Functional Ecology 2006
20, 557–564

Several components of global change alter nitrifying and denitrifying activities in an annual grassland

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Summary

1. The effects of global change on below-ground processes of the nitrogen (N) cycle have repercussions for plant communities, productivity and trace gas effluxes. However, the interacting effects of different components of global change on nitrification or denitrification have rarely been studied *in situ*.

2. We measured responses of nitrifying enzyme activity (NEA) and denitrifying enzyme activity (DEA) to over 4 years of exposure to several components of global change and their interaction (increased atmospheric CO_2 concentration, temperature, precipitation and N addition) at peak biomass period in an annual grassland ecosystem. In order to provide insight into the mechanisms controlling the response of NEA and DEA to global change, we examined the relationships between these activities and soil moisture, microbial biomass C and N, and soil extractable N.

3. Across all treatment combinations, NEA was decreased by elevated CO_2 and increased by N addition. While elevated CO_2 had no effect on NEA when not combined with other treatments, it suppressed the positive effect of N addition on NEA in all the treatments that included N addition. We found a significant CO_2–N interaction for DEA, with a positive effect of elevated CO_2 on DEA only in the treatments that included N addition, suggesting that N limitation of denitrifiers may have occurred in our system. Soil water content, extractable N concentrations and their interaction explained 74% of the variation in DEA.

4. Our results show that the potentially large and interacting effects of different components of global change should be considered in predicting below-ground N responses of Mediterranean grasslands to future climate changes.

Key-words: Denitrification, elevated CO_2, N addition, nitrification, precipitation, warming

Functional Ecology (2006) **20**, 557–564
doi: 10.1111/j.1365-2435.2006.01146.x

Introduction

Many biological processes involved in the N cycle in terrestrial ecosystems are modified by global environmental change (Mosier 1998) and these changes are likely to result in major ecosystem-level changes in plant productivity (Shaw *et al*. 2002), species composition (Zavaleta *et al*. 2003a) and fluxes of atmospherically active gases (Mosier 1998; Hu *et al*. 1999). Our general understanding of the response of the N cycle to several aspects of global change has become clearer over the last decade, including responses to increasing atmospheric CO_2 concentration (Luo *et al*. 1999; Zak *et al*. 2000b), temperature (Rustad *et al*. 2001), precipitation (Jamieson, Monaghan & Barraclough 1999) and N deposition (Matson, Lohse & Hall 2002). However, the response of ecosystems to the interactions among the various components of global change has received less attention (Loiseau & Soussana 2000; Mikan *et al*. 2000; Ollinger *et al*. 2002; Shaw *et al*. 2002). In particular, understanding the interactive effects of components of global change on key processes such as nitrification and denitrification is important, since these processes influence soil inorganic N concentrations, nitrate leaching and the production of N_2O, a highly active greenhouse

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gas that also contributes to stratospheric ozone destruction (IPCC 2001).

The components of global change cited above have the potential, directly or indirectly, to affect nitrification and denitrification. Elevated CO_2 has generally been found to increase soil water content and the availability of below-ground labile C through its effect on plants (Hungate 1999). These CO_2-induced changes are expected to have direct positive effects on the environmental conditions that control denitrification and negative effects on those controlling nitrification, and also have indirect effects on both of these processes, e.g. through their impact of soil inorganic N availability (Hungate 1999). Nitrification and denitrification are affected by changes in temperature in most soil incubation studies (Grundmann *et al.* 1995; Maag & Vinther 1996). However, the few *in situ* studies of the effect of warming on nitrification and denitrification do not show consistent results across experiments, and the underlying mechanisms remain poorly understood (Verburg, Van Loon & Lükewille 1999; Shaw & Harte 2001). Increased precipitation should increase soil water content and soil heterotrophic respiration, thereby reducing soil $[O_2]$ and, under moderately moist soil conditions, is expected to favour denitrifiers while tightening environmental constraints on nitrifiers (Tiedje 1988; Grundmann *et al.* 1995; Maag & Vinther 1996). N addition is expected to increase substrate availability for nitrifiers and denitrifiers, which should result in higher rates of nitrification and denitrification. Finally, little is known of the mechanisms of interaction of the components of global change on nitrification or denitrification, since only a few studies have measured significant effects of treatment interactions (see review in Barnard, Leadley & Hungate 2005).

Measurements of nitrifying and denitrifying enzyme activity (NEA and DEA, respectively) provide a measure of the amount of functionally active enzyme associated with these processes in the soil (e.g. Smith & Tiedje 1979). These assays reflect the environmental constraints on nitrifying and denitrifying communities in the soil, such as N substrate availability, soil aeration, labile C availability, temperature and pH (Tiedje 1988; Paul & Clark 1989). It is not the goal of this study to estimate *in situ* N transformation rates, but to understand the environmental constraints on the microbial enzymatic activities. In the present study, we measured the effect of four interacting components of global change – increased elevated atmospheric CO_2 concentrations, temperature, precipitation and N addition – on NEA and DEA in an annual grassland, together with measurements of soil moisture, microbial biomass C and N, and soil extractable N.

The objectives of this study were to: (i) quantify the amplitude of changes in NEA and DEA in response to components of global changes with particular reference to interaction effects; and (ii) identify the mechanisms that underlie the response of NEA and DEA to these components of global climate change during peak biomass period, a key period in the growing season of these Mediterranean grasslands.

Materials and methods

Experimental set-up and soil sample collection

This study was conducted at Stanford University's Jasper Ridge Biological Preserve in central California (37°24′N, 122°14′W, elevation 150 m), in a moderately fertile, natural Mediterranean grassland dominated by annual grasses (*Avena barbata* Link and *Bromus hordeaceus* L.) and forbs (*Geranium dissectum* L. and *Erodium botrys* (Cav.) Bertol.). The Jasper Ridge Global Change Experiment provided full factorial combination of four, two-level global change treatments and was initiated in November 1998. A detailed description of the experimental set-up and management is given by Shaw *et al.* (2002). In brief, 32 experimental plots (2 m diameter) were organized as a split-plot design, each plot being divided into four 0·78 m^2 quadrants. Treatments were atmospheric CO_2 concentration (ambient and 680 μmol mol^{-1}), temperature (ambient and ambient + 80 W m^{-2} thermal radiation, resulting in a soil-surface temperature increase of 0·8–1 °C), precipitation (ambient and ambient + 50% + 3-week growing-season elongation) and N addition (ambient and ambient + 7 g N–$Ca(NO_3)_2$ m^{-2} $year^{-1}$). These treatments and their combinations are referred to as CO_2, T, W, N and their combinations, respectively. Treatments were applied using CO_2 emitter rings, infrared heaters, spray/drip systems and slow-release fertiliser. The addition of N with slow-release fertiliser was intended to mimic in a crude way atmospheric N deposition. Each of the 16 combinations of CO_2, temperature, N addition and precipitation was replicated eight times.

Two soil cores (2·2 cm diameter × 15 cm deep) were sampled in each plot quadrant on 25–26 April 2003, pooled and homogenized. Sampling date corresponds to the period of peak biomass in this Mediterranean annual grassland (Zavaleta *et al.* 2003a). A subsample of the soil was used to determine gravimetric soil water content. Soil was stored at 4 °C until the enzyme assays began (as observed by Luo *et al.* (1996) a few days of storage did not affect enzyme activities).

Nitrifying and denitrifying enzyme activity measurements

NEA was measured at soil pH using the method described in Lensi *et al.* (1986). This method is similar to other methods for measuring NEA (Hart *et al.* 1994), with the difference that nitrate concentration is measured after biological reduction into N_2O by denitrification as opposed to measurements of nitrate concentration by chemical assays. This has the advantage of providing a more sensitive measure of NEA. The measurement protocol is as follows. Two 5-g subsamples from each

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sample were placed in 150 ml flasks. One flask of each pair was immediately sealed with a rubber stopper and its atmosphere replaced by a 90:10 He–C_2H_2 mixture. Five millilitres of suspension of a denitrifying *Pseudomonas fluorescens* bacteria ($OD_{580} = 2$) in a solution containing 1 mg C-glucose g^{-1} dry soil, 1 mg C–glutamic acid g^{-1} dry soil were then added. This subsample served as a control (i.e. to measure initial soil [NO_3^-]) for the second sample. The second subsample was enriched with 1·4 ml of a $(NH_4)_2SO_4$ solution (final soil N content: 0·2 mg g^{-1} dry soil) in order to ensure moisture content equivalent to 80% water-holding capacity and no limitation by ammonium (high [NH_4^+] should also limit NO_3^- assimilation by microorganisms). The flask was then sealed with Parafilm© and incubated at 25 °C for 24 h. After this aerobic incubation which allowed nitrate to accumulate, the soil was enriched with 3·6 ml *P. fluorescens* suspension in a glucose and glutamic acid solution (same concentrations as above). Anaerobiosis and N_2O-reductase inhibition were obtained in the flask as described above. After complete conversion of NO_3^- into N_2O, N_2O concentrations were analysed by gas chromatography. NEA was calculated as the difference between NO_3^- contents in the first and second subsamples, normalised by the mass of soil used (oven dry equivalent).

We measured DEA (Smith & Tiedje 1979; modified by Patra *et al*. 2005) at soil pH over a short period by making all factors affecting denitrification non-limiting. Five grams equivalent dry soil were placed in a 150 ml plasma flask containing 1 mg C-glucose g^{-1} dry soil, 1 mg C–glutamic acid g^{-1} of dry soil, and 0·1 mg N–NO_3^- g^{-1} dry soil. The atmosphere of each tube was replaced by a 90:10 He–C_2H_2 mixture providing anaerobic conditions and inhibition of N_2O-reductase activity. N_2O efflux was measured in this flask after 0, 4 and 7 h, to make sure no *de novo* synthesis of denitrifying enzyme took place. N_2O concentrations were analysed on a gas chromatograph equipped with an electron capture detector (Varian Star 3400CX, Varian Chromatography Group, Palo Alto, CA).

MICROBIAL BIOMASS N AND C, SOIL EXTRACTABLE N, SOIL NO_3^- AND NH_4^+

Soil microbial N and C were measured by chloroform fumigation-extraction (Brookes *et al*. 1985). Soil samples (10–15 g) were fumigated for 5 days with chloroform vapour. Control samples were not fumigated. Control and fumigated samples were extracted in 0·5 M K_2SO_4 by shaking for 60 min. Samples were frozen until analysis for total carbon using a total organic C analyser (Shimadzu TOC-5000 A, Shimadzu Corp., Tokyo, Japan). Total N in the extracts was later obtained by persulphate digestion and analysis on a continuous flow autoanalyser (Astoria 2, Astoria Pacific, Clackamas OR). Microbial biomass N was calculated as [(total N in fumigated soil) – (total N in non-fumigated soil)]/ 0·54 (Brookes *et al*. 1985). Microbial biomass C was calculated in a similar way, with an extraction efficiency coefficient of 0·45 (Wu *et al*. 1990). Soil extractable N was measured in the unfumigated extracts.

Bags containing 1·5 g combination anion–cation ion-exchange resin (Bio-Rad™ AG-1-X8 CL^- form and Baker™ HCR-W2 H^+ form) were placed in the field from 1 April 2003 to 1 June 2003, at the end of a PVC tube inserted into the soil at a 45° angle such that the bag was at approximately 25 cm depth. When removed, the bags were rinsed in de-ionised water and each bag was extracted in 15 ml of a 2 M KCl solution by shaking for 30 min. The extracts were frozen until analysis in October 2004.

STATISTICAL ANALYSIS

We analysed our data with a split-plot, randomised complete block analysis of variance ($n = 8$ for all measurements), using SAS 8·02 (SAS Institute, Cary, NC). To correct non-equal variance, NEA data were square-root transformed; DEA and microbial biomass C and N data were log-transformed. A least significant difference test (LSD) was performed to determine differences of means between groups. Relative treatment effects were calculated as follows: % effect = 100 × [treatment – control]/control. We also checked for correlations and performed stepwise regressions for NEA and DEA on treatment means for the 16 treatment combinations. The explanatory variables in the stepwise regressions were gravimetric soil water content, soil extractable N, and microbial biomass N and C.

Results

NITRIFYING AND DENITRIFYING ENZYME ACTIVITIES

Across all treatments, elevated CO_2 depressed NEA by 46% (Fig. 1, Table 1). Under ambient CO_2, N addition increased NEA, but this effect was not apparent at elevated CO_2 (significant $CO_2 \times N$ interaction). We found no significant overall effects of the precipitation and temperature treatments.

Increased precipitation and N addition significantly increased DEA (respectively +45 and +68%, Fig. 2, Table 1). Elevated CO_2 increased DEA at high N addition and ambient temperature ($CO_2 \times N$ interaction), but at high N addition and warming, elevated CO_2 had no effect on DEA ($CO_2 \times T \times N$ interaction). Pairwise comparisons showed that at elevated CO_2, the N, WN and TWN treatments significantly increased DEA, respectively, by 77, 34 and 163%. We found no significant overall effects of CO_2 or temperature treatments.

MICROBIAL BIOMASS N AND C, SOIL EXTRACTABLE N, SOIL NO_3^- AND NH_4^+

Microbial biomass N was significantly increased by elevated N addition (+11%), an effect that was attenuated

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Table 1. Effect and interaction of the different treatments (CO_2: elevated CO_2, T: elevated temperature, N: increased N addition, W: increased precipitation) on soil water content (SWC), nitrifying enzyme activity (NEA), denitrifying enzyme activity (DEA), microbial biomass N and C, soil extractable N, and nitrate and ammonium trapped on resins. % effect = 100 × [elevated – ambient]/ambient. Significant responses are indicated in bold.

	SWC		NEA		DEA		Microbial biomass N		Microbial biomass C		Soil extractable N		Resin NH_4^+		Resin NO_3^-	
Treatment	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value	% effect	*P*-value
Main plot effects																
CO_2	1	0·60	**–46**	**<0·001**	12	0·44	–5	0·41	–1	0·95	–4	0·57	2	0·13	–34	0·93
T	–1	0·70	21	0·24	–2	0·65	–3	0·46	–3	0·92	1	0·87	–9	0·62	–38	0·37
CO_2 × T		0·60		0·35		0·70		0·054		0·27		0·62		0·86		0·92
Sub-plot effects																
N	**4**	**0·017**	**48**	**0·006**	**68**	**<0·001**	**11**	**0·014**	3	0·35	**28**	**<0·001**	**79**	**0·005**	**1176**	**<0·001**
W	**5**	**0·003**	6	0·85	**45**	**<0·001**	–6	0·16	–2	0·16	0	0·75	–14	0·71	81	0·37
CO_2 × N		0·99		**0·002**		**0·031**		0·062		0·53		0·62		0·51		0·91
CO_2 × W		0·95		0·24		0·29		0·29		0·39		0·13		0·40		0·79
T × N		0·32		0·18		0·27		0·42		0·71		0·57		**0·049**		0·71
T × W		0·44		0·27		0·42		0·055		0·79		0·89		0·94		0·99
W × N		0·74		0·19		0·32		**0·019**		0·063		0·99		0·92		0·99
CO_2 × T × N		0·20		0·79		**0·009**		0·077		0·39		0·99		0·26		0·29
CO_2 × T × W		0·79		0·82		0·19		0·065		0·64		0·37		0·46		0·45
CO_2 × N × W		0·55		0·46		0·85		0·22		0·52		0·12		0·24		0·77
T × W × N		0·75		0·86		0·22		0·51		0·71		0·97		0·37		0·21

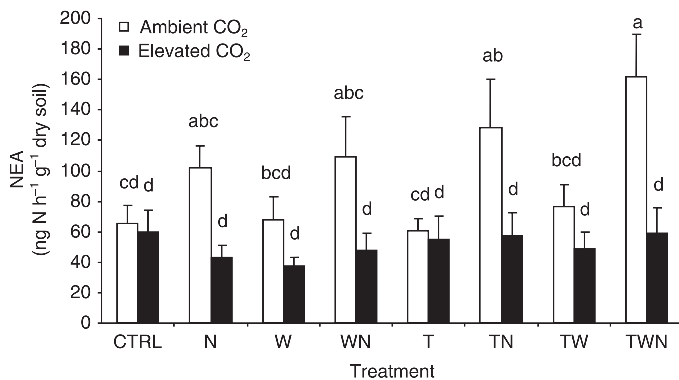

Fig. 1. Nitrifying enzyme activity (NEA) in each treatment combination. Treatments are ambient CO_2 (open bars) and elevated CO_2 (closed bars), N addition (N), precipitation (W) and temperature (T), and their combinations. In the control (CTRL) treatment, all other treatments are ambient. Bars indicate mean ± SE, bars sharing a letter are not significantly different ($P \leq 0{\cdot}05$, LSD test).

Table 2. Microbial biomass N and C, and soil extractable N in the main treatment plots (T: temperature, N: N addition, W: precipitation). Values indicate mean ± SE. Refer to Table 1 for the statistical analysis of the complete data set.

Treatment	Microbial biomass N (µg N g^{-1} dry soil)	Microbial biomass C (µg C g^{-1} dry soil)	Soil extractable N (µg N g^{-1} dry soil)
Ambient CO_2	38·8 ± 1·5	394·6 ± 19·8	9·0 ± 0·4
Elevated CO_2	36·6 ± 1·2	395·2 ± 18·6	8·8 ± 0·4
Ambient T	38·2 ± 1·4	401·6 ± 19·7	8·9 ± 0·4
Elevated T	37·2 ± 1·4	387·7 ± 18·6	8·9 ± 0·4
Ambient N	35·8 ± 1·4	388·2 ± 19·6	7·8 ± 0·2
Elevated N	39·5 ± 1·3	401·6 ± 18·8	10·0 ± 0·5
Ambient W	38·9 ± 1·3	397·4 ± 16·7	9·0 ± 0·4
Elevated W	36·6 ± 1·4	392·5 ± 21·2	8·8 ± 0·3

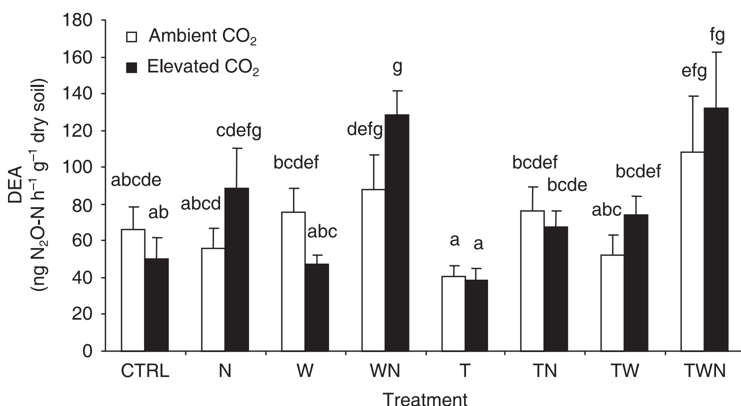

Fig. 2. Denitrifying enzyme activity (DEA) in each treatment combination. Treatments are ambient CO_2 (open bars) and elevated CO_2 (closed bars), N addition (N), precipitation (W) and temperature (T), and their combinations. In the control (CTRL) treatment, all other treatments are ambient. Bars indicate mean ± SE, bars sharing a letter are not significantly different ($P \leq 0{\cdot}05$, LSD test).

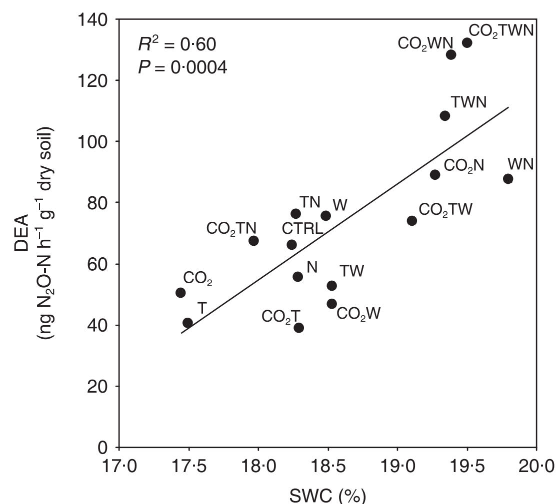

Fig. 3. Correlation between denitrifying enzyme activity (DEA) and soil water content (SWC) in each treatment mean (treatments are CTRL: control, CO_2: elevated CO_2, T: elevated temperature, N: increased N addition, W: increased precipitation, and their combinations).

in combination with increased precipitation (Table 1). Microbial biomass C was not significantly affected by any treatment (Table 1). Increased N addition significantly increased soil extractable N (+28%, Table 1). Increased N addition resulted in a significant increase of NH_4^+ and a large significant increase of NO_3^- trapped in soil resin bags (Table 1). We also measured a significant T × N interaction for NH_4^+ in resin extracts, the positive effect of elevated temperature at ambient N becoming negative with N addition. The values of microbial biomass N and C, and soil extractable N in the main treatment plots are given in Table 2.

SOIL WATER CONTENT

The precipitation and N addition treatments both caused a slight but significant increase in soil water content in our samples (+5%, $P = 0{\cdot}003$ and +4%, $P = 0{\cdot}02$, respectively, Table 1), but other treatments did not alter soil water content. Across all treatments, average soil water content was 18·7%.

CORRELATIONS

We found no significant correlation between NEA and DEA measurements and other measurements performed at the same time (i.e. above-ground biomass, laboratory-incubated soil N_2O and CH_4 effluxes, data not shown). We found significant positive correlations for treatment means between DEA and soil water content ($R^2 = 0{\cdot}60$; $P = 0{\cdot}0004$, Fig. 3).

The variation of NEA was not significantly explained by the explanatory variables that we measured. Stepwise regression showed that soil water content, soil extractable N and their interaction explained 74% of the variation of DEA, with soil water content explaining 60% ($P < 0{\cdot}001$), soil extractable N an additional 9% ($P = 0{\cdot}02$) and their interaction 7% ($P = 0{\cdot}04$).

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Discussion

NITRIFYING ENZYME ACTIVITY

At ambient CO_2, N addition showed a large positive effect on NEA across all treatments. Although information on the effect of moderate N addition on NEA in natural grassland ecosystems is lacking, measures of gross and net nitrification fluxes generally indicate a positive response to N addition (Barnard *et al.* 2005). The positive effect of N addition on the amount of NH_4^+ trapped on resins suggests that long-term N addition, even in the form of NO_3^-, enhanced substrate availability for nitrification. We did not, however, find any significant correlations of NEA with measured soil N availability.

The absence of a response of NEA to warming in our study is consistent with other field experiments that have addressed the effect of warming on nitrification in herbaceous ecosystems. These studies have shown no significant response of net nitrification (Verburg *et al.* 1999; Shaw & Harte 2001) or gross nitrification (Shaw & Harte 2001). The scarcity of experiments calls attention to the need for studying the response of nitrification with *in situ* warming experiments.

When not combined with other treatments, elevated CO_2 had no effect on NEA. These results are consistent with those of other studies in grassland ecosystems, in which NEA has been found to decrease or be left unchanged at elevated CO_2 (Niklaus *et al.* 2001; Barnard *et al.* 2004a,b). Across all treatments, however, elevated CO_2 suppressed the positive effect of N addition on NEA in all the treatments that included N addition. The response of nitrification to elevated CO_2 and N addition in multifactorial experiments has so far been addressed in only a few studies. Zak *et al.* (2000a) found a significant stimulation of gross and net nitrification at high N availability, but no significant effect of elevated CO_2 and no significant $CO_2 \times N$ availability interaction. However, in grassland mesocosms using soil from Jasper Ridge, Hungate *et al.* (1997) found a pattern of response of gross nitrification that was similar to that measured for NEA in our study. In their experiment, elevated CO_2 had no effect on gross nitrification in unfertilised conditions, but decreased gross nitrification under nutrient enrichment.

This strong CO_2–N interaction in Jasper Ridge experiments is puzzling, especially since we cannot explain the patterns in NEA using soil properties that we hypothesised would be driving responses to global change factors (e.g. soil NH_4^+ concentrations and soil water content). We have several possible explanations for this interaction, but none can be clearly demonstrated with our data. First, Hungate *et al.* (1997) suggested that fertilisation with slow-release fertilizer pellets, which were also used in our experiment, might cause a more homogeneous distribution of N in the soil, resulting in a tighter coupling of nitrification and gross microbial immobilization of NH_4^+. This could accentuate the effects of elevated CO_2 on microbial competition for NH_4^+. The marginally significant $CO_2 \times N$ interaction for microbial biomass N – elevated CO_2 consistently increased soil microbial N only in the high N treatments – lends some credence to this explanation, but we did not measure rates of microbial N immobilisation. Second, elevated CO_2 and N addition can have strong synergistic effects on microbial respiration (Niklaus 1998) which would reduce soil [O_2]. There is a wide range of other possible explanations, including decreased mineralisation rates (Zak *et al.* 2000b), increased plant NH_4^+ uptake (Bassirirad, Gutschick & Lussenhop 2001) or increased root density (Raynaud & Leadley 2004; see also Niklaus *et al.* 2001 for additional hypotheses).

Unlike DEA (see below), our values of NEA were not correlated with a variety of potential explanatory variables that we measured. There are two possible explanations for this lack of correlation: (i) we did not measure key explanatory variables (e.g. NH_4^+ consumption rates, soil [O_2]) or (ii) our measurements of explanatory variables did not integrate over a long enough time period. Concerning this second point, nitrifying bacteria are known to have low growth rates as compared to denitrifying bacteria (Prosser 1989). Thus, our point measurements of explanatory variables, e.g. extractable N and soil water content, may provide an adequate picture of environmental constraints on DEA, but not NEA. For example, elevated CO_2 has been shown to substantially increase soil water content in the Jasper Ridge experiment (Zavaleta *et al.* 2003b), but did not have an effect on soil water content at our sampling date, presumably because of abundant rainfall prior to sampling.

DENITRIFYING ENZYME ACTIVITY

Increased soil water content, extractable N concentrations and their interaction explained much of the variation in DEA in this experiment. This corresponds to our hypotheses that DEA should be tightly coupled to N availability and to soil water content (the latter affecting DEA through its effects on soil [O_2], Tiedje 1988). These factors clearly explain the large positive responses of DEA to the N addition (where N was added as NO_3^-) and increased precipitation treatments. In particular, soil water content explained 60% of the variance in DEA across all treatments, and appears to be a major factor controlling DEA across a wide array of components of global change and their interactions in this experiment.

Increased temperature showed no significant effect on DEA in our experiment. The significant three-way interaction between CO_2, temperature and N treatments is challenging to interpret. Elevated CO_2 and temperature tended to decrease DEA, unless in combination with N addition, while N addition tended to increase DEA only when in combination with elevated temperature or CO_2. Other experimental results suggest

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interactive effects of CO_2 and temperature on C availability in the soil that would affect DEA (Tscherko, Kandeler & Jones 2001), but the underlying mechanisms are still unknown in field conditions.

The unresponsiveness of DEA to elevated CO_2 is consistent with other undisturbed grassland studies that have found little effect of elevated CO_2 on DEA (Kammann 2001; Barnard *et al*. 2004a). The response of DEA showed a significant $CO_2 \times N$ interaction in our study, with a positive effect of elevated CO_2 on DEA only in the treatments that included N addition. This suggests that the absence of an elevated CO_2 effect on DEA in treatments without N addition might be explained by N-limitation of denitrifiers. The few other experiments that measured the response of denitrifiers to both elevated CO_2 and N addition found no significant interaction between these treatments, but the systems studied differed from our grassland field study (Ambus & Robertson 1999: poplar mesocosms; Martín-Olmedo, Rees & Grace 2002: barley microcosms).

Conclusion

Our study shows that different components of global changes can have large and interacting effects on N cycling in grasslands. In particular, N addition drove the response of NEA at ambient CO_2, but elevated CO_2 suppressed this effect; the determinism of this response still remains to be identified. The mechanisms that drove the response of DEA across a broad range of treatments are the factors that determine soil [O_2] and N substrate availability. Further investigations are in progress to determine to what extent the patterns observed in the present study may show intra- and interannual variability. This study stresses the importance of multifactorial experimental designs in revealing such interactions that are necessary to predict the overall effects of global changes on N dynamics.

Acknowledgements

We would like to thank Nona Chiariello and Chris Field for their help in making this work possible, Jeff Dukes, Lisa Moore and the people who were involved with the soil sampling, Nadine Guillaumaud for her support with the enzymatic measurements, Hans-Rudolf Roth for precious help with the statistical model, and Yuka Otsuki Estrada for handling the JRGCE results database. The Jasper Ridge Global Change Experiment was supported by the National Science Foundation, the David and Lucile Packard Foundation, the Morgan Family Foundation, and the Jasper Ridge Biological Preserve. This work was funded by CNRS support of the Laboratoire d'Ecologie, Systématique et Evolution (UMR CNRS 8079), CNRS and INRA supports of the Laboratoire d'Ecologie Microbienne (UMR CNRS 5557; USC INRA 1193) and the US National Science Foundation (DEB-0092642). E.E.C. was supported by a US Department of Energy Graduate Research in Environment Fellowship. R.B. was supported in part by the Schneider-Forest Grant of the Chancellerie des Universités de Paris.

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Received 29 March 2006; revised 5 May 2006; accepted 8 May 2006
Editor: Maria Jesus Briones

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