Competitive interactions shape plant responses to nitrogen fertilization and drought: evidence from a microcosm experiment with Lilium bulbiferum L. and Secale cereale L.

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Abstract Many recent studies have analysed plant species responses to environmental change, but interactive effects of global change drivers and how they are modulated by biotic interactions are still poorly understood. In a mesocosm experiment, we studied the interactive effects of nitrogen (N) fertilization and drought events on plant growth and how these effects are shaped by competitive interactions, using a segetal plant community typical of the lowlands of central Europe (composed of Lilium bulbiferum (segetal species) and Secale cereale (crop species)). We expected that N fertilization increases the drought sensitivity of Lilium (negative interaction effect), and that these effects are shaped by interspecific competition with Secale. Secale and Lilium showed opposing responses to N fertilization (second year of the experiment): Whilst Secale aboveground and belowground biomass almost doubled with N fertilization, Lilium aboveground and belowground biomass showed no response or decreased, respectively, providing Secale with a competitive advantage. Lilium aboveground tissue dieback (as a proxy for growth vigour) was 22% in N and 35% in drought treatments (control: 6%), but reached 91% when combining these treatments. Increasing Lilium tissue dieback was strongly related to decreasing belowground (root) biomass, caused by both negative direct effects of combined treatments (N fertilization + drought), and negative indirect effects acting via treatment-induced increase in Secale biomass. Our results demonstrate that competitive interactions can shape the effects of global change drivers on plant growth. This knowledge in turn could be important for plant species.

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conservation, particularly in the face of ongoing shifts in environmental conditions.

**Keywords**  
δ¹³C signature · Global change · Interspecific competition · Root:shoot ratio · Segetal plants

**Introduction**

Climate change and ecosystem eutrophication with nitrogen (N) are two major components of global change, with far-reaching consequences for biodiversity and ecosystem functioning on global and local scales (Sala et al. 2000; Valliere et al. 2017; IPBES 2019). Recent climate projections predict rising mean annual temperatures, changing precipitation regimes, and shifts in the frequency and magnitude of extreme weather events, including more severe summer droughts (IPCC 2013). The deposition of reactive nitrogen compounds has tripled on a global scale since the beginning of the industrialization in the nineteenth century (Galloway et al. 2004). As a consequence, both climate change and N fertilization may influence plant species coexistence due to effects on ecophysiological processes controlling plant growth rates and competitive interactions (Fowler et al. 2013; Ferrarini et al. 2017; Baattrup-Pedersen et al. 2018; Rowland et al. 2021).

Drought events affect ecosystem functions such as primary production and carbon allocation, but also the functional composition of plant communities due to diversity-specific trait spectra and concomitant differences in morpho-physiological adaptations of species to drought stress (Zandalinas et al. 2018). Recent studies have demonstrated that drought resistance of plants is strongly controlled by traits that are directly associated with physiological processes (stomatal conductance, hydraulic pressure, carboxylation capacity), rather than morphological traits (Rowland et al. 2021). These findings suggest that drought events might act as a filter favouring species that better tolerate or cope with hydraulic stress, which in turn provides a competitive advantage during periods of water shortage. Ecosystem eutrophication with N is a further important driver of global change (Galloway et al. 2004; Fowler et al. 2013). Since N is a (co-)limiting nutrient in many ecosystems, N fertilization affects primary productivity and interactions between plant species, but also their sensitivity to abiotic and biotic stressors such as drought, frost events, and herbivory (Bobbink et al. 2010). In many cases, N fertilization causes a shift in plant species composition to the benefit of fast growing and highly productive species, which in turn outcompete the less productive ones (Bobbink et al. 2010; Friedrich et al. 2011). In this way, N fertilization constitutes one of the most substantial drivers of current global biodiversity loss (Drewniak and González-Meler 2017; Valliere et al. 2017).

Whilst N fertilization has affected ecosystem processes over the past decades, the impact of climate change is expected to increase in importance over the course of this century (IPCC 2013). This means that ecosystems with already altered N cycles and levels are now subject to climate shifts, and both factors will continue to act upon ecosystems in the coming decades (Greaver et al. 2016). Thus, alteration of N cycles via eutrophication may in turn alter the response of ecosystems to climate change. Combinations of abiotic stressors, however, often are non-additive, and thus cannot be inferred from approaches in which environmental factors were assessed singularly (Dieleman et al. 2012; Xu et al. 2018). For example, it is conceivable that plant responses to climate change may interact with N fertilization, likely due to fertilization effects on plant traits such as height growth or root:shoot ratios (Dziedek et al. 2016a). Increasing above-ground investments are often accompanied by increasing transpiration rates and thus by increasing evaporative demands, whereas lowered below-ground investments may constrain water uptake and thus a plant’s water supply (Meyer-Grünefeldt et al. 2015a, b). In this way, N fertilization has the potential to increase the drought sensitivity of plants, particularly when N fertilization predates drought events (Turner and Henry 2009; Meyer-Grünefeldt et al. 2015a; Dziedek et al. 2016a). Moreover, nitrogen concentrations are increasing over the years, whereas drought events often occur as sporadic extreme events. Thus, drought events represent a further environmental stressor on top of chronic changes in the nutritional status of an ecosystem.

In addition to the effects of abiotic environmental changes, biotic factors such as competitive interactions also may shape species and community responses to global change. Competitive interactions
are often mediated by plant traits that determine a species’ competitive performance in a given plant community (Guittar et al. 2016; Xu et al. 2018). For example, field experiments of Valliere et al. (2017) showed that experimental N additions exacerbated the loss of native shrub species in a Mediterranean shrubland and accelerated the encroachment of non-natives, especially annual grasses, all of which strongly benefited from increasing N availability. An important trait supporting the competitiveness of (nonnative) grasses over shrubs was that grass species were characterized by high biomass investments to shallow roots (particularly under increasing N availability), which rapidly decreased soil moisture in the upper soil profile to the detriment of deeper-rooted perennials (Wood et al. 2006; Valliere et al. 2017). Contrasting traits in terms of N use may thus co-determine plant community responses to global change (Mendoza et al. 2016; Ferrarini et al. 2017).

To improve our understanding of interactive effects of co-occurring drivers of global change, the present study analysed combined effects of N fertilization and drought events on plant growth and how these effects are mediated by interspecific competition of plant species.

We performed a 2-year mesocosm experiment to compare the effects of drought events on N-fertilized and non-fertilized plants, taking a segetal plant community typical of the lowlands of central Europe. This plant community often consists of only two species, *Lilium bulbiferum* L. as the segetal plant species and *Secale cereale* L. as crop species (Sclerantho annui-Arnoseridetum minimae; Ellenberg and Leuschner 2010; Bos 2012). Referring to this community we considered *Lilium bulbiferum* L. as the target species in our experiment. Nowadays, *Lilium bulbiferum* is highly endangered due to continuous habitat loss, management intensification, and more frequent drought events (Ludwig and Schnittler 1996; van Elsen et al. 2006; Bos 2012; Meyer et al. 2013, 2015). Current conservation measures aim at a long-term protection of remnant *Lilium bulbiferum* population by establishing rye fields that are subject to organic farming, using a typical lowland rye breed adapted to sandy and low-nutrient soils (so-called Champagne rye; Bos 2012). Since *Lilium bulbiferum* populations are still decreasing, we selected this plant community as an example to scrutinize the effects of currently acting global change drivers in relation to competitive interactions of both plant species.

Our goal behind this experimental setting was to analyse how N-fertilized plants respond to subsequent drought events, simulating field conditions in which plant communities with already altered N levels (due to fertilizer application or atmospheric N deposition) are nowadays subject to climate change. Specifically, we wanted to know how combined effects of N fertilization and drought events affect growth of *Lilium bulbiferum* and how these effects are modulated by interspecific competition with the crop species *Secale cereale*. Growth responses were measured in terms morphological traits (aboveground and belowground biomass, root:shoot ratios, and tissue dieback) and physiological traits (aboveground and belowground tissue δ13C signatures and C:N ratios). We hypothesized that (i) N fertilization increases the drought sensitivity (i.e. relative shifts in trait values in response to treatments, such as decreasing biomass productivity and tissue C:N ratios, and increasing tissue dieback and δ13C signatures) of *Lilium bulbiferum* (i.e. non-additive response), and that (ii) *Lilium bulbiferum* responses to treatments are shaped by competitive interactions with the crop species *Secale cereale*.

**Methods**

**Experimental design**

Our mesocosm experiment was conducted in a greenhouse in the experimental garden of the University of Lüneburg (Lüneburg, Germany, 53°14′23.8″N, 10°24′45.5″E). The mesocosm pots used were 0.3 × 0.3 × 0.3 m³ in dimension (volume: 27 L) and were filled with soil material originating from the natural habitat (i.e. low-intensively managed arable fields) of *Lilium bulbiferum* in the river Elbe valley in Lower Saxony (53°10′08″N, 11°00′04″E) to simulate a high degree of natural site conditions (soil characteristics of the upper mineral soil: texture: slightly loamy sand, pH(H₂O): 4.8).

In October 2015 five bulbils of *Lilium bulbiferum* (sampled from the leaf axils from natural populations and at the same sites where soil samples were taken; henceforth referred to as *Lilium*) and 20 seeds of winter rye (*Secale cereale*; seed farm Hof Tütsberg,
Schneverdingen, Germany; henceforth referred to as *Secale*) were introduced into each mesocosm. The ratio of five *Lilium* bulbils and 20 *Secale* seeds was chosen to simulate the ratio of these species as they appear in the real segetal plant community (Bos 2012). Four *Lilium* bulbils were placed in the corners (corner distance 8–10 cm) and one in the centre of the mesocosms. *Secale* seeds were sown in two rows with 2.5 cm between-seed distance and a sowing row distance of 12.5 cm (corresponding to the sowing density used in the arable fields; seed row distance to the mesocosm’s edge was 8.7 cm at each side; planting design see Fig. 1). This design was used to simulate growth conditions of *Lilium* in its natural habitat (i.e. in a rye field). We established a total of 40 mesocosms, 20 of which were randomly assigned to two treatments in March 2016 (start of growing season): control and N fertilization treatment (henceforth referred to as control and N treatment, respectively). In the N treatment, N was applied as NH₄NO₃ dissolved in deionized water and at 11 occasions from March to July 2016 (0.2 L solution per occasion). The total amount of N applied was equivalent to 40 kg ha⁻¹ year⁻¹, which corresponds to both current maximum N deposition rates in the lowlands of central Europe (Vivanco et al. 2018) or restricted N fertilizer application rates in arable fields subject to plant species protection targets under organic farming (Kuhn 2017). Non-fertilized mesocosms received 0.2 L deionized water at the same time when N solutions were applied. In August 2016, all plants were harvested by clipping stalks above the soil surface to imitate a late-summer field harvesting. The below-ground biomass (and particularly the bulbs of *Lilium*) remained untouched during harvest (and all plants survived and regrew in the second year of the experiment; see below).

In October 2016, 20 *Secale* seeds were again introduced into each mesocosm, following the procedure applied in 2015 (since *Secale cereale* is an annual species). In March 2017, mesocosms were subject to a total of four treatments (i.e. *n* = 10 per treatment). To this end, 10 out of the 20 control mesocosms from 2016 were randomly assigned to a control, and the remaining 10 mesocosms to a drought treatment (henceforth referred to as D treatment). In addition, 10 out of the 20 N treated mesocosms from 2016 were randomly assigned to an N treatment (i.e. continuation of the N fertilization), and the remaining 10 mesocosms to an N + D treatment (i.e. a combination of N and drought treatments, henceforth referred to as ND treatment). N treatments were performed in the same way as in 2016, but N was applied on 8 occasions between March and June to allow for a timely start of the subsequent drought treatments. In the D and ND treatments, mesocosms were subject to a drought period (no watering of plants) starting in the mid of June and lasting for four weeks. Drought treatments resulted in a decrease of the mesocosms’ soil water content by 31.7% (mean percentage loss compared to field capacity at the end of the drought period; mean amount of soil water in the mesocosm pots under field capacity: 2.7 L, mean amount of soil water after the drought treatment: 1.84 L; water loss in relation to field capacity: 31.7%). Water loss was quantified by comparing pre- and post-treatment mesocosm weights. With the exception of the drought period, mesocosms were regularly watered (both years) and the soil moisture content was kept close to field capacity (about 25% of the total soil pore volume). All pots were randomly relocated every two weeks to avoid position effects (both years). The mean

![Fig. 1](https://example.com/fig1.png)  
*Fig. 1* Planting design of the mesocosm experiment. Mesocosm pots were 30 cm × 30 cm in width (black frame). In total, 20 seeds of *Secale cereale* (blue circles) and five bulbils of *Lilium bulbiferum* (orange circles) were planted in the first year of the experiment (and *Secale cereale* seeds again in the second year of the experiment; further explanations see text)
temperature in the greenhouse was 16.5 °C and 17.2 °C, and the mean relative humidity was 67.6% and 69.4% in the first (2016) and second year (2017) of the experiment, respectively (means from the beginning of March to the end of August).

Plant harvest and measurements of response variables

In 2016, the following response variables were quantified: aboveground biomass dry weight of Secale and Lilium after harvest. In 2017, the experiment was completed in August and the following response variables quantified: aboveground and belowground biomass dry weight of Secale and Lilium, necrotic/ dead aboveground tissue of Lilium, C:N ratio and δ13C signatures of the aboveground biomass, coarse roots, and bulbs of Lilium (for the relevance of the response variables measured in the context of climate change and fertilization experiments see Dziedek et al 2016b; Meyer-Grünefeldt et al. 2015b). Aboveground biomass dry weight was determined after harvest of all aboveground plant parts of Secale and Lilium (clipping stalks above the soil surface). Belowground biomass was quantified by carefully wet sieving roots until soil residues were removed as far as possible. Subsequently, roots were towelled down and separated for each of the two species using a stereomicroscope. For Lilium, belowground biomass (produced by the plants over the 2 years of the experiment) was differentiated for bulbs, coarse, and fine roots, considering a root diameter of 2 mm as threshold between fine and coarse roots (Rose et al. 2009). As the separation and cleaning procedure of Secale roots (produced by the plants in 2017) and Lilium fine roots was very laborious, we quantified these variables for a subset of five randomly selected mesocosms per treatment only (i.e. \( n = 5 \) per treatment).

All biomass samples were dried to a constant weight at 40 °C and afterwards the dry weight was quantified (for Secale in g per mesocosm, for Lilium in g per individual per mesocosm, calculated as mean of the respective five individuals). For both species we quantified root:shoot ratios (RSR; \( n = 5 \) per treatment in 2017), defined as the quotient of aboveground biomass dry weight and (coarse + fine) root biomass dry weight (Friedrich et al. 2011). At the end of the D treatment, the amount of necrotic/dead aboveground tissue (henceforth referred to as ‘tissue dieback’) of each Lilium individual was visually estimated (in % necrotic/damaged tissue per plant individual; i.e. leaf cells were damaged or dead, recognizable by a corresponding discoloration (brownish or black) and tissue dieback per microcosm was quantified as the mean of individual responses (using the relative importance of each individual in terms of its contribution to total tissue dieback of all Lilium plants per mesocosm).

Analyses of morphological responses were complemented by the analyses of physiological responses for Lilium (tissue C:N ratios and δ13C signatures, only for living tissue and for 2017). To this end, biomass samples of the aboveground biomass, coarse roots, and bulbs were ground in a centrifugal mill (ZM 200, Retsch, Haan, Germany) and re-dried at 40 °C for 3 days. Tissue C:N ratios and δ13C signatures were measured using a continuous flow elemental analyzer-isotope mass spectrometer (vario EL cube, Elementar, Hanau, Germany, coupled to an Isoprime Isotope-ratio mass spectrometer, Isoprime Ltd., Cheadle Hulme, UK). Isotope signatures were presented in the delta notation (in ‰) as a relative deviation from an international standard (PeeDee Belemnite). The relative precision of repeated analyses of an International Atomic Energy Agency-standard (IAEA-CH-3) was ± 0.1‰.

Statistics

As a first step we assigned all responses variables measured to a group of morphological and a group of physiological response variables and tested by means of a MANOVA, whether grouped variables were differently affected by treatments (N, D, ND) and Secale aboveground biomass (multivariate response; Quinn and Keough 2002; Crawley 2007). Grouped morphological variables were as follows: Lilium aboveground biomass, Lilium belowground biomass, Lilium root:shoot ratio, and amount of necrotic/dead tissue. Grouped physiological variables were: Lilium aboveground biomass δ13C signature, Lilium coarse root δ13C signature, Lilium aboveground biomass C:N ratio, and Lilium coarse root C:N ratio (data from the second year of the experiment). These response variables were selected in correspondence to those considered in the linear models (see below). We transformed the variables ‘tissue dieback’ and ‘Lilium aboveground biomass’ (log- and square root-
transformation, respectively) to meet model assumptions (Crawley 2007).

Second, we used linear models to explore the effects of experimental treatments and of interspecific competition on morphological and physiological response variables of Lilium (data for 2017; using the morphological and physiological response variables mentioned above). In the full model we considered the treatments N, D, ND, and Secale aboveground biomass as explanatory variables and included all possible two-way interaction terms between treatments and Secale aboveground biomass (to test for possible treatment effects acting upon Lilium via shifts in Secale aboveground biomass, i.e. treatment-induced shifts in the interspecific competition between Lilium and Secale). We omitted the variable Secale belowground biomass from the models due to its high positive correlation with Secale aboveground biomass ($r = 0.90$). The variables 'tissue dieback' and 'Secale aboveground biomass' were log-transformed to meet model assumptions (Crawley 2007). Determination of the most parsimonious model was based on the Akaike information criterion (AIC), and the model with the smallest AIC was chosen as the best-fitting model (Burnham and Anderson 2002; Tredennick et al. 2021). Model assumptions were visually checked and confirmed according to Zuur et al. (2009).

Third, we explored the multiple relationships (direct and indirect effects) between N fertilization, drought, interspecific competition, and target species performance using structural equation models (SEMs; applied to second-year data). Specifically, we tested whether (i) treatments (N, D, ND) have a direct effect on growth (total biomass) and RSR (biomass allocation) of Lilium, but also (ii) an indirect effect related to treatment-induced shifts in Secale’s biomass and thus competitive Secale-Lilium interactions, which in turn could mediate treatment effects on Lilium growth and performance (tissue dieback). Thus, we were interested in the following pathways: (i) N, D, ND effects on Secale biomass, (ii) N, D, ND and Secale biomass effects on Lilium biomass, (iii) N, D, ND, and Lilium biomass effects on Lilium RSR ratios, and (iv) effects of Secale biomass, Lilium biomass, and Lilium RSR on Lilium tissue dieback (SEM model). In this way, SEM path analyses were considered to complement outcomes of linear models (ideally, our design should have included monocultures of Lilium and Secale in response to treatments, but this would have tripled the number of microcosms and was not feasible due to logistical constraints). Tissue dieback was used as an indicator for Lilium’s growth vigour and competitive performance (Dziedek et al. 2016b), and the aboveground biomass of Secale was used as a proxy for its competitive strength, assuming that an increase in Secale’s aboveground biomass will lead to a reduction in the competitive ability of Lilium. To support the interpretation of Lilium growth responses to treatments and Secale biomass, we also calculated SEMs in which the variable “total Lilium biomass” was replaced by “Lilium aboveground biomass” and “Lilium belowground biomass” (see Supplementary Material Fig. S1 and S2, respectively). Treatments (N, D, ND) were coded as binary variables (0 = no treatment, 1 = treatment) and were treated as numeric predictors in the model (Rosseel 2012). Tissue dieback was log-transformed to improve linearity with other variables in the model (Grace et al. 2010). Model fit statistics were evaluated following Kline (2014): Chi-square test with associated $P$-value ($P > 0.05$ indicates that sample and observed covariance matrices are statistically not different), standardized root mean square residual (SRMR) and comparative fit index (CFI). Low values of SRMR ($< 0.10$) and high values of CFI ($\geq 0.90$) indicate a good model fit. All analyses were conducted in R (version 3.3.1) using the package lavaan (Rosseel 2012).

**Results**

**Treatment effects on Secale biomass**

Secale aboveground and belowground biomass increased in N-fertilized mesocosms ($P < 0.05$; Table 1). In the N treatments, aboveground biomass almost doubled in comparison to the control (second year). In contrast, the D treatment had no effect on the Secale aboveground and belowground biomass. In addition, Secale root:shoot ratios remained unaffected across treatments (Table 1).

**Treatment effects on morphological response variables of Lilium**

Grouped morphological response variables of Lilium significantly responded to the D treatment ($P = 0.036$), the ND treatment ($P < 0.001$), and to
Secale aboveground biomass ($P = 0.061$; Table 2). In addition, grouped morphological variables were significantly affected by interactions of Secale aboveground biomass with N and ND treatments ($P = 0.020$ and 0.029, respectively; Table 2).

If single response variables are considered, Lilium aboveground biomass positively responded to N fertilization in the first year of the experiment (i.e. of one-year old plants; $P < 0.05$; Table 1). In the second year, Lilium aboveground biomass was not affected in N-fertilized mesocosms, and fine and coarse root biomass decreased (i.e. of two-year old plants; Fig. 2a, b). This decrease in belowground biomass was particularly strong in the ND treatment (including biomass of bulbs; by 32% compared to the control; $P < 0.05$; Table 1, Fig. 2b). In addition, Lilium RSR showed a large decrease in N-fertilized mesocosms (by 33% in the N and by 29% in the ND treatment compared to the control; Fig. 2c). Lilium tissue dieback (necrotic/dead aboveground tissue) was

### Table 1 Treatment effects on morphological and physiological response variables (first year: means of $n = 20$; second year: means of $n = 5$ for Secale/Lilium (fine) root/total belowground biomass and root:shoot ratios, and $n = 10$ for all other variables, ± 1 SE in brackets)

| Treatment Response variables | Control | N | D | ND |
|-----------------------------|---------|---|---|----|
|                            | Mean (SE) | Mean (SE) | Mean (SE) | Mean (SE) |
| **First year** | | | | |
| Secale aboveground biomass (g per pot) | 12.51$^a$ (0.39) | 16.90$^b$ (0.62) | | |
| Lilium aboveground biomass (g per plant) | 0.015$^a$ (0.001) | 0.022$^b$ (0.002) | | |
| **Second year** | | | | |
| Secale aboveground biomass (g per pot) | 11.39$^a$ (1.18) | 22.19$^b$ (2.02) | 9.28$^a$ (0.67) | 20.80$^b$ (1.47) |
| Secale belowground biomass (g per pot) | 8.59$^{ab}$ (1.09) | 15.07$^c$ (2.98) | 4.48$^a$ (0.58) | 11.97$^{bc}$ (1.17) |
| Secale root:shoot ratio | 0.64$^a$ (0.06) | 0.71$^a$ (0.14) | 0.46$^a$ (0.02) | 0.51$^a$ (0.04) |
| Lilium necrotic/dead tissue (%) | 5.78$^a$ (2.34) | 21.62$^{ab}$ (7.17) | 35.48$^b$ (8.11) | 91.00$^c$ (8.15) |
| Lilium aboveground biomass (g per plant) | 0.57$^a$ (0.08) | 0.55$^a$ (0.09) | 0.59$^a$ (0.03) | 0.39$^a$ (0.05) |
| Lilium total belowground biomass (g per plant) | 10.49$^a$ (1.82) | 6.58$^{ab}$ (2.02) | 7.36$^{ab}$ (0.35) | 3.42$^b$ (0.85) |
| Lilium fine root biomass (g per plant) | 3.82$^a$ (0.79) | 1.58$^b$ (0.62) | 1.28$^{ab}$ (0.12) | 0.81$^b$ (0.17) |
| Lilium coarse root biomass (g per plant) | 2.15$^a$ (0.50) | 0.72$^b$ (0.13) | 0.95$^b$ (0.08) | 0.52$^b$ (0.08) |
| Lilium bulb biomass (g per plant) | 5.12$^a$ (0.75) | 4.20$^{ab}$ (0.73) | 5.44$^a$ (0.26) | 2.38$^b$ (0.40) |
| Lilium root:shoot ratio | 12.05$^a$ (2.69) | 3.97$^b$ (0.45) | 4.19$^b$ (0.21) | 3.45$^b$ (1.00) |
| Lilium aboveground biomass C:N ratio | 23.28$^a$ (1.73) | 23.37$^a$ (1.89) | 49.14$^b$ (1.05) | 39.56$^b$ (1.21) |
| Lilium coarse root C:N ratio | 50.14$^a$ (7.32) | 42.31$^b$ (2.10) | 48.45$^a$ (4.96) | 38.46$^b$ (1.46) |
| Lilium bulb C:N ratio | 68.77$^a$ (1.30) | 43.20$^b$ (0.51) | 84.00$^a$ (4.60) | 38.91$^b$ (2.19) |
| Lilium aboveground biomass $\delta^{13}$C signature (%) | $-30.14^a$ (0.10) | $-29.48^b$ (0.20) | $-29.77^{ab}$ (0.10) | $-29.69^{ab}$ (0.17) |
| Lilium coarse root $\delta^{13}$C signature (%) | $-29.94^a$ (0.15) | $-29.01^b$ (0.21) | $-28.90^b$ (0.13) | $-29.17^b$ (0.21) |
| Lilium bulb $\delta^{13}$C signature (%) | $-29.94^a$ (0.14) | $-28.87^b$ (0.12) | $-29.09^b$ (0.11) | $-29.32^{ab}$ (0.17) |

D = drought treatment, N = nitrogen treatment, ND = combined nitrogen and drought treatment; DW = dry weight. Lower case letters indicate significant between-treatment differences (first year: ANOVA; second year: Tukey post hoc test; not underlined and underlined lower case letters indicate $P < 0.05$ and $P < 0.1$, respectively).
particularly high in the ND treatment (91%; Table 1; Fig. 2d).

The D treatment (performed in the second year of the experiment) had no effect on the aboveground biomass of *Lilium*, but significantly decreased *Lilium* fine and coarse root biomass and thus RSRs (Fig. 2b, c). However, the sole effect of drought on both *Lilium* biomass and tissue dieback was comparatively low compared to the ND treatment (Table 1; Fig. 2).

### Treatment effects on physiological response variables of *Lilium*

Grouped physiological response variables were affected by all treatments (N, D, ND; *P* < 0.002 and < 0.001, respectively) and *Secale* aboveground biomass (*P* = 0.048), but we found no significant interaction terms (Table 2). *Lilium* aboveground biomass C:N ratios were not affected in the N treatment (*P* > 0.05), but showed a remarkable increase in the drought treatments (by 211% compared to the control; *P* < 0.05; Table 1; second year data). In contrast, N fertilization significantly lowered tissue C:N ratios of coarse roots and bulbs.

Tissue δ^{13}C signatures of *Lilium* (above- and belowground) increased across treatments, but this trend was less pronounced for the aboveground tissue in the D and ND treatments (Table 1). Between-treatment differences of tissue δ^{13}C signatures were not significant.

### Treatments effects in relation to *Secale*-Lilium competition

Results of the linear models showed that *Lilium* aboveground biomass decreased with increasing *Secale* aboveground biomass across treatments (slope estimate: −0.485; *P* < 0.001; Table 3; second-year data). Moreover, we found significant interactions between *Secale* aboveground biomass and the N treatment, which increased *Lilium* tissue dieback (slope estimate: 3.166; *P* = 0.045; Table 3; Fig. 3a, b), and decreased *Lilium* belowground biomass (slope estimate: −9.953; *P* = 0.029; Table 3) and *Lilium* RSRs (slope estimate: −20.231; *P* < 0.001; Table 3). *Lilium* RSRs were also negatively affected by interactions between *Secale* aboveground biomass and the D and ND treatments (slope estimate: −20.920; *P* = 0.003, slope estimate: −15.945; *P* = 0.012, respectively; Table 3). N fertilization thus exacerbated *Lilium* tissue dieback and reduced *Lilium* RSRs via increasing aboveground biomass of *Secale*.

For tissue C:N ratios and δ^{13}C signatures, only N, D, and ND treatments were significant predictors in the linear models, and all two-way interactions were excluded in the most parsimonious models (with the

### Table 2 Results of the MANOVA summarizing effects of treatments (N, D, ND) and *Secale* aboveground biomass on grouped morphological (a) and physiological (b) response variables (multivariate response) of *Lilium*

|                | (a) Grouped morphological response variables | (b) Grouped physiological response variables |
|----------------|--------------------------------------------|--------------------------------------------|
|                | F-value | P-value | F-value | P-value |
| N              | 1.851   | 0.196   | 5.724   | 0.002   |
| D              | 3.945   | < 0.001 | 12.721  | < 0.001 |
| ND             | 16.094  | < 0.001 | 20.349  | < 0.001 |
| *Secale* above | 3.207   | 0.061   | 2.744   | 0.048   |
| N*Secale* above| 4.791   | 0.020   | 1.070   | 0.390   |
| D*Secale* above| 0.810   | 0.547   | 0.480   | 0.750   |
| ND*Secale* above| 4.242  | 0.029   | 0.042   | 0.996   |

The response variables selected correspond to those considered in the linear models (Table 3). Grouped morphological variables: *Lilium* aboveground biomass, *Lilium* belowground biomass, *Lilium* root:shoot ratio, amount of necrotic/dead tissue; grouped physiological variables: *Lilium* aboveground biomass δ^{13}C signature, *Lilium* coarse root δ^{13}C signature, *Lilium* aboveground biomass C:N ratio, *Lilium* coarse root C:N ratio, (data of the second year of the experiment). Abbreviations of treatments: N = nitrogen treatment, D = drought treatment, ND = combined nitrogen and drought treatment, *Secale* above = *Secale* aboveground biomass; significant effects and marginally significant effects (*P* < 0.05 and *P* < 0.1, respectively) are indicated in bold.
Table 3 Results of the most parsimonious linear models for treatment and Secale effects on responses of Lilium bulbiferum (for data of the second year of the experiment)

| Morphological variables | Necrotic/dead tissue | Lilium aboveground | Lilium belowground | Lilium root:shoot ratio |
|-------------------------|----------------------|---------------------|---------------------|-------------------------|
|                         | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value |
| Intercept               | 2.411     | 1.139    | 0.263   | 1.732     | 6.183    | < 0.001 | 15.615    | 2.086    | 0.055   | 44.207    | 3.533    | 0.004   |
| N                       | - 8.213   | - 1.876  | 0.069   | 0.303     | 2.708    | 0.010   | 27.607    | 2.332    | 0.034   | 45.895    | 3.258    | 0.006   |
| D                       | 1.684     | 3.177    | 0.003   | - 0.072   | - 0.854  | 0.399   | - 3.773   | - 2.031  | 0.060   | 45.895    | 3.258    | 0.006   |
| ND                      | 3.493     | 4.699    | < 0.001 | 0.117     | 1.083    | 0.286   | - 6.121   | - 2.896  | 0.011   | 30.568    | 1.971    | 0.070   |
| Secale above            | - 0.491   | - 0.562  | 0.577   | - 0.485   | - 4.222  | < 0.001 | - 1.986   | - 0.693  | 0.499   | 20.120    | 5.167    | < 0.001 |
| N*Secale above          | 3.166     | 2.079    | 0.045   | - 9.953   | - 2.412  | 0.029   | - 20.231  | - 4.426  | < 0.001 |
| D*Secale above          | - 20.920  | - 3.592  | 0.003   | 20.120    | 5.167    | < 0.001 |
| ND*Secale above         | - 15.945  | - 2.918  | 0.012   | - 15.945  | - 2.918  | 0.012   |

| Physiological variables | Lilium aboveground | Lilium coarse root | Lilium aboveground | Lilium coarse root |
|-------------------------|---------------------|--------------------|---------------------|--------------------|
|                         | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value | Estimate  | t-value  | P-value |
| Intercept               | - 30.140  | - 221.491 | < 0.001 | 29.937    | - 209.232 | < 0.001 | 23.277    | 8.823    | < 0.001 | 53.582    | 6.935    | < 0.001 |
| N                       | 0.661     | 3.435    | 0.002   | 0.928     | 4.462     | < 0.001 | 0.094     | 0.025    | 0.980   | 35.286    | 2.205    | 0.035   |
| D                       | 0.371     | 1.925    | 0.062   | 1.041     | 5.145     | < 0.001 | 25.854    | 6.932    | < 0.001 | - 1.975   | - 1.011  | 0.319   |
| ND                      | 0.452     | 2.346    | 0.025   | 0.761     | 3.761     | < 0.001 | 16.280    | 4.363    | < 0.001 | - 10.780  | - 3.970  | < 0.001 |
| Secale above            | - 1.440   | - 0.452  | 0.654   | - 13.804  | - 2.478   | 0.018   |
| N*Secale above          | - 13.804  | - 2.478  | 0.018   |

Abbreviations of treatments: N = nitrogen treatment, D = drought treatment, ND = combined nitrogen and drought treatment, Secale above = Secale aboveground biomass; significant effects and marginally significant effects (P < 0.05 and P < 0.1, respectively) are indicated in bold.
exception of Lilium root C:N ratios, for which we found a significant N × Secale aboveground biomass interaction; slope estimate: −13.804; \( P = 0.018 \); Table 3).

The SEM provided a good fit to the data\( (P(\chi^2) = 0.118; \quad CFI = 0.956; \quad SRMR = 0.052; \quad P = 0.018)\); D and ND treatments strongly reduced total Lilium biomass \( (R^2: 0.60) \) and RSR \( (R^2: 0.65; \) direct treatment effect), which in turn enhanced the plants’ tissue dieback \( (R^2: 0.60) \). In contrast, N and ND treatments positively affected Secale aboveground biomass, which in turn had negative effects on Lilium belowground biomass \( (R^2: 0.65; \) indirect treatment effect; \( P = 0.73) \). In this way, N and ND treatments enhanced the tissue dieback of Lilium due to competitive interactions between Secale and Lilium. SEMs provided the same findings \( (P = 0.018) \) when the variable “total Lilium biomass” was replaced by “Lilium aboveground biomass” or “Lilium belowground biomass” \( (P = 0.018) \) (supplementary material, Fig. S1 and S2).

Discussion

Our findings confirm our first hypothesis that N fertilization has the potential to significantly increase the drought sensitivity of Lilium plants, in this instance mainly due to effects on morphological attributes of the target species such as biomass productivity or traits such as RSR \( (P = 0.018) \) (indicating aboveground/belowground allocation patterns). Increasing sensitivity \( (P = 0.018) \)
relative changes in response variables in relation to treatments) was reflected by a distinct decline of the belowground biomass of *Lilium* and a concomitant increase in tissue dieback in mesocosms subject to the ND treatment. Moreover, our study demonstrates that this strong dieback is partly driven by the combined effects of N fertilization and drought, but also by the effects of increasing biomass productivity of *Secale* as a result of N fertilization. Thus, direct (i.e. treatment) and indirect (i.e. competitive interactions) effects explain *Lilium* responses to shifts in abiotic conditions.

Non-additive effects of N fertilization and drought on *Lilium* growth can be explained by two mechanisms. First, N fertilization may increase a plant’s aboveground investments in relation to belowground biomass formation, resulting in decreasing RSR (according to the ‘resource optimization hypothesis’; Ågren and Franklin 2003; cf. Table 3 for N effects on RSR, *P* = 0.004). Decreasing RSR, as a result of N fertilization, has been shown for several plant species and across different life forms (Friedrich et al. 2012; Meyer-Grünefeldt et al. 2015b; Dziedek et al. 2016a). Lowered RSR may increase a plant’s evaporative demands (per unit root biomass) and transpiration costs, making it more prone to drought stress (Gordon et al. 1999; Meyer-Grünefeldt et al. 2015a). Second, N fertilization often alters root morphology in that roots show higher extension rates but lower tissue density (Eissenstat 1991; Mendoza et al. 2016; Wang et al. 2018). As a result, root mortality increases when soils start to dry out (Meier and Leuschner 2008; Noguchi
et al. 2013). In our experiment, the dieback of fine roots could therefore have contributed to the lowered fine root biomass in N-fertilized mesocosms (Dziedek et al. 2016b). In this context it is important to note that *Lilium* dieback in mesocosms subject to a sole D treatment was low compared to the ND treatment, indicating a higher drought resistance of non-fertilized plants and amplifying (non-additive) negative effects of combined N and D treatments. Strong effects of ND treatments on overall plant growth and performance are also reflected by significant responses of both grouped morphological and physiological variables (MANOVA).

However, as stated in our second hypothesis, adverse effects of N fertilization on *Lilium* growth were also related to fertilization-induced shifts in the interspecific competition with *Secale*. This interpretation is supported by findings of the linear models and SEM, according to which N and ND treatments strongly fostered *Secale* biomass productivity, which in turn strengthened its competitive superiority over *Lilium*. Importantly, *Secale* and two-year old *Lilium* exhibited opposing responses to N fertilization (i.e. growth benefit vs. no effect or even inhibition, respectively; Mendoza et al. 2016), particularly with regard to the ND treatment, in which about 90% of the *Lilium* aboveground tissue necrotized. Although our experimental design did not allow for a clear detection of the mechanisms causing the dieback of *Lilium* in the ND microcosms, it is likely that both aboveground competition for light and belowground competition (for nutrients and water) contributed to this growth response: On the one hand, increasing *Secale* aboveground productivity reduced both above- and belowground biomass of *Lilium*, a mechanism that was fostered by N fertilization (N/ND × *Secale* biomass interaction; indicated by both significant multivariate responses of grouped morphological variables (MANOVA) und univariate responses (linear models)). On the other hand, two-year old *Lilium* plants proved to be the inferior competitor for belowground resources such as N, since its aboveground biomass C:N ratios remained unaltered or even increased in the N and ND treatment, respectively, indicating that *Lilium* could not benefit from an increased soil N availability in N-fertilized mesocosms (N accumulation in belowground biomass, indicated by decreasing C:N ratios; von Oheimb et al. 2010). The same holds true for belowground resources such as water: In all mesocosms in which *Secale* developed a high belowground (root) biomass, tissue δ13C signatures of *Lilium* increased significantly. This suggests that *Secale* also was the superior competitor for water. This interpretation is supported by the finding that tissue δ13C signatures of *Lilium* were more related to *Secale* belowground biomass than to *Lilium* RSRs (i.e. 13C discrimination of *Lilium* seems to be more affected by competition for water than by transpiration rates).

Between-species competitive interactions, however, emerged only in the second year of the experiment (i.e. when *Lilium* plants were 2 years old), as suggested by between-year comparisons of aboveground biomass responses of both species to N fertilization. Probably, one-year old *Lilium* plants were too small to cause such interactions being effective aboveground or belowground (e.g. *Lilium* aboveground biomass was almost 40-times higher for two-year old compared to 1-year old plants). This finding might also point to some shortcomings of our experiment: Since competitive interactions were observed only over two years, it is conceivable that our findings might be different for longer-term experiments. In addition, indirect effects of global change drivers on plant growth via interspecific interactions and the underlying mechanisms might be better detected, if experiments include monocultures of all plant species typical of a focal plant community. However, interactions effects between N fertilization and interspecific competition were also found in experiments of Friedrich et al. (2011) and Valliere et al. (2017), but the outcomes of our experiment suggest that impacts of co-occurring drivers of global change (here: N fertilization and drought) are mediated by interspecific competition.

Contrary to decreasing RSR of *Lilium* under N fertilization, *Secale* RSR remained unchanged, indicating balanced aboveground-belowground investments even under high N availability (and contrasting the prediction of the resource optimization hypothesis). This strategy seems to be typical of many grass species, providing them a competitive advantage for belowground resources over other species or life forms, particularly under increasing N availability (Wood et al. 2006; Friedrich et al. 2011; Mendoza et al. 2016).

Our findings that both ‘direct’ (abiotic) and ‘indirect’ (biotic) pathways may influence plant responses
to environmental changes might have implications for the long-term preservation of plant diversity, particularly in N-limited environments. Many target species of conservation measures may suffer from direct and non-additive effects of N fertilization and drought events. These drivers might act via abiotic and biotic interactions, and in turn often are responsible for the encroachment of non-target species (e.g. grasses) in protected environments (Phoenix et al. 2012; Henning et al. 2017; Valliere et al. 2017). In this context, mitigation strategies need to counteract (airborne) N inputs (e.g. by means of biomass or soil removal) in order to achieve balanced ecosystem N budgets in the long term.

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**Data availability** All data will be available in the “Dryad Digital Repository” after manuscript acceptance.

**Code availability** Not applicable.

**Declarations**

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