

Direct and indirect plant-soil feedbacks

1 Intraspecific variation in indirect plant-soil feedbacks as a driver of a wetland plant invasion

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15 ABSTRACT

16 Plant-soil feedbacks (PSFs) can influence plant competition via direct interactions with
17 pathogens and mutualists or indirectly via apparent competition/mutualisms (i.e., spillover to co-
18 occurring plants) and soil legacy effects. Presently, it is unknown how intraspecific variation in
19 PSFs interacts with the environment (e.g., nutrient availability) to influence competition between
20 native and invasive plants. We conducted a fully crossed multi-factor greenhouse experiment to
21 determine the effects of soil biota, interspecific competition, and nutrient availability on biomass
22 of replicate populations from one native and two invasive lineages of common reed (*Phragmites*
23 *australis*) and a single lineage of native smooth cordgrass (*Spartina alterniflora*). Harmful soil
24 biota consistently dominated PSFs involving all three *P. australis* lineages, reducing biomass by
25 10%, regardless of nutrient availability or *S. alterniflora* presence as a competitor. Spillover of
26 soil biota derived from the rhizosphere of the two invasive *P. australis* lineages reduced *S.*
27 *alterniflora* biomass by 7%, whereas soil biota from the native *P. australis* lineage increased *S.*
28 *alterniflora* biomass by 6%. Interestingly, regardless of lineage, *P. australis* soil biota negatively
29 affected *S. alterniflora* biomass when grown alone (i.e., a soil legacy), but had a positive impact
30 when grown with *P. australis*, suggesting that *P. australis* is preferred by harmful generalist soil
31 biota or facilitates *S. alterniflora* via spillover (i.e., apparent mutualism). Soil biota also reduced
32 the negative impacts of interspecific competition on *S. alterniflora* by 13%, although it remained
33 competitively inferior to *P. australis* across all treatments. Moreover, competitive interactions
34 and the response to nutrients did not differ among *P. australis* lineages, indicating that
35 interspecific competition and nutrient deposition may not be key drivers of *P. australis* invasion
36 in North America. Taken together, although soil biota, interspecific competition, and nutrient
37 availability appear to have no direct impact on the success of invasive *P. australis* lineages in

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38 North America, indirect spillover and soil legacies from *P. australis* occur and may have
39 important implications for co-occurring native species and restoration of invaded habitats. Our
40 study integrates multiple factors linked to plant invasions, highlighting that indirect interactions
41 are likely commonplace in driving successful invasions and their impacts on the local
42 community.

43

44 **KEYWORDS:** indirect interactions, interspecific competition, invasive species, nutrients,
45 *Phragmites australis*, soil biota, soil legacy, spillover, *Spartina alterniflora*

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46 INTRODUCTION

47 Plant species influence the community composition and function of soil biota, which in
48 turn can impact fitness of host plant species, a reciprocal interaction commonly referred to as a
49 plant-soil feedback (PSF) (Kulmatiski et al. 2008). The net impact of PSFs on host plants
50 depends on the balance between beneficial (nitrogen-fixing bacteria, mycorrhizal fungi, and
51 other mutualists) and harmful (soil-borne pathogens, parasites, and herbivores) interactions with
52 soil biota (Klironomos 2002, Reinhart and Callaway 2006). PSFs have clear implications for the
53 success of invasive plant species (van der Putten et al. 2013). For example, invasive plants could
54 experience less positive (i.e., weaker associations with mutualists) or more negative (i.e., greater
55 attack by local natural enemies) PSFs relative to closely-related native species, suggesting some
56 biotic resistance of the native soil community (Elton 1958, Callaway et al. 2013). Alternatively,
57 invasive plants may generate more positive or less negative PSFs than closely-related native
58 species, potentially resulting in dominance for the invader. Several empirical studies, meta-
59 analyses, and reviews support this latter scenario (Klironomos 2002, Agrawal et al. 2005,
60 Kulmatiski et al. 2008, Suding et al. 2013). Interestingly, generalist soil biota cultivated by
61 invaders also interact with co-occurring native plant species, resulting in indirect effects of the
62 invasive species mediated through PSFs (i.e., pathogen/mutualist spillover, more generally
63 known as apparent competition/mutualism) (Eppinga et al. 2006, Mangla et al. 2008). Moreover,
64 other plant species may also be inhibited by soil biota even after removal of the invasive plant
65 (i.e., a soil legacy) (Corbin and D'Antonio 2012, Grove et al. 2015).

66 Little is known about how PSFs interact with other factors linked to plant invasions such
67 as competitive interactions with native species and nutrient availability. For example, modeling
68 and experimental studies have demonstrated that even low strength PSFs can alter interspecific

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69 competition (Bever 2003, Casper and Castelli 2007, Hodge and Fitter 2013), itself a key
70 mechanism thought to underlie the success of invasive species (reviewed by Gioria and Osborne
71 2014). Likewise, anthropogenic nutrient deposition is a major component of global
72 environmental change and a facilitating factor in many plant invasions (Dukes and Mooney
73 1999). Nutrient availability can alter competitive interactions (Wilson and Tilman 1993), activity
74 of plant mutualists and pathogens in the soil (Johnson et al. 2008), and thus the direction and
75 magnitude of PSFs (Manning et al. 2008). However, such a multi-factor approach has rarely
76 been used to study the role of PSFs in invasion success (but see Larios and Suding 2015).

77 Finally, intraspecific genetic variation is an important part of ecological and evolutionary
78 processes (see Bolnick et al. 2011 for review) and is known to alter the effects of nutrients (e.g.,
79 Saltonstall and Stevenson 2007) and competitors (e.g., Howard et al. 2008, Gomola et al. 2017)
80 on plant fitness, and to influence community composition of soil biota (Schweitzer et al. 2008,
81 Nelson and Karp 2013, Lamit et al. 2016, Bowen et al. in review), yet experiments examining
82 intraspecific variation in PSFs remain rare (but see Bukowski and Petermann 2014, Maron et al.
83 2015, Wagg et al. 2015). Biological invasions are often characterized by multiple introduction
84 events, which can lead to the presence of multiple genetic lineages in the introduced range (e.g.,
85 Durka et al. 2005, Meyerson et al. 2012, Gomola et al. 2017). Cryptic invasions have also been
86 described (e.g., Saltonstall 2002, Tyler et al. 2007), where invasive genotypes or hybrids co-
87 occur with native genotypes. Because many studies of invasive species assume that no
88 intraspecific variation exists in their interactions with the environment and resident community,
89 this can result in misleading insights and management recommendations, particularly if different
90 genotypes employ different mechanisms of invasion (Meyerson and Cronin 2013, Gomola et al.
91 2017).

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92 The goal of this study was to investigate the effects of plant intraspecific genetic
93 variation, soil biota, and nutrient availability on competitive interactions between common reed,
94 *Phragmites australis* (Cav.) Trin. ex Steudel, and a dominant, co-occurring, native marsh grass
95 species, smooth cordgrass (*Spartina alterniflora* Loisel.). In North America, there is a
96 widespread endemic native lineage of *P. australis* as well as two invasive lineages (European
97 and Gulf) (Saltonstall 2002, Meyerson et al. 2009, Lambertini et al. 2012, Meyerson et al. 2012).
98 We grew nine *P. australis* populations (three of each lineage) and a single population of *S.*
99 *alterniflora* in pots containing live or sterilized soil inoculum from the rhizosphere of the
100 respective *P. australis* population, at two nutrient levels, and with the two plant species either
101 alone or together. Based on invasion biology dogma, we tested the following predictions: 1)
102 invasive *P. australis* lineages have more positive PSFs than the native lineage; 2) indirect
103 spillover and soil legacies of soil biota on *S. alterniflora* are more negative from invasive than
104 native *P. australis* lineages; 3) the direction and strength of PSFs, spillover, and soil legacies
105 depend upon the presence of an interspecific competitor and nutrient availability; 4) nutrient
106 availability and lineage-specific PSFs alter interspecific competition between *P. australis* and *S.*
107 *alterniflora*; and 5) invasive *P. australis* lineages exhibit stronger interspecific competitive
108 ability and response to nutrient availability than native lineages and *S. alterniflora*.

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MATERIALS AND METHODS

111 *Study organisms*

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Phragmites australis is a model organism for studying plant invasions (Meyerson et al. 2016) and is one of the most widely distributed plants in the world. Multiple genetic lineages of *P. australis* grow sympatrically in North America (Saltonstall 2002, Meyerson et al. 2009,

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115 Lambertini et al. 2012, Meyerson et al. 2012, Meyerson and Cronin 2013). The native lineage is
116 endemic to North America and consists of at least fourteen different haplotypes (Saltonstall
117 2002, Meadows and Saltonstall 2007). An invasive lineage of *P. australis* from Europe has
118 spread aggressively in wetlands of North America since first appearing in herbarium records
119 ~150 years ago (Saltonstall 2002, Howard et al. 2008, Meyerson et al. 2012). This invasive
120 European lineage is mostly comprised of a single haplotype (*M*) and forms large, dense,
121 monospecific populations that negatively impact hydrology, native plant diversity, habitat quality
122 for fauna, and ecosystem function (reviewed by Meyerson et al. 2009). An additional lineage
123 (known as Gulf) is widely distributed along the Gulf of Mexico and west to California
124 (Lambertini et al. 2012, Meyerson et al. 2012) and is likely a recent arrival from Mexico or
125 Central America (Colin and Eguiarte 2016). Although its mode of introduction to North America
126 remains unknown, we classify it as invasive (following Richardson et al. 2000) due to its rapidly-
127 growing populations (Bhattarai and Cronin 2014) and the speed with which it has spread
128 (Meyerson et al. 2012).

129 Recent studies with *P. australis* have described distinct oomycete, archaea, and bacteria
130 communities from rhizosphere soil of native and European *P. australis* lineages in North
131 America (Nelson and Karp 2013, Crocker et al. 2015, Yarwood et al. 2016, Bowen et al. in
132 review), suggesting that the net impact of soil biota (i.e., PSF) may also differ among *P. australis*
133 lineages. However, virtually all studies to date have focused on describing community structure
134 of soil biota, whereas the direction and magnitude of their impact on each *P. australis* lineage
135 remain relatively unknown. The exception is the study by Crocker et al. (2015), which
136 demonstrated that virulence of some isolated *Pythium* oomycetes differed between native and

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137 European lineages. Furthermore, the ecology, trophic interactions, or microbial community of
138 the Gulf lineage remains virtually unknown (but see Bowen et al. in review).

139 The widely distributed perennial grass *S. alterniflora* is native to salt marshes on the East
140 and Gulf Coasts of North America, but invasive in other locations, such as the West Coast of
141 North America (Tyler et al. 2007) and China (Zhao et al. 2010, Li et al. 2014). We selected *S.*
142 *alterniflora* as a standardized competitor because it is a dominant plant in many coastal marshes,
143 where it co-occurs with *P. australis* (Bertness 1991, Medeiros et al. 2013) and even shares
144 pathogens (Li et al. 2014). The response to abiotic factors and competitive ability of *S.*
145 *alterniflora* have been well described; specifically, *S. alterniflora* has a strong positive response
146 to increased nutrient availability (Tyler et al. 2007, Zhao et al. 2010) and is generally an inferior
147 competitor to *P. australis* and other salt marsh plants, except in environments with high abiotic
148 stress (Bertness 1991, Pennings et al. 2005, Medeiros et al. 2013).

149

150 *Greenhouse experiment design*

151 We conducted a greenhouse experiment to examine the interactive effects of soil biota,
152 interspecific competition, and nutrient availability on clonal growth (i.e., above- and
153 belowground biomass) of the three *P. australis* lineages in North America and native *S.*
154 *alterniflora*. The experimental design consisted of four treatments: 1) live/sterile soil biota - live
155 or sterilized soil inoculum collected *in situ* from the rhizosphere of each *P. australis* population
156 was added to each pot (10% of total soil weight to minimize variation in abiotic soil properties
157 and nutrient flushes following soil sterilization). Soil biota was always combined with its
158 associated *P. australis* population such that no mixing of soil and *P. australis* sources occurred.
159 2) presence/absence of an interspecific competitor - pots were planted with either *P. australis*, *S.*

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160 *alterniflora*, or both species combined. 3) high/low nutrient levels - nutrient levels were
161 manipulated to represent nutrient-rich and nutrient-poor environments. 4) *P. australis* lineage -
162 plants and corresponding soil inoculum from populations of the native, European, and Gulf
163 lineages of *P. australis* were used for the experiment. These four treatments were fully crossed
164 (36 total treatment combinations) and replicated among clones from three distinct *P. australis*
165 populations within each lineage (Table 1). We planted ten replicates of each of the treatment
166 combinations for all nine *P. australis* populations, resulting in a total of 1,080 pots. Plants were
167 grown in a greenhouse located at Louisiana State University (30.36° N, -91.14° W) and pots
168 were arranged in a randomized blocked design with five blocks to account for possible gradients
169 in the greenhouse environment. A more detailed description of the experimental treatments and
170 design is provided in Appendix S1.

171

172 *Data collection*

173 Harvesting was completed from 5 to 13 December, 2015. At this southern climate, plants
174 were still growing and had not reached the flowering stage, which generally follows the second
175 year of growth or later when propagating from rhizome cuttings. Above- and belowground
176 biomass was harvested for each plant species from each pot, oven-dried to constant mass at 60
177 °C, and weighed to the nearest 0.1 g. Because no plants produced a panicle, we used above- and
178 belowground biomass (i.e., clonal growth) and root:shoot ratio (i.e., biomass allocation) as
179 proxies for fitness. As these variables all demonstrated similar results, we focus on total biomass
180 for our results and discussion, but report fully on other variables in Appendices S3, S4, and S5.

181

182 *Data analysis*

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183 To examine how response variables for each plant species (*P. australis*, *S. alterniflora*)
184 were influenced by *P. australis* lineage (native, European, Gulf), live/sterile soil inoculum,
185 presence/absence of an interspecific competitor, and high/low nutrient availability, we used
186 Akaike's Information Criteria corrected for finite sample size (AICc) to select the most
187 informative mixed-effect model from a set of candidate models (Burnham and Anderson 2010).
188 The full model included the variables above and all two-, three-, and four-way interactions as
189 fixed effects (fifteen total variables). *Phragmites australis* population and greenhouse block were
190 included as random effects to account for within-lineage variation and possible greenhouse
191 environment gradients, respectively. We report AICc weights that indicate the proportional
192 strength of support for model *i* being the best model given the set of plausible models ($\Delta\text{AICc} \leq$
193 2). For our interpretations, we estimated least-squares means (back-transformed) based on the
194 most likely model for each response variable and focused on effect sizes (i.e., proportional
195 differences in means) (Burnham and Anderson 2010). For brevity, only results for models with
196 AICc weight ≥ 0.30 are reported (i.e., the top model for each response variable) and we focus the
197 discussion on the interesting yet poorly-understood interaction effects (see Appendix S2).

198

199

RESULTS

200 *Factors affecting total biomass of Phragmites australis*

201 Soil inoculum, interspecific competition, and nutrient availability were identified as
202 influential in explaining variation in *P. australis* total biomass using AICc model selection. Four
203 candidate models received adequate support ($\Delta\text{AICc} \leq 2$) and all included these same three main
204 effects (cumulative AICc weight = 1) or various interactions between them. The top model

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205 (AICc weight = 0.429, Table 2) included the main effects only and had two times the support of
206 the other three models (second top model: AICc weight = 0.218).

207 Based on the top model, average biomass was 10% lower for *P. australis* grown in pots
208 containing live (43.47 ± 0.5 g, back-transformed least-squares mean \pm S.E.) than sterile soil
209 inoculum (48.47 ± 0.5 g) (Fig. 1A), regardless of the *P. australis* lineage, presence of an
210 interspecific competitor, or nutrient availability (no influential interactions in the top model).
211 Similarly, competition with *S. alterniflora* reduced biomass of *P. australis* by 13% (42.72 ± 0.5
212 g) relative to when grown alone (49.27 ± 0.5 g) (Fig. 1B), but this effect did not differ among
213 lineages or interact with soil biota or nutrient availability. Finally, although high nutrient
214 availability doubled biomass production of *P. australis* (from 31.26 ± 0.5 g to 63.44 ± 0.5 g, Fig.
215 1C), this effect also did not interact with the other variables.

216

217 *Factors affecting total biomass of Spartina alterniflora*

218 The variables influential in explaining *S. alterniflora* biomass were: *P. australis* lineage,
219 soil inoculum, interspecific competition, nutrient availability, and the lineage \times soil inoculum,
220 lineage \times nutrient availability, interspecific competition \times soil inoculum, and interspecific
221 competition \times nutrient availability interactions (top model: AICc weight = 0.714, Table 2). The
222 second top model (AICc weight = 0.286) included these variables and additional interactions but
223 had less than half the support of the top model.

224 Most interestingly, the direction of the impact of *P. australis* soil inoculum on *S.*
225 *alterniflora* biomass depended upon the *P. australis* lineage the soil inoculum was sourced from
226 (lineage \times soil inoculum interaction) as well as the presence/absence of *P. australis* as a
227 competitor (interspecific competition \times soil inoculum interaction). The impact of soil biota on *S.*

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228 *alterniflora* biomass was in the opposite direction for invasive (a 7% decrease relative to sterile
229 soil; European: 23.32 ± 0.06 g to 20.29 ± 0.06 g; Gulf: 23.78 ± 0.06 g to 20.68 ± 0.06 g) and
230 native (a 6% increase from 18.14 ± 0.06 g to 20.44 ± 0.06 g) *P. australis* lineages, an overall
231 difference in biomass of 13% (Fig. 2). Moreover, when grown alone, *S. alterniflora* biomass was
232 12% lower in pots with live (28.63 ± 0.04 g) than sterile (32.67 ± 0.04 g) *P. australis* soil
233 inoculum (Fig. 3A). Conversely, when competing with *P. australis*, *S. alterniflora* plants in live
234 soil inoculum had 6% higher biomass (13.68 ± 0.04 g) than those in sterile inoculum ($12.92 \pm$
235 0.04 g), an 18% difference between treatments. Soil biota from *P. australis* also altered the
236 effects of interspecific competition, reducing *S. alterniflora* biomass by 52% in live soil
237 inoculum (from 28.63 ± 0.04 g to 13.68 ± 0.04 g) and 60% in sterile soil inoculum pots (from
238 32.67 ± 0.04 g to 12.92 ± 0.04 g) (Fig. 3A). The effects of interspecific competition also
239 interacted with nutrient availability; biomass of *S. alterniflora* decreased by 53% (from $17.37 \pm$
240 0.04 g to 8.09 ± 0.04 g) and 58% (from 47.60 ± 0.04 g to 19.79 ± 0.04 g) in nutrient-poor and
241 nutrient-rich pots, respectively (Fig. 3B). Increased nutrient availability had a strong effect on *S.*
242 *alterniflora* biomass, which was 174% (from 17.37 ± 0.04 g to 47.60 ± 0.04 g) and 145% (from
243 8.09 ± 0.04 g to 19.79 ± 0.04 g) higher than in nutrient-poor pots when grown alone and with *P.*
244 *australis* as a competitor, respectively (interspecific competition \times nutrient availability
245 interaction) (Fig. 3B). Finally, in nutrient-poor pots, differences in *S. alterniflora* biomass among
246 pots with soil inoculum from different *P. australis* lineages were relatively small ($< 4\%$, range of
247 12.10 to 12.52 ± 0.06 g). However, in nutrient-rich pots, *S. alterniflora* grown with soil inoculum
248 from the invasive lineages of *P. australis* had 19% higher biomass (European: 34.30 ± 0.06 g;
249 Gulf: 34.62 ± 0.06 g) than pots with soil inoculum or plants from the native lineage (27.89 ± 0.06

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250 g) (lineage \times nutrient availability interaction). This pattern was consistent regardless of the
251 presence of *P. australis* as a competitor or whether the soil inoculum was live or sterile.

252

253 *Factors affecting other response variables*

254 The top models for other *P. australis* and *S. alterniflora* response variables (aboveground
255 biomass, belowground biomass, root:shoot ratio, see Appendices S3, S4, and S5) were
256 remarkably similar to those for total biomass. However, lineage-specific effects were more
257 prevalent and three additional terms were identified as influential, which we focus on describing
258 below. For *P. australis*, the negative impact of live soil biota on aboveground biomass of the
259 European lineage was 64% and 75% less than the native and Gulf lineages (lineage \times soil
260 inoculum interaction), respectively (Appendix S5, Fig. S1). The European lineage also had the
261 greatest root:shoot ratio in nutrient-poor pots, but this changed to the native lineage in nutrient-
262 rich pots (lineage \times nutrient availability interaction) (Appendix S5, Fig. S2). Finally, for the
263 additional *S. alterniflora* traits, the tripartite lineage \times interspecific competition \times soil inoculum
264 interaction was the only other variable identified as influential that was not already present in the
265 top model for total biomass. For this interaction, the presence of *P. australis* increased the
266 strength of soil biota impacts on *S. alterniflora* root:shoot ratio across all three lineages, but the
267 direction of this effect varied by *P. australis* lineage (Gulf: a 3% to 34% decrease in root:shoot
268 ratio; European: 6% to 23% decrease; native: 9% to 27% increase) (Appendix S5, Fig. S3).

269

270

DISCUSSION

271 Our study suggests that interactions with soil biota do not directly influence the success
272 of invasive *P. australis* lineages, with this view supported by a similar reduction (10%) in total

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273 biomass of native and invasive (European and Gulf) lineages through interactions with their
274 respective soil biota, irrespective of the presence of an interspecific competitor or nutrient
275 availability. This consistent negative impact of soil biota on *P. australis* supports the established
276 view that conspecific PSFs are predominantly negative (Bever 2003, Kulmatiski et al. 2008). On
277 the other hand, soil biota from invasive *P. australis* populations reduced biomass of native *S.*
278 *alterniflora*, whereas soil biota from native *P. australis* populations had a positive effect,
279 suggesting the potential to exclude and facilitate co-occurring native plant species, respectively
280 (Bever et al. 1997, Klironomos 2002, van der Putten et al. 2013). Interestingly, PSFs involving
281 *P. australis* soil biota were negative for *S. alterniflora* grown alone (i.e., a negative soil legacy)
282 but positive when grown in the presence of *P. australis*, suggesting that *P. australis* may be
283 preferred by harmful generalist soil biota, or facilitates *S. alterniflora* via apparent mutualism. To
284 our knowledge, this is the first study to demonstrate that the direction of indirect PSFs differs
285 among conspecific native and invasive plant taxa, and can change depending upon the
286 presence/absence of the initial host plant of the soil biota (i.e., between spillover and soil legacy).
287 Consistent with previous studies (Bertness 1991, Pennings et al. 2005), we also found *P.*
288 *australis* to be a dominant interspecific competitor and that *S. alterniflora* had a stronger
289 response to increased nutrient availability, which also mediated the impact of interspecific
290 competition on *S. alterniflora*. However, contrary to expectations, we found little support for the
291 hypothesis that invasive *P. australis* lineages have superior interspecific competitive ability or
292 response to nutrients when compared to the native lineage. Taken together, these results suggest
293 that rather than direct PSFs, interspecific competitive ability, and response to nutrient deposition,
294 the more nuanced indirect effects of *P. australis* soil biota on *S. alterniflora* via spillover of
295 pathogens and mutualists (i.e., apparent competition and mutualism), soil legacy effects, and

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296 altered interspecific competition strength appear to be the key differences among *P. australis*
297 lineages that may ultimately influence their relative success in North American salt marshes.
298 Finally, our study, which integrates multiple factors linked to invasion success, highlights how
299 indirect interactions can underpin successful invasions and their impact, and could inform
300 approaches to management and restoration of areas invaded by *P. australis*.

301

302 *Direct PSFs of Phragmites australis lack intraspecific variation or context dependency*

303 Despite strong lineage-specific differences in rhizosphere microbial communities (Nelson
304 and Karp 2013, Yarwood et al. 2016, Bowen et al. in review), impact of *Pythium* spp. pathogens
305 (Crocker et al. 2015), and soil biota effects on aboveground biomass (this study), we found that
306 the impact of soil biota was consistently negative for all three *P. australis* lineages - a 10%
307 reduction in total biomass. Thus, contrary to previous studies (Klironomos 2002, Agrawal et al.
308 2005, Kulmatiski et al. 2008, Suding et al. 2013, but see Callaway et al. 2013) and our first
309 prediction, invasive *P. australis* lineages do not benefit from more positive PSFs than the native
310 lineage, indicating that soil biota does not directly facilitate the relative success of invasive *P.*
311 *australis* lineages in North America. This unexpected result is consistent with that of Bowen et
312 al. (in review), who used structural equation modeling to show that rhizosphere bacterial
313 richness, activity, and metabolism did not mediate *P. australis* biomass. One possible reason for
314 the lack of differences in PSF strength among lineages could simply be that although lineages
315 differ in their microbial communities, their net effects on the plant are the same. However,
316 studies in other systems contradict this explanation, such as that of Wagg et al. (2015) who
317 demonstrated that differences in PSFs of two populations of *Trifolium pratense* were related to
318 corresponding differences in the rhizosphere microbe community. Finally, in contrast to our third

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319 prediction, the impact of soil biota on *P. australis* biomass was unaffected by the presence of *S.*
320 *alterniflora* as a competitor or nutrient availability, suggesting there is little context dependency
321 of *P. australis* PSFs regarding these variables. Intriguingly, these results are similar to those of
322 the only other study to take such a multi-factor approach to the role of PSFs in plant invasions,
323 where Larios and Suding (2015) found that negative PSFs of invasive wild oat (*Avena fatua*)
324 were also largely unaffected by competition or nutrient availability.

325

326 *Lineage-specific indirect PSFs influence Phragmites australis invasion and impact*

327 In support of our second prediction, we found that generalist soil biota from the
328 rhizosphere of populations of the two invasive *P. australis* lineages had a net negative impact on
329 *S. alterniflora* biomass, whereas soil biota from populations of the native lineage had a net
330 positive impact, regardless of the presence of *P. australis* plants or nutrient availability. The
331 large extent and density of populations of the invasive *P. australis* lineages relative to the native
332 lineage and other native wetland plants means that even small invasion-induced changes in PSFs
333 could be widespread and important in invaded habitats. Thus, our study represents the first to
334 demonstrate intraspecific variation in spillover and provides support for its importance as a
335 potential mechanism driving plant invasions. One possible explanation for the negative impact
336 on *S. alterniflora* could be that invasive *P. australis* accumulates local generalist soil pathogens,
337 which spillover onto *S. alterniflora*, overwhelming any positive impacts from beneficial
338 organisms (Borer et al. 2007, Mangla et al. 2008). Similarly, beneficial soil biota may spillover
339 to *S. alterniflora* from soil associated with the native *P. australis* lineage, representing a possible
340 explanation for why the native *P. australis* lineage usually co-occurs with a diverse suite of other
341 native species (Meyerson et al. 2009). These indirect interactions are representative of apparent

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342 competition and mutualism, respectively, whereby shared natural enemies or mutualists mediate
343 interactions between two or more species. There is growing support for apparent competition
344 involving herbivores and pathogens as an important driver of plant invasions (Borer et al. 2007,
345 Dangremond et al. 2010), including for *P. australis* (Bhattarai et al. 2017a). Interestingly, Li et
346 al. (2014) demonstrated pathogen spillover between *P. australis* and *S. alterniflora* in the
347 Yangtze River estuary in China, but the roles of the species were reversed; there, *S. alterniflora*
348 is invasive and spillover of the fungal pathogen *Fusarium palustre* resulted in significant dieback
349 of native *P. australis*. Unfortunately, our experimental design did not allow us to assess how soil
350 biota sourced from *S. alterniflora* populations affects *P. australis* in North America, although a
351 reciprocal transplant experiment using soil biota from *S. alterniflora* and other native plants is a
352 logical next step.

353 Furthermore, live *P. australis* soil biota decreased native *S. alterniflora* biomass by 12%
354 when grown alone, but this changed to a 6% increase when competing with *P. australis*. We
355 observed similar interspecific competition \times soil inoculum interaction effects for all *S.*
356 *alterniflora* response variables, although the direction of this impact on biomass allocation
357 (root:shoot ratio) also differed among lineages, further supporting our predictions of strong
358 interplay among these three factors. Similarly, Larios and Suding (2015) demonstrated that
359 native purple needlegrass (*Stipa pulchra*) exhibited a positive PSF when grown alone at low
360 nutrient levels, but that this PSF became neutral when competing with *A. fatua* or in high nutrient
361 environments. At least two scenarios could explain the effects we observed for *S. alterniflora*.
362 First, harmful generalist soil biota may interact preferentially with *P. australis*, only switching
363 hosts to *S. alterniflora* when *P. australis* is absent. Such a preference is not entirely unexpected
364 given that the soil inoculum was originally collected from naturally occurring *P. australis*

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365 populations and likely contained organisms coadapted to that population, lineage, and species
366 (Nelson and Karp 2013, Yarwood et al. 2016, Bowen et al. in review). Thus, we suggest that *P.*
367 *australis* generates a negative soil legacy whereby harmful generalist soil biota switch to native
368 host species when *P. australis* is no longer present. Negative soil legacies appear to be relatively
369 common among invasive species and are widely-recognized to prevent establishment of native
370 plants and improve chances of invader recolonization (D'Antonio and Meyerson 2002, Corbin
371 and D'Antonio 2012, Grove et al. 2015). Second, our findings could be indicative of spillover of
372 beneficial soil biota cultivated by *P. australis* to *S. alterniflora* (i.e., an apparent mutualism),
373 suggesting that *P. australis* may indirectly facilitate the growth of co-occurring native plants.
374 However, these underlying mechanisms cannot easily be disentangled without identifying the
375 organisms involved, which was unfortunately outside the scope of this study.

376

377 *Effects of interspecific competition and nutrient availability*

378 Superior competitive ability has long been recognized as a common trait of invasive plant
379 species (Elton 1958, reviewed by Gioria and Osborne 2014) and is often cited as one of the main
380 reasons the European *P. australis* lineage has become so prevalent in North America. In support
381 of this view and our fifth prediction, we found that interspecific competition decreased biomass
382 of *P. australis* and *S. alterniflora* by 13% and 57%, respectively. This result is consistent with
383 studies showing that native *S. alterniflora* tends to be restricted to lower marsh areas due to its
384 poor competitive ability but superior tolerance of abiotic stress factors such as high salinity and
385 flooding (Bertness 1991, Pennings et al. 2005). Several studies have also indicated that European
386 *P. australis* is a stronger competitor than the native and Gulf lineages (Howard et al. 2008,
387 Holdredge et al. 2010). However, we failed to find any differences in total biomass, interspecific

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388 competitive ability, or impact on *S. alterniflora* biomass among the three *P. australis* lineages.

389 Thus, we suggest that interspecific competitive ability may not be a key factor explaining the

390 predominance of European relative to native and Gulf *P. australis* in North America.

391 Several studies have found that soil biota and nutrient availability can significantly alter

392 the outcome of interspecific competition (Casper and Jackson 1997, Casper and Castelli 2007,

393 Hodge and Fitter 2013, but see Maron et al. 2016). In our study, live soil biota and nutrient-poor

394 conditions both reduced the negative impact of interspecific competition on biomass of *S.*

395 *alterniflora* but not *P. australis*, partially supporting our fourth prediction. The effect of soil

396 biota on interspecific competition can likely be attributed to the consistent negative PSF suffered

397 by *P. australis*, which may decrease its competitive ability or the strength of beneficial spillover

398 affecting *S. alterniflora*. Moreover, our findings contrast with earlier studies that found nutrient

399 addition reduces negative impacts of interspecific competition on *S. alterniflora* (Levine et al.

400 1998, Emery et al. 2001). However, these experiments did not use *P. australis* as a competitor, a

401 species possessing one of the highest nitrogen use efficiencies of all land plants (Mozdzer et al.

402 2013). Furthermore, at high levels of nutrient availability, light becomes the main limiting

403 resource in plant competition (Casper and Jackson 1997, Aerts 1999), meaning that the taller *P.*

404 *australis* would continue to outcompete the shorter *S. alterniflora*. Perhaps most importantly, no

405 differences in the impact of these competitive interactions on *S. alterniflora* total biomass were

406 detected among *P. australis* lineages, indicating they are unlikely to be important in explaining

407 the relative success of invasive versus native *P. australis* lineages in North America.

408 Increased nutrient deposition via disturbance and anthropogenic modification is

409 considered a major contributing factor to *P. australis* invasion success (Bertness et al. 2002,

410 Holdredge et al. 2010) and plant invasions in general (Dukes and Mooney 1999). Unsurprisingly,

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411 nutrient availability had a strong effect on biomass of both our study species, but this was greater
412 for *S. alterniflora* than *P. australis* (Zhao et al. 2010), which may help explain why *S.*
413 *alterniflora* has become an invasive plant in salt marshes on the West Coast of North America
414 (Tyler et al. 2007), China (Zhao et al. 2010, Li et al. 2014), and elsewhere. However, the strength
415 of nutrient effects did not vary among *P. australis* lineages, which could be considered unusual,
416 given that European invasive *P. australis* enjoys a higher maximum nutrient uptake ability than
417 the native lineage (Mozdzer et al. 2010). However, differences may be more subtle, such as the
418 stronger plasticity in biomass allocation (root:shoot ratio) in response to nutrient addition that we
419 observed for the European invasive lineage, which may impact other measures of fitness (i.e.,
420 sexual reproduction) or biomass over more than one growing season. Additionally, *S.*
421 *alterniflora* grown in pots containing soil inoculum from the native *P. australis* lineage did not
422 respond as positively to nutrient additions as plants associated with soil inoculum from the
423 invasive *P. australis* lineages. This effect was independent of the presence of *P. australis* and
424 soil biota sterilization, suggesting that abiotic factors of the original soil inoculum may have
425 affected nutrient uptake of *S. alterniflora* in nutrient-rich pots, which is surprising given the low
426 soil inoculum ratio of 10% of total soil weight. Furthermore, the influence of nutrient availability
427 on *P. australis* was unaffected by the presence of live soil biota and interspecific competitors,
428 suggesting that the harmful effects of negative PSFs and interspecific competition do not impact
429 the response of *P. australis* to changes in nutrient availability. For *S. alterniflora*, however, the
430 biomass increase in response to nutrients was highest when grown on its own. This is
431 unsurprising, given that *P. australis* is the superior competitor and its presence should reduce the
432 ability of *S. alterniflora* to fully utilize resources. Importantly, this effect also did not vary
433 among *P. australis* lineages, and taken together, our results suggest that nutrient deposition may

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434 not directly contribute to the spread of invasive *P. australis* lineages into wetlands and marshes
435 dominated by native *P. australis* or *S. alterniflora*.

436

437 *Management implications and future directions*

438 The identity and impact of the soil community should be an important consideration
439 when attempting to restore habitat occupied by invasive plant species (D'Antonio and Meyerson
440 2002, Corbin and D'Antonio 2012). Thus, we suggest microbial inoculation (e.g., Middleton and
441 Bever 2012), topsoil removal (e.g., Hölzel and Otte 2003), and planting of the native *P. australis*
442 lineage or other native plants as potentially useful approaches to reduce the effects of harmful
443 soil biota, promote cultivation of beneficial soil biota, and facilitate the development of a diverse
444 native community in areas where invasive *P. australis* is being managed. Successful restoration
445 may be crucial to preventing re-establishment of invasive *P. australis* by improving resistance to
446 colonization by seedlings and vegetative spread (Byun et al. 2013). Management of the
447 surrounding matrix to minimize propagule pressure from neighboring invasive *P. australis*
448 populations would also be beneficial when considering such an approach. Furthermore, future
449 studies should focus on the identification of lineage-specific pathogens and mutualists which
450 may be useful in novel management efforts with the goal of controlling invasive *P. australis*
451 lineages and restoring the native lineage, respectively (Kowalski et al. 2015). Finally, because
452 invasive species interact directly and indirectly with a complex community of organisms and
453 abiotic conditions, expanding PSF studies to multitrophic and community-level interactions, and
454 continuing to address context dependency, is critical to furthering our understanding of the role
455 of PSFs in plant invasions.

456

457

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647 Phenotypic plasticity of *Spartina alterniflora* and *Phragmites australis* in response to
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- 649 **Table 1.** List of *Phragmites australis* field populations used for the greenhouse experiment.

Population name, state (ID code)	Latitude	Longitude	Lineage	Status
Palm Canyon Road, CA (PCN)	33.83	-116.62	Native	Endemic
Little Caliente Hot Springs, CA (LCN)	34.54	-119.62	Native	Endemic
Mackay Island, NC (NCN)	36.51	-75.95	Native	Endemic
East Cameron, LA (ECM)	29.77	-93.29	European	Invasive
I-40, AZ (I40M)	34.72	-114.49	European	Invasive
Mackay Island, NC (NCM)	36.51	-75.95	European	Invasive
Okeehetee Park, FL (FLI)	26.65	-80.16	Gulf	Invasive
Intracoastal City, LA (ICI)	29.78	-92.20	Gulf	Invasive

Direct and indirect plant-soil feedbacks

Creole, LA (CRI)	29.83	-93.11	Gulf	Invasive
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650

651 **Table 2.** AICc best models ($\Delta\text{AICc} \leq 2$) explaining variation in total biomass (square root transformed) for each plant species
 652 (*Phragmites australis* or *Spartina alterniflora*). Explanatory variables: L = *P. australis* lineage (native, European, Gulf), C =
 653 presence/absence of an interspecific competitor, N = high/low nutrient availability, and S = live/sterile soil inoculum. \times denotes
 654 interactions between explanatory variables.

Response variables	Models	AICc	ΔAICc	AICc weight
<i>Phragmites australis</i>				
Total biomass	C N S	2611.6	0.00	0.429
	C N S C \times S	2612.9	1.35	0.218
	C N S N \times S	2613.3	1.73	0.181
	C N S C \times N	2613.4	1.82	0.173
<i>Spartina alterniflora</i>				
Total biomass	C L N S C \times N C \times S L \times N L \times S	2373.5	0.00	0.714
	C L N S C \times N C \times S L \times N L \times S N \times S C \times N \times S	2375.4	1.83	0.286

655

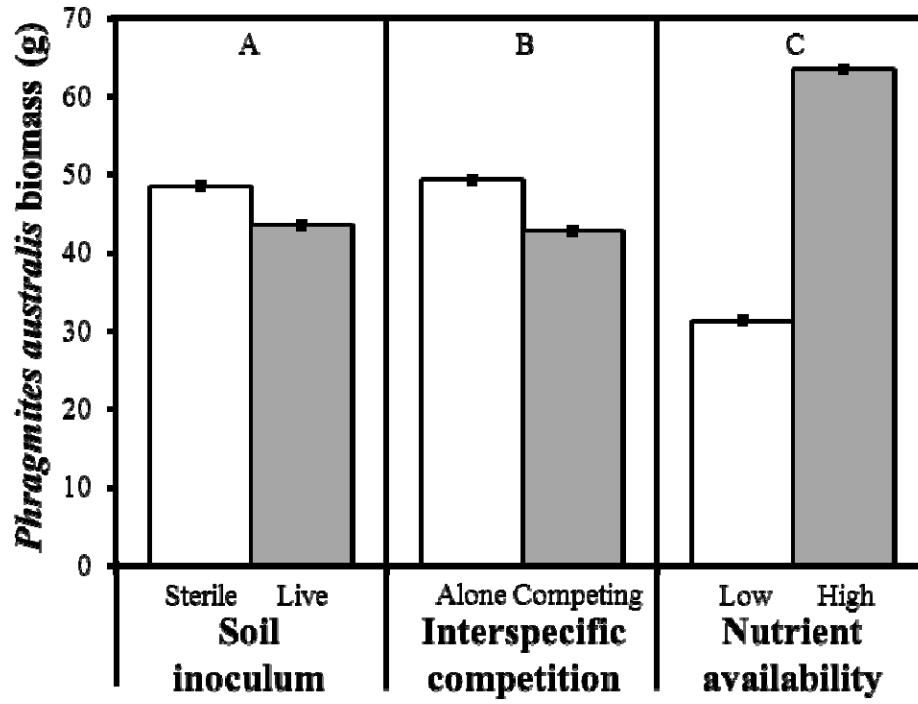
Soil biota, competition, and nutrients

656 **Figure 1.** Influential main effects on *Phragmites australis* biomass (g) (least squares mean \pm
657 S.E.) identified using mixed-effect model selection: A) live or sterilized *P. australis* soil
658 inoculum, B) presence or absence of competition with *Spartina alterniflora*, and C) high or low
659 nutrient availability. No interactions among main effects were included in the top model (see
660 Table 2).

661
662 **Figure 2.** Indirect impact of live or sterilized soil inoculum obtained from the three *Phragmites*
663 *australis* lineages on biomass (g) (least squares mean \pm S.E.) of *Spartina alterniflora*. The
664 interaction between soil inoculum and *P. australis* lineage was identified as influential using
665 mixed-effect model selection (see Table 2). Some error bars are obscured due to their small size.

666
667 **Figure 3.** Influential interaction effects of competition with *Phragmites australis* on biomass (g)
668 (least squares mean \pm S.E.) of *Spartina alterniflora* grown in A) live or sterilized *P. australis* soil
669 inoculum and B) with high or low nutrient availability. These interactions were identified as
670 influential using mixed-effect model selection (see Table 2). Error bars are obscured due to their
671 small size.

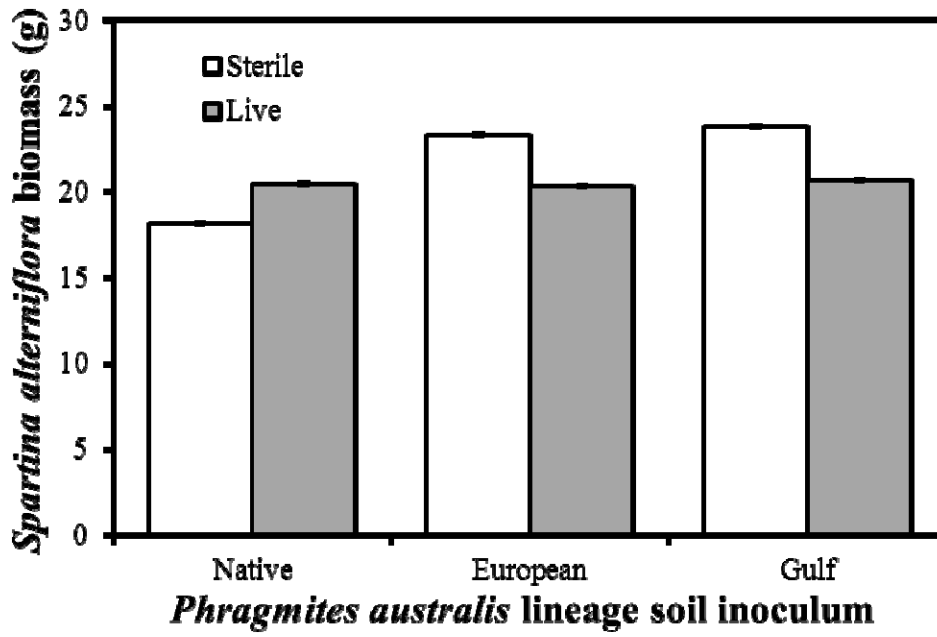
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672

673 Figure 1.

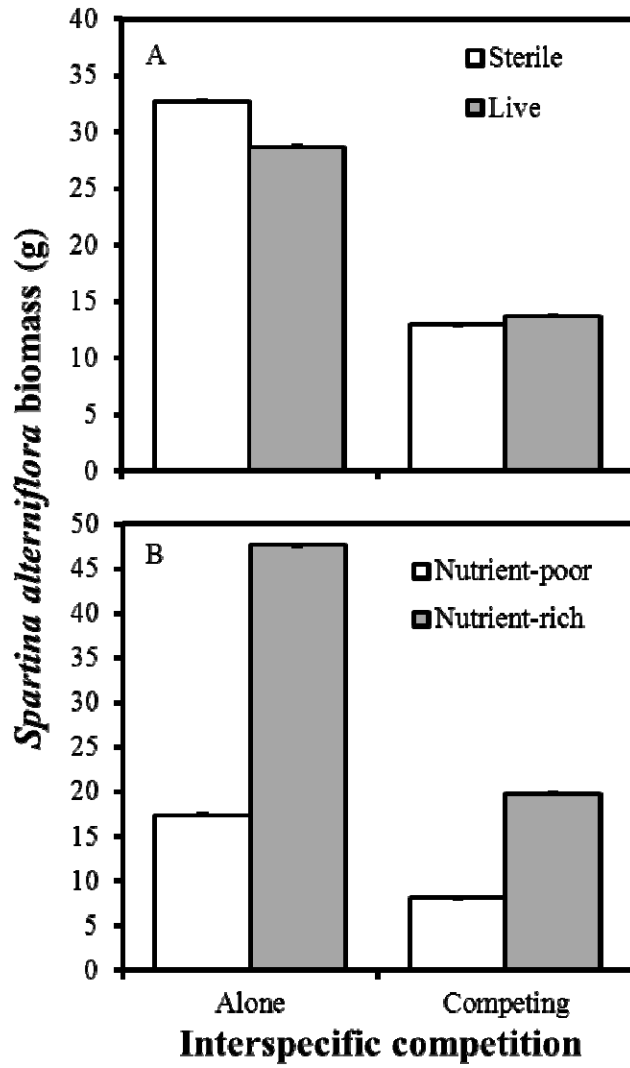
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674

675 **Figure 2.**

Soil biota, competition, and nutrients



676

677 **Figure 3.**