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

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1   **Abstract**

2   Few studies have investigated whether responses to nutrient supply of mixed plant  
3   communities change under combined elevated CO<sub>2</sub> and climate warming. In this  
4   study we analyzed the response of constructed temperate grassland communities to  
5   five levels of nitrogen (N) supply, ranging from 0 to 150 kg N ha<sup>-1</sup>, under two climate  
6   scenarios. Biomass of the plant communities responded positively to N supply in the  
7   current climate, but was insensitive to N supply in the future climate. This altered  
8   response was not the result of a changing response from a single species, but all  
9   species seemed to contribute to it. The weaker response in the future climate was  
10   caused by changes in N uptake rather than by changes in nitrogen use efficiency, as  
11   community N stocks showed the same response pattern as community biomass.  
12   Climate change apparently modified the relation between fertilizer N addition and  
13   plant available N.

14

15   Keywords: biomass; climate warming; elevated CO<sub>2</sub>; nitrogen; grassland species

16

1    **Abbreviations**

2	SOM	soil organic matter
3	T <sub>air</sub>	air temperature
4	PAR	photosynthetically active radiation
5	SD	standard deviation
6	vpd	vapour pressure deficit
7	ET	evapotranspiration
8	SWC	soil water content
9	ANCOVA	analysis of covariance
10	SEN	soil extractable nitrogen
11		

1    **Introduction**

2       The fertilizing effect of nutrients on biomass production and crop yield is widely  
3       established by a wealth of empirical evidence (see a.o. Vitousek et al. 1997). Being an  
4       important macro nutrient, nitrogen (N) is often one of the most limiting for production  
5       (Vitousek et al. 1997). On the other hand, ecosystem productivity will, already in the  
6       near future, be affected by changing climate and increasing CO<sub>2</sub> concentrations.

7       Future climate will influence plant production not only through direct effects  
8       (Ainsworth and Long 2005; Rustad et al. 2001) but, also via indirect effects, e.g. by  
9       interaction with the N cycle (Soussana and Luscher 2007).

10      In grasslands, increased CO<sub>2</sub> consistently stimulates growth and productivity  
11      (Ainsworth and Long 2005), while effects of experimental warming on productivity  
12      vary between positive and negative, with the mean response around zero (Rustad et al.  
13      2001). The few experiments on combined effects of elevated CO<sub>2</sub> and higher  
14      temperatures on grassland ecosystems are also not equivocal, with productivity  
15      responses varying from additive to strongly interactive (Norby and Luo 2004; Shaw et  
16      al. 2002). This makes the response of grasslands to combined climatic changes  
17      difficult to forecast, while Earth System Models do predict concurrent increases in  
18      CO<sub>2</sub> and temperature in all IPCC SRES (Intergovernmental Panel on Climate Change  
19      Special Reports Emission Scenarios) scenarios (IPCC 2007).

20      The problem is similar for interactions between climate change and nitrogen  
21      supply, which have likewise predominantly been studied for single factors effects on  
22      plant production. Under elevated CO<sub>2</sub>, plant demand for N increases, and hence N can  
23      become more limiting for plant productivity (Oren et al. 2001). The combined effects  
24      of elevated CO<sub>2</sub> and N supply have therefore been studied frequently (Luo et al. 2004;  
25      Oren et al. 2001; Soussana and Luscher 2007). However, concurrent warming could

1 help overcome the progressive N limitation, by stimulating mineralization (Hovenden  
2 et al. 2008). Unfortunately, combined CO<sub>2</sub> - temperature - N addition studies are still  
3 very rare. In the only study conducted so far, Dukes et al. (2005) found that nitrate  
4 deposition increased biomass production in contrast to and irrespective of elevated  
5 CO<sub>2</sub> and/or warming and/or precipitation.

6 Impacts of climate change arise through complex pathways, several of which can  
7 affect the N cycle. For example, climate change induced alterations of the N cycle  
8 could operate through the soil mineral N pool. Both mineralization of soil organic  
9 matter (SOM) and immobilization of N in SOM are driven by microbial activity that  
10 can be affected directly or indirectly by climate change (Zak et al. 2000). The balance  
11 between the changes in both processes will determine how and if plant N availability  
12 is altered, and thus also the potential productivity response of grasslands in a future  
13 climate. Another possible pathway via which N cycling interacts with climate change  
14 involves a climate-driven change in the fraction of N<sub>2</sub>-fixing species. N<sub>2</sub>-fixing plants  
15 can assimilate atmospheric N and thus increase ecosystem N stocks (Hogh-Jensen and  
16 Schjoerring 2000). If climate change alters the composition of plant communities  
17 (Zavaleta et al. 2003), the available amount of N at the community level could  
18 change. Such pathways of climate change responses combining species interactions  
19 with nutrient cycles in complex systems are still largely unexplored.

20

21 The mediating role of nitrogen in these various response mechanisms suggests not  
22 only that plant communities may react differently to climate change in the presence or  
23 absence of N addition but, looking at it from a different angle, that their response to N  
24 addition might change in a future climate. Addressing this question is hampered by

1 the fact that the majority of studies on ecosystem responses still focus on single  
2 components of change (Norby and Luo 2004).

3 To detect the effect of a future climate on biomass responses to N addition,  
4 synthetic grassland communities were subjected to different levels of N supply either  
5 under current conditions or under simultaneous elevated CO<sub>2</sub> and warming. These  
6 conditions were created in climate-controlled chambers as a cost-effective method to  
7 detect changes, which can subsequently be explored further in more expensive, large-  
8 scale field set-ups with combined free-air CO<sub>2</sub> enrichment and free-air temperature  
9 increase.

10 We exposed synthetic plant communities with multiple species (6), such that  
11 responses arose both from the species-specific sensitivities and from interspecific  
12 interactions. The drawback of this choice is that the intrinsic response of the species in  
13 the absence of competitors or facilitators cannot be determined, but this is outweighed  
14 by two advantages: the complexity of real communities is approximated, and changes  
15 in species composition of the communities can be studied. Because of the multiple  
16 possible interactions between climate change and N-cycling, the response of plant  
17 biomass production to N addition may differ in a future climate where the balance  
18 between microbial and plant N uptake may be altered. Therefore the following  
19 hypotheses were tested:

20 1) N addition stimulates biomass production of synthetic grassland communities  
21 differently in future versus current climate;  
22 2) this alteration is mediated by shifts in plant species composition.  
23

1    **Material and Methods**

2    Experimental set-up

3       This research is part of a larger experimental platform in which newly established  
4       grassland communities are subjected to different stressors in a future climate. This  
5       paper will focus on N and differences in the availability of this resource. The platform  
6       is located at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium ( $51^{\circ} 09'$   
7       N,  $04^{\circ} 24'$  E), where average annual precipitation is 776 mm (evenly distributed  
8       throughout the year) and average annual air temperature is  $10.8^{\circ}\text{C}$ . The grassland  
9       communities were grown in six sunlit, climate-controlled chambers, facing south. The  
10      distances between the chambers were maximized (2.2 and 3.1 m) to avoid mutual  
11      shading. The interior surface area was  $1.5 \times 1.5$  m, the height at the north side 1.5 m  
12      and at the south side 1.2 m. The top of the chambers consisted of a colorless  
13      polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film  
14      (200  $\mu\text{m}$  thick), both UV transparent. Three of the six chambers tracked the current  
15      climate with current air temperature ( $T_{\text{air}}$ ) and CO<sub>2</sub> concentration, while the other  
16      three chambers were exposed to a future climate scenario with  $3^{\circ}\text{C}$  warming and a  
17      target CO<sub>2</sub> concentration of 620 ppm (further referred to as ‘current’ and ‘future  
18      climate’, respectively). Because the experimental platform involved a large number of  
19      factors, a completely factorial design with warming and CO<sub>2</sub> as separate treatments  
20      was not feasible. We therefore chose to combine CO<sub>2</sub> and warming in a single  
21      treatment as characterization of a future climate.

22       The CO<sub>2</sub> concentration was measured and regulated with a CO<sub>2</sub> control group with  
23       a CO<sub>2</sub> analyzer (WMA-4, PPSystems, Hitchin, UK). In the current climate chambers  
24       the concentration was  $375 \pm 17$  ppm (SD) while in the future climate chambers it was  
25        $657 \pm 105$  ppm (SD). Every half hour,  $T_{\text{air}}$  was monitored with a combined humidity–

1 temperature sensor (Siemens, type QFA66, Erlangen, Germany) and  
2 photosynthetically active radiation (PAR) with a quantum sensor (SDEC, type  
3 JYP1000, Tours, France). During the experiment (7 May – 4 October, 2007), monthly  
4 average  $T_{air}$  was 14.5, 17.6, 17.4, 17.4 and 14.6 °C in May, June, July, August and  
5 September, respectively. In the current climate chambers  $T_{air}$  was on average  $0.3 \pm 1.6$   
6 °C (SD) higher than outside, while the future climate chambers were  $3.3 \pm 2.1$  °C  
7 (SD) warmer than outside. Average vapour pressure deficit (vpd) was  $0.28 \pm 0.31$  and  
8  $0.66 \pm 0.41$  kPa (SD) in current and future climate, respectively. The average daily  
9 PAR sum was  $26.9 \text{ mol m}^{-2} \text{ d}^{-1}$  and differed very little between the two climates ( $2.1 \pm$   
10  $0.6 \text{ mol m}^{-2} \text{ d}^{-1}$  (SD))

11 Each chamber contained 30 grassland communities with the same species  
12 composition in PVC containers (24 cm inner diameter, 40 cm height), filled with  
13 sandy soil (93.23% sand, 4.59% silt, 2.19% clay; field capacity  $0.13 \text{ m}^3 \text{ m}^{-3}$ ; pH 7.6;  
14 total Kjeldahl-N  $0.42 \text{ g kg}^{-1}$ ). The containers were placed side-by-side so that a closed  
15 canopy could form, and were buried in the soil to avoid unnatural soil temperatures.  
16 Irrigation was calculated from the monthly rainfall over the period 1995-2005 and  
17 corrected for differences in evapotranspiration (ET) inside and outside the chambers.  
18 To this end, De Boeck et al. (2006) calculated ET inside current climate chambers  
19 from changes in soil water content (SWC) and the amount of administered water, and  
20 the outside ET with Hamon's equation (Haith and Shoemaker 1987) based on day  
21 length, vapour pressure and  $T_{air}$ . The containers were watered every two days  
22 according to the 10 year average of 14 to 15 raining days per month during the  
23 growing season. Total monthly irrigation matched 61.5, 64.4, 85.1, 80.2, 80.9 and  
24 69.7 mm in May, June, July, August, September and October, respectively. The future  
25 climate chambers received the same amount of water as the current climate chambers,

1 so that any enhanced consumption would result in aggravated soil drought. Water  
2 could freely drain from the containers while capillary rise was prevented by a  
3 drainage system placed below the chambers. Profile probe tubes for the PR2 soil  
4 moisture sensor (Delta-T Devices Ltd., UK) were installed in each community and  
5 SWC was measured every 10 days between 22 June and 29 September 2007. Soil  
6 water content was not altered by the climate scenario ( $F_{1,4.82} = 2.93$ ,  $p = 0.150$ ;  
7 ANCOVA; see *Data analyses*), decreased slightly during the growing season ( $F_{1,4.91} =$   
8 14.96,  $p = 0.012$ ) from 11.6 to 10.5%, but remained well above wilting point (6.9%)  
9 and below field capacity (13%). The decrease did not differ between the climates (no  
10 significant interaction day of year  $\times$  climate;  $F_{1,3.96} = 0.21$ ,  $p = 0.668$ ).

11 In each chamber, seven of the 30 containers were randomly chosen to develop five  
12 levels of N addition. Four containers received either 0 g N m<sup>-2</sup>, 1 g N m<sup>-2</sup>, 3 g N m<sup>-2</sup> or  
13 7 g N m<sup>-2</sup>, and three of them received 15 g N m<sup>-2</sup> (corresponding with 0 to 150 kg N  
14 ha<sup>-1</sup>). This yields three replicates (chambers) per N treatment, only the highest level  
15 had two additional containers per chamber. The fertilizer was applied as NH<sub>4</sub>NO<sub>3</sub>. In  
16 addition all communities (also those that received no N) were fertilized with 7.5 g m<sup>-2</sup>  
17 P<sub>2</sub>O<sub>5</sub> and 15 g m<sup>-2</sup> K<sub>2</sub>O and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The amounts of  
18 P and K were kept constant so that only effects of the N treatments were measured.  
19 All of the fertilizer was given dissolved in water (assuring no percolation), in four  
20 equal amounts evenly spread over the growing season.

21  
22 Grassland communities

23 The plant communities were established between 7 and 10 May 2007 by  
24 transplanting five-week-old seedlings. Each community contained 18 plants and was  
25 composed of six equally represented species from three functional groups (two

1 species per group): grasses (*Lolium perenne* L., *Poa pratensis* L.), non-N<sub>2</sub>-fixing  
2 dicots (*Plantago lanceolata* L., *Rumex acetosa* L.) and N<sub>2</sub>-fixing dicots (*Lotus*  
3 *corniculatus* L., *Medicago lupulina* L.). All these species have a perennial life cycle,  
4 co-occur in temperate European grasslands and have comparable heights. The 18  
5 plants (three per species) were placed in a hexagonal grid with a 4.5 cm interspace.  
6 Interspecific interactions were maximized by avoiding clumping and by using five  
7 different designs. The five designs were randomly distributed over the treatments.

8

## 9 Biomass and nitrogen

10 Above-ground (shoot and stubble) and below-ground (root) biomass were  
11 harvested at the end of the growing season (1-4 October, 2007). Above-ground  
12 biomass was subdivided by species. Total below-ground biomass per community was  
13 estimated from 12 soil cores (2 cm diameter) per container. To adequately represent  
14 the total root biomass in the soil, six cores were taken directly below the plants (one  
15 per species) and six cores in the middle of a triangle between three plant positions.  
16 Root samples were washed until they were free of soil. All plant material was dried at  
17 70 °C for 48 hours and then weighed.

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

25

1 Data analysis

2 The experiment had a split plot design with climate (future vs. current) as the main  
3 plot factor and N treatment (further referred to as ‘nitrogen’) as the subplot factor.

4 Analyses of covariance (ANCOVA) were performed in SAS (version 9.1, SAS  
5 Institute Inc., Cary, NC) using the mixed procedure (Littell et al. 1996) with climate,  
6 nitrogen (co-variable) and, if appropriate species, as fixed factors, and chamber as a  
7 random factor nested within climate. Soil water content was analyzed with repeated  
8 measures ANCOVA with time as co-variable (repeated in time). Non-significant  
9 treatment factors (climate, nitrogen, species) were excluded from the ANCOVAs. In  
10 case of significant interactions a posteriori analyses of the parameter estimates were  
11 performed with a t-test. The random factor chamber was never significant (except on  
12 SWC) but was nevertheless kept in the model to account for undetected chamber  
13 effects. In this way, we assured that degrees of freedom of tests of the fixed effects  
14 were not inappropriately high in any analysis (Hurlbert 1984; Oksanen 1999).

15 ‘Kenwardroger’ corrections for degrees of freedom were used (Kenward and Roger  
16 1997), to account for correlations between the data within individual chambers and  
17 make use of this degree of dependency to approximate the effective number of  
18 degrees of freedom.

19

20 **Results**

21 Community biomass

22 The total biomass of the grassland communities increased with increasing N supply  
23 in the current climate, but did not respond to the applied N in the future climate  
24 (significant climate × nitrogen interaction, Fig. 1, Table 1; a posteriori analyses per  
25 climate, respectively  $t_{31,3} = 5.27$ ,  $p < 0.0001$  and  $t_{31,2} = 1.03$ ,  $p = 0.309$ ). This

1 difference in response to N supply was also significant in root biomass (Fig. 1, Table  
2 1) and Figure 1 suggests a similar pattern above-ground (although not significant;  
3 Table 1).

4 To determine the possible impact of changes in species composition on these  
5 surprising results, above-ground biomass was analyzed by species (Fig. 2, note that  
6 the species were not discernable below-ground). Within species, the slopes of the  
7 biomass responses to N supply did not differ significantly between the climates (no  
8 climate  $\times$  nitrogen interaction, Table 2). However, when the species specific  
9 responses to N addition in the future climate were plotted against those in the current  
10 climate (Fig. 3), all species were located below the 1:1 line on this graph. This  
11 suggests that small and statistically insignificant differences between the slopes in the  
12 two climates, pointing in the same direction for all species, may have contributed to  
13 the significantly weaker response of total community biomass to N supply in a future  
14 climate (Fig. 1).

15 Regardless of the climate, with increasing N supply there was a shift in above-  
16 ground biomass between the three most abundant species. *Medicago lupulina*, a N<sub>2</sub>-  
17 fixer, declined, while *P. lanceolata*, and especially *L. perenne* (a fast growing species  
18 with a high N demand) became more abundant (reflected in significant nitrogen  $\times$   
19 species interaction, Fig. 2, Table 2). We therefore assume that *M. lupulina* was  
20 competitively suppressed at high N, but this remains speculative.

21  
22 Plant and soil N stocks

23 The previous section indicated that species shifts occurred in response to N  
24 addition, but that these did not differ between the climates. Thus, the contrasting  
25 sensitivities of community biomass to N supply between the two climates (Fig. 1)

1 most likely did not originate from climate-driven changes in species composition.  
2 Two possible causes thus remain: the contrasting sensitivities either arose from  
3 differences in N uptake (i.e. impaired uptake in the future climate at higher N supply,  
4 yielding the flat biomass-N curve for this treatment in Fig. 1), or uptake was not  
5 affected by future climate but N use efficiency was. Fig. 4 shows that N uptake was  
6 responsible, as the differences in community N stocks between the treatments mirror  
7 those in community biomass shown in Fig. 1. Likewise to biomass, community N  
8 stocks increased with N supply in the current climate, while in the future climate they  
9 did not change significantly (significant climate  $\times$  nitrogen interaction, Table 1; a  
10 posteriori analyses per climate, respectively  $t_{7.29} = 2.45$ ,  $p = 0.042$  and  $t_{7.38} = -0.92$ ,  $p$   
11 = 0.386). Both above-ground and root N stocks showed this pattern (Fig. 4),  
12 suggesting that there were no shifts in N allocation, although the interaction was  
13 statistically significant only for roots (Table 1).

14 To test whether the changes in plant N acquisition with climate might originate  
15 from altered N availability in the soil we determined the SEN. At the start of the  
16 experiment SEN was  $20.2 \text{ mg N kg}^{-1}$  air dry soil or  $9.4 \text{ g N m}^{-2}$ . At the end of the  
17 season, after the harvest, there were, no differences in SEN between the climates or  
18 the five N treatments (Fig. 5, Table 1). Values at the end were  $19.2 \pm 0.3 \text{ mg N kg}^{-1}$   
19 air dry soil or  $8.9 \pm 0.1 \text{ g N m}^{-2}$  (mean  $\pm$  SE;  $n=29$ ).

20

## 21 Discussion

22 Community biomass production did not respond to N supply in the future climate,  
23 while it increased ( $47.5 \text{ g g}^{-1}$  N added) in the current climate (Fig. 1). This difference  
24 in productivity response to N between the climates could not be attributed to changes  
25 in species composition between the climates. The species that responded positively to

1 N addition in the current climate did so in the future climate (*L. perenne*); likewise for  
2 the species that responded negatively (*M. lupulina*) or had no response (Fig. 2).  
3 However, all species seemed to have contributed slightly to the altered community  
4 response to N in the future climate (Fig. 3). Similar results were reported for a  
5 Mediterranean grassland where community biomass production responded less to a 7  
6 g m<sup>-2</sup> N supply in future than in current climate, although the authors did not test for  
7 this (Fig. 6 in Dukes et al (2005); comparing the difference in biomass increase from  
8 (i) ambient CO<sub>2</sub> to (ii) ambient CO<sub>2</sub> + nitrate deposition with, on the other hand, from  
9 (iii) elevated CO<sub>2</sub> + increased temperature to (iv) elevated CO<sub>2</sub> + increased  
10 temperature + nitrate deposition).

11 What may have caused the grassland communities to respond less to N supply in a  
12 warmer environment with more atmospheric CO<sub>2</sub>? To answer this question we have to  
13 consider the N balance of the mesocosms. This N balance consists of fluxes into - and  
14 out of the SEN (soil extractable N) pool and the vegetation (Fig. 6). Mineral N enters  
15 the SEN by mineralization of SOM and by added fertilizer. Outputs of N from the  
16 SEN are uptake by the vegetation and losses of N through immobilization by  
17 microorganisms in the soil and through leaching and gaseous emissions by microbial  
18 processes (Thornley and Cannell 2000). In addition, N enters the vegetation through  
19 N<sub>2</sub>-fixation. At the end of our experiment, the SEN equaled 95% of the pool at the  
20 start and did not differ among treatments, whatever the amount of N added (Fig. 5).  
21 The extractable mineral N pool being unaltered by climate change or nitrogen  
22 addition, it is clear that only shifts in the component fluxes can explain the differences  
23 in community N stocks.

24 In both climates, in the absence of additional N, the only input fluxes of N to the  
25 plant and SEN pools were N<sub>2</sub>-fixation and mineralization of SOM. When adding a

1 third input flux, fertilizer N, the plant communities in the current climate enhanced  
2 their total N stock, as expected. This increase in total plant N ( $11.1 \text{ g N m}^{-2}$  in current  
3 climate) reflected the major part of the  $15 \text{ g m}^{-2}$  added fertilizer (Fig. 4). However, in  
4 the future climate the plant community N stock remained the same upon fertilizer  
5 addition. Given the fact that the soil mineral N pool was never affected, and  
6 respecting the N balance, two explanations seem feasible (i) the added fertilizer was  
7 absorbed by the plants in the future climate, but plant community N stocks  
8 nevertheless remained the same because atmospheric N<sub>2</sub>-fixation declined  
9 accordingly; (ii) in the future climate, the balance between mineralization and  
10 immobilization was altered, changing the N availability for the plants.

11 Active nodules (pink to red colour) were observed during harvest, confirming N<sub>2</sub>-  
12 fixation, although it was not quantified. Nitrogen addition has been found to decrease  
13 N<sub>2</sub>-fixation (Carlsson et al. 2009). Explanation (i), however, implies a different  
14 response of N<sub>2</sub>-fixation to N addition between the climates. This seems unlikely  
15 because the only N<sub>2</sub>-fixing species that reduced its biomass upon fertilization, *M.*  
16 *lupulina*, did so to a similar extent in current and future climate (Fig. 2). Explanation  
17 (ii), in contrast, is very plausible if in the future climate additional labile C inputs  
18 provided extra carbon and energy for the microbes, thus enhancing the N demand of  
19 the growing and more active microbial population (de Graaff et al. 2006) that converts  
20 plant available N into plant unavailable microbial products. Stimulated labile carbon  
21 inputs via rhizodeposition (root turnover, root exudation and mycorrhizal turnover)  
22 have been reported for elevated CO<sub>2</sub> (Allard et al. 2006; Cheng 1997; Godbold et al.  
23 2006; Johansson et al. 2009; Meier et al. 1997; Uselman et al. 2000) and warming  
24 (Meharg and Killham 1989; Uselman et al. 2000; Whipps 1984).

25

1 In conclusion, this study, which is one of the few studies that combine warming,  
2 elevated CO<sub>2</sub> and nitrogen supply, provides the striking result that N fertilization does  
3 not stimulate productivity of synthetic grassland communities in a future climate. In  
4 spite of the somewhat artificial context of the experiment (small scale, disturbed soil  
5 and artificial soil hydrology), the net primary productivity (NPP) of the grassland  
6 communities was between 1500 and 2500 g biomass m<sup>-2</sup> (Fig. 1), which is close to the  
7 European average grassland NPP of 1500 g biomass m<sup>-2</sup> (Schulze et al. 2009). This  
8 indicates the value of our experiment as a screening study. Moreover, the fact that  
9 similar results were found for a different biome (Mediterranean grasslands, Dukes et  
10 al. 2005), stresses the need for further validation in large-scale set-ups with field  
11 swards exposed to combined free-air CO<sub>2</sub> enrichment and free-air temperature  
12 increase. In addition, studies should be conducted on a longer timescale, since e.g.  
13 species composition may not be stable yet, if seed production and recruitment are  
14 affected. On the other hand, changes in N availability under elevated CO<sub>2</sub> are likely to  
15 be progressive (especially at lower N supplies) (Luo et al. 2004) and interactive  
16 effects of warming may likewise develop over time (Hovenden et al. 2008). In further  
17 exploration, emphasis will need to be on soil processes which determine N availability  
18 for plants. Nevertheless, the observed altered response to N addition in a future  
19 climate could have important agricultural implications.

20

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6

1    **References**

- 2    Ainsworth EA and Long SP (2005) What have we learned from 15 years of free-air  
3    CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of  
4    photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. New  
5    Phytol. 165:351-371
- 6    Allard V, Robin C, Newton PCD, Lieffering M and Soussana JF (2006) Short and  
7    long-term effects of elevated CO<sub>2</sub> on *Lolium perenne* rhizodeposition and its  
8    consequences on soil organic matter turnover and plant N yield. Soil Biol.  
9    Biochem. 38:1178-1187
- 10    Carlsson G, Palmborg C, Jumpponen A, Scherer-Lorenzen M, Hogberg P and Huss-  
11    Danell K (2009) N<sub>2</sub> fixation in three perennial *Trifolium* species in  
12    experimental grasslands of varied plant species richness and composition.  
13    Plant Ecol. 205:87-104
- 14    Cheng WX (1997) Rhizosphere feedbacks in elevated CO<sub>2</sub>. Tree Physiology 19:313-  
15    320
- 16    De Boeck HJ, Lemmens C, Bossuyt H, Malchaire S, Carnol M, Merckx R, Nijs I and  
17    Ceulemans R (2006) How do climate warming and plant species richness  
18    affect water use in experimental grasslands? Plant Soil 288:249-261
- 19    de Graaff MA, van Groenigen KJ, Six J, Hungate B and van Kessel C (2006)  
20    Interactions between plant growth and soil nutrient cycling under elevated  
21    CO<sub>2</sub>: a meta-analysis. Glob. Change Biol. 12:2077-2091
- 22    Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR, Thayer S, Tobeck T,  
23    Mooney HA and Field CB (2005) Responses of grassland production to single  
24    and multiple global environmental changes. PLoS. Biol. 3:1829-1837

- 1 Godbold DL, Hoosbeek MR, Lukac M, Cotrufo MF, Janssens IA, Ceulemans R, Polle  
2 A, Velthorst EJ, Scarascia-Mugnozza G, De Angelis P, Miglietta F and  
3 Peressotti A (2006) Mycorrhizal hyphal turnover as a dominant process for  
4 carbon input into soil organic matter. *Plant Soil* 281:15-24
- 5 Haith DA and Shoemaker LL (1987) Generalized watershed loading functions for  
6 stream-nutrients. *Water Resources Bulletin* 23:471-478
- 7 Hogh-Jensen H and Schjoerring JK (2000) Below-ground nitrogen transfer between  
8 different grassland species: Direct quantification by  $^{15}\text{N}$  leaf feeding compared  
9 with indirect dilution of soil  $^{15}\text{N}$ . *Plant Soil* 227:171-183
- 10 Hovenden MJ, Newton PCD, Carran RA, Theobald P, Wills KE, Schoor JKV,  
11 Williams AL and Osanai Y (2008) Warming prevents the elevated  $\text{CO}_2$   
12 induced reduction in available soil nitrogen in a temperate, perennial  
13 grassland. *Glob. Change Biol.* 14:1018-1024
- 14 Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments.  
15 *Ecol. Monogr.* 54:187-211
- 16 IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of  
17 Working Group I to the Fourth Assessment Report of the Intergovernmental  
18 Panel on Climate Change. Cambridge University Press, Cambridge
- 19 Johansson EM, Fransson PMA, Finlay RD and van Hees PAW (2009) Quantitative  
20 analysis of soluble exudates produced by ectomycorrhizal roots as a response  
21 to ambient and elevated  $\text{CO}_2$ . *Soil Biol. Biochem.* 41:1111-1116
- 22 Kenward MG and Roger JH (1997) Small sample inference for fixed effects from  
23 restricted maximum likelihood. *Biometrics* 53:983-997
- 24 Littell RC, Milliken GA, Stroup WW and Wolfinger RD (1996) SAS System for  
25 mixed models. SAS Institute Inc., Cary, North Carolina.

- 1   Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE,  
2           Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR and Field CB (2004)  
3           Progressive nitrogen limitation of ecosystem responses to rising atmospheric  
4           carbon dioxide. *Bioscience* 54:731-739
- 5   Meharg AA and Killham K (1989) Distribution of assimilated carbon within the plant  
6           and rhizosphere of *Lolium perenne* - influence of temperature. *Soil Biol.*  
7           *Biochem.* 21:487-489
- 8   Meier M, Saurer M, Haldemann C and Fuhrer J (1997) Effect of elevated CO<sub>2</sub> on the  
9           carbon balance of a grass-clover mixture. *Acta Oecol.-Int. J. Ecol.* 18:313-317
- 10   Norby RJ and Luo YQ (2004) Evaluating ecosystem responses to rising atmospheric  
11           CO<sub>2</sub> and global warming in a multi-factor world. *New Phytol.* 162:281-293
- 12   Oksanen L 1999 Logic of experiments in ecology: is pseudoreplication a pseudoissue?  
13           In *Oikos Seminar on Costs and Gains of Recent Progress in Ecology*. pp 27-  
14           38. Munksgaard Int Publ Ltd, Hallnas, Sweden.
- 15   Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR,  
16           McCarthy H, Hendrey G, McNulty SG and Katul GG (2001) Soil fertility  
17           limits carbon sequestration by forest ecosystems in a CO<sub>2</sub>-enriched  
18           atmosphere. *Nature* 411:469-472
- 19   Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE,  
20           Cornelissen JHC and Gurevitch J (2001) A meta-analysis of the response of  
21           soil respiration, net nitrogen mineralization, and aboveground plant growth to  
22           experimental ecosystem warming. *Oecologia* 126:543-562
- 23   Schulze ED, Luyssaert S, Ciais P, Freibauer A, Janssens IA, Soussana JF, Smith P,  
24           Grace J, Levin I, Thiruchittampalam B, Heimann M, Dolman AJ, Valentini R,  
25           Bousquet P, Peylin P, Peters W, Rodenbeck C, Etiope G, Vuichard N,

- 1 Wattenbach M, Nabuurs GJ, Poussi Z, Nieschulze J and Gash JH (2009)
- 2 Importance of methane and nitrous oxide for Europe's terrestrial greenhouse-
- 3 gas balance. Nat. Geosci. 2:842-850
- 4 Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA and Field CB (2002)
- 5 Grassland responses to global environmental changes suppressed by elevated
- 6 CO<sub>2</sub>. Science 298:1987-1990
- 7 Soussana JF and Luscher A (2007) Temperate grasslands and global atmospheric
- 8 change: a review. Grass Forage Sci. 62:127-134
- 9 Thornley JHM and Cannell MGR (2000) Dynamics of mineral N availability in
- 10 grassland ecosystems under increased CO<sub>2</sub>: hypotheses evaluated using the
- 11 Hurley Pasture Model. Plant Soil 224:153-170
- 12 Uselman SM, Qualls RG and Thomas RB (2000) Effects of increased atmospheric
- 13 CO<sub>2</sub>, temperature, and soil N availability on root exudation of dissolved
- 14 organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). Plant Soil
- 15 222:191-202
- 16 Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW,
- 17 Schlesinger WH and Tilman GD (1997) Human alteration of the global
- 18 nitrogen cycle: Sources and consequences. Ecol. Appl. 7:737-750
- 19 Whipps JM (1984) Environmental factors affecting the loss of carbon from the roots
- 20 of wheat and barley seedlings. J. Exp. Bot. 35:767-773
- 21 Zak DR, Pregitzer KS, King JS and Holmes WE (2000) Elevated atmospheric CO<sub>2</sub>,
- 22 fine roots and the response of soil microorganisms: a review and hypothesis.
- 23 New Phytol. 147:201-222

1 Zavaleta ES, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, Field CB and  
2 Mooney HA (2003) Grassland responses to three years of elevated  
3 temperature, CO<sub>2</sub>, precipitation, and N deposition. *Ecol. Monogr.* 73:585-604  
4  
5

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

5

Biomass	total plant			above-ground			roots			soil		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
nitrogen	1, 31.2	19.85	<b>0.001</b>	1, 10.4	5.23	<b>0.044</b>	1, 31.5	12.42	<b>0.001</b>			
climate	1, 14.9	5.32	<b>0.036</b>	1, 3.5	0.08	0.793	1, 15.3	4.20	0.058			
climate x nitrogen	1, 31.2	8.96	<b>0.005</b>	1, 6.7	3.33	0.113	1, 31.5	4.26	<b>0.047</b>			
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	<b>0.018</b>	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	<b>0.045</b>	1, 25	0.06	0.804
climate x nitrogen	1, 7.3	5.67	<b>0.047</b>	1, 6.3	1.93	0.211	1, 6.3	6.82	<b>0.038</b>	1, 25	0.63	0.454

6

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

4

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	<b>&lt;.0001</b>
nitrogen x species	5, 195	30.32	<b>&lt;.0001</b>
climate x species	5, 21.8	2.45	0.066
climate x nitrogen	1, 11.6	2.93	0.113
climate x nitrogen x species	5, 192	0.48	0.794

5

6

7

8

1   **Fig. 1** Biomass of synthetic grassland communities exposed to five levels of N supply  
2   (between 0 and 15 g N m<sup>-2</sup>) and to current (□, ---) or future (■, —) climate. Means ±  
3   SE (n = 3 (chambers); at 15 g N m<sup>-2</sup>, n = 9 (3 chambers, 3 replicates per chamber) for  
4   total, above-ground, and root biomass. Individual pot values were used for linear  
5   regressions (n = 21)

6

7   **Fig. 2** Above-ground biomass of the individual species of the synthetic grassland  
8   communities exposed to five levels of N supply (between 0 and 15 g N m<sup>-2</sup>) and to  
9   current (□, ---) or future (■, —) climate. Means ± SE (n = 3 (chambers); but, at 15 g  
10   N m<sup>-2</sup>, n = 9 (3 chambers, 3 replicates per chamber)). Individual pot values were used  
11   for linear regressions (n = 21)

12

13   **Fig. 3** Slopes of the above-ground biomass responses of individual grassland species  
14   to increasing N supply (Fig. 2) in future climate plotted against the same slopes in  
15   current climate. The 1:1 line indicates no effect of climate on the biomass response to  
16   nitrogen supply. Species below this line undergo a negative effect of future climate  
17   and species above the line a positive effect

18

19   **Fig. 4** N stock of synthetic grassland communities exposed to five levels of N supply  
20   (between 0 and 15 g N m<sup>-2</sup>) and to current (□, ---) or future (■, —) climate. Means ±  
21   SE (n = 3 (chambers)) for total, above-ground, and root N stock. Individual pot values  
22   were used for linear regressions (n = 15)

23

24   **Fig. 5** Soil extractable nitrogen in synthetic grassland ecosystems exposed to five  
25   levels of N supply (between 0 and 15 g N m<sup>-2</sup>) and to current (□, ---) or future (■,

1 —) climate. Means  $\pm$  SE ( $n = 3$  (chambers)). Individual pot values were used for  
2 linear regressions ( $n = 15$ )

3

4 **Fig. 6** Schematic presentation of the N balance of the synthetic grassland  
5 communities. SEN: soil extractable N pool. Modified from (Thornley and Cannell  
6 2000)

7