Biogeochemistry **37:** 89–109, 1997.

Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland

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Accepted 21 September 1996

Key words: annual grassland, elevated CO_2, first autumn rains, gross mineralization, gross nitrification, ^{15}N, ^{15}N pool dilution, NO, N_2O, NPK fertilizer, trace gases

Abstract. We examined the effects of growth carbon dioxide (CO_2) concentration and soil nutrient availability on nitrogen (N) transformations and N trace gas fluxes in California grassland microcosms during early-season wet-up, a time when rates of N transformation and N trace gas flux are high. After plant senescence and summer drought, we simulated the first fall rains and examined N cycling. Growth at elevated CO_2 increased root production and root carbon:nitrogen ratio. Under nutrient enrichment, elevated CO_2 increased microbial N immobilization during wet-up, leading to a 43% reduction in gross nitrification and a 55% reduction in NO emission from soil. Elevated CO_2 increased microbial N immobilization at ambient nutrients, but did not alter nitrification or NO emission. Elevated CO_2 did not alter soil emission of N_2O at either nutrient level. Addition of NPK fertilizer (1:1:1) stimulated N mineralization and nitrification, leading to increased N_2O and NO emission from soil. The results of our study support a mechanistic model in which elevated CO_2 alters soil N cycling and NO emission: increased root production and increased C:N ratio in elevated CO_2 stimulate N immobilization, thereby decreasing nitrification and associated NO emission when nutrients are abundant. This model is consistent with our basic understanding of how C availability influences soil N cycling and thus may apply to many terrestrial ecosystems.

Introduction

The effects of increasing atmospheric CO_2 concentration on the fluxes of other greenhouse and reactive trace gases are largely unknown, yet these interactions are critical to our understanding of biological feedbacks to changes in atmospheric chemistry (Robertson et al. 1989). Elevated CO_2 could increase or decrease trace gas fluxes to the atmosphere, thereby altering their effects on atmospheric chemistry. For example, elevated CO_2 stimulated methane efflux from a salt-marsh ecosystem (Dacey et al. 1993). Because methane is an important greenhouse gas, increased methane emissions from wetlands in response to elevated CO_2 could enhance CO_2-induced global warming.

To date, no studies have examined whether elevated CO_2 alters soil emissions of nitrous oxide (N_2O) and nitric oxide (NO), yet both are important trace gases. N_2O is a potent greenhouse gas, 180 times more efficient at infrared absorption than CO_2 (Lashof & Ahuja 1990), and is also involved in stratospheric ozone destruction (Cicerone 1987). NO catalyzes ozone production in the troposphere and also forms nitric acid, an important component of acid rain (Williams et al. 1992). The soil is an important source of NO, accounting for as much as 40% of total global NO sources (Williams et al. 1992). Atmospheric concentrations of N_2O have been rising over the last 20 years (Houghton et al. 1990) Increased biomass burning and agricultural fertilization are thought to be increasing soil emissions of NO to the atmosphere (Davidson 1991).

Elevated CO_2 could affect soil emissions of N_2O and NO by altering substrate availability to nitrifying and denitrifying bacteria (Figure 1), the major producers of these N trace gases (Firestone & Davidson 1989). Elevated CO_2 often stimulates plant production and thus total litter input to soil (van Veen et al. 1991). Additionally, plant litter C:N can increase under elevated CO_2 (Bazzaz 1990; Coûteaux et al. 1991; Cotrufo et al. 1994, but see Curtis et al. 1989). Both increased litter input and higher litter C:N in elevated CO_2 could increase microbial N demand and stimulate heterotrophic N immobilization, decreasing NH_4^+ and NO_3^- availability to nitrifiers and denitrifiers. Lower nitrification rates should depress N_2O and NO fluxes both directly, as nitrification produces N_2O and NO, and indirectly, by reducing NO_3^- availability for denitrification, which also produces N_2O and NO. However, where NO_3^- is in excess, elevated CO_2 could stimulate denitrification by increasing C availability to denitrifiers, potentially increasing soil emissions of NO and N_2O where denitrification is an important source of these N trace gases.

Fluxes of N_2O and NO from soil vary across ecosystems and through time (Williams et al. 1992). Soil moisture is one of the critical environmental variables controlling NO and N_2O fluxes, especially in seasonally dry ecosystems, where NO and N_2O emissions increase dramatically with the first rains after the dry season (Davidson 1991 and citations therein). In California's mediterranean climate, the first rains after the summer drought stimulate soil microbial activity, soil N transformations, and N trace gas fluxes (Schimel et al. 1989).

In this experiment, we tested the hypothesis that elevated CO_2 alters internal N transformations and fluxes of N_2O and NO during early season wet-up. Specifically, we hypothesized that elevated CO_2, by increasing soil C availability, would stimulate heterotrophic N immobilization, leading to decreased nitrification and decreased NO and N_2O fluxes (Figure 1). Increased C availability in elevated CO_2 could also stimulate or inhibit denitrification

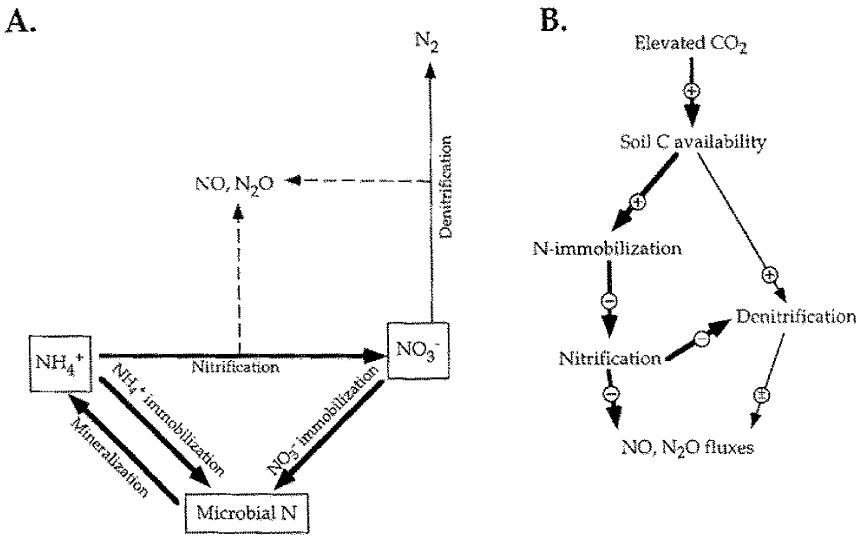

Figure 1. (A) Microbial N transformations relevant to this study. Boxes indicate soil N pools. Arrows between boxes indicate gross fluxes of N within the soil, whereas arrows to NO, N_2O, and N_2 indicate net fluxes of N gases from the soil. (As measured in this study, gross NH_4^+ consumption is nitrification plus NH_4^+ immobilization, and gross NO_3^- consumption is NO_3^- immobilization plus denitrification. Mineralization and nitrification were measured directly.) (B) Postulated effects of elevated CO_2 on soil C status, N transformations, and N trace gas fluxes during early season wet-up. Arrows indicate causal relationships among parameters and expected changes resulting from elevated CO_2. The symbols "+" and "−" on the arrows indicate the expected direction of change in the parameter to which the arrow points due to the change in the parameter from which the arrow originates. Arrow thickness indicates the expected magnitude of effect.

(Figure 1). However, nitrification is a much larger component of the N cycle than denitrification during early season wet-up in these grasslands (Schimel et al. 1989), so we expected CO_2 effects on nitrification to be more important than effects on denitrification in altering NO and N_2O fluxes.

Methods

This research was part of the elevated CO_2 experiment located at the Jasper Ridge Biological Preserve near Stanford, CA (37°24′ N, 122°13′ W, 100 m elevation). The climate is mediterranean, with cool, wet winters and warm, dry summers. The experiments described here took place in the project's Micro-Ecosystems for Climate Change Analysis (MECCA), an outdoor facility consisting of 20 open-top chambers (1.3 m^2 by 3 m tall), 10 with ambient and 10 with elevated (ambient + 360 ppm) CO_2 treatments. Each chamber

contains 28–32 polyvinyl chloride tubes (0.95 m tall $\times$ 0.2 m diameter) filled with soil and supporting grassland plant communities typical of California. For further experimental details see Field et al. (1996).

We examined N dynamics during the early season wet-up that typically occurs in late September through early November using a subset of the MECCA tubes from the 1993–1994 growing season. The plant community in these tubes comprised *Avena barbata* and *Bromus hordeaceus*, introduced (European) annual grasses and the co-dominants in the community; *Nassela pulchra*, a native, perennial grass; *Lotus wrangelianus*, an annual N fixer; and *Hemizonia congesta*, a late-blooming annual forb. Species densities in these tubes approximated the natural densities at which they occur in the field.

Experimental design

We used a 2-way factorial design (nutrients by CO_2) with 2 levels of each factor and 6 replicates of each treatment for measurements after experimental wet-up, and 3 replicates for pre-wet-up measurements. CO_2 treatments, maintained throughout the entire experiment, were ambient and elevated CO_2 (ambient + 360 μL L^{-1}) as described in Field et al. (1996). To release plant growth from mineral nutrient limitation in the high nutrient treatment, tubes in five chambers at each CO_2 level received 20 g m^{-2} each of N (as urea), P, and K, applied as 120-day Osmocote slow-release fertilizer in October 1993.

We seeded tubes in October 1993 and allowed plants to senesce over the summer of 1994. In early September 1994, we inserted one 10-cm diameter by 12-cm deep section of galvanized steel stove pipe ("collar") in each tube to isolate part of each tube for a 9-day ^{15}N-partitioning experiment (see below). On 1 September 1994, we harvested 3 tubes from each treatment to determine microbial biomass and plant detrital mass prior to experimental wet-up. We clipped detrital shoots (shoots produced during the previous growing season) from each tube, then cut each tube open and divided soil into 0–15, 15–45, and 45–90 cm layers. From the 0–15 cm layer, we removed a 200 g soil subsample for microbial biomass (see below). From each layer, we washed detrital roots (roots produced during the previous growing season) from the soil (see *Final Harvest* below).

On 3 September 1994 we simulated the first rains after the summer dry season by adding 1 L distilled H_2O to each tube 3 times at 0.5-hr intervals, simulating a 9-cm rain event. This amount is larger than the average first rain after the dry summer, but not unrealistic: 13 cm of rain fell during a two-day storm in November 1995, the first substantial rain of the 1995–1996 growing season. Furthermore, adding this amount compensated for the very high evaporative demand during the experiment and ensured that the wet-up event caused a reasonably large change in soil moisture. Soil moisture did

not exceed field capacity (0.35 g H_2O g $soil^{-1}$) during the experiment, so although the water addition was large, the changes in N cycling we observed should be qualitatively similar to those following a natural rain event.

Internal N cycling measurements

We measured the gross rates of mineralization, nitrification, and NH_4^+ and NO_3^- consumption (see Figure 1A) using the mixed-soil ^{15}N pool-dilution technique (Schimel et al. 1989). From an area outside the 10-cm diameter collar, we removed a 3-cm diameter $\times$ 10-cm deep soil core from each tube. We removed large plant fragments and then removed two 100 g soil samples from each core, placing them into two plastic bags, one for NH_4^+ and one for NO_3^-. We then added 5 ml of either 0.67 mM $(NH_4)_2SO_4$ or of 0.27 mM as KNO_3 (both 99 atom % ^{15}N) to each bag, an addition of approximately 1 μg ^{15}N as NH_4^+ and 0.2 μg ^{15}N as NO_3^- per gram soil. We added less $^{15}NO_3^-$ than $^{15}NH_4^+$ because we expected the soil extractable pools of NO_3^- to be considerably smaller than pools of NH_4^+ (e.g. Davidson et al. 1990). We added the ^{15}N in 0.5 ml increments, mixing the solution into the soil with each addition. Immediately after adding the ^{15}N (t0), we extracted 20–30 g of soil from each bag in 0.5 M K_2SO_4. We then placed each plastic bag back in the hole from which the soil was removed. After 24 hours (t24), we extracted another 20–30 g soil with 0.5 M K_2SO_4. We conducted this experiment three times (with separate soil cores and ^{15}N additions) after experimental wet-up, during the 0–24, 72–96, and 192–216 hour intervals following water addition. We filtered all extracts through pre-rinsed Whatman #1 Qualitative paper filters, collected the filtrate in specimen cups, and immediately froze the extracts on dry ice until analysis, approximately two months later.

We determined NO_3^- and NH_4^+ concentration in the extractions colorometrically on a Lachat autoanalyzer. We used a diffusion procedure to collect NH_4^+ and NO_3^- on acidified filter discs (Brooks et al. 1989) and then analyzed the discs for ^{15}N content by direct combustion mass spectroscopy (Europa Scientific Tracermass). We calculated gross mineralization, nitrification, and NO_3^- and NH_4^+ consumption using the equations from Wessel and Tietema (1992).

We conducted a 9-day ^{15}N-partitioning experiment to determine changes in N cycling integrated over the entire wet-up event. After the first 1-L water addition, we added 0.82 mg $^{15}NH_4^+$-N to the section of each tube enclosed by the 10-cm diameter collar, approximately 1 μg ^{15}N per g soil to a depth of 10 cm. We added the $^{15}NH_4^+$ in 50 ml of 0.55 mM ^{15}N-$(NH_4)_2SO_4$ solution (99 atom % ^{15}N). We used a syringe to spread the solution directly over the soil surface, taking care to avoid placing the $^{15}NH_4^+$ on the standing dead plants.

Soil moisture

We used the remainder of the soil in each bag for t0 and t24 gravimetric moisture determination. We converted gravimetric moisture to percent water-filled pore space using measured bulk density of 0.90 g cm^{-3} for the top 15 cm of soil in these microcosms (BA Hungate, unpublished data), and assuming a particle density of 2.65 g cm^{-3} (Brady 1990).

N_2O and NO flux measurements

In 4 tubes of each treatment, we measured N_2O and NO fluxes 2 hours before, and 0.5 h, 3 h, and 1, 2, 4, and 9 days after wet-up. We used a static chamber to determine N_2O flux, by covering each tube with a 3-L plastic container whose lip, coated with Apiezon grease, sealed with the rim of the PVC tubes. We sampled the headspace in the containers at 0, 15, and 30 minutes using nylon syringes, and measured N_2O concentrations by gas chromatography within 12 hours of sample collection. We used a 3-L plastic container covered with aluminum foil sealed to the PVC tubes to measure NO flux. The container was plumbed to a chemoluminescent NO_x detector, as described in Davidson et al. (1991). We did not distinguish between NO and NO_2, but soil production of NO_2 is typically negligible (Williams et al. 1992), so we refer only to NO flux in this paper. We recorded the mV reading from the detector every 15 seconds for 4–8 minutes, longer when fluxes were very low (and unstable). At the beginning of each measurement period, we calibrated the detector using a tank of known NO concentration. N_2O and NO accumulation in the chamber headspace was linear ($r > 0.9$ for 90% of the measurements); where NO fluxes were extremely low and unstable, linear regression most simply described changes in chamber headspace concentration. Thus, we used linear regression to determine the rates of N_2O and NO flux, regressing N_2O or NO concentration with time over the measurement interval.

Microbial biomass

We sent approximately 50 g soil from the 3-day soil core and the soil from the collar from the final harvest (see below) to the Soil Microbial Biomass Service (Oregon State University, Corvalis, OR) for direct microscopic determination of active and total bacterial and fungal biomass, using selective fluorescent staining of soil samples diluted in phosphate buffer (Ingham et al. 1989). We also sent samples from tubes that were harvested on 1 September, but did not receive water addition, to establish baseline biomass levels. All active counts were conducted, and total counts prepared (and thus fixed) within 48 hours of sample collection.

Final harvest

On 12 September 1994, we removed tubes from the MECCA boxes, cut open each tube, and divided the soil into 3 layers: 0–15 cm, 15–45 cm and 45–90 cm. We kept the material (detritus, plants, and soil) in the collar separate from the rest of the 0–15 cm layer. We collected 200 g of well-mixed soil from each of the two deeper layers and from the soil in the collar, removing detrital roots from the soil samples by hand. We then washed detrital roots from the remaining soil in the two deeper layers. We clipped standing dead plants from the collar, but left seedlings attached to roots. We washed the soil in the collar and separated live plants from detrital roots. We dried plant and detrital material at 65 °C and then weighed it. We determined N concentration and ^{15}N enrichment in live plants and in detrital shoots and roots from the collar by analyzing a finely ground subsample by combustion, gas chromatography, and isotope-ratio mass spectroscopy (Europa Scientific, UK).

We extracted soil samples from each layer using the same protocol for the pool-dilution extractions, and analyzed for concentrations and ^{15}N enrichments of NH_4^+ and NO_3^- using the same techniques. In the soil from the collar, we also measured microbial biomass N and ^{15}N using the chloroform-fumigation direct extraction technique (Brookes et al. 1985). We exposed 25–35 g soil to chloroform vapors for 24 h in a glass desiccator, then extracted the soil with 0.5 M K_2SO_4. We converted all the N in the fumigated and non-fumigated extractions to NH_4^+ by Kjeldahl digestion, then determined total N colorometrically (Lachat Instruments, Inc., Milwaukee, WI). We collected the NH_4^+ on acidified filter discs using a diffusion procedure (Brooks et al. 1989), then analyzed the discs for ^{15}N content by mass spectroscopy. We calculated microbial biomass N and ^{15}N (chloroform-labile N and ^{15}N) as the difference in total N or ^{15}N (in μg g^{-1} dry soil) between fumigated and non-fumigated samples, dividing by 0.54 to correct for extraction efficiency (Brookes et al. 1985). We expressed microbial N and ^{15}N on a m^2 basis, using the soil bulk density in our microcosms of 0.9, and extrapolating to 10 cm depth. We calculated the amount of ^{15}N remaining in the "soil fixed" pool as ^{15}N recovered in the soil minus ^{15}N recovered in the microbial and extractable N pools.

Statistical analyses

We sampled the same replicates through time for the N-trace gas fluxes, gross N-transformations, and microbial counts. Hence, we used 2-way repeated measures ANOVAs to test for overall effects of CO_2 and nutrients, effects of time (after wet-up), and interactions between time, nutrients and CO_2. To test for net differences over the entire wet-up event, we calculated the weighted

average rate for each tube. Because we did not have an *a priori* biological model for the dynamics of trace gas production and N transformations after wet-up, we used simple linear interpolation to calculate weighted averages, weighting each measurement by the time interval it represents. We analyzed these weighted averages in 2-way ANOVAs with nutrients and CO_2 as main effects. We also used 2-way ANOVAs to test for treatment effects on ^{15}N recovery in plants, microbes and soil, on microbial biomass N, and on plant and detrital C:N. We used a 3-way ANOVA (CO_2 by nutrients by time) to analyze detrital root mass, including data from both the initial harvest on 1 September and the final harvest on 12 September.

Results

Detrital root mass and C:N

Plant growth at elevated CO_2 resulted in greater detrital root mass and C:N ratios at both nutrient levels (Table 1). Hence, elevated CO_2 caused increased C input to soil, while also increasing the relative availability of C to N in detrital roots to soil microorganisms. These results are consistent with our initial hypothesis, setting the stage for altering N dynamics with early season wet-up (Figure 1B).

Soil moisture, seedling growth, microbial biomass

The simulated rain event increased soil moisture from 3 to 30% (gravimetric) immediately after wetting (Figure 2A). Soil moisture then declined to 20% after 96 hours, and to 15% by the final harvest. There were no significant differences between CO_2 and nutrient treatments in soil moisture values (data not shown). Plant seedlings appeared 96 hours after wetting and were about 4 cm tall at the final harvest, 9 days after wet-up. Bacterial and fungal biomass responded rapidly to water addition, increasing after 24 hours and peaking after 96 hours (Figure 2B). Fungal biomass was 3–4 times greater than bacterial biomass, and showed a much larger response to water addition. Nutrients and CO_2 did not substantially alter any components of the microbial biomass we examined, so we show only the overall means for simplicity (Figure 2B).

NO and N_2O fluxes

Wet-up immediately stimulated NO flux (Table 2, Figure 3A). Nutrient addition increased NO flux throughout the experiment. At high nutrients, NO

Table 1. Detrital root mass and C:N ratios. Values are means ± standard errors, and significance level (*P*) from ANOVAs for the effects of CO_2, NPK and their interaction. Detrital root mass values (n = 9) are a combination of results from the harvest on 1 September preceding the experimental wet-up and from the final harvest on 12 September. There was no significant difference in detrital root mass between these two dates, nor any significant interaction of harvest date with CO_2 and/or nutrients ($P > 0.5$ in all cases). Detrital root C:N values (mean ± standard errors, N = 6) were determined only for the final harvest on 12 September.

	Treatment				*P*		
Response variable	Ambient CO_2 − NPK	Elevated CO_2 − NPK	Ambient CO_2 + NPK	Elevated CO_2 + NPK	CO_2	NPK	CO_2 × NPK
Mass (g m^{-2})	150 ± 9	194 ± 18	216 ± 19	259 ± 28	0.04	0.003	0.98
C:N (g C g N^{-1})	52 ± 2	56 ± 2	31 ± 1	37 ± 2	0.009	<0.001	0.68

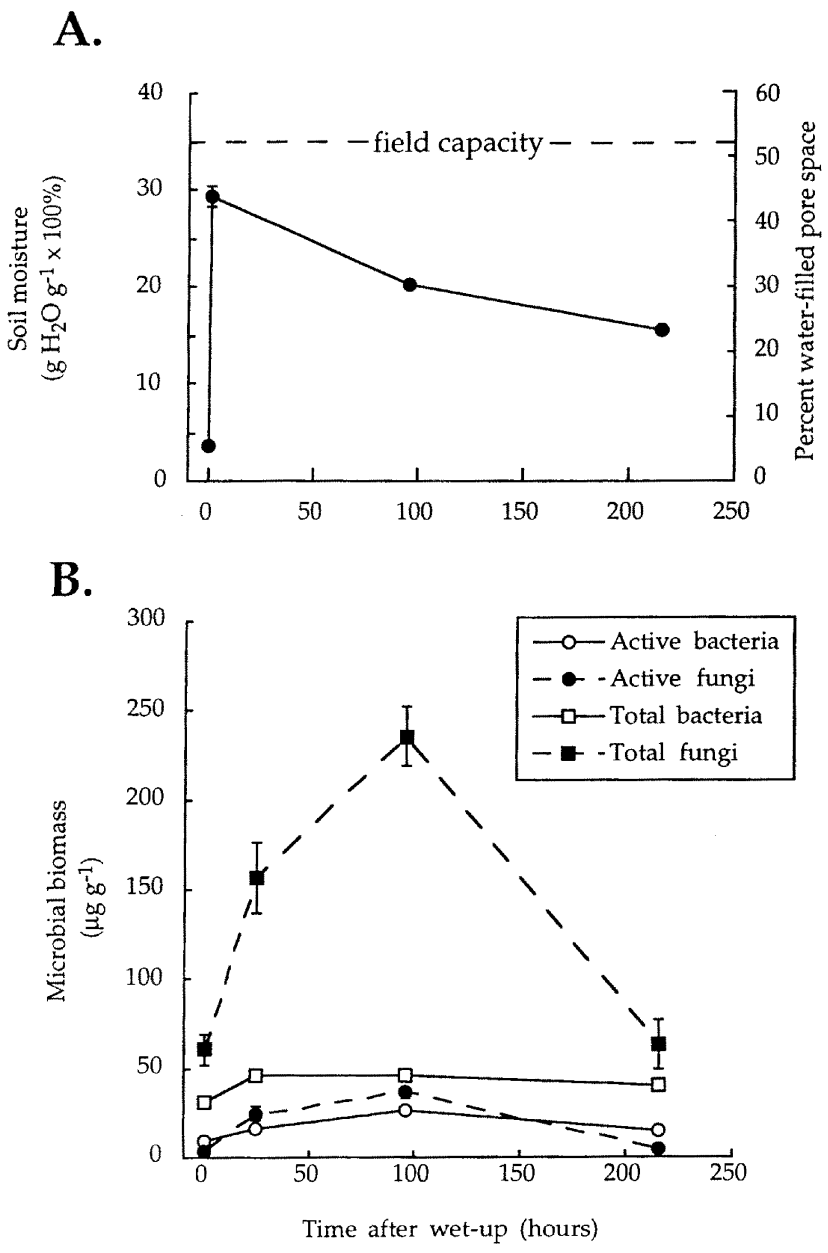

Figure 2. (A) Time course after wet-up of soil moisture, expressed on a soil dry weight basis and converted to percent water-filled pore space (using measured bulk density of 0.9, and assuming a particle density of 2.65). (B) Time course after wet-up of components of the microbial biomass determined by direct microscopic counts. Values are means ± 1 standard error.

flux reached maximum rates after 24–48 h. Even after 192 hours, NO fluxes at high nutrients were 8 times higher than fluxes before water addition. In contrast, NO flux at low nutrients peaked after 0.5 hr and returned to pre-water addition rates after 48 hours. At high nutrients, elevated CO_2 caused decreased NO flux over the 9-day period (Figure 3A and C). Elevated CO_2 did not significantly alter NO flux at low nutrients, but NO fluxes at low nutrients were often at detection limits. Hence, a CO_2 effect on NO flux of similar magnitude to that observed at high nutrients could not have been detected.

N_2O flux also increased immediately after water addition (Figure 3B), reaching maximum rates after 24–72 hours. For all treatments, N_2O fluxes returned to pre-water addition rates after 192 hours. Nutrients increased N_2O flux, but the increase was not as large as for NO (Table 2, Figure 3B and C). In contrast to NO, elevated CO_2 did not alter total N_2O flux throughout the 9-day period, but caused slight changes in the dynamics of N_2O production following water addition. Elevated CO_2 caused an initial increase in N_2O fluxes (0.5 and 3.5 hours after wet-up), but then decreased N_2O flux at high nutrients at 24 hours, and at low nutrients at 72 hours.

Internal N cycling

Nutrient addition increased gross mineralization and NH_4^+ consumption (Table 2, Figure 4A). Elevated CO_2 had no effect on gross mineralization and NH_4^+ consumption (Table 2, Figure 4A). Our observation that CO_2 did not alter gross NH_4^+ consumption is consistent with our hypothesis that CO_2 stimulates N immobilization: gross NH_4^+ consumption includes both NH_4^+ immobilization and nitrification (Figure 1A), so compensatory changes in these two processes would result in no change in gross NH_4^+ consumption (see nitrification and $^{15}NH_4^+$ partitioning results, below). Gross mineralization was highest during the first 24 hours following wet-up at high nutrients, then declined 3-fold after 72 hours and remained constant thereafter (Table 2, Figure 4A). In contrast, gross mineralization at low nutrients and gross NH_4^+ consumption at both nutrient levels were constant throughout the 9-day period.

Nutrient addition stimulated gross nitrification and gross NO_3^- consumption (Figure 4B, Table 2). At high nutrients, elevated CO_2 decreased gross nitrification during all three measurement intervals, causing a 43% reduction in total nitrification during the 9-day period. Elevated CO_2 did not alter gross NO_3^- consumption in either nutrient treatment, nor gross nitrification at low nutrients. Throughout the 9-day period, nitrification was a major component of gross NH_4^+ consumption: 47–71% of the NH_4^+ consumption was by nitrifiers (Table 3). This percentage was not significantly affected by nutrients or CO_2. 42–89% of the total mineralized N was nitrified; this proportion was

Table 2. Significance levels (*P*) from 2-way repeated measures ANOVAs (RMA) and 2-way ANOVAs for weighted averages (Average) for N_2O and NO fluxes and for gross N transformations. The factors are CO_2 and nutrients (NPK), with time (after wet-up) as the repeated measure. For treatment means and standard errors, see Figures 3 and 4.

Response variable		*P*						
		CO_2	NPK	$CO_2 \times$ NPK	Time	Time $\times$ CO_2	Time $\times$ NPK	Time $\times$ NPK $\times$ CO_2
NO flux	RMA	0.05	<0.001	0.04	0.03	0.02	0.001	0.03
	Average	0.003	<0.001	0.006				
N_2O flux	RMA	0.90	0.001	0.41	<0.001	0.10	0.005	0.26
	Average	0.61	0.002	0.81				
Nitrification	RMA	0.02	<0.001	0.03	0.001	0.73	0.04	0.72
	Average	0.05	<0.001	0.07				
NO_3^- cons.	RMA	0.49	<0.001	0.53	0.48	0.96	0.10	0.86
	Average	0.51	<0.001	0.68				
Mineralization	RMA	0.91	<0.001	0.92	0.06	0.89	0.05	0.87
	Average	0.73	<0.001	0.77				
NH_4^+ cons.	RMA	0.57	<0.001	0.80	0.33	0.11	0.33	0.20
	Average	0.88	<0.001	0.68				

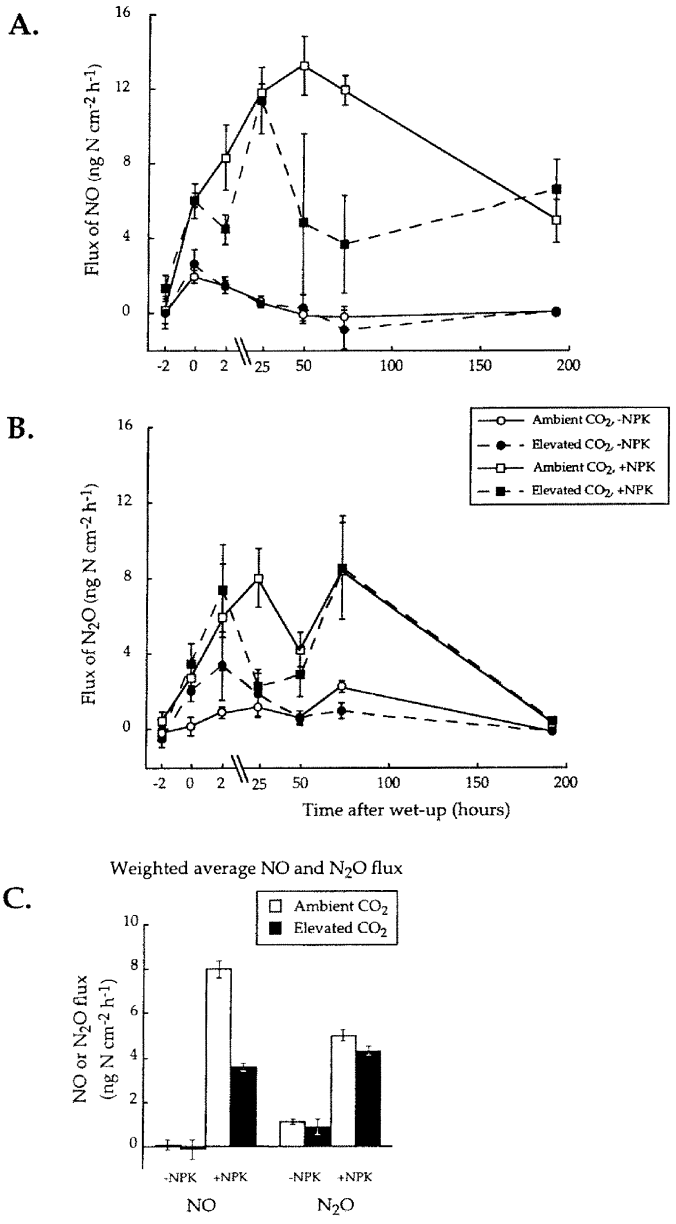

Figure 3. Time course after wet-up for the fluxes from soil of (A) NO and (B) N_2O, in ng N cm^{-2} h^{-1} and (C) the weighted averages of these fluxes during the 9-day experiment. Values are means $\pm$ 1 standard error. Positive values indicate net flux from the soil to the atmosphere, and negative values net uptake by the soil from the atmosphere. Note the change in scale on the X-axis 4 hours after wet-up.

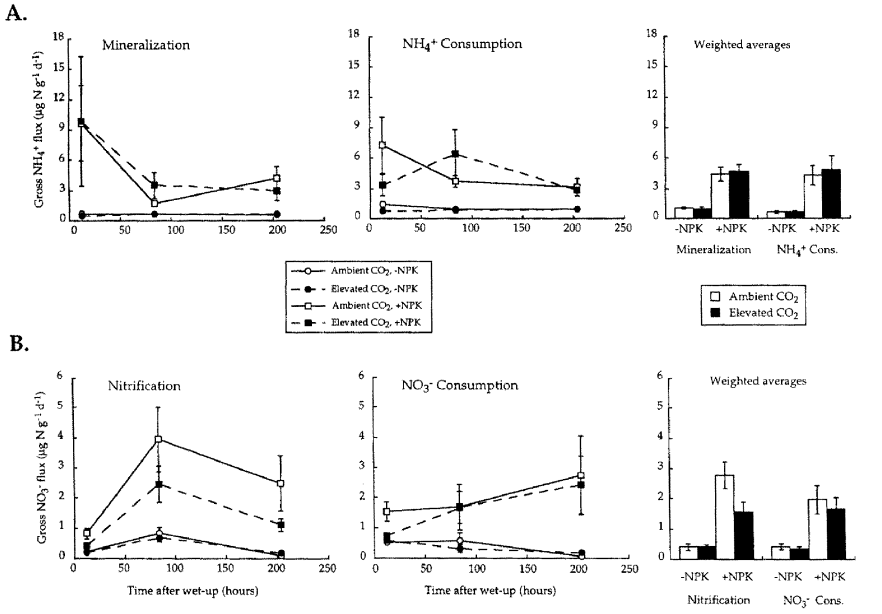

Figure 4. Time courses after wet-up and weighted averages over the 9-day period for the gross rates of (A) NH_4^+ transformations (mineralization and NH_4^+ consumption) and (B) NO_3^- transformations (nitrification and NO_3^- consumption), in μg N g dry soil^{-1} day^{-1}. These processes are presented schematically in Figure 1. Values are means $\pm$ 1 standard error. Note the different scales for NH_4^+ and NO_3^- transformation rates.

lower under elevated CO_2 (Table 3), reflecting the depression by CO_2 of nitrification at high nutrients (Figure 4B, Table 2).

9-day $^{15}NH_4^+$ partitioning

67 $\pm$ 5% of the total ^{15}N applied was recovered (mean $\pm$ standard error, pooled across all treatments). This percentage was not significantly affected by the nutrient or CO_2 treatments ($P > 0.05$). No $^{15}NH_4^+$ or $^{15}NO_3^-$ was recovered in soil from the 15–45 and 45–90 cm depths nor did the wetting front exceed 30-cm deep (data not shown), indicating that ^{15}N losses were primarily due to gaseous N losses, including losses as NO and N_2O.

Microbial ^{15}N uptake during the 9-day partitioning experiment is an integrative measure of N immobilization, including both $^{15}NH_4^+$ immobilization, and immobilization of $^{15}NO_3^-$ produced through nitrification (Figure 1A). Elevated CO_2 caused a significant increase in microbial ^{15}N uptake (Figure 5, Table 4), consistent with our hypothesis that elevated CO_2 would increase N immobilization after wet-up (Figure 1B). Although elevated CO_2 did not alter ^{15}N immobilization in detritus (Figure 5, Table 4), C originating from

Table 3. The percentage of mineralized N that is subsequently nitrified, and nitrification as a percentage of gross NH_4^+ consumption (cons.). Means ± standard errors (n = 5–6) are for the weighted average flux rates over the 9-day period. Significance levels (P) are from 2-way ANOVAs for the effects of CO_2, NPK, and their interaction.

	Treatment means and standard errors				*P*		
	Ambient CO_2 − NPK	Elevated CO_2 − NPK	Ambient CO_2 + NPK	Elevated CO_2 + NPK	CO_2	NPK	CO_2 × NPK
% of mineralized N that is nitrified	88 ± 9%	64 ± 13%	89 ± 28%	42 ± 7%	0.06	0.59	0.57
Nitrification as % of gross NH_4^+ cons.	52 ± 10%	60 ± 24%	71 ± 15%	47 ± 15%	0.63	0.87	0.37

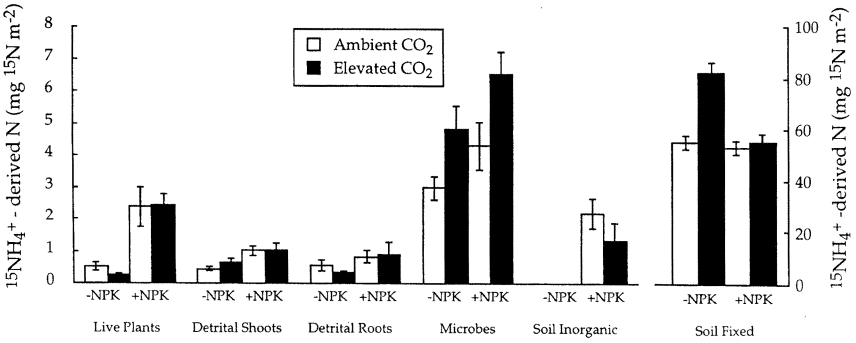

Figure 5. ^{15}N enrichment (in mg ^{15}N m^{-2}) in plants, detritus, microbes, soil inorganic and soil fixed N pools at the final harvest, 9 days after wet-up. $^{15}NH_4^+$ (99 atom % ^{15}N) was added to the soil surface 0.5 hours after wet-up. Values are means $\pm$ 1 standard error. Note the different scale for the fixed soil ^{15}N pool.

detritus during the wet-up event and detrital root fragments remaining in the soil from the previous year could drive the large increase in bulk soil microbial N immobilization that we observed under elevated CO_2. At low nutrients, the amount of ^{15}N that was not extractable from soil was significantly higher under elevated CO_2 (Figure 5, Table 4). Since chloroform fumigation probably targets microbial cytoplasm, much of the ^{15}N that was not extractable from soil could have been in microbial cell walls. Elevated CO_2 did not alter plant ^{15}N uptake (Figure 5, Table 4). Plant ^{15}N uptake was significantly higher under nutrient addition (Figure 5, Table 4), indicating a stronger plant N sink when nutrients are abundant. Microbial ^{15}N uptake was also higher under nutrient addition, but to a lesser extent than for plant uptake.

Discussion

Both the nutrient addition and elevated CO_2 treatments substantially altered N cycling during the 9 day wet-up event. Nutrient addition stimulated N transformation rates and N_2O and NO fluxes. Elevated CO_2 stimulated N immobilization, decreased nitrification, and decreased NO fluxes from soil under nutrient enrichment, results that are consistent with our hypothesis (Figure 1B). Elevated CO_2 did not substantially alter N_2O fluxes, but fluxes of NO were larger than fluxes of N_2O. Thus, under nutrient enrichment, elevated CO_2 increased ecosystem N retention during early season wet-up, a period when N losses through NO and N_2O fluxes are high.

The increases in NO and N_2O production after experimental wet-up that we observed are consistent with many studies that have documented increased

Table 4. Results from 2-way ANOVAs for the $^{15}NH_4^+$ partitioning experiment. Shown are significance levels (*P*) for the main effects of CO_2 and NPK and for their interaction. Response variables are the N pools in which ^{15}N recovery was assessed 9 days after adding tracer $^{15}NH_4^+$ to each experimental microcosm.

Response variable	*P*		
	CO_2	NPK	$CO_2 \times$ NPK
Live plants	0.81	<0.001	0.68
Detrital shoots	0.53	0.008	0.53
Detrital roots	0.79	0.09	0.53
Microbes	0.005	0.03	0.77
Extractable NH_4^+	0.17	<0.001	0.10
Extractable NO_3^-	0.79	<0.001	0.79
Soil fixed	0.08	0.21	0.17

NO and N_2O production after rain (Davidson et al. 1991 and citations therein). Water addition to dry soil causes a flush of labile organic C and N from dead microbial cells (Bottner 1985), conditions to which bacterial and fungal biomass (Figure 2B), nitrification (Figure 4B), and denitrification (Rudaz et al. 1991) quickly respond.

50–80% of mineralized N was nitrified during this period of high microbial activity. In contrast, nitrification accounted for 12–46% of N mineralized during an entire growing season in a California grassland (Davidson et al. 1990), and for 30% of the N mineralized six weeks after experimental water addition (Schimel et al. 1989). Our results show that nitrification plays a larger role during the first 9 days following the onset of fall rains than during other times of the year in California grasslands.

By directly releasing NO and N_2O, and by producing NO_3^- for subsequent denitrification and leaching, nitrification is the major biological N transformation that regulates ecosystem N loss (Robertson 1989). NH_4^+ availability strongly influences nitrification (Robertson 1989; Davidson et al. 1990), so microbial immobilization of N can buffer against N losses by decreasing NH_4^+ availability to nitrifiers. In our experiment, elevated CO_2 stimulated microbial N immobilization and led to decreased nitrification and decreased N trace gas losses, suggesting that elevated CO_2 could increase ecosystem N retention. In ecosystems dominated by annual plants, including crop systems, this mechanism may be especially important during the early season, when plants are small sinks for N and cannot buffer against N losses.

When soil moisture is below field capacity (as in our study), nitrification is probably the major source of NO emission from soil (Davidson 1992). The lower NO fluxes under elevated CO_2 that we observed at high nutrients

are likely a direct consequence of the lower nitrification rates under elevated CO_2, rather than a depression of NO fluxes via denitrification.

It is not immediately clear why elevated CO_2 did not depress nitrification in the low nutrient treatment. Coefficients of variation for nitrification, the presumed source of NO, were about 60% for both nutrient levels. Thus, we should have been able to detect a CO_2 depression of nitrification at low nutrients, had it occurred, comparable to that observed at high nutrients. It seems unlikely that nitrifiers were more NH_4^+ limited at high nutrients, because nitrification accounted for about 70% of the N mineralized at both nutrient levels (Table 3). Alternatively, if NH_4^+ distribution was more homogenous at high nutrients due to a relatively even distribution of the Osmocote fertilizer, NH_4^+ immobilization and nitrification could have been more tightly coupled at high nutrients, but less so at low nutrients, where increased immobilization may have occurred in microsites where nitrification was negligible. Consistent with this interpretation, a simple model showed that the rate of nitrification becomes increasingly sensitive to changes in NH_4^+ immobilization when soil microsites where NH_4^+ immobilization is occurring are homogeneously distributed in the soil (Davidson & Hackler 1994).

There are several factors that could explain why elevated CO_2 did not substantially alter N_2O fluxes. First, below field capacity, both nitrification and denitrification (the latter likely occurring in anaerobic microsites) can contribute to N_2O flux in similar California grasslands (Rudaz et al. 1991; Davidson 1992). Even if N_2O losses via nitrification were substantially lower under elevated CO_2 due to decreased nitrification, increased N_2O losses via denitrification could have compensated to cause no net change in N_2O efflux. Although decreased nitrification under elevated CO_2 could depress denitrification by reducing NO_3^- availability, increased C input to soil in elevated CO_2 should stimulate denitrification (Figure 1B) by providing necessary organic C, the electron donor for denitrification. Second, although soil moisture was below field capacity, suggesting low denitrification rates, denitrification may have been the dominant source of N_2O in our experiment and may not have responded to elevated CO_2. Even though CO_2 caused decreased nitrification, nitrification rates were very high in this experiment, so denitrification may have been limited by factors other than NO_3^- availability. If so, the decreased nitrification rates we observed would not have altered denitrification rates and associated N_2O fluxes. Third, the ratio of NO:N_2O produced during nitrification decreases as O_2 partial pressure decreases (Hutchinson & Davidson 1993). Increased soil C availability in elevated CO_2 could have reduced O_2 partial pressure (Rudaz et al. 1991), favoring N_2O as an end product of nitrification. This mechanism could offset any decreases in N_2O flux caused by decreased nitrification.

Whatever the mechanism, these results have important implications for emissions of NO and N_2O in a high-CO_2 world. N_2O is an important greenhouse gas (Lashof & Ahuja 1990), and emissions from soil are an important contribution to the observed increase in atmospheric N_2O concentration over the last century (Matson & Vitousek 1990). Interactions between elevated CO_2 and greenhouse gas fluxes can occur, as shown by the CO_2 stimulation of CH_4 flux from wetlands (Dacey et al. 1993). However, we found no evidence that elevated CO_2 alters soil emissions of N_2O during wet-up events.

Our finding that elevated CO_2 depressed NO fluxes by 55% in the high nutrient treatment demonstrates that elevated CO_2 can substantially alter the fluxes of reactive trace gases. Furthermore, our results provide a simple mechanistic explanation for reduced NO efflux: higher root production and root C:N in elevated CO_2 increased N immobilization, reducing NH_4^+ availability, and thereby decreasing nitrification and associated NO production. This model may be general to many terrestrial ecosystems, especially to seasonally dry ecosystems where soil NH_4^+ availability is high (e.g. P-limited systems, or those affected by N deposition), and to annual crop systems, where roots remain in the soil after harvest and where soil nutrient availability is high. In these systems, increases in root production and detrital root C:N in elevated CO_2 may stimulate microbial NH_4^+ immobilization, depress nitrification, and thereby decrease NO fluxes following wet-up of dry soils.

Acknowledgements

We thank Anne Blanche Adams, Tina Billow, Paul Brooks, Josep Canadell, Zoe Cardon, Nona Chiariello, Erik Nelson, Julie desRosier, Chris Field, Sharon Hall, Don Herman, Rob Jackson, Geeske Joel, Jane Marks, Heather Reynolds, Ted Schuur, Sue Thayer, Hailin Zhong, and Zabihullah Wardak for field and laboratory assistance. We thank Tina Billow and Sharon Hall for help with the NO_x calculations. Carla D'Antonio, Eric Davidson, Chris Field, Mary Firestone, Beth Holland, Tom Jordan, Jane Marks, and Pam Matson provided valuable advice and helpful comments on the manuscript. The Jasper Ridge CO_2 Experiment is supported by grants from the US National Science Foundation to the Carnegie Institution of Washington, Stanford University, and the University of California at Berkeley. BAH was supported by a National Defense Science and Engineering Graduate Fellowship and a National Science Foundation Dissertation Improvement Grant. CPL was supported by the US Department of Energy's Graduate Fellowships for Global Change Program administered by ORISE. HLP was supported by a National Science Foundation Fellowship. This is CIWDPB paper number 1285.

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