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

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

The available research addressing the impact of exotic *Phragmites* on ecosystem properties suggests that its presence unequivocally contributes to an increase in plant biomass and productivity because exotic *Phragmites* produces more shoots, has a higher growth rate, generally grows taller, and produces more biomass than the native strain (Table 1; Lelong et al. 2007; Jodoin et al. 2008; Mozdzer et al. 2013). Although more *Phragmites* biomass usually correlates with higher soil organic matter (SOM) which could support denitrifying soil microbes and thus enhance denitrification, some studies reported no difference in SOM or denitrification between the exotic *Phragmites* strain and an area having vegetation other than exotic *Phragmites* (Table 1). More importantly, there are no studies to date that have compared SOM content and denitrification rates between the exotic and native *Phragmites* strains (i.e., there are no bolded citations on Table 1 under denitrification).

There are two contrasting scenarios that may explain the relationship between *Phragmites* and soil nutrient concentrations (nitrate (NO_3), ammonium (NH_4), and phosphate (PO_4)). Studies have indicated that exotic *Phragmites* has a higher nutrient demand compared to the native strain (Holdredge et al. 2010; Mozdzer and Zieman 2010; Mozdzer et al. 2013). In addition, if the exotic strain has higher plant biomass than the native strain (e.g., Table 1) and if nutrients are bound in those plant tissues (as reported in several studies for Biomass [N] in Table 1), then soil nutrient concentrations will be smaller under exotic *Phragmites* stands compared to those under native stands. In contrast, soil nutrient concentrations may be larger under exotic *Phragmites* stands if interactions with microbial communities enhance nutrient mineralization rates. This latter pattern of increased soil nutrients has been documented in studies of other invasive wetland plants such as exotic and hybrid *Typha* (Angeloni et al. 2006; Larkin et al. 2011; Geddes et al. 2014). However, our review of the literature shows only two studies (Price et al. 2014; Yarwood et al. 2016) directly compared soil nutrient concentrations between exotic and native *Phragmites* stands and their results showed variable results (i.e., larger, smaller, or equal 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 x 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 abundance ($P = 0.134$; data not shown). Specifically, we found positive relationships between *Phragmites* stem density and ammonium ($P < 0.001$), SOM ($P = 0.012$), phosphate ($P = 0.047$), and denitrification potential rates ($P = 0.003$), and negative relationships with temperature ($P = 0.043$) and water level ($P < 0.001$) (Fig. 3). Lastly, linear regression analysis using stem density of exotic and native *Phragmites* combined as the explanatory variable to address if stem density *per se*, irrespective of strain, was responsible for the observed patterns revealed significant correlations that matched those of the native *Phragmites* stem density alone, suggesting the native strain was the one that had the greatest influence over the significant results (data not shown).

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

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

DISCUSSION

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

In addition, water level showed strong correlations with many measured parameters in native *Phragmites* sites, including native *Phragmites* stem density, suggesting this abiotic driver may have mediated the responses we observed with stem density. However, we acknowledge that our measurements of water level were limited to single time points and to surface water. More sophisticated techniques such as wells, piezometers, and/or graduated staff gauges, as well as incorporation of groundwater level estimates, would have provided more detailed information on the hydrology of these sites. Furthermore, multiple measurements over an extended period of time (hydrographs or time series) prior to our sampling date would have provided additional insight into the potential effects of hydrology on the biotic and abiotic variables measured in our study. Our surface water level measurement represents one time point that could potentially reflect conditions of only a couple of days before sampling, as opposed to more long-term water dynamics. Therefore, although several variables in our study show strong correlations with water 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 counterparts. A similar finding was documented by Price et al. (2014) for soil ammonium and nitrate, but not for phosphate. However, due to the correlational nature of this study, it is also likely that we observed higher native *Phragmites* stem density in areas where soil ammonium and phosphate concentrations were larger as these are important nutrients that limit plant growth and control stem density (e.g., Meyerson et al. 2000a; Welch, Davis, and Gates 2006; Saltonstall and Stevenson 2007; Engloner 2009; Eid et al. 2010). In contrast, water levels negatively correlated with all measured nutrients: nitrate, ammonium, and phosphate (Fig. 5), suggesting that increased water levels may have slowed microbial decomposition of organic matter and mineralization of inorganic nutrients due to decreased oxygen availability.

Although the exotic *Phragmites* strain has been considered a useful plant in remediation studies due to its ability to remove excess nutrients and improve water quality (e.g., Araki et al. 2005; Ruiz-Rueda et al. 2009; Rodríguez and Brisson 2015), results from our study suggest that it was the native strain that exhibited a positive correlation between *Phragmites* stem density and denitrification (Fig. 3). Rodríguez and Brisson's study (2015) and our study are the only two examples that we know of that show significant effects of the native strain on nutrient removal – phosphate in their study; nitrate through denitrification in ours– when compared to the exotic one, perhaps as a result of native stand stem densities being on the highest end of those reported in the literature. Yet it is important to exercise caution when interpreting these data as another explanation may involve the reverse pattern: if there are higher stem densities in areas with higher levels of soil nitrate, then denitrification rates may be higher due to higher soil nitrate concentrations, and not necessarily due to the higher native stem densities. However, we found 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 australis* 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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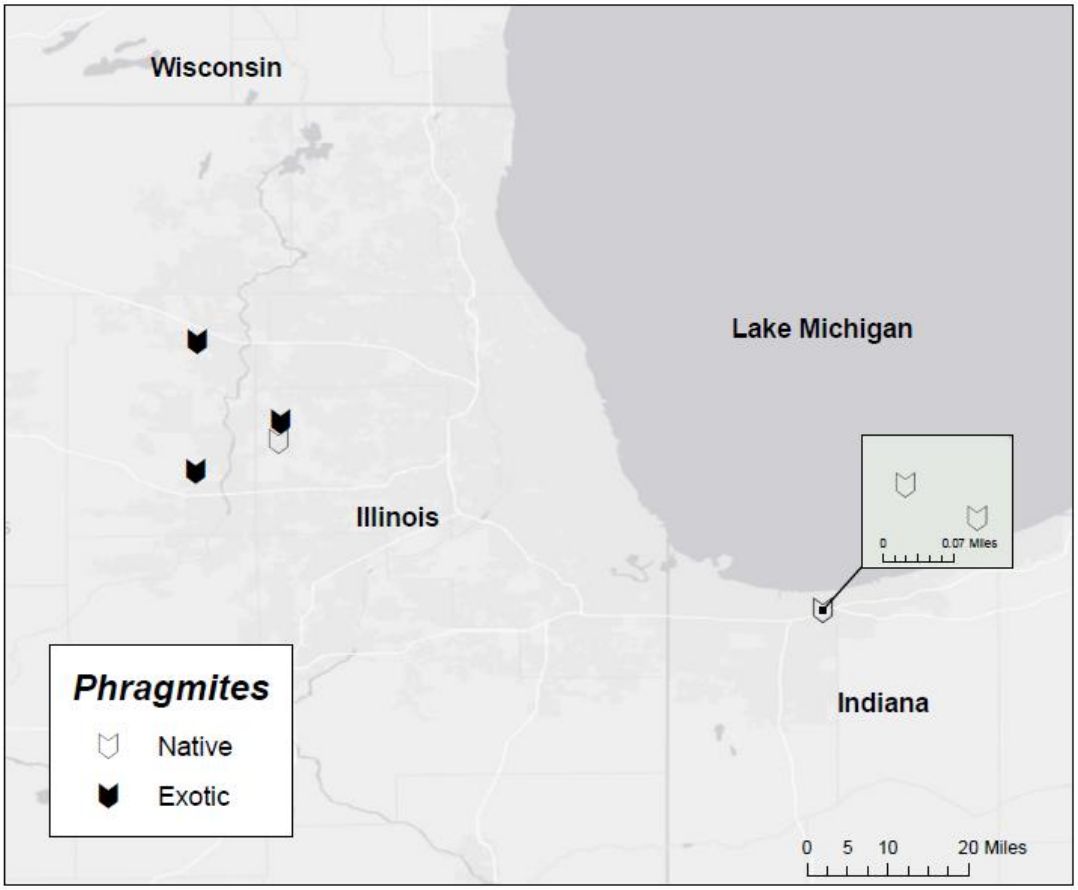
List of Figures

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

Fig. 1



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

Fig. 2

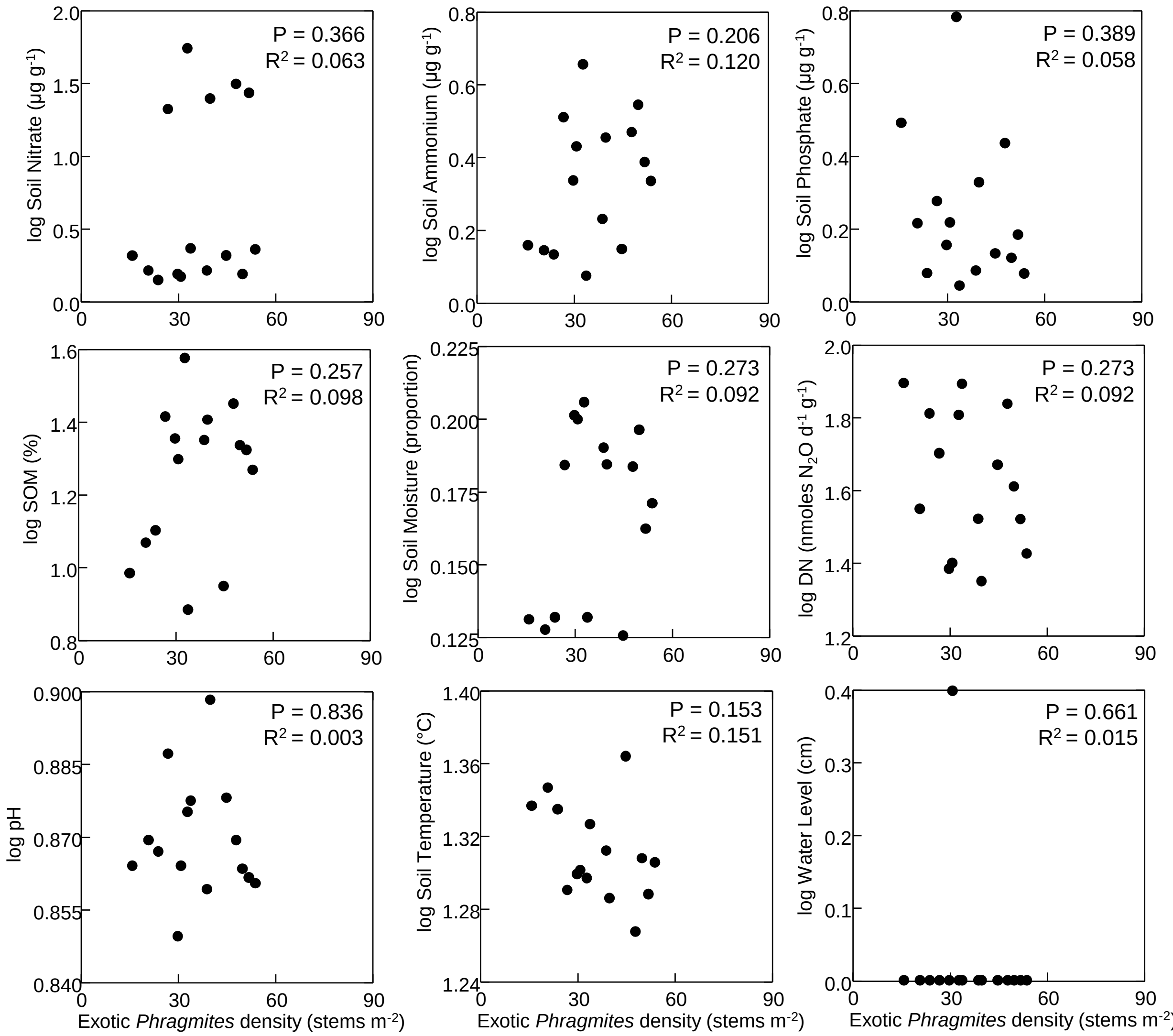
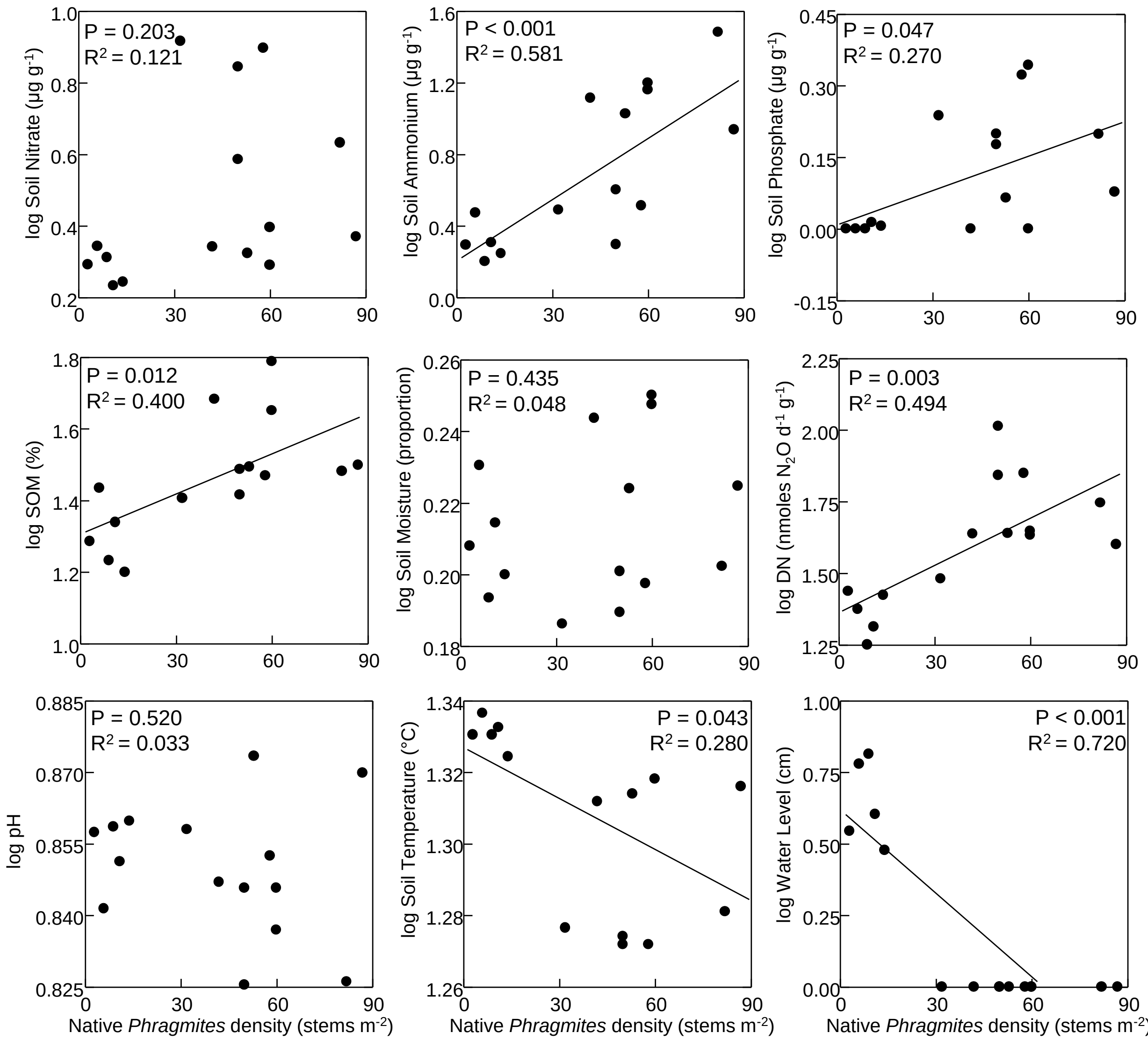
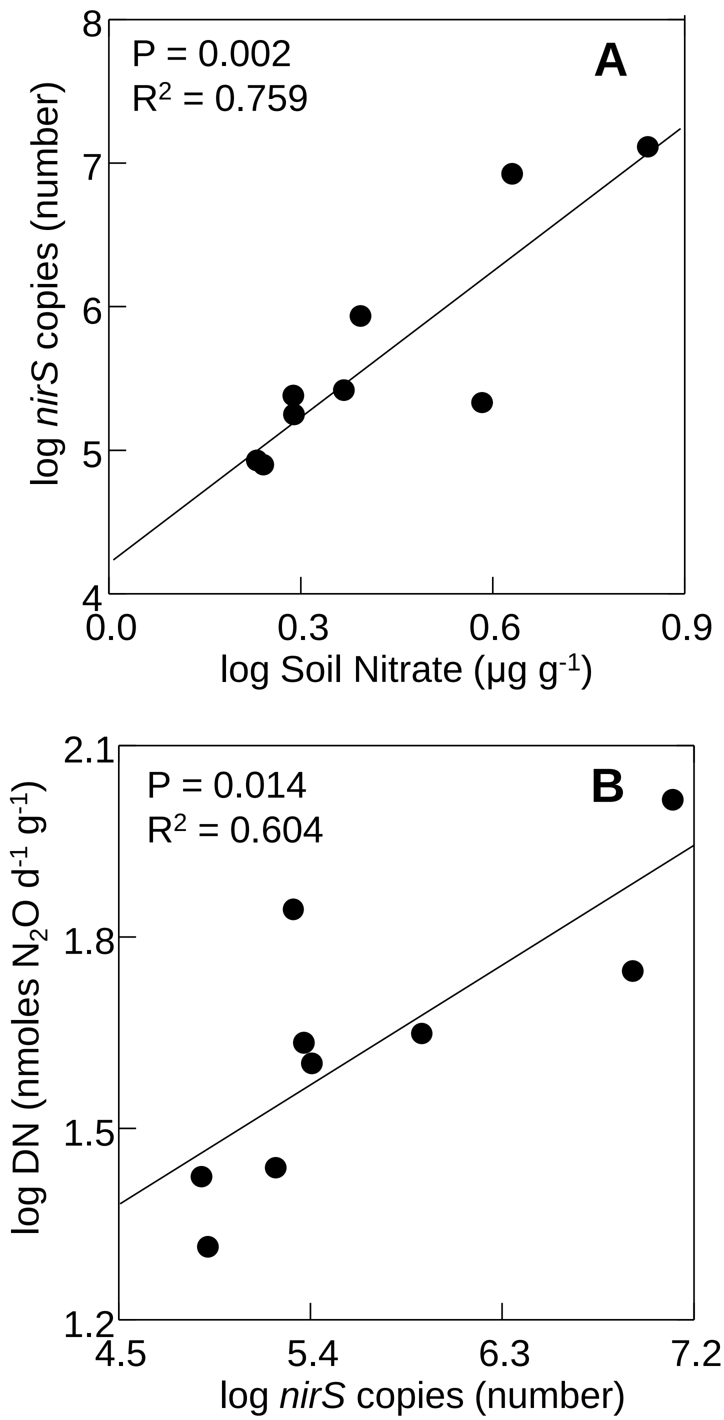

P = 0.366
R² = 0.063
log Soil Nitrate (µg g⁻¹)
2.0
1.5
1.0
0.5
0.0
P = 0.206
R² = 0.120
log Soil Ammonium (µg g⁻¹)
0.8
0.6
0.4
0.2
0.0
P = 0.389
R² = 0.058
log Soil Phosphate (µg g⁻¹)
0.8
0.6
0.4
0.2
0.0
P = 0.257
R² = 0.098
log SOM (%)
1.6
1.4
1.2
1.0
0.8
P = 0.273
R² = 0.092
log Soil Moisture (proportion)
0.225
0.200
0.175
0.150
0.125
P = 0.273
R² = 0.092
log DN (nmoles N₂O d⁻¹ g⁻¹)
2.0
1.8
1.6
1.4
1.2
P = 0.836
R² = 0.003
log pH
0.900
0.885
0.870
0.855
0.840
P = 0.153
R² = 0.151
log Soil Temperature (°C)
1.40
1.36
1.32
1.28
1.24
P = 0.661
R² = 0.015
log Water Level (cm)
0.4
0.3
0.2
0.1
0.0
0
30
60
90
Exotic Phragmites density (stems m⁻²)
Exotic Phragmites density (stems m⁻²)
Exotic Phragmites density (stems m⁻²)

Fig. 3



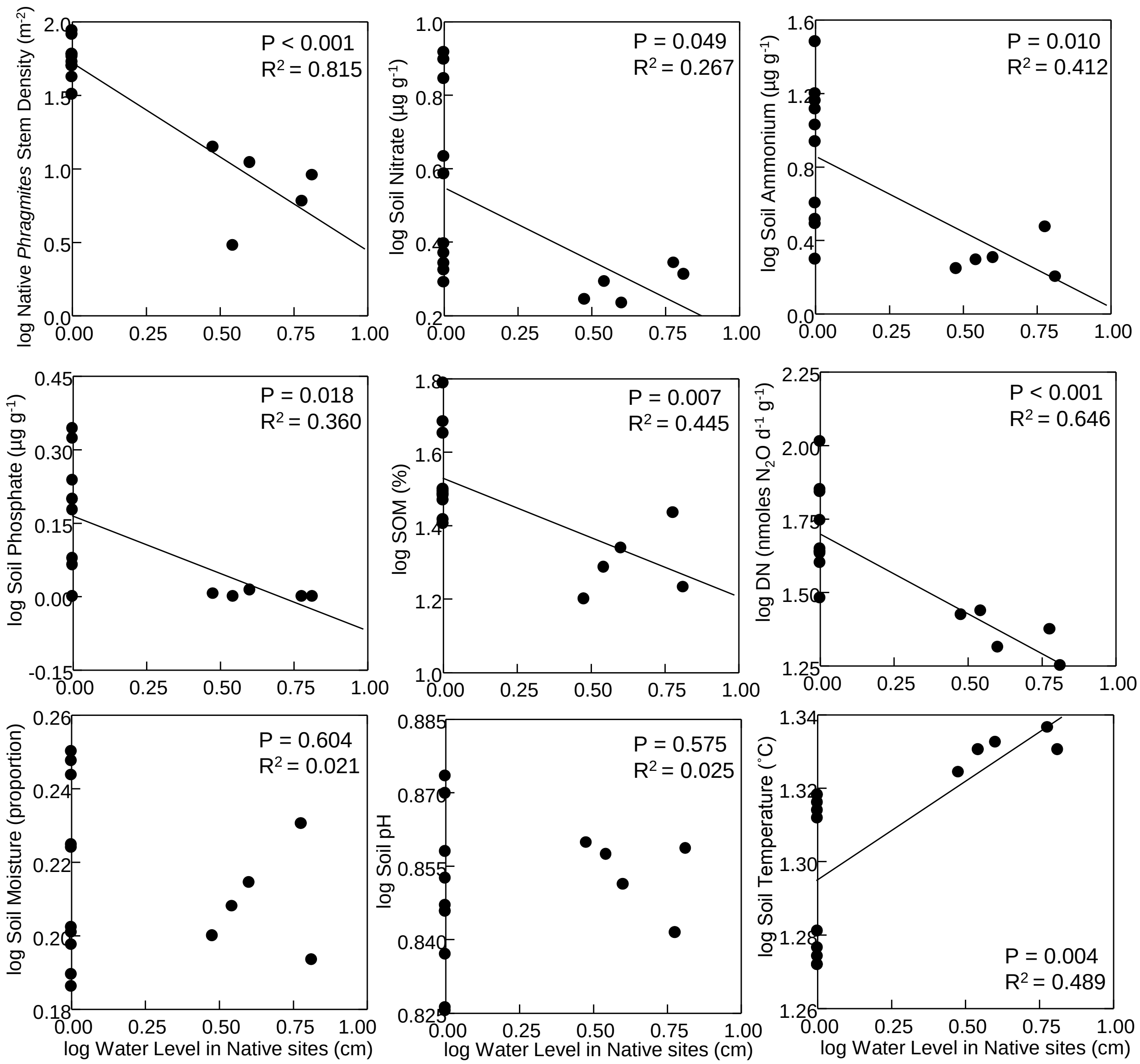
P = 0.203
R2 = 0.121
log Soil Nitrate (µg g-1)
P < 0.001
R2 = 0.581
log Soil Ammonium (µg g-1)
P = 0.047
R2 = 0.270
log Soil Phosphate (µg g-1)
P = 0.012
R2 = 0.400
log SOM (%)
P = 0.435
R2 = 0.048
log Soil Moisture (proportion)
P = 0.003
R2 = 0.494
log DN (nmoles N2O d-1 g-1)
P = 0.520
R2 = 0.033
log pH
P = 0.043
R2 = 0.280
log Soil Temperature (°C)
P < 0.001
R2 = 0.720
log Water Level (cm)
Native Phragmites density (stems m-2)

Fig. 4



P = 0.002
R2 = 0.759
A
log nirS copies (number)
8
7
6
5
4
0.0
0.3
0.6
0.9
log Soil Nitrate (µg g-1)
P = 0.014
R2 = 0.604
B
log DN (nmoles N2O d-1 g-1)
2.1
1.8
1.5
1.2
4.5
5.4
6.3
7.2
log nirS copies (number)

Fig. 5


log Native *Phragmites* Stem Density (m^{-2})
P < 0.001
R^2 = 0.815
log Soil Nitrate (µg g^{-1})
P = 0.049
R^2 = 0.267
log Soil Ammonium (µg g^{-1})
P = 0.010
R^2 = 0.412
log Soil Phosphate (µg g^{-1})
P = 0.018
R^2 = 0.360
log SOM (%)
P = 0.007
R^2 = 0.445
log DN (nmoles N_2O d^{-1} g^{-1})
P < 0.001
R^2 = 0.646
log Soil Moisture (proportion)
P = 0.604
R^2 = 0.021
log Soil pH
P = 0.575
R^2 = 0.025
log Soil Temperature (˚C)
P = 0.004
R^2 = 0.489
log Water Level in Native sites (cm)
log Water Level in Native sites (cm)
log Water Level in Native sites (cm)