

Global change, nitrification, and denitrification: A review

Romain Barnard¹ and Paul W. Leadley

Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud XI, Orsay, France

Bruce A. Hungate

Department of Biological Sciences/Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona, USA

Received 15 April 2004; revised 29 October 2004; accepted 7 December 2004; published 26 January 2005.

[1] We reviewed responses of nitrification, denitrification, and soil N₂O efflux to elevated CO₂, N availability, and temperature, based on published experimental results. We used meta-analysis to estimate the magnitude of response of soil N₂O emissions, nitrifying enzyme activity (NEA), denitrifying enzyme activity (DEA), and net and gross nitrification across experiments. We found no significant overall effect of elevated CO₂ on N₂O fluxes. DEA and NEA significantly decreased at elevated CO₂; however, gross nitrification was not modified by elevated CO₂, and net nitrification increased. The negative overall response of DEA to elevated CO₂ was associated with decreased soil [NO₃⁻], suggesting that reduced availability of electron acceptors may dominate the responses of denitrification to elevated CO₂. N addition significantly increased field and laboratory N₂O emissions, together with gross and net nitrification, but the effect of N addition on field N₂O efflux was not correlated to the amount of N added. The effects of elevated temperature on DEA, NEA, and net nitrification were not significant: The small number of studies available stress the need for more warming experiments in the field. While N addition had large effects on measurements of nitrification and denitrification, the effects of elevated CO₂ were less pronounced and more variable, suggesting that increased N deposition is likely to affect belowground N cycling with a magnitude of change that is much larger than that caused by elevated CO₂.

Citation: Barnard, R., P. W. Leadley, and B. A. Hungate (2005), Global change, nitrification, and denitrification: A review, *Global Biogeochem. Cycles*, 19, GB1007, doi:10.1029/2004GB002282.

1. Introduction

[2] Modifications of atmospheric composition and climate have large effects on both the structure and functioning of terrestrial ecosystems. Our understanding of aboveground plant responses to environmental change is becoming clearer [Ward *et al.*, 1999; Rustad *et al.*, 2001; Matson *et al.*, 2002], although their responses to interacting changes are less well characterized and often surprising [Ollinger *et al.*, 2002; Shaw *et al.*, 2002]. The impacts of global environmental change on belowground microbial processes are less well understood [Panikov, 1999; Mikan *et al.*, 2000; Zak *et al.*, 2000b; Asner *et al.*, 2001; Rustad *et al.*, 2001; Matson *et al.*, 2002], especially for key soil N transformations such as nitrification and denitrification.

[3] Nitrification and denitrification play key roles in regulating the concentration of inorganic N in soil, leaching of nitrate, and the production of N₂O, a potent greenhouse gas that also contributes to stratospheric ozone destruction

[Smith, 1997; Intergovernmental Panel on Climate Change, 2001]. Thus changes in nitrification and denitrification in response to increasing CO₂, increased temperature, and N deposition can directly feed back to atmospheric and climatic change. Furthermore, by mediating N losses from ecosystems, nitrification and denitrification influence ecosystem N stocks over decades to centuries. Because the availability of N in ecosystems may limit C sequestration [Loiseau and Soussana, 1999; Oren *et al.*, 2001], changes in nitrification and denitrification could alter terrestrial C storage and atmospheric CO₂ concentrations.

[4] Nitrification and denitrification are potentially affected by CO₂, temperature, and N through a wide variety of complex, interacting mechanisms. Some of the effects are direct (e.g., N addition increases substrate availability for both processes), but many are indirect. For example, nitrification is aerobic and denitrification is anaerobic, so that indirect effects of environmental change on soil O₂ concentrations play a key role in controlling these processes. Increased CO₂ and temperature have been shown to have strong effects on soil water content and soil biological activity in many field experiments [Rustad *et al.*, 2001; Zak *et al.*, 2000b], thereby exerting strong control over soil O₂ concentrations.

¹Now at Institute of Plant Sciences, Eidgenössische Technische Hochschule, Zurich, Switzerland.

[5] Nitrification is generally favored by increasing the availability of NH_4^+ , the initial substrate for nitrification. It is favored at moderate pH and in well-aerated soils, but declines as soils become very dry. The temperature response of nitrification is approximately bell-shaped with an optimum between $\sim 20^\circ\text{C}$ and 35°C . The decline at higher temperatures may be partially due to increased biological O_2 consumption [Linn and Doran, 1984; Paul and Clark, 1989; Prosser, 1989; Grundmann et al., 1995; Parton et al., 2001; Avrahami et al., 2003]. Denitrification is generally favored by high availability of labile C as a source of energy and of NO_3^- as an electron acceptor. It is favored in poorly aerated soils, with a pH close to neutrality. The response of denitrification to temperature is similar to that of nitrification, but can have a higher temperature maximum [Tiedje, 1988; Paul and Clark, 1989; Merrill and Zak, 1992; Weier et al., 1993; Strong and Fillery, 2002; Simek and Cooper, 2002].

[6] Both nitrification and denitrification can produce N_2O . During denitrification, NO_3^- is reduced to NO_2^- and then to the gases NO , N_2O , or N_2 , the latter being the most reduced form. Increasing soil anoxia, labile C availability, NO_3^- availability, pH, and temperature shift gaseous emissions toward the more reduced forms [Tiedje, 1988; Paul and Clark, 1989; Weier et al., 1993; Bollmann and Conrad, 1998; Parton et al., 2001; Simek et al., 2002]. During nitrification, some NO , N_2O , and N_2 can be released through two pathways, the best documented of which is nitrifier denitrification [Webster and Hopkins, 1996; Wrage et al., 2001, 2004]. Nitrification-associated N_2O efflux is generally a small fraction of total nitrification N flux, but can often make a major contribution to total soil N_2O emissions [Webster and Hopkins, 1996; Kester et al., 1997; Bollmann and Conrad, 1998; Wolf and Brumme, 2002]. There is some preliminary evidence that the fraction of N_2O emissions associated with nitrification declines with increasing temperature [Avrahami et al., 2003]. N_2O production is therefore a complex process that cannot be easily be related to either total denitrification or nitrification fluxes per se [Webster and Hopkins, 1996; Wolf and Russow, 2000; Wrage et al., 2001, 2004], although some recently developed approaches may provide interesting insights into the metabolic origin of N_2O [Yoshida and Toyoda, 2000; Schmidt et al., 2004]. We have examined the responses of nitrification, denitrification, and N_2O efflux to elevated CO_2 , N addition, and warming, based on a review of published experimental results.

2. Materials and Methods

2.1. Data Analysis

[7] In our literature survey, we limited our analysis to experiments that examined the effects of elevated CO_2 , warming, and N addition on natural or seminatural communities. We have attempted to be exhaustive, especially for studies published in the last decade. We did not take into account the studies that measured N_2O fluxes from agricultural soils, as these data have been extensively reviewed [Bouwman et al., 2002]. The data were sorted by treatment (elevated CO_2 , N addition, warming), process measured (field and laboratory N_2O emissions, net and

gross nitrification, nitrifying enzyme activity (NEA), net and gross denitrification, denitrifying enzyme activity (DEA)), type of ecosystem (woody or herbaceous), type of experiment (field or mesocosm), and duration of treatment. CO_2 treatments ranged from 550 to 750 $\mu\text{mol mol}^{-1}$ in the experiments we assessed, but we considered all of these as a common treatment in our analysis primarily because of low sample size. By contrast, N addition treatments ranged from 25 to 420 $\text{kg N ha}^{-1} \text{yr}^{-1}$, so in addition to the meta-analysis we examined the relationship between N_2O emissions and the amount of N added. N was generally added as NH_4NO_3 in the experiments that we analyzed, but N was also added as urea [Mosier et al., 1991; Castro et al., 1994; Hungate et al., 1997b], atmospheric deposition [Skiba et al., 1998; Lovett and Rueth, 1999], mixing of soils with different N availability [Ambus and Robertson, 1999; Zak et al., 2000a], or NH_4SO_4 [Brumme and Beese, 1992]. We considered only the warming studies in the field or using mesocosms: We did not include soil incubation studies.

[8] We restricted our analyses to experimental results for which the measurement error was available, either from reported values or figures in published articles, or from data provided as personal communications. On the basis of control and treatment means (\bar{X}_c and \bar{X}_t , respectively), standard deviations (S_c and S_t), and sample sizes (n_c and n_t), we used the response ratio $r = \bar{X}_t/\bar{X}_c$ as a metric. Following Curtis and Wang [1998], the log-transformation of r is $\text{lr} = \ln(r)$, approximately normally distributed if \bar{X}_c and \bar{X}_t are normally distributed and \bar{X}_c is unlikely to be negative. The mean of lr is approximately the true response ratio, and its variance v is equal to

$$v = \frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2}. \quad (1)$$

The 95% confidence interval for the logged response ratio is then

$$95\% \text{ CI} = \text{lr} - 1.96\sqrt{v} \text{ to } \text{lr} + 1.96\sqrt{v}. \quad (2)$$

The confidence limits for the unlogged response ratio are obtained by computing their respective antilogs. From the mean and confidence limits of this unlogged response ratio, the mean and 95% confidence limits for the relative effect (%effect = $(r - 1) \times 100$) can then be calculated. Note that the significance levels based on the 95% confidence interval calculated this way may differ slightly from those in the original papers, due to possible data transformations in these papers and elements of the experiments that were not taken into account in our analysis. The data were analyzed to check whether mean control values and percent effect of treatment might be correlated, since the range of background values was often quite large. No correlation between mean control values and % effect of treatment was found for any of the variables measured.

[9] When several measurements in time were available, we used the overall mean, weighted by the number of replicates at each measurement. In that case, for i repeated measures with n_i replicates and SE_i standard errors at each measurement time, pooled standard error SE was calculated

as follows. The equation of analysis of variance [Fourgeaud and Fuchs, 1967] shows that for j groups, each composed of measures i repeated n_i times, the total sum of squares of means (TSS) is the sum of within-groups sum of squares of means (ISS) and between-groups sum of squares of means (WSS),

$$\text{TSS} = \text{ISS} + \text{WSS} = \sum_i \sum_j (X_{ij} - \bar{X}_{..})^2 = \sum_i \sum_j (X_{ij} - \bar{X}_i)^2 + \sum_i n_i (\bar{X}_i - \bar{X}_{..})^2, \quad (3)$$

where X_{ij} is the measure i of group j , and $\bar{X}_{..}$ is the value of the mean over all groups.

[10] In the data we collected, the samples are small, and the unbiased variance among the means σ^2 is

$$\sigma^2 = \frac{\text{TSS}}{N - 1}, \quad (4)$$

where N is the total number of measurements added over time.

[11] Pooled standard error is expressed as

$$SE = \frac{\sigma}{\sqrt{N}}. \quad (5)$$

From equations (3) and (4), we can calculate

$$\sigma^2 = \frac{\sum_i (n_i - 1)\sigma^2 + \sum_i n_i (\bar{X}_i - \bar{X}_{..})^2}{N - 1}. \quad (6)$$

Following equation (5), we then obtain

$$SE^2 = \frac{\sum_i (n_i - 1)n_i SE^2 + \sum_i n_i (\bar{X}_i - \bar{X}_{..})^2}{N - 1}. \quad (7)$$

The pooled standard error is then

$$SE = \sqrt{\frac{\sum_i n_i (n_i - 1) SE_i^2 + \sum_i n_i (\bar{X}_i - \bar{X}_{..})^2}{N(n_i - 1)}}. \quad (8)$$

[12] Meta-analysis was performed on the data, following Hedges et al. [1999], to estimate the mean effect size (magnitude of response of the processes measured) across experiments, and whether this effect was significantly different from zero. In brief, we used the response ratio r as a metric of effect size [Hedges et al., 1999], and each experiment was weighted by its within-experiment variance to calculate overall mean effect size and 95% confidence interval. Similarly as described above, the results are presented as mean and 95% confidence interval limits of the relative effect of treatment. Hedges et al. [1999] warn that when the number of studies (k) used in a meta-analysis is small (e.g., $k \leq 20$), the calculated 95% confidence interval may actually be as low as 91%. In this case, caution is warranted in the interpretation of results where a limit of

the 95% confidence interval is close to the zero response ratio.

2.2. Processes

[13] Nitrifying enzyme activity (NEA, also called potential nitrification, measured in the laboratory) reflects the enzymatic potential of the soil nitrifying bacteria to oxidize NH_4^+ into NO_2^- or NO_3^- under optimal conditions [Lensi et al., 1986]. In the absence of de novo synthesis of nitrifying enzymes during the laboratory incubation, NEA measurements provide a measure of the environmental constraints on soil nitrifiers prior to the NEA assay. Grundmann et al. [1995] have shown that changes in NEA are correlated with modifications of the major environmental constraints on nitrification, such as temperature, ammonium availability, and soil aeration. Gross nitrification is the amount of NO_3^- produced by nitrification, while net nitrification is the difference between gross nitrification and microbial NO_3^- consumption. Net nitrification was measured here by isotopic methods [Bengtsson and Bergwall, 2000; Zak et al., 2000a], laboratory incubation [Lovett and Rueth, 1999; Finzi et al., 2001; Carnol et al., 2002], or in situ buried-bag techniques [Kjonaas et al., 1998]. Gross nitrification was measured by isotope pool dilution [Hungate et al., 1997b; Zak et al., 2000a].

[14] We considered denitrifying enzyme activity (DEA, or potential denitrification) to reflect the size of the pool of functionally active denitrifying enzymes in the soil. Measured in the laboratory, the assay reflects the enzymatic potential of the soil denitrifying bacteria to reduce NO_3^- to N oxides or N_2 under optimal conditions [Tiedje, 1994], and in the absence of de novo synthesis of denitrifying enzymes during the laboratory incubation, the environmental constraints on soil denitrifiers prior to the DEA assay will then be indicated by their enzymatic capacity under the optimal assay conditions [Smith and Tiedje, 1979]. We used only studies of DEA in which soil incubation was no longer than 8 hours, due to the high probability of de novo synthesis of enzymes during longer incubations (X. Le Roux, personal communication, 2000). DEA has been shown to be correlated with annual denitrification rates in some studies [Groffman and Tiedje, 1989; Watson et al., 1994]. Net denitrification (i.e., NO_3^- transformed to N_2O in field conditions) was measured with static field chambers and ethylene inhibition [Phoenix et al., 2003], or by isotopic method [Bengtsson and Bergwall, 2000].

[15] We also examined N_2O fluxes measured in the field (using static chambers) or under laboratory conditions. The measured N_2O flux represents total emissions from both nitrification and denitrification, as these processes can occur simultaneously [Abbasi and Adams, 2000; Wolf and Brumme, 2002].

3. Results

3.1. CO_2

[16] Elevated CO_2 (Figure 1a) decreased NEA over 11 experiments (-18% mean effect size [Niklaus et al., 2001; Barnard et al., 2004b, 2005, unpublished data, 2003]) and increased net nitrification over five experiments (33%

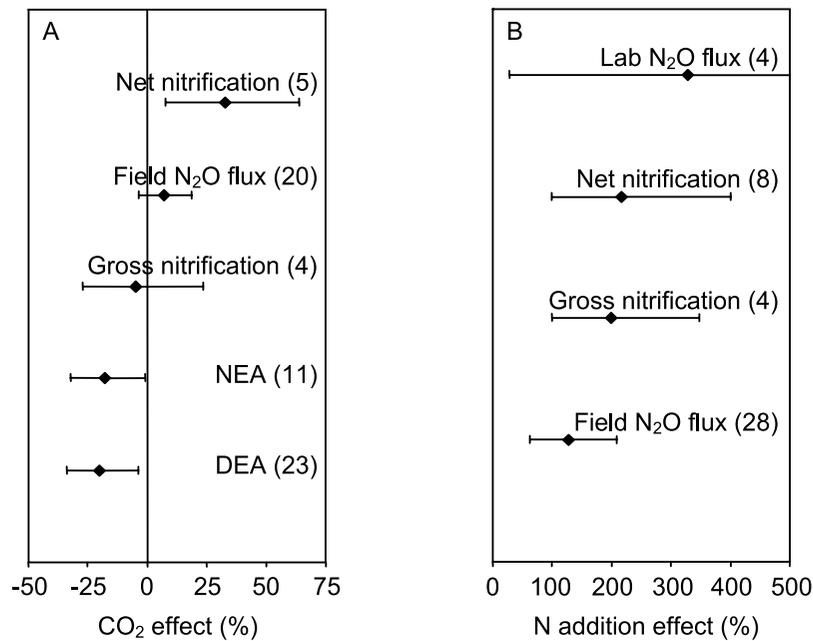


Figure 1. Meta-analysis of the effect of (a) elevated CO₂ and (b) N addition on several nitrification and denitrification measurements. Bars show 95% confidence interval of the overall mean effect size, based on the number of experiments indicated in parentheses.

mean effect size [Zak *et al.*, 2000a; Finzi *et al.*, 2001; Kammann, 2001; Carnol *et al.*, 2002]), but these should be viewed with caution since they are based on a small number of studies. Gross nitrification was not affected by elevated CO₂ (low nutrient treatment [Hungate *et al.*, 1997b]) [Zak *et al.*, 2000a].

[17] Over all ecosystems ($k = 23$), elevated CO₂ significantly decreased DEA (Figure 1a; mean effect size was -20% over all systems, and -24% in herbaceous systems), though the variance between and within studies was quite high and some individual studies documented significant increases in DEA (Figure 2). Across the experiments for which data were available, the effect of elevated CO₂ on DEA was not significantly correlated with the effect of elevated CO₂ on soil [NH₄⁺], soil [NO₃⁻], soil microbial N (11 experiments), or soil water content (eight experiments). Elevated CO₂ did not significantly alter N₂O fluxes measured either in the field or in the laboratory in herbaceous or forest ecosystems, and effect sizes were quite small (generally lower than 30%) (Figures 1a and 3).

3.2. N Addition

[18] In contrast to the small effects of elevated CO₂, added N substantially increased all nitrification variables measured (Figure 1b). Net nitrification (forest systems only) [Kjonaas *et al.*, 1998; Lovett and Rueth, 1999; Bengtsson and Bergwall, 2000; Zak *et al.*, 2000a] and gross nitrification [Hungate *et al.*, 1997b; Zak *et al.*, 2000a] were significantly increased by N addition (respectively, 217% and 200% mean effect size).

[19] The very large differences in variation between studies that measured the effect of N addition on DEA did not warrant combination of these results for meta-analysis [Hedges *et al.*, 1999]. However, all six experiments show a

positive effect size [Mohn *et al.*, 2000; Ambus and Robertson, 1999; R. Barnard *et al.*, unpublished data, 2003] (data not shown), and this effect was significant in four studies. In two studies, net denitrification declined [Bengtsson and Bergwall, 2000] or did not respond significantly [Phoenix *et al.*, 2003] to N addition.

[20] N addition significantly stimulated soil N₂O efflux measured in the field (Figure 4) and in the laboratory [MacDonald *et al.*, 1997; Sitaula *et al.*, 2001], with mean effect sizes of 128% in the field and 328% in the laboratory. In the field, N addition caused similar increases in soil N₂O efflux in both herbaceous (151%) and forest systems (105%). We found no significant relationship between the amount of fertilizer N added and its effect on the amount of N-N₂O released between control and fertilized plots (Figure 5) or on net nitrification (data not shown). There was no correlation between the percent stimulation by N addition and the background rate of N₂O efflux.

3.3. Temperature

[21] In the three studies examining the response of net nitrification to temperature, one documented significantly increased rates (113%, [Hart and Perry, 1999]), while two found nonsignificantly increased (+50%, [Verburg *et al.*, 1999]) or decreased rates (-28% , [Shaw and Harte, 2001]). In the only documented study that measured the effect of elevated temperature on NEA in the field, the values of NEA reported were too low to allow comparison [Barnard *et al.*, 2004a].

[22] Of the six experiments that measured the response of DEA to elevated temperature (ambient+2 to +3°C), only one found a significant effect (+44% [Tschirko *et al.* [2001] at elevated temperature and elevated CO₂). The other studies measured a mean effect of elevated temperature on

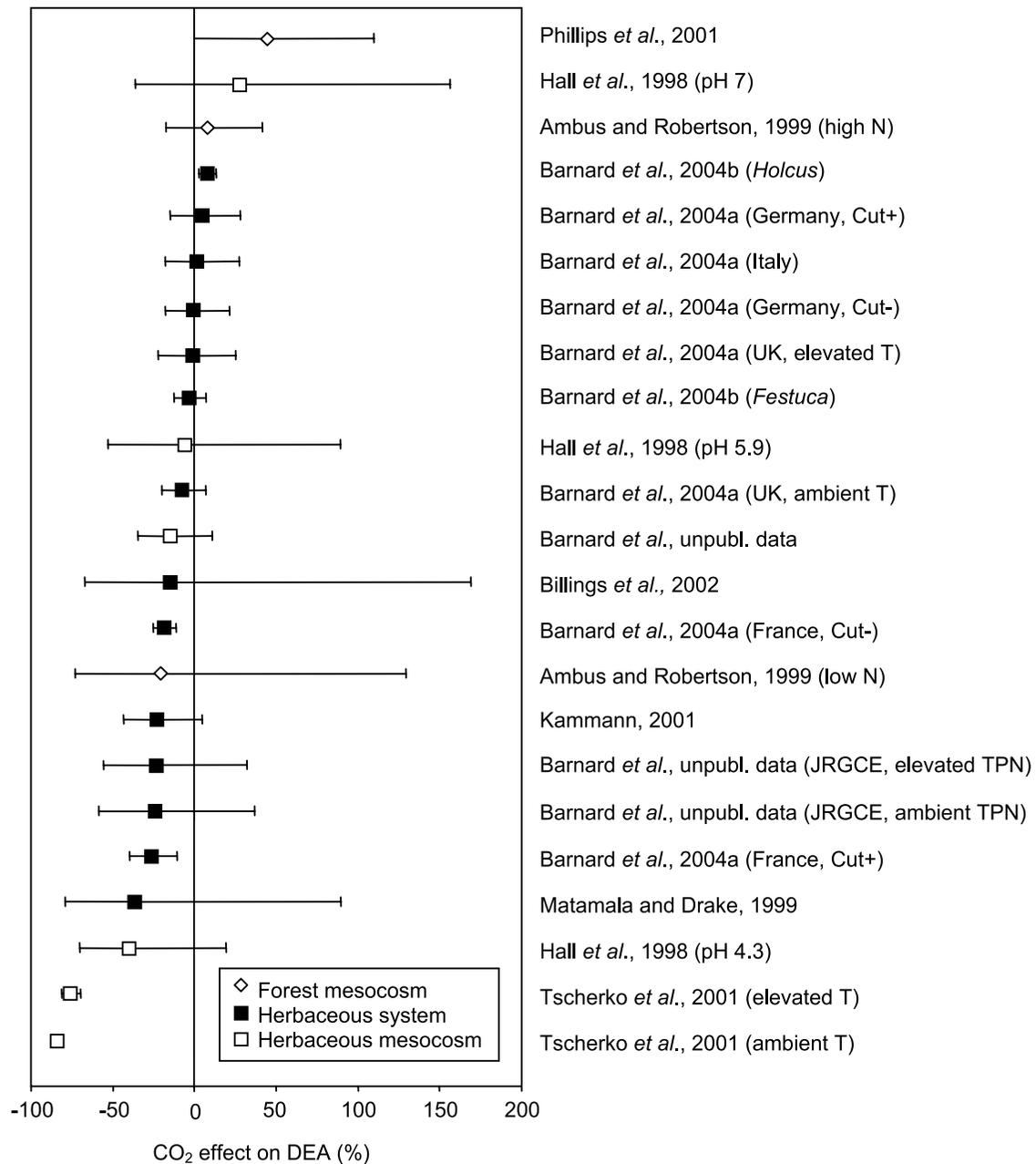


Figure 2. Effect of elevated CO₂ on denitrifying enzyme activity (DEA). Bars show 95% confidence interval of the effect size. In certain studies, elevated CO₂ was crossed with other treatments that are given here: high or low cutting frequency (Cut+ and Cut-), soil N content (high N and low N), temperature level (ambient T and elevated T), soil pH, plant species, or study site. Note that the use of log transformations makes the confidence intervals asymmetrical. The Barnard (R. Barnard *et al.*, unpublished data, JRGCE, 2003) references indicate measurements that were made in the Jasper Ridge Global Change Experiment (see *Shaw et al.* [2002] for a description of the experimental setup).

DEA that was smaller than 20% and not significant (Tscherko *et al.* [2001] at ambient CO₂ [see also Barnard *et al.*, 2004a, unpublished data, 2003]).

3.4. Interaction Between Treatments

[23] Most multiple treatment studies report no significant interaction between treatments (Table 1): Only four experiments out of 25 measured a significant interaction between

treatments. Among these four, three measured a significant interaction between CO₂ and N addition treatments.

4. Discussion

4.1. Nitrification

[24] Increased availability of NH₄⁺ should increase nitrification. Our analysis shows that N addition substantially

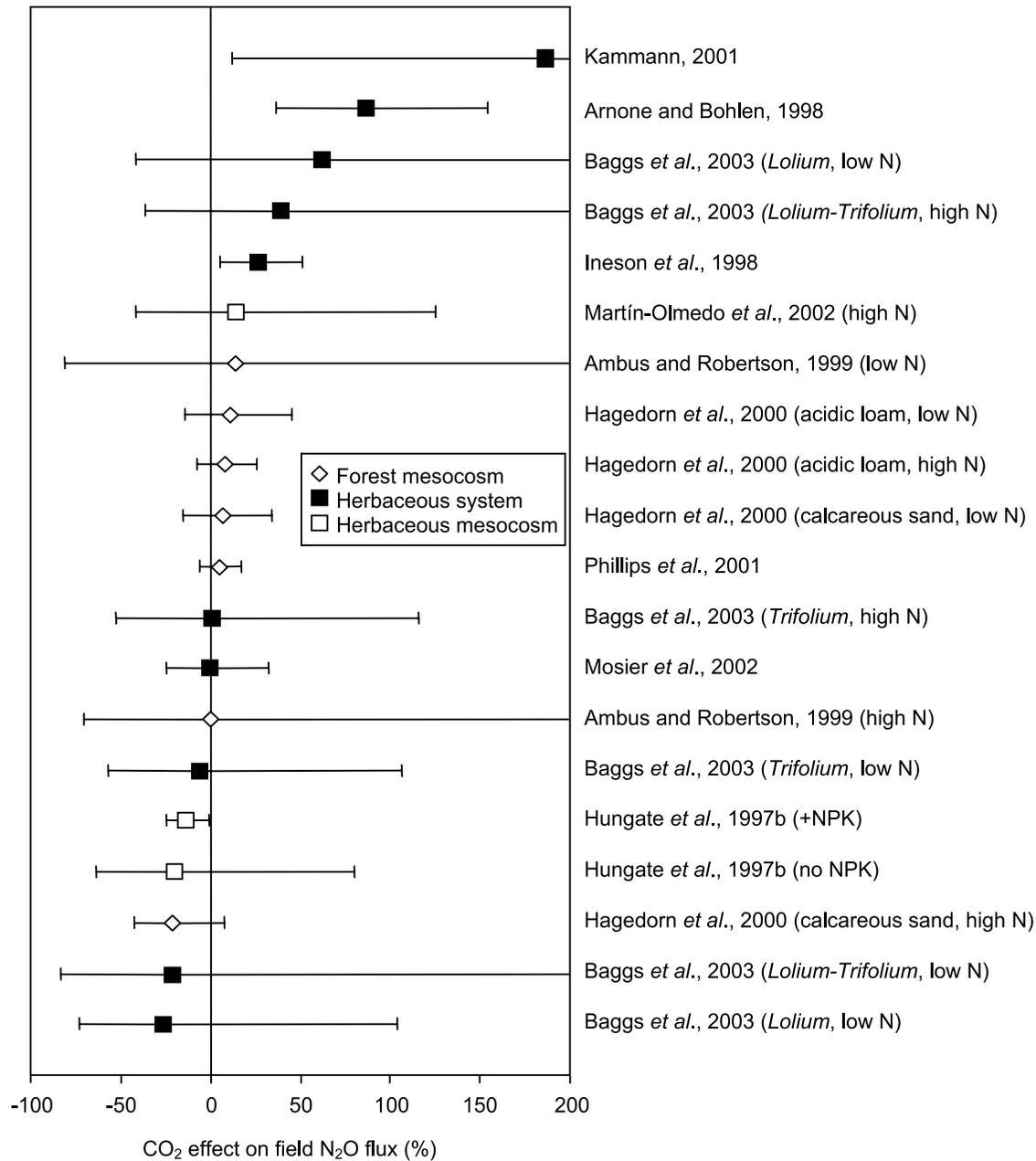


Figure 3. Effect of elevated CO_2 on field N_2O flux. Bars show 95% confidence interval of the effect size. In certain studies, elevated CO_2 was crossed with other treatments that are given here: soil N content (low N and high N, no NPK and +NPK), plant cover or type of soil. Note that the use of log transformations makes the confidence intervals asymmetrical.

increases net nitrification, gross nitrification, and NEA. Elevated CO_2 could potentially affect nitrification through modifications of NH_4^+ availability because it has been shown to modify gross mineralization in a number of studies, but the magnitude and direction of changes in mineralization vary considerably between studies [Zak *et al.*, 2000b]. Many studies have shown that CO_2 does not affect soil $[\text{NH}_4^+]$ [Arnone and Bohlen, 1998; Niklaus *et al.*, 1998a, 2001; Johnson *et al.*, 2001], although a few studies have shown reductions [Berntson and Bazzaz, 1998; Matamala and Drake, 1999], and one has shown an increase [Barnard *et al.*, 2004b]. Given that nitrification

responds positively to direct increases in N availability, it is essential that we gain better insight into the indirect effects of elevated CO_2 on NH_4^+ availability, if possible using better proxies of NH_4^+ availability than soil $[\text{NH}_4^+]$.

[25] Nitrification is inhibited at low soil $[\text{O}_2]$. Elevated CO_2 is often reported to lead to increased soil water content through reduced stomatal conductance [Knapp *et al.*, 1996; Hungate *et al.*, 1997a; Arnone and Bohlen, 1998; Niklaus *et al.*, 1998b; Hungate *et al.*, 2002]. Thus, decreased NEA at elevated CO_2 is consistent with this expected indirect effect of elevated CO_2 that would reduce soil $[\text{O}_2]$. Barnard *et al.* [2004b] have suggested that the effect of CO_2 on soil

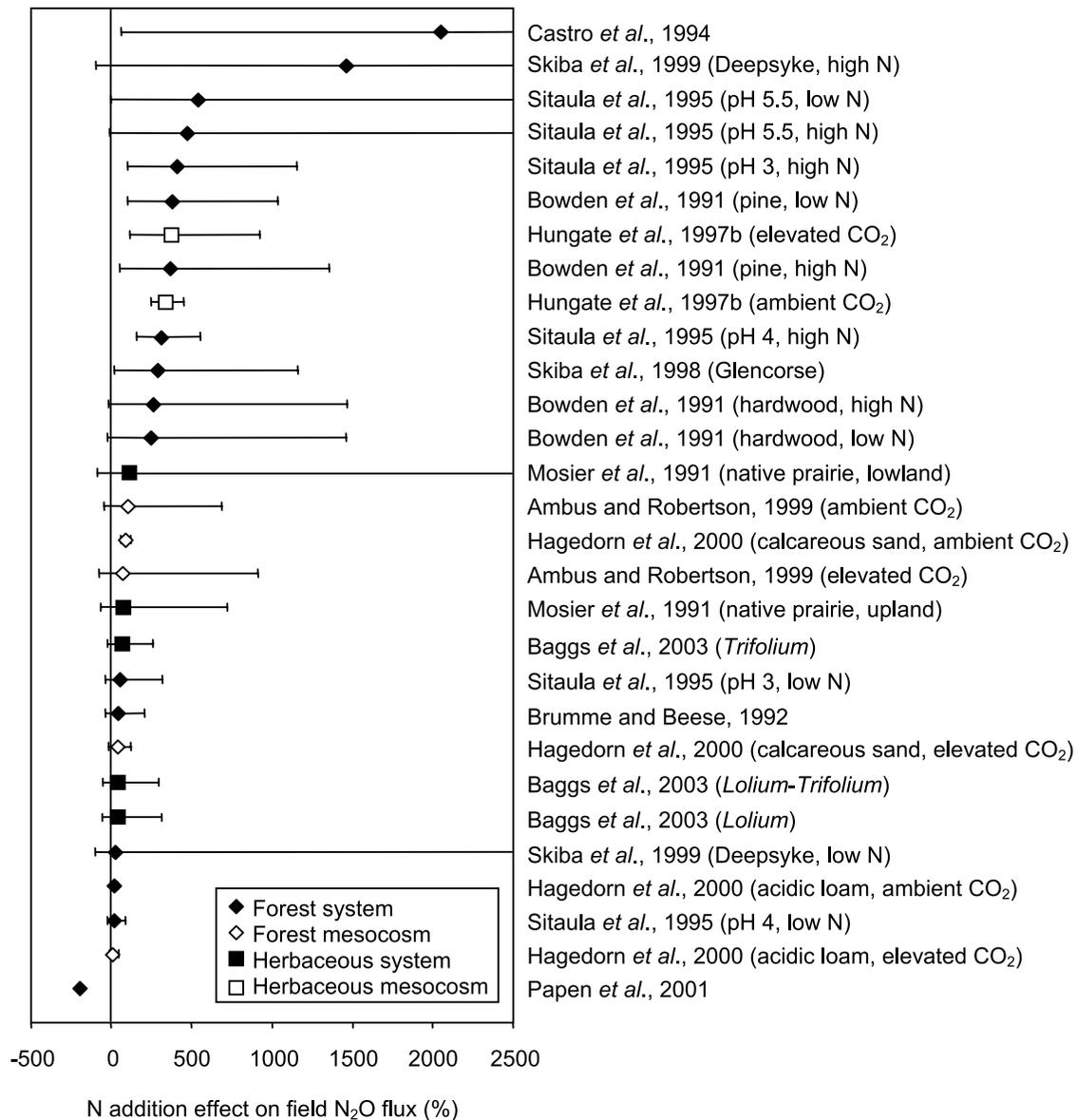


Figure 4. Effect of N addition on field N₂O fluxes. Bars show 95% confidence interval of the effect size. Confidence interval for *Papen et al.* [2001] could not be calculated because the control N₂O flux was negative. In certain studies, N addition was crossed with other treatments that are given here: CO₂ concentration (ambient CO₂ and elevated CO₂), soil N content (high N and low N), soil pH, plant species, or study site. Note that the use of log transformations makes the confidence intervals asymmetrical.

moisture alone is not sufficient to explain the response of NEA to elevated CO₂, and that the indirect effect of elevated CO₂ on soil heterotrophic activity through increased rhizodeposition should be included (see *Arnone and Bohlen* [1998] for a comparable mechanism for denitrification). The CO₂ responses of net and gross nitrification are not consistent with the NEA response or the low soil [O₂] hypothesis. There are several possible explanations for this discrepancy. First, the number of net and gross nitrification studies is small. Thus differences between NEA and net and gross nitrification could reflect qualitatively different responses among ecosystems. Second, these different measures do not provide the same information about nitrification. NEA measures the quantity of functionally active

nitrification enzyme in the soil, i.e., the nitrification potential of the soil, while net and gross nitrification are flux measurements that take into account the in situ environmental constraints on this potential. Third, net nitrification cannot be compared directly with NEA or gross nitrification, because it includes NO₃⁻ sinks (denitrification and microbial immobilization). On the basis of the NEA and gross nitrification data, we are inclined to believe that elevated CO₂ will generally have either little effect or a negative effect on gross nitrification. If so, this would be one of the possible explanations for the generally observed nonresponsiveness [*Arnone and Bohlen*, 1998] or decreases [*Niklaus et al.*, 1998a, 2001; *Johnson et al.*, 2001] in soil [NO₃⁻] at elevated CO₂ [*Niklaus et al.*, 2001].

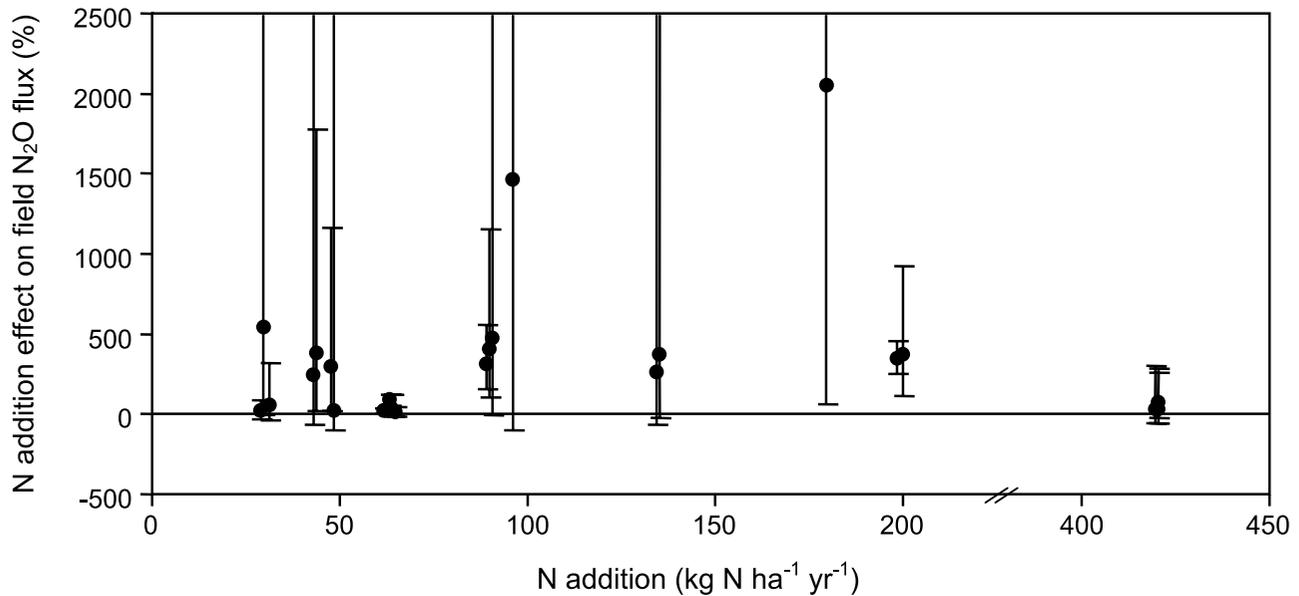


Figure 5. Relation between the amount of fertilizer N added and the difference in the amount of N-N₂O released between control and fertilized plots.

[26] Given that three out of the four studies that we reviewed for warming experiments were conducted in ecosystems from cold climates, we had expected that the direct effect of temperature would generally increase nitrification. Instead, the response of nitrification to warming was highly variable. We do not have any clear hypotheses that would explain this. The response of nitrification to

warming in the field is an area in which considerably more experimental work is required.

4.2. Denitrification

[27] Increased availability of NO₃⁻ should increase denitrification. It is therefore surprising that denitrification

Table 1. Significance of Interaction Between Treatments in Multiple-Treatment Studies^a

Measure	Treatment							Significance of Interaction	Reference
	CO ₂	N	T	Cut	pH	W	Pl.		
Field N ₂ O	X	X						ni	Hagedorn et al. [2000] (calcareous sand)
Field N ₂ O	X	X						ni	Hagedorn et al. [2000] (acidic loam)
Field N ₂ O	X	X						ns	Hungate et al. [1997b]
Field N ₂ O	X	X						ni	Ambus and Robertson [1999]
Field N ₂ O	X	X						ni	Martin-Olmedo et al. [2002]
Field N ₂ O		X			X			ns	Sitaula et al. [1995]
Field N ₂ O	X	X						ni	Baggs et al. [2003] (<i>Lolium</i>)
Field N ₂ O	X	X						ns	Baggs et al. [2003] (<i>Trifolium</i>)
Field N ₂ O	X	X						ni	Baggs et al. [2003] (<i>Lolium-Trifolium</i>)
Lab N ₂ O		X			X		X	a, Pl. × pH	Sitaula et al. [2001]
DEA	X		X					ni	Tscherko et al. [2001]
DEA	X				X			ns	Hall et al. [1998]
DEA	X	X						ni	Martin-Olmedo et al. [2002]
DEA	X	X						ni	Ambus and Robertson [1999]
DEA	X		X					ns	Barnard et al. [2004a] (Wales)
DEA	X			X				ns	Barnard et al. [2004a] (Germany)
DEA	X			X				ns	Barnard et al. [2004a] (France)
DEA	X	X	X			X		a, CO ₂ × N a, CO ₂ × T × N	R. Barnard et al. (unpublished data, 2003) (JRGCE)
NEA	X		X					ns	Barnard et al. [2004a] (Wales)
NEA	X			X				ns	Barnard et al. [2004a] (Germany)
NEA	X			X				ns	Barnard et al. [2004a] (France)
NEA	X	X	X			X		b, CO ₂ × N	R. Barnard et al. (unpublished data, 2003) (JRGCE)
Gross nitrification	X	X						a, CO ₂ × NPK	Hungate et al. [1997b]
Gross nitrification	X	X						ns	Zak et al. [2000a]
Net nitrification	X	X						ns	Zak et al. [2000a]

^aThe treatments are atmospheric CO₂ concentration (CO₂), soil N (N), temperature (T), cutting frequency (Cut), pH level (pH), water regime (W), and presence of plants (Pl). "X" indicates the presence of the corresponding treatment, and empty cells indicate that the treatment was not applied. Significance of interaction: ns, P > 0.05; a, 0.05 > P > 0.01; b, 0.01 > P > 0.001. For experiments in which no statistical information on interactions is indicated (ni), we did not see any obvious indications of strong interactions in the data. We assume that significant interactions would generally be indicated, but this may not always be the case.

showed such extremely variable responses to N addition, ranging from highly positive responses of DEA in some studies to highly negative responses of net denitrification in others. Mineral N addition in agricultural systems generally leads to increased denitrification, but can also have highly variable effects on denitrification (measured as DEA or net denitrification) ranging from small to highly positive responses [Tiedje, 1988; Stevens and Laughlin, 1997]. It has been suggested that the occasional lack of response of denitrification to fertilization occurs because labile C availability can be limiting in fertile mineral soils [Tiedje, 1988]. Negative responses to N addition are more difficult to explain, but might arise from increased competition between heterotrophic bacteria for labile C, where denitrifiers lose out to other heterotrophs.

[28] Despite the lack evidence for a direct effect of N addition on denitrification, elevated CO₂ could potentially affect denitrification through modifications of NO₃⁻ availability. Over the 23 studies that measured the effect of elevated CO₂ on DEA, 11 also measured soil [NO₃⁻]. Meta-analysis of these soil [NO₃⁻] data shows a significant decrease of soil [NO₃⁻] at elevated CO₂, with a mean effect of -35% (95% confidence interval limits are -50% and -14%). Soil [NO₃⁻] has often been shown to decrease at elevated CO₂ in other studies [Niklaus et al., 1998a, 2001; Johnson et al., 2001] but may also be unaffected [Arnone and Bohlen, 1998]. Reduced [NO₃⁻] may or may not reflect reduced NO₃⁻ availability, but reduced [NO₃⁻] has been used to explain decreased DEA at elevated CO₂ in several studies [Tschirko et al., 2001; Barnard et al., 2004a, 2004c]. We found no significant correlation between the effects of elevated CO₂ on DEA and soil [NO₃⁻] in our meta-analysis, but this may be due to the number of factors that are likely to simultaneously affect DEA or that NO₃⁻ concentrations may not reflect NO₃⁻ availability for denitrifiers. Our review does not provide strong evidence for a NO₃⁻-mediated response of denitrification to CO₂; however, we feel that there is sufficient circumstantial evidence to suggest that this could be an important mechanism and should be investigated further.

[29] Denitrification should be favored at low soil [O₂]. Thus, decreased DEA at elevated CO₂ is not consistent with the expected indirect effects of elevated CO₂ on soil [O₂] (see section 4.1). A lack of responsiveness of DEA to elevated CO₂ could be explained by the very low soil [O₂] required for the functioning of denitrifying enzymes. Some studies, but certainly not all, suggest that the effect of elevated CO₂ on soil water content is most pronounced in moderately dry soils and small in wet soils [e.g., Niklaus et al., 1998b; Barnard et al., 2004b]. Thus the effect of CO₂ on soil wetness may occur outside of the range of soil moisture where denitrification is strongly affected. Other mechanisms, such as a reduction in NO₃⁻ availability, must, however, be invoked to explain decreased DEA under elevated CO₂.

[30] Many soil incubation experiments show a positive direct effect of warming on denitrification [de Klein and van Logtestijn, 1996; Maag and Vinther, 1996; Castaldi, 2000; Dobbie and Smith, 2001]. Temperature could also indirectly affect denitrification in the soil by influencing the availability of N and C substrates and soil [O₂] [Holtan-Hartwig et

al., 2002; Loiseau and Soussana, 2000]. In particular, the indirect effect of temperature on soil [O₂] through increased soil respiration should favor denitrification [Castaldi, 2000]. Tschirko et al. [2001] suggested that increased dissolved organic C at higher temperature was a major factor explaining increased DEA, but is the only study (of six) that showed increased DEA with increased temperature. The discrepancy between the frequently observed positive response of denitrification to temperature in laboratory incubated soil (not part of this study) and the general unresponsiveness of denitrification in the field or mesocosm experiments considered in this study suggests that the mechanisms of response to temperature in the field remain poorly understood.

4.3. N₂O Flux

[31] N addition was expected to increase N₂O flux, which it did in both field and laboratory experiments. This is consistent with Bouwman et al. [2002], whose review of N₂O fluxes in agricultural systems points to a strong increase of N₂O emissions accompanying N application rates. N addition increased N₂O flux in both herbaceous and forest systems. Thus the major differences in growth forms between these two ecosystem types did not cause their responses to N addition to diverge. Nevertheless, differences between herbaceous and forest systems could possibly be hidden by the large measurement errors that were associated with field N₂O flux measurements. The positive response of N₂O flux to N addition could potentially be explained by increases in nitrifier-associated N₂O flux or denitrification since N addition increased net nitrification, gross nitrification, and DEA (but is not in agreement with two studies of net denitrification).

[32] The response of N₂O flux to N addition was highly variable, and there was no clear correlation with the amount of N added. N saturation of ecosystems may be one of the explanations for this high variability, especially in studies in northern European forests (that account for 11 out of the 31 field N₂O flux studies presented here). In such sites, N₂O fluxes may be already at near maximum rates due to N saturation of the system.

[33] For the 20 experiments that we reviewed, field N₂O fluxes were not substantially altered by elevated CO₂. However, large CO₂ effects were measured by Kammann [2001], Baggs et al. [2003], and Arnone and Bohlen [1998]. The latter concluded that the significant 87% stimulation of field N₂O fluxes was mainly attributable to an 18% increase in soil water content in their high-CO₂ plots. Elevated CO₂ often leads to increased soil water content and soil C inputs; both of these indirect effects of CO₂ should favor nitrifier-denitrification and denitrification. So why is N₂O flux generally insensitive to elevated CO₂? First, CO₂ may not affect soil water content at levels of soil moisture where these processes are sensitive to changes in soil water content. Second, DEA and [NO₃⁻] generally decline at elevated CO₂. This may mean that N₂O emissions associated with denitrifier activity are generally reduced at elevated CO₂. Third, a wide variety of positive effects (e.g., decreased soil aeration) may be counterbalanced by a wide

variety of negative effects (e.g., shifts of denitrifying flux toward N_2 at very low soil aeration).

[34] Warming has not been found to have large direct effects on field N_2O emissions [Peterjohn *et al.*, 1994; McHale *et al.*, 1998], while soil incubation experiments show a positive response of N_2O emissions to warming at low or moderate temperatures [Clayton *et al.*, 1997; Smith *et al.*, 1998; Dobbie and Smith, 2001]. Maag and Vinther [1996] have shown that the nitrification-associated N_2O fluxes decrease with temperature while denitrification-associated N_2O fluxes increase with temperature. Indeed, the contribution of nitrification and denitrification to N_2O emissions is sensitive to temperature, also depending on soil type [Gödde and Conrad, 1999]. Castaldi [2000] suggested that increased temperature would enhance microbial respiration, depleting $[O_2]$ in the soil and thereby favoring denitrification against nitrification. Temperature does not seem to have a large effect on in situ N_2O emissions, but too few data are available to draw any strong conclusions, and we stress the need for further studies on the effect of global warming on these processes.

[35] Short-term biotic and abiotic variations can modify microbial processes [Mamilov and Dilly, 2002], so what is the appropriate timescale for measuring nitrification and denitrification in order to be as integrative as possible? Some studies show the high variability of these processes in time: Climatic conditions such as rain events [Billings *et al.*, 2002; Mohn *et al.*, 2000] or freeze-thaw cycles [Müller *et al.*, 2002], seasonal variations [Castro *et al.*, 1994; Matamala and Drake, 1999; Mosier *et al.*, 2002] or interannual variations [Bowden *et al.*, 1991; Finzi *et al.*, 2001; Skiba *et al.*, 1999; Zak *et al.*, 2000a] can substantially alter nitrification and denitrification. For example, Mosier *et al.* [2002] present N_2O flux data that vary considerably during the 43 months of measurement at elevated CO_2 . Given the variability of N_2O measurements within that single experiment, the broad range of results over all the experiments presented here is not surprising. The experiments in natural systems and in tree mesocosms were longer term (2.5 to 8 years) than the herbaceous mesocosms experiments (14 days to 9 months); however, there was no experiment duration effect within each experimental system or over all experiments (data not shown). In addition to temporal variation, spatial variability of N_2O fluxes and of denitrification can also be very large [Velthof *et al.*, 1996; Clemens *et al.*, 1999]. A clearer picture will likely emerge when studies include measurements of nitrifier- and denitrifier-associated N_2O flux at appropriate spatial and temporal scales, along with the key drivers of these processes: soil water content, soil labile C, soil $[O_2]$, and NO_3^- and NH_4^+ availabilities.

4.4. Interaction Between Treatments

[36] When several treatments were applied within an individual study, the interaction between treatments in the studies presented was generally nonsignificant for the processes we have examined (Table 1). However, other processes have shown significant interactions between global change treatments. For example, Shaw *et al.* [2002] have found that grassland net primary productivity response to single global change treatments and multiple treatment

combinations (increased CO_2 , temperature, precipitation, and N addition) differs greatly. Although multitreatment experimental setups are very large and costly, long-term multifactorial in situ experiments lead to valuable insights into complex interacting mechanisms controlling nitrification and denitrification, among other biological processes.

4.5. Conclusion

[37] Nitrification, denitrification, and N_2O efflux are controlled by complex, interacting environmental and biological factors that are likely to be modified by elevated CO_2 , N addition, and warming. While the limited number of elevated temperature experiments presented here stresses the need for more warming studies in the field, some patterns emerge from elevated CO_2 and N addition studies. Elevated CO_2 generally has little effect or a negative effect on nitrification, while N addition increases nitrification. Elevated CO_2 may generally decrease denitrification, possibly through decreased soil NO_3^- availability, while the response of denitrification to N addition is highly variable. There is often little response of N_2O fluxes to elevated CO_2 in the field, which might be explained by the balance between positive and negative effects of elevated CO_2 on the environmental and biological processes governing N_2O emissions. The stimulation of field N_2O emissions by N substrate additions is clearly shown, even though the range of response is wide and shows no correlation with the amount of N added. It is becoming clearer that to gain better insight over the complexity of environmental controls on nitrification and denitrification, it is necessary to monitor these processes using a variety of methods, along with their key drivers.

[38] **Acknowledgments.** We wish to thank Karen Dobbie (University of Edinburgh), Per Gundersen (Danish Forest and Landscape Research Institute), Frank Hagedorn (Swiss Federal Institute of Forest, Snow and Landscape Research), Claudia Kammann (University of Giessen), Arvin Mosier (USDA/ARS), Asko Simojoki (University of Helsinki), and Ute Skiba and Lucy Sheppard (CEH Edinburgh), who kindly provided some unpublished data. We would like to thank Jean-Paul Briane for his help. We thank Laure Barthes, Jean-Christophe Lata, Xavier Le Roux, and Xavier Raynaud for numerous insightful discussions. This work was funded by CNRS support of the Laboratoire d'Ecologie, Systématique et Evolution (UMR CNRS 8079) and by National Science Foundation (NSF DEB 0092642). R. B. was supported in part by the Schneider-Forest Grant of the Chancellerie des Universités de Paris.

References

- Abbasi, M. K., and W. A. Adams (2000), Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions, *Soil Biol. Biochem.*, **32**, 1251–1259.
- Ambus, P., and G. P. Robertson (1999), Fluxes of CH_4 and N_2O in aspen stands grown under ambient and twice-ambient CO_2 , *Plant Soil*, **209**, 1–8.
- Arnone, J. A., and P. J. Bohlen (1998), Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO_2 , *Oecologia*, **116**, 331–335.
- Asner, G. P., A. R. Townsend, W. J. Riley, P. A. Matson, J. C. Neff, and C. C. Cleveland (2001), Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen deposition, *Biogeochemistry*, **54**, 1–39.
- Avrahami, S., W. Liesack, and R. Conrad (2003), Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers, *Environ. Microbiol.*, **5**, 691–705.
- Baggs, E. M., M. Richter, U. A. Hartwig, and G. Cadisch (2003), Nitrous oxide emissions from grass swards during the eighth year of

- elevated atmospheric pCO₂ (Swiss FACE), *Global Change Biol.*, *9*, 1214–1222.
- Barnard, R., L. Barthes, X. Le Roux, H. Harmens, A. Raschi, J. F. Soussana, B. Winkler, and P. W. Leadley (2004a), Atmospheric CO₂ elevation has little effect on nitrifying and denitrifying enzyme activity in four European grasslands, *Global Change Biol.*, *10*, 488–497.
- Barnard, R., L. Barthes, X. Le Roux, and P. W. Leadley (2004b), Dynamics of nitrifying activities, denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO₂, *New Phytol.*, *162*, 365–376.
- Barnard, R., P. W. Leadley, R. Lensi, and L. Barthes (2005), Plant, soil microbial and soil inorganic nitrogen responses to elevated CO₂: A study in microcosms of *Holcus Lanatus*, *Acta Oecol.*, in press.
- Bengtsson, G., and C. Bergwall (2000), Fate of ¹⁵N labelled nitrate and ammonium in a fertilized forest soil, *Soil Biol. Biochem.*, *32*, 545–557.
- Berntson, G. M., and F. A. Bazzaz (1998), Regenerating temperate forest microcosms in elevated CO₂: Belowground growth and nitrogen cycling, *Oecologia*, *113*, 115–125.
- Billings, S. A., S. M. Schaeffer, and R. D. Evans (2002), Trace N gas losses and N mineralization in Mojave desert soils exposed to elevated CO₂, *Soil Biol. Biochem.*, *34*, 1777–1784.
- Bollmann, A., and R. Conrad (1998), Influence of O₂ availability on NO and N₂O release by nitrification and denitrification in soils, *Global Change Biol.*, *4*, 387–396.
- Bouwman, A. F., L. J. M. Boumans, and N. H. Batjes (2002), Emissions of N₂O and NO from fertilized fields: Summary of available measurements data, *Global Biogeochem. Cycles*, *16*(4), 1058, doi:10.1029/2001GB001811.
- Bowden, R. D., J. M. Melillo, P. A. Steudler, and J. D. Aber (1991), Effects of nitrogen additions on annual nitrous oxide fluxes from temperate forest soils in the northeastern United States, *J. Geophys. Res.*, *96*, 9321–9328.
- Brumme, R., and F. Beese (1992), Effects of liming and nitrogen fertilization on emissions of CO₂ and N₂O from a temperate forest, *J. Geophys. Res.*, *97*, 12,851–12,858.
- Camol, M., L. Hogenboom, M. E. Jach, J. Remacle, and R. Ceulemans (2002), Elevated atmospheric CO₂ in open top chambers increases net nitrification and potential denitrification, *Global Change Biol.*, *8*, 590–598.
- Castaldi, S. (2000), Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment, *Biol. Fertil. Soils*, *32*, 67–72.
- Castro, M. S., W. T. Peterjohn, J. M. Melillo, P. A. Steudler, H. L. Gholz, and D. H. Lewis (1994), Effects of nitrogen fertilization on the fluxes of N₂O, CH₄, and CO₂ from soils in a Florida slash pine plantation, *Can. J. For. Res.*, *24*, 9–13.
- Clayton, H., I. P. McTaggart, J. Parker, L. Swan, and K. A. Smith (1997), Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertilizer form and environmental conditions, *Biol. Fertil. Soils*, *25*, 252–260.
- Clemens, J., M. P. Schillinger, H. Goldbach, and B. Huwe (1999), Spatial variability of N₂O emissions and soil parameters of an arable silt loam—A field study, *Biol. Fertil. Soils*, *28*, 403–406.
- Curtis, P. S., and X. Z. Wang (1998), A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology, *Oecologia*, *113*, 299–313.
- de Klein, C. A. M., and R. S. P. van Logtestijn (1996), Denitrification in grassland soils in the Netherlands in relation to irrigation, N-application rate, soil water content and soil temperature, *Soil Biol. Biochem.*, *28*, 231–237.
- Dobbie, K. E., and K. A. Smith (2001), The effects of temperature, water-filled pore space and land use on N₂O emissions from an imperfectly drained gleysol, *Eur. J. Soil Sci.*, *52*, 667–673.
- Finzi, A. C., A. S. Allen, E. H. DeLucia, D. S. Ellsworth, and W. H. Schlesinger (2001), Forest litter production, chemistry, and decomposition following two years of free-air CO₂ enrichment, *Ecology*, *82*, 470–484.
- Fourgeaud, C., and A. Fuchs (1967), *Statistique*, Dunod, Paris.
- Gödde, M., and R. Conrad (1999), Immediate and adaptational temperature effects on nitric oxide production and nitrous oxide release from nitrification and denitrification in two soils, *Biol. Fertil. Soils*, *30*, 33–40.
- Groffman, P. M., and J. M. Tiedje (1989), Denitrification in north temperate forest soils: Relationships between denitrification and environmental factors at the landscape scale, *Soil Biol. Biochem.*, *21*, 621–626.
- Grundmann, G. L., P. Renault, L. Rosso, and R. Bardin (1995), Differential effects of soil water content and temperature on nitrification and aeration, *Soil. Sci. Soc. Am. J.*, *59*, 1342–1349.
- Hagedorn, F., J. B. Bucher, D. Tarjan, P. Rusert, and I. Bucher-Wallin (2000), Responses of N fluxes and pools to elevated atmospheric CO₂ in model forest ecosystems with acidic and calcareous soils, *Plant Soil*, *224*, 273–286.
- Hall, J. M., E. Paterson, and K. Killham (1998), The effect of elevated CO₂ concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential, *Global Change Biol.*, *4*, 209–216.
- Hart, S. C., and D. A. Perry (1999), Transferring soils from high- to low-elevation forests increases nitrogen cycling rates: Climate change implications, *Global Change Biol.*, *5*, 23–32.
- Hedges, L. V., J. Gurevitch, and P. S. Curtis (1999), The meta-analysis of response ratios in experimental ecology, *Ecology*, *80*, 1150–1156.
- Holtan-Hartwig, L., P. Dörsch, and L. R. Bakken (2002), Low temperature control of soil denitrifying communities: Kinetics of N₂O production and reduction, *Soil Biol. Biochem.*, *34*, 1797–1806.
- Hungate, B. A., F. S. Chapin, H. Zhong, E. A. Holland, and C. B. Field (1997a), Stimulation of grassland nitrogen cycling under carbon dioxide enrichment, *Oecologia*, *109*, 149–153.
- Hungate, B. A., C. P. Lund, H. L. Pearson, and F. S. Chapin (1997b), Elevated CO₂ and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland, *Biogeochemistry*, *37*, 89–109.
- Hungate, B. A., M. Reichstein, P. Dijkstra, D. Johnson, G. Hymus, J. D. Tenhunen, C. R. Hinkle, and B. G. Drake (2002), Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment, *Global Change Biol.*, *8*, 289–298.
- Ineson, P., P. A. Coward, and U. A. Hartwig (1998), Soil gas fluxes of N₂O, CH₄ and CO₂ beneath *Lolium perenne* under elevated CO₂: The Swiss free air carbon dioxide enrichment experimentation, *Plant Soil*, *198*, 89–95.
- Intergovernmental Panel on Climate Change (2001), *Climate Change 2001: The Scientific Basis—Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, edited by J. T. Houghton et al., 881 pp., Cambridge Univ. Press, New York, NY.
- Johnson, D. W., B. A. Hungate, P. Dijkstra, G. Hymus, and B. G. Drake (2001), Effects of elevated carbon dioxide on soils in a Florida scrub oak system, *J. Environ. Qual.*, *30*, 501–507.
- Kammann, C. (2001), Die Auswirkung steigender atmosphärischer CO₂-Konzentration auf die Flüsse der Klimaspurengase N₂O und CH₄ in einem Grünland-Ökosystem, Ph.D. thesis, Justus-Liebig-Universität Giessen, Giessen, Germany.
- Kester, R. A., M. E. Meijer, J. A. Libochant, W. de Boer, and H. J. Laanbroek (1997), Contribution of nitrification and denitrification to the NO and N₂O emissions of an acid forest soil, a river sediment and a fertilized grassland soil, *Soil Biol. Biochem.*, *29*, 1655–1664.
- Kjønaas, O. J., A. O. Stuanes, and M. Huse (1998), Effects of weekly nitrogen additions on N cycling in a coniferous forest catchment, Gårdsjön, Sweden, *For. Ecol. Manage.*, *101*, 227–249.
- Knapp, A. K., E. P. Hamerlynck, J. M. Ham, and C. E. Owensby (1996), Responses in stomatal conductance to elevated carbon dioxide in 12 grassland species that differ in growth form, *Vegetatio*, *125*, 31–41.
- Lensi, R., S. Mazurier, F. Gourbière, and A. Josserand (1986), Rapid determination of the nitrification potential of an acid forest soil and assessment of its variability, *Soil Biol. Biochem.*, *18*, 239–240.
- Linn, D. M., and J. W. Doran (1984), Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils, *Soil. Sci. Soc. Am. J.*, *48*, 1267–1272.
- Loiseau, P., and J.-F. Soussana (1999), Elevated [CO₂], temperature increase and N supply effects on the accumulation of below-ground carbon in a temperate grassland ecosystem, *Plant Soil*, *212*, 123–134.
- Loiseau, P., and J. F. Soussana (2000), Effects of elevated CO₂, temperature and N fertilization on nitrogen fluxes in a temperate grassland ecosystem, *Global Change Biol.*, *6*, 953–965.
- Lovett, G. M., and H. Rueth (1999), Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient, *Ecol. Appl.*, *9*, 1330–1344.
- Maag, M., and F. P. Vinther (1996), Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures, *Appl. Soil Ecol.*, *4*, 5–14.
- MacDonald, J. A., U. Skiba, L. J. Sheppard, B. Ball, J. D. Roberts, K. A. Smith, and D. Fowler (1997), The effect of nitrogen deposition and seasonal variability on methane oxidation and nitrous oxide emission rates in an upland spruce plantation and moorland, *Atmos. Environ.*, *31*, 3693–3706.
- Mamilov, A. S., and O. A. Dilly (2002), Soil microbial eco-physiology as affected by short-term variations in environmental conditions, *Soil Biol. Biochem.*, *34*, 1283–1290.

- Martin-Olmedo, P., R. M. Rees, and J. Grace (2002), The influence of plants grown under elevated CO₂ and N fertilization on soil nitrogen dynamics, *Global Change Biol.*, *8*, 643–657.
- Matamala, R., and B. G. Drake (1999), The influence of atmospheric CO₂ enrichment on plant-soil nitrogen interactions in a wetland plant community on the Chesapeake Bay, *Plant Soil*, *210*, 93–101.
- Matson, P., K. A. Lohse, and S. J. Hall (2002), The globalization of nitrogen deposition: Consequences for terrestrial ecosystems, *Ambio*, *31*, 113–119.
- McHale, P. J., M. J. Mitchell, and F. P. Bowles (1998), Soil warming in a northern hardwood forest: Trace gas fluxes and leaf litter decomposition, *Can. J. For. Res.*, *28*, 1365–1372.
- Merrill, A. G., and D. K. Zak (1992), Factors controlling denitrification rates in upland and swamp forests, *Can. J. For. Res.*, *22*, 1597–1604.
- Mikan, C. J., D. R. Zak, M. E. Kubiske, and K. S. Pregitzer (2000), Combined effects of atmospheric CO₂ and N availability on the below-ground carbon and nitrogen dynamics of aspen mesocosms, *Oecologia*, *124*, 432–445.
- Mohn, J., A. Schürmann, F. Hagedorn, P. Schleppi, and R. Bachofen (2000), Increased rates of denitrification in nitrogen-treated forest soils, *For. Ecol. Manage.*, *137*, 113–119.
- Mosier, A., D. Schimel, D. Valentine, K. Bronson, and W. Parton (1991), Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands, *Nature*, *350*, 330–332.
- Mosier, A. R., J. A. Morgan, J. Y. King, D. LeCain, and D. G. Milchunas (2002), Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado shortgrass steppe under elevated CO₂, *Plant Soil*, *240*, 201–211.
- Müller, C., M. Martin, R. J. Stevens, R. J. Laughlin, C. Kammann, J. C. G. Ottow, and H.-J. Jäger (2002), Processes leading to N₂O emissions in grassland soil during freezing and thawing, *Soil Biol. Biochem.*, *34*, 1325–1331.
- Niklaus, P. A., P. W. Leadley, J. Stöcklin, and C. Körner (1998a), Nutrient relations in calcareous grassland under elevated CO₂, *Oecologia*, *116*, 67–75.
- Niklaus, P. A., D. Spinnler, and C. Körner (1998b), Soil moisture dynamics of calcareous grassland under elevated CO₂, *Oecologia*, *117*, 201–208.
- Niklaus, P. A., E. Kandeler, P. W. Leadley, B. Schmid, D. Tschirko, and C. Körner (2001), A link between plant diversity, elevated CO₂ and soil nitrate, *Oecologia*, *127*, 540–548.
- Ollinger, S. V., J. D. Aber, P. B. Reich, and R. J. Freuder (2002), Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO₂ and land use history on the carbon dynamics of northern hardwood forests, *Global Change Biol.*, *8*, 545–562.
- Oren, R., et al. (2001), Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere, *Nature*, *411*, 469–472.
- Panikov, N. S. (1999), Understanding and prediction of soil microbial community dynamics under global change, *Appl. Soil Ecol.*, *11*, 161–176.
- Papen, H., M. Daum, R. Steinkamp, and K. Butterbach-Bahl (2001), N₂O- and CH₄-fluxes from soils of a N-limited and N-fertilized spruce forest ecosystem of the temperate zone, *J. Appl. Bot.*, *75*, 159–163.
- Parton, W. J., E. A. Holland, S. J. Del Grosso, M. D. Hartman, R. E. Martin, A. R. Mosier, D. S. Ojima, and D. S. Schimel (2001), Generalized model for NO_x and N₂O emissions from soils, *J. Geophys. Res.*, *106*, 17,403–17,419.
- Paul, E. A., and F. E. Clark (Eds.) (1989), *Soil Microbiology and Biochemistry*, Academic, San Diego, Calif.
- Peterjohn, W. T., J. M. Melillo, P. A. Steudler, K. A. Newkirk, F. P. Bowles, and J. D. Aber (1994), Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures, *Ecol. Appl.*, *4*, 617–625.
- Phillips, R. L., S. C. Whalen, and W. H. Schlesinger (2001), Influence of atmospheric CO₂ enrichment on nitrous oxide flux in a temperate forest ecosystem, *Global Biogeochem. Cycles*, *15*, 741–752.
- Phoenix, G. K., R. E. Booth, J. R. Leake, D. J. Read, J. P. Grime, and J. A. Lee (2003), Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands, *Global Change Biol.*, *9*, 1309–1321.
- Prosser, J. I. (1989), Autotrophic nitrification in bacteria, *Adv. Microb. Physiol.*, *30*, 125–181.
- Rustad, L. E., B. D. Campbell, G. M. Marion, R. J. Norby, M. J. Mitchell, A. E. Hartley, J. H. C. Cornelissen, J. Gurevitch, and GCTE-NEWS (2001), A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming, *Oecologia*, *126*, 543–562.
- Schmidt, H.-L., R. A. Werner, N. Yoshida, and R. Well (2004), Is the isotopic composition of nitrous oxide an indicator for its origin from nitrification or denitrification? A theoretical approach from referred data and microbiological and enzyme kinetic aspects, *Rapid Commun. Mass Spectrom.*, *18*, 2036–2040.
- Shaw, M. R., and J. Harte (2001), Response of nitrogen cycling to simulated climate change: Differential responses along a subalpine ecotone, *Global Change Biol.*, *7*, 193–210.
- Shaw, M. R., E. S. Zavaleta, N. R. Chiariello, E. E. Cleland, H. A. Mooney, and C. B. Field (2002), Grassland responses to global environmental changes suppressed by elevated CO₂, *Science*, *298*, 1987–1990.
- Simek, M., and J. E. Cooper (2002), The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years, *Eur. J. Soil Sci.*, *53*, 345–354.
- Simek, M., L. Jisova, and D. W. Hopkins (2002), What is the so-called optimum pH for denitrification in soil?, *Soil Biol. Biochem.*, *34*, 1227–1234.
- Sitaula, B. K., L. R. Bakken, and G. Abrahamsen (1995), N-fertilisation and soil acidification effects on N₂O and CO₂ emission from temperate pine forest soil, *Soil Biol. Biochem.*, *27*, 1401–1408.
- Sitaula, B. K., J. I. B. Sitaula, A. Aakra, and L. R. Bakken (2001), Nitrification and methane oxidation in forest soil: Acid deposition, nitrogen input and plant effects, *Water Air Soil Pollut.*, *130*, 1061–1066.
- Skiba, U., L. Sheppard, C. E. R. Pitcairn, I. Leith, A. Crossley, S. van Dijk, V. H. Kennedy, and D. Fowler (1998), Soil nitrous oxide and nitric oxide emissions as indicators of elevated atmospheric N deposition rates in seminatural ecosystems, *Environ. Pollut.*, *102*, 457–461.
- Skiba, U., L. J. Sheppard, C. E. R. Pitcairn, S. van Dijk, and M. P. Rossall (1999), The effect of N deposition on nitrous oxide and nitric oxide emissions from temperate forest soils, *Water Air Soil Pollut.*, *130*, 619–624.
- Smith, K. A. (1997), The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils, *Global Change Biol.*, *3*, 327–338.
- Smith, K. A., P. E. Thomson, H. Clayton, I. P. McTaggart, and F. Conen (1998), Effects of temperature, water content and nitrogen fertilization on emissions of nitrous oxide by soils, *Atmos. Environ.*, *32*, 3301–3309.
- Smith, M. S., and J. M. Tiedje (1979), Phases of denitrification following oxygen depletion in soil, *Soil Biol. Biochem.*, *11*, 261–267.
- Stevens, R. J., and R. J. Laughlin (1997), The impact of cattle slurries and their management on ammonia and nitrous oxide emissions from grassland, in *Gaseous Nitrogen Emissions From Grassland*, edited by S. C. Jarvis and B. F. Pain, pp. 233–256, CAB Int., Oxford, UK.
- Strong, D. T., and I. R. P. Fillery (2002), Denitrification response to nitrate concentrations in sandy soils, *Soil Biol. Biochem.*, *34*, 945–954.
- Tiedje, J. M. (1988), Ecology of denitrification and dissimilatory nitrate reduction to ammonium, in *Biology of Anaerobic Microorganisms*, edited by A. J. B. Zehnder, pp. 179–244, John Wiley, Hoboken, N. J.
- Tiedje, J. M. (1994), Denitrifiers, in *Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties*, edited by R. W. Weaver et al., pp. 985–1018, Soil Sci. Soc. of Am., Madison, Wis.
- Tschirko, D., E. Kandeler, and T. H. Jones (2001), Effect of temperature on below-ground N-dynamics in a weedy model ecosystem at ambient and elevated atmospheric CO₂ levels, *Soil Biol. Biochem.*, *33*, 491–501.
- Velthof, G. L., S. C. Jarvis, A. Stein, A. G. Allen, and O. Oenema (1996), Spatial variability of nitrous oxide fluxes in mown and grazed grasslands on a poorly drained clay soil, *Soil Biol. Biochem.*, *28*, 1215–1225.
- Verburg, P. S. J., W. K. P. Van Loon, and A. Lükewille (1999), The CLIMEX soil heating experiment: Soil response after 2 years of treatment, *Biol. Fertil. Soils*, *28*, 271–276.
- Ward, S. J. E., G. F. Midgley, M. H. Jones, and P. S. Curtis (1999), Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO₂ concentrations: A meta-analytic test of current theories and perceptions, *Global Change Biol.*, *5*, 723–741.
- Watson, C. J., C. Jordan, and M. D. B. Allen (1994), Relationships between denitrifying enzyme activity and soil properties, *Proc. R. Irish Acad. Sect. B*, *94*, 237–244.
- Webster, E. A., and D. W. Hopkins (1996), Contributions from different microbial processes to N₂O emissions from soil under different moisture regimes, *Biol. Fertil. Soils*, *22*, 331–335.
- Weier, K. L., J. W. Doran, J. F. Power, and D. T. Walters (1993), Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate, *Soil. Sci. Soc. Am. J.*, *57*, 66–72.
- Wolf, I., and R. Brumme (2002), Contribution of nitrification and denitrification sources for seasonal N₂O emissions in an acid German forest soil, *Soil Biol. Biochem.*, *34*, 741–744.

- Wolf, I., and R. Russow (2000), Different pathways of formation of N_2O , N_2 and NO in black earth soil, *Soil Biol. Biochem.*, *32*, 229–239.
- Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema (2001), Role of nitrifier denitrification in the production of nitrous oxide, *Soil Biol. Biochem.*, *33*, 1723–1732.
- Wrage, K., G. L. Velthof, H. J. Laanbroek, and O. Oenema (2004), Nitrous oxide production in grassland soils: Assessing the contribution of nitrifier denitrification, *Soil Biol. Biochem.*, *36*, 229–236.
- Yoshida, N., and S. Toyoda (2000), Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers, *Nature*, *405*, 330–334.
- Zak, D. R., K. S. Pregitzer, P. S. Curtis, and W. E. Holmes (2000a), Atmospheric CO_2 and the composition and function of soil microbial communities, *Ecol. Appl.*, *10*, 47–59.
- Zak, D. R., K. S. Pregitzer, J. S. King, and W. E. Holmes (2000b), Elevated atmospheric CO_2 , fine roots and the response of soil microorganisms: A review and hypothesis, *New Phytol.*, *147*, 201–222.

R. Barnard, Institute of Plant Sciences, ETH Zürich, Universitätstrasse 2, CH-8092 Zürich, Switzerland. (romain.barnard@ipw.agrl.ethz.ch)

B. A. Hungate, Department of Biological Sciences/Merriam-Powell Center for Environmental Research, Box 5640, Northern Arizona University, Flagstaff, AZ 86011–5640, USA.

P. W. Leadley, Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud XI, UMR CNRS 8079, F-91405 Orsay, France.