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Nitrogen Uptake by Native and Invasive Temperate Coastal Macrophytes: Importance of Dissolved Organic Nitrogen

Thomas J. Mozdzer · Joseph C. Zieman · Karen J. McGlathery

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Abstract We investigated if the success of the invasive common reed *Phragmites australis* could be attributed to a competitive ability to use dissolved organic nitrogen (DON) when compared to the dominant macrophyte *Spartina alterniflora* in tidal wetlands. Short-term nutrient uptake experiments were performed in the laboratory on two genetic lineages of *Phragmites* (native and introduced to North America) and *S. alterniflora*. Our results provide the first evidence for direct assimilation of DON by temperate marsh plants and indicate that amino acids are assimilated intact by all plant types at similar rates. Both *Phragmites* lineages had significantly greater urea–N assimilation rates than *S. alterniflora*, and the affinity for dissolved inorganic nitrogen (DIN) species was the greatest in native *Phragmites* > introduced *Phragmites* > *S. alterniflora*. Field studies demonstrated uptake of both DON and DIN in similar proportion as those determined in the laboratory experiments. Based on these uptake rates, we estimate that DON has the potential to account for up to 47% of N demand for *Phragmites* plants, and up to 24% for *S. alterniflora* plants. Additionally, we suggest that differences in N uptake between native and introduced *Phragmites* lineages explain one mechanism for the success of the introduced type under increasingly eutrophic conditions.

T. J. Mozdzer (✉) · J. C. Zieman · K. J. McGlathery
Department of Environmental Sciences, University of Virginia,
PO Box 400123, 291 McCormick Rd,
Charlottesville, VA 22904-4123, USA
e-mail: mozdzert@si.edu

Present Address:
T. J. Mozdzer
Smithsonian Environmental Research Center,
647 Contees Wharf Rd, PO Box 28, Edgewater, MD 21037, USA

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Introduction

Intertidal marshes of the North American Atlantic coast are dominated by the halophytic smooth cordgrass, *Spartina alterniflora* (Mitsch and Gosselink 1993). Over the past century, the common reed, *Phragmites australis*, (hereafter *Phragmites*) has become pervasive in Atlantic coast tidal marshes, displacing *Spartina* spp. and other intertidal species; its unprecedented expansion is a concern for wetland ecologists working in both tidal and non-tidal wetlands due to changes in faunal use (Meyerson et al. 2000a, Angradi et al. 2001, Buchsbaum et al. 2006), biogeochemical cycles (Windham and Lathrop 1999, Meyerson et al. 2000b, Windham and Ehrenfeld 2003), and reductions in species richness and biodiversity (Chambers et al. 1999, Bertness et al. 2002). *Phragmites* is thought to be one of the world's most widely distributed angiosperms (Holm et al. 1977, Mal and Narine 2004). Although native to North America (Niering et al. 1977, Orson 1999), the recent invasion has been attributed to the introduction of a non-native lineage from Eurasia (Saltonstall 2002).

Several studies have suggested different circumstances by which *Phragmites* can be so ecologically successful in a variety of habitats, including eutrophication of habitats (Chambers et al. 1999, Bertness et al. 2002), shoreline development (Bertness et al. 2002), wrack disturbance (Minchinton 2002a), and increased precipitation (Minchinton 2002b). While these studies did not specifically identify if the populations were native or introduced, it is likely that they were the introduced type because the introduced lineage is the predominant lineage along the North American

Atlantic coast (Saltonstall 2002). Recent studies highlighting differences between the native and introduced types of *Phragmites* indicate that the shift from the native to the introduced lineage may have important ecosystem consequences. For example, the introduced types has been shown to have greater aboveground biomass (League et al. 2006, Saltonstall and Stevenson 2007, Mozdzer and Zieman 2010), greater stem density (League et al. 2006, Mozdzer and Zieman 2010), greater height (League et al. 2006), and greater photosynthetic rates (Mozdzer and Zieman 2010). However, the question still remains as to what makes the introduced Eurasian lineage so successful in invading North American tidal marshes.

Few studies have addressed how a nitrogen (N)-limited plant, such as *Phragmites*, can be so overwhelmingly successful in a N-limited system (Windham 1999, Windham and Meyerson 2003). This is particularly important since introduced *Phragmites* demands up to 50% more N than the species it is replacing (Windham and Ehrenfeld 2003), and inorganic N availability is typically limited by microbial activity. This suggests that *Phragmites* may be getting N from sources other than ammonium (NH_4^+) and nitrate (NO_3^-). Meyerson et al. (2000b) suggested that the ability to use dissolved organic nitrogen (DON) as an alternative N source may give *Phragmites* a competitive advantage and facilitate its expansion. To date, this hypothesis has not been tested.

Tidal marsh habitats are characterized as systems that accumulate organic matter, which may result in significant pools of bioavailable DON, specifically dissolved free amino acids (DFAA). This pool of N is poorly characterized in temperate wetland systems (Gardner and Hanson 1979), although interestingly, the classic study of Valiela and Teal (1979) demonstrated that the bulk of N flowing through a New England salt marsh was in the form of DON. In other estuarine systems, DFAA have been shown to comprise a significant portion of DON in sediments. For example, in Chesapeake Bay sediments, DFAA concentrations ranged from 1–300 μM, with the highest values found just below the surface of the sediment (Burdige and Martens 1990). In fjords in Denmark, concentrations of DON in sediments were as high as 3 mM and up to 82% of the DON was in the bioavailable form of dissolved combined amino acids, DFAA, and urea (Guldberg et al. 2002).

This study is the first study to our knowledge to investigate the direct utilization of DON by the dominant macrophytes *S. alterniflora* and *Phragmites* in temperate tidal marshes. By using a combination of laboratory and field experiments, we sought to determine if either native or introduced *Phragmites* lineages or *S. alterniflora* have the ability to assimilate DON directly, which would allow plants to use this bioavailable N pool. We also asked if differences in root N uptake between native and introduced lineages of *Phragmites* could help explain the success of the introduced lineage and limited distribution of native lineages in North American Atlantic coast wetlands.

Methods

Site Description

Three field sites were used during our study: Oyster Marsh (OM), Occupacia Creek Marsh (OCM), and Eastern Shore National Wildlife Refuge (ES-NWR). Oyster Marsh is a tidal salt marsh located within the Virginia Coast Reserve Long-Term Ecological Research (VCR-LTER) complex in Oyster, VA (37.28° N, 75.95° W). OM is a typical mid-Atlantic salt marsh dominated in the intertidal zone by *S. alterniflora*, with *Phragmites* invading from upland. This site was chosen because it contained genetically identified populations of non-native haplotype M *Phragmites* (Saltonstall 2002), hereafter introduced *Phragmites*, and *S. alterniflora.* OM was the source of introduced *Phragmites* and *S. alterniflora* plants for the laboratory experiments, and as well as the site for porewater N availability. OCM is an oligohaline tidal marsh on Occupacia Creek, a tributary of the Rappahannock river, near Chance VA (38.06° N, 76.94° W). This site contains genetically identified populations of Atlantic coast native, haplotype F, *Phragmites* (Saltonstall 2002), hereafter native *Phragmites*, and was the source of the native *Phragmites* for the laboratory experiments. ES-NWR is a tidal salt marsh in the VCR-LTER (37.14° N, 75.94°), similar to the OM site. ES-NWR was chosen as the field manipulation site because it is a relatively pristine tidal salt marsh found within the US National Wildlife Refuge, and was an area where we could add stable isotopes without interfering with long-term experiments in the OM site. *Phragmites* at the ES-NWR was determined to be the introduced lineage based on morphological characteristics (Blossey 2002) and late senescence.

Laboratory Experimental Conditions

Emergent shoots and rhizome fragments of non-native *Phragmites* and *S. alterniflora* were collected from OM, and native *Phragmites* were harvested from OCM. Plants were collected in the spring 2004 and 2005 for two sets of experiments. After collection, the root systems were washed clean of organic matter and dead root material, and individual shoots were placed in 10-cm diameter pots in clean sand to allow for easy transfer into a hydroponic media. Due to differences in plant distribution, it was not possible to collect all three plant types at one site. We assume that any differences observed between the lineages are attributed to genetic differences between the two types.

Plants were fertilized bi-weekly in a greenhouse with a mixed medium of 300 ppm solution of Peters 20-20-20 (N:P:K) amended with 100 ppm glycine and glutamic acid (Sigma). A mixture of inorganic and organic N fertilizers was used to minimize possible synergistic and antagonistic effects on DON utilization (Henry and Jefferies 2003). Plant soils were kept at field capacity using an electronic moisture control system. Within 8 weeks, individual plants achieved a suitable root mass (>100 mg dw) and a shoot height of 60–100 cm. To minimize potential diurnal effects in nutrient uptake, experiments were conducted at approximately the same time each morning (09:00–11:00).

Single Concentration Uptake Experiments

To investigate the assimilation of DON and dissolved inorganic nitrogen (DIN; as NH_4^+) among all three plants, we conducted a series of experiments by presenting individual plants with different sources of N. Experiments were adapted from Epstein et al. (1963) to calculate assimilation of labeled DON compounds (glycine, glutamic acid, urea) and NH_4^+ into root tissue. Whole plants, rather than excised roots, were used since treatment responses can vary greatly between methodologies using excised roots and whole plants (Falkengren-Grerup et al. 2000). We acknowledge that this laboratory procedure may influence root structure, however, this method is generally accepted and has been used widely (Bradley and Morris 1990, Chambers et al. 1998, Falkengren-Grerup et al. 2000). Glycine and glutamic acid, the dominant amino acids in marsh porewater (Gardner and Hanson 1979), were chosen for use in the laboratory experiments. Additionally, glycine is thought to be a poor substrate for microorganisms (Lipson et al. 1999). Uptake rates of the individual DON compounds and NH_4^+ were determined for 15 replicate plants over a 45-min treatment period. Plant roots were exposed to solutions of 100 μM N for each treatment using uniformly, dual-labeled, ^{15}N, ^{13}C, glycine, glutamic acid, and urea (>98% enriched; Cambridge Isotope Laboratories, Andover, MA, USA) and ^{15}N labeled NH_4Cl (99% enriched; Cambridge Isotope Laboratories, Andover, MA, USA) at pH of 6.5. Use of dual isotope labels allowed us to determine if the whole molecule was assimilated intact. A control treatment (no nutrients) was run identically in order to assess natural isotopic abundances.

At the beginning of all experiments, plants were rinsed free of sand using tap water, then equilibrated for at least 30 min in a solution of 0.50 mM $CaCl_2$ to maintain cell membrane integrity (Epstein 1961) at experimental conditions: temperature 20°C and 400 μmols photons m^{-2} s^{-1} photosynthetically active radiation in a CONVIRON PGR15 environmental chamber (Winnipeg, Canada). After the equilibration period, plant roots were immersed for 45 min in 500 ml of a continuously aerated, well-mixed solution identical to the equilibration medium, but containing either a 100 μM ^{15}N or 100 μM ^{15}N ^{13}C substrate. 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 treatment plants were transferred to flasks containing only the equilibration medium. After exposure to the media, roots were rinsed for 2 min with 1 mM KCl l^{-1} to remove any possible excess labeled substrate in the Donnan free space (Epstein et al. 1963). Whole root systems were then carefully blotted dry and excised. Excised roots were dried to a constant weight at 60°C, weighed, and ground to a fine powder using a mortar and pestle. Rhizomes were not included in the analysis. Root samples were analyzed at the UC Davis Stable Isotope Facility for stable isotope (^{13}C, ^{15}N) concentrations using a Europa Integra continuous flow mass spectrometer.

Michaelis–Menten Parameters

In a separate experiment in 2005, we determined Michaelis–Menten kinetic parameters in *S. alterniflora* and both *Phragmites* haplotypes by exposing individual plants to varying concentrations of labeled media. Plants used in this experiment were collected from the same locations, and were treated identically in the greenhouse as in the experiment described above. Experimental conditions also were the same, except to determine the N uptake kinetic parameters, individual plants were exposed to six different N concentrations for each N compound (glycine, glutamic acid, urea, or NH_4^+) for 30 min (5, 10, 25, 50, 100, and 500 μM) (n=3 individual plants per plant type per N concentration). The reaction volume for these treatments was adjusted (2,000, 1,000, 1,000, 500, 500, and 250 ml respective to concentrations above) to ensure that drawdown would not be more than 10% of starting concentration. At the end of the treatment period, plants were processed as described above to determine isotope incorporation. We did not have enough native *Phragmites* plants to determine glutamic acid uptake kinetics, and we did not have replicates at the 500 μM N treatment. For these values, rates from the non-native type were substituted for parameter estimates since differences between the two plant types were not significant.

Competition Experiments

To determine the interactive effects of N assimilation, we exposed *S. alterniflora* and both *Phragmites* lineages to a combination of both labeled and unlabeled N media. Plants were exposed for 30 min to either [100 $^{15}NH_4$ + 100 μM glycine] or [100 NH_4 + 100 μM $^{15}N^{13}C$-glycine], resulting

in a final N concentration of 200 μM in the reaction vessel (n=3 individual plants per treatment). Experimental conditions were identical to those described above.

Effects of Salinity

To determine effects of salinity on N assimilation rates, *S. alterniflora* and *Phragmites* haplotypes were exposed to 100 μM N treatments ($^{15}NH_4$ or $^{15}N^{13}C$-glycine) at three different salinities (10, 20, and 30 ppt) made with synthetic sea salts (Instant Ocean) (n=3 individual plants per treatment). Experimental conditions were otherwise identical to the experiment above. After a 30-min exposure to the labeled media, plants were prepared and analyzed for isotopic ratios as described above.

In Situ Field Experiment

The field component of this experiment was conducted in a salt marsh located within the ES-NWR. This site was selected because it epitomized the widespread expansion of *Phragmites* into tidal marshes along the North American Atlantic coast. We tested the ability of *S. alterniflora* and *Phragmites* growing adjacent to each other in a tidal marsh to take up both DON and DIN. This experiment consisted of three treatments: control, $^{15}NH_4Cl$, and a combined amino acid treatment of glycine and glutamic acid uniformly labeled with ^{15}N, ^{13}C. For each individual replicate (n=3 per treatment per plant), an 8.85-cm internal diameter, 30-cm length core was inserted flush to the sediment surface in an attempt to eliminate clonal integration and to limit lateral leakage of the introduced substrate. The porewater volume of the core was estimated to be 1.55 l using the porosity of marsh soils (~0.85) near this field site (Thomas 2004). During the falling tide, all cores were amended with either 25 μmols of combined amino acid–N (1:1; ^{15}N, ^{13}C glycine: ^{15}N, ^{13}C glutamic acid), 65 μmols of $^{15}NH_4Cl$, or deionized water using a high-resolution water sampler (Berg and McGlathery 2001) to distribute the tracer uniformly. The cores were inserted on August 2, 2004, and the plant + core remained intact for 4 days, after which the entire plant + core was carefully removed for subsequent analysis.

The entire core was rinsed initially with 0.5 mM $CaCl_2$, as were plant roots in the laboratory, followed by rinsing with a garden hose (tap water) to remove the organic matter from the dense root mat. A conservative sub-sample (mean root mass 874±65 mg dw) of live root material was removed carefully from the core for isotopic analysis. The remainder of the core was sorted to determine total root biomass to calculate root N assimilation. Additionally, the aboveground shoot was rinsed with tap water and prepared for isotopic analysis. Both root and shoot tissue was dried to a constant weight at 60°C, and samples were prepared and analyzed as described above.

Calculations and Statistical Analysis

Assimilation rates of ^{15}N and ^{13}C for all experiments were calculated using a modified equation of Hauck and Bremner (1976) as cited in Knowles and Blackburn (1993):

$$\mu g \text{ assimilated} = [m_1(\text{APE}_{\text{sample}} - \text{APE}_{\text{control}})]/\text{APE}_{\text{treatment}} \quad (1)$$

$$V_{\text{uptake}} = (\mu g \text{ assimilated}/\text{MW}_{\text{treatment}})/(m_2 \times t_{\text{exp}}) \quad (2)$$

Where m_1 is the mass of N or C in the sample in μg, $\text{APE}_{\text{sample}}$ is the atom % excess ^{15}N or ^{13}C of the sample exposed to a labeled substrate, $\text{APE}_{\text{control}}$ is the atom % excess ^{15}N or ^{13}C in the control sample, $\text{APE}_{\text{treatment}}$ is the atom % excess of the labeled ^{15}N or ^{13}C treatment, MW is the molecular weight of C or N isotope assimilated, m_2 is the dry root mass of the sample in g, and t_{exp} is the duration of the exposure to labeled substrate.

All statistical analyses were conducted in SAS (version 9.01; Cary, NC, USA) testing analysis of variance (ANOVA) using proc GLM and post hoc contrast statements unless otherwise noted. The single concentration N uptake experiments were factorial in design with three plant types and four nutrient treatments. The salinity experiment was also factorial with three levels of salinity and three plant types. Michaelis–Menten uptake parameters were determined by fitting measured uptake rates at different N concentrations to the Michaelis–Menten equation using a non-linear model (proc NLIN) in SAS. The calculated kinetic parameters included the maximum uptake rate at saturating nutrient concentrations, V_{max} (μmol ^{15}N g^{-1} root h^{-1}) and the half-saturation constant, K_{m} (μmol ^{15}N L^{-1}). The affinity for each nutrient, α, is described as the ratio of V_{max}:K_{m} (L g^{-1} root h^{-1}). The field experiment was also a factorial with two plant types and two N treatments; post hoc Tukey tests were used to determine differences among treatments.

Arbuscular Mycorrhizal Fungi and Root Mass Determination

To determine if DON assimilation was influenced by mycorrhizal association, 15 plants of *S. alterniflora*, native *Phragmites*, and introduced *Phragmites* from the laboratory experiments in 2004 were sacrificed to determine if plants were colonized by arbuscular mycorrhizal fungi (AMF) using a modification of the technique developed by

Kormanik and McGraw (1982) as described by Burke et al (2002). The trypan blue staining method was used to ensure the results of the present study were comparable with other studies (Cooke et al. 1993, Hoefnagels et al. 1993, Cooke and Lefor 1998, Burke et al. 2002, 2003). Dry root mass was determined on all experimental plants at the completion of experimental treatments after drying samples to a constant mass at 60°C.

Porewater N Determination

On August 16, 2005 bulk porewater at approximately 10 cm in depth was sampled from five *S. alterniflora* and five introduced *Phragmites* locations in the OM marsh using the high-resolution porewater sampler (Berg and McGlathery 2001). Samples were filtered (GHP Pall, 0.45 μm) and frozen for subsequent porewater N determination. NH_4^+ was analyzed using the Lachat QuikChem 8500 (Loveland, Colorado) using QuikChem® method 31-107-06-1-B. Urea was determined using a modification of the methods of Mulvenna and Savidge (1992) and Goeyens et al. (1998). Dissolved free amino acids were determined on a Dionex ICS 3000 (Sunnyvale, CA, USA) using the AccQ-Tag chemistry package from Waters Corporation (Milford, MA, USA). Statistical analysis was performed in SAS (V 9.1) using proc GLM to determine significant differences in N availability between *S. alterniflora* and *Phragmites* plant zones.

Results

Single Concentration Uptake Experiments

^{15}N assimilation was observed in all nutrient treatments and in all plant types with significant differences between N treatments ($P<0.001$), and with an interaction between plant type and N treatment ($P<0.001$) (Table 1). Ammonium-treated plants had the greatest observed N assimilation rates followed by urea, glycine, and glutamic acid (Fig. 1). There were no significant differences in N uptake between the plant types of the same treatment ($P>0.05$). Glycine-treated plants had the greatest N assimilation rates among amino acid treatments at 3.8±0.2 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ for both introduced *Phragmites* and *S. alterniflora*, and 2.9±0.1 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ for native *Phragmites*, with no significant differences observed among plant types. These rates of glycine–N uptake equal up to ~20% of NH_4^+ assimilation rates. Glutamic acid–N assimilation rates were not significantly different ($P>0.05$) than glycine–N assimilation rates. They ranged from 1.5±0.1 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ for introduced *Phragmites* plants to 1.1±0.02 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ in native *Phragmites* plants, and equaled up to ~8% of NH_4^+ assimilation rates.

While amino acid assimilation rates were not significantly different among all plant types, both urea-treated *Phragmites* lineages had significantly higher N assimilation rates than *S. alterniflora* ($P<0.001$). Native and introduced *Phragmites* lineages exhibited similar urea–N assimilation rates of 14.4±0.6 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ and 14.4±0.8 μmols $^{15}N\ g^{-1}dw\ h^{-1}$, respectively. Urea assimilation rates for *S. alterniflora* (2.4±0.1 μmols $^{15}N\ g^{-1}dw\ h^{-1}$) were significantly lower than those of both *Phragmites* types, and were similar to amino acid assimilation rates (Fig. 1). Interestingly, the urea–N assimilation rate of the introduced *Phragmites* was not statistically different than the NH_4^+–N assimilation rates (Fig. 1).

The NH_4^+ assimilation rates obtained by this experiment are comparable to previous studies that have used depletion over time as a proxy for nutrient uptake (Chambers et al. 1998). No significant differences were observed between introduced *Phragmites* and *S. alterniflora* plants (18.9±1.3 and 20.4±1.7 μmols $^{15}N\ g^{-1}dw\ h^{-1}$, respectively). Native *Phragmites* had the greatest NH_4^+ assimilation rates at 38.0±3.9 μmols $^{15}N\ g^{-1}dw\ h^{-1}$ (Fig. 1).

^{13}C assimilation was calculated to determine if organic molecules were assimilated intact. The glycine used in this experiment had two labeled ^{13}C atoms to each labeled ^{15}N atom, and glutamic acid had five labeled ^{13}C atoms to each labeled ^{15}N atom (Fig. 2). *S. alterniflora* plants had a 1.48:1 ratio of ^{13}C:^{15}N, followed by 1.25:1 for introduced *Phragmites*, and 1.17:1 for native *Phragmites* plants (Fig. 2). ^{13}C:^{15}N ratios indicate that at least 60%, and up to 74% of the glycine molecules were taken up intact based on the ^{13}C:^{15}N ratios. ^{13}C:^{15}N ratios from glutamic-acid-treated plants varied from 1.99:1 in *S. alterniflora* to 3.01:1 in native *Phragmites*, indicating at least 40%, and up to 60% was taken up whole. Urea-treated plants did not show any assimilation of ^{13}C (data not presented).

Root mass had no significant effect on N uptake for any N treatment ($P=0.4887$), and there was no interaction effect of plant×treatment ($P=0.5021$) on nutrient uptake. However, introduced *Phragmites* root mass was significantly greater than both native *Phragmites* and *S. alterniflora* root masses ($P<0.0001$; Fig. 3). Root structure varied considerably between *Phragmites* plant types in both mass and morphology. Introduced *Phragmites* plants had the greatest root masses among all plant types (Fig. 3), and consisted of a combination of fine roots (<2 mm) and larger secondary root structures. On the other hand, native *Phragmites* root systems had significantly less mass than introduced *Phragmites* plants, and consisted of primarily of fine roots (<2 mm). *S. alterniflora* roots systems were similar in mass to native *Phragmites* plants, but were morphologically similar to introduced *Phragmites* plants.

Table 1 Results of ANOVA for N uptake experiments on *S. alterniflora*, native *Phragmites*, and introduced *Phragmites*

Variable	Sources	df	*n*	*F*	*P*
Single N concentration					
N uptake	plant	2	180	16.9	<0.0001
	N-treatment	3	180	119.0	<0.0001
	plant × N-treatment	6	180	11.9	<0.0001
root mass	plant	2	180	55.4	<.0001
	N-treatment	3	180	0.81	0.4887
	plant × N-treatment	6	180	11.9	<0.0001
Salinity Treatment					
glycine rate	plant	2	27	3.5	0.0536
	salinity	2	27	19.6	<.0001
	plant × N-treatment	4	27	4.5	0.0106
ammonium rate	plant	2	27	3.6	0.0471
	salinity	2	27	4.0	0.0361
	plant × salinity	4	27	1.6	0.2274
Competition Treatment					
N uptake	plant	2	18	1.0	0.4074
	N-treatment	1	18	64.8	<.0001
Field ^{15}N assimilation					
N uptake	N-treatment	1	12	292.2	0.0025
	plant	1	12	259.8	<.0001
	treatment × plant	1	12	109.8	<.0001

Plant effect indicates differences between *S. alterniflora*, native *Phragmites*, or introduced *Phragmites* plant type; N treatment effects indicate differences in plant N uptake rates

Assimilation Kinetics Experiments

From the uptake kinetics experiments we calculated the parameters of V_{max}, K_m, and α, which represent the maximum potential uptake rate, half-saturation constant, and the affinity for the molecule, respectively. Uptake rates followed saturation kinetics (Fig. 4), NH_4^+ V_{max} was greatest for *S. alterniflora* (60.5±4.6 μmol ^{15}N g^{-1} h^{-1}), followed by introduced *Phragmites* (40.1±2.0 μmol ^{15}N g^{-1} h^{-1}), and native *Phragmites* (24.3±1.2 μmol ^{15}N g^{-1} h^{-1}); however, the affinity for NH_4^+ demonstrated the opposite pattern with native *Phragmites* having the greatest affinity and *S. alterniflora* having the lowest (Table 2). Again, *S. alterniflora* had the greatest V_{max} for glycine uptake at 14.3±2.3 μmol ^{15}N g^{-1} h^{-1}, followed by native *Phragmites* (9.2±1.5 μmol ^{15}N g^{-1} h^{-1}) and introduced *Phragmites* (7.9±2.3 μmol ^{15}N g^{-1} h^{-1}), which did not differ from each other, and no differences were observed in α (Table 2). No significant differences were observed in glutamic acid V_{max} rates or α between *S. alterniflora* and introduced *Phragmites* plants, with rates of 7.4±1.7 μmol ^{15}N g^{-1} h^{-1} and 7.3±0.9 μmol ^{15}N g^{-1} h^{-1}, respectively (Table 2). The half-saturation constant, K_m, was always greater in all N treatments in *S. alterniflora* than either *Phragmites* lineage (Table 2).

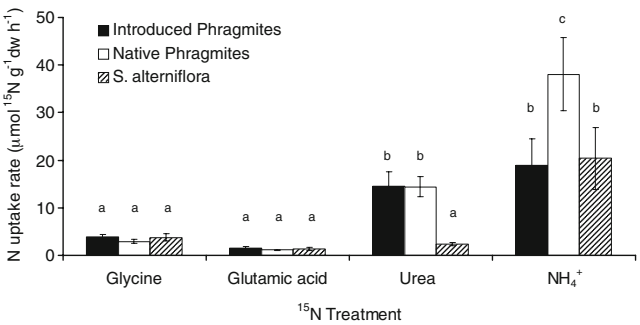

Fig. 1 Laboratory ^{15}N assimilation rates in μmols ^{15}N g^{-1} root dry weight h^{-1}, n=15 individual plants per treatment, total n=180 plants. *Error bars* represent ±SE. Significant differences among means (P< 0.05) are represented by *different letters*

Salinity Treatments

Salinity had a significant negative effect on ^{15}N-glycine uptake rates in both native *Phragmites* (P=0.001) and introduced *Phragmites* (P=0.032). Increasing concentrations of salinity reduced ^{15}N-glycine uptake rates more than

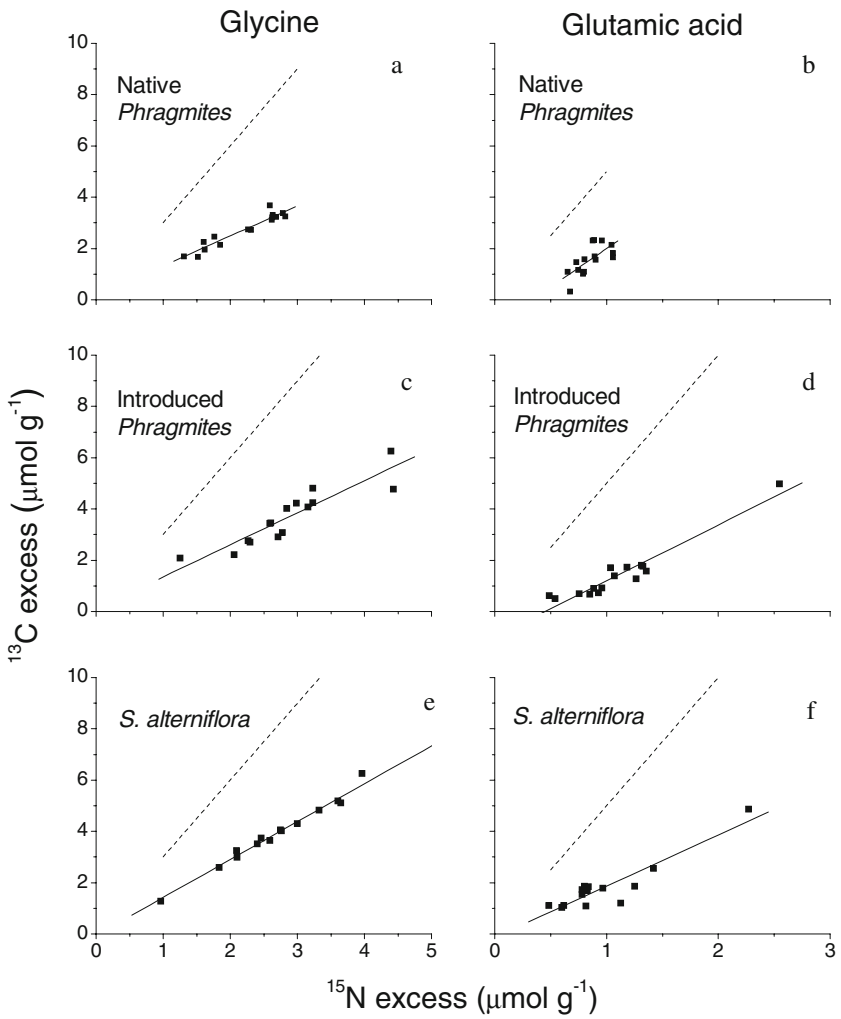

Fig. 2 Relationship between ^{13}C excess and ^{15}N excess in amino-acid-treated plants. Regression lines are the assimilated ratios of ^{13}C:^{15}N for each plant type. Glycine-treated plants n=15 individual plants: **a** native *Phragmites* (slope=1.17, r^2=0.909); **c** introduced *Phragmites* (slope=1.25, r^2=0.828); **e** *S. alterniflora* (slope=1.48, r^2=0.843). Glutamic-acid-treated plants, n=15 individual plants: **b** native *Phragmites* (slope=3.01, r^2=0.482); **d** introduced *Phragmites* (slope=2.17, r^2=0.919); **f** *S. alterniflora* (slope=1.99, r^2=0.843). *Broken lines* indicated theoretical C:N ratio

threefold in native *Phragmites* from 2.49±0.22 μmol ^{15}N g^{-1} h^{-1} to 0.72±0.11 μmol ^{15}N g^{-1} h^{-1} (Table 3). In introduced *Phragmites*, ^{15}N-glycine uptake rates were approximately halved from 1.61±0.21 μmol ^{15}N g^{-1} h^{-1} to 0.89±0.06 μmol ^{15}N g^{-1} h^{-1}. However, increasing levels of salinity did not affect ^{15}N-glycine uptake rates in *S. alterniflora* plants (P=0.52; Table 3).

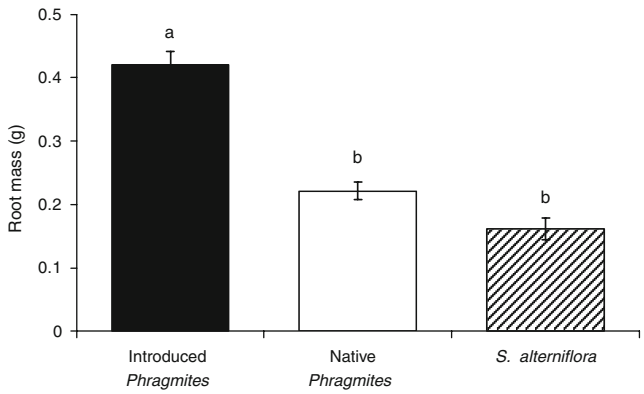

Fig. 3 Plant root mass from laboratory experiment. Mean plant root mass, n=60 per plant type, *error bars* represent ±1 SE in greenhouse grown plants from nutrient uptake experiment. Significant differences (P<0.001) are represented by *different letters*

Salinity also affected both *Phragmites* lineages NH_4^+–N uptake rates negatively (Table 4). Increasing salinity concentration had the greatest effect on native *Phragmites* haplotypes by again reducing N assimilation threefold, from 22.41±5.55 μmol ^{15}N g^{-1} h^{-1} at 10 ppt to 7.63±1.90 μmol ^{15}N g^{-1} h^{-1} (P=0.036) at the 30 ppt treatment; however, rates were not reduced at either the 10 or 20 ppt treatment (Table 4). Introduced *Phragmites* uptake rates were reduced by a factor of two from 14.36±2.88 μmol ^{15}N g^{-1} h^{-1} at 10 ppt to 7.63±1.90 μmol ^{15}N g^{-1} h^{-1} at 30 ppt (Table 4). Again, increasing concentrations of salinity (10–30 ppt) had no effect on DIN assimilation rates in *S. alterniflora* (Table 4).

Competition Treatments

When presented with an unlabeled competing substrate, ^{15}N-glycine was assimilated by all three plant types

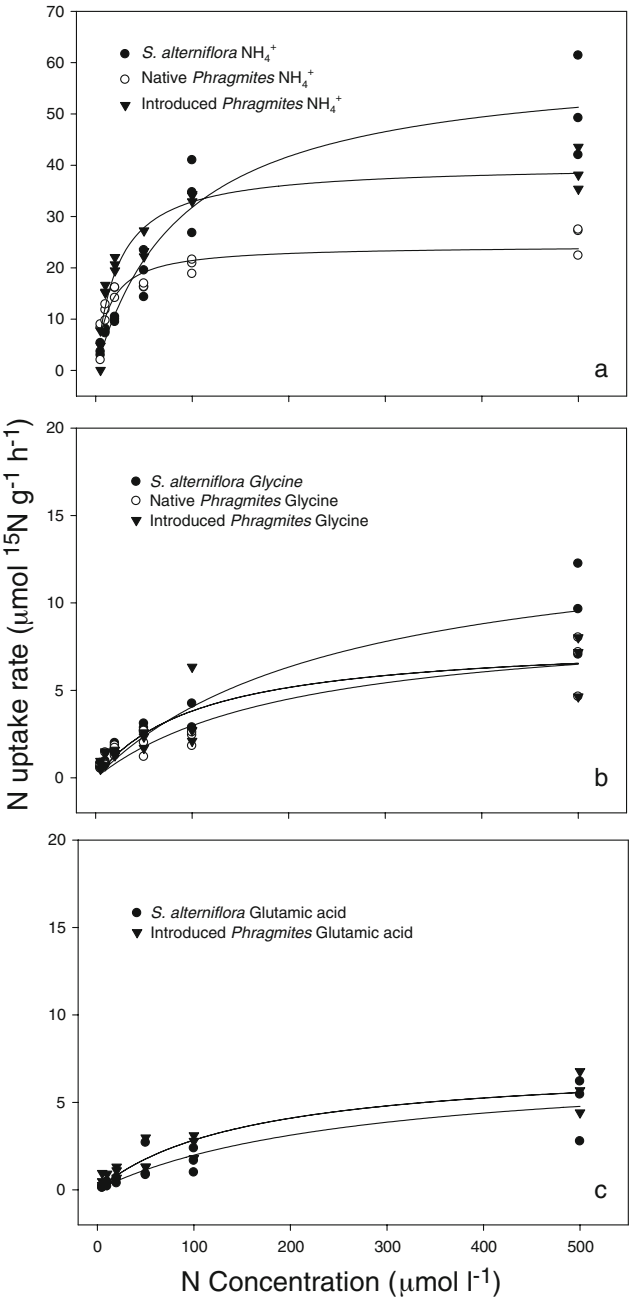

Fig. 4 Plant N uptake of **a** NH_4^+, **b** glycine–N, and **c** glutamic acid–N in *S. alterniflora*, native *Phragmites*, and introduced *Phragmites*. Each value represents the uptake rate for an individual plant, and the *plotted line* is the best fit estimate for the Michaelis–Menten equation for 18 individual plants per N treatment

(Table 5) at rates similar to those without a competing alternative N substrate (Fig. 1). No significant differences in ^{15}N-glycine uptake rates were observed between plant types, and the presence of a competing substrate did not affect ^{15}N-glycine uptake. Interestingly, the presence of a competing unlabeled organic N source in addition to a labeled inorganic N source increased NH_4^+ assimilation rates in *S. alterniflora* (Table 5) relative to those without a

Table 2 Uptake kinetics parameters of ^{15}N-ammonium, ^{15}N-Glycine, and ^{15}N-glutamic acid on root systems of intact plants of *S. alterniflora*, introduced *Phragmites*, and native *Phragmites*

Plant	^{15}N-ammonium				^{15}N-glycine				^{15}N-glutamic acid			
	V_{max} (μmol g^{-1} h^{-1})	K_m (μmol L^{-1})	α (L g^{-1} h^{-1})	r^2	V_{max} (μmol g^{-1} h^{-1})	K_m (μmol L^{-1})	α (L g^{-1} h^{-1})	r^2	V_{max} (μmol g^{-1} h^{-1})	K_m (μmol L^{-1})	α (L g^{-1} h^{-1})	r^2
S. alterniflora	60.5 (4.6)	90 (18)	0.67	0.97	14.3 (2.3)	256 (88)	0.06	0.94	7.4 (1.7)	271 (140)	0.03	0.88
Introduced *Phragmites*	40.1 (1.9)	22.0 (3.7)	1.82	0.98	8.0 (1.1)	109 (40)	0.07	0.90	7.3 (0.8)	157 (46)	0.05	0.95
Native *Phragmites*	24.3 (1.2)	13.6 (2.6)	1.79	0.98	9.2 (1.5)	208 (79)	0.04	0.92				

Uptake parameters are estimated from 18 individual plants, and values in parentheses indicate standard error estimates from the non-linear model

Table 3 Effects of salinity on the uptake of 100 μM ^{15}N glycine by intact root systems of *S. alterniflora*, native *Phragmites*, and introduced *Phragmites*. n=3 individual plants per treatment

Salinity (ppt)	*S. alterniflora* V_{uptake} (μmol g^{-1} h^{-1})	Native *Phragmites* V_{uptake} (μmol g^{-1} h^{-1})	Introduced *Phragmites* V_{uptake} (μmol g^{-1} h^{-1})
10	1.38±0.25	2.49±0.22	1.61±0.21
20	1.33±0.04	1.32±0.18	0.84±0.20
30	1.03±0.29	0.72±0.11	0.89±0.06

competing N substrate (Fig. 1). However, this increase was not significant due to the large error observed within these samples.

Field Experiment

All N-treated plants assimilated ^{15}N during the 4-day exposure to either the NH_4^+ or amino acid treatment, and introduced *Phragmites* plants incorporated three times more of the ^{15}N tracer in both N treatments (P<0.0001). Of the 25 μmols ^{15}N introduced in the DON treatment, *Phragmites* plants incorporated 30% (7.45±0.89 μmol ^{15}N) into their biomass, whereas *S. alterniflora* incorporated only 7% (1.83±0.62 μmol ^{15}N) of the introduced ^{15}N label (Fig. 5). Forty-two percent (27.43±6.22 μmol ^{15}N) of the 65 μmols ^{15}N introduced was recovered in introduced *Phragmites* plants in the NH_4^+ treatment, while only 12% (7.51±1.45 μmol ^{15}N) was recovered in *S. alterniflora* plants (Fig. 5). In addition to differences in the percent assimilation by the plant species, there were also differences in the translocation of the incorporated N within the plant depending on the treatment (P=0.137). In all treatments, *S. alterniflora* translocated a greater proportion of the assimilated N from root to shoot (Fig. 5). For introduced *Phragmites*, 11% of the N in the DON treatment was recovered in the shoot, in contrast to the NH_4^+ treatment, where only 4% was recovered in the shoot. *S. alterniflora*, on the other hand, showed different trends in N allocation with 16% recovered in the shoot in the DON treatment, and 33% in the shoot for the NH_4^+ treatment. When total N assimilated was normalized to g dry root mass, introduced *Phragmites* plants had three times greater (P<0.0001) NH_4^+ uptake than *S. alterniflora* (2.53±0.09 μmol ^{15}N g^{-1}dw and 0.75±0.01 μmol ^{15}N g^{-1}dw, respectively) as well as two times greater DON assimilation (0.54±0.04 μmol ^{15}N g^{-1}dw and 0.19±0.07 μmol ^{15}N g^{-1}dw, respectively). DON assimilation rates in the field were 27% and 24% of ammonium for introduced *Phragmites* and *S. alterniflora*, respectively. These rates are proportionally similar to those determined in the laboratory experiments. There were no significant differences in root mass between either plant species (P=0.53), but introduced *Phragmites* plants from the field experiment had approximately six times more shoot mass than *S. alterniflora* plants (P=0.007).

Table 4 Effects of salinity on the uptake of 100 μM ^{15}N ammonium by intact root systems of *S. alterniflora*, native *Phragmites*, and introduced *Phragmites*. n= 3 individual plants per treatment

Salinity (ppt)	*S. alterniflora* V_{uptake} (μmol g^{-1} h^{-1})	Native *Phragmites* V_{uptake} (μmol g^{-1} h^{-1})	Introduced *Phragmites* V_{uptake} (μmol g^{-1} h^{-1})
10	15.4±7.2	22.4±5.6	14.4±2.9
20	15.4±1.6	22.0±0.8	8.9±2.6
30	14.8±2.3	7.6±1.9	6.3±0.7

AMF Colonization

AMF were not found on any plant roots from the laboratory experiment (n=45).

Porewater N Concentrations

Our porewater data indicate that significant quantities of DON were available to both plant species. Significantly, more plant available N (NH_4^+ + urea + DFAA) was found in *S. alterniflora* porewater (70.8±8.7 μmol N l^{-1}) than in introduced *Phragmites* porewater (45.43±5.1 μmol N l^{-1}; P=0.03). *S. alterniflora* porewater contained 30% more available DON (DFAA + urea; 20.6±3.2 μmol N l^{-1}) than introduced *Phragmites* porewater (14.0±2.0 μmol amino acid–N l^{-1}; P=0.11; Fig. 6). No significant differences were found in the amount of DFAA available in either plant zone (P=0.39); however, the dominant DFAA were different. In *S. alterniflora* porewater, glutamic acid, glycine, asparagine, and alanine contributed the bulk of DFAA, whereas in introduced *Phragmites* porewater, serine, asparagine, glutamic acid, and glycine were the dominant forms. *S. alterniflora* sites had almost double, but not significantly (P=0.14) greater urea–N concentrations (7.8±1.8 μmol N l^{-1}) compared to introduced *Phragmites* (4.2±1.1 μmol N l^{-1}), and greater, but not significantly (P=0.09) greater NH_4^+ concentrations (47.4±7.3 μmol N l^{-1} vs. 31.5±4.4 μmol N l^{-1}, respectively). Combined, DON accounted for approximately 30% of plant available N for both plant species at this site.

Discussion

DON Assimilation

Our study is the first to demonstrate the use of organic N compounds by temperate wetland plants by direct uptake of

Table 5 Uptake rates of ^{15}N-ammonium and ^{15}N-glycine by intact root systems of *S. alterniflora*, native *Phragmites*, and introduced *Phragmites* in the presence of unlabeled competing nitrogen sources

Plant	Substrate [100 μM]	Competing substrate [100 μM]	Uptake rate at 100 μM ^{15}N (μmol g^{-1} h^{-1})
S. alterniflora	^{15}N-ammonium	Glycine	41.1±15.8
Native *Phragmites*	^{15}N-ammonium	Glycine	23.2±1.2
Introduced *Phragmites*	^{15}N-ammonium	Glycine	30.2±1.4
S. alterniflora	^{15}N-glycine	Ammonium	3.8±0.3
Native *Phragmites*	^{15}N-glycine	Ammonium	4.0±1.0
Introduced *Phragmites*	^{15}N-glycine	Ammonium	3.1±0.1

intact amino acids and urea. The NH_4^+ uptake kinetic rates we determined by using stable isotopes as tracers in intact plants are lower, but within the same range to those previously determined using time course depletion experiments (Chambers et al. 1998, Tylova-Munzarova et al. 2005). Our parameter estimates were almost an order of magnitude lower than Romero et al. (1999), which may be attributed to different *Phragmites* genetic lineages or differences in methodologies. The lower values reported by our study are therefore conservative estimates of plant N uptake since they measure plant assimilation, and not depletion of nutrients from the water column which may be associated with rhizosphere bacteria. Although our K_m estimates are greater than those reported in the aforementioned studies that used depletion over time as a proxy for uptake, our K_m estimates for amino acid uptake are in the range of other studies using stable isotopes to estimate kinetic parameters (Kielland 1994, Henry and Jefferies 2003). Most importantly, our study provides a common metric that can be used to compare differences in N uptake parameters. Finally, use of intact plants assured that we did not overestimate assimilation kinetics as is possible in experiments where rates are determined from excised roots (Falkengren-Grerup et al. 2000).

Since the plants were not colonized by AMF, N assimilation rates reported in this study can be attributed to plant processes only. The lack of AMF colonization suggests that such associations do not clearly benefit either *Phragmites* lineage in our study. AMF do not generally form associations with *Phragmites* (Burke et al. 2002), and are unlikely in saturated soils since AMF are obligate aerobes (Cooke and Lefor 1998). Although the ^{13}C:^{15}N were lower than the hypothesized ratios, this may be explained by the high background concentrations of ^{13}C in the plants, and the subsequent dilution of the ^{13}C signal. Lower ^{13}C ratios may also be attributed to amino acid metabolism of C (Schmidt and Stewart 1999) or amino acid transport processes (Oliver 1994, Nasholm et al. 1998). It is possible that even though ^{13}C concentrations were not increased significantly in the urea treatment that the urea molecule was assimilated intact. Merigout et al. (2008) showed for the plant *Arabidopsis* that the urea molecule was hydrolyzed in the root tissue by cytosolic ureases into two amino groups and the resulting $^{13}CO_2$ gas would dissipate and therefore be undetectable in the root biomass.

Our results have potential implications for interactions of *S. alterniflora* and introduced *Phragmites*. At low [N], the high affinity for N by introduced *Phragmites* (Table 2) could potentially give this plant type an advantage at accessing limited N pools. In addition, these plants could take up significantly more N (both DON and DIN) than *S. alterniflora*, which is evidenced by our field experiment and greater N assimilation by introduced *Phragmites*. Numerous studies have shown that *Phragmites* demands

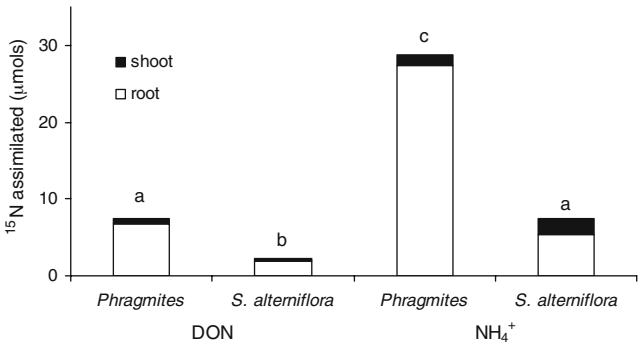

Fig. 5 Mean field ^{15}N assimilation by introduced *Phragmites* and *S. alterniflora* into plant root and shoot, n=3 mesocosms per plant type per treatment. Significant differences (P<0.05) in assimilated ^{15}N are indicated by *different letters*

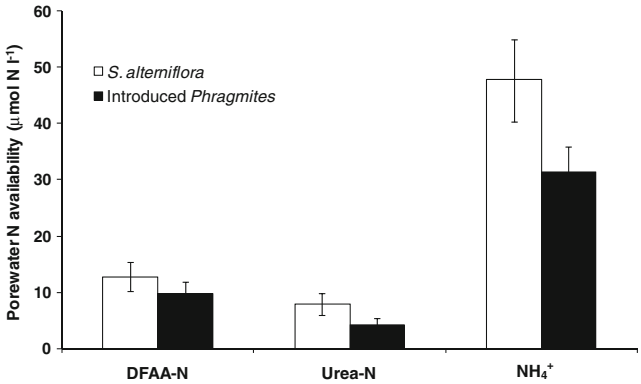

Fig. 6 Mean porewater N concentration of bioavailable N species ± SE, dissolved free amino acids (DFAA-N), urea–N, and NH_4^+ in μmol N l^{-1} in introduced *Phragmites* and *S. alterniflora* marsh zones

>50% more N than the species it displaces in salt, brackish, and tidal fresh marshes (Templer et al. 1998, Windham 2001, Windham and Ehrenfeld 2003, Windham and Meyerson 2003). The high affinity and greater uptake of N could reduce N availability in sediment porewater for the other species. Other studies have reported lower porewater N availability in introduced *Phragmites* marshes compared to *S. alterniflora marshes* (Chambers et al. 1998). When porewater DIN pools are lowered, uptake of DON could provide a competitive advantage. We found that at least 30% of porewater N was in the form of DON in both *S. alterniflora* and introduced *Phragmites* zones, making DON a potentially viable source of plant N for both species.

N uptake kinetics from our study may be used to provide insights in plant nutrient uptake and competition between *Phragmites* and *S. alterniflora.* We acknowledge that N uptake kinetics are not static and change based upon physio-chemistry in the rhizosphere (Mendelssohn and Morris 2000); however, since our plants were grown under identical conditions N uptake kinetics do provide a common metric for comparisons. Although all plant types had a lower affinity for amino acids than NH_4^+, when both nutrient sources were presented in the laboratory experiments, assimilation rates of DON did not differ. Our data indicates that plants take up organic nutrients in the field at similar proportions to those determined in the laboratory experiments. Unfortunately, it is impossible to determine actual uptake rates of ^{15}N in the field from our experiment; however, field N assimilation of DON in both plant species was calculated to be approximately 25% of NH_4^+. This is proportional to experimentally determined uptake rates, and suggests that our N uptake parameters hold proportionally constant in the field.

Given the dominance of *S. alterniflora* and *Phragmites* in wetland habitats worldwide and our finding of significant DON uptake, we suggest that DON utilization should be considered when investigating plant interactions in wetland systems. Studies have shown that DON can be a significant pool of N in temperate estuarine systems (Gardner and Hanson 1979, Valiela and Teal 1979, Burdige and Martens 1988, Burdige and Zheng 1998, Tyler et al. 2001, Guldberg et al. 2002, Verity 2002); however, this pool is rarely quantified into its refractory and bioavailable fractions. DON is especially important since changes in land use, eutrophication, and coastal development have been related to increases in water column nutrients, with the greatest increases in the form of DON (Verity 2002). Also, it has been suggested that atmospheric deposition in marine systems may result in significant increases in DON concentrations in the form of labile amino acids (Cornell et al. 1995).

From our data, we present a potential estimate of the contribution of DON to plant nutrient demand by making the following assumptions: (1) when presented with both DON and NH_4^+, neither uptake mechanism is negatively affected by competition (Table 5), at 100 μM porewater [N] (total porewater [N] from Fig. 6), (2) uptake rates of NH_4^+ are approximately 20 μmol ^{15}N g^{-1} h^{-1} (Fig. 1), (3) uptake rates of DFAA (glycine) are approximately 4 μmol ^{15}N g^{-1} h^{-1} (Fig. 2), then (4) uptake in the presence of both DON and NH_4^+ substrates is the sum of the two rates. This calculation estimates that amino acid–N may contribute up to 17% to plant N demand for both *S. alterniflora* and *Phragmites*. This is a conservative estimate since we only included uptake of glycine alone ignoring other primary amines that are available in the soil solution for plant uptake. Even assuming maximum uptake rate (V_{max}), we also come to a similar metric of approximately 18% of plant N demand coming from DFAA-N alone. However, if we include urea–N, (14.4 μmol ^{15}N g^{-1} h^{-1} for both *Phragmites* haplotypes, and 2.4 μmol ^{15}N g^{-1} h^{-1} for *S. alterniflora*), then DON can account for up to 47% of *Phragmites* N demand and only 24% for *S. alterniflora.* This calculation suggests the possibility of DON utilization by *Phragmites* being a potential mechanism facilitating its expansion in temperate salt marshes. The authors acknowledge that physio-chemistry in the rhizosphere may impact our analysis; however, since measured uptake rates in the field were proportionally constant as the laboratory experiment, it suggests that our experimentally determined rates, hence our estimates of potential N demand, are good estimates for both plant species.

N translocation and assimilation varied with both plant type and N treatment over the 4-day field experiment. Significantly more (17–33%) assimilated N was translocated into the shoot in *S. alterniflora* plants than in introduced *Phragmites* plants (4–11%); however, these results may be confounded by the differences in aboveground biomass between the two plant types. Nonetheless, it demonstrates that assimilated N is very quickly moved into the shoot of *S. alterniflora.* Although *Phragmites* translocated less N to its aboveground shoot, three times more DON was recovered in the shoot when compared to DIN, suggesting that introduced *Phragmites* may rely more upon DON as a N source, in contrast to *S. alterniflora,* which translocated NH_4^+ more rapidly to the shoot. N translocation differences with plant species merits further investigation.

As expected, salinity had less of an effect on the halophytic *S. alterniflora* N uptake parameters than introduced *Phragmites.* Reductions in DIN uptake with increasing salinity are consistent with the results of Bradley and Morris (1991) and Chambers et al. (1998). A potentially confounding factor in the salinity experiment is that our plants were grown initially in freshwater, and were only acclimated to the salinity treatments for 30 min prior

to experimentation. Therefore, the effects of salinity we observed could potentially be greater than in plants that had been grown and acclimated to a saline environment (Bradley and Morris 1991). More studies are suggested to further investigate this interaction.

Differences Between Native (F) and Introduced (M) *Phragmites*

An important finding of this study is the difference in N uptake between the native and introduced lineages of *Phragmites*. To date, studies have reported examples of congener invasive species success out-performing native counterparts (Schweitzer and Larson 1999, McDowell 2002, Deng et al. 2004). Since native and introduced lineages compete for the same ecological niche (Park and Blossey 2008), we suggest that the native type may be more efficient at using N at low concentrations. It is possible that under historically N-limited conditions, the introduced *Phragmites* was more N-limited than the native lineage. Since pre-industrial times, anthropogenic N inputs have doubled along the North American Atlantic coast (Galloway et al. 2004), and at the same time, introduced *Phragmites* has outcompeted the native type (Saltonstall 2002), and has expanded into historically unoccupied habitats throughout North American wetlands (Chambers et al. 1999). Correlative studies have suggested that increased N availability is a factor facilitating the introduced *Phragmites* invasion (Bertness et al. 2002, King et al. 2007), and this shift in N availability is necessary for the invasion since the introduced lineage demands approximately four times more N than the native type in mid-Atlantic tidal marshes (Mozdzer and Zieman 2010). This suggests that there is a threshold of N beyond where the introduced *Phragmites* has an advantage in the field. Our data suggests that above ~20 μM [NH_4^+] (Fig. 4a) the introduced lineage may have a competitive advantage for available N. More detailed field investigations are needed to experimentally determine this threshold.

While our data suggests that the overwhelming success of the introduced *Phragmites* lineage may be attributed to physiological processes belowground, one puzzling result is that native *Phragmites* had greater N uptake rates when compared to the introduced lineage with increasing salinity (Table 4). This result is counter-intuitive since one does not usually find Atlantic coast native types in saline habitats (Saltonstall and Stevenson 2007). One explanation may be that with increasing salinity, there is often an increase in H_2S concentrations which are known to limit the distribution of *Phragmites* (Chambers et al. 1998, Chambers et al. 2003). Since introduced *Phragmites* produced more belowground roots (Fig. 3; Mozdzer unpublished data), increased belowground production may make the introduced lineage more efficient at oxidizing the rhizosphere. Tulbure et al. (2007) reported greater culm oxidation in introduced *Phragmites* which may also explain the increased tolerance of the introduced lineage to elevated hydrogen sulfide.

Conclusions

Our field and laboratory experiments suggest that DON utilization by *Phragmites* may be a factor facilitating its expansion. Due to the high N demand of introduced *Phragmites*, its affinity for N, and its ability to use a variety of N sources, introduced *Phragmites* can potentially exploit both N-rich and N-depleted environments. As coastal systems become nutrient enriched, this may promote the invasion of the introduced *Phragmites* and the displacement of native species. Our study also demonstrates how belowground physiological differences within a species provide one mechanism to help explain the biological invasion and overwhelming success of an introduced genotype. Since native *Phragmites* lineages do not respond similarly to increasing N concentration due to physiological limitations in N uptake, they cannot take advantage of higher N concentrations, which could ultimately limit their production and competitive ability under current and predicted environmental forcings.

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