Global change, nitrification, and denitrification: A review

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[1]   We reviewed responses of nitrification, denitrification, and soil N_2O efflux to elevated CO_2, N availability, and temperature, based on published experimental results. We used meta-analysis to estimate the magnitude of response of soil N_2O emissions, nitrifying enzyme activity (NEA), denitrifying enzyme activity (DEA), and net and gross nitrification across experiments. We found no significant overall effect of elevated CO_2 on N_2O fluxes. DEA and NEA significantly decreased at elevated CO_2; however, gross nitrification was not modified by elevated CO_2, and net nitrification increased. The negative overall response of DEA to elevated CO_2 was associated with decreased soil $[NO_3^-]$, suggesting that reduced availability of electron acceptors may dominate the responses of denitrification to elevated CO_2. N addition significantly increased field and laboratory N_2O emissions, together with gross and net nitrification, but the effect of N addition on field N_2O 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 CO_2 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 CO_2.

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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 [*Wand 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 N_2O, 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 CO_2, 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 CO_2 concentrations.

[4] Nitrification and denitrification are potentially affected by CO_2, 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 O_2 concentrations play a key role in controlling these processes. Increased CO_2 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 O_2 concentrations.

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[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 ~20°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 μmol 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 kg N ha^{-1} 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 ($\overline{X}_c$ and $\overline{X}_t$, respectively), standard deviations (S_c and S_t), and sample sizes (n_c and n_t), we used the response ratio r = $\overline{X}_t/\overline{X}_c$ as a metric. Following *Curtis and Wang* [1998], the log-transformation of r is lr = ln(r), approximately normally distributed if $\overline{X}_c$ and $\overline{X}_t$ are normally distributed and $\overline{X}_c$ is unlikely to be negative. The mean of lr is approximately the true response ratio, and its variance v is equal to

$$v = \frac{S_t^2}{n_t \overline{X}_t^2} + \frac{S_c^2}{n_c \overline{X}_c^2}. \tag{1}$$

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

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

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) × 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 a the sum of within-groups sum of squares of means (ISS) and between-groups sum of squares of means (WSS),

$$\text{TSS} = \text{ISS} + \text{WSS} = \sum_i \sum_j \left(X_{ij} - \overline{X_{..}}\right)^2 = \sum_i \sum_j \left(X_{ij} - \overline{X_{i.}}\right)^2 + \sum_i n_i \left(\overline{X_{i.}} - \overline{X_{..}}\right)^2, \quad (3)$$

where X_{ij} is the measure i of group j, and $\overline{X_{..}}$ 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

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

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

$$\sigma^2 = \frac{\sum_i (n_i - 1)\sigma^2 + \sum_i n_i \left(\overline{X_{i.}} - \overline{X_{..}}\right)^2}{N-1}. \quad (6)$$

Following equation (5), we then obtain

$$SE^2 = \frac{\sum_i (n_i - 1)n_i SE^2 + \sum_i n_i \left(\overline{X_{i.}} - \overline{X_{..}}\right)^2}{N-1}. \quad (7)$$

The pooled standard error is then

$$SE = \sqrt{\frac{\sum_i n_i (n_i - 1)SE_i^2 + \sum_i n_i \left(\overline{X_{i.}} - \overline{X_{..}}\right)^2}{N(n_i - 1)}}. \quad (8)$$

[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 [*Kjønaas 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. CO₂

[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%
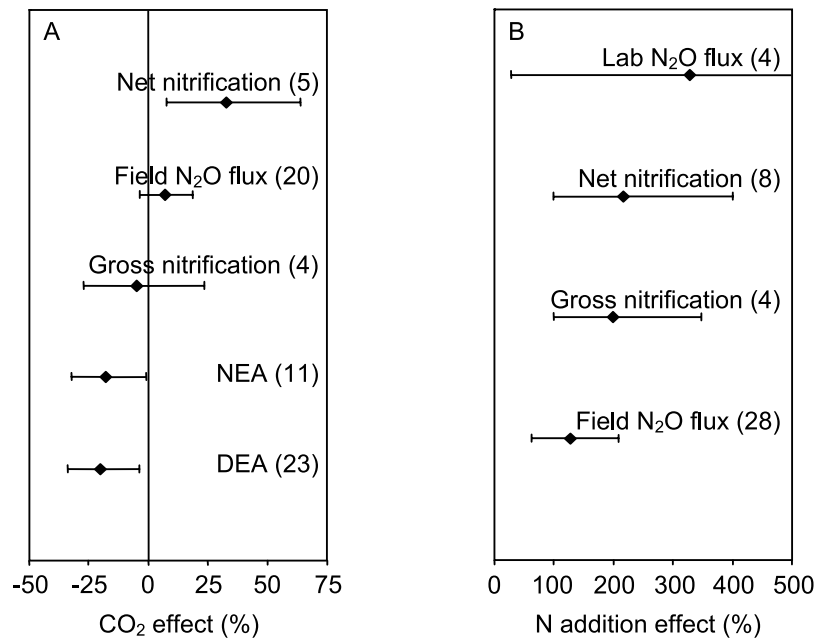
Figure 1. Meta-analysis of the effect of (a) elevated CO_2 and (b) N addition on several nitrification and denitrification measurements. Bars show 95% confidence interval of the overall mean effect size, based on the number of experiments indicated in parentheses.

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 CO_2 (low nutrient treatment [*Hungate et al.*, 1997b]) [*Zak et al.*, 2000a].

[17] Over all ecosystems (k = 23), elevated CO_2 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 CO_2 on DEA was not significantly correlated with the effect of elevated CO_2 on soil [NH_4^+], soil [NO_3^-], soil microbial N (11 experiments), or soil water content (eight experiments). Elevated CO_2 did not significantly alter N_2O 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 CO_2, added N substantially increased all nitrification variables measured (Figure 1b). Net nitrification (forest systems only) [*Kjønaas 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 N_2O 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 N_2O 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-N_2O 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 N_2O 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% (*Tscherko et al.* [2001] at elevated temperature and elevated CO_2). The other studies measured a mean effect of elevated temperature on
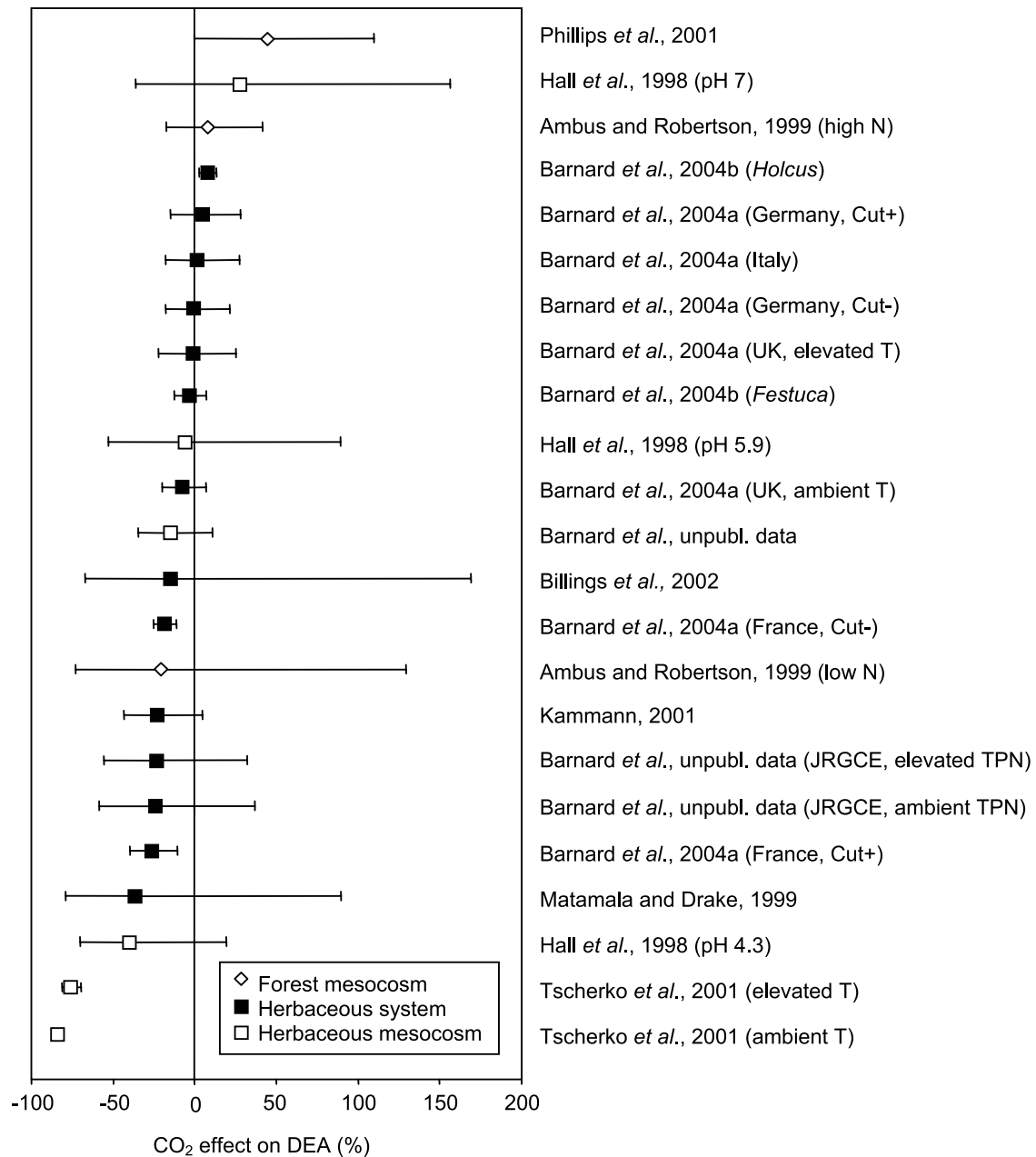
Figure 2. Effect of elevated CO_2 on denitrifying enzyme activity (DEA). Bars show 95% confidence interval of the effect size. In certain studies, elevated CO_2 was crossed with other treatments that are given here: high or low cutting frequency (Cut+ and Cut−), soil N content (high N and low N), temperature 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 CO_2 [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 CO_2 and N addition treatments.

4. Discussion

4.1. Nitrification

[24] Increased availability of NH_4^+ should increase nitrification. Our analysis shows that N addition substantially
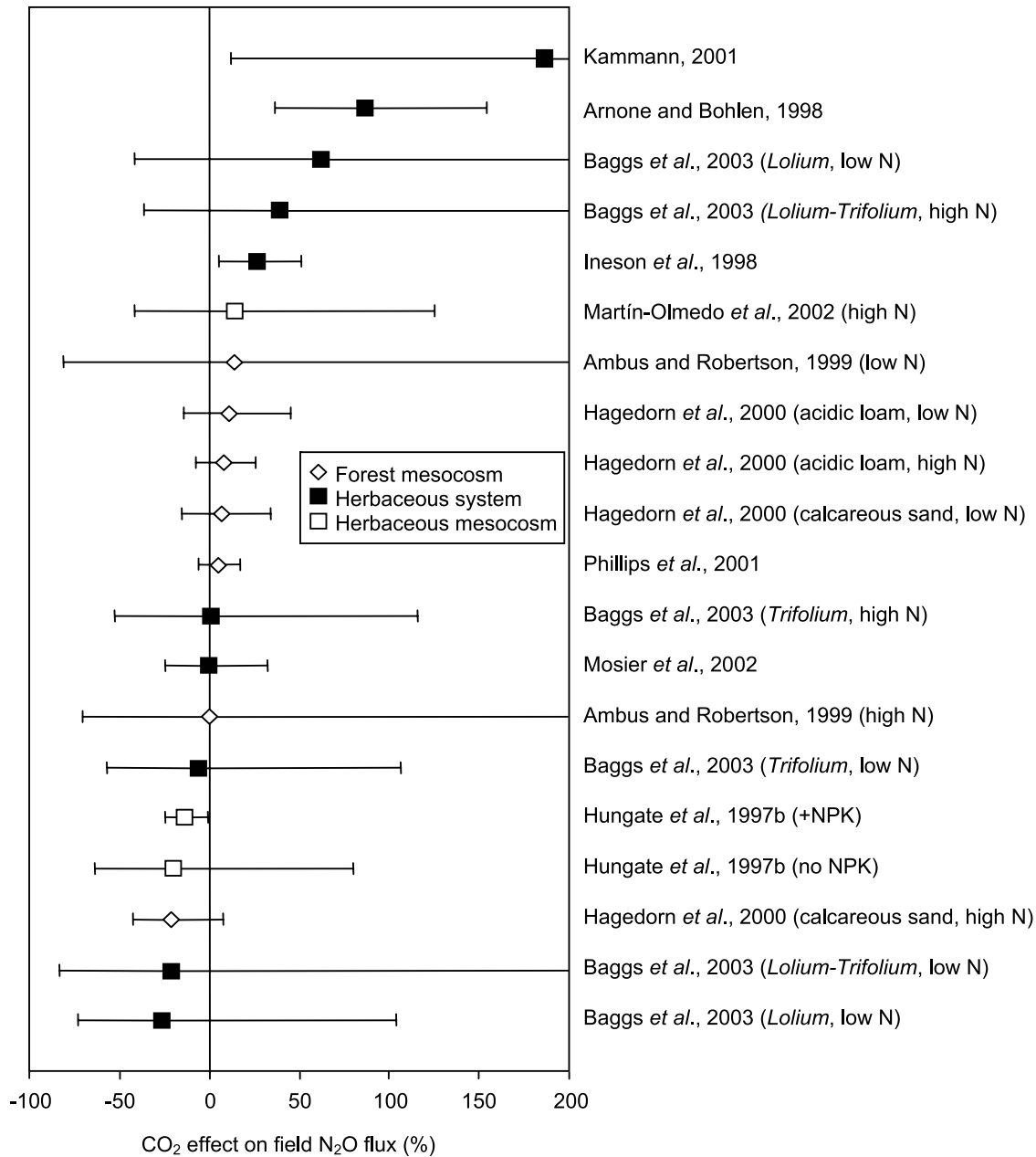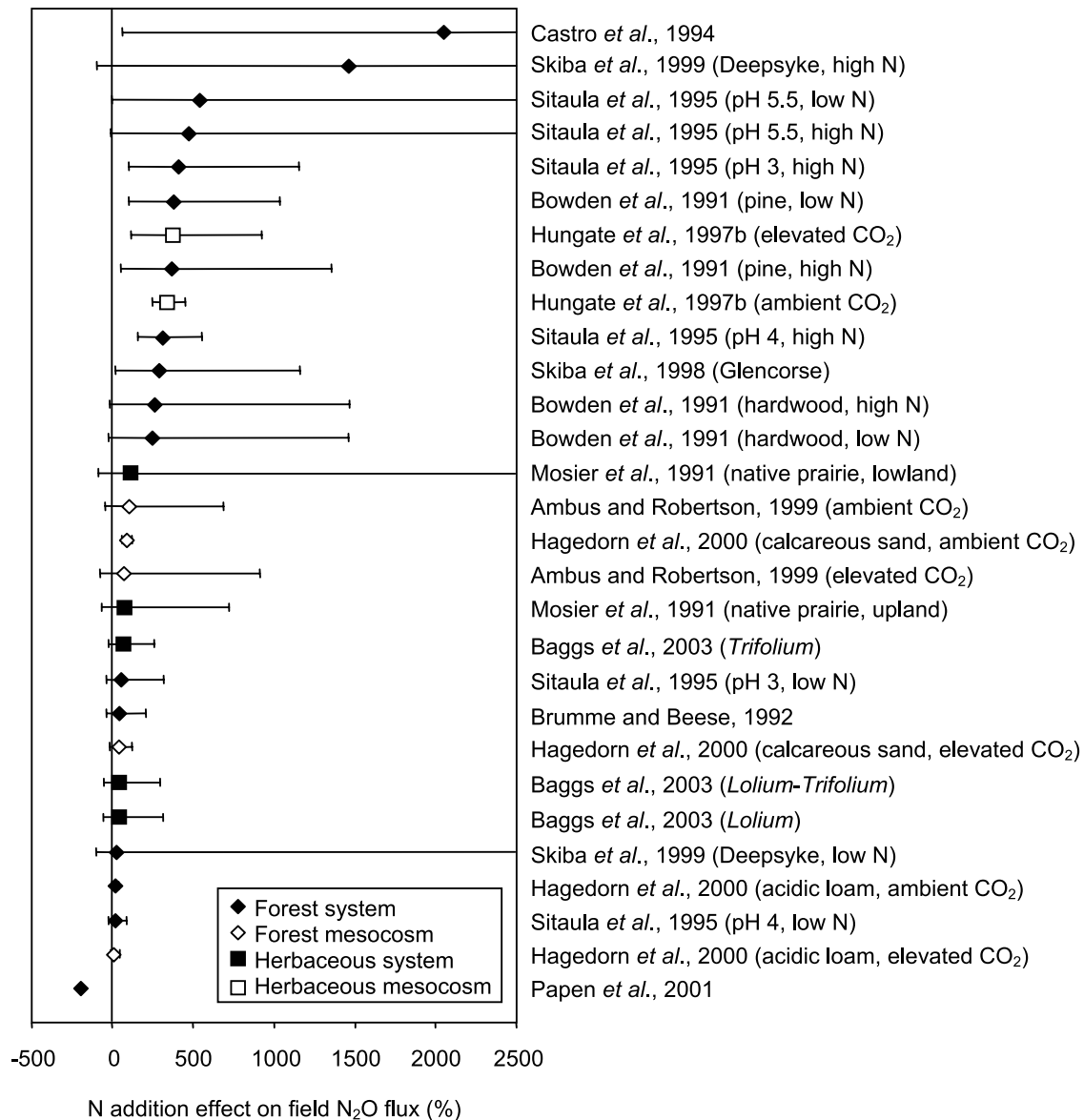
Figure 3. Effect of elevated CO_2 on field N_2O flux. Bars show 95% confidence interval of the effect size. In certain studies, elevated CO_2 was crossed with other treatments that are given here: soil N content (low N and high N, no NPK and +NPK), plant cover or type of soil. Note that the use of log 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 [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 [NH_4^+].

[25] Nitrification is inhibited at low soil [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 [O_2]. *Barnard et al.* [2004b] have suggested that the effect of CO_2 on soil

Figure 4. Effect of N addition on field N_2O fluxes. Bars show 95% confidence interval of the effect size. Confidence interval for *Papen et al.* [2001] could not be calculated because the control N_2O flux was negative. In certain studies, N addition was crossed with other treatments that are given here: CO_2 concentration (ambient CO_2 and elevated CO_2), 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 CO_2, and that the indirect effect of elevated CO_2 on soil heterotrophic activity through increased rhizodeposition should be included (see *Arnone and Bohlen* [1998] for a comparable mechanism for denitrification). The CO_2 responses of net and gross nitrification are not consistent with the NEA response or the low soil $[O_2]$ 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 NO_3^- sinks (denitrification and microbial immobilization). On the basis of the NEA and gross nitrification data, we are inclined to believe that elevated CO_2 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 $[NO_3^-]$ at elevated CO_2 [*Niklaus et al.*, 2001].
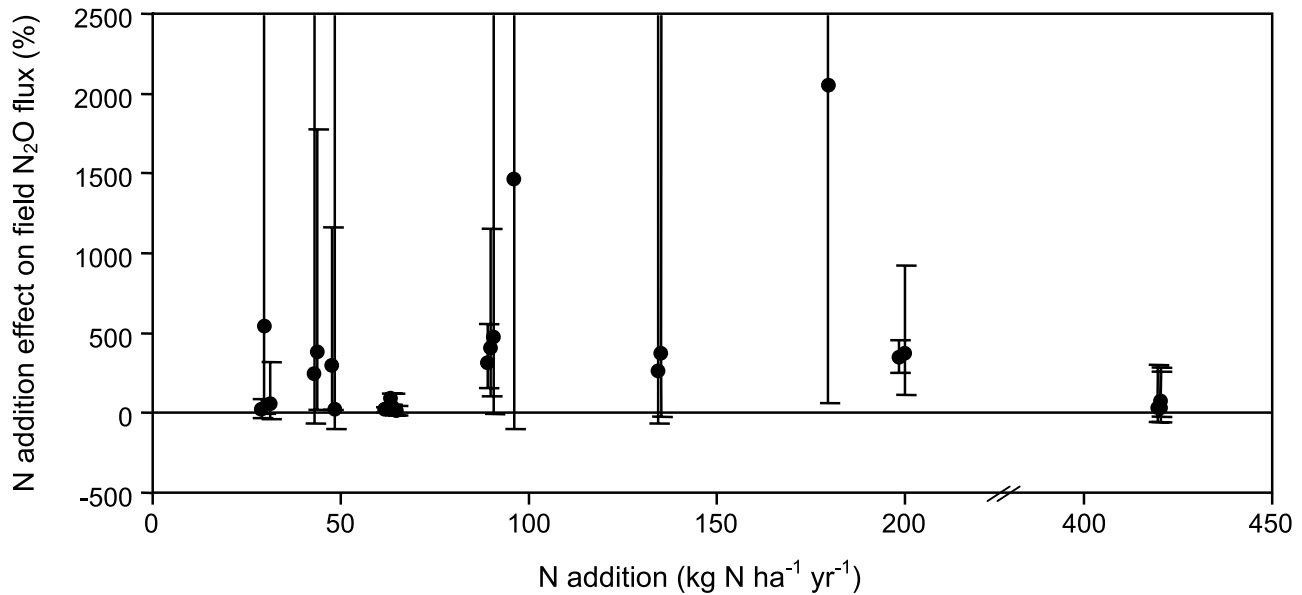
Figure 5. Relation between the amount of fertilizer N added and the difference in the amount of N-N_2O released between control and fertilized plots.

[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 NO_3^- 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_2O	X	X						ni	*Hagedorn et al.* [2000] (calcareous sand)
Field N_2O	X	X						ni	*Hagedorn et al.* [2000] (acidic loam)
Field N_2O	X	X						ns	*Hungate et al.* [1997b]
Field N_2O	X	X						ni	*Ambus and Robertson* [1999]
Field N_2O	X	X						ni	*Martín-Olmedo et al.* [2002]
Field N_2O		X		X				ns	*Sitaula et al.* [1995]
Field N_2O	X	X						ni	*Baggs et al.* [2003] (*Lolium*)
Field N_2O	X	X						ns	*Baggs et al.* [2003] (*Trifolium*)
Field N_2O	X	X						ni	*Baggs et al.* [2003] (*Lolium-Trifolium*)
Lab N_2O		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	*Martín-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) (JGRCE)
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]

[a]The treatments are atmospheric CO_2 concentration (CO_2), 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 CO_2 could potentially affect denitrification through modifications of NO_3^- availability. Over the 23 studies that measured the effect of elevated CO_2 on DEA, 11 also measured soil $[NO_3^-]$. Meta-analysis of these soil $[NO_3^-]$ data shows a significant decrease of soil $[NO_3^-]$ at elevated CO_2, with a mean effect of −35% (95% confidence interval limits are −50% and −14%). Soil $[NO_3^-]$ has often been shown to decrease at elevated CO_2 in other studies [*Niklaus et al.*, 1998a, 2001; *Johnson et al.*, 2001] but may also be unaffected [*Arnone and Bohlen*, 1998]. Reduced $[NO_3^-]$ may or may not reflect reduced NO_3^- availability, but reduced $[NO_3^-]$ has been used to explain decreased DEA at elevated CO_2 in several studies [*Tscherko et al.*, 2001; *Barnard et al.*, 2004a, 2004c]. We found no significant correlation between the effects of elevated CO_2 on DEA and soil $[NO_3^-]$ in our meta-analysis, but this may be due to the number of factors that are likely to simultaneously affect DEA or that NO_3^- concentrations may not reflect NO_3^- availability for denitrifiers. Our review does not provide strong evidence for a NO_3^--mediated response of denitrification to CO_2; 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 $[O_2]$. Thus, decreased DEA at elevated CO_2 is not consistent with the expected indirect effects of elevated CO_2 on soil $[O_2]$ (see section 4.1). A lack of responsiveness of DEA to elevated CO_2 could be explained by the very low soil $[O_2]$ required for the functioning of denitrifying enzymes. Some studies, but certainly not all, suggest that the effect of elevated CO_2 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 CO_2 on soil wetness may occur outside of the range of soil moisture where denitrification is strongly affected. Other mechanisms, such as a reduction in NO_3^- availability, must, however, be invoked to explain decreased DEA under elevated CO_2.

[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 $[O_2]$ [*Holtan-Hartwig et*

al., 2002; *Loiseau and Soussana*, 2000]. In particular, the indirect effect of temperature on soil $[O_2]$ through increased soil respiration should favor denitrification [*Castaldi*, 2000]. *Tscherko 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 N_2O flux, which it did in both field and laboratory experiments. This is consistent with *Bouwman et al.* [2002], whose review of N_2O fluxes in agricultural systems points to a strong increase of N_2O emissions accompanying N application rates. N addition increased N_2O 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 N_2O flux measurements. The positive response of N_2O flux to N addition could potentially be explained by increases in nitrifier-associated N_2O 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 N_2O 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 N_2O flux studies presented here). In such sites, N_2O fluxes may be already at near maximum rates due to N saturation of the system.

[33] For the 20 experiments that we reviewed, field N_2O fluxes were not substantially altered by elevated CO_2. However, large CO_2 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 N_2O fluxes was mainly attributable to an 18% increase in soil water content in their high-CO_2 plots. Elevated CO_2 often leads to increased soil water content and soil C inputs; both of these indirect effects of CO_2 should favor nitrifier-denitrification and denitrification. So why is N_2O flux generally insensitive to elevated CO_2? First, CO_2 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 $[NO_3^-]$ generally decline at elevated CO_2. This may mean that N_2O emissions associated with denitrifier activity are generally reduced at elevated CO_2. 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.

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