Shifts in plant and soil C, N and P accumulation and C:N:P stoichiometry associated with flooding intensity in subtropical estuarine wetlands in China

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A B S T R A C T

Flooding caused by rising sea levels can influence the biogeochemistry of estuarine wetland ecosystems. We studied the relationships of higher flooding intensity with soil carbon (C), nitrogen (N) and phosphorus (P) concentrations in communities of the native sedge *Cyperus malaccensis* var. brevifolius Boecklr. in the wetlands of the Minjiang River estuary in China. The aboveground and total biomasses of *C. malaccensis* were higher in high-flooding habitats than in intermediate- and low-flooding habitats. These differences in plant biomass were accompanied by a lower N:P ratio in the aboveground biomass and a higher N:P ratio in the belowground biomass. Higher intensities of flooding were associated with higher soil N and P concentrations in intermediate and deep soil layers. The higher P concentration under flooding was mainly associated with the higher clay content, whereas the higher N concentration was associated with higher salinity. Flooding intensity did not have a net total effect on soil total C concentration. The positive direct effect of flooding intensity on total soil C concentration was counteracted by its positive effects on CH_4 emissions and soil salinity. The results suggest that *C. malaccensis* wetlands will be able to maintain and even increase the current C, N and P storage capacity of the ecosystem under moderate increases of flooding in the Minjiang River estuary.

Key words: Climate change, storage, C:N, nitrogen, nutrient stoichiometry, N:P, phosphorus, *Cyperus malaccensis*, wetland

1. Introduction

China has a large coastal area, with 1.2×10^4 km^2 of wetlands (Shen and Zhu, 1999; Huang *et al*., 2006) that are cradles of biodiversity upon which countless species of plants and animals depend for survival (Zhou *et al*., 2006). Wetlands are among the world's most productive environments and provide a wide array of ecosystem services. Estuarine wetlands are influenced by river discharge, tidal changes, sedimentary inputs and land uses, so the elemental ratios are among the most important variables in terrestrial ecosystems worldwide (Morse *et al*., 2004; Wang *et al*., 2014a). Estuarine wetlands are also among the ecosystems most affected by anthropogenic changes globally (Ramsar, 2013). Rising ocean levels are now especially critical for coastal wetland ecosystems, in part because of the consequent increase in salinity (Ramsar, 2013). Increases in the sea level can flood most of the present coastal wetlands in China, which would move wetland habitat inland. Much of the coastal wetlands could consequently disappear (Yang et al., 2014), and some previously freshwater tidal wetlands would be seriously affected by the higher flooding and salinity caused by higher sea levels and/or hurricane frequency.

Increased soil salinity is a direct effect of increased flooding with seawater. The combination of water salinity levels with flooding intensity strongly influence wetlands C-storing capacity (DeLaune and White, 2012). Salinity can change the nutrient concentrations of wetland soil (Tong *et al*., 2010; Heagle *et al*., 2013). Carbon (C), nitrogen (N) and phosphorus (P) concentrations could thus be affected by changes in the retention of these elements, which would affect their global cycles (Morrissey et al., 2014). Salinity increase has a strong impact in soil microbial community and thus in mineralization capacity (Xi *et al*., 2014; Morrissey *et al*., 2014; Hu *et al*., 2016). The effects on soil microbial activity can be positive or negative depending on the range of salinity, levels of flooding and the metabolic processes (Xi *et al*., 2014; Morrissey *et al*., 2014; Marks *et al*., 2016). For example, Marks *et al*., (2016), in Mississippi wetlands, observed that denitrification was positively related with salinity at a range of moderate salinity levels whereas it was negatively related with salinity at high ranges of salinity (near the level of seawater). Thus, changes in salinity due to shifts in flooding intensity can severely affect the biogeochemistry of wetlands.

The stoichiometrical shifts in ecosystem C:N:P ratios have an important role in several ecological processes, such as species growth rate, plant and soil community composition, trophic web structure or soil processes such as mineralization rates (Ågren, 2008; Sardans *et al*., 2012a). In the 1950s, Redfield (1958) reported a consistent C, N and P ratio in plankton and seawater, and several researchers have since studied this subject in the plants (McGroddy *et al*., 2004), microbes (Cleveland and Liptzin, 2007) and soils (Tian *et al*., 2010) of aquatic and terrestrial ecosystems. 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), but most studies have focused mainly on nutrient stoichiometry in the top 60 cm of soil or have not distinguished the soil layers at finer intervals (Tian *et al*., 2010; Schipper and Sparling, 2011). This is especially evident in wetlands where most studies have been focused on soil surface, so research on the changes in soil stoichiometry throughout the soil depth profile is warranted for a better management of the ecosystems (Tian *et al*., 2010; Wang *et al*., 2014a; Wang *et al*., 2014b).

The effects of changes in flooding and salinity on the stoichiometric C:N:P balances of soils and plants, the relationships between salinity and other soil properties and the extent of these effects on soil profiles, especially in deep soils, in wetlands warrant study. The study along flooding and salinity gradient of the C:N:P ratios along soil profile altogether and also at the same time with information of other soil and plant variables should provide us more integrated information of the overall shifts in ecosystem basic traits, because different pairwise ratios become informative clues of different basic ecosystem processes (Sardans *et al*., 2012a). For instance, soil C:N and C:P are related with mineralization rates, whereas soil N:P rates is related with growth rates (Sardans *et al*., 2012a).

The current rapid development of the global economy has accelerated the human disturbance of natural ecosystems (by crop fertilization, N deposition, land use change and species invasion). As a result, soil and plant C, N and P biochemical processes are increasingly affected, which causes an imbalance of the C, N and P stoichiometry that is likely to increase in the near future (Sardans *et al*., 2012a,b; Peñuelas *et al*., 2012, 2013). A better understanding of the resulting soil C, N and P stoichiometric shifts and other related plant-soil system related traits in wetlands due to changes in the levels of flooding and salinity linked to the projected increases in sea level (IPCC, 2014) would provide decision makers with the necessary information for developing effective methods to enhance the potential capacity of these ecosystems to fix C, N and P and reduce the impact of the emissions of greenhouse gases (Sardans *et al*., 2012a; Peñuelas *et al*., 2012 and 2013). This information could also improve our understanding of the impacts on the potential uses and regeneration capacity of flooded wetlands by determining the cycles and balances of C, N and P and the fertility of the soil. Flooding intensity enhancement can change C cycle in several ways; changing plant growth (Byun *et al*., 2017; Conner *et al*., 2001) and ecosystem CO_2 fluxes (Han *et al*., 2015), changing soil organic matter decomposition rates (Conner and Day, 1991; Stagg *et al*., 2017), or increasing C losses by rising CH_4 emissions as observed in wetlands with increased annual number of hours of flooding (Torres-Alvaro *et al*., 2005; Jerman *et al*., 2009; Pangala *et al*., 2013). These C loads can also exert a notable influence in C:N:P ratios. Given all these results, we hypothesized that the effects of the changes in the flooding intensity on plant and soil C:N:P stoichiometry should be related with changes in soil texture, pH, salinity and redox potential. We expected less favourable redox conditions for litter decomposition at higher intensity of flooding. Other physico-chemical effects of flooding on soil traits and plant growth altogether can also affect the wetland capacity to accumulate C, N and P at higher levels of flooding intensity. In fact, changes in C and nutrients in plants associated with flooding regime shift have been observed in other wetland areas (Miao *et al*., 1997; Tong *et al*., 2012). For example, higher plant growth and nutrient allocation among different plant organs could affect soil C, N and P soil status under higher flooding intensities.

To understand the effects of different flooding regimes and the possible associated changes in soil traits such as salinity and texture on C, N and P concentrations and stoichiometries in estuarine wetlands, we: (1) measured the C, N and P concentrations, contents and stoichiometry in plant and the possible different allocation of C, N and P in aboveground and belowground organs at different flooding intensities, (2) the C, N and P concentrations, contents and stoichiometry in the soil system at different soil depths at distinct flooding intensities, (3) the possible changes in C, N and P accumulation in plant soil system (4) the relationships between the soil C, N and P concentrations, and C:N, C:P and N:P ratios and the capacity of soil to store C and to emit methane (CH_4) at different flooding intensities and (5) explored the importance of influencing factors such as salinity, texture and redox potential (Eh) on these relationships between flooding intensity and plant and soil C, N and P concentrations, contents and stoichiometry.

2. Material and methods

2.1. Study area

This study was conducted in three Minjiang River estuarine wetlands in southeastern China, between the mid-subtropical and the southern subtropical zones (119°5′36″-119°41′5″E, 25°50′43″-26°9′42″N; Fig. 1). Minjiang River wetlands cover 476 km^2. The climate is relatively warm and wet with a mean annual temperature of 19.7 °C and a mean annual precipitation of 1346 mm (Zheng et al., 2006). We selected three habitats, the Shanyutan, Bianfuzhou and Tajiaozhou wetlands, as high-, intermediate- and low-flooding habitats, respectively (Fig. 1). The dominant plant species in all three wetlands was the sedge *Cyperus malaccensis* var. *brevifolius* Boecklr. The high-flooding habitat is flooded by intermediate tides ca. 240 d y^{-1} and is submerged beneath 10-120 cm of water for 0.5-4 h during each tidal inundation (average of 540 h y^{-1} of inundation). The intermediate-flooding habitat is flooded by intermediate tides ca. 220 d y^{-1} and is submerged beneath 10-100 cm of water for 0.5-3 h during each tidal inundation (average of 385 h y^{-1} of inundation). The low-flooding habitat is flooded by intermediate tides ca. 180 d y^{-1} and is submerged beneath 10-70 cm of water for 0.5-1.5 h during each tidal inundation (average of 180 h y^{-1} of inundation). The soil surface of the entire estuarine wetland is exposed at low tide, but the soil remains flooded in some deep soil layers.

2.2. Collection and measurement of plant and soil samples

The samples were collected in August 2012 from three plots randomly established in each of the habitats and the three plots distance about 20 m among each other. We collected the *C. malaccensis* aboveground biomass in each plot from a large plant growth homogeneous site, by selecting a quadrat (10 × 10 m) and collecting the above-and belowground biomasses (0-60 cm) from a center selected sub-quadrat (1 × 1 m). All plant material was gently washed with deionized water and then oven-dried to a constant mass (80 °C for 24-36 h) and weighed. The total biomass of each plant population was determined from the sum of the above- and belowground biomasses. Soil samples were collected at the same time with a small core sampler (length and diameter of 1.3 and 0.1 m) from each of 20 layers (at intervals of 5 cm from 0 to 100 cm of soil depth). A total of 180 soil samples (three wetland types × 20 soil layers × three replicate plots) were thus collected. The soil samples were air-dried, roots and visible plant remains were removed by hand and the samples were finely ground in a ball mill.

Total soil organic-C concentration was determined by $K_2Cr_2O_7$-H_2SO_4 digestion (Sorrell et al., 1997; Bai et al., 2005), porewater dissolved organic-C (DOC) concentration was measured using an TOC-V CPH total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan), total N concentration was analyzed by the K 370 Kjeldahl method (Buchi Scientific Instruments, Flawil, Switzerland) and porewater (collected by centrifugation at 5000 r min^{-1}) NH_4^+, NO_3^-, SO_4^{2-} and Cl^- concentrations were determined by ICS2100 ion chromatography (American Dionex Production, Sunnyvale, USA). The concentrations of C and N of the plants were determined using a Vario EL III Elemental Analyzer (Elementar Scientific Instruments, Hanau, Germany). Total soil P and plant P concentrations were all determined by the method of described in Lu (1999). Briefly, we placed 0.5-1.0 g of dry material into 100-mL digestion tubes, adding 8 mL of 98% H_2SO_4, adding 10 drops of 72% $HClO_4$ and then incubating this solution for 0.5 h at 300 °C in a Digiblock EHD36 Digestion System (Zhongzi Instruments, Beijing, China). The cooled digest was transferred to a 50-mL volumetric flask, and the pH was adjusted by adding 4 mol L^{-1} $NaHCO_3$ or 0.5 mol L^{-1} H_2SO_4 until the liquid became yellowish. Five milliliters of molybdenum-antimony anti-reagent were added and then deionized water to a volume of 50 mL. The solution stood for 30 min and was then analyzed colorimetrically using a UV-2450 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) at a wavelength of 700 nm (Lu, 1999; Ruban et al., 1999). A standard calibration curve was constructed by placing 0, 1, 2, 3, 4, 5 and 6 mL of a 5 mg L^{-1} P standard solution into 50-mL volumetric flasks, adding 5 mL of molybdenum-antimony anti-reagent and then diluting to 50 mL with deionized water, thereby obtaining 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg L^{-1} P standard solutions. These solutions were analyzed colorimetrically using the UV-2450 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) at a wavelength of 700 nm.

Soil CH_4 production was determined by anaerobic incubation (Wang *et al.*, 2010). Thirty grams of fresh soil were placed into 120-mL incubation bottles, and two volumes of distilled water were added. Soils for incubation were stored cold within two weeks. The incubation bottles were purged with N_2 for 2 min to replace the O_2 and were then sealed with a rubber stopper and incubated at 20 °C for 3 d. Five milliliters of gas were extracted from the headspaces each day (for a total of four times). CH_4 concentration was determined with a GC-2012 gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan).

Bulk density was measured from three 3 × 1 cm cores per soil layer, Eh and pH were measured with an Eh/pH meter (IQ Scientific Instruments, Carlsbad, USA) and salinity was measured using a 2265FS EC Meter (Spectrum Technologies Inc., Paxinos, USA). These measurements were conducted in the field. Soil particle size: clay (< 4 μm), silt (4-63 μm) and sand (> 63 μm) percentages were measured by a Master Sizer 2000 Laser Particle Size Analyser (Master Scientific Instruments, Suffolk, UK) (Wang *et al.*, 2015b).

2.3. Determination of soil C content and soil CH_4 production

The C contents in the 0-100 cm soil profiles were estimated by (Mishra *et al.*, 2010). CH_4 production was estimated by (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 CH_4 production (μg CH_4 g (d.w.soil)$^{-1}$ d^{-1}), *dc/dt* is the recorded change in the mixing ratio of C (CH_4) in the headspace over time (mmol mol^{-1} d^{-1}), V_H is the volume of the headspace (L), *Ws* is the dry weight of the soil (g), *MW* is the molecular weight of CH_4 (g), *MV* is the molecular volume (L), *T* is the temperature (K) and T_{st} is the standard temperature (K).

2.5. Statistical analyses

The significant differences of overall soil variables values due to site were assessed by linear mixed models with site as fixed factor and soil depth as random factor by using "*nlme*" (Pinheiro *et al*., 2016) R package with the "*lme*" function. The data were tested for normality of distribution (Kolmogorov D-test) and homogeneity of variance (modified Levene's test). As all the data met model assumptions, thus untransformed data were used for statistical analysis.

The significance of the differences in soil and plant variables (including C storing and CH_4 emissions) among the soil layers and levels of flooding were assessed by general linear models with Tukey's post-hoc tests. We analyzed the Pearson correlation coefficients between soil physic-chemical variables and soil and plant nutrient concentrations, contents and stoichiometry. These statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, USA). We performed multivariate statistical analyses using general discriminant analysis (GDA) to determine the overall differences at the various flooding intensities of total soil C, N and P concentrations; DOC, NH_4^+, NO_3^- and SO_4^{2-} concentrations and total soil C:N, C:P and N:P ratios. We also considered the portion of the variance due to the different soil layers as an independent categorical variable. The GDAs were performed using Statistica 6.0 (StatSoft, Inc. Tulsa, USA). Soil C:N, C:P and N:P ratios were calculated as mass ratios. To study the effects of flooding intensity on plant and soil C and nutrient concentrations, contents and stoichiometry by indirect effects through soil physic-chemical variables, such as salinity, clay content and soil Eh, we used structural equation modelling. The flooding intensity was estimated as the annual average number of hours the soils were flooded. We fit the different models using the sem R package (Fox *et al.,* 2013) and determined the minimum adequate model using the Akaike information criterion. Standard errors and the significance level (P value) of the total, direct and indirect effects were calculated using bootstrapping (1200 repetitions) (Davison *et al*., 1986; Mitchell-Olds, 1986)

3. Results

3.1. Plant biomass and nutrient characteristics

The aboveground and total biomasses of *C. malaccensis* were higher in the high-flooding habitat than in the intermediate- and low-flooding habitats (P<0.05) but did not differ between the latter two (P>0.05, Fig. 2A). The belowground biomass of *C. malaccensis* was significantly lower with lower flooding (P<0.05, Fig. 2A).

The aboveground P concentration of *C. malaccensis* was significantly higher, and C:P and N:P ratios were significantly lower with high flooding intensity (P<0.05, Table

1). The belowground *C. malaccensis* N concentration was higher and belowground C:N and C:P ratios were lower in the high-flooding habitat than in the low-flooding habitat (P<0.05, Table 1). *C. malaccensis* C concentrations in above- and belowground biomasses did not differ with flooding intensity (Table 1).

The aboveground and total biomasses, and C and N accumulation in aboveground and total biomass of *C. malaccensis* were highest in the high-flooding habitat (P<0.05), with no differences between intermediate- and low-flooding habitats. The belowground biomass and C accumulation of *C. malaccensis* was significantly higher with higher flooding intensity (P<0.05, Fig. 2A, B). The belowground and total N accumulation of *C. malaccensis* was significantly lower with lower flooding intensity (P<0.05, Fig. 2C). The aboveground, belowground and total P accumulation of *C. malaccensis* were significantly higher with higher flooding intensity (P<0.05, Fig. 2D).

3.2. Spatial variation of soil traits in different flooding intensity habitats

Soil C, N, P, NO_3^-, DOC, SO_4^{2-} and Cl^- concentrations; C:N, C:P and N:P ratios; texture; salinity; pH; Eh and bulk density differed significantly among the habitats (P<0.05, Fig. 3, 4 and 5, Table S1 and S2). There was higher N and P concentration in the deeper soil layer of high intensity flooding habitat than that of immediate and low-intensity flooding habitat (Fig. 3). NO_3^- concentrations were higher in all soil layers with high flooding intensity, and NH_4^+ concentrations were higher in the deepest layers with low flooding intensity (Fig. 3). Higher levels of flooding were thus correlated with higher N and P concentrations (Fig. 3). Higher flooding intensity was also correlated with higher clay and silt concentrations and with lower sand concentrations (Fig. 4) and lower N:P, C:N and C:P ratios in most of the soil layers (Fig. 5).

Soil C, N, P, NO_3^-, SO_4^{2-} and Cl^- concentrations, texture, salinity and bulk density differed significantly with soil depth (P<0.05, Table S1 and S2). Soil N, P, SO_4^{2-} and Cl^- concentrations, texture and salinity differed significantly in the interaction of habitat and soil depth (P<0.05, Table S1 and S2). Soil C:N ratios were lower and NO_3^-, SO_4^{2-} and Cl^- concentrations were thus higher with higher salinity at all depths (Table S1 and S2).

Soil C content did not differ significantly with flooding intensity (P>0.05, Fig. 6) but differed significantly with soil depth (P<0.001); deep layers had lower C contents (Fig. 6). Soil CH4 production was significantly greater in soils submitted to high flooding intensity (P<0.001) and also differed significantly for soils collected from different soil depths (P<0.001), with deeper layers having significant lower CH_4 production (Fig. 6). Higher levels of flooding and salinity were thus correlated with higher N and P concentrations but not with the C-storage capacity of the soil.

Clay, silt, NO_3^-, SO_4^{2-} and Cl concentrations and Eh were positively correlated with soil salinity, whereas total soil C concentration, soil C:N and C:P ratios, sand concentration and soil pH were negatively correlated with salinity (Table S3).

3.3. DFA analysis and structural models

The physicochemical traits of the soil differed among the three habitats (Fig. 7), with squared Mahalanobis distances of 27.0 (P<0.0001), 54.0 (P<0.0001) and 13.8 (P<0.0001) between the high- and intermediate-flooding habitats, the high- and low-flooding habitats and the intermediate- and low-flooding habitats, respectively. The soils were more similar between the Bianfuzhou (intermediate flooding) and Tajiaozhou (low flooding) wetlands than with the Shanyutan (high flooding) wetland. Total soil P, salinity, Cl^{-1} NO_3^-, SO_4^{2-} and NH_4^+ concentrations and clay concentration were the soil variables that differed the most among the habitats. The GDA indicated that higher flooding intensity was correlated with higher Cl^-, NO_3^- and SO_4^{2-} concentrations and salinity and with a finer soil texture. The high-flooding habitat, however, did not have a higher Eh potential (Table S4).

Flooding, despite presenting a direct negative relationship, had a positive total relationship with soil P concentration because of its indirect effect through its effect on soil clay concentration (Fig. 8A). Conversely, flooding had a direct positive relationship with N concentration, but this positive relationship was partially counteracted by the indirect negative effect through its effect on salinity, (Fig. 8B). There was no significant total relationship of flooding with soil C concentration because the positive direct relationship of flooding with soil C concentration was counteracted by the indirect effect of flooding through its relationships with salinity, which was negatively related with soil C concentration (Fig. 8C). Flooding was negatively and directly related with soil C:N ratio and had no indirect relationships through its effects on soil salinity, clay concentration and Eh on soil C:N ratio (Fig. 9A). Differently, flooding was positively related with soil C:P ratio, but the total relationship with soil C:P ratio was negative because of the indirect negative relationships through its relationships with soil salinity and clay concentrations (Fig. 9B). The direct relationship of flooding intensity with soil N:P ratio was positive, but the total relationship was not significant through its indirect negative relationships with soil salinity and clay concentrations (Fig. 9C). Flooding had a direct positive relationships with soil NO_3^- and SO_4^{2-} concentrations. In the case of NO_3^- this positive direct relationship was partially counteracted by the indirect effects of flooding through its relationships with soil salinity. In the case of SO_4^{2-} the direct relationships with flooding were even reinforced by the positive indirect relationship of flooding with salinity (Fig. 10). Soil clay concentration was positively correlated with soil NH_4^+ concentration (Fig. 10).

4. Discussion

4.1. Variation of plant nutrient accumulation associated with flooding

In our study, plants in the high flooding habitats accumulated more nutrients than those living in conditions of low flooding. The observed higher N and P accumulation in plants was not consistent with what was expected from the periodic inundation of the soil that can limit the access of the plants to the soil nutrients by the anoxic effects on root growth (Amlin and Rood, 2001; Kirwan and Guntenspergen, 2012), slowing mineralization (Adame *et al*., 2010; Pereira *et al*., 2016), and by high levels of leaching of P and particularly of N (Noe and Hupp, 2007; Kobayashi *et al*., 2009). But, other studies have observed that increases in flooding enhances the litter production (Pereira *et al*., 2016) and that N and P is accumulated in soil by a slow mineralization (Baker *et al*., 2001), which were consistent with our results. Wetlands that become isolated from tides quickly become P-deficient, as observed in Cienaga Grande where flooding retrieve produced a strong die-back associated with a decrease of soil P (Cardona and Botero, 1998), showing the positive relationships between flooding intensity and P-accumulation. Moreover, the N:P ratio was lower with higher N and P concentrations in the aboveground organs', whereas N:P ratio was higher in the belowground organs at the high-flooding intensity. These results were consistent with the expected in the frame of the growth rate hypothesis (Sterner and Elser, 2002; Sardans *et al*., 2012b; Gargallo-Garriga *et al*., 2014). All populations in this study were primary populations in an homogeneous wetland area of *C. malaccensis*, with very short distance among different plots. Thus average plant ages should be very similar among all studied plots, so our results were consistent with higher growth rates expected from lower N:P ratios. The average N:P ratios were 3.94±0.17, 6.08±2.20 and 7.61±2.31 for aboveground organs and 2.88±0.15, 2.73±0.04 and 2.63±0.39 for belowground organs in the high-, intermediate- and low-flooding habitats, respectively. This higher allocation of P to aboveground organs relative to N is consistent with the higher biomass accumulation in higher flooding habitats expected by the growth rate hypothesis (GRH) (Sterner and Elser, 2002; Gargallo-Garriga *et al*., 2014). The N:P ratio was mostly lower than the average N:P ratios (6.7-7.2) of terrestrial and aquatic plants and algae in their natural environments (Elser *et al*., 2000; Güsewell and Koerselman, 2002; Geider and La Roche, 2002; Knecht and Göransson, 2004) and much lower than the average N:P ratio (14-16, by mass) of terrestrial plants (including those of wetlands) in their natural environments (Elser *et al*., 2000; Güsewell and Koerselman, 2002; Güsewell and Bollens, 2003; Knecht and Göransson, 2004). The higher accumulation of biomass as a consequence of the higher growth from the lower N:P ratio in photosynthetic tissues is thus expected by the GRH (Sterner and Elser, 2002). Subtropical zones have high rainfalls and temperatures that favor erosion and the loss of N and P, so nutrients are limited (Olde Venterink *et al*., 2003; Tian *et al*., 2010). The lower values of N:P ratio at higher flooding intensity can be also a consequence, at least partially, of the lower solubility and consequent larger capacity of precipitation of P than N. In fact, recent studies have observed rising N:P and C:P ratios in China freshwater ecosystems, including freshwater plants, are due to the high human N and P inputs resulting from economic development and increasing use of fertilizers (Yan *et al*., 2016). We can not discard, however, that other factors such as differences in herbivore attack can also explain some of these differences under distinct intensities of flooding.

Wang *et al*. (2015) observed higher N:P ratios in plants than in soils, as observed in this study, and thus suggesting that high temperatures and rainfalls in this subtropical study area may contribute to the high rates of N leaching and occlusion in the highly weathered soil (Laird *et al*., 2010). Thus, plants retained more N than P in their biomasses than soils, and N could be limiting. The lower plant and soil N:P ratios, however, were also consistent with the high P-saturation of croplands in China (MacDonald *et al*., 2011). The overall data thus suggest that higher flooding intensity is associated with higher plant production and C and N and mainly P accumulation in plant-soil system associated with human driven N and P pollution. In turn the higher N, P and lower N:P ratios under higher flooding can have a feed-back by enhancing the higher plant production capacity.

4.2. Variation of soil nutrient accumulation along soil depth associated with flooding

Soil C:N, C:P and N:P ratios were generally stable across the soil profile within and between different flooding intensities, because the concentrations of these three elements decreased with depth to similar degrees. Previous studies also found that C:N, C:P and N:P ratios were constant over space in the upper layers (0-10 cm) (Wang *et al*., 2010; Tian *et al*., 2010). The three wetlands in this study were separated by less than 20 km and so had the same climatic traits and similar soil types. Our results for these subtropical wetlands contrast with those for other types of ecosystems, from forest to cropland, where C:N:P ratios differ along soil profile (Tian *et al*., 2010; Lou *et al*., 2012; Wang *et al*., 2014c), but are consistent with other studies in wetland areas that found that C:N, C:P and N:P ratios were constant along soil profile (Wang *et al*., 2010). These constant C:N, C:P and N:P ratios along soil profile in wetland areas may be due to the rapid input of sediments by flooding that can continually increase the depth of wetland soils.

4.3. Differences in soil texture, Eh and salinity with flooding and their relationships with the variation of soil nutrients and stoichiometry

The main effect of increased flooding on the observed higher soil N and P contents may be associated with high plant production capacity and probably to slower litter mineralization but also with higher salt (C and N) and clay (P) concentrations due to the gradient of saltwater, brackish water and freshwater. Moreover, higher soil clay concentrations, as observed in our study at the high-flooding habitat, have been associated with higher nitrification intensity (Aulakh *et al*., 1996). This is also consistent with all results observing that NO_3^- concentration was higher and NH_4^+ concentration was lower in the high-flooding habitat than in the intermediate- and especially in the low-flooding habitats, suggesting that nitrification activity would be more intense with higher flooding intensity in these studied wetlands. High nitrification rates have been reported from high-flooding habitats (Neill, 1995; Gao et al., 2012; Bellinger *et al*., 2014). Plant roots adapted to flooding release O_2 to the rhizosphere, thereby enhancing nitrification (Bloom, 1999; Zhang and Scherer, 2002; Ni *et al*., 2007). Furthermore, other important influencing variables such as SO_4^{2-} and Cl^- concentrations and salinity were higher at higher flooding intensity due to the gradient of saltwater, brackish water and freshwater.

The observed differences of soil texture under different flooding intensities were consistent with the fact that more intensely flooded wetland soils had slower water flow that facilitated the sedimentation of fine particles. Water flow was faster in the habitats further inland influenced more by the river flow, so the particles sedimenting from the water were coarser. Higher rates of sediment deposition have been observed in river areas associated to higher flooding events with sediments composed basically by fine particles (Cahuvel *et al*., 1996).

The soil C:N ratio (11.1, 0-10 cm layer) in the study area was higher than the average ratio for China (10.5, Tian *et al*., 2010) and the global ratio (10.5, Cleveland and Liptzin, 2007), an effect associated to the high plant productivity and N nutrient-use efficiency in subtropical regions (Singh *et al*., 2013). N could have some degree of limitation in this ecosystem, in agreement with a previous study of cropland (rice paddy field) in this area, where 95 kg N ha^{-1} were needed to be applied during the period of growth (Wang *et al*., 2012b). The total soil N concentration, however, decreased in soils under cultivation (Wang *et al*., 2014a). N limitation also played a role in the growth of *Spartina alterniflora* in a similar estuary of the Yangtze River, ca. 800 km north of the Minjiang estuary (Gan *et al*., 2011).

The N and mainly P accumulation in plants and soils were thus correlated with flooding intensity further suggesting that these wetlands act as sinks for P from the river discharge. These lower N:P ratios would be a consequence and thus an example of the current diverse nutrient imbalances triggered by human activity (Peñuelas *et al*., 2012; 2013). In contrast with soil N and P concentrations, C concentration was not higher under high intensities of flooding, so the lower mineralization capacity (by the effects of flooding discussed above) could had been counteracted by the higher CO_2 fluxes from roots that is related to higher productivity of the plant (Nakamura *et al*., 2010; Rewald *et al*., 2016) and soil nutrient availability (Makita *et al*., 2015), so the amount of C lost from flooding and salinity could relate to the productivity of the plant community. Also the observed higher CH_4 emission from more highly flooded soils, accounting for the lower soil C:N and C:P ratios in the more highly flooded habitats could also contribute to the higher soil losses of C. The higher CH_4 emission under more intense flooding has been widely observed in other studies (Torres-Alvaro *et al*., 2005; Pangala *et al*., 2013), as expected in wetlands with increasing annual hours of flooding (Potter, 1997; Jerman *et al*., 2009).

5. Concluding remarks

Higher flooding intensity and salinity may lead to a higher accumulation of C, N and P in plants and N and P in soils. The increases in overall plant biomass were accompanied by a higher proportional allocation of N and P to aboveground biomass, higher N:P ratios in belowground biomass and lower N:P ratios in aboveground biomass. These changes were associated with higher growth rates as expected by the growth rate hypothesis.

Although flooding intensity had a positive direct effect on total soil C concentration, higher flooding intensity did not change total soil C concentrations. The positive direct effect was counteracted by the enhancement of CH_4 emissions and by the increased soil salinity that decreased soil C accumulation.

Some of the effects of flooding on soil stoichiometry were likely due to the effects on salinity and soil texture (mainly clay concentration). Soil clay concentration was positively related with total soil C, N, and mainly P concentrations, whereas soil salinity was negatively related with total soil C and N concentrations and N:P ratios. Eh was less associated with soil stoichiometry.

The results demonstrated that moderate increases in flooding intensity could increase the capacity of wetlands globally to act as C, N and P sinks. This question is relevant to the agricultural and environmental management of this and other regions of the world containing large areas of P-saturated cropland soils, with rivers transporting great amounts of C, N and P rich materials, and exposed to future increases in flooding intensity.

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Tables

Table 1

Plant C, N and P concentrations and n Different letters indicate significant differences within each above ground or belowground. utrient ratios in above- and belowground organs of *C. malaccensis.*

	Habitat	C concentration (mg g^{-1})	N concentration (mg g^{-1})	P concentration (mg g^{-1})	C:N ratio	C:P ratio	N:P ratio
Above ground	High flooding	377.8±3.7	12.0±0.4	3.1±0.2a	31.40±0.67b	123.82±7.58c	3.94±0.17c
	Intermediate flooding	383.5±3.5	10.8±0.9	2.3±0.7b	35.90±2.71ab	228.59±99.73b	6.08±2.20b
	Low flooding	377.5±2.2	9.9±0.8	1.6±0.6c	38.58±3.41a	278.07±69.87a	7.61±2.31a
Below ground	High flooding	365.8±11.7	11.0±0.5a	3.8±0.3	33.42±2.10b	96.19±6.46b	2.88±0.15
	Intermediate flooding	381.2±1.8	9.7±0.5ab	3.6±0.2	39.34±2.08ab	107.35±6.01ab	2.73±0.04
	Low flooding	371.4±9.3	8.0±0.8b	3.1±0.3	47.18±4.26a	122.11±12.58a	2.63±0.39

Different letters indicate significant differences within each above ground or belowground.

Figure captions

Fig. 1. Location of the three sampling habitats, the Shanyutan, Bianfuzhou and Tajiaozhou wetlands, corresponding to the high-, intermediate- and low-flooding habitats, respectively.

Fig. 2. Plant biomass (A), C accumulation (B), N accumulation (C) and P accumulation (D) for *C. malaccensis* in the high-, intermediate- and low-flooding habitats. Different letters indicate significant differences among the habitats ($P<0.05$) by the One-Way ANOVA (n=3). Error bar indicates standard error of the mean of triplicate measurements.

Fig. 3. Concentrations of soil total C (A), N (B), P (C), DOC (D) , NO_3^- (E), NH_4^+ (F), SO_4^{2-} (G) and Cl^- (H) for *C. malaccensis* wetlands at the various soil depths in the high-, intermediate- and low-flooding habitats. Different letter indicates significant differences among the habitats ($P<0.05$) by the One-Way ANOVA (n=3). Error bar indicates standard error of the mean of triplicate measurements.

Fig. 4. Clay (A), silt (B) and sand (C) concentrations and salinity (D), pH (E), Eh (F) and bulk density (G) for *C. malaccensis* wetlands at the various soil depths in the high-, intermediate- and low-flooding habitats. Different letter indicates significant differences among the habitats ($P<0.05$) by the One-Way ANOVA (n=3). Error bar indicates standard error of the mean of triplicate measurements.

Fig. 5. C:N (A), C:P (B) and N:P (C) ratios for *C. malaccensis* wetlands at the various soil depths in the high-, intermediate- and low-flooding habitats. Different letter indicates significant differences among the habitats ($P<0.05$) by the One-Way ANOVA (n=3). Error bar indicates standard error of the mean of triplicate measurements.

Fig. 6. Soil C concentration (A) and CH_4 production (B) for *C. malaccensis* wetland at the various soil depths in the high-, intermediate- and low-flooding habitats. Different letter indicates significant differences among the habitats ($P<0.05$) by the One-Way

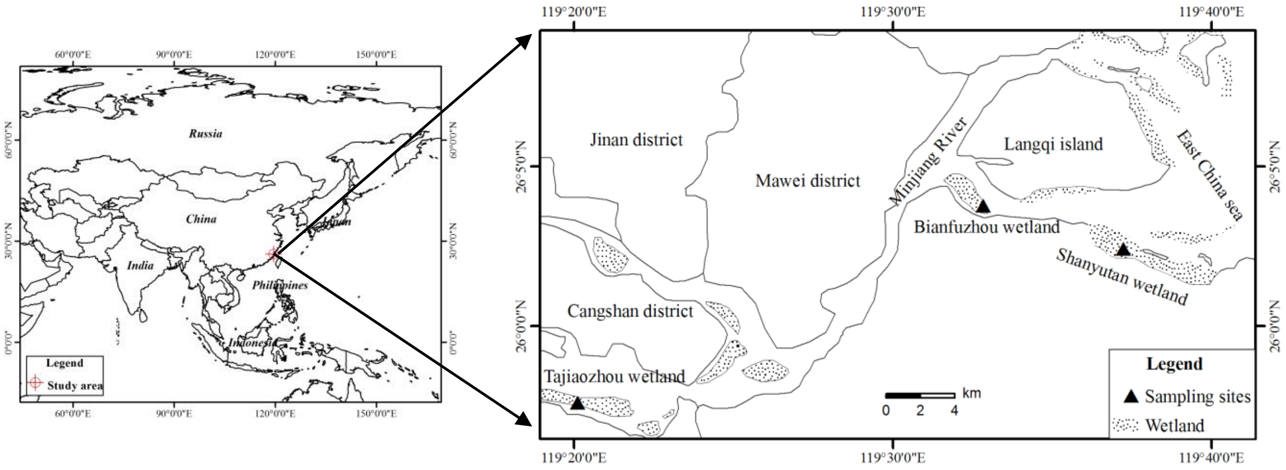
ANOVA (n=3). Error bar indicates standard error of the mean of triplicate measurements.

Fig. 7. Biplots of the standardized canonical discriminate function coefficients for the first two roots representing the soil variables as independent variables and the various grouping dependent factors corresponding to distinct flooding intensities.

Fig. 8. Diagrams of the structural models that best explain the maximum variance of soil P (A), N (B) and C (C) concentrations at different flooding intensities (h y^{-1} of inundation), with direct and indirect effects through the effects on soil traits (pH, clay concentration and salinity). The P values of the corresponding effects are indicated in parentheses. Black arrows refer to positive relationships and red arrows to negative relationships.

Fig. 9. Diagrams of the structural models that best explain the maximum variance of soil C:N (A), C:P (B) and N:P (C) ratios at different flooding intensities (h y^{-1} of inundation), with direct and indirect effects through the effects on soil traits (pH, clay concentration and salinity). The P values of the corresponding effects are indicated in parentheses. Black arrows refer to positive relationships and red arrows to negative relationships.

Fig. 10. Diagrams of the structural models that best explain the maximum variance of soil NO_3^- (A), NH_4^+ (B), and SO_4^{2-} (C) concentrations at different flooding intensities (h y^{-1} of inundation), with direct and indirect effects through the effects on soil traits (pH, clay concentration and salinity). The P values of the corresponding effects are indicated in parentheses. Black arrows refer to positive relationships and red arrows to negative relationships.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Jinan district
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Minjiang River
Langqi island
East China sea
Bianfuzhou wetland
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Sampling sites
Wetland

Fig. 1.

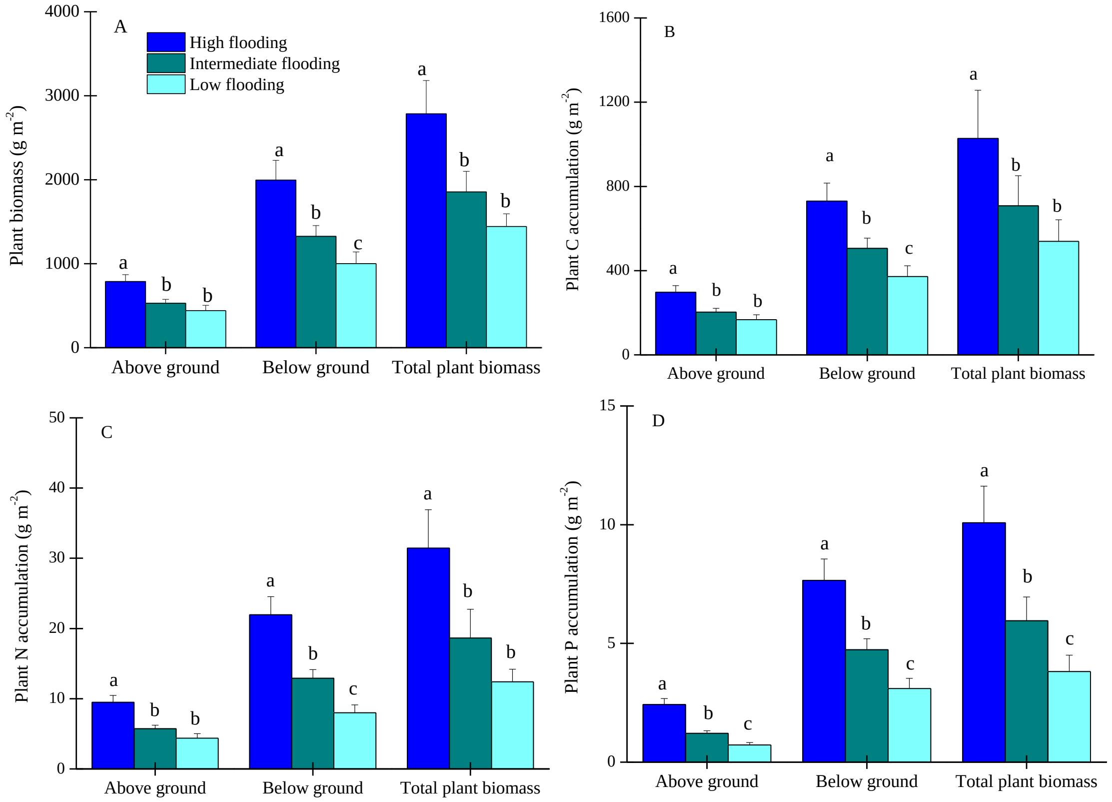
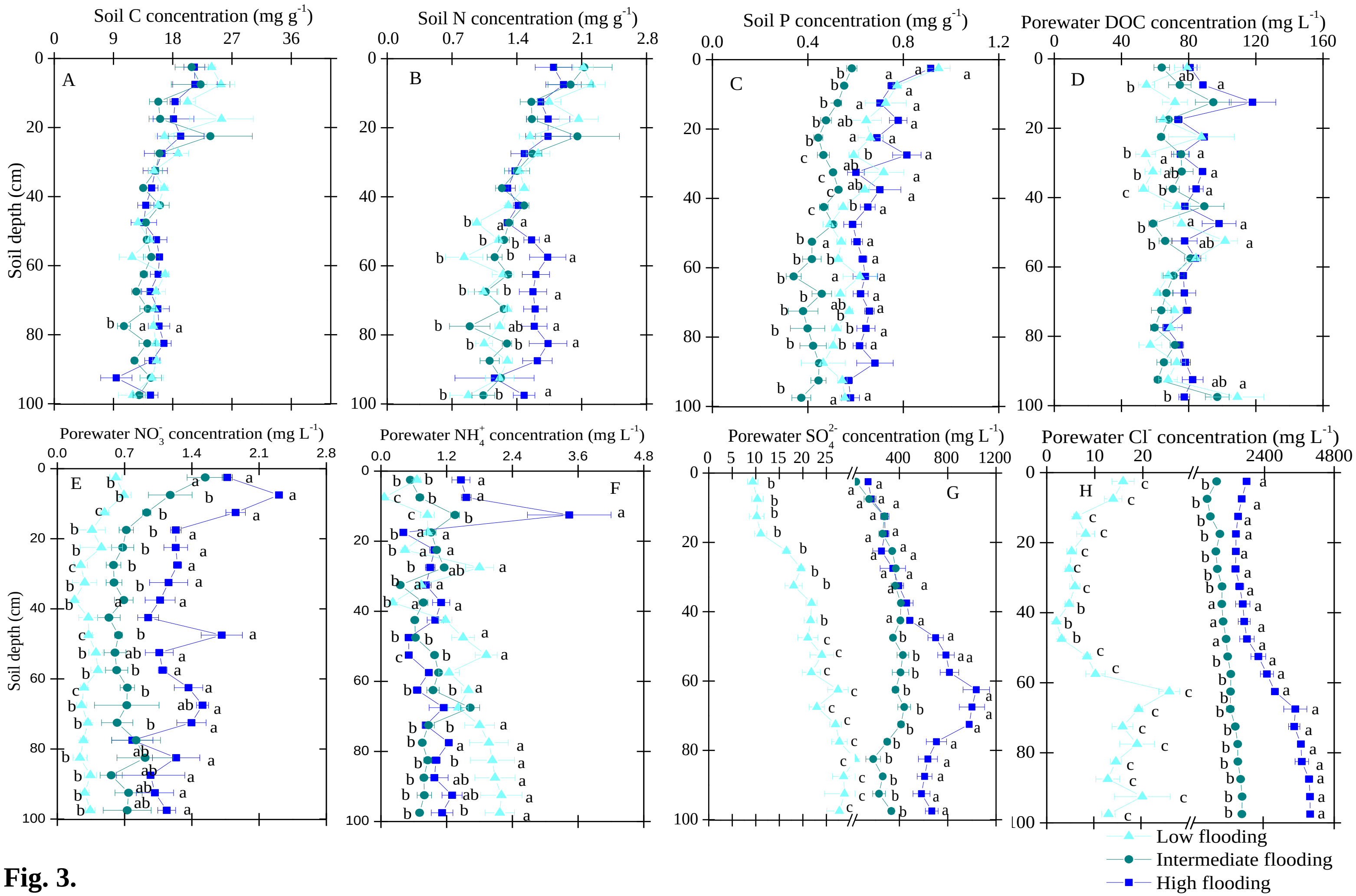

A
High flooding
Intermediate flooding
Low flooding
Plant biomass (g m-2)
4000
3000
2000
1000
0
Above ground
Below ground
Total plant biomass
B
Plant C accumulation (g m-2)
1600
1200
800
400
0
C
Plant N accumulation (g m-2)
50
40
30
20
10
0
D
Plant P accumulation (g m-2)
15
10
5
0

Fig. 2.



Soil C concentration (mg g-1)
Soil N concentration (mg g-1)
Soil P concentration (mg g-1)
Porewater DOC concentration (mg L-1)
Porewater NO3- concentration (mg L-1)
Porewater NH4+ concentration (mg L-1)
Porewater SO42- concentration (mg L-1)
Porewater Cl- concentration (mg L-1)
Soil depth (cm)
A
B
C
D
E
F
G
H
Low flooding
Intermediate flooding
High flooding

Fig. 3.

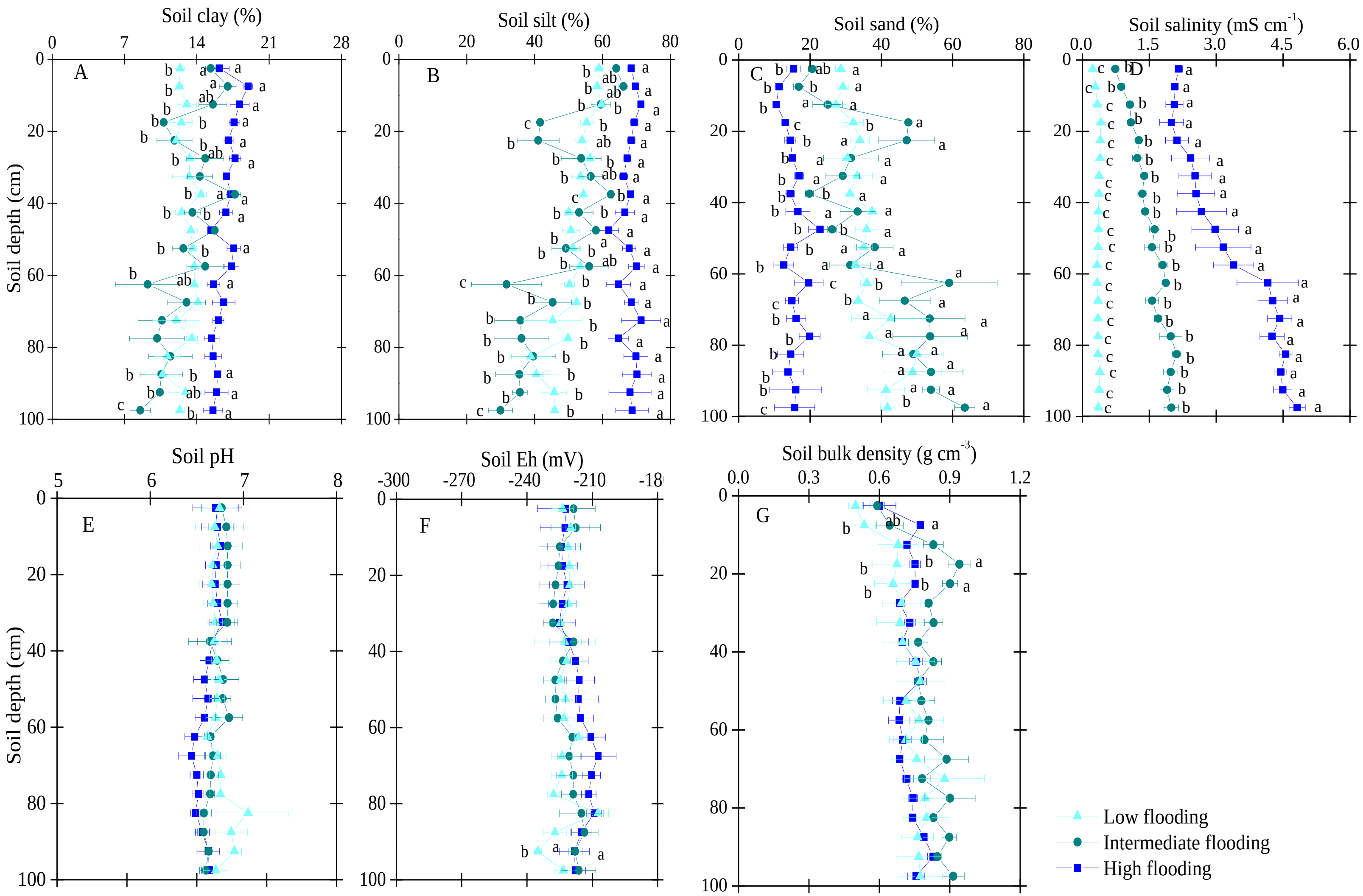
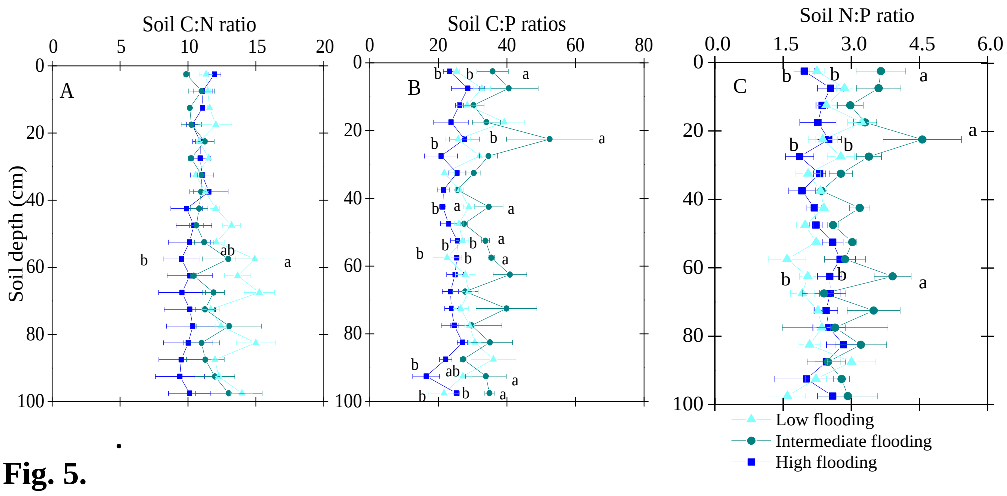
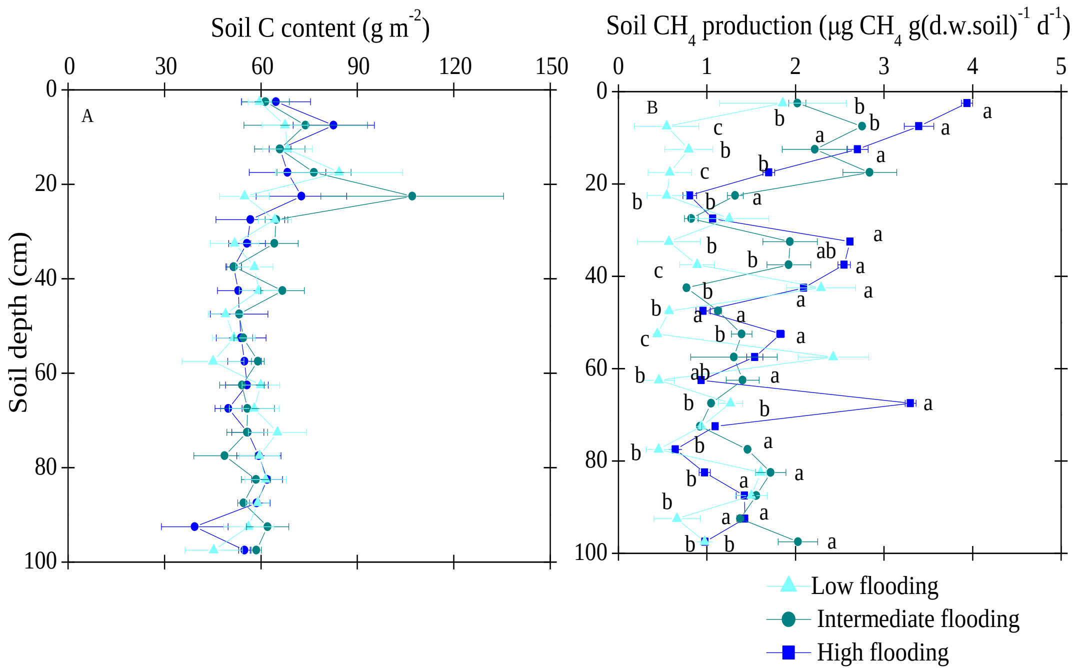

Soil clay (%)
Soil silt (%)
Soil sand (%)
Soil salinity (mS cm-1)
Soil pH
Soil Eh (mV)
Soil bulk density (g cm-3)
Soil depth (cm)
A
B
C
D
E
F
G
Low flooding
Intermediate flooding
High flooding

Fig. 4.



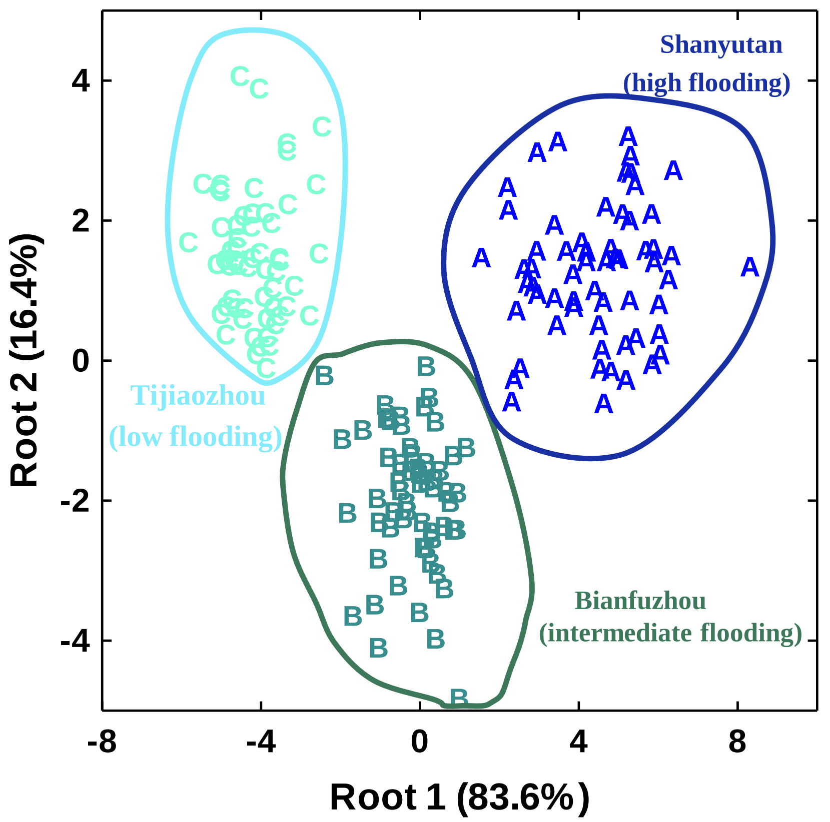
Soil C:N ratio
Soil C:P ratios
Soil N:P ratio
Soil depth (cm)
A
B
C
Low flooding
Intermediate flooding
High flooding

Fig. 5.



Soil C content (g m-2)
A
Soil depth (cm)
Soil CH4 production (µg CH4 g(d.w.soil)-1 d-1)
B
Low flooding
Intermediate flooding
High flooding

Fig. 6.


Shanyutan
(high flooding)
Tijiaozhou
(low flooding)
Bianfuzhou
(intermediate flooding)
Root 2 (16.4%)
Root 1 (83.6%)

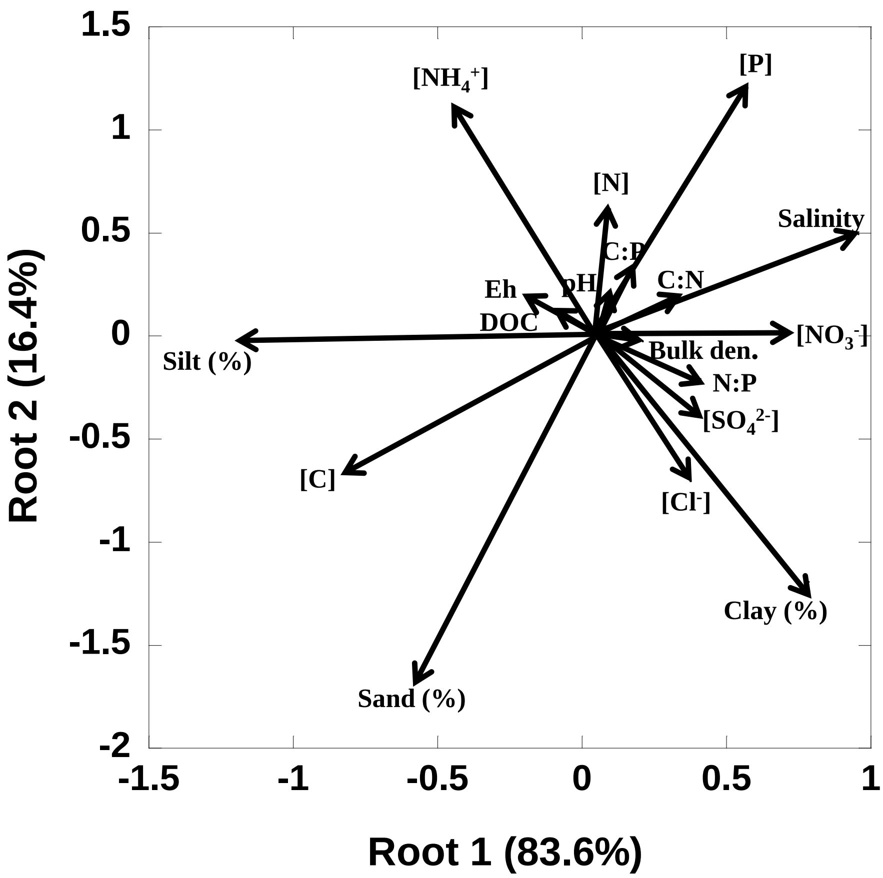
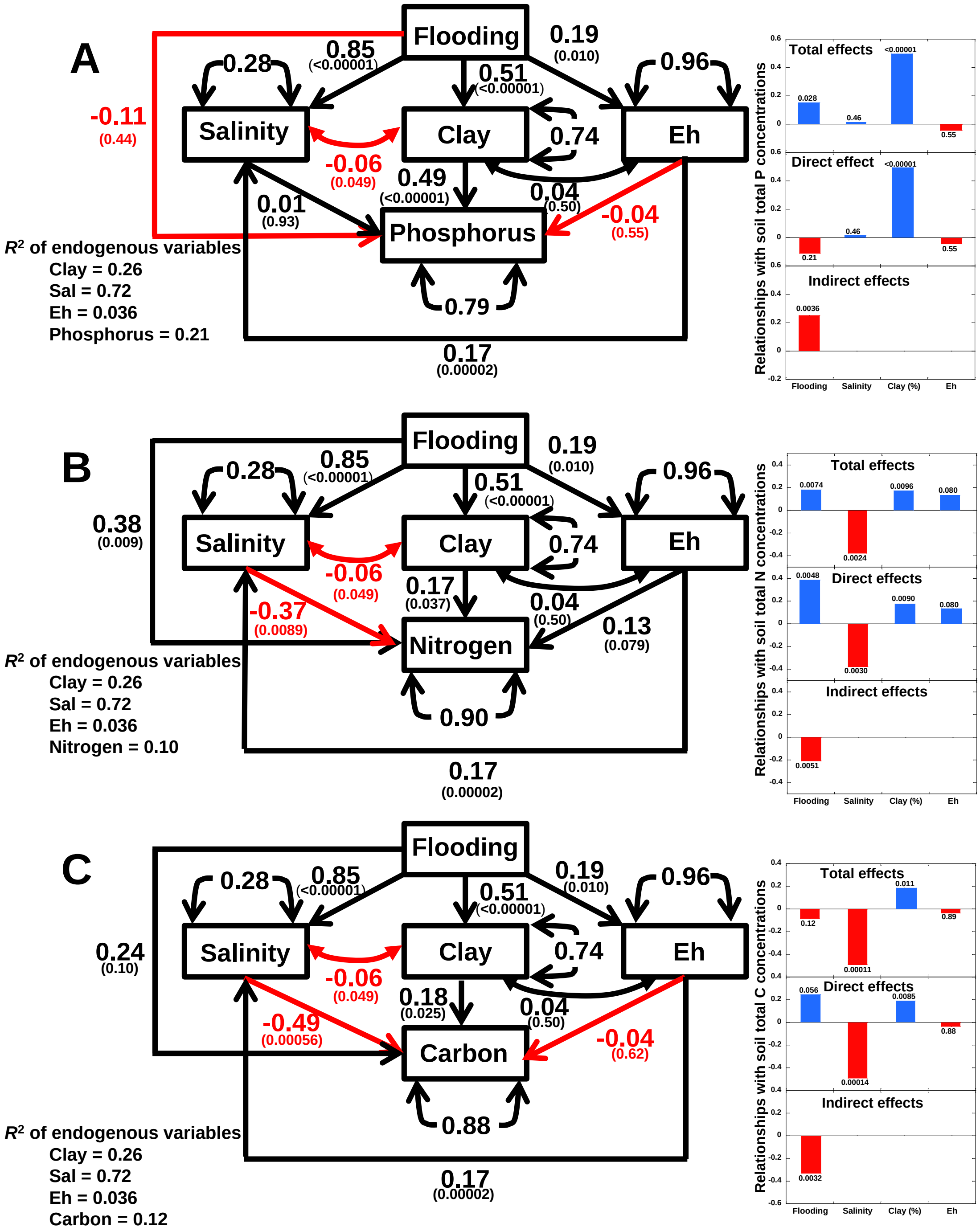

[NH4+]
[P]
[N]
Salinity
C:P
C:N
pH
Eh
DOC
[NO3-]
Silt (%)
Bulk den.
N:P
[SO42-]
[C]
[Cl-]
Clay (%)
Sand (%)
Root 2 (16.4%)
Root 1 (83.6%)

Fig. 7.



A
Flooding
Salinity
Clay
Eh
Phosphorus
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
0.74
-0.11
(0.44)
-0.06
(0.049)
0.49
(<0.00001)
0.01
(0.93)
0.04
(0.50)
-0.04
(0.55)
0.79
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
Phosphorus = 0.21
Relationships with soil total P concentrations
Total effects
Direct effect
Indirect effects
Flooding
Salinity
Clay (%)
Eh
B
Flooding
Salinity
Clay
Eh
Nitrogen
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
0.74
0.38
(0.009)
-0.06
(0.049)
0.17
(0.037)
-0.37
(0.0089)
0.04
(0.50)
0.13
(0.079)
0.90
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
Nitrogen = 0.10
Relationships with soil total N concentrations
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh
C
Flooding
Salinity
Clay
Eh
Carbon
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
0.74
0.24
(0.10)
-0.06
(0.049)
0.18
(0.025)
-0.49
(0.00056)
0.04
(0.50)
-0.04
(0.62)
0.88
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
Carbon = 0.12
Relationships with soil total C concentrations
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh

Fig. 8.

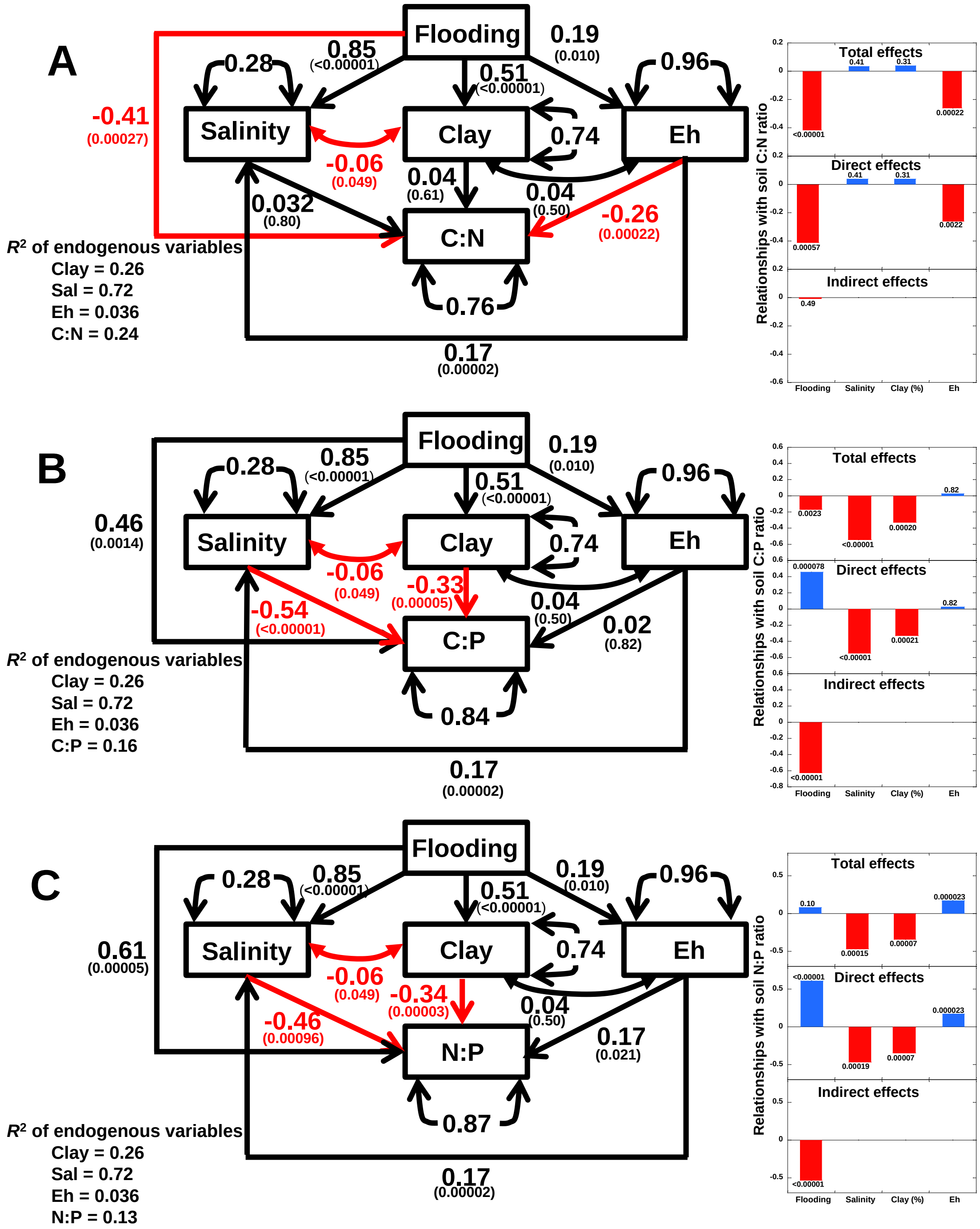

A
Flooding
Salinity
Clay
Eh
C:N
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
-0.41
(0.00027)
0.74
-0.06
(0.049)
0.04
(0.61)
0.04
(0.50)
0.032
(0.80)
-0.26
(0.00022)
0.76
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
C:N = 0.24
Relationships with soil C:N ratio
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh
B
Flooding
Salinity
Clay
Eh
C:P
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
0.46
(0.0014)
0.74
-0.06
(0.049)
-0.33
(0.00005)
-0.54
(<0.00001)
0.04
(0.50)
0.02
(0.82)
0.84
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
C:P = 0.16
Relationships with soil C:P ratio
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh
C
Flooding
Salinity
Clay
Eh
N:P
0.85
(<0.00001)
0.19
(0.010)
0.28
0.96
0.51
(<0.00001)
0.61
(0.00005)
0.74
-0.06
(0.049)
-0.34
(0.00003)
-0.46
(0.00096)
0.04
(0.50)
0.17
(0.021)
0.87
0.17
(0.00002)
R² of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
N:P = 0.13
Relationships with soil N:P ratio
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh

Fig. 9.

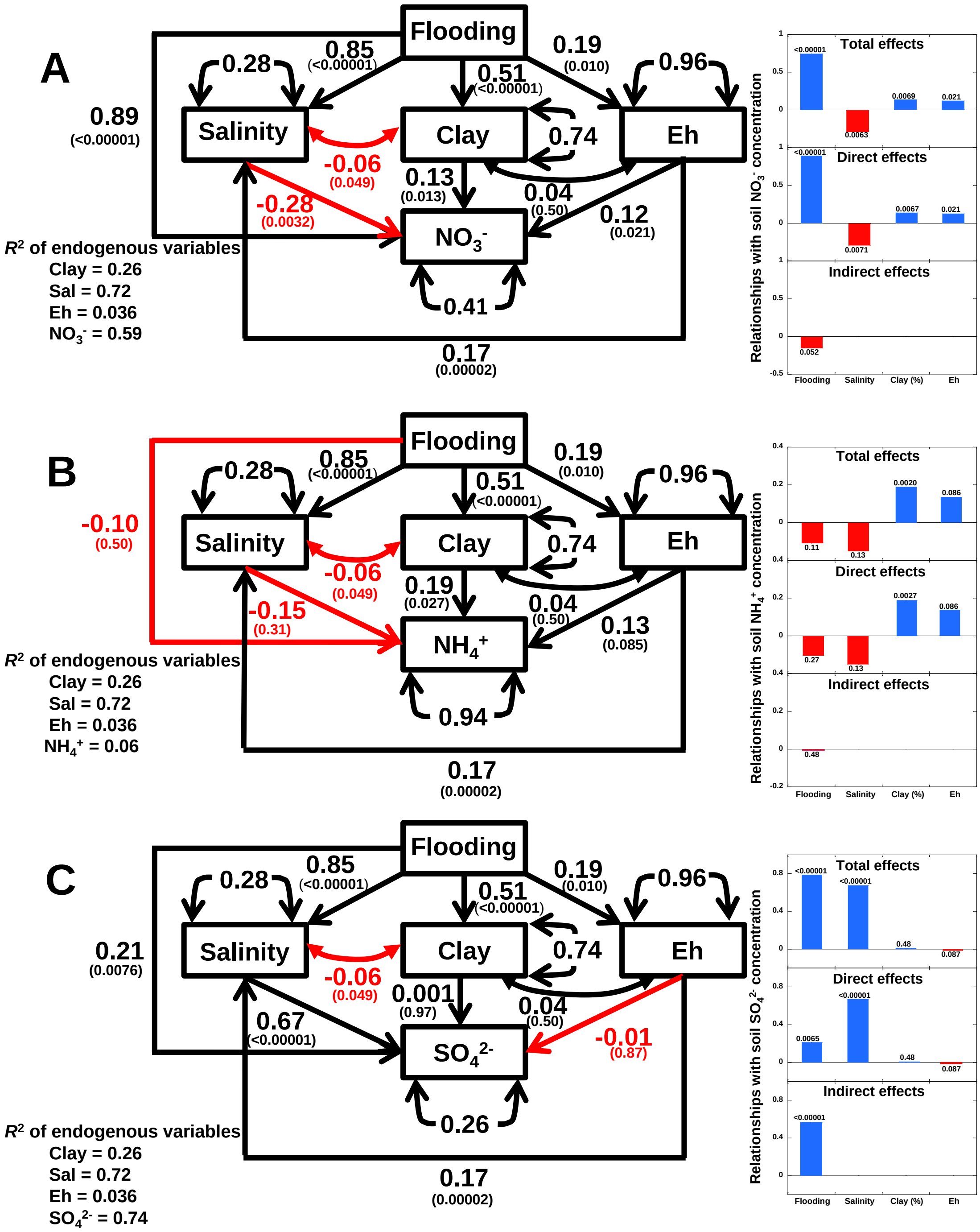

A
Flooding
Salinity
Clay
Eh
NO3-
0.85 (<0.00001)
0.28
0.51 (<0.00001)
0.19 (0.010)
0.96
0.89 (<0.00001)
-0.06 (0.049)
0.74
-0.28 (0.0032)
0.13 (0.013)
0.04 (0.50)
0.12 (0.021)
0.41
0.17 (0.00002)
R2 of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
NO3- = 0.59
Relationships with soil NO3- concentration
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh
B
Flooding
Salinity
Clay
Eh
NH4+
0.85 (<0.00001)
0.28
0.51 (<0.00001)
0.19 (0.010)
0.96
-0.10 (0.50)
-0.06 (0.049)
0.74
-0.15 (0.31)
0.19 (0.027)
0.04 (0.50)
0.13 (0.085)
0.94
0.17 (0.00002)
R2 of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
NH4+ = 0.06
Relationships with soil NH4+ concentration
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh
C
Flooding
Salinity
Clay
Eh
SO42-
0.85 (<0.00001)
0.28
0.51 (<0.00001)
0.19 (0.010)
0.96
0.21 (0.0076)
-0.06 (0.049)
0.74
0.001 (0.97)
0.67 (<0.00001)
0.04 (0.50)
-0.01 (0.87)
0.26
0.17 (0.00002)
R2 of endogenous variables
Clay = 0.26
Sal = 0.72
Eh = 0.036
SO42- = 0.74
Relationships with soil SO42- concentration
Total effects
Direct effects
Indirect effects
Flooding
Salinity
Clay (%)
Eh

Fig. 10.