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REGULAR ARTICLE

# Uptake of organic nitrogen by coastal wetland plants under elevated $CO_2$

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**Abstract**

*Aims* This study was conducted to answer the question of whether elevated $CO_2$ and the presence or absence of inorganic nitrogen ($NH_4^+$) affect the uptake of different forms of organic nitrogen in two dominant saltmarsh species *Schoenoplectus americanus* and *Spartina patens*.

*Methods* *S. americanus* and *S. patens* were grown under elevated and ambient $CO_2$ conditions and a series of hydroponic assays were conducted using dual labelled $^{13}C^{15}N$– glycine, glutamic acid and urea supplied in both the absence and presence of $NH_4^+$.

*Results* Results show rates of glycine and urea uptake were lower under elevated $CO_2$ conditions for both species. Ratios of $^{13}C$ and $^{15}N$ in *S. patens* roots showed that at least 68 and 79% of glycine under ambient and elevated $CO_2$, respectively, was taken up intact. Provision of $NH_4^+$ with organic N caused organic N uptake rates to decline by up to 75% in *S. americanus* and up to 50% in *S. patens* compared with plants that only received organic N.

*Conclusions* The reduction in organic N uptake in the presence of $NH_4^+$ suggests that plants rely primarily on mineral N in the field. In addition, we can deduce that organic N uptake is not likely to supply plants with the additional N required under elevated $CO_2$, and that the repressive effects of elevated $CO_2$ on organic N uptake may have negative consequences for ecosystem productivity and carbon sequestration.

**Keywords** Elevated $CO_2$ · Organic nitrogen · Saltmarsh · *Schoenoplectus americanus* · *Spartina patens* · Carbon sequestration

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## Introduction

Anthropogenic activities are having pervasive impacts on the global climate system, including enrichment of

the atmosphere with increasing levels of $CO_2$. The capacity of terrestrial and marine ecosystems to sequester $CO_2$ from the atmosphere is of critical importance as climate adaptation and mitigation strategies are being developed. Coastal wetlands are amongst the most effective ecosystems at sequestering carbon due to high rates of primary productivity and a unique ability to preserve carbon in sediments (Chmura et al. 2003; Fourqurean et al. 2012). As a key determinant of plant growth, nitrogen (N) plays a pivotal role in controlling carbon sequestration rates in coastal wetlands (Morris et al. 2002). Indeed, it is widely considered to be among the most important factors limiting the productivity response of ecosystems to elevated $CO_2$ (Reich et al. 2006; Huang et al. 2015; Terrer et al. 2016). Understanding how plants utilize nitrogen as the atmosphere becomes increasingly enriched in $CO_2$ is key to ensuring accurate predictions of how coastal wetlands will respond to global change, and also to assess their capacity to sequester carbon.

Plants take up N primarily in the inorganic forms $NH_4^+$ and $NO_3^-$. However, organic forms of soil N can also be rapidly acquired by both mycorrhizal and non-mycorrhizal plants, such as temperate crops (Schobert and Komor 1987; Jones and Darrah 1994; Näsholm et al. 2001), boreal grasses (Näsholm et al. 1998), tropical trees and shrubs (Turnbull et al. 1996), Arctic and alpine grasses, sedges and shrubs (Chapin et al. 1993; Kielland 1994; Raab et al. 1996) and Arctic and temperate saltmarsh species (Henry and Jefferies 2003a; Mozdzer et al. 2010). Indeed, plants possess a wide variety of proteins that facilitate the uptake and partitioning of different forms of organic N such as amino acids, peptides, nucleotides, and urea (Rentsch et al. 2007; Kojima et al. 2006). This versatility of plants in acquiring different forms of N presents challenges to understanding the $CO_2$-induced plant growth enhancement, which has been predicted to diminish as N becomes limited (Luo et al. 2004). Studies have focussed on putative increased uptake and utilisation of organic N under elevated $CO_2$ due to increased inorganic N limitation, but to date have found limited supporting evidence (Hofmockel et al. 2007; Jin and Evans 2010; Chen et al. 2015).

Plant N concentrations generally decline under elevated $CO_2$ (Cotrufo et al. 1998; Long et al. 2004; Taub and Wang 2008). Explanations for this decline include a dilution of Rubisco (Wong 1990; Kuehny et al. 1991; Gifford et al. 2000) and a reduction of transpiration-driven mass flow of N through soils and into plants (McDonald et al. 2002). Recent evidence also suggests that, at least for $C_3$ plants under $NO_3^-$ nutrition, $CO_2$ enrichment can decrease tissue N directly by inhibiting shoot $NO_3^-$ assimilation (Bloom et al. 2010). In contrast, elevated $CO_2$ did not interfere with $NO_3^-$ uptake and assimilation in $C_4$ plants (Bloom et al. 2012). The few studies that have investigated the effect of elevated $CO_2$ on organic N uptake have found diverging results. Elevated $CO_2$ was observed to increase organic N uptake in the desert shrub *Larrea tridentate* (Jin and Evans 2010) but was found to have no effect on the uptake of organic N in grassland species or temperate pine species (Hofmockel et al. 2007; Andresen et al. 2009; Chen et al. 2015). The direct effect of $CO_2$ on N uptake and/or assimilation may have implications for the widely held view that N availability constrains the $CO_2$ fertilization effect. Indeed, evidence from the decennial timescale covered by FACE studies suggests that N limitation of plant growth is associated with negative effects of elevated $CO_2$ on plant N acquisition (a direct effect) at neutral to modest shifts in productivity, rather than with growth dilution of plant N or processes leading to progressive N limitation (indirect effect) (Feng et al. 2015). Likewise, the absence of N limitation is associated with enhanced N acquisition (Finzi et al. 2007; Iversen 2010). Understanding the link between rising $CO_2$ and plant N uptake is particularly important in wetland ecosystems where a large fraction of the available soil N is in organic form, particularly in marshes in mid to high latitudes (Mozdzer et al. 2010, 2014; Fig. S1).

Coastal wetlands provide valuable ecosystem services such as coastal protection and biodiversity support (Zedler and Kercher 2005; De Groot et al. 2006). Recent research from long-term coastal wetland studies has revealed the importance of plant traits in the context of ecosystem responses to global change factors. *Spartina patens*, a $C_4$ grass, dominant in North American coastal wetlands, exhibited modest to neutral shifts in productivity in response to elevated $CO_2$ while *Schoenoplectus americanus*, a $C_3$ sedge showed modest to large shifts in productivity (Erickson et al. 2013). In addition, Langley and Megonigal (2010) demonstrated that plant community shifts can act as a feedback that alters the whole ecosystem response to elevated $CO_2$ concentrations. This was in part due to differences in the kinetics of inorganic N uptake of the two dominant saltmarsh species, *S. patens* and *S. americanus* (Cott et al. 2018), with

greater uptake rates for $NO_3^-$ and $NH_4^+$ recorded for *S. patens,* allowing biomass of *S. patens* to respond strongly to N addition. In addition, *S. patens* has been recorded to have mycorrhizal associations (Cooke et al. 1993; Hoefnagels et al. 1993) which are highlyeffective at acquiring and supplying limiting nutrients suchas N. Hence, differences in how these species utilize N can have important ecosystem-level impacts. Although it has long been known that most plant species can acquire organic N, no studies to date have explored the effects of elevated $CO_2$ on organic N uptake in coastal wetland plant species.

Our objectives were to: (1) experimentally investigate the ability of a $C_4$ saltmarsh grass species *Spartina patens* (Aiton) Muhl. and a $C_3$ saltmarsh sedge *Schoenoplectus americanus* (Pers.) Volkart ex Schinz and R. Keller to directly acquire organic N, (2) to examine the effect of an elevated $CO_2$ atmosphere on organic N uptake, and (3) to determine if the co-provision of inorganic N affected the uptake of organic N. We hypothesized that both species have the ability to acquire organic N, but that *Spartina patens* would have greater organic N uptake rates due to possible mycorrhizal colonization, organisms that are known to effectively take up organic N. Further, we hypothesized that despite an increased demand for N under elevated $CO_2$ conditions, uptake of organic N would be lower under elevated $CO_2$ conditions, particularly for *S. patens,* based on evidence from FACE studies in which elevated $CO_2$ suppressed plant N acquisition at neutral to modest shifts in productivity (Feng et al. 2015). Finally, the co-occurrence of different N compounds in the soil can influence the uptake of both inorganic and organic N (Czaban et al. 2016), so we hypothesized that the presence of $NH_4^+$ (the dominant form of N in wetland soils, Keller et al. 2009) would negatively affect the uptake of organic N.

## Materials and methods

### Chamber set up and site

Nutrient uptake experiments were conducted in a set of six plant growth chambers located at the Smithsonian Environmental Research Center, Maryland, USA. The chambers were 1.0 m wide × 0.7 m deep × 1.0 m high, with wooden frames covered with clear polyester film, and equipped with a blower for drawing ambient air through the chamber. Three chambers were maintained at ambient $CO_2$ and three at elevated $CO_2$ (ambient +400 ppm $CO_2$). Air from each chamber was sampled by an automated manifold system, measured for $CO_2$ concentration using an infrared gas analyzer (Li-7000; Licor, Lincoln, NE, USA), and adjusted manually to a set $CO_2$ concentration target on a daily basis.

### Experimental conditions

Emergent shoots and rhizome fragments of *S. americanus* and *S. patens* were collected from Kirkpatrick Marsh, Edgewater, Maryland (38.8742° N, 76.5474°W) in spring 2015. After collection, material was washed and individual shoots were planted in 6.3 × 6.3 × 25 cm pots filled with clean sand to allow for easy transfer into a hydroponic media. Pots were placed in ambient and elevated $CO_2$ chambers and fertilized weekly with a medium of 300 ppm solution of Peters 20–20-20 (N:P:K; containing 13% Urea; Griffin Greenhouse Supplies Inc.) fertilizer amended with 100 ppm glycine and glutamic acid (Sigma) to induce transporters for potential uptake capacity of these N-containing amino acids. As chambers were located outside, plants were exposed to a range of light conditions up to full sunlight (1500 μmol $m^{-2}$ $s^{-1}$) and experienced natural temperature fluctuations, with a mean monthly average of 23.4 °C. After 3 weeks plants were gradually acclimated to 8 ppt water to reflect the salinity of Kirkpatrick marsh. Within 8 weeks, individual plants achieved a suitable root mass (>100 mg dw).

### Uptake experiments

To investigate the uptake of organic N, we conducted a series of experiments by presenting individual plants with different sources of N. Experiments were adapted from Epstein et al. (1963) and Mozdzer et al. (2010) to calculate uptake and assimilation of labeled organic N compounds into root tissue. Whole plants, rather than excised roots were used since excised roots can underestimate nutrient uptake (Falkengren-Grerup et al. 2000). The amino acids glycine and glutamic acid were chosen along with urea as these compounds are taken up by different transporters, glycine is the third most abundant amino acid at the field site (Table S1), and urea constitutes up to 8% of total nitrogen of porewater in East Coast USA tidal marshes (Mozdzer et al. 2014). In addition, these N forms have been used widely in other

studies. However, the concentration of amino acids and urea used in these experiments are lower than previous studies (Falkengren-Grerup et al. 2000; Mozdzer et al. 2010; Chen et al. 2015; Ma et al. 2018) but in line with the range of concentrations of N found in porewater at Kirkpatrick Marsh (Supplementary Information Fig. S1, S2; Keller et al. 2009). Plants were exposed to elevated and ambient $CO_2$ conditions for the duration of the growth period and in addition all assays took place under elevated and ambient $CO_2$ conditions.

Uptake rates of the individual organic N compounds were determined for 3 replicate plants in each chamber (9 plants in total) over a 45-min treatment period. At the beginning of all experiments, plants were rinsed free of sand using tap water, then equilibrated overnight in a solution of 0.50 mM $CaCl_2$ adjusted to 8 ppt salinity with synthetic sea salts (Instant Ocean) within experimental chambers. After the equilibration period, plant roots were immersed for 45 min in 500 ml of aerated, well-mixed solution identical to the equilibration medium, but containing 50 μM N uniformly dual labelled $^{15}N$ $^{13}C$ glycine, glutamic acid, or urea (>98% enriched; Cambridge Isotope Laboratories, Andover, MA, USA). Preliminary studies measuring depletion of the substrate over time demonstrated a linear uptake of $NH_4^+$ and urea–N for the initial 90 min. Control treatments (only the equilibration medium) were run in parallel in order to assess natural isotopic abundances. After exposure to the media, roots were rinsed thoroughly with 1 mM KCl to remove possible excess isotope label adsorbed to the root surface. To minimize potential diurnal effects in nutrient uptake, experiments were conducted at approximately the same time each morning (09:00–11:00), which coincides with peak photosynthetic activity in situ. Whole root systems were then excised, dried to a constant weight at 60 °C, weighed, and milled in a ball mill to a fine powder. Rhizomes were not included in the analysis. Root samples were analyzed at the Smithsonian Stable Isotope Facility for stable isotope ($^{13}C$, $^{15}N$) concentrations using a GRAVITAS Thermo Scientific Delta V Advantage mass spectrometer coupled with a Costech ECS 4010 elemental analyzer.

A further set of experiments were carried out to determine if organic N uptake took place in the presence of inorganic N. Experimental conditions were identical to above except 50 μM $NH_4^+$ (unlabeled) was added to the solution containing 50 μM $^{15}N$ labeled glycine, glutamic acid or urea.

### Michaelis–Menten parameters

An additional set of assays were carried out to determine the Michaelis-Menten parameters by exposing individual plants to varying concentrations of labelled organic N. Experimental conditions were identical to above except that plants were exposed to 5 different $^{15}N$ concentrations for each compound (glycine, glutamic acid and urea) for 45 min (5, 10, 25, 50 and 100 μM) ($n = 3$ individual plants for each species, per N concentration). Reaction volumes of the assays were adjusted to 2000, 1000, 1000, 500, 500 ml for the above concentrations respectively, to ensure drawdown of the substrate was not more than 10% of the starting concentration. The mean drawdown of nitrogen was 0.04 μM $g^{-1}$ $min^{-1}$ ranging from 0.01 to 0.09 μM $g^{-1}$ $min^{-1}$. Roots were processed as described above.

### Calculations

Uptake rates of $^{15}N$ and $^{13}C$ ($V_{uptake}$) for individual plants were calculated from the mass of labeled isotope that was acquired ($m_{acq}$, in μg) (adapted from Hauck and Bremner 1976; Mozdzer et al. 2010)

$$m_{acq} = \frac{m_1 \left(APE_{samp} - APE_{ctrl}\right)}{APE_{treat}} \quad (1)$$

$$V_{uptake} = \frac{\left(m_{acq}/MW_{treat}\right)}{\left(m_2\, t_{exp}\right)} \quad (2)$$

where $m_1$ is the mass of N or C in the sample (in μg), $APE_{samp}$ is the atom % excess $^{15}N$ or $^{13}C$ of the root sample exposed to a labeled substrate, $APE_{ctrl}$ is the atom % excess $^{15}N$ or $^{13}C$ in the control root sample, $APE_{treat}$ is the atom % excess of the labeled $^{15}N$ or $^{13}C$ treatment (98%), MW is the molecular weight of the N or C isotope, $m_2$ is the dry root mass of the sample (in grams), and $t_{exp}$ is the duration of the exposure to labeled substrate (in minutes).

The $^{15}N$ uptake rates from each exposure series ($n$ = 15 plants) were then fit to the Michaelis-Menten equation in order to derive values of maximal uptake rate ($V_{max}$) and the substrate concentration at which the rate is 50% of $V_{max}$ ($K_m$):

$$V_{uptake} = \frac{V_{max}[c]}{K_m + [c]} \quad (3)$$

where [c] is the concentration of $^{15}$N-glycine, glutamic acid or urea, $V_{max}$ (in μmol $^{15}$N $g^{-1}$ $h^{-1}$) provides a measure of uptake capacity under saturating N conditions, while $K_m$ (in μM of organic N substrate) provides an estimate of the species' affinity for organic N; smaller values corresponding to greater affinity. Curve fitting was carried out in R 3.4 using a non-linear regression function (SSmicmen from the nlstools library). To determine if there were differences among species and/or $CO_2$ levels, we used bootstrapping to compute 95% confidence intervals for all parameter estimates (via nlsBoot, also from nlstools; $n = 999$ iterations). Estimates were considered different if there was no overlap between pairs of bootstrapped 95% confidence intervals (Christiansen et al. 2016)

### Mycorrhizal determination

To determine if species were colonized by arbuscular mycorrhizal fungi (AMF), 12 plants of each species were harvested and tested using the trypan blue staining method modified from Kormanik and McGraw (1982). The roots were autoclaved with 10% KOH at 121 °C for 15 min to clear the roots of colouration and subsequently stained with 0.05% *w/v* trypan blue in lactoglycerol. Colonization was estimated using the grid-line intersection method (Giovannetti and Mosse 1980).

### Data analysis

Differences among experimental factors ($CO_2$, N treatment and species) with respect to $^{15}$N uptake rate were evaluated with ANOVA-type linear models in R version 3.0.2. Separate models were fitted to data for $^{15}$N uptake with and without $NH_4^+$ present. Both models had the same form. The mean of three replicates within each chamber was considered the experimental unit so that $n = 3$ per treatment. Transformations to response variables (natural log) were made if residuals were not normal and homoscedastic. Terms for all possible two and three way interactions were also included. Tukey-adjusted pairwise comparisons were subsequently made among species and $CO_2$ level combinations; the family-wise error rate was held at 0.05. Analysis of covariance (ANCOVA) was used to determine differences in the slopes of the regression lines for $^{13}$C:$^{15}$N ratios under elevated and ambient $CO_2$ conditions.

## Results

We performed a series of dual labelled $^{13}$C$^{15}$N uptake experiments to test if the saltmarsh species *S. americanus* and *S. patens* have the capacity to acquire organic N, and if elevated $CO_2$ conditions and the co-provision of $NH_4^+$ affected organic N uptake rates. $^{15}$N assimilation was observed in both *S. americanus* and *S. patens* for each N treatment (Fig. 1a), with significant differences between species ($p < 0.05$) and N treatments ($p < 0.001$) (Table 1). Species differed in their response to N treatments (Species x N treatment, $p < 0.05$). *S. patens* had higher uptake rates than *S. americanus* for both glycine and glutamic acid, whilst *S. americanus* had the highest urea uptake rates.

Overall, we observed a trend for $CO_2$ to negatively affect $^{15}$N uptake ($p < 0.05$) (Table 1). In pairwise comparisons, glutamic acid uptake was significantly reduced in *S. americanus* under elevated $CO_2$ conditions compared with ambient conditions and similarly for *S. patens,* urea uptake was significantly reduced under elevated $CO_2$ (Fig. 1a). Glycine uptake appeared to be 10% lower under elevated than ambient $CO_2$ conditions for *S. americanus* and *S. patens*, although this effect was not statistically significant (Fig. 1a).

$^{13}$C uptake was measured to determine if the $^{15}$N uptake occurred as intact organic molecules or as mineral $^{15}$N following organic degradation in the hydroponic solution. By comparing the slopes of excess $^{13}$C versus excess $^{15}$N to the expected slope of 2 (glycine) or 5 (glutamic acid), derived from the $^{13}$C:$^{15}$N ratio of the tracer, a conservative estimate of the fraction of nitrogen taken up as amino acid can be obtained (Fig. 2). In *S. americanus* this fraction was 64% and 60% for glycine under ambient and elevated $CO_2$ respectively, whilst in *S. patens* this fraction was 68 and 79% respectively. Glutamic acid was found to be taken up by *S. americanus* 39% intact for both ambient and elevated $CO_2$ conditions. In *S. patens* this fraction was 36 and 39% for glutamic acid under ambient and elevated $CO_2$ conditions respectively. No significant differences were found in the $^{13}$C:$^{15}$N values of ambient and elevated $CO_2$ treated plants (analysis of covariance). Plants exposed to urea were not significantly enriched in $^{13}$C (Fig. 3).

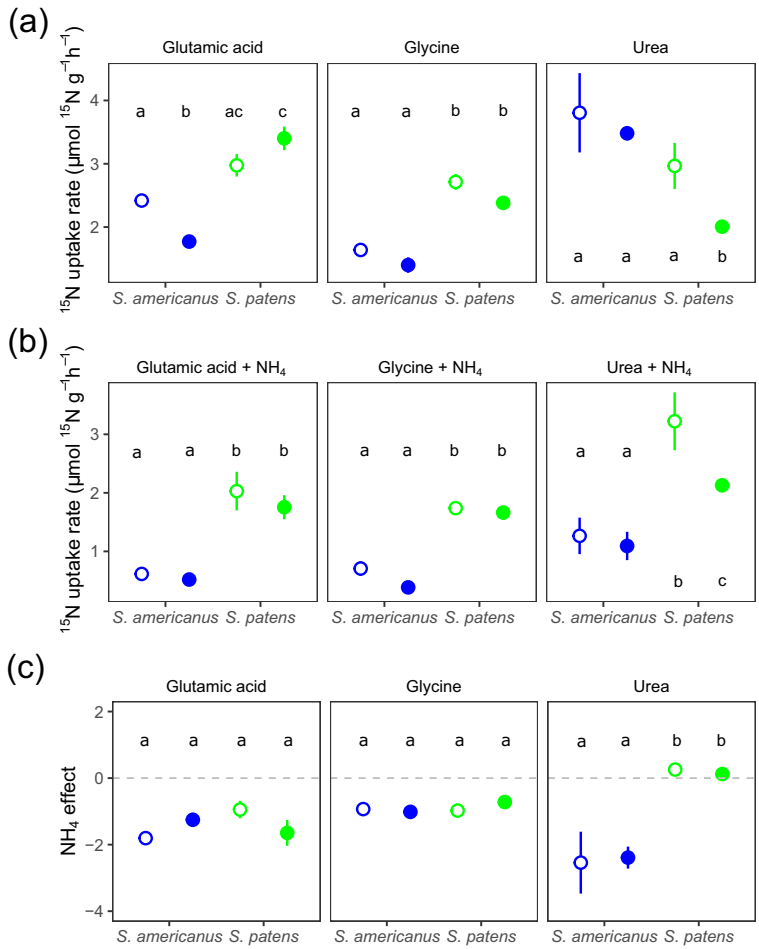


**Fig. 1** **a** $^{15}$N uptake rates in μmol $^{15}$N g$^{-1}$ root dry weight h$^{-1}$ in *S. americanus* and *S. patens* under ambient $CO_2$ conditions (open circles) and elevated $CO_2$ conditions (closed circles), Error bars represent ±SE, $n = 3$ per treatment **b** Uptake rates of $^{15}$N-glycine, glutamic acid and urea in the presence of 50 μM $NH_4^+$ in *S. americanus* and *S. patens* and **c** the effect of $NH_4^+$ on organic $^{15}$N uptake: (μmol $^{15}$N g$^{-1}$ h$^{-1}$) assessed as value with $NH_4^+$ present minus value without $NH_4^+$ present. Open circles represent $^{15}$N uptake under ambient $CO_2$ conditions and closed circles represent uptake under elevated $CO_2$ conditions. Error bars represent ±SE, $n = 3$ per treatment. Group letters that differ within an N form denote statistical separation in pairwise comparisons of means

Michaelis-Menten kinetics revealed that both species had relatively low $V_{max}$ values for each form of organic N with higher values for *S. patens* consistently observed (Table 2). *S. americanus* had lower $K_m$ values for glycine and urea uptake, but similar glutamic acid $K_m$ values were observed in both species. No differences in kinetic parameters between ambient and elevated $CO_2$-treated plants were observed in *S. americanus* or *S. patens*.

An additional set of assays were carried out to determine if inorganic nitrogen affected the uptake of organic N for these species under elevated $CO_2$ conditions. The presence of $NH_4^+$ significantly negatively affected organic N uptake ($p < 0.01$) (Fig. 1b). However, the patterns of $CO_2$, species and N treatment effects on $^{15}$N uptake of organic N held true even in the presence of $NH_4^+$. $CO_2$ had a significant negative effect on $^{15}$N uptake ($p < 0.05$, Table 1). Species and N treatment were also found to be significant ($p < 0.001$) (Table 1).

Plants used in the assays were grown for 8 weeks under elevated $CO_2$ conditions and exhibited responses consistent with known effects of $CO_2$ on $C_3$ and $C_4$ plants. Root biomass was higher in *S. americanus* ($C_3$) under elevated $CO_2$ conditions with no difference in *S. patens* ($C_4$) root biomass between treatments (Supporting Information Fig. S3). Total root N content was higher in *S. americanus* but the percent root N content was higher in *S. patens* (Supporting Information Fig. S4–5). The percent root N content in *S. americanus* was lower under elevated $CO_2$ conditions ($p < 0.05$).

All specimens of *S. patens* harvested were found to have AMF present and colonization ranged from 4 to 18% (data not shown). No evidence of AMF colonization was found for *S. americanus*.

**Table 1** Statistical results for the linear models describing $^{15}N$ uptake rates as a function of species and treatments

| Model | Term | d.f. | F | p |
|---|---|---|---|---|
| $^{15}N$ uptake | | | | |
| | $CO_2$ | 1 | 6.4 | 0.018 |
| | Species | 1 | 5.54 | 0.027 |
| | N treatment | 2 | 19.17 | <0.001 |
| | $CO_2$ x Species | 1 | 0.17 | 0.67 |
| | $CO_2$ x N treatment | 2 | 1.3 | 0.28 |
| | Species x N treatment | 2 | 29.27 | <0.001 |
| | $CO_2$ x Species x N treatment | 2 | 3.4 | 0.049 |
| $^{15}N$ uptake in the presence of $NH_4$ | | | | |
| | $CO_2$ | 1 | 7.49 | 0.011 |
| | Species | 1 | 141.5 | <0.001 |
| | N treatment | 2 | 17.96 | <0.001 |
| | $CO_2$ x Species | 1 | 0.1 | 0.75 |
| | $CO_2$ x N treatment | 2 | 0.5 | 0.6 |
| | Species x N treatment | 2 | 0.14 | 0.86 |
| | $CO_2$ x Species x N treatment | 2 | 1.71 | 0.2 |

## Discussion

Nitrogen plays a pivotal role in regulating the global carbon cycle and increasing evidence suggests that the form of N uptake will be a critical factor in determining plant performance under anticipated future environmental conditions (Rubio-Asensio and Bloom 2017; Reich et al. 2018). Here, we show that two dominant saltmarsh plant species, the $C_4$ grass *S. patens* and the $C_3$ sedge *S. americanus*, are capable of taking up organic nitrogen, and that elevated $CO_2$ conditions and the co-provision of $NH_4^+$ have a negative effect on uptake.

### Organic N uptake

This is the first record of organic N utilization in the common North American coastal wetland plant species *S. patens* and *S. americanus*. These species have been the focus of an ongoing elevated $CO_2$ experiment for over three decades (began 1986; Curtis et al. 1989; Drake 2014), an elevated $CO_2$ x N addition experiment for over one decade (began 2006; Pastore et al. 2017), and ongoing $CO_2$ x N x Phragmites invasion experiment (began 2011; Mozdzer and Caplan 2018), and on ongoing $CO_2$ x warming experiment (began 2016; Noyce et al. 2019). Therefore, an extensive body of data on tidal wetland responses to elevated $CO_2$ is based on the traits of these "model species". Uptake rates for glycine, glutamic acid and urea are in the same range as those found in previous studies on the saltmarsh species *Puccinellia phryganodes, Phragmites australis and Spartina alterniflora* (Henry and Jefferies 2003a; Mozdzer et al. 2010; Quintã et al. 2015) suggesting that the findings of this study have broad relevance. In our experiment, a significant proportion of the supplied tracer was absorbed as intact amino acid (Fig. 3), with the highest proportion (79%) recorded for glycine taken up by *S. patens* under elevated $CO_2$ conditions. In comparison, the proportion of amino acids taken up as intact organic molecules has ranged from 5 to 11% in arctic marsh species (Henry and Jefferies 2003a), 12–52% in temperate grassland species (Näsholm et al. 2000; Bardgett et al. 2003), up to 91% in boreal forest species (Näsholm et al. 1998), and 100% in desert species *Larrea trendata* (Jin and Evans 2010). Thus, we conclude that both these tidal wetland species can utilize N in its organic form.

The technique we adopted to determine the acquisition of intact organic molecules with dual labelled amino acids has been widely used (Näsholm et al. 1998; Henry and Jefferies 2003a; Persson et al. 2003; Quintã et al. 2015). However, underestimation of intact organic N uptake due to plant respiratory losses of $^{13}CO_2$ may occur (Schimel and Chapin 1996; Warren 2012). For example, compound-specific analysis of $^{13}C^{15}N$-asparagine revealed rapid plant metabolism within minutes of uptake resulting in underestimation of the quantity of amino acid acquired by white clover (*Trifolium repens*) (Czaban et al. 2016). It has been speculated that the rapid shift in the $^{13}C$:$^{15}N$ ratio from 2:1 towards 1:1 during glycine uptake is due to loss of the carboxyl group following incorporation into the plant (Näsholm et al. 1998). Incorporation of intact molecules can also be overestimated when the molecules were mineralized prior to uptake, then taken up as separate inorganic compounds in proportion to the initial organic molecule (Sauheitl et al. 2009; Rasmussen et al. 2010). As the saltmarsh species in our study have access to atmospheric $CO_2$ there should be little reliance on inorganic C in the root medium, therefore the likelihood is low of acquiring substantial amounts of $^{13}C$ (from $^{13}CO_2$/$H^{13}CO_3^-$) in parallel with $^{15}N$. In fact, it is possible that the incorporation of intact molecules is underestimated. Plants exposed to urea were not significantly enriched in

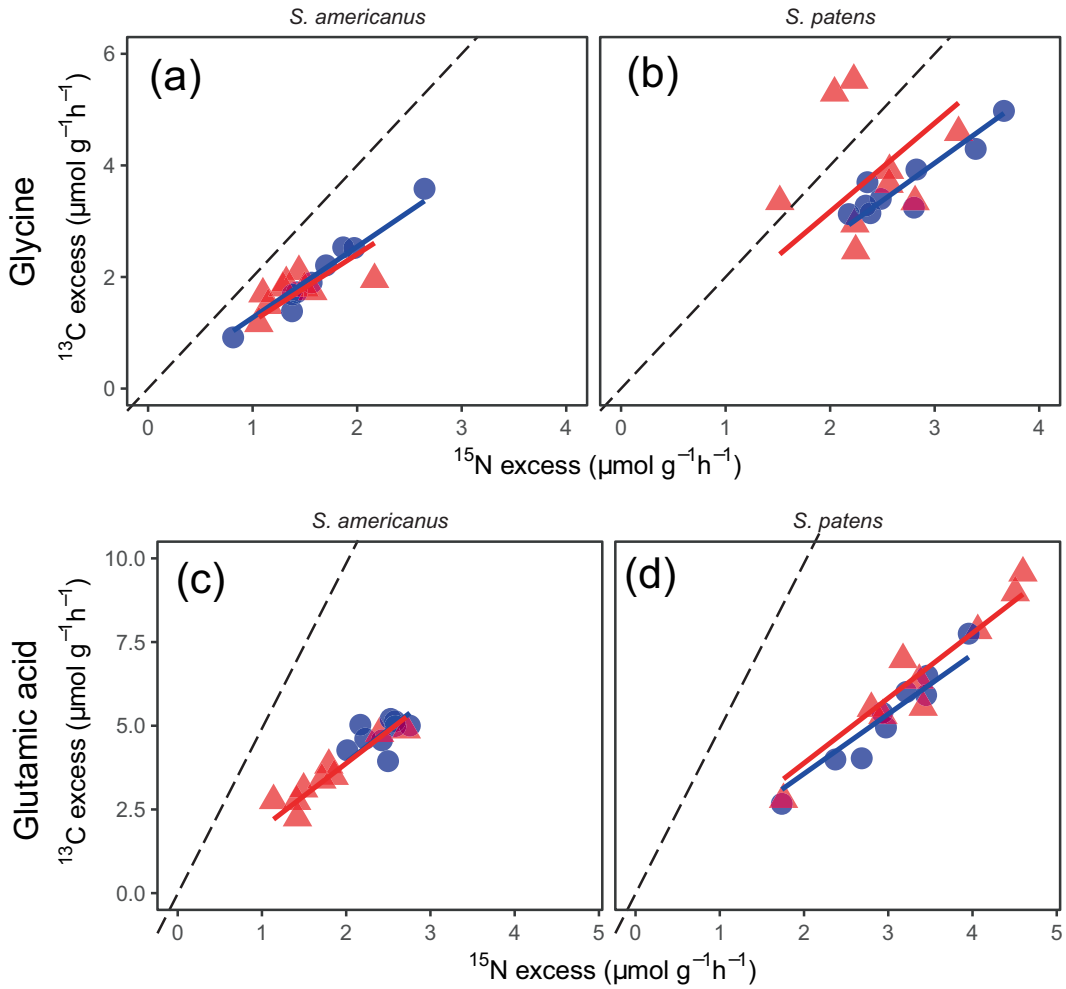


**Fig. 2** Relationship between $^{13}$C excess and $^{15}$N excess in amino acid treated plants under ambient $CO_2$ (blue circles) and elevated $CO_2$ conditions (red triangles). Regression lines are the assimilated ratios of $^{13}$C:$^{15}$N for each plant type. Glycine-treated plants (**a**, **b**) **a** *S. americanus* under ambient conditions (slope = 1.27, $r^2 = 0.99$) and elevated conditions (slope = 1.2, $r^2 = 0.97$); **b** *S. patens* under ambient conditions (slope = 1.35, $r^2 = 0.99$) and elevated conditions (slope = 1.59, $r^2 = 0.92$). Glutamic acid treated plants (**c**, **d**) **c** *S. americanus* under ambient conditions (slope = 1.95, $r^2 = 0.99$) and elevated conditions (slope = 1.94, $r^2 = 0.88$); and **d** S. *patens* under ambient conditions (slope = 1.78, $r^2 = 0.99$) and elevated conditions (slope = 1.94, $r^2 = 0.99$). Broken lines represent the theoretical $^{13}$C:$^{15}$N ratio of the glycine (2:1) and glutamic acid (5:1)

$^{13}$C (Fig. 3), but the urea molecule may have been assimilated intact nonetheless. Mérigout et al. (2008) showed that in *Arabidopsis* the urea molecule was hydrolyzed in the root tissue by cytosolic ureases into two amino groups and the resulting $^{13}CO_2$ gas dissipated quickly such that it was undetectable in the root biomass.

We demonstrated the capacity of saltmarsh plant species to take up organic N in non-sterile hydroponic solutions, but did not investigate the capacity for organic N uptake in competition with soil microbes. Studies performed under field conditions have found that soil microbes compete strongly for organic N (Andresen et al. 2009; Bardgett et al. 2003; Chen et al. 2015). For example, in loblolly pine field experiments recovery of $^{15}$N-alanine 3 h after application was <2% in fine roots versus 24% in microbial biomass (Hofmockel et al. 2007). Although it is likely that label recovery would have been lower under field conditions at our study site due to the greater abundance of microorganisms, we argue that the patterns observed in the capacity for uptake between plant species under elevated $CO_2$ treatments are robust. Such was the case in Hofmockel et al. (2007) where the pattern of lower uptake of organic N

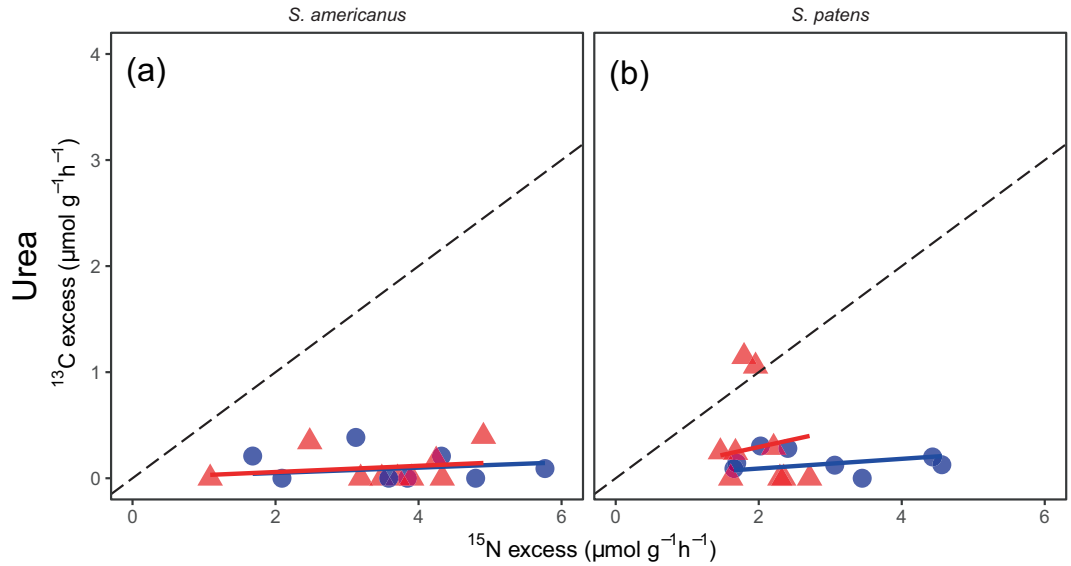


**Fig. 3** Relationship between $^{13}$C excess and $^{15}$N excess in urea treated plants under ambient $CO_2$ (blue circles) and elevated $CO_2$ conditions (red triangles). Regression lines are the assimilated ratios of $^{13}$C:$^{15}$N for each plant type. Broken lines represent the theoretical $^{13}$C:$^{15}$N ratio of the urea (1:2)

by fine roots under elevated $CO_2$ conditions was evident despite competition with soil microbes.

## Interspecific differences

An important finding of this study is the interspecific differences in organic N uptake. *S. patens* had higher uptake rates than *S. americanus* for glycine and glutamic acid when organic N was provided alone, and higher rates for both amino acids and urea when organic N was supplemented with $NH_4^+$. This difference between species may be driven by colonization of *S. patens* roots by AMF. We cannot deduce how much of the $^{13}$C and $^{15}$N label was located in either plant or fungal cells, but as the fungus is an integral part of the mycorrhizal root, this distinction is not critical in the context of species-level responses to global change (Näsholm et al. 1998).

The Michaelis-Menten kinetics revealed that both species have a high affinity for amino acids with the lowest $K_m$ values recorded for glutamic acid uptake in

**Table 2** Mean of bootstrapped estimates for the Michaelis-Menten parameters $V_{max}$ (μmol g$^{-1}$ h$^{-1}$) and $K_m$ (μM) ± standard error

| N form | Species | $CO_2$ treatment | $V_{max}$ (μmol g$^{-1}$ h$^{-1}$) | $K_m$ (μM) |
|---|---|---|---|---|
| Glutamic acid | *S. patens* | Ambient | 3.9 ± 0.6 (2.9–5.3) | 5.6 ± 4.1 (0.04–16.8) |
| | | Elevated | 3.4 ± 0.3 (2.7–4.2) | 4.1 ± 2.3 (0.3–9.9) |
| | *S. americanus* | Ambient | 1.9 ± 0.3 (1.3–2.7) | 7.4 ± 6.4 (0.5–25.1) |
| | | Elevated | 1.2 ± 0.2 (0.9–1.6) | 4.7 ± 3.7 (0.04–14.2) |
| Glycine | *S. patens* | Ambient | 4.4 ± 0.7 (3.4–6.1) | 29.3 ± 12.1 (12.5–57.3) |
| | | Elevated | 8.9 ± 3.7 (5.8–15.5) | 99.1 ± 75.2 (42.8–226.1) |
| | *S. americanus* | Ambient | 1.6 ± 0.1 (1.3–1.9) | 6.3 ± 2.5 (2.7–12.2) |
| | | Elevated | 1.2 ± 0.1 (0.9–1.5) | 7.1 ± 4.1 (1.7–16.5) |
| Urea | *S. patens* | Ambient | 18.4 ± 31.7 (6.4–66.3) | 300.5 ± 639 (56.3–1274) |
| | *S. americanus* | Elevated | 30.1 ± 62.3 (7.2–136.6) | 613 ± 1429 (87.4–3000) |
| | *S. patens* | Ambient | 5.3 ± 6.8 (2.3–13.9) | 41.7 ± 111.8 (0.1–188.5) |
| | *S. americanus* | Elevated | 2.8 ± 0.9 (1.7–4.8) | 14.9 ± 18.0 (0.2–51.6) |

Values in parentheses represent the 95% confidence interval of estimates from across all bootstrapped fits ($n = 999$)

*S. patens*. These kinetic parameters are in the same range as those recorded for other saltmarsh species (Kielland 1994; Henry and Jefferies 2003a; Mozdzer et al. 2010). *S. patens* consistently had higher $V_{max}$ values than *S. americanus* corresponding to interspecific differences observed for inorganic N uptake (Cott et al. 2018). Niche partitioning for different N forms has been proposed as a mechanism allowing species coexistence (Tilman 1994; Harrison et al. 2007). While this study was not specifically designed to examine preferential use of different N forms by coexisting plants, our results suggest that at low levels of inorganic N *S. patens* has a higher capacity (i.e. higher $V_{max}$) to acquire organic N than *S. americanus* (Fig. 1b). This ability may confer a physiological advantage because the assimilation cost of amino acids is less than that of inorganic N, as they can be directly assimilated into proteins in the root without reductive steps (Pate 1986). This energy bonus makes it more beneficial for plants to take up organic than inorganic N, even when organic N availability to the roots is much lower. Small plants with high N concentration that are not light limited were found to benefit most from this effect (Franklin et al. 2017). In addition, niche partitioning has been found to be mediated by mycorrhizal symbiosis (Gerz et al. 2018). Indeed *S. patens* is smaller, has a higher N concentration than *S. americanus* (Supporting Information Fig. S5), and is colonized by AMF. Therefore, it may be that under field conditions niche partitioning is realized with *S. patens* taking advantage of a relative wide range of N forms. The concentration of total free amino acids in porewater was found to be significantly lower under *S. patens* communities than *S. americanus* communities in a long-term experiment at the Global Change Research Wetland (Supporting Information Fig. S2), which may reflect drawdown of this N source by *S. patens.*

### $CO_2$ effect

Despite a putative increase in the N demand under elevated $CO_2$ conditions, we hypothesized that elevated $CO_2$ would negatively affect uptake of organic N based on evidence from FACE studies that elevated $CO_2$ suppresses plant N acquisition at neutral to modest shifts in productivity (Feng et al. 2015). The effect of elevated $CO_2$ on the model species in our study is well documented, with *S. patens* exhibiting small increases in NEP (net ecosystem productivity) in response to $CO_2$ that averaged 13% across 19 years (Erickson et al. 2013). The $CO_2$ stimulation of NEP in the *S. americanus* community averaged 35% but was highly variable with a range of 0 to 88% (Erickson et al. 2013). Despite differences in their productivity response to $CO_2$ our findings indicate that elevated $CO_2$ caused a repression in the overall uptake of organic N in both species. Pairwise comparison showed that $CO_2$ had a significant effect on the uptake of some forms of organic N, but not on that of others. Uptake rates in *S. americanus* were between 10 and 30% lower under elevated $CO_2$ conditions. In *S. patens* glycine and urea uptake were 17 and 33% lower under elevated $CO_2$ conditions respectively, whilst no significant differences were found for glutamic acid-treated *S. patens*. Similarly, Hofmockel et al. (2007) found that loblolly pine roots assimilated significantly more alanine under ambient $CO_2$ than elevated $CO_2$ one week after tracer application. Elevated $CO_2$ also appeared to repress the organic N uptake kinetics of *S. americanus*, which is consistent with its negative effect on inorganic nitrogen uptake in this species (Cott et al. 2018).

The mechanisms underlying the repression of uptake of some forms of N under elevated $CO_2$ remain speculative and are beyond the scope of this study. The reduction in transpiration-driven mass flow of N through soil is one hypothesis proposed to cause the reduction in N tissue concentration (McDonald et al. 2002), as elevated $CO_2$ consistently reduces evapotranspiration (ET) (Bernacchi et al. 2007; Li et al. 2010). If the reduction in evapotranspiration was a contributing factor for the reduction in $^{15}N$ uptake under elevated $CO_2$ conditions observed in this study, we would expect that elevated $CO_2$ would repress uptake to a greater extent in *S. patens* than *S. americanus* because elevated $CO_2$ has been shown to reduce ET by 50% more in *S. patens* than *S. americanus* (Li et al. 2010). Indeed, it has been suggested that a nitrogen deficit induced by reduced transpiration in elevated $CO_2$ treatments caused the reduction in shoot density and biomass of *S. patens* in the long-term study at our site (Drake 2014). However, we found that elevated $CO_2$-driven suppression of N uptake was not higher in *S. patens* and conclude that evapotranspiration was not the primary cause of lower $^{15}N$ uptake under elevated $CO_2$ conditions.

Metabolites such as glutamine have been shown to suppress the expression of genes associated with $NO_3^-$ and $NH_4^+$ uptake (Rawat et al. 1999; Zhuo et al. 1999; Glass et al. 2002). Glucose supply to plant roots can inhibit the induction of glutamate dehydrogenase and

asparagine synthetase (Oaks and Hirel 1985), both of which are involved in N metabolism (Good et al. 2004). The fact that C and N metabolism are tightly linked is inescapable (Coruzzi and Bush 2001), specifically in the case of organic N, and it may be that increased carbohydrate supply to roots as a result of elevated $CO_2$ exposure acts directly or indirectly on plant nitrogen pools, ultimately causing a downregulation of genes associated with N uptake.

### $NH_4^+$ effect

One aspect causing uncertainty about the importance of organic N compounds as a N source for plants is the possible interaction between organic and inorganic N forms during absorption by plant roots (Thornton and Robinson 2005). We hypothesized that the presence of $NH_4^+$ (the dominant form of N in wetland soils) would negatively affect the uptake of organic N. Our results show that the co-provision of $NH_4^+$ resulted in the decrease of glycine and glutamic acid uptake for both species. Glycine uptake in *S. americanus* decreased by 70% whilst urea uptake decreased by 65%. These results corroborate other studies that found asparagine was utilized to a greater extent by white clover when inorganic N was absent (Czaban et al. 2016). Similarly, urea uptake in *Arabidopsis* was inhibited by the presence of $NH_4^+$ (Mérigout et al. 2008).

Amino acid transporters have best been described in *Arabidopsis*, with AAP1, AAP5 and LHT1 each displaying a different specificity and affinity for various amino acids (Hirner et al. 2006; Lee et al. 2007; Svennerstam et al. 2008). Urea transporters include a high-affinity DUR3 transporter (Liu et al. 2003; Kojima et al. 2006), whilst passive urea transport has been shown to be mediated by aquaporins (TIPs PIPs and NIPs) (Witte 2011). Root uptake of $NH_4^+$ involves the AMT family of high-affinity transporters (Nacry et al. 2013). Although N transporters have not been specifically characterized in the saltmarsh species in our study, we assume the above transporters mediate uptake of the various N forms, and uptake of $NH_4^+$ is not competing with the same transporters for organic N uptake. However, our results suggest there is an interaction. It may be that there is a common mechanism involved in the integrated control of root N uptake (Nacry et al. 2013), allowing the plant under mixed N nutrition to coordinate concurrent uptake of different N sources to optimise its overall N acquisition. Our results confirm that *S. americanus* and *S. patens* can acquire organic N in the presence of inorganic N, which emphasizes the potential for these saltmarsh species to take up amino acids and urea under typical conditions in which the soil solution is a mixture of different N forms.

### Implications for marsh responses to global change

The balance of plant N demand and microbial N supply responds strongly to perturbation and often dictates the direction and magnitude of ecosystem responses to global change (Craine et al. 2018). Previous studies at the Global Change Research Wetland have shown that elevated $CO_2$ favours plant N demand over supply (net demand), drawing down porewater inorganic N concentrations and progressively creating an N limitation on the size of the elevated $CO_2$ response (Langley and Megonigal 2010; Lu et al. 2019). Warming favours net N demand at 2 °C above ambient but favours net N supply with additional warming up to 5 °C (Noyce et al. 2019). In all cases, allocation to roots increases with net plant N demand, affecting important ecosystem properties such as soil surface elevation gain and soil carbon sequestration (Langley et al. 2009). Here we demonstrated that organic N is taken up by dominant species at this site, uptake kinetics varies among species, and organic N uptake responds to elevated $CO_2$. Further research is needed to establish the extent to which organic N dynamics mediates ecosystem responses to global change factors that have hitherto been attributed solely to inorganic N. However, the superior affinity of *S. patens* for organic N may explain a variety of observations, such as it's ability to persist in low-N environments in competition with a species that is more competitive for inorganic N (Cott et al. 2018), and the fact that *S. patens* and *S. americanus*-dominated communities respond differently to shifts in N demand and supply caused by warming (Noyce et al. 2019).

## Conclusions

We found that *S. americanus* and *S. patens* are capable of taking up organic N, adding to the growing body of evidence that organic N contributes to the N demand of plants. *S. patens* was found to have a greater ability to take advantage of organic N, which may in part be due to mycorrhizal colonization. Despite differences in their productivity response to $CO_2$ our data indicate that

elevated $CO_2$ repressed uptake of some forms of organic N in both species. In addition, the co-provision of $NH_4^+$ caused the downregulation of organic N uptake. The 70% reduction in organic N uptake in the presence of $NH_4^+$ suggests that plants likely still rely primarily on mineral N in the field, yet even so, dissolved organic N may account for a non-trivial fraction of N demand in natural settings based on these results. However, we can deduce that organic N uptake is not likely to supply plants with the additional N they demand when exposed to elevated $CO_2$, and that the repressive effects of elevated $CO_2$ on organic N uptake may have negative consequences for ecosystem productivity and carbon sequestration. This may be particularly relevant in high to mid-latitude marshes where organic N constitutes a significant portion of available N (Henry and Jefferies 2003b; Mozdzer et al. 2014, Fig. S1). Further field-relevant studies are needed to understand the role of organic N in alleviating N limitation on coastal wetland ecosystem responses to elevated $CO_2$ (Langley and Megonigal 2010), and therefore feedback on rising atmospheric $CO_2$ concentrations.

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