

1 **Plant invasive success associated with higher N-use efficiency and**
2 **stoichiometric shifts in the soil-plant system in the Minjiang River**
3 **tidal estuarine wetlands of China**

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13 **Abstract** The tidal estuarine wetlands of China are rich in plant diversity, but several
14 human-driven processes, such as species invasion, can affect the biogeochemical
15 cycles of these ecosystems, and by changing soil conditions can inhibit the
16 regeneration of native vegetation. We seasonally analyzed the carbon (C), nitrogen (N)
17 and phosphorus (P) concentrations in soils and in leaves, stems and roots of the
18 invasive species *Spartina alterniflora* and of the native species *Cyperus malaccensis*
19 var. *brevifolius* Boeckeler. This latter species was analyzed both in natural
20 non-invaded stands and in stands that had been invaded by *Spartina* but from which it
21 had been removed and replaced by *Cyperus*. The aim was to investigate the effect of
22 plant invasion, subsequent removal and replanting with a native species on C, N and P
23 stoichiometry of the plant-soil system in the tidal wetlands of the Minjiang River. C
24 and N concentrations averaged across seasons did not differ significantly among the
25 plant species. P concentration was lower in the stems of *Spartina* than in the stems of
26 the native species *Cyperus* but was not significantly different in the roots of the two
27 species. The soil C and N concentrations were higher in the *Spartina* stand than in the
28 *Cyperus* stand, whereas the soil P concentrations were not significantly different. The
29 invasive species had a higher N-resorption capacity, N:P ratios in stem and roots,
30 biomass, absolute growth and biomass N and had a lower relative growth rate and
31 litter production than the native species. After the removal of the invasive plants, the
32 regenerating native plants have a higher capacity to resorb N and lower relative
33 growth rates. All these traits show that a conservative strategy and a high N-use
34 efficiency and internal plant control of the N in the ecosystem underlie the invasive

35 success of *Spartina* in this N-limited wetland. Relative growth rate was associated
36 with lower plant N:P ratios, whereas absolute growth rate was associated with higher
37 nutrient-use efficiency and lower C and N turnover and storage capacities in the
38 biomass. Changes in soil properties produced by the establishment of an invasive
39 plant can condition the later regeneration of native plants.

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41 **Keywords:** Carbon · ecological stoichiometry · nitrogen · N:P ratio · N resorption

42 · phosphorus · wetlands

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57 **Introduction**

58 Tidal estuarine wetlands cover an estimated 12 000 km² of China's 18 000 km of
59 coastline (Shen and Zhu 1999; Huang et al. 2006). These tidal wetlands are generally
60 rich in animal and plant biodiversity (Zhou et al. 2006) and have important
61 biogeochemical roles within the entire estuarine ecosystem (Zeng et al. 2009a; Zeng
62 et al. 2009b; Wang et al. 2010a; Wang et al. 2010b; Tong et al. 2010). The Minjiang
63 River estuary in southeastern China is an important tidal wetland ecosystem due to its
64 unique location at the transition of the central and southern subtropical climatic zones
65 (Zheng et al. 2006).

66 *Spartina alterniflora* and *Cyperus malaccensis* var. *brevifolius* Boeckeler
67 comprise much of the emergent macrophytic biomass in the Minjiang River estuary
68 (Liu et al. 2006). Some stands of *Cyperus* have been invaded over the past 10 years by
69 *Spartina*, which is now the most prevalent plant species in the wetland area. This
70 change in dominance may be affecting the biogeochemical cycles of the estuarine
71 wetland, because the rates of litter decomposition and the soil profiles in the stands of
72 *Spartina* and *Cyperus* are known to differ (Zhang et al. 2008; Jia et al. 2008; Zeng et
73 al. 2009a; Tong et al. 2009).

74 The elemental composition of plant tissues is tightly associated with the nutrient
75 concentrations of litter, which in turn can feed back to the soil (McClougherty et al.
76 1985; Bridgham et al. 1995; Ehrenfeld et al. 2005; Townsend et al. 2007; Aragon et al.
77 2014). Higher ratios of carbon (C) to other nutrients in litter can increase C storage
78 and reduce the mobility and rates of mineralization of key nutrients (Wang et al.

79 2010b; Wang and Yu 2008). Such effects appear to be caused by the increasing
80 nutrient limitation of the soil microbial communities when provided with
81 nutrient-poor organic material. Moreover, plants can have different capacities to use
82 and resorb nutrients (Mulder et al. 2013). Nutrient-resorption capacity has been
83 observed to be related to plant invasive success in some studies (Sardans and Peñuelas
84 2012; Wang et al. 2014). Plant-litter-soil interactions have been extensively modeled
85 (Vitousek and Peter 1984; Northup et al. 1998; Meier and Bowman 2008), observed
86 in numerous ecosystems (Cebrian 1999; Cebrian and Lartigue 2004; Güsewell and
87 Verhoeven 2006; Wurzbürger and Hendrick 2009) and experimentally examined
88 (Jobbágy and Jackson 2001; Hawlena and Schmitz 2010) in terrestrial ecosystems, but
89 little is known about the effect of invasive success and its relationships with nutrient
90 fluxes and stoichiometries in wetland plant-soil systems.

91 Variable foliar ratios of C to nitrogen (N) (C:N) and to phosphorus (P) (C:P) are
92 assumed to be caused by the physiological adjustment of plant species to the local
93 supplies of nutrients (Broadley et al. 2004; Kerkhoff et al. 2006; Demars and Edwards
94 2007; Townsend et al. 2007; Elser et al. 2010; Peñuelas et al. 2010; Sardans and
95 Peñuelas 2013). Evidence, however, is accumulating that intraspecific differences in
96 terrestrial plants can match or exceed interspecific variability (Wright et al. 2004;
97 Elser et al. 2010; Peñuelas et al. 2010; Sardans and Peñuelas 2013). These
98 species-specific patterns of elemental composition likely reflect important differences
99 in plant functional traits that have unique biochemical, and hence elemental,
100 requirements (Sardans et al. 2014). The elemental composition of *Cyperus* may thus

101 differ from that of *Spartina*, even for individuals growing under very similar
102 environmental conditions, and thereby may affect the dynamics of soil nutrients by
103 affecting the elemental composition of litter and/or the capacity to take up nutrients.

104 Shifts in nutrient stoichiometry have frequently been associated with the success
105 of invasive plants (Sardans and Peñuelas 2012). Successful invasive species in
106 nutrient-rich environments usually have low C:nutrient ratios (Peñuelas et al. 2010)
107 and high N:P ratios (Neves et al. 2010) in their tissues, but the effect of N:P ratios on
108 the success of invasive plants is still unclear. Moreover, the positive relationship
109 between N:P ratio and invasive success has seldom been reported for nutrient-poor
110 environments. Contrasting patterns would be associated with environments with some
111 important constraints to plant production (Kunk and Vitousek 2007; Sardans and
112 Peñuelas 2012) such as the wetlands of China (Wang et al. 2014). Furthermore, some
113 studies have observed that changes in soil nutrient status are related to plant invasive
114 success in wetlands (Currie et al., 2014; Geddes et al., 2014). Wetland macrophyte
115 plants are frequently limited by nutrients (Subedi et al., 2012; Currie et al., 2014)
116 and in particular by N in China (Wang et al., 2010; Sun et al., 2012) including the
117 studied wetland area of Minjiang River (Wang et al., 2014). Thus, we hypothesized
118 that different nutrient use and consequently changes in plant-soil nutrient
119 concentrations and stoichiometry should be underlying and related with invasive
120 species success of *Spartina* in marsh wetlands of Minjiang River. Moreover, the
121 effects of the changes in soil nutrient concentrations and stoichiometries that invasive
122 plants can produce and the subsequent role of these changes in the soil on the

123 regenerative capacity of native species remain to be investigated.

124 We investigated the relationships between invasive success and the changes in
125 nutrient cycles and stoichiometries in the plant-soil system. We also studied the
126 success of re-established native *Cyperus* after the removal of the invasive species.
127 Specifically, we have examined the effects of the invasion of *Spartina* and regenerated
128 communities of *Cyperus* on the seasonal variation of the stoichiometries of C, N and P
129 in the plant-soil system in natural in the subtropical tidal wetlands of the Minjiang
130 River in China. Our aims were (1) to describe the C:N, C:P and N:P ratios of the
131 leaves, stems and roots of the invasive *Spartina*, the native *Cyperus* and the
132 regenerated *Cyperus* over the growing season, (2) to determine if plant-specific tissue
133 stoichiometry translates into differences between the nutrient concentrations of the
134 litter and soil, (3) to examine the relationships between the success of plant invasion
135 and the nutrient concentrations and stoichiometries of the plants, litter and soils, (4) to
136 study the relationships of plant nutrient concentrations and stoichiometry with growth
137 and nutrient resorption and (5) to determine if the changes in soil nutrient
138 concentrations of C, N and P and in their stoichiometries produced during *Spartina*
139 invasion can thereafter affect the regeneration of *Cyperus*.

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145 **Methods**

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147 Study area

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149 This study was conducted in the Shanyutan wetland (26° 01' 46" N, 119° 37' 31"
150 E; Fig. 1), the largest tidal wetland (approximately 3120 ha) in the estuary of the
151 Minjiang River. The climate in this region is relatively warm and wet with a mean
152 annual temperature of 19.6 °C and a mean annual precipitation of 1346 mm (Zheng et
153 al. 2006). The soil surface is submerged across the study site beneath 10-120 cm of
154 water for 3-3.5 h during each tidal inundation. The soil surfaces of the entire wetland
155 are exposed at low tide, but the soil remains flooded at some depths. The average
156 annual weight percentage of water in the soil and the soil redox potential are 116%
157 [(soil wet weight- soil dry weight/soil dry weight · 100)] and 12.6 mV, respectively.
158 The average salinity of the tidal water between May and December 2007 was 4.2 ±
159 2.5‰.

160 *S. alterniflora* and *C. malaccensis* are the two dominant species of plants. They
161 are typically found in the upper (mid to high) portions of mudflats. *Spartina* is an
162 invasive plant. The decomposition rates of the litter of *Spartina* are slower than those
163 of *Cyperus* (Tong et al. 2009). Wetland soils in areas dominated by *Spartina* biomass
164 generally have a lower pH and bulk density than do areas dominated by *Cyperus* (Jia
165 et al. 2008). *Cyperus* is a perennial herb that grows from March to September, with
166 the root and some stems remaining during winter. *Spartina* is also a perennial herb. It

167 grows from the April to October, with the root and most stems remaining during
168 winter. We studied and compared three different mono-species stands types: *Cyperus*,
169 the native plant, *Spartina*, the invasive plant (communities more than 10 years old)
170 and regenerated *Cyperus* stands where the invasive *Spartina* was removed three years
171 previously and subsequently planted with *Cyperus*. In regenerated *Cyperus* stands,
172 *Spartina* was removed by cutting the above ground and shallow below ground (0-20
173 cm) plant material, and then the native plant species *Cyperus* was planted in 2009
174 (seedlings 50 cm high with a density is 150 m⁻²). The root systems of the two studied
175 species have similar biomass distribution across soil depth with significant biomass at
176 soil depths layers under 50 cm, but with the higher biomass fraction in the upper 0-15
177 cm of soil layer (Tong et al., 2011).

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179 Sample collection and measurements

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181 Soil samples were collected in July 2012, period of strong growth (Fig. 1). Sampling
182 locations were established in the *Cyperus* (native plant), *Spartina* (invaded more than
183 10 years ago) and regenerated *Cyperus* (three years after removal of *Spartina*)
184 communities. Three plots were randomly selected at each location, and soil profiles
185 (width, 1 m; length, 1 m; depth, 0.6 m) were excavated. Samples were collected with
186 a small sampler (length, 0.3 m; diameter, 0.1 m) from each of six soil layers (0-10,
187 10-20, 20-30, 30-40, 40-50 and 50-60 cm) at the center and both sides of the soil pit.
188 These three samples were bulked to form one sample per layer. A total of 54 soil

189 samples (three plant communities × three plots × six soil layers) were thus collected.
190 In the laboratory, the samples were air-dried, roots and visible plant remains were
191 removed and the samples were finely ground in a ball mill.

192 Total soil organic C was determined by the $K_2Cr_2O_7-H_2SO_4$ digestion method
193 (Sorrell et al. 1997; Bai et al. 2005), total soil N concentration was analyzed by the K
194 370 Kjeldahl method (Buchi Scientific Instruments, Switzerland) and total soil P
195 concentration was determined by perchloric-acid digestion followed by
196 ammonium-molybdate colorimetry and measurement using a UV-2450
197 spectrophotometer (Shimadzu Scientific Instruments, Japan). Soil parameters were
198 also determined. Soil salinity was measured by DDS-307 conductivity (Boqu
199 Scientific Instruments, China), pH was measured with an 868 pH meter (Orion
200 Scientific Instruments, USA), soil particle size was measured by a Master Sizer 2000
201 Laser Particle Size Analyser (Master Scientific Instruments, UK) and soil water
202 content was measured gravimetrically (Lu 1999).

203 Plant samples were collected in May, July, September and December 2012,
204 corresponding to grass buds, stem elongation, budding blossom, and seed maturation
205 stages, in order to capture potential seasonal differences in chemical composition.
206 Most plant growth occurs between April and October, and litter is produced largely
207 toward the end of the growing season into early winter. Plant samples were collected
208 from a consistent height to reduce the potential effects of site-specific confounding
209 variables. We selected stands of the three plant communities for the collection of
210 aboveground biomass, randomly established one large quadrat (10 × 10 m) in each

211 stand and sampled the aboveground biomass from three randomly selected
212 sub-quadrats (1 × 1 m). The harvested aboveground biomass was sorted into living
213 and dead (litter) material. The living and litter fractions were then sorted into stems
214 and leaves. The leaves of *Cyperus* were difficult to collect because they had degraded
215 and fell easily from the plants (Liu et al. 2006) and so had very limited biomass (Zeng
216 et al. 2009b). This material did not represent a major part of the aboveground biomass
217 and so was not collected.

218 Belowground biomass was also harvested from these sample sub-quadrats. All
219 plant material was gently washed with water and then oven-dried to a constant mass
220 (80 °C for 24-36 h) and weighed. The total numbers of analyzed samples of plants and
221 litters were 30 and 24, respectively, for the *Spartina* community and 33 and 15,
222 respectively, for the natural and regenerated *Cyperus* communities.

223 The concentrations of C and N of the plants and litters were determined using a
224 Vario EL III Elemental Analyzer (Elementar Scientific Instruments, Germany). P
225 concentrations of the plants and litters were determined using the molybdate-blue
226 reaction (Lu 1999) with a UV-2450 spectrophotometer (Shimadzu Scientific
227 Instruments, Japan).

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229 Measurements of resorption and growth

230

231 The nutrient resorption efficiency (NRE) was estimated as the percentage of N
232 withdrawn from all green biomass before abscission:

233 $NRE = 100\% \times [(N_{\text{biomass}} - N_{\text{litter}}) / N_{\text{biomass}}]$

234 where N_{biomass} and N_{litter} are the concentrations of N in all biomass and litter,
235 respectively (Huang et al. 2008).

236 Absolute growth rate (AGR) is the increase in biomass over time regardless of
237 plant size, whereas the relative growth rate (RGR) is the rate of biomass increase per
238 unit size and time. Its units are mass per mass and time:

239 $RGR = 1/B \cdot (dB/dt) = (Ln B_2 - Ln B_1) / t_2 - t_1$

240 where B is the dry weight of the biomass. We thus calculated RGR and AGR by the
241 formulae (Foster and Gross 1997; Zhang et al. 2008):

242 $RGR = (Ln B_{i+1} - Ln B_i) / (t_{i+1} - t_i)$

243 $AGR = (B_{i+1} - B_i) / (t_{i+1} - t_i)$

244 where t_i is the collection time and B_i and B_{i+1} are the biomasses at times t_i and t_{i+1} .

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246 Data analysis

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248 We calculated average C, N and P concentrations and C:N, C:P and N:P ratios (on a
249 molar basis) of the live plants, litters and soils and performed two-way analyses of
250 variance (ANOVAs) to compare the concentrations and ratios among the three plant
251 communities and six soil depths. We analyzed the Pearson correlation coefficients
252 between soil parameters (pH, salinity and water content), total soil C, N and P
253 concentrations and total soil C:N, C:P and N:P ratios. All univariate analyses were
254 performed using SPSS 13.0 (SPSS Inc., Chicago, USA).

255 We used discriminant function analysis (DFA) to determine the impacts of the
256 various plots on overall soil elemental composition (total soil C, N and P
257 concentrations and total soil C:N, C:P and N:P ratios) and to discriminate between the
258 effects of climate and taxonomy (including differences at the species level) on the
259 elemental concentrations, stoichiometries and allocations between leaves and wood.
260 DFA is a supervised statistical algorithm that derives an optimal separation between
261 groups established a priori by maximizing between-group variance while minimizing
262 within-group variance (Raamsdonk et al. 2001). DFA is thus an adequate tool for
263 identifying the variables most responsible for the differences among groups. The
264 DFAs were performed using Statistica 6.0 (StatSoft, Inc. Tule, Oklahoma, USA).

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277 **Results**

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279 Effect of plant invasion and removal on soil C, N and P concentrations and
280 stoichiometries

281

282 The concentrations of total soil C, N and P concentrations were positively correlated
283 ($P < 0.05$) (Fig. S1A-C). The C, N and P concentrations generally varied with soil
284 depth, *Spartina* invasion and removal and the interaction of soil depth with *Spartina*
285 invasion and removal ($P < 0.01$, Table 1, Figs. S2A-C); P concentrations were not
286 significantly affected by the interaction of soil depth with plant invasion and removal.

287 Soil C and N concentrations were generally higher in the *Spartina* community than in
288 the natural and regenerated *Cyperus* communities ($P < 0.01$, Table 2). Soil P
289 concentration was lower in the regenerated *Cyperus* community than in the *Spartina*
290 and natural *Cyperus* communities ($P < 0.01$).

291 The C:N ratios varied significantly with soil depth ($P < 0.01$, Table 1, Fig. S3A)
292 similarly in all communities. The C:P and N:P ratios also varied significantly with soil
293 depth ($P < 0.01$, Table 1, Figs. S3B and S3C). Soil C:P and N:P ratios were
294 significantly lower in the natural *Cyperus* community than in the *Spartina* and
295 regenerated *Cyperus* communities ($P < 0.01$, Table S1).

296

297 Effect of plant invasion and removal on soil parameters

298

299 Soil pH and salinity were significantly lower in the *Spartina* community than in the
300 natural and regenerated *Cyperus* communities ($P < 0.01$) (Table 1 and S1, Figs. 2 and
301 S4A, 4C). Soil water content did not differ significantly among the three communities
302 ($P > 0.05$, Table 1 and S1, Fig. S4B), but soil clay content did ($P < 0.01$, Table 1 and
303 S1, Fig. S4D).

304

305 Effects of soil parameters on total soil C, N and P concentrations and stoichiometries

306

307 In all three communities, total soil C and N concentrations were negatively correlated
308 with pH, and total soil P concentration was negatively correlated with salinity. The
309 C:N ratio was positively correlated with salinity, and the C:P ratio was correlated
310 negatively with pH and positively with salinity. The N:P ratio was correlated
311 negatively with pH and positively with water content (Table 3).

312

313 Effects of seasonality and plant invasion and regeneration on plant C, N and P
314 concentrations and stoichiometries

315

316 The C concentrations of foliar, stems, litters and roots varied with season ($P < 0.05$,
317 Figs. S2, S5, S6 and S6, Table 4). Stem C concentrations were higher in *Spartina* than
318 in *Cyperus* ($P < 0.05$). Stem N concentrations varied with season, and N
319 concentrations were lower in stems and higher in litter in *Spartina* than in the native
320 species ($P < 0.05$). P stem and litter concentrations varied with season, and the P

321 concentrations of stems and roots were higher in the natural *Cyperus* stands than in
322 *Spartina* ($P < 0.05$).

323 Stem and root C:N ratios were lower and N:P ratios were higher in spring ($P <$
324 0.05 , Figs. S6 and S7, Table 5). The stem C:N ratio was higher in *Spartina* than in the
325 native species ($P < 0.05$). Stem and litter N:P ratios were lower in the natural *Cyperus*
326 community than in the regenerated community and in *Spartina* ($P < 0.05$).

327

328 N and P resorption

329

330 The average seasonal rates of N resorption for natural and regenerated *Cyperus* and
331 for *Spartina* were $16.3 \pm 5.7\%$, $23.2 \pm 6.2\%$ and $57.2 \pm 3.3\%$, respectively, and the
332 rates of P resorption were $45.0 \pm 8.0\%$, $39.4 \pm 7.0\%$ and $55.3 \pm 8.4\%$, respectively.
333 The rates of both N and P resorption were thus higher for *Spartina* than for natural
334 and regenerated *Cyperus*, particularly for N ($P < 0.05$, Fig. 3).

335

336 Growth rate

337

338 The average seasonal RGRs for natural and regenerated *Cyperus* and for *Spartina*
339 were 0.0035 ± 0.0004 , 0.0023 ± 0.0003 and $0.0010 \pm 0.0003 \text{ g g}^{-1}\text{d}^{-1}$, respectively.
340 The RGRs were higher for both natural and regenerated *Cyperus* than for *Spartina*,
341 and the RGR was higher for natural than for regenerated *Cyperus* ($P < 0.05$, Fig. 4A).

342 The average seasonal AGRs for natural and regenerated *Cyperus* and for *Spartina*

343 were 1.35 ± 0.66 , 2.08 ± 0.76 and $4.84 \pm 1.17 \text{ g m}^{-2}\text{d}^{-1}$, respectively. The AGRs were
344 lower for both natural and regenerated *Cyperus* than for *Spartina* ($P < 0.05$, Fig. 4B)
345 but did not differ significantly between natural and regenerated *Cyperus* ($P > 0.05$).

346

347 Litter production

348

349 The total annual litter productions for natural and regenerated *Cyperus* and for
350 *Spartina* and were 747 ± 62 , 646 ± 53 and $653 \pm 41 \text{ g m}^{-2}$, respectively. The litter
351 production was higher for natural *Cyperus* than for regenerated *Cyperus* and *Spartina*
352 ($P < 0.05$, Fig. 5) but did not differ significantly between regenerated *Cyperus* and
353 *Spartina* ($P > 0.05$).

354

355 Multivariate analysis

356

357 The multivariate analysis confirmed the overall differences in soil properties and in
358 plant elemental compositions among the three communities. The differences between
359 the invaded stands and the natural and regenerated native stands were larger than the
360 differences between the natural and regenerated native stands (Fig. 6). The DFAs of
361 the soil parameters identified differences in N concentration, salinity, soil water
362 content, clay content and pH among the three communities (Table 6). The squared
363 Mahalanobis distances between *Spartina* and natural *Cyperus*, regenerated *Cyperus*
364 and natural *Cyperus* and *Spartina* and regenerated *Cyperus* were $F = 5.18$ ($P <$

365 0.0019), $F = 4.21$ ($P < 0.001$) and $F = 16.2$ ($P < 0.001$), respectively. In a PCA of
366 plant elemental compositions and soil parameters in the samples collected in July, the
367 first PC axis separated invasive *Spartina* stands from both natural ($P < 0.001$) and
368 regenerated ($P < 0.0001$) *Cyperus* stands by higher soil C, N and P concentrations,
369 higher soil N:P and C:P ratios and higher stem C concentrations and C:N and C:P
370 ratios. The natural *Cyperus* stands, however, were significantly separated ($P < 0.0001$)
371 from the regenerated stands mainly due to higher N:P ratios in stems and litter in the
372 regenerated stands.

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387 **Discussion**

388 The invasive species (*Spartina*) had higher C:N, C:P and N:P ratios than the native
389 species difference that is consistent with the observed higher capacity of the invasive
390 species to resorb N and thus to have a more conservative use and use efficiency than
391 the native species. The invasive species had higher litter N concentrations but
392 produced more biomass and much less litter than the native species. The invasive
393 species consequently lost less N in its litter than the native species (Figure 7).
394 Moreover, by having higher N resorption it had more total N content and lost a much
395 lower proportion of N of its total stocks than the native species and produced much
396 more new biomass per unit of N lost. These results indicated a much more efficient
397 use of N, the limiting nutrient (Wang et al., 2014), in the invasive than in the native
398 species. Interspecific differences in the C:N, C:P and N:P ratios may likely reflect
399 differences in plant morphology, nutrient-use efficiency and photosynthetic capacity
400 between the Poaceae (*Spartina*) and Cyperaceae (*Cyperus*) plants. The lower N losses
401 by litter together with the higher N in soils suggest slower N mineralization rates in
402 soils under the invasive species *Spartina* than in soils under *Cyperus* (Figure 7) such
403 as observed by Tong et al. (2009).

404 The C:N ratios of the litter were strongly correlated with the rates of litter
405 decomposition in the communities, with lower C:N ratios usually associated with
406 higher rates of decomposition (Windham 2001). The litter C:N ratios of *Spartina* were
407 higher than those of *Cyperus*. These results are consistent with the low rates of litter
408 decomposition in the Minjiang River estuary (Tong and Liu 2009) and with the

409 negative correlation between rate of decomposition of soil C and the C:N ratio in this
410 estuary (Wang et al. 2010b). Our results thus support the C:N ratio as an indicator of
411 litter and organic-matter decomposition (Elser et al. 2003) and suggest that the rates
412 of litter decomposition can be lower in invaded than in native stands (Tong and Liu
413 2009). The C:P and N:P ratios were lower in the native plants than in the invasive
414 plants in summer (the growing season), with a consistently higher RGR for *Cyperus*
415 than for the invasive *Spartina*. The RGRs of *Cyperus* and *Spartina* were 0.004 and
416 0.001 g g⁻¹ d⁻¹, respectively. Lower C:P and N:P ratios have been associated with
417 higher growth rates (Elser et al. 2003; Peñuelas et al. 2013). Conversely, AGR (the
418 new total biomass produced per unit time) was higher in the invasive species,
419 coinciding with its much higher biomass (allowing a lower RGR), higher N
420 concentrations and contents and lower losses of N in the litter, all indicating a high
421 retention and conservative use of N in the invasive species.

422 The invasive plant species in our study thus grows more slowly than the native
423 species (Zhang et al. 2008; Zeng et al. 2009a; Zeng et al. 2009b), with low C and N
424 turnovers. The lower litter production and the trend to lower respiration rates in
425 *Spartina* than in the native *Cyperus* observed in other studies (Tong et al. 2014) are
426 also consistent with the lower RGR of the invasive species and the more conservative
427 strategy of stress tolerance of *Spartina* than of *Cyperus*. Most studies in environments
428 with no limitations of resources such as water, light or nutrients generally find that
429 plant invasion is frequently dependent on higher rates of nutrient uptake and cycling
430 (Sardans and Peñuelas 2012). The strategy for plant success in terrestrial

431 environments where at least one important resource is clearly limiting has not been
432 clearly defined, but despite the low number of studies and frequent contradictory
433 results, most studies suggest that a more conservative use, higher uptake and storage
434 capacity of the limiting resource underlie plant success (Funk and Vitousek 2007;
435 Sardans and Peñuelas 2012).

436 The soil of the *Spartina* community had lower clay content, related to the high
437 capacity of the community to trap larger sediments, which can improve soil aeration
438 during the periods between flooding and could explain the lower salinity, lower
439 capacity to retain salts and higher drainage capacity of the soil. These factors can also
440 contribute to improving the capacity of the plants to take up N by generating more
441 favorable conditions for root activity by more equilibrate soil texture, allowing for
442 example higher capacity of soil enzyme activity in conditions of better soil ventilation
443 (Renella et al., 2006; Vasconcellos et al., 2013). Lower clay content probably allows
444 to better mixing of litter with soil preventing litter losses with tidal water fluxes
445 favoring higher organic soil C concentrations such as been observed.

446 The average N:P ratios (on a molar basis) were 28.7 ± 5.1 and 16.2 ± 1.7 for
447 *Spartina* (leaves, stems and roots) and *Cyperus* (stems and roots), respectively, which
448 were higher than the average N:P ratios (14.8-15.9) of terrestrial and aquatic plants
449 and algae in their natural environments (Elser et al. 2000; Güsewell and Koerselman
450 2002; Geider and La Roche 2002; Knecht and Göransson 2004). The foliar N:P ratio
451 is often used to represent nutrient limitation during plant growth (Tessier and Raynal
452 2003; Wang and Yu 2008), and a high N:P ratio suggests that P can be also limiting

453 (the foliar N:P ratio was 38.3 for *Spartina*). In contrast, the wetland soils of our study
454 had particularly low N:P ratios (4.1-4.3 on a molar basis) compared to the soils from
455 other ecosystems (Cleveland and Liptzin 2007; Tian et al. 2010), indicating that the
456 limiting nutrient was N in the soil of this wetland area (Fig. 3). A high N:P ratio has
457 also been observed in the invasive plant *Phragmites australis* in an area near the
458 Minjiang estuary (Wang et al. 2014). The N:P ratio and N-resorption capacity were
459 higher in this invasive species than in the native species, and the soils had lower N:P
460 ratios.

461 Nutrient limitation is especially significant in tidal wetlands, likely because the
462 periodic inundation of the soil limits the access of the plants to the soil nutrients by
463 slowing mineralization (Adame et al. 2010), by the anoxic effects on root growth
464 (Amlin and Rood 2001; Kirwan and Guntenspergen 2012) and by high levels of
465 leaching of P and particularly of N (Noe and Hupp 2007; Kobayashi et al. 2009).
466 Subtropical zones have high precipitation and temperatures that favor the erosion and
467 loss of N and P, which can also limit nutrient levels (Olde et al. 2003; Tian et al.
468 2010).

469 To summarize, we found lower N and P concentrations in soils than in plants in
470 the tidal estuarine wetlands of the Minjiang River, indicating that plants retain
471 nutrients, especially N. We also observed higher N:P ratios in the plants than in the
472 soils. *Spartina* was more efficient than the native *Cyperus* in storing more N (the
473 limiting nutrient) in the biomass, in accordance with its invasive success. These
474 results are consistent with the few previous similar studies, indicating that the success

475 of invasive plants in nutrient-poor soils depends on conservative strategies, such as
476 the more efficient use, storage and retention of the limiting resource (Funk and
477 Vitousek 2007; González et al. 2010; Matzek 2011; Wang et al. 2014), allowing
478 longer nutrient residence times (Laungani and Knops 2009). Notably, our results
479 clearly linked plant N:P ratios with growth rates. The results of this study are
480 consistent with the growth rate hypothesis, with a clear relationship between low N:P
481 ratio and high RGR, indicating that the new biomass produced relative to the total
482 plant biomass is associated with lower N:P ratios but not with AGR, which should
483 also depend on the turnover of biomass and on resource-use efficiency. All these
484 results are also consistent with the higher litter production of the invasive *Spartina*
485 than of the native *Cyperus*.

486 *Cyperus* replanted after the removal of *Spartina* had soil and plant elemental
487 compositions different than those for the natural *Cyperus* community. These
488 differences were mainly due to the higher stem and litter N:P ratios and lower RGR in
489 the regenerated than in the natural *Cyperus* community. The shift toward higher soil
490 and root N:P ratios in the invaded community may thus be associated with the
491 subsequent higher stem and litter N:P ratios and lower RGR in the regenerated
492 relative to the natural *Cyperus* community. Moreover, soil P is lower in *Cyperus*
493 replanted than in the natural *Cyperus* community, likely as a result of the lower
494 concentration of P in the litter of *Cyperus* replanted than in the natural *Cyperus*
495 community. Invasion shifted the overall plant-soil nutrient concentrations,
496 distributions and stoichiometries, especially those linked to N, and these shifts further

497 influenced the plant-soil nutrient status and limited the RGR of the native species in
498 the early to middle stages of the regeneration of the native species.

499

500 **Conclusions**

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502 The nutrient compositions and stoichiometries in the plants, litter and soils, the great
503 N resorption and previous studies (Wang et al., 2014) indicated that N was the
504 limiting factor in this tidal estuarine wetland. The success of plant invasion under
505 these environmental conditions was related to a low RGR and to a high capacity to
506 resorb, store and efficiently use nutrients, in this case N. Plant invasion was thus
507 associated with a more conservative use of nutrients, as suggested by other studies
508 under conditions of nutrient limitation. RGR was associated with lower plant N:P
509 ratios, whereas AGR was associated with higher nutrient-use efficiency and lower C
510 and N turnover and storage capacities in the biomass. The physical removal of the
511 invasive species and restoration with a native species tended to reestablish the soil
512 properties to some extent, but some significant differences remained between the
513 natural and regenerated communities three years after the removal of the invasive
514 plants, indicating that the presence of the invasive plants had changed the soil
515 properties and affected the regeneration.

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762 **Tables**

763 **Table 1** Summary of factorial ANOVAs of the effects of plant invasion and removal and soil depth
764 on soil nutrient concentrations, stoichiometry and soil parameters.

	df	F	P
C concentration			
Soil depth	5,36	7.198	<0.001
Plant invasion and removal	2,36	8.319	0.002
Plant invasion and removal × Soil depth	10,36	3.431	0.003
N concentration			
Soil depth	5,36	27.187	<0.001
Plant invasion and removal	2,36	5.609	0.008
Plant invasion and removal × Soil depth	10,36	4.635	<0.001
P concentration			
Soil depth	5,36	42.395	<0.001
Plant invasion and removal	2,36	14.691	<0.001
Plant invasion and removal × Soil depth	10,36	1.715	0.115
C:N ratio			
Soil depth	5,36	8.664	<0.001
Plant invasion and removal	2,36	1.262	0.295
Plant invasion and removal × Soil depth	10,36	0.896	0.546
C:P ratio			
Soil depth	5,36	7.474	<0.001
Plant invasion and removal	2,36	4.327	0.021
Plant invasion and removal × Soil depth	10,36	3.154	0.005
N:P ratio			
Soil depth	5,36	5.405	0.001
Plant invasion and removal	2,36	3.705	0.034
Plant invasion and removal × Soil depth	10,36	4.504	<0.001
pH			
Soil depth	5,36	0.568	0.724
Plant invasion and removal	2,36	11.611	<0.001
Plant invasion and removal × Soil depth	10,36	0.995	0.465
Water content			
Soil depth	5,36	0.588	0.709
Plant invasion and removal	2,36	0.341	0.713
Plant invasion and removal × Soil depth	10,36	1.301	0.267
Salinity			
Soil depth	5,36	3.963	0.006
Plant invasion and removal	2,36	6.301	0.005
Plant invasion and removal × Soil depth	10,36	0.630	0.778
Clay content			
Soil depth	5,36	7.830	<0.001
Plant invasion and removal	2,36	41.322	<0.001
Plant invasion and removal × Soil depth	10,36	5.349	<0.001

765 **Table 2** Soil (average of soil depths) C, N and P (mean \pm S.E.) concentrations (mg g^{-1}) in the three
766 communities.

Nutrient	Natural <i>C. malaccensis</i>	<i>S. alterniflora</i>	Regenerated <i>C. malaccensis</i>
C	20.9 \pm 1.0 b	23.4 \pm 2.0a	21.2 \pm 1.6b
N	1.27 \pm 0.06 a	1.37 \pm 0.12b	1.26 \pm 0.08a
P	0.69 \pm 0.04 a	0.70 \pm 0.03a	0.64 \pm 0.03a

767 Different letters within a row indicate significant differences ($P < 0.05$).

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800 **Table 3** Pearson correlation coefficients of soil nutrient concentrations and ratios with soil
 801 parameters.

Community	Index	pH	Water content	Salinity	Clay content
Natural <i>C. malaccensis</i> (n = 18)	[C]	-0.422	0.585*	-0.113	0.855**
	[N]	-0.391	0.472*	-0.409	0.664**
	[P]	-0.084	0.287	-0.404	0.400
	C:N	0.154	-0.067	0.501*	-0.102
	C:P	-0.288	0.269	0.328	0.384
<i>S. alterniflora</i> (n = 18)	[C]	-0.484*	0.400	-0.166	0.559*
	[N]	-0.233	0.746**	0.453	0.051
	[P]	-0.299	0.729**	0.118	0.000
	[P]	-0.356	0.334	-0.112	-0.093
	C:N	0.185	-0.092	0.651**	0.118
Regenerated <i>C. malaccensis</i> (n = 18)	C:P	-0.060	0.715**	0.605**	0.127
	N:P	-0.195	0.817**	0.204	0.058
	[C]	-0.680**	-0.031	0.559*	-0.238
	[N]	-0.259	0.388	-0.156	-0.070
	[P]	0.478*	0.272	-0.690**	-0.011
Total (n = 54)	C:N	-0.511*	-0.345	0.745**	-0.174
	C:P	-0.706**	-0.154	0.769**	-0.096
	N:P	-0.724**	0.018	0.655**	-0.010
	[C]	-0.453**	0.207	0.140	-0.004
	[N]	-0.356**	0.192	-0.167	0.073
	[P]	-0.082	-0.031	-0.469**	-0.063
	C:N	-0.117	-0.004	0.495**	-0.110
	C:P	-0.359**	0.225	0.493**	0.086
	N:P	-0.369**	0.285*	0.225	0.191

802 * significant at $P < 0.05$, ** significant at $P < 0.01$

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818 **Table 4** C, N and P concentrations in plant tissues and litter.

Community	Sample	Element	Mean \pm S.E. (mg g ⁻¹)
Natural <i>C. malaccensis</i>	Leaf	C	—
	Stem	C	396.0 \pm 5.2
	Root	C	363.1 \pm 19.1
	Litter	C	385.3 \pm 6.6
<i>S. alterniflora</i>	Leaf	C	406.8 \pm 5.9
	Stem	C	408.8 \pm 14.2
	Root	C	357.8 \pm 9.4
	Litter	C	377.1 \pm 17.6
Regenerated <i>C. malaccensis</i>	Leaf	C	—
	Stem	C	395.7 \pm 5.3
	Root	C	381.3 \pm 6.1
	Litter	C	388.7 \pm 2.4
Natural <i>C. malaccensis</i>	Leaf	N	—
	Stem	N	12.09 \pm 1.53
	Root	N	7.78 \pm 0.18
	Litter	N	10.56 \pm 0.46
<i>S. alterniflora</i>	Leaf	N	17.49 \pm 1.81
	Stem	N	9.97 \pm 5.47
	Root	N	7.35 \pm 0.34
	Litter	N	11.30 \pm 2.34
Regenerated <i>C. malaccensis</i>	Leaf	N	—
	Stem	N	12.43 \pm 2.07
	Root	N	8.45 \pm 0.88
	Litter	N	10.17 \pm 0.89
Natural <i>C. malaccensis</i>	Leaf	P	—
	Stem	P	1.90 \pm 0.22
	Root	P	1.05 \pm 0.15
	Litter	P	1.01 \pm 0.13
<i>S. alterniflora</i>	Leaf	P	1.15 \pm 0.18
	Stem	P	0.99 \pm 0.34
	Root	P	0.91 \pm 0.21
	Litter	P	0.83 \pm 0.06
Regenerated <i>C. malaccensis</i>	Leaf	P	—
	Stem	P	1.13 \pm 0.15
	Root	P	0.99 \pm 0.16
	Litter	P	0.86 \pm 0.07

Factorial ANOVA statistics	Stem	Litter	Root
C			
Season	F = 19.6 P < 0.001	F = 16.2 P < 0.001	F = 3.09 P = 0.046
Plant invasion and removal	F = 9.19 P = 0.001	F = 2.96 P = 0.07	F = 2.00 P = 0.16

Season × Plant invasion and removal	$F = 11.2$ $P < 0.001$	$F = 6.92$ $P < 0.001$	$F = 1.70$ $P = 0.17$
N			
Season	$F = 119$ $P < 0.001$	$F = 17.5$ $P < 0.001$	$F = 1.43$ $P = 0.26$
Plant invasion and removal	$F = 8.13$ $P = 0.002$	$F = 1.63$ $P = 0.22$	$F = 2.13$ $P = 0.14$
Season × Plant invasion and removal	$F = 24.2$ $P < 0.001$	$F = 7.32$ $P < 0.001$	$F = 2.49$ $P = 0.052$
P			
Season	$F = 21.6$ $P < 0.001$	$F = 0.57$ $P = 0.64$	$F = 4.29$ $P = 0.014$
Plant invasion and removal	$F = 94.4$ $P < 0.001$	$F = 3.91$ $P = 0.034$	$F = 0.53$ $P = 0.60$
Season × Plant invasion and removal	$F = 25.6$ $P < 0.001$	$F = 4.56$ $P = 0.003$	$F = 4.49$ $P = 0.052$

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846 **Table 5** C, N and P ratios in plant tissues and litter.

Community	Sample	Ratio	Mean \pm S.E. (mg g ⁻¹)
Natural <i>C. malaccensis</i>	Leaf	C:N	—
	Stem	C:N	40.4 \pm 5.2
	Root	C:N	55.0 \pm 3.5
	Litter	C:N	43.3 \pm 2.3
<i>S. alterniflora</i>	Leaf	C:N	28.1 \pm 2.6
	Stem	C:N	89.1 \pm 25.0
	Root	C:N	58.3 \pm 3.8
	Litter	C:N	44.3 \pm 8.4
Regenerated <i>C. malaccensis</i>	Leaf	C:N	—
	Stem	C:N	40.4 \pm 5.4
	Root	C:N	55.6 \pm 5.7
	Litter	C:N	47.2 \pm 4.4
Natural <i>C. malaccensis</i>	Leaf	C:P	—
	Stem	C:P	564 \pm 59
	Root	C:P	1006 \pm 164
	Litter	C:P	1070 \pm 184
<i>S. alterniflora</i>	Leaf	C:P	1028 \pm 140
	Stem	C:P	1574 \pm 563
	Root	C:P	1197 \pm 171
	Litter	C:P	1253 \pm 54
Regenerated <i>C. malaccensis</i>	Leaf	C:P	—
	Stem	C:P	983 \pm 131
	Root	C:P	1151 \pm 255
	Litter	C:P	1212 \pm 107
Natural <i>C. malaccensis</i>	Leaf	N:P	—
	Stem	N:P	14.2 \pm 0.8
	Root	N:P	18.3 \pm 2.9
	Litter	N:P	24.6 \pm 3.6
<i>S. alterniflora</i>	Leaf	N:P	38.3 \pm 8.6
	Stem	N:P	27.2 \pm 120
	Root	N:P	20.7 \pm 3.4
	Litter	N:P	32.4 \pm 7.5
Regenerated <i>C. malaccensis</i>	Leaf	N:P	—
	Stem	N:P	27.3 \pm 7.9
	Root	N:P	22.6 \pm 6.1
	Litter	N:P	27.3 \pm 4.4

Factorial ANOVA statistics	Stem	Litter	Root
C:N			
Season	$F = 31.2$ $P < 0.001$	$F = 10.1$ $P = 0.002$	$F = 3.18$ $P = 0.042$
Plant invasion and removal	$F = 60.7$	$F = 0.84$	$F = 0.45$

	$P < 0.001$	$P = 0.44$	$P = 0.64$
Season × Plant invasion	$F = 10.6$	$F = 4.86$	$F = 2.88$
and removal	$P < 0.001$	$P = 0.002$	$P = 0.029$
C:P			
Season	$F = 27.4$	$F = 0.92$	$F = 5.63$
	$P < 0.001$	$P = 0.45$	$P = 0.0046$
Plant invasion and removal	$F = 79.1$	$F = 1.51$	$F = 1.42$
	$P < 0.001$	$P = 0.24$	$P = 0.26$
Season × Plant invasion	$F = 38.1$	$F = 3.52$	$F = 5.86$
and removal	$P < 0.001$	$P = 0.012$	$P < 0.001$
N:P			
Season	$F = 63.7$	$F = 13.6$	$F = 7.12$
	$P < 0.001$	$P < 0.001$	$P = 0.0014$
Plant invasion and removal	$F = 29.8$	$F = 5.40$	$F = 1.05$
	$P < 0.001$	$P = 0.012$	$P = 0.36$
Season × Plant invasion	$F = 22.8$	$F = 8.47$	$F = 2.83$
and removal	$P < 0.001$	$P < 0.001$	$P = 0.032$

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873 **Table 6** Statistics (Wilks' λ and P) of the discriminant functional analysis of the soils with pH;
874 salinity; depth; moisture and clay contents; total C, N and P concentrations and C:N, C:P and N:P
875 ratios as variables. Bold type indicates a significant effect of the variable in the model ($P < 0.05$).

	Wilks' λ	F	P
[C]	0.941	1.16	0.33
[N]	0.799	4.07	0.014
[P]	0.951	0.947	0.40
pH	0.612	11.7	0.0001
Water content	0.797	4.72	0.015
Salinity	0.604	12.1	<0.0001
Clay content	0.702	7.87	0.0014
C:N ratio	0.776	4.62	0.0086
C:P ratio	0.993	0.138	0.87
N:P ratio	0.963	0.708	0.50
Depth	0.678	1.59	0.13

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Figure captions

Fig. 1 Location of the sampling sites.

Fig. 2 Comparison of average pHs (mean \pm S.E.) at the various soil depths in the three communities. Different letters indicate significant differences between communities ($P < 0.05$).

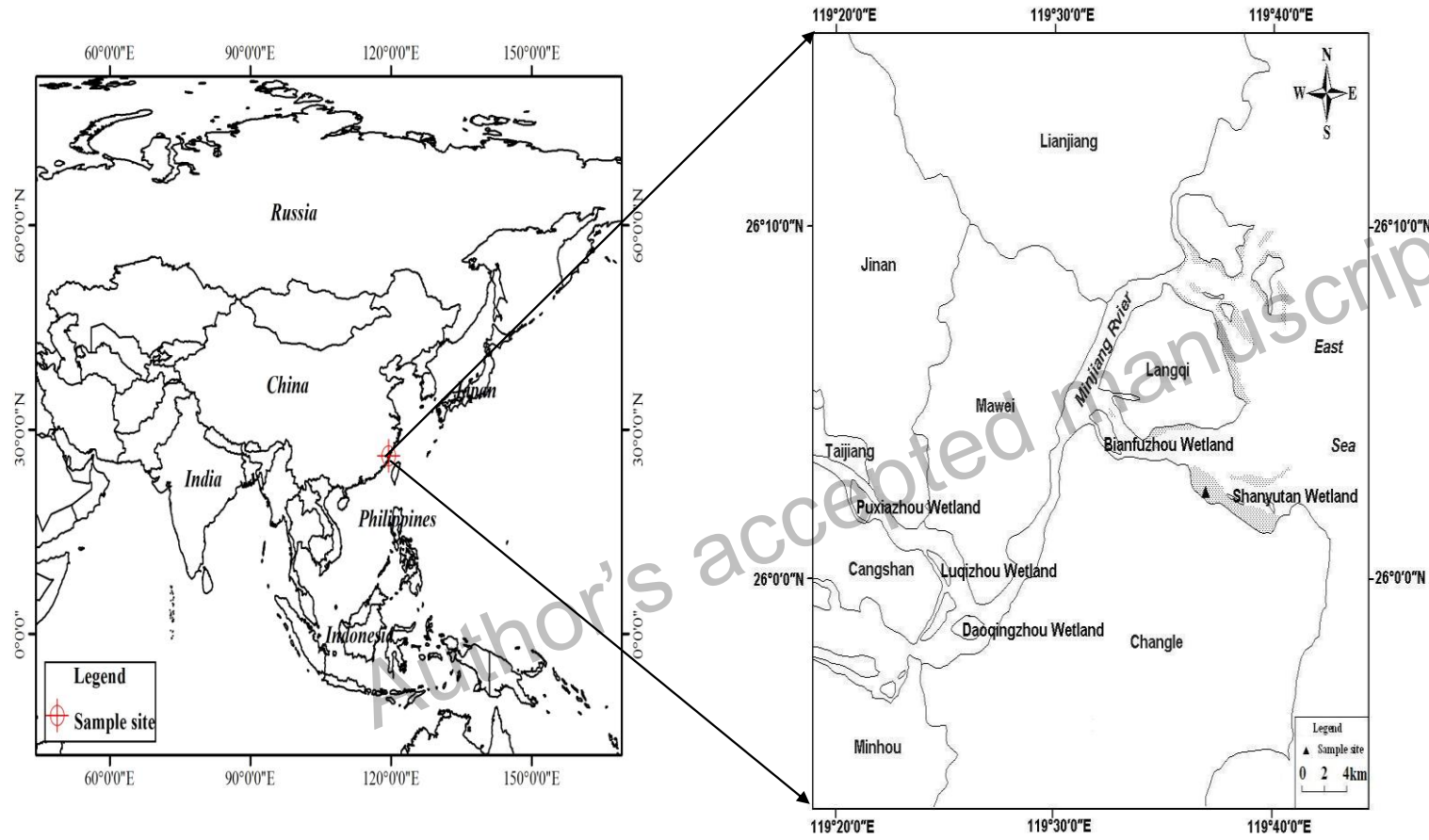
Fig. 3 Nutrient-resorption rates (mean \pm S.E.) for N and P in the three communities. Different letters indicate significant differences between communities ($P < 0.05$).

Fig. 4 Relative (A) and absolute (B) growth rates (mean \pm S.E.) in the three communities. Different letters indicate significant differences between communities ($P < 0.05$).

Fig. 5 Annual litter production in the three communities. Different letters indicate significant differences between communities ($P < 0.05$).

Fig. 6 Biplots of the PCAs conducted with soil, litter, root and stem data for July (summer) as variables for the natural *Cyperus* community (C), invasive *Spartina* community (S) and regenerated *Cyperus* community after removal of invasive *Spartina* (CR). Arrows indicate significant differences of the PC scores ($P < 0.05$) among the communities.

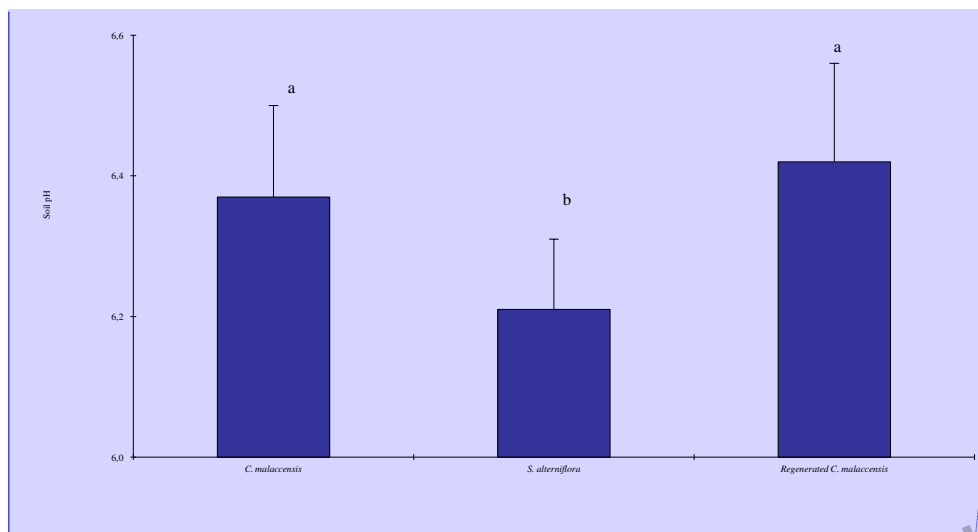
Fig. 7 N-cycle in plant-soil system in native *Cyperus* stands and in invasive *Spartina* stands.



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2 **Fig. 1**

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Comentari [j1]: Please weiqi Figures from 2 to 5 make considerable greater the legends of the axes "X" and "Y" in this case "Soil pH" "*C. malaccensis*" and so on.

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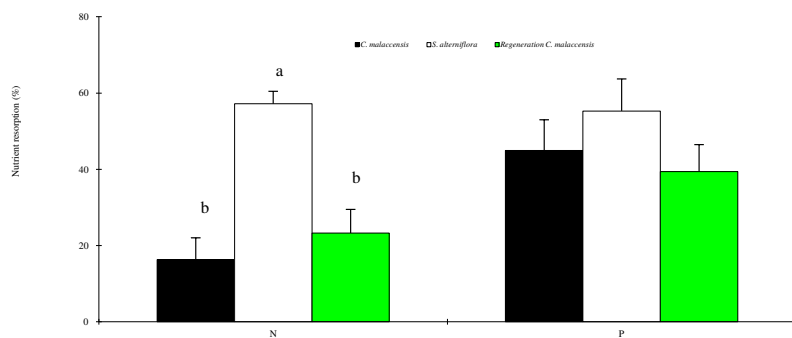
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Fig. 2

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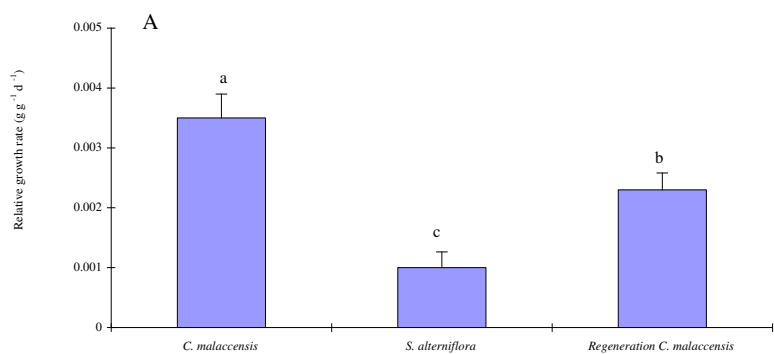


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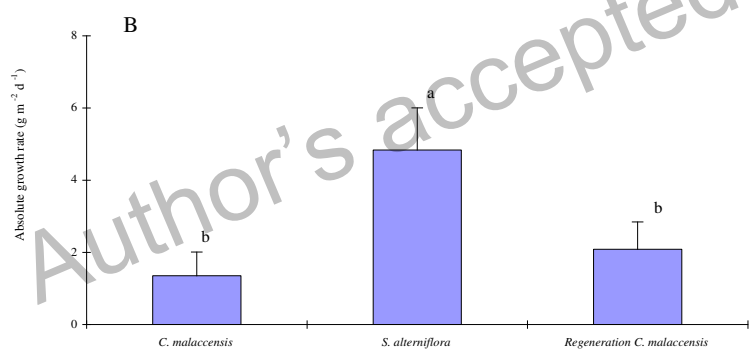
Fig. 3

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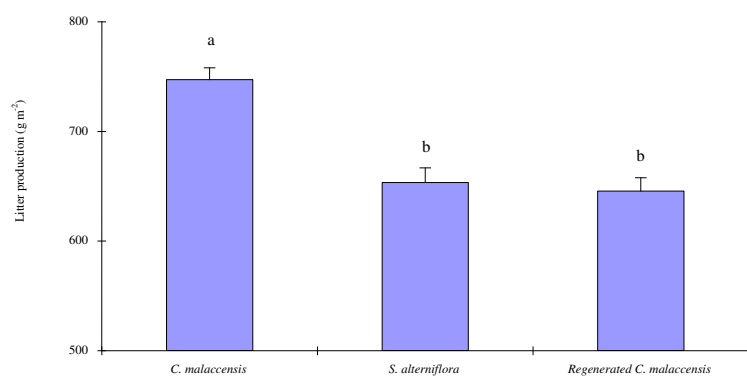
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Fig. 4

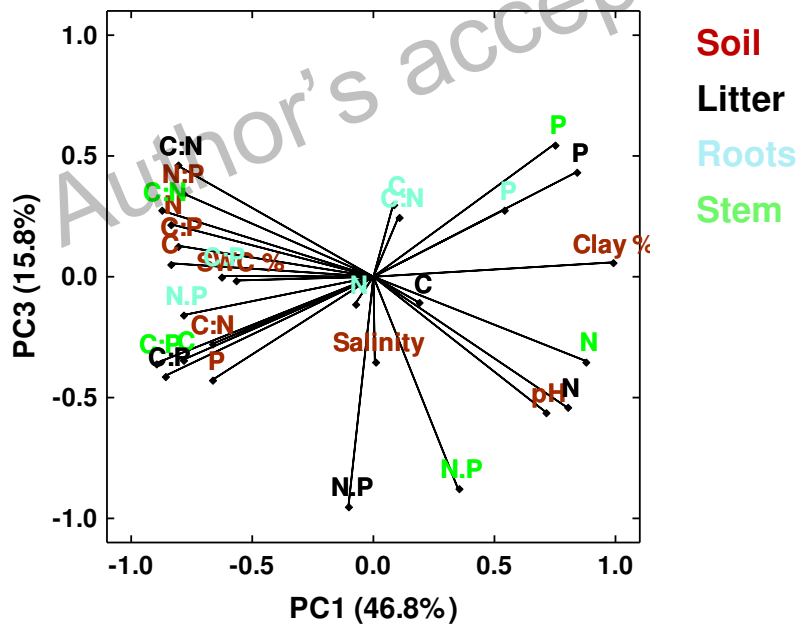
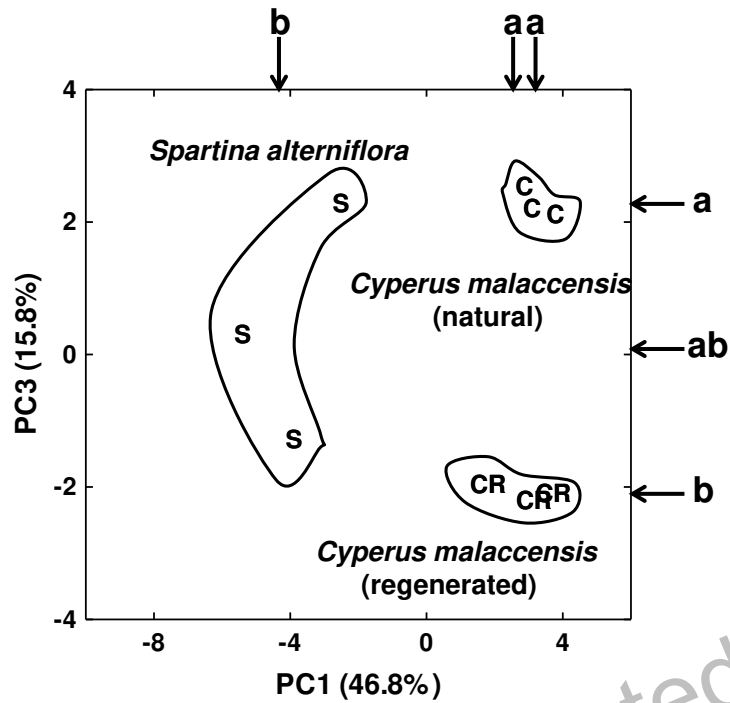
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Fig. 5

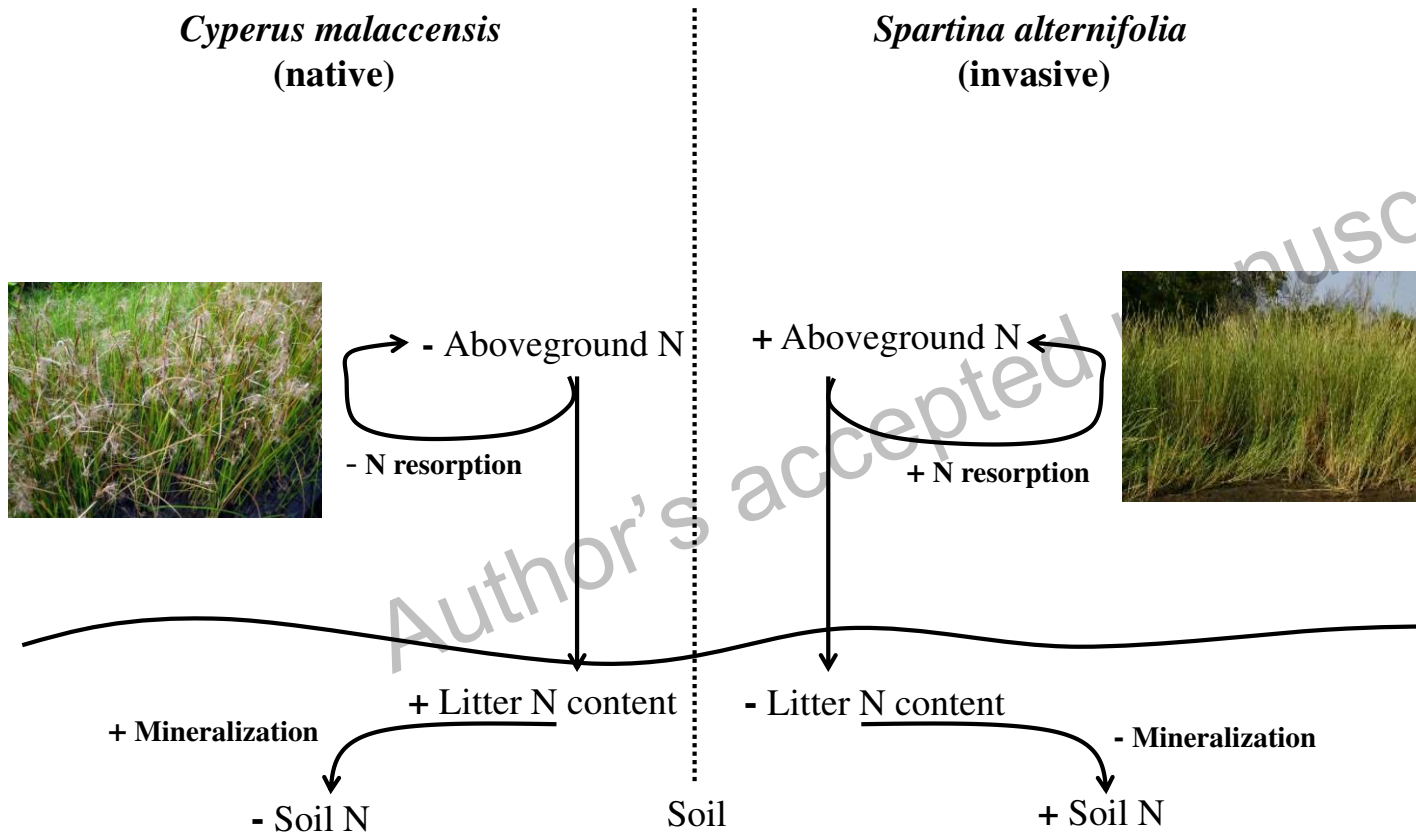
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89 Fig. 6

Comparison of plant-soil N cycle in invaded and native stands



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91 **Figure 7**