

1 **Agricultural land use decouples soil nutrient cycles in a subtropical**  
2 **riparian wetland in China**

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

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26 **ABSTRACT**

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

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

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## 50 **1.Introduction**

51 Anthropogenic activities can strongly alter the nutrient pools of carbon (C), nitrogen (N),  
52 phosphorus (P) and potassium (K) in soils by many processes including increasing nutrient  
53 inputs, N deposition, drought, species invasion or increases in atmospheric CO<sub>2</sub> (Tian et al.,  
54 2010; Sardans et al., 2012b; Sardans and Peñuelas, 2012). These shifts are frequently  
55 associated to changes in the structure of plant communities and/or in nutrient outputs (e.g.  
56 crop harvesting and weathering) (Sardans et al., 2012a). Land-use changes due to agronomic  
57 practices and livestock production generate soil stoichiometric shifts in forests (Falkengren-  
58 Gerup et al., 2006; Sardans and Peñuelas, 2013), shrublands (Sardans and Peñuelas, 2013),  
59 grasslands (Mulder and Elser, 2009) and steppes (Jiao et al., 2013). The status of the C:N:P  
60 ratio in wetland soil under different intensities of human disturbance, however, remains  
61 unknown.

62         Recent stoichiometric ecological studies have shown that K is even more associated  
63 than are N or P with stoichiometric differences among various plant ecotypes (Sardans et al.,  
64 2012c; Sardans and Peñuelas, 2014) or with stoichiometric shifts in response to  
65 environmental changes (Rivas-Ubach et al., 2012). The strong link between plant K  
66 concentrations and water availability (Yavitt et al., 2004; Sardans et al., 2012c) justifies the  
67 study of K and its stoichiometric relationships with other nutrients.

68         Changes in soil stoichiometry can influence the capacity to regenerate natural  
69 vegetation after the abandonment of human activities, delaying it for many decades  
70 (Falkengren-Gerup et al., 2006; Jiao et al., 2013). This impact can be especially critical in  
71 sensitive diversity-rich ecosystems, such as wetlands, that are severely affected by changes in  
72 land use (Ramsar, 2013). The effect of land-use change on the stoichiometry of wetlands has  
73 received little attention (Koerselman and Meuleman, 1996). Wetlands occupy  $5.7 \times 10^6$  km<sup>2</sup>  
74 worldwide, are cradles of biodiversity upon which countless species of plants and animals

75 depend for survival and are among the world's most productive environments, being a sink of  
76 C in the form of peat and plant matter and providing a wide array of benefits (Mitsch and  
77 Gosselink, 2007; Ramsar, 2013). In the current context of global change, wetlands continue to  
78 be among the most threatened ecosystems, and yet we lack information about the impact of  
79 anthropogenic changes on their abiotic and biotic environments (Mitsch and Gosselink, 2007;  
80 Ramsar 2013). The ability of wetlands to adapt to changing conditions and to the current  
81 accelerating rates of global change will be crucial to world biodiversity conservation. A better  
82 understanding of the resulting soil C, N, P and K ecological stoichiometries in wetlands  
83 submitted to land use changes would provide decision makers with the necessary information  
84 for developing effective methods to enhance the potential capacity of these ecosystems to fix  
85 C and reduce the impact of emissions of greenhouse gases (Peñuelas et al., 2013). It would  
86 also and provide information on the impacts of anthropogenic activity on the regenerative  
87 capacity of wetlands by determining the cycles and balances of C, N, P and K and the fertility  
88 of the soil. We expect that human activities changing nutrient balances (fertilization and  
89 harvesting), species composition and water fluxes should exert a great impact on soil  
90 elemental composition. This should change C fluxes and hinder further ecosystem restoration  
91 processes by shifting soil condition far from the optimum from that of natural wetlands. China  
92 has a coastal zone approximately 18,000 km in length, much of which is occupied by tidal  
93 wetlands in estuaries, estimated at more than  $1.2 \times 10^4$  km<sup>2</sup> (Huang et al., 2006). These areas  
94 are characterized by rapid economic development, and by the fast replacement of natural  
95 undisturbed areas by areas disturbed by crops, livestock and tourism. The loads of N and P to  
96 rivers caused by human activities and further transported downstream to the wetlands  
97 (Howarth et al., 1996) cause water eutrophication (Anderson et al., 2002) that threatens the  
98 health of wetlands (An et al., 2007) and decreases ecosystemic services (Lee et al., 2006).  
99 Research in these areas, however, has been scarce, and studies on various spatial and temporal

100 scales are therefore needed.

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

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125 **2. Material and methods**

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127 *2.1. Study area and experimental design*

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129 This work was conducted in the Difengjiang wetland of the Minjiang River estuary (China)  
130 (25°58'53.50"-25°59'46.01"N, 119°17'52.60"-119°20'25.67"E Fig. 1). The climate is  
131 subtropical with mean annual temperatures and precipitation of 19.7 °C and 1348.8 mm,  
132 respectively. The soil surface of the riparian wetland is submerged across the study site for 1-  
133 2.5 h during each tidal inundation. The large perennial grass, *Phragmites australis* (mature  
134 height of 2 m at 150 stems m<sup>-2</sup>), is one of the most important plant species and is typically  
135 found from the upstream to the downstream regions of the Minjiang River tidal wetland (Liu  
136 et al., 2006).

137 To determine the associations between different agricultural land-use changes and the  
138 concentrations and ratios of soil C, N, P and K, we established plots on a wide range of land  
139 uses: natural *P. australis* wetland (control), flower (Jasmine) cultivation (*P. australis* plants  
140 removed eight years previously), intertidal mudflat culture (the aerial parts of *P. australis*  
141 plants removed 10 years previously), rice cultivation (*P. australis* plants removed 20 years  
142 previously), vegetable cultivation (*P. australis* plants removed 30 years previously), and fruit  
143 (Longan) cultivation (*P. australis* plants removed 40 years previously). The natural *P.*  
144 *australis* wetland and intertidal mudflat culture plots have not been fertilized. The plots of  
145 flower, rice, vegetable and fruit cultivations were fertilized (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O=16-16-16%; Keda  
146 Fertilizer Co., Ltd.) with dosages of 225, 235, 150, 300 kg ha<sup>-1</sup> y<sup>-1</sup> respectively.

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

150 cultivation plots had changed from wetland to paddy soil.

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

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## 161 *2.2. Collection and measurement of soil samples*

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163 Three plots were randomly selected in each of the locations, and soil profiles (width, 1 m;  
164 length, 1 m; depth, 0.5 m) were excavated. Samples were collected with a small sampler  
165 (length, 0.3 m; diameter 0.1 m) from each of five soil layers (0-10, 10-20, 20-30, 30-40 and  
166 40-50 cm) at the center and on both sides of the soil pits. These three samples from each layer  
167 were bulked to form one sample per layer. A total of 90 soil samples (six types of land  
168 use×three plots×five layers) were thus collected. In the laboratory, the samples were air-dried,  
169 roots and visible plant remains were removed and the samples were finely ground in a ball  
170 mill. Total soil organic C was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> digestion method (Lu 1999),  
171 total soil N concentration was analyzed by the K 370 Kjeldahl method (Buchi Scientific  
172 Instruments, Switzerland), total soil P concentration was measured by perchloric-acid  
173 digestion followed by ammonium-molybdate colorimetry and measurement using an UV-  
174 2450 spectrophotometer (Shimadzu Scientific Instruments, Japan), total K concentration was

175 determined by FP 640 flame photometry (Shanghai Electronic Technology Instruments, China,  
176 Wang et al., 2014).

177 Soil parameters that can potentially be changed by human activities and that could be  
178 factors influencing the status and processes of soil nutrients were also determined. Bulk  
179 density was measured from three 5×3 cm cores per layer, salinity was measured with a DDS-  
180 307 conductivity meter (Boqu Scientific Instruments, China), pH was measured with an 868  
181 pH meter (Orion Scientific Instruments, USA), soil-particle size (clay, silt and sand) was  
182 measured by a Mastersizer 2000 laser particle size analyser (Malvern Scientific Instruments,  
183 UK), soil-water content was measured by the drying method (Lu, 1999) and soil carbon (CO<sub>2</sub>)  
184 release was determined by the incubation method (Wang et al., 2010). Thirty g of fresh soil  
185 were placed into 120 ml incubation bottles, and then bottles were sealed with a rubber stopper,  
186 and incubated at 20 °C during three days, 5 ml gases were extracted from headspace every day  
187 (four times). CO<sub>2</sub> concentration was determined by the GC-2014 gas chromatograph  
188 instrument (Shimadzu Scientific Instruments, Japan).

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### 190 2.3. C storage and release

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192 C storages for the 0-50 cm profiles were estimated using the equation (Mishra et al. 2010):

$$193 \quad C_S = \sum_{j=1}^n c_m \times \rho_b \times D$$

194 where  $C_S$  is C storage (kg m<sup>-2</sup>),  $j$  is the soil-depth interval (1, 2, ... n),  $C_m$  is the C  
195 concentration (g kg<sup>-1</sup>),  $\rho_b$  is the bulk density (kg m<sup>-3</sup>),  $D$  is the thickness of each layer (m) and  
196  $n$  is the number of layers.

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

$$198 \quad P = \frac{dc}{dt} \cdot \frac{V_H}{W_S} \cdot \frac{MW}{MV} \cdot \frac{T_{st}}{T_{st} + T}$$



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200 where  $P$  is the rate of C release ( $\text{mg}^{-1} \text{g}^{-1} \text{d}^{-1}$ ),  $dc/dt$  is the recorded change in the mixing ratio  
201 of C ( $\text{CO}_2$ ) in the headspace over time ( $\text{mmol mol}^{-1} \text{d}^{-1}$ ),  $V_H$  the volume of the headspace (L),  
202  $W_s$  the dry weight of the soil (g),  $MW$  is the molecular weight of  $\text{CO}_2$  (g),  $MV$  the molecular  
203 volume (L),  $T$  is the temperature (K) and  $T_{st}$  is the standard temperature (K). As in a previous  
204 study in this same site we observed that in the wetlands most carbon release from soil was in  
205 the form of  $\text{CO}_2$  (Wang et al., 2010), and in the present study, some of the land use types were  
206 not wetlands, and thus had not anaerobic periods, we expected that  $\text{CH}_4$  emissions not to be  
207 the main C release form, and thereby we only determined  $\text{CO}_2$  release.

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#### 209 *2.4. Statistical analyses*

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211 The differences in the soil variables among sites with different land uses (land use $\times$ soil layer)  
212 were assessed by two-way ANOVAs with Tukey's post-hoc tests. We used Pearson's  
213 correlation to examine relationships among factors. A Kolmogorov-Smirnov (KS) test was  
214 performed on each variable to test for normality. The studied soil variables were normally  
215 distributed. We used discriminant functional analysis (DFA) to associate the various levels of  
216 disturbance with overall elemental composition and stoichiometry. DFA is a supervised  
217 statistical algorithm that derives an optimal separation between groups established a priori by  
218 maximizing between-group variance while minimizing within-group variance (Raamsdonk et  
219 al., 2001). Univariate analyses were performed using SPSS 13.0 software (SPSS Inc.,  
220 Chicago, Illinois). The DFAs were performed using Statistica 6.0 (StatSoft, Inc. Tule,  
221 Oklahoma, USA). C:N:P:K ratios were calculated as molar ratios.

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224 **3.Results**

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226 *3.1.Effect of agricultural land use on C, N, P and K concentrations and stoichiometry*

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

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

249 vegetable and fruit cultivation ( $P<0.05$ ), higher than those for flower cultivation ( $P<0.05$ ) but  
250 not significantly different from those for intertidal mudflat culture and rice cultivation (Table  
251 S4).

252

### 253 *3.2.Effect of land-use on C storage and release*

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

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

261

### 262 *3.3.Effect of land use on soil parameters*

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

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275 *3.4.Effect of soil parameters on C, N, P and K concentrations and stoichiometries*

276 Total soil N and especially P and K concentrations were negatively correlated with bulk  
277 density (Table 1). The total soil C:N and C:P ratios were correlated positively with pH, water  
278 content, salinity and clay percentage and negatively with bulk density and sand percentage  
279 (Table 1). The total soil C:K ratio was correlated positively with pH and salinity and  
280 negatively with bulk density (Table 1). The total soil N:P ratio was correlated positively with  
281 water content and salinity and negatively with pH. The total soil N:K ratio was correlated  
282 positively with pH and sand percentage and negatively with bulk density, clay percentage and  
283 silt percentage (Table 1). The total soil P:K ratio was correlated positively with pH and sand  
284 percentage and negatively with water content, bulk density, salinity, clay percentage and silt  
285 percentage (Table 1).

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287 *3.5.Effect of soil parameters and nutrient stoichiometries on C balance*

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

292

293 *3.6.Multivariate DFA analyses*

294 A general DFA with soil depth as a categorical independent variable and with total soil C, N,  
295 P and K concentrations and soil C:N:P:K ratios as continuous independent variables  
296 significantly separated the soils of the various land uses (Tables 3 and 4, Fig. 3). All sites pair-  
297 wise squared Mahalanobis distances were significant (Table 4). The independent variables  
298 with significant effects in the model were total soil C, N, P and K concentrations and C:N,

299 C:K and P:K ratios (Table 3). The soils under human land-use generally had different  
300 elemental compositions than the wetland control soil, mainly because they had higher total P  
301 concentrations (Fig. 3). The soil for fruit cultivation had a different chemical composition  
302 mainly due to a higher total soil N concentration and soil P:K ratio (Fig. 3).

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

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## 322 **4.Discussion**

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### 324 *4.1.Decoupling of soil C, N, P and K concentrations associated with land-use change*

325 Our results strongly suggest that this wetland area is N-limited, although the N fertilization  
326 was applied in the cultivation plots, the soil N concentration remains lower in the soils of  
327 human managed areas similar to non-fertilized natural wetlands. Furthermore, N limitation is  
328 also especially significant in tidal wetlands, likely because of the periodic inundation of the  
329 soil that limits the access of plants to soil nutrients by the anoxic effects on root growth, by  
330 slowing mineralization, and by high levels of leaching of N. After several years of cropland  
331 management, including fertilization, total soil P was higher in the most cases, whereas N and  
332 K contents did not differ from those of the *P. australis* wetland. N limitation also played a role  
333 in the growth of *Spartina alterniflora* in a similar estuary of the Yangtze River, which is ~800  
334 km north of the Minjiang estuary (Gan et al., 2011).

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

344 Our results are partially consistent with the premise that human activity, by creating more-  
345 productive ecosystems, tends to favor ecosystems with higher total soil P concentrations and  
346 lower total soil C:P, C:N and N:P ratios that are able to support species with high growth

347 rates, which is in line with the growth rate hypothesis at the level of ecosystems (Sterner and  
348 Elser, 2002). Except in the case of the rice cropland (that fix-N<sub>2</sub>) (Herridge et al., 2008), the  
349 croplands had lower total soil N:P ratios than the natural wetland, an effect that is probably  
350 related to high P concentrations from the accumulation of P from fertilization and from its  
351 lower mobilization capacity relative to N. This lack of effect on soil N concentrations in the  
352 rice cropland was probably due to the capacity of this cropland to fix N<sub>2</sub> by the symbiotic  
353 association between the aquatic fern *Azolla* and by free-living cyanobacteria (Herridge et al.,  
354 2008).

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

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

368

#### 369 *4.2.C balance and the response to changes in nutrient stoichiometry*

370 Our data suggest that cultivation can increase soil C release through respiration, due at least in  
371 part to the effects of cropland management reducing water content and salinity. Agricultural

372 management has eliminated tidal flooding, so water contents and salinities have declined.  
373 Thus, lower soil water content by eliminating tidal flooding can increase C release, as  
374 reported in a previous study (Nomura et al., 2013). Soil C release by respiration, apart from  
375 being correlated with plant productivity, may thus also be associated with other environmental  
376 parameter, such as soil-water content. Our results also suggest that the use of land for crop  
377 production in subtropical wetlands can increase soil CO<sub>2</sub> emissions, as has been observed in  
378 previous studies (Shang et al., 2013). The negative relationship between salinity and C release  
379 may be due to the inhibition of growth and activity of soil microorganisms by osmotic stress  
380 at higher salinities (Rietz and Haynes, 2003). Previous studies have observed that soil C  
381 release was negatively correlated with salinity (Setia et al., 2011).

382 Total soil N:P and N:K ratios were positively correlated with total soil C, consistent  
383 with similar previous studies (Hessen et al., 2004) and the growth rate hypothesis (Sterner and  
384 Elser, 2002), where high N:P ratios are associated with low soil microbial activity and rates of  
385 nutrient and carbon cycles. Soil respiration has been associated with soil C storage and plant  
386 productivity (Dias et al., 2010; De Deyn et al., 2011), but we observed that soil C storage and  
387 release were generally not statistically correlated (only the soil for fruit cultivation had a  
388 significant correlation). The transformation of these natural wetlands to croplands may thus  
389 increase soil CO<sub>2</sub> emissions without clear changes in C storage.

390

## 391 **5. Conclusions**

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

395



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

400

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

405

406 Soil CO<sub>2</sub> emissions were higher in the croplands without clear differences in C storage. Crop  
407 production would load more organic matter to the soil but would also enhance the  
408 decomposition of organic matter, an effect likely linked to lower soil salinity and better soil  
409 aeration (less soil clay and water content).

410

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

416

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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  $P < 0.01$

568 **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  $P < 0.01$

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617 **Table 3** Statistics (Wilks' Lambda and *P*-value) of the discriminant functional analysis among soils of the  
 618 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  
 619 soil depth as variables. Bold type indicates a significant effect of the variable in the model (*P*<0.05)  
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Soil variables	Wilk's Lambda	<i>F</i>	<i>P</i>
<b>[C]</b>	<b>0.654</b>	<b>7.51</b>	<b>&lt;0.0001</b>
<b>[N]</b>	<b>0.763</b>	<b>4.41</b>	<b>0.0015</b>
<b>[P]</b>	<b>0.356</b>	<b>25.7</b>	<b>&lt;0.0001</b>
<b>[K]</b>	<b>0.842</b>	<b>2.66</b>	<b>0.029</b>
<b>C:N ratio</b>	<b>0.743</b>	<b>4.90</b>	<b>0.00066</b>
C:P ratio	0.922	1.20	0.32
<b>C:K ratio</b>	<b>0.759</b>	<b>4.51</b>	<b>0.0013</b>
N:P ratio	0.912	1.37	0.25
N:K ratio	0.919	1.26	0.29
<b>P:K ratio</b>	<b>0.408</b>	<b>20.6</b>	<b>&lt;0.0001</b>
Depth	0.869	0.511	0.96

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661 **Table 4** Squared Mahalanobis distances among soils of the land uses in the discriminant functional analysis  
 662 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  
 663 depth as variables

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

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**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	<i>F</i>	<i>P</i>
Respiration	0.999	0.111	0.99
<b>pH</b>	<b>0.097</b>	<b>140</b>	<b>&lt;0.001</b>
<b>Water content</b>	<b>0.433</b>	<b>19.6</b>	<b>&lt;0.001</b>
<b>Bulk density</b>	<b>0.671</b>	<b>7.36</b>	<b>&lt;0.001</b>
<b>Salinity</b>	<b>0.079</b>	<b>174</b>	<b>&lt;0.001</b>
C release	0.999	0.111	0.98
<b>C storage</b>	<b>0.496</b>	<b>15.2</b>	<b>&lt;0.001</b>
Depth	0.728	1.25	0.21

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752 **Table 6** Squared Mahalanobis distances among soils of the land uses in the discriminant functional analysis  
 753 with soil C storage, C release, bulk density, pH, respiration, salinity, water content and depth as variables  
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	intertidal mudflat culture	Vegetable cultivation	Flower cultivation	Fruit cultivation	Rice cultivation
Wetland control	305 <i>P</i> <0.0001	379 <i>P</i> <0.0001	424 <i>P</i> <0.0001	392 <i>P</i> <0.0001	322 <i>P</i> <0.0001
intertidal mudflat culture		37.6 <i>P</i> <0.0001	30.0 <i>P</i> <0.0001	110 <i>P</i> <0.0001	31.9 <i>P</i> <0.0001
Vegetable cultivation			12.7 <i>P</i> <0.0001	84.9 <i>P</i> <0.0001	27.8 <i>P</i> <0.0001
Flower cultivation				124 <i>P</i> <0.0001	41.3 <i>P</i> <0.0001
Fruit cultivation					29.6 <i>P</i> <0.0001

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776 **Figure captions**

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

779 **Fig. 2** Relationships among soil nutrient concentrations in the five types of land use. C vs. N

780 (A), C vs. P (B), C vs. K (C), N vs. P (D), N vs. K (E) and P vs. K (F)

781 **Fig. 3** (A) Biplot representing the standardized canonical discriminant function coefficients

782 for the first two roots representing the soil samples from the five types of land use in the space

783 generated by the first two roots of the discriminant functional analysis of total soil C, N, P and

784 K concentrations and total soil C:N, C:P, C:K, N:P, N:K and P:K ratios. (B) Biplot

785 representing the scores (mean  $\pm$  S.E.) of the analysis in A

786 **Fig. 4** (A) Biplot representing the standardized canonical discriminant function coefficients

787 for the first two roots representing the soil samples from the five types of land use in the space

788 generated by the first two roots of the discriminant functional analysis of soil C storage, C

789 release, bulk density, pH, respiration, salinity and water content. (B) Biplot representing the

790 scores (mean  $\pm$  S.E.) of the analysis in A

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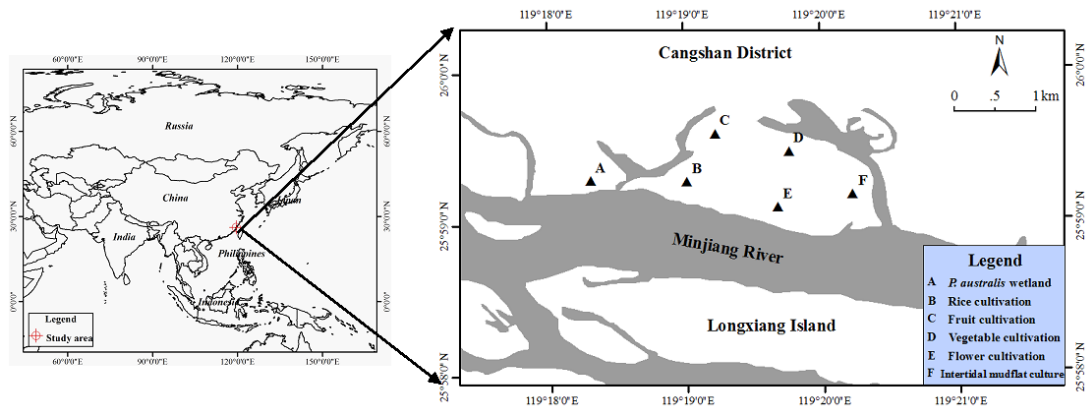
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838 **Fig.1**

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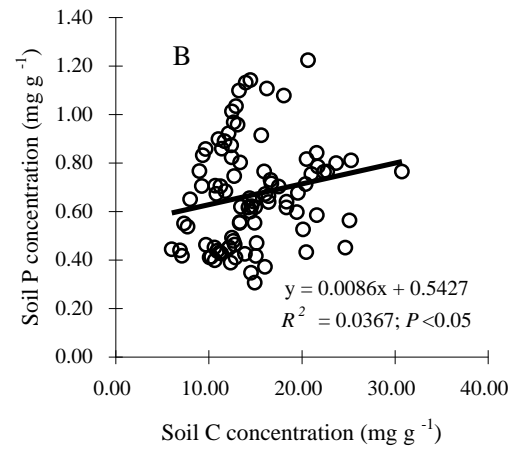
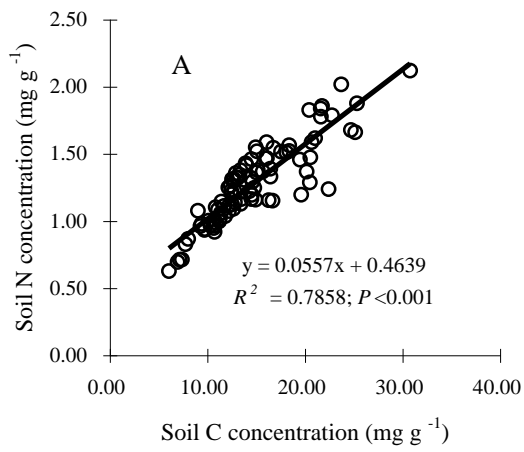
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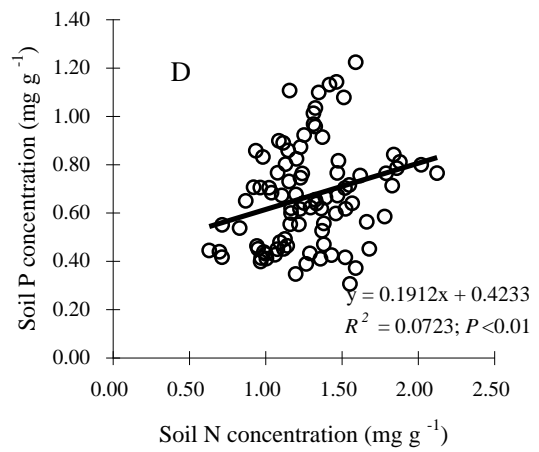
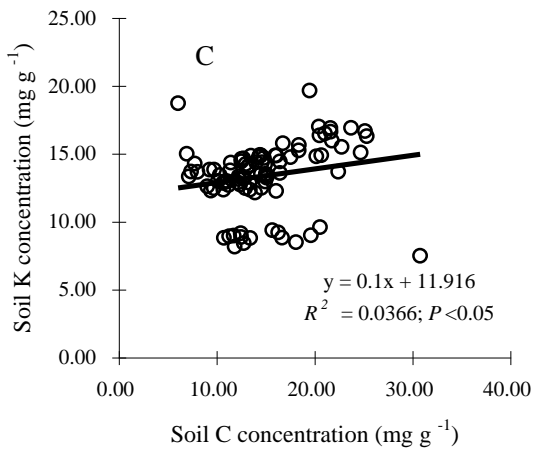
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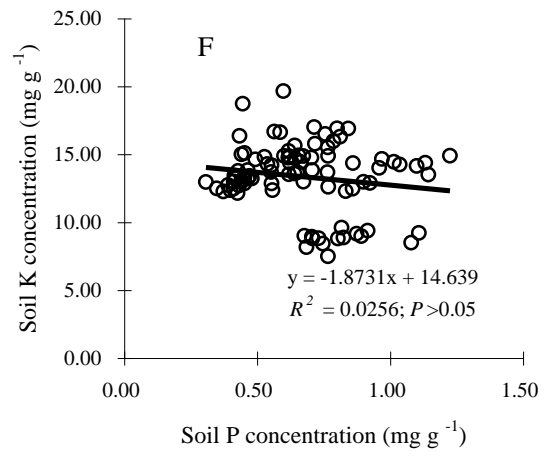
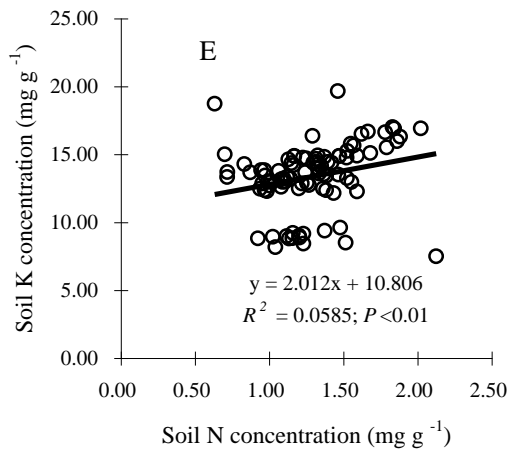
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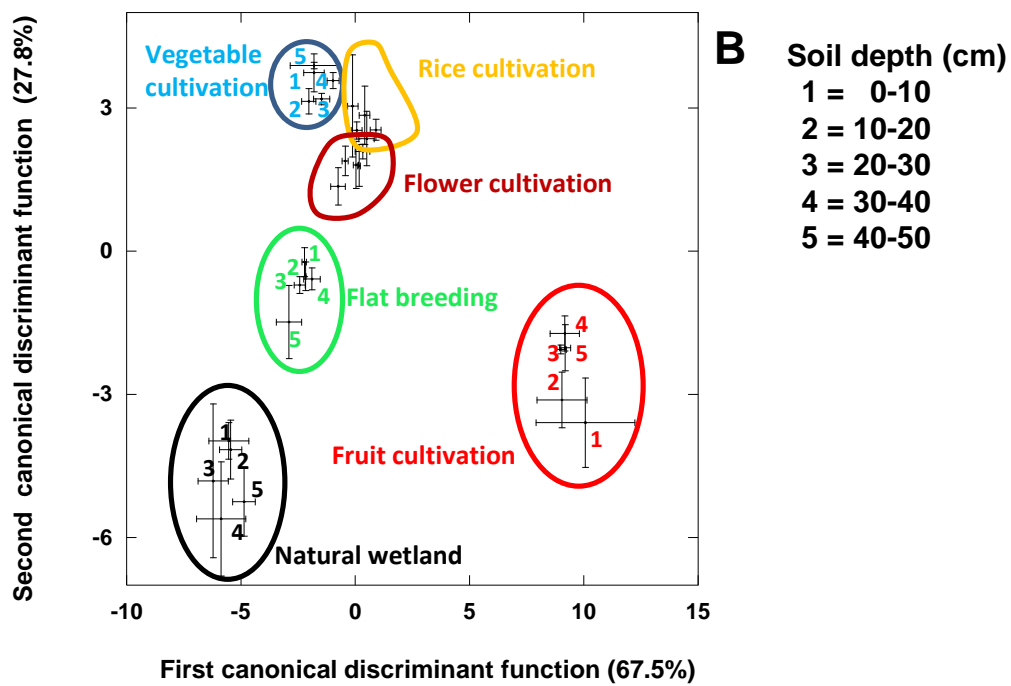
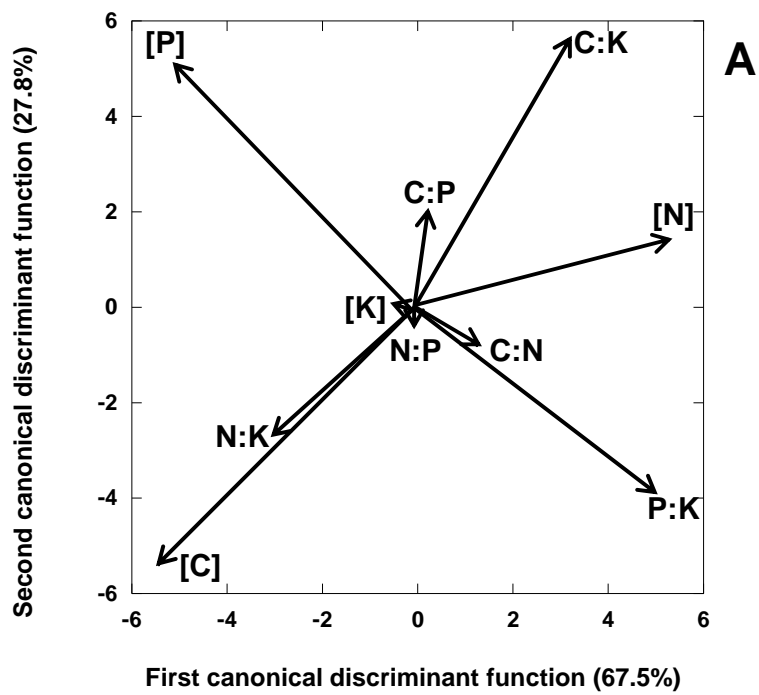
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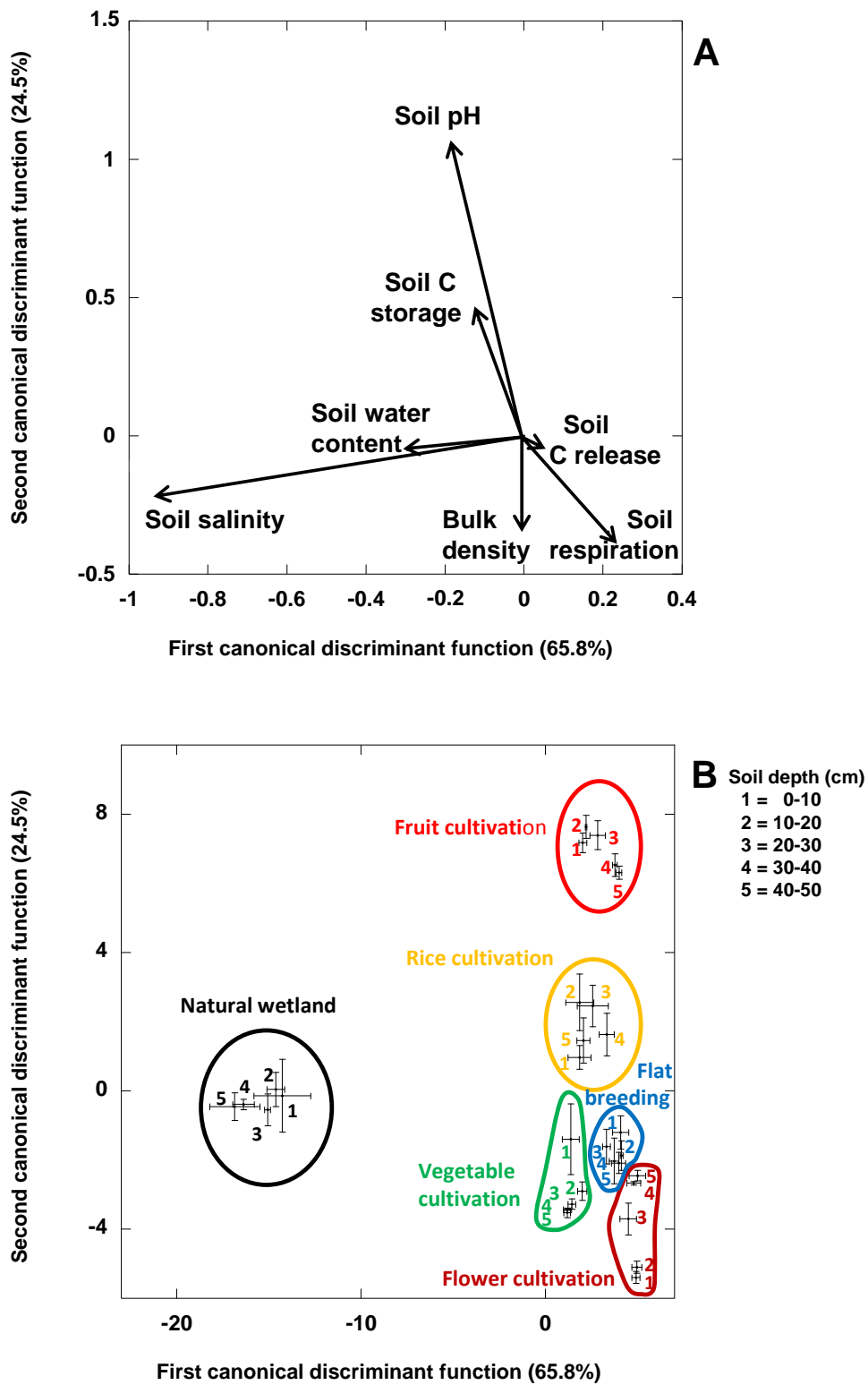
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**Fig. 2**





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864 **Fig. 3**  
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869 **Fig. 4**