Responses of soil nutrient concentrations and stoichiometry to different human land uses in a subtropical tidal wetland

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ABSTRACT

We studied the impacts of anthropogenic changes in land use on the stoichiometric imbalance of soil carbon (C), nitrogen (N), phosphorus (P) and potassium (K) in *Phragmites australis* wetlands in the Minjiang River estuary. We compared five areas with different land uses: *P. australis* wetland (control), grassland, a mudskipper breeding flat, pond aquaculture and rice cropland. Human activity has affected the elemental and stoichiometric compositions of soils through changes in land use. In general, soil C and N concentrations were lower and total soil K concentrations were higher at the sites under human land uses relative to the control site, and total soil P concentrations were generally not significantly different. The close relationship between total soil C and N concentrations in all cases, including fertilization with N, suggested that N was the most limiting nutrient in these wetlands. Lower soil N concentrations and similar soil P concentrations and higher soil K concentrations under human land-use activities suggest that human activity has increased the role of N limitation in these wetlands. Only grassland use increases soil N contents (only in the 0-10 cm of soil). Despite N fertilization, lower soil N concentrations were also observed in the rice cropland, indicating the difficulty of avoiding N limitation in these wetlands. The observed lower soil N:P ratio, together with higher soil P and K availabilities in rice croplands, is consistent with the tendency of human activity to change the competitive relationships of plants, in this case favoring species adapted to high rates of growth (low N:P ratio) and/or favoring plants with high demands for P and K. Both, soil C storage and respiration were higher in grasslands, likely due to the introduction of grasses, which led to a high density of plants, increased grazing activity and soil compaction. Soil C storage and respiration were lower under human land uses, except in the rice cropland, with respect to natural wetland. Using overall data, soil C storage and respiration were correlated, indicating that soil respiration was correlated with plant productivity. In this wetland area the impacts of different human land-uses on soil stoichiometry and C-cycle can be very different depending on the activity. Further regeneration of natural communities can be determined by the previous type of land-use.

Keywords**:** C:N; imbalance of nutrients; Nitrogen; N:P; phosphorus; potassium

1.Introduction

The quantity and relative supply of nutrients in agricultural soils have important implications for human nutrition and global biogeochemical cycles. Human interventions can strongly alter soils and the nutrient pools of carbon (C), nitrogen (N) and phosphorus (P) by increasing nutrient inputs (e.g. fertilization and increased weathering), by changing the structure of plant communities and by changing nutrient export (e.g. crop harvesting and increased erosion). Whether and how humans affect the relative balance of soil nutrients (C, N and P) through induced changes in land use remain unclear, especially in terrestrial ecosystems. Previous studies have shown a close relationship between human disturbances, such as N deposition, climate change, species invasion or increases in atmospheric CO_2, and elemental and stoichiometric shifts in plants and soils (Melillo et al., 2003; Vitousek et al., 2004; Tian et al., 2010; Sardans et al., 2012a and 2012b; Sardans and Peñuelas, 2012). Much less information, in contrast, is available on the impact of land-use changes on soil stoichiometry (Sardans et al., 2012b).

C, N and P are strongly intertwined biochemically. The relative dynamics of these elements, however, are poorly quantified, and dependencies between elements have not been well investigated (Ågren, 2008**)**. Well-balanced C:N:P ratios of 186:13:1 and 60:7:1 for soil and soil organisms, respectively, have been determined on a global scale (Cleveland and Liptzin, 2007), or recently for wetland soils 539:28:1 (Xu et al., 2013). Whether wetland soil also has a balanced C:N:P ratio under different intensities of human disturbance or not, however, remains unknown.

Estuarine wetland is influenced by rivers and tides, so the elemental ratios appear to be more variable than in other ecosystems worldwide. The human impact on the stoichiometry of these types of ecosystems in estuarine wetlands has received little attention (Koerselman and Meuleman, 1996). The study of the C, N and P concentrations and stoichiometries of wetland soil would be useful for determining the cycles and balances of C, N and P and the fertility of the soil. The current rapid development of the global economy stimulates human disturbance of natural ecosystems and hence their soil C, N and P biochemical processes (Peñuelas et al., 2012; 2013). Anthropogenic inputs of N and P increased from the 1860s to this century to the point that they reached the levels of the natural global N and P fluxes and caused an imbalance of C, N and P stoichiometry that is likely to increase in the near future (Peñuelas et al., 2012, 2013).

Ecological stoichiometric studies in terrestrial ecosystems have mainly focused on N and P (Sardans et al., 2011; 2012a). Recent stoichiometric studies have observed that potassium (K) is even more associated than is N or P with stoichiometric differences among various plant ecotypes (Sardans et al., 2012c; Sardans and Peñuelas, 2014) or with stoichiometric shifts in response to environmental changes (Sardans et al., 2012c; Rivas-Ubach et al., 2012). The strong link between plant K concentrations and water availability (Yavitt et al., 2004; Sardans et al., 2012c) justifies the study of K and its stoichiometric relationships with other nutrients. This focus would more strongly integrate the dimension of water availability in the study of terrestrial ecological stoichiometry and would better characterize biogeochemical niches. Recent ecological stoichiometric studies have observed that K plays a more fundamental role than does N or P in the differences in elemental composition between and within species, depending on the environmental conditions of growth, especially those related to water availability (Lawniczak et al., 2009; Sardans et al., 2012c).

Because of the intensity of local-scale disturbances, both horizontal and vertical heterogeneity will change the elemental composition of soil. A better knowledge of the resulting soil C, N and P ecological stoichiometries would provide decision makers with the necessary information for developing effective methods to enhance the potential capacity of soil to fix C and reduce the emissions of greenhouse gases (Peñuelas et al., 2013).

China has a coastal zone approximately 18 000 km in length, much of which is occupied by tidal wetlands in estuaries, estimated at more than 1.2×10^4 km^2 (Shen and Zhu, 1999; Huang et al., 2006). These areas are characterized by rapid economic development, and the intensity of human disturbance is higher than in other ecosystems, with much replacement of natural undisturbed areas by areas disturbed by crops, livestock, pollution and tourism. N and P loads to rivers caused by human activities and further transported by upstream rivers to the wetlands (Howarth et al., 1996) cause water eutrophication (Anderson et al., 2002), which threatens the health of wetlands (An et al., 2007) and decreases ecosystem services (Lee et al., 2006). Research, however, has been scarce, and studies are therefore needed on different spatial and temporal scales.

To further understand the effects of human disturbances on soil C, N, P and K concentrations and stoichiometries in wetlands, we here aimed to: (1) clarify the changes in soil C, N, P and K concentrations associated with human disturbance and determine the relationships among C, N, P and K concentrations at different soil depths in estuarine tidal wetlands, (2) explore the influencing factors and (3) discuss the relationships between the

C:nutrient, N:P, N:K and P:K ratios and the capacity of soil to fix C.

2. Material and methods

2.1. 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 Minjiang River estuary. 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 across the study site is submerged beneath 10-120 cm of water for 3-3.5 h during each tidal inundation. At low tide, soil surfaces of the entire estuarine wetland are exposed, and the annual average weight of the water content (ratio of water weight to dry-soil weight) and the soil redox potential are 116% and 12.6 mV, respectively. The soil remains flooded at some depths. The average salinity of the tidal water from May to December 2007 is 4.2 ± 2.5 ‰. *Phragmites australis* is one of the most important plant species (Liu et al., 2006) in the area and is typically found in the upper (mid to high) portions of mudflats, which are a main component of the Shanyutan tidal wetland.

P. australis is a C_3 plant (mature height of 2 m with 150 stems m^{-2}). The above- and belowground biomasses of *P. australis* in the study area are 1500 and 2322 g m^{-2}, respectively, and the above- and belowground C, N and P storages by the plants are 0.24 and 0.85 kg m^{-2}, 16.7 and 21.8 g m^{-2} and 0.60 and 1.97 g m^{-2}, respectively (Tong et al., 2011). The rate of decomposition of litter from these plants is 0.00384 d^{-1}, and the amounts of C, N and P released account for 53.1, 79.6 and 79.1%, respectively, of the initial litter during the 280-day period of decomposition (Wang et al., 2012a).

The areas of natural wetlands are gradually decreasing as human disturbance increases. We have studied the following types of human disturbance: (1) natural *P. australis* wetland with very limited or no human disturbance was defined as the control, (2) grassland established in *P. australis* wetlands where cattle have been bred for six years, (3) mudflats where mudskippers have been bred for 10 years (hereafter referred to as flat breeding), (4) pond aquaculture where fish have been bred for 10 years and (5) cropland where rice has been cultivated for 70 years was defined as very high disturbance; the rice cropland received annual applications of N, P and K fertilizers at 95, 30 and 58 kg ha^{-1}, respectively (Wang et al., 2012b).

2.2. Soil-sample collection and measurement

The soil samples were collected in October 2007. Sampling locations were established in the *P. australis* wetland, grassland, flat breeding, pond aquaculture and rice cropland (Fig. 1). Three plots were randomly selected in each of the locations, and soil profiles (width, 1 m; length, 1 m; depth, 0.5 m) were excavated. Samples were collected with a small sampler (length and diameter were 0.3 and 0.1 m) from each of five soil layers (0-10, 10-20, 20-30, 30-40 and 40-50 cm) at the center and both sides of the soil pit. These three samples from each layer were bulked to form one sample per layer. A total of 75 soil samples (five types of land-use x three plots x five soil layers) were thus collected. In the laboratory, the soil samples were air-dried, roots and visible plant remains were removed and the soil 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), total soil P concentration was measured by perchloric-acid digestion followed by ammonium-molybdate colorimetry, available-P concentration was determined by extraction with acidic ammonium fluoride and measurement using an UV-2450 spectrophotometer (Shimadzu Scientific Instruments, Japan), total K concentration was determined by FP 640 flame photometry (Shanghai Electronic Technology Instruments, China) and available-N concentration was measured by the alkaline-hydrolysis diffusion method (Lu, 1999).

Environmental influencing factors were also determined. Bulk density was measured from three 5 × 3 cm cores per soil layer, salinity was measured by DDS-307 conductivity (Boqu Scientific Instruments, China), pH was measured with an 868 pH meter (Orion Scientific Instruments, USA).

For CO_2 measurements, sampling locations were established in the P. australis wetland, grassland, flat breeding, pond aquaculture and rice cropland. Three plots were randomly selected in each of the locations, and total soil respiration includes the autotrophic respiration. It was determined with the Li-8100 soil CO_2-flux system (Licor Instruments, USA) in 10th July and 10th December, i.e. in summer and winter with low tide when soil surfaces of the entire estuarine wetland are exposed. Linear curve was chosen to fit the data of soil respiration.

2.3. Statistical analyses

The C storage for the 0-50 cm soil profiles were estimated using the equation (Mishra et al., 2010):

$$C_S = \sum_{j=1}^{n} c_{\mathrm{m}} \times \rho_{\mathrm{b}} \times D$$

where C_S is C storage (kg m^{-2}), j is soil-depth interval (1, 2, … n), C_m is the C concentration (kg kg^{-1}), ρ_{b} is the soil bulk density (kg m^{-3}), D is the thickness of each soil layer (m) and n is the number of soil layers.

All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, Illinois). The significance of the differences among treatments (types of land-use) on soil variables were assessed by one-way analyses of variance with Tukey's post-hoc tests. We analyzed the Pearson correlation coefficients between environmental factors and total soil C, N, P and K concentrations; available-N and -P concentrations; total soil C:N, C:P, N:P, C:K, N:K and P:K concentration ratios and available N:P ratio. We also analyzed the effects the types of land-use on soil C storage and respiration and the Pearson correlation coefficients between total soil C:N, C:P, N:P, C:K, N:K and P:K concentration ratios; available N:P ratio and soil C storage and respiration. We used discriminant functional analysis (DFA) to determine the importance of total soil C, N, P and K concentrations; available-N and -P concentrations; total soil C:N, C:P, N:P, C:K, N:K and P:K concentration ratios and available N:P ratio in the separation of the chemical soil composition of the plots at the various types of land-use. 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). We used major axis regression (MA) and standardized major axis (SMA) using the SMATR package (http://www.bio.mq.edu.au/ecology/SMATR) to compare differences in regression slopes between the total soil C versus N, C versus P and C versus K concentrations; between the total soil N versus C, N versus P and N versus K concentrations; between the total soil P versus C, P versus N and P versus K concentrations and between the total soil N versus P and available soil N versus P concentrations. Soil C:N, C:P, N:P, C:K, N:K, P:K and available N:P ratios were calculated as molar ratios. Soil respiration was the averaged value for summer and winter.

3. Results

3.1. Effect of human disturbance on concentrations of total soil C, N, P, K and available N and P

C concentrations were significantly higher in the grassland than in the control plots only at a soil depth of 0-10 cm. C concentrations in soils at flat breeding and pond aquaculture plots were lower than in the control, especially in the deepest layers (below 20 cm, Fig. 2A). Total soil N concentrations followed similar patterns (Fig. 2B). Total soil P concentrations did not differ significantly with soil depth under different land uses, and the total soil P concentrations did not generally differ between the control and the other land uses (Fig. 2C). Total soil K concentrations were significantly higher in the human land uses plots relative to the controls but did not differ significantly with soil depth among the different human land uses (Fig. 2D).

The concentrations of available N were significantly higher in flat breeding plots relative to the control but were significantly lower in soil layers above 30 cm in grassland and pond aquaculture plots relative to the control (Fig. 2E). The concentrations of available P were significantly higher in the plots of rice cropland relative to the control and were significantly lower in the treatment of pond aquaculture at depths between 10 and 30 cm. The concentrations of available P in the other disturbed plots were not significantly different from the control (Fig. 2F). The concentrations of available P under the various intensities of disturbance did not significantly change with soil depth.

In summary, our data suggest that human land uses decreased total soil C and N concentrations, increased soil available-P and total soil K concentrations but had no clear general effect on total soil P concentrations. The different responses of C, N, P and K concentrations to human activities may result from alterations in soil pH, bulk density or salinity due to human disturbance (Table 1, Table 2). pH was negatively correlated with total soil C, N, P and available-N concentrations and was positively correlated with K concentration. Bulk density was lower in the disturbed plots and was thus negatively correlated with C, N, P and available-N concentrations and positively correlated with K concentration. Salinity was lower in all disturbed plots except the flat breeding plots, where salinity was positively correlated with total soil C, N, P and available-N concentrations and negatively correlated with total soil K concentration.

3.2. Relationships among the concentrations of total soil C, N, P, K and available N and P

The concentrations of C and N and of N and P were positively correlated ($P<0.01$) (Fig. 3A, Fig.3D). C and P concentrations were also positively correlated ($P<0.05$) (Fig. 3B). The concentrations of C and K and of N and K were negatively correlated ($P<0.05$) (Fig. 3C, Fig.3E). The concentrations of P and K and of available N and available P were not significantly correlated ($P>0.05$) (Fig. 3F, Fig.3G).

SMA tests of common slopes revealed differences among nutrient correlations. The slopes of the correlations among nutrients were significantly different ($P<0.001$), except for total soil N versus P concentrations relative to available-N versus available-P concentrations ($P>0.05$).

3.3. Effect of human disturbance on the ratios among total soil C, N, P and K concentrations and on soil available-N and available-P stoichiometry

Total soil C:N ratios were not significantly different in the various types of land-use across the soil profile (Fig. 4A). Total soil C:P ratios increased significantly in the grassland only at a soil depth of 0-10 cm, and the C:P ratios were lower for flat breeding and pond aquaculture (Fig. 4B). Total soil C:K ratios were significantly lower for grassland relative to the control plots in soil layers below 10 cm ($P<0.05$), and the C:K ratios for flat breeding and pond aquaculture were lower than those of the control plots at all soil depths ($P<0.05$) (Fig. 4C). Total soil N:P ratios were significantly higher for grassland in the 0-10 cm layer and were lower for flat breeding and pond aquaculture (Fig. 4D). Total soil N:K ratios were significantly lower for grassland in the 10-50 cm soil layers and were significantly lower for flat breeding and pond aquaculture in the 0-50 cm soil layers (Fig. 4E). Total soil P:K ratios were significantly lower in all soil layers in the plots of all types of land uses respect to natural *P. australis* wetland (Fig. 4F). The available soil N:P ratios were higher for flat breeding in the 30-50 cm soil layers. Available N:P ratios were lower for grassland and pond aquaculture, especially above 30 cm (Fig. 4G).

In summary, when comparing different soil layers under different land-use, soil C:N ratios were similar under different land-uses; they had low coefficients of variation (Table 3), whereas soil C:K, C:P, N:P, N:K and P:K ratios were strongly dependent of land-use and had higher coefficients of variation than C:N (Table 3, Figure 3). Our data suggest that the soil influencing factors were changed by changes in land-use (Table 1) and that they were also related to the variation in nutrient stoichiometry (Tables 4). Bulk density and pH were correlated negatively with total soil C:N, C:P, C:K, N:P and N:K ratios and with available N:P ratios.

The overall chemical compositions of the soils were significantly different among all types of studied human land uses. The squared Mahalanobis distances between the soils of the various disturbed sites were significantly different in all pairwise comparisons (Table 5). The total soil P and K concentrations, total soil C:P and P:K concentration ratios, soil available-P concentration and available N:P concentration ratio were the significant variables in the model (Table 6). As indicated in the biplot space originated by the first two roots of the FDA (explaining 86.6% of the total variance), total soil P and K concentrations were higher and N:P, C:P and P:K ratios were lower in the soils of the croplands (Fig. 5A, Fig. 5B).

3.4. Soil C storage and respiration

Soil C storage and respiration varied with the land-use type (Fig. 6). C storages of the control plot (*P. australis* wetland) and the grassland, flat breeding, pond aquaculture and rice cropland were 136 ± 5, 197 ± 7, 122 ± 6, 68.8 ± 4.8 and 85.3 ± 4.4 Mg hm^{-2}, respectively, and soil respirations were 1.37 ± 0.08, 6.61 ± 0.33, 0.79 ± 0.08, 2.30 ± 0.11 and 1.29 ± 0.12 μmol m^{-2} s^{-1}, respectively. Soil C storage thus followed the decreasing order: grassland > *P. australis* wetland > flat breeding > pond aquaculture > rice cropland and soil respiration followed the order: grassland > cropland > *P. australis* wetland > pond aquaculture > flat breeding.

4. Discussion

4.1. Imbalances among soil C, N, P, K and available-N and -P concentrations induced by human disturbance

Our data suggest that N was the limiting nutrient in the study area. N limitation was especially significant in the rice cropland, where 95 kg N ha^{-1} were applied during the period of growth (Wang et al., 2012b). The total soil N concentration, however, did not increase (Fig. 2B). N limitation also played a role in the growth of *Spartina alterniflora* in the similar estuary of the Yangtze River, which is about 800 km north of the Minjiang estuary (Gan et al., 2011). In other near wetland area *P. australis* invasive success has been observed to be related with its higher capacity to resorb N and increase N use efficiency (Wang et al., 2014). The results of this study further suggest N-limitation in these wetland areas, because despite the increase in N of soil by fertilization application, the plant-soil system did not diminish the C:N ratio indicating that N is limiting, and more N available translated in more C fixation. In contrast, P and K fertilization produce lower C:K and C:P indicating a decrease in K and P use efficiency because they are not the limiting factor, and more P and K did not translate in higher C fixation. Moreover, whereas soil N-availability decreased under the most human uses, P-availability did not. Nutrient limitation is especially significant in tidal wetlands, likely because of the periodic inundation of the soil that limits the access of plants to soil nutrients by the anoxic effects on root growth (Amlin and Rood, 2001; Kirwan and Guntenspergen, 2012), by slowing mineralization (Adame et al., 2010), and by high levels of leaching of P and particularly of N (Noe and Hupp, 2007; Kobayashi et al., 2009). Moreover, the N:P ratio (4.4 in molar basis) of the studied wetland soils is much lower than the average value of 28 for a set of different wetlands across the world (Xu et al., 2013) fact that also show the N-limitation in this wetland.

The soil C and N concentrations did not differ significantly in croplands relative to the control plots but were lower in rice croplands. P concentrations were higher in under rice croplands, pond aquaculture and flat breeding plots. Soil K concentrations were also significantly higher under human land uses. Higher soil available P and total K concentrations associated with rice cropland, which have been linked to the management of fertilization (Wang et al., 2012b). The different responses of total soil C, N, P and K concentrations to human land uses may also be due to alterations in soil pH, bulk density and salinity caused by the human management (Tables 1 and 2), as observed in a previous study (Haugwitz et al., 2011; Li et al., 2012).

The C:N and C:P ratios (0-10 cm layer) in the study area were higher than the average ratios for China and the global ratios, an effect related to the limitation of those nutrients, especially of N, but also linked with higher plant productivity capacity per unit of nutrient (higher nutrient-use efficiency) in this subtropical wet regions (LeBauer and Treseder, 2008; Hidaka and Kitayama, 2009; Cleveland et al., 2013; Singh et al., 2013). High C:N and C:P ratios mean high C concentrations in the soil (Ladd et al., 2013). The high temperatures and amounts of precipitation in our subtropical study area (Minjiang River estuary) may contribute to high rates of N and P leaching and occlusion in the highly weathered soil (Laird et al., 2010), but the low soil N:P ratios may be due to the higher solubility of N than of P, exacerbated by the continuous tidal flooding in this area. A previous study in this area observed that plants retained N in their biomass more than other nutrients (Wang et al., 2014), indicating the natural role of N limitation in this area. N:P ratios were similar to those of other areas (Table 7), which may have contributed to the simultaneous variation in N and P concentrations (Fig. 2B, Fig. 2C).

Both the concentrations and ratios of C, N, P and K varied with soil depth (Figs. 2 and 4), consistent with previous studies (Cleveland and Liptzin, 2007; Yang et al., 2011; Li et al., 2012), but only the soil C:N ratios in our study were stable across the soil profile under different land uses (Table 3), in agreement with earlier reports (Schipper and Sparling, 2011; Tian et al., 2010). The observed N limiting role would be the cause of the closer and general relationship between C and N than the relationships among the other nutrient pairs.

In summary, different types of land-use appear to have altered the general elemental compositions of the soils. Higher concentrations of soil available P and total K coincided with lower soil N:P ratios (total and available) in rice croplands. Despite the decrease in available-N concentrations, our results are partially consistent with the premise that humans, by creating more-productive ecosystems, tend to favor ecosystems with low N:P ratios able to support species with high growth rates, which is in line with the growth rate hypothesis at the level of ecosystems (Sterner and Elser, 2002). This effect is also probably related to lower soil P mobility than to soil N mobility, which under soil N and P fertilization tends to imbalance N and P by decreasing soil N:P ratios, as observed in other parts of the world (Cech et al., 2008; Peñuelas et al., 2009; 2013), and is related to higher levels of N than of P leaching (Arbuckle and Downing, 2001; Gundersen et al., 2006). Moreover, in these wetlands, this increase in soil N:P can be also due to the higher uptake of N than of P loaded by fertilizers due to N limiting role.

Human land-uses increased soil bulk density and pH and except in the case of flat breeding decrease salinity. Human land-uses by its management reduce tidal impact and thus the constant leaching and salinization. Moreover, the more flooded soils have more fine texture, the reasons were that in the more flooded habitats, the water flow speed was slow and soils remained more time under water, making that fine particles transported by water have more time to sediment. This reason is related to the fact that human land-uses by reducing tidal impact increases soil bulk density. Moreover, the increases of soil pH and the decreases of leaching is related with the observed increase in K soil concentrations under different human land uses with respect to natural wetland.

4.2. Soil C balance and the response to changes in nutrient stoichiometry

Our data suggest that the introduction of grasses (light disturbance) increased both soil C storage and respiration, which led to a high density of plants, increased grazing activity and soil compaction. Soil C storage and respiration decreased for the other land uses except the rice cropland (Fig. 6). Soil C storage and respiration were correlated, in agreement with previous studies (Dias et al., 2010; Carbone et al., 2011; De Deyn et al., 2011), indicating that soil respiration could be correlated with plant productivity (Caprez et al., 2012). Both soil C storage and respiration were higher grassland plots, likely due to the introduction of grasses, which led to a high density of plants, increased grazing activity and soil compaction. Thus, in grasslands the higher plant productivity, with higher belowground and aboveground C storage would increase by higher root (autotrophic) respiration. Soil C storage and respiration were lower at the other disturbed sites except under rice croplands. Human disturbance can increase soil CO_2 emissions (Shang et al., 2013), but soil respiration in the present study was not higher at all disturbed sites, only in in grassland and rice cropland communities (Fig. 6). When comparing the different soil uses, the relationship between soil C storage and soil C released by respiration is not clear, showing that distinct land-uses exerted different impacts on soil C-cycle. Taking out grassland communities, C respiration and C storage in soil were inversely correlated when comparing different sites, with rice cropland having the highest C respiration and lowest soil C storage and with flat breeding and pond aquaculture having the contrary patterns. Thus, our findings are not completely consistent with previous studies conducted in other ecosystems such as subtropical forests, where soil respiration decreased when plant productivity decreased (Sheng et al., 2010).

Total soil C:N, C:P and N:P ratios were positively correlated with soil C storage and respiration ($P<0.05$, Table 8), consistent with similar previous studies (Hessen et al., 2004; Kirkby et al., 2013). These results showed that N was the limiting nutrient in these ecosystems; when the soil concentration of N was lower with respect to C and P soil respiration decreases thus indicating a limitation of soil biological activity under low N concentration. Notably, under the different land uses, the changes on N:P ratios were mainly due to changes in soil N concentrations since total soil P remained more or less constant.

5. Conclusions and implications

1. Human land-use activities were associated with lower soil N concentrations and higher total soil K and available-P concentrations.

2. The stoichiometric changes and their relationships with other soil properties such as soil

respiration suggest the limitation of N in the ecosystems of this estuary. A soil N:P ratio lower than global ratios and a lower soil N concentration in rice cropland despite N fertilization also suggest N limitation.

3. Anthropogenic transformations of land use were associated with a lower available N:P ratio, an effect related to increases in fertilization under a natural N limitation and also in accordance with the lower P solubility than N and with the tendency of human activities to favor more-productive ecosystems with low N:P ratios able to support species with high rates of growth.

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Table 1. Soil pH, bulk density and salinity in wetlands (mean ± S.E.) under different types of land use.

Influencing factor	*Phragmites australis* wetland	Grassland	Flat breeding	Pond aquaculture	Rice cropland
pH	5.49±0.05a	6.11±0.05b	6.33± 0.04c	6.98±0.22d	6.43±0.18c
Bulk density (g cm^{-3})	0.72±0.01a	1.04±0.04b	0.86±0.03c	1.46±0.03d	1.37±0.01e
Salinity (μS cm^{-1})	299±2.77a	274±1.32b	305±1.29a	146±6.57c	230±11.5d

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

Table 2. Pearson correlation coefficients between soil nutrient concentration and influencing factors.

Nutrient concentration	Influencing factor	*Phragmites australis* wetland (n=15)	Grassland (n=15)	Flat breeding (n=15)	Pond aquaculture (n=15)	Rice cropland (n=15)	Total types (n=75)
C	pH	-0.707**	-0.732**	0.234	-0.994**	-0.891**	-0.742**
	bulk density	0.078	-0.957**	-0.359	-0.470*	-0.769**	-0.830**
	salinity	0.800**	-0.321	-0.492*	0.666**	0.846**	0.747**
N	pH	0.324	-0.685**	0.323	-0.983**	-0.583*	-0.730**
	bulk density	-0.420	-0.935**	-0.406	-0.418	-0.194	-0.841**
	salinity	-0.364	-0.367	-0.541*	0.654**	0.373	0.713**
P	pH	0.202	-0.511*	0.747**	-0.998**	-0.950**	-0.496**
	bulk density	-0.730**	-0.889**	-0.278	-0.326	-0.870**	-0.449**
	salinity	-0.684**	-0.528*	-0.532*	0.608**	0.945**	0.467**
K	pH	-0.525*	-0.465*	0.395	-0.322	-0.365	0.685**
	bulk density	0.366	-0.571*	-0.194	-0.255	0.204	0.610**
	salinity	0.494*	0.037	-0.730**	0.215	0.083	-0.429**
Available N	pH	0.695**	-0.648**	0.514*	-0.962**	-0.940**	-0.251
	bulk density	-0.690*	-0.939**	-0.213	-0.706**	-0.653**	-0.682**
	salinity	-0.969**	-0.442*	-0.719**	0.898**	0.868**	0.630**
Available P	pH	0.694**	-0.499*	0.734**	-0.969**	-0.991**	0.084
	bulk density	-0.558*	-0.863**	-0.305	-0.876**	-0.342	0.253
	salinity	-0.842**	-0.471*	-0.604**	0.985**	0.622**	-0.383*

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

Table 3. Total soil C:N, C:P, C:K, N:P, K:N, K:P and available N:P ratios for all studied sites (mean ± S.E.) at different soil depths. Different letters means statistical differences (*P*<0.05).

Layer	C:N	C:P	C:K	N:P	N:K	P:K	Available N:P ratio	n
0-10 cm	6.34±1.57%b	28.53±2.58%	29.90±3.89%	25.32±3.52%a	28.22±1.83%	9.55±1.09%c	138.08±24.36%a	15
10-20 cm	7.26±0.64%b	22.11±1.88%	31.10±4.69%	18.04±1.21%b	29.59±1.93%	13.07±2.37%b	40.92±8.06%b	15
20-30 cm	7.77±0.81%b	21.57±2.41%	33.87±3.51%	16.02±0.65%b	29.61±3.03%	16.12±3.72%ab	45.52±9.41%b	15
30-40 cm	10.87±1.53%a	23.52±3.15%	36.63±5.36%	15.76±0.69%b	32.34±2.71%	18.51±2.34%a	47.97±10.21%b	15
40-50 cm	7.80±0.86%b	22.46±2.18%	36.45±2.33%	16.89±0.98%b	32.94±3.19%	17.36±1.18%ab	48.22±7.87%b	15
Average	8.01±0.76%	23.64±1.26%	33.59±1.37%	18.41±1.77%	30.54±0.88%	14.92±0.85%	64.14±18.53%	15

Different letters within different depth indicate significant differences (*P*<0.05).

Table 4. Pearson correlation coefficients between nutrient ecological stoichiometry and soil pH, bulk density and salinity.

Type	Ratio	*Phragmites australis* wetland (n=15)	Grassland (n=15)	Flat breeding (n=15)	Pond aquaculture (n=15)	Rice cropland (n=15)	Total types (n=75)
pH	C:N	-0.678**	-0.197	0.089	-0.748**	-0.618**	-0.742**
	C:P	-0.360	-0.816**	-0.209	0.408	0.617**	-0.722**
	N:P	0.016	-0.733**	-0.528*	0.711**	0.922**	-0.664**
	C:K	-0.248	-0.688**	-0.103	-0.928**	-0.901**	-0.849**
	N:K	0.713**	-0.655**	-0.219	-0.189	-0.840**	-0.826**
	P:K	0.368	-0.158	-0.162	-0.965**	-0.961**	-0.849
	Available N:P	0.557*	-0.604**	-0.698**	0.753**	0.855**	-0.369*
Bulk density	C:N	0.429	0.027	-0.230	-0.364	-0.508*	-0.693**
	C:P	0.634**	-0.889**	-0.206	0.944**	-0.313	-0.785**
	N:P	0.552*	-0.857**	-0.154	0.563*	-0.008	-0.772**
	C:K	-0.329	-0.899**	-0.405	-0.850**	-0.504*	-0.873**
	N:K	-0.761**	-0.875**	-0.501*	-0.011	-0.532*	-0.862**
	P:K	-0.826**	-0.451*	0.612**	-0.868**	-0.444*	-0.776**
	Available N:P	-0.739**	-0.825**	0.361	0.871**	0.066	-0.713**
Salinity	C:N	0.767**	0.425	-0.295	-0.011	0.534*	0.854**
	C:P	0.825**	-0.117	-0.183	-0.720**	-0.031	0.704**
	N:P	0.559*	-0.261	0.005	-0.275	-0.326	0.626**
	C:K	0.366	-0.340	0.200	0.896**	0.663**	0.701**
	N:K	-0.753**	-0.360	0.931**	-0.014	0.657**	0.657**
	P:K	-0.818**	-0.536*	0.026	0.969**	0.725**	0.616**
	Available N:P	-0.901**	-0.417	0.137	-0.871**	-0.285	0.666**

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

Table 5. Test statistics for squared Mahalanobis distances among soils of varying land use in the discriminant functional analysis of total soil C, N, P, and K concentrations; total soil C:N, C:P, C:K, N:P, K:N and K:P ratios; available-N concentration; available-P concentration and available N:P ratio.

Level of human disturbance	Grassland	Flat breeding	Pond aquaculture	Rice cropland
Wetland	**901** ***P*<0.001**	**1835** ***P*<0.001**	**1388** ***P*<0.001**	**1537** ***P*<0.001**
Grassland		**969** ***P*<0.001**	**529** ***P*<0.001**	**554** ***P*<0.001**
Flat breeding			**292** ***P*<0.001**	**872** ***P*<0.001**
Pond aquaculture				**206** ***P*<0.001**

Table 6. Statistics (Wilks' Lambda and *P*-value) of the discriminant functional analysis among soils of varying land use with total soil C, N, P and K concentrations; total soil C:N, C:P, C:K, N:P, K:N and K:P ratios; available-N and available-P concentrations and available N:P ratio as variables. Bold type indicates a significant effect of the variable in the model ($P<0.05$).

Soil variable	Wilks' Lambda	*P*
C	0.427	0.11
N	0.429	0.11
P	**0.103**	**<0.01**
K	**0.126**	**<0.01**
Available N	0.601	0.34
Available P	**0.359**	**0.05**
C:N ratio	0.863	0.86
C:P ratio	**0.331**	**0.04**
C:K ratio	0.771	0.68
N:P ratio	0.507	0.20
N:K ratio	0.511	0.20
P:K ratio	**0.109**	**<0.01**
Available N:P ratio	**0.217**	**<0.01**

Table 7. Comparison of C:N, C:P and N:P ratios (molar) in the 0-10 cm layers of various soil types.

Soil type	C:N	C:P	N:P	Reference
Chinese temperate desert soil	10.5	12.4	1.2	Tian et al.2011
Chinese frigid highland soil	11.7	24.0	2.7	Tian et al.2010
Chinese Histosols	14.9	132	8.0	Tian et al.2010
Chinese Aridisols	9.6	11.2	1.2	Tian et al.2010
Chinese land soil	12.3	52.7	3.9	Tian et al.2010
Global grassland soils	11.8	64.3	5.6	Cleveland and Liptzin, 2007
Global forest soil	12.4	81.9	6.6	Cleveland and Liptzin, 2007
Global land soil	12.3	72.0	5.9	Cleveland and Liptzin, 2007
Wetland soil	23.6	103	4.4	Present study
Wetland soils	14.8	539	28	Xu et al., 2013

The data for wetland soil were the averages of the 0-10 cm soil layers at all sites.

Table 8. Pearson correlation coefficients between nutrient stoichiometric ratios and soil respiration and carbon storage (n=15).

Variable	C:N	C:P	C:K	N:P	N:K	P:K	Available N:P
Soil respiration	-0.246	0.489*	0.112	0.448*	0.044	-0.332	-0.587*
Soil carbon storage	0.598**	0.909**	0.642**	0.858**	-0.352	-0.593*	0.100

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

Figure captions

Fig. 1. Location of the five sampling sites with different land use: (1) natural *Phragmites australis* wetland (control), (2) grassland where cattle have been bred for six years in *P. australis* wetland, (3) flat breeding where mudskippers have been bred for 10 years, (4) pond aquaculture where fish have been bred for 10 years and (5) rice cropland where rice has been cultivated for 70 years.

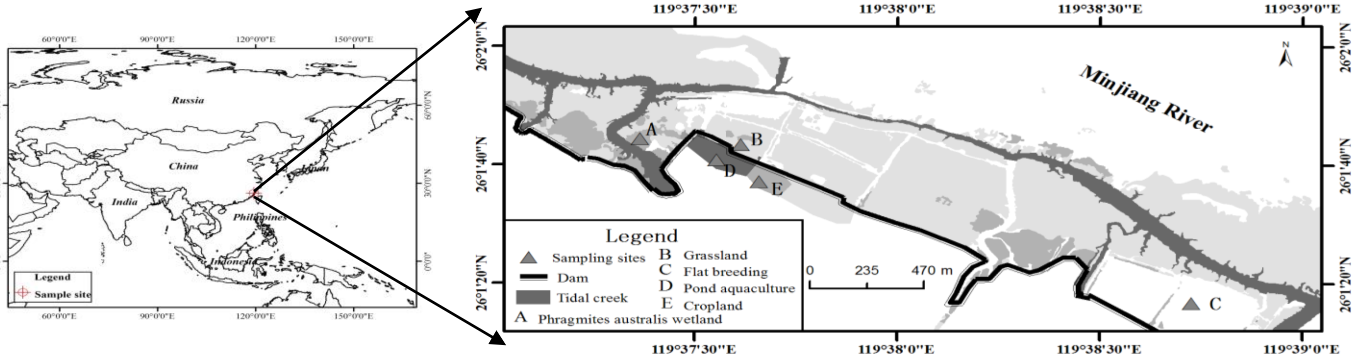
Fig. 2. Concentrations of C (A), N (B), P (C), K (D), available N (E) and available P (F) at the various soil depths in sites with different land use. Different letters indicate significant differences between sites ($P<0.05$).

Fig. 3. Relationships among soil nutrient concentrations in sites with different land-use.

Fig. 4. C:N (A), C:P (B), C:K (C), N:P (D), N:K (E), P:K (F) and available N:Available P (G) at various soil depths at sites with different land use. Different letters indicate significant differences between sites ($P<0.05$).

Fig. 5. (A) Biplot representing the scores of the soil samples from the various types of land-use in the space generated by the first two roots of the discriminant functional analysis (FDA) of total soil C, N, P and K concentrations; total soil C:N, C:P, C:K, N:P, K:N and K:P ratios; available-N and available-P concentrations and available N:P ratio. (B) Biplot representing the standardized canonical discriminate function coefficients for the first two roots of this FDA.

Fig. 6. Soil respiration and C storage in sites with different land use.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26°2'0"N
26°1'40"N
26°1'20"N
Minjiang River
N
A
B
C
D
E
Legend
Sampling sites
Dam
Tidal creek
A Phragmites australis wetland
B Grassland
C Flat breeding
D Pond aquaculture
E Cropland
0
235
470 m

Fig. 1

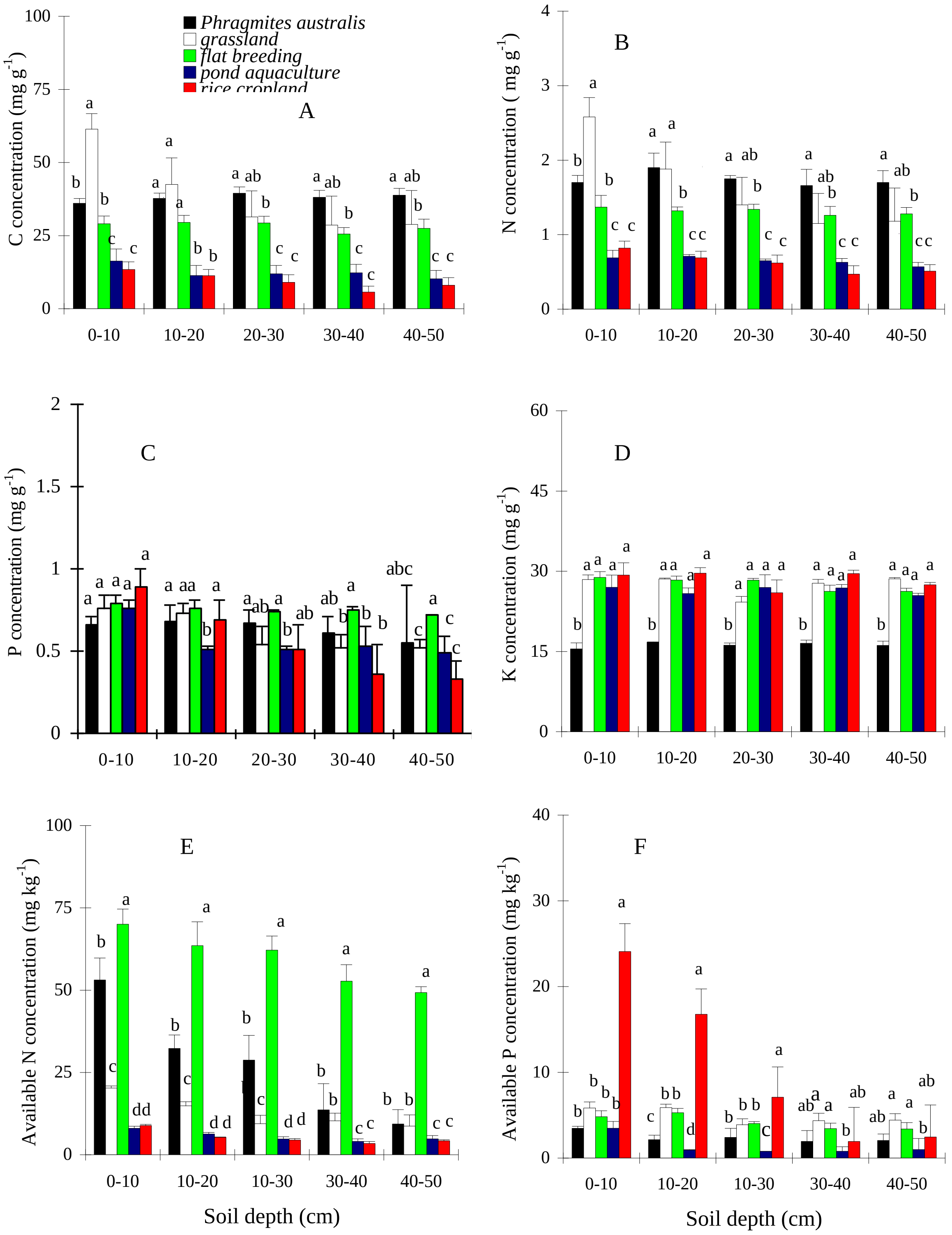
Phragmites australis
grassland
flat breeding
pond aquaculture
rice cropland
A
C concentration (mg g-1)
100
75
50
25
0
B
N concentration (mg g-1)
4
3
2
1
0
C
P concentration (mg g-1)
2
1.5
1
0.5
0
D
K concentration (mg g-1)
60
45
30
15
0
E
Available N concentration (mg kg-1)
100
75
50
25
0
F
Available P concentration (mg kg-1)
40
30
20
10
0
0-10
10-20
20-30
10-30
30-40
40-50
Soil depth (cm)
Soil depth (cm)

Fig. 2

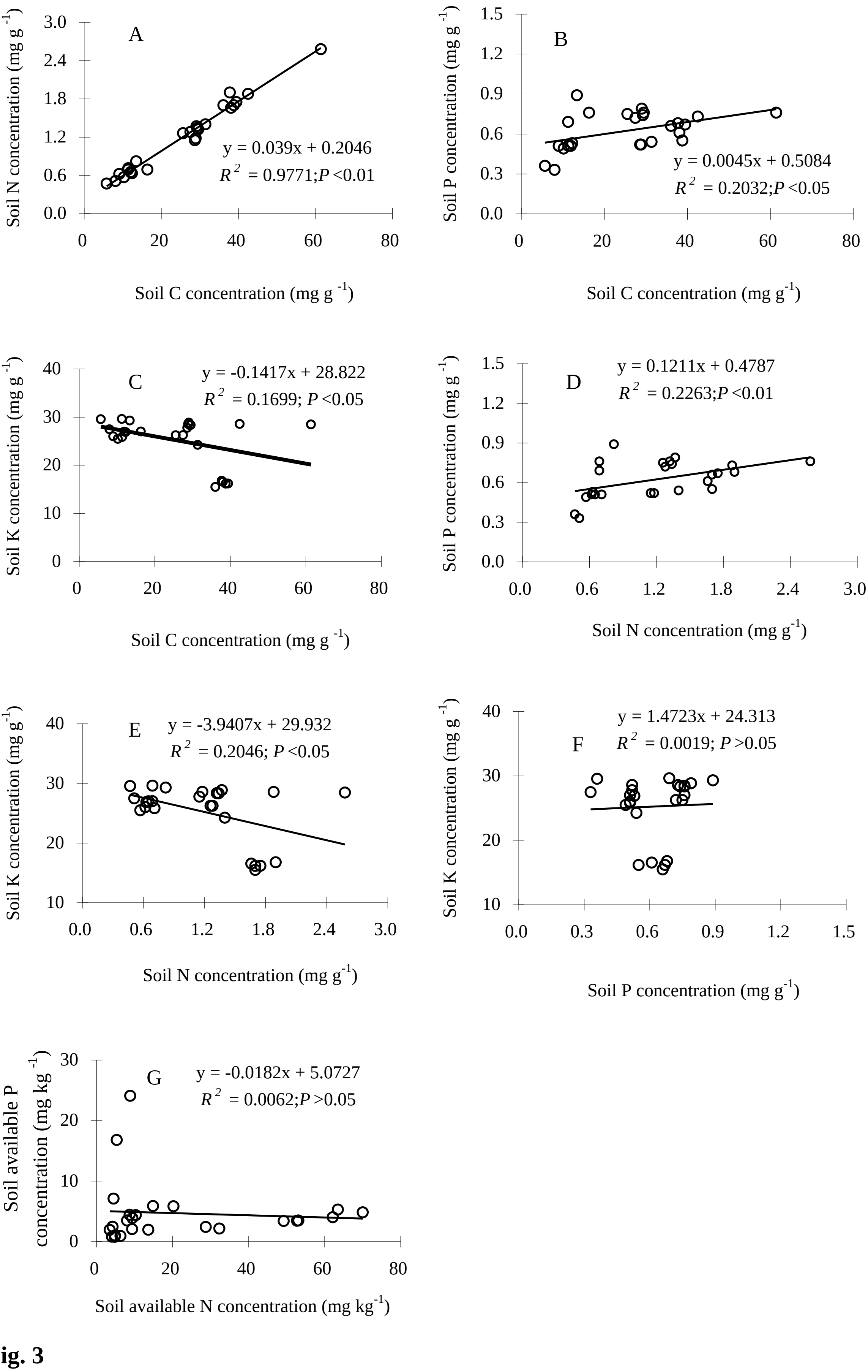
A
y = 0.039x + 0.2046
R 2 = 0.9771;P <0.01
Soil N concentration (mg g -1)
3.0
2.4
1.8
1.2
0.6
0.0
0
20
40
60
80
Soil C concentration (mg g -1)
B
y = 0.0045x + 0.5084
R 2 = 0.2032;P <0.05
Soil P concentration (mg g -1)
1.5
1.2
0.9
0.6
0.3
0.0
0
20
40
60
80
Soil C concentration (mg g-1)
C
y = -0.1417x + 28.822
R 2 = 0.1699; P <0.05
Soil K concentration (mg g -1)
40
30
20
10
0
0
20
40
60
80
Soil C concentration (mg g -1)
D
y = 0.1211x + 0.4787
R 2 = 0.2263;P <0.01
Soil P concentration (mg g -1)
1.5
1.2
0.9
0.6
0.3
0.0
0.0
0.6
1.2
1.8
2.4
3.0
Soil N concentration (mg g-1)
E
y = -3.9407x + 29.932
R 2 = 0.2046; P <0.05
Soil K concentration (mg g-1)
40
30
20
10
0.0
0.6
1.2
1.8
2.4
3.0
Soil N concentration (mg g-1)
F
y = 1.4723x + 24.313
R 2 = 0.0019; P >0.05
Soil K concentration (mg g -1)
40
30
20
10
0.0
0.3
0.6
0.9
1.2
1.5
Soil P concentration (mg g-1)
G
y = -0.0182x + 5.0727
R 2 = 0.0062;P >0.05
Soil available P concentration (mg kg -1)
30
20
10
0
0
20
40
60
80
Soil available N concentration (mg kg-1)

Fig. 3

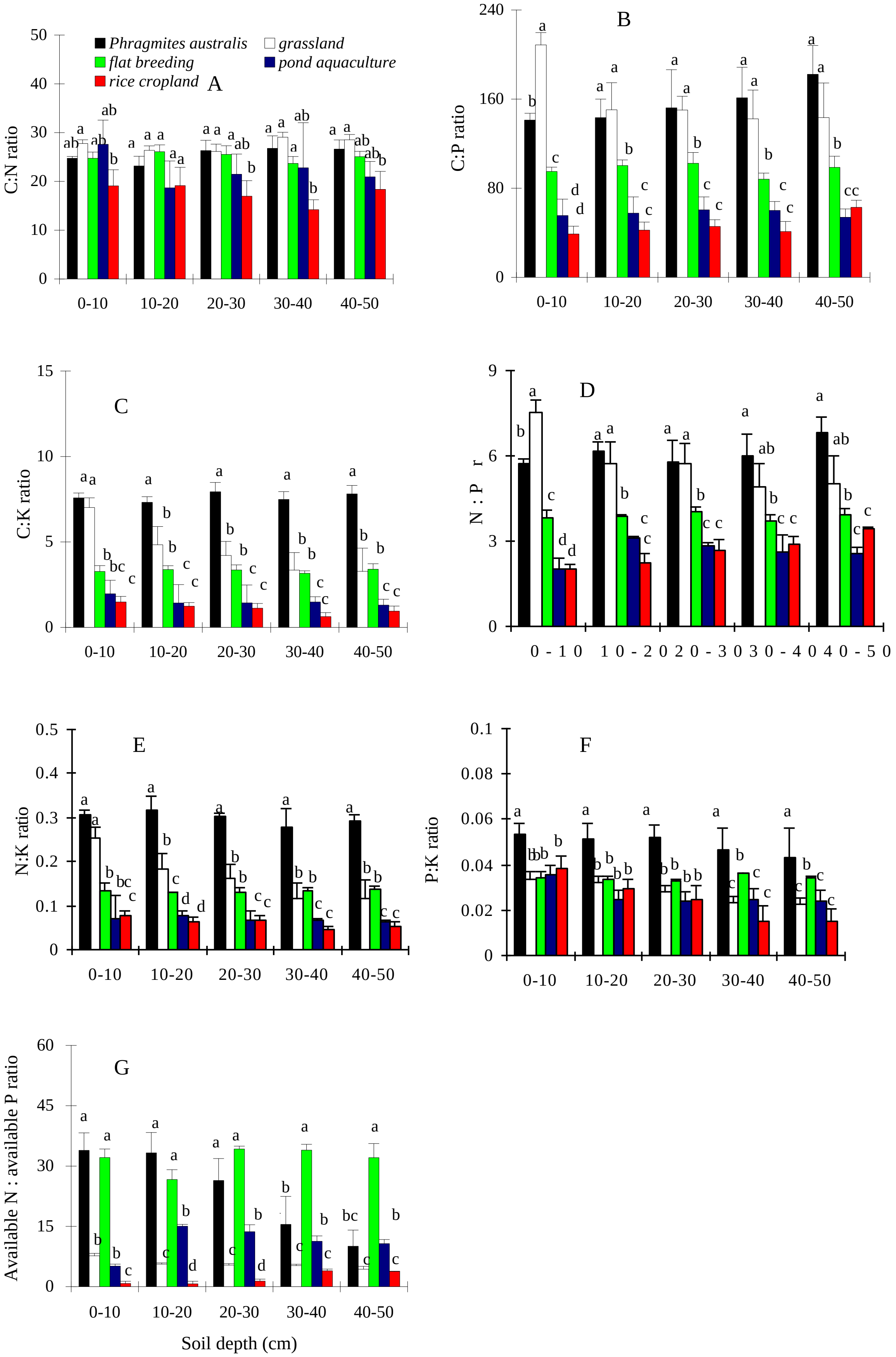
A
B
C
D
E
F
G
■ Phragmites australis
□ grassland
■ flat breeding
■ pond aquaculture
■ rice cropland
C:N ratio
C:P ratio
C:K ratio
N : P r
N:K ratio
P:K ratio
Available N : available P ratio
0-10
10-20
20-30
30-40
40-50
Soil depth (cm)

Fig. 4

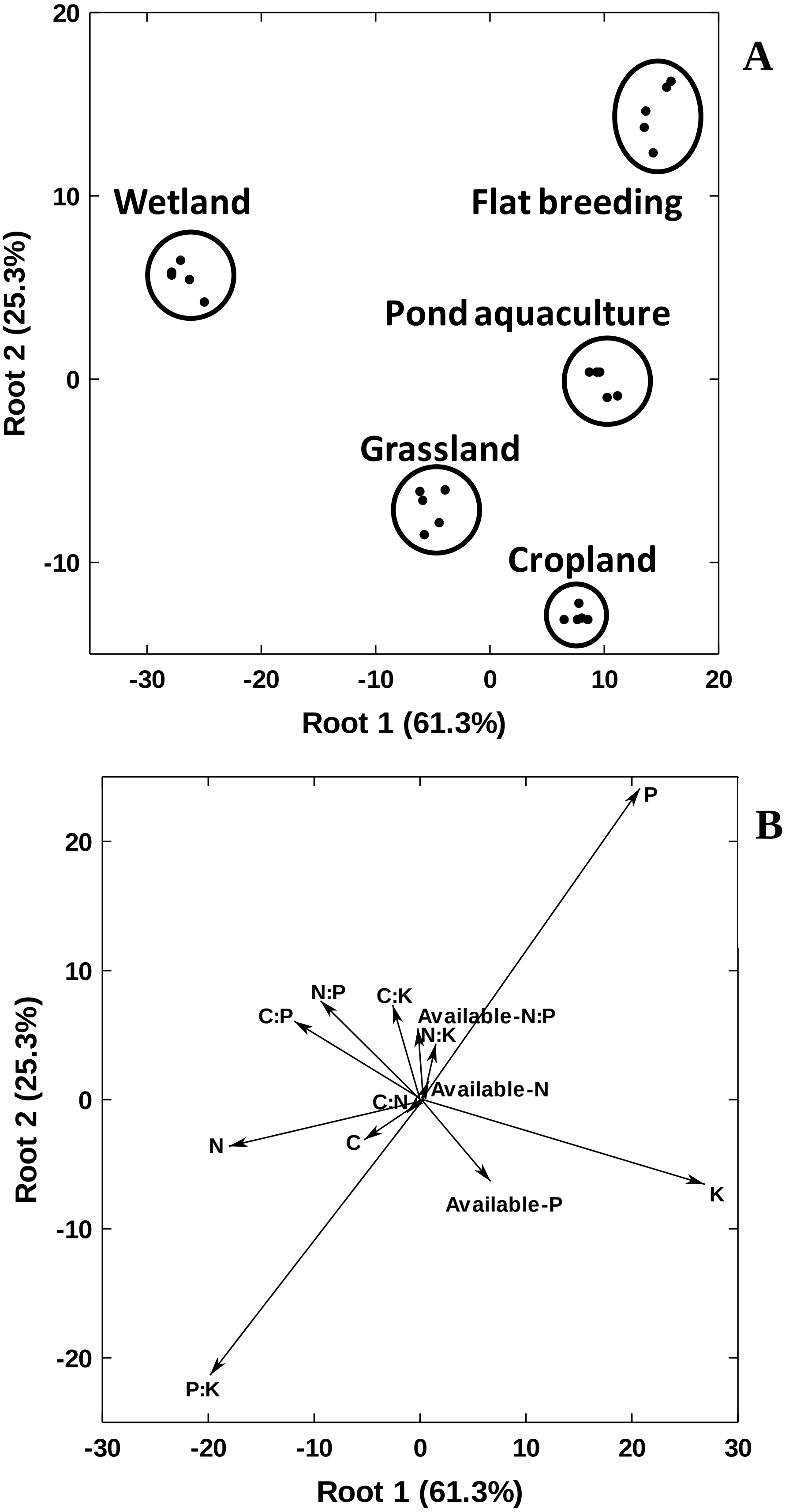
A
Wetland
Flat breeding
Pond aquaculture
Grassland
Cropland
Root 2 (25.3%)
Root 1 (61.3%)
20
10
0
-10
-30
-20
-10
0
10
20
B
P
C:K
N:P
C:P
Available-N:P
N:K
Available-N
C:N
N
C
Available-P
K
P:K
Root 2 (25.3%)
Root 1 (61.3%)
20
10
0
-10
-20
-30
-20
-10
0
10
20
30

Fig. 5

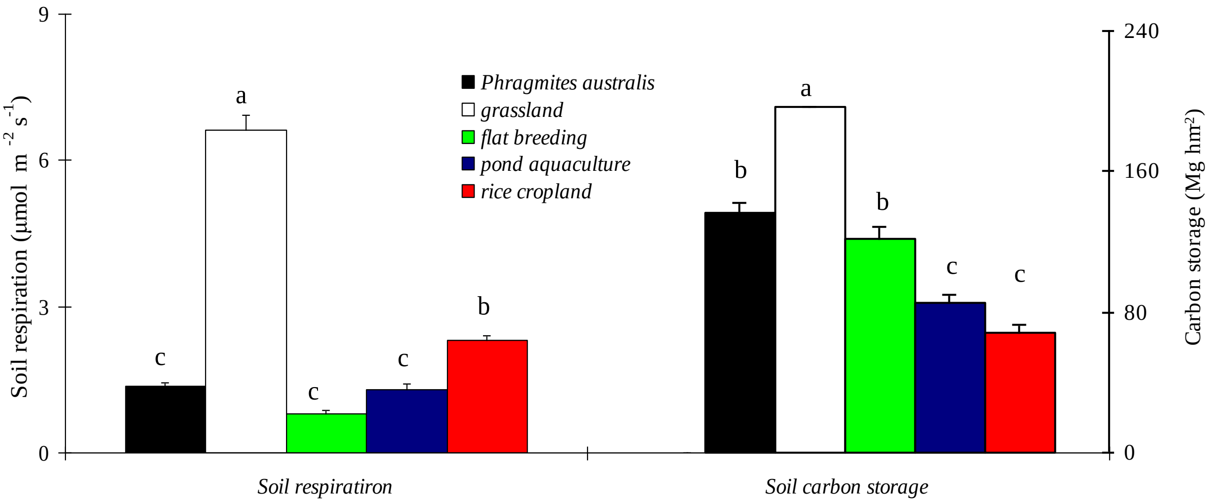
9
6
3
0
Soil respiration (μmol m-2 s-1)
240
160
80
0
Carbon storage (Mg hm-2)
■ Phragmites australis
□ grassland
🟩 flat breeding
■ pond aquaculture
🟥 rice cropland
c
a
c
c
b
b
a
b
c
c
Soil respiratiron
Soil carbon storage

Fig.6