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Stimulation of grassland nitrogen cycling under carbon dioxide enrichment

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Abstract Nitrogen (N) limits plant growth in many terrestrial ecosystems, potentially constraining terrestrial ecosystem response to elevated CO_2. In this study, elevated CO_2 stimulated gross N mineralization and plant N uptake in two annual grasslands. In contrast to other studies that have invoked increased C input to soil as the mechanism altering soil N cycling in response to elevated CO_2, increased soil moisture, due to decreased plant transpiration in elevated CO_2, best explains the changes we observed. This study suggests that atmospheric CO_2 concentration may influence ecosystem biogeochemistry through plant control of soil moisture.

Key words N mineralization · Elevated CO_2 · Annual grasslands · Soil moisture

Introduction

Nitrogen limits plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991). Elevated CO_2 could increase or decrease N availability to plants. The conclusion from laboratory and microcosm studies is that increased soil C availability causes these changes. Increased labile C inputs to soil resulting from higher root exudation or turnover under elevated CO_2 can stimulate (Zak et al. 1993) or depress (Díaz et al. 1993) N availability to plants, and stimulate leaching losses of N (Körner and Arnone 1993). Also, increased C:N ratio in litter produced under elevated CO_2 can slow nutrient release during decomposition (Coûteaux et al. 1991; Field et al. 1992).

Elevated CO_2 can also cause increased soil moisture as a result of decreased plant transpiration (Field et al. 1995). The consequences of increased soil moisture for ecosystem processes, including N cycling (Kuikman et al. 1990; Tietema et al. 1992), have not been considered in elevated CO_2 studies. The work presented here is the first to directly measure changes in ecosystem N cycling in an intact ecosystem under elevated CO_2 and to postulate soil moisture as the driving mechanism.

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Materials and methods

We conducted this research in two grasslands at the Jasper Ridge Biological Preserve of Stanford University, California, United States (37°24′N, 122°14′W; elevation 150 m). The climate is mediterranean, with cool, wet winters and hot, dry summers. Serpentine and sandstone annual grasslands occur adjacent to one another but differ dramatically in productivity and nutrient limitation (Field et al. 1996). On each grassland, open-top chambers maintain either ambient or elevated (ambient+350 ppm) CO_2 atmospheres over ten replicate plots, each covering 0.3-m^2 ground area (Jackson et al. 1994; Field et al. 1996). Here we present results from the first growing season (1992) after establishing the CO_2 treatments.

In April 1992 (when plant biomass is near its seasonal maximum), we measured the gross rates of microbial production and consumption of NH_4^+ and plant NH_4^+ uptake using the ^{15}N pool dilution technique (Davidson et al. 1991). We added $^{15}NH_4^+$ to each plot by evenly distributing 3 mg ^{15}N [as 1.5 mmol l^{-1} aqueous $(^{15}NH_4)_2SO_4$, 99 atom% ^{15}N] over the soil surface of a 200-cm^2 area in each plot, and then simulating a 5-mm rainfall event to wash the $^{15}NH_4^+$ into the soil. Then, 24 h later, we clipped plant shoots in an 80-cm^2 area centered within the labeled 200-cm^2 area. We removed one 1.9-cm diameter soil core from each plot – 15 cm deep in the sandstone soil and 10 cm deep in the more shallow serpentine. We removed roots from each soil core by hand. We extracted soil solution N from a subsample from each soil core using 0.5 M K_2SO_4 and immediately froze the extracts. We determined NH_4^+ and NO_3^- concentrations in the extractions colorimetrically (Lachat 1990). We determined extractable N by Kjehldahl diges-

tion and NH_4^+ in the digestion by colorimetry (Lachat 1990). We concentrated the NH_4^+ from the digested sample using a diffusion technique (Brooks et al. 1989) and then determined ^{15}N content by combustion isotope-ratio mass spectroscopy.

We calculated gross mineralization using the equations of Kirkham and Bartholomew (1954), with two additional assumptions: (1) that the ^{15}N recovered from Kjehldahl digestion and diffusion of the non-fumigated extraction was $^{15}NH_4^+$, and (2) that the pool size of soil solution NH_4^+ did not change. Production of $^{15}NO_3^-$ through nitrification during the 24-h labeling period should not violate the first assumption because Kjehldahl digestion typically does not reduce NO_3^- (Dalal et al. 1984), and the diffusion procedure collects only NH_4^+ (Brooks et al. 1989). Also, in similar experiments on the same soils, we have found no differences in the direction or magnitude of change in NH_4^+ pool size between CO_2 treatments (B.A. Hungate unpublished work), validating the second assumption. We calculated ^{15}N enrichments at 0 h as the amount of ^{15}N added divided by the total NH_4^+ pool size. We measured ^{15}N enrichment and N concentration in plants by direct combustion and mass spectroscopy, and in microbes by chloroform-fumigation extraction followed by direct combustion and mass spectroscopy (Davidson et al. 1991). We calculated gross NH_4^+ uptake rates for plants and microbes using the measured enrichment and an exponential model of ^{15}N decline in the NH_4^+ pool during the 24-h period (Davidson et al. 1991). We express gross mineralization and immobilization on a ground area basis, using measured bulk densities of 0.97 g soil cm^{-3} for the serpentine and 1.18 g soil cm^{-3} for the sandstone (Hungate et al., in press). For each plot, we also calculated the proportion of mineralized NH_4^+ that plants took up and the proportion that microbes immobilized. In the short-term (24-h) assay, these proportions can be larger than 1 (see e.g., Table 1) due to stimulation of $^{15}NH_4^+$ uptake caused by the $^{15}NH_4^+$ addition (Davidson et al. 1991).

We calculated total ^{15}N recovery as the sum of ^{15}N recovered in soil and plant components at the end of the 24-h period. Across all treatments, we recovered 88±4% of the total $^{15}NH_4^+$ applied. Two-way analysis of variance (ANOVA) showed that this percentage did not differ between CO_2 treatments (P=0.24) nor between the serpentine and sandstone grasslands (P=0.94), and the interaction was not significant (P=0.77). Nitrification and subsequent denitrification or leaching of $^{15}NO_3^-$ are the likely pathways through which losses of ^{15}N occurred, reducing ^{15}N recovery. In these soils, the pH is 5.5–6.5 (Luo et al. in press), and soil NH_4^+ concentrations in this study were relatively low, <2 µg N g^{-1} soil (data not shown). Thus, we do not expect NH_3 volatilization to be an important fate of ammonium.

We measured soil moisture gravimetrically and microbial biomass C using the chloroform-fumigation technique (Voroney and Paul 1984) from the same soil cores used for the ^{15}N pool dilution. We removed three 5- to 15-g subsamples from each core. In one, we determined gravimetric soil moisture as weight loss after drying for 48 h at 70° C. We fumigated one of the two remaining subsamples with chloroform vapors for 24 h in a glass desiccator. We incubated each fumigated and non-fumigated subsample in 1-l mason jars for 10 days in the dark. At the end of the incubation, we analyzed the headspace in each jar for CO_2 concentration by gas chromatography (Shimadzu), then determined microbial biomass C as the difference in CO_2 production between fumigated and non-fumigated subsamples, divided by 0.41 to correct for C assimilation during the 10-day incubation (Anderson and Domsch 1978).

We determined relative water use efficiency for the four most common species on each grassland using the $\delta^{13}C$ technique, using the approach and equations described in Jackson et al. (1994). We measured $\delta^{13}C$ by isotope-ratio mass spectroscopy (Europa Scientific, UK) of homogenized shoot material, and calculated carbon isotope discrimination (Δ):

$$\Delta=(\delta_{air}-\delta_{leaf})/(1+\delta_{air}),$$

where δ_{air}=–8‰ in ambient CO_2. In elevated CO_2, the seasonal average δ_{air} was –18.4‰ (monitored in March, April, and May, and adjusted for plant growth in ambient air before chamber placement on 6 January). Then, we determined c_i/c_a (the ratio of intercellular to external CO_2 concentration) using the equations of Farquhar et al. (1989), and calculated the ratio of water-use efficiencies for two leaves experiencing the same leaf-to-air vapor concentration gradient:

$$(A_1/E_1)/(A_2/E_2)=(c_{a1}-c_{i1})/(c_{a2}-c_{i2}),$$

where A/E is the ratio of photosynthesis to transpiration (for leaf 1 or leaf 2). Thus, relative WUE for ambient CO_2 is defined as 1.

Plant production occurs in two phases in these ecosystems (Chiariello 1989). Early season annuals germinate in October and flower and senesce from March to May. Late season annuals also germinate in October, but flower late in the summer and senesce as late as November. In order to characterize total N uptake by the vegetation and its intraannual pattern, we measured plant N pools for early season (April) and estimated plant N pools for late season production. We calculated total plant N uptake for the early season by summing the product of plant mass (shoots and roots) and N concentration in April. In the serpentine, we estimated late-season annual plant biomass in June using an indirect approach based on plant height:biomass relationships (data from Field et al. 1996). We did not measure N concentration in June, but we have found no evidence for CO_2 effects on N concentrations in the late season annuals during vegetative growth (April 1992), nor after seed set (January 1994 and 1995; B.A. Hungate, unpublished work). Thus, we calculated N uptake by late-season annuals as biomass in June times N concentration in April; we calculated total N uptake as early plus late-season N uptake.

Standing litter in January includes both early and late season production from the previous growing season. Because we had no separate measure of late season N uptake in the sandstone, we used the total N pool in standing litter in January 1993 as an index of total annual plant N uptake for 1992. We measured total standing dead plant mass in January by clipping plant shoots in an 80-cm^2 in each plot and weighing the dried material; we also measured N concentration by combustion gas-chromatography. We estimated total N uptake as the mass of standing litter times N concentration. We estimated late season N uptake as the difference between total N pool in standing litter in January 1993 and plant N pools in April 1992 (early season). Because some N is lost with seed release and litterfall over the summer, this underestimates both total N uptake and late season N uptake (see e.g., values for the ambient CO_2 treatment, Table 2). However, this approach provides an index of the effect of elevated CO_2 on total plant N uptake during the 1992 growing season.

When we had comparable measurements in the two ecosystems, we tested for the effects of elevated CO_2 using two-way ANOVA with CO_2 and ecosystems as the main effects. To test for CO_2 effects within each ecosystem for late season plant N uptake, we used single factor ANOVAs. We used analysis of covariance (ANCOVA) to investigate the potential mechanisms of the effects of elevated CO_2 on gross mineralization, using soil moisture and microbial biomass C as covariants. For this analysis, we used data expressed on a per gram soil basis for all three variables.

Results and discussion

Elevated CO_2 caused an increase in the gross rate of NH_4^+ mineralization on both serpentine and sandstone grasslands (two-way ANOVA, P=0.088, Fig. 1A) when plants were approaching their maximum biomass. Elevated CO_2 did not alter gross microbial NH_4^+ immobilization (two-way ANOVA, P=0.866, Fig. 1B). The proportion of mineralized NH_4^+ that was immobilized (two-way ANOVA, P=0.075, Table 1) decreased in elevated CO_2, indicating greater NH_4^+ availability. In no case was the CO_2 by ecosystem interaction significant (two-way ANOVAs, P>0.57, Fig. 1).

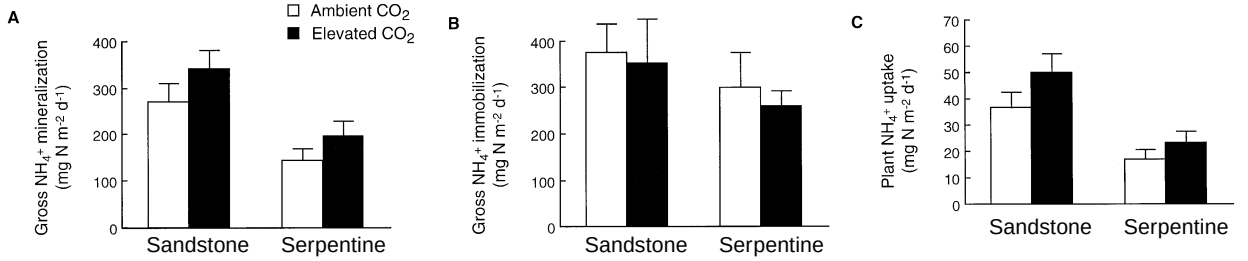

Fig. 1 **A** Gross NH_4^+ mineralization, **B** gross NH_4^+ immobilization, and **C** plant NH_4^+ uptake for 2 April 1992 in serpentine and sandstone grasslands in ambient and elevated CO_2 treatments. Values presented are means±standard errors (n=10)

Plant NH_4^+ uptake increased in elevated CO_2 (two-way ANOVA, P=0.081, Fig. 1C). Though this could occur as a result of a direct CO_2 stimulation of plant NH_4^+ uptake, elevated CO_2 did not alter the proportion of mineralized N that plants took up (two-way ANOVA, P=0.69, Table 1). This suggests that the increase in plant NH_4^+ uptake occurred in response to greater NH_4^+ availability caused by increased NH_4^+ mineralization. Increased plant NH_4^+ uptake accounts for only a part of the extra NH_4^+ mineralized in elevated CO_2. While NH_4^+ mineralization increased by 53–60 mg N m^{-2} day^{-1} in elevated CO_2 (Fig. 1, Table 1), plant uptake only increased by 5–13 mg N m^{-2} day^{-1} (Fig. 1, Table 1). Thus, we cannot account for 75–92% of the extra NH_4^+ mineralized in elevated CO_2. Nitrification and subsequent N losses through nitrate leaching and denitrification are plausible fates for this NH_4^+ (Robertson 1989).

Elevated CO_2 had no effect on total plant N uptake during the early part of the growing season (two-way ANOVA, P=0.96, Table 2) but substantially increased N uptake by the late season vegetation in the serpentine (one-way ANOVA, P=0.04, Table 2) and N pools in litter in January, our index of total N uptake in the sandstone (one-way ANOVA, P=0.01, Table 2). Hence, elevated CO_2 increased plant N acquisition late in the growing season.

We observed no dilution of N in plant tissue in elevated CO_2 in late-season annuals during vegetative growth (two-way ANOVA, P=0.98, Table 2), and found no effect of elevated CO_2 on N concentrations in litter at the end of the growing season (two-way ANOVA, P=0.99, Table 2). Thus, the increase in N uptake shown here is proportional to the CO_2 stimulation of late-season plant production (Field et al. 1996): these plants are able to obtain sufficient N to meet increased growth in elevated CO_2 and do not show sings of increased N stress. A sustained increase in the availability of NH_4^+ – the dominant source of N for these annual plants (Jackson et al. 1989; Jackson and Reynolds 1996) – is the likely cause of the increased annual N uptake by plants with no dilution of N in plant tissue.

Table 1 The proportion of gross NH_4^+ mineralization that plants take up and that microbes immobilize, and absolute changes in plant NH_4^+ uptake and gross NH_4^+ mineralization in response to elevated CO_2. Elevated CO_2 did not alter the proportion of mineralized NH_4^+ that plants take up, but reduced the proportion of mineralized NH_4^+ that is subsequently immobilized. Values for proportions are means±SEM (n=10). Values for absolute changes in response to elevated CO_2 are the differences in means (n=10) between elevated and ambient CO_2 treatments

Ecosystem	CO_2	Proportion of gross mineralization as		Increase in elevated CO_2 (in mg N m^{-2} day^{-1})	
		Plant uptake	Immobilization	Plant uptake	Mineralization
Serpentine	Ambient	0.17±0.05	2.93±0.83	4.6	59.3
	Elevated	0.11±0.02	1.48±0.26		
Sandstone	Ambient	0.15±0.02	1.53±0.30	12.8	52.5
	Elevated	0.17±0.03	1.19±0.33		

Table 2 N concentration (g N per 100 g plant) in late-season annuals during vegetative growth (April) and in litter at the end of the 1992 growing season (January 1993), and early season, late season, and total aboveground plant N uptake for 1992 (g N m^{-2}). Elevated CO_2 caused a 50% stimulation of aboveground N uptake in serpentine and sandstone grasslands. Increased N uptake occurred during the late part of the growing season, the time when the effects of elevated CO_2 on increased soil moisture are most pronounced

Ecosystem	CO_2	N concentration (%)		Plant N uptake for 1992 (g N m^{-2})		
		Late annuals in April	Litter in January	Early N uptake	Late N uptake	Annual N uptake
Serpentine	Ambient	1.33±0.08	0.97±0.03	1.38±0.09	0.47±0.15	1.85±0.17
	Elevated	1.31±0.06	0.97±0.05	1.35±0.08	1.37±0.37	2.73±0.42
Sandstone	Ambient	1.37±0.13	0.74±0.02	2.55±0.49	0	2.10±0.18
	Elevated	1.39±0.06	0.74±0.04	2.33±0.45	0.85	3.18±0.34

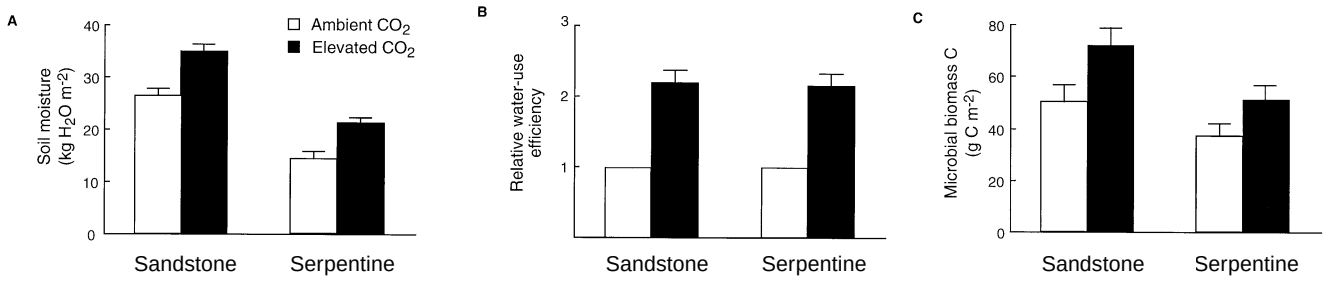

Fig. 2 **A** Soil moisture, **B** relative water use efficiency, and **C** microbial biomass carbon for 2 April 1992. Values presented are means±standard errors (**A** and **C**, n=10; **B** n=4)

We investigated the possible mechanism for increased NH_4^+ availability in April 1992, the time of peak ecosystem biomass. Soil moisture was 40% higher in the elevated CO_2 treatment in both sandstone and serpentine grasslands (two-way ANOVA, P<0.001, Fig. 2A). This reflects decreased stomatal conductance and transpiration (Jackson et al. 1994), and a doubling of whole plant water-use efficiency in the dominant plants on both grasslands (Fig. 2B). Decreased transpiration per unit of leaf area could be compensated by increased leaf area or increased evaporation from the soil surface, but these compensations, if they occurred, were too small to eliminate the effect of elevated CO_2 on soil moisture. Elevated CO_2 also increased microbial biomass C by 27% on the serpentine and by 48% on the sandstone (two-way ANOVA, P=0.011, Fig. 2C). Thus, there was evidence that CO_2 altered both soil moisture and soil C availability.

Analysis of covariance provides correlative support for the hypothesis that increased soil moisture caused the increase in gross NH_4^+ mineralization we observed. The main effect of CO_2 on gross NH_4^+ mineralization was not significant (two-way ANCOVA, P=0.607) when soil moisture and microbial biomass C were included as covariants, indicating that they accounted for the CO_2 effect. However, whereas microbial biomass C was not a significant covariant for gross NH_4^+ mineralization (two-way ANCOVA, P=0.246), soil moisture was a significant covariant (two-way ANCOVA, P=0.016). The stronger relationship between soil moisture and gross mineralization (Pearson correlation, r=0.435, P=0.005) than between microbial biomass C and gross mineralization (Pearson correlation, r=0.070, P=0.668) suggests that increased soil moisture is more likely to be the cause of the higher gross mineralization in elevated CO_2.

These results cannot unequivocally show that increased soil moisture is the mechanism causing increased gross mineralization because we did not independently manipulate soil moisture and CO_2 concentration. Nevertheless, we suggest that increased soil moisture in elevated CO_2 is a plausible and simple explanation for the changes we observed, and that our ANCOVA analysis provides correlative support for this hypothesis. Increased soil moisture can enhance bacterial motility and accessibility to substrates (Hamdi 1971), stimulate protozoan grazing and associated N mineralization (Kuikman et al. 1991), and increase substrate diffusion (Davidson et al. 1990). Any one or combination of these could explain the results we observed.

The changes in N cycling under elevated CO_2 were qualitatively similar in serpentine and sandstone grasslands, though these grasslands differ in many characteristics (Field et al. 1996). The similar patterns in contrasting grassland ecosystems suggest that stimulated N cycling resulting from increased soil moisture under elevated CO_2 may occur in many grassland ecosystems, and possibly in other ecosystems where plant canopies control soil moisture. To date, increased soil moisture or decreased plant water stress under elevated CO_2 has been observed in grassland (Field et al. 1995) and agricultural forb (Clifford et al. 1993) ecosystems, and it is likely to occur in a broad range of water-limited grassland, shrubland, and forest ecosystems (Field et al. 1995). Repeated monitoring at Jasper Ridge using time-domain reflectometry (Topp et al. 1980) in 1993–1995 indicates consistently increased soil moisture in the sandstone grassland under elevated CO_2, both late in the growing season and during midseason dry spells (Field et al. 1995). Some of the measurements indicate smaller CO_2 effects on soil moisture on serpentine than sandstone (A.L. Fredeen, C.P. Lund, and C.B. Field, unpublished work), perhaps as a result of greater evaporation in the serpentine, with a large amount of bare soil (Schulze et al. 1994).

The long-term effects of elevated CO_2 on plant production will depend on a number of factors, including mechanisms that operate on a longer time scale than this study addressed. The direct CO_2 stimulation of photosynthesis is the primary mechanism favoring increased plant production, but nutrient limitation may counteract this response by constraining the CO_2 stimulation of production from the outset, and by exacerbating N limitation through decreased litter quality and reduced N availability to plants (Mooney et al. 1991). Also, increased turnover of roots and exudation of labile C in elevated CO_2 can alter N availability (Zak et al. 1993; Díaz et al. 1993; Körner and Arnone 1993), though the importance of this in natural ecosystems is unknown.

In this study, we suggest that elevated CO_2 stimulated soil N mineralization through increased soil moisture. Though we can not rule out the possibility that increased N mineralization and soil moisture also enhanced N losses (Davidson and Swank 1986; Robertson 1989), we found that greater N mineralization in elevated CO_2 led to increased plant N uptake. In N-limited ecosystems, this CO_2-stimulation of N mineralization through increased soil moisture could release plant growth from N limitation and stimulate plant production.

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