# Agricultural land use decouples soil nutrient cycles in a subtropical riparian wetland in China

Weiqi Wang[a]*, Chun Wang[a], Jordi Sardans[b,c]*, Qingwen Min[d], Congsheng Zeng[a], Chuan Tong[a], Josep Penuelas[b,c]

[a] *Institute of Geography, Fujian Normal University, Fuzhou 350007, China*

[b] *CSIC, Global Ecology CREAF-CSIC-UAB, Cerdanyola del Valles, 08193 Barcelona, Catalonia, Spain*

[c] *CREAF, Cerdanyola del Valles, 08193 Barcelona, Catalonia, Spain*

[d] *Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China.*

*Correspondence: W. Wang. E-mail: wangweiqi15@163.com; J. Sardans. E-mail: j.sardans@creaf.uab.cat

**Handling title: Land use changes soil stoichiometry**

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## ABSTRACT

We examined the impact of human changes in land use on the concentrations and stoichiometric relationships among soil carbon (C), nitrogen (N), phosphorus (P) and potassium (K) in a *Phragmites australis* riparian wetland (Minjiang River estuary, China). We compared a natural (unaltered) wetland with five altered land uses: intertidal mudflat culture, and vegetable, flower, fruit and rice cultivations. All these land uses decreased C, N and K soil concentrations relative to those in the *P. australis* wetland. The close relationship between total soil C and N concentrations, under all land uses, suggested that N was the most limiting nutrient in these wetlands. The lower N concentrations, despite the use of N fertilizers, indicated the difficulty of avoiding N limitation in the agricultural land. Croplands, except rice cultivation, had lower soil N:P ratios than the original *P. australis* wetland, consistent with the tendency of favoring species adapted to high rates of growth (low N:P ratio). The release of soil C was less and the soil C:N and C:P ratios higher in the natural *P. australis* riparian wetland than in the croplands, whereas C storage was more similar. The levels of soil C storage were generally opposite to those of C release, indicating that C release by respiration was the most important factor controlling C storage. Cropland soil management promotes faster nutrient and C cycles and changes in soil nutrient stoichiometry. These impacts can further hinder the regeneration of natural vegetation by nutrient imbalances and increases C-cycling and C emissions.

Keywords: Nitrogen; Phosphorus; N:P; Land-use change; Decoupling of nutrient; Stoichiometry

## 1.Introduction

Anthropogenic activities can strongly alter the nutrient pools of carbon (C), nitrogen (N), phosphorus (P) and potassium (K) in soils by many processes including increasing nutrient inputs, N deposition, drought, species invasion or increases in atmospheric $CO_2$ (Tian et al., 2010; Sardans et al., 2012b; Sardans and Peñuelas, 2012). These shifts are frequently associated to changes in the structure of plant communities and/or in nutrient outputs (e.g. crop harvesting and weathering) (Sardans et al., 2012a). Land-use changes due to agronomic practices and livestock production generate soil stoichiometric shifts in forests (Falkengren-Gerup et al., 2006; Sardans and Peñuelas, 2013), shrublands (Sardans and Peñuelas, 2013), grasslands (Mulder and Elser, 2009) and steppes (Jiao et al., 2013). The status of the C:N:P ratio in wetland soil under different intensities of human disturbance, however, remains unknown.

Recent stoichiometric ecological studies have shown that K is even more associated than are 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 (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.

Changes in soil stoichiometry can influence the capacity to regenerate natural vegetation after the abandonment of human activities, delaying it for many decades (Falkengren-Gerup et al., 2006; Jiao et al., 2013). This impact can be especially critical in sensitive diversity-rich ecosystems, such as wetlands, that are severely affected by changes in land use (Ramsar, 2013). The effect of land-use change on the stoichiometry of wetlands has received little attention (Koerselman and Meuleman, 1996). Wetlands occupy $5.7\times10^6$ $km^2$ worldwide, are cradles of biodiversity upon which countless species of plants and animals depend for survival and are among the world’s most productive environments, being a sink of C in the form of peat and plant matter and providing a wide array of benefits (Mitsch and Gosselink, 2007; Ramsar, 2013). In the current context of global change, wetlands continue to be among the most threatened ecosystems, and yet we lack information about the impact of anthropogenic changes on their abiotic and biotic environments (Mitsch and Gosselink, 2007; Ramsar 2013). The ability of wetlands to adapt to changing conditions and to the current accelerating rates of global change will be crucial to world biodiversity conservation. A better understanding of the resulting soil C, N, P and K ecological stoichiometries in wetlands submitted to land use changes would provide decision makers with the necessary information for developing effective methods to enhance the potential capacity of these ecosystems to fix C and reduce the impact of emissions of greenhouse gases (Peñuelas et al., 2013). It would also and provide information on the impacts of anthropogenic activity on the regenerative capacity of wetlands by determining the cycles and balances of C, N, P and K and the fertility of the soil. We expect that human activities changing nutrient balances (fertilization and harvesting), species composition and water fluxes should exert a great impact on soil elemental composition. This should change C fluxes and hinder further ecosystem restoration processes by shifting soil condition far from the optimum from that of natural wetlands. 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 $1.2\times10^4$ km$^2$ (Huang et al., 2006). These areas are characterized by rapid economic development, and by the fast replacement of natural undisturbed areas by areas disturbed by crops, livestock and tourism. The loads of N and P to rivers caused by human activities and further transported downstream to the wetlands (Howarth et al., 1996) cause water eutrophication (Anderson et al., 2002) that threatens the health of wetlands (An et al., 2007) and decreases ecosystemic services (Lee et al., 2006). Research in these areas, however, has been scarce, and studies on various spatial and temporal scales are therefore needed.

To solve this lack of knowledge, we aimed to determine: (1) the changes in C, N, P and K concentrations and stoichiometry associated with land-use changes at various soil depths in riparian tidal wetlands, (2) the relationships of soil influencing factors and (3) the capacity of soil to store C with soil C, N, P and K ratios shifts and land-use changes.

## 2.Material and methods

### *2.1.Study area and experimental design*

This work was conducted in the Difengjiang wetland of the Minjiang River estuary (China) (25°58′53.50″-25°59′46.01″N, 119°17′52.60″-119°20′25.67″E Fig. 1). The climate is subtropical with mean annual temperatures and precipitation of 19.7 ºC and 1348.8 mm, respectively. The soil surface of the riparian wetland is submerged across the study site for 1-2.5 h during each tidal inundation. The large perennial grass, *Phragmites australis* (mature height of 2 m at 150 stems $m^{-2}$), is one of the most important plant species and is typically found from the upstream to the downstream regions of the Minjiang River tidal wetland (Liu et al., 2006).

To determine the associations between different agricultural land-use changes and the concentrations and ratios of soil C, N, P and K, we established plots on a wide range of land uses: natural *P. australis* wetland (control), flower (Jasmine) cultivation (*P. australis* plants removed eight years previously), intertidal mudflat culture (the aerial parts of *P. australis* plants removed 10 years previously), rice cultivation (*P. australis* plants removed 20 years previously), vegetable cultivation (*P. australis* plants removed 30 years previously), and fruit (Longan) cultivation (*P. australis* plants removed 40 years previously). The natural *P. australis* wetland and intertidal mudflat culture plots have not been fertilized. The plots of flower, rice, vegetable and fruit cultivations were fertilized ($N$-$P_2O_5$-$K_2O$=16-16-16%; Keda Fertilizer Co., Ltd.) with dosages of 225, 235, 150, 300 kg $ha^{-1}$ $y^{-1}$ respectively.

The soil types for *P. australis* wetland and intertidal mudflat culture plots were wetland soil, the soil types for vegetable cultivation, flower (Jasmine) cultivation and fruit (Longan) cultivation plots had changed from wetland to krasnozem soil and the soil types for rice cultivation plots had changed from wetland to paddy soil.

In our study, three plots (1 $m^2$ each one) were randomly selected at each location. These plots were separated 100 m among them in each site with different land use. *P. australis* wetland is the control plot of the experiment. *P. australis* wetlands are water sources in the region, and they are protected by government, so the human influence was very limited. Sampling locations were established in the *P. australis* riparian wetland and at sites of intertidal mudflat culture, vegetable, flower, fruit and rice cultivations (Fig. 1). Soil samples were collected in March 2013. Under natural conditions (without any human activity) all studied sites that currently have a human activity should be a *P. australis* wetland such as is the control.

## *2.2.Collection and measurement of soil samples*

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, 0.3 m; diameter 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 on both sides of the soil pits. These three samples from each layer were bulked to form one sample per layer. A total of 90 soil samples (six types of land use×three plots×five 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 (Lu 1999), 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 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, Wang et al., 2014).

Soil parameters that can potentially be changed by human activities and that could be factors influencing the status and processes of soil nutrients were also determined. Bulk density was measured from three 5×3 cm cores per layer, salinity was measured with a DDS-307 conductivity meter (Boqu Scientific Instruments, China), pH was measured with an 868 pH meter (Orion Scientific Instruments, USA), soil-particle size (clay, silt and sand) was measured by a Mastersizer 2000 laser particle size analyser (Malvern Scientific Instruments, UK), soil-water content was measured by the drying method (Lu, 1999) and soil carbon ($CO_2$) release was determined by the incubation method (Wang et al., 2010). Thirty g of fresh soil were placed into 120 ml incubation bottles, and then bottles were sealed with a rubber stopper, and incubated at 20 ℃ during three days, 5 ml gases were extracted from headspace every day (four times). $CO_2$ concentration was determined by the GC-2014 gas chromatograph instrument (Shimadzu Scientific Instruments, Japan).

### 2.3.C storage and release

C storages for the 0-50 cm 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 the soil-depth interval (1, 2, … n), $C_m$ is the C concentration (g kg$^{-1}$), $\rho_{\mathrm{b}}$ is the bulk density (kg m$^{-3}$), $D$ is the thickness of each layer (m) and $n$ is the number of layers.

C release was estimated using the equation (Wassmann et al., 1998):

$$P = \frac{dc}{dt} \cdot \frac{V_H}{W_S} \cdot \frac{MW}{MV} \cdot \frac{T_{st}}{T_{st} + T}$$

where $P$ is the rate of C release (mg$^{-1}$ g$^{-1}$ d$^{-1}$), $dc/dt$ is the recorded change in the mixing ratio of C ($CO_2$) in the headspace over time (mmol mol$^{-1}$ d$^{-1}$), $V_H$ the volume of the headspace (L), $Ws$ the dry weight of the soil (g), $MW$ is the molecular weight of $CO_2$ (g), $MV$ the molecular volume (L), $T$ is the temperature (K) and $T_{st}$ is the standard temperature (K). As in a previous study in this same site we observed that in the wetlands most carbon release from soil was in the form of $CO_2$ (Wang et al., 2010), and in the present study, some of the land use types were not wetlands, and thus had not anaerobic periods, we expected that $CH_4$ emissions not to be the main C release form, and thereby we only determined $CO_2$ release.

### *2.4.Statistical analyses*

The differences in the soil variables among sites with different land uses (land use×soil layer) were assessed by two-way ANOVAs with Tukey's post-hoc tests. We used Pearson's correlation to examine relationships among factors. A Kolmogorov-Smirnov (KS) test was performed on each variable to test for normality. The studied soil variables were normally distributed. We used discriminant functional analysis (DFA) to associate the various levels of disturbance with overall elemental composition and stoichiometry. 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). Univariant analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, Illinois). The DFAs were performed using Statistica 6.0 (StatSoft, Inc. Tule, Oklahoma, USA). C:N:P:K ratios were calculated as molar ratios.

## 3.Results

### *3.1.Effect of agricultural land use on C, N, P and K concentrations and stoichiometry*

Total soil C, N and P concentrations varied significantly with land use and soil depth (Table S1, Fig. S1). We also observed a significant interaction between these two factors on total soil C, N and P concentrations (Table S1, Fig. S1). Total soil K concentration varied among land-use type, but land-use type was not associated with soil depth (Table S1, Fig. S1). C, N and K concentrations were generally higher in the natural wetland than in the agricultural plots ($P<0.05$), but the P concentration in the natural wetland was higher than in the plots of flower and rice cultivation (Table S2). Total soil C, N, P and K were all them correlated less the concentrations of P and K (Fig. 2).

Total soil C:N and P:K ratios significantly diverged among land-use types and in the association of soil depth with land-use type. These ratios did not differ significantly with soil depth (Table S3, Fig. S2). The C:P and N:P ratios significantly diverged among land-use types ($P<0.001$,) but not with soil depth or in the association of soil depth and land-use type (Table S3, Fig. S2). Total soil C:K, C:P, N:P and N:K ratios significantly differed among depths and land-use types and in the association of depth and land-use type (Table S3, Fig. S2). Soil C:N and C:P ratios were higher in the natural wetland than in the agricultural plots ($P<0.05$) (Table S4). Total soil C:K ratios in the natural wetland were also higher than for all agricultural plots ($P<0.05$) except for fruit cultivation (Table S4). Total soil N:P ratios in the natural wetland were higher than those for vegetable and fruit cultivation ($P<0.05$) but not for the other agricultural land-use types (Table S4). Total soil N:K ratios in the natural wetland were higher than those for vegetable and flower cultivation ($P<0.05$), lower than those for fruit cultivation ($P<0.05$) but not significantly different from those for intertidal mudflat culture and rice cultivation (Table S4). Total soil P:K ratios in the natural wetland were lower than those for vegetable and fruit cultivation ($P$<0.05), higher than those for flower cultivation ($P$<0.05) but not significantly different from those for intertidal mudflat culture and rice cultivation (Table S4).

### *3.2.Effect of land-use on C storage and release*

Soil C storage and release differed among the various agricultural land-uses (Fig. S3). C storage and release differed significantly with soil depth and land-use types and in the association of depth and land-use type (Table S5, Fig. S3).

The overall C storage was significantly higher in the natural wetland than for vegetable cultivation lower than for intertidal mudflat culture and not different from the other agricultural land-uses (Table S6). C release was lower in the natural wetland than in all agricultural plots ($P$<0.05) except those for rice cultivation (Table S6).

### *3.3.Effect of land use on soil parameters*

Soil pH and water content differed significantly among the land-use types and in the association of soil depth and land-use type (Table S7, Fig. S4) but not with depth. pH was higher in the natural wetland than for intertidal mudflat culture and vegetable and flower cultivation and lower than for fruit and rice cultivation (Table S8). Soil salinity differed significantly among the land-use types (Table S7, Fig. S4) and in the association of soil depth and land-use type (Table S7, Fig. S4) but not with soil depth. Soil salinity was significantly higher in the natural wetland than for all agricultural land uses ($P$<0.05). The clay, the sand and the bulk density percentage were higher in the natural wetland than in all agricultural land uses ($P$<0.05). The silt percentage was higher in the natural wetland than for flower, fruit and rice cultivations but was not different for intertidal mudflat culture and vegetable cultivation (Table S8).

### *3.4.Effect of soil parameters on C, N, P and K concentrations and stoichiometries*

Total soil N and especially P and K concentrations were negatively correlated with bulk density (Table 1). The total soil C:N and C:P ratios were correlated positively with pH, water content, salinity and clay percentage and negatively with bulk density and sand percentage (Table 1). The total soil C:K ratio was correlated positively with pH and salinity and negatively with bulk density (Table 1). The total soil N:P ratio was correlated positively with water content and salinity and negatively with pH. The total soil N:K ratio was correlated positively with pH and sand percentage and negatively with bulk density, clay percentage and silt percentage (Table 1). The total soil P:K ratio was correlated positively with pH and sand percentage and negatively with water content, bulk density, salinity, clay percentage and silt percentage (Table 1).

### *3.5.Effect of soil parameters and nutrient stoichiometries on C balance*

C release was correlated positively with pH and negatively with water content. C storage was positively correlated with water content (Table 1). C release was correlated positively with C:K, N:K and P:K ratios and negatively with C:P and N:P ratios. C storage was positively correlated with C:N, C:P, C:K, N:P and N:K ratios (Table 2).

### *3.6.Multivariate DFA analyses*

A general DFA with soil depth as a categorical independent variable and with total soil C, N, P and K concentrations and soil C:N:P:K ratios as continuous independent variables significantly separated the soils of the various land uses (Tables 3 and 4, Fig. 3). All sites pair-wise squared Mahalanobis distances were significant (Table 4). The independent variables with significant effects in the model were total soil C, N, P and K concentrations and C:N, C:K and P:K ratios (Table 3). The soils under human land-use generally had different elemental compositions than the wetland control soil, mainly because they had higher total P concentrations (Fig. 3). The soil for fruit cultivation had a different chemical composition mainly due to a higher total soil N concentration and soil P:K ratio (Fig. 3).

The DFA with soil depth as a categorical independent variable and C storage, C release, bulk density, pH, salinity and water content as continuous independent variables significantly separated the soils of the various land uses (Tables 5 and 6, Fig. 4). All sites pair-wise squared Mahalanobis distances were significant (Table 6). The independent variables with significant effects in the model were total soil C, N, P and K concentrations and C:N, C:K and P:K ratios (Table 5). Soils under natural wetland were different than soils under human land-use mainly by present higher salinity and water content (Fig. 4). The soil for fruit cultivation had a chemical composition different from that of the other sites due to a higher pH (Fig. 4).

# 4.Discussion

## *4.1.Decoupling of soil C, N, P and K concentrations associated with land-use change*

Our results strongly suggest that this wetland area is N-limited, although the N fertilization was applied in the cultivation plots, the soil N concentration remains lower in the soils of human managed areas similar to non-fertilized natural wetlands. Furthermore, N limitation is also 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, by slowing mineralization, and by high levels of leaching of N. After several years of cropland management, including fertilization, total soil P was higher in the most cases, whereas N and K contents did not differ from those of the *P. australis* wetland. N limitation also played a role in the growth of *Spartina alterniflora* in a similar estuary of the Yangtze River, which is ~800 km north of the Minjiang estuary (Gan et al., 2011).

Both the concentrations and ratios of C, N, P and K varied with soil depth, consistent with previous studies (Cleveland and Liptzin, 2007). Only the C:N ratios were stable across the soil profile under the various levels of disturbance (Table S4), in agreement with earlier reports (Tian et al., 2010). The mean molar C:N (13.4) and C:P (67.2) ratios in the 0-10 cm layers of all sites in the study area were similar to the average ratios for China (12.3 and 52.7, respectively; Tian et al., 2010) and to global ratios (12.3 and 72.0, respectively; Cleveland and Liptzin, 2007). The lower soil N:P ratios observed in soils, between 3 and 6 depending on site and soil depth, may be due to the higher solubility of N than of P and the consequent higher losses of N (and also K) than of P by the continuous tidal flooding in this area.

Our results are partially consistent with the premise that human activity, by creating more-productive ecosystems, tends to favor ecosystems with higher total soil P concentrations and lower total soil C:P, C:N and N:P ratios that are 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). Except in the case of the rice cropland (that fix-$N_2$) (Herridge et al., 2008), the croplands had lower total soil N:P ratios than the natural wetland, an effect that is probably related to high P concentrations from the accumulation of P from fertilization and from its lower mobilization capacity relative to N. This lack of effect on soil N concentrations in the rice cropland was probably due to the capacity of this cropland to fix $N_2$ by the symbiotic association between the aquatic fern *Azolla* and by free-living cyanobacteria (Herridge et al., 2008).

Our results thus demonstrated soil imbalances among C, N, P and K under crop production associated with land management, as observed in croplands in non-wetland areas in other parts of the world under intensive management, including constant inputs of fertilizer (Cech et al., 2008; Peñuelas et al., 2009). Imbalances have also been associated with a higher leaching of N than of P in other wetland areas (Arbuckle and Downing, 2001). Moreover, the high precipitation in our subtropical study area may contribute to high rates of nutrient leaching in the highly weathered soil (Laird et al. 2010), which would affect N and K (more soluble) more than P (less soluble).

The natural wetlands of southern China dominated by *P. australis* have a large capacity to retain N (Wang et al., 2014). Their replacement by cropland species less able to retain N, and the removal of biomass in the harvest, decrease soil N concentrations despite N fertilization and thus lead to a high limitation of N. This limitation could be a constraint to the potential regenerative capacity of the natural wetlands.

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

Our data suggest that cultivation can increase soil C release through respiration, due at least in part to the effects of cropland management reducing water content and salinity. Agricultural management has eliminated tidal flooding, so water contents and salinities have declined. Thus, lower soil water content by eliminating tidal flooding can increase C release, as reported in a previous study (Nomura et al., 2013). Soil C release by respiration, apart from being correlated with plant productivity, may thus also be associated with other environmental parameter, such as soil-water content. Our results also suggest that the use of land for crop production in subtropical wetlands can increase soil $CO_2$ emissions, as has been observed in previous studies (Shang et al., 2013). The negative relationship between salinity and C release may be due to the inhibition of growth and activity of soil microorganisms by osmotic stress at higher salinities (Rietz and Haynes, 2003). Previous studies have observed that soil C release was negatively correlated with salinity (Setia et al., 2011).

Total soil N:P and N:K ratios were positively correlated with total soil C, consistent with similar previous studies (Hessen et al., 2004) and the growth rate hypothesis (Sterner and Elser, 2002), where high N:P ratios are associated with low soil microbial activity and rates of nutrient and carbon cycles. Soil respiration has been associated with soil C storage and plant productivity (Dias et al., 2010; De Deyn et al., 2011), but we observed that soil C storage and release were generally not statistically correlated (only the soil for fruit cultivation had a significant correlation). The transformation of these natural wetlands to croplands may thus increase soil $CO_2$ emissions without clear changes in C storage.

## 5.Conclusions

Increasing human activity was associated with lower soil C, N and K concentrations in the soil layers.

The stoichiometric changes and their relationships with other soil properties such as soil C release suggest a limitation of N in the ecosystems of this estuary. An N:P ratio lower than the global ratio and a lower N concentration in the croplands despite N fertilization also suggest N limitation.

Anthropogenic transformations of land use were associated with lower total soil N concentrations and N:P ratios in some croplands, which is consistent with the tendency of croplands to favor species adapted to high rates of growth (low soil N:P ratios), such as vegetables and fruits.

Soil $CO_2$ emissions were higher in the croplands without clear differences in C storage. Crop production would load more organic matter to the soil but would also enhance the decomposition of organic matter, an effect likely linked to lower soil salinity and better soil aeration (less soil clay and water content).

Human activities transforming natural wetlands to croplands (including fertilization) can promote a large imbalance between the concentrations of mobile elements (N and K), and the concentrations of relatively immobile elements (P) with the trend to increase. This can difficult the reestablishment of natural wetlands and/or reduce the long-term capacity of crop production.

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# Tables

**Table 1** Pearson correlation coefficients between nutrient concentrations, stoichiometry, C balance and soil properties (n=90)

| Nutrient | pH | Water content | Bulk density | Salinity | Clay percentage | Silt percentage | Sand percentage |
|---|---|---|---|---|---|---|---|
| C | 0.184* | 0.630** | -0.733** | 0.716** | 0.507** | 0.244** | -0.365** |
| N | 0.133 | 0.577** | -0.636** | 0.573** | 0.445** | 0.201* | -0.312** |
| P | 0.382** | -0.011 | -0.336** | 0.066 | 0.093 | -0.012 | -0.030 |
| K | -0.660** | 0.720** | -0.250** | 0.537** | 0.762** | 0.630** | -0.718** |
| C:N | 0.177* | 0.425** | -0.575** | 0.588** | 0.356** | 0.156 | -0.246** |
| C:P | -0.107 | 0.440** | -0.306** | 0.495** | 0.265** | 0.136 | -0.197* |
| C:K | 0.568** | 0.053 | -0.434** | 0.228* | -0.066 | -0.162 | 0.131 |
| N:P | -0.177* | 0.286** | -0.082 | 0.260** | 0.091 | 0.032 | -0.058 |
| N:K | 0.634** | -0.098 | -0.296** | 0.043 | -0.233* | -0.302** | 0.290** |
| P:K | 0.706** | -0.363** | -0.186* | -0.189* | -0.322** | -0.364** | 0.366** |
| C release | 0.225* | -0.409** | 0.113 | -0.338** | -0.163 | -0.008 | 0.072 |
| C storage | 0.118 | 0.198* | 0.012 | 0.172 | 0.017 | -0.030 | 0.012 |

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

**Table 2** Pearson correlation coefficients of C balance with nutrient ratios (n=90)

| C balance | C:N | C:P | C:K | N:P | N:K | P:K |
|---|---|---|---|---|---|---|
| C release | -0.155 | -0.342** | 0.280** | -0.343** | 0.377** | 0.532** |
| C storage | 0.404** | 0.536** | 0.650** | 0.442** | 0.630** | 0.033 |

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

**Table 3** Statistics (Wilks' Lambda and *P*-value) of the discriminant functional analysis among soils of the land uses with total soil C, N, P and K concentrations; total soil C:N, C:P, C:K, N:P, N:K and P:K ratios and soil depth as variables. Bold type indicates a significant effect of the variable in the model (*P*<0.05)

| Soil variables | Wilk's Lambda | *F* | *P* |
|---|---|---|---|
| **[C]** | **0.654** | **7.51** | **<0.0001** |
| **[N]** | **0.763** | **4.41** | **0.0015** |
| **[P]** | **0.356** | **25.7** | **<0.0001** |
| **[K]** | **0.842** | **2.66** | **0.029** |
| **C:N ratio** | **0.743** | **4.90** | **0.00066** |
| C:P ratio | 0.922 | 1.20 | 0.32 |
| **C:K ratio** | **0.759** | **4.51** | **0.0013** |
| N:P ratio | 0.912 | 1.37 | 0.25 |
| N:K ratio | 0.919 | 1.26 | 0.29 |
| **P:K ratio** | **0.408** | **20.6** | **<0.0001** |
| Depth | 0.869 | 0.511 | 0.96 |

**Table 4** Squared Mahalanobis distances among soils of the land uses in the discriminant functional analysis with total soil C, N, P, and K concentrations; total soil C:N, C:P, C:K, N:P, N:K and P:K ratios and soil depth as variables

| | Intertidal mudflat culture | Vegetable cultivation | Flower cultivation | Fruit cultivation | Rice cultivation |
|---|---|---|---|---|---|
| Wetland control | 30.5<br>*P*<0.0001 | 86.7<br>*P*<0.0001 | 76.0<br>*P*<0.0001 | 227<br>*P*<0.0001 | 91.6<br>*P*<0.0001 |
| intertidal mudflat culture | | 23.3<br>*P*<0.0001 | 13.8<br>*P*<0.0001 | 140<br>*P*<0.0001 | 23.6<br>*P*<0.0001 |
| Vegetable cultivation | | | 12.4<br>*P*<0.0001 | 157<br>*P*<0.0001 | 13.1<br>*P*<0.0001 |
| Flower cultivation | | | | 112<br>*P*<0.0001 | 4.68<br>*P*=0.020 |
| Fruit cultivation | | | | | 108<br>*P*<0.0001 |

**Table 5** Statistics (Wilks' Lambda and *P*-value) of the discriminant functional analysis among soils of the land uses with soil C storage, C release, bulk density, pH, respiration, salinity, water content and depth as variables. Bold type indicates a significant effect of the variable in the model ($P$<0.05)

| Variable | Wilk's lambda | *F* | *P* |
|---|---|---|---|
| Respiration | 0.999 | 0.111 | 0.99 |
| **pH** | **0.097** | **140** | **<0.001** |
| **Water content** | **0.433** | **19.6** | **<0.001** |
| **Bulk density** | **0.671** | **7.36** | **<0.001** |
| **Salinity** | **0.079** | **174** | **<0.001** |
| C release | 0.999 | 0.111 | 0.98 |
| **C storage** | **0.496** | **15.2** | **<0.001** |
| Depth | 0.728 | 1.25 | 0.21 |

**Table 6** Squared Mahalanobis distances among soils of the land uses in the discriminant functional analysis with soil C storage, C release, bulk density, pH, respiration, salinity, water content and depth as variables

| | intertidal mudflat culture | Vegetable cultivation | Flower cultivation | Fruit cultivation | Rice cultivation |
|---|---|---|---|---|---|
| Wetland control | 305<br>*P*<0.0001 | 379<br>*P*<0.0001 | 424<br>*P*<0.0001 | 392<br>*P*<0.0001 | 322<br>*P*<0.0001 |
| intertidal mudflat culture | | 37.6<br>*P*<0.0001 | 30.0<br>*P*<0.0001 | 110<br>*P*<0.0001 | 31.9<br>*P*<0.0001 |
| Vegetable cultivation | | | 12.7<br>*P*<0.0001 | 84.9<br>*P*<0.0001 | 27.8<br>*P*<0.0001 |
| Flower cultivation | | | | 124<br>*P*<0.0001 | 41.3<br>*P*<0.0001 |
| Fruit cultivation | | | | | 29.6<br>*P*<0.0001 |

## Figure captions

**Fig. 1** Location of the five types of land use

**Fig. 2** Relationships among soil nutrient concentrations in the five types of land use. C vs. N (A), C vs. P (B), C vs. K (C), N vs. P (D), N vs. K (E) and P vs. K (F)

**Fig. 3** (A) Biplot representing the standardized canonical discriminant function coefficients for the first two roots representing the soil samples from the five types of land use in the space generated by the first two roots of the discriminant functional analysis of total soil C, N, P and K concentrations and total soil C:N, C:P, C:K, N:P, N:K and P:K ratios. (B) Biplot representing the scores (mean ± S.E.) of the analysis in A

**Fig. 4** (A) Biplot representing the standardized canonical discriminant function coefficients for the first two roots representing the soil samples from the five types of land use in the space generated by the first two roots of the discriminant functional analysis of soil C storage, C release, bulk density, pH, respiration, salinity and water content. (B) Biplot representing the scores (mean ± S.E.) of the analysis in A

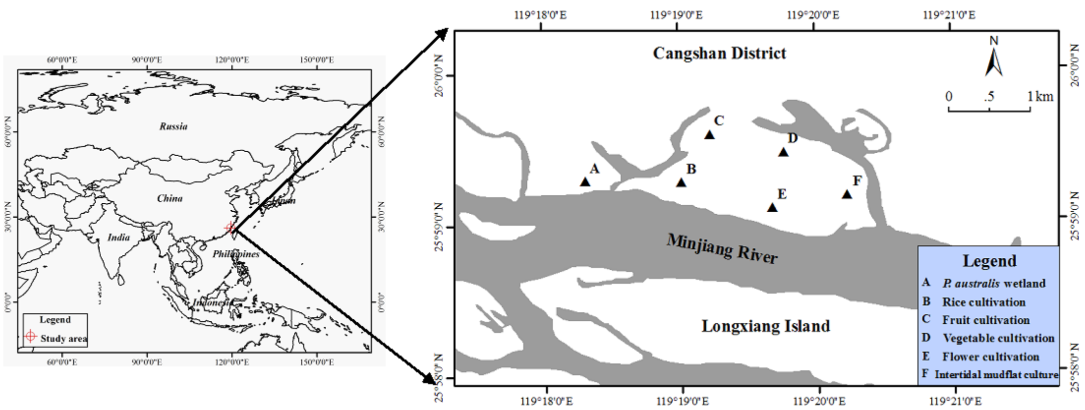
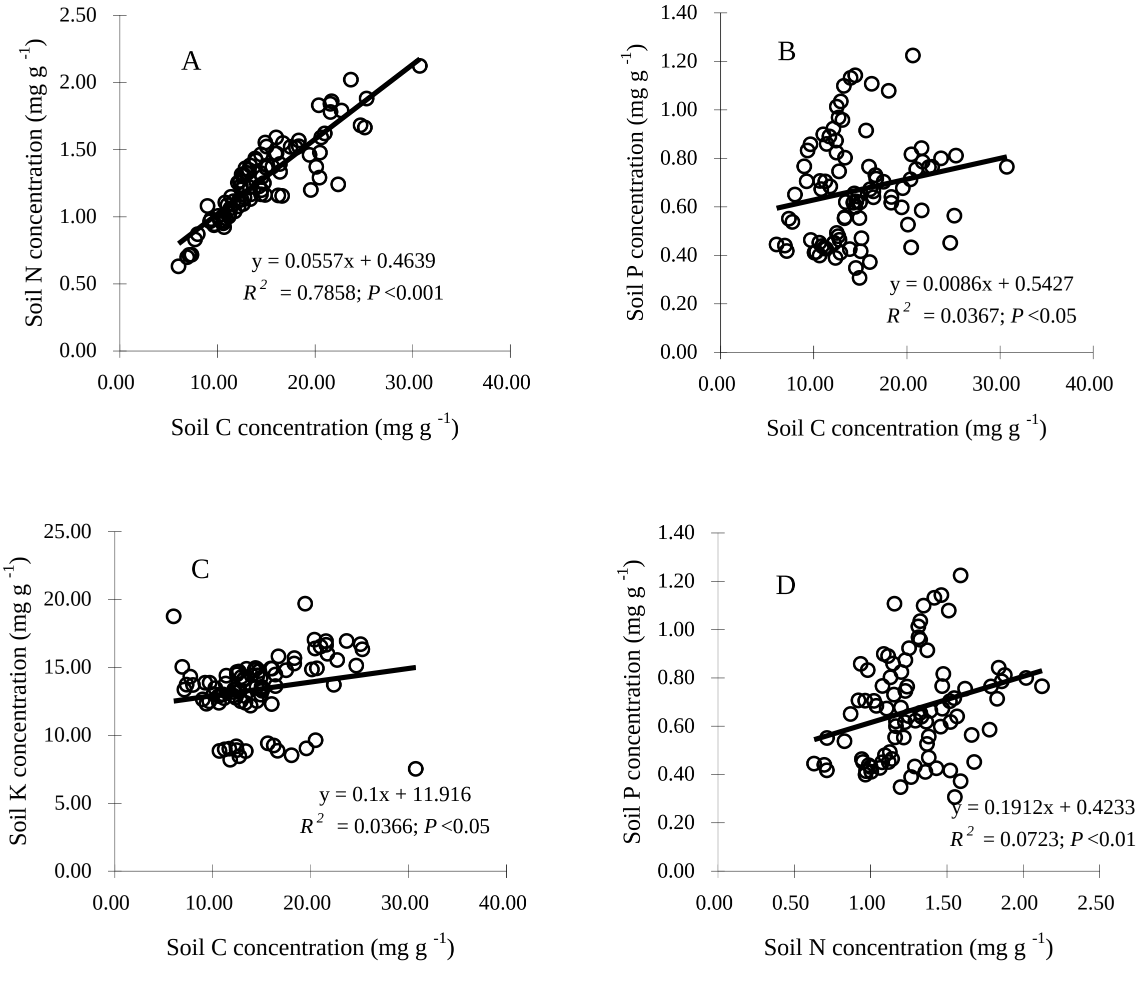

60°0'0"E
90°0'0"E
120°0'0"E
150°0'0"E
60°0'0"N
30°0'0"N
0°0'0"
Russia
China
India
Japan
Philippines
Indonesia
Legend
Study area
119°18'0"E
119°19'0"E
119°20'0"E
119°21'0"E
26°0'0"N
25°59'0"N
25°58'0"N
Cangshan District
N
0
.5
1 km
A
B
C
D
E
F
Minjiang River
Longxiang Island
Legend
A *P. australis* wetland
B Rice cultivation
C Fruit cultivation
D Vegetable cultivation
E Flower cultivation
F Intertidal mudflat culture

**Fig.1**


A
Soil N concentration (mg g -1)
2.50
2.00
1.50
1.00
0.50
0.00
0.00
10.00
20.00
30.00
40.00
Soil C concentration (mg g -1)
y = 0.0557x + 0.4639
R 2 = 0.7858; P <0.001
B
Soil P concentration (mg g -1)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
10.00
20.00
30.00
40.00
Soil C concentration (mg g -1)
y = 0.0086x + 0.5427
R 2 = 0.0367; P <0.05
C
Soil K concentration (mg g -1)
25.00
20.00
15.00
10.00
5.00
0.00
0.00
10.00
20.00
30.00
40.00
Soil C concentration (mg g -1)
y = 0.1x + 11.916
R 2 = 0.0366; P <0.05
D
Soil P concentration (mg g -1)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.50
1.00
1.50
2.00
2.50
Soil N concentration (mg g -1)
y = 0.1912x + 0.4233
R 2 = 0.0723; P <0.01

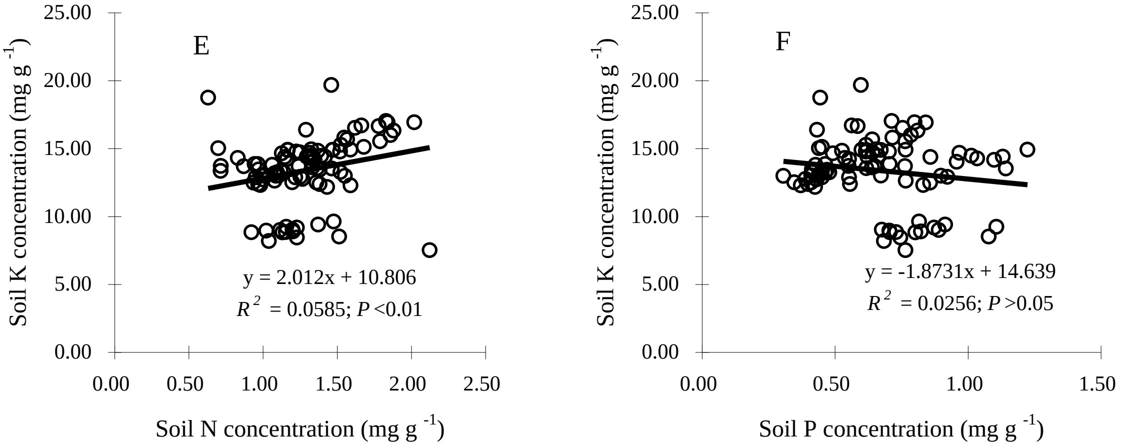

E
Soil K concentration (mg g -1)
25.00
20.00
15.00
10.00
5.00
0.00
0.00
0.50
1.00
1.50
2.00
2.50
Soil N concentration (mg g -1)
y = 2.012x + 10.806
R 2 = 0.0585; P <0.01
F
Soil K concentration (mg g -1)
25.00
20.00
15.00
10.00
5.00
0.00
0.00
0.50
1.00
1.50
Soil P concentration (mg g -1)
y = -1.8731x + 14.639
R 2 = 0.0256; P >0.05

**Fig. 2**

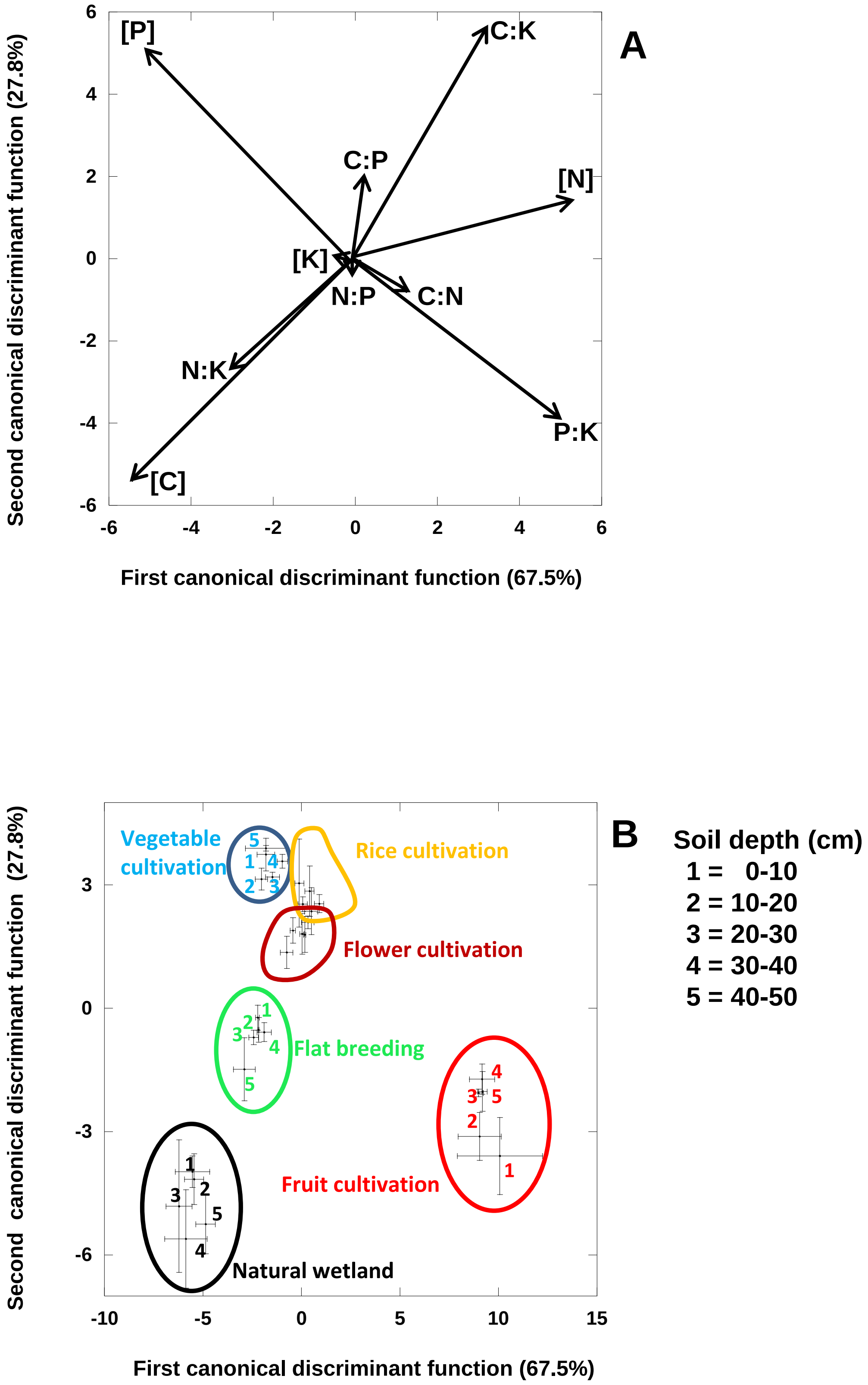

A
[P]
C:K
C:P
[N]
[K]
N:P
C:N
N:K
P:K
[C]
6
4
2
0
-2
-4
-6
Second canonical discriminant function (27.8%)
First canonical discriminant function (67.5%)
B
Soil depth (cm)
1 =   0-10
2 = 10-20
3 = 20-30
4 = 30-40
5 = 40-50
Vegetable cultivation
Rice cultivation
Flower cultivation
Flat breeding
Fruit cultivation
Natural wetland
3
0
-3
-6
-10
-5
5
10
15
Second canonical discriminant function (27.8%)
First canonical discriminant function (67.5%)

**Fig. 3**

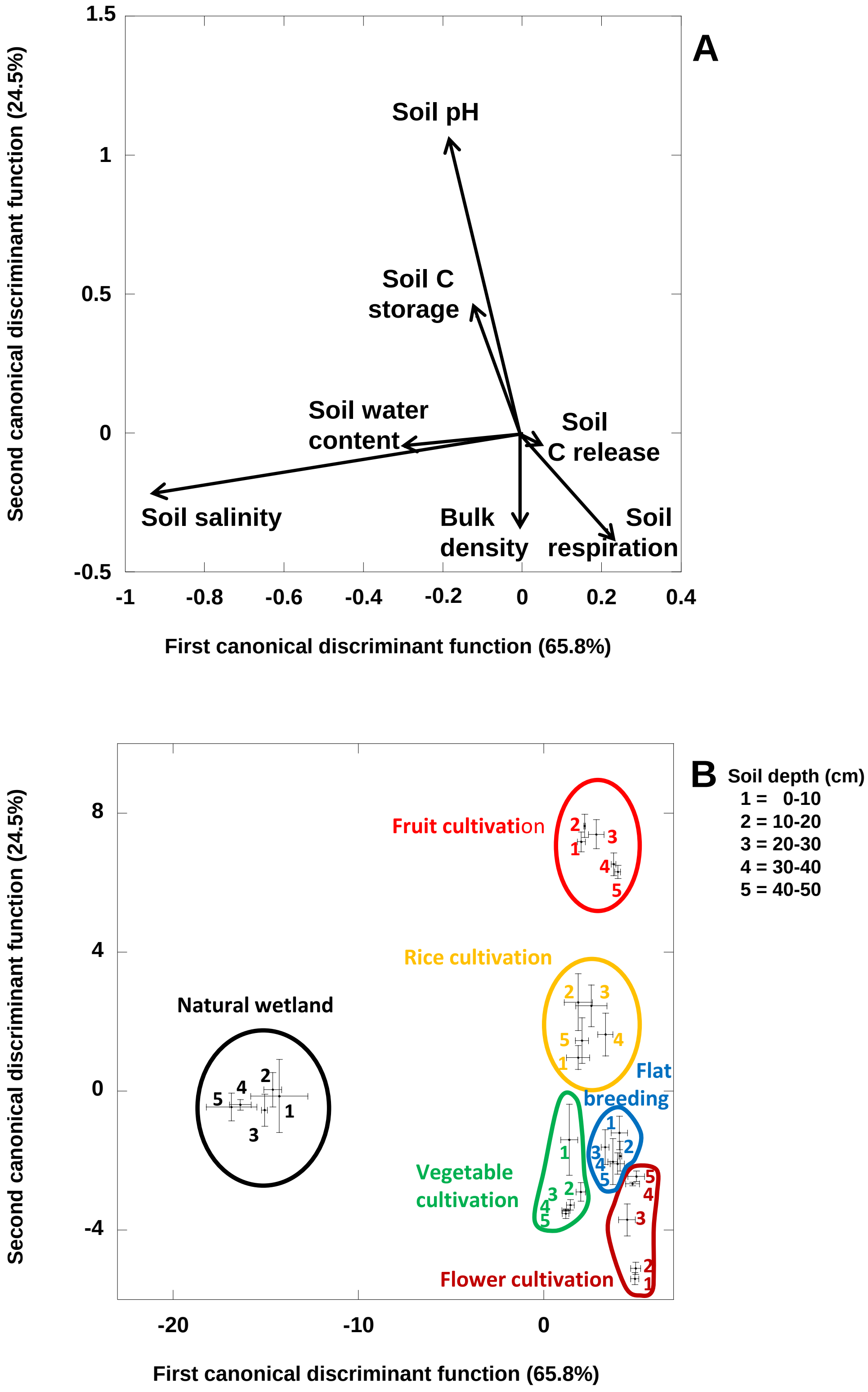

A
Second canonical discriminant function (24.5%)
First canonical discriminant function (65.8%)
1.5
1
0.5
0
-0.5
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
Soil pH
Soil C storage
Soil water content
Soil C release
Soil salinity
Bulk density
Soil respiration
B
Soil depth (cm)
1 = 0-10
2 = 10-20
3 = 20-30
4 = 30-40
5 = 40-50
8
4
0
-4
-20
-10
0
Fruit cultivation
Rice cultivation
Natural wetland
Flat breeding
Vegetable cultivation
Flower cultivation

**Fig. 4**