Plant Soil (2012) 353:59–71
DOI 10.1007/s11104-011-1008-3

REGULAR ARTICLE

Nitrogen deposition increases susceptibility to drought - experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench

**Uta Friedrich · Goddert von Oheimb ·
Wolf-Ulrich Kriebitzsch · Kristina Schleßelmann ·
Malte Sebastian Weber · Werner Härdtle**

Abstract
Aims We investigated the response of the perennial grass *Molinia caerulea* (L.) Moench to combined effects of fertilization (N, P) and drought events. We hypothesized that N fertilization increases, and drought decreases productivity, but that N addition strengthens negative effects caused by drought.

Methods Within a full-factorial 2-year greenhouse experiment we measured biomass productivity and allocation, tissue nutrient concentrations and nitrogen allocation patterns using ^{15}N as a tracer.

Results N fertilization caused a strong increase in productivity, but effects of drought were almost insignificant. However, we found strongly interrelated, non-additive effects of fertilization and drought, expressed by a strong increase of necrotic tissue. Dead aboveground biomass showed the highest values for N and ^{15}N.

Conclusions Accelerated productivity of aboveground tissue under N fertilization resulted in increased evaporative demands and thus higher drought susceptibility. In addition ^{15}N allocation patterns showed that fertilization-drought treatments disenabled plants' control of their N allocation. *Molinia* was unable to withdraw leaf N during the dieback of aboveground tissue. Due to the lack of an adaptive strategy to the combined effects of fertilization and drought, increasing summer drought may weaken the competitive performance of species with traits comparable to those of *Molinia* in N-fertilized environments.

Keywords Biomass allocation · Climate change · Heathland · *Molinia caerulea* · ^{15}N tracer · Nutrient allocation

Responsible Editor: Harry Olde Venterink.

U. Friedrich (✉) · G. von Oheimb · K. Schleßelmann ·
M. S. Weber · W. Härdtle
Institute of Ecology, University of Lüneburg,
Scharnhorststr. 1,
21335 Lüneburg, Germany
e-mail: uta.friedrich@leuphana.de

W.-U. Kriebitzsch
Institute for World Forestry, Johann Heinrich von Thünen
Institute (vTI), Federal Research Institute for Rural Areas,
Forestry and Fisheries,
Leuschnerstraße 91,
21031 Hamburg, Germany

Introduction

Atmospheric nitrogen (N) deposition and climate change are (beside land use changes) important drivers of biodiversity loss and may affect ecosystem functioning on global and regional scales (Sala et al. 2000; Tylianakis et al. 2008). Atmospheric N deposition has risen since the beginning of industrialization, with an upward trend expected in the near future (Galloway et al. 2004). N loads affect ecosystem nutrient levels and cycles, the growth of plants and their competitive interactions, and are responsible for shifts in the species composition of many plant communities (Alonso et al. 2001; Bobbink et al. 1998; Britton et al. 2001; Marcos et al. 2003; Schmidt et al. 2004; Stevens et al. 2006; von Oheimb et al. 2010). Even low levels of chronic N inputs may have long-lasting impacts on ecosystems, as demonstrated by Clark and Tilman (2008) using the example of prairie grasslands. While airborne N loads have affected ecosystems in recent decades, impacts of climate change are predicted to increase in importance, particularly in the course of this century. The Intergovernmental Panel on Climate Change (IPCC 2007) predicts rising mean annual temperatures, changes in precipitation patterns and alterations of the frequency and magnitude of extreme weather events (e.g. summer drought). On the ecosystem level, these changes can alter primary productivity, carbon sequestration and nutrient cycling (Gorissen et al. 2004; Grime et al. 2000; Walther 2010). On the species level, shifts in climate may affect the performance and range of species, but also species interactions such as competition for light and nutrients (Andresen et al. 2010; Fotelli et al. 2005; Gorissen et al. 2004; Grime et al. 2000; Walther 2010).

Assessments and predictions of species responses, however, are often difficult due to largely unknown interrelations between simultaneously acting drivers of global change. It is conceivable, for example, that species responses to climate change could be both mitigated and strengthened by other global change drivers such as N deposition. Species and their environments, however, may face both climate shifts and increasing N loads in the course of this century (Baeten et al. 2010; Tylianakis et al. 2008). Results from grassland experiments have shown that the combination of different drivers can produce non-additive interrelated effects. Barnard et al. (2006) found that nitrifying enzyme activity in grassland soils was not affected by elevated CO_2 levels, but increased as a result of N addition. However, if both treatments were combined, the positive effects of N addition were no longer visible. Ritchie (2000) showed that thermal conditions and N availability interacted to influence herbivore abundance. In addition, positive effects of N loads on herbivore abundance were most pronounced under high rainfall conditions (Boyer et al. 2003). Since such interactions are sometimes unexpected and may remain undetected if single factors are analyzed in isolation (Betson et al. 2007), studies aiming to predict species responses to global change may be more meaningful when these factors are analyzed in combination, for example by means of full-factorial experimental approaches.

Shifts in water availability (e.g. due to summer droughts) and increasing N supply may have a particularly strong impact on plant growth and competition. Studies on the effects of summer droughts often found a reduction in plant growth and productivity (Baeten et al. 2010; Damgaard et al. 2009; Peñuelas et al. 2004; Shah and Paulsen 2003). N additions, by contrast, stimulated plant growth, but also increased the plants' shoot-root ratios (Aerts et al. 1991; Boot 1989; Ericsson 1995; Thornton 1991). The combination of both factors may implicate interactive effects on plant growth and competition: Biomass allocation to the shoots enables plants to compete more effectively for aboveground resources (Aerts and Chapin 2000; Goldberg 1990). Therefore, this strategy is beneficial to plants as long as the nutrient and water supply is sufficient. The risk of water shortage during drought events because of an increasing transpiring surface, however, may result in a trade-off in biomass allocation (Aerts and Bobbink 1999). Thus, improved N availability could strengthen adverse effects of drought when both factors act simultaneously (Betson et al. 2007).

In a study by Gordon et al. (1999), increasing N availability in combination with drought strengthened growth reductions (due to drought) of *Calluna vulgaris*, but not of *Pteridium aquilinum*. These results indicate that plant responses are species-specific, and the consideration of functional types may be helpful to detect general response patterns. MacGillivray et al. (1995) found that the plants' ability to tolerate nutrient deficiency correlated positively with the plants' resistance to extreme events such as frost or drought, but negatively with their resilience (i.e. speed of recovery). Hence, fast-growing species with high nutrient requirements and competitiveness tended to be more sensitive to drought, but may recover faster than plants characterized by a high nutrient stress tolerance. Such differences in drought sensitivity may mediate the competitive relationships between different functional plant types. Morecroft et al. (2004), for example, found a decrease in the percentage cover of perennial grasses as a result of drought in grassland ecosystems, while the cover of short-lived ruderal species increased.

For the prediction of potential changes in plant growth and competition as a result of environmental shifts it is, thus, crucial to understand and to quantify plant responses (in terms of their biomass and nutrient allocation) to combined effects of global change drivers (i.e. N deposition, increase of drought events) and in relation to functional types. In the present study, we investigated effects of simulated drought events and N deposition on the performance of a common European grass species in a full-factorial 2-year pot experiment. We selected *Molinia caerulea* (L.) Moench (henceforth referred to as *Molinia*) as a focal species for several reasons. Firstly, *Molinia* is a fast-growing perennial grass, which has considerably increased in frequency and cover in various European habitats of high conservation value, for example in wet and dry heaths, but also in mires and moorlands (Aerts et al. 1991; Brys et al. 2005; Chambers et al. 1999; Diemont and Heil 1984; Falk et al. 2010). Thus, the analysis of *Molinia* responses may contribute to an understanding of mechanisms underlying the ongoing process of *Molinia* encroachment under current and prospective environmental shifts. Secondly, our findings may help to characterize and predict response patterns with regard to global change typical of perennial grasses (as an important functional type) which show high competitive vigour and expansiveness. Since high N loads may cause a shortage of phosphorus (P) in primarily N limited systems (Härdtle et al. 2009; Verhoeven et al. 1996), a P-addition treatment was included in our experiment. Nutrient treatments were performed during two growing seasons, while the drought treatment started in the second year of the experiment. In this way, drought took effect on already fertilized plants, hence simulating possible impacts of drought events on systems that have already undergone atmospheric inputs of N (i.e. drought will affect plant growth in addition to the already existing N deposition). Growth responses were measured in terms of biomass allocation and tissue N and P concentrations. Our analyses were complemented by a ^{15}N tracer experiment (second year) in order to determine N allocation patterns resulting from treatments. We hypothesize that (i) N addition increases shoot-root ratios of *Molinia*, (ii) drought treatments reduce above- and belowground growth, and (iii) N addition increases the species' susceptibility to drought (expressed by decreasing productivity).

Materials and methods

Experimental design

Molinia caerulea (L.) Moench seeds were collected in the Lüneburger Heide nature reserve (NW Germany) and transferred to germination dishes in a non-tempered greenhouse in September 2007. Seedlings emerged in the middle of March 2008 and were transferred to plant pots ($12\times12\times12$ cm^3) that contained nutrient-poor sand (of 7 cm thickness) covered by a humus layer (of 4 cm thickness; the thickness of the humus and sand layers was chosen in order to mimic natural site conditions typical of heaths of NW Germany). The sand was taken from a local sand pit (i.e. C-material typical of podzols; soil ecological characteristics: pH$_{H2O}$: 7.1, base saturation: 100%, S-value: 4.8 mval 100 g^{-1}, N content: 100 mg kg^{-1}, P content: 95 mg kg^{-1}). The humus material was collected from the upper soil horizons of a podzol in the Lüneburger Heide nature reserve (soil ecological characteristics: pH$_{H2O}$: 3.8, base saturation: 21.5%, S-value: 1.7 mval 100 g^{-1}, N content: 800 mg kg^{-1}, P content: 147 mg kg^{-1}). 16 plant individuals were planted together in one pot (equidistantly in 4 rows and lines).

All pots were randomly assigned to a full-factorial combination of the following treatments: N addition, P addition, and drought (treatments are henceforth referred to as N, P, and D treatment, respectively). The following treatments were applied in the first year (2008): control, N, P and N+P (i.e. combination of N and P) using 30 pots per treatment. At the end of the 2008 growing season, 10 of the 30 pots per treatment were harvested (see description below). The following treatments were carried out in the second year (2009): control, N, P, N+P, D, N+D, P+D, N+P+D, using 10 pots respectively of the pots remaining from the corresponding treatments in 2008 (i.e. 10 pots per treatment and year). N-treated pots received 48 kg N ha^{-1} yr^{-1} (NH_4NO_3), P-treated pots received 4 kg P ha^{-1} yr^{-1} (Na_2HPO_4) and N+P-treated pots received a combination of both. Controls received deionised water only. Nutrient solutions were applied weekly from May through the end of August over two growing seasons. Pots were kept in a greenhouse and their arrangement was changed monthly at random. All pots were watered regularly to prevent water stress, with the exception of pots receiving drought treatments. Drought treatments were carried out three times during the growing season (first week of June, first week of July, last week of July). Each drought period started after the weekly fertilizer addition and lasted for 1 week until the next fertilizer addition. During the growing season, D-treated pots received approximately 18% less water than controls.

^{15}N tracer additions

^{15}N tracer additions were performed during the second growing season (i.e. 2009). ^{15}N tracer was added three times to all pots. The ^{15}N tracer addition was given at the June 2, June 30 and July 21, that is always 1 day before a drought period was initiated. Each pot received 0.1 mg $^{15}NH_4{}^{15}NO_3$ (99.22 atom%) dissolved in 70 ml deionized water. The total amount of 0.3 mg $^{15}NH_4{}^{15}NO_3$ was calculated to achieve a distinct ^{15}N tracer signal in the plant biomass, but was too small to cause a fertilization effect in non-N treated pots (Friedrich et al. 2011a).

Harvesting and chemical analyses

In 2008, 10 pots (of the 30 pots per treatment) were harvested at the beginning of September (when plants were still in a fresh and green status). A second harvest (of all remaining pots) took place after the second growing season in 2009. For both years we quantified aboveground and belowground biomass production and related biomass allocation patterns. We separated the aboveground biomass (leaves and flower stalks) into (i) living tissue, (ii) dead tissue, and (iii) basal internodes. In addition, we determined the shoot-root ratio, N and P biomass concentrations and the ^{15}N tracer recovery to assess ^{15}N allocation patterns (in 2009).

After harvest, biomass samples were dried at 80°C for 12 h and weighed. Subsequently, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany) or ground with a mixer mill (MM 400, Retsch, Haan, Germany), and re-dried at 105°C before weighing. N and ^{15}N concentrations were analyzed using a continuous flow elemental analyzer-isotopic ratio mass spectrometer (vario El cube, Elementar, Hanau, Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK). For P determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550°C for 3 h. Ash samples were dissolved in an HCl solution, which was evaporated to dryness. Residues were again dissolved in an HCl solution (Schlichting et al. 1995) and analysed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Calculation of ^{15}N recovery and ^{15}N allocation patterns

^{15}N tracer recovery in the plant parts (living and dead aboveground biomass, basal internodes and belowground biomass) was calculated as follows:

$$^{15}N_{rec} = N_{tot}{}^{*}\left(\text{atom\%}^{15}N_{labeled} - \text{atom\%}^{15}N_{ref}\right) / \left(\text{atom\%}^{15}N_{tracer} - \text{atom\%}^{15}N_{ref}\right),$$

where $^{15}N_{rec}$ is the mass of ^{15}N tracer recovered in the plant tissue of a given plant part of labeled plants (mg N per plant), N_{tot} is the total mass of N in the plant tissue of labeled plants (mg N per plant), atom%$^{15}N_{labeled}$ is the atom%^{15}N in the plant tissue of labeled plants, atom%$^{15}N_{ref}$ is the atom%^{15}N in the plant tissue of non-labeled plants, and atom%$^{15}N_{tracer}$ is the atom%^{15}N of the added ^{15}N tracer (modified equation according to Nadelhoffer et al. (2004)). We used atom%^{15}N of biomass samples from non-labeled, but N- and P-treated plants harvested in the first year as atom%$^{15}N_{ref}$. In this way, we accounted for differences in natural ^{15}N abundances caused by N additions during the first year of the study. ^{15}N allocation to a given plant part was calculated as the percentage of ^{15}N recovered in the plant part relative to the total amount of ^{15}N recovered in the total plant.

Data evaluation and statistics

Treatment effects on biomass dry weights (dw) of the different plant parts, shoot-root ratios, tissue N and P concentrations and ^{15}N allocation were compared by means of one-way ANOVA with a Tukey's post hoc test ($P<0.05$). Interrelated treatment effects (i.e. type of fertilization, drought) were tested using Generalized Linear Models (GLM). Each GLM included the main effects of N, P and drought as well as the first order interaction terms (N x P, N x D, P x D).

Analyses were conducted with SPSS 17.0 (SPSS Inc., Chicago, IL).

Data evaluation for the first growing season was restricted to an analysis of shoot-root ratios as affected by treatments (using living plant material). All data in Figures and Tables are given on a per plant basis (with the exception of Figures referring to ^{15}N allocation (%) and shoot-root ratios). In order to evaluate nutrient limitation of plant growth, tissue nutrient concentrations were plotted as a function of nutrient contents following the approach of Timmer and Stone (1978, cf. Fig. 4). To this end, the total aboveground biomass per plant was multiplied by the tissue nutrient concentration (biomass means per plant) to calculate the total nutrient contents per plant. Trajectories of shifts in nutrient concentration and nutrient content as a result of fertilization were plotted in a nutrient content-nutrient concentration space which is compartmentalized by an upward and a horizontal line. The upward line is determined by the respective data point obtained from the control treatment and by the origin of the coordinate system. The horizontal line is determined by the respective data point obtained from control treatment and runs parallel to the x-axis. The trajectories indicate whether an increase in nutrient contents occurred because a nutrient was limiting (a shift into the sector between the upward and the horizontal line, i.e. increases in both nutrient concentrations and biomass) or due to luxury consumption (shifts of values along the upward line, i.e. increased nutrient concentrations without any gain in biomass).

Results

Treatment effects on biomass production and allocation

Aboveground biomass of *Molinia* increased by a factor of 5 and 6 as a result of N and N+P fertilization, respectively (Fig. 1a). This increase was apparent for all plant parts, of which internodes showed the highest increase (seven- and six-fold in the N and N+P treatment, respectively; calculated from data in Table 1). P treatments had no effect on the aboveground biomass (Fig. 1a), but N x P interactions were significant for internodes and dead aboveground biomass (Table 1). D treatment alone slightly, but significantly reduced the aboveground biomass (Fig. 1a), but this reduction was insignificant if single plant parts are considered (i.e. internodes, living and dead aboveground biomass; significances for the respective plant parts not shown in Fig. 1). By contrast, drought in combination with N strongly reduced both total and living aboveground biomass in comparison to the N treatment (living biomass was 59 mg (dw) and 123 mg (dw) in the N+D and N treatment, respectively; Table 1, Fig. 1a). Correspondingly, dead aboveground biomass increased by a factor of 6 and 10 in N+D and N+P+D pots, respectively, in comparison to controls. N x D interactions were significant for all aboveground plant parts (Table 1).

Treatments had similar effects on the belowground biomass. This is reflected in a sixfold and sevenfold biomass increase resulting from N and N+P fertilization, respectively (Fig. 1b). D treatments had no

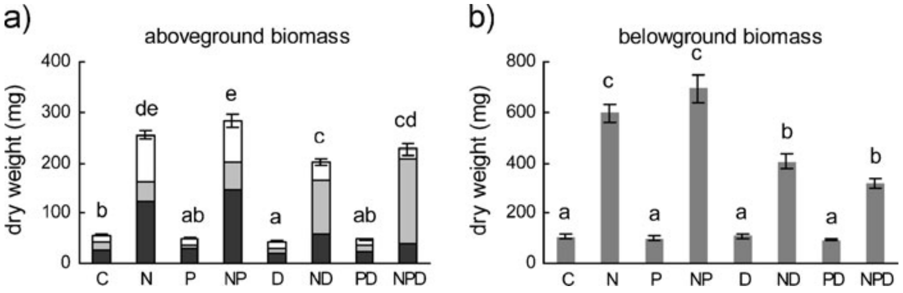

Fig. 1 Effects of fertilization and drought treatments on biomass dry weights (in mg; for abbreviations see legend of Table 1). Data refer to plant individuals and show means and standard errors (*error bars*) of aboveground biomass (**a**) and belowground biomass (**b**) harvested in 2009. Aboveground biomass is separated into living biomass (*black*), dead biomass (*grey*) and basal internodes (*white*). Different letters indicate significant differences found for the total aboveground biomass according to one-way ANOVA ($P<0.05$)

Table 1 Means and standard errors (in parentheses) of the plant parts analyzed (above alive: living aboveground biomass, above dead: dead aboveground biomass, internodes: biomass of basal internodes, below: belowground biomass; all in mg dry weight per plant (dw)), shoot-root ratios, tissue N and P concentrations (in mg g^{-1} dw) and ^{15}N allocation (in % of total recovered ^{15}N) in relation to treatments (C: control; N: nitrogen; P: phosphorus; N+P: nitrogen and phosphorus; D: drought; N+D: nitrogen and drought; P+D: phosphorus and drought; N+P+D: nitrogen, phosphorus and drought). Results of the GLM analyses with interactions of nitrogen (N), phosphorus (P) and drought (D) are given where significant: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

	C	N	P	N+P	D	N+D	P+D	N+P+D	Interactions
Biomass (mg dw)									
Above alive	25.5	123.2	27.7	147.5	20.2	59.0	22.2	38.6	N x D*** P x D*
	(0.8)	(4.5)	(1.4)	(4.3)	(1.2)	(9.6)	(0.9)	(9.8)	
Above dead	17.0	41.0	9.5	55.5	10.0	107.5	12.6	170.7	N x P*** N x D*** P x D***
	(1.0)	(2.1)	(0.9)	(2.7)	(0.6)	(13.5)	(0.7)	(6.3)	
Internodes	13.9	91.0	13.0	81.3	12.8	34.9	12.6	16.9	N x P* N x D***
	(0.6)	(3.2)	(0.6)	(4.0)	(0.7)	(7.5)	(0.5)	(6.1)	
Below	107.2	596.5	100.8	693.8	108.7	404.7	94.0	317.1	N x D***
	(8.9)	(37.5)	(7.6)	(55.8)	(9.1)	(29.7)	(4.2)	(21.2)	
Shoot-root ratio	0.4	0.4	0.4	0.3	0.3	0.2	0.4	0.2	N x P* N x D**
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	
N concentration (mg g^{-1}dw)									
Above alive	10.9	12.7	11.8	10.9	12.4	18.0	12.0	21.5	N x D***
	(0.2)	(0.5)	(0.3)	(0.3)	(0.3)	(1.5)	(0.3)	(1.5)	
Above dead	3.7	3.1	3.6	3.8	3.4	7.4	3.8	9.1	N x P* N x D***
	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.7)	(0.1)	(0.6)	
Internodes	3.7	4.8	4.0	5.0	4.5	8.4	5.5	11.7	N x D*
	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.8)	(0.3)	(1.4)	
Below	4.3	5.3	5.0	5.2	4.8	5.9	5.1	6.3	
	(0.3)	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.4)	
P concentration (mg g^{-1} dw)									
Above alive	1.2	0.5	1.6	0.7	1.3	1.0	1.8	2.2	N x D*** P x D*
	(0.1)	(0.0)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.3)	
Above dead	0.5	0.1	0.7	0.2	0.4	0.3	0.8	0.5	N x D**
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.1)	
Internodes	1.3	0.4	1.5	0.7	1.3	1.0	1.6	2.3	N x P* N x D***
	(0.1)	(0.0)	(0.1)	(0.0)	(0.1)	(0.2)	(0.1)	(0.3)	P x D**
Below	0.9	0.3	1.1	0.5	1.0	0.3	1.3	0.5	
	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	
^{15}N allocation (%)									
Above alive	35.1	28.0	41.2	25.6	31.1	24.7	30.6	21.3	N x P* N x D***
	(1.8)	(1.1)	(1.5)	(1.0)	(0.9)	(2.6)	(0.8)	(3.5)	
Above dead	3.2	1.4	1.7	2.4	1.0	11.7	2.9	22.9	N x P* N x D***
	(0.3)	(0.2)	(0.2)	(0.2)	(0.1)	(2.8)	(0.3)	(2.9)	P x D**
Internodes	7.4	9.5	7.7	8.7	8.2	6.4	9.9	3.7	N x P** N x D***
	(0.4)	(0.2)	(0.4)	(0.7)	(0.4)	(0.9)	(0.5)	(0.8)	
Below	54.3	61.1	49.5	62.9	59.8	57.3	55.8	52.1	N x D***
	(2.4)	(0.8)	(1.6)	(1.3)	(1.0)	(2.4)	(0.6)	(2.9)	

significant effects on belowground biomass, and fertilization effects were reduced under drought (fourfold and threefold increase in the N+D and N+P+D treatments, respectively). Accordingly, N x D interactions were significant (Fig. 1b, Table 1). P addition had no effect on belowground biomass.

During the first growing season (2008), shoot-root ratios doubled as a result of N and N+P addition, but were unaffected by P (Fig. 2a). In 2009, shoot-root ratios decreased (in comparison to 2008), and differences caused by treatments in 2008 were balanced. Drought reduced shoot-root ratios in combination with N (i.e. N+D and N+P+D treatments; Fig. 2b), and this was mirrored by significant N x D interactions (Table 1).

Treatment effects on nutrient concentrations (2008 and 2009)

N fertilization increased N concentrations in combination with D treatments (i.e. N+D and N+P+D), but not in the N and N+P treatments (with the exception of internodes; Fig. 3). Increases were significant for all plant parts, but were highest for the internodes (two- and three-fold increase in the N+D and N+P+D treatment, respectively; Fig. 3c). Combined effects of N and D treatments resulted in significant N x D interactions (Table 1). P fertilization had no effect on N concentrations.

P concentrations increased for all plant parts in the P and P+D treatments, but declined in the N and N+P treatments in comparison to controls (Table 1). In the N+P+D treatment, P concentrations increased in the living aboveground tissue and in the internodes, but decreased in the belowground biomass.

N accumulation was associated with increasing biomass in N and N+P treatments, while N concentrations increased only slightly (Fig. 4a). By contrast, P fertilization increased P concentrations, but biomass increased only when N was also added (Fig. 4b). In D-treated pots N fertilization caused an increase in both biomass and N concentrations (Fig. 4c). P fertilization combined with drought increased P concentrations, but did not affect biomass production (Fig. 4d). In summary, the nutrient content – concentration analyses showed that drought in combination with fertilization caused higher tissue nutrient concentrations and lower biomass increases.

Treatment effects on ^{15}N allocation (2009)

Treatments clearly affected ^{15}N allocation patterns to the different plant parts (Fig. 5), in which a mean 25% of added tracer was recovered.

^{15}N allocation to the living aboveground biomass (Fig. 5a) was reduced in the N and N+P treatment, but increased in the P treatment relative to the control (in the latter, 35% of recovered tracer was found). In contrast, D treatment resulted in insignificant differences in allocation patterns when combined with fertilization (i.e. N+D, P+D, N+P+D).

In the dead aboveground biomass (Fig. 5b), ^{15}N sequestration was particularly high in the N+D and N+P+D treatments, but differences were insignificant for all the other treatments.

In the internodes (Fig. 5c), ^{15}N sequestration did not differ for the N, P, and N+P treatments, but significantly decreased when N and D treatments were combined (i.e. N+D, N+P+D).

^{15}N allocation to the belowground biomass (Fig. 5d) was higher than for other plant parts (relative allocation of ^{15}N was >50%). ^{15}N sequestration was highest in the N and N+P treatment, but differences were insignificant for the D treatment in combination with fertilization (i.e. N+D, P+D, N+P+D).

N x D interactions were significant for all plant parts (Table 1). The same applied to N x P

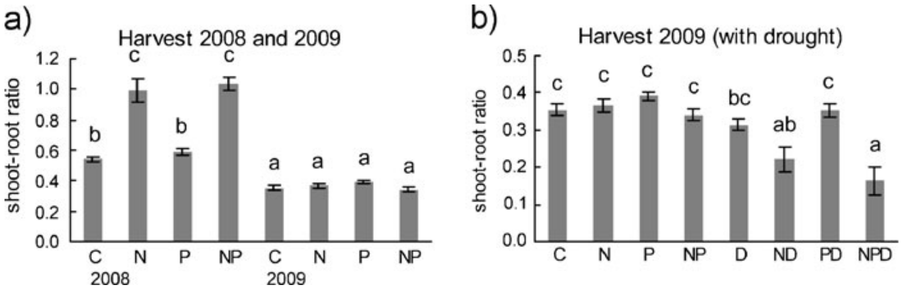

Fig. 2 Means and standard errors (*error bars*) of shoot-root ratios of biomass harvests after fertilization treatments in 2008 and 2009 (**a**), and biomass harvest after the combination of fertilization and drought treatments in 2009 (**b**); for abbreviations see legend of Table 1. Different letters indicate significant differences ($P<0.05$) according to one-way ANOVA

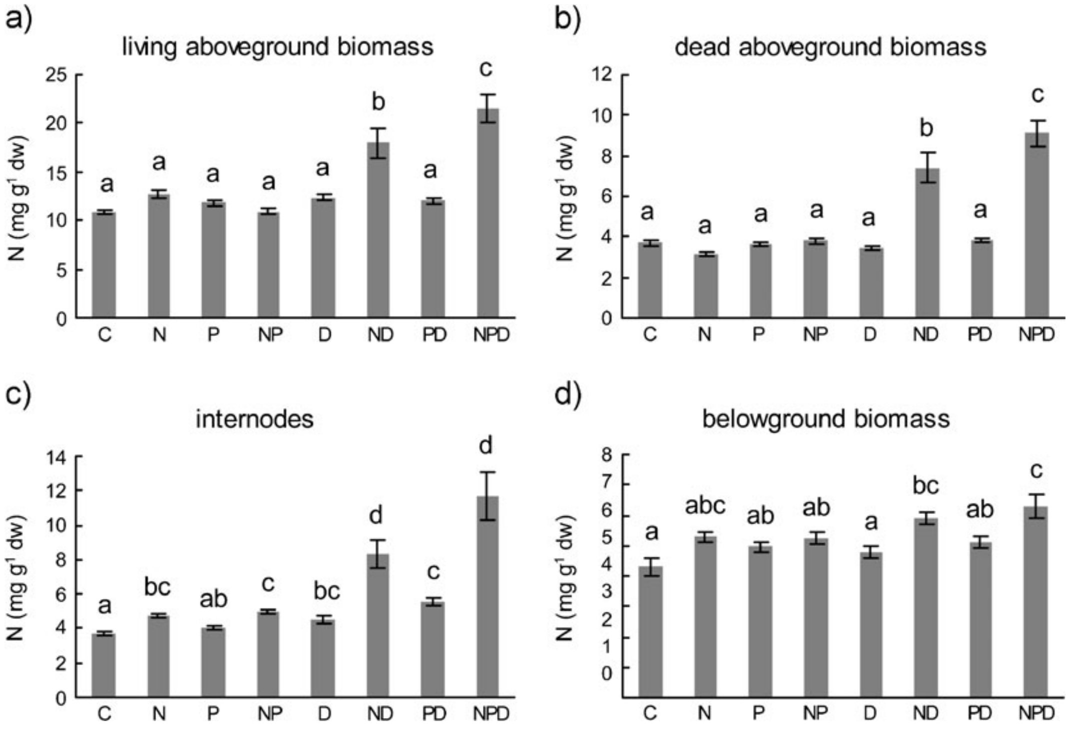

Fig. 3 Effects of fertilization and drought treatments on tissue N concentrations (in mg N g^{-1} dw). Data refer to plant individuals and show means and standard errors (*error bars*) of: living aboveground biomass (**a**), dead aboveground biomass (**b**), basal internodes (**c**), and belowground biomass (**d**); for abbreviations see legend of Table 1. Different letters indicate significant differences ($P<0.05$) according to one-way ANOVA

interactions, with the exception of ^{15}N allocation to belowground biomass.

Discussion

Treatment effects on biomass production and allocation

Biomass of *Molinia* increased due to N fertilization, accompanied by increasing shoot-root ratios in the first, but not in the second growing season (Figs. 1 and 2). Higher belowground allocation in the second year should have compensated for differences in shoot-root ratios found in the first year. We hypothesize that increasing belowground allocation in the second year was attributable to an age but not to a fertilization effect (Müller et al. 2000). This interpretation is supported by the finding that shoot-root ratios declined across treatments in 2009, and thus also in the controls, indicating a process independent of nutrient availability.

In contrast to N fertilization, D treatment (without fertilization) had only a slight effect on total productivity, and effects were insignificant for the respective plant parts (such as living aboveground biomass, internodes, and belowground biomass; Fig. 1). This indicates that the experimentally imposed drought was not enough to cause a strong growth decline. Response patterns of *Molinia* to drought in our experiment coincide with the species' performance within a wide range of (partly artificial) habitats with strong gradients in water supply, a finding attributable to the species' morphological and physiological plasticity (Aerts et al. 1991; Salim et al. 1988; Taylor et al. 2001). In addition, *Molinia* is characterized by comparatively low shoot-root ratios (about 0.5 in unfertilized environments; Aerts et al. 1991; Fig. 2) that may support the species' ability to satisfy its water demands even during periods of drought (Aerts and Chapin 2000; Goldberg 1990). However, if N additions have positive, and D treatments only slight (or even insignificant) effects on biomass production,

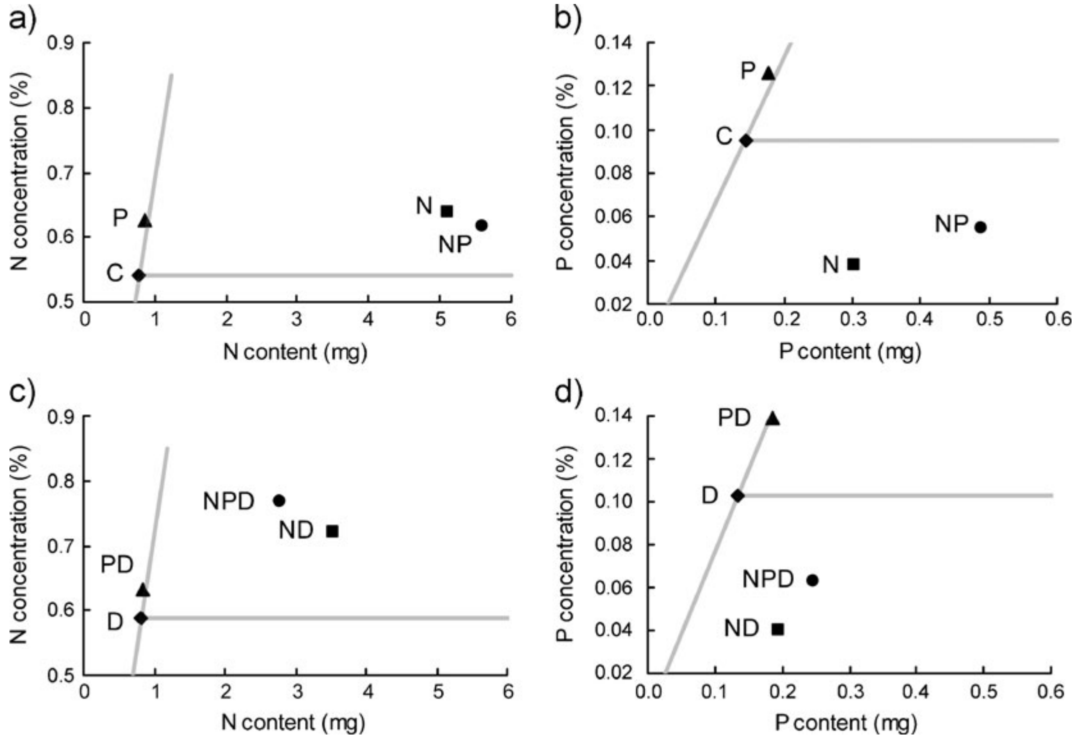

Fig. 4 Nutrient concentrations (%) vs. nutrient contents (mg) of total biomass per plant individual for nitrogen (N) and phosphorus (P) in regularly watered (**a**: N; **b**: P) and drought (D) treated pots (**c**: N; d: P). Shifts of values along the *upward line* indicate increased nutrient accumulation without gains in biomass (luxury consumption), while shifts of values along the *horizontal line* indicate increased nutrient contents and biomass without changes in concentrations. A shift into the sector between both lines denotes increases in both nutrient concentrations and biomass, indicating that the initial nutrient level was growth limiting. A shift into the sector below the *horizontal line* indicates that the nutrient concentration has been diluted due to additional growth

then a decrease in productivity (compared to N treatments) accompanied by a distinct increase of necrotic biomass (six- to ten-fold; Fig. 1a) is at first an unexpected response to the combination of these treatments, and is indicative of non-additive interrelated effects (Barnard et al. 2006; Fig. 1). We hypothezise that growth responses to combined effects of N and D were the result of water shortage attributable to the higher aboveground productivity (following N fertilization) and thus increased water requirements of plants due to higher transpiration rates (van Heerwaarden et al. 2005). Thus, if N fertilization takes place in combination with drought, *Molinia* is unable to meet its evaporative demands, thus ultimately leading to leaf wilting and the formation of necrotic tissue (Brooks and Coulombe 2009; Gordon et al. 1999; Nilsen 1995). This process should affect N-limited plants in particular, since N inputs may then cause a disproportionate increase in leaf biomass, and hence an increased probability of water stress (Högberg et al. 1993). As a consequence, N fertilization may strengthen a plant's susceptibility to periods of drought in N-limited environments. This result is in agreement with the observations of Betson et al. (2007), who found an increasing susceptibility of *Pinus sylvestris* to drought events in forests exposed to moderate to high N deposition (using the trees' δ^{13}C foliage signature as a response variable). However, our results are inconsistent with those of Saneoka et al. (2004), who found that higher levels of N nutrition increased the drought tolerance of the grass *Agrostis palustris*.

In contrast to the morphological and physiological plasticity with which *Molinia* responds to environmental gradients (Thornton 1991), N-fertilized plants exhibited no adaptive strategy to D treatments in our experiment, for example by increased belowground productivity (Asseng et al. 1998; Fotelli et al. 2002),

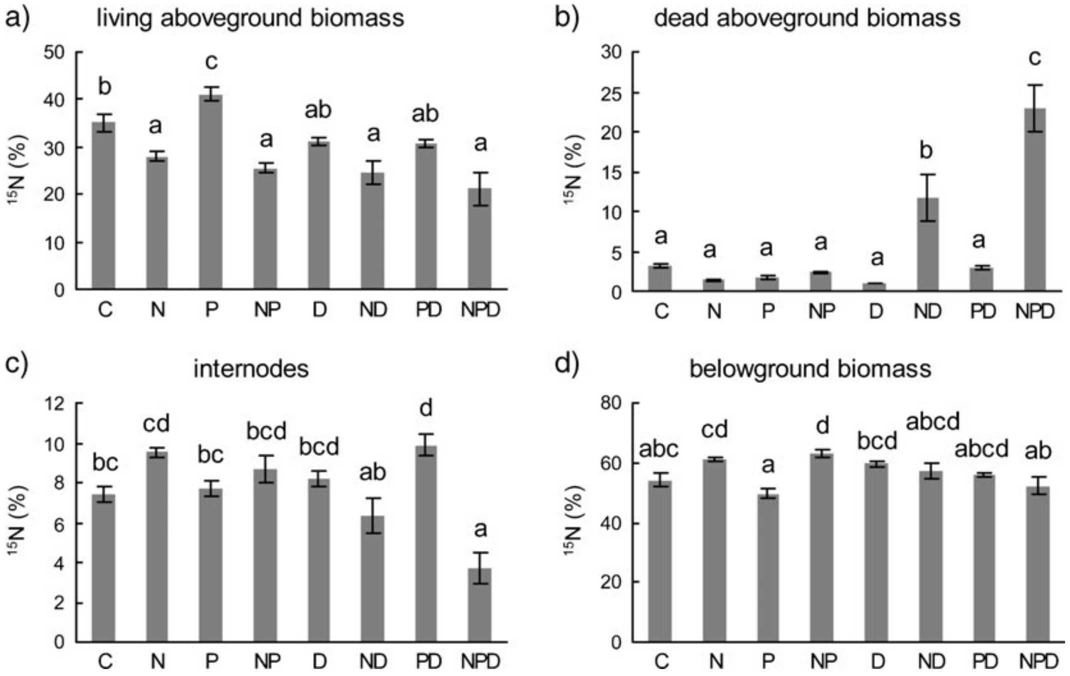

Fig. 5 Effects of fertilization and drought treatments on ^{15}N allocation (in % of total recovered ^{15}N per plant). Data show means and standard errors (*error bars*) of: living aboveground biomass (**a**), dead aboveground biomass (**b**), basal internodes (**c**), and belowground biomass (**d**); for abbreviations see legend of Table 1. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA

decreasing shoot-root ratios (Gonzalez-Dugo et al. 2010; Kahmen et al. 2005; Lösch 2001), or "compensation growth" that may counterbalance losses of photosynthetic active tissue (Ericsson 1995; Xu et al. 2009). This result may be partly ascribed to our experimental design, in which plants were fertilized in the first, and exposed to both fertilization and drought in the second year. Since *Molinia* is characterized by a highly efficient N (re)cycling and storage strategy (see discussion below), a high proportion of N stored in roots and internodes (in 2008) may have contributed to the fast production of aboveground tissue in spring 2009 (Thornton 1991), even before the first D treatment was applied. As a consequence, fertilized plants showed increased evaporative demands (compared to controls) and thus higher drought susceptibility, despite their higher belowground allocation in the second year. This interpretation in turn supports the hypothesis that N fertilization of (primarily) N-limited plants may foster their productivity in the early growing season and thereby their water requirements in summer, increasing the probability of water shortage during drought events (Högberg et al. 1993).

Treatment-related nutrient concentrations and ^{15}N allocation

Both patterns of productivity and nutrient concentrations indicated that growth of *Molinia* was limited by N, a finding that is in agreement with studies by Aerts (1990), Thornton (1991), and Falk et al. (2010; Figs. 1, 3 and 4). This is reflected in insignificant differences in tissue N concentrations across treatments (Fig. 3; with the exception of N+D and N+P+D), since sequestered N contributed to a significant increase in productivity (Fig. 4). P, in turn, had no effect on productivity and thus was not a growth-limiting nutrient (Fig. 4). D treatments, by contrast, caused a lowered productivity of N-fertilized plants (i.e. N+D and N+P+D treatments). This is reflected in the higher tissue N concentrations (Figs. 3a, c), since N was partly accumulated in leaves and internodes due to the reduced biomass. We hypothesize that two mechanisms may have contributed to reduced productivity in N+D and N+P+D treatments. Firstly, plants closed their stomata and hence decreased their photosynthetic rate during the experimentally imposed drought (Gonzalez-Dugo et al. 2010; Lösch 2001; Shah and Paulsen 2003). Second, a high proportion of necrotic tissue may have weakened the growth vigour of plants due to a shortage of photosynthates, which in turn may appear when shoot-root ratios of about 0.2 are achieved (see Fig. 2c; Chapin et al. 1987; Gordon et al. 1999; Shah and Paulsen 2003; Ward et al. 1999).

A striking finding, however, was that dead aboveground biomass showed the highest values for N and ^{15}N (Figs. 3b, 5b). This indicates that *Molinia* was unable to withdraw N during the drought-induced dieback of aboveground tissue. This is in contrast to experimental findings, which showed *Molinia* to have a highly efficient N-resorption strategy which, for example, enables plants to withdraw about 85% of N from senescing leaves (van Heerwaarden et al. 2005). Obviously, the formation of necrotic tissue occurred too fast to allow for an efficient resorption of leaf N. Thus, the combined effects of fertilization and drought disenabled *Molinia*'s control of its N cycling and allocation. This interpretation is supported by the result that ^{15}N allocation patterns to aboveground and belowground biomass differed significantly between fertilization treatments and controls, but differences were insignificant when fertilization and drought were applied simultaneously (i.e. no "controlled" allocation; Fig. 5a, d). This response pattern was unexpected, since *Molinia* is known for its high (e.g. morphological) adaptability to environmental gradients and shifts (Aerts et al. 1991; Thornton 1991). Our results, by contrast, indicate that *Molinia* lacks an adaptive strategy to environmental conditions in which drought events follow fertilization. It is, thus, conceivable that climate shifts (such as increasing summer drought) in combination with airborne N loads may weaken the competitive performance of this grass species in its current habitats. Our findings support the hypothesis of MacGillivray et al. (1995) that plant traits which promote high productivity (in the case of *Molinia* the usage of stored and recycled N for accelerated productivity) lead to a correlated susceptibility to extreme events (such as drought). This in turn may cause shifts in the species composition of affected environments (Morecroft et al. (2004).

In this context it is important to note that the establishment success of seedlings of *Calluna* and *Molinia* (particularly during the rejuvenation phase of a heath) may determine the course of a heath's vegetation development in the medium term (Friedrich et al. 2011b). This may demonstrate the importance of competition experiments (e.g. by means of pot experiments) with *Molinia* and *Calluna* seedlings under different trophic and water conditions.

In conclusion the results of the present study demonstrate that combinations of N fertilization and drought may result in strongly interrelated, non-additive effects on plant growth. Results of treatment-related biomass and nutrient allocation patterns show that *Molinia* suffered severely from environmental conditions in which drought events followed N fertilization. Thus, despite its adaptability to a wide range of environmental conditions, the species lacks an adaptive strategy to combined effects of fertilization and drought. As a consequence, increasing summer drought (as currently predicted by climatic models) may weaken the species' competitiveness in N-fertilized environments (e.g. due to airborne N inputs). This may also apply to other perennial, fast-growing plant species with traits similar to those of *Molinia* such as the mobilization of stored N for a rapid increase in aboveground biomass during the early growing season.

References

Aerts R (1990) Nutrient use efficiency in evergreen and deciduous species from heathlands. Oecologia 84:391–397

Aerts R, Bobbink R (1999) The impact of atmospheric nitrogen deposition on vegetation processes in terrestrial, non-forest ecosystems. In: Langan SJ (ed) The impact of nitrogen deposition on natural and semi-natural ecosystems. Kluwer, Dordrecht, pp 85–122

Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67

Aerts R, Boot RGA, van der Aart PJM (1991) The relation between above- and belowground biomass allocation patterns and competitive ability. Oecologia 87:551–559

Alonso I, Hartley SE, Thurlow M (2001) Competition between heather and grasses on Scottish moorlands: interacting effects of nutrient enrichment and grazing regime. J Veg Sci 12:249–260

Andresen LC, Michelsen A, Jonasson S, Schmidt IK, Mikkelsen TN, Ambus P, Beier C (2010) Plant nutrient mobilization in temperate heathland responds to elevated CO_2, temperature and drought. Plant Soil 328:381–396

Asseng S, Ritchie JT, Smucker AJM, Robertson MJ (1998) Root growth and water uptake during water deficit and recovering in wheat. Plant Soil 201:265–273

Baeten L, De Frenne P, Verheyen K, Graae BJ, Hermy M (2010) Forest herbs in the face of global change: a single-species-multiple-threats approach for *Anemone nemorosa*. Plant Ecol Evol 143:19–30

Barnard R, Le Roux X, Hungate BA, Cleland EE, Blankinship JC, Barthes L, Leadley PW (2006) Several components of global change alter nitrifying and denitrifying activities in an annual grassland. Funct Ecol 20:557–564

Betson NR, Johannisson C, Löfvenius MO, Grip H, Granström A, Högberg P (2007) Variation in the $\delta^{13}C$ of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of a 32-year chronology. Glob Change Biol 13:2317–2328

Bobbink R, Hornung M, Roelofs JGM (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. J Ecol 86:717–738

Boot RGA (1989) The significance of size and morphology of root systems for nutrient acquisition and competition. In: Lambers H (ed) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic Publishing, The Hague, pp 299–311

Boyer AG, Swearingen RE, Blaha MA, Fortson CT, Gremillion SK, Osborn KA, Moran MD (2003) Seasonal variation in top-down and bottom-up processes in a grassland arthropod community. Oecologia 136:309–316

Britton AJ, Pakeman RJ, Carey PD, Marrs RH (2001) Impacts of climate, management and nitrogen deposition on the dynamics of lowland heathland. J Veg Sci 12:797–806

Brooks JR, Coulombe R (2009) Physiological responses to fertilization recorded in tree rings: isotopic lessons from a long-term fertilization trial. Ecol Appl 19:1044–1060

Brys R, Jacquemyn H, De Blust G (2005) Fire increases aboveground biomass, seed production and recruitment success of *Molinia caerulea* in dry heathland. Acta Oecol-Int J Ecol 28:299–305

Chambers FM, Mauquoy D, Todd PA (1999) Recent rise to dominance of *Molinia caerulea* in environmentally sensitive areas: new perspectives from palaeoecological data. J Appl Ecol 36:719–733

Chapin FS III, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. Bioscience 37:49–57

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712–715

Damgaard C, Riis-Nielsen T, Schmidt IK (2009) Estimating plant competition coefficients and predicting community dynamics from non-destructive pin-point data: a case study with *Calluna vulgaris* and *Deschampsia flexuosa*. Plant Ecol 201:687–697

Diemont WH, Heil GW (1984) Some long-term observations on cyclical and seral processes in Dutch heathlands. Biol Conserv 30:283–290

Ericsson T (1995) Growth and shoot: root ratio of seedlings in relation to nutrient availability. Plant Soil 168–169:205–214

Falk K, Friedrich U, von Oheimb G, Mischke K, Merkle K, Meyer H, Härdtle W (2010) *Molinia caerulea* responses to N and P fertilisation in a dry heathland ecosystem (NW-Germany). Plant Ecol 209:47–56

Fotelli MN, Rennenberg H, Geßler A (2002) Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ^{15}N uptake and partitioning, responses of amino acids and other N compounds. Plant Biol 4:311–320

Fotelli MN, Rudolph P, Rennenberg H, Geßler A (2005) Irradiance and temperature affect the competitive interference of blackberry on the physiology of European beech seedlings. New Phytol 165:453–462

Friedrich U, Falk K, Bahlmann E, Marquardt T, Meyer H, Niemeyer T, Schemmel S, von Oheimb G, Härdtle W (2011a) Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study. Glob Change Biol 17:1549–1559

Friedrich U, von Oheimb G, Dziedek C, Kriebitzsch WU, Selbmann K, Härdtle W (2011b) Mechanisms of *Molinia caerulea* encroachment in dry heathland ecosystems with chronic nitrogen inputs. Environ Pollut. doi:10.1016/j.envpol.2011.08.010

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Goldberg DE (1990) Components of resource competition in plant communities. In: Grace JB, Tilman D (eds) Perspectives on plant competition. Academic, San Diego, pp 27–49

Gonzalez-Dugo V, Durand J-L, Gastal F (2010) Water deficit and nitrogen nutrition of crops. A review. Agron Sustain Dev 30:529–544

Gordon C, Woodin SJ, Alexander IJ, Mullins CE (1999) Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytol 142:243–258

Gorissen A, Tietema A, Joosten NN, Estiarte M, Penuelas J, Sowerby A, Emmett BA, Beier C (2004) Climate change affects carbon allocation to the soil in shrublands. Ecosystems 7:650–661

Grime JP, Brown VK, Thompson K, Masters GJ, Hillier SH, Clarke IP, Askew AP, Corker D, Kielty JP (2000) The response of two contrasting limestone grasslands to simulated climate change. Science 289:762–765

Härdtle W, von Oheimb G, Gerke AK, Niemeyer M, Niemeyer T, Assmann T, Drees C, Matern A, Meyer H (2009) Shifts in N and P budgets of heathland ecosystems: effects of management and atmospheric inputs. Ecosystems 12:298–310

Högberg P, Johannisson C, Hällgren J-E (1993) Studies of ^{13}C in the foliage reveal interactions between nutrients and water in forest fertilization experiments. Plant Soil 152:207–214

IPCC (2007) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Kahmen A, Perner J, Buchmann N (2005) Diversity-dependent productivity in semi-natural grasslands following climate perturbations. Funct Ecol 19:594–601

Lösch R (2001) Wasserhaushalt der Pflanzen. Quelle & Meyer, Wiebelsheim

MacGillivray CW, Grime JP, Band SR, Booth RE, Campbell B, Hendry GAF, Hillier SH, Hodgson JG, Hunt R, Jalili A, Mackey JML, Mowforth MA, Neal AM, Reader R, Rorison IH, Spencer RE, Thompson K, Thorpe PC (1995) Testing predictions of the resistance and resilience of vegetation subjected to extreme events. Funct Ecol 9:640–649

Marcos E, Calvo L, Luis-Calabuig E (2003) Effects of fertilization and cutting on the chemical composition of vegetation and soils of mountain heathlands in Spain. J Veg Sci 14:417–424

Morecroft MD, Masters GJ, Brown VK, Clarke IP, Taylor ME, Whitehouse AT (2004) Changing precipitation patterns alter plant community dynamics and succession in an ex-arable grassland. Funct Ecol 18:648–655

Müller I, Schmid B, Weiner J (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspect Plant Ecol Evol Syst 3:115–127

Nadelhoffer KJ, Colman BP, Currie WS, Magill A, Aber JD (2004) Decadal-scale fates of ^{15}N tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). For Ecol Manage 196:89–107

Nilsen P (1995) Effect of nitrogen on drought strain and nutrient uptake in Norway spruce *Picea abies* (L.) Karst.) trees. Plant Soil 172:73–85

Peñuelas J, Gordon C, Llorens L, Nielsen T, Tietema A, Beier C, Bruna P, Emmett B, Estiarte M, Gorissen A (2004) Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north-south European gradient. Ecosystems 7:598–612

Ritchie ME (2000) Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. Ecology 81:1601–1612

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Biodiversity-global biodiversity scenarios for the year 2100. Science 287:1770–1774

Salim KA, Carter PL, Shaw S, Smith CA (1988) Leaf abscission zones in *Molinia caerulea* (L.) Moench, the purple moor grass. Ann Bot 62:429–434

Saneoka H, Moghaieb REA, Premachandra GS, Fujita K (2004) Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris* Huds. Environ Exp Bot 52:131–138

Schlichting E, Blume H-P, Stahr K (1995) Bodenkundliches Praktikum: Eine Einführung in pedologisches Arbeiten für Ökologen, insbesondere Land- und Forstwirte, und für Geowissenschaftler, 2nd edn. Blackwell, Berlin

Schmidt IK, Tietema A, Williams D, Gundersen P, Beier C, Emmett BA, Estiarte M (2004) Soil solution chemistry and element fluxes in three European heathlands and their responses to warming and drought. Ecosystems 7:638–649

Shah NH, Paulsen GM (2003) Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil 257:219–226

Stevens CJ, Dise NB, Gowing DJG, Mountford JO (2006) Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Glob Change Biol 12:1823–1833

Taylor K, Rowland AP, Jones HE (2001) *Molinia caerulea* (L.) Moench. J Ecol 89:126–144

Thornton B (1991) Effect of nutrition on the short-term response of *Molinia caerulea* to defoliation. Ann Bot 68:569–576

Timmer VR, Stone EL (1978) Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Soil Sci Soc Am J 42:125–130

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. Ecol Lett 11:1351–1363

van Heerwaarden LM, Toet S, van Logtestijn RSP, Aerts R (2005) Internal nitrogen dynamics in the graminoid *Molinia caerulea* under higher N supply and elevated CO_2 concentrations. Plant Soil 277:255–264

Verhoeven JTA, Koerselman W, Meuleman AFM (1996) Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends Ecol Evol 11:494–497

von Oheimb G, Power SA, Falk K, Friedrich U, Mohamed A, Krug A, Boschatzke N, Härdtle W (2010) N:P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands. Ecosystems 13:317–327

Walther GR (2010) Community and ecosystem responses to recent climate change. Philos Trans R Soc B 365:2019–2024

Ward JK, Tissue DT, Thomas RB, Strain BR (1999) Comparative responses of model C3 and C4 plants to drought in low and elevated CO_2. Glob Change Biol 5:857–867

Xu Z, Zhou G, Shimizu H (2009) Are plant growth and photosynthesis limited by pre-drought following rewatering in grass? J Exp Bot 60:3737–3749