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

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Abstract The tidal estuarine wetlands of China are rich in plant diversity, but several human-driven processes, such as species invasion, can affect the biogeochemical cycles of these ecosystems, and by changing soil conditions can inhibit the regeneration of native vegetation. We seasonally analyzed the carbon (C), nitrogen (N) and phosphorus (P) concentrations in soils and in leaves, stems and roots of the invasive species *Spartina alterniflora* and of the native species *Cyperus malaccensis* var. *brevifolius* Boeckeler. This latter species was analyzed both in natural non-invaded stands and in stands that had been invaded by *Spartina* but from which it had been removed and replaced by *Cyperus*. The aim was to investigate the effect of plant invasion, subsequent removal and replanting with a native species on C, N and P stoichiometry of the plant-soil system in the tidal wetlands of the Minjiang River. C and N concentrations averaged across seasons did not differ significantly among the plant species. P concentration was lower in the stems of *Spartina* than in the stems of the native species *Cyperus* but was not significantly different in the roots of the two species. The soil C and N concentrations were higher in the *Spartina* stand than in the *Cyperus* stand, whereas the soil P concentrations were not significantly different. The invasive species had a higher N-resorption capacity, N:P ratios in stem and roots, biomass, absolute growth and biomass N and had a lower relative growth rate and litter production than the native species. After the removal of the invasive plants, the regenerating native plants have a higher capacity to resorb N and lower relative growth rates. All these traits show that a conservative strategy and a high N-use efficiency and internal plant control of the N in the ecosystem underlie the invasive success of *Spartina* in this N-limited wetland. Relative growth rate was associated with lower plant N:P ratios, whereas absolute growth rate was associated with higher nutrient-use efficiency and lower C and N turnover and storage capacities in the biomass. Changes in soil properties produced by the establishment of an invasive plant can condition the later regeneration of native plants.

Keywords: Carbon · ecological stoichiometry · nitrogen · N:P ratio · N resorption · phosphorus · wetlands

Introduction

Tidal estuarine wetlands cover an estimated 12 000 km^2 of China's 18 000 km of coastline (Shen and Zhu 1999; Huang et al. 2006). These tidal wetlands are generally rich in animal and plant biodiversity (Zhou et al. 2006) and have important biogeochemical roles within the entire estuarine ecosystem (Zeng et al. 2009a; Zeng et al. 2009b; Wang et al. 2010a; Wang et al. 2010b; Tong et al. 2010). The Minjiang River estuary in southeastern China is an important tidal wetland ecosystem due to its unique location at the transition of the central and southern subtropical climatic zones (Zheng et al. 2006).

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

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

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

Variable foliar ratios of C to nitrogen (N) (C:N) and to phosphorus (P) (C:P) are assumed to be caused by the physiological adjustment of plant species to the local supplies of nutrients (Broadley et al. 2004; Kerkhoff et al. 2006; Demars and Edwards 2007; Townsend et al. 2007; Elser et al. 2010; Peñuelas et al. 2010; Sardans and Peñuelas 2013). Evidence, however, is accumulating that intraspecific differences in terrestrial plants can match or exceed interspecific variability (Wright et al. 2004; Elser et al. 2010; Peñuelas et al. 2010; Sardans and Peñuelas 2013). These species-specific patterns of elemental composition likely reflect important differences in plant functional traits that have unique biochemical, and hence elemental, requirements (Sardans et al. 2014). The elemental composition of *Cyperus* may thus differ from that of *Spartina*, even for individuals growing under very similar environmental conditions, and thereby may affect the dynamics of soil nutrients by affecting the elemental composition of litter and/or the capacity to take up nutrients.

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

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

Methods

Study area

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

S. alterniflora and *C. malaccensis* are the two dominant species of plants. They are typically found in the upper (mid to high) portions of mudflats. *Spartina* is an invasive plant. The decomposition rates of the litter of *Spartina* are slower than those of *Cyperus* (Tong et al. 2009). Wetland soils in areas dominated by *Spartina* biomass generally have a lower pH and bulk density than do areas dominated by *Cyperus* (Jia et al. 2008). *Cyperus* is a perennial herb that grows from March to September, with the root and some stems remaining during winter. *Spartina* is also a perennial herb. It grows from the April to October, with the root and most stems remaining during winter. We studied and compared three different mono-species stands types: *Cyperus*, the native plant, *Spartina*, the invasive plant (communities more than 10 years old) and regenerated *Cyperus* stands where the invasive *Spartina* was removed three years previously and subsequently planted with *Cyperus*. In regenerated *Cyperus* stands, *Spartina* was removed by cutting the above ground and shallow below ground (0-20 cm) plant material, and then the native plant species *Cyperus* was planted in 2009 (seedlings 50 cm high with a density is 150 m^{-2}). The root systems of the two studied species have similar biomass distribution across soil depth with significant biomass at soil depths layers under 50 cm, but with the higher biomass fraction in the upper 0-15 cm of soil layer (Tong et al., 2011).

Sample collection and measurements

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

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

Plant samples were collected in May, July, September and December 2012, corresponding to grass buds, stem elongation, budding blossom, and seed maturation stages, in order to capture potential seasonal differences in chemical composition. Most plant growth occurs between April and October, and litter is produced largely toward the end of the growing season into early winter. Plant samples were collected from a consistent height to reduce the potential effects of site-specific confounding variables. We selected stands of the three plant communities for the collection of aboveground biomass, randomly established one large quadrat (10 × 10 m) in each stand and sampled the aboveground biomass from three randomly selected sub-quadrats (1 × 1 m). The harvested aboveground biomass was sorted into living and dead (litter) material. The living and litter fractions were then sorted into stems and leaves. The leaves of *Cyperus* were difficult to collect because they had degraded and fell easily from the plants (Liu et al. 2006) and so had very limited biomass (Zeng et al. 2009b). This material did not represent a major part of the aboveground biomass and so was not collected.

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

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

Measurements of resorption and growth

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

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

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

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

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

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

$$RGR = (LnB_{i+1} - LnB_i)/(t_{i+1} - t_i)$$

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

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}.

Data analysis

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

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

Results

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

The concentrations of total soil C, N and P concentrations were positively correlated ($P < 0.05$) (Fig. S1A-C). The C, N and P concentrations generally varied with soil depth, *Spartina* invasion and removal and the interaction of soil depth with *Spartina* invasion and removal ($P < 0.01$, Table 1, Figs. S2A-C); P concentrations were not significantly affected by the interaction of soil depth with plant invasion and removal. Soil C and N concentrations were generally higher in the *Spartina* community than in the natural and regenerated *Cyperus* communities ($P < 0.01$, Table 2). Soil P concentration was lower in the regenerated *Cyperus* community than in the *Spartina* and natural *Cyperus* communities ($P < 0.01$).

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

Effect of plant invasion and removal on soil parameters

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

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

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

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

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

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

N and P resorption

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

Growth rate

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

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

Litter production

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

Multivariate analysis

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

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

Discussion

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

The C:N ratios of the litter were strongly correlated with the rates of litter decomposition in the communities, with lower C:N ratios usually associated with higher rates of decomposition (Windham 2001). The litter C:N ratios of *Spartina* were higher than those of *Cyperus*. These results are consistent with the low rates of litter decomposition in the Minjiang River estuary (Tong and Liu 2009) and with the negative correlation between rate of decomposition of soil C and the C:N ratio in this estuary (Wang et al. 2010b). Our results thus support the C:N ratio as an indicator of litter and organic-matter decomposition (Elser et al. 2003) and suggest that the rates of litter decomposition can be lower in invaded than in native stands (Tong and Liu 2009). The C:P and N:P ratios were lower in the native plants than in the invasive plants in summer (the growing season), with a consistently higher RGR for *Cyperus* than for the invasive *Spartina*. The RGRs of *Cyperus* and *Spartina* were 0.004 and 0.001 g g^{-1} d^{-1}, respectively. Lower C:P and N:P ratios have been associated with higher growth rates (Elser et al. 2003; Peñuelas et al. 2013). Conversely, AGR (the new total biomass produced per unit time) was higher in the invasive species, coinciding with its much higher biomass (allowing a lower RGR), higher N concentrations and contents and lower losses of N in the litter, all indicating a high retention and conservative use of N in the invasive species.

The invasive plant species in our study thus grows more slowly than the native species (Zhang et al. 2008; Zeng et al. 2009a; Zeng et al. 2009b), with low C and N turnovers. The lower litter production and the trend to lower respiration rates in *Spartina* than in the native *Cyperus* observed in other studies (Tong et al. 2014) are also consistent with the lower RGR of the invasive species and the more conservative strategy of stress tolerance of *Spartina* than of *Cyperus*. Most studies in environments with no limitations of resources such as water, light or nutrients generally find that plant invasion is frequently dependent on higher rates of nutrient uptake and cycling (Sardans and Peñuelas 2012). The strategy for plant success in terrestrial environments where at least one important resource is clearly limiting has not been clearly defined, but despite the low number of studies and frequent contradictory results, most studies suggest that a more conservative use, higher uptake and storage capacity of the limiting resource underlie plant success (Funk and Vitousek 2007; Sardans and Peñuelas 2012).

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

The average N:P ratios (on a molar basis) were 28.7 ± 5.1 and 16.2 ± 1.7 for *Spartina* (leaves, stems and roots) and *Cyperus* (stems and roots), respectively, which were higher than the average N:P ratios (14.8-15.9) of terrestrial and aquatic plants and algae in their natural environments (Elser et al. 2000; Güsewell and Koerselman 2002; Geider and La Roche 2002; Knecht and Göransson 2004). The foliar N:P ratio is often used to represent nutrient limitation during plant growth (Tessier and Raynal 2003; Wang and Yu 2008), and a high N:P ratio suggests that P can be also limiting (the foliar N:P ratio was 38.3 for *Spartina*). In contrast, the wetland soils of our study had particularly low N:P ratios (4.1-4.3 on a molar basis) compared to the soils from other ecosystems (Cleveland and Liptzin 2007; Tian et al. 2010), indicating that the limiting nutrient was N in the soil of this wetland area (Fig. 3). A high N:P ratio has also been observed in the invasive plant *Phragmites australis* in an area near the Minjiang estuary (Wang et al. 2014). The N:P ratio and N-resorption capacity were higher in this invasive species than in the native species, and the soils had lower N:P ratios.

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

To summarize, we found lower N and P concentrations in soils than in plants in the tidal estuarine wetlands of the Minjiang River, indicating that plants retain nutrients, especially N. We also observed higher N:P ratios in the plants than in the soils. *Spartina* was more efficient than the native *Cyperus* in storing more N (the limiting nutrient) in the biomass, in accordance with its invasive success. These results are consistent with the few previous similar studies, indicating that the success of invasive plants in nutrient-poor soils depends on conservative strategies, such as the more efficient use, storage and retention of the limiting resource (Funk and Vitousek 2007; González et al. 2010; Matzek 2011; Wang et al. 2014), allowing longer nutrient residence times (Laungani and Knops 2009). Notably, our results clearly linked plant N:P ratios with growth rates. The results of this study are consistent with the growth rate hypothesis, with a clear relationship between low N:P ratio and high RGR, indicating that the new biomass produced relative to the total plant biomass is associated with lower N:P ratios but not with AGR, which should also depend on the turnover of biomass and on resource-use efficiency. All these results are also consistent with the higher litter production of the invasive *Spartina* than of the native *Cyperus*.

Cyperus replanted after the removal of *Spartina* had soil and plant elemental compositions different than those for the natural *Cyperus* community. These differences were mainly due to the higher stem and litter N:P ratios and lower RGR in the regenerated than in the natural *Cyperus* community. The shift toward higher soil and root N:P ratios in the invaded community may thus be associated with the subsequent higher stem and litter N:P ratios and lower RGR in the regenerated relative to the natural *Cyperus* community. Moreover, soil P is lower in *Cyperus* replanted than in the natural *Cyperus* community, likely as a result of the lower concentration of P in the litter of *Cyperus* replanted than in the natural *Cyperus* community. Invasion shifted the overall plant-soil nutrient concentrations, distributions and stoichiometries, especially those linked to N, and these shifts further influenced the plant-soil nutrient status and limited the RGR of the native species in the early to middle stages of the regeneration of the native species.

Conclusions

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

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Tables

Table 1 Summary of factorial ANOVAs of the effects of plant invasion and removal and soil depth 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

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

Nutrient	Natural *C. malaccensis*	*S. alterniflora*	Regenerated *C. malaccensis*
C	20.9 ± 1.0 b	23.4 ± 2.0a	21.2 ± 1.6b
N	1.27 ± 0.06 a	1.37 ± 0.12b	1.26 ± 0.08a
P	0.69 ± 0.04 a	0.70 ± 0.03a	0.64 ± 0.03a

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

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

Community	Index	pH	Water content	Salinity	Clay content
Natural *C. malaccensis* (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
	N:P	-0.484*	0.400	-0.166	0.559*
S. alterniflora (n = 18)	[C]	-0.233	0.746**	0.453	0.051
	[N]	-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
	C:P	-0.060	0.715**	0.605**	0.127
	N:P	-0.195	0.817**	0.204	0.058
Regenerated *C. malaccensis* (n = 18)	[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
	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
Total (n = 54)	[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

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

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

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

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

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

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

Factorial ANOVA statistics	*Stem*	*Litter*	*Root*
C:N			
Season	$F = 31.2$	$F = 10.1$	$F = 3.18$
	$P < 0.001$	$P = 0.002$	$P = 0.042$
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	$P < 0.001$	$P < 0.001$	$P = 0.032$

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

	Wilk's 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

Figure captions

Fig. 1 Location of the sampling sites.

Fig. 2 Comparison of average pHs (mean ± 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 ± 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 ± 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.

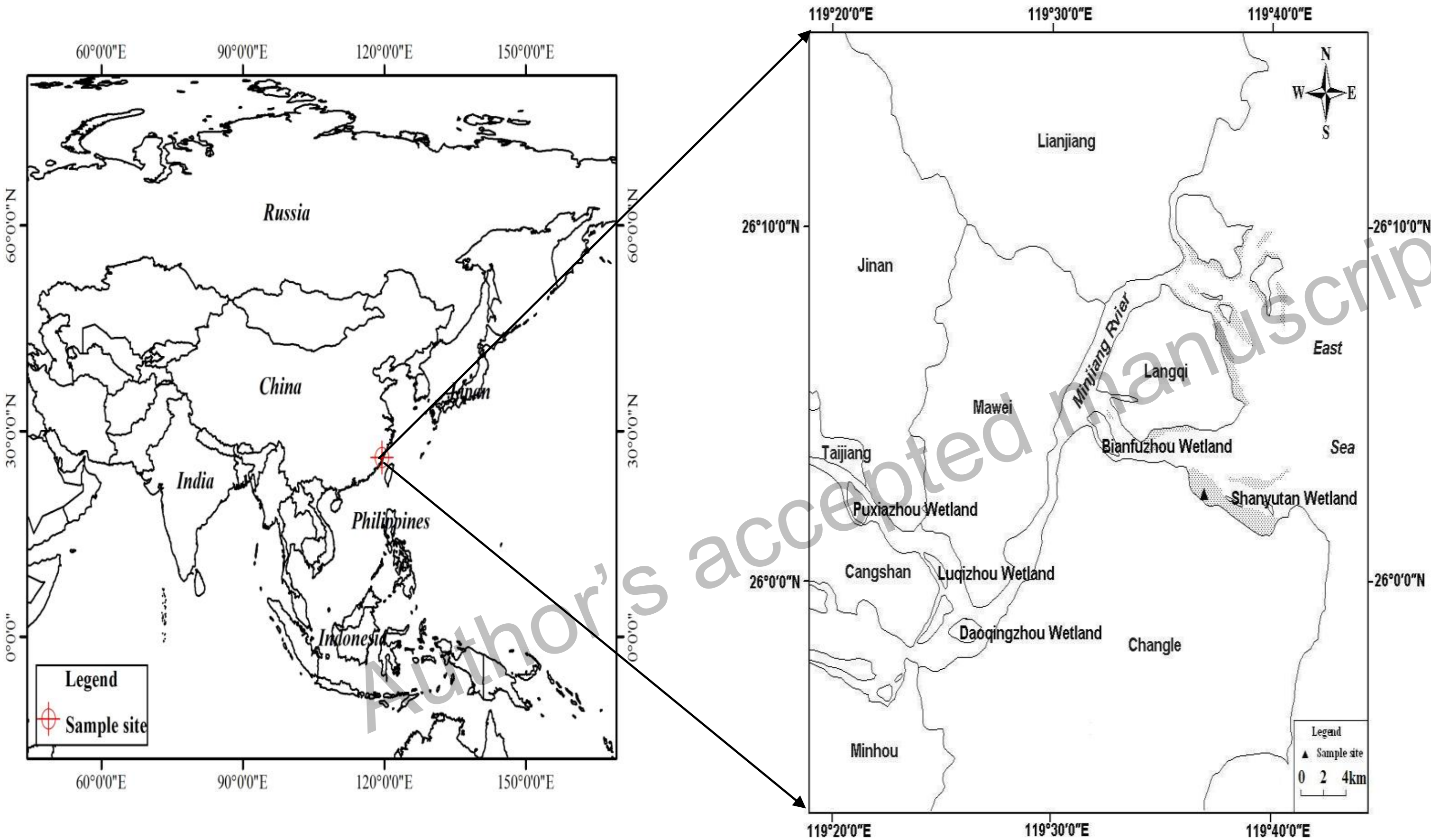
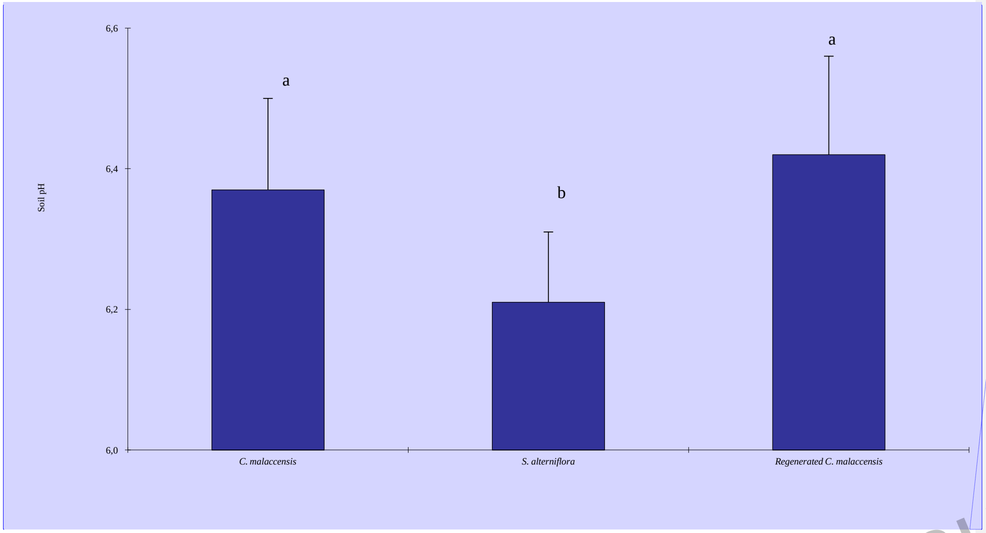

60°0'0"E
90°0'0"E
120°0'0"E
150°0'0"E
60°0'0"N
30°0'0"N
0°0'0"
Russia
China
Japan
India
Philippines
Indonesia
Legend
Sample site
119°20'0"E
119°30'0"E
119°40'0"E
26°10'0"N
26°0'0"N
N
W
E
S
Lianjiang
Jinan
Minjiang Rvier
Langqi
East
Sea
Mawei
Taijiang
Bianfuzhou Wetland
Shanyutan Wetland
Puxiazhou Wetland
Cangshan
Luqizhou Wetland
Daoqingzhou Wetland
Changle
Minhou
Legend
Sample site
0 2 4km

Fig. 1



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.

Fig. 2

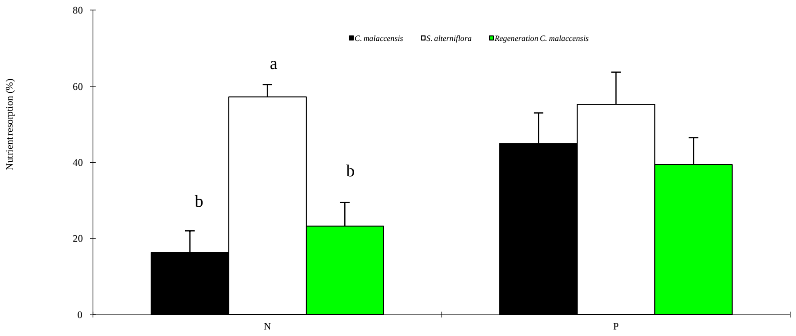

C. malaccensis
S. alterniflora
Regeneration C. malaccensis
Nutrient resorption (%)
80
60
40
20
0
a
b
b
N
P

Fig. 3

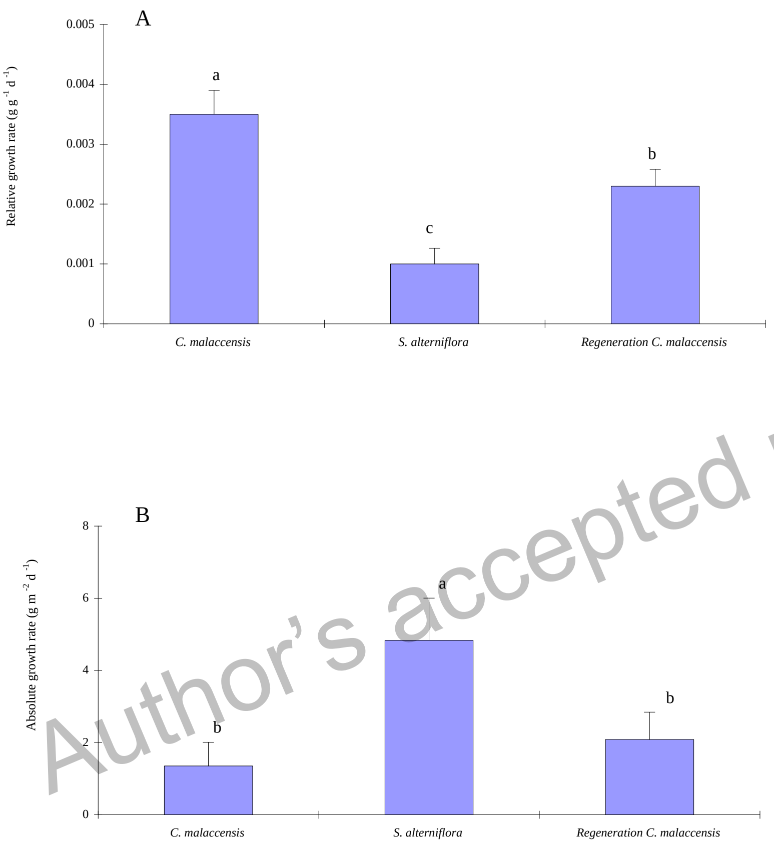

A
Relative growth rate (g g -1 d -1)
0.005
0.004
0.003
0.002
0.001
0
a
c
b
C. malaccensis
S. alterniflora
Regeneration C. malaccensis
B
Absolute growth rate (g m -2 d -1)
8
6
4
2
0
b
a
b
C. malaccensis
S. alterniflora
Regeneration C. malaccensis

Fig. 4

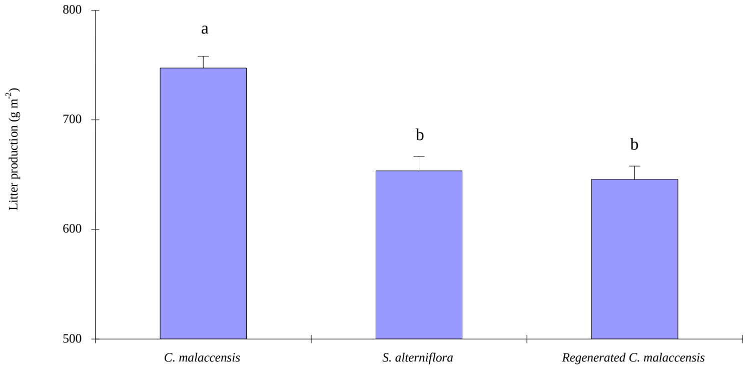

800
700
600
500
Litter production (g m-2)
a
b
b
C. malaccensis
S. alterniflora
Regenerated C. malaccensis

Fig. 5

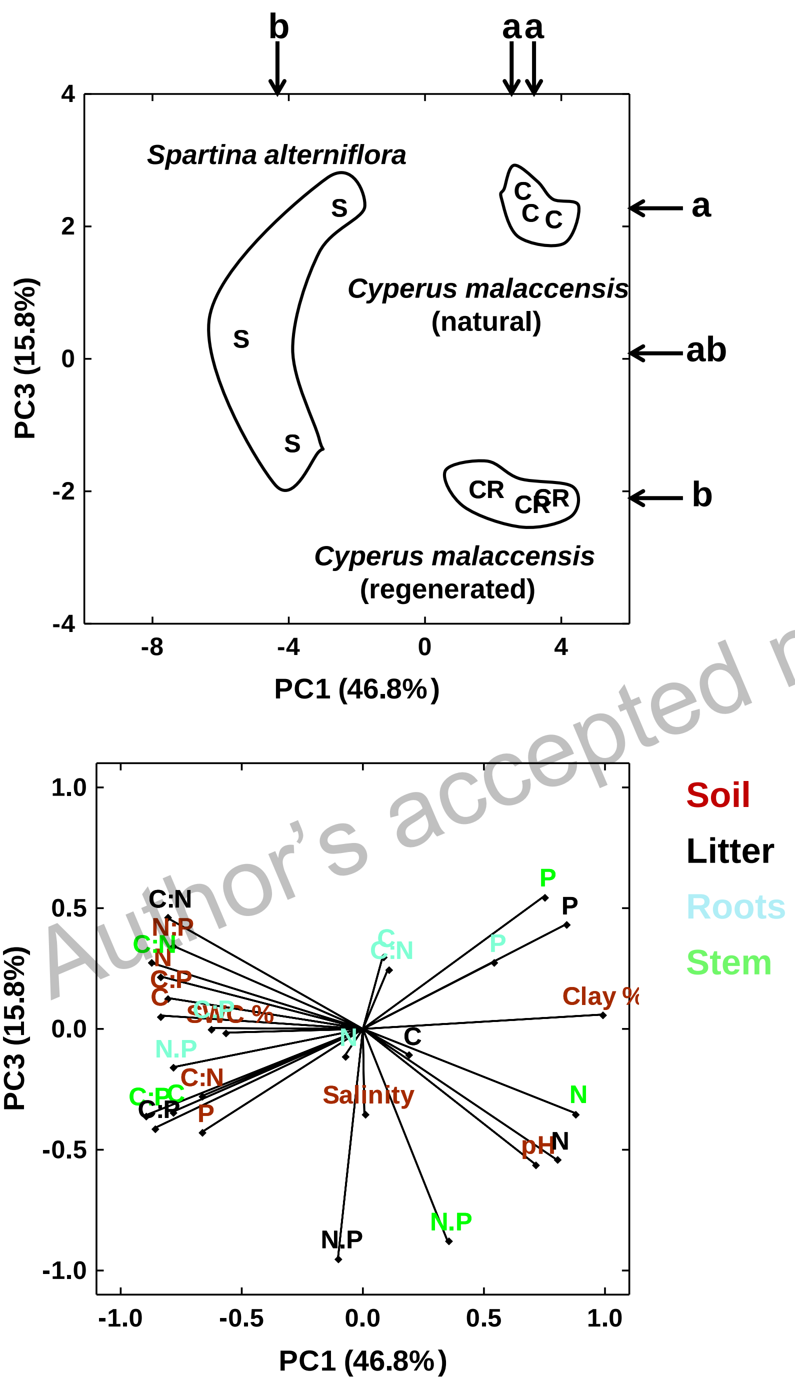

b
a a
a
ab
b
Spartina alterniflora
S
S
S
C
C C
Cyperus malaccensis
(natural)
CR
CR CR
Cyperus malaccensis
(regenerated)
4
2
0
-2
-4
PC3 (15.8%)
-8
-4
0
4
PC1 (46.8%)
Soil
Litter
Roots
Stem
1.0
0.5
0.0
-0.5
-1.0
PC3 (15.8%)
-1.0
-0.5
0.0
0.5
1.0
PC1 (46.8%)
C:N
N:P
C:N
N
C:P
C
SWC %
C:P
N.P
C:N
C:P
C
C:P
P
C
C:N
P
P
P
Clay %
N
C
Salinity
N
pH
N
N.P
N.P

Fig. 6

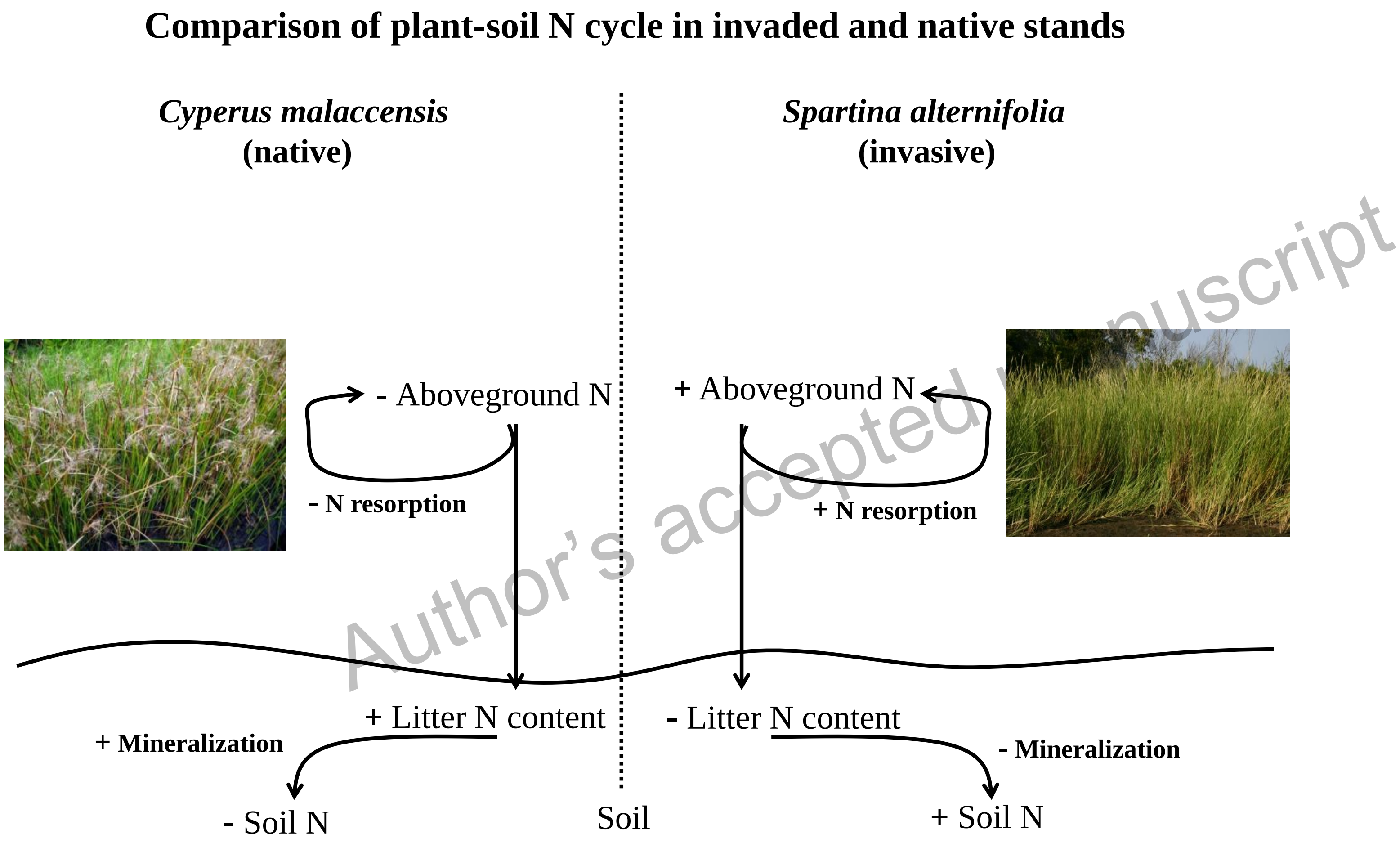

Comparison of plant-soil N cycle in invaded and native stands
Cyperus malaccensis
(native)
Spartina alternifolia
(invasive)
- Aboveground N
- N resorption
+ Aboveground N
+ N resorption
+ Litter N content
+ Mineralization
- Soil N
Soil
- Litter N content
- Mineralization
+ Soil N

Figure 7