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Mining of Deep Nitrogen Facilitates *Phragmites australis* Invasion in Coastal Saltmarshes

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Abstract

Phragmites australis (or common reed), one of the most widespread invasive species in wetlands of North America, has a high nitrogen (N) demand compared to native species. However, it is unclear how *P. australis* is able to meet this N demand, especially in systems with low soil N and limited N inputs. We evaluated whether deep rooting could be a mechanism by which *P. australis* accesses otherwise unused N pools, allowing it to circumvent nutrient competition and acquire the "missing N" needed to meet its N demand. We examined above and belowground N pools, as well as depth profiles of belowground biomass (to 3 m), in native and *P. australis*-dominated plant communities in two brackish marshes of the Chesapeake Bay. To evaluate whether deep roots contribute to *P. australis* meeting its high N demand, we amended soil porewater with ^{15}N at four soil depths (10, 20, 40, and 80 cm) and measured the rate of ^{15}N assimilation into plant biomass. We found that *P. australis* had 6–8 × the aboveground standing stock N, 2–3 × the total belowground biomass, and roots up to 3 × deeper than native plant communities (some exceeding 3 m). Our ^{15}N tracer study demonstrated that *P. australis* acquired N from all measured soil depths, whereas N uptake by native plants was minimal below 20 cm. Our results demonstrate that deep rooting is a mechanism by which *P. australis* can access previously buried N pools, helping to satisfy its high N demand and thus fuel its invasion. Moreover, these results fundamentally challenge our understanding of biogeochemical processes deep within the soil profile given the presence of active roots at depths where they were previously thought to be largely inert.

Keywords *Phragmites australis* · Nitrogen · ^{15}N · Deep rooting · *Spartina patens* · *Schoenoplectus americanus*

Introduction

Many tidal and non-tidal wetlands in North America are rapidly converting to near-monocultures of an invasive lineage of the common reed *Phragmites australis* (Cav.) Trin. ex Steud. (Chambers et al. 1999; Saltonstall 2002; Kettenring et al. 2012). The invasive lineage of this species is widely recognized as an ecosystem engineer capable of altering the structure and function of ecosystems. It degrades faunal habitat (Meyerson et al. 2000a), reduces biodiversity (Chambers et al. 1999; Bertness et al. 2002), and alters biogeochemical cycles (Windham and Lathrop 1999; Meyerson et al. 2000b; Windham and Ehrenfeld 2003). Its competitive advantage over native plant communities are driven, in large part, by its rapid rates of photosynthesis (Mozdzer et al. 2013; Mozdzer and Caplan 2018), prolific growth both above and belowground (Mozdzer and Zieman 2010; Mozdzer et al. 2010; Mozdzer and Megonigal 2012), and copious production of seeds and other propagules (Kettenring et al. 2011).

P. australis' high productivity comes with a high demand for nitrogen (N). In most North American wetlands, plant N demand far exceeds supply, making N the foremost nutrient-limiting productivity (Valiela et al. 1973; Bedford et al. 1999). This appears to be the case with respect to *P. australis* colonization; gradients in N pollution correlate well with *P. australis* occurrence at the landscape scale (Bertness et al. 2002;

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King et al. 2007). However, multiple studies have indicated that the N requirements of mature *P. australis* surpass those of the native species in the ecosystems it invades (Windham and Meyerson 2003; Mozdzer and Zieman 2010). The source of the N necessary to sustain mature clones of *P. australis* in marshes with minimal N pollution has eluded scientists (Meyerson et al. 2000b) and has hindered our understanding of *P. australis* invasion dynamics in such systems.

P. australis invasion itself has a number of consequences for soil nitrogen pools. For example, it can cause more N to be retained within the system through plant uptake, microbial immobilization, and burial (Neubauer et al. 2005; Mozdzer et al. 2016). Early studies reported that *P. australis* has a higher standing stock biomass and N content than many marsh plant species including *Typha angustifolia* (Findlay et al. 2002), *Spartina pectinata* (Rickey and Anderson 2004), *P. australis* ssp. *americanus* (a lineage native to North America) (Mozdzer et al. 2013), and *Spartina patens* (Meyerson et al. 2000a). *Phragmites australis* therefore has a higher demand for N than these native plant species (Windham and Ehrenfeld 2003; Mozdzer et al. 2010). In acquiring N to meet this higher demand, *P. australis* likely reduces the availability of N to native species. This greater N demand must be met by either an increase in N from an import process (N fixation, atmospheric deposition, uptake from the water column), from an increase in N mineralization within the system, or accessing nutrient pools previously unavailable or accessible to native communities (Mozdzer et al. 2016).

Species shifts brought about by plant invasion are often associated with changes in functional traits that can have cascading effects on ecosystem structure and function. Native plant species in brackish tidal wetlands of the North American Atlantic and Gulf Coasts (e.g., *S. patens*, *S. americanus*, and *Distichlis spicata*) are typically shallow-rooted with a majority of the belowground biomass concentrated in the upper 30 cm of the soil profile, sharply decreasing with depth (Saunders et al. 2006). Due to the shallow rooting profiles in native wetland plant communities, few studies have examined rooting profiles or the influences of plant roots below a depth of 30 cm. However, changes in plant community composition by novel invaders may change root depth distributions and associated biogeochemical processes (Mozdzer et al. 2016). For example, *P. australis* belowground biomass has been shown to be significantly greater than several native plants species in the upper 50 cm of the soil (Windham 2001), including *S. patens* (quantified over upper 40 cm), *S. americanus* (65 cm) (Saunders et al. 2006), *S. alterniflora* (50 cm) (Blum 1993), and *T. angustifolia* (50 cm) (Templer et al. 1998).

Deeper rooting profiles may allow communities to access new nutrient-rich soil pools (Pimentel et al. 2005), circumvent nutrient competition (Faillace et al. 2018), and access deeper water sources with lower salinity (Lissner and Schierup 1997). Deep rooting may also provide a source of carbon for microbial communities (Raich and Nadelhoffer 1989; Windham and Lathrop 1999; Bernal et al. 2017), increase redox potentials at deeper depths (Armstrong et al. 1996), and lead to increases in plant productivity through feedbacks that accelerate invasion (Mozdzer et al. 2016). However, previous studies have not examined the role of rooting depth and associated nutrient uptake in wetland ecosystems as a trait that may enable invasive *P. australis* to proliferate.

We sought to determine if mining deep N could be a primary mechanism by which *P. australis* meets its high demand for N. We compared the amount and depth distribution of belowground biomass, N pools, and plant N uptake between *P. australis* and native-dominated plant communities. We predicted that a deeper distribution of belowground biomass, specifically below the depth of native plant species, provides access to deeper, more nutrient-rich porewater, providing *P. australis* access to N at depths where it is unavailable to more shallowly rooted native species.

Methods

Study Site

This study was conducted in Edgewater, MD, USA, which is in the Rhode River sub-estuary of the Chesapeake Bay. We used two brackish tidal marshes within the jurisdiction of the Smithsonian Environmental Research Center, namely Fox Creek and Corn Island. These marshes are approximately 1 km apart and have similar surface elevations. Wetlands in this area experience a mean tidal range of 44 cm, and salinity typically varies seasonally between 4 and 15 PSU (Langley et al. 2009). These high marsh platform sites were selected because they contained functionally distinct native plant communities at similar high marsh elevations and because they were in close proximity to monotypic stands of *P. australis*. Corn Island was dominated by the C_3 sedge *S. americanus* (with *S. patens* subdominant), while Fox Island was dominated by the C_4 grasses *S. patens* and *D. spicata*. *P. australis* became established in both sites after about 1990 (McCormick et al. 2010). Soils in the area are comprised of peat (> 80% organic matter) than can exceed several meters in depth.

Aboveground Biomass and Standing Stock N

At both sites, we estimated aboveground biomass and standing stock N following Windham and Lathrop (1999). In 2012 and 2013, aboveground biomass was harvested from six, 0.125-m^2 quadrats, sampled 5 m apart along a transect in each plant community and site ($n = 24$ quadrats in total), dried to constant mass at 60° C, and weighed. The transect was established parallel to the creek edge and parallel to both

plant communities to account for factors associated with tidal inundation. To improve our N budget for *Phragmites*, we also randomly selected 20 *Phragmites* stems, which were subsequently separated by plant organ (leaf, stem, and flower), dried to constant mass, and weighed to determine the allocation of biomass. Samples were homogenized using a ball mill (SPEX SamplePrep 8000D, Metuchen, NJ, USA) and analyzed for C and N content on an EAI CE-440 Elemental Analyzer (Exeter Analytical, North Chelmsford, MA, USA). Mean tissue N content was multiplied by total plot biomass to calculate aboveground standing stock N.

Belowground Biomass and Depth Distribution

Belowground biomass and its distribution with depth were determined from two soil cores (5-cm diameter) per plant community and site ($n = 8$). Cores were collected using a Russian Peat Borer (Aquatic Research Instruments, Hope, ID, US). All cores extended to 3-m depth except for one of the *P. australis* cores from Fox Creek, which extended to 3.5 m. One core was taken per day, allowing us to divide each of the eight cores into 27 sections in the field. Section thicknesses were as follows: 5-cm sections from 0 to 30 cm depth, 10-cm sections from 30 to 100 cm depth, 20-cm sections from 100 to 200 cm, and 25-cm sections at depths beyond 200 cm. Core sections were immediately wrapped in plastic to limit exposure to ambient air and transported to the lab within 1 h of collection for porewater extraction (described below). After porewater was extracted, core sections were washed through a 2 mm and a 1-mm sieve to remove sediment and loosen roots and rhizomes. Belowground biomass samples were sorted into living and dead categories based on visual interpretation of color and turgidity (Windham 2001). Live roots were further separated into categories for fine roots, C_3 rhizome, C_4 rhizome, C_3 red roots (Saunders et al. 2006), and *P. australis* rhizomes. Sorted samples were dried at 60° C to constant mass and weighed. Elemental N content was determined as described above to determine standing stock belowground plant N. Given the number of analyses and related processing of the core sections anaerobically, it was only possible to collect two cores per plant community.

Belowground biomass distribution was characterized for each core using the asymptotic equation of Gale and Grigal (1987):

$$Y = 1 - \beta^d$$

where Y is the cumulative biomass fraction, ranging from 0 at the surface to 1 at depth d (in cm), and β is a coefficient describing the shape of that relationship. Larger β values indicate that a greater proportion of biomass was found at depth, while smaller β values indicate that a greater proportion was found near the surface. Fitting to determine β values was performed using nonlinear least squares (via function *nls*) in R version 4.1.2 (R Project for Statistical Computing, Vienna, Austria).

Porewater Ammonium

Porewater was pressed by hand from each core section in an anaerobic chamber (COY Lab Products, Grass Lake, MI, USA) within 1 h of sample collection. It was then filtered (0.45 µm) and frozen for later analysis. Ammonium (NH_4) concentrations were determined using the indophenol blue method (Solorzano 1969). We measured only ammonium because the soils at this site are anaerobic (as is typical for salt marshes) and levels of nitrate are typically below limits of detection (Keller et al. 2009).

Nitrogen Uptake

To evaluate whether *P. australis* actively uses nitrogen from deep soil, we conducted an isotope tracing experiment at the Fox Creek site using a method modified from McKinley et al. (2009). We established 4 plots (1 m^2 each) in the *P. australis* community and 5 plots in the native community, randomly assigning them to a reference group (where ^{15}N was at natural abundance) or one of the following enrichment treatments: 10 cm (native community only), 20 cm, 40 cm, and 80 cm. The 10-cm labeling plot was used in the native community because a large portion of the belowground biomass was < 30 cm; the additional treatment therefore increased our ability to measure ^{15}N uptake. Plots were not replicated due to the prohibitively high costs of the ^{15}N-substrate. We underscore that the experiment was not designed to characterize variation in assimilation rates but as proof of the concept that *P. australis* mines deep soil N. Two days prior to ^{15}N addition, the youngest leaf that was fully expanded and non-budding was collected from every stem within each *P. australis* plot. In the native plots, 10 full *S. patens* stems were haphazardly collected to quantify ^{15}N ratios at time zero.

On 26 July 2012, we introduced $^{15}NH_4Cl$ (99 at % ^{15}N, Cambridge Isotope Laboratory, Andover, MA, USA) to each plot using a hollow stainless steel rod (4.8-mm outer diameter) with 20 holes that were 0.16-mm diameter drilled into the lowermost 5 cm of the rod. We prepared solutions containing 1 g, 1 g, 2 g, and 6 g of $^{15}NH_4Cl$, each dissolved in 8.1 L of deionized water, for injection into the 10, 20, 40, and 80-cm depth plots, respectively. Concentrations increased with depth to strengthen our ability to detect the ^{15}N tracer (McKinley et al. 2009). Each plot was injected with 100 mL of the tracer solution in a grid of 9 rows × 9 columns (10 cm spacings) to ensure uniform labeling. After each addition, the rod was flushed with 50 mL of deionized water into a waste bucket.

We used allometric relationships to determine plant biomass in the *P. australis* plots (plant height and basal stem

diameter) and small clip plots (10 × 10 cm) to estimate biomass in the native plant community. Plant biomass was used to determine the total amount of ^{15}N assimilated by plants in each plot.

Two weeks after ^{15}N addition, we collected a newly expanded leaf from each stem in the *P. australis* plots and 10 full *S. patens* shoots from each native plot to provide a conservative estimate of plant uptake. These plant samples and those collected prior to tracer addition were dried to constant mass and homogenized using a ball grinder (SPEX SamplePrep 8000D, Metuchen, NJ, USA). Subsamples were sent to the UC Davis Stable Isotope Facility for ^{15}N determination.

The amount of ^{15}N label taken up in each plot (^{15}N *uptake*, in percent) was calculated as:

$$^{15}N\ uptake = \left(\frac{(N_{lab} - N_{\text{pre}}) \times TN_{lf}}{Mass\ ^{15}N\ injected} \right) \times 100$$

where N_{lab} is the ^{15}N concentration in the labeled sample (in atom percent), N_{pre} is the ^{15}N concentration in the vegetation prior to labeling (in atom percent), and TN_{lf} is the total leaf N in each plot. ^{15}N uptake is a measure of how much of the introduced label was assimilated by the vegetation in each plot and provides a conservative estimate of uptake that can be compared between the two plant communities.

Data Analysis

For most response variables (excluding ^{15}N uptake), mixed effects linear models were used to determine whether means differed by community (*P. australis* vs. native, a fixed effect); response variables included biomass, standing stock N, porewater NH_4, and β values describing rooting depth distributions. For biomass and standing stock N models, additional fixed effects were included for location (aboveground vs. belowground) and the community × location interaction. In all models, site was included as a random effect. This allowed us to evaluate fixed effects using data from the two sites together, thus maximizing statistical power and the generalizability of our results. Log transformations were applied to all response variables except β to ensure that residuals were distributed normally (checked with quantile–quantile plots). χ^2 statistics from Wald tests with type III sums of squares were used to assess the importance of main effects. For ^{15} N uptake, median tracer assimilation by *P. australis* vs. the native community was compared using a Wilcoxon rank sum test. Associations between uptake fraction and belowground biomass abundance were evaluated with Pearson correlation tests. Statistical analyses were carried out in R version 4.1.2 (R Project for Statistical Computing, Vienna, Austria) including the code libraries *lme4* and *car*.

Results

Biomass and Standing Stock N

Biomass was greater for the *P. australis* community than for the native community (effect of community: $\chi^2 = 67.5$, $p < 0.001$; Fig. 1, Table 2) though the size of this difference was greater for aboveground biomass than belowground (community × location interaction: $\chi^2 = 6.9$, $p = 0.009$, Table 2). On average, *P. australis* had 5 × greater aboveground biomass than the native community (2945 vs. 575 g m^{-2}) and 2.1 × greater belowground biomass (2738 vs. 1294 g m^{-2}). Despite modest differences in sample means between Fox Creek and Corn Island (Tables 1 and 2), patterns were largely consistent between sites. The most notable exception was that the belowground biomass of the native community was likely greater at Corn Island than at Fox Creek (Fig. 1), due mainly to the presence of C_3 rhizomes and red roots (both of which originate from *S. americanus*) at the site.

Standing stock N was also greater in the *P. australis* community, both aboveground and below (effect of community: $\chi^2 = 111$, $p < 0.001$, effect of location: $\chi^2 = 8.2$, $p = 0.004$; Fig. 2, Table 2). Aboveground, the discrepancy between communities was more extreme than for biomass; *P. australis* held 8 × the aboveground standing stock N than did the native community (36.9 vs. 4.8 g N m^{-2}). Belowground, the difference was smaller, with *P. australis* holding 1.3 × the standing stock N than that held in the native community (19 vs. 15 g N m^{-2}). While the ratio was relatively consistent between sites aboveground, belowground standing stock N in the native community was noticeably greater at Corn Island than at Fox Creek (Fig. 2). To a still greater extent than for biomass, this was due to N held in C_3 rhizomes and red roots belonging to *S. americanus* (Table 1).

Although leaves accounted for only 30% of *P. australis* biomass, they constituted nearly 66% of the aboveground standing stock N, pointing to the high N investment made in photosynthetic tissues (Table 1). In the native community, *S. patens* accounted for 87% of aboveground standing stock N at Fox Creek but only 44% at Corn Island (where *S. americanus* was dominant) and accounted for 52% of the aboveground N (Table 1).

Porewater Ammonium

Porewater NH_4 concentrations were greater in the native community than the *P. australis* community overall (effect of community: $\chi^2 = 384$, $p < 0.001$, Table 2). However, the magnitude of this difference varied strongly by site (community × site interaction: $\chi^2 = 147$, $p < 0.001$; Fig. 3, Table 2). At Fox Creek, the native community's porewater NH_4 increased

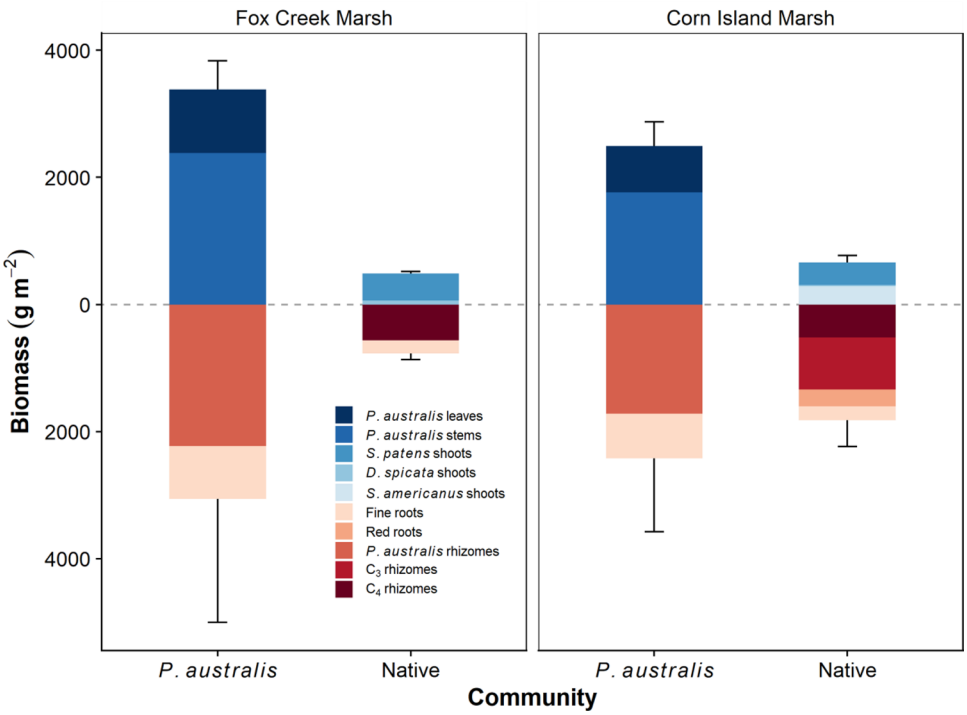

Fig. 1 Mean (±SE) above- and below-ground biomass (g m^{-2}) in the *P. australis* and native plant communities; $n=4$ in the *P. australis* community and 5 in the native community

from about 40 µmol L^{-1} at the surface to over 1200 µmol L^{-1} at 3-m depth, whereas in the *P. australis* community, it remained relatively low and invariant with depth. At Corn Island, mean porewater NH_4 was only slightly greater in the native community and in neither community did NH_4 vary substantially with depth. That said, relative to concentrations in the uppermost 50 cm, NH_4 was elevated in both communities between 50 and 200 cm (Fig. 3).

Belowground Biomass Depth Distributions

Invasive *P. australis* distributed belowground biomass more deeply than native vegetation, yielding greater β values (Fig. 4; effect of community: $\chi^2=13.0$, $p=0.001$, Table 2). More than 50% of *P. australis* belowground biomass was found below 30 cm, with live biomass found as deep as 387 cm at Fox Creek and 280 cm at Corn Island (Fig. 4A).

Table 1 Community composition and biomass partitioning at Fox Creek and Corn Island marshes estimated from 0.125 m^2 plot measurements (means of 4 plots in the *P. australis* community and 5 plots in the native community). Coarse roots were combined with fine roots in this table. Red roots have been previously identified by coloration methods and isotopic ($\delta^{13}C$) signature to be *S. americanus* (Saunders et al. 2006)

Community		*N* (%)	Fox Creek Marsh		Corn Island Marsh	
			Total biomass (%)	Total *N* (%)	Total biomass (%)	Total *N* (%)
***Phragmites australis* community**						
Aboveground	Leaves	2.796	29.5	65.9	29.2	65.9
	Stems	0.605	70.2	34.1	70.5	34.1
Belowground	Fine roots	1.195	27.1	47.1	29.1	49.5
	Rhizomes	0.490	72.9	52.9	70.9	50.5
Native community						
Aboveground	*S. patens* shoots	0.714	86.7	86.3	53.9	44.0
	S. americanus shoots	1.233	0.0	0.0	43.3	53.8
	D. spicata shoots	0.711	13.2	13.7	2.80	2.10
Belowground	Fine roots	1.155	26.4	27.7	12.0	11.6
	Red roots	1.400	0.0	0.0	14.6	17.2
	C_3 Rhizomes	1.201	0.0	0.0	45.0	45.4
	C_4 Rhizomes	1.080	73.6	72.3	28.4	25.7

Table 2 Summary of mixed effects models used in this study. *df*, degrees of freedom; R^2m, R^2 for fixed effects only; R^2c, R^2 of the full model (fixed and random effects)

Response	Transform	Random term	Fixed terms	χ^2	df	*P*	R^2m	R^2c
Biomass	log	Site	Location	0.7	1	0.403	0.73	0.73
			Community	67.5	1	< 0.001		
			Location × community	6.9	1	0.009		
Standing stock N	log	Site	Location	8.2	1	0.004	0.80	0.81
			Community	111.1	1	< 0.001		
			Location × community	25.2	1	< 0.001		
Porewater N	log	Group	Site	56.2	1	< 0.001	0.73	0.73
			Community	384.7	1	< 0.001		
			Site × community	146.7	1	< 0.001		
β	-	Site	Community	13.0	1	< 0.001	0.57	0.70

This corresponded to β values (mean ± SD) of 0.979 ± 0.006 at Fox Creek and 0.966 ± 0.005 at Corn Island (Fig. 4B). In contrast, the native community at Fox Creek held 99% of belowground biomass in the upper 30 cm of the soil, and the deepest live root was found within 75 cm of the soil surface (β = 0.798 ± 0.039). At Corn Island, 97% of the native community's belowground biomass was in the upper 30 cm, with the deepest live root observed around 110 cm (β = 0.917 ± 0.004). Belowground biomass from the native C_3 and C_4 communities at Corn Island had similar depth distributions.

Nitrogen Uptake

P. australis assimilated ^{15}N more rapidly than the native vegetation at all depths, with tracer uptake ranging from 18.3 to 27.8% for *P. australis* and 0.1 to 15.7% for the natives (Fig. 5; $W = 0$, $p = 0.029$). The greatest ^{15}N uptake rate occurred at 40-cm depth for *P. australis*, which was approximately the depth at which the greatest proportion of belowground biomass was situated (Fig. 5). Uptake declined towards the surface and more deeply, yielding a moderately strong correlation with belowground biomass (ρ = 0.88; $p = 0.312$). In contrast, ^{15}N uptake rates in the native community were greatest near the surface (10 cm) and decreased with depth, paralleling the distribution of belowground biomass (ρ = 0.94; $p = 0.057$).

Discussion

Our results indicate that the invasion of *P. australis* into native plant communities is facilitated by its deep roots providing it access to N that is not typically accessed by native

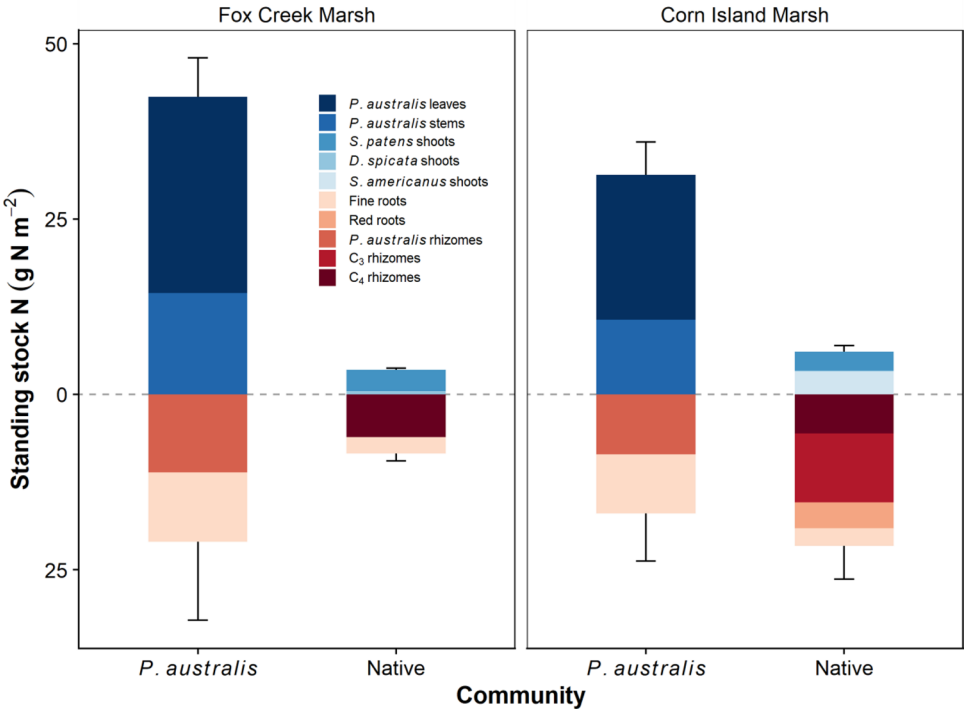

Fig. 2 Mean (±SE) above- and below-ground nitrogen content (i.e., standing stock N; g m^{-2}) in the *P. australis* and native plant communities; $n = 4$ in the *P. australis* community and 5 in the native community

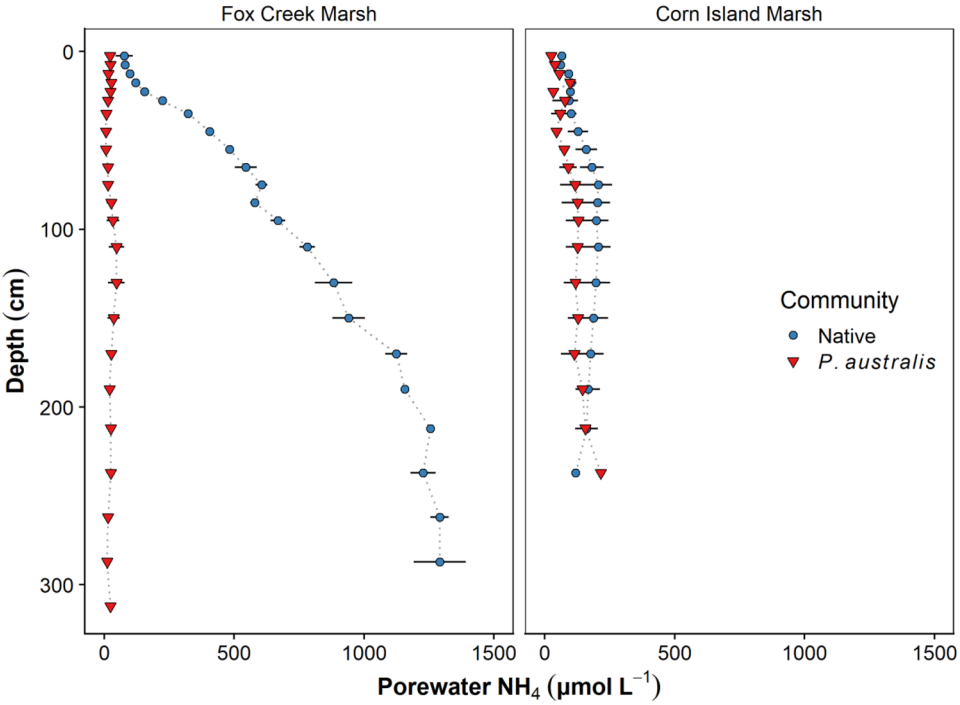

Fig. 3 Mean (±SE) porewater ammonium (NH_4) in the native and *P. australis* communities at Fox Creek (*S. patens*-dominated) and Corn Island (*S. americanus*–dominated) marshes. $n = 2$ cores per plant community

plant communities. This study may therefore answer a long-standing question in *P. australis* invasion science (Meyerson et al. 2000): what is the source of the missing N that allows *P. australis* to flourish? We demonstrated with an ^{15}N tracer that native plants do not use the deep nutrient pools that invasive *P. australis* uses, providing evidence in support of

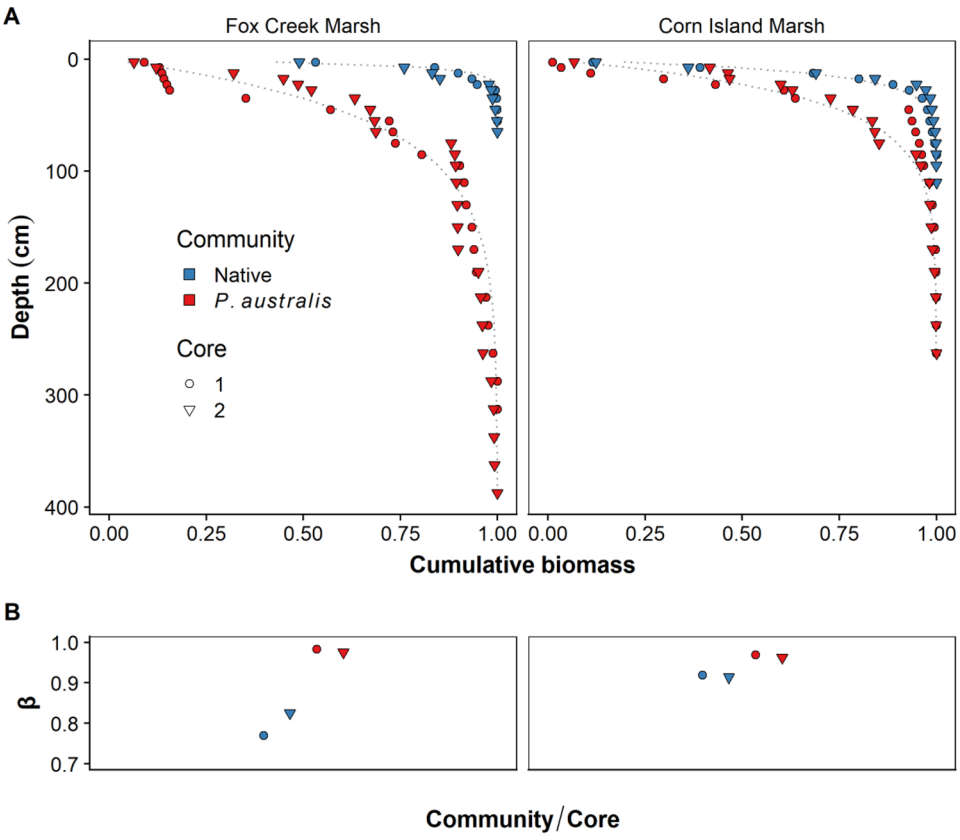

Fig. 4 **A** Cumulative belowground biomass distribution as a function of soil depth and **B** their associated β values

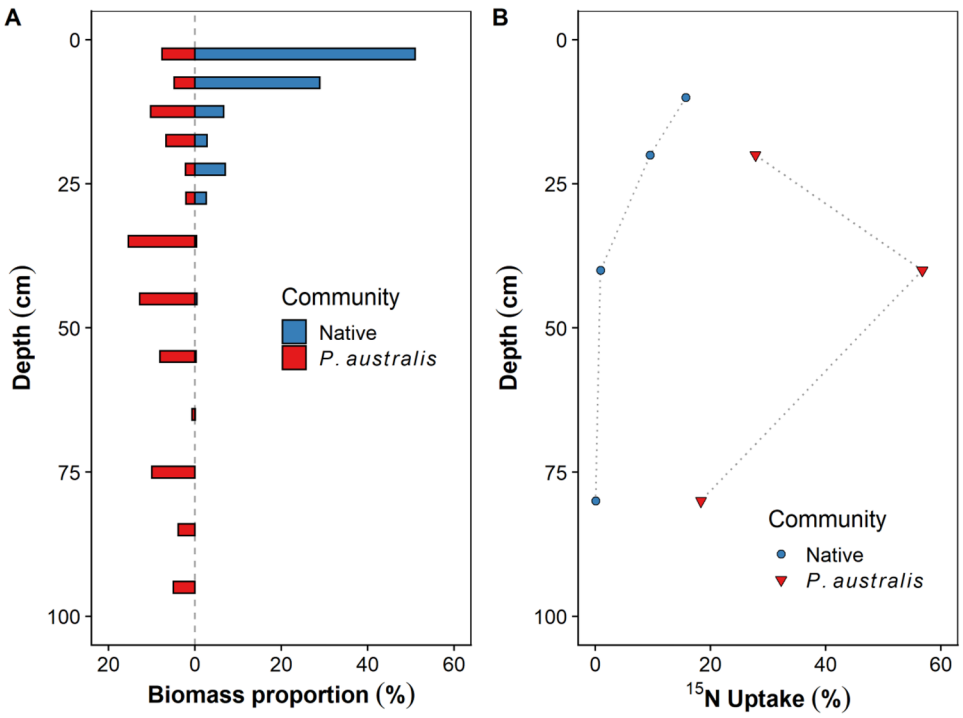

Fig. 5 **A** Proportion of total rooting biomass at each depth increment in the invasive *P. australis* and native communities. **B** ^{15}N uptake from plots in the native and *P. australis* communities at Fox Creek marsh

hypothesized niche separation between *P. australis* and its competitors (Mozdzer et al. 2016). Further, by accessing deep N pools, *P. australis* likely creates positive feedbacks when it transports this limiting nutrient from a sequestered location to the surface (Bernal et al. 2017; Mozdzer et al. 2016), specifically by increasing near-surface N pools and thus facilitating rapid clonal expansion and seedling growth.

Deep soil N pools provide *P. australis* an N source that does not require competition and can help to satisfy its high N demand in N-limited ecosystems. Given that N assimilation from our ^{15}N labeling experiment mirrored root depth distributions in both native and introduced species, the results support our interpretation that deep roots contributed to plant N acquisition. Similar results have been reported in other species. For instance, McKinley et al. (2009) reported similarly deep N uptake in a coastal terrestrial forest using nearly identical methods, and Faillace et al. (2018) found that shrubs that grew larger could access N more readily than competitors. Our data likewise indicate that the rooting profile of *P. australis* is more similar to that of trees and shrubs ($\beta \approx 0.97–0.98$) than to that of grasses ($\beta \approx 0.94–0.95$) (Jackson et al. 1996). Further, *P. australis* showed greater N uptake compared to plants in the native community at all depths. Given the presence of live roots at depths exceeding 3 m, this suggests that *P. australis* is capable of extracting N at lower depths than shallow-rooted native plant species. Given that soil N pools typically increase with depth at our sites (Fig. 5) and at other tidal wetlands (Chambers et al. 1998; Langley and Megonigal 2010; Mozdzer et al. 2016), access to deep soil N pools evidently provides *P. australis* a mechanism of avoiding direct N competition with native species and ultimately allowing it to establish and spread in low-N systems.

Deep rooting may be a strategy by which *P. australis* gains a competitive advantage over native, shallow-rooted plant communities (Mozdzer et al. 2016). With a deeper rooting profile, *P. australis* accesses nutrient pools below the typical rooting zone of native plants (Fig. 4) (Saunders et al. 2006), allowing it to bypass belowground competition with the native species. Additionally, the deeper, more extensive rooting system may be a method of accessing freshwater in otherwise saline habitats (Lissner and Schierup 1997), ameliorating the well-established salinity effects on *P. australis* (Chambers et al. 1998, 2003). However, the importance of this mechanism remains to be tested.

Changes in plant community composition brought about by *P. australis* invasion can fundamentally alter N processes in coastal wetlands by mobilizing deep N pools. The 2- to 3-fold greater belowground biomass and approximately 3 × deeper roots increased plant N stocks by 6–8×. These findings are consistent with previous studies reporting significant increases in N retention and N demand with *P. australis* invasion (Windham and Meyerson 2003, Templer et. al. (1998). In N-limited wetland ecosystems, mobilization of deep soil N is a parsimonious explanation for the means by which *P. australis* meets its high N demand and accumulates large N pools. Indeed, we have previously shown that *P. australis*, but not native plants, can stimulate (i.e., prime) the decomposition of deep soil organic matter, increasing N mineralization rates (Bernal et al. 2017). The extreme depth

to which *P. australis* can root and thereby access N (> 3 m) is, to our knowledge, a trait not possessed by any native wetland plant species in North American tidal marshes, all of which have relatively shallow rooting systems (Windham and Lathrop 1999; Windham 2001; Saunders et al. 2006).

Differences between our sites demonstrate that the magnitude of deep soil N mining by *P. australis* can vary due to differences in plant community composition and invasion history. Consistent with other studies (Chambers et al. 1998; Templer et al. 1998; Meyerson et al. 2000a; Windham 2001, Windham and Meyerson 2003, Mozdzer et al. 2010), soils colonized by *P. australis* contained lower porewater N at both sites. We attribute this to *P. australis*' high N demand (Fig. 2), which is a well-recognized phenomenon in *P. australis*-invaded wetlands (Meyerson and Cronin 2013). Two factors could explain the large differences in porewater N pools between *P. australis*-dominated and native-dominated communities at the two locations in our study. First, lower porewater N at Corn Island could be due to the far greater abundance of *S. americanus*, which has a greater ability to drawn down soil N pools than other dominant marsh species such as *S. patens* (Langley and Megonigal 2010; Cott et al. 2018). In addition, standing stock N in the native plant community (both aboveground and belowground) is greater at Corn Island than at Fox Creek, where *S. americanus* is absent. Second, it is possible that *P. australis* invaded Corn Island earlier and has already drawn down available N pools. Although we know *P. australis* became established at both sites within the last 30 years (Kettenring et al. 2010), we lack the high-resolution historical data needed to date the colonization. Regardless of the underlying factor, our data demonstrate that biomass and standing stock N in the vegetation are high when pools of soil N are high. In turn, high productivity enhances the ability of *P. australis* to capture light (Caplan et al. 2015), presumably allowing it to competitively exclude native plants and likely contributing to its dominance in tidal marshes across North America and beyond (Eller et al. 2017).

Conclusions

We demonstrated that *P. australis* actively uses soil N pools extending to 3-m depth below the marsh surface, providing a source of N needed to support an aboveground biomass 3–5 times greater than that of the native plant communities it displaces. Our study answers a longstanding question: how and where does *P. australis* gain access to the N needed to support its high N demand? We propose that mining previously buried soil N brings that N into the surficial soil nitrogen cycle, which increases ecosystem N availability and ecosystem productivity for decades or more. Although deep soil N pools are finite, the rooting depth of *P. australis* can be expected to increase under elevated CO_2 (Mozdzer et al. 2016), sustaining this competitive advantage in the long-term as concentrations of atmospheric CO_2 increase. In cases where N pools are lower, *P. australis* also enhances soil organic matter decomposition deep within the soil profile (Bernal et al. 2017, Bernal et al. 2023), thereby also increasing plant N availability. Together, our data suggest that *P. australis* shares an important trait with trees and shrubs invading terrestrial grasslands (Rundel et al. 2014), namely the ability to develop deeper root systems than more shallowly rooted native competitors. We propose that deep rooting and mining of N deep within the soil profile is a primary factor facilitating the invasion of *P. australis* in North American wetlands.

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Data Availability Data from this study are available in the Smithsonian Institution figshare repository (https://smithsonian.figshare.com) under https://doi.org/10.25573/serc.22116053.

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