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The influence of atmospheric CO_2 enrichment on plant-soil nitrogen interactions in a wetland plant community on the Chesapeake Bay

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Abstract

We investigated plant and soil nitrogen pools and soil processes in monospecific stands of the C_3 sedge *Scirpus olneyi* and the C_4 grass *Spartina patens* grown in the field in open top chambers in a brackish marsh on the Chesapeake Bay. Stands of *S. olneyi* responded to eight years of elevated CO_2, by increased rates of net ecosystem gas exchange and a large stimulation of net ecosystem production. We conducted our study in the summer of 1994 and 1995 when soil cores were collected and aboveground biomass was estimated. Nitrogen concentration in elevated CO_2 treatments was reduced 15% in stems of *S. olneyi* and 8% in the upper 10 cm of the soil profile. While total plant nitrogen per unit of land area remained the same between treatments, total soil nitrogen showed a non-significant tendency to decrease in the upper 10 cm of the soil profile in elevated CO_2 both years of study. A significant decrease in soil bulk density largely contributed to the observed decrease in soil nitrogen. Exchangeable nitrogen and potential denitrification rates were also reduced in elevated CO_2, but net nitrogen mineralization was unchanged by elevated CO_2 treatment in *S. olneyi* both years. Plants and soils in a pure stand of the C_4 grass, *S. patens*, showed none of these effects of elevated CO_2 treatment. Our data provides evidence of changes in nitrogen dynamics of an ecosystem exposed to elevated CO_2 for eight years; however due to the variability in these data, we cannot say if or how these changes are likely to impact the effect of rising CO_2 on primary production or carbon accumulation in this ecosystem in the future.

Introduction

Since the beginning of the industrial revolution, human activity has had the potential to alter biogeochemical cycling and climate patterns through increases in CO_2 and other greenhouse gases in the atmosphere (Watson et al., 1990). A major goal of current research is to understand how terrestrial ecosystems will respond to this changing environment. Of particular concern is how these changes will affect terrestrial *C* and *N* dynamics.

Carbon and *N* cycles are closely coupled in terrestrial ecosystems. For example, *N* supply often controls the rate of net primary production (NPP). Because both gross primary production (photosynthesis) and plant respiration represent biochemical processes that are catalyzed by *N*-rich enzymes, the rate of these processes depends, in part, on the *N*-content of tissue (McGuire et al., 1995). Within species, there are strong linear relationships between tissue *N*-content and both RuBP carboxylase and chlorophyll content (Evans, 1989; Field and Mooney, 1986). In elevated CO_2 atmospheres, the *N*-content of the tissues is often reduced, reflecting changes in protein concentration, at the level of Rubisco (Van Oosten and Besford, 1995; Jacob et al., 1995) or respiratory proteins (Azcón-Bieto et al., 1994) and greater accumulation of starch and other carbon storage compounds (Drake et al., 1997). Thus, the effect of elevated CO_2 on the concentration of *N* in plant tissues may induce changes in litter quality which in turn influence decomposition and mineralization rates (Melillo et al., 1982),

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altering soil *N* availability. Although elevated CO_2 often results in decreased *N* concentration in green tissues (Drake et al., 1997), litter *N* is not always consequently decreased. The increase in C/N ratios in the litter due to a decrease of its *N* concentration is occasionally seen (Cotrufo et al., 1994) and is species dependent (Kemp et al., 1994). However, there is no conclusive evidence from field experiments for such a change in the rate of litter decay or nutrient cycling as a consequence of elevated CO_2 (O'Neill and Norby, 1996).

On the other hand, increased production in response to elevated CO_2 enhances root growth (Rogers et al., 1994). This effect can lead to a greater exploration of the soil volume, which might increase nutrient uptake. The turnover of a larger root biomass would also increase the availability of additional carbon to the microbes (Diaz et al., 1993; Zak et al., 1993). Reports of enhancement of methanogenesis (Dacey et al., 1994) and soil respiration (Ginkel et al., 1997; Johnson et al., 1994; Körner and Arnone, 1992) support this idea. Also, if elevated CO_2 causes greater inputs of carbon from roots into the soil, *N* availability may increase by enhanced *N* fixation (Luxmoore, 1981) or *N* mineralization (Zak et al., 1993). However, Diaz et al. (1993) showed that an increase in substrates released into the rhizosphere due to elevated CO_2 may lead to nutrient sequestration by microorganisms and a consequent nutritional limitation on plant growth.

We have carried out measurements in order to construct a *N* budget for an ecosystem. Our aim was to determine whether chronic exposure to elevated CO_2 leads to depletion of exchangeable ammonium and, as a consequence, to regulation of the effect of elevated CO_2 on production in a brackish wetland exposed to elevated atmospheric CO_2 over the course of eight years.

Materials and methods

Study site and aboveground measurements

The experimental site is located in a brackish marsh of the Rhode River, a sub-estuary of the Chesapeake Bay where *Scirpus olneyi* (C_3) and *Spartina patens* (C_4) are the two dominant plant species and form monospecific stands. The CO_2 enrichment study has been running since 1987 (Curtis et al., 1989). Within each community five chambers received no CO_2 additions (A) while five chambers were enriched with CO_2 concentrations of 340 μL L^{-1} above ambient levels (E) in open top chambers. Each year CO_2 treatments began in late April before growth of the plants started and terminated at the end of the growing season in mid November.

Aboveground biomass in the *S. olneyi* community was determined in August 1994 and 1995 as described by Curtis et al. (1989). The morphology of *S. olneyi* shoots allows for calculating a direct linear relationship between shoot area and shoot dry weight. After all shoots in a chamber were counted and their length and width measured, six to ten shoots were harvested, measured, and compared to the confidence limits of the regression equations described by Curtis et al. (1989). This relationship was applied to the rest of the shoots in each chamber to estimate the dry weight of the non harvested shoots. *S. patens* biomass was determined in 1994 by harvesting and counting all the shoots from five randomly selected squares of 25 cm^2 in each chamber. The harvested plants were used to calculate the average shoot dry weight per area. Total biomass was estimated by directly extrapolating the weight of the shoots in the harvested area to biomass per square meter of land (see also Drake et al., 1996). Between 5 and 10 shoots were pooled from each chamber, dried at 60 °C for three to four days, and analyzed for total C and N using a CHNS/O analyzer.

Belowground analysis for the growing season of 1994

Two soil cores of 2 cm in diameter and 30 cm depth, were taken from each chamber in late July in the *S. olneyi* and *S. patens* communities (a total of 40). Each core was divided in three segments starting from the top to obtain 0–10, 10–20 and 20–30 cm deep soil samples. Samples were kept in ice during sampling and quickly brought to the lab where they were homogenized and stored at −20 °C until processed. Five subsamples for each depth and CO_2 treatment were used for measurement of exchangeable nitrogen. Ammonium (NH_4^+) was measured in subsamples of 10–15 g fresh weight that were placed in flasks with 100 mL of 2 *M* KCl and were shaken for one hour. The solution was then filtered and the extract was determined colorimetrically by the indophenol blue method (Keeney and Nelson, 1982). Nitrate (NO_3^-) from the extracts was reduced to nitrite by passage through a colum of copperized Cd, and the resulting nitrite was quantitated by a modified Griess-Ilosvay method (Keeney and Nelson, 1982).

Roots were separated from the soil fraction in five samples for each depth and CO_2 treatment. Samples

were rinsed with distilled water and then passed through a 250 μm sieve. We obtained a root fraction composed of fine roots and a fraction composed of peat and mineral soil, which we will call soil. We calculated bulk density as the total dry mass recovered from the core divided by the known core volume. Roots were dried at 60 °C and soils dried at 105 °C until constant weight was obtained, then the material was ground and analyzed for *C* and *N* using a CHNS/O analyzer.

Belowground analysis for the growing season of 1995

Two soil cores of 2 cm in diameter and 10 cm depth were taken from each chamber in either June, August or October from the *S. olneyi* community (a total of 60). Samples were kept in ice during sampling in the field and brought to the lab where samples were homogenized and analyzed immediately for exchangeable N, potential net mineralization and denitrification rates. Subsamples of 10 g fresh soil were analyzed for NH_4^+ and NO_3^- as indicated above. Net *N* mineralization was measured by incubation of 10–15 g of fresh soil, mixed with 5 g of quartz sand, in plastic vessels at 35 °C for 14 days (Keeney, 1982). Soil moisture was determined for each sample and distilled water was added until soil was saturated. After the incubation, soil samples were suspended in 100 mL of 2 *M* KCl and soil inorganic *N* was analyzed as described above. Net *N* mineralization was then calculated by subtracting the pre-incubation soil inorganic-*N* concentrations from the post-incubation soil inorganic-*N* concentrations. Denitrification rates were measured by the acetylene block method by estimating soil denitrifier enzyme concentrations (Tiedje, 1982). Subsamples of 15 g soil fresh weight were placed into 125 mL Erlenmeyer flasks and closed with rubber serum stoppers. Immediately, 5 mL of 5 mg g^{-1} chloramphenicol was added and Erlenmeyers were flushed with N_2 gas for 10 min to make the sample anaerobic. Then, 5 more mL were added containing 0.2 *mM* potassium nitrate and 1.8 *M* glucose and 12 mL of air was replaced by acetylene in the Erlenmeyers flasks. Microbial nitrous oxide (N_2O) evolution was measured over two hours period in which samples were constantly shaked and maintained at 30 °C. Nitrous oxide was detected using a gas chromatograph equipped with a Porapak *Q* column and an electron capture detector that was maintained at 375 °C.

Statistical analysis

All statistical analysis were performed using the Statistical Analysis System (SAS). Multiple analysis of variance was used to test for main effects and interactions of plant species, depth and CO_2 level on *N* concentration and *N*-content of plant tissues and soil, bulk density and *N*-availability in 1994. Two way analysis of variance was used to test for main effects and interaction of CO_2 level and time of the season on all above described variables plus *N*-mineralization and denitrification rates in 1995. Contrast statements were used to determine the significance between interacting variables and differences were considered significant at the $p < 0.1$ level. All reported values are means and standard errors of 5 replicates.

Results

Belowground analysis for the growing season of 1994

The effects of eight years of continuous CO_2 enrichment of pure canopies of *S. olneyi* and *S. patens* on root and soil properties were concentrated on the upper 10 cm of the soil profile (results of the season of 1994, Table 1). Depth was the major factor affecting the levels of *N* concentrations of the soil and in plant roots and of bulk density ($p < 0.001$). Species strongly influenced the soil and root *N* concentration values ($p < 0.001$). A significant interaction between CO_2 level, species and depth was seen for exchangeable *N* (p=0.012), soil *N* concentration (p=0.016) and bulk density (p=0.100).

The concentration of exchangeable *N*, measured as NH_4^+ (NO_3^- was less than 0.05% of NH_4^+ in all treatments, data not shown), reached its maximum values in the upper 10 cm of the soil profile in both plant communities. Exchangeable N was lower in the elevated CO_2 treatment at all depths studied. The reduction in exchangeable N was larger for *S. olneyi* (28–55%) than for *S. patens* communities (10–30%) but was statistically significant only in the 0–10 cm of the soil profile of *S. olneyi* community (p=0.069; Table 1).

The soil *N* concentration was higher in the *S. olneyi* than in the *S. patens* communities. Both communities had the highest *N* concentration in the upper 10 cm of the soil profile, except for *S. olneyi* growing at elevated CO_2, for which soil *N* concentration was significantly lower (p=0.006; Table 1). The total soil *N* content in the upper 10 cm was reduced by 45.2 g N m^{-2} in the *S. olneyi* community at high CO_2 (P=0.006; Table 1).

Table 1. Exchangeable NH_4^+, *N* concentration of soil and roots, total *N* content of soil and roots on a ground area basis and bulk density of intact soil cores of *S. olneyi* and *S. patens* communities. Plants were growing in open top chambers in the field at either ambient (A) or elevated CO_2 (ambient plus 340 μL L^{-1} CO_2, E). Samples were taken in July 1994. Values are averages and standard errors of 5 replicates

Depth cm	NH_4-N μg NH_4^+ g^{-1} dw	[N] soil mg g^{-1} dw	Soil N content g N m^{-2}	[N] Root mg g^{-1} dw	Root N content g N m^{-2}	Bulk density g cm^{-3}
Scirpus olneyi						
0–10						
A	88.4±20.8	2.99±0.03	154.4±21.6	1.64±0.02	129.0±3.7	0.14±0.01
E	39.9±11.8*	2.74±0.06*	109.2±11.2*	1.54±0.06	125/2±9.8	0.11±0.00*
10–20						
A	62.6±14.2	2.65±0.04	287.6±4.6	1.50±0.09	89.2± 9.1	0.17±0.00
E	35.5±8.5	2.59±0.07	255.3±18.0	1.28±0.12	92.4±11.1	0.17±0.07
20–30						
A	48.5±7.6	2.75±0.08	215.5±15.3	1.71±0.15	103.2±9.6	0.15±0.01
E	34.7±3.4	2.76±0.08	227.3±29.3	1.65±0.11	97.4±4.5	0.14±0.01
Spartina patens						
0–10						
A	114.7±20.8	2.24±0.07	104.0±12.0	1.06±0.04	58.4±9.0	0.09±0.01
E	102.1±13.9	2.32±0.05	122.5±15.2	1.02±0.03	62.2±9.5	0.11±0.02
10–20						
A	96.1±11.1	2.02±0.05	162.7±14.6	1.42±0.05	127.3±9.4	0.17±0.02
E	67.1±6.2	2.04±0.02	160.7±14.9	1.15±0.05*	101.4±21.2	0.15±0.02
20–30						
A	87.4±11.1	2.18±0.08	142.1±7.2	1.46±0.11	127.3±24.6	0.15±0.01
E	64.2±11.2	2.03±0.08	151.8±6.8	1.65±0.09	115.8±11.4	0.14±0.01

With similar root mass between ambient and elevated CO_2 (not shown), only 20% of the reduction in total soil *N* content was accounted for the reduced soil *N* concentration. Soil *N* concentration and soil *N* content in the *S. patens* community were not affected by elevated CO_2 at any depth studied (Table 1).

Elevated CO_2 had little effect on the *N* concentration of roots (Table 1). While root *N* concentration of *S. olneyi* was similar across the soil profile, the root *N* concentration of *S. patens* plants increased with depth (Table 1). The total amount of root *N* in the top 30 cm of soil on area basis was not affected by either CO_2 levels or plant species (Table 1). However, nearly 40% of total root *N* content in the *S. olneyi* communities grown at either ambient or elevated CO_2 was allocated on the top 10 cm of soil, whereas only 20% of total root *N* of *S. patens* was seen in the first 10 cm of soil.

Soil bulk density was significantly lower (21%) in the upper 10 cm in the *S. olneyi* community in the elevated CO_2 treatment (p=0.100; Table 1). Bulk density was lower in the upper 10 cm when compared to deeper soil profiles in the *S. patens* community, but it was no effect of elevated CO_2 (Table 1). CO_2 effects on bulk density were not seen at depths below 10 cm at either plant community. Soil water content was 89% in the *S. olneyi* and 85% in the *S. patens* community and it was not changed by CO_2 treatment or soil depth.

Belowground analysis for the growing season of 1995

Throughout the growing season of 1995, soil analysis were made only in the 0–10 cm of the soil profile in the *S. olneyi* community, because results obtained in 1994 revealed that the main effects of elevated CO_2 were occurring in the upper soil layer of this species. Sampling

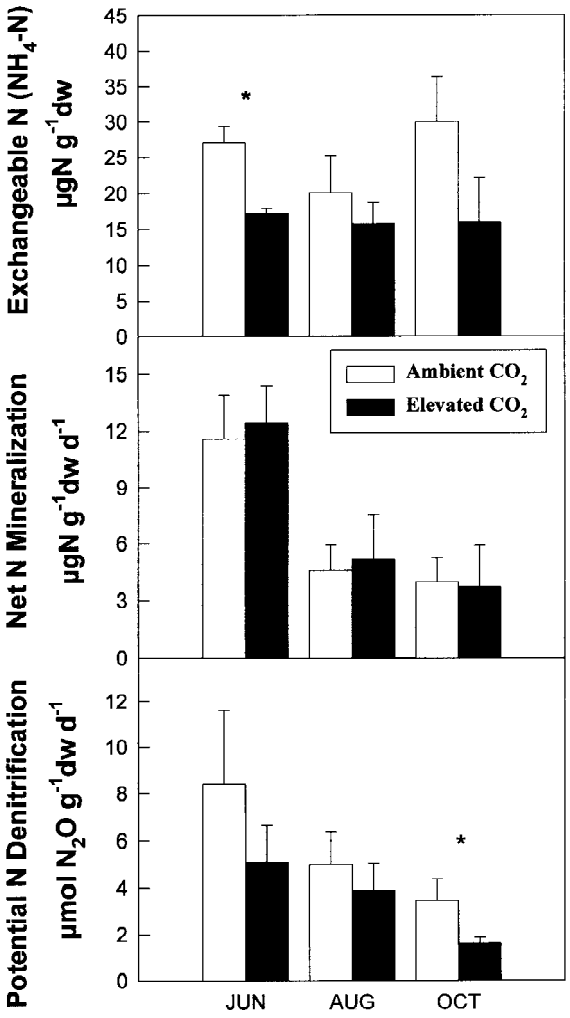

Figure 1. Exchangeable NH_4^+, Net N mineralization and Potential denitrification rates in *S. olneyi* community in 1995. Plants were exposed to either ambient (open bars) or elevated CO_2 (ambient plus 340 μL L^{-1} CO_2; solid bars) in open top chambers in the field. Samples were taken in June, August and October 1995. Values are averages of 5 replicates±standard error. * indicates significant differences.

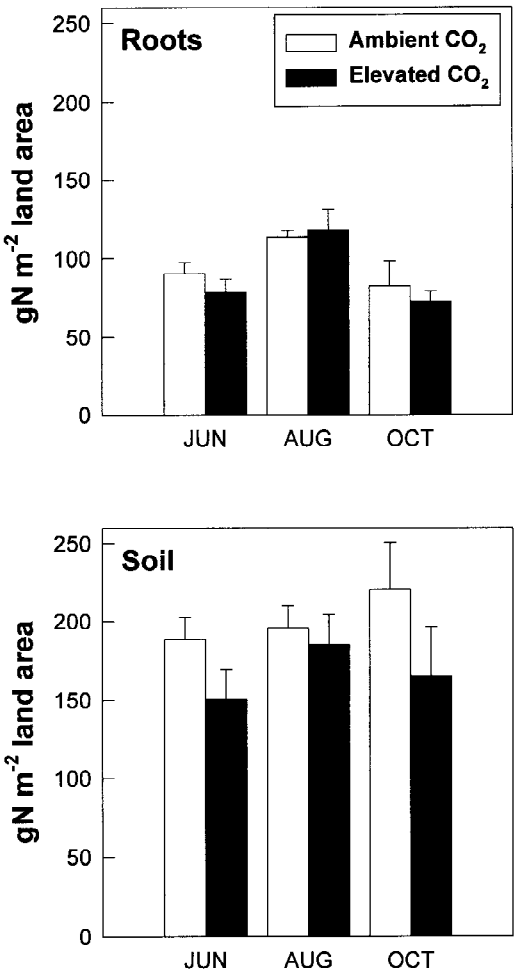

Figure 2. Total N-content per unit of land area in roots and soil of *S. olneyi* community in 1995. Samples were collected under *S. olneyi* plants exposed to either ambient (open bars) or elevated CO_2 (ambient plus 340 μL L^{-1} CO_2; solid bars) in open top chambers in the field. Values are averages of 5 replicates±standard error.

time during the growing season significantly affected *N* mineralization (p=0.016), soil and root *N* concentrations (p <0.005) and bulk density (p=0.048). No interaction between growing season sampling time and CO_2 level was found.

Net *N* mineralization rates were higher in June than August or October and they were not affected by elevated CO_2 (Figure 1). The exchangeable soil *N* concentration was not influenced by season but it was always lower in elevated CO_2 and it was significantly lower in June (p=0.066; Figure 1). Potential denitrification rates were marginally affected by season and they were lower in elevated CO_2 treatments throughout the season being significantly different in October (p=0.100; Figure 1).

Elevated CO_2 affected soil *N* concentration that was reduced by an average of 6.5% throughout the season and it was significantly lower in August and October (p <0.100; Table 2). The *N* concentration in the root fraction did not change significantly with CO_2 treatments (Table 2). Soil bulk density was reduced by CO_2 enrichment throughout the season averaging 19% and being significantly lower in June and October (p <0.100; Table 2). Mean total root *N* content was affected by sampling time but not by CO_2 treatment

Table 2. N concentration [N] of root and soil and soil bulk density of *S. olneyi* in intact soil cores taken in June, August and October 1995 to a depth of 10 cm. *S. olneyi* was growing in open top chambers in the field at either ambient (A) or elevated CO_2 (ambient plus 340 μL L^{-1} CO_2, E). Values are averages and standard errors of 5 replicates

Year Period	[N] Soil mg g^{-1} dw	[N] Root mg g^{-1} dw	Bulk density g cm^{-3}
June			
A	2.46±0.06	1.16±0.08	0.15±0.016
E	2.47±0.01	1.13±0.01	0.12±0.005*
August			
A	2.56±0.03	1.38±0.05	0.17±0.001
E	2.44±0.05*	1.36±0.04	0.15±0.007
October			
A	2.29±0.06	1.36±0.08	0.16±0.016
E	2.16±0.07*	1.31±0.03	0.12±0.024*

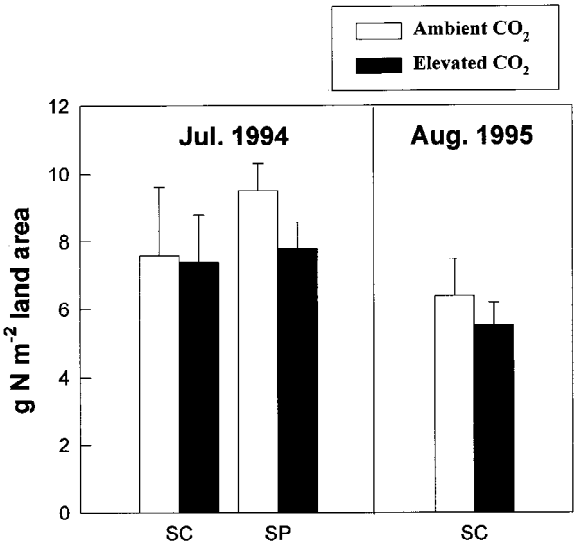

Figure 3. Total N-content per unit of land area in July of 1994 and August 1995 in *S. olneyi* and *S. patens* communities exposed to either ambient (open bars) or elevated CO_2 (ambient plus 340 μL L^{-1}; solid bars) in open top chambers in the field. Values are averages of 5 replicates±standard error.

(p=0.023 and p=0.1855 respectively) and mean total soil *N* content was not affected by sampling time but it was 19% lower in elevated CO_2 (p <0.100) (Figure 2). These results were similar to the ones found for 1994 (Table 1).

Aboveground analysis in the growing season of 1994 and 1995

Elevated CO_2 did not affect aboveground biomass per unit of land area in the 1994 and 1995 growing seasons at either plant community (Table 3). However, elevated CO_2 in *S. olneyi* resulted in a significant reduction in the shoot *N* concentration of 13% in the season of 1994 and of 19% in 1995 (Table 3). Leaf and shoot *N* concentration of *S. patens* were unchanged in 1994 (Table 3). The carbon concentration of aboveground tissues of *S. olneyi* and *S. patens* did not change between growing seasons or within CO_2 treatments (not shown). Therefore, the C/N ratios of shoots of *S. olneyi* increased significantly in the two growing seasons studied in plants grown at elevated CO_2, but they were not affected by elevated CO_2 in *S. patens* (Table 3). Despite the decrease in tissue *N* concentration in *S. olneyi*, total aboveground *N* content per unit of land area was the same for ambient and elevated CO_2 (Figure 3).

Discussion

A decrease in exchangeable soil N was consistently observed for both plant communities in 1994 and for *S. olneyi* in 1995. Although, owing largely to the high variability of the values, this was a statistically significant effect only in the upper 10 cm of the soil profile in the *S. olneyi* plant community in 1994 and in June of 1995. At any time, the exchangeable NH_4^+

Table 3. Aboveground biomas per unit of land area, concentration of N and C/N ratio in green tissues for stands of *S. olneyi* and *S. patens* during August. Plants were growing in open top chambers in the field at either ambient (A) or elevated CO_2 (ambient plus 340 μL L^{-1} CO_2, E). Values are averages and standard errors of 5 replicates

$[CO_2]$ μL L^{-1}	Biomass g m^{-2}		[N] Shoot mg g^{-1}dw		C/N	
	1994	1995	1994	1995	1994	1995
Scirpus olneyi						
A	511±113	291±67	1.5±0.1	1.6±0.10	29.4±1.7	27.0±1.4
E	586± 74	368±42	1.3±0.1*	1.3±0.01*	34.8±1.8*	33.3±1.7*
Spartina patens						
A	1006±91		0.94±0.03		48.1±1.6	
E	852±87		0.91±0.01		49.9±0.8	

concentration in the soil represent the net result of *N* mineralization, plant uptake, immobilization by microorganism, fixation in clay material and nitrification (Schlesinger, 1997). In our study, as much as 18% of the reduction in soil NH_4^+ could be due to the reduction of bulk density and the associated reduction in the substrate where NH_4^+ is tied up in the *S. olneyi* community. The decrease in bulk density in the elevated chambers is due to a small increase in root biomass and to a large decrease in peat dry matter (Matamala, 1997). Still, after taking in account the effect of loss of peat, exchangeable NH_4^+ in elevated CO_2 is reduced by 35 and 18.5% in 1994 and 1995 respectively. Elevated atmospheric CO_2 treatment did not alter the potential for *N* mineralization in soils maintaining *S. olneyi*. Other studies have shown no changes in *N* mineralization rates and *N* availability in elevated CO_2 (Ross et al., 1995). However, Zak et al. (1993) reported increased *N* mineralization under elevated CO_2, suggesting that increases in microbial populations, mediated by an increase in root exudates, would increase *N* availability for plants growing in elevated CO_2 atmospheres. Other authors have concluded that root-induced *N* mineralization could not contribute substantially towards plant *N* acquisition (Griffiths and Robinson, 1992; Griffiths, 1993). Since the total standing crop *N* did not change in *S. olneyi* in elevated CO_2 (see also Curtis et al., 1989), the enhancement of microbial processes such as *N*-fixation (Drake, unpublished data), methane production (Dacey et al., 1994) and heterotrophic respiration (Ball and Drake, 1997; Gonzàlez-Meler, 1995) would suggest that soil microorganisms incorporate and immobilize *N* in soils of *S. olneyi* stands exposed to elevated CO_2.

We also investigated potential denitrification rates in elevated CO_2 treatments as a mechanism for the observed reduced *N* availability in *S. olneyi*. Denitrification is the most important mechanism returning *N* from terrestrial ecosystems to the atmosphere and is a major loss of *N* in most parts of a salt marsh ecosystem (Kaplan et al., 1979). Since CO_2 enrichment appears to increase the rhizosphere carbon (Kuikman et al., 1991; Lekkerkerk et al., 1990) elevated CO_2 would increase denitrification rates contributing to the release of *N* compounds to the atmosphere and subsequent loss of *N* in soil fractions. Ineson et al. (1998) found higher N_2O-N production beneath *Lolium perenne* growing under high N inputs and elevated CO_2. However, our results show that potential denitrification rates were reduced in soil cores taken from *S. olneyi* community exposed to elevated CO_2. Denitrification rates are often dependent on availability of reduced organic compounds in the soil, but, other resources (e.g. NO_3) can limit denitrification (Haider et al., 1987). It is likely that nitrification rates would be inhibited because of the decrease in *N* availability. Hungate et al. (1997) showed similar results in a California grassland where greater *N* immobilization in microbes lead to a decrease in nitrification rates in nutrient-rich soils exposed to elevated CO_2.

Diaz et al. (1993) suggested that high CO_2 exposure would produce a reduction in nutrient availability due to a greater immobilization of nutrients by microbes which would produce a consequent nutritional limitation for plant growth, limiting the CO_2 response. However, *S. olneyi* stands grown in elevated CO_2 have shown enhanced ecosystem photosynthesis rates since the beginning of the project in 1987 (Arp and Drake, 1991; Long and Drake, 1992; Drake et al., 1996) and

in 1994, ecosystem photosynthesis was increased by 51% in *S. olneyi* grown in elevated CO_2 (Drake et al., 1997). This increase in net ecosystem CO_2 photosynthesis results in an increase in the *N*-use efficiency because there was no increase in *N*-uptake. Moreover, photosynthetic tissues of *S. olneyi* grown in the field showed acclimation of photosynthesis to elevated CO_2 mediated by a decrease in the Rubisco-*N*-content and in total soluble proteins. Despite lower enzyme contents, *S. olneyi* exposed to elevated CO_2, maintained higher rates of photosynthesis per unit of leaf area (Jacob et al., 1995) indicating greater *N* use efficiency.

Long-term atmospheric CO_2 enrichment resulted in a decrease of the total soil *N*-content by 29.3% in the upper 10 cm of the soil of the community dominated by the sedge *S. olneyi* in July 1994, and similar values were obtained throughout the season of 1995. This reduction in soil *N*-content is the result of the combined effects of a reduction in the bulk density and a decrease in the *N* concentration of the soil fraction. As much as 80% of the observed decrease in soil *N*-content can be explained by the decrease in bulk density in the upper 10 cm of soil in the *S. olneyi* community in the elevated CO_2 treatment. As peat is the fraction with the highest percentage of *N* in soils of this salt marsh, any reduction in the amount of peat will produce a marked decrease in total soil *N*. We do not have a unique explanation for the loss of peat. Lower soil density could be due to a larger peat decomposition, higher soil respiration rates in the elevated CO_2 chambers as it was shown by Gonzàlez-Meler (1995) during the summer of 1992 could be the indirect evidence of this effect. Also, lower soil density could be the result of a larger root biomass, for example Curtis et al. (1990) reported an increase of 83% in root biomass in *S. olneyi* community using regrowth cores. The increase in fine root biomass in regrowth cores has generally been maintained throughout the years of CO_2 exposure of the salt marsh ecosystem (Drake et al., 1996). Such an increase reflects a great potential of the belowground parts of *S. olneyi* to respond to elevated CO_2 as well as for changes in the root-soil volume relationships. Both effects together could produce changes in marsh elevation that could explain this reduction in bulk density.

Despite the possible reduction in *N* availability for plants exposed to elevated CO_2 in this wetland ecosystem, there is no evidence that net ecosystem carbon assimilation is limited after eight years of CO_2 exposure. We can not conclude if this ecosystem has reached a new equilibrium or if these shifts in *N* dynamics will determine long term responses of salt marshes to elevated CO_2.

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