Impacts of drought and nitrogen addition on *Calluna* heathlands differ with plant life-history stage

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Summary

1. Climate change and atmospheric deposition of nitrogen (N) affect the biodiversity patterns and functions of ecosystems world-wide. While many single-factor studies have quantified ecosystem responses to single global change drivers, less is known about the interaction effects of these drivers on ecosystem functions.

2. Here, we present the results of a three-year field and a two-year glasshouse experiment, in which we assessed responses of *Calluna vulgaris* heathlands to the single and combined effects of drought events (D) and N fertilization (D: 25% precipitation reduction in the field experiment and 20–50% soil water content reduction in the glasshouse experiment; N fertilization: 35 kg N ha^{-1} year^{-1}).

3. We examined the effects of D and N treatments on growth responses of the dominant dwarf shrub *Calluna vulgaris* (in terms of biomass production and allocation, tissue δ^{13}C signatures and C:N ratios) in relation to two plant life-history stages and different 'ecotypes' (sub-Atlantic vs. subcontinental heathlands).

4. Plant responses varied strongly with life-history stage, and the interaction of N and D showed lower effects than would be expected based on additive responses to single factors. While D treatments had no effects on *Calluna* in the building phase (ca. ten-year-old plants), seedlings (particularly one-year-old plants) were highly susceptible to drought. Differences in response patterns were attributable to the high shoot–root ratios typical of young *Calluna* plants. These ratios decreased with progressing life history as a result of increasing below-ground biomass investments. Below-ground biomass production and shoot–root ratios differed between plants from the different heathland sites.

5. Tissue δ^{13}C signatures decreased and C:N ratios increased with plant age as a result of decreasing evaporative demands (per unit root biomass). N fertilization increases the shoot–root ratios and thereby the drought susceptibility of *Calluna* plants.

6. *Synthesis.* Our findings suggest that plant responses to global change are difficult to anticipate by means of single-factor studies or by focusing on a single life-history stage. This highlights the need for global change research to include multiple factors and life-history stages when assessing an ecosystem's susceptibility to shifts in environmental conditions.

Key-words: biomass allocation, *Calluna vulgaris*, climate change, drought, nitrogen deposition, plant–climate interactions, δ^{13}C

Introduction

Climate change and nitrogen (N) atmospheric deposition are amongst the most important drivers of biodiversity loss and shifts in ecosystem functions (Sala *et al.* 2000). Climate extremes such as summer drought events are projected to become more frequent and severe in central Europe in the course of this century (IPCC 2013) and have the potential to seriously affect biodiversity patterns and ecosystem functions such as primary productivity and nutrient cycling (Gorissen

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et al. 2004; Albert *et al.* 2011; Jentsch *et al.* 2011). Airborne N deposition has tripled since the beginning of industrialization (Galloway *et al.* 2004) and is considered responsible for biodiversity loss due to its effects on plant growth and competition, particularly in low-N environments (Bobbink *et al.* 2010; Duprè *et al.* 2010; Jones & Power 2012). In W-Europe, N deposition has slightly decreased in recent decades, but is still high compared to pre-industrial levels (Waldner *et al.* 2014).

Despite the strong effects that these drivers will have on global biodiversity and ecosystem functions, little is known about possible interactions between them (Zavaleta *et al.* 2003; Fagúndez 2013). Anticipating ecosystem responses is crucial for biodiversity conservation and guiding ecosystem management, particularly in the face of ongoing climate shifts and likely interactions of multiple environmental stressors (Lindenmayer *et al.* 2010). There are two ways in which global change drivers might affect ecosystem responses. First, co-occurring global change drivers can be additive (i.e. the interaction between drivers is statistically not significant). In this case, ecosystem responses can be predicted directly from single-factor studies (Zavaleta *et al.* 2003). Secondly, effects of global change drivers can be non-additive. This could result in dampening (antagonistic) or amplifying (synergistic) interaction effects (i.e. interactions are statistically significant; Meyer-Grünefeldt *et al.* 2015). In the second case, ecosystem responses can only be predicted from multifactor studies. In our particular system, there is evidence that the drought susceptibility of plants may increase under high N availability because of insufficient water supply resulting from N-induced changes in shoot–root ratios or mycorrhizal infection rates (Aerts, Boot & van der Aart 1991; Hofland-Zijlstra & Berendse 2009).

In this study, we analysed heathland ecosystem responses to the combined effects of drought and N fertilization. Heathlands – dominated by the dwarf shrub *Calluna vulgaris* (L.) Hull (henceforth referred to as *Calluna*) – are amongst the oldest cultural landscapes in Europe, host a huge proportion of the biodiversity typical of open acidic sites, and are thus of high conservation value (e.g. recognized by international frameworks such as the NATURA 2000 habitat directive; Southon *et al.* 2012). As heathlands are typical low-N environments and predominantly found in (sub-) Atlantic climate (Gimingham 1972; Loidi *et al.* 2010), they are considered highly vulnerable to both increasing frequency and severity of summer droughts and airborne N inputs (von Oheimb *et al.* 2010a,b; Southon *et al.* 2012). Although a considerable amount of research has addressed the ecological implications of elevated N inputs in heathland ecosystems (see Aerts *et al.* 1990; Marcos, Calvo & Luis-Calabuig 2003; Calvo *et al.* 2007; Jones & Power 2012), little is known about heathland responses to the combined effects of N fertilization and climatic variables (Fagúndez 2013). Moreover, due to the large area covered by heathlands in NW Europe, it is conceivable that *Calluna* responses to environmental changes (particularly climatic ones) might differ between 'provenances' or 'ecotypes' typical of a particular region (Beierkuhnlein *et al.*

2011; Härdtle *et al.* 2013). *Calluna* from drier sites at the southern and eastern margins of the European distribution range might be less susceptible to drought events, and thus also to water shortage, than wetter sites due to evolutionary active selection processes. This has been found for other species such as *Fagus sylvatica* (Rose *et al.* 2009).

Plant age is a further factor that controls for a plant's susceptibility to environmental shifts and one that has not been investigated for *Calluna* to date. According to Watt (1947, 1955), the structure of heathlands is characterized by different developmental phases, each of which is associated with a specific life-history stage of *Calluna*. Different life-history stages, in turn, are often linked to shifts in biomass allocation patterns (also described as 'partitioning'), which are reflected by plant traits such as shoot–root ratios or relative investments in reproductive tissue (Weiner 2004). Thus, the susceptibility of *Calluna* to climatic events may vary with plant age, mediated by accompanied shifts in shoot–root ratios and rooting depth.

To study the impacts of N fertilization and reduced summer precipitation (henceforth referred to as 'drought') in relation to plant age and ecotype on growth responses of *Calluna* (as the key dwarf shrub of lowland heaths), we performed a three-year field and a two-year glasshouse experiment, each with a full-factorial combination of N fertilization and drought. The field experiment was conducted in two different *Calluna* heaths, both in the building phase, representing two climatic counterparts within the lowlands of northern Germany. In the glasshouse experiment, *Calluna* plants were raised from seeds originating from the two heathland sites to analyse both ecotype effects (sub-Atlantic vs. subcontinental origin) and age effects (one-year-old vs. two-year-old plants) on growth responses to drought and N addition. Growth responses were measured in terms of biomass production and allocation. In addition, we analysed tissue $\delta^{13}C$ signatures [as an indicator of the plants' (water) stress] and tissue C:N ratios (as an indicator of the plants' nutritional status) as affected by treatments. We hypothesized that (i) plants in early life stages are more sensitive than plants in later life stages, (ii) sub-Atlantic heaths would be more susceptible to drought than subcontinental heaths, and (iii) N fertilization has the potential to increase the plants' drought susceptibility due to an increased above-ground biomass allocation (in relation to the below-ground allocation; according to the 'resource optimization hypothesis'; Ågren & Franklin 2003).

Materials and methods

STUDY SITES

The two study sites represented dry heathland ecosystems (Friedrich *et al.* 2011) and were characterized by different climates. Mean annual precipitation of the sub-Atlantic site ('Lueneburg Heath nature reserve') was 827 mm and of the subcontinental site ('Nemitz Heath nature reserve') 611 mm. The sub-Atlantic and the subcontinental heaths (henceforth referred to as SAH and SCH, respectively) were dominated by *Calluna* (monospecific stands) with a structure typical

of the building phase (ca. ten-year-old plants; Watt 1947, 1955; for site characteristics see Table 1).

DESIGN OF THE FIELD EXPERIMENT

In the field experiment, we used a randomized block design (with seven replicate blocks) and with two treatments in factorial combinations (drought treatment, henceforth referred to a D treatment; N fertilization, henceforth referred to as N treatment). Each block was divided into four plots (2 m × 2 m in size), which were assigned randomly to these treatments (i.e. control, D treatment, N treat-

ment, D+N treatment). D treatments were carried out using fixed rainout shelters (3 m × 3 m; modified design based on Yahdjian & Sala 2002) and reduced the growing season precipitation by about 25% to mimic currently projected climate change scenarios for the study area (IPCC 2013). The effects of reduced precipitation on the soil water content were quantified by means of volumetric water content sensors (measurements based on capacitance/frequency domain technology; Decagon Devices, Washington, USA). Sensors were inserted in the upper soil horizons (with the highest root density of *Calluna*), and measurements of the volumetric soil water contents were conducted in the growing season in the year 2010 (Table 1). The measurements indicated that the D treatment

Table 1. Site characteristics of the heathlands analysed (sub-Atlantic and subcontinental heaths: SAH and SCH, respectively; Vischer-Leopold, Balzer & Ssymank 2008)

	SAH			SCH		
Topography						
Location	53°15′N, 09°58′E			52°58′N, 11°20′E		
Altitude (m a.s.l.)	105			25		
Size (ha)	About 5500			About 400		
Climate	Sub-Atlantic			Subcontinental		
Site protection and vegetation types						
Protection	Protected under the EU Conservation of Wild Birds and the Fauna-Flora-Habitat Directive Directive since 2006, Nature reserve since 1922			Protected under the EU Conservation of Wild Birds and the Fauna-Flora-Habitat Directive Directive since 2006		
Vegetation type (dominant)	*Calluna* heathlands, > 80% cover of *C. vulgaris* (monospecific stands)			*Calluna* heathlands, > 80% cover of *C. vulgaris* (monospecific stands) mmonosonospecificstands		
Vegetation types (others)	Beech-oak/spruce forests Dunes Dry grasslands Mires			Pine forests Dunes Dry grasslands		
Soil characteristic						
Soil type	Podzol			Podzol		
pH (H$_2$O)	3.0–3.5*			3.9–4.3		
	Organic layer	*A-horizon*		*Organic layer*	*A-horizon*	
Thickness (cm)	0.0–5.0	1.0–10.0		0.0–2.5	1.5–9.0	
P$_t$ (in mg g^{-1})	0.42*	0.10*		0.61	0.09	
N$_t$ (in mg g^{-1})	12.8	0.8		13.3	0.5	
C (in mg g^{-1})	363	21		412	15	
Climate characteristic[†]						
Climate	Sub-Atlantic			Subcontinental		
Long-term mean temperature (°C)[†]	9.0			9.2		
Long-term mean precipitation (mm yr^{-1})[†]	827.0			611.0		
Annual values	*2009*	*2010*	*2011*	*2009*	*2010*	*2011*
Mean precipitation (mm)	771.2	707.1	809.7	581.2	628.2	504.2
Precipitation growing season (mm)[‡]	265.8	356.6	455.9	219.7	385.8	308.6
Mean temperature (°C)	9.5	7.9	9.7	9.6	8.1	9.9
Temperature growing season (°C)[‡]	15.7	15.3	15.6	16.2	15.8	16.2

Topography, Site protection and vegetation types, soil characteristics (for organic layer and A-horizon), climate characteristics and annual climate values (for each study year) are shown.
*According to von Oheimb *et al.* (2010a,b).
[†]German Weather Service (www.dwd.de); values represent 30-year means.
[‡]Growing season: May to September.

reduced the soil water content under the rainout shelters by about 35% (percentage loss compared to field capacity, averaged over the growing season). Rainout shelters were set up from May to October (2009–2011). N-treated plots received 35 kg N ha^{-1} year^{-1} (as NH_4NO_3, dissolved in deionized water). The same amount of deionized water was applied in the control and D plots. N solutions were applied annually on eight occasions from May to September (2009–2011).

DESIGN OF THE GLASSHOUSE EXPERIMENT

For the glasshouse experiment, *Calluna* seeds were collected in September 2009 (from both heathland sites) and sown in germination dishes in a glasshouse in January 2010. The glasshouse was constructed with glasshouse film (UV-B Window, folitec, Germany) and removable side parts to ensure as natural climatic conditions as possible. Seedlings emerged in May and were transplanted into pots (6 cm in diameter and 4.6 cm in height) in mid-June (four individuals per pot with a planting distance of 2 cm). Pots contained humus collected from the upper soil horizon of a podzol in the Lueneburg Heath nature reserve (soil chemical characteristics: pH_{H2O}: 4.1, base saturation: 21%, salt-extractable base cations: 0.9 $mmol_c$ 100 g^{-1}, N_{tot} content 600 mg kg^{-1}, P_{tot} content: 57 mg kg^{-1}). In May 2011, plants were transplanted to larger pots (7.5 × 7.5 × 8 cm^3) to prevent pot effects due to intraspecific competition. Position effects in the glasshouse were avoided by relocating the pots every three weeks throughout the experiment. Eighty pots per heathland site (SAH, SCH) were assigned randomly to the following treatments: control, D treatment, N treatment and D+N treatment (i.e. total no. of pots per site = 320). Controls were regularly watered and the soil moisture content was kept near to field capacity (corresponding to about 23% soil water content). For D treatments, plants were exposed to two drought periods per growing season (i.e. no watering of plants in August and September 2010, and in July and August 2011). D treatments lasted for 10 and 8 days in 2010 and resulted in a decrease in the pots' soil water content by about 30% and 20%, respectively (percentage loss compared to field capacity at the end of the drought period). In 2011, drought treatments lasted for 5 and 4 days and resulted in a decrease in the soil water content by about 50% and 46%, respectively (calculated by means of pre- and post-treatment pot weights). Plants were regularly watered between the two D treatments in both years (for about 4 weeks with soil water contents near to field capacity). To avoid plant dieback in the experiment, drought treatments were ended when shoot tips showed the first symptoms of wilting (and not after a previously defined water loss of pots). N-treated pots received 35 kg N ha^{-1} year^{-1} (as NH_4NO_3; analogous to the field experiment) dissolved in deionized water, and controls and D treatments received the same amount of deionized water (weekly applications from July to September in 2010 and June to September in 2011).

SAMPLING AND CHEMICAL ANALYSES

Growth responses were measured in terms of biomass production and allocation, tissue $\delta^{13}C$ signatures and C:N ratios in the above-ground and below-ground tissue. In the field experiment, above-ground biomass production was quantified following von Oheimb et al. (2010a,b), that is a calliper gauge was used to measure the increment of the five longest current season's shoots on one main stem of seven randomly selected *Calluna* plants per plot. Measurements were

performed annually in each plot during the first week of October. Subsequently, we harvested 50 shoots per plot (randomly selected). Shoots were air-dried for chemical analyses. Below-ground biomass production was analysed by means of ingrowth cores (modified design based on Steingrobe, Schmid & Claassen 2000). Two ingrowth cores per plot (polyethylene mesh bags, mesh size 2 mm, 7 cm in diameter and 5 cm in height) were inserted vertically in the soil from December 2009 to October 2010 (for further processing of ingrowth cores see description below). After harvest, roots in ingrowth cores were washed (to remove adherent soil particles), dried (at 40 °C for 48 h) and weighed.

In the glasshouse experiment, 40 pots per treatment and heathland site, respectively, were harvested in October of each study year. After harvest, the biomass of four pots (of one treatment) was pooled in one sample to obtain sufficient plant material for analyses (resulting in 10 replicates per treatment). Biomass samples were dried at 40 °C for 48 h, and above-ground and below-ground biomasses were weighed.

In addition to biomass production, we analysed above-ground and below-ground tissue $\delta^{13}C$ signatures and C: N ratios. Tissue $\delta^{13}C$ signatures can be used as a proxy for a plant's water status, as they are related to intrinsic water-use efficiency – the ratio of net CO_2 assimilation and stomatal conductance (i.e. A/g_s; Farquhar, O'Leary & Berry 1982; Farquhar, Ehleringer & Hubick 1989; Mariotte et al. 2013). For analyses, samples were ground in an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany; above-ground biomass samples from the field experiment) or a mixer mill (MM 400, Retsch, Haan, Germany; biomass samples from the glasshouse experiment and below-ground biomass samples from the field experiment) and redried at 105 °C (12 h) and 40 °C (48 h) for C:N and $\delta^{13}C$ analyses, respectively. Both variables were measured using a continuous flow elemental analyser-isotope mass spectrometer (vario EL cube, Elementar, Hanau, Germany, coupled to an Isoprime 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 IAEA standards (IAEA-CH-3) was ± 0.1‰.

STATISTICAL ANALYSIS

The results of the field experiment were evaluated using repeated measures linear mixed models (RM-LMM) that took account of the time-series measurements in the years 2009–2011 (i.e. temporal pseudoreplication; Crawley 2007). Each RM-LMM included 'time', 'site', 'D treatment', 'N treatment' and all interaction terms as fixed factors, and 'Block' as random factor. Treatment effects on below-ground response variables in the field experiment (only measured in 2010) were analysed by means of linear mixed models (LMM). Each LMM included treatments (site, D, N) and all interaction terms as fixed factors, and 'Block' as a random factor. Treatment effects on response variables in the glasshouse experiment were analysed with general linear models (LM) with 'site', 'D treatment', 'N treatment' and interaction terms as fixed factors. Simplification of all models was performed by stepwise backward selection of fixed factors and the respective interaction terms, until only significant factors/interaction terms remained (with $P < 0.05$; following the procedure described in Crawley 2007). We used post hoc tests (Tukey) to compare mean values of response variables as related to treatments. All analyses were conducted in R 2.12.0 (http://www.R-project.org) with the packages LME4 (Bates et al. 2014) and lmerTest (Kuznetsova, Brockhoff & Christensen 2014).

Results

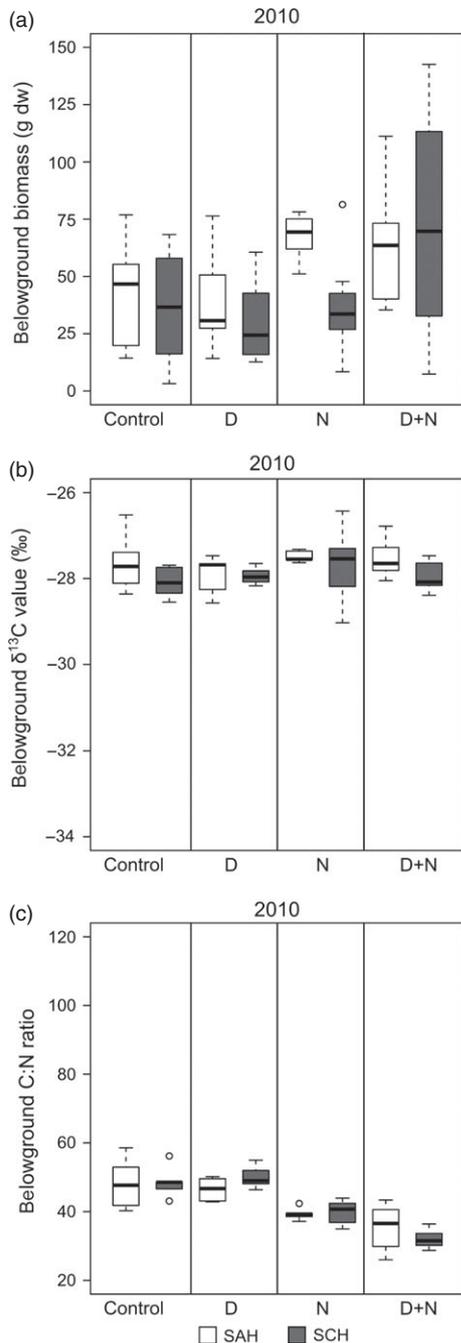## TREATMENT EFFECTS ON BIOMASS PRODUCTION AND ALLOCATION

In the field experiment, both above-ground and below-ground biomass production increased with N fertilization in both study sites (Figs 1a and 2a, Table 2). In addition, we found differences in the above-ground biomass production between the three study years (year effect) as well as interactions of year with N (i.e. year-related responses of above-ground biomass production to N fertilization; Table 2). D treatments had no effect on biomass production.

In contrast to the field experiment, D treatments negatively affected the biomass production of one-year-old plants, but not of two-year-old plants in the glasshouse experiment (Table 2; Fig. 3). N fertilization increased biomass increment (with the exception of below-ground biomass of one-year-old plants; Fig. 3, Table 3). For two-year-old plants, above-ground biomass more than doubled as a result of N addition, and below-ground biomass increased by a factor of 1.2. Moreover, we found a significant site effect for below-ground production.
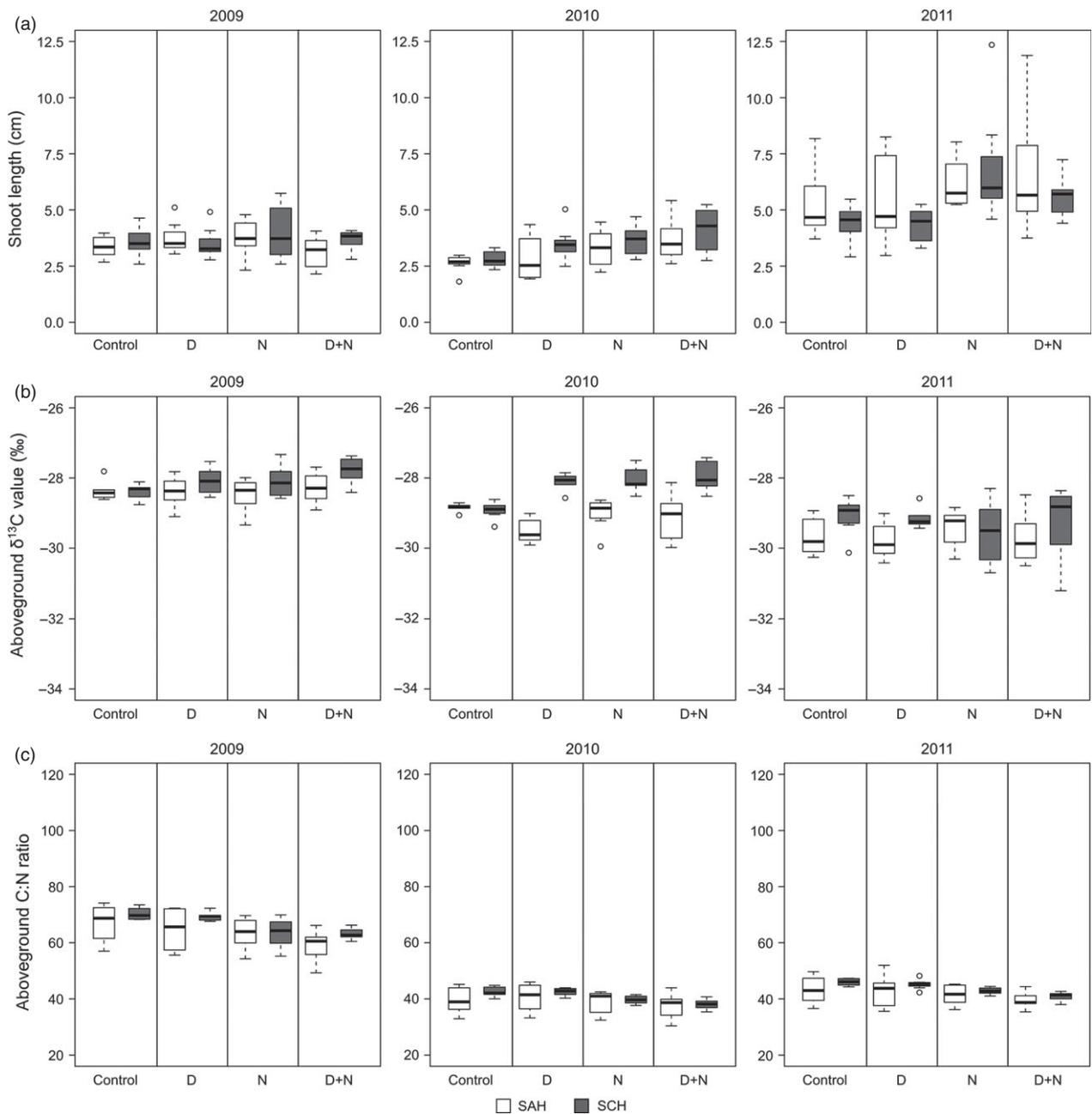
Fig. 1. Effects of treatments (control; D: drought; N: nitrogen; D+N: drought and nitrogen) on (a) above-ground biomass production measured as the increment of current season's shoots (cm), (b) above-ground biomass $\delta^{13}C$ values (‰) and (c) above-ground C:N ratios of *Calluna* plants in the field experiment for each study year (2009–2011). Box and whisker plots show the treatment effects separately for the sub-Atlantic (SAH) and subcontinental (SCH) heathland site (for significances of main and interaction effects see Table 2).

Fig. 2. Effects of treatments (control; D: drought; N: nitrogen; D+N: drought and nitrogen) on (a) below-ground biomass production (dw), (b) below-ground biomass $\delta^{13}C$ values (‰) and (c) below-ground C:N ratios of *Calluna* plants in the field experiment for the study year 2010. Box and whisker plots show the treatment effects separately for the sub-Atlantic (SAH) and subcontinental (SCH) heathland site (for significances of main and interaction effects see Table 2).

Biomass shoot–root ratios (glasshouse experiment) were high for one-year-old *Calluna* (Fig. 3c), and distinctly decreased for two-year-old plants (by about 60%; one-way ANOVA, $P < 0.001$). Shoot–root ratios increased strongly as a result of N fertilization, particularly for the two-year-old plants (by about 79% and 69% for SAH and SCH plants, respectively). D treatments only affected shoot–root ratios of

one-year-old plants (slight increase). Moreover, plants originating from SCH had significantly higher shoot–root ratios (Table 3). Plants in the D+N treatment had a lower shoot–root ratio than would be expected based on the main effects (significant N × D interaction term in Table 3).

TREATMENT EFFECTS ON TISSUE $\delta^{13}C$ SIGNATURES

In the field experiment, $\delta^{13}C$ signatures of the above-ground tissue varied strongly with study year and site (i.e. year and site effects; Fig. 1b, Table 2). Above-ground tissue $\delta^{13}C$ values were less negative for SCH plants across treatments, and differences between SAH and SCH were most pronounced in 2010 (except for the control). N treatments had weak and D treatments had no effects on above-ground tissue $\delta^{13}C$ signatures, but we found a significant site α D interaction (i.e. higher $\delta^{13}C$ values in the D treatment for SCH; Table 2; Fig. 1b).

In the glasshouse experiment, significant differences were found for tissue $\delta^{13}C$ signatures (above-ground and below-ground) between one- and two-year-old plants (with a decrease of about 2‰ for two-year-old plants; one-way ANOVA, $P < 0.001$; Fig. 4). D treatments only caused an increase in the $\delta^{13}C$ values of one-year-old plants, whereas N treatments had a strong effect on the $\delta^{13}C$ signatures of two-year-old plants (increase of values; Table 3). Moreover, we found a weak site x N interaction on the above-ground tissue of two-year-old plants (i.e. N treatments caused a stronger increase of $\delta^{13}C$ signatures at SAH sites).

TREATMENT EFFECTS ON TISSUE C:N RATIOS

In the field experiment, above-ground biomass C:N ratios varied strongly with the study year (Fig. 1c; Table 2: year effect). N treatments lowered C:N ratios and varied with the study year (year x N interaction, Table 2). D treatments also affected above-ground and below-ground tissue C:N ratios. Furthermore, N x D interactions dampened single treatment effects of the below-ground tissue C:N ratios.

In the glasshouse experiment, tissue C:N ratios increased strongly with increasing plant age (from 32.4 to 81.0 and 34.2 to 77.2 for the above-ground tissue of control plants from SAH and SCH, respectively; Tukey's post hoc test, $P < 0.001$; Fig. 5). D treatments lowered tissue C:N ratios, but only for one-year-old plants, whereas N treatments affected C:N ratios across sites and plant age. Plants in the D+N treatment had lower C:N ratios than would be expected based on the main effects (i.e. significant N x D interaction; Table 3, Fig. 5a, one-year-old plants).

Discussion

TREATMENT EFFECTS ON BIOMASS PRODUCTION AND ALLOCATION

Calluna plants showed remarkable differences in their drought susceptibility between the field and glasshouse experiments. Whereas D treatments had no effect in the field experiment,

Table 2. Treatment effects on biomass production, $\delta^{13}C$ signatures and C:N ratios of *Calluna* plants in the field experiment (building phase; based on RM-LMM for above-ground biomass measurements in 2009–2011, and LMM for below-ground biomass measurements in 2010; only significant factors/interaction terms of the minimal adequate models were considered). Above-ground biomass production was measured as the increment of the current season's shoots (cm) in each study year (2009–2011). Treatments effects on $\delta^{13}C$ signatures were only significant for the above-ground, but not for the below-ground biomass

| | Biomass production | | | | $\delta^{13}C$ signature | | C:N ratio | | | |
| | Above-ground | | Below-ground | | Above-ground | | Above-ground | | Below-ground | |
	F	P	F	P	F	P	F	P	F	P
Year	16.81	**<0.001**	na	na	23.47	**<0.001**	1120.52	**<0.001**	na	na
Site	0.82	0.38			14.28	**0.003**	2.51	0.14	0.68	0.41
N	32.47	**<0.001**	9.55	**0.003**	5.32	**0.02**	102.98	**<0.001**	67.17	**<0.001**
D	0.04	0.85			0.77	0.38	8.78	**0.004**	5.24	**0.03**
Year × N	9.07	**<0.001**					3.72	**0.03**		
Site × D					6.34	**0.01**				
N × D									6.19	**0.02**

Site, sub-Atlantic vs. subcontinental heath sites; N, nitrogen fertilization; D, drought treatment; na, year effects were not analysed for below-ground biomass.
Significant *P*-values are indicated in bold characters.

biomass production of one-year-old plants was highly sensitive to drought in the glasshouse, despite the comparatively minor drought plants experienced in the glasshouse in 2010 (in terms of soil water loss; cf. method section). We hypothesize that these findings are attributable to differences in shoot–root ratios as a core trait that highly determines a plant's growth response to drought (Weiner 2004). In principle, increasing above-ground investments are accompanied by increasing transpiration rates and thus by increasing evaporative demands (Gordon *et al.* 1999a), whereas low below-ground investments (resulting in low root biomass) constrain water uptake and thus a plant's water supply. One-year-old *Calluna* plants were characterized by the highest shoot–root ratios, and ratios decreased by about 60% for the two-year-old individuals. With progressing life history, *Calluna* continuously increases its relative below-ground investments, and in the building and mature phase plants achieve shoot–root ratios below 1 (Gimingham 1972; Wallèn 1987). In addition, old *Calluna* plants achieve a deeper rooting depth than young plants. In well-drained sandy soils, the rooting zone of old plants may extend to nearly 50 cm, although often more than 80% of the roots are concentrated in the upper 10 cm of the soil (Gimingham 1960; Genney, Alexander & Hartley 2002). As a consequence, drought susceptibility of *Calluna* decreases with plant age. This interpretation is supported by both our findings for tissue $\delta^{13}C$ signatures (see below) and field observations, according to which extreme natural drought events may cause a severe dieback of *Calluna* seedlings, whereas older plants (building or mature phase) proved to be highly resilient (Britton *et al.* 2001; Kongstad *et al.* 2012). Moreover, losses in soil water content above which we observed wilting symptoms differed significantly for one- and two-year-old plants (one-year-old plants wilted at about 18% and two-year-old plants at about 12% soil water content). We rule out the possibility that the different responses of *Calluna* plants in the field and glasshouse experiments were related to different effects of D

treatments on the soil water availability, as in both experiments the soil water content was reduced to a similar extent (cf. method section). Moreover, it is very likely that water shortage was even higher for plants in the field than for plants in the glasshouse, as natural drought events in the field might have strengthened the experimentally induced drought. As a result, heath podzols often achieve soil water losses of more than 70% (relative to field capacity) in the course of the growing season (Leuschner 2002). We also rule out the possibility of an effect of the soil medium on the plants' shoot–root ratios, as the soil used in the glasshouse experiment was collected at the field experimental sites (cf. method section). In the field experiment, natural drought events might have reduced the contrast between controls and D treatments. Spring and early summer precipitation was below average in 2009 and 2010, which in turn might have dampened effects of D treatments (see also interpretation of $\delta^{13}C$ signatures below). In conclusion, our findings support our first hypothesis (drought susceptibility of *Calluna* plants would depend on the plants' age), but this applied in particular to one-year-old plants. Contradicting our second hypothesis, we found no evidence for a higher drought susceptibility of SAH vs. SCH plants. Given that age-related changes in drought sensitivity also occur in other ecosystems, predictions of ecosystem responses to climate change could be biased when based on the assumption that effects of climate change are independent of plant age (cf. Luo & Chen (2013) and conclusion section).

N treatments affected the biomass increment of two-year-old plants in particular (glasshouse experiment; cf. Power, Ashmore & Cousins 1998; von Oheimb *et al.* 2010a,b; Phoenix *et al.* 2012). However, as N addition strongly facilitated above-ground biomass allocation of *Calluna* plants, N treatments caused a significant increase in shoot–root ratios (up to a factor of 1.8; Aerts, Boot & van der Aart 1991; Friedrich *et al.* 2011; Meyer-Grünefeldt *et al.* 2015). This in turn means that – based on the previous discussion – N fertilization has
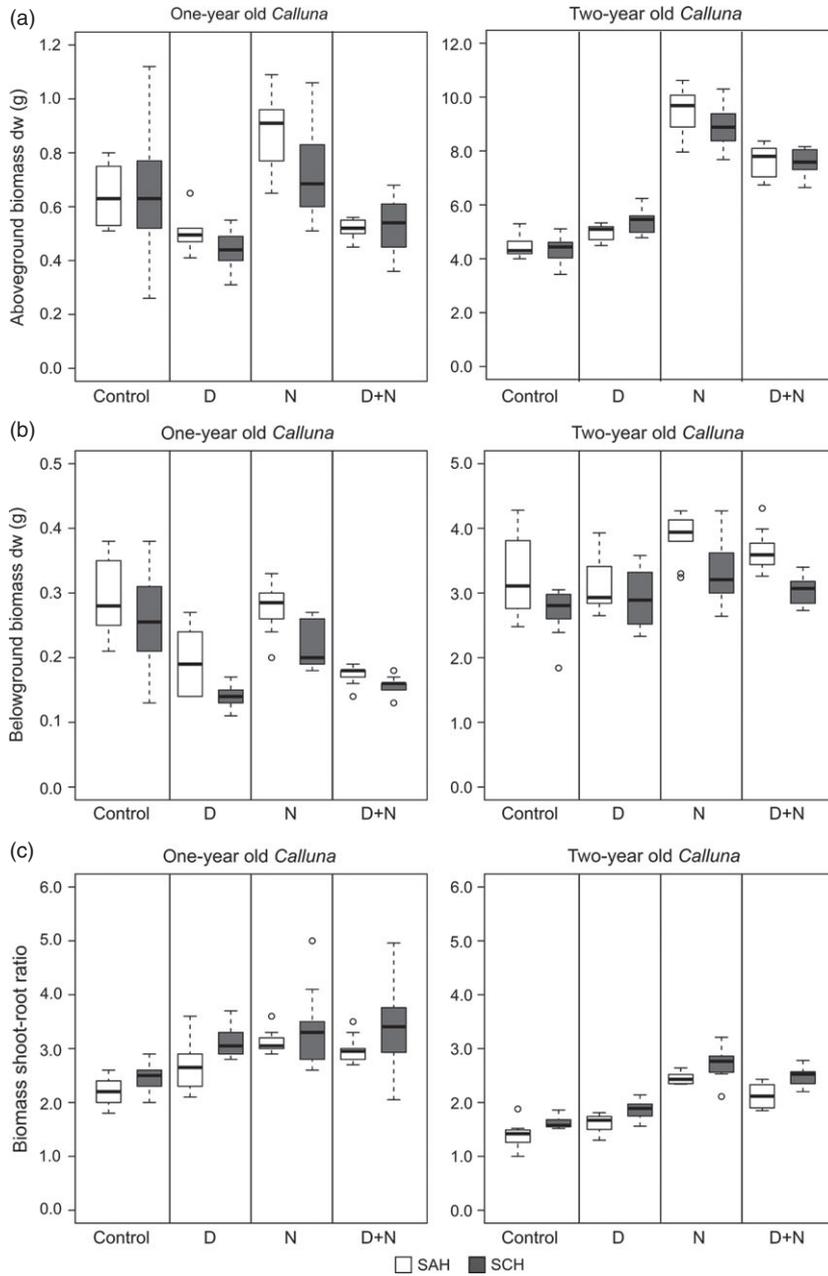
Fig. 3. Effects of treatments (control; D: drought; N: nitrogen; D+N: drought and nitrogen) (a) on above-ground and (b) below-ground biomass dry weights (dw, in g) and (c) biomass shoot–root ratios of one- and two-year-old *Calluna* plants in the glasshouse experiment. Box and whisker plots show the treatment effects separately for the sub-Atlantic (SAH) and subcontinental (SCH) heathland site (for significances of main and interaction effects see Table 3).

the potential to strongly increase a plant's drought susceptibility, particularly when N fertilization predates drought events (as compared to the simultaneous impact on N fertilization and drought). This supports our third hypothesis (N fertilization has the potential to increase the plants' drought susceptibility due to an increased above-ground biomass allocation) and is in agreement with the findings of Friedrich *et al.* (2012) and Southon *et al.* (2012); see also discussion on $\delta^{13}C$ signatures below. However, in our experiment, N and D treatments were applied simultaneously, and we found non-additive (dampening) effects on shoot–root ratios. Hence, shoot–root ratios were lower in the D+N treatment than would be expected based on adding the effects of the single-factor treatments. These findings suggest that the extent to which environmental stressors (such as N fertilization and drought events) affect plant growth patterns strongly depends on the temporal sequence in which

they appear. We hypothesize that plant growth tended to be carbon limited in the D+N treatment due to the closure of stomata resulting from water shortage (Wilson, Baldocchi & Hanson 2000; Muller *et al.* 2011; Schwalm *et al.* 2012).

Both below-ground biomass production and shoot–root ratios differed between plants originating from the different heathland sites (SAH and SCH). This suggests differences in allocation patterns between ecotypes, an interpretation which is supported by our findings for $\delta^{13}C$ signatures (see below).

TREATMENT EFFECTS ON TISSUE $\Delta^{13}C$ SIGNATURES

In the field experiment, tissue $\delta^{13}C$ signatures (above-ground) were not affected by D treatments, but values differed between study years and sites ('year effects' and 'site effects'). These findings support our previous interpretation

Table 3. Treatment effects on biomass production, shoot–root ratios, $\delta^{13}C$ signatures and C:N ratios of one-year-old and two-year-old *Calluna* plants in the glasshouse experiment (pioneer phase; calculated by means of LM; only significant factors/interaction terms of the minimal adequate models were considered)

| | Biomass production | | | | | | $\delta^{13}C$ signature | | | | C:N ratio | | | |
| | Above-ground | | Below-ground | | Shoot-root ratio | | Above-ground | | Below-ground | | Above-ground | | Below-ground | |
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
One-year-old plants														
Site			18.84	**<0.001**	10.74	**0.002**					0.19	0.66	10.95	**0.001**
N	11.93	**<0.001**			30.90	**<0.001**			11.71	**0.001**	125.48	**<0.001**	151.54	**<0.001**
D	55.61	**<0.001**	100.48	**<0.001**	6.14	**0.02**	137.50	**<0.001**	92.17	**<0.001**	29.12	**<0.001**	40.59	**<0.001**
N × D					8.80	**0.004**					22.27	**<0.001**	5.95	**0.02**
Site × N											1.01	0.32		
Site × D											1.58	0.21		
Site × N × D											8.72	**0.004**		
Two-year-old plants														
Site			20.23	**<0.001**	30.01	**<0.001**	0.36	0.55	8.26	**0.005**	19.00	**<0.001**	1.34	0.25
N	269.94	**<0.001**	36.86	**<0.001**	225.51	**<0.001**	15.91	**<0.001**	50.49	**<0.001**	814.30	**<0.001**	258.08	**<0.001**
D					1.84	0.18	0.93	0.34	5.61	**0.02**	0.17	0.68	0.26	0.61
N × D					4.02	**0.048**								
Site × N							4.09	**<0.05**			19.90	**<0.001**	21.07	**<0.001**
Site × D											18.64	**<0.001**		
Site × N × D									12.68	**<0.001**				

Site, sub-Atlantic vs. subcontinental heathland sites; N, nitrogen fertilization; D, drought treatment.
Significant *P*-values are indicated in bold characters.

that the weather conditions in a respective study year reduced the contrast between controls and D treatments. In fact, between-year shifts in $\delta^{13}C$ signatures were higher than the (non-significant) treatment effects on $\delta^{13}C$ signatures.

However, tissue $\delta^{13}C$ signatures of plants in the glasshouse experiment strongly support our hypothesis that shoot–root ratios constitute a key trait that strongly determines a plant's susceptibility to drought. Along with age-related decreases in shoot-root ratios we found decreasing $\delta^{13}C$ values for both the above-ground and below-ground tissue. This indicates that plants with higher shoot–root ratios are characterized by higher evaporative demands, stronger stomata closure and, thus, lower ^{13}C discrimination (Farquhar, Ehleringer & Hubick 1989; Gordon *et al.* 1999b; Llorens *et al.* 2004). Moreover, the D treatment only affected the $\delta^{13}C$ signatures of one-year-old plants.

N addition strongly increased the above-ground investments, particularly of two-year-old plants. As a consequence, the plants' shoot–root ratios and thus their evaporative demands increased. This was mirrored by increasing tissue $\delta^{13}C$ signatures in the N treatment, which in turn suggests a higher drought susceptibility of N fertilized plants (and again supports our third hypothesis).

Tissue $\delta^{13}C$ signatures of the below-ground biomass of two-year-old plants were significantly different between sites ('site' as main effect, with lower values found for SCH). This 'site effect' suggests that *Calluna* plants originating from the two heathland sites (SAH and SCH) represent different ecotypes. We hypothesize that both the more continental climate and soils with low water capacity (as are typical of the SCH site) might have selected ecotypes that are better adapted to low precipitation and summer drought events than plants from the SAH (Härdtle *et al.* 2013). It follows from this that 'site

effects' found in our study may be attributable to differences in ecotype, climate and soil properties. Significant site x D interactions on above-ground tissue $\delta^{13}C$ signatures also indicate that D treatments caused a higher drought stress at sites typical of the SCH.

TREATMENT EFFECTS ON TISSUE C:N RATIOS

In the field experiment, the tissue C:N ratios of current season's shoots responded strongly to between-year variations in weather conditions. This is in agreement with findings for $\delta^{13}C$ signatures, which were also affected by between-year variations in weather conditions. We hypothesize that weather conditions affected the lignification rates of the current season's shoots, which in turn was reflected by shifts in tissue C:N ratios (Terzi *et al.* 2013).
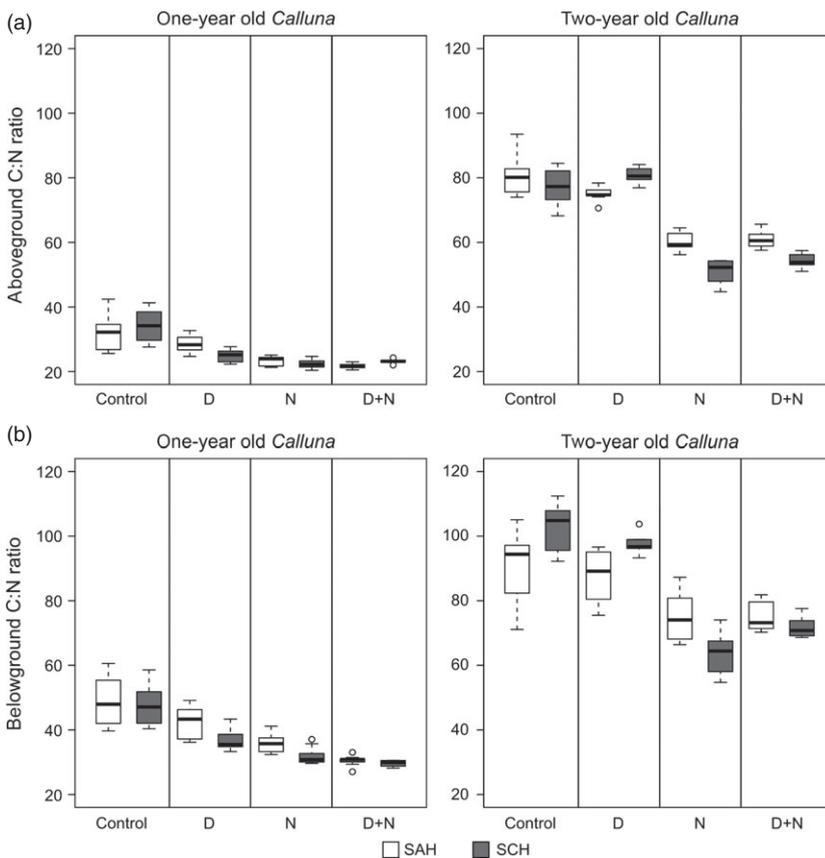
In the glasshouse experiment, tissue C:N ratios more than doubled between one- and two-year-old plants across treatments, suggesting a strong lignification that started in two-year-old *Calluna* plants (note that tissue C:N ratios of field and glasshouse plants are not comparable because different parts of the plants were sampled). As lignification of plant tissue is accompanied by a decrease in drought susceptibility (Terzi *et al.* 2013), tissue C:N ratios also indicate an age-dependent response of *Calluna* plants to drought.

Conclusion

Calluna heathlands proved to be highly resistant (in the sense of Enright *et al.* 2014) to drought events in the building phase (despite lower precipitation values during the experiment compared to long-term means). This is in agreement with the
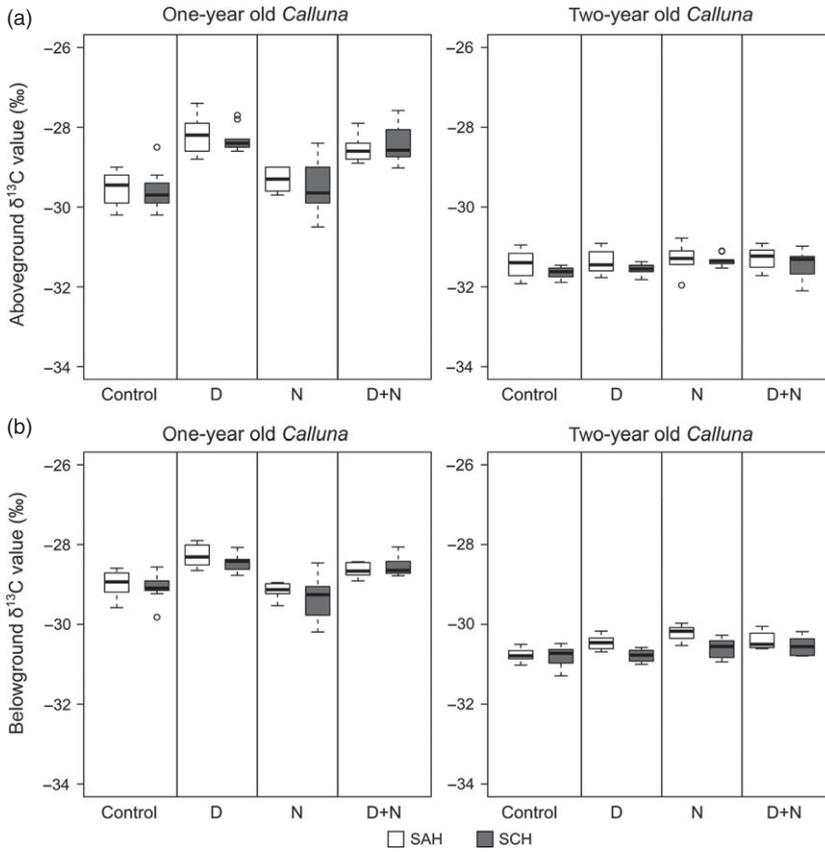
Fig. 4. Effects of treatments (control; D: drought; N: nitrogen; D+N: drought and nitrogen) (a) on above-ground and (b) below-ground biomass $\delta^{13}C$ values (‰) of one- and two-year-old *Calluna* plants in the glasshouse experiment. Box and whisker plots show the treatment effects separately for the sub-Atlantic (SAH) and subcontinental (SCH) heathland site (for significances of main and interaction effects see Table 3).

Fig. 5. Effects of treatments (control; D: drought; N: nitrogen; D+N: drought and nitrogen) (a) on above-ground and (b) below-ground biomass C:N ratios of one- and two-year-old *Calluna* plants in the glasshouse experiment. Box and whisker plots show the treatment effects separately for the sub-Atlantic (SAH) and subcontinental (SCH) heathland site (for significances of main and interaction effects see Table 3).

studies by Llorens *et al.* (2004) and Kongstad *et al.* (2012). However, this does not mean that heathland ecosystems are resistant to climate shifts, and it should be taken into account that certain phases typical of a heath's developmental cycle are more susceptible to drought than others (in this context, it is also important to note that several studies have modified and complemented the original four-phased cyclic model of Watt (1947); in oceanic heaths, for example, layering – that is the adventitious rooting of *Calluna* stems – can also act as an alternative regeneration mechanism; Marrs 1986, 1988; Marrs & Diemont 2013). Our results suggest that the pioneer phase is the most susceptible developmental phase of (lowland) heaths, as *Calluna* seedlings (and one-year-old plants in particular) are characterized by high shoot–root ratios and low tissue lignification, making them particularly susceptible to drought events.

As age-related changes in biomass allocation patterns were found for different plant life forms (Weiner 2004), it is likely that life-stage-related changes in drought sensitivity also apply for many other plant species typical of a wide range of ecosystems. Ettinger & HilleRisLambers (2013) found that tree mortality in forest ecosystems is strongly affected by stand development processes. Climate change effects (in terms of decreasing precipitation and increasing temperature) on tree growth significantly decreased with stand age. A study by Luo & Chen (2013) also confirmed that climate change-associated increases in tree mortality were significantly higher in young than old forests due to the higher sensitivity of young trees to regional warming and drought. This suggests that life-stage-related responses of trees to climate change have not been sufficiently studied, and observations from old forests may underestimate the sensitivity of forest ecosystems to climate change. This could also apply to other ecosystems (including heathlands) and would affect the reliability of predictions regarding ecosystem responses to environmental change scenarios.

Nutrient availability and N deposition affect the shoot–root ratio of plants, and this is likely to affect the vulnerability drought and responses to climatic change (e.g. in terms of tissue dieback). The two factors analysed in our study (i.e. drought and N fertilization) interacted non-additively on plant growth. This suggests that multifactor approaches with different treatment levels (i.e. different 'intensity-effects' of a single factor; Britton *et al.* 2003) are needed to better predict impacts of co-occurring global change drivers on ecosystem functions. Although such experiments would mean a higher effort, they would represent an ideal approach to address interactions effects of global change drivers in relation to different intensity levels of such factors. Outcomes of such approaches would also be important for the guidance of management and conservation efforts to counteract or mitigate long-term effects of global change.

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Data accessibility

All data is publicly available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.k7d23.

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