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Invasion History and Soil Depth Shape Microbial Functional Groups and Methanogen Communities in *Phragmites australis*Coastal Wetlands

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Abstract

Plant invasions can substantially alter soil microbial processes and biogeochemical cycling in coastal wetlands. We investigated how *Phragmites australis* invasion and invasion history influence microbial processes associated with soil organic matter decomposition, methanogenesis, and denitrification compared with native *Spartina*-dominated marshes. We measured extracellular enzyme activities and microbial functional gene abundances across soil depths and characterized methanogen community composition at historically and recently invaded sites. *Phragmites* invasion significantly reduced β-glucosidase activity, indicating lower microbial decomposition potential for labile carbon, whereas phenol oxidase activity showed less consistent responses. These effects varied with soil depth and invasion history, suggesting shifts in microbial activity and substrate availability following invasion. Methanogen abundance was higher in *Phragmites*-invaded soils, particularly in deeper soil layers, while methanogen community composition differed between historically and recently invaded sites. Historically invaded sites showed a greater relative contribution of hydrogenotrophic methanogens, suggesting a shift in methanogenic pathways with invasion history. In contrast, the abundances of denitrification genes (nirS, nirK, and nosZ) were generally higher in *Spartina* soils than in *Phragmites* soils, particularly at recently invaded sites. Together, these results demonstrate that *Phragmites* invasion alters the vertical distribution and composition of microbial functional groups involved in carbon and nitrogen cycling and that these responses vary with invasion history. Our findings highlight the importance of microbial community responses for understanding the long-term biogeochemical consequences of plant invasion in coastal wetlands.

Introduction

Coastal wetlands play an important role in global carbon cycling by storing substantial amounts of organic carbon, while also serving as sources of greenhouse gases (GHGs), including methane (CH_4) and nitrous oxide (N_2O). The production and consumption of these gases are strongly regulated by soil microbial processes under the water-saturated and frequently anaerobic conditions characteristic of wetland soils [1]. Wetland vegetation can further regulate these processes by modifying substrate availability, soil redox conditions, and microbial communities involved in methane production, oxidation, and nitrogen transformations [2, 3].

Plant invasion can alter these plant–soil–microbe interactions by changing nutrient cycling, soil physicochemical properties, organic matter inputs, and microbial community-composition. *Phragmites australis* (hereafter *Phragmites*) has aggressively expanded in salt and brackish marshes along the U.S. East Coast, largely following the introduction of a highly competitive European lineage [4, 5]. Its expansion has displaced native marsh vegetation, including *Spartina* species, and has altered belowground carbon and nitrogen cycling. For example, *Phragmites*-dominated sites can exhibit lower pore-water ammonium concentrations than native *Spartina* sites [6]. Such changes in the belowground environment may alter the abundance and activity of microbial functional groups involved in organic matter decomposition, methanogenesis, and denitrification.

The effects of *Phragmites* invasion on methane cycling may arise through several interacting mechanisms. *Phragmites* has an extensive belowground root and rhizome system that can alter both carbon availability and soil redox conditions. Root-derived carbon and plant biomass turnover can provide substrates for methanogenic microorganisms, whereas aerenchyma-mediated oxygen transport can modify the rhizosphere environment and microbial activity. In addition, the well-developed aerenchyma of *Phragmites* provides an efficient pathway for transporting CH_4 from anaerobic soil to the atmosphere [7–9]. Previous studies have reported higher CH_4 fluxes in *Phragmites*-dominated wetlands than in native vegetation [10–12], suggesting that invasion may alter both methanogenic communities and the environmental conditions regulating methane cycling.

Phragmites invasion may also influence microbial nitrogen cycling, particularly denitrification. Root-mediated oxygen transport can increase oxygen availability in the rhizosphere and thereby alter the balance between aerobic and anaerobic nitrogen transformations. Because denitrification is controlled by both environmental conditions and the abundance and composition of functional microbial groups, changes in vegetation may affect microorganisms carrying genes involved in N_2O production and reduction, including nirS, nirK, and nosZ. However, reported effects of *Phragmites* invasion on N_2O dynamics have been inconsistent. Changes in sediment aeration may suppress heterotrophic denitrification, whereas shifts in nitrogen availability and microsite redox conditions may alter N_2O production and consumption [13]. Examining denitrifier functional genes can therefore provide insight into how plant invasion alters the microbial potential for nitrogen transformations.

Plant effects on microbial processes are particularly pronounced in the rhizosphere, where roots supply organic substrates, nutrients, and oxygen that shape microbial activity and community composition [14–16]. The extensive and deep root system of *Phragmites* may extend these effects into deeper soil layers. Previous studies have shown that *Phragmites* can stimulate microbial processing of previously protected or relatively inactive deep-soil carbon pools [17, 18]. Consequently, microbial responses to invasion may vary substantially with soil depth, yet vertical patterns in microbial functional groups in *Phragmites*-invaded marshes remain comparatively poorly understood.

The duration of invasion may further influence these belowground responses. As *Phragmites* persists, cumulative changes in plant inputs, soil organic matter, rhizosphere chemistry, and microbial communities may result in microbial communities at historically invaded sites that differ from those at more recently invaded sites. In the Rhode River sub-estuary of Chesapeake Bay, introduced *Phragmites* expanded substantially between 1971 and 2007, with the number of patches increasing from 5 to 212 and total area increasing from 0.73 to 18 ha [19]. This invasion history provides an opportunity to compare microbial responses between marshes invaded for different periods and native *Spartina*-dominated marshes. Despite numerous studies of microbial communities associated with wetland vegetation, relatively little is known about how invasion history and soil depth interact to structure microbial functional groups involved in carbon and nitrogen cycling.

In this study, we investigated how *Phragmites* invasion, invasion history, and soil depth influence microbial processes associated with organic matter decomposition, methanogenesis, and denitrification in Chesapeake Bay marshes. We compared extracellular enzyme activities, the abundances of microbial functional genes associated with carbon and nitrogen cycling, and methanogen community composition among historically invaded *Phragmites* sites, recently invaded *Phragmites* sites, and native *Spartina*-dominated sites. We hypothesized that (1) *Phragmites* invasion would alter extracellular enzyme activity because of differences in the quantity and quality of plant-derived carbon inputs; (2) methanogen abundance and community composition would differ between *Phragmites* and *Spartina* soils and vary with soil depth and invasion history; and (3) the abundance of denitrification-related functional genes would differ between *Phragmites* and *Spartina* soils as a consequence of invasion-induced changes in the rhizosphere environment. By integrating microbial activity, functional gene abundance, community composition, soil depth, and invasion history, this study provides insight into the microbial mechanisms through which invasive plants alter carbon and nitrogen cycling in coastal wetlands.

Materials and methods

Study sites and sampling

This study was conducted in two *Phragmites*-invaded brackish marsh systems located along the Rhode River, a sub-estuary of Chesapeake Bay (38°53'N, 76°33'W), Maryland, USA. Fox Creek Marsh and Kirkpatrick Marsh are part of the Smithsonian Global Change Research Wetland (GCREW), operated by the Smithsonian Environmental Research Center. Salinity at the study sites ranges from 4 to 15 ppt, and the mean tidal range is 44 cm. The high-marsh platform is 40–60 cm above mean low water level, and the soils are predominantly organic (> 80%) to a depth of 5 m. Mean daily air temperatures range from −4 to 31°C, and mean annual precipitation is 1,080 mm. We selected marsh sites dominated by *Spartina* and *Phragmites*.

Historically, Kirkpatrick Marsh was primarily dominated by the native species *Spartina patens* and *Distichlis spic*ata [20, 21]. However, over approximately the past 40 years, an invasive lineage of *Phragmites* has expanded substantially in this area, increasing its coverage by more than 25-fold [19]. Consequently, we selected sites representing both long-term and more recent *Phragmites* invasion. Marshes along the Rhode River included sites that had long been dominated by invasive *Phragmites* (hereafter “historically invaded” sites; established before 1971 [19]), Fox Creek marshes that were more recently invaded by *Phragmites* (hereafter “recently invaded” sites; established between 1971 and 2007 [19]), and marshes that remained dominated by native vegetation (*Spartina patens*; hereafter “native *Spartina*”).

Soil samples were collected during summer, when plant biomass was at its peak. At each site, soil cores (50 mm diameter) were collected from randomly selected locations after removing a small amount of surface litter. Cores were divided into three depth intervals: 0–15, 15–30, and 30–50 cm. Three replicate samples, each consisting of nine pooled soil cores, were collected at each site. Field-moist soils were

sieved through a 2-mm mesh, and large pieces of plant material and soil animals were removed. The sieved soils were stored at 4°C until enzyme analysis, which was completed within four days of sampling. For molecular analyses, samples were immediately transported to the laboratory and stored at − 80°C until DNA extraction.

Microbial activity

We determined the microbial activity of the lignin-degrading enzymes phenol oxidase, and the cellulolytic enzyme β-glucosidase in the soil samples. β-glucosidase activity was measured using 50 mM pNP-β-glucopyranoside as substrate and absorbance was measured at 410 nm [22]. The activity of phenol oxidase was measured using 10 mM of L-DOPA as substrate and reading absorbance at 460 nm [23, 24] using a Thermo Scientific UV/VIS Spectrometer Evolution 220 (Thermo science Inc., USA).

Microbial abundance

Soil DNA was extracted using the PowerSoil Kit (Q-BIOgene, USA) according to the manufacturer's instructions. DNA quality and quantity were assessed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., USA). Gene copy numbers of microbial functional groups were quantified by real-time quantitative PCR (qPCR) using a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR Green detection. Two independent qPCR assays were performed for each soil DNA extract.

Fungal abundance was quantified using the ITS1F and ITS4R primers [25], and laccase gene abundance was determined using the Cu1F and Cu2R primers [26]. For fungal ITS regions, amplification consisted of 40 cycles of denaturation at 94°C for 25 s, primer annealing at 50°C for 25 s, and extension at 72°C for 25 s. Laccase genes were amplified using a three-step PCR protocol [27], with 40 cycles including denaturation at 94°C for 1 min and primer annealing at 54°C for 2 min.

Methanogenic archaea were quantified by targeting the mcrA gene [28]. Amplification consisted of 40 cycles with denaturation at 95°C for 25 s, primer annealing at 64.5°C, and extension at 72°C for 25 s [28].

Denitrifying bacteria were quantified by targeting the nirS, nirK, and nosZ genes [29–31]. Amplification followed a three-step PCR protocol consisting of 40 cycles of denaturation at 95°C for 25 s, primer annealing, and extension at 72°C for 25 s. Primer annealing temperatures were 65°C for nirS and 56°C for nosZ. The reported annealing condition for nirK was 63°C.

Standard curves were generated using 10-fold serial dilutions of plasmids containing the target genes amplified from environmental samples. Gene abundances were expressed as gene copy numbers per gram of dry soil.

Methanogen Community Analysis

Methanogen community composition was analyzed using terminal restriction fragment length polymorphism (T-RFLP). Approximately 300 ng of purified PCR product was digested in a final volume of 25 μL containing 10 U of HhaI restriction enzyme. The reaction mixture was incubated at 37°C for 4 h. The digested products were analyzed using an ABI 377 automated sequencer, and terminal restriction fragments were identified using GeneScan software.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM SPSS Statistics, USA). One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used to evaluate differences in extracellular enzyme activities and microbial gene abundances among treatments. Two-way ANOVA was used to evaluate the effects of vegetation type, soil depth, and their interaction on extracellular enzyme activities and microbial gene abundances. Statistical significance was determined at $p < 0.05$.

Results

Microbial enzyme activity

β-Glucosidase activity did not differ significantly between vegetation types in the upper soil layers (0–15 and 15–30 cm) at the historically invaded site. However, at 30–50 cm depth, β-glucosidase activity was lower in *Phragmites* soils than in *Spartina* soils (Fig. 1a). At the recently invaded site, β-glucosidase activity was significantly higher in Spartina soils, whereas *Phragmites* soils exhibited lower activity across soil depths (Depth: $p < 0.01$, Plant: $p < 0.05$, Depth × Plant: $p < 0.01$; Fig. 1b).

Phenol oxidase activity also varied with vegetation type and soil depth. At the historically invaded site, *Phragmites* soils generally exhibited lower phenol oxidase activity, whereas *Spartina* soils showed reduced activity at 0–15 cm depth (Depth: $p < 0.01$, Plant: $p < 0.01$, Depth × Plant: $p < 0.01$; Fig. 1c). At the recently invaded site, phenol oxidase activity was higher in *Spartina* soils than in *Phragmites* soils only at 0–15 cm depth.

Microbial abundance

Fungal and laccase gene abundances were not significantly affected by vegetation type at the historically invaded site (Fig. 2). In addition, neither fungal nor laccase gene abundance differed significantly among soil depths. At the recently invaded site, however, fungal and laccase gene abundances were higher in *Phragmites* soils than in *Spartina* soils at 30–50 cm depth.

At the historically invaded site, the abundances of the denitrification genes nirS and nirK did not differ significantly between *Spartina* and *Phragmites* soils (Fig. 3a, b). The abundance of nosZ showed little variation with soil depth but was higher in *Phragmites* soils at 30–50 cm depth. In contrast, at the

recently invaded site, the abundances of nirS, nirK, and nosZ decreased with soil depth in *Spartina* soils, whereas nirS abundance remained relatively consistent across soil depths in *Phragmites* soils (Fig. 3a–c). Overall, the abundances of nirS, nirK, and nosZ were higher in *Spartina* soils than in *Phragmites* soils at the recently invaded site (Depth: $p < 0.001$, Plant: $p < 0.001$, Depth × Plant: $p < 0.001$).

Methanogen abundance was higher in *Phragmites* soils than in *Spartina* soils at both historically and recently invaded sites (Fig. 4; Depth: $p < 0.001$, Plant: $p < 0.001$, Depth × Plant: $p < 0.001$). In *Phragmites* soils, methanogen abundance increased with soil depth, whereas it remained relatively consistent across soil depths in *Spartina* soils. Methanogen abundance was also higher at the recently invaded site than at the historically invaded site.

Methanogen Community Composition

Methanogen community composition was characterized using DNA-based T-RFLP analysis in soils from historically invaded *Phragmites* sites, recently invaded *Phragmites* sites, and native *Spartina* sites (Supplementary Table 1). Methanogen diversity and community composition differed among the sites. At the historically invaded site, hydrogenotrophic methanogens were the most abundant group across soil depths (Fig. 5a). In contrast, acetoclastic methanogens accounted for more than 70% of the methanogen community at the recently invaded site (Fig. 5b). In native *Spartina* soils, methanogen community composition varied with soil depth, although similar patterns were observed at 0–15 and 30–50 cm depths.

Discussion

Effects of Phragmites Invasion on Soil Organic Matter Decomposition

Our results indicate that *Phragmites* invasion alters microbial processes associated with soil organic matter decomposition, although the magnitude of these effects varied with soil depth and invasion history. β-Glucosidase activity was generally lower in *Phragmites* soils than in native *Spartina* soils, particularly at the recently invaded site and in deeper soil at the historically invaded site. Because β-glucosidase is involved in the degradation of labile cellulose-derived carbon, lower activity suggests reduced microbial potential for labile carbon decomposition in *Phragmites*-invaded soils.

Differences in plant-derived carbon inputs may contribute to these patterns. *Phragmites* produces substantial above- and belowground biomass, while differences in litter quality and root-derived carbon inputs between *Phragmites* and native marsh vegetation can alter substrate availability for soil microorganisms. *Phragmites* litter has relatively high lignin content and decomposes slowly compared with litter from other wetland plants [32]. More broadly, invasive plants can alter soil nutrient pools and nutrient cycling through changes in litter inputs and soil properties [33, 34]. In addition, *Phragmites* has an extensive and relatively deep root and rhizome system [18], which may modify microbial activity beyond the surface soil. Such effects may contribute to the observed variation in β-glucosidase activity with soil depth.

In contrast to β-glucosidase, phenol oxidase activity showed less consistent responses to *Phragmites* invasion. Phenol oxidase is involved in the degradation of more chemically complex organic compounds, including phenolic compounds. The contrasting responses of β-glucosidase and phenol oxidase therefore suggest that *Phragmites* invasion may affect different components of soil organic matter decomposition differently rather than uniformly increasing or decreasing overall decomposition.

The effects of invasion also differed between historically and recently invaded sites. The stronger differences in β-glucosidase activity observed at the recently invaded site suggest that microbial responses to *Phragmites* may vary with invasion history. Over longer periods of invasion, cumulative changes in plant inputs, soil organic matter, and microbial communities may modify the effects of vegetation replacement on microbial decomposition. Together, these results suggest that the effects of *Phragmites* invasion on soil carbon cycling depend on both soil depth and invasion history, emphasizing the importance of considering the vertical and temporal dimensions of plant invasion when evaluating its effects on wetland soil carbon dynamics.

Effects of Phragmites Invasion on Methanogen Abundance and Community Composition

Phragmites invasion was associated with substantial changes in both methanogen abundance and community composition. Methanogen abundance was higher in *Phragmites* soils than in native *Spartina* soils and increased with soil depth, whereas abundance remained relatively consistent across depths in *Spartina* soils. Previous studies have suggested that methanogen abundance can be associated with CH_4 flux in soil ecosystems [8], and greater methanogen abundance has also been reported in deeper soils of *Phragmites*-dominated marshes [35]. The extensive belowground biomass and deep rooting of *Phragmites* [18] may increase carbon inputs to deeper soil layers and create conditions favorable for methanogenic communities. Thus, the greater abundance of methanogens observed in deeper *Phragmites* soils suggests increased potential for methanogenesis following invasion.

Invasion history was also associated with differences in methanogen community composition. Historically invaded *Phragmites* soils were characterized by a greater relative contribution of hydrogenotrophic methanogens, whereas acetoclastic methanogens accounted for more than 70% of the methanogen community at the recently invaded site. In addition, hydrogenotrophic Methanomicrobiales increased with soil depth at the historically invaded site. These contrasting patterns suggest that the dominant pathways of methanogenesis may change as the belowground environment develops following *Phragmites* invasion.

Differences in substrate availability may contribute to these shifts in methanogen community composition. Methanogenic groups differ in their use of substrates, and changes in plant-derived carbon inputs can therefore alter their relative abundance. *Phragmites* has high belowground biomass and can influence carbon availability through root inputs and biomass turnover. Previous work has shown that changes in carbon availability can alter methanogen community composition [36]. The predominance of acetoclastic methanogens at the recently invaded site may therefore reflect greater availability of acetate or other labile substrates during earlier stages of invasion, whereas the greater relative

contribution of hydrogenotrophic methanogens at the historically invaded site may reflect longer-term changes in carbon availability and anaerobic soil conditions. However, because substrate concentrations and pathway-specific methane production were not measured in this study, the mechanisms underlying these community shifts require further investigation.

Phragmites may also influence methane dynamics through mechanisms other than changes in methanogen communities. Its high primary productivity and extensive root and rhizome system can provide carbon substrates to soil microbial communities, while its aerenchyma can facilitate gas transport between the soil and atmosphere [7, 9]. At the same time, oxygen transport through roots may locally suppress methanogenesis by increasing rhizosphere oxygen availability. Therefore, the net effect of *Phragmites* invasion on methane dynamics likely reflects interactions among carbon inputs, soil redox conditions, methanogen abundance and community composition, and plant-mediated gas transport. Our results demonstrate that *Phragmites* invasion alters methanogenic communities, particularly in deeper soil layers, and that these changes differ with invasion history.

Effects of Phragmites Invasion on Denitrification-Related Microbial Communities

Phragmites invasion was associated with changes in the abundance of microbial functional genes involved in denitrification. At the recently invaded site, the abundances of nirS, nirK, and nosZ were consistently higher in *Spartina* soils than in *Phragmites* soils and generally decreased with soil depth. In contrast, differences between vegetation types were less pronounced at the historically invaded site. These patterns suggest that the effects of *Phragmites* invasion on denitrifier communities vary with both soil depth and invasion history.

The nirS and nirK genes encode nitrite reductases involved in the production of nitric oxide during denitrification, whereas nosZ encodes nitrous oxide reductase, which catalyzes the reduction of N_2O to N_2 [37]. Thus, changes in the abundance of these functional genes indicate shifts in the microbial community with the genetic potential to produce and consume N_2O. However, gene abundance alone does not directly represent process rates or N_2O fluxes because denitrification is also regulated by environmental factors such as oxygen availability, nitrogen availability, organic carbon supply, and soil redox conditions.

Differences in rhizosphere conditions between *Spartina* and *Phragmites* may contribute to the observed patterns. Plant species can influence nitrogen transformations by altering oxygen transport, nutrient availability, and carbon inputs to the rhizosphere. Previous studies have reported differences in pore-water nutrient concentrations between *Phragmites*- and *Spartina*-dominated marshes [6]. The lower abundance of denitrification-related genes in *Phragmites* soils at the recently invaded site may therefore reflect changes in the belowground environment following vegetation replacement. The decline in denitrifier gene abundance with depth in *Spartina* soils further indicates that the vertical distribution of these functional groups is strongly influenced by soil depth.

Interestingly, differences in denitrifier gene abundance between *Spartina* and *Phragmites* were less evident at the historically invaded site than at the recently invaded site. This pattern suggests that the initial effects of vegetation change on denitrifier communities may become less pronounced with increasing invasion duration, potentially as soil conditions and microbial communities adjust to long-term *Phragmites* dominance. However, because denitrification rates and N_2O fluxes were not directly measured in this study, the consequences of these shifts for ecosystem-level N_2O emissions cannot be determined from functional gene abundance alone. Direct measurements of denitrification and N_2O production and consumption would be needed to determine whether the observed microbial changes translate into changes in nitrogen gas fluxes.

Declarations

Author Contribution

S. Kim conducted the experiments, analyzed the data, and wrote the original manuscript. T.M and J.P Megongial contributed to the experimental design. M. McCormick contributed to the laboratory experiments and data interpretation. Y. Kim contributed to the study design and interpretation of the results. H. Kang supervised the research, contributed to the study conception and design, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. Also, the data supporting the findings of this study are included in the article and its supplementary information files.

References

1. IPCC (2014) Climate Change 2014: Synthesis Report. Intergovernmental Panel on Climate Change, Geneva, Switzerland

2. Whalen SC (2005) Biogeochemistry of methane exchange between natural wetlands and the atmosphere. Environ Eng Sci 22:73–94
3. Koelbener A, Ström L, Edwards PJ, Venterink HO (2010) Plant species from mesotrophic wetlands cause relatively high methane emissions from peat soil. Plant Soil 326:147–158
4. Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proc Natl Acad Sci USA 99:2445–2449
5. Price A, Fant JB, Larkin DJ (2014) Ecology of native vs. introduced *Phragmites australis* (common reed) in Chicago-area wetlands. Wetlands 34:369–377
6. Chambers RM, Mozdzer TM, Ambrose JC (1998) Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. Aquat Bot 62:161–169
7. Brix H, Sorrell BK, Lorenzen B (2001) Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases? Aquat Bot 69:313–324
8. Kim J, Lee S, Jang I, Jeong S, Kang H (2015) Can abundance of methanogen be a good indicator for CH_4 flux in soil ecosystems? Environ Geochem Health 37:1007–1015
9. Bernal B, Kim S, Mozdzer TJ (2023) Species shifts induce soil organic matter priming and changes in microbial communities. Sci Total Environ 859:159956
10. Tong C, Wang WQ, Huang JF, Gauci V, Zhang LH, Zeng CS (2012) Invasive alien plants increase CH_4 emissions from a subtropical tidal estuarine wetland. Biogeochemistry 111:677–693
11. Mozdzer TJ, Megonigal JP (2013) Increased methane emissions by an introduced *Phragmites australis* lineage under global change. Wetlands 33:609–615
12. Gao GF, Li PF, Shen ZJ, Qin YY, Zhang X, Ghoto K, Zhu XY, Zheng HL (2018) Exotic *Spartina alterniflora* invasion increases CH_4 while reduces CO_2 emissions from mangrove wetland soils in southeastern China. Sci Rep 8:9243
13. Emery MD, Fulweiler RW (2014) *Spartina alterniflora* and invasive *Phragmites australis* stands have similar greenhouse gas emissions in a New England marsh. Aquat Bot 116:83–92
14. Liu X, Lin TC, Vadeboncoeur MA, Yang Z, Chen S, Xiong D, Yang Y (2019) Root litter inputs exert greater influence over soil C than does aboveground litter in a subtropical natural forest. Plant Soil 444:489–499
15. Farrer EC, Van Bael SA, Clay K, Smith MKKH (2022) Plant-microbial symbioses in coastal systems: their ecological importance and role in coastal restoration. Estuaries Coasts 45:1805–1822
16. Rolando JL, Kolton M, Song T, Liu Y, Pinamang P, Conrad R, Morris JT, Konstantinidis KT, Kostka JE (2024) Sulfur oxidation and reduction are coupled to nitrogen fixation in the roots of the salt marsh foundation plant *Spartina alterniflora*. Nat Commun 15:3607
17. Bernal B, Megonigal JP, Mozdzer TJ (2017) An invasive wetland grass primes deep soil carbon pools. Glob Change Biol 23:2104–2116
18. Mozdzer TJ, Langley JA, Mueller P, Megonigal JP (2016) Deep rooting and global change facilitate spread of invasive grass. Biol Invasions 18:2619–2631

19. McCormick MK, Kettenring KM, Baron HM, Whigham DF (2010) Extent and reproductive mechanisms of *Phragmites australis* spread in brackish wetlands in Chesapeake Bay, Maryland (USA). Wetlands 30:67–74
20. Baldwin AH, Kettenring KM, Whigham DF (2010) Seed banks of *Phragmites australis*-dominated brackish wetlands: relationships to seed viability, inundation, and land cover. Aquat Bot 93:163–169
21. Drake BG (2014) Rising sea level, temperature, and precipitation impact plant and ecosystem responses to elevated CO_2 on a Chesapeake Bay wetland: review of a 28-year study. Glob Change Biol 20:3329–3343
22. Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1–24
23. Pind A, Freeman C, Lock MA (1994) Enzymic degradation of phenolic materials in peatlands—measurement of phenol oxidase activity. Plant Soil 159:227–231
24. Wright AL, Reddy KR (2001) Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci Soc Am J 65:588–595
25. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322
26. Luis P, Walther G, Kellner H, Martin F, Buscot F (2004) Diversity of laccase genes from basidiomycetes in a forest soil. Soil Biol Biochem 36:1025–1036
27. Lauber CL, Sinsabaugh RL, Zak DR (2009) Laccase gene composition and relative abundance in oak forest soil is not affected by short-term nitrogen fertilization. Microb Ecol 57:50–57
28. Lueders T, Friedrich MW (2003) Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and mcrA genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. Appl Environ Microbiol 69:320–326
29. Liu X, Tiquia SM, Holguin G, Wu L, Nold SC, Devol AH, Luo K, Palumbo AV, Tiedje JM, Zhou J (2003) Molecular diversity of denitrifying genes in continental margin sediments within the oxygen-deficient zone off the Pacific Coast of Mexico. Appl Environ Microbiol 69:3549–3560
30. Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L (2004) Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. J Microbiol Methods 59:327–335
31. Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative detection of the nosZ gene encoding the nitrous oxide reductase and comparison of the abundance of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl Environ Microbiol 72:5181–5189
32. Ágoston-Szabó E, Dinka M (2008) Decomposition of Typha angustifolia and *Phragmites australis* in the littoral zone of a shallow lake. Biologia 63:1104–1110
33. Meyerson LA, Saltonstall K, Windham L, Kiviat E, Findlay S (2000) A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. Wetl Ecol Manag 8:89–103

34. Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523
35. Kim S, Kang J, Megonigal JP, Kang H, Seo J, Ding W (2018) Impacts of *Phragmites australis* invasion on soil enzyme activities and methanogens of tidal marshes. Microb Ecol 76:782–790
36. Liu Y, Liu X, Cheng K, Li L, Zhang X, Zheng J, Pan G (2016) Responses of methanogenic and methanotrophic communities to elevated atmospheric CO_2 and temperature in a paddy. Front Microbiol 7:1895
37. Thomson AJ, Giannopoulos G, Pretty J, Baggs EM, Richardson DJ (2012) Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philos Trans R Soc Lond B Biol Sci 367:1157–1168

Figures

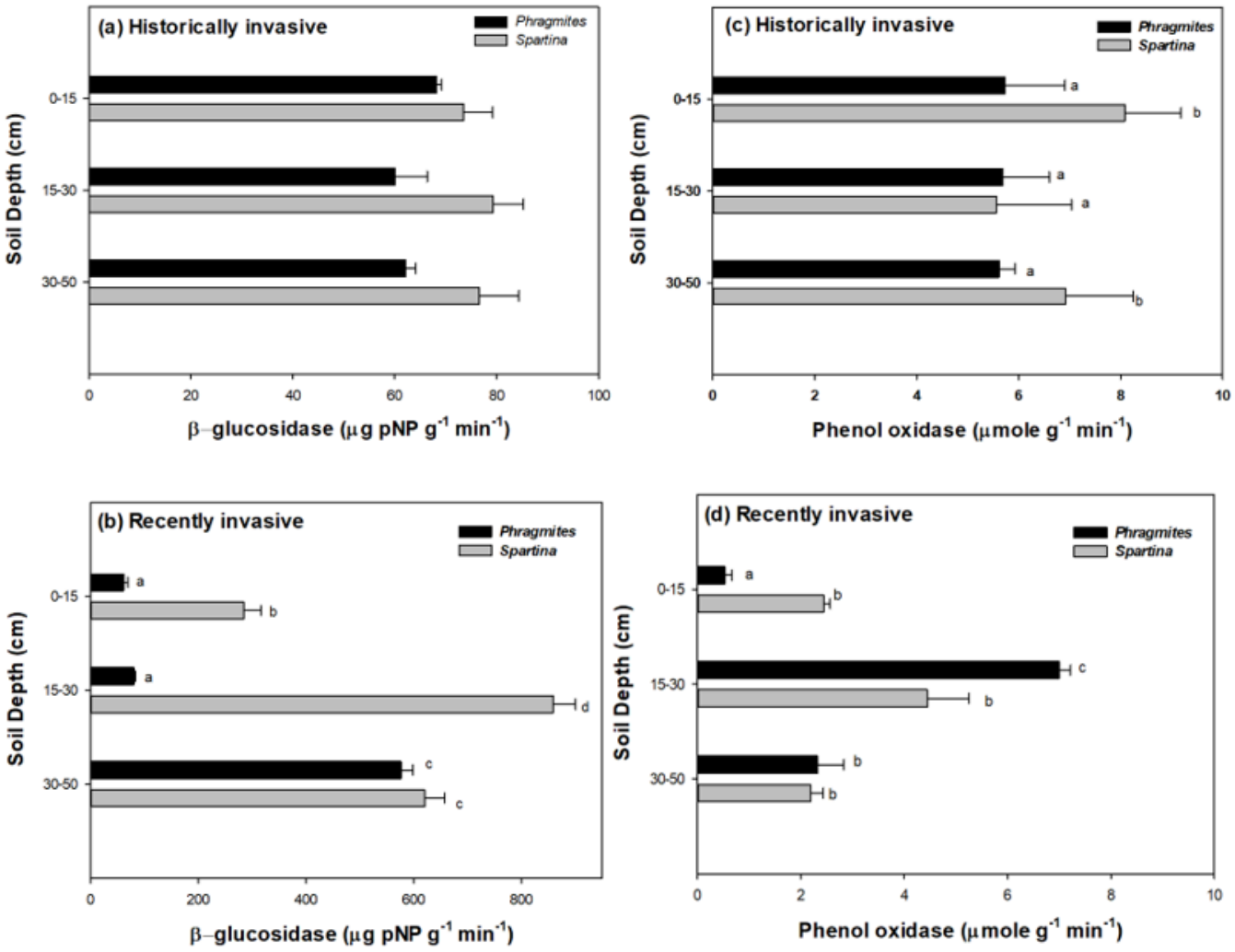

Fig. 1

Figure 1

A depth profile of β-glucosidase (a), and phenol oxidase activity (b) in two different sites with different plant types.

Different letters denote significant differences between sites and season at P<0.05 level based on two-way ANOVA

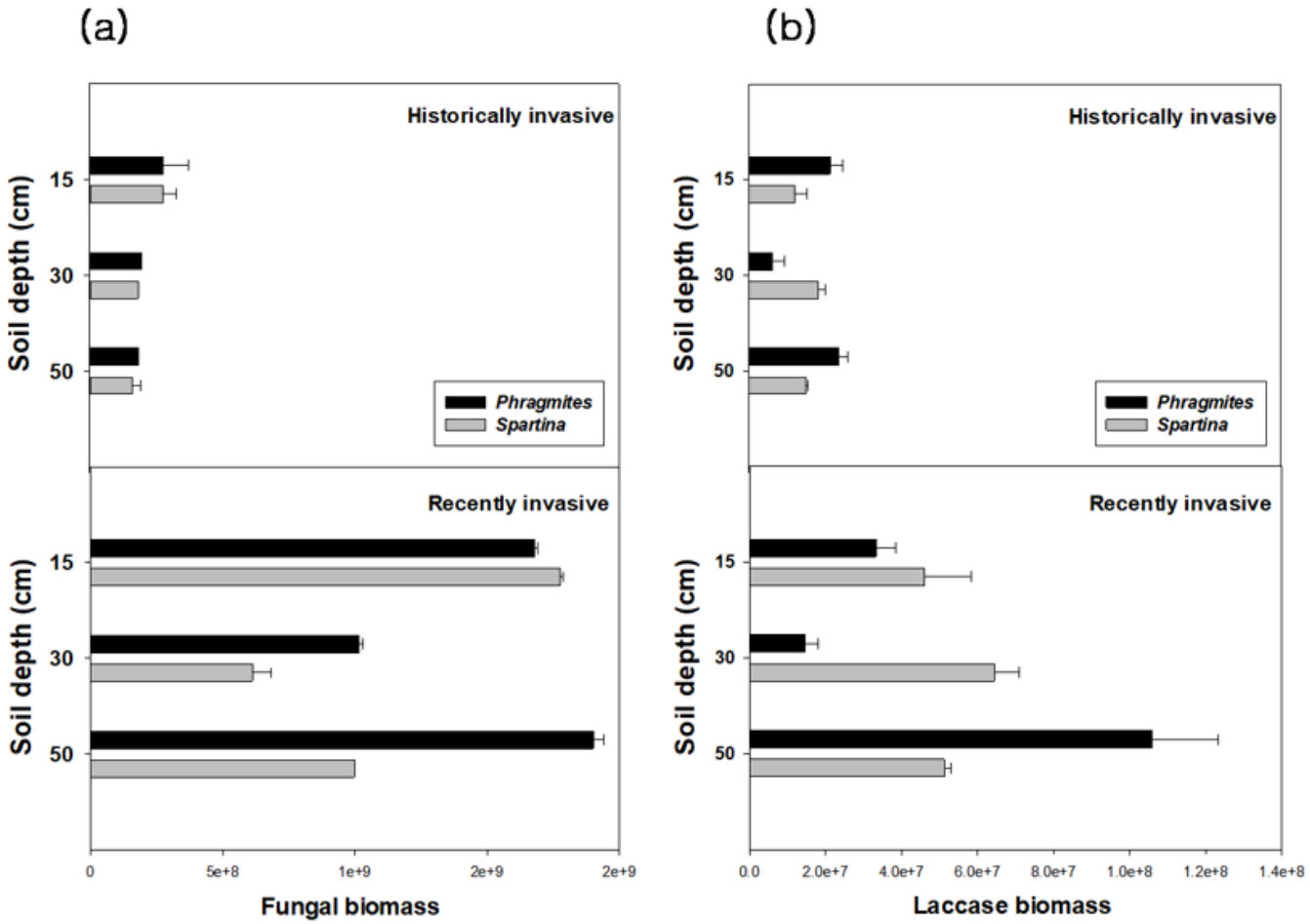

Fig. 2

Figure 2

A depth profile of fungal (a), and laccase abundance (b) in two different sites with different plant types.

Different letters denote significant differences between sites and season at P<0.05 level based on two-way ANOVA

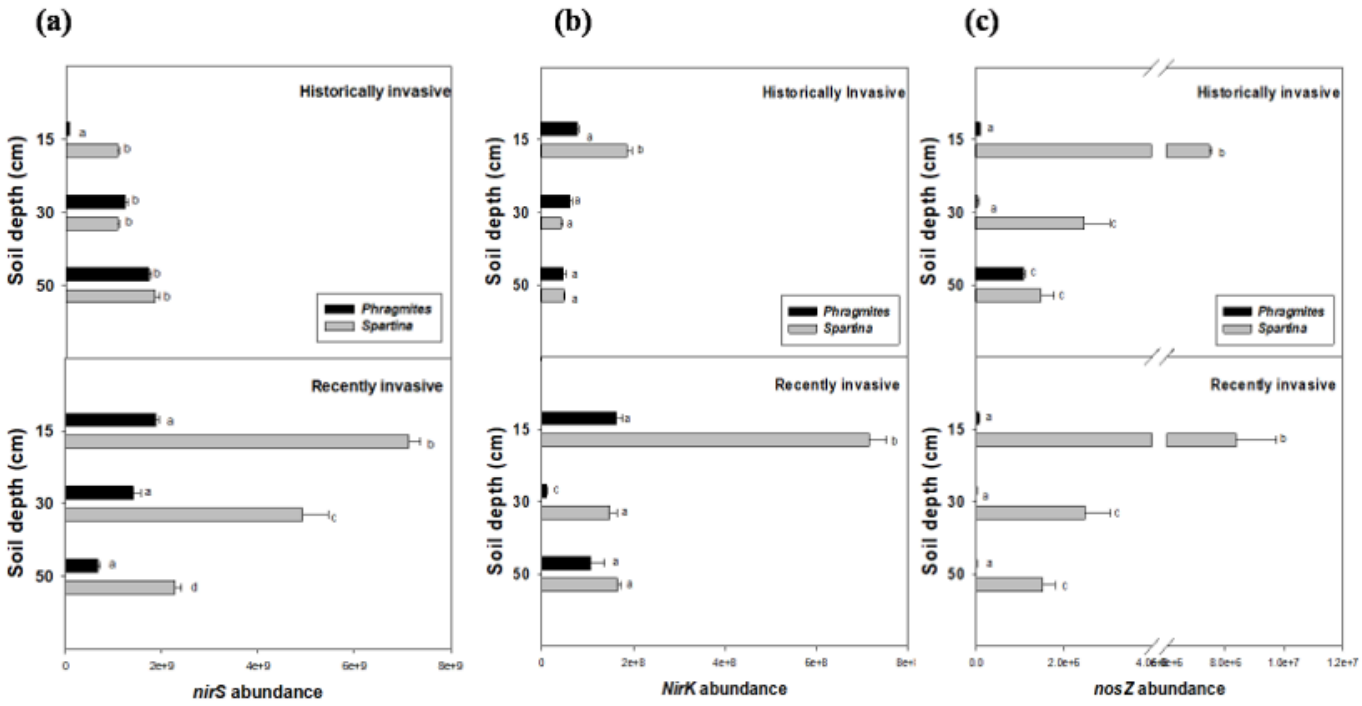

Fig. 3

Figure 3

A depth profile of denitrifier*nirK*(a) denitrifier*nirS* (b), andnosZ (c) in two different sites with different plant types.

Different letters denote significant differences between sites and season at P<0.05 level based on two-way ANOVA

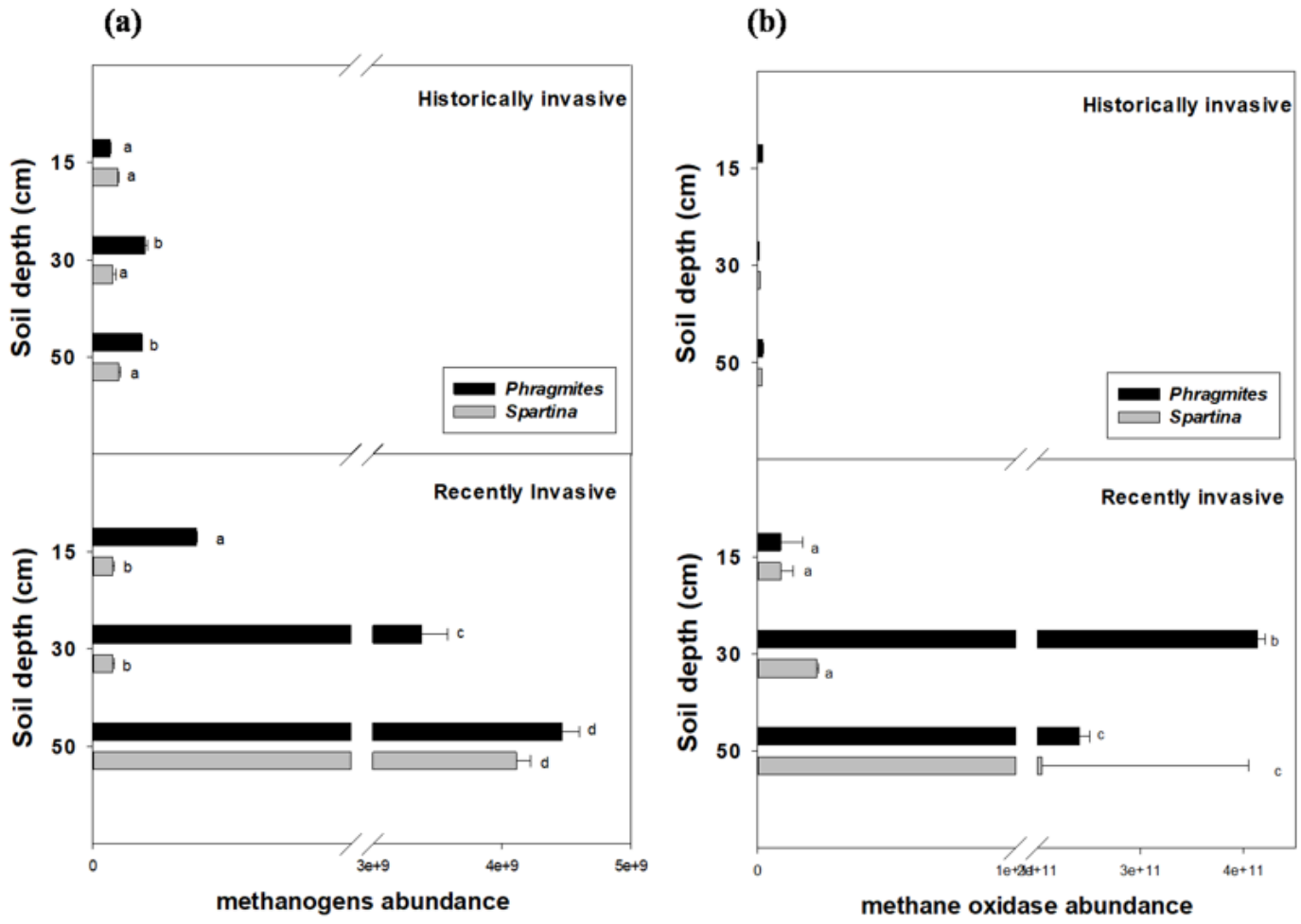

Fig. 4

Figure 4

A depth profile of methanogens abundance mcrA (a), methanotrophpmo*K*(b) in two different sites with different plant types.

Different letters denote significant differences between sites and season at P<0.05 level based on two-way ANOVA

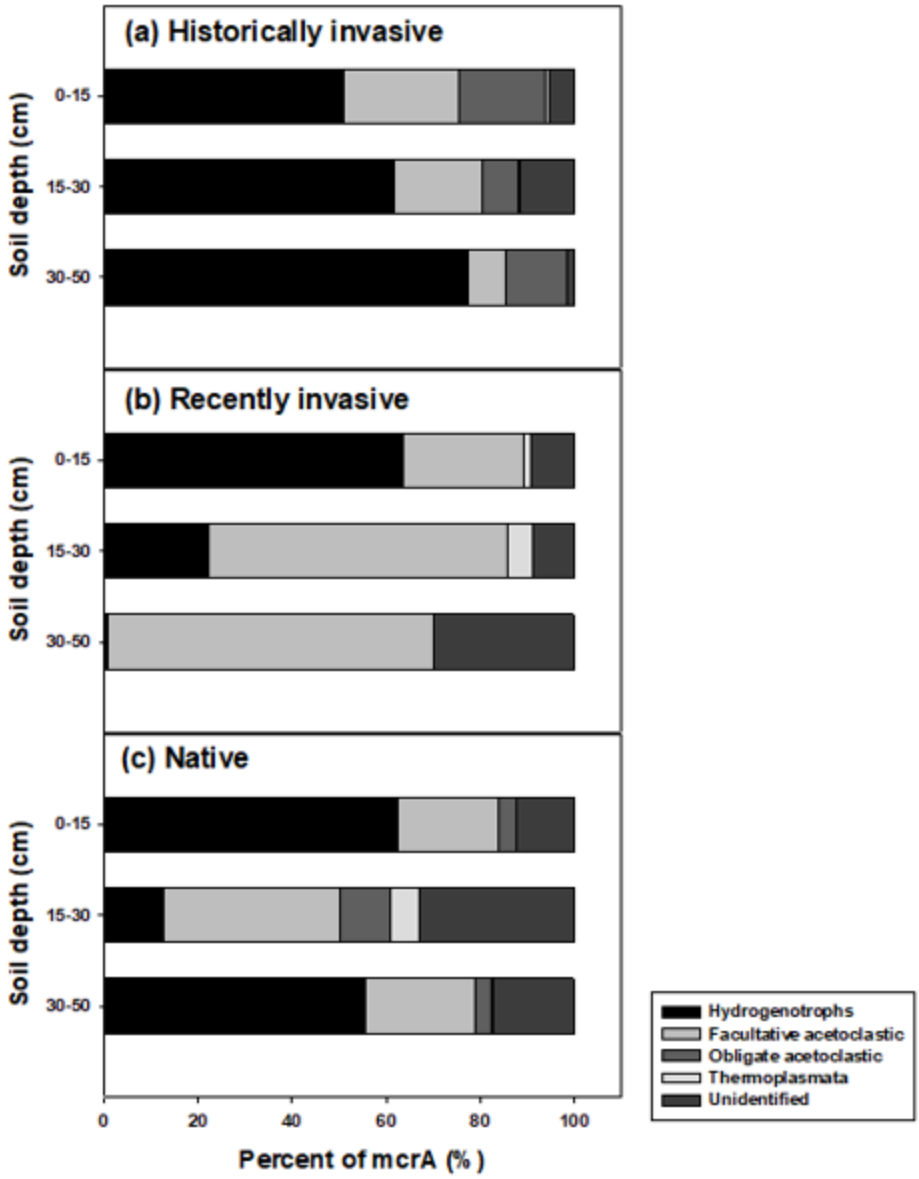

Fig. 5

Figure 5

Relative percentage of mcrAphylotypes across three sites (historically invasive, recently invasive, and native sites) and three different soil depths.

Supplementary Files

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- Supplementarymaterial.docx