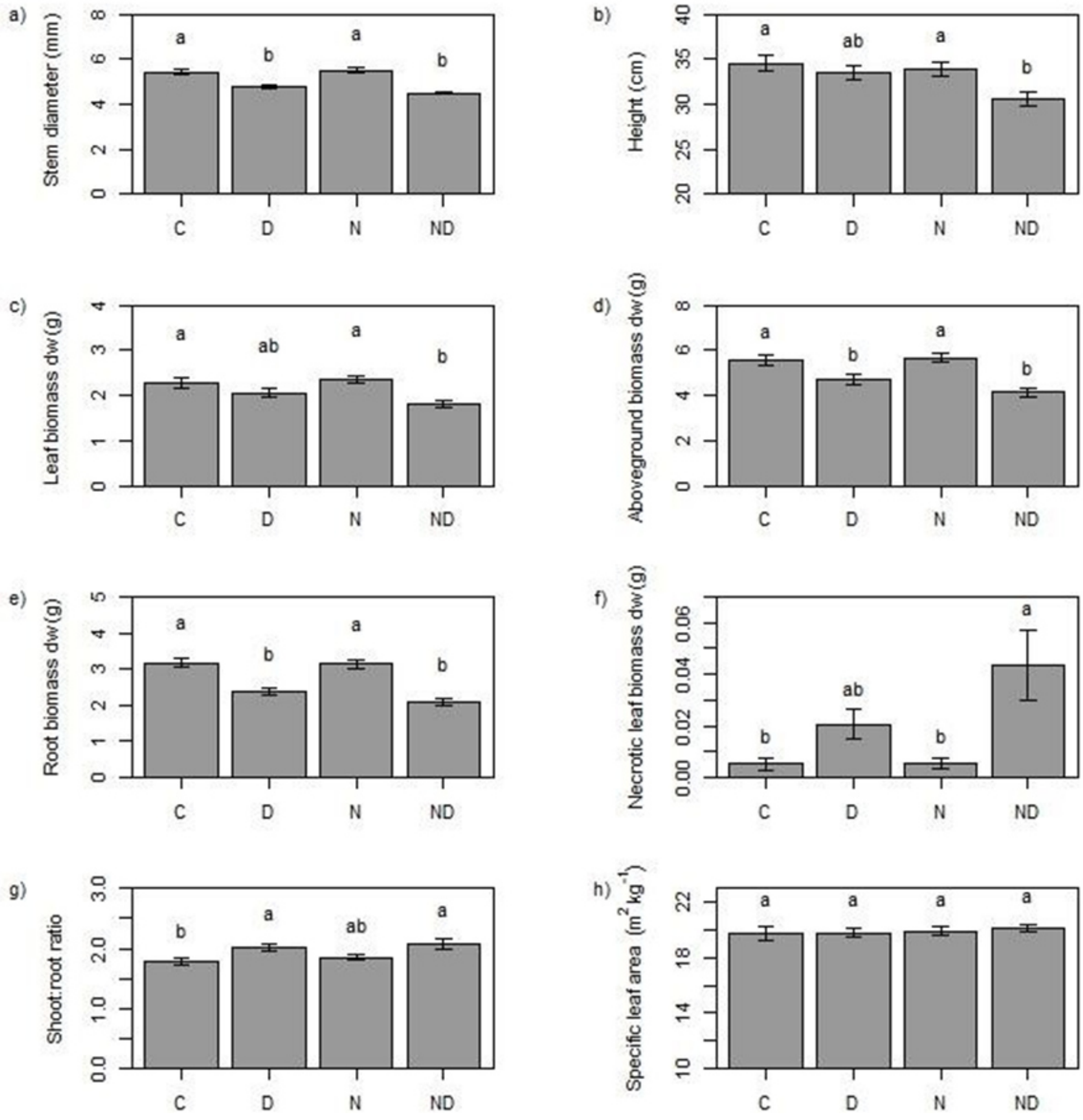


Fig. 2 Treatment effects on morphological response variables of beech seedlings. Different letters indicate significant differences ($P<0.05$); Tukey's post hoc test; error bars show ± 1SE)

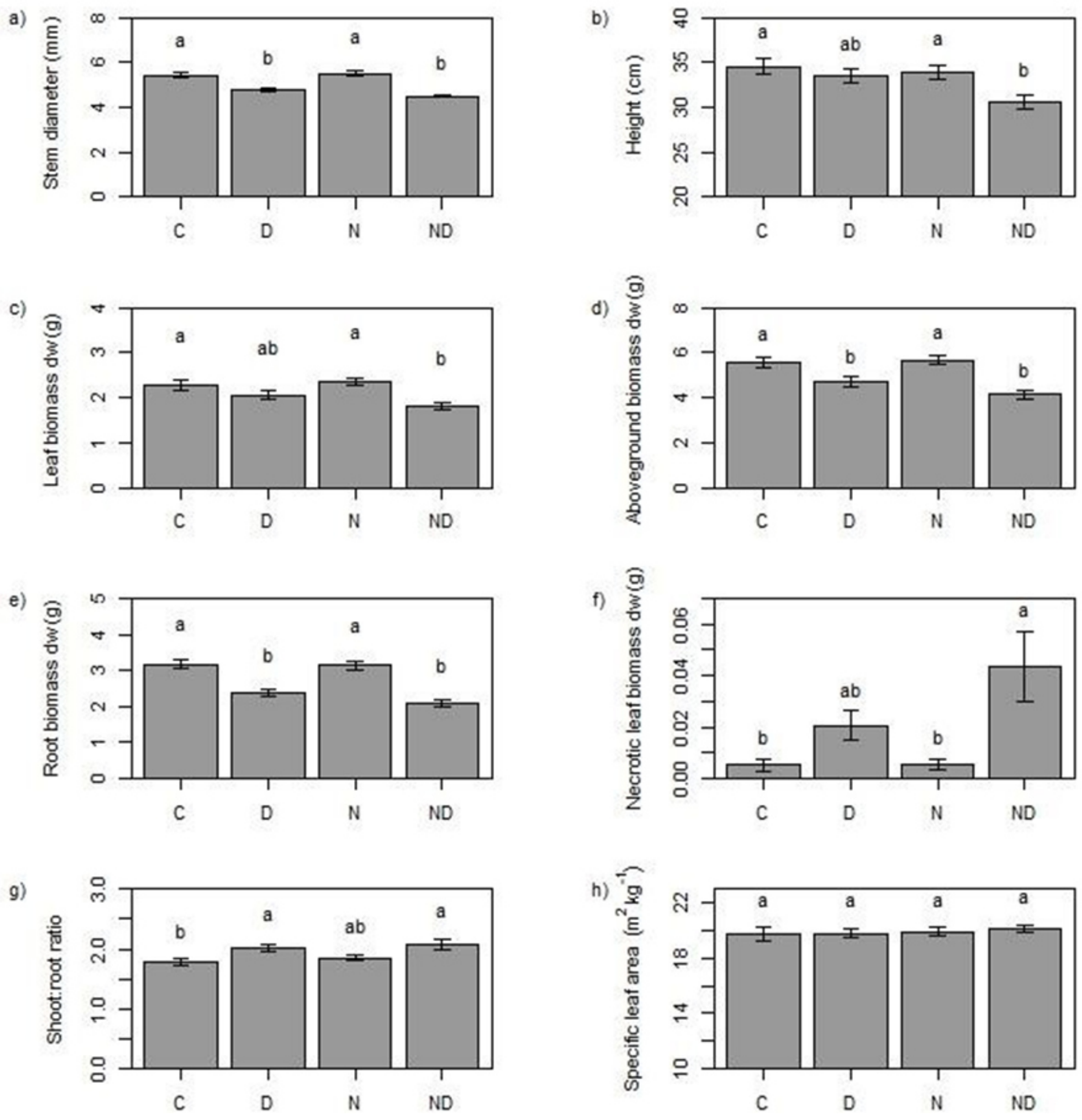


Fig. 3 Treatment effects on chemical response variables of beech seedlings. Different letters indicate significant differences (*P<0.05*); Tukey's post hoc test; error bars show ± 1SE)

4. Discussion

4.1. 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 $\delta^{13}C$ 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 (Garcia-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; Löf et al. 2005; Rose et al. 2009).

4.2. N fertilization effects in 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. Fig. 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. N × D interactions).

4.3. 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 N × 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) N × 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 (Meyer 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).

4.4. Family effects on response variables

Seed family significantly affected all the response variables analyzed. 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 × Family, N × Family, N × D × 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 δ^{13}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 analyzed.

4.5. 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).

5. 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 two 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.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix 1

Table 1 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 δ13C *	-1.8	-0.1	-1.9
Root δ13C *	-2.1	-0.3	-2.7

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

* Note that negative effect size values indicate an increase in δ^{13}C signatures

Abbreviations: N: nitrogen fertilization, D: drought treatment. ND: nitrogen fertilization and drought treatment.

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Phenotypic Plasticity Explains Response Patterns of European Beech (*Fagus sylvatica* L.) Saplings to Nitrogen Fertilization and Drought Events

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Abstract: Climate and atmospheric changes affect forest ecosystems worldwide, but little is known about the interactive effects of global change drivers on tree growth. In the present study, we analyzed single and combined effects of nitrogen (N) fertilization and drought events (D) on the growth of European beech (*Fagus sylvatica* L.) saplings in a greenhouse experiment. We quantified morphological and physiological responses to treatments for one- and two-year-old plants. N fertilization increased the saplings' aboveground biomass investments, making them more susceptible to D treatments. This was reflected by the highest tissue dieback in combined N and D treatments and a significant N × D interaction for leaf $\delta^{13}C$ signatures. Thus, atmospheric N deposition can strengthen the drought sensitivity of beech saplings. One-year-old plants reacted more sensitively to D treatments than two-year-old plants (indicated by D-induced shifts in leaf $\delta^{13}C$ signatures of one-year-old and two-year-old plants by +0.5‰ and −0.2‰, respectively), attributable to their higher shoot:root-ratios (1.8 and 1.2, respectively). In summary, the saplings' treatment responses were determined by their phenotypic plasticity (shifts in shoot:root-ratios), which in turn was a function of both the saplings' age (effects of allometric growth trajectories = apparent plasticity) and environmental impacts (effects of N fertilization = plastic allometry).

Keywords: allometric growth; apparent plasticity; $\delta^{13}C$; global change; plastic allometry; shoot:root ratio

1. Introduction

Many ecosystems are currently subject to unprecedented shifts in environmental conditions on both regional and global scales [1]. This is true of forest ecosystems in particular, since trees are characterized by long life-cycles, and growth processes are mediated by the environment over centuries [2]. Among the currently active drivers of global change, climate and atmospheric changes (such as altered precipitation regimes and the deposition of reactive forms of nitrogen) have been shown to be amongst the major drivers of biodiversity loss and shifts in ecosystem functions [3]. Current climate projections assume rising mean annual temperatures, changing precipitation patterns, and shifts in the frequency and magnitude of extreme weather events (including more severe summer drought events [4]). In forest ecosystems, this may affect ecosystem functions such as primary production and carbon sequestration or the diversity and functional composition of tree species [5–7]. Atmospheric nitrogen (N) deposition has tripled in the past century, with an upward trend expected for the coming decades [8,9]. Airborne N loads are considered to be responsible for enhanced radial increment of trees, but also for adverse effects on the biodiversity of forests [10,11]. While critically high loads of airborne N have affected ecosystem processes over the past decades, the impact of climate change is expected to increase in importance over the course of this century. This means that ecosystems which already have altered nitrogen levels are now subject to climate change, and both factors will continue to act upon ecosystems in the coming decades [12].

Although an increasing body of research has addressed ecosystem responses to environmental shifts by means of single-factor approaches, little is known about the interactive effects of co-occurring global change drivers and how these may affect ecosystem processes and services in the future [13,14]. It is, for example, conceivable that tree growth responses to climate change could be strengthened by the deposition of reactive forms of N, probably due to fertilization effects on morphological traits such as shoot:root ratios ("plastic allometry" [15]). Thus, the extent to which climate shifts may alter tree growth patterns over time will depend on how N deposition will interact with climate warming or drought events [16]. Recent experiments have demonstrated that the combination of summer drought and N fertilization resulted in non-additive effects on plant growth and vitality [17–19]. As a consequence, N-fertilized plants may exhibit higher drought sensitivity as compared to non-fertilized ones.

A further but—with regard to many tree species—not adequately considered factor influencing a plant species' response to environmental shifts is plant age. Many trees follow allometric growth trajectories that are characterized by age-related shifts in biomass allocation patterns ("partitioning"; [15,20,21]). This is reflected by traits such as shoot:root ratios, which are expected to decrease with increasing sapling age. As a consequence, a sapling's drought sensitivity may decrease with an age-related increase of belowground investments ("apparent plasticity" [15]). Thus, a tree's phenotypic plasticity (in terms of both plastic allometry and apparent plasticity) may influence its growth responses to environmental change [22].

In the present study, we analyzed growth responses of tree saplings to combined effects of N fertilization and drought events, taking *Fagus sylvatica* L. as an example. We focused on this tree species because *Fagus sylvatica* is the most abundant and dominating broad-leaved tree species in many parts of Western and Central Europe, and, therefore, is of particular importance from an ecological and economic point of view [23]. Although several studies have investigated the drought sensitivity of beech provenances along precipitation gradients in Central Europe and the Mediterranean region (for an overview see [24]), little is known about the drought sensitivity of beech populations of the south-western range margin (i.e., the Iberian Peninsula), an area that is considered one of the glacial refugia of *Fagus sylvatica* [25]). Since the genotypic plasticity and allelic richness of beech trees in this region are expected to be particularly high [25,26], 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 [24,27–29]. However, it remains unclear how sensitive these beech populations are in responding to co-occurring drivers of global change such as drought events and N deposition.

In the present study, we performed a two-year greenhouse experiment in which we altered the temporal combinations of N fertilization and summer drought, taking beech saplings originating from populations of the Cantabrian Mountain as an example. Specifically, we asked to what extent growth responses of tree saplings to single and combined effects of these drivers of global change were influenced by the saplings age (i.e., we compared the growth responses of one-year-old and two-year-old plants). Beech saplings were subjected to N fertilization in the first and second year of the experiment, but some of the N-fertilized saplings were also exposed to drought events in the second year (i.e., drought took effect on already fertilized plants). Growth responses of saplings were measured in terms of morphological response variables (increment of height, stem diameter, and leaf biomass, total dry weight of aboveground and belowground biomass, tissue die-back) and physiological response variables (leaf C and N concentrations, leaf C:N ratios, and leaf $\delta^{13}C$ signatures as a proxy for the plants' intrinsic water use efficiency [30]). To test for age-related responses, we compared growth responses of one- and two-year-old plants. We hypothesized that (i) N-fertilized plants would exhibit higher drought sensitivity than non-fertilized plants; and (ii) one-year-old plants would be more sensitive to drought treatments than two-year-old plants.

2. Materials and Methods

2.1. Seed Collection

Fagus sylvatica seeds were collected across seven forest sites on north-facing slopes in the Cantabrian Mountains (NW Spain; Figure 1) in autumn 2009 (distances between sampling sites were 3–50 km; for forest site characteristics see Table S1 and Dziedek et al. [31]).

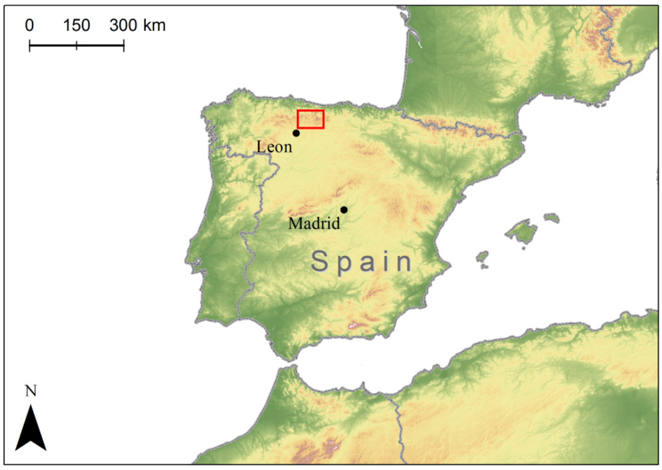

Figure 1. Location of the forest area in the Cantabrian Mountains (North Spain), in which seeds of beech trees were sampled (rectangle).

2.2. Sapling Cultivation And Treatments

The experiment was performed in a greenhouse at the Thünen-Institute (Hamburg, Germany) from spring 2010 to autumn 2011. After stratification in winter 2009/2010, seeds were raised in small pots (so-called "Jiffy Strips", Meyer KG, Rellingen, Germany) and seedlings were transplanted into circular plastic pots in May 2010 (one seedling per pot with 1 L of volume; cultivation substrate: TKS 2, Floragard, Oldenburg, Germany). In the second year of the experiment (2011), saplings were transplanted into circular 3 L plastic pots (one sapling per pot) to account for an increasing belowground biomass (cf. cultivation methods described by [24,31]).

In 2010, a total of 336 pots were randomly assigned to the two treatments applied in the first year: control and nitrogen treatment (i.e., 168 pots per treatment). In 2011, the pots from the 2010 control treatment were randomly assigned to a control and a drought treatment (n = 84 pots in each new treatment). Similarly, the 168 pots from the 2010 nitrogen treatment were randomly assigned to a nitrogen treatment and a combined nitrogen-drought treatment performed in 2011. Thus, 84 pots, respectively, were subjected to four different treatments in 2011: control, nitrogen treatment, drought treatment, and a combined nitrogen-drought treatment (henceforth referred to as control, D, N, and ND treatment, respectively). Pots in the control and N treatment (applied in 2010 and 2011) were well watered during the experiment to avoid drought effects (ca. 40% soil water content). Plants in the D and ND treatments were subjected to two drought periods (applied in 2011), during which no watering took place and the soil water content was reduced to ca. 10%. Both drought periods lasted for about two weeks in June and August 2011 (the duration depended on the development of the pots' soil moisture, quantified by daily weighing during the D treatment). After D treatments, plants were again regularly watered (i.e., 40% soil water content). In the N and ND treatments, N was applied (as NH_4NO_3) in both study years in a quantity equivalent to 50 kg·N·ha^{-1}·year^{-1} (as solution in deionized water; corresponding to current maximum N deposition rates at the natural sites). Nutrient solutions were applied every two weeks from 15th July to 15th September (in 2010 and 2011, except for the two-week drought periods in the ND treatment in 2011). Controls and D treatments received the same amount of deionized water. The mean temperature in the greenhouse was 17.5 °C and 19.0 °C, and the mean relative humidity was 77% and 71% in the first year and second year of the experiment, respectively (means from July to October in 2010 and May to September in 2011). All pots were randomly relocated every four weeks to avoid position effects.

2.3. Measurement of Response Variables

In 2010, the following response variables were measured at the end of the growing season (October): stem diameter (measured 5 cm above the root collar in N-S and E-W direction), plant height (measured from the root collar to the shoot apex), and total leaf biomass (inferred from the number of leaves per tree individual and the mean biomass of a single leaf, determined after leaf harvest at the end of the experiment). In September 2011, all saplings were harvested and the following variables were measured: stem diameter, plant height, number of dead branches, and number of necrotic leaves (more than two-third of the leaf area with necrotic tissue). The root biomass was sampled by carefully wet sieving roots until soil residues were removed as far as possible. As this cleaning procedure was very laborious, belowground biomass was quantified for a subset of randomly selected saplings only (n = 28 per treatment). All biomass samples (shoots, leaves, and roots) were dried to a constant weight at 40 °C, and the following variables were determined: leaf biomass (dry weight = DW of all leaves), aboveground biomass (DW of shoots and leaves), root biomass DW, and shoot:root ratios (aboveground biomass:belowground biomass ratio; n = 28 per treatment). Using both years' data, we also calculated the annual stem, height, and total leaf biomass increment.

Analyses of morphological responses were complemented by the analyses of physiological responses (i.e., leaf C and N concentrations, leaf C:N rations, leaf $\delta^{13}C$ signatures; cf. [24,31]). To this end, leaf samples (one sample comprised all leaves of a tree individual) were ground in a centrifugal mill (ZM 200, Retsch, Haan, Germany) and re-dried at 40 °C for 3 days. C and N concentrations and $\delta^{13}C$ signatures were measured using a continuous flow elemental analyzer-isotope mass spectrometer (vario EL cube, Elementar, Hanau, Germany), coupled to an Isoprime Isotope-ratio mass spectrometer (IRMS, Isoprime Ltd., Cheadle Hulme, UK). Isotope signatures were presented in the delta (δ) notation (in per mil; ‰) as a relative deviation from an international standard (PeeDee Belemnite). The relative precision of repeated analyses of an International Atomic Energy Agency-standard (IAEA-CH-3) was ±0.1‰.

To assess the effect of sapling age on shoot:root ratios and leaf $\delta^{13}C$ signatures, we compared data from the present study with measurements from Dziedek et al. ([31]; one-year-old plants).

2.4. Statistical Analyses

We applied linear mixed-effects models (LMMs) to analyze the effects of treatment (control, D, N, ND) on morphological and physiological responses. To account for variability between forest sites, we used forest site as a random effect. We used the same mixed-model approach to analyze age-related effects (one-year old vs. two-year-old plants) of N and D treatments and their interactions on shoot:root ratio and leaf $\delta^{13}C$ signatures. As some response variables were count data (i.e., number of dead branches and necrotic leaves), we fitted generalized linear mixed-effects models (GLMMs) assuming a Poisson error distribution with a logit link function. We found no indication for overdispersion. The response variables "belowground biomass" and "shoot:root ratio" were log-transformed to meet model assumptions [32]. Model selection (determination of the best-fitting and most parsimonious model) was based on the Akaike information criterion (AIC) using maximum likelihood (ML) estimations and the model with the smallest AIC was chosen as the best-fitting model [33]. Parameter estimates of the best-fitting models were based on the restricted maximum likelihood (REML) method. All statistical analyses were conducted with R 3.1.2 (R Project for Statistical Computing; http://www.R-project.org) with the packages lme4 [34] and lmerTest [35].

3. Results

3.1. Effects of N, D, and ND Treatments on Two-Year-Old Saplings

N and ND treatments caused a significant increase in the aboveground biomass production (in terms of stem increment, leaf biomass increment, and total aboveground biomass), but had no significant effect on the belowground biomass (Table 1 and Table S2). Moreover, the plants' height increment increased in the ND treatment in comparison to the controls. The relative increase in the aboveground biomass allocation resulted in significantly higher shoot:root ratios in the N and ND treatments (Table S2). Branch dieback was significant only in the ND treatment ($p = 0.006$), whereas N fertilization reduced the number of necrotic leaves (negative estimate; Table 1). N fertilization caused a distinct increase in N concentrations, which in turn resulted in decreasing C:N ratios (Tables 1 and S2). Leaf δ^{13}C signatures were not significantly affected by N fertilization. D treatments had no effect on morphological responses, but caused decreasing values for C concentrations, N concentrations, and leaf δ^{13}C signatures (Tables 1 and S2).

3.2. Effects of Sapling-Age

On average, one-year-old plants showed significantly higher shoot:root ratios than two-year-old plants (Table 2; $p < 0.001$). In the controls, shoot:root ratios decreased from 1.78 in the first year to 1.20 in the second year (Figure 2a). The effects of the N treatments on shoot:root ratios were not affected by plant age (i.e., no significant N × Age interaction; Table 2). This indicates that N fertilization caused an increase in shoot:root ratios irrespective of the plants age.

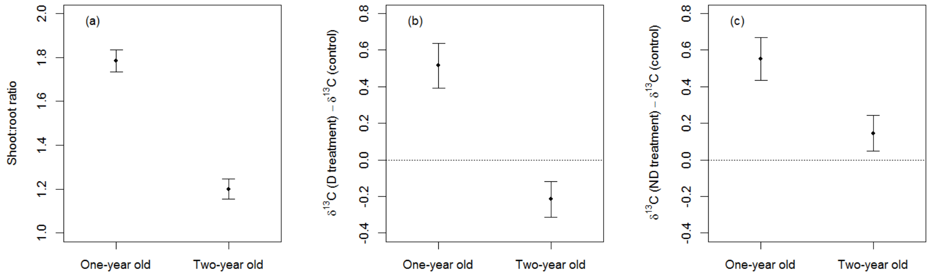

Figure 2. (**a**) Shoot:root ratios (mean ± 1SE) of one-year-old and two-year-old *Fagus sylvatica* plants in the control treatment. Age-related differences are significant at $\alpha = 0.001$. Differences in tissue δ^{13}C signatures (mean ± 1SE) between (**b**) drought treatments (D) and the control ($p < 0.001$) and (**c**) combined nitrogen and drought treatments (ND) and the control ($p = 0.008$) of one-year-old and two-year-old *Fagus sylvatica* plants.

In addition, we found age-related responses of leaf δ^{13}C signatures to D and ND treatments, indicated by a significant D × Age interaction for leaf δ^{13}C signatures (Table 2). Whereas D treatments caused an increase in leaf δ^{13}C values of one-year-old plants by about 0.53‰ (from −29.36‰ to −28.83‰), leaf δ^{13}C values of two-year-old plants decreased by about 0.19‰ (from −29.05‰ to −29.24‰; Figure 2b). In the ND treatment, we found a significant increase in leaf δ^{13}C values by about 0.57‰ for one-year-old plants (from −29.36‰ to −28.79‰), whereas shifts in δ^{13}C values were non-significant for two-year-old plants (increase by about 0.09‰ from −29.05‰ to −28.96‰; Figure 2c). Importantly, N treatments strengthened an increase in leaf δ^{13}C signatures following D treatments, indicated by significant D × N interaction (and a positive estimate) for both one-year-old and two-year-old plants ($p = 0.042$).

Table 1. Results of mixed-effects models (LMM and GLMM) for treatment effects in response to morphological and physiological variables. Abbreviations of treatments: D = drought treatment, N = nitrogen treatment, ND = combined nitrogen and drought treatment; significant effects ($p < 0.05$) are indicated in bold.

Morphological Variables	**Stem Increment**			**Height Increment**			**Leaf Biomass Increment**			**Aboveground Biomass**		
	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value
Intercept	1.867	16.828	**<0.001**	3.837	3.617	**0.004**	2.546	9.986	**<0.001**	18.121	19.998	**<0.001**
D	0.026	0.260	0.793	0.048	0.054	0.957	0.158	1.158	0.248	0.609	0.709	0.479
N	0.566	5.649	**<0.001**	2.021	2.292	0.023	0.397	2.932	**0.004**	2.712	3.170	**0.002**
ND	0.567	5.675	**<0.001**	2.599	2.956	**0.003**	0.461	3.413	**<0.001**	3.593	4.211	**<0.001**
Morphological Variables	**Belowground Biomass**			**Shoot:Root Ratio**			**No. of Necrotic Leaves**			**No. of Dead Branches**		
	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value	Estimate	χ^2	*p*-value	Estimate	χ^2	*p*-value
Intercept	2.669	41.668	**<0.001**	0.073	3.690	**0.002**	0.550	2.363	**0.018**	0.072	−0.270	0.787
D	0.047	0.599	0.550	0.008	−0.375	0.709	0.464	1.675	0.094	0.079	−0.386	0.700
N	0.068	0.866	0.389	0.059	2.857	**0.005**	−0.620	−2.130	**0.033**	0.216	1.101	0.270
ND	0.109	0.079	0.169	0.043	2.061	**0.041**	−0.330	−1.163	0.245	0.512	2.724	**0.006**
Physiological Variables	**C Concentration Leaves**			**N Concentration Leaves**			**C:N Ratio**			**Leaf δ^{13}C Signature**		
	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value	Estimate	*t*-value	*p*-value
Intercept	463.205	1.987	**<0.001**	15.413	27.799	**<0.001**	30.777	29.853	**<0.001**	29.049	−167.820	**<0.001**
D	−3.738	2.931	**0.004**	−1.354	−4.296	**<0.001**	2.420	4.634	**<0.001**	−0.197	−2.175	**0.030**
N	−2.466	1.934	0.054	3.138	9.954	**<0.001**	−5.380	10.302	**<0.001**	−0.094	−0.134	0.302
ND	−2.482	1.945	0.053	2.557	8.107	**<0.001**	−4.718	−9.031	**<0.001**	0.083	0.920	0.358

Table 2. Best-fitting mixed-effects models for (a) shoot:root ratio and (b) leaf δ^{13}C signatures of *Fagus sylvatica* saplings. Shoot:root ratios and leaf δ^{13}C signatures were modelled as a function of the saplings' age (one-year old vs. two-year-old plants), drought treatments (D, ND), nitrogen treatments (N, ND), and their interactions. The best-fitting model was selected using the Akaike information criterion (AIC).

Fixed Effects	Estimate	*t*-Value	*p*-Value
(a) Shoot:root ratio			
Intercept	1.747	22.185	<0.001
D	0.023	4.649	<0.001
N	0.089	2.078	0.038
Age (1 year vs. 2 years)	−0.456	−4.392	<0.001
D × Age	−0.258	−2.621	0.009
(b) Leaf δ^{13}C signature			
Intercept	−29.688	−159.014	<0.001
D	0.442	5.431	<0.001
N	−0.034	−0.516	0.606
Age (1 year vs. 2 years)	1.058	8.188	<0.001
D × N	0.191	2.035	0.042
D × Age	−0.546	−5.804	<0.001

4. Discussion and Conclusions

4.1. Treatment (N, D, ND) Effects on Sapling Growth

Beech saplings significantly increased their aboveground investments in N and ND treatments and hence followed the "resource optimization hypothesis" [36], according to which plants increase their aboveground biomass allocation as a result of improved nutrient supply. This observation is in agreement with other studies [17,18,37,38], and was also mirrored by increased shoot:root ratios in the N and ND treatments. However, only plants from the ND treatment showed a significant biomass dieback. This finding supports our first hypothesis ("N-fertilized plants exhibit higher drought sensitivity") and suggests that combined effects of N fertilization and drought may adversely affect the vitality of beech saplings. This interpretation is supported by the finding that N treatments strengthened the effect of drought on leaf δ^{13}C signatures (D × N interaction; Table 2). This might be explained with the observed shifts in biomass allocation patterns (i.e., increasing shoot:root ratios), as has also been documented by other studies. [39,40]. An indication of drought stress based on the plants' leaf δ^{13}C signatures in the ND treatment was particularly pronounced for one-year-old plants (see paragraph on "effects of sapling age"). We cannot rule out the possibility that other factors, such as nutrient imbalances or a failure of photoassimilate transport, may have also contributed to the dieback of branches of two-year-old plants, because these factors are impaired by N fertilization [41].

4.2. Effects of Sapling Age

Comparisons of leaf δ^{13}C signatures showed that plant responses to treatments were strongly influenced by sapling age. This supports our second hypothesis that one-year-old plants would react more sensitively to treatments than two-year-old plants, particularly with regard to drought events. We hypothesize that differences in drought sensitivity were mainly related to the plants' shoot:root ratios [22], which significantly differed for one-year-old and two-year-old beech saplings (i.e., two-year-old plants showed relatively higher belowground investments than one-year-old plants). As a consequence of these age-related shifts in biomass allocation patterns (i.e., apparent plasticity; according to Weiner [15]), two-year-old plants may be less drought sensitive and may experience less constraints in their water supply, particularly in periods of drought [42,43]. This interpretation is supported by the finding that leaf δ^{13}C signatures of one-year-old plants increased by 0.53‰ and 0.57‰

in the D and ND treatment, respectively, whereas two-year-old plants showed no significant shifts or even decreasing values in leaf δ^{13}C signatures in response to the ND and D treatments, respectively.

Given that the biomass allocation patterns of many plant species follow allometric trajectories and are therefore a function of plant age [15], our findings on age-related responses might have general implications for predictions of plant responses to environmental stressors such as climate or atmospheric changes. In forest ecosystems, for example, biomass allocation patterns strongly depend on tree age [44], but relationships between the trees' life-stages and their sensitivity to climate and atmospheric changes have not been well investigated or are even unknown [45]. Ettinger and HilleRisLambers [46] found that climate change-related tree mortality in forest ecosystems was strongly affected by stand development processes, and effects of decreasing precipitation or increasing temperatures on tree growth significantly decreased with stand age. A study by Luo and Chen [45] confirmed that climate change-associated increases in tree mortality were significantly higher in young compared to old forests due to the higher sensitivity of young trees to regional warming and drought. Thus, observations from mature forest stands might underestimate climate change effects on tree mortality. Luo and Chen [45] concluded that life-stage related analyses of tree growth are crucial to better understand and predict forest responses to climate change. This might also apply to an assessment of interaction effects of drought and N deposition on tree growth: given that one-year-old seedlings have the highest shoot:root ratios (according to age-related allometric trajectories; [15]) and that N fertilization further increases shoot:root ratios (according to the resource optimization hypothesis), then N-fertilized one-year-old seedlings should exhibit the highest sensitivity to drought events (also suggested by the significant D × N interaction for leaf δ^{13} signatures). This conclusion is supported by the experiments of Dziedek et al. [31], which showed that a combination of N fertilization and drought negatively affected the total biomass production and strongly increased the formation of necrotic leaf tissue.

In conclusion, our experiments provided evidence that nitrogen fertilization has the potential to increase the drought sensitivity of beech saplings due to its impact on biomass partitioning, with consequences for the plants' shoot:root ratios (i.e., plastic allometry of tree saplings). However, this increase in drought sensitivity is confounded with sapling age, because sapling development seems to follow allometric growth trajectories in which partitioning patterns are also life-stage dependent (i.e., apparent plasticity of tree saplings). As a consequence, predictions of tree growth responses to atmospheric and climate changes should consider the effects related to both the plastic allometry and apparent plasticity of a tree species' development. We are aware that greenhouse experiments are limited with regard to a generalization of findings, and our study does not allow us to directly infer growth response of naturally regenerated trees to global change effects. However, allometric growth trajectories should also apply to naturally regenerated tree saplings. This would indicate that assessments of tree growth responses to global change should include life-stage related shifts in a tree's sensitivity to co-occurring global change drivers.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/8/3/91/s1, Table S1: Site characteristics (from Dziedek et al. [31]), Table S2: Summary of treatment effects on the response variables measured, Table S3: Data for two-year-old trees, Table S4: Data for one-year-old and two-year-old trees (comparisons of shoot:root ratios and leaf δ^{13} signatures). References [47,48] are cited in the supplementary materials").

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Supplemental Materials:

Christoph Dziedek, Andreas Fichtner, Leonor Calvo, Elena Marcos, Kirstin Jansen, Matthias Kunz, David Walmsley, Goddert von Oheimb and Werner Härdtle (2017) Phenotypic Plasticity Explains Response Patterns of European Beech (*Fagus Sylvatica* L.) Saplings to Nitrogen Fertilization and Drought Events. Forests, 8, 91.

2. Materials and Methods

2.1. Seed Collection

Fagus sylvatica seeds were collected across seven forest sites on north-facing slopes in the Cantabrian Mountains (NW Spain; Figure 1) in autumn 2009 (distances between sampling sites were 3–50 km; for forest site characteristics see Table S1 and Dziedek et al. [31]).

Table S1. Climatic conditions for the seven forest locations in the Cantabrian Mountains (North Spain), from which *Fagus sylvatica* seeds were sampled in 2009 (from Dziedek et al. [31]).

Location	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)

(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). Distances between sampling sites were 3–50 km, and distances between weather stations and sampling were between 5 and 11 km; the prevailing soil type at the forest sites sampled is a humic cambisols; chemical soil properties based on means of four samples (±1 SE): litter layer depth: 8.6 cm (1.8), $pH_{(H2O)}$: 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. [1]; plant community: Blechno spicanti-Fagetum sensu Rivas-Martinez [2].

3. Results

3.1. Effects of N, D, and ND Treatments on Two-Year-Old Saplings

N and ND treatments caused a significant increase in the aboveground biomass production (in terms of stem increment, leaf biomass increment, and total aboveground biomass), but had no significant effect on the belowground biomass (Tables 1 and S2). Moreover, the plants' height increment increased in the ND treatment in comparison to the controls. The relative increase in the aboveground biomass allocation resulted in significantly higher shoot:root ratios in the N and ND treatments (Table S2). Branch dieback was significant only in the ND treatment ($p = 0.006$), whereas N fertilization reduced the number of necrotic leaves (negative estimate; Table 1). N fertilization caused a distinct increase in N concentrations, which in turn resulted in decreasing C:N ratios (Tables 1 and S2). Leaf $\delta^{13}C$ signatures were not significantly affected by N fertilization. D treatments had no effect on morphological responses, but caused decreasing values for C concentrations, N concentrations, and leaf $\delta^{13}C$ signatures (Tables 1 and S2).

Table S2. Treatment effects on response variables (means, ± SE in brackets) of two-year-old beech saplings. Abbreviations: D = drought treatment, N = nitrogen treatment, ND = combined nitrogen and drought treatment; DW = dry weight.

Response variables	Control		D		N		ND	
Stem increment (mm yr^{-1})	1.86	(0.07)	1.90	(0.06)	2.46	(0.08)	2.47	(0.08)
Height increment (cm yr^{-1})	3.90	(0.69)	3.88	(0.59)	5.90	(0.69)	6.45	(0.68)
Leaf biomass increment (g DW yr^{-1})	2.56	(0.11)	2.71	(0.10)	2.95	(0.12)	3.01	(0.13)
Aboveground biomass (g DW)	18.13	(0.60)	18.77	(0.52)	20.84	(0.69)	21.73	(0.70)
Belowground biomass (g DW)3	15.13	(0.81)	15.57	(0.72)	16.16	(1.00)	16.90	(0.96)
Shoot:root ratio	1.20	(0.05)	1.18	(0.04)	1.37	(0.04)	1.34	(0.06)
No. necrotic leaves	2.67	(0.93)	3.00	(0.97)	1.04	(0.19)	1.31	(0.27)
No. dead branches	1.15	(0.20)	1.01	(0.14)	1.38	(0.21)	2.01	(0.54)
Leaf C concentration (g kg^{-1})	463.23	(0.98)	459.48	(0.93)	460.75	(0.91)	460.76	(1.00)
Leaf N concentration (g kg^{-1})	15.36	(0.27)	14.08	(0.21)	18.57	(0.31)	18.01	(0.25)
Leaf CN ratio	30.87	(0.52)	33.17	(0.47)	25.37	(0.44)	25.98	(0.38)
Leaf $\delta^{13}C$ signature	-29.05	(0.09)	-29.24	(0.08)	-29.14	(0.08)	-28.97	(0.06)

Supplementary Materials: The following are available online at http://www.mdpi.com/journal/forests, Table S1: Site characteristics (from Dziedek et al. [31]), Table S2: Summary of treatment effects on the response variables measured, Table S3: Data for two-year-old trees, Table S4: Data for one-year-old and two-year-old trees (comparisons of shoot:root ratios and leaf δ^{13} signatures). References [1,2] are cited in the supplementary materials").

Table S3: Data for two-year-old trees

Ident_no	Forest-site	Treatment	Stem_Diam_2010	Height_2010	Stem_Diam_2011	Height_2011	Stem_incr	Height_incr	Leaf_biom_incr	DW_Stem	DW_Leaves	DW_Above	DW_Below	No_Branches_2011	No_Dead_Branches_2011	No_necr_leeaves	Leaf_N	Leaf_C	Leaf_CN	Leaf_d13C
1	1	C	5.4	44	7.03	48	1.63	4	0.13	9.42	2.87	12.29	14.23	5	3	2	18.9	471.45	24.92	-29.37
2	1	C	5.15	46.2	6.54	41.2	1.39	-5	1.1	6.72	2.45	9.17	8.65	11	9	6	20.3	466.7	23	-29.06
3	1	C	5.4	50.5	7.08	59.3	1.68	8.8	1.38	11.63	3.3	14.93	NA	18	1	0	16.3	472.2	29.04	-28.66
4	1	C	5.68	50.6	7.45	43.8	1.77	-6.8	1.49	13.04	3.87	16.91	12.7	15	5	2	19	471.5	24.81	-28.11
5	1	C	5.32	43.9	6.8	44.2	1.48	0.3	1.98	8.91	3.33	12.24	NA	18	4	1	20.4	471.2	23.11	-28.56
6	1	C	5.56	63.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	1	C	6.36	47.5	9.68	47.5	3.32	0	2.98	18.96	5.25	24.21	NA	22	0	0	17.7	461.6	26.03	-30.13
8	1	C	6.52	39.5	8.49	46.5	1.97	7	2.54	13.14	4.59	17.73	NA	19	0	0	18.6	469.5	25.29	-28.64
9	1	C	5.94	47.5	7.72	48.4	1.78	0.9	2.53	12.35	4.24	16.59	NA	20	4	4	17.3	464.1	26.79	-29.07
10	1	C	4.87	38.9	6.47	41.5	1.6	2.6	0.42	6.41	1.86	8.27	5.47	14	4	6	19.8	478.4	24.14	-29.29
11	1	C	4.78	41.3	6.54	31.3	1.76	-10	-0.76	6.55	2.07	8.62	NA	13	7	6	20.1	475.5	23.66	-27.45
12	1	C	6.07	39.4	7.73	45.4	1.66	6	2.1	12.89	4.6	17.49	NA	25	2	10	17.1	465.1	27.13	-28.39
13	2	C	6.07	50.4	7.23	50.5	1.16	0.1	1.92	10.62	2.92	13.54	NA	18	0	3	14.9	468.6	31.47	-28.6
14	2	C	7.21	73.6	8.56	75.5	1.35	1.9	3.02	18.11	4.95	23.06	NA	22	0	0	11.8	475.8	40.27	-27.85
15	2	C	6.66	64.3	7.63	60.8	0.97	-3.5	2.5	13.93	5.11	19.04	13.4	27	1	0	12.4	455.9	36.84	-29.87
16	2	C	6.09	51.2	7.3	50	1.21	-1.2	1.68	10.16	2.93	13.09	NA	19	0	0	13.1	453.3	34.71	-27.91
17	2	C	5.44	42.2	6.47	46.8	1.03	4.6	1.28	9.48	3.53	13.01	NA	10	0	0	12.9	456.5	35.5	-29.29
18	2	C	6.29	53.7	8.39	55.8	2.1	2.1	3.69	16.73	5.04	21.77	NA	13	2	1	11.5	458.2	39.85	-29.35
19	2	C	6.78	53.4	8.51	53.2	1.73	-0.2	2.41	14.49	3.89	18.38	NA	23	0	2	12.5	457.8	36.77	-29.97
20	2	C	5.72	50.5	7.02	50.4	1.3	-0.1	2.55	12.85	4.34	17.19	11.32	20	0	2	11.7	463.6	39.76	-29.86

21	2	C	6.96	40.4	8.55	44.5	1.59	4.1	2.68	14.74	5.09	19.83	18.52	13	0	1	12.95	463.9	35.93	-28.54
22	2	C	5.73	42.4	7.35	46.5	1.62	4.1	3.36	12.45	4.56	17.01	NA	19	1	3	14.5	465.6	32.19	-29.73
23	2	C	6.33	34.5	8.15	37.7	1.82	3.2	2.36	12.36	4.2	16.56	16.52	25	0	0	15.2	460.3	30.3	-28
24	2	C	5.42	54.4	6.74	52.8	1.32	-1.6	2.19	9.34	3.22	12.56	NA	19	1	0	13.3	464.5	34.94	-28.91
25	3	C	7.25	45.7	8.88	56.3	1.63	10.6	4.18	17.63	6.42	24.05	NA	22	2	0	13.8	465.5	33.67	-30.92
26	3	C	7.67	49.4	8.55	60.2	0.88	10.8	2.75	15.24	5.02	20.26	NA	20	0	0	13.1	464.4	35.54	-29.01
27	3	C	5.7	42.5	7.5	61.8	1.8	19.3	3.4	15.83	5.37	21.2	15.31	21	0	7	12.7	442.3	34.79	-29.95
28	3	C	7.74	45.4	8.57	46.5	0.83	1.1	3.09	13.88	4.67	18.55	14.98	23	3	1	13.5	457.1	33.85	-29.33
29	3	C	4.49	29.5	6.78	34.2	2.29	4.7	2.05	7.91	2.88	10.79	NA	10	0	0	16.6	454.4	27.31	-29.25
30	3	C	7.24	41.2	8.89	48.4	1.65	7.2	3.16	16.85	5.34	22.19	NA	29	0	0	15.4	471.2	30.69	-28.58
31	3	C	7.4	57	9.4	65	2	8	3.43	19.04	5.13	24.17	NA	25	0	0	12.9	465.9	36.17	-29.16
32	3	C	6.07	36.2	7.53	34.4	1.46	-1.8	3.54	11.13	3.94	15.07	13.91	12	0	0	15.2	464.1	30.57	-28.5
33	3	C	5.06	26.3	7.22	30.5	2.16	4.2	2.51	8.4	3.07	11.47	NA	7	1	8	17.6	472.4	26.84	-29.52
34	3	C	5.92	36.9	8.1	41.1	2.18	4.2	3.29	12.45	4.42	16.87	16.7	16	0	0	14.6	469	32.21	-30.48
35	3	C	7.18	59.4	8.07	59.2	0.89	-0.2	4.2	16.79	5.66	22.45	NA	21	0	0	11.8	467.2	39.57	-29.6
36	3	C	6.58	44.3	7.61	46.7	1.03	2.4	2.54	12.11	4.64	16.75	NA	17	0	0	13.7	498.7	36.4	-29.18
37	4	C	5.01	55.8	7.95	51	2.94	-4.8	1.11	12.46	2.92	15.38	NA	17	1	2	16	463.6	28.92	-27.97
38	4	C	5.96	35.1	7.85	45.3	1.89	10.2	3.44	12.13	5.02	17.15	NA	27	1	6	13.3	460.1	34.49	-27.99
39	4	C	5.84	46.2	NA	NA	NA	NA	NA	NA	NA	NA	14.25	NA	NA	NA	NA	NA	NA	NA
40	4	C	6.09	45.8	9.52	61	3.43	15.2	4.51	21.37	6.66	28.03	17.66	32	0	1	13.25	457	34.46	-29.25
41	4	C	5.64	48.8	7.64	50.7	2	1.9	2.75	12.65	3.99	16.64	15.39	21	0	0	13.5	455.7	33.72	-28.06
42	4	C	7.05	58.4	10.47	64.5	3.42	6.1	3.85	25.51	5.75	31.26	NA	20	0	0	14.9	448.4	30.11	-29.31
43	4	C	6.83	66.6	9.07	58.4	2.24	-8.2	2.63	17.6	4.22	21.82	NA	13	5	2	17.5	471.7	26.94	-27.62
44	4	C	6.62	55.5	10.1	60	3.48	4.5	2.94	20.6	5.65	26.25	NA	26	0	1	12.5	454	36.36	-28.27
45	4	C	6.15	62.4	7.72	63.8	1.57	1.4	2.49	13.81	5.61	19.42	NA	36	0	73	11.6	453.1	39.13	-29.57
46	4	C	5.62	52.8	7.44	49.4	1.82	-3.4	1.85	10.22	3.4	13.62	NA	15	5	1	16.9	451.6	26.76	-28.58

47	4	C	7.29	60.5	9.04	65.4	1.75	4.9	4.17	22.2	6.33	28.53	15.1	29	1	0	13.3	447.1	33.72	-29.75
48	4	C	6.78	41.3	8.29	43.4	1.51	2.1	2.31	14.12	4	18.12	NA	24	5	18	13.7	458	33.51	-28.51
49	5	C	5.03	24.9	6.59	29.5	1.56	4.6	1.85	6.62	2.37	8.99	NA	15	0	0	15.3	456.3	29.91	-29.06
50	5	C	7	43.2	9.42	52.8	2.42	9.6	3.54	18	5	23	19.14	27	0	2	12.7	461.4	36.32	-28.81
51	5	C	7.05	52.8	10.04	63.3	2.99	10.5	3.54	25.17	6.1	31.27	NA	21	0	0	13.3	462	34.68	-28.91
52	5	C	6.76	39.5	8.71	48.8	1.95	9.3	3.28	13.55	5.54	19.09	NA	26	1	3	16.5	454.8	27.56	-28.28
53	5	C	6.11	49	7.68	50.8	1.57	1.8	2.98	14.15	4.33	18.48	13.79	19	0	0	13.9	460.3	33.1	-28.45
54	5	C	6.8	47.4	8.06	47.9	1.26	0.5	2.73	13.26	4.62	17.88	NA	24	0	3	12.7	466.6	36.64	-28.53
55	5	C	6.04	49.6	7.43	55.5	1.39	5.9	3.21	12.84	4.35	17.19	NA	15	3	3	16.4	459.5	27.98	-29.21
56	5	C	7.35	41.5	8.87	47	1.52	5.5	3.63	15.34	4.55	19.89	NA	35	0	0	16.8	453.8	26.99	-28.41
57	5	C	6.8	43.9	8.5	41	1.7	-2.9	3.26	14.03	4.18	18.21	NA	17	2	2	16.6	459.4	27.63	-28.74
58	5	C	7.17	42.4	9.56	54.6	2.39	12.2	4	18.53	5.63	24.16	19.54	28	0	0	12.8	455	35.6	-29.57
59	5	C	6.69	41.7	7.44	42.2	0.75	0.5	2.32	9.11	3.57	12.68	NA	17	4	3	17	466	27.47	-28.51
60	5	C	7.21	44.7	8.64	53.2	1.43	8.5	3.61	14.78	5.3	20.08	16.16	18	1	2	14.5	444.2	30.58	-28.97
61	6	C	4.7	37.7	7.08	44.3	2.38	6.6	1.54	7.88	2.46	10.34	NA	15	0	0	14.9	469.4	31.52	-29.15
62	6	C	6.41	43.2	9.1	60.5	2.69	17.3	NA	NA	NA	NA	NA	31	1	2	14.2	471.6	33.18	-30.36
63	6	C	6.94	54.2	9.52	84.8	2.58	30.6	3.19	22.58	5.27	27.85	NA	23	0	0	15.4	465.9	30.16	-30.27
64	6	C	5.65	43.2	8.27	51.5	2.62	8.3	2.51	14.85	3.86	18.71	14.98	24	1	1	NA	NA	NA	NA
65	6	C	5.48	40	7.5	38.4	2.02	-1.6	1.98	10.54	3.46	14	NA	25	1	5	17.4	465.1	26.7	-29.35
66	6	C	4.98	46.7	6.98	53.5	2	6.8	1.36	9	2.65	11.65	10.6	14	0	0	16.1	475.7	29.47	-28.54
67	6	C	6.76	50	9.19	63.4	2.43	13.4	2.42	17.96	4.12	22.08	NA	26	0	3	15.8	462.3	29.27	-30.04
68	6	C	6.75	44.5	8.33	46.7	1.58	2.2	1.53	12.04	3.27	15.31	14.6	17	0	0	16.2	462.1	28.47	-29.57
69	6	C	7.06	55.3	8.93	58.8	1.87	3.5	3.11	18.09	4.65	22.74	NA	27	1	0	15.4	475.5	30.92	-29.41
70	6	C	5.79	38.8	8.12	43.8	2.33	5	1.59	11.34	3.13	14.47	NA	26	0	5	16.8	473.8	28.13	-30.3
71	6	C	5.14	39.7	7.4	50.4	2.26	10.7	1.15	10.27	2.83	13.1	NA	14	0	0	17.6	466.2	26.45	-30.79
72	6	C	6.54	46.9	7.82	43.2	1.28	-3.7	1.16	12.75	4	16.75	17.83	25	0	2	14.6	479.7	32.86	-30.45

73	7	C	7.25	47.1	8.99	53.6	1.74	6.5	2.76	15.96	5.11	21.07	19.47	18	2	0	13.5	455.8	33.88	-29.73
74	7	C	3	27.5	4.95	31.9	1.95	4.4	0.72	3.82	1.34	5.16	6.35	7	0	0	20.2	459.9	22.76	-30.19
75	7	C	6.91	55.5	9.97	61.8	3.06	6.3	3.46	21.93	5.59	27.52	25.42	28	1	0	15.4	465.5	30.15	-28.37
76	7	C	6.95	45.7	8.64	43.6	1.69	-2.1	2.44	14.22	3.87	18.09	NA	22	3	0	19.2	462.4	24.07	-27.78
77	7	C	7.21	50.3	9.38	48.1	2.17	-2.2	3.48	18.98	5.05	24.03	NA	24	0	1	NA	NA	NA	NA
78	7	C	5.77	42.4	7.25	49.4	1.48	7	2.05	9.42	3.15	12.57	NA	17	1	0	20.7	461.3	22.29	-28.2
79	7	C	6.42	44.3	7.61	46.7	1.19	2.4	2.54	11.14	4.13	15.27	NA	17	0	0	14.6	461.3	31.67	-28.59
80	7	C	5.4	45.4	8.45	50.5	3.05	5.1	2.27	14.21	3.98	18.19	NA	19	0	0	16.5	463.6	28.18	-29.17
81	7	C	7.56	54.8	8.98	47.9	1.42	-6.9	3.35	17.16	5.36	22.52	21.56	22	3	13	NA	NA	NA	NA
82	7	C	7.02	46.8	9.2	49.3	2.18	2.5	3.07	14.95	4.94	19.89	NA	20	1	0	14.4	453.2	31.48	-28.41
83	7	C	7.32	57.2	8.83	66.5	1.51	9.3	3.24	16.48	4.38	20.86	NA	25	0	0	19.9	464.8	23.38	-28.9
84	7	C	6.6	61.1	8.67	61.4	2.07	0.3	2.86	16	5.57	21.57	NA	30	0	0	16.8	456.8	27.22	-29.25
85	1	D	6.28	58.1	8.5	61.3	2.22	3.2	2.49	16.58	4.7	21.28	NA	20	1	2	15.3	467.2	30.63	-28.33
86	1	D	5.66	47.6	8.06	55.4	2.4	7.8	2.1	12.46	3.8	16.26	NA	15	2	1	17.4	462.5	26.59	-29.38
87	1	D	6.39	54	7.9	51.8	1.51	-2.2	1.82	12.03	3.34	15.37	NA	15	2	34	14.1	458	32.51	-29.13
88	1	D	6.9	48.8	9.36	48.3	2.46	-0.5	1.52	18.27	4.48	22.75	NA	19	2	0	15.7	463.1	29.45	-29.19
89	1	D	6.8	57.8	8.84	52.6	2.04	-5.2	1.61	17.43	4.62	22.05	16.7	18	4	1	16.1	455.8	28.23	-28.38
90	1	D	4.55	38.5	6.44	45	1.89	6.5	1.83	7.21	2.5	9.71	NA	17	1	0	17.4	467.3	26.9	-27.91
91	1	D	6.5	55.5	8.7	63.4	2.2	7.9	1.81	14.66	4.18	18.84	12.11	19	0	2	13.2	448.4	33.91	-29.98
92	1	D	6.7	53.1	8.82	56.4	2.12	3.3	1.32	16.92	4.71	21.63	NA	15	2	1	16.4	471.3	28.79	-29.08
93	1	D	6.73	53.5	8.6	54.6	1.87	1.1	1.75	15.44	3.82	19.26	NA	20	3	1	18.4	458.8	25	-28.59
94	1	D	5.84	37.2	8.09	37.8	2.25	0.6	1.02	8.81	2.76	11.57	11.43	12	4	5	17.2	456.5	26.52	-28.25
95	1	D	5.64	53.3	7.65	53.7	2.01	0.4	2.34	14.06	4.43	18.49	14.38	19	1	1	13.4	468.4	34.84	-29.54
96	1	D	5.92	59.4	7.66	53	1.74	-6.4	1.36	13.12	3.51	16.63	NA	19	3	3	17.7	468.2	26.41	-29.49
97	2	D	6.94	50.2	8	48.8	1.06	-1.4	2.83	13.87	5.02	18.89	15.34	15	3	1	12.5	453.7	36.21	-29.36
98	2	D	5.63	44.1	6.8	45.7	1.17	1.6	2.21	9.51	3.76	13.27	17.06	12	0	0	12.2	456.7	37.42	-29.45

99	2	D	7.68	70.2	8.89	67	1.21	-3.2	3.31	18.04	5.31	23.35	NA	22	1	2	11.7	464.3	39.84	-29.4
100	2	D	5.44	41.7	6.83	42.1	1.39	0.4	1.74	9.1	3.67	12.77	NA	9	0	0	11.5	460.9	40.19	-30.01
101	2	D	6.22	47.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
102	2	D	5.84	40.5	7.72	49.7	1.88	9.2	2.88	13.34	4.18	17.52	NA	24	2	0	13.8	468.7	33.95	-28.63
103	2	D	5.64	48.4	6.91	53.8	1.27	5.4	0.94	10.44	1.95	12.39	10.88	16	0	42	12.8	460.5	36.11	-30.17
104	2	D	5.75	43.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
105	2	D	4.81	34.4	6.02	37	1.21	2.6	2.01	6.6	3.05	9.65	10.49	10	0	20	13.2	461.2	34.84	-29.97
106	2	D	5.21	63.5	7.3	58.7	2.09	-4.8	1.93	10.46	3.92	14.38	NA	14	3	0	12	454.35	37.81	-30.72
107	2	D	6.18	58.5	7.41	55	1.23	-3.5	1.78	11.47	3.5	14.97	NA	18	0	0	13.9	469.2	33.73	-28.39
108	2	D	5.47	51.3	6.22	56.3	0.75	5	2.61	8.46	3.56	12.02	NA	12	0	0	11.7	465.9	39.67	-29.4
109	3	D	6.28	44.9	8.03	54.1	1.75	9.2	4.38	15.3	5.72	21.02	21.74	30	0	4	12	459.1	38.29	-29.02
110	3	D	6	32	7.73	36.8	1.73	4.8	4.08	11.36	4.61	15.97	14.04	24	0	2	12.6	463.5	36.91	-29.41
111	3	D	6.64	51	9.15	60.4	2.51	9.4	3.79	19.29	5.69	24.98	NA	19	0	0	12.3	476	38.76	-29.77
112	3	D	6.54	54.2	7.94	57.7	1.4	3.5	3.1	14.27	5.65	19.92	21.24	17	0	4	10.8	450.1	41.6	-29.53
113	3	D	6.25	27.5	8.97	38.6	2.72	11.1	3.67	12.03	4.96	16.99	NA	16	0	0	12.3	454.2	37.01	-29.62
114	3	D	7.44	47.7	9.31	60.4	1.87	12.7	4.75	19.78	6.27	26.05	NA	21	1	1	12.6	465.8	36.91	-30.61
115	3	D	5.3	29	7.07	35.4	1.77	6.4	2.31	7.5	2.89	10.39	NA	13	0	1	15.2	466	30.76	-28.94
116	3	D	6.66	37	8.63	44.2	1.97	7.2	4.09	15.19	5.56	20.75	NA	20	1	0	13.7	457.4	33.49	-30.2
117	3	D	8.35	56.9	10.56	61.5	2.21	4.6	3.72	27.22	6.75	33.97	NA	26	0	4	13.4	465	34.8	-29.84
118	3	D	6.75	52.8	8.37	54.1	1.62	1.3	4.5	17.44	6.35	23.79	NA	18	0	0	13.6	461.9	33.88	-30.15
119	3	D	7.34	38.5	9.41	44.3	2.07	5.8	4.87	16.93	6.96	23.89	21.65	30	0	1	12	447.95	37.39	-30.13
120	3	D	5.91	37.1	7.12	37.2	1.21	0.1	3.15	10.44	4.32	14.76	NA	13	0	0	13	479.4	37.01	-29.36
121	4	D	5.65	39.6	8.4	49.6	2.75	10	2.51	15.07	4.43	19.5	NA	26	1	0	15.6	458.4	29.3	-29.04
122	4	D	5.59	56.4	8.7	58	3.11	1.6	3.43	16.47	5.96	22.43	15.17	19	3	0	12.4	442.1	35.64	-28.18

123	4	D	7.06	51.4	8.28	52.9	1.22	1.5	3.54	17.86	5.42	23.28	18.82	27	0	0	12.5	466.3	37.2	-28.26
124	4	D	7.16	53.5	8.48	57.9	1.32	4.4	2.52	14.23	4.39	18.62	10.8	30	2	56	15.1	461.5	30.48	-28.45
125	4	D	6.73	58.4	8.35	59.7	1.62	1.3	2.86	15.73	5.43	21.16	17.35	24	1	0	13.5	469.1	34.82	-28.7
126	4	D	6.69	42.3	9.14	47	2.45	4.7	2.87	16.56	4.62	21.18	NA	27	0	0	14.5	459.3	31.7	-28.63
127	4	D	6.74	65.2	9.7	63.2	2.96	-2	3.53	19.82	5.42	25.24	NA	17	2	0	13.15	457.7	34.77	-28.2
128	4	D	6.9	72.4	8.69	70.4	1.79	-2	2.01	18.88	5.27	24.15	NA	27	3	1	12.8	470.2	36.77	-29.48
129	4	D	6.48	59.4	8.52	62.4	2.04	3	3.54	17.85	5.68	23.53	NA	25	0	0	14.9	474.6	31.82	-28.58
130	4	D	6.46	46.9	8.16	45	1.7	-1.9	2.32	13.16	4.32	17.48	NA	14	1	0	13.7	459.2	33.45	-29.08
131	4	D	6.2	73.9	8.62	69.7	2.42	-4.2	3.29	15.14	5.1	20.24	NA	22	1	0	12.1	446.9	37.01	-29.03
132	4	D	7.03	49.7	8.48	56.7	1.45	7	3.93	19.96	5.84	25.8	NA	23	1	0	11.9	468.8	39.4	-28.82
133	5	D	7.26	46.9	9.42	59.3	2.16	12.4	2.83	16.55	4.15	20.7	NA	27	0	0	16.5	454.2	27.55	-28.7
134	5	D	6.29	41.8	7.37	43.9	1.08	2.1	2.62	11.74	4.17	15.91	NA	23	0	3	13.8	460.65	33.36	-28.88
135	5	D	6.1	37	7.94	46.6	1.84	9.6	3.24	13.16	3.97	17.13	NA	17	1	3	15.8	462	29.25	-28.95
136	5	D	6.68	45.2	7.87	47.2	1.19	2	2.81	14.36	4.75	19.11	NA	26	2	2	12.5	448.1	35.77	-28.11
137	5	D	6.69	36.6	7.76	42	1.07	5.4	2.82	11.52	3.99	15.51	NA	24	0	1	15.2	447.1	29.49	-29.67
138	5	D	7.25	47.8	9.69	52.1	2.44	4.3	2.56	16.92	5.49	22.41	21.95	25	0	0	12.8	448	34.92	-28.66
139	5	D	6.71	44.2	9.06	51.5	2.35	7.3	2.84	15.97	5.23	21.2	NA	24	1	1	15.7	437.9	27.87	-28.46
140	5	D	6.16	41.8	7.17	39.4	1.01	-2.4	2.35	9.62	3.36	12.98	11.06	27	6	18	17.2	452.5	26.34	-28.86
141	5	D	6.72	41.8	8.3	48.9	1.58	7.1	NA	NA	NA	NA	NA	28	0	1	13.4	452.8	33.92	-27.78
142	5	D	6.1	41	8.52	45.3	2.42	4.3	3.44	12.86	5.32	18.18	15.73	18	0	1	12.5	447.5	35.68	-28.81
143	5	D	7.06	55	8.96	52	1.9	-3	2.65	16.89	4.81	21.7	NA	28	4	4	13.6	462.9	34.14	-28.78
144	5	D	5.93	43.3	7.49	47.9	1.56	4.6	3.06	12.3	4.39	16.69	13.09	18	1	2	13.65	457.65	33.58	-29
145	6	D	5.77	43.7	7.85	46.6	2.08	2.9	1.79	12.49	3.24	15.73	NA	20	1	0	12.9	460.4	35.67	-29.93
146	6	D	6.01	64.1	7.93	71.6	1.92	7.5	2.98	18.35	4.34	22.69	NA	27	0	0	12.8	473.4	37.12	-30.39

147	6	D	6.55	54.4	NA	NA	NA	NA	NA	NA	NA	NA	16.69	NA	NA	NA	NA	NA	NA	NA
148	6	D	6.5	46.7	9.27	46.3	2.77	-0.4	2.85	18.92	4.85	23.77	NA	38	1	2	14.7	461.2	31.32	-29.68
149	6	D	4.76	46.8	6.75	47.9	1.99	1.1	1.43	8.92	2.1	11.02	10.44	17	0	0	16.9	478.5	28.36	-29.53
150	6	D	6.4	41.3	8.09	39.7	1.69	-1.6	1.97	12.07	3.65	15.72	NA	26	1	0	15.8	459.2	28.97	-30.5
151	6	D	6.59	38.4	9.15	46.3	2.56	7.9	2.2	15.83	4.61	20.44	15.41	23	1	1	14.9	461.2	31.01	-30.52
152	6	D	5.5	50.6	7.18	54.9	1.68	4.3	2.15	10.68	3.69	14.37	14.46	19	0	0	11.2	455.4	40.57	-29.91
153	6	D	7	50.9	8.32	47.5	1.32	-3.4	3.12	18.84	4.94	23.78	NA	21	1	0	12.9	457.4	35.56	-29.89
154	6	D	6.26	38	7.95	39.8	1.69	1.8	3.23	13.49	5.17	18.66	NA	23	0	0	12.4	460.4	37	-30.15
155	6	D	4.5	35.2	7.23	37.2	2.73	2	1.9	8.52	2.53	11.05	NA	12	0	0	12	456.3	38.15	-31.32
156	6	D	6.02	45.4	7.97	54.4	1.95	9	2.2	11.2	3.1	14.3	NA	18	1	0	13.85	459.85	33.2	-29.73
157	7	D	5.27	30	7.78	38.5	2.51	8.5	2.54	9.71	3.91	13.62	NA	29	0	0	17.2	455.2	26.45	-29.18
158	7	D	6.57	51.4	8.45	57.8	1.88	6.4	2.34	15.05	4.36	19.41	NA	18	2	0	14.7	451.1	30.64	-28.6
159	7	D	6.31	46.4	8.88	66.7	2.57	20.3	2.32	17.26	5.49	22.75	NA	15	0	0	14.9	445.1	29.81	-28.89
160	7	D	5.7	44.3	7.26	54.2	1.56	9.9	2.81	11.2	4.4	15.6	NA	26	0	2	14.85	460.95	30.99	-28.66
161	7	D	6.42	45.2	7.85	51.5	1.43	6.3	2.89	11.83	4.52	16.35	16.78	18	1	0	NA	NA	NA	NA
162	7	D	7.48	49	9.62	53.2	2.14	4.2	3.17	19.43	4.68	24.11	22.31	16	2	0	16.3	452.7	27.81	-28.26
163	7	D	6.58	41.5	8.26	53.1	1.68	11.6	3.86	14.94	5.49	20.43	NA	26	1	3	13.8	449.6	32.57	-30.15
164	7	D	5.76	31.4	7.82	31.4	2.06	0	2.59	10.3	3.91	14.21	NA	21	1	0	16.2	461.2	28.47	-29.33
165	7	D	5.46	37.1	7.97	45.3	2.51	8.2	2.34	13.58	4.06	17.64	NA	17	0	0	17.3	459.6	26.57	-28.87
166	7	D	6.47	41.7	8.69	35.8	2.22	-5.9	1.3	12.57	3.03	15.6	11.41	20	1	2	NA	NA	NA	NA
167	7	D	7.4	48.7	9.35	56.5	1.95	7.8	2.97	18.35	4.44	22.79	17.49	20	2	3	15.35	451.25	29.39	-28.58
168	7	D	6.91	44.6	9.63	66.3	2.72	21.7	3.06	21.47	6.17	27.64	NA	18	0	0	15.2	456.4	29.98	-29.44
169	1	N	5.82	51.5	8.36	58	2.54	6.5	2.72	15.17	4.93	20.1	16.96	21	4	0	18.9	450.5	23.9	-29.15

170	1	N	6.18	45	9.03	45.4	2.85	0.4	2.44	15.81	4.5	20.31	NA	19	5	3	20.4	468.8	22.96	-28.41
171	1	N	5.1	47.6	7.4	53.8	2.3	6.2	1.75	12.88	3.16	16.04	NA	15	3	0	21.1	461.1	21.84	-28.88
172	1	N	5.25	41.5	7.38	37.4	2.13	-4.1	2.17	11.18	3.12	14.3	NA	16	2	0	22	458.3	20.8	-28.93
173	1	N	5.51	41.4	7.93	48.8	2.42	7.4	2.03	12.85	3.96	16.81	NA	17	3	1	20.7	457.3	22.09	-29.39
174	1	N	6.35	40.5	9.01	42.4	2.66	1.9	0.94	13.99	4.34	18.33	12	19	3	9	20.8	443	21.28	-29.2
175	1	N	5.95	45.1	8.89	43.6	2.94	-1.5	2.09	14.14	3.95	18.09	NA	22	6	8	21.2	448.7	21.15	-28.97
176	1	N	6.45	63.5	9.34	57.8	2.89	-5.7	2.22	18.32	4.92	23.24	14.95	24	11	6	19.9	459.8	23.16	-28.29
177	1	N	4.44	29.5	7.22	31.6	2.78	2.1	2.33	7.96	3.12	11.08	NA	21	2	1	18.4	451.5	24.48	-28.78
178	1	N	4.98	35.5	7.86	43.1	2.88	7.6	1.89	9.6	2.95	12.55	10.34	13	1	1	19.5	449.4	23.02	-28.47
179	1	N	6.77	56.5	9.48	56.4	2.71	-0.1	2.92	20.78	5.6	26.38	NA	18	1	3	17.7	470.8	26.65	-28.77
180	1	N	6.56	52.1	10.18	47.7	3.62	-4.4	2.04	19.13	4.52	23.65	NA	14	2	1	20.7	469	22.65	-29.49
181	2	N	5	35.4	6.93	37.2	1.93	1.8	2.13	7.44	2.99	10.43	NA	18	2	0	15.5	462.2	29.87	-28.69
182	2	N	7.28	48.9	9.07	62.6	1.79	13.7	4.02	18.14	6.11	24.25	17.18	27	0	1	14.4	462.9	32.13	-30.94
183	2	N	7.64	57	8.49	54.3	0.85	-2.7	3	16.72	4.69	21.41	13.9	NA	0	5	25.8	477	18.5	-28.46
184	2	N	6.4	47	8.65	57.3	2.25	10.3	4.23	16.33	5.78	22.11	NA	23	1	0	17.7	459.2	25.91	-29.45
185	2	N	5.25	33.1	7.82	42	2.57	8.9	2.41	12.49	4.28	16.77	NA	17	2	0	13.3	463.6	34.93	-29.5
186	2	N	6.38	46.7	8.64	48	2.26	1.3	2.67	15.93	5.39	21.32	NA	26	0	0	14.9	463	31.08	-28.61
187	2	N	7.11	62.4	9.63	74.4	2.52	12	3.25	21.75	5.49	27.24	NA	22	3	2	16.1	452.5	28.15	-28.61
188	2	N	5.55	42.1	8.46	46	2.91	3.9	2.43	12.1	4.25	16.35	NA	16	2	1	19	461.1	24.22	-29.65
189	2	N	7.41	57	9.46	59.3	2.05	2.3	3.29	20.45	5.67	26.12	16.6	29	1	0	16.7	459.2	27.52	-28.54
190	2	N	6.3	49.3	8.18	51.3	1.88	2	2.54	14.82	4.64	19.46	NA	19	6	5	17.6	467	26.58	-28.72
191	2	N	7.16	45.9	8.98	50	1.82	4.1	4.09	16.66	6.64	23.3	NA	27	1	0	NA	NA	NA	NA
192	2	N	7.09	41.3	8.33	48.2	1.24	6.9	3.07	15.04	4.79	19.83	14.4	23	1	1	19.35	467.6	24.18	-29.43
193	3	N	6.46	45.5	8.99	59.5	2.53	14	4.35	21.93	7.2	29.13	NA	32	0	3	13.3	468.2	35.21	-29.78
194	3	N	6.6	52.3	8.94	61	2.34	8.7	4.33	19.27	5.88	25.15	NA	20	0	1	13.4	465.1	34.81	-28.78
195	3	N	7.4	57.4	10.79	70.4	3.39	13	4.72	31.8	7.74	39.54	27.49	24	0	0	16.1	470.5	29.21	-29.86

196	3	N	6.09	43.4	8.63	43.7	2.54	0.3	4.18	20.34	5.85	26.19	NA	33	0	2	17.65	469.4	26.55	-29.46
197	3	N	7.36	55.4	10.3	79.2	2.94	23.8	5.92	26.24	7.84	34.08	NA	27	0	1	14.6	471.4	32.23	-30.47
198	3	N	5.36	28.1	8.01	38.4	2.65	10.3	1.87	9.76	4.59	14.35	NA	10	0	0	17.7	465.7	26.29	-30
199	3	N	7.1	48.8	9.58	54.3	2.48	5.5	4.87	20.47	7.95	28.42	19.79	21	1	1	12.9	453.2	35.08	-29.79
200	3	N	5.54	37	7.86	47	2.32	10	2.77	10.48	4.4	14.88	NA	11	0	0	19.8	454.2	22.92	-29.68
201	3	N	4.79	47.2	10.2	56.4	5.41	9.2	4.25	23.16	6.19	29.35	19.73	28	3	4	18.85	460.2	24.44	-29.85
202	3	N	6.56	26.9	6.62	42.7	0.06	15.8	2.96	8.38	3.61	11.99	NA	10	0	0	14.9	464.9	31.2	-28.91
203	3	N	6.95	48.2	9.5	57.4	2.55	9.2	4.71	17.77	5.92	23.69	NA	15	2	1	16.9	450	26.56	-29.46
204	3	N	5.48	36.2	7.57	34.8	2.09	-1.4	3.01	10.01	4.27	14.28	13.35	19	1	0	18.4	467.1	25.45	-29.89
205	4	N	7.38	48.4	9.95	69.1	2.57	20.7	3.06	20.07	4.43	24.5	NA	27	0	0	17	460	27.02	-29.13
206	4	N	5.93	45.3	8.28	54.7	2.35	9.4	3.38	16.19	4.94	21.13	NA	25	0	3	15.4	455.5	29.59	-28.78
207	4	N	5.52	47.3	8.49	53.6	2.97	6.3	3.96	15.47	5.69	21.16	NA	21	1	0	14.4	459.6	31.95	-29.06
208	4	N	7.04	54	10.2	57.5	3.16	3.5	2.86	22.3	5.21	27.51	NA	27	1	0	19.2	462.1	24.04	-28.59
209	4	N	4.21	43.2	6	38.4	1.79	-4.8	-0.27	6.23	1.6	7.83	NA	11	4	0	24.6	470.7	19.16	-28.97
210	4	N	7.24	54	10.5	70.1	3.26	16.1	4.5	27.24	6.03	33.27	NA	27	0	0	15.8	456.8	28.92	-28.42
211	4	N	5.9	33.2	7.63	36.8	1.73	3.6	2.23	10.59	3.24	13.83	10.38	21	0	0	23.3	474	20.37	-28.5
212	4	N	6.12	45.5	9.93	51	3.81	5.5	2.55	16.75	4.32	21.07	13.6	25	0	0	16.3	441.5	27.02	-29.17
213	4	N	7.45	61.4	10.89	60.7	3.44	-0.7	3.03	23.49	5.16	28.65	20.91	32	5	1	19.6	457.3	23.33	-28.23
214	4	N	6.36	59.4	7.97	65.1	1.61	5.7	2.17	15.62	2.89	18.51	12.01	19	0	0	24.7	480	19.46	-27.88
215	4	N	5.6	48.7	8.03	54.4	2.43	5.7	2.28	13.63	3.27	16.9	NA	25	0	1	23.7	476.7	20.14	-28.2
216	4	N	5.52	60.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
217	5	N	7.13	48.7	9.72	48.3	2.59	-0.4	3.62	15.35	5.52	20.87	18.23	19	0	0	17.5	454.7	25.93	-29.49
218	5	N	7.22	44	9.51	56.6	2.29	12.6	3.38	19.1	5.28	24.38	NA	22	0	0	19.1	446.4	23.38	-28.92
219	5	N	6.55	48.9	8.33	49.6	1.78	0.7	3.54	12.46	4.52	16.98	NA	17	1	1	18.6	464.3	24.98	-27.81
220	5	N	6.35	46.1	8.65	62.8	2.3	16.7	3.3	17.28	5.29	22.57	NA	19	0	0	18.7	449.5	24	-28.38
221	5	N	6.57	39.9	9.53	44.2	2.96	4.3	2.67	15.16	4.49	19.65	12.43	20	1	1	18.7	445	23.77	-29.21

222	5	N	6.47	39	8.83	43	2.36	4	2.23	10.67	3.09	13.76	NA	23	0	0	22.2	463.2	20.85	-28.16
223	5	N	6.07	39.9	8.59	43.5	2.52	3.6	2.91	12.42	4.25	16.67	NA	21	0	2	19.35	457.6	23.65	-28.87
224	5	N	7.21	48.4	9.9	47	2.69	-1.4	4.1	19.16	6.01	25.17	19.98	32	4	1	17.1	460.8	26.88	-28.68
225	5	N	6.58	54	10.45	66.5	3.87	12.5	4.32	23.13	6.08	29.21	NA	22	1	0	17.9	461.3	25.72	-28.9
226	5	N	5.18	33.3	6.7	37.1	1.52	3.8	1.98	8.55	3.06	11.61	NA	13	0	0	20.8	471.6	22.63	-28.17
227	5	N	7.75	38.4	9.92	47.8	2.17	9.4	3.64	18.54	5.78	24.32	NA	27	1	0	19.3	460.7	23.85	-29.39
228	5	N	5.28	37.2	6.49	41.3	1.21	4.1	2.11	8.28	2.94	11.22	9.47	18	1	0	17.55	462.1	26.31	-29.22
229	6	N	6.28	47.4	9.13	59.9	2.85	12.5	2.57	19.21	4.52	23.73	NA	23	1	0	20.4	467.4	22.97	-29.94
230	6	N	5.97	57	8.31	62	2.34	5	2.22	15.2	4.05	19.25	15.37	20	0	0	19.5	457.3	23.46	-29.23
231	6	N	6.12	43.2	8.65	50.2	2.53	7	2.63	15.3	3.61	18.91	NA	19	0	0	20.6	456.1	22.13	-29.96
232	6	N	5.04	32.7	8.36	38.7	3.32	6	2.17	11.71	3.21	14.92	NA	25	0	0	18.4	467.85	25.48	-29.74
233	6	N	6.29	41.4	8.65	56.1	2.36	14.7	2.06	15.43	3.77	19.2	11.15	31	0	3	21	448.8	21.42	-29.42
234	6	N	4.85	37.6	7.02	39.1	2.17	1.5	1.41	9.08	2.9	11.98	NA	15	1	0	21.7	472.9	21.77	-30.71
235	6	N	5.66	38.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
236	6	N	6.15	43	8.83	58.2	2.68	15.2	2.54	16.07	3.81	19.88	NA	21	0	0	16.1	461.3	28.68	-29.61
237	6	N	8.11	57.5	9.52	61	1.41	3.5	3.87	22.56	5.99	28.55	NA	33	1	1	15	467.6	31.17	-30.52
238	6	N	6.6	47.3	9.34	57.7	2.74	10.4	2.78	20.95	4.54	25.49	15.71	34	2	0	19	457.9	24.1	-30.27
239	6	N	6.13	48.1	8.2	50	2.07	1.9	2.01	13.29	3.31	16.6	NA	18	1	0	21	458.3	21.78	-29.99
240	6	N	5.92	45.4	9.18	60.4	3.26	15	3	18.45	4.21	22.66	15.83	24	3	0	18.6	458	24.63	-30.96
241	7	N	7.45	50	9.58	58.6	2.13	8.6	2.36	17.59	4.98	22.57	17.13	31	2	1	20.5	465.1	22.69	-28.97
242	7	N	6.5	51.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
243	7	N	7.99	49.5	10.55	55	2.56	5.5	3.91	17.58	6.54	24.12	NA	38	0	1	16.7	449.6	26.86	-28.7
244	7	N	6.62	45.6	8.62	52.6	2	7	3.09	14.88	4.95	19.83	NA	19	1	0	19.2	458.3	23.91	-29.1
245	7	N	7.92	57	10.29	70.4	2.37	13.4	4.78	22.71	7.87	30.58	NA	19	0	0	16.5	459.8	27.88	-29.22
246	7	N	6.24	36	8.83	36.6	2.59	0.6	2.98	13.61	4.23	17.84	NA	43	3	0	20.8	457.1	21.98	-28.99

247	7	N	8.23	55.7	10.78	56.7	2.55	1	4.01	24.12	6.77	30.89	NA	28	0	2	NA	NA	NA	NA
248	7	N	5.86	47.5	6.67	39.9	0.81	-7.6	1.44	7.96	2.47	10.43	NA	15	2	1	24.2	463	19.13	-28.37
249	7	N	8.31	47.5	11.05	53.9	2.74	6.4	5.19	21.84	7.01	28.85	33.01	28	1	1	17.5	462.6	26.42	-28.77
250	7	N	7.62	52.2	9.29	55.1	1.67	2.9	3.38	18.56	5.75	24.31	NA	23	2	3	18.1	453.3	25.08	-29.54
251	7	N	5.55	38.6	7.77	42.6	2.22	4	1.38	9.15	3.44	12.59	10.09	15	0	1	17	468.8	27.58	-27.93
252	7	N	6.59	45.2	9.02	54.7	2.43	9.5	2.65	16.56	5.34	21.9	20.47	22	0	0	18.2	453.7	24.95	-28.45
253	1	ND	3.96	32.2	6.21	34.2	2.25	2	-0.2	5.12	1.72	6.84	6.94	7	4	3	19.95	451.65	22.63	-29.02
254	1	ND	5.52	49.5	7.86	53	2.34	3.5	2.32	13.22	3.75	16.97	NA	16	3	2	21	471.1	22.41	-29.08
255	1	ND	6.9	53.2	10.05	53.2	3.15	0	2.74	18.98	5.09	24.07	17.96	25	4	4	20.3	451.1	22.24	-29.12
256	1	ND	5.87	46.5	8.6	46.7	2.73	0.2	1.56	16.02	3.65	19.67	NA	20	2	3	19.9	459.3	23.03	-28.8
257	1	ND	6.18	43.6	9.38	34.4	3.2	-9.2	0.81	15.39	3.96	19.35	12.56	30	44	16	20.6	455	22.12	-29.14
258	1	ND	5.95	55.3	9.6	59.4	3.65	4.1	2.59	20.88	4.95	25.83	NA	22	7	0	19.2	463.95	24.14	-29.15
259	1	ND	5.26	45.6	8.24	44.8	2.98	-0.8	1.78	14.47	4.66	19.13	NA	16	5	0	20.2	462.3	22.84	-28.83
260	1	ND	4.97	53.4	7.93	48.2	2.96	-5.2	0.39	13.44	2.99	16.43	NA	10	5	1	20.2	466.5	23.12	-28.85
261	1	ND	6.46	48.1	9.98	51.3	3.52	3.2	2.55	19.19	4.36	23.55	20.77	20	2	0	19.9	465.7	23.4	-27.68
262	1	ND	6.28	47.5	9.18	50.4	2.9	2.9	2.12	15.69	4.45	20.14	NA	18	1	1	20.1	448.5	22.32	-28.68
263	1	ND	5.49	47.2	8.8	48.7	3.31	1.5	1.06	16.23	3.69	19.92	NA	15	3	1	18.15	456.35	25.13	-29.41
264	1	ND	4.28	33.7	6.7	38.6	2.42	4.9	0.27	7.36	2.43	9.79	NA	12	1	2	22.6	455.8	20.2	-28.81
265	2	ND	6.89	51.9	9.64	52.9	2.75	1	3.92	17.39	5.25	22.64	23.46	33	1	0	NA	NA	NA	NA
266	2	ND	7.35	44.7	9.11	45.5	1.76	0.8	4.25	19.33	5.76	25.09	NA	24	1	0	17.4	468.8	26.88	-29.16
267	2	ND	6.08	48.3	8.86	56.7	2.78	8.4	3.77	15.83	4.84	20.67	NA	23	0	3	16.8	458.6	27.35	-28.59
268	2	ND	4.34	35.5	6.23	42.9	1.89	7.4	1.25	7.54	1.8	9.34	NA	14	0	1	24	471.6	19.65	-29.26
269	2	ND	4.97	41.7	7.3	42.1	2.33	0.4	3	9.6	4.04	13.64	NA	18	0	2	15.5	459.7	29.72	-28.81

270	2	ND	6.6	51.1	8.33	65.4	1.73	14.3	2.4	15.76	4.35	20.11	NA	23	0	0	16	451.4	28.21	-28.69
271	2	ND	7.91	54	10.22	68.2	2.31	14.2	3.02	19.73	5.2	24.93	NA	23	0	0	14.9	463.2	31.17	-30.1
272	2	ND	7.03	49.7	8.77	64.4	1.74	14.7	3.12	20.03	5.04	25.07	NA	21	1	1	17.4	476.3	27.36	-29.12
273	2	ND	6.23	53.1	NA	NA	NA	NA	NA	NA	NA	NA	22.55	NA	NA	NA	NA	NA	NA	NA
274	2	ND	6.63	46.7	9.44	54.7	2.81	8	3.31	17.5	4.87	22.37	14.1	21	1	0	17.05	448.8	26.32	-28.56
275	2	ND	6.94	49.3	8.85	65	1.91	15.7	3.99	19.94	5.85	25.79	18.27	27	0	1	NA	NA	NA	NA
276	2	ND	6.34	47.4	8.13	56.3	1.79	8.9	3.3	13.72	4.88	18.6	NA	17	0	1	15	479.6	31.9	-29.3
277	3	ND	7.74	77.4	9.99	86	2.25	8.6	5.47	29.72	9.1	38.82	NA	28	1	0	13.5	473.4	35.05	-29.45
278	3	ND	6.37	34	9.01	40.3	2.64	6.3	4	12.82	5.16	17.98	NA	14	2	1	18.1	473.8	26.11	-28.82
279	3	ND	6.96	48.5	11.85	54.9	4.89	6.4	4.37	25.92	6.57	32.49	NA	30	7	6	19	470.6	24.76	-30
280	3	ND	9.55	39.7	8.54	48.4	-1.01	8.7	2.66	15.25	4.49	19.74	NA	18	1	1	17.1	468.85	27.47	-29.07
281	3	ND	9.74	59.7	9.8	72.3	0.06	12.6	2.94	22.1	4.43	26.53	17.83	15	1	5	19.5	461.4	23.63	-29.02
282	3	ND	6.5	46.5	9.84	63	3.34	16.5	5.31	25.52	7.49	33.01	18.9	29	0	0	15.9	472.4	29.72	-28.87
283	3	ND	6.75	41.4	9.98	52.1	3.23	10.7	5.43	17.13	7.06	24.19	20.46	19	0	1	14.4	445.1	30.82	-29.7
284	3	ND	6.85	55.6	9.33	71.8	2.48	16.2	3.54	21.56	5.36	26.92	NA	27	1	0	18.6	475.7	25.58	-28.77
285	3	ND	5.91	35.6	8.42	50.2	2.51	14.6	4.72	15.71	6.68	22.39	NA	26	0	4	15.9	458.95	28.82	-29.81
286	3	ND	6.49	56.4	9.18	56	2.69	-0.4	4.72	18.08	6.55	24.63	NA	22	1	0	16.4	463.9	28.26	-29.97
287	3	ND	6.46	58.8	8.34	61.3	1.88	2.5	4.97	17.94	6.27	24.21	NA	21	0	0	13.3	460.3	34.66	-28.95
288	3	ND	5.81	38	8.54	48.4	2.73	10.4	2.69	13.14	3.78	16.92	12.32	17	0	4	17.9	447.6	24.95	-29.05
289	4	ND	6.31	43.7	9.7	47	3.39	3.3	5.25	21.27	7.87	29.14	NA	24	4	1	15.6	459	29.33	-28.41
290	4	ND	6.07	61.3	9.52	61.8	3.45	0.5	2.98	20.59	6.4	26.99	NA	19	2	1	15.5	460.3	29.65	-29.92
291	4	ND	6.08	48.4	8.76	56.3	2.68	7.9	3.94	16.15	5.32	21.47	18.04	23	3	2	18	475.5	26.44	-28.19
292	4	ND	6.73	74.8	10.52	71.6	3.79	-3.2	4.01	30.22	6.61	36.83	17.2	29	1	0	18.4	470.2	25.55	-28.46
293	4	ND	7.01	47	9.66	63.4	2.65	16.4	5.64	23.84	7.24	31.08	NA	32	1	2	16.4	462.2	28.2	-29.53

294	4	ND	5.9	58	8.19	55.1	2.29	-2.9	2.6	16.94	5.17	22.11	NA	30	2	0	18.05	457.65	25.39	-27.95
295	4	ND	6.59	54.1	9.41	63	2.82	8.9	2.34	20.37	4.58	24.95	15.15	24	1	0	19.7	476.3	24.18	-28.57
296	4	ND	6.05	58.2	8.45	60.3	2.4	2.1	2.75	16.53	4.26	20.79	NA	23	0	1	20.3	469.4	23.12	-27.76
297	4	ND	6.52	67.4	9.18	65.2	2.66	-2.2	2.99	22.94	7.4	30.34	15.81	27	1	0	14.9	448.2	30.18	-28.59
298	4	ND	6.11	59.5	9.39	67	3.28	7.5	4.82	22.99	7.32	30.31	NA	31	1	0	16.3	467.7	28.62	-28.22
299	4	ND	5.85	43.6	7.51	49.6	1.66	6	1.99	12.46	3.23	15.69	NA	24	2	0	19.8	463.1	23.42	-28.85
300	4	ND	6.28	49.9	8.92	58	2.64	8.1	3.1	18.23	4.07	22.3	NA	26	3	1	19	468.4	24.68	-28.31
301	5	ND	7	41.6	8.79	42.4	1.79	0.8	2.45	13.82	3.75	17.57	NA	18	0	0	20	450.1	22.52	-28.85
302	5	ND	5.77	36.2	8.23	37.7	2.46	1.5	1.97	11.64	4	15.64	NA	19	0	0	18.4	448.2	24.31	-28.63
303	5	ND	6.43	43.8	8.08	43.4	1.65	-0.4	2.65	11.85	4.88	16.73	16.73	18	5	3	17.5	463.3	26.48	-29.13
304	5	ND	5	32.1	7.05	36	2.05	3.9	1.14	7.22	2.96	10.18	9.1	12	2	1	20.9	457.1	21.85	-27.97
305	5	ND	5.9	40.7	9.04	55.5	3.14	14.8	3.18	17.24	4.85	22.09	22.31	26	1	3	17.8	476.25	26.78	-28.38
306	5	ND	6.98	40	9.39	51.9	2.41	11.9	2.88	18.4	5.22	23.62	NA	27	2	0	17.2	438.2	25.48	-29.16
307	5	ND	5.41	40.2	8.09	46.4	2.68	6.2	2.97	13.55	4.74	18.29	NA	27	2	3	19.2	451.1	23.5	-29.22
308	5	ND	6.27	42.5	8.63	45.8	2.36	3.3	2.17	13.61	3.89	17.5	18.54	19	6	5	21.8	457.8	20.98	-28.48
309	5	ND	6.7	54.7	9.25	75	2.55	20.3	4.41	21.69	5.97	27.66	NA	23	0	0	13	446.8	34.27	-29.47
310	5	ND	5.84	39.4	7.89	55	2.05	15.6	3.4	13.28	4.14	17.42	NA	20	1	1	16.7	457.2	27.37	-28.49
311	5	ND	6.97	39.6	8.79	45.8	1.82	6.2	2.41	15.31	5.11	20.42	NA	19	1	0	17.3	445.2	25.78	-29.44
312	5	ND	6.09	43	8.82	45.1	2.73	2.1	3.69	13.6	4.49	18.09	NA	24	3	0	20.5	452.2	22.05	-28.45
313	6	ND	7.56	47.6	10.55	64	2.99	16.4	3.76	22.95	5.91	28.86	NA	32	0	0	16.6	465.5	28.11	-29.04
314	6	ND	7.19	41.7	10.27	52	3.08	10.3	2.66	17.67	4.85	22.52	18.3	18	2	0	16.1	471.6	29.24	-29.95
315	6	ND	5.77	45.5	8.44	62.7	2.67	17.2	2.04	14.84	3.69	18.53	11.15	NA	2	11	19.35	458	23.71	-29.66
316	6	ND	6.08	52.5	9.05	59.8	2.97	7.3	3.13	17.77	4.38	22.15	NA	25	1	0	17.2	466.8	27.21	-30.28
317	6	ND	5.61	40.4	7.92	51.8	2.31	11.4	2.64	13.06	3.27	16.33	NA	20	1	0	19.4	463.6	23.96	-28.12

318	6	ND	7.6	50.1	9.2	55	1.6	4.9	3.53	19.97	5.39	25.36	NA	27	1	0	16.5	462.3	28.02	-29.26
319	6	ND	7.52	48.5	8.94	60.2	1.42	11.7	2.82	16.43	4.39	20.82	13.45	22	0	1	16.7	458	27.4	-29.84
320	6	ND	6.64	49	8.59	55.3	1.95	6.3	2.94	16.14	4.81	20.95	NA	33	1	1	19.7	453.1	22.97	-29.17
321	6	ND	7.46	43.5	9.28	52.3	1.82	8.8	2.47	17.69	4.6	22.29	NA	32	0	0	19.6	464.7	23.74	-28.55
322	6	ND	7.6	73.4	11.07	92	3.47	18.6	5.03	33.24	6.56	39.8	24.59	38	1	1	15.5	467.7	30.26	-29.73
323	6	ND	5.87	43.8	7.26	44.4	1.39	0.6	1.61	11.58	2.79	14.37	NA	25	3	0	20.3	461.7	22.74	-28.61
324	6	ND	5.48	33.5	7.92	51.1	2.44	17.6	3.6	14.63	4.84	19.47	NA	23	0	0	15.5	463.2	29.97	-29.04
325	7	ND	6.92	45.5	9.67	50.3	2.75	4.8	4.42	18.82	7.54	26.36	NA	21	2	0	14.3	450.9	31.47	-28.92
326	7	ND	7.72	58.4	9.98	65.5	2.26	7.1	3.41	27.62	6.73	34.35	NA	26	0	1	16.3	458.8	28.19	-28.6
327	7	ND	6.85	42.3	9.08	45.7	2.23	3.4	2.11	12.43	4.29	16.72	NA	18	0	0	17.9	454.4	25.43	-29.12
328	7	ND	5.23	39	6.28	41.8	1.05	2.8	0.95	7.22	1.99	9.21	8.03	14	0	0	NA	NA	NA	NA
329	7	ND	6.04	39.4	7.88	51.5	1.84	12.1	2.93	11.14	3.78	14.92	NA	28	0	0	22	461.1	20.98	-28.89
330	7	ND	6.76	45.6	8.73	50.7	1.97	5.1	3.85	18.49	6.35	24.84	29.27	25	1	0	17.2	458.5	26.67	-29.11
331	7	ND	6.75	47.3	8.9	42.9	2.15	-4.4	1.92	13.56	4.33	17.89	14.15	19	3	4	18.9	458.6	24.3	-28.09
332	7	ND	6.17	41	8.05	48.2	1.88	7.2	2.99	14.05	4.95	19	15.42	24	1	1	17.8	447.3	25.12	-29.54
333	7	ND	6.3	38.4	8.14	40.3	1.84	1.9	2.62	13.38	4.22	17.6	NA	15	1	0	NA	NA	NA	NA
334	7	ND	6.37	45.8	9.04	49.3	2.67	3.5	3.4	15.99	6.08	22.07	NA	21	2	0	19.4	462	23.84	-29.18
335	7	ND	6.55	39.2	10.29	44.6	3.74	5.4	2.68	15.49	4.97	20.46	NA	23	0	0	18.65	454.85	24.34	-29.33
336	7	ND	6.27	39.8	7.82	42.2	1.55	2.4	2.28	9.99	3.55	13.54	NA	23	2	1	19.9	458.6	23.05	-28.29

Table S4: Data for one-year-old and two-year-old trees (comparisons of shoot:root ratios and leaf δ^{13} signatures).

Forest_site	Age	Treatment	SRR	Leaf_d13C
1	1	C	1.218	-29.955
1	1	C	1.818	-30.124
1	1	C	1.576	-30.574
1	1	C	1.5	-28.112
1	1	C	1.558	-30.353
1	1	C	1.803	-29.947
1	1	C	1.62	-30.241
1	1	C	1.397	-30.347
1	1	C	1.601	-30.084
1	1	C	1.727	-30.123
2	1	C	2.592	-29.794
2	1	C	2.961	-29.935
2	1	C	2.247	-29.647
2	1	C	1.908	-30.34
2	1	C	1.61	-29.52
2	1	C	2.348	-30.274
2	1	C	2.591	-30.097
2	1	C	1.566	-30.319
2	1	C	2.692	-30.291
2	1	C	2.662	-30.28
3	1	C	1.289	-28.726
3	1	C	1.784	-30.087
3	1	C	1.361	-29.543
3	1	C	1.994	-29.788
3	1	C	1.765	-29.277
3	1	C	1.918	-29.676
3	1	C	1.168	-30.129
3	1	C	2.079	-30.578
3	1	C	1.602	-29.933
3	1	C	1.62	-29.77
4	1	C	1.274	-29.299
4	1	C	1.73	-29.486
4	1	C	2.509	-28.568
4	1	C	1.788	-28.829
4	1	C	1.737	-29.332
4	1	C	1.76	-28.768

4	1	C	1.405	-29.11
4	1	C	1.868	-28.389
4	1	C	1.618	-29.209
4	1	C	1.381	-28.984
5	1	C	1.4	-28.896
5	1	C	2.185	-29.925
5	1	C	1.835	-28.741
5	1	C	1.761	-28.746
5	1	C	1.996	-28.171
5	1	C	1.947	-29.705
5	1	C	1.735	-28.456
5	1	C	2.011	-28.899
5	1	C	1.982	-28.697
5	1	C	2.039	-28.463
6	1	C	1.791	-28.26
6	1	C	1.739	-28.641
6	1	C	1.762	-28.658
6	1	C	2.194	-29.28
6	1	C	1.663	-29.877
6	1	C	1.406	-26.767
6	1	C	0.994	-28.118
6	1	C	1.08	-28.651
6	1	C	0.762	-27.201
6	1	C	0.89	-29.181
7	1	C	1.188	-28.57
7	1	C	1.771	-28.74
7	1	C	1.612	-30.21
7	1	C	1.5	-29.587
7	1	C	1.864	-29.313
7	1	C	2.239	-30.054
7	1	C	1.48	-28.91
7	1	C	2.312	-30.066
7	1	C	0.656	-27.322
7	1	C	1.629	-29.115
8	1	C	1.349	-29.039
8	1	C	1.811	-29.642
8	1	C	2.58	-28.694
8	1	C	2.217	-29.943
8	1	C	2.559	-29.71
8	1	C	1.71	-29.637
8	1	C	2.263	-29.779

8	1	C	2.022	-30.058
8	1	C	2.31	-29.173
8	1	C	1.786	-29.958
1	1	D	1.848	-29.775
1	1	D	2.066	-29.861
1	1	D	1.914	-29.98
1	1	D	1.865	-28.089
1	1	D	1.707	-29.109
1	1	D	2.382	-28.945
1	1	D	2.061	-29.041
1	1	D	2.105	-29.747
1	1	D	2.254	-29.133
1	1	D	2.102	-29.478
2	1	D	2.623	-28.417
2	1	D	2.469	-28.725
2	1	D	2.249	-30.244
2	1	D	2.852	-28.537
2	1	D	1.373	-30.711
2	1	D	1.69	-29.776
2	1	D	2.357	-29.046
2	1	D	2.218	-29.102
2	1	D	2.634	-29.154
2	1	D	2.406	-28.846
3	1	D	1.379	-29.509
3	1	D	1.995	-28.662
3	1	D	1.941	-28.944
3	1	D	2.331	-29.255
3	1	D	2.308	-28.785
3	1	D	2.36	-28.289
3	1	D	1.468	-29.049
3	1	D	2.44	-28.138
3	1	D	2.448	-28.373
3	1	D	1.657	-29.853
4	1	D	2.551	-27.809
4	1	D	1.783	-27.74
4	1	D	2.998	-28.087
4	1	D	2.775	-26.937
4	1	D	2.744	-27.839
4	1	D	3.821	-27.555
4	1	D	1.705	-27.897
4	1	D	1.762	-29.354

4	1	D	2.398	-28.678
4	1	D	1.783	-28.191
5	1	D	1.738	-28.036
5	1	D	1.935	-28.568
5	1	D	1.916	-29.297
5	1	D	2.234	-28.577
5	1	D	2.74	-28.042
5	1	D	2.539	-28.671
5	1	D	1.776	-28.005
5	1	D	1.32	-28.624
5	1	D	2.168	-28.358
5	1	D	2.558	-28.84
6	1	D	1.566	-28.082
6	1	D	1.762	-27.045
6	1	D	1.36	-28.564
6	1	D	1.432	-28.705
6	1	D	2.472	-27.174
6	1	D	2.545	-27.735
6	1	D	2.02	-27.913
6	1	D	1.978	-27.926
6	1	D	2.012	-28.394
6	1	D	1.21	-28.097
7	1	D	1.401	-30.555
7	1	D	1.368	-28.983
7	1	D	1.499	-29.359
7	1	D	2.002	-29.326
7	1	D	1.789	-29.083
7	1	D	1.555	-29.291
7	1	D	1.472	-29.737
7	1	D	2.435	-28.694
7	1	D	1.432	-28.837
7	1	D	2.005	-29.126
8	1	D	1.796	-29.886
8	1	D	1.838	-29.558
8	1	D	1.471	-29.831
8	1	D	1.597	-28.974
8	1	D	1.702	-29.859
8	1	D	1.015	-30.751
8	1	D	3.539	-27.552
8	1	D	1.611	-29.432
8	1	D	1.473	-28.201

8	1	D	1.563	-29.854
1	1	N	1.529	-30.298
1	1	N	1.646	-29.791
1	1	N	1.66	-30.894
1	1	N	1.871	-30.4
1	1	N	1.814	-30.304
1	1	N	2.93	-30.755
1	1	N	1.579	-29.851
1	1	N	1.619	-29.716
1	1	N	1.635	-29.395
1	1	N	1.368	-28.46
2	1	N	2.066	-29.14
2	1	N	2.637	-30.194
2	1	N	2.324	-29.902
2	1	N	2.212	-30.286
2	1	N	2.255	-29.742
2	1	N	3.108	-29.644
2	1	N	2.664	-30.526
2	1	N	1.968	-29.996
2	1	N	2.748	-30.296
2	1	N	2.534	-29.828
3	1	N	1.389	-28.631
3	1	N	1.428	-29.267
3	1	N	1.463	-29.575
3	1	N	2.183	-28.363
3	1	N	2.102	-28.077
3	1	N	2.375	-29.09
3	1	N	2.025	-28.971
3	1	N	2.094	-28.714
3	1	N	2.159	-28.656
3	1	N	1.586	-28.789
4	1	N	1.482	-29.165
4	1	N	1.515	-28.213
4	1	N	1.698	-29.274
4	1	N	1.835	-30.029
4	1	N	2.746	-28.954
4	1	N	1.947	-29.451
4	1	N	1.53	-29.694
4	1	N	1.655	-28.791
4	1	N	1.7	-28.781
4	1	N	1.429	-28.192

5	1	N	1.646	-27.838
5	1	N	1.888	-28.849
5	1	N	2.436	-28.908
5	1	N	1.539	-29.496
5	1	N	1.344	-28.977
5	1	N	2.521	-28.267
5	1	N	2.098	-28.396
5	1	N	2.198	-28.976
5	1	N	2.32	-29.177
5	1	N	1.597	-28.379
6	1	N	1.443	-29.168
6	1	N	1.76	-28.047
6	1	N	1.072	-27.802
6	1	N	0.981	-28.17
6	1	N	1.267	-29.109
6	1	N	1.519	-28.387
6	1	N	1.786	-27.795
6	1	N	2.043	-29.3
6	1	N	1.604	-29.846
6	1	N	1.6	-29.239
7	1	N	1.895	-29.908
7	1	N	1.147	-29.767
7	1	N	1.285	-29.894
7	1	N	1.24	-30.12
7	1	N	1.326	-29.001
7	1	N	1.869	-30.846
7	1	N	2.075	-29.535
7	1	N	2.336	-29.222
7	1	N	2.584	-29.955
7	1	N	1.721	-30.484
8	1	N	1.643	-29.919
8	1	N	1.924	-29.095
8	1	N	2.261	-30.141
8	1	N	1.348	-29.179
8	1	N	1.468	-29.44
8	1	N	1.548	-29.078
8	1	N	1.685	-30.351
8	1	N	3.051	-28.947
8	1	N	1.576	-29.997
8	1	N	1.206	-29.748
1	1	ND	0.683	-29.666

1	1	ND	1.72	-29.591
1	1	ND	1.416	-30.46
1	1	ND	1.583	-29.986
1	1	ND	2.022	-29.492
1	1	ND	1.73	-29.511
1	1	ND	2.118	-29.566
1	1	ND	1.538	-30.23
1	1	ND	2.338	-30.233
1	1	ND	1.557	-29.728
2	1	ND	3.189	-30.103
2	1	ND	2.376	-29.241
2	1	ND	2.027	-29.761
2	1	ND	2.647	-29.264
2	1	ND	2.327	-28.938
2	1	ND	1.971	-29.657
2	1	ND	2.139	-29.87
2	1	ND	2.951	-28.724
2	1	ND	2.188	-28.815
2	1	ND	2.017	-28.492
3	1	ND	2.48	-28.35
3	1	ND	2.062	-28.217
3	1	ND	2.423	-28.446
3	1	ND	2.952	-27.863
3	1	ND	1.87	-29.466
3	1	ND	1.982	-27.852
3	1	ND	2.073	-28.133
3	1	ND	2.826	-27.405
3	1	ND	1.578	-28.486
3	1	ND	2.387	-28.476
4	1	ND	2.808	-28.367
4	1	ND	3.401	-27.174
4	1	ND	1.714	-27.59
4	1	ND	3.163	-28.375
4	1	ND	2.939	-28.636
4	1	ND	2.956	-27.823
4	1	ND	2.025	-28.416
4	1	ND	2.819	-28.441
4	1	ND	2.49	-27.177
4	1	ND	1.53	-29.012
5	1	ND	3.029	-28.626
5	1	ND	2.735	-27.885

5	1	ND	2.06	-27.792
5	1	ND	1.899	-28.811
5	1	ND	1.696	-28.699
5	1	ND	4.574	-28.122
5	1	ND	1.455	-27.504
5	1	ND	1.922	-27.371
5	1	ND	4.786	-28.153
5	1	ND	2.305	-28.956
6	1	ND	1.6	-27.782
6	1	ND	2.032	-27.953
6	1	ND	2.471	-27.912
6	1	ND	1.275	-27.599
6	1	ND	0.883	-28.064
6	1	ND	2.227	-27.402
6	1	ND	0.948	-28.483
6	1	ND	1.96	-27.834
6	1	ND	1.058	-28.565
6	1	ND	1.623	-27.771
7	1	ND	1.505	-29.357
7	1	ND	1.758	-29.482
7	1	ND	1.09	-29.165
7	1	ND	1.613	-28.464
7	1	ND	1.743	-29.569
7	1	ND	1.872	-29.209
7	1	ND	1.809	-29.352
7	1	ND	1.281	-30.52
7	1	ND	1.394	-29.508
7	1	ND	1.331	-28.963
8	1	ND	2.321	-28.311
8	1	ND	1.536	-29.759
8	1	ND	1.9	-29.934
8	1	ND	2.628	-30.465
8	1	ND	2.308	-29.495
8	1	ND	1.853	-29.376
8	1	ND	1.259	-29.557
8	1	ND	1.465	-27.661
8	1	ND	1.811	-29.499
8	1	ND	2.22	-29.401
1	2	C	0.86	-29.37
1	2	C	1.06	-29.058
1	2	C	NA	-28.658

1	2	C	1.33	-28.114
1	2	C	NA	-28.557
1	2	C	NA	-30.135
1	2	C	NA	-28.639
1	2	C	NA	-29.068
1	2	C	1.51	-29.293
1	2	C	NA	-27.445
1	2	C	NA	-28.386
11	2	C	NA	-28.604
11	2	C	NA	-27.853
11	2	C	1.42	-29.872
11	2	C	NA	-27.907
11	2	C	NA	-29.289
11	2	C	NA	-29.351
11	2	C	NA	-29.965
11	2	C	1.52	-29.856
11	2	C	1.07	-28.54
11	2	C	NA	-29.728
11	2	C	1	-28
11	2	C	NA	-28.909
13	2	C	NA	-30.918
13	2	C	NA	-29.014
13	2	C	1.38	-29.953
13	2	C	1.24	-29.332
13	2	C	NA	-29.251
13	2	C	NA	-28.579
13	2	C	NA	-29.165
13	2	C	1.08	-28.502
13	2	C	NA	-29.519
13	2	C	1.01	-30.48
13	2	C	NA	-29.602
13	2	C	NA	-29.18
14	2	C	NA	-27.967
14	2	C	NA	-27.993
14	2	C	1.59	-29.25
14	2	C	1.08	-28.062
14	2	C	NA	-29.31
14	2	C	NA	-27.622
14	2	C	NA	-28.272
14	2	C	NA	-29.574
14	2	C	NA	-28.581

14	2	C	1.89	-29.751
14	2	C	NA	-28.514
16	2	C	NA	-29.064
16	2	C	1.2	-28.811
16	2	C	NA	-28.907
16	2	C	NA	-28.276
16	2	C	1.34	-28.454
16	2	C	NA	-28.53
16	2	C	NA	-29.209
16	2	C	NA	-28.413
16	2	C	NA	-28.74
16	2	C	1.24	-29.575
16	2	C	NA	-28.512
16	2	C	1.24	-28.969
17	2	C	NA	-29.154
17	2	C	NA	-30.363
17	2	C	NA	-30.266
17	2	C	1.25	NA
17	2	C	NA	-29.354
17	2	C	1.1	-28.542
17	2	C	NA	-30.038
17	2	C	1.05	-29.572
17	2	C	NA	-29.409
17	2	C	NA	-30.301
17	2	C	NA	-30.786
17	2	C	0.94	-30.455
19	2	C	1.08	-29.731
19	2	C	0.81	-30.186
19	2	C	1.08	-28.374
19	2	C	NA	-27.785
19	2	C	NA	-28.199
19	2	C	NA	-28.588
19	2	C	NA	-29.174
19	2	C	1.04	NA
19	2	C	NA	-28.413
19	2	C	NA	-28.904
19	2	C	NA	-29.249
1	2	D	NA	-28.334
1	2	D	NA	-29.383
1	2	D	NA	-29.125
1	2	D	NA	-29.187

1	2	D	1.32	-28.378
1	2	D	NA	-27.914
1	2	D	1.56	-29.982
1	2	D	NA	-29.078
1	2	D	NA	-28.594
1	2	D	1.01	-28.247
1	2	D	1.29	-29.537
1	2	D	NA	-29.487
11	2	D	1.23	-29.356
11	2	D	0.78	-29.452
11	2	D	NA	-29.399
11	2	D	NA	-30.009
11	2	D	NA	-28.63
11	2	D	1.14	-30.17
11	2	D	0.92	-29.972
11	2	D	NA	-30.72
11	2	D	NA	-28.391
11	2	D	NA	-29.397
13	2	D	0.97	-29.016
13	2	D	1.14	-29.411
13	2	D	NA	-29.774
13	2	D	0.94	-29.53
13	2	D	NA	-29.62
13	2	D	NA	-30.611
13	2	D	NA	-28.939
13	2	D	NA	-30.205
13	2	D	NA	-29.838
13	2	D	NA	-30.151
13	2	D	1.1	-30.13
13	2	D	NA	-29.356
14	2	D	NA	-29.036
14	2	D	1.48	-28.18
14	2	D	1.24	-28.262
14	2	D	1.72	-28.45
14	2	D	1.22	-28.704
14	2	D	NA	-28.63
14	2	D	NA	-28.2
14	2	D	NA	-29.478
14	2	D	NA	-28.579
14	2	D	NA	-29.076
14	2	D	NA	-29.027

14	2	D	NA	-28.82
16	2	D	NA	-28.698
16	2	D	NA	-28.88
16	2	D	NA	-28.948
16	2	D	NA	-28.11
16	2	D	NA	-29.666
16	2	D	1.02	-28.665
16	2	D	NA	-28.46
16	2	D	1.17	-28.855
16	2	D	NA	-27.776
16	2	D	1.16	-28.81
16	2	D	NA	-28.778
16	2	D	1.28	-29
17	2	D	NA	-29.931
17	2	D	NA	-30.39
17	2	D	NA	-29.677
17	2	D	1.06	-29.525
17	2	D	NA	-30.501
17	2	D	1.33	-30.517
17	2	D	0.99	-29.908
17	2	D	NA	-29.887
17	2	D	NA	-30.15
17	2	D	NA	-31.322
17	2	D	NA	-29.73
19	2	D	NA	-29.181
19	2	D	NA	-28.596
19	2	D	NA	-28.888
19	2	D	NA	-28.664
19	2	D	0.97	NA
19	2	D	1.08	-28.256
19	2	D	NA	-30.15
19	2	D	NA	-29.328
19	2	D	NA	-28.869
19	2	D	1.37	NA
19	2	D	1.3	-28.583
19	2	D	NA	-29.438
1	2	N	1.19	-29.152
1	2	N	NA	-28.413
1	2	N	NA	-28.884
1	2	N	NA	-28.934
1	2	N	NA	-29.39

1	2	N	1.53	-29.196
1	2	N	NA	-28.969
1	2	N	1.55	-28.286
1	2	N	NA	-28.776
1	2	N	1.21	-28.474
1	2	N	NA	-28.768
1	2	N	NA	-29.491
11	2	N	NA	-28.691
11	2	N	1.41	-30.938
11	2	N	1.54	NA
11	2	N	NA	-29.45
11	2	N	NA	-29.5
11	2	N	NA	-28.606
11	2	N	NA	-28.61
11	2	N	NA	-29.654
11	2	N	1.57	-28.536
11	2	N	NA	-28.723
11	2	N	1.38	-29.43
13	2	N	NA	-29.781
13	2	N	NA	-28.78
13	2	N	1.44	-29.855
13	2	N	NA	-29.46
13	2	N	NA	-30.472
13	2	N	NA	-30.003
13	2	N	1.44	-29.792
13	2	N	NA	-29.676
13	2	N	1.49	-29.85
13	2	N	NA	-28.914
13	2	N	NA	-29.462
13	2	N	1.07	-29.89
14	2	N	NA	-29.131
14	2	N	NA	-28.781
14	2	N	NA	-29.062
14	2	N	NA	-28.588
14	2	N	NA	-28.97
14	2	N	NA	-28.418
14	2	N	1.33	-28.499
14	2	N	NA	-28.463
14	2	N	1.55	-29.168
14	2	N	1.37	-28.23
14	2	N	1.54	-27.879

14	2	N	NA	-28.2
16	2	N	1.14	-29.492
16	2	N	NA	-28.92
16	2	N	NA	-27.807
16	2	N	NA	-28.377
16	2	N	1.58	-29.208
16	2	N	NA	-28.156
16	2	N	NA	-28.87
16	2	N	1.26	-28.684
16	2	N	NA	-28.902
16	2	N	NA	-28.167
16	2	N	NA	-29.392
16	2	N	1.18	-29.22
17	2	N	NA	-29.941
17	2	N	1.25	-29.231
17	2	N	NA	-29.961
17	2	N	NA	-29.739
17	2	N	1.72	-29.419
17	2	N	NA	-30.71
17	2	N	NA	-29.608
17	2	N	NA	-30.523
17	2	N	1.62	-30.271
17	2	N	NA	-29.995
17	2	N	1.43	-30.964
19	2	N	1.32	-28.968
19	2	N	NA	-28.697
19	2	N	NA	-29.096
19	2	N	NA	-29.218
19	2	N	NA	-28.986
19	2	N	NA	-28.374
19	2	N	0.87	-28.77
19	2	N	NA	-29.536
19	2	N	1.25	-27.929
19	2	N	1.07	-28.451
1	2	ND	0.99	-29.02
1	2	ND	NA	-29.079
1	2	ND	1.34	-29.12
1	2	ND	NA	-28.798
1	2	ND	1.54	-29.144
1	2	ND	NA	-29.15
1	2	ND	NA	-28.829

1	2	ND	NA	-28.846
1	2	ND	1.13	-27.681
1	2	ND	NA	-28.676
1	2	ND	NA	-29.41
1	2	ND	NA	-28.809
11	2	ND	0.97	NA
11	2	ND	NA	-29.16
11	2	ND	NA	-28.593
11	2	ND	NA	-29.26
11	2	ND	NA	-28.813
11	2	ND	NA	-28.692
11	2	ND	NA	-30.096
11	2	ND	NA	-29.125
11	2	ND	1.59	-28.56
11	2	ND	1.41	NA
11	2	ND	NA	-29.301
13	2	ND	NA	-29.446
13	2	ND	NA	-28.821
13	2	ND	NA	-30
13	2	ND	NA	-29.07
13	2	ND	1.49	-29.015
13	2	ND	1.75	-28.87
13	2	ND	1.18	-29.702
13	2	ND	NA	-28.772
13	2	ND	NA	-29.81
13	2	ND	NA	-29.967
13	2	ND	NA	-28.947
13	2	ND	1.37	-29.045
14	2	ND	NA	-28.407
14	2	ND	NA	-29.924
14	2	ND	1.19	-28.19
14	2	ND	2.14	-28.457
14	2	ND	NA	-29.532
14	2	ND	NA	-27.95
14	2	ND	1.65	-28.572
14	2	ND	NA	-27.756
14	2	ND	1.92	-28.591
14	2	ND	NA	-28.217
14	2	ND	NA	-28.85
14	2	ND	NA	-28.312
16	2	ND	NA	-28.849

16	2	ND	NA	-28.629
16	2	ND	1	-29.126
16	2	ND	1.12	-27.966
16	2	ND	0.99	-28.38
16	2	ND	NA	-29.164
16	2	ND	NA	-29.218
16	2	ND	0.94	-28.478
16	2	ND	NA	-29.468
16	2	ND	NA	-28.487
16	2	ND	NA	-29.438
16	2	ND	NA	-28.449
17	2	ND	NA	-29.038
17	2	ND	1.23	-29.946
17	2	ND	1.66	-29.66
17	2	ND	NA	-30.282
17	2	ND	NA	-28.116
17	2	ND	NA	-29.256
17	2	ND	1.55	-29.845
17	2	ND	NA	-29.168
17	2	ND	NA	-28.549
17	2	ND	1.62	-29.733
17	2	ND	NA	-28.607
17	2	ND	NA	-29.04
19	2	ND	NA	-28.922
19	2	ND	NA	-28.603
19	2	ND	NA	-29.123
19	2	ND	NA	-28.886
19	2	ND	0.85	-29.112
19	2	ND	1.26	-28.093
19	2	ND	1.23	-29.544
19	2	ND	NA	-29.177
19	2	ND	NA	-29.326
19	2	ND	NA	-28.29

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Nitrogen Addition Enhances Drought Sensitivity of Young Deciduous Tree Species

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Understanding how trees respond to global change drivers is central to predict changes in forest structure and functions. Although there is evidence on the mode of nitrogen (N) and drought (D) effects on tree growth, our understanding of the interplay of these factors is still limited. Simultaneously, as mixtures are expected to be less sensitive to global change as compared to monocultures, we aimed to investigate the combined effects of N addition and D on the productivity of three tree species (*Fagus sylvatica*, *Quercus petraea*, *Pseudotsuga menziesii*) in relation to functional diverse species mixtures using data from a 4-year field experiment in Northwest Germany. Here we show that species mixing can mitigate the negative effects of combined N fertilization and D events, but the community response is mainly driven by the combination of certain traits rather than the tree species richness of a community. For beech, we found that negative effects of D on growth rates were amplified by N fertilization (i.e., combined treatment effects were non-additive), while for oak and fir, the simultaneous effects of N and D were additive. Beech and oak were identified as most sensitive to combined N+D effects with a strong size-dependency observed for beech, suggesting that the negative impact of N+D becomes stronger with time as beech grows larger. As a consequence, the net biodiversity effect declined at the community level, which can be mainly assigned to a distinct loss of complementarity in beech-oak mixtures. This pattern, however, was not evident in the other species-mixtures, indicating that neighborhood composition (i.e., trait combination), but not tree species richness mediated the relationship between tree diversity and treatment effects on tree growth. Our findings point to the importance of the qualitative role ('trait portfolio') that biodiversity play in determining resistance of diverse tree communities to environmental changes. As such, they provide further understanding for adaptive management strategies in the context of global change.

Keywords: climate change, complementarity, ecosystem functioning, insurance hypothesis, nitrogen deposition, plant–climate interactions, temperate forest, tree growth

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INTRODUCTION

Forest ecosystems are currently facing unprecedented shifts in environmental conditions, with implications for biodiversity patterns, ecosystem functions and services (Anderson-Teixeira et al., 2015). Important drivers of environmental shifts are, among others, climate change and atmospheric changes, for example the deposition of reactive forms of nitrogen (Vitousek et al., 1997; Sala et al., 2000). Climate change, accompanied by increasing temperatures and more frequent drought events (IPCC, 2013), is expected to severely affect carbon and water cycles of forest ecosystems (Grossiord et al., 2014). Moreover, drought events and increasing summer temperatures may impose constraints on growth and competitiveness of trees species that are considered sensitive to water shortage (Geßler et al., 2007; Grossiord et al., 2014). On the other hand, atmospheric deposition of nitrogen (N) has tripled since 1860 and is expected to further increase in coming decades (Galloway et al., 2004). In forest ecosystems, N deposition is considered responsible for accelerated biomass increment in recent decades, because tree growth is often limited by the availability of N (Rennenberg et al., 1998; Nadelhoffer, 2000; Pretzsch et al., 2014). Long-term N loading has also been shown to alter soil nutrient cycling and promote soil acidification, leaching of nitrate and soil cations (Magill et al., 1997; Aber et al., 1998; Rennenberg et al., 1998). Consequently, both an increase in nitrogen deposition and drought events may have severe consequences for forest community dynamics, and thus for ecosystem functioning and services.

Due to the global importance of forest ecosystems, there is a bulk of research that addressed the effects of global change drivers on various ecosystem functions (for a global overview see Allen et al., 2010; Bobbink et al., 2010). Many studies, however, have focused on single-factor approaches, whereas analyses on interaction effects are scarce (Zavaleta et al., 2003; Yang et al., 2013), particularly for combined N and D effects (Nilsen, 1995; Meyer-Grünefeldt et al., 2015b,a). It is conceivable, for example, that co-occurring drivers of global change do not act additively (i.e., the summation of single effects), but have non-additive effects on ecosystem responses (i.e., show antagonistic or synergistic interactions; Meyer-Grünefeldt et al., 2015b). This implies that ecosystem responses to multiple environmental shifts cannot be inferred from single-factor studies alone, and emphasizes the need for concomitant multi-factor approaches (Lindenmayer et al., 2010; Ochoa-Hueso et al., 2014; Hautier et al., 2014).

Next to the physiological response of individual trees, the structure and composition of forest ecosystems is central for allowing forest to adapt to global environmental changes (Coomes et al., 2014; De Frenne et al., 2015). In this context, species diversity is assumed to mitigate climate change effects on forest productivity, because diverse forests are expected to react less sensitively to environmental shifts as compared to monocultures (Filotas et al., 2014). Overall, there is increasing evidence that biodiversity promotes various ecosystem functions and services (e.g., Cardinale et al., 2012), and three main mechanisms have been proved to drive diversity-functioning relationships: complementarity (i.e., resource partitioning and facilitation), selection (or sampling) effects (i.e., the higher likelihood that mixtures contain highly productive species) and ecological insurance (Loreau and Hector, 2001; Scherer-Lorenzen, 2014). Many recent biodiversity-ecosystem functioning experiments provided evidence that increasing diversity can reduce the variability of ecosystem properties, and thus increase the temporal stability (e.g., in terms of resistance or resilience) at the ecosystem level (Tilman et al., 2006; Hector et al., 2010; Proulx et al., 2010; Isbell et al., 2015). For instance, observational and simulational studies have shown a positive relationship between tree species richness and the stability of wood production (Jucker et al., 2014; Morin et al., 2014). This beneficial stabilizing effect of biodiversity, also termed as 'insurance hypothesis' (Yachi and Loreau, 1999), can arise from overyielding (i.e., the productivity of mixtures is higher than the average of the monocultures or most productive monoculture), the spatial (i.e., niche partitioning), or temporal (i.e., species asynchrony) complementarity between species or facilitative plant-interactions (Loreau, 2010; Hector et al., 2010; McIntire and Fajardo, 2014). Thus, biodiversity related 'insurance effects' imply that diverse forests are composed of tree species that (i) differ with regard to intrinsic responses to environmental change, (ii) differ with regard to the speed with which they respond to environmental disturbances, or (iii) show a reduction in the strength of competition (Loreau and de Mazancourt, 2013).

We evaluated how N addition and drought interactively affect tree growth in monocultures and mixtures. In a 4-year field experiment with juvenile trees, in which we altered species combinations and species richness levels, we exposed monocultures and mixtures to full-factorial combinations of summer drought and N fertilization. Experiments were conducted with three different tree species: European beech (*Fagus sylvatica*), Sessile oak (*Quercus petraea*), and Douglas fir (*Pseudotsuga menziesii*), henceforth referred to as beech, oak, and fir, respectively. These species differ in key functional traits that are linked to productivity and shade tolerance (e.g., specific leaf area, leaf longevity, and wood density) and are considered to be ecologically and/or economically important from a European perspective (Valladares and Niinemets, 2008; Ellenberg and Leuschner, 2010; Lasky et al., 2014). Specifically, we asked, how sole and combined effects of N fertilization and drought mediate (i) tree-level growth in relation to species identity (of the target and neighboring trees), (ii) stand-level growth in relation to species combination and richness, and (iii) complementarity and selection effects and thus net biodiversity effects of tree communities.

MATERIALS AND METHODS

Study Area

All experimental sites were established in near-natural broad-leaved forest ecosystems typical of the lowlands of NW Germany (Lower Saxony, 53° 8′ 7.827″ N 10° 22′ 20.96″). Soils of the study area developed from sediments of the penultimate glacial period, and prevailing soil types are acidic Cambisols or Luvisols (according to the WRB system, 2006). Mean pH_{H2O}-values in the upper mineral (A−) horizon ranged between 3.9 and 4.7. The natural forest communities at these sites are acidic beech forests that belong to the Galio odorati-Fagetum (Ellenberg and Leuschner, 2010). The climate is of a sub-oceanic type. Mean precipitation is 718 mm yr^{-1}, and the annual mean temperature is 9.2°C.

Experimental Design and Plant Material

In April 2010 we established a 4-year field experiment using a randomized block design (with seven replicate blocks). Blocks were established under larger canopy gaps (0.25–0.50 ha in size) to simulate a quasi-natural regeneration situation under an opened canopy. All blocks were fenced during the experiment to exclude grazing effects. Each block consisted of six plots with different species combinations, where three target species (beech, oak, and fir) were grown, either in monoculture, 2-species or 3-species mixtures (for species combinations see **Table 1**). Each plot was divided into four subplots (1 m × 1 m) with 0.5 m wide buffer strips, and each subplot was randomly assigned to one of the following treatments: control, nitrogen (N) fertilization, drought treatment, and a combination of N fertilization and drought treatment (henceforth referred to as control, N treatment, D treatment, and N+D treatment, respectively). The experiment thus comprised six species combinations and four treatment levels, resulting in a total of 24 experimental combinations (each 7 × replicated).

In April 2010, each subplot was planted with 25 3-year-old tree saplings (planting distance: 20 cm), which originated from a local forest nursery. In mixed-species subplots, trees were planted in a systematic species alteration pattern (e.g., beech-oak-fir-beech-oak-fir etc.). To account for edge effects, only the central nine individuals were considered as target trees for subsequent analyses. All treatments started in the year 2012, i.e., 2 years after sapling planting. This delayed start was chosen to avoid confounding effects between experimental treatments and planting.

In the N treatments (i.e., N and N+D), N was applied (as NH_4NO_3) in a quantity equivalent to 50 kg N ha^{-1} yr^{-1} (as solution in deionized water). This treatment strength was chosen to simulate the effects of atmospheric N deposition which some forest ecosystems currently receive in NW Europe (with 50 kg N ha^{-1} yr^{-1} representing the upper range limit of current deposition rates; Galloway et al., 2004; Bobbink et al., 2010).

To simulate summer drought events (D treatments; i.e., D and N+D) we installed rain-out shelters (2–3 m aboveground) with UV transparent foil (UV-B Window, folitec GmbH, Westerburg, Germany) in the respective subplots to exclude any precipitation. The rain-out shelters were installed from July 9th to July 31st and August 13th to September 7th in 2012, and from July 5th to September 5th in 2013. Effects of D treatments on soil water contents were determined by means of volumetric soil water content sensors (based on Time Domain Reflectometry; Decagon Devices, Pullman, WA, USA) that were installed in four representative blocks in 2012 and 2013 in the upper mineral soil (0–5 cm). Measurements of volumetric soil water contents indicated that D treatments reduced the soil water content by about 20% (volumetric losses compared to field capacity, achieved during the last week of the D treatments), which corresponds to a moderate-severe drought event in the study region (Rose et al., 2009).

Tree Measurements

For all trees, height and biomass were determined. Tree height (measured from the root collar to the top) was recorded at the beginning of the treatment application (April 2012) and at the end of the experiment in September 2013, which corresponded to a 2-year growing period. For each tree we calculated relative growth rate (RGR) of tree height as $RGR = (\ln H_2 - \ln H_1)/(t_2 - t_1)$, where H_1 and H_2 are the tree heights at the beginning (t_1) and end (t_2) of the experiment. We used RGR instead of absolute growth rates as a response variable to model individual tree growth, because RGR is less sensitive toward differences in initial size (Mencuccini et al., 2005). After tree harvest (September 2013), we additionally measured the stem biomass (including branches) and the biomass of leaves or needles for all tree individuals. Biomass samples were dried at 40°C for 3 days (until weight constancy) and

TABLE 1 | Design of the experiment.

Species	Diversity level	Species combination	No. trees
Fagus sylvatica (beech)	mono	–	252
Quercus petraea (oak)	mono	–	252
Pseudotsuga menziesii (fir)	mono	–	252
Fagus sylvatica	mix2	beech-oak	140
Fagus sylvatica	mix2	beech-fir	140
Quercus petraea	mix2	beech-oak	112
Pseudotsuga menziesii	mix2	beech-fir	112
Fagus sylvatica	mix3	beech-oak-fir	84
Quercus petraea	mix3	beech-oak-fir	84
Pseudotsuga menziesii	mix3	beech-oak-fir	84
Total			1512

Number of planted target trees of each diversity level and species combination. Mono, monoculture; mix2, 2-species mixture; mix3, 3-species mixture.

TABLE 2 | Target tree characteristics of the three study species.

	Mean (*SD*)	Range
Fagus sylvatica		
Initial tree height (cm)	89.4 (16.5)	52.0–141.0
AGB (g)	51.5 (41.4)	4.5–305.1
AGR (cm $year^{-1}$)	16.7 (11.4)	0.0–55.5
RGR (cm cm^{-1} $year^{-1}$)	0.15 (0.08)	0.0–0.37
Quercus petraea		
Initial tree height (cm)	101.0 (25.2)	38.0–178.0
AGB (g)	53.0 (48.3)	0.1–323.8
AGR (cm $year^{-1}$)	17.2 (12.3)	0.0–59.0
RGR (cm cm^{-1} $year^{-1}$)	0.13 (0.08)	0.0–0.36
Pseudotsuga menziesii		
Initial tree height (cm)	118.6 (25.9)	62.0–202.0
AGB (g)	150.3 (107.7)	16.2–683.2
AGR (cm $year^{-1}$)	28.6 (13.7)	0.0–87.0
RGR (cm cm^{-1} $year^{-1}$)	0.19 (0.07)	0.0–0.37

Absolute (AGR) and relative growth rate (RGR) of tree height refer to a 2-year census interval; AGB, aboveground biomass at the end of the census interval.

subsequently weighted. Target tree characteristics are provided in **Table 2**.

Data Analysis

Individual tree growth analyses was focused on 1291 target trees in total (beech: 558, oak: 320, fir: 413). Due to mortality, 12% of the original 1512 target trees were not available to be measured at the end of the experiment. Oak showed highest mortality, followed by fir and beech, but we found no statistically significant treatment effect across species (beech: $P = 0.10$; oak: $P = 0.91$; fir: $P = 0.83$; Supplementary Figure S1). Moreover, observations with negative growth rates (3% of the surviving trees) were assumed to be damage-related (e.g., due to planting failures or falling large-sized branches) or to have measurement error, and therefore omitted in the subsequent height growth analysis to avoid biased estimates. However, trees with zero increments were retained.

To examine the tree size, treatment, and species diversity (measured as species richness) dependence of RGR of the three target species, we applied linear mixed models using block, plot and treatment as nested random factors. We fitted several alternative models for each target species separately including initial height, treatment, species combination, and their interactions as fixed effects. To address the skewed response and heteroscedasticity of the beech and oak growth data, the residual error was modeled using a variance function based on the power of the fitted values (Pinheiro and Bates, 2004). Models were selected based on the Akaike Information Criterion (AIC) and maximum likelihood (ML) estimations. Moreover, we ranked the models based on Akaike weights (w_i), which are the relative likelihood of each model to be the best-fitting model, given the complete set of candidate models (Burnham and Anderson, 2002). Only models with an AIC difference (ΔAIC) ≤ 2 (compared with the best-fitting model) were considered as models with substantial support (Burnham and Anderson, 2002), and for each species the model with the highest Akaike weights was chosen as the most parsimonious model. Parameter estimates of the best-fitting models were based on the restricted maximum likelihood (REML) method.

The strength of each treatment effect on RGR rates was determined by the magnitude of treatment effect (MTE). MTE was calculated as $\mathrm{MTE} = (X_T - X_C)/(X_T + X_C)$, where X_T is the predicted response of target tree *i* in the global change driver treatments (N, D, N+D) and X_C the predicted response in the control (C) treatment. This index ranges from –1 (negative global change driver influence) to +1 (positive global change driver influence) for each species, thus facilitating between-species comparisons. Differences in MTE among species were evaluated by analysis of variance (ANOVA) with a *post hoc* performance (Tukey HSD test).

Total aboveground biomass (all woody compartments and leaves; AGB) was used as a response for tree vigor. For trees that died during the experiment we used the average species-specific AGB of each treatment and species combination. We applied the additive partitioning method according to Loreau and Hector (2001) to quantify the net biodiversity effect (NE) on AGB of species mixtures, which we further partitioned into the complementarity (CE), and selection effect (SE). NE, CE, and SE were calculated using the following equations:

$$\mathrm{NE} = \Sigma \mathrm{Y} - \overline{\mathrm{M}}$$

$$\mathrm{CE} = \mathrm{N} \times \overline{\mathrm{M}} \times \overline{\Delta \mathrm{RY}}$$

$$\mathrm{SE} = \mathrm{N} \times \mathrm{cov}(\mathrm{M}, \Delta \mathrm{RY})$$

where Y is the observed AGB for each species in mixture and M is the yield of a species growing in monoculture. N is the number of species and ΔRY the deviation from the expected relative yield of a species in mixture ($\Delta \mathrm{RY} = (\mathrm{Y/M}) - (1/\mathrm{N})$).

To account for size differences of the species-mixtures, and thus allow for inter-site comparisons, diversity components were standardized dividing NE, CE, and SE by the expected AGB based on monocultures (see Morin et al., 2011). For the subsequent analysis these values were square-root transformed to meet the model assumptions while preserving the original positive and negative signs (Loreau and Hector, 2001). For each species combination we fitted a linear-mixed effects model using treatment as fixed effect and block as random factor to account for potential differences in site conditions. All statistical analyses were performed in R (version 3.1.0[1]) using the packages *nlme* and *MuMIn*.

RESULTS

Effects of Nitrogen Fertilization and Drought on Tree-Level Height Growth

For all species the minimum adequate models according to the AIC included tree size, treatment and species composition effects (**Table 3**). For beech, the treatment effects significantly depended on tree size ($P < 0.01$; **Table 4**), with treatment effects becoming more pronounced with increasing height. For oak and fir, the RGR-treatment relationships were consistent across the observed height range. Compared to control plots, RGR of oak was significantly lower in the N+D treatment ($P < 0.05$), and marginally significant lower in the N treatment ($P \leq 0.1$), while a significant decline in RGR of fir was induced by drought ($P < 0.05$). Moreover, a significant species composition effect on the shape of the size response was observed for beech ($P < 0.001$) and oak ($P < 0.01$), while for fir, the species composition effect ($P < 0.01$) was independent of tree size (**Table 4**). There was no support for a statistically significant three-way interaction effect on RGR, showing that for each species the size-treatment relationship did not shift with species composition (**Table 3**). Graphical validation plots indicated unbiased estimates. The best-supported models explained between 41% (beech), 44% (oak), and 51% (fir) of the variation in RGR of height.

The positive RGR-size relationship was most pronounced for beech with a greater increase in growth rates when growing in mixture with fir (**Figure 1**). Similarly, RGR of oak trees in monoculture increased with size. In contrast, the influence of

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[1] http://www.R-project.org

TABLE 3 | Model selection statistics (Akaike Information Criterion ΔAIC and Akaike weights w_i) for various candidate models describing the RGR of tree height as a function of initial tree height (H), treatment (T), and species composition (C) effects of European beech (*Fagus sylvatica*), Sessile oak (*Quercus petraea*), and Douglas fir (*Pseudotsuga menziesii*).

Model	Fixed effects							*Fagus sylvatica*		*Quercus petraea*		*Pseudotsuga menziesii*	
	H	T	C	H × T	H × C	T × C	H × T × C	ΔAIC	w_i	ΔAIC	w_i	ΔAIC	w_i
1	×	×						23.99	0.00	4.47	0.08	11.57	0.00
2	×		×					15.72	0.00	7.94	0.01	2.38	0.09
3		×	×					73.76	0.00	9.46	0.01	7.21	0.01
4	×	×	×					19.45	0.00	7.25	0.02	**0.01**	**0.30**
5	×	×	×	×				13.67	0.00	10.08	0.00	0.00	0.30
6	×	×	×		×			10.82	0.00	**0.13**	**0.50**	1.20	0.16
7	×	×	×			×		25.29	0.00	8.32	0.01	7.38	0.01
8	×	×	×	×	×			**0.00**	**0.91**	3.51	0.07	1.75	0.12
9	×	×	×	×		×		19.64	0.00	8.55	0.00	7.81	0.01
10	×	×	×		×	×		14.00	0.00	0.00	0.26	8.42	0.00
11	×	×	×	×	×	x		4.74	0.08	2.55	0.05	9.46	0.00
12	×	×	×	×	×	×	x	15.61	0.00	5.20	0.00	15.21	0.00

The best-supported models with the highest Akaike weights are highlighted in bold. For Douglas fir the more parsimonious model that included a marginal significant height-treatment interaction (P = 0.09) was rejected, since the main effects-only model fit the data equally well (ΔAIC = 0.01, w_i for both models = 30%).

size was less evident for oak growing in 2- or 3-species mixtures and fir growing either in monoculture or mixture. The mode of growth response to treatment effects, however, was significantly different among species and tree sizes (**Figure 2**). Oak and fir showed an additive response (summation of the single effects) to simultaneous N addition and drought, whereas the response of beech was non-additive (i.e., an antagonistic response of smaller and a synergistic response of larger individuals). This trend was consistent along the investigated diversity gradient, since we did not observe interacting effects of treatment and species composition (**Table 3**).

Compared to the control, RGR of small trees in the N treatment was lower for beech and oak, but higher for fir (**Figure 2**). In contrast to oak, growth rates of large beech and fir trees were enhanced by nitrogen enrichment ($P_{\text{adj.}} < 0.01$). In contrast, drought reduced height growth of all species and sizes with effects being strongest for large-sized beech trees ($P_{\text{adj.}} < 0.001$). The combination of N addition and drought was negative for all species, but size-dependency was strongest for beech. The sensitivity of oak and fir to N+D was equally high for small and large trees, with effects being much stronger for oak. Large beech trees, however, suffered most from N+D, resulting in a sevenfold decline in growth rates compared to small individuals. Thus, growth reductions induced by combined N and D effects of large individuals significantly increased within the series fir < oak < beech (all comparisons: $P_{\text{adj.}} < 0.05$; **Figure 2**).

TABLE 4 | Best-fitting mixed-effects models for RGR of tree height of (a) European beech (*Fagus sylvatica*), (b) Sessile oak (*Quercus petraea*) and (c) Douglas fir (*Pseudotsuga menziesii*).

Fixed effects	d.f.	*L*-ratio	*P*-value
(a) *Fagus sylvatica*			
Initial tree height (H)	1	56.3	<0.001
Treatment (T)	3	2.3	0.517
Species composition (C)	3	10.5	0.014
H × T	3	15.3	0.002
H × C	3	20.9	<0.001
(b) *Quercus petraea*			
Initial tree height (H)	1	4.2	0.040
Treatment (T)	3	6.7	0.082
Species composition (C)	2	1.2	0.543
H × C	2	10.0	0.007
(c) *Pseudotsuga menziesii*			
Initial tree height (H)	1	15.6	<0.001
Treatment (T)	3	8.4	0.039
Species composition (C)	2	9.2	0.002

P-values were derived from likelihood-ratio tests based on maximum likelihood (ML) estimations.

AGB was closely related to RGR, and the strength of the relationship did not significantly differ among species (Supplementary Figure S2).

Effects of Nitrogen Fertilization and Drought on Stand-Level Biomass Production

In the absence of D or N treatments the mixture effect on overall stand productivity was positive for all species mixtures (**Figure 3**). Overyielding was evident in 81% of the control plots and in 69% of the sites (blocks) across treatments (Supplementary Table S1), but we observed a large variation across sites (Supplementary Table S2).

The impact of global change drivers (D, N, or N+D) on the net biodiversity effect was driven by species identity rather than species diversity. Regardless of treatment, the average net diversity effects of beech-fir and beech-oak-fir stands remained positive and did not statistically differ from the control (**Figure 3**). In contrast, for beech-oak mixtures the magnitude and direction of diversity effects differed between treatments. N addition reduced the positive effect of species mixture to become

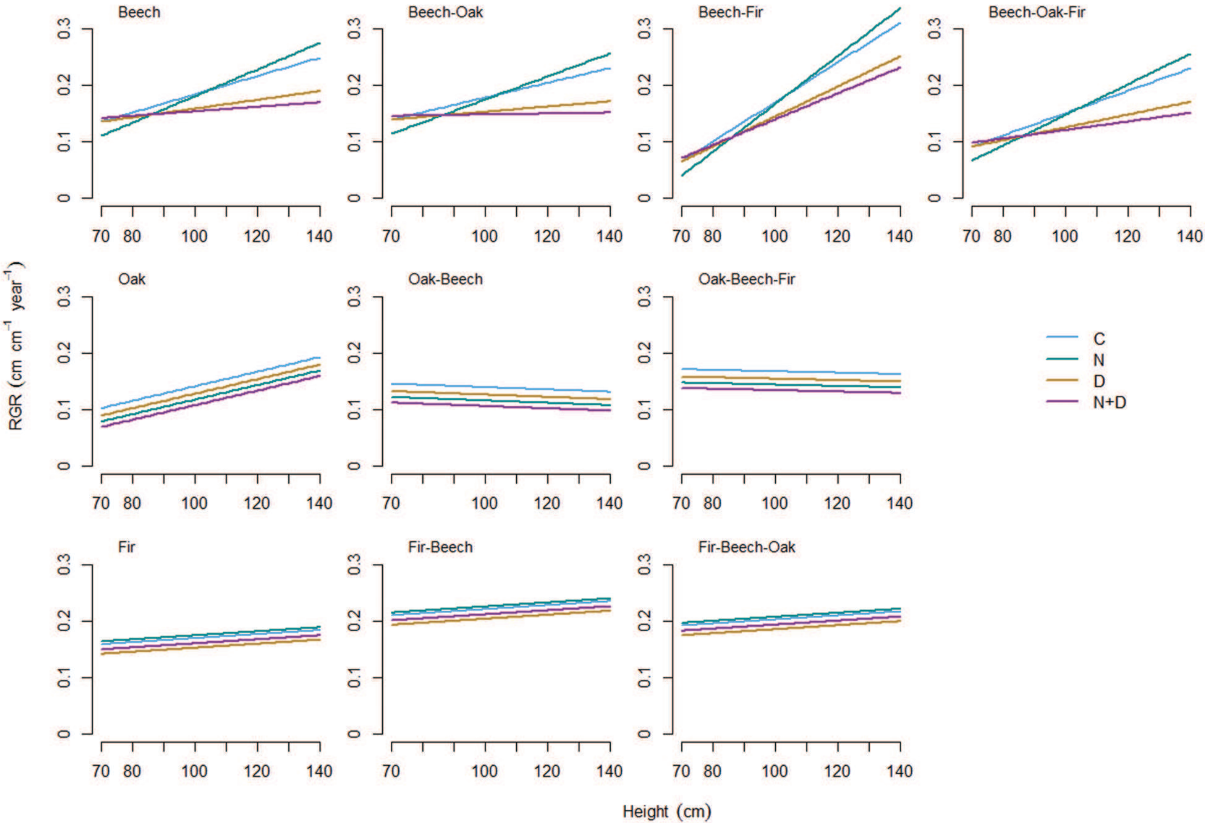

FIGURE 1 | Relationship between tree size (initial height), global change effects (C, control; N, nitrogen addition; D, drought; N+D: nitrogen addition plus drought), species diversity (monoculture; 2-species mixture, and 3-species mixture) and relative growth rate (RGR) of tree height for European beech (*Fagus sylvatica;* upper row), Sessile oak (*Quercus petraea;* middle row) and Douglas fir (*Pseudotsuga menziesii*; lower row). Regression lines are based on the predictions of the best-fitted models in **Table 4**.

neutral ($P = 0.1$), whereas the combined effects of N addition and drought caused a significant loss of biodiversity effects and underyielding, respectively ($P < 0.05$; **Figure 3**). This pattern can be primarily attributed to the loss of complementarity effects with regard to the N+D treatment ($P < 0.05$) and selection effects in relation to the N treatment ($P < 0.1$). Similarly, different underlying complementarity and selection effects were obvious for beech-fir and beech-oak-fir mixtures. In 61% of the beech-fir sites the selection effect was greater than the complementarity effect, particularly in the D treatment. Thus, high stand biomass productivities can be mainly ascribed to fir. In the 3-species mixture the selection effect became negative in the N+D treatment ($P = 0.1$) and neutral in the N treatment, but CE were always greater than SE (**Figure 3**).

DISCUSSION

Species-Specific Growth Response to Combined Effects of Nitrogen Addition and Drought

Our results show that tree growth response to treatments was mainly driven by species identity rather than species diversity, and the combined effects of N and D treatments proved to be both additive and non-additive. In the first case, the combined effects of N+D on RGR of tree height corresponded with the sum of the single effects (oak and fir), but in the latter case the combination of both factors caused negative growth responses, with mutually amplifying effects (for large beech trees, despite the positive single effect of N fertilization). This finding suggests that – at least in the case of beech – growth responses to environmental shifts are difficult to infer from species responses to single factors (Zavaleta et al., 2003). Several mechanisms may account for the non-additive effects of N+D treatments. First, N fertilization often results in a shift in biomass allocation patterns (in favor of aboveground biomass), resulting in a concomitant increase of biomass shoot-root ratios (Thomas and Hilker, 2000; Meyer-Grünefeldt et al., 2015b). For example, leave biomass investments of coniferous tree species increased with N fertilization (Högberg et al., 1993), and can thus increase the water consumption and probability of water stress (Nilsen, 1995). The responses described above are in agreement with the ‘resource optimization hypothesis’, according to which plants show (relatively) higher aboveground investments (and hence higher shoot-root ratios) with increasing nutrient availability

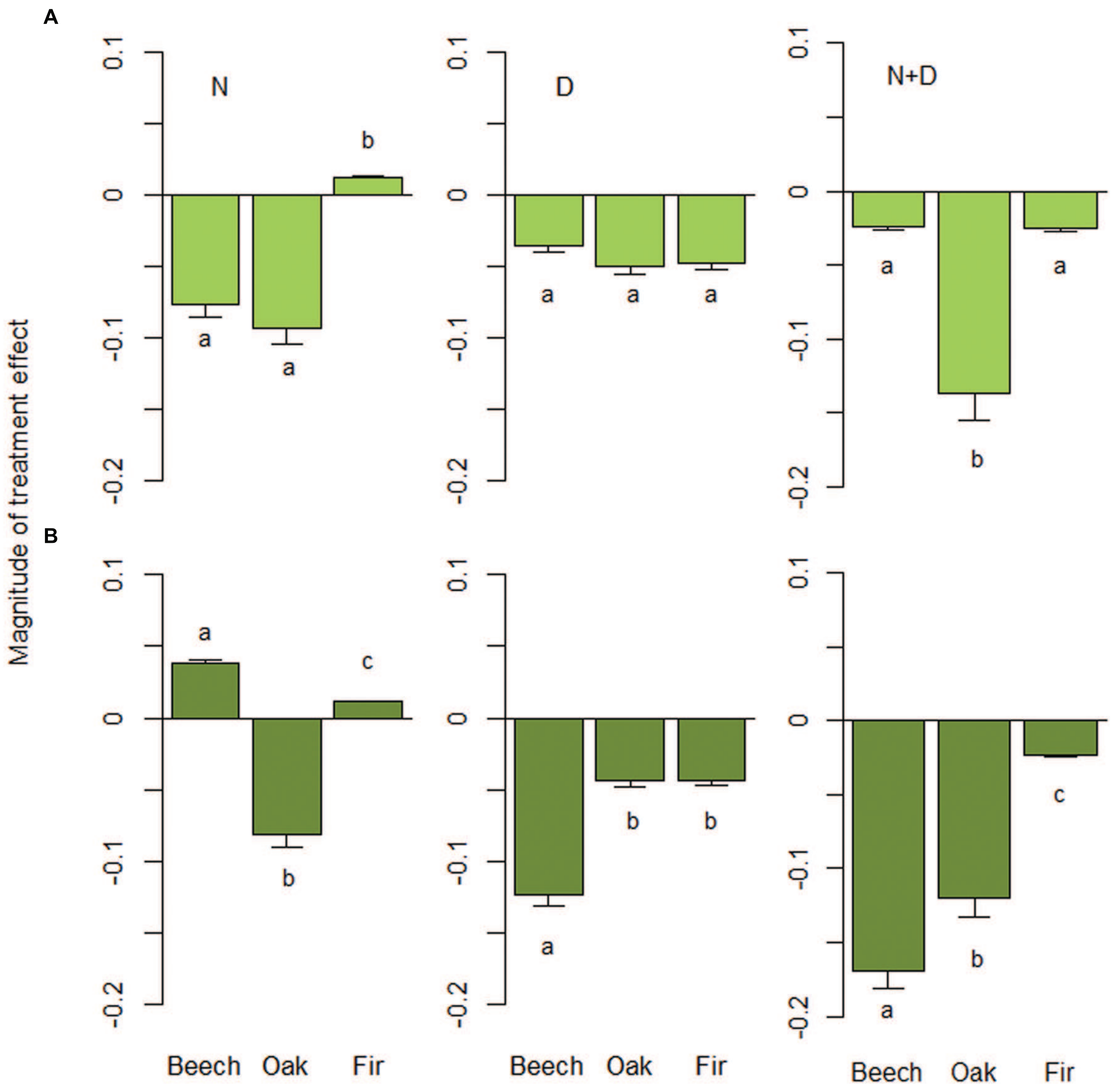

FIGURE 2 | Magnitude of treatment effect (MTE) for nitrogen addition (N), drought (D) and their combination (N+D). For each species (European beech: *Fagus sylvatica*, Sessile oak: *Quercus petraea,* and Douglas fir: *Pseudotsuga menziesii*), MTEs were predicted for **(A)** small-sized (initial height of 80 cm) and **(B)** large-sized trees (initial height of 130 cm) based on our best-fitting models (see **Table 4**). Error bars show standard errors based on across-species combination response. Different letters indicate significant differences (Tukey-test: $P < 0.05$) among species.

(McConnaughay and Coleman, 1999; Ågren and Franklin, 2003). High shoot-root ratios, in turn, can lead to increasing evaporative demands and thus a higher sensitivity to drought events (Meyer-Grünefeldt et al., 2015b). Second, N fertilization can increase fine- and coarse-root mortality and decrease the mycorrhiza colonization, both of which can impair supply and therefore increase their drought sensitivity (Hendricks et al., 2000; Nadelhoffer, 2000; Teste et al., 2012). Third, as trees can optimize the fine root and branch hydraulic system in water-limited environments (Hertel et al., 2013; Schuldt et al., 2016), an increasing N availability might prevent such adaptation mechanisms and therefore increase the suspectibility to drougth.

Tree species also responded differently to N fertilization, with a facilitation of (large) beech and fir trees, but adverse effects on oak. Deleterious effects of N fertilization on juvenile oak trees have also been reported in the study of BassiriRad et al. (2015), without a clear indication of the underlying mechanisms. In our study, species-specific responses are likely related to their traits and competitive hierarchy. Oak trees are light-demanding and may suffer from an unfavorable light environment when overgrown from larger neighbors, particularly at N-fertilized sites (Ellenberg and Leuschner, 2010). In this context, the strong size-asymmetry of treatment effects for beech suggests that our findings are related to size-asymmetric competition, because larger individuals mostly obtain a disproportionate share of

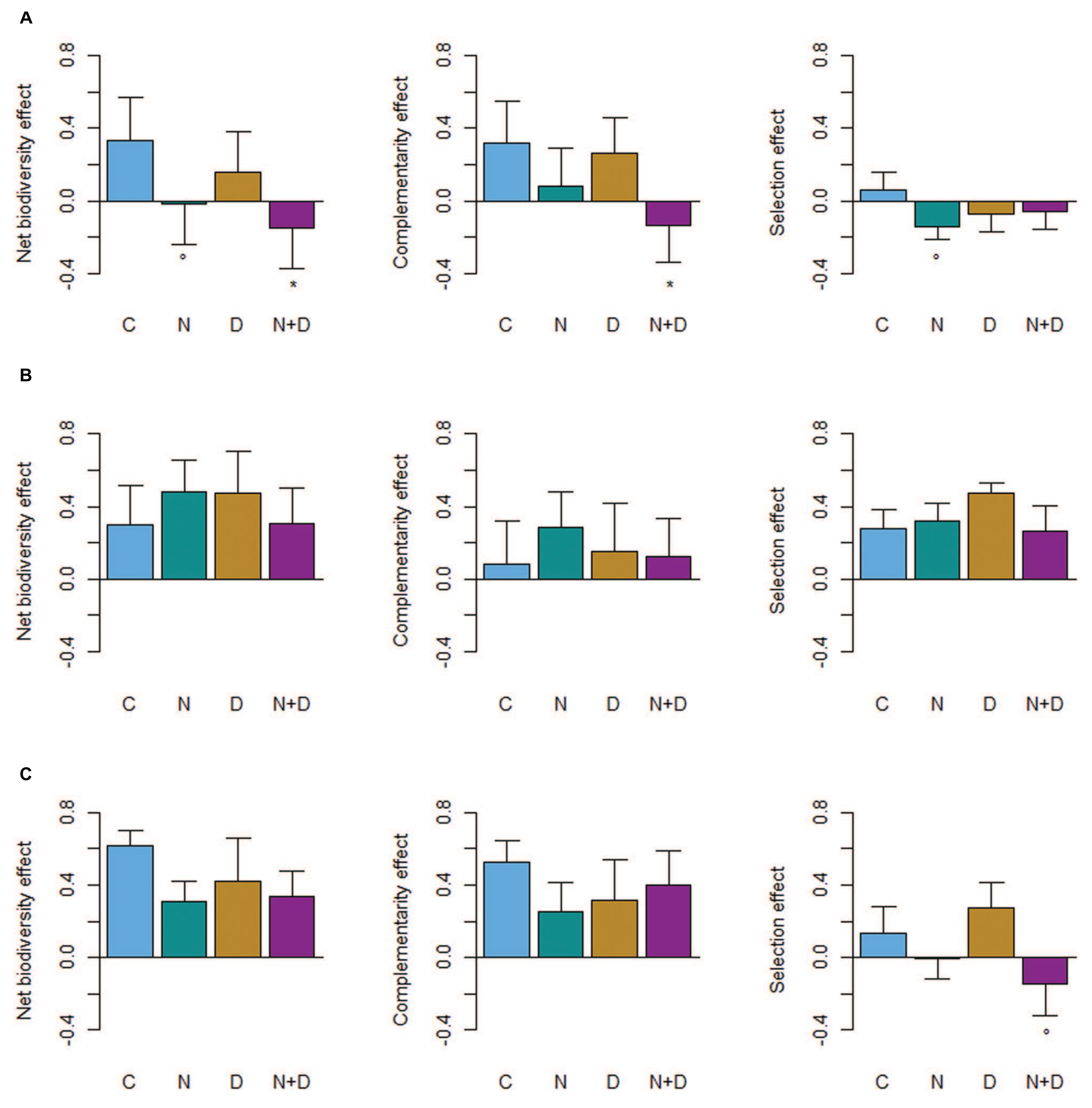

FIGURE 3 | Changes in net biodiversity, complementarity and selection effects with global change drivers (C, control; N, nitrogen addition; D, drought; N+D: nitrogen addition and drought) for (A) beech-oak stands (*Fagus sylvatica–Quercus petraea*), (B) beech-fir stands (*Fagus sylvatica–Pseudotsuga menziesii*), and (C) beech-oak- fir stands (*Fagus sylvatica–Quercus petraea–Pseudotsuga menziesii*). Values are square-root transformed to meet model assumptions while preserving the original positive and negative signs. Asterisks indicate significant differences ($P < 0.05$) and open circles marginal significant differences ($P \leq 0.1$) between the control (C) plots and global change driver (N, D, N+D) treatments.

resources and thus suppress the growth of smaller individuals (Potvin and Dutilleul, 2009). As a consequence, larger trees have a competitive advantage in resource acquisition over smaller individuals, and thus benefit most from additional nutrients, explaining the N-induced height growth decline of smaller oak and beech trees.

Species differences in the sensitivity to drought, as shown for larger individuals in our study, coincides with the well-known ecophysiology of these species (see for example Thomas, 2000; Geßler et al., 2007; Meier and Leuschner, 2008; Friedrichs et al., 2009; Härdtle et al., 2014). In a study of five temperate adult tree species, Zimmermann et al. (2015) found that beech is most susceptible to drought, which is in line with our observed increasing drought sensitivity as beech trees grew larger. Thus, species-specific differences in drought sensitivity might result in shifts in the competitive hierarchy in mixed-species tree communities. Our study, however, provided no evidence for changes of treatment effects depending on community composition. This suggests that treatment effects at the scale of individual trees were highly species-specific, and growth responses of juvenile trees to treatments were strongly mediated by the species' trait characteristics (also see discussion below) and local neigborhood conditions (Lübbe et al., 2015, 2016). An additional explanation to the statistically non-significant three-way interaction (H × T × C) and two-way interaction (T × C) is that diversity effects may need time to fully evolve in long-living plant communities such as forests, and therefore may become more pronounced as trees become larger.

We found that tree size-related changes in RGR were context-specific (neighborhood composition) and varied with species identity. Species interactions leading to a spatial complementarity in resource use due to differences in leaf habit (e.g., Coomes et al., 2009) are likely to be important in beech-fir mixtures. As a result, species mixing can mitigate drought susceptibility of mature beech trees by reducing intra-specific competition (Metz et al., 2016). In contrast, oak trees (as the most light-demanding species) proved to be week competitors (at least under the given experimental settings), and benefitted most from growing with conspecific neighbors. Thus, positive mixture effects in our study may be primarily the result of trait induced competitive hierarchies (Kunstler et al., 2012) and the species' trait characteristics also accounted for the observed interacting effects of tree size and species composition.

Functional Composition of Forests Modulate the Effects of Nitrogen Addition and Drought on Stand Productivity

Overyielding was evident for almost all plots across treatments, which is in agreement with many previous studies reporting a positive effect of tree diversity on forest productivity (e.g., Paquette and Messier, 2011; Vilà et al., 2013; Forrester and Bauhus, 2016). However, in our experiment the NE on stand-level productivity strongly depended on both the species composition and the species-specific responses to treatments. In the beech-oak mixture, we found a significant underyielding in the N+D treatment, attributable to negative N+D effects on CE. We hypothesize that the negative NE was brought about by the negative responses of beech and oak to N+D treatments already observed at the tree-level. This, in turn, would indicate that stand-level, and tree-level responses to 'environmental shifts' are closely related, or, more specifically, may depend on the trait characteristics of the species included in a mixture (Lübbe et al., 2015). This interpretation is supported by the result that we found no NE and a negative SE for beech-oak mixtures in the N treatment, likely brought about by the strong negative response of oak trees to N fertilization. We conclude that the resistance of a species mixture to environmental shifts may be more determined by the traits typical of the species included in a mixture than by the mere complementarity of the traits (or the functional dissimilarity) of these species (as given in the case of beech and oak). Biodiversity thus would not serve *per se* as an 'insurance' for the mitigation of global change effects on ecosystem functions (Lübbe et al., 2015), but would act in terms of a 'trait portfolio' that preserves a broad spectrum of functional traits enabling a species' resistance to environmental stressor (comparable to a lock-and-key model, according to which only particular traits ensure higher resistance of plant communities to environmental shifts; Polley et al., 2013). This perspective emphasizes the importance of both the quantity and quality of biodiversity for ecosystem resistance to environmental change (Mouillot et al., 2013).

The hypothesis provided above also supports the interpretation of treatment responses of those mixtures in which fir was included (i.e., beech-fir and beech-oak-fir mixtures). In these mixtures we found positive NE across treatments, suggesting that fir acted as a kind of 'buffer' mitigating the (partly negative) effects of N fertilization and drought. In the beech-fir mixture, positive NE were mainly attributable to SE, particularly in the D treatment. Obviously, the low sensitivity of fir to D and N+D treatments (of small and small + large trees, respectively; see Bansal et al., 2015) was conveyed to the stand-level, resulting in the observed positive NE across treatments. In the 3-species mixture, fir obviously mitigated the adverse effects of N and N+D observed for the beech-oak mixture, resulting in positive CE (substantially contributing to the NE). We hypothesize that trait-characteristics of fir mainly concurred to the observed response pattern (e.g., its low drought sensitivity; Bansal et al., 2015), resulting in an increased stand-level resistance of the tree-mixture. In summary, stand-level responses to treatments (and corresponding NE) were strongly mediated by species composition and the species' functional trait characteristics included in a mixture. This finding is in line with our observation on the individual tree level and matches observations in other tree diversity experiments, according to which species identity often proved to be as influential as species richness effects on productivity patterns (Jacob et al., 2010; Lang et al., 2012; Grossiord et al., 2013; Ratcliffe et al., 2015).

CONCLUSION

Our results highlight the importance of assessing interacting effects of nitrogen addition and drought to evaluate forest productivity in response to global environmental change. We are aware of the limitation to generalize results from juvenile tree field-experiments to adult tree communities, but manipulations of N and D treatments are hardly achievable in later forest development stages due to the longevity of trees. Hence, our experimental framework provides a unique opportunity to enhance our mechanistic understanding of tree growth in the context of global change by disentangling the effects of various global change drivers and their interactions unequivocally.

We found evidence that the magnitude and direction of combined global change driver effects depend on species identity and neighborhood composition (i.e., trait combination) rather than the level of tree species richness. Thus, species diversity might not mitigate *per se* the impact of drought and increasing N deposition in long-living plant communities. Instead, the occurrence of certain trait combinations ('trait portfolio') in diverse communities might act as an 'insurance' for the mitigation of global change effects on ecosystem functions. This suggests that the quality of trait composition ('lock-and-key principle') is a main component of the ecological insurance hypothesis.

AUTHOR CONTRIBUTIONS

WH and GvO conceived the study. CD performed the field and laboratory work, and AF analyzed the data. AF, CD, WH, and GvO wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2016.01100

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Supplementary Material

Christoph Dziedek, Werner Härdtle, Goddert von Oheimb and Andreas Fichtner (2016) Nitrogen Addition Enhances Drought Sensitivity of Young Deciduous Tree Species. Frontiers in Plant Science, 7, 1100.

Table S1 Treatment specific overyielding, complementarity (CE) and selection (SE) effects for each species mixture.

Treatment	n (sites)	% overyielding	% SE > CE
Beech-Oak			
C	7	71.4	14.3
N	7	42.9	0.0
D	7	57.1	14.3
N+D	7	28.6	28.6
Mean		50.0	14.3
Beech-Fir			
C	7	57.1	71.4
N	7	85.7	57.1
D	7	71.4	57.1
N+D	7	71.4	57.1
Mean		75.0	60.7
Beech-Oak-Fir			
C	7	100.0	14.3
N	7	71.4	28.6
D	7	85.7	28.6
N+D	7	85.7	14.3
Mean		85.7	21.4
Mean (all stands)	84	69.0	32.1

Table S2 Intraclass correlation coefficients for the site (block) effect based on biomass production models for each diversity effect-species mixture combination. NE: net biodiversity effect; CE: complementarity effect; SE: selection effect.

Effect	Beech-Oak	Beech-Fir	Beech-Oak-Fir
NE	0.55	0.03	0.36
CE	0.57	0.04	0.21
SE	0.28	0.00	0.35

Figure S1 Treatment-specific observed mortality for (a) European beech (*Fagus sylvatica*), (b) Sessile oak (*Quercus petraea*) and (c) Douglas fir (*Pseudotsuga menziesii*). Across all species, the effect of treatment on mortality rates was not significant ($P > 0.05$) as indicated by generalized mixed effect models (GLMMs with a logit-link function and binomial distribution using block, plot and treatment as nested random factors).

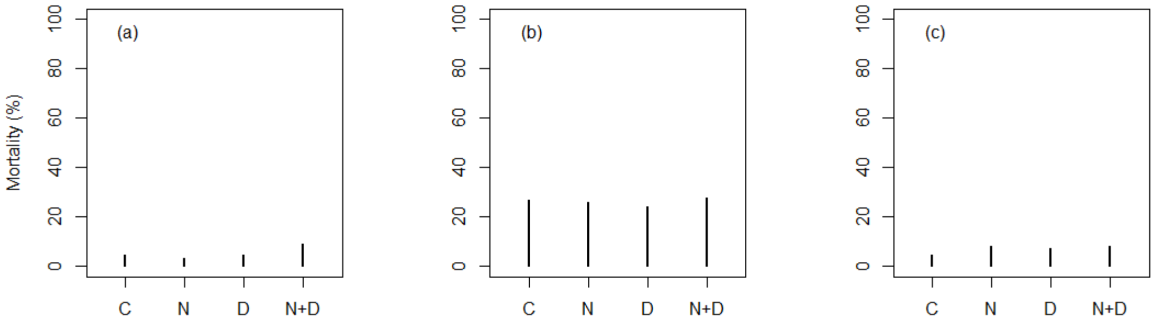

Figure S2 Relationship between aboveground biomass (AGB) and relative growth rate (RGR) of tree height for (a) European beech (*Fagus sylvatica*), (b) Sessile oak (*Quercus petraea*) and (c) Douglas fir (*Pseudotsuga menziesii*). Grey areas denote the 95% confidence intervals. Panel (d) shows variation in the strength of the AGB-RGR relationship (standardized slope with 95% confidence interval) with species.