



6-2018

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Recommended Citation

Kelly, John; Volesky, L. A.; Iqbal, S.; and Geddes, P. Relationships of Native and Exotic Strains of *Phragmites Australis* to Wetland Ecosystem Properties. *Wetlands*, 38, 3: 577-589, 2018. Retrieved from Loyola eCommons, Biology: Faculty Publications and Other Works, <http://dx.doi.org/10.1007/s13157-018-1001-1>

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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

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

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

33

34 INTRODUCTION

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

44 Wetland degradation and loss because of urbanization and agriculture are important
45 factors that have led to major losses of wetland area worldwide and to the disruption of wetland
46 structure and function (Ehrenfeld 2000, Zedler 2003). Specifically, the invasion of wetlands by
47 exotic plant species can affect ecosystem properties and their ability to perform ecosystem
48 services such as denitrification positively, neutrally, or negatively (Theuerkauf et al. 2017).
49 Several studies have shown that invasive plants can diminish denitrification potential rates (e.g.,
50 Evans et al. 2001, Dassonville et al. 2011, Carey et al. 2017) or enhance them (e.g., Ehrenfeld
51 2003, Zedler 2003, Lishawa et al. 2014). A few studies, however, have documented no change in
52 denitrification potential when comparing soils under exotic and native plant stands (e.g.,
53 Ehrenfeld 2003).

54 Exotic *Phragmites australis* is one of the four introduced species of concern (which also
55 include *Lythrum salicaria*, *Typha x glauca*, and *Phalaris arundinacea*) that have spread
56 throughout North American temperate wetlands (Galatowitsch et al. 1999). Historically, native
57 *Phragmites* was found in North America within heterogeneous plant communities in coastal and
58 inland marshes (Meyerson et al. 2009). By the 1970s, however, the presence of extensive
59 *Phragmites* stands in all lower 48 US states led to the suspicion that an exotic strain of
60 *Phragmites* might be responsible for this expansive spread (Meyerson et al. 2009). By 2007,
61 three genetic lineages of *Phragmites* were identified in North America based on genetic
62 sequence data: 1) *P. australis* subspecies *americanus* (native *Phragmites* hereafter), 2) *P.*
63 *australis* subspecies *berlandieri* (Gulf Coast *Phragmites* hereafter), and 3) *P. australis* haplotype
64 M (exotic *Phragmites* hereafter) (Saltonstall 2002; Saltonstall et al. 2004; Saltonstall and Hauber
65 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 (NO₃), ammonium (NH₄), and phosphate
95 (PO₄)). 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).

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

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

130 have all been shown to control *Phragmites* stem attributes such as density, height, diameter, and
131 biomass (e.g., Chambers, Meyerson and Saltonstall 1999; Meyerson et al. 2000a; Vretare et al.
132 2001; Chambers et al. 2003; Welch, Davis and Gates 2006; Saltonstall and Stevenson 2007; Eid
133 et al. 2010), where stem attributes correlate positively with increased fertility and negatively with
134 increased salinity (Englone 2009). Responses of *Phragmites* stem attributes to hydrological
135 variation such as water depth or flooding frequency yielded more ambiguous results in previous
136 studies (Englone 2009). Similarly to other correlational studies involving invasive species,
137 assigning causality can be difficult (e.g., Geddes et al. 2014; Price et al. 2014). Nevertheless,
138 correlational studies such as ours will enable the development of specific hypotheses regarding
139 the effects of exotic and native *Phragmites* on ecosystem properties that can be tested via
140 controlled manipulative experiments.

141 **Materials and Methods**

142 We measured environmental attributes, soil nutrient concentrations, denitrification, and
143 denitrifier abundance during the summer of 2011 in three sites dominated by native *Phragmites*
144 and in three sites dominated by exotic *Phragmites*; all stands had at least 95% *Phragmites* cover.
145 Study sites were located in DuPage and Kane Counties in Illinois, and Lake County in Indiana
146 (Fig. 1). The exotic stands were located at Dick Young Forest Preserve, Burnidge Forest
147 Preserve, and Pratts Wayne Woods Forest Preserve, and the native stands were located at
148 Calumet Prairie (2 sites) and West Chicago Prairie (Fig. 1). Stands were identified as native or
149 exotic using genetic analysis (Price et al. 2014) following the methodology of Saltonstall et al.
150 (2004).

151 We collected samples from Illinois sites on July 26, 2011 and from Indiana sites on July
152 27, 2011. All variables were measured at 5 randomly selected plots in each of the 6 sites, for a
153 total of 30 plots. Plots were spaced at 5-7 m intervals beginning 10 meters from the stand edge.
154 At each plot, we measured several variables *in situ* (see below) and we took a soil core (~6-8 cm
155 in diameter, ~10-14 cm deep) using a serrated knife to cut through the roots and two trowels to
156 extract the core, placed it in a Ziploc bag, and immediately stored it on ice. Soil cores were
157 placed in a refrigerator until analysis.

158

159 ***Phragmites* Density and Environmental Attributes**

160 *Phragmites* stem density was quantified by counting only new, green *Phragmites* aerial
161 stems using a 1 m x 0.5 m quadrat (total area sampled = 0.5 m²). Brown, senesced stems from
162 the previous season(s) were not included in the counts. Soil temperature was taken using a Fisher
163 Scientific Traceable Lollipop Waterproof/Shockproof Thermometer by inserting it 10 cm into
164 the soil. Depth of standing water was measured with a meter stick. Soil pH was determined in the
165 lab by mixing 15 g of soil with 30 mL DI water. The slurry was stirred and allowed to stand for
166 30 minutes for CO₂ equilibration after which pH was read with an ORION model 310 pH meter
167 (Robertson et al. 1999). Soil moisture was calculated as the difference between dry and wet mass
168 of 10 g of wet soil sample that had been weighed and dried to constant weight in a drying oven at
169 105°C.

170 **Nutrient Concentrations: Carbon, Nitrogen, Phosphorus**

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

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

183 Soil ammonium was measured using the phenol-hypochlorite method (Wetzel and Likens
184 1991), in KCl-extracted samples. Absorbance was recorded using a Shimadzu UV-Vis
185 spectrophotometer at 630 nm in 1 cm quartz cuvettes. Nitrate was measured in KCl-extracted
186 samples following the cadmium-reduction method on a Seal Analytical AQ2+ Discrete Auto-
187 analyzer. Soil orthophosphate was determined using the ascorbic acid method (Wetzel and
188 Likens 1991), using Troug's solution as the extractant (Mehlich 1953). Absorbance was recorded
189 using a Shimadzu UV-Vis spectrophotometer at 885 nm in 1 cm quartz cuvettes.

190 **Denitrification Potential**

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

199 The principle behind the acetylene inhibition technique is based on the fact that N₂O
200 reductase, the enzyme used by denitrifying bacteria in the last step of the denitrification pathway
201 to convert nitrous oxide to nitrogen gas, is inhibited by acetylene. Thus, this inhibition allows a
202 measurement of nitrous oxide concentration as a proxy for how much denitrification is possible
203 by the soil microbes under controlled lab conditions. The differences in nitrous oxide produced
204 were then used to compare the ability of soils to perform denitrification under native and exotic
205 stands of *Phragmites*.

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

212 vials. Gas samples were quantified for nitrous oxide using a Shimadzu gas chromatograph (GC-
213 2014) equipped with an Electron Capture Detector (ECD) and a HayeSep Q stainless steel
214 column. Ultrapure nitrogen was the carrier gas, and the detector, oven, and injector temperatures
215 were set at 300 °C, 40 °C and 60 °C, respectively.

216 **Molecular Analyses of Soil Denitrifier Communities**

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

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

233 followed the approach described by Geets et al. (2007) except that the annealing temperature was
234 changed to 57 °C and the extension temperature was changed to 72 °C. All qPCR experiments
235 were run using an MJ Research DNA Engine Opticon1 thermal cycler equipped with Opticon
236 Monitor software version 3.1 (Biorad, Hercules, CA). Conditions for all qPCR reactions were as
237 follows: 12.5 µl QuantiTect SYBR Green PCR Master Mix (Qiagen, Valencia, CA), 0.5 µM final
238 concentration of each primer, 5 µl template, and water were added to a final 25 µl volume. qPCR
239 was carried out using primers cd3AF (GTSAACGTSAAGGARACSSG) and R3cd
240 (GASTTCGGRTGSGTCTTGA), which produce a 425 base pair amplicon (Throbäck et al.
241 2004). All reactions were performed in low-profile 0.2 mL white strip tubes with optical ultra-
242 clear strip caps (Bio-Rad). Thermal cycling was as follows: initial denaturation at 95 °C for 10
243 min, 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 57 °C for 1 min, extension
244 at 72 °C for 1 min, hold at 78 °C for 1 sec, and plate read. Finally, a melting curve was run from
245 50–95 °C with a read every 1 °C and a hold of 1 sec between reads. Specificity of qPCR
246 reactions was confirmed by melting curve analysis and agarose gel electrophoresis.

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

255 $\mu\text{g/mL}$ kanamycin, grown overnight at 37 °C, and PCR-screened for the presence of inserts of
256 appropriate size using M13F and M13R primers. Plasmids containing the appropriately sized
257 inserts were isolated using PureLink Quick Plasmid Miniprep Kit (Invitrogen). Plasmids were
258 digested with EcoRI (New England BioLabs) according to the manufacturer's instructions and
259 the digestion reaction was run on an agarose gel. The fragment containing *nirS* was cut out from
260 the gel and purified using QIAquick Gel Extraction Kit (Qiagen). The concentration of this *nirS*-
261 containing fragment was determined by Quant-iT DNA Assay Kit (Invitrogen). Standard curves
262 for qPCR reactions were generated using a 10-fold dilution series ranging from 1.37×10^6 to 137
263 copies of *nirS*. *nirS* copy numbers were normalized based on grams of soil.

264 **Data Analysis**

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

274 **RESULTS**

275 Contrary to our expectations, there were no statistically significant differences in any of
276 the measured variables between the exotic *Phragmites* and native *Phragmites* sites, with the
277 exception of pH. Soils associated with the exotic strain had higher soil pH (7.41) than that
278 associated with the native strain (7.08) ($P = 0.048$). There was also no significant difference in
279 *Phragmites* stem density between exotic and native *Phragmites* sites ($P = 0.787$). Exotic
280 *Phragmites* sites had a stem density mean of 36.26 stems m^{-2} (± 11.8 SD) whereas the native
281 sites had a mean of 41.13 stems m^{-2} (± 27.41 SD). Although not significant, the sites dominated
282 by native *Phragmites* had slightly higher stem numbers than sites dominated by their exotic
283 counterpart, a result that contradicted our expectations. Additionally, there was large variability
284 in *Phragmites* stem densities across sites, and this variability was much greater in native
285 *Phragmites* sites (range: 8.6-60.4 stems m^{-2}) than in the exotic *Phragmites* sites (range: 28-40.8
286 stems m^{-2}). Similarly to stem density, surface water levels had greater variability in native
287 *Phragmites* sites (range: 0-5 cm, SD = 1.94) than in exotic *Phragmites* sites (range: 0-1.5 cm, SD
288 = 0.39).

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

294 In contrast, native *Phragmites* stem density showed significant correlations with all
295 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

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

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

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

364 We found negative correlations between native *Phragmites* stem density and water level
365 and soil temperature. High native *Phragmites* stem densities may have correlated with low water
366 levels because native *Phragmites* is presumably less tolerant of standing water than exotic
367 *Phragmites* (Meyerson et al. 2009; Price et al. 2014) and therefore selectively invades areas with
368 lower water levels. Alternatively, native *Phragmites* could be responsible for more efficient
369 water uptake than its exotic counterpart and/or enhanced evapotranspiration rates, keeping water
370 levels low. The negative correlation between native *Phragmites* stem density and soil
371 temperature was likely due to the height and leaf surface area that *Phragmites* can achieve
372 (Meyerson et al. 2009; Saltonstall et al. 2010; Mozdzer and Zieman 2010; Hirtreiter and Potts
373 2012; Price et al. 2014). In denser native *Phragmites* stands, shading of the understory could
374 have resulted in lower soil temperatures. A similar phenomenon was observed in exotic
375 *Phragmites* stands in other studies, where standing water temperatures decreased due to the
376 shading from the plant canopy (Rogalski and Skelley 2012) or from accumulated litter
377 (Holdredge and Bertness 2011). However, we found a positive correlation between standing
378 water levels and soil temperature (Fig. 5). Although we observed no significant correlations
379 between native or exotic *Phragmites* stem density or water level and pH, the significant
380 difference we found in pH when comparing native and exotic stands may imply that 1) there may
381 be a systematic preference of the exotic strain for alkaline soils, 2) the exotic strain has not been

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

384 Native *Phragmites* density also correlated positively with soil organic matter. Given
385 *Phragmites*' ability to produce high amounts of biomass, dead plant matter can accumulate
386 rapidly, decreasing light availability (Holdredge and Bertness 2011; Hirtreiter and Potts 2012)
387 and eventually decomposing into soil organic matter. As expected, SOM negatively correlated
388 with water level, as decomposition of organic matter depends on an oxic environment. It has
389 been documented that *Phragmites* accumulates so much SOM that it tends to terrestrialize the
390 wetland ecosystems that it invades (Chambers et al. 1999; Windham 2001; Rooth et al. 2003;
391 Meyerson et al. 2009), even changing habitat characteristics for fauna (Derr 2008; Meyerson et
392 al. 2010). This trend of increased SOM has also been documented in other exotic species such as
393 *Typha x glauca* (Angeloni et al. 2006; Larkin et al. 2011; Mitchell et al. 2011; Geddes et al.
394 2014). The positive correlation between increasing SOM and increasing native *Phragmites* stem
395 density found in this study corroborates these latter claims and points to effects of the native
396 strain being similar to or even greater than those of the exotic strain, at least in our study sites.
397 Because SOM has not been reported to be an important determinant of stem density in previous
398 research (e.g., Engloner 2009), we believe native *Phragmites* density was likely a driver for
399 SOM production.

400 Our finding that native *Phragmites* stem density had a positive correlation with soil
401 ammonium and phosphate concentrations may provide support for the claim that native plant
402 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

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

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

443 **CONCLUSION**

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

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

460

461 **ACKNOWLEDGMENTS**

462 We would like to thank our collaborators at the Chicago Botanic Garden, Dan Larkin, Jeremie
463 Fant, Amy Price, and Paul Hartzog, for genetically identifying *Phragmites* and allowing us to
464 conduct nitrate analyses. In addition, we would like to acknowledge the support of Kane,
465 DuPage, and Lake (IN) County Forest Preserves, and IL Nature Preserves Commission for
466 permits and access to sites. We thank John Kasmer and Joel Olfelt for their editorial

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

471

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728

729 **List of Figures**

730 **Figure 1.** Map of sampling sites. GPS coordinates for the 6 sampling sites: Lake County, IN: N
731 41 35.400' - W 087 14.838' and N 41 35.428' - W 087 14.9'; DuPage County, IL: N 41 53.499' -
732 W 088 13.412' and N 41 55.632' - W 088 13.208'; and Kane County, IL: N 42 04.263' - W 088
733 22.210' and N 41 50.212' - W 088 22.354'.

734 **Figure 2.** Relationships between stem density of exotic *Phragmites* to environmental attributes,
735 soil nutrients, and denitrification.

736 **Figure 3.** Relationships between stem density of native *Phragmites* to environmental attributes,
737 soil nutrients, and denitrification.

738 **Figure 4.** Relationships between soil NO₃ and *nirS* copies (A) and between *nirS* copies and
739 denitrification (B) in sites containing native *Phragmites*.

740 **Figure 5.** Relationships between water level in native *Phragmites* sites to stem density,
741 environmental attributes, soil nutrients, and denitrification.

742

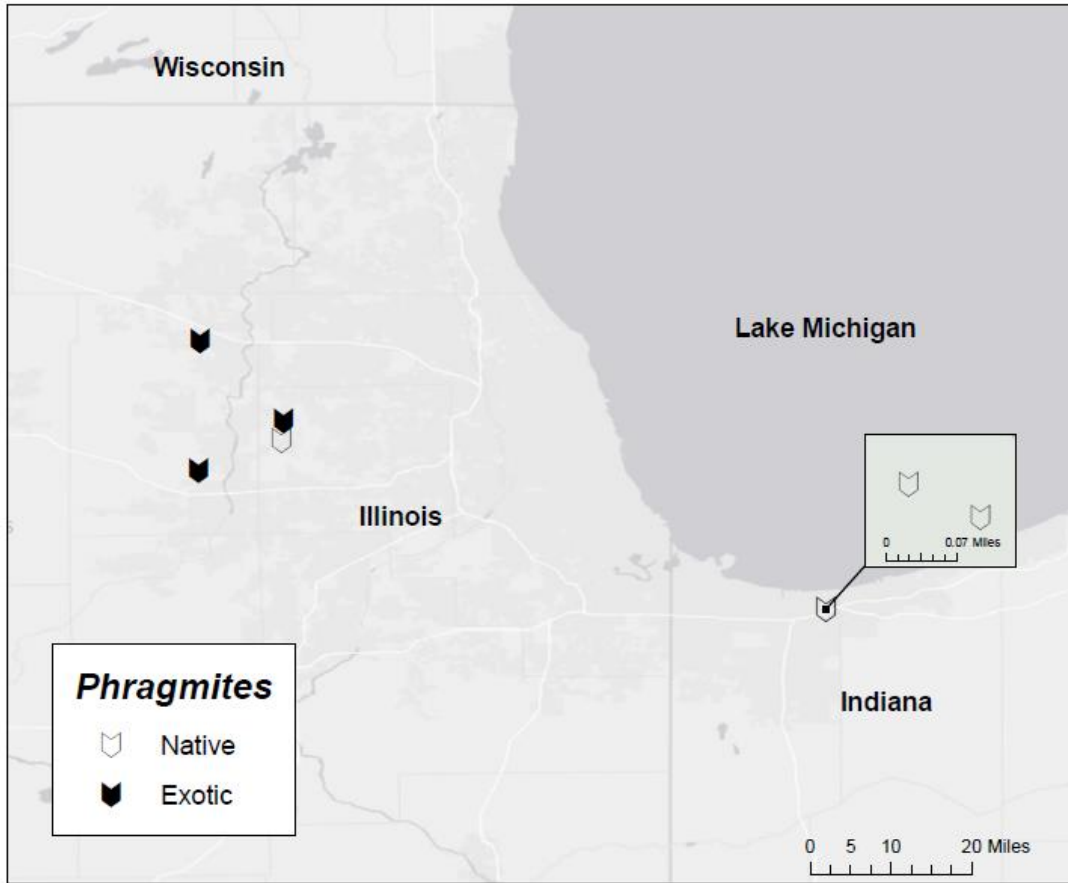
743 **Table 1.** Summary of studies that examine the effect of exotic *P. australis* (haplotype M) on several
744 ecosystem attributes. A plus (+) indicates that there was an increase in the ecosystem attribute for a site
745 with exotic *Phragmites* (putative; not necessarily genetically identified) 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	+	Allred 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]	+	Allred 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 NH₄ 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	+	Allred 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

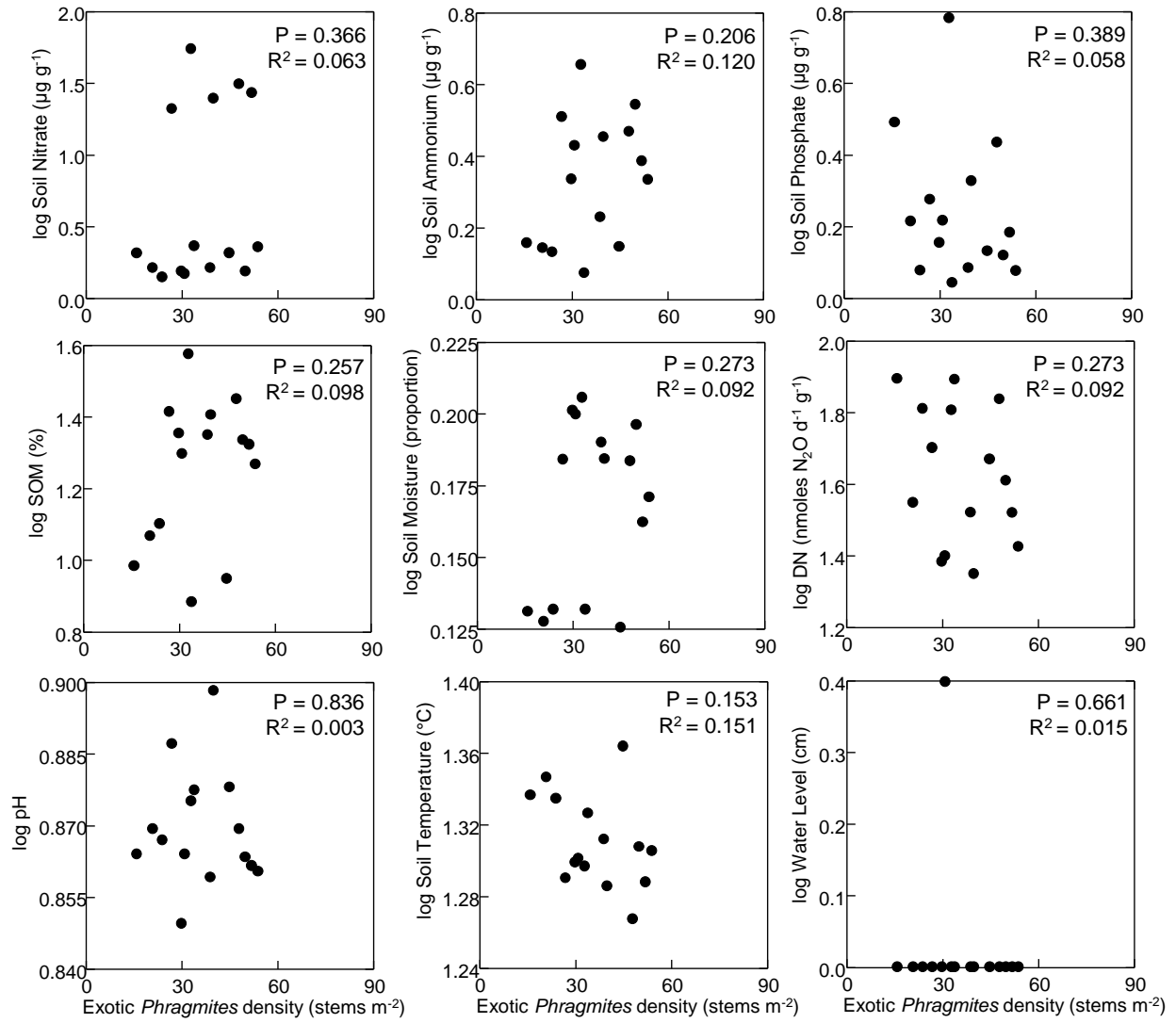
Fig. 1

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755 Fig. 2



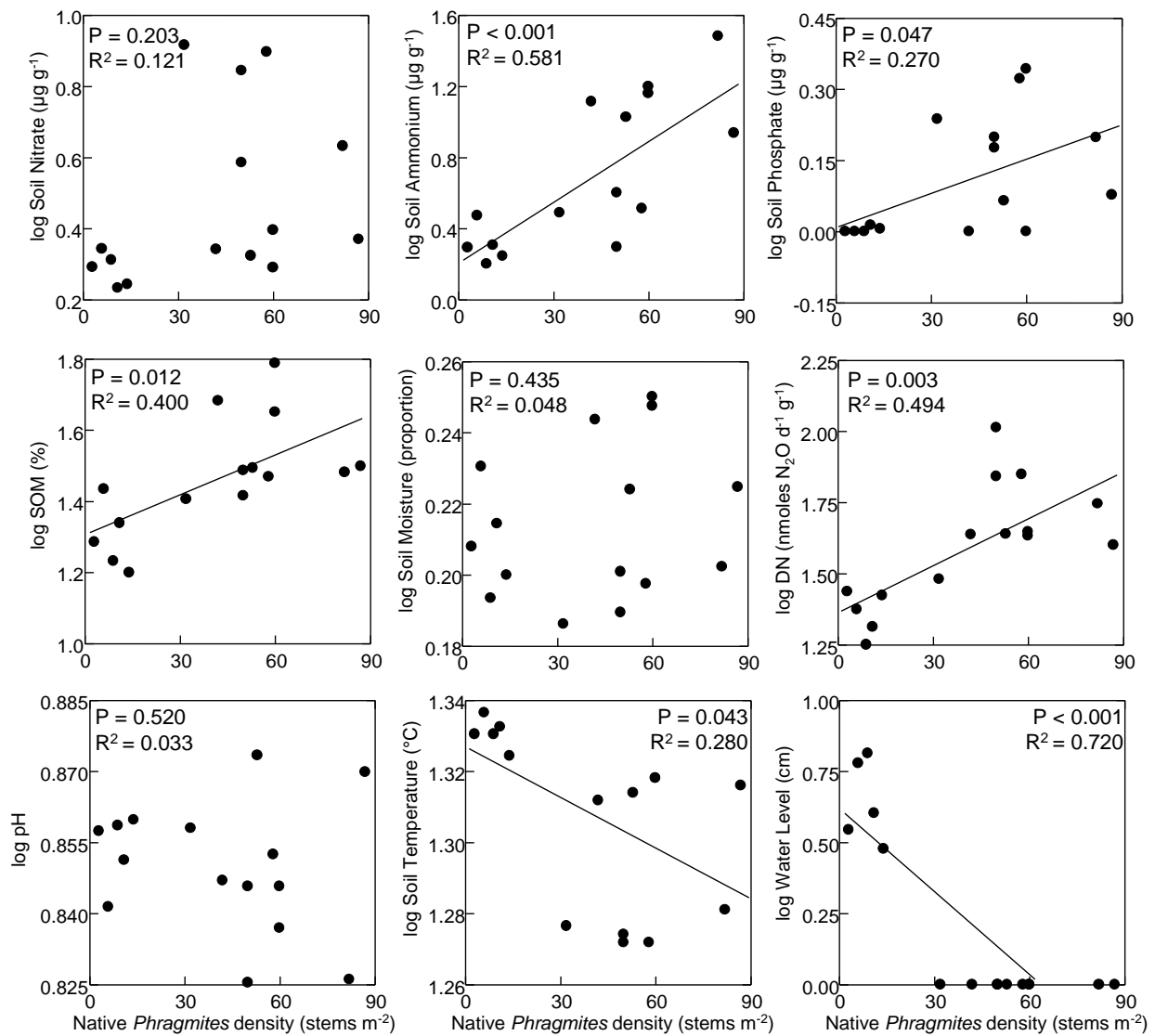
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760 Fig. 3

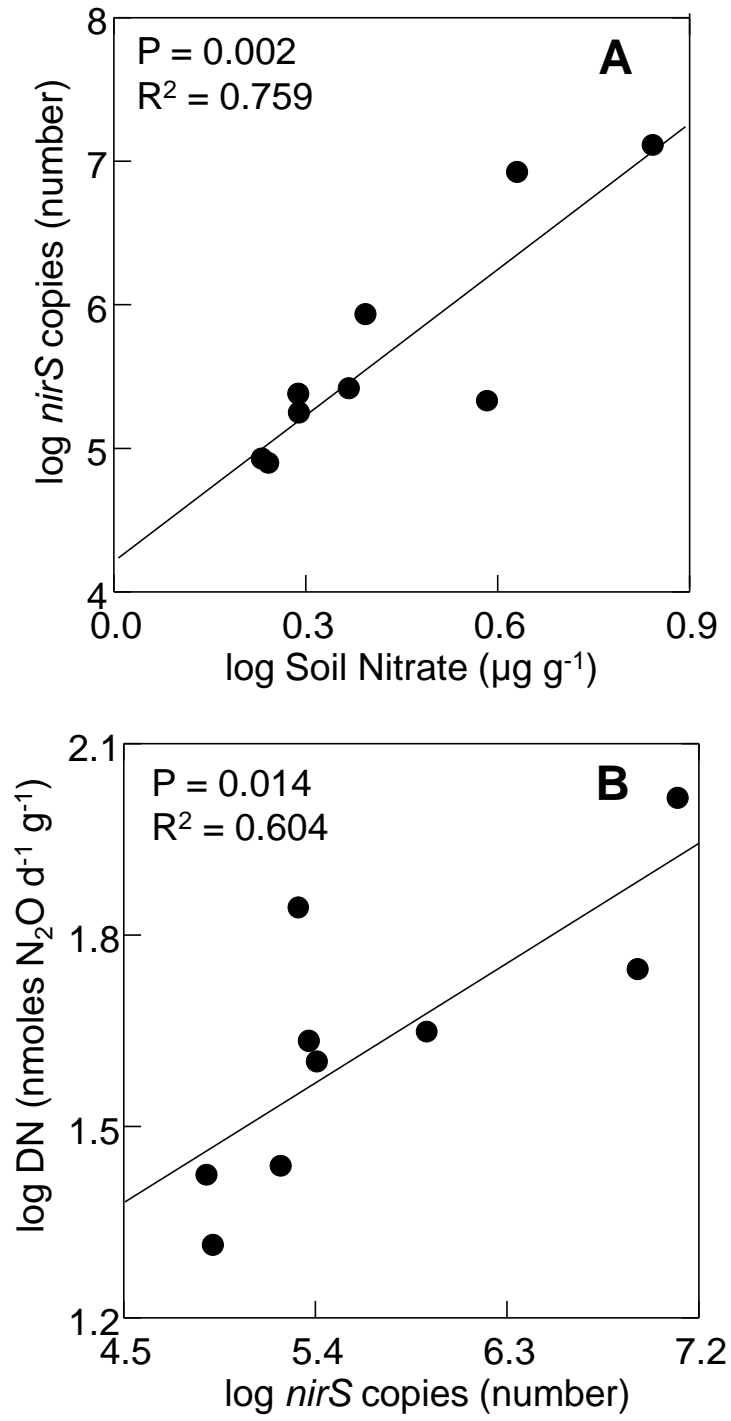


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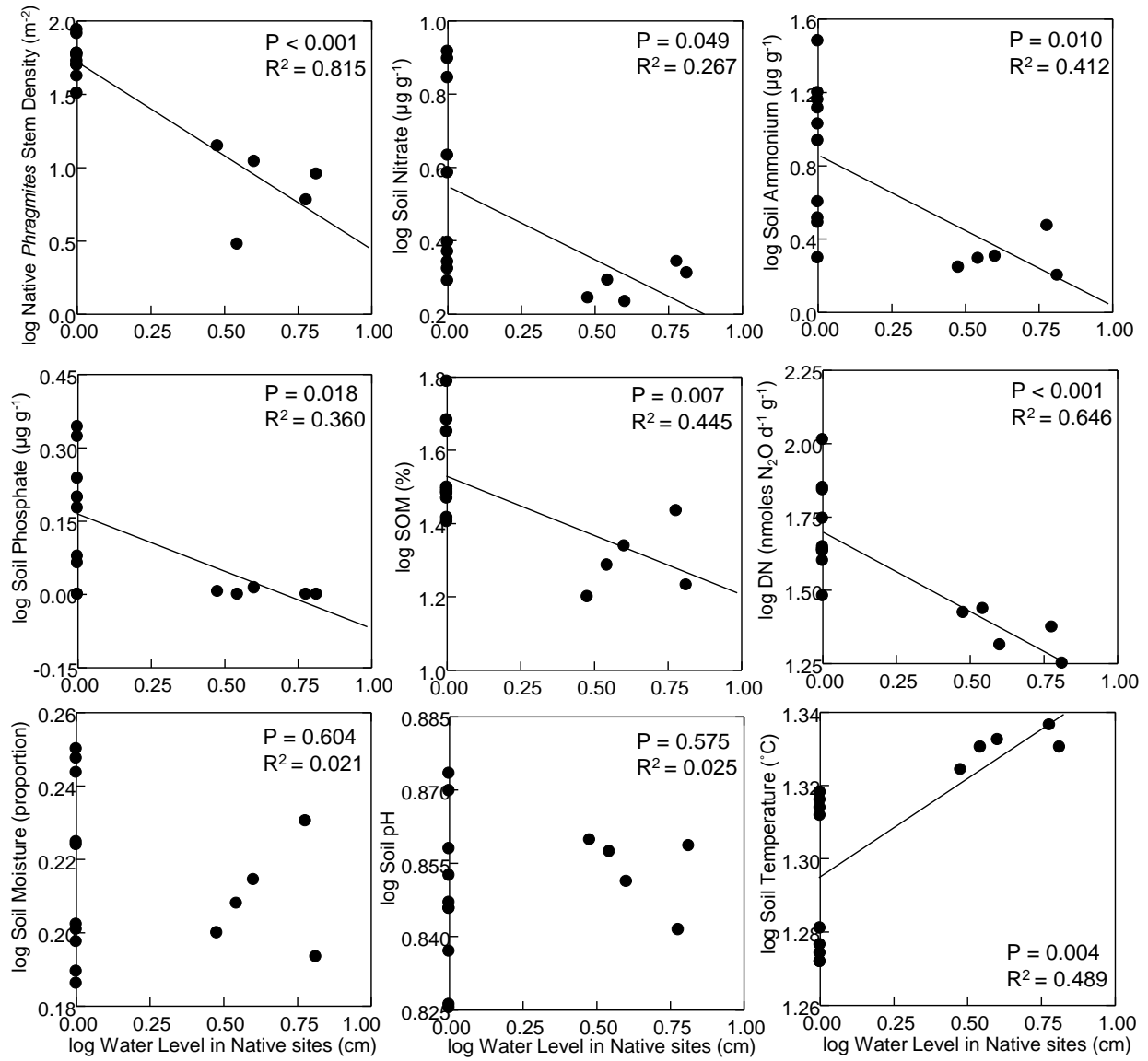
764 Fig. 4



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766 Fig. 5

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