**Biogeosciences
Discussions**

Altered response to nitrogen supply of mixed grassland communities in a future climate: a controlled environment microcosm study

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Altered response to N supply in a future climate

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Abstract

Few studies have investigated whether responses to nutrient supply of mixed plant communities change under combined elevated CO_2 and climate warming. In this study we analyzed the response of constructed temperate grassland communities to five levels of nitrogen (N) supply, ranging from 0 to 150 kg N ha^{-1}, under two climate scenarios. Biomass of the plant communities responded positively to N supply in the current climate, but was insensitive to N supply in the future climate. This altered response was not the result of a changing response from a single species, but all species seemed to contribute to it. The weaker response in the future climate was caused by changes in N uptake rather than by changes in nitrogen use efficiency, as community N stocks showed the same response pattern as community biomass. Climate change apparently modified the relation between fertilizer N addition and plant available N.

1 Introduction

Ecosystem responses to climate change are largely determined by the availability of water and nutrients (Cannell and Thornley, 1998). In regions were water is not limiting, nitrogen (N) is one of the key limiting elements (Vitousek et al., 1997). The question arises whether the impact of climate change will differ between N poor and N rich systems (e.g. Oren et al., 2001).

Impacts of climate change can arise through complex pathways, several of which involve interaction with N. For example, in natural grassland communities N_2-fixing and non-N_2-fixing plants co-occur. N_2-fixers have access to atmospheric N via N_2-fixation, and provide N not only to themselves but also to other species via transfer (Hogh-Jensen and Schjoerring, 2000). Since climate change can alter interspecific interactions and thus the composition of plant communities (Zavaleta et al., 2003), the available amount of N at the community level could change if the proportion of N_2-fixers

is affected. Such pathways of climate change impact that combine species interactions with nutrient cycles in complex systems are still poorly explored.

Other possible pathways of climate change impact could result in an alteration of the soil mineral N pool. The mineral N pool is mainly determined by mineralization of soil organic matter (SOM) and immobilization of N in SOM, in addition to uptake of N by the vegetation (Thornley and Cannell, 2000). Both mineralization and immobilization are driven by microbial activity and can be affected directly or indirectly by climate change (Zak et al., 2000). The balance between the changes in both processes will determine how and if N availability for the vegetation alters, and thus also the potential productivity response of grasslands in a future climate.

The mediating role of nitrogen in these various response mechanisms suggests that plant communities may react differently to climate change in N poor versus N rich environments. Addressing this question is hampered by the fact that the majority of studies on ecosystem responses still focus on single components of change (Norby and Luo, 2004). In grasslands, increased CO_2 fairly consistently stimulates growth and productivity (Ainsworth and Long, 2005), while effects of experimental warming vary between positive and negative, with the mean response around zero (Rustad et al., 2001). The few experiments on combined effects of elevated CO_2 and higher temperatures, however, give contrasting results, varying from additive to strongly interactive responses (Norby and Luo, 2004; Shaw et al., 2002). This makes the response of grasslands to combined climatic changes difficult to forecast, while all of the predicted IPCC SRES scenarios simulate concurrent increases in CO_2 and temperature (IPCC, 2007). The problem is similar for interactions between climate change and nitrogen supply, which have likewise predominantly been studied for single factors of change. Nitrogen can limit productivity under elevated CO_2 (Soussana and Luscher, 2007; Oren et al., 2001), but warming could overcome this limitation by stimulating mineralization (Hovenden et al., 2008). Contrary to effects of combined CO_2 and N supply, effects of combined warming and N supply have been less investigated, and combined CO_2 – temperature – N studies are even rarer.

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Interactive Discussion

To detect the role N will play in biomass responses to a future climate, grassland communities were subjected to different levels of N supply either under current conditions or under simultaneous elevated CO_2 and warming. These conditions were created in climate-controlled chambers as a cost-effective method to detect changes, which can subsequently be explored further in more expensive, large-scale field setups with combined free-air CO_2 enrichment and free-air temperature increase.

We exposed plant communities with multiple species, such that responses arose both from the species-specific sensitivities and interspecific interactions. The drawback of this choice is that the intrinsic response of the species in the absence of competitors or facilitators cannot be determined, but this is outweighed by two advantages: the complexity of real communities is approximated, and changes in species composition of the communities can be studied. The following hypotheses were tested:

(1) climate change alters biomass responses of grassland communities to N supply;

(2) this alteration is mediated by shifts in species composition.

2 Material and methods

2.1 Experimental set-up

This research is part of a larger experimental platform in which newly established grassland communities are subjected to different stressors in a future climate. This paper will focus on N and differences in the availability of this resource. The platform is located at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium (51°09′ N, 04°24′ E), where average annual precipitation is 776 mm (evenly distributed throughout the year) and average annual air temperature 10.8 °C. The grassland communities were grown in six sunlit, climate-controlled chambers, facing south. The distances between the chambers were maximized to avoid mutual shading. The interior surface area was

$150 \times 150\,cm^2$, the height at the north side 150 cm and at the south side 120 cm. The top of the chambers consisted of a colorless polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film (200 mm thick), both UV transparent. Three of the six chambers tracked the current climate with current air temperature (T_{air}) and CO$_2$ concentration, while the other three chambers were exposed to a future climate scenario with 3 °C warming and a target CO$_2$ concentration of 620 ppm (further referred to as "current" and "future climate", respectively). Because the experimental platform involved a large number of factors, a completely factorial design with warming and CO$_2$ as separate treatments was not feasible. We therefore chose to combine CO$_2$ and warming in a single treatment as characterization of a future climate.

The CO$_2$ concentration was measured and regulated with a CO$_2$ control group with a CO$_2$ analyzer (WMA-4, PPSystems, Hitchin, UK). In the current climate chambers the concentration was 375±17 ppm (SD) while in the future climate chambers it was within 10% and 20% of the target of 620 ppm during 84.4% and 95.6% of the time, respectively. Every half hour, T_{air} was monitored with a combined humidity–temperature sensor (Siemens, type QFA66, Erlangen, Germany) and photosynthetically active radiation (PAR) with a quantum sensor (SDEC, type JYP1000, Tours, France). During the experiment (7 May–4 October 2007), monthly average T_{air} was 14.5, 17.6, 17.4, 17.4 and 14.6 °C in May, June, July, August and September, respectively. In the current climate chambers T_{air} was on average 0.3±1.6 °C (SD) higher than outside, while the future climate chambers were 3.3±2.1 °C (SD) warmer than outside. Differences between chambers were negligible as compared to the achieved difference between the climates. Side-by-side comparison of T_{air} amounted to 0.1±1.2, 0.3±0.9 and 0.4±0.7 °C (SD) between the three current climate chambers and to −0.4±0.8, −0.4±1.0 and 0.1±0.8 °C (SD) between the three future climate chambers. The average daily PAR sum was 26.9 mol m^{-2} d^{-1} and differed very little between the two climates (2.1±0.6 mol m^{-2} d^{-1} – SD) nor did it differ greatly between the chambers. Side-by-side comparison amounted to −0.2±0.2, 0.1±0.1 and 0.3±0.2 mol m^{-2} d^{-1} (SD) between the three current climate chambers, and to −0.1±0.2, −1.0±0.2 and

$-1.0\pm0.2\,\mathrm{mol\,m^{-2}\,d^{-1}}$ (SD) between the three future climate chambers. Average vapour pressure deficit (vpd) was 0.28 ± 0.31 and 0.66 ± 0.41 kPa (SD) in current and future climate, respectively. Differences between chambers were small: side-by-side comparison of vpd equalled -0.04 ± 0.13, 0.07 ± 0.10 and 0.03 ± 0.12 kPa (SD) between the current climate chambers, and -0.14 ± 0.12, 0.08 ± 0.26 and -0.06 ± 0.25 kPa (SD) between the future climate chambers.

Each chamber contained 30 grassland communities with the same species composition in PVC containers (24 cm inner diameter, 40 cm height), filled with sandy soil (93.23% sand, 4.59% silt, 2.19% clay; field capacity $0.13\,\mathrm{m^3\,m^{-3}}$; pH 7.6; total Kjeldahl-N $0.42\,\mathrm{g\,kg^{-1}}$). The containers were placed side-by-side so that a closed canopy could form, and were buried in the soil to avoid unnatural soil temperatures. Irrigation was calculated from the monthly rainfall over the period 1995–2005 and corrected for differences in evapotranspiration (ET) inside and outside the chambers. To this end, De Boeck et al. (2006) calculated ET inside current climate chambers from changes in soil water content (SWC) and the amount of administered water, and the outside ET with Hamon's equation (Haith and Shoemaker, 1987) based on day length, vapour pressure and T_{air}. The containers were watered every two days according to the 10 year average of 14 to 15 raining days per month during the growing season. Total monthly irrigation matched 61.5, 64.4, 85.1, 80.2, 80.9 and 69.7 mm in May, June, July, August, September and October, respectively. The future climate chambers received the same amount of water as the current climate chambers, so that any enhanced consumption would result in aggravated soil drought. Water could freely drain from the containers while capillary rise was prevented by a drainage system placed below the chambers. Profile probe tubes for the PR2 soil moisture sensor (Delta-T Devices Ltd., UK) were installed in each community and SWC was measured every 10 days between 22 June and 29 September 2007. Soil water content was not altered by the climate scenario ($F_{1,4.82}$ = 2.93, p = 0.150; ANCOVA; see Sect. 2.4 Data analyses), decreased slightly during the growing season ($F_{1,4.91}=14.96$, $p=0.012$) from 11.6 to 10.5%, but remained well above wilting point (6.9%) and below field capacity (13%). The decrease did not differ

between the climates (no significant interaction day of year × climate; $F_{1,3.96}=0.21$, $p=0.668$).

In each chamber five containers were randomly chosen to develop five N treatments, yielding three replicates (chambers) per treatment. The highest N level had two additional containers per chamber available from other experiments where this level was the control. The five N fertilization levels ($0\,g\,N\,m^{-2}$, $1\,g\,N\,m^{-2}$, $3\,g\,N\,m^{-2}$, $7\,g\,N\,m^{-2}$, $15\,g\,N\,m^{-2}$; corresponding with 0 to $150\,kg\,N\,ha^{-1}$) were applied as NH_4NO_3. In addition all communities (also those which received no N) were fertilized with $7.5\,g\,m^{-2}\,P_2O_5$ and $15\,g\,m^{-2}\,K_2O$ and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The amounts of P and K were kept constant so that only effects of the N treatments were measured. All of the fertilizer was given dissolved in water (assuring no percolation), in four equal amounts evenly spread over the growing season.

2.2 Grassland communities

The plant communities were established between 7 and 10 May 2007 by transplanting five-week-old seedlings. Each community contained 18 plants and was composed of six equally represented species from three functional groups (two species per group): grasses (*Lolium perenne* L., *Poa pratensis* L.), non-N_2-fixing dicots (*Plantago lanceolata* L., *Rumex acetosa* L.) and N_2-fixing dicots (*Lotus corniculatus* L., *Medicago lupulina* L.). All these species have a perennial life cycle, co-occur in temperate European grasslands and have comparable heights. The 18 plants (three per species) were placed in a hexagonal grid with a 4.5 cm interspace. Interspecific interactions were maximized by avoiding clumping and by using five different designs. The five designs were randomly distributed over the treatments.

2.3 Biomass and nitrogen

Above-ground (shoot and stubble) and below-ground (root) biomass were harvested at the end of the growing season (1–4 October 2007). Above-ground biomass was

subdivided by species. Total below-ground biomass per community was estimated from 12 soil cores (2 cm diameter) per container. To adequately represent the total root biomass in the soil, six cores were taken directly below the plants (one per species) and six cores in the middle of a triangle between three plant positions. Root samples were washed until they were free of soil. All plant material was dried at 70 °C for 48 h and then weighed.

Total community N stock at the end of the growing season was determined on each community by multiplying N concentration with biomass, separately for the above-ground and the root compartment. Nitrogen concentration was measured with a CN element analyser (NC-2100, Carlo Erba Instruments, Italy) after grinding the samples. The soil extractable N content (SEN, nitrate and ammonium) of each community at the end of the season and, of a soil sample from the start of the experiment, was determined on 70 °C oven dried soil with KCl extraction.

2.4 Data analysis

The experiment had a split plot design with climate (future vs. current) as the main plot factor and N treatment (further referred to as "nitrogen") as the subplot factor. Analyses of covariance (ANCOVA) were performed in SAS (version 9.1, SAS Institute Inc., Cary, NC) using the mixed procedure (Littell et al., 1996) with climate, nitrogen (co-variable) and, if appropriate species, as fixed factors, and chamber as a random factor nested within climate. Soil water content was analyzed with repeated measures ANCOVA with time as co-variable (repeated in time). Non-significant treatment factors (climate, nitrogen, species) were excluded from the ANCOVAs. In case of significant interactions post hoc comparisons of the parameter estimates were performed with a t-test. The random factor chamber was never significant (except on SWC) but was nevertheless always kept in the model to account for undetected chambers effects. In this way, we assured that degrees of freedom of tests of the fixed effects were not inappropriately high in any analysis (Hurlbert, 1984; Oksanen, 1999). "Kenwardroger" corrections for degrees of freedom were used (Kenward and Roger, 1997), to account

for correlations between the data within individual chambers and make use of this degree of dependency to approximate the effective number of degrees of freedom.

3 Results

3.1 Community biomass

The total biomass of the grassland communities increased with increasing N supply in the current climate but did not respond to the applied N in the future climate (significant climate × nitrogen interaction, Fig. 1, Table 1; a posteriori comparison per climate, respectively $t_{31.3}=5.27$, $p<0.0001$ and $t_{31.2}=1.03$, $p>0.05$). This difference in response to N supply was also significant in root biomass (Fig. 1, Table 1) and Fig. 1 suggests a similar pattern above-ground (although not significant; Table 1).

To determine the possible impact of changes in species composition on these results, above-ground biomass was analyzed by species (Fig. 2, note that the species were not discernable below-ground). The slopes of the biomass responses to N supply, per species, did not differ significantly between the climates (no climate × nitrogen interaction, Table 2). However, when the slope values in the future climate were plotted against those in the current climate (Fig. 3), all the points on this graph were below the 1:1 line. This suggests that small differences between the slopes in the two climates, pointing in the same direction for all species, may have contributed to the significantly weaker response of total community biomass to N supply in a future climate in Fig. 1.

There was no difference in biomass between the climates (Table 2). Regardless of the climate, with increasing N supply there was a shift in above-ground biomass between the most abundant species. *Medicago lupulina*, a N_2-fixer, declined, while *P. lanceolata*, and especially *L. perenne*, a fast growing species with a high N need, became more abundant (reflected in significant nitrogen × species interaction, Fig. 2, Table 2), suggesting that *M. lupulina* was competitively suppressed at high N.

3.2 Plant and soil N stocks

The previous section indicated that the contrasting sensitivities of community biomass to N supply between the two climates (Fig. 1) most likely did not originate from changes in species composition. Two possible causes thus remain: the contrasting sensitivities either arose from differences in N uptake (i.e. impaired uptake in the future climate at higher N supply, yielding the flat biomass-N curve for this treatment in Fig. 1), or uptake was not affected by future climate but N use efficiency was. Figure 4 shows that N uptake was responsible, as the differences in community N stocks between the treatments mirror those in community biomass shown in Fig. 1. Likewise to biomass, community N stocks increased with N supply in the current climate, while in the future climate they did not change significantly (significant climate × nitrogen interaction, Table 1; a posteriori comparison per climate, respectively $t_{7.29}=2.45$, $p<0.05$ and $t_{7.38}=-0.92$, $p>0.05$). Both above-ground and root N stocks showed this pattern (Fig. 4), suggesting that there were no shifts in N allocation, though the interaction was only significant for roots (Table 1).

The changes in plant N acquisition with climate might derive from altered N availability in the soil. The soil extractable nitrogen (SEN) at the start of the experiment was 20.2 mg N kg^{-1} air dry soil or 9.4 g N m^{-2}. At the end of the season, after the harvest, there were, however, no differences in SEN between the climates or the five N treatments (Fig. 5, Table 1). Values at the end were 19.2±0.3 mg N kg^{-1} air dry soil or 8.9±0.1 g N m^{-2} (mean ± SE; $n=29$).

4   Discussion

Community biomass production did not respond to N supply in the future climate, while it did so in the current climate (Fig. 1). This difference in response to N could not be attributed to changes in species composition between the two climates (Fig. 2), which might, for example, arise from increased abundance of a very (un)responsive species. In contrast, all species seemed to contribute to the altered N response (Fig. 3). Similar results were reported for a Mediterranean grassland where community biomass production responded less to a $7\,g\,m^{-2}$ N supply in future than in current climate, although the authors did not test for this (Fig. 6 in Dukes et al., 2005, comparing the treatments: (i) ambient CO_2 with ambient CO_2 + nitrate deposition and (ii) elevated CO_2 + increased temperature with elevated CO_2 + increased temperature + nitrate deposition).

What may have caused the grassland communities to respond less to N supply in a warmer environment with more atmospheric CO_2? To answer this question we have to consider the N balance of the mesocosms. This N balance consists of fluxes to – and from the SEN pool and the vegetation (Fig. 6). Mineral N enters the SEN by mineralization of SOM and by added fertilizer. Outputs of N from the SEN are uptake by the vegetation and losses of N through immobilization by microorganisms in the soil, leaching and gaseous emissions by microbial processes (Thornley and Cannell, 2000). In addition, N enters the vegetation through N_2-fixation. At the end of our experiment, the SEN equaled 95% of the pool at the start and did not differ among treatments (Fig. 5). The extractable mineral N pool being unaltered by climate change or nitrogen addition, it is clear that only shifts in the fluxes can explain the differences in community N stocks.

In both climates, in the absence of additional N, the only input fluxes of N to the plant and SEN pools were N_2-fixation and mineralization of SOM. When adding a third input flux, fertilizer N, the plant communities in the current climate enhanced their total N stock, as expected. This increase in plant N reflected the major part of the $15\,g\,m^{-2}$

added fertilizer (Fig. 4). However, in the future climate the plant community N stock remained the same upon fertilizer addition. Given the fact that the soil mineral N pool was never affected, and respecting the N balance, two explanations seem feasible (i) the added fertilizer was absorbed by the plants in the future climate, but plant community N stocks nevertheless remained the same because atmospheric N_2-fixation declined accordingly; (ii) in the future climate, the balance between mineralization and immobilization was altered, changing the N availability for the plants.

Active nodules (pink to red colour) were observed during harvest which confirms N_2-fixation, although it was not quantified. Nitrogen addition has been found to decrease N_2-fixation (Carlsson et al., 2009). Explanation (i), however, implies a different response of N_2-fixation to N addition between the climates. This seems unlikely because the only N_2-fixing species that reduced its biomass upon fertilization, *M. lupulina*, did so to a similar extent in current and future climate (Fig. 2). Explanation (ii), in contrast, is not implausible, but hinges on the assumption that rhizodeposition via root turnover, root exudation, and mycorrhizal turnover, increases in the future climate treatment. Stimulated soil inputs of labile carbon compounds via rhizodeposition have been reported for elevated CO_2 (Johansson et al., 2009, but see Uselman et al., 2000; Allard et al., 2006; Cheng, 1997; Meier et al., 1997; Godbold et al., 2006) and warming (Meharg and Killham, 1989; Uselman et al., 2000; Whipps, 1984). These additional labile C inputs in the future climate may have provided extra carbon and energy for the microbes, thus enhancing the N demand of the growing and more active microbial population (de Graaff et al., 2006).

When N is limiting, additional input of easily decomposable substrates can prime the decomposition of native SOM (Kuzyakov et al., 2000; Fontaine et al., 2004, 2007). This can either enhance soil N availability for the plants (de Graaff et al., 2009) or, the available N can be immobilized by increased immobilization in the growing microbial biomass and necromass pools (e.g. Allard et al., 2006). Hence, our observation that, at low N, plant N contents were similar between current and future climate is consistent with the findings of Allard et al. (2006).

With increasing N supply, we found that plant N contents (and plant biomass) increased in the ambient climate, but remained unaltered in the future climate. In the ambient climate, microbial populations were most likely C-limited, and all added N remained available to the plants. In the future climate, we hypothesize that N availability remained constant, despite the increasing addition of fertilizer N, because of two, mutually non-exclusive mechanisms: enhanced immobilization in the growing microbial biomass and/or reduced gross mineralization of native SOM. Such negative responses of labile carbon additions on mineralization of SOM under high N, relative to low N availabilities, were previously reported by Fontaine et al. (2004). Allard et al. (2006) also reported unchanged N availability for plants under elevated CO_2 regardless of N supply, because of a counterbalance between immobilization and mineralization. We therefore hypothesize that in our study, future climate enhanced C inputs in the soil, which, at high N supply, led to reduced mineralization and/or enhanced immobilization, and compensated for the extra N provided via fertilization. Thus, N availability for plants did not change upon N addition in a future climate, explaining why plant biomass and total N content did not respond to the N supply. The different responses of plant N availability to altered rhizodeposition reported by de Graaff et al. (2009), compared to the study of Allard et al. (2006) and our study, stress the need for further research to disentangle the soil processes determining N availability for plants (mineralization, immobilization, competition between microbes and plants, preferential substrates for microbes,. . .).

The observed shifts in species composition in response to N supply suggest that these shifts were mediated predominantly by competition (rather than allelopathy or facilitation). For example, the N_2-fixer *M. lupulina* would not be expected to intrinsically decline at higher N (Hebeisen et al., 1997). Hence, it was probably competitively suppressed by *P. lanceolata* and *L. perenne*, which both became more productive by N addition. This may also be the reason why three other species did not respond to added N. *Lolium perenne* combines a high N uptake rate with a low N use efficiency, which is advantageous in a high-N environment (Soussana et al., 2005), but in N-limited environments it is frequently outcompeted by less N limited species (Del

Pozo et al., 2000), such as *M. lupulina* in this study. Climate change did not affect the species' biomasses. The fact that the combined elevated CO_2 and warming led to no net change in biomass, confirms the contrasting impacts of these two global changes reported in the literature (Shaw et al., 2002; Ainsworth and Long, 2005; Rustad et al., 2001). It is therefore all the more striking that the future climate modified the response of these mixed communities to nitrogen addition.

5 Conclusions

In conclusion, this study, which is one of the few studies that combine warming, elevated CO_2 and nitrogen supply, provides the striking result that grassland communities do not significantly respond to N addition in a future climate. In spite of the somewhat artificial context of the experiment (small scale, disturbed soil and artificial soil hydrology), the net primary productivity – NPP) of the grassland communities was between 1500 and 2500 g biomass m^{-2} (Fig. 1), which is close to the European NPP average for grasslands of 1500 g biomass m^{-2} (Schulze et al., 2009). This indicates its value as a screening experiment. Moreover, the fact that similar results were reported for a different biome (Mediterranean grasslands; Dukes et al., 2005), stresses the need for further validation in large-scale set-ups with field swards exposed to combined free-air CO_2 enrichment and free-air temperature increase. Given that the observed altered response to N addition in a future climate could have important implications for agricultural economics, it should also be investigated on a longer timescale, particularly since changes in N availability under elevated CO_2 are likely to be progressive and interactive effects of warming may likewise develop over time (Hovenden et al., 2008). Besides, botanical composition may not be stable yet either, if seed production and recruitment are affected. In further exploration, emphasis will need to be on soil processes which determine N availability for plants.

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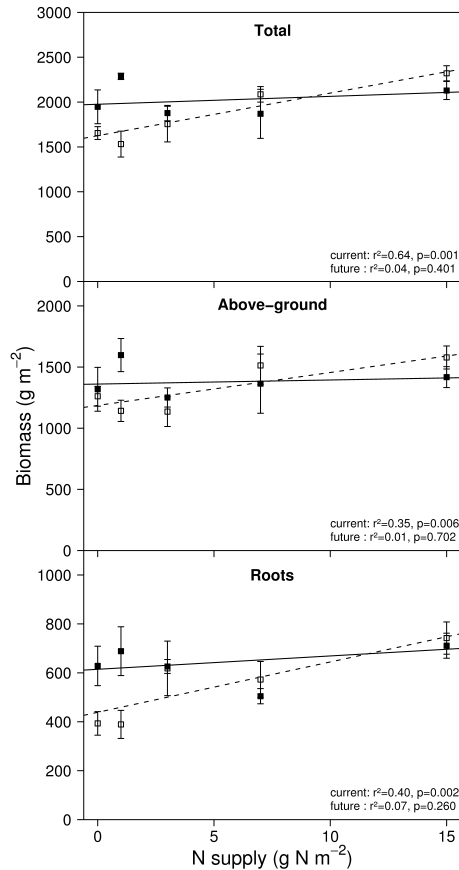Full Screen / Esc

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Table 1. Statistical analysis (ANCOVA) of community biomass and N stock (total, above-ground – shoot and stubble – roots and soil) of grassland communities subjected to five levels of N supply (between 0 and 15 g N m^{-2}) and two climates (current climate and future climate with elevated CO_2 and higher air temperatures). N supply is the covariate. Degrees of freedom (DF), F values and significance levels (p); p-values are presented in bold when significant (<0.05).

	total plant			above-ground			roots			soil		
Biomass	DF	F	p	DF	F	p	DF	F	p			
nitrogen	1, 31.2	19.85	**0.001**	1, 10.4	5.23	**0.044**	1, 31.5	12.42	**0.001**			
climate	1, 14.9	5.32	**0.036**	1, 3.5	0.08	0.793	1, 15.3	4.20	0.058			
climate × nitrogen	1, 31.2	8.96	**0.005**	1, 6.7	3.33	0.113	1, 31.5	4.26	**0.047**			
N stock	DF	F	p	DF	F	p	DF	F	p	DF	F	p
nitrogen	1, 7.3	1.16	0.316	1, 7.1	0.01	0.919	1, 6.3	10.05	**0.018**	1, 25	1.44	0.258
climate	1, 6.6	0.80	0.401	1, 3.7	0.35	0.590	1, 6.5	6.14	**0.045**	1, 25	0.06	0.804
climate x nitrogen	1, 7.3	5.67	**0.047**	1, 6.3	1.93	0.211	1, 6.3	6.82	**0.038**	1, 25	0.63	0.454

Table 2. Statistical analysis (ANCOVA) of above-ground biomass (shoot and stubble) by species. Degrees of freedom (DF), F values and significance levels (p); p-values are presented in bold when significant (<0.05).

Biomass	DF	F	p
nitrogen	1, 8.5	4.88	0.056
climate	1, 17.6	0.00	0.987
species	5, 76.1	53.94	**<.0001**
nitrogen × species	5, 195	30.32	**<.0001**
climate × species	5, 21.8	2.45	0.066
climate × nitrogen	1, 11.6	2.93	0.113
climate × nitrogen × species	5, 192	0.48	0.794

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Fig. 1. Biomass of grassland communities exposed to five levels of N supply (between 0 and 15 g N m^{-2}) and to current (□, —) or future (■, —) climate. Means ± SE (n=3 (chambers); at 15 g N m^{-2}, n=9 (3 chambers, 3 replicates per chamber) for total, above-ground, and root biomass. Individual pot values were used for linear regressions (n=21).
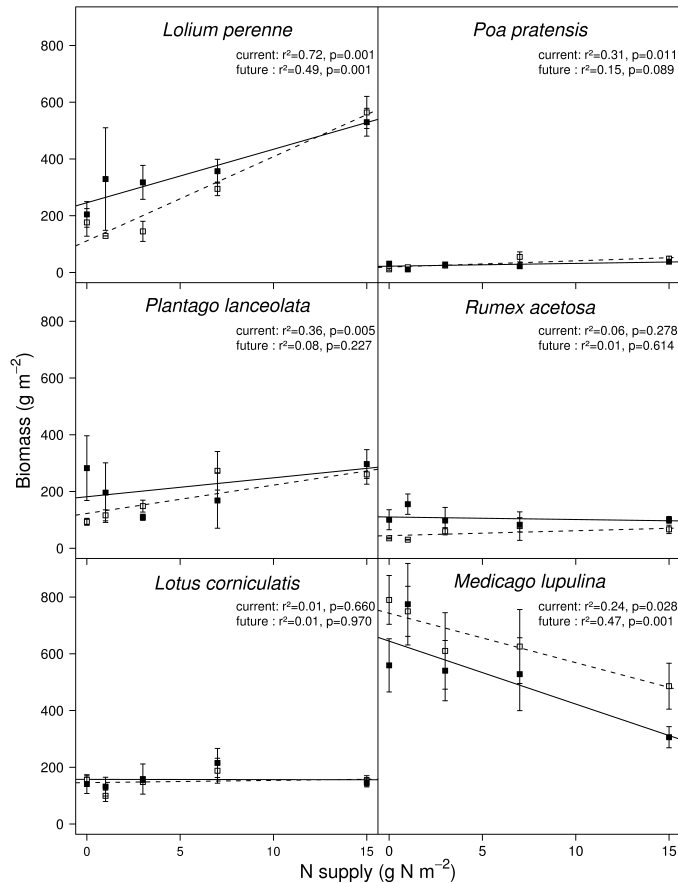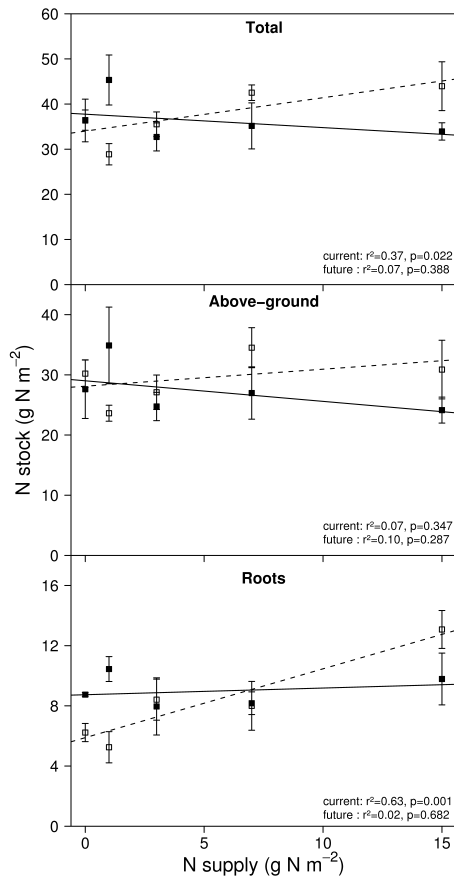
Fig. 2. Above-ground biomass of the individual species of the grassland communities exposed to five levels of N supply (between 0 and 15 g N m^{-2}) and to current ($\square$, —) or future ($\blacksquare$, —) climate. Means ± SE (n=3 (chambers); at 15 g N m^{-2}, n=9 (3 chambers, 3 replicates per chamber). Individual pot values were used for linear regressions (n=21).
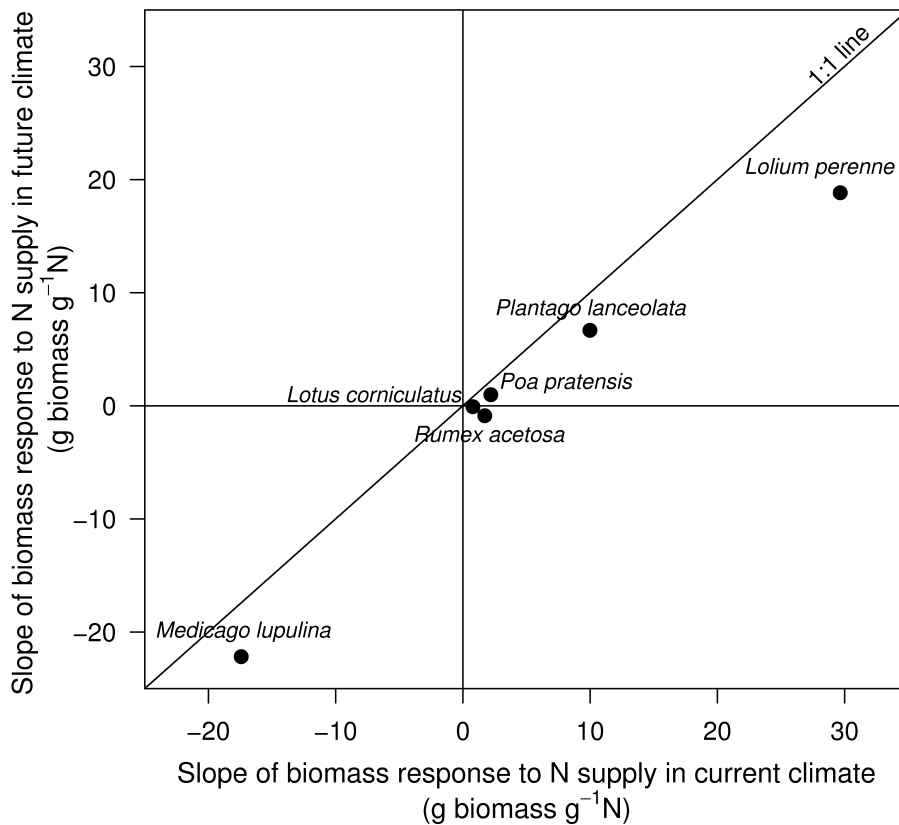
Fig. 3. Slopes of the above-ground biomass responses of individual grassland species to increasing N supply (Fig. 2) in future climate plotted against the same slopes in current climate. The 1:1 line indicates no effect of climate on the biomass response to nitrogen supply. Species below this line undergo a negative effect of future climate and species above the line a positive effect.

Fig. 4. N stock of grassland communities exposed to five levels of N supply (between 0 and 15 g N m^{-2}) and to current ($\square$, —) or future ($\blacksquare$, —) climate. Means ± SE (n=3 (chambers)) for total, above-ground, and root N stock. Individual pot values were used for linear regressions (n=15).

current: r²=0.01, p=0.852
future : r²=0.01, p=0.769
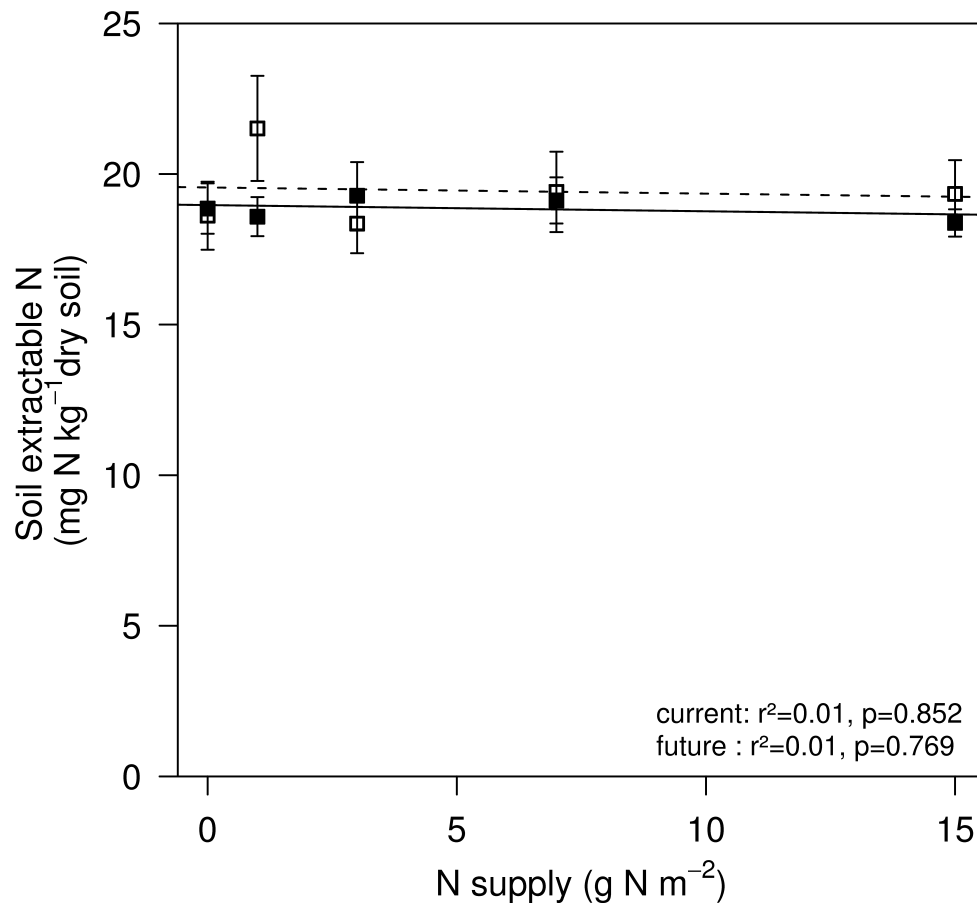
Fig. 5. Soil extractable nitrogen in grassland ecosystems exposed to five levels of N supply (between 0 and 15 g N m^{-2}) and to current ($\square$, —) or future ($\blacksquare$, —) climate. Means ± SE (n=3 (chambers)). Individual pot values were used for linear regressions (n=15).
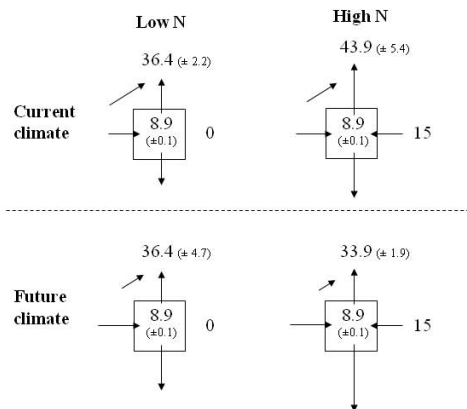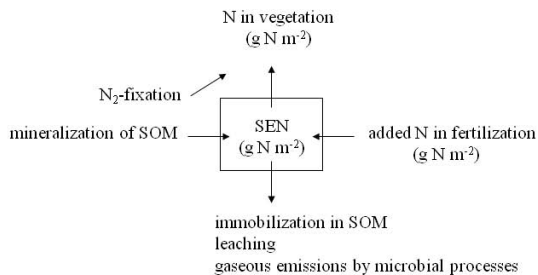
Fig. 6. Schematic presentation of the N balance of the grassland communities in current and future climate at low ($0\,\mathrm{g\,N\,m^{-2}}$) and high ($15\,\mathrm{g\,N\,m^{-2}}$) N supply. The soil extractable N pool (SEN) was calculated as the mean over all the treatments (five N treatments and two climates; means ± SE, $n=28$); N in the vegetation is calculated for each treatment (climate and N supply; low $N=0\,\mathrm{g\,N\,m^{-2}}$, high $N=15\,\mathrm{g\,N\,m^{-2}}$; means ± SE, $n=3$). Arrows represent fluxes and are not proportionate but indicate increase or decrease. Only fluxes of the same type should be compared. Increase or decrease is either known because measured or derived from literature and through deduction.

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