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RELATIONSHIPS OF NATIVE AND EXOTIC STRAINS OF *PHRAGMITES AUSTRALIS* TO WETLAND ECOSYSTEM PROPERTIES

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ABSTRACT

Invasions by exotic plant species like *Phragmites australis* can affect wetlands and the services they provide, including denitrification. Native and exotic *Phragmites* strains were genetically verified in 2002 but few studies have compared their ecosystem effects. We compared relationships between native and exotic *Phragmites* and environmental attributes, soil nutrient concentrations, and abundance and activity of soil denitrifying bacteria. There were no significant differences for any measured variables between sites with exotic and native strains. However, there were significant positive correlations between native *Phragmites* stem density and soil nutrient concentrations and denitrification rates. Furthermore, denitrifying bacterial abundance was positively correlated with nitrate concentration and denitrification rates. Additionally, there were significant negative correlations between water levels in native

Phragmites sites and native stem density, nutrient concentrations, and denitrification rates. Surprisingly, we found no significant relationships between exotic stem density or water level and measured variables. These results suggest 1) the native strain may have important ecosystem effects that had only been documented for exotic *Phragmites*, and 2) abiotic drivers such as water level may have mediated this outcome. Further work is needed to determine if the stem density gradients were a consequence, rather than a cause, of pre-existing gradients of abiotic factors.

Keywords: *Phragmites australis*; denitrification; soil nutrients; *Phragmites australis* subspecies *americanus*; exotic haplotype M; *nirS*

INTRODUCTION

Wetlands are important ecosystems because they provide habitat for numerous species and are responsible for essential ecosystem services such as flood abatement and nutrient cycling. For example, wetlands provide ideal conditions for denitrification, a microbially-driven process that can transform excess nitrate from surface and groundwater into gaseous forms of nitrogen (nitrous oxide and nitrogen), thus improving water quality (Zedler 2003). Emergent wetland plants can enhance denitrification by producing high levels of soil organic matter, which provides energy to soil microbes that catalyze denitrification (e.g., Bastviken et al. 2005). Denitrification services provided by natural wetlands have been well documented, and estimates indicate they can remove up to 80% of nitrate from water (Zedler 2003).

Wetland degradation and loss because of urbanization and agriculture are important factors that have led to major losses of wetland area worldwide and to the disruption of wetland structure and function (Ehrenfeld 2000, Zedler 2003). Specifically, the invasion of wetlands by exotic plant species can affect ecosystem properties and their ability to perform ecosystem services such as denitrification positively, neutrally, or negatively (Theuerkauf et al. 2017). Several studies have shown that invasive plants can diminish denitrification potential rates (e.g., Evans et al. 2001, Dassonville et al. 2011, Carey et al. 2017) or enhance them (e.g., Ehrenfeld 2003, Zedler 2003, Lishawa et al. 2014). A few studies, however, have documented no change in denitrification potential when comparing soils under exotic and native plant stands (e.g., Ehernfeld 2003).

Exotic *Phragmites australis* is one of the four introduced species of concern (which also include *Lythrum salicaria, Typha* x *glauca*, and *Phalaris arundinacea*) that have spread throughout North American temperate wetlands (Galatowitsch et al. 1999). Historically, native *Phragmites* was found in North America within heterogeneous plant communities in coastal and inland marshes (Meyerson et al. 2009). By the 1970s, however, the presence of extensive *Phragmites* stands in all lower 48 US states led to the suspicion that an exotic strain of *Phragmites* might be responsible for this expansive spread (Meyerson et al. 2009). By 2007, three genetic lineages of *Phragmites* were identified in North America based on genetic sequence data: 1) *P. australis* subspecies *americanus* (native *Phragmites* hereafter), 2) *P. australis* subspecies *berlandieri* (Gulf Coast *Phragmites* hereafter), and 3) *P. australis* haplotype M (exotic *Phragmites* hereafter) (Saltonstall 2002; Saltonstall et al. 2004; Saltonstall and Hauber 2007; see Saltonstall 2016 for a thorough review of the many *Phragmites* haplotypes). Because

66 of its ability to thrive in commonly disturbed environments and to outcompete native plants,
67 exotic *Phragmites* is considered one of the worst invaders of North American wetlands
68 (Meyerson et al. 2009), costing about $4.6 million annually in control and eradication efforts
69 (Martin and Blossey 2013).

70 Understanding how native and exotic plants can impact ecosystems and microbially-
71 mediated nutrient cycling processes, especially denitrification, is necessary for properly
72 managing wetland ecosystems to help mitigate eutrophication. However, not much is known
73 about whether native and exotic *Phragmites* strains differ in their relationships to environmental
74 attributes, soil nutrients, denitrification rates, and their association with denitrifier microbes.
75 Because reliable identification of the different strains of *Phragmites* was not possible prior to the
76 molecular work of Saltonstall (2002), few studies have focused on ecological impacts of
77 different *Phragmites* strains. Our search of the literature identified several studies that examined
78 the effect of *Phragmites* on certain ecosystem attributes (Table 1). Of those studies, however,
79 only 11 addressed ecosystem impacts of genetically verified *Phragmites* strains (native versus
80 exotic; see bolded entries in Table 1), and more than half of those addressed differences in only
81 one variable (plant biomass), warranting further studies.

82 The available research addressing the impact of exotic *Phragmites* on ecosystem
83 properties suggests that its presence unequivocally contributes to an increase in plant biomass
84 and productivity because exotic *Phragmites* produces more shoots, has a higher growth rate,
85 generally grows taller, and produces more biomass than the native strain (Table 1; Lelong 2007;
86 Jodoin et al. 2008; Mozdzer et al. 2013). Although more *Phragmites* biomass usually correlates

87 with higher soil organic matter (SOM) which could support denitrifying soil microbes and thus
88 enhance denitrification, some studies reported no difference in SOM or denitrification between
89 the exotic *Phragmites* strain and an area having vegetation other than exotic *Phragmites* (Table
90 1). More importantly, there are no studies to date that have compared SOM content and
91 denitrification rates between the exotic and native *Phragmites* strains (i.e., there are no bolded
92 citations on Table 1 under denitrification).

93 There are two contrasting scenarios that may explain the relationship between
94 *Phragmites* and soil nutrient concentrations (nitrate (NO_3), ammonium (NH_4), and phosphate
95 (PO_4)). Studies have indicated that exotic *Phragmites* has a higher nutrient demand compared to
96 the native strain (Holdredge et al. 2010; Mozdzer and Zieman 2010; Mozdzer et al. 2013). In
97 addition, if the exotic strain has higher plant biomass than the native strain (e.g., Table 1) and if
98 nutrients are bound in those plant tissues (as reported in several studies for Biomass [N] in Table
99 1), then soil nutrient concentrations will be smaller under exotic *Phragmites* stands compared to
100 those under native stands. In contrast, soil nutrient concentrations may be larger under exotic
101 *Phragmites* stands if interactions with microbial communities enhance nutrient mineralization
102 rates. This latter pattern of increased soil nutrients has been documented in studies of other
103 invasive wetland plants such as exotic and hybrid *Typha* (Angeloni et al. 2006; Larkin et al.
104 2011; Geddes et al. 2014). However, our review of the literature shows only two studies (Price et
105 al. 2014; Yarwood et al. 2016) directly compared soil nutrient concentrations between exotic and
106 native *Phragmites* stands and their results showed variable results (i.e., larger, smaller, or equal
107 soil nutrient concentrations between exotic and native stands; Table 1).

The objectives of this study were to quantify and compare 1) environmental attributes (soil temperature, water level, soil moisture, and soil pH), 2) soil nutrient concentrations (carbon as soil organic matter, nitrate, ammonium, and phosphate), and 3) soil denitrification rates and abundance of denitrifiers (as determined by *nirS* copy numbers) between stands dominated by exotic *Phragmites* versus those dominated by native *Phragmites*. We hypothesized that sites dominated by exotic *Phragmites* would have larger soil nutrient concentrations and higher rates of denitrification than sites dominated by native *Phragmites.* Additionally, we predicted that the abundance of denitrifying bacteria (as estimated by *nirS* copy numbers) would positively correlate with nitrate concentration, because nitrate is used as the electron acceptor for denitrification. We expected these latter relationships to be stronger in areas dominated by exotic *Phragmites* than in areas dominated by the native strain.

In addition to comparing differences in ecosystem attributes between stands dominated by exotic *Phragmites* versus those dominated by native *Phragmites*, we also examined relationships between measured ecosystem attributes and *Phragmites* stem density of both strains as well as water level using a regression approach. We acknowledge that invasive species are likely to affect environmental attributes of the sites they invade (i.e., invasive species are the *cause* of the measured changes), but they are also likely to invade areas that had certain environmental conditions to begin with (i.e., the invasion is a *consequence* of pre-existing conditions such as abiotic factors or nutrient concentrations). Specifically for *Phragmites*, previous work has determined that several abiotic factors affect stem density, and hence these abiotic gradients in combination with stem density may be responsible for the observed patterns we report in our results. For example, salinity, nutrient availability, and hydrology/water level

have all been shown to control *Phragmites* stem attributes such as density, height, diameter, and biomass (e.g., Chambers, Meyereson and Saltonstall 1999; Meyerson et al. 2000a; Vretare et al. 2001; Chambers et al. 2003; Welch, Davis and Gates 2006; Saltonstall and Stevenson 2007; Eid et al. 2010), where stem attributes correlate positively with increased fertility and negatively with increased salinity (Engloner 2009). Responses of *Phragmites* stem attributes to hydrological variation such as water depth or flooding frequency yielded more ambiguous results in previous studies (Engloner 2009). Similarly to other correlational studies involving invasive species, assigning causality can be difficult (e.g., Geddes et al. 2014; Price et al. 2014). Nevertheless, correlational studies such as ours will enable the development of specific hypotheses regarding the effects of exotic and native *Phragmites* on ecosystem properties that can be tested via controlled manipulative experiments.

Materials and Methods

We measured environmental attributes, soil nutrient concentrations, denitrification, and denitrifier abundance during the summer of 2011 in three sites dominated by native *Phragmites* and in three sites dominated by exotic *Phragmites*; all stands had at least 95% *Phragmites* cover. Study sites were located in DuPage and Kane Counties in Illinois, and Lake County in Indiana (Fig. 1). The exotic stands were located at Dick Young Forest Preserve, Burnidge Forest Preserve, and Pratts Wayne Woods Forest Preserve, and the native stands were located at Calumet Prairie (2 sites) and West Chicago Prairie (Fig. 1). Stands were identified as native or exotic using genetic analysis (Price et al. 2014) following the methodology of Saltonstall et al. (2004).

We collected samples from Illinois sites on July 26, 2011 and from Indiana sites on July 27, 2011. All variables were measured at 5 randomly selected plots in each of the 6 sites, for a total of 30 plots. Plots were spaced at 5-7 m intervals beginning 10 meters from the stand edge. At each plot, we measured several variables *in situ* (see below) and we took a soil core (~6-8 cm in diameter, ~10-14 cm deep) using a serrated knife to cut through the roots and two trowels to extract the core, placed it in a Ziploc bag, and immediately stored it on ice. Soil cores were placed in a refrigerator until analysis.

***Phragmites* Density and Environmental Attributes**

Phragmites stem density was quantified by counting only new, green *Phragmites* aerial stems using a 1 m x 0.5 m quadrat (total area sampled = 0.5 m^2). Brown, senesced stems from the previous season(s) were not included in the counts. Soil temperature was taken using a Fisher Scientific Traceable Lollipop Waterproof/Shockproof Thermometer by inserting it 10 cm into the soil. Depth of standing water was measured with a meter stick. Soil pH was determined in the lab by mixing 15 g of soil with 30 mL DI water. The slurry was stirred and allowed to stand for 30 minutes for CO_2 equilibration after which pH was read with an ORION model 310 pH meter (Robertson et al. 1999). Soil moisture was calculated as the difference between dry and wet mass of 10 g of wet soil sample that had been weighed and dried to constant weight in a drying oven at 105°C.

Nutrient Concentrations: Carbon, Nitrogen, Phosphorus

SOM, nitrogen (nitrate and ammonium), and phosphorus concentrations were measured from soil cores from each of the 30 plots. Soil cores were kept separate for all analyses. Roots, twigs, and debris were removed from each soil core, and cores were then manually homogenized and mixed within each individual Ziploc bag (i.e., cores were kept separate for analyses). Subsamples from each soil core were then taken to determine soil nutrient content. All nutrient concentrations were measured within 36 hours of sample collection.

SOM was measured as mass loss on ignition and quantified as ash-free dry mass (AFDM). Ten grams of each wet soil sample were placed in an aluminum pan, weighed, and dried to constant weight in a drying oven at 105°C. Dry samples were then ashed in a muffle furnace at 550°C for two hours to obtain AFDM values. SOM (%) was calculated as a percentage of soil dry mass (g) by dividing AFDM by soil dry mass and multiplying by 100 (APHA 2005).

Soil ammonium was measured using the phenol-hypochlorite method (Wetzel and Likens 1991), in KCl-extracted samples. Absorbance was recorded using a Shimadzu UV-Vis spectrophotometer at 630 nm in 1 cm quartz cuvettes. Nitrate was measured in KCl-extracted samples following the cadmium-reduction method on a Seal Analytical AQ2+ Discrete Auto-analyzer. Soil orthophosphate was determined using the ascorbic acid method (Wetzel and Likens 1991), using Troug's solution as the extractant (Mehlich 1953). Absorbance was recorded using a Shimadzu UV-Vis spectrophotometer at 885 nm in 1 cm quartz cuvettes.

Denitrification Potential

Soil microbe denitrification potential was measured using the DEA (denitrification enzyme activity) assay, based on the acetylene inhibition technique (Groffman et al. 1999). Although this technique has some caveats, it is a technique that is accessible in terms of cost, allows large number of samples to be run simultaneously, and is still widely used (Groffman et al. 2006). The technique involves the measurement of nitrous oxide concentration as a proxy for potential denitrification. Therefore, comparative studies like this one that measure relative denitrification potential rather than absolute denitrification fluxes are likely to be less affected by the technique's caveats (e.g., Alldred et al. 2016).

The principle behind the acetylene inhibition technique is based on the fact that N_2O reductase, the enzyme used by denitrifying bacteria in the last step of the denitrification pathway to convert nitrous oxide to nitrogen gas, is inhibited by acetylene. Thus, this inhibition allows a measurement of nitrous oxide concentration as a proxy for how much denitrification is possible by the soil microbes under controlled lab conditions. The differences in nitrous oxide produced were then used to compare the ability of soils to perform denitrification under native and exotic stands of *Phragmites.*

Canning jars (230 mL) were fitted with butyl septa and 60 mL of soil were placed in each jar along with water and an amendment that included glucose (as a carbon source; 120 mg l^{-1}) and nitrate (140 mg l^{-1}) (Groffman et al. 1999) to form a slurry. Jars were flushed with helium for five minutes to remove oxygen and then equilibrated to atmospheric pressure. 10 mL of acetylene were then added to each jar and 4 mL gas samples were collected from the headspace in jars at 30, 60, 90, and 180 minutes after acetylene addition and stored in gas-tight evacuated

vials. Gas samples were quantified for nitrous oxide using a Shimadzu gas chromatograph (GC-2014) equipped with an Electron Capture Detector (ECD) and a HayeSep Q stainless steel column. Ultrapure nitrogen was the carrier gas, and the detector, oven, and injector temperatures were set at 300 °C, 40 °C and 60 °C, respectively.

Molecular Analyses of Soil Denitrifier Communities

For the quantification of soil denitrifiers, we analyzed soil from 3 replicate cores chosen randomly from the 5 replicate cores collected from each site, for a total of 18 samples (3 exotic *Phragmites* sites x 3 plots each and 3 native *Phragmites* sites x 3 plots each). The abundance of denitrifying bacteria in the sediments was assessed based on quantification of copy numbers of *nirS* genes via real-time quantitative polymerase chain reaction (qPCR). The *nirS* gene encodes the cytochrome-containing version of nitrite reductase (Braker et al. 1998), the enzyme that catalyzes the reduction of nitrite to nitric oxide, which is the first committed step of denitrification (Zumft 1997). The *nirK* gene, which encodes a functionally redundant version of nitrite reductase (Braker et al. 1998), was not quantified. The *nirS* gene was chosen for this study because previous work has shown that *nirS*-containing denitrifiers are abundant in wetlands, and the copy number of *nirS* genes is commonly used as an indicator of the abundance of denitrifying bacteria (e.g., Angeloni et al. 2006; Geddes et al. 2014).

Genomic DNA was isolated from each of the soil samples (~0.5 g) with the UltraClean Soil DNA Kit (MoBio Laboratories, Salana Beach, CA). Successful DNA isolation was confirmed by agarose gel electrophoresis. The amount of DNA isolated from each sample was determined with the Quant-iT DNA Assay Kit (Invitrogen, Carlsbad, CA). The *nirS* qPCR assay

followed the approach described by Geets et al. (2007) except that the annealing temperature was changed to 57 ºC and the extension temperature was changed to 72 ºC. All qPCR experiments were run using an MJ Research DNA Engine Opticon1 thermal cycler equipped with Opticon Monitor software version 3.1 (Biorad, Hercules, CA). Conditions for all qPCR reactions were as follows: 12.5 µl QuantiTect SYBR Green PCR Master Mix (Qiagen, Valencia, CA), 0.5 µM final concentration of each primer, 5 µl template, and water were added to a final 25 µl volume. qPCR was carried out using primers cd3AF (GTSAACGTSAAGGARACSGG) and R3cd (GASTTCGGRTGSGTCTTGA), which produce a 425 base pair amplicon (Throbäck et al. 2004). All reactions were performed in low-profile 0.2 mL white strip tubes with optical ultra-clear strip caps (Bio-Rad). Thermal cycling was as follows: initial denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 57 °C for 1 min, extension at 72 °C for 1 min, hold at 78 °C for 1 sec, and plate read. Finally, a melting curve was run from 50–95 °C with a read every 1 °C and a hold of 1 sec between reads. Specificity of qPCR reactions was confirmed by melting curve analysis and agarose gel electrophoresis.

The standard used for qPCR reactions was a cloned *nirS* gene from *Paracoccus denitrificans* (ATCC 13543). *P. denitrificans* was grown according to ATCC guidelines and DNA was extracted using the UltraClean Microbial Isolation Kit (MoBio). *nirS* genes were amplified from this DNA using the cd3aF and R3cd primers and the PCR conditions described by Throbäck et al. (2004). PCR amplicons were cloned with the TOPO-TA cloning kit (Invitrogen) using vector pCR4 and transformed into chemically competent *Escherichia coli.* Transformed *E. coli* were grown overnight on LB agar plates containing 50 µg/mL kanamycin. Several randomly selected colonies were transferred to LB broth containing 50

μg/mL kanamycin, grown overnight at 37 ºC, and PCR-screened for the presence of inserts of appropriate size using M13F and M13R primers. Plasmids containing the appropriately sized inserts were isolated using PureLink Quick Plasmid Miniprep Kit (Invitrogen). Plasmids were digested with EcoRI (New England BioLabs) according to the manufacturer's instructions and the digestion reaction was run on an agarose gel. The fragment containing *nirS* was cut out from the gel and purified using QIAquick Gel Extraction Kit (Qiagen). The concentration of this *nirS*-containing fragment was determined by Quant-iT DNA Assay Kit (Invitrogen). Standard curves for qPCR reactions were generated using a 10-fold dilution series ranging from 1.37×10^6 to 137 copies of *nirS*. *nirS* copy numbers were normalized based on grams of soil.

Data Analysis

We compared all measured variables between sites with native *Phragmites* strains and those with exotic strains using t-tests (n=3). Additionally, to address the relationship between density of *Phragmites* and the measured environmental attributes, soil nutrient concentrations, denitrification, and soil denitrifier abundance (*nirS* copy numbers), we conducted separate regression analyses using the number of *Phragmites* stems per square meter or water level as the independent variable, combining both strains together, as well as separately for each strain (i.e., exotic and native). All dependent variables were log-transformed to conform to assumptions of homoscedasticity. Data analyses were performed using Systat v. 11 (Systat Software, Inc., San Jose, CA). Alpha ≤ 0.05 was used to evaluate significance.

RESULTS

Contrary to our expectations, there were no statistically significant differences in any of the measured variables between the exotic *Phragmites* and native *Phragmites* sites, with the exception of pH. Soils associated with the exotic strain had higher soil pH (7.41) than that associated with the native strain (7.08) ($P = 0.048$). There was also no significant difference in *Phragmites* stem density between exotic and native *Phragmites* sites ($P = 0.787$). Exotic *Phragmites* sites had a stem density mean of 36.26 stems m^{-2} (± 11.8 SD) whereas the native sites had a mean of 41.13 stems m^{-2} (± 27.41 SD). Although not significant, the sites dominated by native *Phragmites* had slightly higher stem numbers than sites dominated by their exotic counterpart, a result that contradicted our expectations. Additionally, there was large variability in *Phragmites* stem densities across sites, and this variability was much greater in native *Phragmites* sites (range: 8.6-60.4 stems m^{-2}) than in the exotic *Phragmites* sites (range: 28-40.8 stems m^{-2}). Similarly to stem density, surface water levels had greater variability in native *Phragmites* sites (range: 0-5 cm, SD = 1.94) than in exotic *Phragmites* sites (range: 0-1.5 cm, SD = 0.39).

Linear regression analysis using stem density of exotic *Phragmites* as the explanatory variable revealed no significant correlations for any of the measured variables (nitrate, ammonia, phosphate, SOM, soil moisture, denitrification potential, soil temperature, soil pH, or water level) (Fig. 2). Denitrifier abundance was also not significantly correlated with exotic *Phragmites* stem density ($P = 0.448$; data not shown).

In contrast, native *Phragmites* stem density showed significant correlations with all measured variables except for nitrate, soil moisture, and pH (Fig. 3), as well as for denitrifier

296 abundance (P = 0.134; data not shown). Specifically, we found positive relationships between
297 *Phragmites* stem density and ammonium (P < 0.001), SOM (P = 0.012), phosphate (P = 0.047),
298 and denitrification potential rates (P = 0.003), and negative relationships with temperature (P =
299 0.043) and water level (P < 0.001) (Fig. 3). Lastly, linear regression analysis using stem density
300 of exotic and native *Phragmites* combined as the explanatory variable to address if stem density
301 *per se*, irrespective of strain, was responsible for the observed patterns revealed significant
302 correlations that matched those of the native *Phragmites* stem density alone, suggesting the
303 native strain was the one that had the greatest influence over the significant results (data not
304 shown).

305 For soils under the native *Phragmites* strain, we found that *nirS* copy numbers were
306 significantly correlated with soil nitrate concentrations and denitrification potential rates.
307 Specifically, there was a positive correlation between *nirS* copy numbers and nitrate
308 concentrations (P = 0.002, R^2 = 0.759, Fig. 4A) as well as for denitrification rates (P = 0.014, R^2
309 = 0.604, Fig. 4B). These relationships were not significant for soils under the exotic strain.

310 When water level in sites with native *Phragmites* was compared with the measured
311 variables, we found significant negative correlations with native *Phragmites* stem density (P <
312 0.001), nitrate (P = 0.049), ammonium (P = 0.010), phosphate (P = 0.018), SOM (P = 0.007),
313 and denitrification (P < 0.001), and a positive correlation with soil temperature (P = 0.004) (Fig.
314 5). However, we found no correlations between water level from sites with exotic *Phragmites*
315 and any of the measured variables (data not shown).

316 **DISCUSSION**

317 Over the past century, exotic *Phragmites* has successfully invaded all of the lower 48 US
318 states (Meyerson et al. 2009), yet little is known about whether sites that have experienced this
319 invasion versus sites with a native *Phragmites* strain possess different relationships to ecosystem
320 properties (Meyerson et al. 2009). Our study addressed information gaps concerning differences
321 in environmental attributes, soil nutrient concentrations, and denitrification in soils of native and
322 exotic *Phragmites* stands. Contrary to previous studies and to our own expectations, this study
323 revealed no differences in measured variables when comparing native versus exotic sites, and
324 that native *Phragmites* exhibited stronger correlations with the measured parameters than exotic
325 *Phragmites* when stem density was considered.

326 In addition, water level showed strong correlations with many measured parameters in
327 native *Phragmites* sites, including native *Phragmites* stem density, suggesting this abiotic driver
328 may have mediated the responses we observed with stem density. However, we acknowledge
329 that our measurements of water level were limited to single time points and to surface water.
330 More sophisticated techniques such as wells, piezometers, and/or graduated staff gauges, as well
331 as incorporation of groundwater level estimates, would have provided more detailed information
332 on the hydrology of these sites. Furthermore, multiple measurements over an extended period of
333 time (hydrographs or time series) prior to our sampling date would have provided additional
334 insight into the potential effects of hydrology on the biotic and abiotic variables measured in our
335 study. Our surface water level measurement represents one time point that could potentially
336 reflect conditions of only a couple of days before sampling, as opposed to more long-term water
337 dynamics. Therefore, although several variables in our study show strong correlations with water
338 level, we recognize the shortcomings of our measurements. Ultimately, our results may reflect

differences in water level or other abiotic gradients that themselves affect and control stem density. Yet it is possible that the reverse is true: stem density may lead to marked differences in plant evapotranspiration rates and accumulation of plant litter, both of which can affect surface water levels. We thus discuss our findings providing possible alternative explanations where appropriate. Despite this caveat, we contend that these results provide novel information regarding the effects of the native *Phragmites* strain at high stem densities, a seemingly rare occurrence given the reported values of native *Phragmites* stem density in the literature (see below). Teasing apart if the invasive species are the cause or the consequence of the change in environmental attributes can ultimately be achieved through controlled experimentation, and we strongly argue for this experimental approach for a more mechanistic understanding of the effects of exotic and native *Phragmites* on ecosystems.

Previous research suggests that exotic *Phragmites* develops more dense stands than native *Phragmites* (e.g., League et al. 2006; Hansen et al. 2007; Saltonstall and Stevenson 2007; Meyerson et al. 2009; Price et al. 2014). We found *Phragmites* stem density was highly variable, especially for the native strain, and that water levels in the native *Phragmites* sites negatively correlated with stem density. Our small sample size of selected sites (n=3) may have affected our ability to detect significant differences between *Phragmites* strains. However, similar to our findings, a few other studies have also indicated that native *Phragmites* stands can exhibit high stem densities (Lynch and Saltonstall 2002; Meyerson et al. 2009; Saltonstall et al. 2010). It is likely that the native strain may indeed have important ecosystem effects once a threshold stem density (or biomass) is reached. A wide range of native *Phragmites* densities have been reported in the literature: 22.3 stems m^{-2} (Price et al. 2014), 37.3 stems m^{-2} (Mozdzer and Zieman 2010),

and 55 stems m^{-2} (Rodríguez and Brisson 2015). In comparison, we found average native stand stem densities of 41.13 stems m^{-2}; the maximum density in native stands was 82 stems m^{-2}, whereas in exotic stands the maximum was 54 stems m^{-2}.

We found negative correlations between native *Phragmites* stem density and water level and soil temperature. High native *Phragmites* stem densities may have correlated with low water levels because native *Phragmites* is presumably less tolerant of standing water than exotic *Phragmites* (Meyerson et al. 2009; Price et al. 2014) and therefore selectively invades areas with lower water levels. Alternatively, native *Phragmites* could be responsible for more efficient water uptake than its exotic counterpart and/or enhanced evapotranspiration rates, keeping water levels low. The negative correlation between native *Phragmites* stem density and soil temperature was likely due to the height and leaf surface area that *Phragmites* can achieve (Meyerson et al. 2009; Saltonstall et al. 2010; Mozdzer and Zieman 2010; Hirtreiter and Potts 2012; Price et al. 2014). In denser native *Phragmites* stands, shading of the understory could have resulted in lower soil temperatures. A similar phenomenon was observed in exotic *Phragmites* stands in other studies, where standing water temperatures decreased due to the shading from the plant canopy (Rogalski and Skelley 2012) or from accumulated litter (Holdredge and Bertness 2011). However, we found a positive correlation between standing water levels and soil temperature (Fig. 5). Although we observed no significant correlations between native or exotic *Phragmites* stem density or water level and pH, the significant difference we found in pH when comparing native and exotic stands may imply that 1) there may be a systematic preference of the exotic strain for alkaline soils, 2) the exotic strain has not been

established long enough to acidify the soil to the extent of the native strain, or 3) that some other disturbance in the sites with the exotic strain led to systematic increases in pH.

Native *Phragmites* density also correlated positively with soil organic matter. Given *Phragmites*' ability to produce high amounts of biomass, dead plant matter can accumulate rapidly, decreasing light availability (Holdredge and Bertness 2011; Hirtreiter and Potts 2012) and eventually decomposing into soil organic matter. As expected, SOM negatively correlated with water level, as decomposition of organic matter depends on an oxic environment. It has been documented that *Phragmites* accumulates so much SOM that it tends to terrestrialize the wetland ecosystems that it invades (Chambers et al. 1999; Windham 2001; Rooth et al. 2003; Meyerson et al. 2009), even changing habitat characteristics for fauna (Derr 2008; Meyerson et al. 2010). This trend of increased SOM has also been documented in other exotic species such as *Typha* x *glauca* (Angeloni et al. 2006; Larkin et al. 2011; Mitchell et al. 2011; Geddes et al. 2014). The positive correlation between increasing SOM and increasing native *Phragmites* stem density found in this study corroborates these latter claims and points to effects of the native strain being similar to or even greater than those of the exotic strain, at least in our study sites. Because SOM has not been reported to be an important determinant of stem density in previous research (e.g., Engloner 2009), we believe native *Phragmites* density was likely a driver for SOM production.

Our finding that native *Phragmites* stem density had a positive correlation with soil ammonium and phosphate concentrations may provide support for the claim that native plant strains can have the ability to modify nutrient concentrations similarly to invasive exotic

403 counterparts. A similar finding was documented by Price et al. (2014) for soil ammonium and
404 nitrate, but not for phosphate. However, due to the correlational nature of this study, it is also
405 likely that we observed higher native *Phragmites* stem density in areas where soil ammonium
406 and phosphate concentrations were larger as these are important nutrients that limit plant growth
407 and control stem density (e.g., Meyerson et al. 2000a; Welch, Davis, and Gates 2006; Saltonstall
408 and Stevenson 2007; Engloner 2009; Eid et al. 2010). In contrast, water levels negatively
409 correlated with all measured nutrients: nitrate, ammonium, and phosphate (Fig. 5), suggesting
410 that increased water levels may have slowed microbial decomposition of organic matter and
411 mineralization of inorganic nutrients due to decreased oxygen availability.

412 Although the exotic *Phragmites* strain has been considered a useful plant in remediation
413 studies due to its ability to remove excess nutrients and improve water quality (e.g., Araki et al.
414 2005; Ruiz-Rueda et al. 2009; Rodríguez and Brisson 2015), results from our study suggest that
415 it was the native strain that exhibited a positive correlation between *Phragmites* stem density and
416 denitrification (Fig. 3). Rodríguez and Brisson's study (2015) and our study are the only two
417 examples that we know of that show significant effects of the native strain on nutrient removal –
418 phosphate in their study; nitrate through denitrification in ours– when compared to the exotic
419 one, perhaps as a result of native stand stem densities being on the highest end of those reported
420 in the literature. Yet it is important to exercise caution when interpreting these data as another
421 explanation may involve the reverse pattern: if there are higher stem densities in areas with
422 higher levels of soil nitrate, then denitrification rates may be higher due to higher soil nitrate
423 concentrations, and not necessarily due to the higher native stem densities. However, we found
424 no relationship between soil nitrate and increasing native stem density (Fig. 3), weakening the

support for this latter explanation. Our study also showed that denitrification rates were negatively correlated with water level (Fig. 5) and thus we contend that water level may have been a driver of denitrification rates alone or in combination with stem density. Lastly, we found a positive correlation between soil nitrate under the native strain with the number of copies of the *nirS* gene, an indicator of denitrifier abundance (Fig. 4A). In turn, copies of the *nirS* gene positively correlated with denitrification rates (Fig. 4B). Our study is novel in that the microbial composition difference between these strains can shed light on ecosystem functioning. However, more studies are needed that compare the microbial communities under native versus exotic strains (but see Yarwood et al. 2016).

If one of the goals of preserving wetland integrity while maximizing water purification functions is to maintain or increase denitrification rates, our study suggests lowering water levels and/or preserving the native strain when in highly dense stands might be a viable option. Similarly, Rodríguez and Brisson (2015) have suggested utilizing the native strain of *Phragmites* for phosphate removal. However, management of wetlands that have both native and exotic strains poses problems because identification of strains is difficult morphologically and usually relies on molecular analyses that are not widely accessible to managers. Further experimental tests are required before research can effectively inform management practices regarding this species.

CONCLUSION

Our research showed that although exotic *Phragmites australi*s has been extensively documented as an aggressive wetland invader, gradients in native *P. australis* stem density and

water level exhibited significant correlations with environmental attributes, soil nutrient concentrations, and denitrification in our study sites, whereas the exotic strain did not. The fact that we did not detect any correlations between exotic *Phragmites* stem density and measured variables but did so for the native strain implies that 1) there is something inherently different about the two strains, with the native strain being the cause of the observed correlations, 2) the native strain selectively invaded sites that had certain pre-existing environmental attributes that controlled stem density and, as a consequence, it showed correlations with those environmental attributes, and/or 3) water levels may drive the observed patterns alone or in combination with other factors, and can thus mediate the responses observed. Further experimental work that compares genetically identified native and exotic *Phragmites* as well as controls for pre-existing environmental attributes to avoid confounding interpretations are needed to provide further insight into whether the two strains have different ecosystem impacts. Additionally, given the high variability likely found in many variables associated with *Phragmites* stands, studies with high stand replication covering a broader geographic scope are warranted.

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contributions to the manuscript, and Lynnette Murphy for creating the map of sampling sites. Financial support for this study was provided by a Research Starter Grant to P.G. from the National Science Foundation (DEB 1034855). Work by S.I. was supported by a Loyola University Chicago WISER Fellowship.

REFERENCES

Alldred M, Baines SB, Findlay, S (2016) Effects of invasive-plant management on nitrogen removal services in freshwater marshes. PLoS One 11(2):e0149813; doi:10.1371/journal.pone.0149813

Angeloni NL, Jankowski KJ, Tuchman NC, Kelly JJ (2006) Effects of an invasive cattail species (*Typha* x *glauca)* on sediment nitrogen and microbial community composition in a freshwater wetland. FEMS Microbiology Letters 263:86-92

APHA (2005) Standard Methods for the Examination of Water and Wastewater, 21st edn. American Public Health Association, Washington, DC

Araki R, Mori M, Mori M, Hasegawa H (2005) Genetic differences in nitrate uptake in two clones of the common reed, *Phragmites australis*. Breeding Science 55:297-302

488 Bastviken SK, Eriksson PG, Premrov A, Tonderski K (2005) Potential denitrification in wetland
489 sediments with different plant species detritus. Ecological Engineering 25:183-190
490
491 Braker G, Fesefeldt A, Witzel KP (1998) Development of PCR primer systems for amplification
492 of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental
493 samples. Applied and Environmental Microbiology 64: 3769–3775
494
495 Carey CJ, Blankinship JC, Eviner VT, Malmstrom CM, Hart SC (2017) Invasive plants decrease
496 microbial capacity to nitrify and denitrify compared to native California grassland communities.
497 Biological Invasions DOI 10.1007/s10530-017-1497-y :1-17
498
499 Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal
500 wetlands of North America. Aquatic Botany 64:261-273
501
502 Chambers RM, Osgood DT, Bart DJ, Montalto F (2003) *Phragmites australis* invasion and
503 expansion in tidal wetlands: Interactions among salinity, sulfide, and hydrology. Estuaries
504 26(2B):398-406
505
506 Dassonville N, Guillaumaud N, Piola F, Meerts P, Poly F (2011) Niche construction by the
507 invasive Asian knotweeds (species complex *Fallopia*): Impact on activity, abundance
508 and community structure of denitrifiers and nitrifiers. Biological Invasions 13:1115-1133
509

510 Derr JF (2008) Common reed (*Phragmites australis)* response to mowing and herbicide
511 application. Invasive Plant Science and Management 1:12-26
512
513 Duke S, Francoeur SN, Judd KE (2015) Effects of *Phragmites australis* invasion on carbon
514 dynamics in a freshwater marsh. Wetlands 35(2): 311-321
515
516 Ehrenfeld JG (2000) Evaluating wetlands within an urban context. Ecological Engineering
517 15:253-265
518
519 Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes.
520 Ecosystems 6:503-523
521
522 Eid ME, Shaltout KH, Al-Sodany YM, Jensen K (2010) Effects of abiotic conditions on
523 *Phragmites australis* along geographic gradients in Lake Burullus, Egypt. Aquatic Botany
524 92(2):86-92
525
526 Engloner AI (2009) Structure, growth dynamics and biomass of reed (*Phragmites australis*) – A
527 review. Flora 204:331-346
528
529 Evans RD, Rimer R, Sperry L, Belnap J (2001) Exotic plant invasion alters nitrogen dynamics in
530 an arid grassland. Ecological Applications 11(5):1301-1310
531

532 Galatowitsch SM, Anderson NO, Ascher PO (1999) Invasiveness in wetland plants in temperate
533 North America. Wetlands 19:733-755
534
535 Geddes P, Grancharova T, Kelly JJ, Treering D, Tuchman NC (2014) Effects of invasive *Typha*
536 x *glauca* on wetland nutrient pools, denitrification, and bacterial communities are influenced by
537 time since invasion. Aquatic Ecology 48:247-258
538
539 Geets J, de Cooman M, Wittebolle L, Heylen K, Vanparys B, De Vos P, Verstraete W, Boon N
540 (2007) Real-time PCR assay for the simultaneous quantification of nitrifying and denitrifying
541 bacteria in activated sludge. Applied Microbiology and Biotechnology 75(1):211-21
542
543 Groffman, PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin
544 AE, Kana TM, Nielsen LP, Voytek MA (2006) Methods for measuring denitrification: Diverse
545 approaches to a difficult problem. Ecological Applications 16(6):2091-2122
546
547 Groffman PM, Holland EA, Myrold DD, Robertson GP, Zou X (1999) Denitrification. In:
548 Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds) Standard soil methods for long-term
549 ecological research. Oxford University Press, New York, pp 272-288
550
551 Hansen DL, Lambertini C, Jampeetong A, Brix H (2007) Clone-specific differences in
552 *Phragmites australis*: Effects of ploidy level and geographic origin. Aquatic Botany 86:269-279
553

554 Hirtreiter JN, Potts DL (2012) Canopy structure, photosynthetic capacity and nitrogen
555 distribution in adjacent mixed and monospecific stands of *Phragmites australis* and *Typha*
556 *latifolia*. Plant Ecology 213:821–829
557
558 Holdredge C, Bertness MD (2011) Litter legacy increases the competitive advantage of invasive
559 *Phragmites australis* in New England wetlands. Biological Invasions 13:423-433
560
561 Holdredge C, Bertness MD, von Wettberg E, Silliman BR (2010) Nutrient enrichment enhances
562 hidden differences in phenotype to drive a cryptic plant invasion. Oikos 119:1776-1784
563
564 Jodoin Y, Lavoie C, Villeneuve P, Theriault M, Beaulieu J, Belzile F (2008) Highways as
565 corridors and habitats for the invasive common reed *Phragmites australis* in Quebec, Canada.
566 Journal of Applied Ecology 45:459-466
567
568 Kulmatiski A, Beard KH, Meyerson LA, Gibson JR, Mock KE (2010) Nonnative *Phragmites*
569 *australis* invasion into Utah wetlands. Western North American Naturalist 70(4):541-552
570
571 Larkin D, Freyman MJ, Lishawa SC, Geddes P, Tuchman NC (2011) Mechanisms of dominance
572 by the invasive cattail *Typha* x *glauca.* Biological Invasions 14:827–838
573

574 League MT, Colbert EP, Seliskar DM, Gallagher JL (2006) Rhizome growth dynamics of native
575 and exotic haplotypes of *Phragmites australis* (common reed). Estuaries and Coasts 29(2):269-
576 276
577
578 Lelong B, Lavoie C, Jodoin Y, Belzile F (2007) Expansion pathways of the exotic common reed
579 (*Phragmites australis*): A historical and genetic analysis. Diversity and Distributions 13:430-437
580
581 Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon
582 and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706-714
583
584 Lishawa, SC, Jankowski KJ, Geddes P, Larkin DJ, Monks AM, Tuchman NC (2014)
585 Denitrification in a Laurentian Great Lakes coastal wetland invaded by hybrid cattail (*Typha* ×
586 *glauca*). Aquatic Sciences 76:483-495
587
588 Lynch EA, Saltonstall K (2002) Paleoecological and genetic analyses provide evidence for
589 recent colonization of native *Phragmites australis* populations in a Lake Superior wetland.
590 Wetlands 22(4):637-646
591
592 Martin LJ, Blossey B (2013) The runaway weed: costs and failures of *Phragmites australis*
593 management in the USA. Estuaries and Coasts 36(3):626–632
594

595 Mehlich A (1953) Determination of P, Ca, Mg, K, Na, and NH_4. Soil Testing Div. Publ. 1-53.
596 North Carolina Dept. Agriculture, Raleigh, NC
597
598 Meyerson LA, Saltonstall K, Chambers RM (2009) *Phragmites australis* in eastern North
599 America: A historical and ecological perspective. In: Silliman BR, Grosholz E, Bertness MD
600 (eds) Salt marshes under global siege. University of California Press, California, pp 57-82
601
602 Meyerson LA, Saltonstall K, Windham L, Kiviat E, Findlay S (2000a) A comparison of
603 *Phragmites australis* in freshwater and brackish marsh environments in North America.
604 Wetlands Ecology and Management 8:89-103
605
606 Meyerson LA, Viola DA, Brown RN (2010) Hybridization of invasive *Phragmites australis* with
607 a native subspecies in North America. Biological Invasions 12:103-111
608
609 Meyerson LA, Vogt KA, Chambers RM (2000b) Linking the success of *Phragmites* to the
610 alteration of ecosystem nutrient cycles. In: Weinstein MP, Kreeger DA (eds) Concepts and
611 controversies in tidal marsh ecology. Kluwer Academic Publishers, Norwell, MA pp 827-844
612
613 Mitchell ME, Lishawa SC, Geddes P, Larkin DJ, Treering D, Tuchman NC (2011) Time-
614 dependent impacts of cattail invasion in a Great Lakes coastal wetland complex. Wetlands
615 31:1143-1149
616

617 Mozdzer TJ, Brisson J, Hazelton ELG (2013) Physiological ecology and functional traits of
618 North American native and Eurasian introduced *Phragmites australis* lineages. AoB PLANTS
619 5:plt048. doi: 10.1093/aobpla/plt048
620
621 Mozdzer TJ, Langley JA, Mueller P, Megonigal JP (2016) Deep rooting and global change
622 facilitate spread of invasive grass. Biological Invasions 18:2619-2631
623
624 Mozdzer TJ, Megonigal JP (2012) Jack-and-master trait responses to elevated CO2 and N:
625 A comparison of native and introduced *Phragmites australis*. PLoS ONE 7(10): e42794.
626 doi:10.1371/journal.pone.0042794
627
628 Mozdzer TJ, Zieman JC (2010) Ecophysiological differences between genetic lineages facilitate
629 the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands.
630 Journal of Ecology 98:451-458
631
632 Nijburg JW, Laanbroek HJ (1997) The fate of ^{15}N-Nitrate in healthy and declining *Phragmites*
633 *australis* stands. Microbial Ecology 34:254-262
634
635 Packett CR, Chambers RM (2006) Distribution and nutrient status of haplotypes of the marsh
636 grass *Phragmites australis* along the Rappahannock River in Virginia. Estuaries and Coasts
637 29:1222-1225
638

639 Price AL, Fant JB, Larkin DJ (2014) Ecology of native vs. introduced *Phragmites australis*
640 (common reed) in Chicago-area wetlands. Wetlands 34:369-377
641
642 Robertson GP, Coleman DC, Bledsoe CS, Sollins P (1999) Standard soil methods for long-term
643 ecological research. Oxford University Press, New York
644
645 Rodríguez M, Brisson J (2015) Pollutant removal efficiency of native versus exotic common
646 reed (*Phragmites australis*) in North American treatment wetlands. Ecological Engineering
647 74:364-370
648
649 Rogalski MA, Skelly DK (2012) Positive effects of nonnative invasive *Phragmites australis* on
650 larval bullfrogs. PLoS ONE 7(8):e44420. doi:10.1371/journal.pone.0044420
651
652 Rooth JE, Stevenson JC, Cornwell JC (2003) Increased sediment accretion rates following
653 invasion by *Phragmites australis*: The role of litter. Estuaries 26(2B):475-483
654
655 Rothman E, Bouchard V (2007) Regulation of carbon processes by macrophyte species in a
656 Great Lakes coastal wetland. Wetlands 27(4):1134-1143
657
658 Ruiz-Rueda O, Hallin S, Baneras L (2009) Structure and function of denitrifying bacterial
659 communities in relation to the plant species in a constructed wetland. FEMS Microbiology
660 Ecology 67(2):308-319

661

662 Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites*
663 *australis*, into North America. Proceedings of the National Academy of Sciences USA 99:2445-
664 2449

665

666 Saltonstall K (2016) The naming of *Phragmites* haplotypes. Biological Invasions 18:2433-2441

667

668 Saltonstall K, Hauber D (2007) Notes on *Phragmites australis* (Poaceae: Arundinoideae) in
669 North America Journal of Botanical Research Institute of Texas 1:385-388

670

671 Saltonstall K, Lambert A, Meyerson LA (2010) Genetics and reproduction of common and giant
672 reed. Invasive Plant Science and Management 3:495-505

673

674 Saltonstall K, Peterson PM, Soreng RJ (2004) Recognition of *Phragmites australis* subsp.
675 *americanus* (Poaceae: Arundinoideae) in North America: Evidence from morphological and
676 genetic analysis. SIDA 21(2):683-692

677

678 Saltonstall K, Stevenson JC (2007) The effect of nutrients on seedling growth of native and
679 introduced *Phragmites australis*. Aquatic Botany 86:331-336

680

681 Theuerkauf SJ, Puckett BJ, Theuerkauf KW, Theuerkauf EJ, Eggleston DB (2017) Density-
682 dependent role of an invasive marsh grass, Phragmites australis, on ecosystem service provision.
683 PLoS One 12(2): e0173007. doi: 10.1371/journal.pone.0173007
684
685 Throbäck IN, Enwall K, Jarvis S, Hallin S (2004) Reassessing PCR primers targeting *nirS*, *nirK*
686 and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS
687 Microbiology Ecology 49:401–417
688
689 Tulbure MG, Johnston KA (2010) Environmental conditions promoting non-native *Phragmites*
690 australis expansion in Great Lakes Coastal Wetlands. Wetlands 30:577-587
691
692 Vretare V, Weisner SEB, Strand JA, Granéli W (2001) Phenotypic plasticity in *Phragmites*
693 *australis* as a functional response to water depth. Aquatic Botany 69:127-145
694
695 Wang WQ, Sardans J, Wang C, Zeng CS, Tong C, Asensio D, Peñuelas J (2015) Ecological
696 stoichiometry of C, N, and P of invasive *Phragmites australis* and native *Cyperus malaccensis*
697 species in the Minjiang River tidal estuarine wetlands of China. Plant Ecology 216(6): 809-822
698
699 Welch BA, Davis CB, Gates RJ (2006) Dominant environmental factors in wetland plant
700 communities invaded by *Phragmites australis* in East Harbor, Ohio, USA. Wetlands Ecology
701 and Management 14(6):511-525
702

703 Wetzel RG, Likens GE (1991) Limnological analyses. Springer-Verlag, New York
704
705 Windham L (2001) Comparison of biomass production and decomposition between *Phragmites*
706 *australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New
707 Jersey, USA. Wetlands 21(2):179-188
708
709 Windham L, Ehrenfeld JG (2003) Net impact of a plant invasion on nitrogen-cycling processes
710 within a brackish tidal marsh. Ecological Applications 13(4):883-897
711
712 Windham L, Lathrop RG (1999) Effects of *Phragmites australis* (common reed) invasion on
713 aboveground biomass and soil properties in brackish tidal marsh of the Mullica River, New
714 Jersey. Estuaries 22(4):927-935
715
716 Windham L, Meyerson LA (2003) Effects of common reed (*Phragmites australis)* expansions on
717 nitrogen dynamics of tidal marshes of the Northern US. Estuaries 26(2B):452-464
718
719 Yarwood SA, Baldwin AH, Gonzalez Mateu M, Buyer JS (2016) Archaeal rhizosphere
720 communities differ between the native and invasive lineages of the wetland plant *Phragmites*
721 *australis* (common reed) in a Chesapeake Bay subestuary. Biological Invasions 18:2717-2728
722
723 Zedler JB (2003) Wetlands at your service: Reducing impacts of agriculture at the watershed
724 scale. Frontiers in Ecology and the Environment 1(2):65-72

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-536

List of Figures

743 **Table 1.** Summary of studies that examine the effect of exotic *P. australis* (haplotype M) on several
744 ecosystem attributes. A plus (+) indicates that there was an increase in the ecosystem attribute for a site
745 with exotic *Phragmites* (putative; not necessarily genetically identified) when compared to a site without
746 exotic *Phragmites* (i.e., with vegetation other than exotic *Phragmites*), a minus (-) indicates a decrease in
747 the ecosystem attribute, and an equal sign (=) indicates there was no difference between the two sites.
748 **Bolded entries** designate studies that compared genetically identified *Phragmites*. A plus (+) indicates
749 that there was an increase in the ecosystem attribute for a site with exotic (haplotype M) *Phragmites*
750 relative to the native *Phragmites* subspecies *americanus*, a minus (-) indicates a decrease in the ecosystem
751 attribute, and an equal sign (=) indicates there was no difference between the exotic and native strains.

Variable	Trend	Citation
Plant biomass	+	Alldred et al. 2016, **Mozdzer et al. 2013, Mozdzer and Megonigal 2012, Holdredge et al. 2010, Kulmatiski et al. 2010,** Rothman and Bouchard 2007, **Saltonstall and Stevenson 2007, League et al. 2006,** Ehrenfeld 2003, Windham 2001, Meyerson et al. 2000a,b, Windham and Lathrop 1999
Soil Organic Matter (SOM)	=	Ehrenfeld 2003
	+	Rooth et al. 2003, Nijburg and Laanbroek 1997
Decomposition rate	+	Duke 2015, Mozdzer et al. 2016
	–	Rothman and Bouchard 2007, Windham 2001
	+ or –	Ehrenfeld 2003
	– or =	Liao et al. 2008
Biomass [N]	+	Alldred et al. 2016, Wang et al. 2015, **Mozdzer and Zieman 2010, Packett and Chambers 2006,** Windham and Meyerson 2003, Meyerson et al. 2000a
	+ or –	Ehrenfeld 2003, Windham and Ehrenfeld 2003
	+ or =	**Rodríguez and Brisson 2015 (− for Biomass [P])**
Total soil N	=	Ehrenfeld 2003
	+	**Yarwood et al. 2016**, Nijburg and Laanbroek 1997

Extractable inorganic N (ammonium, nitrate)	– or =	Ehrenfeld 2003, Meyerson et al. 2000a
	=	Tulbure and Johnston 2010
	–	**Price et al. 2014 (both NH_4 and NO_x)**
Mineralization and nitrification	+	Ruiz-Rueda et al. 2009, Ehrenfeld 2003, Windham and Ehrenfeld 2003, Meyerson et al. 2000a
	+ or =	Windham and Meyerson 2003
Denitrification	+	Alldred et al. 2016, Ruiz-Rueda et al. 2009
	+ or =	Ehrenfeld 2003, Windham and Ehrenfeld 2003, Windham and Meyerson 2003
	=	Meyerson et al. 2000a
Phosphate	=	**Price et al. 2014**, Tulbure and Johnston 2010

752

Fig. 1

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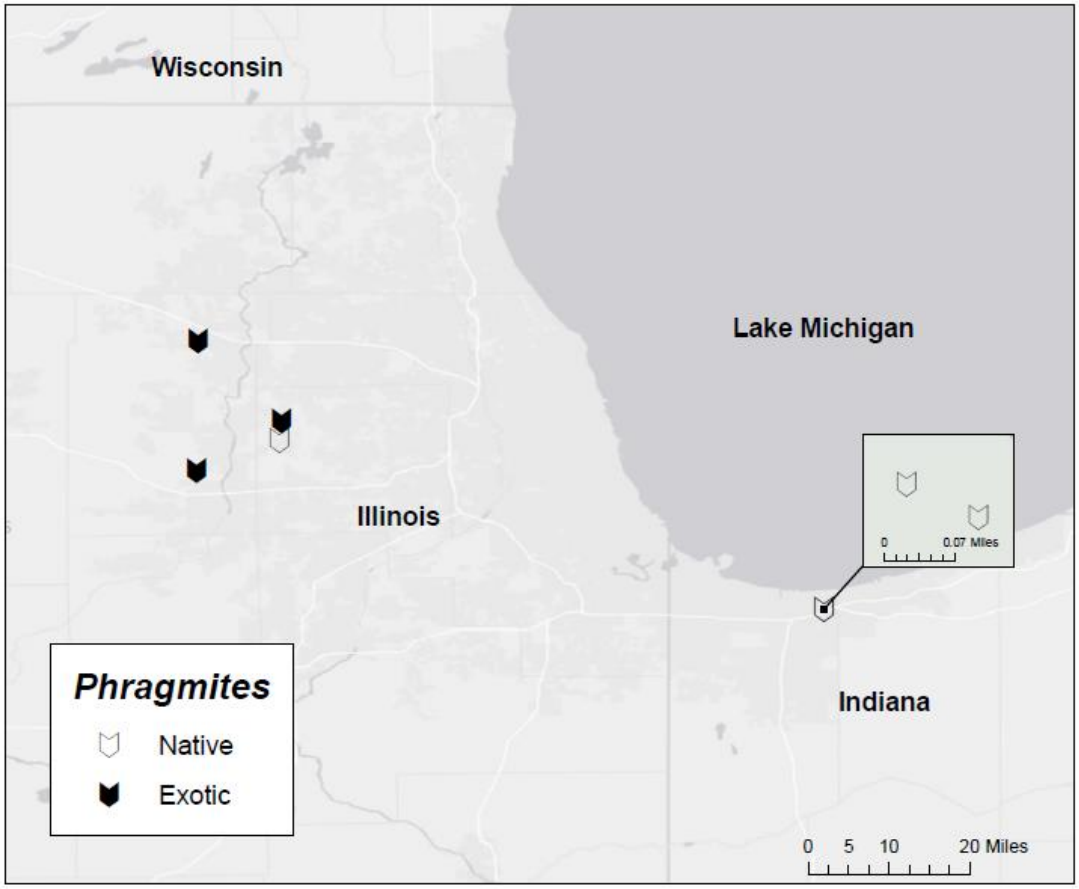
Wisconsin
Lake Michigan
Illinois
Indiana
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Phragmites
Native
Exotic
0 5 10 20 Miles

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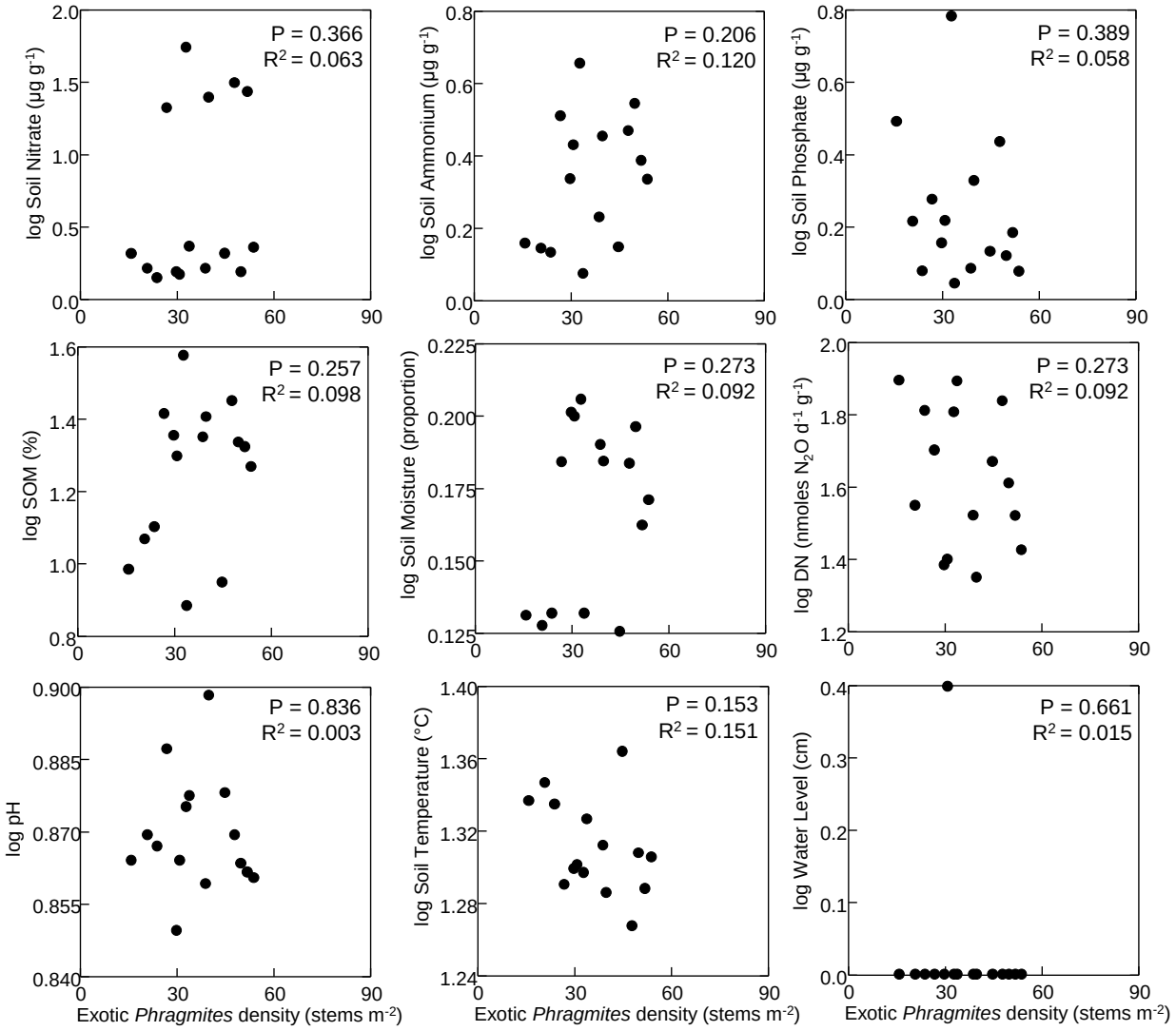
755 Fig. 2



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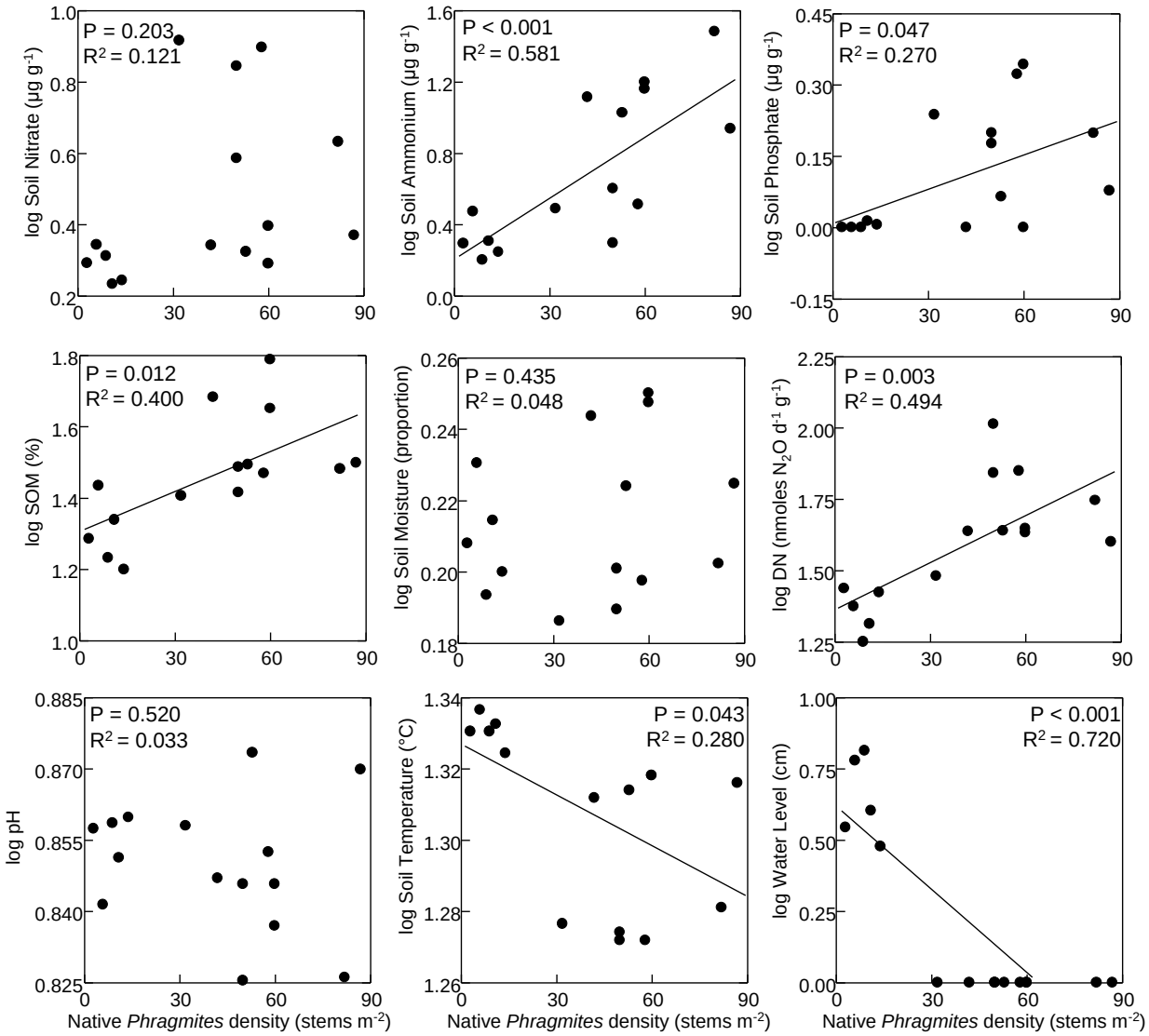
760 Fig. 3



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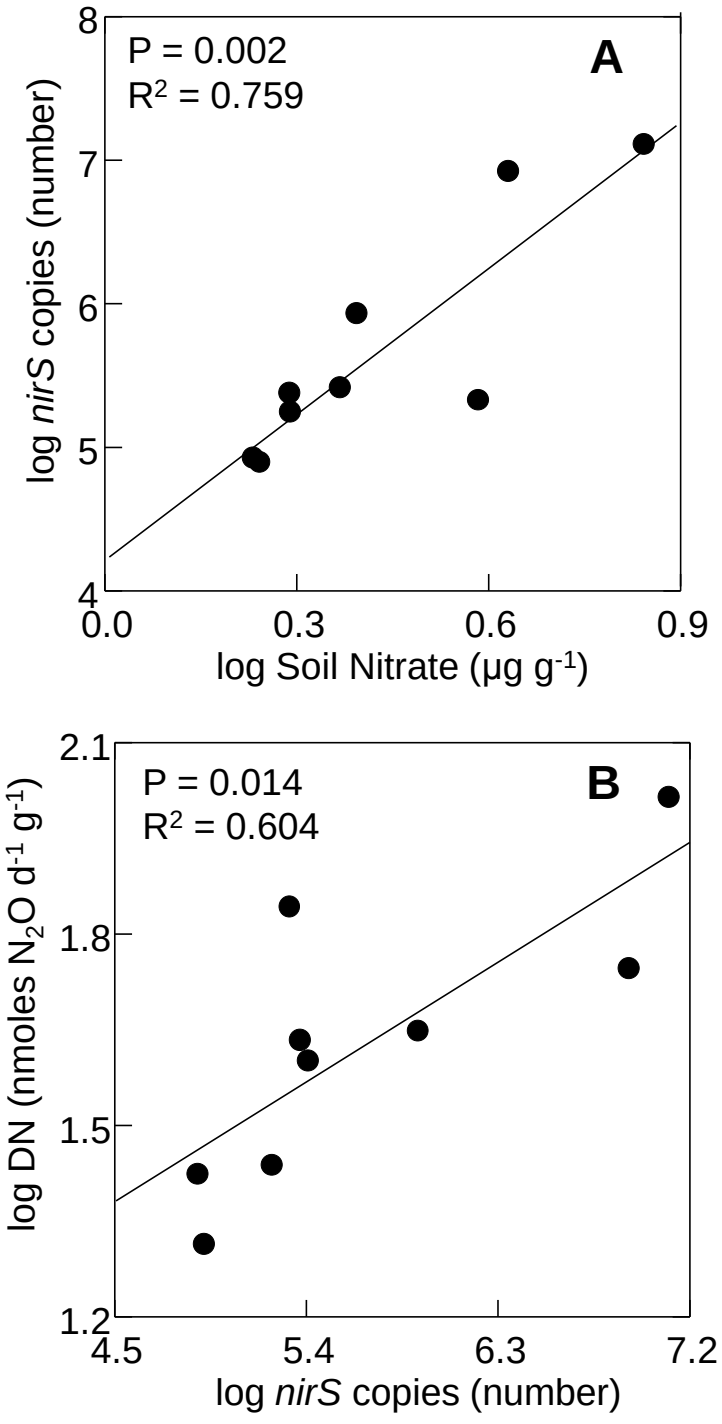
764 Fig. 4



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766 Fig. 5

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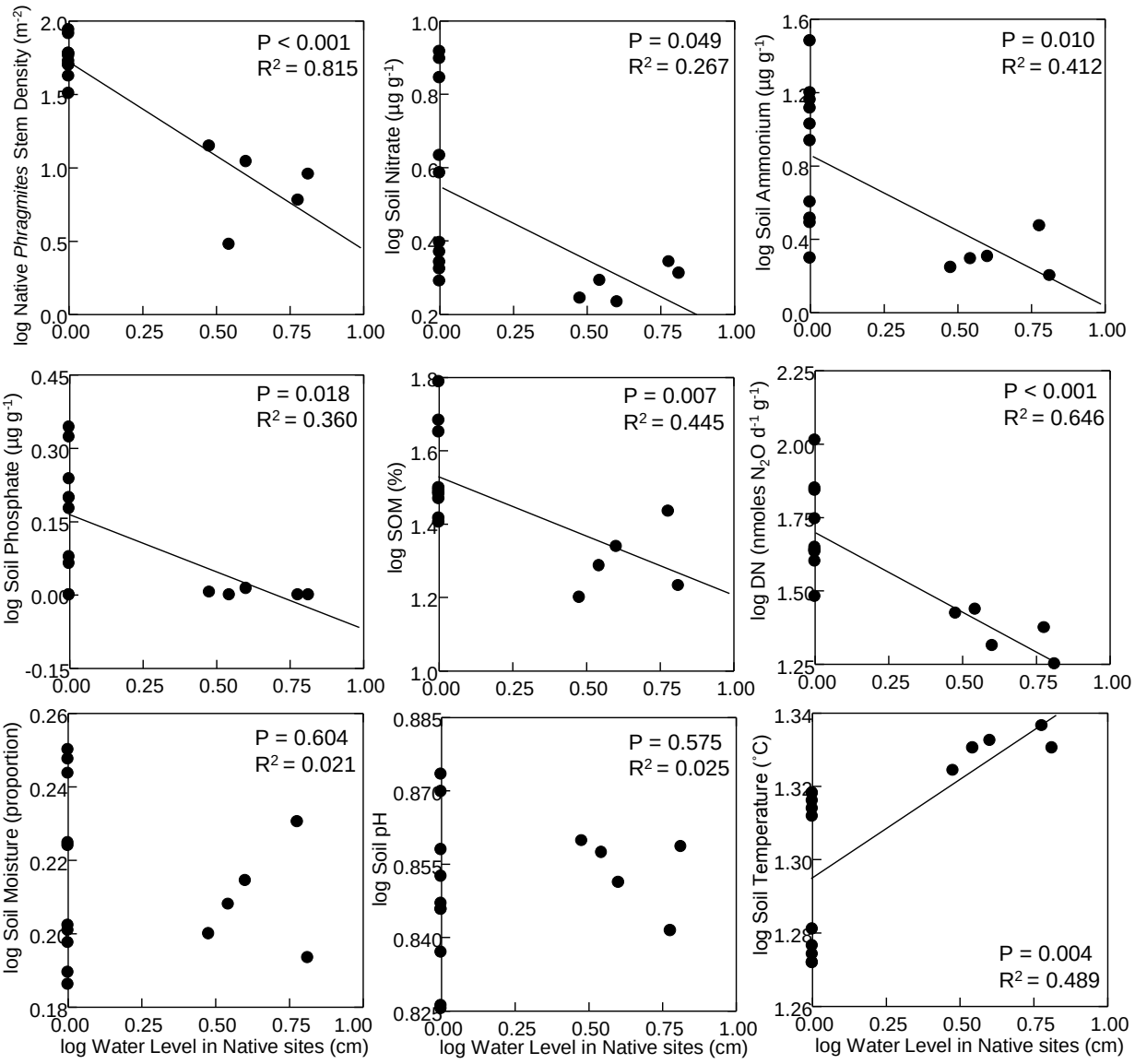

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