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REGULAR ARTICLE

Responses of soil nitrogen cycling to the interactive effects of elevated CO_2 and inorganic N supply

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Abstract Despite increasing interest in the effects of climate change on soil processes, the response of nitrification to elevated CO_2 remains unclear. Responses may depend on soil nitrogen (N) status, and inferences may vary depending on the methodological approach used. We investigated the interactive effects of elevated CO_2 and inorganic N supply on gross nitrification (using ^{15}N pool dilution) and potential nitrification (using nitrifying enzyme activity assays) in *Dactylis glomerata* mesocosms. We measured the responses of putative drivers of nitrification (NH_4^+ production, NH_4^+ consumption, and soil environmental conditions) and of potential denitrification, a process functionally linked to nitrification. Gross nitrification was insensitive to all treatments, whereas potential nitrification was higher in the high N treatment and was further stimulated by elevated CO_2 in the high N treatment. Gross mineralization and NH_4^+ consumption rates were also significantly increased in response to elevated CO_2 in the high N treatment, while potential denitrification showed a significant increase in response to N addition. The discrepancy between the responses of gross and potential nitrification to elevated CO_2 and inorganic N supply suggest that these measurements provide different information, and should be used as complementary approaches to understand nitrification response to global change.

Keywords CO_2 and N interactions · Gross mineralization · Gross nitrification · Potential nitrification · Potential denitrification

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Introduction

Increasing atmospheric CO_2 concentrations could strongly affect soil nitrogen (N) cycling, leading to potentially large feedbacks to climate: first, rising atmospheric CO_2 could alter N losses from terrestrial ecosystems including the production of nitrous oxide (N_2O) (Arnone and Bohlen 1998; Ineson et al. 1998), a potent greenhouse gas (Forster et al. 2007); second, by altering soil N availability (Diaz et al. 1993; Zak et al. 1993), elevated CO_2 could constrain net primary productivity and the long-term response of ecosystems to climate change (Hu et al. 2006; Hungate et al. 2003; Reich et al. 2006a). The response of nitrification to elevated CO_2 is of particular importance, since nitrification contributes to ecosystem N losses directly by releasing NO and N_2O in the atmosphere (Wrage et al. 2001), and indirectly by producing NO_3^- for leaching and denitrification (Tiedje 1988). However, the effects of rising atmospheric CO_2 on nitrification remain unclear (see review by Barnard et al. (2005)).

The response of nitrification to elevated CO_2 may depend on soil N status (Hungate et al. 1997), since the effects of elevated CO_2 on main factors controlling nitrification depend on soil N availability. Indeed, elevated CO_2 may alter soil substrate availability for nitrifiers by altering NH_4^+ production rates as a consequence of higher labile carbon inputs to the soil, but the magnitude and direction of changes in mineralization vary considerably between studies (Hoosbeek et al. 2006; Hungate 1999; Reich et al. 2006b). In addition, elevated CO_2 may alter soil substrate availability for nitrifiers by altering competition for NH_4^+ between nitrifiers and other NH_4^+ consumers: CO_2 can stimulate plant and heterotrophic microbial growth and associated NH_4^+ plant and microbial uptake (Barnard et al. 2006a; Hu et al. 2001). Such effects are likely to depend on soil N status, since plant and microbial growth are often N limited (Hu et al. 2006; Vitousek and Howarth 1991). Finally, elevated CO_2 could alter soil environmental conditions to which nitrifiers are sensitive, by increasing soil water content and thereby altering soil oxygen status (Barnard et al. 2006b). Given that changes in soil water are mediated by plants (Field et al. 1995), and that plant growth is often N limited (Vitousek and Howarth 1991), CO_2-induced effects on soil environmental conditions for nitrifiers are likely to depend on soil N availability. The large range of responses of the drivers of nitrification to elevated CO_2 underscores the importance of experimentally manipulating soil N supply when assessing the impacts of elevated CO_2 on nitrification. Nevertheless, very few studies have examined the combined effects of elevated CO_2 and N addition on nitrification, and among the few studies conducted, contrasting responses have been reported (Barnard et al. 2006b; Hungate et al. 1997; Lagomarsino et al. 2008; Zak et al. 2000), which highlights the need for further investigation.

Inferences about the response of nitrification to elevated CO_2 may also depend on the methodological approach used to assess nitrification, as suggested by a recent meta-analysis on the effects of global change on nitrification, where elevated CO_2 was found to have no effect on gross nitrification, but to decrease potential nitrification (Barnard et al. 2005). The discrepancy between responses of gross and potential nitrification to elevated CO_2 could be due to qualitatively different responses among ecosystems sampled in the meta-analysis, or/and to differences in the information provided by gross and potential rate measurements on the response of nitrification to elevated CO_2. Indeed, gross nitrification is a measure of gross NO_3^- production rate in the soil, while potential nitrification is a measure of optimal NO_3^- production rate in the soil when all the factors potentially limiting nitrification are removed (Barnard et al. 2005; Hart et al. 1994). To reduce our uncertainty and improve our understanding of nitrification response to elevated CO_2, the responses of both gross and potential nitrification to elevated CO_2 should be assessed in the same study; however, to our knowledge, this has never been done.

We investigated the interactive effects of elevated CO_2 and inorganic N supply on nitrification in *Dactylis glomerata* mesocosms, and used two complementary methodological approaches to measure nitrification (Hart et al. 1994): ^{15}N isotope pool dilution technique (Kirkham and Bartholomew 1954), a tool for measuring gross nitrification; and nitrifying enzyme activity assays under optimal conditions for nitrifiers, a tool for studying the nitrification potential of the soil (Pinay et al. 2007). To provide insight into the mechanisms controlling the responses of gross and potential nitrification, we also measured the responses of the main drivers of nitrification by which elevated CO_2 and N could

affect nitrification: gross NH_4^+ production (i.e. gross mineralization) and gross NH_4^+ consumption rates; microbial and plant N contents, as indicators of microbial N immobilization and plant N uptake; soil respiration and root dry mass, as indicators of heterotrophic microbial and root activity; soil water content and soil respiration, as indicators of soil oxygen status. Finally, potential denitrification was measured as denitrification is functionally linked to nitrification (Hayatsu et al. 2008). Our objectives were: (i) to assess the interactive effects of elevated CO_2 and inorganic N supply on soil N cycling and in particular test whether gross and potential nitrification rates respond similarly to treatments; (ii) to identify the variables which could drive the response of gross and potential nitrification in our study.

Materials and methods

Experimental design and treatments

The experiment was conducted on grass monocultures of *Dactylis glomerata*, a fast-growing perennial grass common to a wide variety of grassland habitats. Deep PVC pots (15×20×50 cm) were filled with a layer of expanded clay pellets to improve drainage, and then with a reconstituted sandy-loam soil (see Bloor et al. (2009) for a full description). The soil/sand mix had a total N content of 0.23 g kg^{-1}, a total C content of 2.46 g kg^{-1}, an organic matter content of 4.26 g kg^{-1} (determined by dry combustion), a cation exchange capacity of 1.81 cmol kg^{-1}, and a pH of 8.5 (INRA, Arras, France).

Seeds of *Dactylis glomerata* (obtained from Arbiotech, St Gilles, France) were sown on 23 February 2006 at a density of 2,000 m^{-2} resulting in a total of 60 seeds per pot. Pots were assigned to one of 12 naturally-lit chambers (wooden frame and clear plastic walls, 65×65×100 cm high) set up inside a large glasshouse at the University of Paris XI (Orsay, France). One month later, when the seedlings had fully emerged, we initiated two CO_2 treatments (ambient and 645 µmol mol^{-1}) crossed with two N treatments (ambient and + 10 g N m^{-2}). Six chambers were ventilated with ambient air taken from outside the glasshouse, and the six others with ambient air enriched with CO_2. Average CO_2 concentration was 381±6 µmol mol^{-1} in the ambient treatment and 645±9 µmol mol^{-1} in the elevated CO_2 treatment (CO_2 concentrations were monitored throughout the experiment using a portable carbon dioxide analyser (Carbocap, GM 70, Vaisala, Helsinki, Finland), see Bloor et al. (2008) for further details). Within each chamber, two N treatments were applied: pots in the high N treatment received six 200 ml applications of a 7.9 m*M* NH_4NO_3 l^{-1} solution at 2-week intervals, whereas pots in the low N treatment received the equivalent amount of distilled water (four pots were present per chamber, two in the low N treatment and two in the high N treatment, resulting in a total of 48 pots). Plants grew under the four treatment combinations for 10 weeks, and pots were watered every 3 days throughout the experimental period.

Soil water content and soil respiration

Soil water content and soil respiration were measured in the layer in which soil was sampled for nitrification measurements. Volumetric soil water content was measured in each pot at three dates towards the end of the experimental period: 31 May 2006, 6 June 2006, and at the time of final harvest (7–9 June 2006). Measurements were made at a depth of 5 cm using a SM200 soil humidity probe (Delta-T, Burwell, UK). Immediately prior to final harvest, a soil core (0–6 cm) was taken from each pot and placed in a flask sealed with a septum to determine soil respiration rates. The flask was maintained at 15°C in a water bath and CO_2 accumulation was measured during two minutes using an IR analyser (EGM1, PP system, UK).

Plant harvesting and soil sampling

Plants were harvested 7–9 June 2006 and separated into below- and above-ground material. At this time, roots had fully colonized soil, and shoots were about 50 cm high. Root and shoot dry mass were measured after drying plant material at 60°C for at least 72 h. Soil from the top 10 cm was collected in each pot. Soil from the two equivalent pots of each chamber was pooled, homogenized and sieved using a 2 mm mesh, leading to a total of 24 samples (two CO_2 treatments × two N treatments × 6 replicates). The freshly sieved soil was used to determine microbial N pool, and gross and potential N transformation rates (see below).

Plant and microbial N pools

Total N concentration in roots and shoots was measured using an N elemental analyser (CNRS, Solaize, France), and plant N content was determined as the sum of root and shoot N contents. Soil microbial N was measured using the chloroform fumigation-extraction method (Brookes et al. 1985). Soil samples (5 g) were fumigated for 24 h with chloroform vapour, whereas control samples (5 g) were not fumigated. After extraction in 0.5 *M* K_2SO_4, total N in the extracts was analyzed by dry combustion (INRA, Arras, France), and microbial biomass N was estimated as: [(N in fumigated soil)–(N in non-fumigated soil)] / 0.54 (Brookes et al. 1985).

Gross N fluxes

Gross N transformation rates were determined using ^{15}N pool dilution (Hart et al. 1994), and paired labelled treatments were conducted to assess gross mineralization and NH_4^+ consumption rates, as well as gross nitrification and NO_3^- consumption rates. $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ solutions were used to ensure that the gross NH_4^+ production and consumption rates estimated from the soil samples labelled with $^{15}NH_4NO_3$ could be used as reliable estimates of the gross NH_4^+ production and consumption rates which occurred in the soil samples labelled with $NH_4{}^{15}NO_3$ and used to assess gross nitrification rates (Murphy et al. 2003). Therefore, two 100-g subsamples from each soil sample were placed in two plastic bags, and 6 ml of either 7 µmol of $^{15}NH_4NO_3$ or 7 µmol of $NH_4{}^{15}NO_3$ were applied to each (both 98 atom % ^{15}N), resulting in an addition of 1 µg ^{15}N-NH_4^+ per gram of soil in the samples labelled with $^{15}NH_4NO_3$, and of 1 µg ^{15}N-NO_3^- per gram of soil in the samples labelled with $NH_4{}^{15}NO_3$. The application of $NH_4{}^{15}NO_3$ to assess gross nitrification rates probably lead to an overestimation of in situ rates of nitrification, since 1 µg N-NH_4^+ per gram of soil were provided as additional substrates for nitrifiers (Murphy et al. 2003). Just after the addition of the labelling solutions to the soil, the mixture was well homogenized to reduce sample heterogeneity and avoid preferential use of either the added label or the indigenous soil N (Barraclough 1995). A 15-g subsample was then immediately extracted with 40 ml of 1 *M* KCl. After a 24 h incubation period at 27°C, a second 15-g subsample was taken and equally extracted.

Extracts were filtered, pooled and analyzed colorimetrically for NH_4^+ and NO_3^- concentrations using an autoanalyzer (Lachat Quickchem FIA+8000). A diffusion procedure onto acid traps was used to separate NH_4^+ and NO_3^- in soil extracts for ^{15}N analysis (Brooks et al. 1989). Acid traps were constructed of glass fiber discs, acidified with 20 ml 0.5 *M* $KHSO_4$, and then sealed between two pieces of Teflon tape. For the ammonium diffusion, one acid trap was placed in each KCl extract, floating on top of the solution; 300 mg MgO per 100 ml solution were then added and the extraction cup was sealed. Solutions were incubated at 30°C in a shaking incubator for 7 days. Acid traps were then removed, individually wrapped in labelled aluminium foil packets, and placed in a dessiccator to dry. New acid traps were added to each extract for the nitrate diffusion, along with 200 mg finely ground Devarda's alloy (which converts NO_3^- into NH_4^+), and were incubated, retrieved after 7 days, and dried in a dessiccator as above. Filter disks were analyzed for ^{15}N content by isotope ratio mass spectrometry at the Colorado Plateau Stable Isotope Laboratory (www.isotope.nau.edu).

Gross mineralization (m) and gross NH_4^+ consumption (cA) were calculated from the following equations (Hart et al. 1994) based on samples where NH_4^+ concentrations and atom % ^{15}N-NH_4^+ at time 0 and time 24 were detectable (n=5 instead of 6 in the control, the high N, and the high CO_2 treatments):

$$m = \frac{[NH_4^+]_{t0} - [NH_4^+]_{t24}}{t} x \frac{\log\left([^{15}NH_4^+]_{t0} x [NH_4^+]_{t24} / [^{15}NH_4^+]_{t24} x [NH_4^+]_{t0}\right)}{\log\left([NH_4^+]_{t0} / [NH_4^+]_{t24}\right)}$$

$$cA = m - \frac{[NH_4^+]_{t24} - [NH_4^+]_{t0}}{t}$$

where $[NH_4^+]_{t0}$ is the total NH_4^+ concentrations at time 0; $[NH_4^+]_{t24}$ the total NH_4^+ concentrations at the end of the 24-h incubation time; $[^{15}NH_4^+]_{t0}$ the $^{15}NH_4^+$ concentrations at time 0; $[^{15}NH_4^+]_{t24}$ the $^{15}NH_4^+$ concentrations at the end of the 24-h incubation time; and t the length of incubation time.

Gross nitrification (n) and gross NO_3^- consumption (cN) were calculated from the following equations (Hart et al. 1994) based on samples where NO_3^- concentrations and atom % ^{15}N-NO_3^- at time 0 and

time 24 were detectable (n=5 instead of 6 in high N and high CO_2 treatment):

$$n = \frac{[NO_3^-]_{t0} - [NO_3^-]_{t24}}{t} x \frac{\log\left([^{15}NO_3^-]_{t0} x [NO_3^-]_{t24} / [^{15}NO_3^-]_{t24} x [NO_3^-]_{t0}\right)}{\log\left([NO_3^-]_{t0} / [NO_3^-]_{t24}\right)}$$

$$cN = n - \frac{[NO_3^-]_{t24} - [NO_3^-]_{t0}}{t}$$

where $[NO_3^-]_{t0}$ is the total NO_3^- concentrations at time 0; $[NO_3^-]_{t24}$ the total NO_3^- concentrations at the end of the 24-h incubation time; $[^{15}NO_3^-]_{t0}$ the $^{15}NO_3^-$ concentrations at time 0; $[^{15}NO_3^-]_{t24}$ the $^{15}NO_3^-$ concentrations at the end of the 24-h incubation time; and t the length of incubation time.

Potential N fluxes

Potential nitrification is a measure of the nitrifying enzyme concentration present in a soil sample. This measurement is based on the principle that nitrification rate is proportional to enzyme concentrations when all the factors potentially limiting nitrification are removed, and when no de novo enzyme synthesis occurred (Pinay et al. 2007). Similarly, potential denitrification is a measure of the denitrifying enzyme concentration present in a soil sample, where all factors affecting denitrification are made non-limiting, and where no de novo enzyme synthesis occurred (Tiedje 1982).

Potential nitrification was measured using the method described by Lensi et al. (1986). Two 5-g soil subsamples were placed in 150 ml plasma flasks. One subsample was used to estimate the initial NO_3^- content, and the second to determine NO_3^- accumulation under optimal conditions for nitrification. The first subsample was immediately supplied with a suspension of a denitrifying strain of *Pseudomonas fluorescens*, a solution containing 1 mg C-glucose g^{-1} dry soil and 1 mg C-glutamic acid g^{-1} dry soil, and an atmosphere of He and C_2H_2 (90 : 10) to ensure anaerobic conditions and inhibition of N_2O reductase. This subsample was set to denitrify during four days at 27°C to allow the complete conversion of the initial soil NO_3^- present in the soil into N_2O, and N_2O concentration was measured by gas chromatography (Agilent Micro GC, P200). The second subsample was enriched with 1.4 ml of a $(NH_4)_2SO_4$ solution in order to ensure moisture content equivalent to 80% water-holding capacity, and no limitation by ammonium (final soil N-NH_4^+ concentration 200 µg g^{-1} dry soil). After an aerobic incubation at 27°C for 24 h in a horizontal position to ensure good aeration of the soil, samples were inoculated and incubated under denitrifying conditions as described above, and N_2O concentration was measured by gas chromatography (Agilent Micro GC, P200). Potential nitrification was calculated by subtracting the amount of nitrate initially present in the soil from that accumulated after aerobic incubation.

Potential denitrification was measured using the procedure described by Smith and Tiedje (1979). Five grams equivalent dry soil were placed in 150 ml plasma flasks sealed with rubber stoppers. All the factors affecting denitrification were made non-limiting by adding a solution containing 1 mg C-glucose g^{-1} dry soil, 1 mg C-glutamic acid g^{-1} dry soil and 0.1 mg N-NO_3^- g^{-1} dry soil, and the atmosphere of the flask was replaced by a He : C_2H_2 mixture (90 : 10) to ensure anaerobic conditions and inhibition of N_2O reductase. Flasks were incubated at 27°C for 8 h, and the N_2O concentration was measured every 2 h on a gas chromatograph equipped with an electron capture detector (Agilent Micro GC, P200).

Statistical analysis

Treatment effects were analyzed with a full factorial split-plot model using the PROC MIXED in SAS 9.1 (SAS institute, Cary, NC). Elevated CO_2 was included as a whole-plot effect, and N addition as a split-plot effect. For analyses of repeated measurements in time for soil water content, we used PROC MIXED to run a repeated version of the split-plot model. Unless otherwise indicated, values were not transformed (soil water content data were log-transformed to correct non-normality and unequal variances). Effects with $p<0.05$ are referred to as significant, and effects with $0.5 \leq p < 0.1$ as marginally significant. Relative treatment effects were calculated as: % effect = 100% × [elevated–ambient] / ambient.

Linear regressions were carried out using PROC REG in SAS, and were used to investigate relationships between gross and potential nitrification and putative drivers, including gross mineralization, plant dry mass, plant N content, microbial biomass N, soil respiration and soil water content (average of soil water content data collected on the week preceding the harvest). Relationships between gross and potential nitrification and potential denitrification were also

examined. In addition, we conducted paired comparisons for NH_4^+ contents at both time 0 and time 24 in the paired treatments using the paired statement of PROC TTEST in SAS. This was done to verify that our paired treatments were similar in their NH_4^+ contents at both time 0 and time 24, so that the gross NH_4^+ production and consumption rates calculated from the soil samples labelled with $^{15}NH_4NO_3$ could be used as reliable estimates of the gross NH_4^+ production and consumption rates that occurred in the soil samples labelled with $NH_4{}^{15}NO_3$.

Results

Plant growth, plant and microbial N pools

Elevated CO_2 significantly increased *Dactylis* root and shoot dry mass, but only in the high N treatment (significant CO_2 × N interactions, Table 1, Fig. 1). *Dactylis* root and shoot dry mass were also significantly increased by N addition (Table 1, Fig. 1). Elevated CO_2 had no significant effect on total plant, root, or shoot N content (Table 1). In contrast, N addition strongly increased both root and shoot N contents, resulting in a 274% increase of total plant N content (Table 1, Fig. 1). Similarly, microbial biomass N was not affected by CO_2 enrichment but increased significantly in response to N addition (Table 1, Fig. 1). No significant CO_2 × N interactions were found on plant and microbial N pools (Table 1).

Soil water content and soil respiration

Elevated CO_2 tended to increase soil water content (repeated measures analysis; +8%, p=0.06), whereas N addition decreased soil water content (repeated measures analysis; −16%, p<0.0001). The CO_2-induced effects on soil water content differed depending on N (significant CO_2 × N interaction, repeated measures analysis; p=0.0003): elevated CO_2 mitigated the negative effect of N addition on soil water content, but had no apparent effect in the low N treatment (Fig. 1). Treatment effects on soil water content were similar for the three dates of measurements (CO_2 × Time, p=0.93, N × Time, p=0.52, CO_2 × N× Time, p=0.97).

Elevated CO_2 significantly increased soil respiration (Table 1, Fig. 1), especially at high N (+19% in the low N treatment vs. +57% in the high N treatment, marginally significant CO_2 × N interaction, Table 1, Fig. 1). Soil respiration was also strongly increased by N addition (Table 1, Fig. 1). Root dry mass explained 36% of the variation in soil respiration (positive correlation, p=0.002).

Gross N fluxes

Gross mineralization and NH_4^+ consumption rates were determined based on the NH_4^+ concentrations and atom % ^{15}N-NH_4^+ given in Table 2A, and gross nitrification and NO_3^- consumption rates based on the NO_3^- concentrations and atom % ^{15}N-NO_3^- given in

Table 1 Summary of p-values from two-way split-plot analyses of variance testing for the effects of treatments (elevated CO_2, N addition, and their combination, CO_2 × N) on plant dry mass; plant and microbial N pools; soil respiration and soil water content at harvest date

	CO_2		N		CO_2 × N
	% effect	p-value	% effect	p-value	p-value
Root dry mass	**+22**	**0.02***	**+181**	**<0.0001*****	**0.02***
Shoot dry mass	**+13**	**0.006****	**+287**	**<0.0001*****	**0.003****
Root N content	−26	0.55	**+128**	**<0.0001*****	0.10
Shoot N content	+10	0.94	**+382**	**<0.0001*****	0.26
Plant N content	−3	0.91	**+274**	**0.0007*****	0.46
Microbial biomass N	+6	0.44	**+25**	**0.01***	0.96
Soil respiration	**+48**	**0.03***	**+103**	**0.0003*****	**0.06**$^{\#}$
Soil water content	+10	0.11	**−11**	**0.03***	**0.02***

Significant and marginally significant responses are indicated in bold ($^{\#}$, p<0.1; *, p<0.05; **, p<0.01; ***, p<0.001). Relative treatment effects were calculated as: % effect = 100% × [elevated–ambient] / ambient

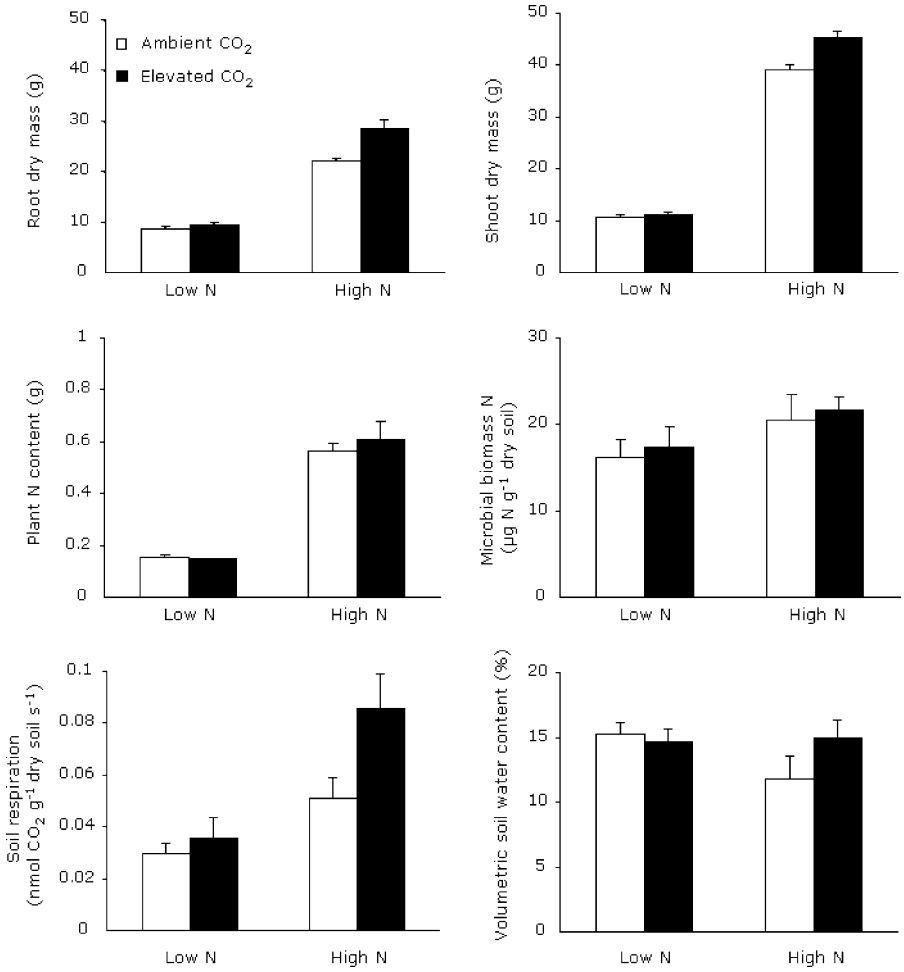

Fig. 1 Root and shoot dry mass, plant N content, microbial biomass N, soil respiration and volumetric soil water content at harvest date for each treatment combination. Treatments are CO_2 (ambient CO_2: open bars, elevated CO_2: closed bars) and Nitrogen (low N: low Nitrogen treatment, high N: high Nitrogen treatment). Means and standard error are presented

Table 2 (A) NH_4^+ concentrations and atom % ^{15}N-NH_4^+ at time 0 (t0) and after the 24-h incubation time (t24) in the soil samples labelled with $^{15}NH_4NO_3$ and used to determine gross mineralization and gross NH_4^+ consumption rates in each treatment combination (+ N: high N treatment; + CO_2: elevated CO_2 treatment) (B) NO_3^- concentrations and atom % ^{15}N-NO_3^- at time 0 (t0) and after the 24-h incubation time (t24) in the soil samples labelled with $NH_4{}^{15}NO_3$ and used to determine gross nitrification and gross NO_3^- consumption rates in each treatment combination (+ N: high N treatment; + CO_2: elevated CO_2 treatment)

	(A) $^{15}NH_4^+$ pool dilution				(B) $^{15}NO_3^-$ pool dilution			
	$[NH_4^+]_{t0}$ µg N-NH_4^+ g^{-1} dry soil	^{15}N-$NH_4^+{}_{t0}$ atom %	$[NH_4^+]_{t24}$ µg N-NH_4^+ g^{-1} dry soil	^{15}N-$NH_4^+{}_{t24}$ atom %	$[NO_3^-]_{t0}$ µg N-NO_3^- g^{-1} dry soil	^{15}N-$NO_3^-{}_{t0}$ atom %	$[NO_3^-]_{t24}$ µg N-NO_3^- g^{-1} dry soil	^{15}N-$NO_3^-{}_{t24}$ atom %
Control	0.82±0.06	40.64±0.85	0.16±0.03	1.83±0.18	1.02±0.03	43.52±2.07	1.74±0.06	25.19±1.61
+ N	0.65±0.07	39.96±1.82	0.17±0.04	1.20±0.13	1.04±0.12	45.33±0.94	0.42±0.15	15.05±3.22
+ CO_2	0.84±0.09	40.58±2.35	0.14±0.04	1.84±0.11	1.02±0.03	44.77±5.04	1.75±0.11	24.93±1.72
+ CO_2, + N	0.92±0.09	32.53±2.02	0.22±0.06	1.29±0.18	1.03±0.05	45.82±1.75	0.53± 0.19	18.09±4.85

Values are means ± standard errors

Table 2B. Paired labellings were similar in their ammonium contents at both time 0 and time 24 (p= 0.11 and 0.75 respectively), so that the gross mineralization and NH_4^+ consumption rates determined in the samples labelled with $^{15}NH_4NO_3$ are indicative of these same rates in the samples labelled with $NH_4{}^{15}NO_3$.

Elevated CO_2 had no effect on gross mineralization in the low N treatment, but increased gross mineralization in the high N treatment (+25%, significant CO_2 × N interaction, Table 3, Fig. 2). Thus, in the low N treatment, gross mineralization averaged 1.24± 0.13 mg N kg^{-1} dry soil d^{-1} under ambient CO_2 and 1.18±0.13 mg N kg^{-1} dry soil d^{-1} under elevated CO_2, while in the high N treatment, gross mineralization averaged 1.21±0.05 mg N kg^{-1} dry soil d^{-1} under ambient CO_2 but 1.51±0.14 mg N kg^{-1} dry soil d^{-1} under elevated CO_2. Similarly, elevated CO_2 increased gross NH_4^+ consumption but only in the high N treatment (+31%, significant CO_2 × N interaction, Table 3, Fig. 2): in the low N treatment, gross NH_4^+ consumption averaged 1.89±0.14 mg N kg^{-1} dry soil d^{-1} under ambient CO_2 and 1.88±0.16 mg N kg^{-1} dry soil d^{-1} under elevated CO_2, while in the high N treatment gross NH_4^+ consumption averaged 1.69± 0.09 mg N kg^{-1} dry soil d^{-1} under ambient CO_2 but 2.21±0.17 mg N kg^{-1} dry soil d^{-1} under elevated CO_2.

In contrast, gross nitrification was not affected by any of the treatments (Table 3, Fig. 2), and averaged 0.73±0.07 mg N kg^{-1} dry soil d^{-1} across all treatments. Gross NO_3^- consumption was not affected by elevated CO_2, but strongly increased with N addition (Table 3, Fig. 2), averaging 0.02±0.12 mg N kg^{-1} dry soil d^{-1} at low N and 1.28±0.18 mg N kg^{-1} dry soil d^{-1} at high N.

Potential N fluxes

Elevated CO_2 alone had no effect on potential nitrification (Table 3, Fig. 2), but tended to increase potential nitrification when combined with N addition from 3.06±0.42 mg N kg^{-1} dry soil d^{-1} under ambient CO_2 to 4.55±0.53 mg N kg^{-1} dry soil d^{-1} under elevated CO_2 (+49%, marginally significant CO_2 × N interaction, Table 3, Fig. 2). N addition increased potential nitrification (Table 3, Fig. 2) from 2.37±0.18 mg N kg^{-1} dry soil d^{-1} at low N to 3.80± 0.39 mg N kg^{-1} dry soil d^{-1} at high N.

Potential denitrification was not affected by elevated CO_2 irrespective of N treatment, but strongly increased with N addition (Table 3, Fig. 2), averaging 0.62±0.04 mg N kg^{-1} dry soil d^{-1} at low N and 2.17± 0.19 mg N kg^{-1} dry soil d^{-1} at high N.

Correlations between gross and potential nitrification, putative drivers of nitrification, and potential denitrification

Gross and potential nitrification were not correlated (p=0.88). Gross nitrification was not significantly correlated with any of the variables measured as putative drivers of nitrification (including gross mineralization, plant dry mass and N content, microbial biomass N, soil respiration and soil water content)—and was not correlated with potential denitrification. In contrast, potential nitrification was positively correlated with plant dry mass (R^2=0.36, p=0.002), plant N content (R^2=0.26, p=0.01), soil respiration (R^2=0.24, p=0.02), and potential denitrification (R^2=0.25, p=0.03).

Table 3 Summary of p-values from two-way split-plot analyses of variance testing for the effects of treatments (elevated CO_2, N addition, and their combination, CO_2 × N) on gross and potential N transformation rates

	CO_2		N		CO_2 × N
	% effect	p-value	% effect	p-value	p-value
Gross nitrification	+3	0.93	−5	0.52	0.68
Potential nitrification	+28	0.15	**+60**	**0.005****	**0.09**$^{\#}$
Gross mineralization	+11	0.13	**+14**	**0.07**$^{\#}$	**0.03***
Potential denitrification	+21	0.22	**+260**	**<0.0001*****	0.34
Gross NH_4^+ consumption	+15	0.12	+5	0.66	**0.05***
Gross NO_3^- consumption	−14	0.74	**+5485**	**0.0002*****	0.63

Significant and marginally significant responses are indicated in bold ($^{\#}$, p<0.1; *, p<0.05; **, p<0.01; ***, p<0.001). Relative treatment effects were calculated as: % effect = 100% × [elevated–ambient] / ambient

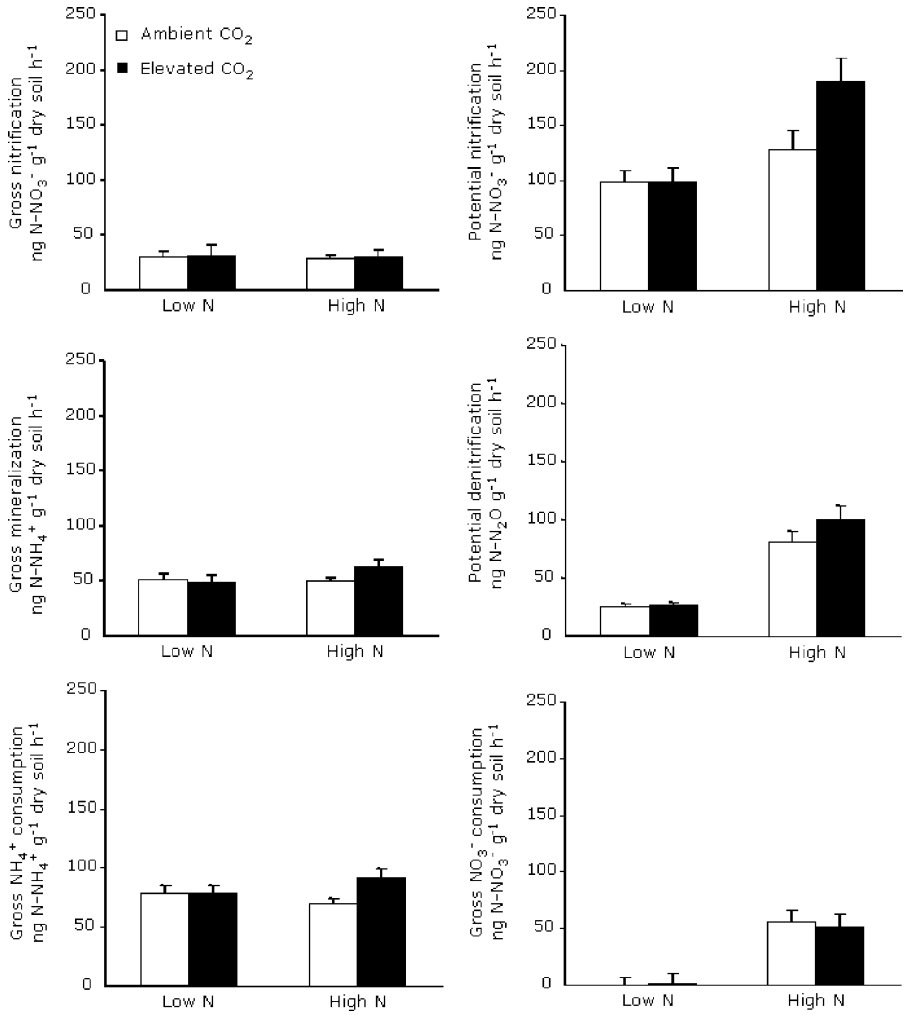

Fig. 2 Gross and potential N transformation rates for each treatment combination. Treatments are CO_2 (ambient CO_2: open bars, elevated CO_2: closed bars) and Nitrogen (low N: low Nitrogen treatment, high N: high Nitrogen treatment). Means and standard error are presented

Discussion

The responses of potential and gross nitrification to CO_2 and N treatments differed substantially. We compare these responses to previous work, examine their relationship to drivers of nitrification and to denitrification, and discuss explanations for strong differences between potential and gross nitrification.

Potential nitrification responded positively to inorganic N supply in agreement with numerous other studies (Barnard et al. 2005, 2006b). Also consistent with past work, potential denitrification increased in response to N addition (Barnard et al. 2005, 2006b), and was positively correlated to potential nitrification. Our finding that potential nitrification responded positively to elevated CO_2, but only with added N, contrasts with those of previous studies where the effect of elevated CO_2 on nitrification was negative, both without (Hungate et al. 1997; Lagomarsino et al. 2008) or with N addition (Barnard et al. 2006b). Negative responses of nitrification to rising atmospheric CO_2 have been attributed to increased competition for NH_4^+ (Hungate et al. 1997; Lagomarsino et al. 2008) or to lower soil O_2 concentrations (Barnard et al. 2005, 2006b). Our measurements of gross NH_4^+ consumption rates and soil respiration are consistent with the idea of increased competition for NH_4^+ in the elevated CO_2 and high N treatment, but this may have been offset by concomitant increases in gross mineralization. Indeed, we found that gross mineralization was increased in the elevated CO_2 treatment at high N. This increase in gross mineralization in the high CO_2 and high N treatment was likely driven by higher C

inputs by roots to the soil (Dijkstra et al. 2005; Hoosbeek et al. 2006), since we found that the high CO_2 and high N treatment stimulated root growth and soil respiration, both indicating higher C availability. Higher soil labile C may have stimulated growth of C-limited microbes (Hu et al. 2006), and led to an increase in organic matter mineralization (Fontaine et al. 2003). This hypothesis is also supported by the positive correlation between potential nitrification and indicators of C availability in the soil (root dry mass and soil respiration). In addition, since soil moisture in the high CO_2 and high N treatment was similar to the ambient treatment, a decrease in soil oxygen content resulting from higher soil water content under elevated CO_2 might not have occurred. Thus, our study supports previous work suggesting that potential nitrification is a sensitive means of detecting the effects of environmental constraints on nitrifying bacteria (Barnard et al. 2004; Le Roux et al. 2008; Pinay et al. 2007). However, the most serious shortcoming of potential nitrification measurements is that we do not know how to relate them to nitrate production in situations where ammonium availability is controlled by in situ processes such as gross mineralization rates and competition with other microbial processes that consume ammonium. Simultaneous measurements of gross nitrification could potentially provide insight into the role of these in situ processes in controlling nitrification, but these measurements have rarely been directly compared.

Gross nitrification was unresponsive to N and CO_2 treatments despite significant effects of one or both of these treatments on virtually all of our other measures of pools and fluxes in the N cycle, including potential nitrification. Furthermore, gross nitrification was neither correlated to known drivers of nitrification, nor to potential nitrification. This lack of response is especially surprising in the high N treatment since we added substantial quantities of the substrate for nitrification (NH_4^+) in this treatment and since significant increases in gross nitrification with N additions are well documented (Barnard et al. 2005; Booth et al. 2005; Hungate et al. 1997). The unresponsiveness of gross nitrification is also surprising in the high CO_2 and high N treatment since our results indicate higher NH_4^+ production rates in this treatment, and since positive relationships between gross mineralization and gross nitrification are often reported (Booth et al. 2005). There are several possible explanations for this: some related to characteristics of the measurement and others related to mechanisms that may have led to homeostasis in gross nitrification rates.

One possible explanation is that ^{15}N pool dilution measurements do not account for remineralization of added ^{15}N (i.e., rapid recycling of ^{15}N by soil microorganisms). There is good evidence that remineralization occurs over very short time scales and can lead to substantial underestimations of gross N fluxes measured using pool dilution methods (Mary et al. 1998; Murphy et al. 2003). In gross nitrification measurements, this underestimation is strongest when there is substantial microbial uptake and recycling of ^{15}N labelled NO_3^- (Mary et al. 1998). Our measurements of gross NO_3^- consumption show that NO_3^- uptake was negligible in the low N treatment, so that remineralization was likewise insignificant; but NO_3^- uptake was large in the high N treatment, so that remineralization may have led to a substantial underestimation of gross nitrification under high N conditions. This could explain the lack of responsiveness of gross nitrification to N addition in our experiment. A second possible explanation for the lack of responsiveness of gross nitrification to treatments is that in situ nitrification rates may have been limited by NH_4^+ availability and that we may have overwhelmed treatment differences by adding NH_4^+ at concentrations that were substantial compared to the native NH_4^+ concentrations at the time of measurement and to the amount of NH_4^+ produced by mineralization during the 24-h incubation time. Based on the NH_4^+ concentrations at the initial and final extraction time and on the rates of gross mineralization, we estimated that about two thirds of the NH_4^+ consumed during the incubation came from gross mineralization and the remainder from draw down of soil NH_4^+ concentrations. Thus, the addition of NH_4^+ in the gross nitrification measurements made them less representative of in situ nitrification rates and might have diminished treatment effects driven by gross mineralization.

There are, however, several lines of evidence that other mechanisms may explain the discrepancy between potential and gross nitrification responses to CO_2 and N treatments. First in their review, Barnard et al. (2005) reported marked differences in the effects of elevated CO_2 on gross nitrification (non-respon-

sive) and potential nitrification (responsive), so the patterns we find are also reflected in this broader analysis. Second, our potential nitrification measurements suggest that more nitrifying enzymes were present in the soil at high N, especially in the elevated CO_2 and high N treatment. These differences in the amount of functionally active nitrifying enzymes between the treatments were not expressed in the gross nitrification measurements, suggesting that either environmental factors (e.g. O_2) or substrate availability (e.g. NH_4^+) were limiting. There is evidence for the latter: higher soil microbial biomass N in the high N treatment may be indicative of stronger competition for NH_4^+ during the gross nitrification incubations, potentially offsetting higher nitrifying enzyme activities. Similarly, higher gross NH_4^+ consumption in the elevated CO_2 and high N treatment might have counteracted higher gross NH_4^+ production in this treatment, resulting in no changes in gross nitrification even though more nitrifying enzymes were present. Our gross nitrification measurements thus provide evidence that large differences in enzyme activities do not necessarily alter nitrification rates.

This difference in potential and gross nitrification responses may also be related to differences in the temporal scale over which potential and gross assays of nitrification operate. Potential nitrification appears to respond to environmental drivers on the time scale of weeks to months (Le Roux et al. 2008; Pinay et al. 2007), in part due to the slow growth rates of nitrifying bacteria (Hayatsu et al. 2008), while gross nitrification is a short-term measurement (24 h in our study) providing information on NO_3^- production over the incubation period. Hence, potential nitrification in our study provides insight into the integrated responses of nitrifiers to N and CO_2 treatments over periods when NH_4^+ limitations most certainly varied greatly as a result of N flushes following fertilization events and increasing competition for N with plants and heterotrophic bacteria over the course of the experiment. In contrast, gross nitrification measurements in our experiment may provide insight into the effects of N and CO_2 treatments on NO_3^- production at a time when competition for NH_4^+ was particularly intense. The complementary information provided by these two types of measurements clearly merits further exploration focusing on their temporal dynamics.

Conclusions

We found that gross and potential nitrification showed contrasting responses to elevated CO_2 and N addition. Gross nitrification was insensitive to all treatments, whereas potential nitrification was higher in the high N treatment and was further stimulated by elevated CO_2 in the high N treatment. Gross nitrification and potential nitrification measurements appear to provide very different information on nitrification and should be used as complementary approaches. Further studies of both gross and potential nitrification responses to future environmental changes and with multiple measurements in time are necessary if we wish to improve our understanding of the nitrification response to global change.

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References

Arnone JA, Bohlen PJ (1998) Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO_2. Oecologia 116:331–335. doi:10.1007/s004420050594

Barnard R, Barthes L, Le Roux X, Leadley PW (2004) Dynamics of nitrifying activities, denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO_2. New Phytol 162:365–376. doi:10.1111/j.1469-8137.2004.01038.x

Barnard R, Leadley PW, Hungate BA (2005) Global change, nitrification, and denitrification: a review. Glob Biogeochem Cycles 19:1–13. doi:10.1029/2004GB002282

Barnard R, Barthes L, Leadley PW (2006a) Short-term uptake of ^{15}N by a grass and soil micro-organisms after long term exposure to elevated CO_2. Plant Soil 280:91–99. doi:10.1007/s11104-005-2553-4

Barnard R, Le Roux X, Hungate BA, Cleland EE, Blankinship JC, Barthes L, Leadley PW (2006b) Several components of global change alter nitrifying and denitrifying activities in an annual grassland. Funct Ecol 20:557–564. doi:10.1111/j.1365-2435.2006.01146.x

Barraclough D (1995) ^{15}N isotope dilution techniques to study soil nitrogen transformations and plant uptake. Fert Res 42:185–192. doi:10.1007/BF00750513

Bloor JMG, Barthes L, Leadley PW (2008) Effects of elevated CO_2 and N on tree-grass interactions: an experimental test using *Fraxinus excelsior* and *Dactylis glomerata*. Funct Ecol 22:537–546. doi:10.1111/j.1365-2435.2008.01390.x

Bloor JMG, Niboyet A, Leadley PW, Barthes L (2009) CO_2 and inorganic N supply modify competition for N between co-occurring grass plants, tree seedlings and soil microorganisms. Soil Biol Biochem 41:544–552. doi:10.1016/j.soilbio.2008.12.013

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecol Monogr 75:139–157. doi:10.1890/04-0988

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842. doi:10.1016/0038-0717(85)90144-0

Brooks PD, Stark JM, McInteer BB, Preston T (1989) Diffusion methods to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soc Am J 53:1707–1711

Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364:616–617. doi:10.1038/364616a0

Dijkstra FA, Hobbie SE, Reich PB, Knops JMH (2005) Divergent effects of elevated CO_2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. Plant Soil 272:41–52. doi:10.1007/s11104-004-3848-6

Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO_2: implications from the plant to the global scale. Plant Cell Environ 18:1214–1225. doi:10.1111/j.1365-3040.1995.tb00630.x

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? Soil Biol Biochem 35:837–843. doi:10.1016/S0038-0717(03)00123-8

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, Haywood J, Lean J, Lowe DC, Myhre G, Nganga J, Prinn R, Raga G, Schulz M, Van Dorland R (2007) Changes in atmospheric constitutents and in radiative forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, USA, pp 129–234

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: SSSo America (Ed) Methods of soil analysis Part 2 Microbiological and Biochemical Properties. Wisconsin, USA, pp 985–1018

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45

Hoosbeek M, Li Y, Scarascia-Mugnozza G (2006) Free atmospheric CO_2 enrichment (FACE) increased labile and total carbon in the mineral soil of a short rotation poplar plantation. Plant Soil 281:247–254. doi:10.1007/s11104-005-4293-x

Hu S, Chapin FS, Firestone MK, Field CB, Chiariello NR (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. Nature 109:188–191. doi:10.1038/35051576

Hu S, Tu C, Chen X, Gruver JB (2006) Progressive N limitation of plant response to elevated CO_2: a microbial perspective. Plant Soil 289:47–58. doi:10.1007/s11104-006-9093-4

Hungate BA (1999) Ecosystems responses to rising atmospheric CO_2: feedbacks through the Nitrogen cycle. In: Luo Y, Mooney HA (eds) Carbon dioxide and environmental stress. Academic, San Diego, USA, pp 265–285

Hungate BA, Lund CP, Pearson HL, Chapin FS III (1997) Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. Biogeochem 37:89–109. doi:10.1023/A:1005747123463

Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB (2003) Nitrogen and climate change. Science 302:1512–1513. doi:10.1126/science.1091390

Ineson P, Coward PA, Hartwig UA (1998) Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: the swiss free air carbon dioxide enrichment experiment. Plant Soil 198:89–95. doi:10.1023/A:1004298309606

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Proc 18:33–34

Lagomarsino A, Moscatelli MC, Hoosbeek MR, De Angelis P, Grego S (2008) Assessment of soil nitrogen and phosphorus availability under elevated CO_2 and N-fertilization in a short rotation poplar plantation. Plant Soil 308:131–147. doi:10.1007/s11104-008-9614-4

Le Roux X, Poly F, Currey P, Commeaux C, Hai B, Nicol GW, Prosser JI, Scholter M, Attard E, Klumpp K (2008) Effects of aboveground grazing on coupling among nitrifier activity, abundance and community structure. ISME J 2:221–232. doi:10.1038/ismej.2007.109

Lensi R, Mazurier S, Gourbière F, Josserand A (1986) Rapid determination of the nitrification potential of an acid forest soil and assessment of its variability. Soil Biol Biochem 18:239–240. doi:10.1016/0038-0717(86)90035-0

Mary B, Recous S, Robin D (1998) A model for calculating nitrogen fluxes in soil using ^{15}N tracing. Soil Biol Biochem 30:1963–1979. doi:10.1016/S0038-0717(98)00068-6

Murphy DV, Recous S, Stockdale EA, Fillery IRP, Jensen LS, Hatch DJ, Goulding KWT (2003) Gross nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution techniques. Adv Agron 79:69–118. doi:10.1016/S0065-2113(02)79002-0

Pinay G, Barbera P, Carreras-Palou A, Fromin N, Sonié L, Couteaux MM, Roy J, Philoppot L, Lensi R (2007) Impact of atmospheric CO_2 and plant life forms on soil microbial activities. Soil Biol Biochem 39:33–42. doi:10.1016/j.soilbio.2006.05.018

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J (2006a) Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440:922–925. doi:10.1038/nature04486

Reich PB, Hungate BA, Luo Y (2006b) Carbon-Nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu Rev Ecol Evol Syst 37:611–636. doi:10.1146/annurev.ecolsys.37.091305.110039

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. Soil Biol Biochem 11:261–267. doi:10.1016/0038-0717(79)90071-3

Tiedje JM (1982) Denitrification. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. American Society of Agronomy, Madison, pp 1011–1026

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB (ed) Environmental microbiology of anaerobes. Wiley, New York, pp 179–244

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochem 13:87–115. doi:10.1007/BF00002772

Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol Biochem 33:1723–1732. doi:10.1016/S0038-0717(01)00096-7

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117. doi:10.1007/BF00010791

Zak DR, Pregitzer KS, Curtis PS, Holmes WE (2000) Atmospheric CO_2 and the composition and function of soil microbial communities. Ecol Appl 10:47–59