Testing interactive effects of global environmental changes on soil nitrogen cycling

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Abstract. Responses of soil nitrogen (N) cycling to simultaneous and potentially interacting global environmental changes are uncertain. Here, we investigated the combined effects of elevated CO_2, warming, increased precipitation and enhanced N supply on soil N cycling in an annual grassland ecosystem as part of the Jasper Ridge Global Change Experiment (CA, USA). This field experiment included four treatments—CO_2, temperature, precipitation, nitrogen—with two levels per treatment (ambient and elevated), and all their factorial combinations replicated six times. We collected soil samples after 7 and 8 years of treatments, and measured gross rates of N mineralization, N immobilization and nitrification, along with potential rates of ammonia oxidation, nitrite oxidation and denitrification. We also determined the main drivers of these microbial activities (soil ammonium and nitrate concentrations, soil moisture, soil temperature, soil pH, and soil CO_2 efflux, as an indicator of soil heterotrophic activity). We found that gross N mineralization responded to the interactive effects of the CO_2, precipitation and N treatments: N addition increased gross N mineralization when CO_2 and precipitation were either both at ambient or both at elevated levels. However, we found limited evidence for interactions among elevated CO_2, warming, increased precipitation, and enhanced N supply on the other N cycling processes examined: statistically significant interactions, when found, tended not to persist across multiple dates. Soil N cycling responded mainly to single-factor effects: long-term N addition increased gross N immobilization, potential ammonia oxidation and potential denitrification, while increased precipitation depressed potential nitrite oxidation and increased potential ammonia oxidation and potential denitrification. In contrast, elevated CO_2 and modest warming did not significantly affect any of these microbial N transformations. These findings suggest that global change effects on soil N cycling are primarily additive, and therefore generally predictable from single factor studies.

Key words: ammonia oxidation; denitrification; elevated CO_2; enhanced N supply; grasslands; increased precipitation; interactions; N immobilization; N mineralization; nitrification; nitrite oxidation; warming.

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INTRODUCTION

Human activities are profoundly altering the composition of the atmosphere and climate with large effects on the functioning of terrestrial ecosystems (IPCC 2007a). These alterations include an increase in global atmospheric CO_2 concentration and air temperature, changes in precipitation regimes (IPCC 2007b), as well as rising atmospheric nitrogen (N) deposition (Galloway et al. 2008). Understanding the response of the N cycle to these global environmental changes is a priority, since N limits primary productivity in many terrestrial ecosystems (Vitousek and Howarth 1991). Responses of N mineralization, microbial N immobilization, nitrification and denitrification to global environmental changes are of particular importance. Indeed, the balance between N mineralization and microbial N immobilization affects inorganic N availability to plants (Schimel and Bennett 2004), while nitrification and denitrification contribute to ecosystem N losses by producing nitrate which can be easily leached, or by releasing N-containing gases in the atmosphere (Wrage et al. 2001, Smith 2010). Numerous studies have investigated the effects of single global environmental changes on N mineralization, N immobilization, nitrification or denitrification (reviewed in Hungate 1999, Zak et al. 2000b, Rustad et al. 2001, Barnard et al. 2005, de Graaff et al. 2006), but few studies have examined the simultaneous and interactive effects of elevated CO_2, temperature, precipitation and N supply on these microbial activities.

Single factor experiments have revealed a large variability in responses of N cycling processes to elevated CO_2, warming, increased precipitation or enhanced N supply. Elevated CO_2 increased net and gross N mineralization rates as a result of increased carbon (C) input to the soil and soil moisture (Hungate et al. 1997a, Ebersberger et al. 2003), and increased gross N immobilization rates due to increased microbial N demand (de Graaff et al. 2006). However, the responses of gross N mineralization and N immobilization to elevated CO_2 greatly varied between studies, and the net effect of CO_2-induced changes in the balance between N mineralization and N immo-

bilization remains unclear (see reviews by Hungate 1999, Zak et al. 2000b, de Graaf et al. 2006, Hu et al. 2006, Reich et al. 2006). Gross and potential nitrification rates generally decreased in response to elevated CO_2 due to decreased ammonium availability for nitrifiers (Hungate et al. 1997b, Niklaus et al. 2001, Lagomarsino et al. 2008), or remained unchanged (Zak et al. 2000a, Barnard et al. 2004, Pinay et al. 2007). Finally, denitrification increased under elevated CO_2 as a result of higher soil labile C availability and soil moisture (Arnone and Bohlen 1998, Ineson et al. 1998, Baggs et al. 2003), or decreased due to reduced nitrate availability (Barnard et al. 2005). Warming generally increased net or gross N mineralization and immobilization rates (Rustad et al. 2001, Shaw and Harte 2001), while the response of nitrification to increased temperature was highly variable, and the response of denitrification was generally non-significant (Barnard et al. 2005). Changes in precipitation regimes, through changes in soil moisture, significantly altered rates of N cycling processes in field studies (Barnard et al. 2006, Dijkstra et al. 2010, Larsen et al. 2010): in particular, increased soil moisture can result in enhanced N mineralization, N immobilization, and nitrification rates under water-limiting conditions (Jamieson et al. 1999, Avrahami and Bohannan 2007, Dijkstra et al. 2010), or in increased denitrification rates by enhancing anaerobic conditions (Barnard et al. 2006). Finally, enhanced N deposition increased gross and potential N mineralization rates through increases in primary productivity and decreases in C/N ratio of the organic matter (Booth et al. 2005, Vourtilis et al. 2007) and enhanced gross and potential nitrification and denitrification rates through increases in soil inorganic N availability (Barnard et al. 2005).

Among the remaining uncertainties with respect to the response of soil N cycling to global change are the simultaneous effects of multiple global environmental changes. Indeed, the effects of increases in CO_2, temperature, precipitation and N supply could be non-additive and therefore not predictable from single-factor experiments (Dukes and Shaw 2007, Norby et al. 2007). In addition, large and mostly unexplained variability of results from single factor experi-

ments may be partly caused by interactive effects among environmental factors: for instance, several studies have shown that the effects of elevated CO_2 on N cycling processes may depend on N addition (Hungate et al. 1997b, Barnard et al. 2006, Hu et al. 2006, Niboyet et al. 2010). As such, multi-factorial global change experiments are critical to understand and predict soil N cycling responses to concurrent changes in the environment (IPCC 2007b, Galloway et al. 2008). Furthermore, the few in situ studies that have assessed the interactive effects between at least two global environmental changes on soil N cycling have reported unexpected and divergent interactive effects, which clearly highlights the need for further investigations. For example, Hovenden et al. (2008) found that elevated CO_2 and warming had antagonistic effects on soil inorganic N availability (warming prevented the reduction in soil inorganic N found under elevated CO_2) in a temperate grassland, while Dijkstra et al. (2010) reported additive effects of elevated CO_2 and warming on soil inorganic N availability in a semiarid grassland.

Here, we investigated the interactive effects between elevated CO_2, increased temperature, increased precipitation and enhanced N supply on soil N cycling in an annual grassland. We studied the responses of N mineralization, N immobilization, nitrification (including the two distinct steps of nitrification—ammonia and nitrite oxidation) and denitrification, and performed measurements of gross or/and potential rates of these microbial activities, along with measurements of their main drivers. Our objectives were (1) to determine the response of soil N cycling to increases in CO_2, temperature, precipitation and N supply, and (2) to investigate the potential interactions between elevated CO_2, temperature, precipitation and N supply on soil N cycling.

Methods

Study site and experimental design

This study was conducted at the Jasper Ridge Biological Preserve (37°24′ N, 122°14′ W, CA, USA). The site experiences a Mediterranean-type climate with a cool, wet growing season from November to March, and a hot, dry summer from June to October. From 1998–2006, mean annual air temperature was 13.3°C, and the site received an annual average of 787 mm precipitation with more than 80% of mean annual precipitation falling between November and March. The dominant species were annual grasses (especially *Avena barbata* and *Bromus hordeaceus*) and annual forbs (especially *Geranium dissectum* and *Erodium botrys*) (Zavaleta et al. 2003). The soil was a fine, mixed, Typic Haploxeralf developed from Franciscan complex alluvium sandstone (Gutknecht et al. 2010).

The Jasper Ridge Global Change Experiment (JRGCE) was initiated in November 1998 and designed to assess the interactive effects of four global environmental changes—elevated CO_2, warming, increased precipitation and enhanced N supply—at levels projected for the second half of the 21st century (Shaw et al. 2002, Zavaleta et al. 2003, Dukes et al. 2005) in an annual grassland. The experiment provided a complete factorial design with four treatments, each at two levels (ambient vs. elevated): atmospheric CO_2 concentration (ambient vs. 680 µmol mol^{-1}), temperature (ambient vs. +80 W m^{-2} thermal radiation, resulting in a soil surface warming of approximately 0.8–1.0°C), precipitation (ambient vs. +50% above ambient precipitation + 3-week elongation of rainy season) and N addition (ambient vs. +7 g N m^{-2} yr^{-1}). CO_2 was elevated with a free-air CO_2 enrichment (FACE) system delivering pure CO_2 at plant height. Temperature was increased using overhead infrared heaters. Precipitation was enhanced at first with drip irrigation (1998–2000) and then with overhead sprinklers (2001–2006). N was applied twice per year as $Ca(NO_3)_2$, with an initial application of 2 g N-$Ca(NO_3)_2$ m^{-2} in solution early in the growing season (each November) and an additional application of 5 g N-$Ca(NO_3)_2$ m^{-2} as slow-release fertilizer (Nutricote 12-0-0, Agrivert, Riverside, CA, USA) later in the growing season (each January). The experiment was organized as a randomized block split-plot design, with CO_2 and temperature treatments applied at the plot level (circular plots, 2 m diameter) and precipitation and N additions manipulated at the subplot level (each plot being divided into four 0.78 m^2 quadrants with 0.5 m solid belowground barriers and mesh aboveground partitions). Each of the 16 possible treatment combinations was

replicated eight times (i.e., 32 plots in total). However, two of the eight replicates were excluded from the present analysis, since they were affected by an accidental fire in July 2003 (Henry et al. 2006, Gutknecht et al. 2010); each of the 16 treatments was thus replicated six times for the present analysis.

Soil sampling

Soil cores (5 cm diameter $\times$ 5 cm deep) were sampled in each quadrant during the 7th and 8th growing seasons of the experiment: on 23 February 2005 (at mid-vegetative stage), 26 April 2005 (at the time of peak plant biomass of the 7th growing season) and 26–27 April 2006 (at the time of peak plant biomass of the 8th growing season). Soil sampling occurred during the growing season since many of the putative treatment effects (e.g., those of the CO_2 treatment) on the processes examined are mediated by plants.

At each sampling date, large roots and rocks were removed by hand, and soil samples were thoroughly mixed by hand through plastic bags before being partitioned for measurements of gross rates of N mineralization, N immobilization and nitrification, potential rates of ammonia oxidation, nitrite oxidation and denitrification, and main drivers of these microbial activities. These drivers included soil NH_4^+ and NO_3^- concentrations, soil moisture, soil pH, and soil laboratory-incubated CO_2 efflux, as an indicator of soil heterotrophic microbial activity.

Gross rates of N mineralization, N immobilization and nitrification

Gross rates of N mineralization, N immobilization and nitrification were determined in February 2005 and April 2005 using ^{15}N pool dilution (Hart et al. 1994). At each date, 50-g soil sub-samples from each quadrant were placed in thin plastic bags and 3 mL of either ^{15}N-$(NH_4)_2SO_4$ or ^{15}N-$Ca(NO_3)_2$ were added (99 atom % ^{15}N), producing target concentrations of 1 µg ^{15}N per gram of soil. Just after addition of the labelling solution to the soil, the ^{15}N label was well homogenized with the soil by 15 min thorough mixing. A 10-g sub-sample was then taken and extracted with 25 mL 0.25 M K_2SO_4 for determination of the initial inorganic N pools. The remaining soil was returned to the field in

the plastic bag, buried in its original location (i.e., at the place where the 0–5 cm soil core was taken), and covered with a thin layer of soil taken from surrounding area in the plots. After a 24-h incubation in the field, a second 10-g sub-sample was taken and extracted as above for determination of the final inorganic N pools. Extracts were filtered and analyzed colorimetrically for NH_4^+ and NO_3^- concentrations using an autoanalyzer (Lachat Quickchem FIA+8000, Lachat Instruments, Milwaukee, WI, USA).

Nitrogen isotope composition ($\delta^{15}N$) of NH_4^+ and NO_3^- was determined using an elemental analyzer coupled to an isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (⟨http://www.mpcer.nau.edu/isotopelab/⟩). NH_4^+ and NO_3^- were separated by diffusion following the procedure described by Stark and Hart (1996). In short, acid traps were made of glass fiber discs, acidified with 20 µL 0.5 M $KHSO_4$, sealed between two pieces of Teflon tape, and floated on top of the extract solution. 300 mg MgO per 100 mL solution was added increasing the pH, and the solution was incubated for 7 days in a shaking incubator, allowing ammonium to accumulate on the glass fiber. Then, after replacing the glass fiber disk, NO_3^- was reduced to NH_4^+ by adding 200 mg finely ground Devarda's alloy. Solutions were again incubated in a shaking incubator for 7 days. Afterwards, acid traps were placed in a dessicator to dry and analyzed for ^{15}N content by isotope ratio mass spectrometer. International standards (IAEA 311 and 305B) and ^{15}N-enriched laboratory standards were similarly diffused and used for quality control.

Gross rates of N mineralization, N immobilization and nitrification were determined using pool dilution equations described in Hart et al. (1994). In brief, gross N mineralization was calculated based on NH_4^+ and ^{15}N-NH_4^+ concentrations at time 0 and 24 h, and gross microbial N immobilization was calculated as the difference between gross N mineralization and net N mineralization. Gross nitrification was calculated based on NO_3^- and ^{15}N-NO_3^- concentrations at time 0 and 24 h.

Potential rates of ammonia oxidation, nitrite oxidation and denitrification

Potential rates of ammonia oxidation, nitrite

oxidation and denitrification were determined in February 2005, April 2005 and April 2006. Measurements of potential rates are proxies of measurements of the concentrations of the ammonia-oxidizing, nitrite-oxidizing or denitrifying enzymes in soils (Tiedje 1982, Hart et al. 1994). These enzyme concentrations (1) are functions of the in situ environmental constraints to which ammonia-oxidizers, nitrite-oxidizers and denitrifiers were exposed in the field prior to soil sampling (Pinay et al. 2007, McGill et al. 2010, Niboyet et al. 2010), and (2) are measured in laboratory incubations under non-limiting substrate and optimal environmental conditions, over time periods where de novo synthesis of enzymes does not occur (Tiedje 1982). Measurements of potential rates thus reflect the direction and magnitude of the environmental constraints in the field on ammonia oxidation, nitrite oxidation and denitrification. Potential rates are thought to be more constant over time than in situ rates which are highly temporally variable (McGill et al. 2010). Though they do not indicate actual rates in the field, they provide information on the impacts of environmental changes on the size of the ammonia-oxidizing, nitrite-oxidizing, and denitrifying microbial communities. Potential N rates were measured on fresh soil stored a few days at 4°C, which does not significantly alter microbial enzyme activities (Luo et al. 1996). For each individual assay, measurements were conducted on 5 g equivalent dry soil, as determined using measurements of gravimetric soil water contents.

Potential ammonia oxidation rates were measured as NO_2^- production rates from soil samples amended with NH_4^+ and $NaClO_3$, an inhibitor of the oxidation of NO_2^- to NO_3^- (Belser and Mays 1980). At each date, 5 g equivalent dry soil were amended with 50 mL of a solution of 0.18 mM $(NH_4)_2SO_4$, 0.8 mM K_2HPO_4, 0.1 mM KH_2PO_4, and 0.01 M $NaClO_3$, which ensured excess NH_4^+ substrate (final concentration 50 µg $N-NH_4^+$ g^{-1} dry soil). Samples were incubated at 28°C for 9 h with constant agitation at 150 rpm. NO_2^- concentrations were measured after 0, 3, 6 and 9 h on a spectrophotometer (Uvikon 800, Leeds, UK) at 520 nm using the Griess reagent. A constant rate of NO_2^- production was always observed during the ammonia-oxidation assays (data not shown).

Potential nitrite oxidation rates were measured as NO_2^- consumption rates from soil samples amended with NO_2^- (Wertz et al. 2007). At each date, 5 g equivalent dry soil were amended with 50 mL of a solution of 0.36 mM $NaNO_2$, which ensured excess NO_2^- substrate (final concentration 50 µg $N-NO_2^-$ g^{-1} dry soil). Samples were incubated at 28°C for 30 h with constant agitation at 150 rpm. NO_2^- concentrations were measured after 0, 9, 24 and 30 h as described above. During the assays, actual NO_2^- production by ammonia oxidizers was not inhibited as it was negligible compared to potential NO_2^- consumption by nitrite oxidizers: given the low background of NH_4^+ in our soil samples (concentration ~ 3 µg $N-NH_4^+$ g^{-1} dry soil), actual NO_2^- production rate was less than 4% of potential NO_2^- consumption rate (X. Le Roux, *personal observation*). A constant rate of NO_2^- consumption was always observed during the nitrite-oxidation assays (data not shown).

Potential denitrification rates were measured as N_2O production rates from soil samples amended with NO_3^- and labile C, and in which N_2O reductase was inhibited with acetylene (Smith and Tiedje 1979). At each date, 5 g equivalent dry soil were placed in 150 mL flasks, which were immediately sealed with rubber stoppers. Headspace atmosphere was replaced by a $He:C_2H_2$ mixture (90:10) to ensure anaerobic conditions and inhibition of N_2O reductase. Soil samples were amended with a solution containing 0.1 mg $N-NO_3^-$ g^{-1} dry soil, 1 mg C-glucose g^{-1} dry soil and 1 mg C-glutamic acid g^{-1} dry soil, which ensured no limitation of denitrification by NO_3^- or C. Flasks were incubated at 27°C for 8 h. N_2O concentration was measured after 2, 4, 6 and 8 h on a gas chromatograph equipped with an electron capture detector (Agilent P200 Micro GC, Agilent Technologies, Palo Alto, CA, USA). A constant rate of N_2O production was always observed during the denitrification assays (data not shown).

Soil NH_4^+ and NO_3^- concentrations

Soil NH_4^+ and NO_3^- concentrations were measured on soil samples collected in February 2005 and April 2005. At each date, NH_4^+ and NO_3^- were extracted in 25 mL of 0.25 M K_2SO_4 from 10 g soil sub-samples, which were vigorously shaken for 30 min. Extracts were then

filtered, and NH_4^+ and NO_3^- concentrations were analyzed colorimetrically using an autoanalyzer (Lachat Quickchem FIA+8000, Lachat Instruments, Milwaukee, WI, USA).

Soil moisture, soil temperature and soil pH

Soil water content was determined gravimetrically at each sampling date by comparing the mass of a 5-g soil sub-sample before and after drying at 105°C for 24 h. Soil temperature data were obtained at hourly intervals from thermocouples buried at 2 cm and 10 cm below the soil surface in each quadrant, and averaged over each sampling date. Soil pH was measured on soil samples collected in April 2006 in 1:1 mixture of soil and distilled water.

Soil laboratory-incubated CO_2 efflux

Soil CO_2 fluxes were measured in February 2005, April 2005 and April 2006 by incubating soil at standardized moisture and temperature. At each date, 15-g soil sub-samples were placed in 250-mL screw-top glass serum bottles and soil moisture was adjusted (0.21 g H_2O g^{-1} dry soil). Bottles were then sealed with screw caps lined with airtight Teflon-silicone septa and incubated for 48 h in the dark at 25°C. Rates of CO_2 production were calculated from three 15-mL headspace samples taken 30–60 min, 24 h, and 48 h after the incubation started. Gas samples were immediately injected into sealed pre-evacuated 12-mL glass vials capped with 20-mm butyl rubber stoppers and analyzed for CO_2 concentrations on a gas chromatograph (Agilent 6890 GC System, Agilent Technologies, Palo Alto, CA, USA). A constant rate of CO_2 production was always observed during the 48-h assays (data not shown).

Statistical analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). We analyzed our data with PROC MIXED using a repeated four-way split-plot analysis of variance in order to assess the overall effects of treatments among the several sampling dates, as well as the temporal variability of these treatment effects. CO_2 and temperature (T) treatments were included as whole-plot effects, and precipitation (W) and N treatments as sub-plot effects. We also assessed the effects of treatments at individual dates using a full factorial split-plot mixed model in order to test exhaustively for interactions among treatments, and to compare our findings with past work at our site where some of the same processes (potential nitrification and denitrification) were measured once (Barnard et al. 2006). We tested for treatment effects in PROC MIXED with the restricted maximum likelihood method, using the containment method for determining degrees of freedom. The numerator degree of freedom was equal to 1 for each of the treatment combinations tested. As the treatments were organized as a split-plot design, the denominator degree of freedom varied depending on the level to which the treatments were applied, and was 15 for the treatments applied at the plot level (i.e., CO_2, T and $CO_2 \times T$), but 60 for the treatments applied at the sub-plot level (i.e., W, N and all interactions involving W and N). Data were log or square-root transformed prior to analysis to ensure homogeneity of variance. Effects with $p < 0.05$ are referred to as significant, and effects with $0.05 \leq p < 0.1$ as marginally significant.

We then conducted a retrospective statistical power analysis using PROC POWER in SAS to determine the statistical power ($1 - \beta$, where β is the probability of erroneously failing to reject the null hypothesis) to detect relative effect sizes (% effect, expressed as [Treatment − Control]/[Control] $\times 100\%$) on each N cycling process. The aim was to test whether non-significant results were due to absence of ecologically significant treatments effects or to a lack of statistical power (Peterman 1990, Steidl et al. 1997). We analyzed statistical power at the plot (degree of freedom = 15) and at the subplot (degree of freedom = 60) levels using two-sided two-sample t-tests. We set the α-level at 0.05, the sample size per group at 6, and determined the standard deviation at the plot and subplot levels using the ESTIMATE statement of PROC MIXED. For the discussion, we considered statistical power to be 'acceptable' when above 0.8, but provide an analysis of statistical power over a range of 0 to 50% effect sizes for each N cycling process.

RESULTS

Treatment effects on gross N mineralization

Gross N mineralization was affected by the

interactive effects of elevated CO_2, increased precipitation and N addition (significant $CO_2 \times W$ and $CO_2 \times W \times N$ interactions; Table 1, Fig. 1). Overall, long-term N addition significantly increased gross N mineralization rates (+29% on average; Table 1, Fig. 2); however, increases in gross N mineralization with added N occurred only when CO_2 and precipitation were either both at ambient or both at elevated levels (Fig. 1). In other words, elevated CO_2 had no effect on gross N mineralization under ambient precipitation, irrespective of the N level, whereas elevated CO_2 increased gross N mineralization under elevated precipitation when N was added (Fig. 1). Furthermore, in February 2005, elevated CO_2 reduced the negative effect of the warming treatment on gross N mineralization ($CO_2 \times T$, p = 0.05; Fig. 3), while in April 2005, elevated CO_2 reversed the negative effect of the precipitation treatment on gross N mineralization ($CO_2 \times W$, p = 0.02; Fig. 3).

Treatment effects on gross N immobilization

Long-term N addition significantly increased gross microbial N immobilization (+40% on average; Table 1, Fig. 2). Gross N immobilization exhibited one marginally significant interactive response to the treatments ($W \times N$ interaction; Table 1), significant for April 2005. At this date, increased precipitation enhanced gross N immobilization, but only when no N was added ($W \times N$, p = 0.05; Fig. 4).

Treatment effects on gross nitrification

Gross nitrification was not significantly affected by any of the treatments or combinations of treatments (Table 1, Fig. 2) except for February 2005, when elevated CO_2 increased gross nitrification when combined with added N at ambient temperature, but decreased gross nitrification when combined with added N at elevated temperature ($CO_2 \times T \times N$, p = 0.03; Fig. 5).

Treatment effects on potential ammonia and nitrite oxidation

Across all dates, long-term N addition increased potential ammonia oxidation (+59% on average; Table 2), but this effect was significant only at the end of the growing season (in April 2005 and 2006; Fig. 6). Increased precipitation tended to increase potential ammonia oxidation

in repeated measures analysis (+15% on average, marginally significant effect; Table 2). Potential ammonia oxidation exhibited two marginally significant interactive responses to the treatments ($CO_2 \times T \times W$ and $T \times W \times N$ interactions; Table 2), significant for April 2006. At this date, elevated CO_2 had a positive effect on potential ammonia oxidation when combined with increased precipitation ($CO_2 \times W$, p = 0.02; Fig. 7), especially at elevated temperature ($CO_2 \times T \times W$, p = 0.05; Fig. 7). Finally, the positive effect of N addition was not observed when temperature and precipitation were both at elevated levels ($T \times W \times N$, p = 0.03; Fig. 7).

Across all dates, increased precipitation reduced potential nitrite oxidation (−10% on average; Table 2), but this effect was significant only at the end of the growing season (in April 2005 and 2006; Fig. 6). Potential nitrite oxidation exhibited three marginally significant interactive responses to the treatments ($CO_2 \times W$, $CO_2 \times W \times N$ and $T \times W \times N$ interactions; Table 2). Furthermore, in April 2005, elevated CO_2 had a positive effect on potential nitrite oxidation when combined with added N ($CO_2 \times N$, p = 0.02; Fig. 8) and precipitation ($CO_2 \times W \times N$, p = 0.002; Fig. 8). Averaged across all treatments, potential nitrite oxidation rates were 5 to 7 times greater than potential ammonia oxidation rates for the three measurement dates (Fig. 7; Fig. 8).

Treatment effects on potential denitrification

Across all dates, long-term N addition and increased precipitation significantly increased potential denitrification (+34% on average with N addition and +22% on average with increased precipitation; Table 2). The positive effect of N addition on potential denitrification was significant at each sampling date (Fig. 6), while the effect of increased precipitation on potential denitrification was significant only at the end of the growing season (in April 2005 and 2006; Fig. 6). Elevated CO_2 and warming had no significant effect on potential denitrification (Table 2, Fig. 6). Potential denitrification exhibited no significant interactions to treatments (Table 2, Fig. 9).

Treatment effects on soil NH_4^+ and NO_3^- concentrations

Long-term N addition significantly increased soil NH_4^+ concentrations (+82% on average, p <

Table 1. Treatment effects on gross N mineralization, N immobilization and nitrification rates.

Treatment	Gross N mineralization		Gross N immobilization		Gross nitrification	
	% effect	p-value	% effect	p-value	% effect	p-value
Main plot effects						
CO_2	+4	0.93	−2	0.48	+6	0.82
T	−4	0.13	−3	0.16	+1	0.57
$CO_2 \times T$		0.29		0.13		0.84
Sub-plot effects						
W	−3	0.30	+3	0.87	+8	0.22
N	**+29**	**0.0001**	**+40**	**0.0001**	+5	0.86
$CO_2 \times W$		**0.005**		0.38		0.33
$CO_2 \times N$		0.75		0.69		0.30
$T \times W$		0.42		0.56		0.73
$T \times N$		0.76		0.17		0.35
$W \times N$		0.66		0.08		0.81
$CO_2 \times W \times N$		**0.03**		0.16		0.40
$T \times W \times N$		0.25		0.99		0.83
$CO_2 \times T \times W$		0.83		0.43		0.12
$CO_2 \times T \times N$		0.21		0.31		0.42
$CO_2 \times T \times W \times N$		0.84		0.62		0.70
Time effects						
Time		**0.003**		0.38		**0.02**
Time $\times CO_2$		0.17		0.51		0.97
Time $\times$ T		0.96		0.56		0.87
Time $\times CO_2 \times T$		0.08		0.57		0.22
Time $\times$ W		0.36		0.75		0.72
Time $\times$ N		0.26		0.14		0.31
Time $\times CO_2 \times W$		0.43		0.85		0.65
Time $\times CO_2 \times N$		0.55		0.62		0.37
Time $\times T \times W$		0.48		0.65		0.69
Time $\times T \times N$		0.61		0.20		0.52
Time $\times W \times N$		**0.03**		**0.04**		0.68
Time $\times CO_2 \times W \times N$		0.42		0.53		0.38
Time $\times T \times W \times N$		0.92		0.37		0.73
Time $\times CO_2 \times T \times W$		0.18		0.78		0.39
Time $\times CO_2 \times T \times N$		0.59		0.10		**0.01**
Time $\times CO_2 \times T \times W \times N$		0.89		0.94		0.24

Notes: The table is a summary of p-values from four-way split-plot analysis of variance with repeated measurements in time testing for the effects of treatments on gross N rates. Significant responses are indicated in bold ($p < 0.05$). Effects of each main treatment (CO_2: elevated CO_2, T: increased temperature, W: increased precipitation, N: N addition) were calculated as: % effect $= 100\% \times$ [elevated − ambient]/ambient (in the ambient and elevated treatments, $n = 48 \times 2$ measurement dates). Numerator degrees of freedom are equal to 1 for the main plot, sub-plot and time effects. Denominator degrees of freedom are equal to 15 for the main plot effects, to 60 for the sub-plot effects, to 5 for time, to 15 for the interactions between time and main plot effects, and to 60 for the interactions between time and sub-plot effects.

0.0001), especially in the plots under ambient temperature (T $\times$ N, p = 0.02). N addition also significantly increased soil NO_3^- concentrations (+252% on average, p < 0.0001). Other treatments had no significant effect on soil NH_4^+ or NO_3^- contents.

Treatment effects on soil moisture, soil temperature and soil pH

Enhanced precipitation significantly increased soil water content (+6% on average, p < 0.0001), but this effect was found only at the end of the growing season (+10% in April 2005, p = 0.0002; +11% in April 2006, p = 0.0002). Other treatments did not significantly alter soil moisture. Warming significantly increased soil temperature at 2 cm

and 10 cm depth (+0.72°C on average at 2 cm depth, p = 0.01; +0.70°C on average at 10 cm depth, p = 0.007), while N addition significantly reduced soil temperature at both depths (−0.46°C on average at 2 cm depth, p < 0.0001; −0.42°C on average at 10 cm depth, p < 0.0001). At 2 cm depth, the negative effect of the N treatment was not observed when CO_2 and precipitation were both at ambient levels ($CO_2 \times W \times N$, p = 0.04), while at 10 cm depth, the negative effect of the N treatment was not observed with ambient CO_2 and increased temperature ($CO_2 \times T \times N$, p = 0.009). Other treatments had no significant effect on soil temperature. Increased precipitation had a small, positive effect on soil pH in April 2006 (+0.1, p = 0.02). Warming also slightly increased
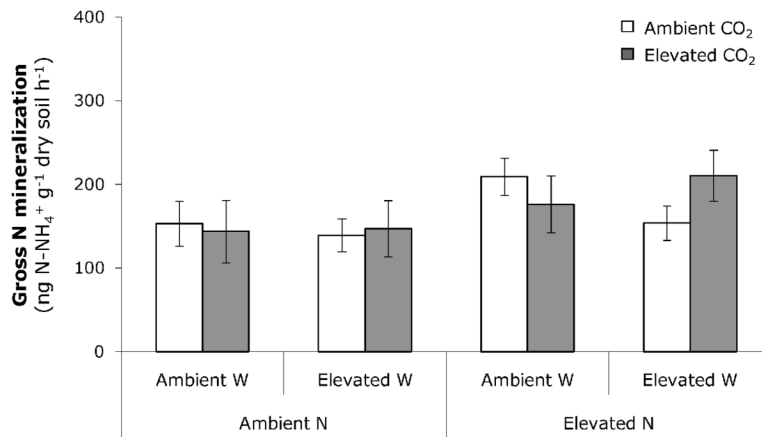
Fig. 1. Interactive effects of CO_2, precipitation and nitrogen treatments on gross N mineralization rates. The mean rates of gross N mineralization are grouped by CO_2 (ambient CO_2: open bars, elevated CO_2: closed bars), precipitation (W), and nitrogen (N) treatments; error bars indicate pooled standard errors (n = 12 × 2 measurement dates).

soil pH when combined with elevated CO_2 and added N (+0.1, CO_2 × T × N, p = 0.03). Other treatments had no significant effect on soil pH.

Treatment effects on soil laboratory-incubated CO_2 efflux

Soil CO_2 fluxes in laboratory incubations were not altered by any of the main treatments investigated, and repeated measures analysis did not reveal significant interactions among treatments (p > 0.05 in all cases).

Statistical power analysis

Retrospective statistical power analysis revealed acceptable power (i.e., $1 - \beta > 0.8$) to detect effect sizes (expressed as [Treatment − Control]/[Control] × 100%) at the sub-plot level of 8% to 16%, depending on the N cycling process examined (Fig. 10A). Sub-plot effects for all N processes could be detected with very high power (i.e., $1 - \beta > 0.99$) when effect sizes were greater than ca. 25% (Fig. 10A). Retrospective statistical power analysis further showed acceptable power (i.e., $1 - \beta > 0.8$) to detect effect sizes at the plot level of 13% to 17% for gross N rates, and of 25% to 29% for potential N rates (Fig. 10B). The differences in statistical power between sub-plot and main plot effects reflect our split-plot design, with more degrees of freedom to test subplot effects (60 compared to 15).

Discussion

Our major findings include: (1) two significant interactive effects among CO_2, precipitation and N treatments on gross N mineralization, (2) some interactive effects on gross N immobilization, gross nitrification, and potential nitrification for specific measurement dates, (3) significant effects of N addition and increased precipitation on soil N cycling, and (4) a general lack of effects of warming and elevated CO_2 on soil N cycling. Here, we discuss these responses of soil N cycling to the single and combined effects of the four global environmental changes.

Responses of soil N cycling to multiple global environmental changes

The Jasper Ridge Global Change Experiment was expressly designed to assess the interactive effects among elevated CO_2, warming, increased precipitation, and N addition. Yet, we found no clear evidence that interactive effects dominated responses of soil N cycling to these global environmental changes. Most interactions, when present, did not persist over time.

The main exceptions were the interactive effects of CO_2, precipitation and N treatments on gross N mineralization: significant increases in gross N mineralization rates occurred with added N when combined with ambient levels of precipitation and CO_2, or with elevated levels of
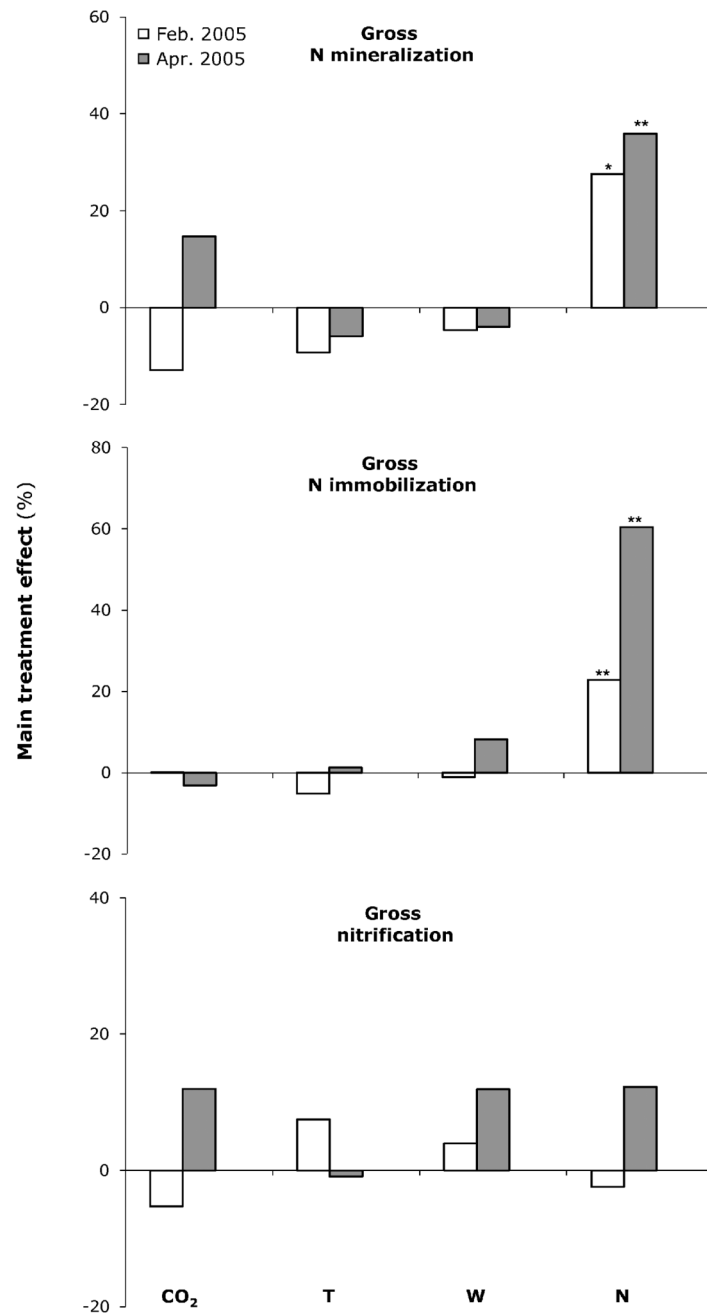
Fig. 2. Effects of elevated CO_2 (CO_2), increased temperature (T), increased precipitation (W) and N addition (N) on gross rates of N mineralization, N immobilization and nitrification. For each measurement date, the effect of each main treatment was calculated as: % effect $= 100\% \times$ [elevated − ambient]/ambient (n $= 48$ in the ambient and elevated treatments). Significant effects are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

precipitation and CO_2 ($CO_2 \times$ W and $CO_2 \times$ W $\times$ N interactions; Table 1, Fig. 1). The positive effect of the N treatment on gross N mineralization rates confirmed results from other experiments (Booth et al. 2005, Dijkstra et al. 2005, Vourtilis et al. 2007), and likely resulted from increases in soil organic matter quality (e.g., increases in litter N content) (Henry et al. 2005), increases in litter
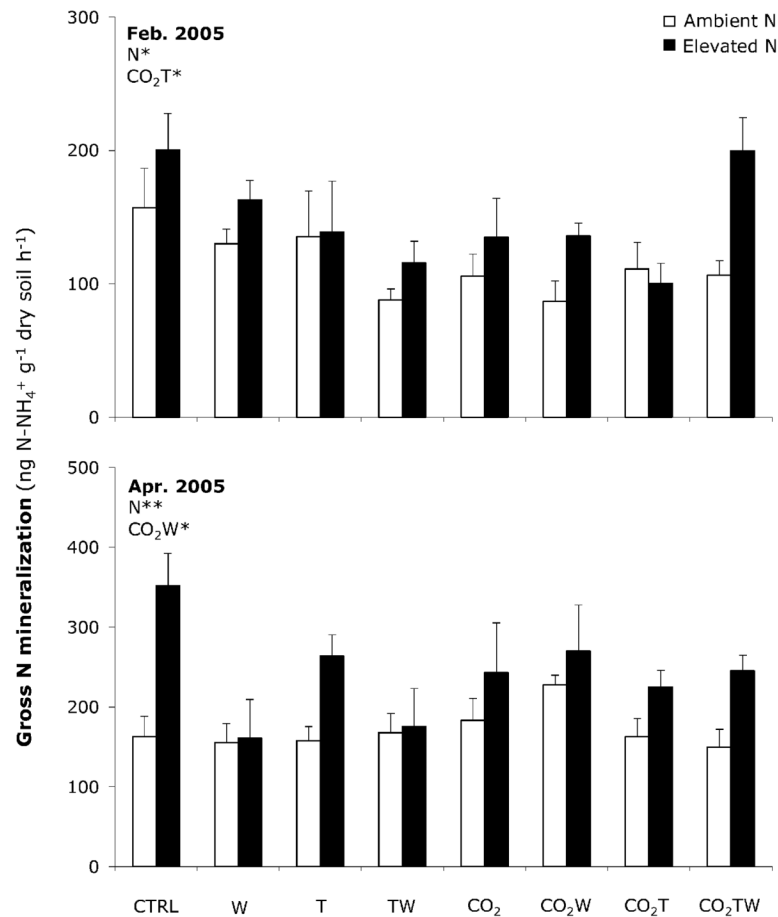
Fig. 3. Gross N mineralization rates in each treatment combination for the two measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors (n = 6). Letters identify treatment effects from mixed model analysis of gross N mineralization data, and interactions are presented as multiple letters (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

input (Dukes et al. 2005), or from relaxation of microbial N limitation (Hu et al. 2001). The positive effect of elevated CO_2 on gross N mineralization rates when combined with added N and elevated precipitation (Fig. 1) indicated that heterotrophic microbial activity did benefit from the likely increase in soil labile C with elevated CO_2 (Pendall et al. 2004), but only when N and water constraints were relaxed (Hungate et al. 1997a, Hu et al. 2001, Ebersberger et al. 2003). Finally, the negative effect of the precipitation treatment on gross N mineralization rates with added N at ambient CO_2 (Fig. 1) may be a consequence of decreased root production at

high precipitation at our site (Dukes et al. 2005): decreased root-C inputs to the soil may have constrained heterotrophic microbes, so they could not benefit from higher N availability with added N at high precipitation (except when CO_2 was elevated, off-setting this effect).

Repeated measures analysis did not reveal any other significant interactions on N cycling processes (Tables 1 and 2). Six out of the total of sixty-six interactions tested were marginally significant (W × N on N immobilization, Table 1; CO_2 × T × W and T × W × N on ammonia oxidation; CO_2 × W, CO_2 × W × N and T × W × N on nitrite oxidation, Table 2). Some interactions
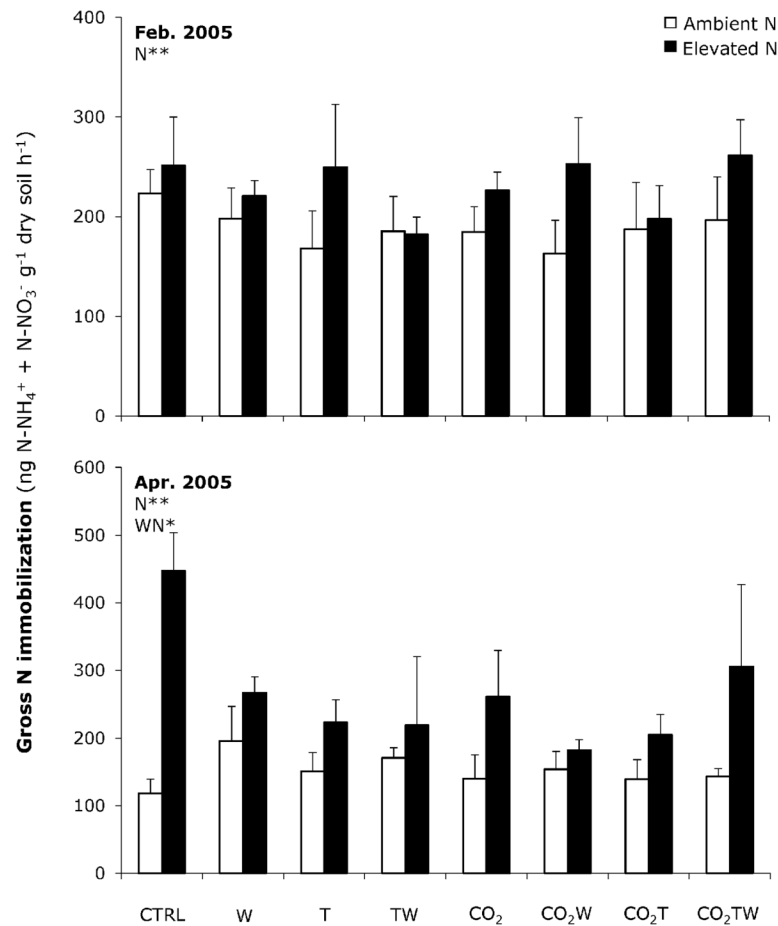
Fig. 4. Gross N immobilization rates in each treatment combination for the two measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors (n = 6). Letters identify treatment effects from mixed model analysis of gross N immobilization data, and interactions are presented as multiple letters (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

were also found on gross N immobilization, gross nitrification and potential nitrification for specific measurement dates, but the significance of these interactive effects appeared to be limited when multiple measurement dates were considered. Furthermore, potential denitrification did not exhibit any interactive responses to treatments. Other studies have also reported that interactions among global change treatments on soil N cycling were rare. In a synthesis of interactions between global change treatments on nitrification, denitrification and N_2O emissions, Barnard et al. (2005) reported that most multiple treatment studies found no significant

interactions (4 out of 25 measured a significant interaction between treatments). Similarly, Larsen et al. (2010) found only few significant interactions among elevated CO_2, warming, and summer drought in a semi-natural Danish heathland ecosystem (15 out of 188 interactions tested on 47 N-related variables were significant).

Thus, except for N mineralization, we found little clear evidence for interactive effects between treatments on soil N cycling at our site that persisted over time. Interactive effects of elevated CO_2 and N addition on potential nitrification and denitrification were observed earlier in our experiment: CO_2 suppressed the positive effect
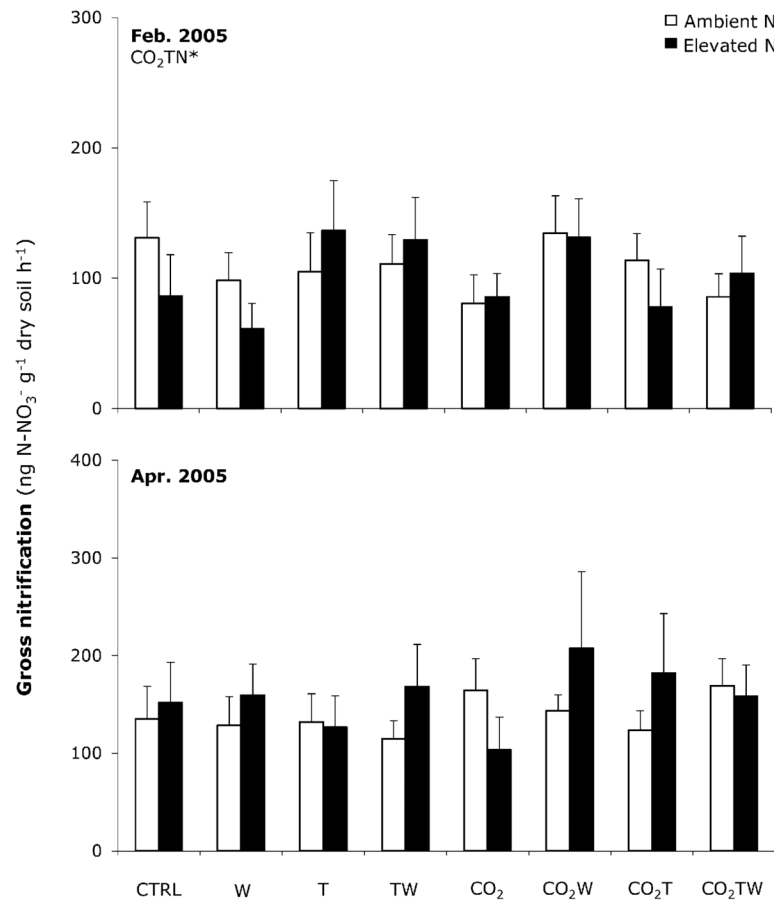
Fig. 5. Gross nitrification rates in each treatment combination for the two measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors (n = 6). Letters identify treatment effects from mixed model analysis of gross nitrification data, and interactions are presented as multiple letters (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

of N on potential nitrification and amplified the positive effect of N on potential denitrification at the end of the fifth growing season (Barnard et al. 2006). Our analysis indicates that these interactive effects were transient. These findings are consistent with the transient appearance of interactive effects on plant growth in this experiment (Shaw et al. 2002, Dukes et al. 2005). The limited number of statistically significant interactive effects on soil N cycling could potentially belie ecologically important interactions that we lacked statistical power to detect. Our power analysis suggests that interactive effects, if they occurred, were small compared to main effects of the N and precipitation treatments. Although small changes in gross rates of N cycling may be ecologically significant (Reich et al. 2006), our design was sufficient to detect most interactive effects at a reasonable threshold (~20%), particularly in light of the high natural variability of these gross rates of N cycling (Corre et al. 2002). Our split-plot design was stronger for detecting sub-plot effects of N, precipitation, and interactions involving the N and precipitation treatments (i.e., 10 out of the 11 interactions tested) compared to main plot effects of CO_2, temperature, and the interaction between CO_2 and temperature treatments, particularly for potential N rates. Still, the absence of significant main plot effects cannot be entirely ascribed to

Table 2. Treatment effects on potential ammonia oxidation, nitrite oxidation and denitrification rates.

Treatment	Potential ammonia oxidation		Potential nitrite oxidation		Potential denitrification	
	% effect	p-value	% effect	p-value	% effect	p-value
Main plot effects						
CO_2	+14	0.42	+16	0.16	+1	0.84
T	+2	0.79	+6	0.46	+2	0.99
$CO_2 \times$ T		0.47		0.14		0.54
Sub-plot effects						
W	+15	0.05	−10	**0.005**	+22	**0.002**
N	+59	**<0.0001**	+3	0.23	+34	**<0.0001**
$CO_2 \times$ W		0.10		0.08		0.13
$CO_2 \times$ N		0.81		0.24		0.12
T $\times$ W		0.78		0.44		0.88
T $\times$ N		0.98		0.94		0.57
W $\times$ N		0.20		0.36		0.76
$CO_2 \times$ W $\times$ N		0.17		0.06		0.28
T $\times$ W $\times$ N		0.05		0.09		0.80
$CO_2 \times$ T $\times$ W		0.05		0.39		0.71
$CO_2 \times$ T $\times$ N		0.36		0.81		0.39
$CO_2 \times$ T $\times$ W $\times$ N		0.15		0.85		0.30
Time effects						
Time		0.47		0.50		**<0.0001**
Time $\times$ CO_2		0.25		0.70		0.19
Time $\times$ T		0.66		0.31		0.84
Time $\times$ $CO_2 \times$ T		0.84		0.45		0.06
Time $\times$ W		0.77		0.09		0.29
Time $\times$ N		**<0.0001**		0.40		0.27
Time $\times$ $CO_2 \times$ W		0.22		0.59		0.26
Time $\times$ $CO_2 \times$ N		**0.02**		0.06		0.76
Time $\times$ T $\times$ W		**0.03**		0.74		0.49
Time $\times$ T $\times$ N		0.97		**0.05**		0.55
Time $\times$ W $\times$ N		0.99		0.86		0.20
Time $\times$ $CO_2 \times$ W $\times$ N		0.63		**0.04**		0.49
Time $\times$ T $\times$ W $\quad \times$ N		0.56		0.52		0.76
Time $\times$ $CO_2 \times$ T $\times$ W		0.70		0.69		0.68
Time $\times$ $CO_2 \times$ T $\times$ N		0.96		0.90		0.32
Time $\times$ $CO_2 \times$ T $\times$ W $\times$ N		0.40		0.71		0.99

Notes: The table is a summary of p-values from four-way split-plot analysis of variance with repeated measurements in time testing for the effects of treatments on potential N rates. Significant responses are indicated in bold ($p < 0.05$). Effects of each main treatment (CO_2: elevated CO_2, T: increased temperature, W: increased precipitation, N: N addition) were calculated as: % effect = $100\% \times$ [elevated − ambient]/ambient (in the ambient and elevated treatments, $n = 48 \times 3$ measurement dates). Numerator degrees of freedom are equal to 1 for the main plot and sub-plot effects, and to 2 for the time effects. Denominator degrees of freedom are equal to 15 for the main plot effects, to 60 for the sub-plot effects, to 10 for time, to 30 for the interactions between time and main plot effects, and to 120 for the interactions between time and sub-plot effects.

low power: our design was sufficient to detect effects of elevated CO_2 and warming, if these had caused changes comparable to those caused by added N and altered precipitation (i.e., changes larger than 20% in gross N rates and 30% in potential N rates). We conclude that low power did not substantially limit the strength of our inferences about interactive effects of global change on the N cycling processes measured.

Responses of soil N cycling to single global environmental changes: enhanced N supply and increased precipitation

Long-term N addition substantially increased gross N immobilization, potential ammonia oxidation and potential denitrification, while increased precipitation increased potential ammonia oxidation and denitrification and depressed potential nitrite oxidation. Increased gross N mineralization rates with N addition likely contributed to the observed increase in soil NH_4^+ concentrations, which in turn led to increased potential ammonia oxidation. Similarly, increased substrate availability for denitrifiers at high N, resulting from the direct addition of NO_3^- or from increased nitrification rates, likely contributed to increased potential denitrification. The marginally significant increase in potential ammonia oxidation with increased soil moisture may reflect reduced water stress for ammonia oxidizers, and is consistent with other studies (Stark and Firestone 1995, Avrahami and Bo-
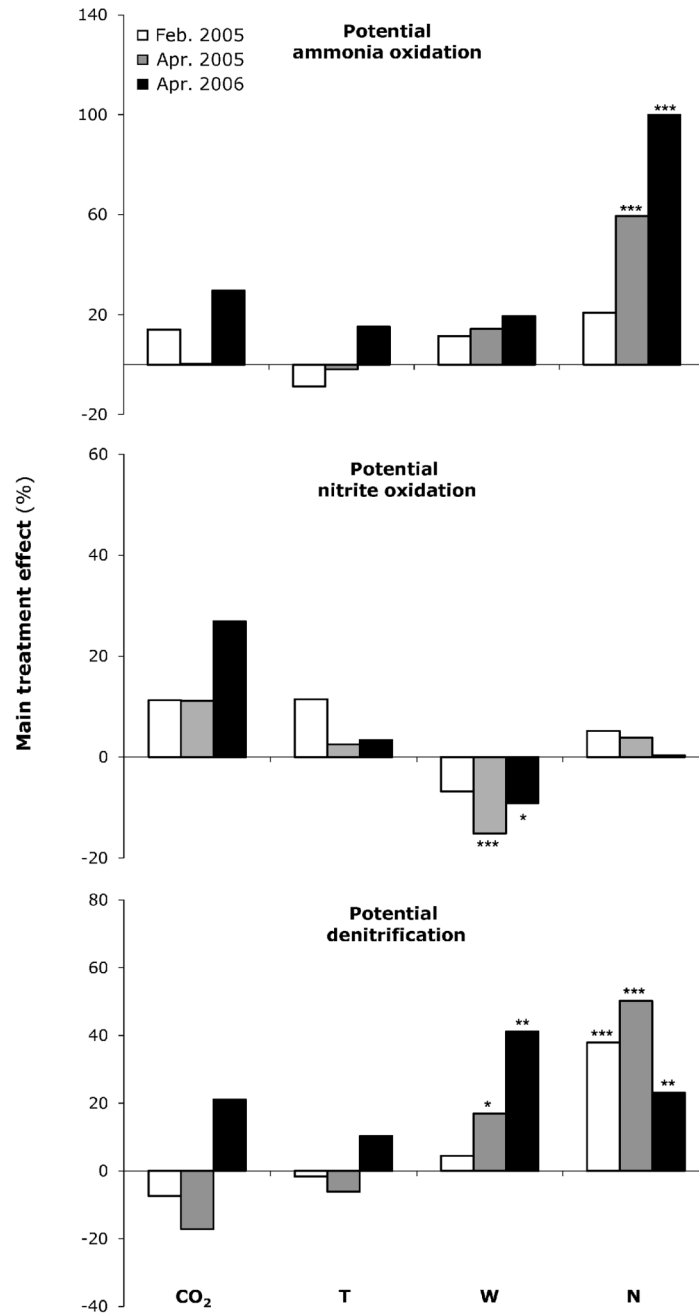
Fig. 6. Effects of elevated CO_2 (CO_2), increased temperature (T), increased precipitation (W) and N addition (N) on potential rates of ammonia oxidation, nitrite oxidation and denitrification. For each measurement date, the effect of each main treatment was calculated as: % effect $= 100\% \times$ [elevated $-$ ambient]/ambient (n $=$ 48 in the ambient and elevated treatments). Significant effects are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

hannan 2007). Finally, increased potential denitrification with increased precipitation likely resulted from higher soil moisture known to induce denitrification through increases in substrate (NO_2^- and NO_3^-) diffusion and decreases in soil oxygen content (Tiedje 1988). These
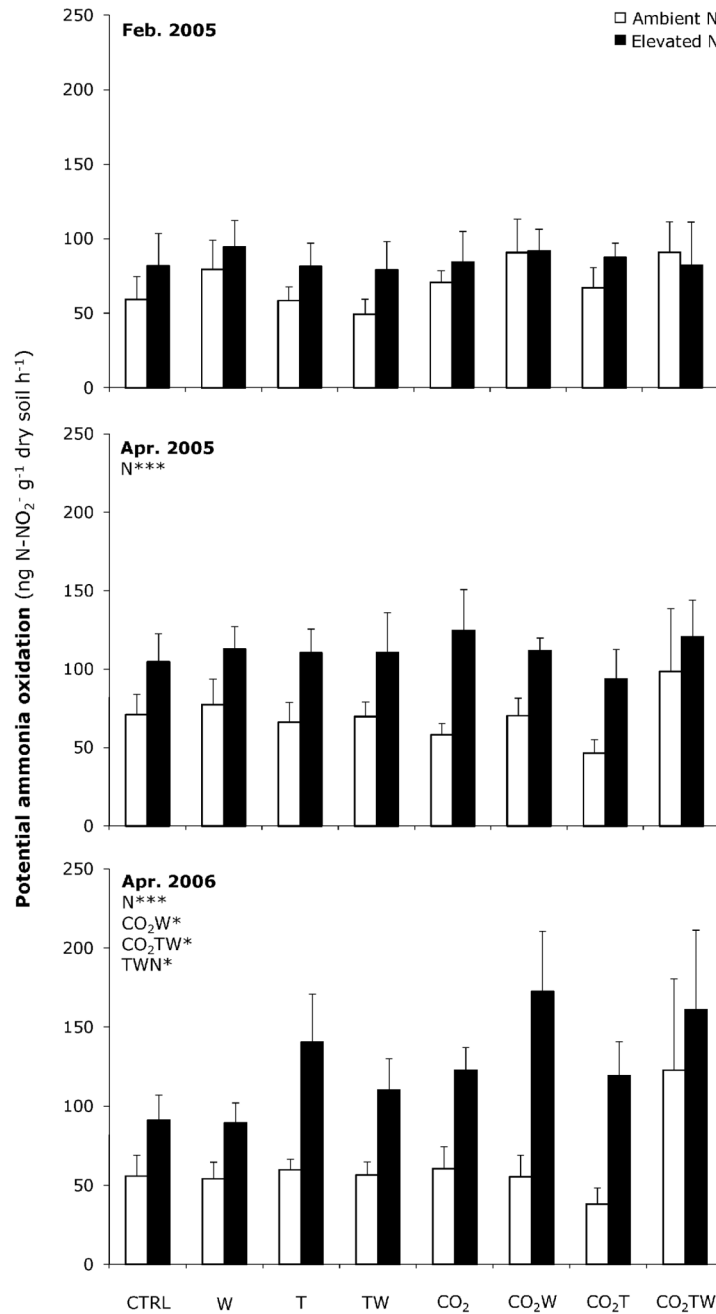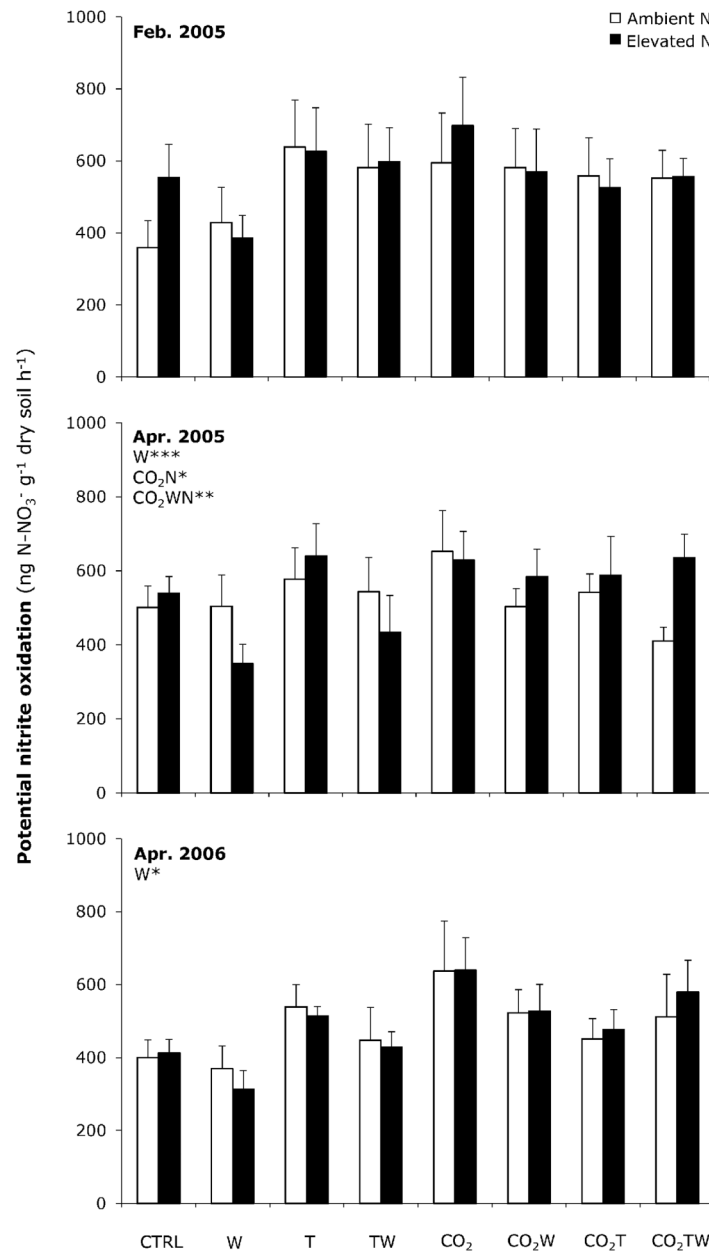
Fig. 7. Potential ammonia oxidation rates in each treatment combination for the three measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors (n = 6). Letters identify treatment effects from mixed model analysis of potential ammonia oxidation data, and interactions are presented as multiple letters (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Fig. 8. Potential nitrite oxidation rates in each treatment combination for the three measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors ($n = 6$). Letters identify treatment effects from mixed model analysis of potential nitrite oxidation data, and interactions are presented as multiple letters (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

positive responses of potential ammonia oxidation and denitrification to N and precipitation treatments confirm previous observations at our site (Barnard et al. 2006). Results presented here indicated that these responses were consistent over long time periods.
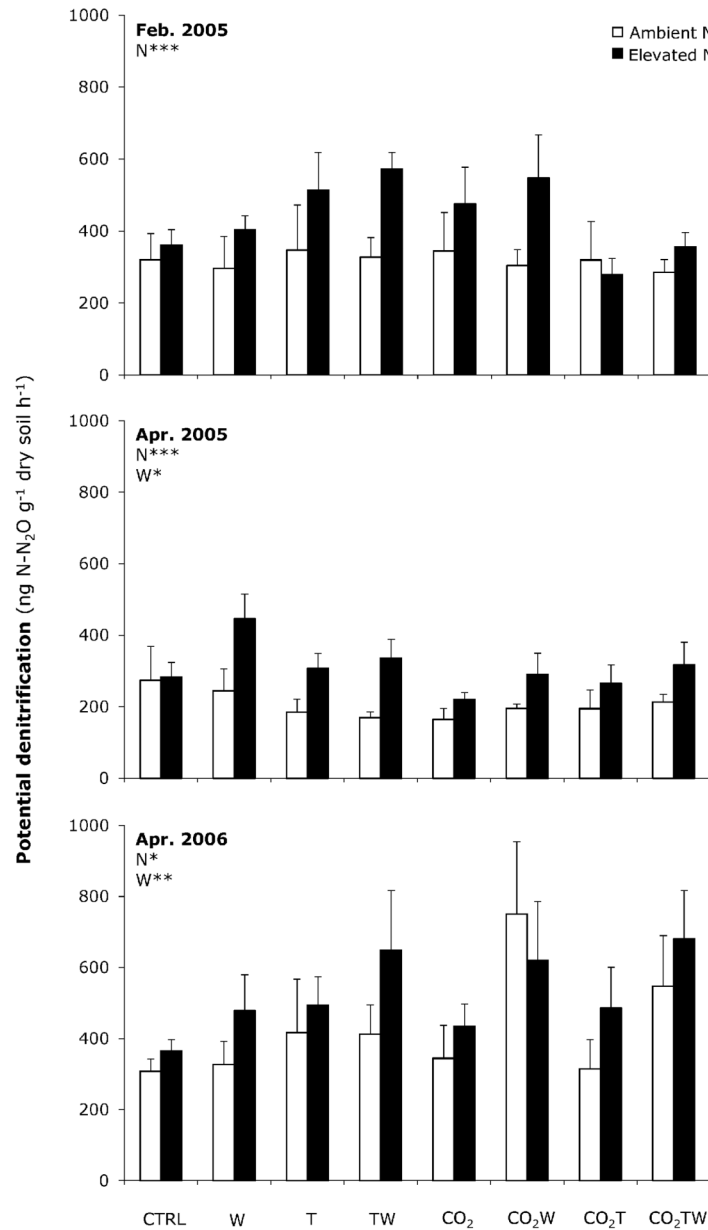
Fig. 9. Potential denitrification rates in each treatment combination for the three measurement dates. Treatments are N addition (ambient N: open bars, elevated N: closed bars), increased precipitation (W), increased temperature (T), elevated CO_2 (CO_2) and all their combinations. In the control treatment (CTRL), all treatments are at ambient levels. Error bars indicate standard errors (n = 6). Letters identify treatment effects from mixed model analysis of potential denitrification data (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Gross nitrification did not respond to the N treatment. This lack of response is striking. Significant increases in gross nitrification with mineral N supply have been reported (see the reviews by Barnard et al. 2005 and Booth et al. 2005), and our results indicated higher NH_4^+ availability and greater potential ammonia oxidation (i.e., greater abundance of ammonia-oxidizing enzymes) at high N, so that increased gross nitrification was expected. A similar ab-
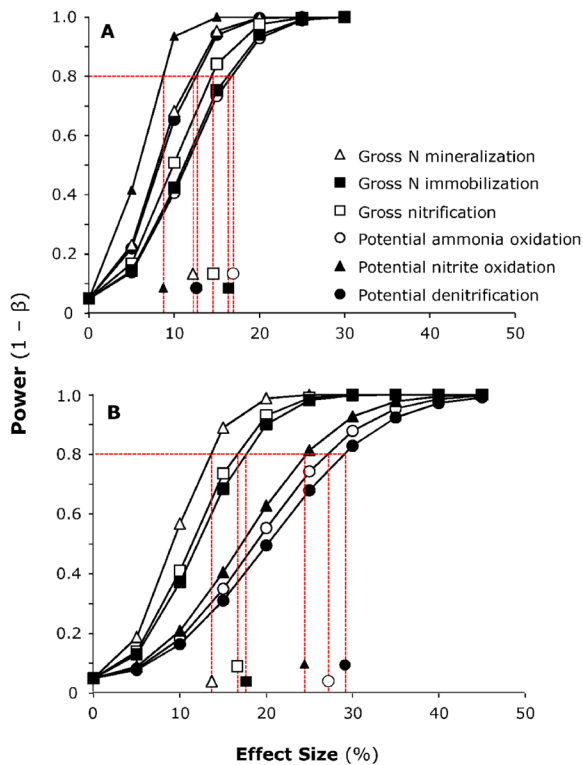
Fig. 10. Statistical power $(1 - \beta)$ as a function of effect sizes (expressed as [Treatment − Control]/ [Control] × 100%) for sub-plot effects (A) and main plot effects (B) of global change treatments and interactions. Red dashed lines show effect size thresholds at which power reaches 0.8 for each response variable. Results are from two-sided two-sample t-tests, with $\alpha = 0.05$ and $n = 6$.

sence of response of gross nitrification to added N despite greater potential nitrification was also found by Niboyet et al. (2010), and suggests that ammonia-oxidizers were limited by environmental factors or by substrate availability at the time of measurements, so that greater abundance of ammonia-oxidizing enzymes at high N did not translate to greater gross nitrification (or that the ^{15}N pool dilution technique for measuring gross nitrification is insufficiently sensitive to meaningful variation captured by the potential measurements). Gross and potential nitrification could provide complementary information on nitrification response to global change: gross rates may provide insights into the response of nitrification to treatments at the time of soil sampling, i.e., over short-time periods since

nitrification is known to be highly temporally variable (Corre et al. 2002), while potential rates may provide insights into the response of the nitrifying microorganisms to the environmental constraints to which they were exposed prior to soil sampling—likely at the scale of weeks due to their slow growth rates (Pinay et al. 2007, Le Roux et al. 2008, Niboyet et al. 2010).

In this work, we investigated the responses of the two steps of nitrification to global change. Most past work has focused on the response of ammonia oxidation, the assumed rate-limiting step of nitrification (Horz et al. 2004), although nitrite oxidation may become limiting for nitrification in disturbed ecosystems (Gelfand and Yakir 2008, Roux-Michollet et al. 2008). We found that ammonia and nitrite oxidation responded differently to treatments: potential nitrite oxidation decreased with increased precipitation and did not respond to added N, while potential ammonia oxidation increased with increased precipitation and N addition. Our results thus provide evidence that the distinct microbial communities involved in ammonia and nitrite oxidation (Hayatsu et al. 2008) are sensitive to different environmental drivers. The negative response of potential nitrite oxidation to elevated precipitation may be mediated by changes in the soil environment (e.g., decreases in oxygen content or increases in soil pH) that constrained nitrite-oxidizers but not ammonia-oxidizers. The absence of response of potential nitrite oxidation to added N despite increases in potential ammonia oxidation is however striking, and does not corroborate previous work reporting a positive correlation between potential nitrite oxidation and N availability (Attard et al. 2010). The most straightforward explanation for this is that nitrite-oxidizing enzymes were in excess compared to ammonia-oxidizing enzymes so that increases in potential ammonia oxidation did not induce increases in potential nitrite oxidation. Consistent with this, potential nitrite oxidation was 5 to 7 times greater than potential ammonia oxidation. An alternative explanation is that denitrifiers and not nitrite-oxidizers have benefited from higher NO_2^- availability. In agreement with this idea, we observed greater potential denitrification with added N. If so, knowledge about NO_2^- dynamics in soil may be important for understanding responses of N

Responses of soil N cycling to single global environmental changes: warming and elevated CO_2

Warming did not significantly affect any of the N cycling processes measured, likely because the temperature increase applied was small (+0.8–1.0°C at the soil surface, +0.7°C at 2 cm and 10 cm soil depth), and too slight to induce significant changes in the microbial activities examined. Indeed, other in situ warming experiments where increases in soil temperature were higher did report increases in N mineralization (see the review by Rustad et al. 2001: +2.4°C on average) and in N immobilization (Shaw and Harte 2001, Larsen et al. 2010). In contrast, the absence of responses of nitrification and denitrification to experimental warming is in agreement with several other field studies (Shaw and Harte 2001, Barnard et al. 2004, Barnard et al. 2006), although positive responses of nitrification (Larsen et al. 2010, Malchair et al. 2010) and denitrification (Tscherko et al. 2001, Larsen et al. 2010) have also been reported among the few studies conducted to date.

Overall, elevated CO_2 had little effect on soil N cycling at our site. First, elevated CO_2 increased gross N mineralization but only when combined with added N and precipitation, and did not significantly affect gross N immobilization or soil NH_4^+ and NO_3^- contents. Our results thus contrast with other field studies that have reported reduced soil inorganic N availability under CO_2 enrichment due to increased plant N (Hu et al. 2001) or microbial N demand (Diaz et al. 1993, Dijkstra et al. 2010). An increase in plant N uptake is however unlikely in the present experiment as elevated CO_2 did not induce significant increases in plant biomass production (Dukes et al. 2005). Furthermore, we found no increase in microbial N immobilization (i.e., no evidence for increased microbial N demand), probably because elevated CO_2 did not markedly alter soil labile C availability (elevated CO_2 did not induce significant increases in soil laboratory-incubated CO_2 efflux), nor soil moisture in our study, in contrast to the studies where elevated CO_2 induced increases in N immobilization (Diaz et al. 1993, Dijkstra et al. 2010). Second, elevated CO_2 modified responses of gross or potential nitrification to other global environmental chang-es (e.g., CO_2 increased ammonia oxidation when precipitation was increased), but the significance of these interactive effects was limited when multiple measurement dates were considered. This weak response of nitrification to elevated CO_2 is consistent with other field experiments (see Hungate et al. 1997b at ambient N, Barnard et al. 2004, Pinay et al. 2007, Larsen et al. 2010). However, it contrasts with a meta-analysis on nitrification response to elevated CO_2 which reported decreases in potential nitrification due to decreases in soil NH_4^+ or oxygen content (Barnard et al. 2005). Third, elevated CO_2 did not significantly alter denitrification, and did not substantially affect any of the main drivers of denitrification (i.e., soil CO_2 efflux, soil moisture or soil NO_3^-) in our study. This result contrasts with field studies where increases in denitrification were reported associated with higher soil labile C or soil moisture content (Arnone and Bohlen 1998, Ineson et al. 1998, Baggs et al. 2003) and to a meta-analysis on denitrification response to elevated CO_2 that reported depressed potential denitrification due to reduced nitrate availability (Barnard et al. 2005).

Treatments vs. predicted global environmental changes

The Jasper Ridge Global Change Experiment was designed to explore a wide range of possible futures within the next century, since all combinations between the ambient and elevated levels of the CO_2, temperature, precipitation, and N treatments were investigated (Shaw et al. 2002, Zavaleta et al. 2003, Dukes et al. 2005). As discussed here, some of the elevated levels of the treatments were more conservative than the others, which is necessary to take into account when interpreting our results. Atmospheric CO_2 concentration was elevated to 680 $\mu mol\ mol^{-1}$, which is close to the middle of the range of the IPCC scenarios for 2100 (IPCC 2007b), and comparable to the CO_2 concentration used in many global change studies (see among reviews on CO_2 effect on soil N cycling Zak et al. 2000b, Barnard et al. 2005, de Graaff et al. 2006). In contrast, our warming treatment resulted in an increase of approximately 0.8–1°C at the soil surface, which is at the low end of the IPCC prediction for the next century (IPCC 2007b). The lack of response of soil N cycling to the warming

treatment in our study may thus reflect the magnitude of the treatment imposed more than the sensitivity of N cycling processes to projected temperature change. Precipitation was elevated by increasing each rain event by 50% and by extending the rainy season by three weeks, which is in the range of predicted changes for California (Dukes et al. 2005). Finally, our N "deposition" treatment consisted of an initial application of 2 g N m^{-2} in solution early in the growing season to mimic the pulse of accumulated dry N deposition that enters the system with the first rains after the summer, and an additional application of 5 g N m^{-2} as slow-release fertilizer to simulate the N input throughout the season. N supply was thus elevated by 7 g N m^{-2} yr^{-1}, while this California grassland currently received 0.5 g N m^{-2} yr^{-1} (Dukes et al. 2005, Dukes and Shaw 2007). This N "deposition" treatment is high, e.g., compared to other grassland studies where N deposition was mimicked by the addition of 4 g N m^{-2} yr^{-1} (Reich et al. 2001). Nevertheless, such a high N deposition rate already occurs in a few industrialized regions of the world (Holland et al. 1999, Galloway et al. 2008), and was applied to approximate projected increase in N deposition for many industrialized areas in coming decades (Galloway et al. 2008).

Conclusions and implications

The N cycling processes examined responded mainly to N addition and altered precipitation: enhanced N supply significantly increased gross N immobilization, potential ammonia oxidation and potential denitrification, while increased precipitation depressed potential nitrite oxidation and increased potential ammonia oxidation and denitrification. In contrast, elevated CO_2 and modest warming did not significantly affect any of these microbial N transformations. Except for gross N mineralization, we found weak evidence of non-additive effects of elevated CO_2, warming, increased precipitation, and enhanced N supply on soil N cycling: statistically significant interactions, when found, tended not to persist across multiple dates. These findings suggest that global change effects on N cycling are primarily additive, and therefore generally predictable from single factor studies.

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