

Marginal *Calluna* populations are more resistant to climate change, but not under high-nitrogen loads

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Abstract The dominant plant species of European heathlands *Calluna vulgaris* is considered vulnerable to drought and enhanced nitrogen (N) loads. However, impacts may vary across the distribution range of *Calluna* heathlands. We tested the hypothesis that *Calluna* of southern and eastern marginal populations (MP) are more resistant to drought events than plants of central populations (CP), and that this is mainly due to trait differences such as biomass allocation patterns. Furthermore, we hypothesised that N fertilisation can offset differences in drought susceptibility between CP and MP. We conducted a full-factorial 2-year

greenhouse experiment with *Calluna* plants of CP and MP and quantified growth responses in terms of biomass production, allocation and tissue $\delta^{13}\text{C}$ signatures. Biomass production, shoot–root ratios and tissue $\delta^{13}\text{C}$ values of 1-year-old plants were higher for CP than for MP, indicating a higher drought susceptibility of CP. These trait differences were not observed for 2-year-old plants. N fertilisation increased shoot–root ratios of 1- and 2-year-old plants and across populations due to a stimulation of the aboveground biomass allocation. As a consequence, population-related differences in drought susceptibility were offset for N-fertilised plants. We concluded that *Calluna* plants originating from different populations developed adaptive traits to local climates, which determined their drought sensitivity. However, the higher drought resistance of MP can be attenuated by an N-induced increase in shoot–root ratios. This suggests that analyses on plant growth responses to global change should include multi-factor approaches with a focus on different populations throughout a species' distribution range.

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Introduction

Climate change is considered an important driver of biodiversity loss (Sala et al. 2000). Precipitation

patterns worldwide are changing, and it is predicted that the frequency of summer drought events in various parts of Europe will increase in this century (Schär et al. 2004; IPCC 2013). Drought events have been shown to alter, amongst other things, carbon (C) sequestration, primary productivity and biomass allocation patterns (Gorissen et al. 2004; Albert et al. 2011; Friedrich et al. 2012) and may therefore threaten the functioning and resilience of ecosystems. Plants evolve different strategies to mitigate negative drought impacts, which may imply tolerance or avoidance mechanisms (Chaves et al. 2002). High stomatal sensitivity enables plants to preserve their water balance in dry conditions (Picon et al. 1996; Yordanov et al. 2000). In contrast, drought tolerance mechanisms, e.g. osmotic adjustments or changes in biomass partitioning, enable plants to be less sensitive to drought (Gieger and Thomas 2002; Aranda et al. 2010). These different mechanisms are not mutually exclusive and plants may evolve strategies including both drought tolerance and avoidance mechanisms.

An individual's physiological adaptability to water shortage depends on the extent of its phenotypic plasticity, which is predefined by the genotype of the plant and is thus heritable. The plant's genotype, in turn, is affected by the environmental conditions at a given site, and these determine whether or not the phenotypic plasticity of specific traits is advantageous or not (Aspelmeier and Leuschner 2004). Thus, the genetic variability of a plant species is strongly mediated by the degree of environmental heterogeneity within its distribution range (Peuke and Rennenberg 2004; Rose et al. 2009). Considering the whole distribution range of a species, it is probable that perennial species with a broad range evolve genetic adaptations to their local habitat and thus exhibit local ecotypes, i.e. genetically different populations depending on the environmental conditions of their provenance (Macel et al. 2007; Kuster et al. 2013). Consequently, it is likely that the geographical position of a population within a species' distribution range is decisive for the drought susceptibility of individuals due to local adaptation. For example, individuals of the European beech (*Fagus sylvatica* L.) of southern, marginal populations from drier environments showed lower relative growth rates, lower biomass shoot–root ratios and under drought conditions lower leaf $\delta^{13}\text{C}$ signatures than individuals from

central populations (Rose et al. 2009). These traits typically indicate a better adaptation to dry conditions and a lower susceptibility to drought events. Knowledge about the specific drought susceptibility of individuals from different provenances and their specific adaptability is required to improve conservation strategies.

Besides climate change, increasing rates of airborne nitrogen (N) loads are amongst the most important global change drivers of biodiversity loss (Sala et al. 2000). N-induced changes in plant physiology, biomass allocation patterns and mycorrhizal colonisation rates may increase the plants' susceptibility to other stressors such as drought events (Bobbink et al. 2010; Southon et al. 2012). The simultaneous impacts of drought events and N deposition might be non-additive, resulting in attenuated or amplified effects that cannot be projected on the basis of single-factor studies. Multi-factor studies are important to understand and predict species or ecosystem responses to co-occurring global change drivers (Zavaleta et al. 2003).

Heathland ecosystems with the dominant ericaceous dwarf shrub *Calluna vulgaris* (L.) Hull (henceforth referred to as *Calluna*) are widely distributed over Western Europe (Gimingham 1972; Vandvik et al. 2014). These nutrient-poor (mostly N-limited) environments have a high conservation value (e.g. Alonso and Hartley 1998; NATURA 2000 Habitat Directive; Borchard et al. 2014; Vandvik et al. 2014), because they host a huge proportion of the biodiversity typical of open acidic sites and are amongst the oldest cultural landscapes in Europe (Gimingham 1972). The distribution range of *Calluna* heathlands is characterised by a humid climate, mild temperatures and moderate summer drought events (Loidi et al. 2010). Both the climatic conditions and the low nutrient availability make heathlands particularly susceptible to the concurrent impacts of changes in the precipitation patterns and of high airborne N loads. Up to now, research on *Calluna* heathlands has focused mainly on the single effects of these two important global change drivers. A recent study, however, examined the interaction effects of experimentally imposed drought and N addition and showed that drought susceptibility of *Calluna* increases with N availability (Meyer-Grünefeldt et al. 2015). N fertilisation increases the biomass shoot–root ratios

of *Calluna*, resulting in higher evaporative demands (Gordon et al. 1999a; Sæbø et al. 2001). Consequently, biomass shoot–root ratios are considered a crucial trait for the plants' drought susceptibility (Meyer-Grünefeldt et al. 2015).

However, to the best of our knowledge, there has been no study on the plants' response to the simultaneous impacts of drought events and enhanced N availability related to the position of the population within the range of *Calluna* heathlands. Due to the broad distribution range of *Calluna* heathlands, it is likely that individuals from different populations have evolved different adaptive traits depending on the prevailing environmental conditions. Especially plants of marginal populations (i.e. the southern and eastern range margins from a European perspective), where summer drought events can limit a further range extension (Loidi et al. 2010), are probably better adapted to drier conditions than plants of the populations in the centre of the distribution range (i.e. central Europe). Characterising these intraspecific variations of *Calluna* is crucial for anticipating the susceptibility of different populations or ecotypes to the impacts of global change and thus for adapting management strategies at these sites.

In this study, we analysed single and combined effects of experimentally imposed drought and N fertilisation on growth responses of *Calluna* in a full-factorial 2-year greenhouse experiment. In order to test whether the plants' responses to the impacts of these global change drivers are provenance specific and thus depend on the local environmental conditions, we investigated the treatment responses of two populations from central provenances and two populations from marginal provenances. We measured biomass production and allocation as growth responses. Additionally, we analysed tissue $\delta^{13}\text{C}$ values as a proxy for water-use efficiency to examine the plants' water stress. We assumed that *Calluna* plants of marginal populations are more resistant to drought than plants of the central populations. Specifically, we hypothesise that (i) the higher drought resistance of the marginal populations is linked to biomass allocation patterns (in terms of shoot–root ratios) and that (ii) N fertilisation can offset the differences in drought susceptibility between CP and MP (due to an N-induced increase of shoot–root ratios across populations).

Materials and methods

Populations and plant material

Calluna seeds of two central (CP) and two marginal (MP) populations were collected in autumn of 2009. Both CP are situated in the centre of the European range of *Calluna* heathlands and are located in Northern Germany (central population west and east, henceforth referred to as CP_W and CP_E; for further site characteristics, see Table 1). The MP are located at the eastern and the southern European range margin of *Calluna* heathlands (henceforth referred to as MP_E and MP_S, respectively). The MP_E is situated in East Germany and the MP_S in North Spain (Cantabrian Mountains; for further site characteristics, see Table 1).

Experimental design

The present study was carried out over two growing seasons (2010–2011) in a non-tempered greenhouse at the University of Lüneburg, Germany (53°13'N, 10°24'E). *Calluna* seeds were sown in germination dishes in January 2010. Seedlings emerged in May and were transplanted into round pots (6 cm in diameter, 4.6 cm height) in the middle of June (4 individuals per pot with a planting distance of 2 cm). Pots contained humus collected from the upper soil horizon of a podsol in the nature reserve Lüneburger Heide (soil ecological characteristics: pH_{H2O}: 4.1, base saturation: 21 %, *S* value: 0.9 mmol_c 100 g⁻¹, N content 600 mg kg⁻¹, P content: 57 mg kg⁻¹). To prevent pot size effects caused by an increase in biomass in the first growing season, plants were re-potted into larger square pots (7.5 × 7.5 × 8 cm³) before the second growing season in May 2011. Position effects in the greenhouse were avoided by relocating the pots every 3 weeks during the two growing seasons.

At the beginning of the first growing season (May 2010), 320 pots per population (CP_W, CP_E, MP_E, MP_S) were randomly assigned to one of the four following treatments (resulting in 80 pots per treatment and population, and a total of 1280 pots): (1) control (2) drought (henceforth referred to as D treatment) (3) N fertilisation (henceforth referred to as N treatment) and (4) a combined D and N treatment (D + N treatment).

In the D treatments, plants were exposed to two drought periods per growing season (August and

Table 1 Site characteristics of the central and the marginal populations

	Central Populations (CP)		Marginal Populations (MP)	
	West	East	East	South
Abbreviation	CP _W	CP _E	MP _E	MP _S
Population name	Lüneburger Heide, Germany	Nemitzer Heide, Germany	Oranienbaumer Heide, Germany	La Majua, Spain
Location	53°09'N 09°56'E	52°58'N 11°21'E	51°46'N 12°21'E	43°01'N 6°05'W
Altitude (m a.s.l.)	105	25	70	1767***
General climatic conditions	Sub-Atlantic	Sub-Continental	Continental	Sub-Mediterranean-Atlantic
Annual precipitation (mm year ⁻¹)	827.0	611.0	604.0	1126
Annual mean temperature (°C)	9.0	8.6	8.7	8.0
Climatic site characteristics				
Length of drought periods (days/year)	21	24	26	>30
Precipitation July–August (mm)	152	120	112	75
Heavy summer rain events (>50 mm/day)	No	No	Yes	Yes
Days per year with temperatures >25 °C	29	36	50	n/a
Sunshine hours (July)	208	214	243	264

Explanatory notes: Length of drought periods: number of consecutive days with <1 mm precipitation; n/a: data not available. Climatic data compiled from

Germany: German Weather Service (www.dwd.de), Härdtle et al. (2007)

Spain: Instituto Tecnológico Geominero de España (1995), Agencia Estatal de Meteorología (AEMET)

September in 2010, July and August in 2011). To avoid dieback, D treatments lasted until the shoot tips showed first wilting symptoms. In the first growing season, D treatments resulted in a decrease of soil water content of 30 and 20 % after 10 and 8 days, respectively (percentage loss compared to field capacity). In the second growing season, the decrease of soil water content was 50 and 46 % after 5 and 4 days, respectively. Soil water losses were determined by comparing pre- and post-treatment pot weights. N-treated pots (i.e. N and D + N treatments) received 35 kg N ha⁻¹ year⁻¹ (as NH₄NO₃) dissolved in deionised water, and control pots received the same amount of deionised water only. The solutions were applied weekly from July to September in 2010 and June to September in 2011.

Sampling and chemical analyses

We analysed biomass production and allocation as well as tissue δ¹³C values of aboveground and

belowground biomass after each growing season. At harvest, 40 pots per treatment and population were separated into aboveground and belowground biomass after one (2010) and two (2011) growing seasons, respectively. Biomass samples of four pots were randomly assigned to one replicate, resulting in 10 replicates per treatment, population and year. Biomass samples were dried at 40 °C for 48 h and weighed to determine biomass production. The biomass allocation was calculated as the biomass shoot–root ratio (henceforth referred to as shoot–root ratio) from the aboveground and belowground dry weight.

Tissue δ¹³C values were used as a proxy to assess the plant's water status. Values are related to the intrinsic water-use efficiency, the ratio of net assimilation and stomatal conductance (Farquhar et al. 1982). Under water stress, plants close their stomata to minimise transpiration losses. This leads to a decrease in the intercellular partial pressure of CO₂. The discrimination against the heavier C isotope (¹³C)

declines and, thus, the $\delta^{13}\text{C}$ values and the water-use efficiency increase (Farquhar et al. 1989).

Samples were ground in an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany) or a mixer mill (MM 400, Retsch, Haan, Germany) and re-dried at 40 °C (48 h) for ^{13}C analyses. $\delta^{13}\text{C}$ values 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). The C isotope composition of the samples was calculated as the $\delta^{13}\text{C}$ value (in ‰) as follows:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000,$$

where R_{sample} and R_{standard} are the $^{13}\text{C}:^{12}\text{C}$ ratios in the sample or the PeeDee Belemnite standard, respectively. The relative precision of repeated analyses of IAEA standards (IAEA-CH-3) was ± 0.1 ‰.

Statistical analysis

To analyse the treatment effects of N fertilisation and drought on response variables dependent on the position of the population within the distribution range of *Calluna* heathlands (henceforth referred to as Range effect), we compared responses of CP and MP (for CP we reassessed data from Meyer-Grünefeldt et al. 2015). Treatment effects on response variables were tested with linear mixed effects models (LMM) for both years of the greenhouse experiments (i.e. 2010 and 2011 for 1- and 2-year-old plants, respectively). Each LMM included the main-factor effects (i.e. Range effect, N, and D) and all interaction terms, and ‘population’ as a random factor. Prior to analysis, the following response variables were log-transformed to meet model assumptions: aboveground biomass, belowground biomass, shoot–root ratio (for 1-year-old plants), and shoot–root ratio (for 2-year-old plants). A post hoc test (Tukey’s HSD test) was performed for multiple between-population comparisons of response variables in relation to treatments ($\alpha = 0.05$). Simplification of all models was done by stepwise backward selection of fixed factors and the respective interaction terms, until only significant factors/interaction terms remained (with $P < 0.05$; Crawley 2007). Model robustness was assessed by Levene test and visual diagnostic test (Q–Q plots). All analyses were conducted in R 2.12.0 ([http://www.R-](http://www.R-project.org)

[project.org](http://www.R-project.org)) using the packages lme4 (Bates et al. 2014), lmerTest (Kuznetsova et al. 2014), and multcomp (Bretz et al. 2010).

Results

Biomass production and allocation

Aboveground biomass production and shoot–root ratios showed a significant Range effect for 1-year-old, but not for 2-year-old plants (Table 2). In the first year, the aboveground biomass production of plants from the CP was about twice as high as the aboveground biomass production of plants from the MP (Fig. 1a), resulting in 30 % higher shoot–root ratios for CP plants than MP plants (Fig. 1c).

D (i.e. D and D + N) treatments decreased the biomass production of 1-year-old plants (with stronger effects on the belowground biomass), but increased the aboveground biomass of 2-year-old plants (Fig. 1a, b; Table 2). As a consequence, the shoot–root ratios increased for both 1- and 2-year-old plants.

N fertilisation caused a shift in biomass allocation patterns in favour of aboveground biomass (particularly of 2-year-old plants), resulting in a significant increase of shoot–root ratios in both years and across populations (Table 2). N fertilisation particularly increased the aboveground biomass production of 2-year-old *Calluna* from the MP (Table 2), resulting in a significant Range \times N interaction. The increase in aboveground biomass production amounted to +210 and +110 % for MP plants than CP plants, respectively (Fig. 1a).

Range \times D \times N effects were found for the biomass production of 2-year-old plants, but not for the plants’ shoot–root ratios (both years). The combined effects of the D and N treatments were non-additive, with a stronger antagonistic (dampening) interaction for MP plants than for CP plants (Table 2; Fig. 1). Furthermore, antagonistic D \times N interaction effects were found for the aboveground biomass production and allocation for 1- and 2-year-old plants (Table 2).

Tissue $\delta^{13}\text{C}$ signatures

Tissue $\delta^{13}\text{C}$ values of 1- and 2-year-old plants differed between *Calluna* from the CP and the MP, with higher

values found for CP (Table 2; Fig. 2 with the exception of the aboveground tissue $\delta^{13}\text{C}$ signatures of 2-year-old plants).

D (and D + N) treatments increased tissue $\delta^{13}\text{C}$ values for both years and across populations (with the exception of the aboveground tissue $\delta^{13}\text{C}$ signatures of 2-year-old plants), for example, by about 1.0 and 0.5 ‰ in the aboveground tissue of 1- and 2-year-old plants, respectively (Fig. 2). Range \times D effects on tissue $\delta^{13}\text{C}$ values were only significant for the aboveground biomass of 2-year-old plants (Table 2), resulting from an inconsistent response to D treatments between plants from the CP and the MP: Aboveground tissue $\delta^{13}\text{C}$ values of *Calluna* were not influenced by D treatments for CP plants, but decreased for MP plants (Fig. 2a).

Range \times N effects were significant for aboveground tissue $\delta^{13}\text{C}$ values in both years (increasing values), and for belowground tissue $\delta^{13}\text{C}$ values of 2-year-old plants (decreasing values; Table 2). Range \times D \times N effects were found for belowground tissue $\delta^{13}\text{C}$ values of 2-year-old *Calluna*. Range-independent D \times N interactions were significant for aboveground tissue and belowground tissue $\delta^{13}\text{C}$ values of

Fig. 1 Effects of treatments (control; D: drought; N: nitrogen; D + N: drought and nitrogen) and populations (CP_W: central population west; CP_E: central population east; MP_E: marginal population east; MP_S: marginal population south) on aboveground (a) and belowground (b) biomass dry weights (dw, in g) and biomass shoot–root ratios (c) of 1- and 2-year-old *Calluna vulgaris*. Lower case letters indicate significant between-population differences (Tukey's HSD test; $\alpha = 0.05$)

1- and 2-year-old plants, respectively (antagonistic effects; Table 2; Fig. 2a).

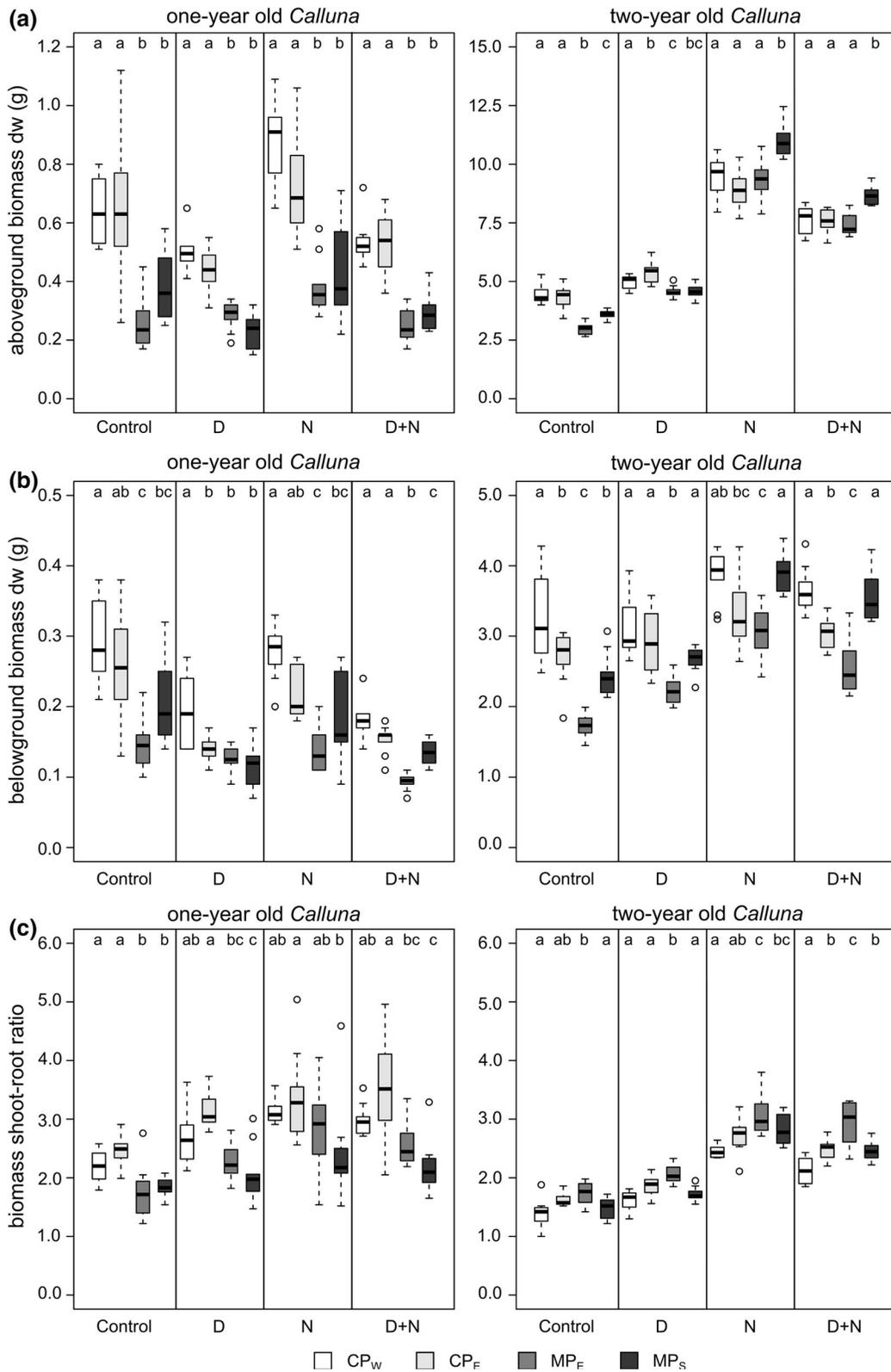
Differences within central and marginal populations

Differences in within-population responses to treatments (i.e. within CP and MP) were mostly non-significant, particularly for 1-year-old plants (cf., for example, controls of 1-year-old plants in Figs. 1, 2). However, we observed that within- and between-population differences became increasingly similar for 2-year-old plants. This was, for example, obvious for response variables such as aboveground biomass production, shoot–root ratios, and aboveground tissue $\delta^{13}\text{C}$ signatures (Figs. 1, 2).

Table 2 Summary of the mixed effects models showing significant main effects and interactions of Range effects (R; central vs. marginal populations), drought (D) and nitrogen

(N) treatments for 1- and 2-year-old *Calluna* plants; numbers show parameter estimates and asterisks the corresponding significances: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

	Aboveground biomass		Belowground biomass		Shoot–root ratio		Aboveground $\delta^{13}\text{C}$		Belowground $\delta^{13}\text{C}$	
1–2 old plants										
Intercept	−0.495	**	−1.593	**	0.852	**	−29.533	***	−29.121	***
R	−0.690	*		*	−0.283	*	−1.888	***	−1.346	*
D	−0.230	***	−0.397	**	0.198	***	1.247	***	0.751	***
N	0.251	**		***	0.332	***			−0.136	*
R \times N							0.389	**		
D \times N	−0.166	*		***	−0.232	***	−0.302	*		
2-year-old plants										
Intercept	4.409	**	2.987	**	0.399	*	−31.542	***	−30.780	***
R									−0.601	*
D	0.785	***		***	0.152	***			0.162	**
N	4.783	***	0.604	***	0.540	***			0.367	***
R \times D			0.329	*			−0.275	*		
R \times N	2.081	***	0.804	***	0.064	*	0.286	*	−0.226	**
D \times N	−2.363	***		***	−0.257	***			−0.214	*
R \times D \times N	−1.025	**	0.502	*					0.378	**



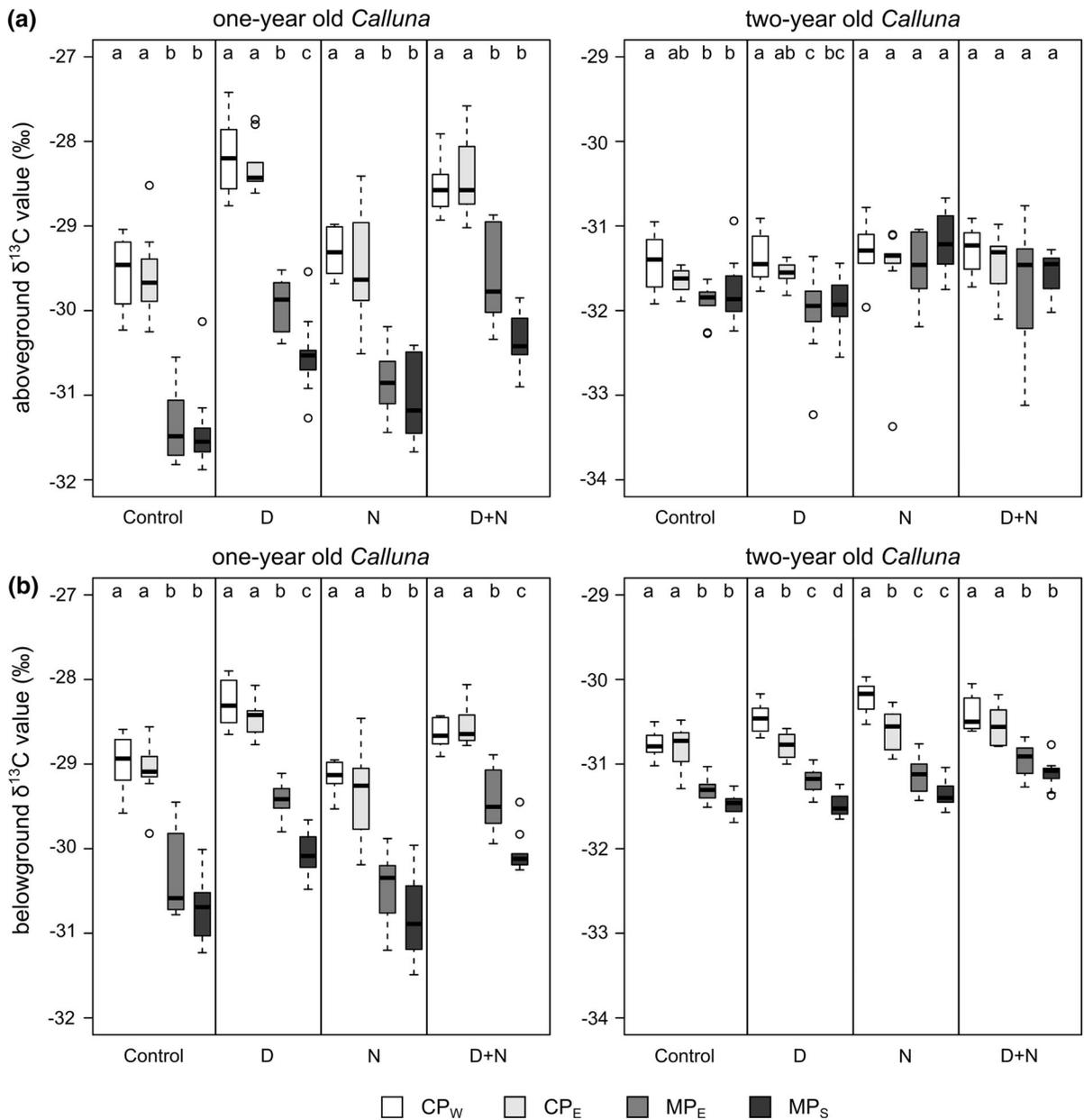


Fig. 2 Effects of treatments and populations (for abbreviations, see Fig. 1 legend) on aboveground (a) and belowground (b) tissue $\delta^{13}\text{C}$ values (in ‰) of 1- and 2-year-old *Calluna*

vulgaris. Lower case letters indicate significant between-population differences (Tukey’s HSD test; $\alpha = 0.05$)

Discussion

Range-related susceptibility of *Calluna* plants to drought

Aboveground biomass production, shoot–root ratios and tissue $\delta^{13}\text{C}$ values of *Calluna* showed a strong

Range effect for 1-year-old plants. MP plants exhibited lower shoot–root ratios and higher discrimination against ^{13}C than CP plants. These trait characteristics suggest a higher resistance to drought, and thus support our general assumption that *Calluna* from the MP is more resistant to drought. In contrast, 1-year-old plants originating from the CP grew faster

(i.e. showed a higher aboveground biomass production and allocation) and had higher shoot–root ratios than MP plants. Shoot–root ratios are considered as a core trait that indicates a plant's drought susceptibility (Weiner 2004), because shoot–root ratios are positively correlated with the plants' transpiration rates and evaporative demand (Gordon et al. 1999a). Therefore, smaller shoot–root ratios provide a selective advantage for the establishment of seedlings, particularly at sites with drought periods during the growing season (Rose et al. 2009). This drought tolerance strategy is not essential for plants from the CP because of the moderate Atlantic climate prevailing at their sites.

This interpretation is supported by findings for the tissue $\delta^{13}\text{C}$ values, which were lower for plants from the MP than for those of the CP. The discrimination against ^{13}C was still higher for plants from the MP even after the D treatments. The relatively low aboveground biomass of 1-year-old MP plants resulted in reduced evaporative demands, and most likely in a higher stomatal conductance than CP plants in a better water status (Lauteri et al. 2004). Therefore, lower tissue $\delta^{13}\text{C}$ values may indicate further adaptive mechanisms to water shortage which allows juvenile plants to cope better with drought periods during the growing season (Lauteri et al. 2004).

We hypothesise that trait differences (e.g. in terms of shoot–root ratios and tissue ^{13}C signatures) between plants from different provenances (i.e. CP and MP) are related to adaptive processes to the climatic conditions typical of the respective sites (Table 1, Macel et al. 2007; Kuster et al. 2013). At sites of the southern range margin (MP_S), plants regularly experience prolonged drought events during summer (i.e. >30 days), and July–August precipitation contributes little to the total annual precipitation (75 mm of 1126 mm). Under continental climate (typical of MP_E), the length of drought periods also is longer than at CP sites (corresponding to the total sunshine hours in July), and days with temperatures >25 °C are more frequent. In addition, MP sites are characterised by heavy summer rain events (e.g. during thunderstorms), which are often related to a high surface runoff and thus not sufficiently replenish the soil water storage. Water shortage in summer particularly affects *Calluna* rejuvenation (i.e. 1-year-old plants), because of its low tissue lignification and unfavourable shoot–root ratios (Meyer-Grünefeldt et al. 2015). The above-mentioned

trait differences, therefore, can be interpreted as adaptations to prevailing climatic conditions (Aranda et al. 2010). Similar observations were reported for tree species, according to which marginal provenances also exhibited a better drought adaptation (Rose et al. 2009).

Interestingly, plants did not adjust biomass allocation to belowground biomass as a result of the D treatments in the second growing season. Different water availabilities were very likely not regulated by allocation patterns but by stomatal responses or the reduction of photosynthetic and nutrient uptake (and thus growth) rates (McConnaughay and Coleman 1999). Different water availabilities were very likely not regulated by allocation patterns but by plastic stomatal responses, or the reduction of photosynthetic rate and nutrient uptake decreased equally and thus an adaptation of allocation patterns was not essential (McConnaughay and Coleman 1999).

Effects of N fertilisation on the plants' drought susceptibility

Our findings indicate that N fertilisation has the potential to foster the drought susceptibility of *Calluna* plants. Plant growth was primarily N-limited, since N addition resulted in an increase in biomass production across populations (except for the belowground biomass production of 1-year-old plants; von Oheimb et al. 2010; Phoenix et al. 2012). Furthermore, higher N availability caused a strong shift in biomass allocation patterns in favour of aboveground biomass (also reflected by increased shoot–root ratios), likely because plants tended to optimise the relation between nutrient and C acquisition in order to maximise growth rates (McConnaughay and Coleman 1999). Increasing shoot–root ratios, in turn, has the potential to increase the plants' drought sensitivity (see discussion above). Shifts in shoot–root ratios (resulting from N fertilisation) were more pronounced for 2-year-old plants and MP (R × N interaction for 2-year-old plants). This response coincided with a stronger increase of aboveground tissue $\delta^{13}\text{C}$ values of MP in the N treatments, likely resulting from an adjustment of the plants' water-use efficiency to counteract the increasing evaporative demands (Farquhar et al. 1989; Gordon et al. 1999b).

Although only the biomass production of 1-year-old *Calluna* was negatively affected by D treatments,

we found antagonistic (dampening) $D \times N$ effects on aboveground biomass production and allocation for both growing seasons (Meyer-Grünefeldt et al. 2015). Thus, N-fertilised plants showed lower aboveground biomass production and hence lower shoot–root ratios than could be inferred from single (D and N) treatment responses. This could be attributable to the fact that N uptake was reduced as a result of lowered transpiration rates under the simultaneously applied D treatments (Meyer-Grünefeldt et al. 2015). Interestingly, even if effects of the single N and D treatment on biomass production differed between the plants from the CP and those from the MP, we found no $Range \times D \times N$ effects for the plants' shoot–root ratios. This supports our second hypothesis that N fertilisation can offset differences in drought sensitivity between CP and MP, because $D \times N$ interactions proved to be range-independent (given the value of shoot–root ratios to assess responses of *Calluna* plants to water shortage; see discussion above). Even if the physiology of MP plants is particularly well adapted to drier environments, the higher drought resistance of these populations can be attenuated by an N-induced increase in shoot–root ratios.

In summary, *Calluna* plants from the southern or eastern range margin proved to be better adapted to drought events than those from the centre of the European range, but our results suggest that N deposition can offset differences in drought sensitivity between *Calluna* plants originating from different European provenances.

Population-specific effects

Despite the geographic distance between the populations (especially in the case of the MP) response patterns to treatments were remarkably similar within the CP and the MP. This indicates that the adaptation of plants to the environmental conditions of their provenance is a decisive factor for the treatment responses and depends on the position of the population within the distribution range of *Calluna* heathlands (see discussion above). Although some differences in treatment responses were found within the CP and the MP, the performances of the 1-year-old plants were particularly similar. In view of the fact that this stage of life is of great importance for the conservation of heathland ecosystems (Marrs 1986; Marrs and Diemont 2013), it is essential to distinguish

central and marginal populations, although not necessarily the individual populations, in order to predict the impacts of multiple global change drivers in terms of drought and N loads across the broad distribution range of heathlands.

In conclusion, atmospheric N deposition might hamper the success of nature conservation measures such as “assisted migration” that consider marginal populations as a source of drought-tolerant ecotypes in the face of climate change (Richter et al. 2012). In order to improve management and conservation efforts, future research on heathland ecosystems should focus on multi-factor studies to anticipate possible interaction effects of different global change drivers (Andresen et al. 2010; Arndal et al. 2013), taking into account that plant responses might differ depending on their respective provenance and age.

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