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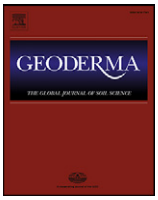

Influences of anthropogenic cultivation on C, N and P stoichiometry of reed-dominated coastal wetlands in the Yellow River Delta

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ABSTRACT

Motivated by the previous studies that indicated well-constrained carbon:nitrogen:phosphorus (C:N:P) ratios in planktonic biomass, and their importance to improve our understanding on the biological processes and nutrient cycling in marine ecosystems, ecologists have endeavored to search for similar patterns and relationship in terrestrial ecosystems. Recent analyses indicated that "Redfield-like" ratios existed in plants; such data might provide insight into the nature of nutrient limitation in terrestrial ecosystems. We attempted to determine if analogous C:N:P stoichiometrical ratios exist in the soil and plant in the reed-dominated coastal wetlands of the Yellow River Delta (YRD). Under the influences of anthropogenic cultivation in the YRD, the reed-dominated wetlands could be classified into three categories, new-born wetland (NW), farmland converted into wetland (FW) and cotton wetland (CW). In these three wetland categories, our results showed that atomic C:N:P ratios (R_{CNP}) in both the soil (42.6:1.6:1, 71.2:2.0:1 and 63.2:1.9:1, respectively) and the plant (1753:22.4:1, 1539:23.0:1 and 1196:23.8:1, respectively) were not well-constrained. Though C:N ratios (R_{CN}) and C:P ratios (R_{CP}) were of relatively large variation among different wetland soils and plants, average atomic N:P ratios (R_{CN}) in both the soil (~1.9:1) and the plant (~23:1) were well-constrained in the reed-dominated wetlands at the YRD scale, suggesting that the N limitation and P limitation were found in the soils and the plants, respectively. The results potentially provide a useful reference for ongoing wetland conservation and restoration in the YRD.

1. Introduction

All organisms are composed of multiple chemical elements which were brought together in non-arbitrary proportions. Carbon (C), nitrogen (N) and phosphorus (P) are the three main chemical elements that exist in different ratios in living organisms. The key characteristics of organisms and ecosystems could be determined by dynamics of element ratios (Sterner and Elser, 2002). In ecological contexts, stoichiometry has been usually applied to elemental composition, with an emphasis on the major constituents of living organisms, particularly on C, N, and P (Michaels, 2003). Redfield (1958) observed that, on average, planktonic biomass contains C, N and P in an atomic ratio of 106:16:1, which is similar to the ratio of C, N and P in marine water. The elegant simplicity of this stoichiometric relationship—the Redfield ratio—belies its incredible utility (Cleveland and Liptzin, 2007). The predictive power of the Redfield ratio has prompted ecologists to search for similar patterns and relationships in terrestrial ecosystems, and has even inspired a new discipline of ecological stoichiometry to understand the balance of multiple chemical elements in ecological interactions (Elser et al., 2000). Ecological stoichiometry has already been proven to be valuable in understanding various connections between trophic interactions and nutrient cycling (Elser and Hassett, 1994; Elser et al., 1996, 2001, 2003; Sterner and Elser, 2002). Ecological stoichiometric theories have integrated the first law of thermodynamics, natural selection during biological evolutions, and the central dogma of molecular biology (Elser et al., 2000; Zhang et al., 2013), resulting in the successful link of various studies on different scales from molecules to organisms and from ecosystems to the biosphere (Cleveland and Liptzin, 2007; Michaels, 2003; Sardans et al., 2012).

Phragmites australis, commonly named as reed grass, has received considerable attention in the last decade (Havens, 2002). *P. australis* is a cosmopolitan plant found throughout the world. A single plant can spread over 0.05 ha in 2 years. Its rapid vegetative propagation and ability to suppress other graminoids by shading and litter mat formation (Havens et al., 2003; Windham, 2001) give it a distinct advantage over other species. *P. australis* can survive in most wet habitats. The wetlands

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in Yellow River Delta are not the exception. The Yellow River Delta (YRD) is the fastest growing delta in the world. The wetland of the YRD is not only the most complete estuarine wetland, but also the youngest wetland ecosystem in the warm temperate zone in China (Li et al., 2009). The Yellow River Delta National Nature Reserve was established in October 1992 by the State Council of China, and covered 153 000 ha including 58 000 ha of core area. Reed marsh covers ~26 000 ha of the core area of the reserve. Out of the Reserve, most of *P. australis* wetlands were reclaimed farmlands which were mainly used to cultivate cotton plant, while part of the cotton plant farmland was converted to reed grassland by following the policy of reverting cultivated land to grassland and forests. Cotton is classified as one of the most salt-tolerant crops and considered a pioneer crop in reclamation of saline soils (Maas, 1990) and has a complete self-protection system against salinity (Ashraf, 2002). To date, we have not found any relevant published studies on anthropogenic cultivation over the soil and plant C, N and P stoichiometry of reed-dominated wetland in the YRD. The goal of the study was to investigate C, N, and P stoichiometry characteristics in reed-dominated wetland soils and plants, as well as to analyze and confirm influencing factor of anthropogenic cultivation. The objectives were (1) to determine the soil and plant C, N, and P stoichiometry of typical reed-dominated wetland and cotton farm land, (2) to characterize R_{CN}, R_{CP} and R_{NP} distribution patterns in wetland soil profiles and plant tissues, and (3) to identify the influence of anthropogenic cultivation on C:N:P ratios of the reed-dominated costal wetland.

2. Material and methods

2.1. Study region

The studied sites are located in the Yellow River Delta Ecological Research Station of Coastal Wetland, Chinese Academy of Sciences, in the Yellow River Delta Natural Reserves (Fig. 1), which was established in 1992 to preserve the habitat for birds and unique estuarine coastal wetland ecosystems. The YRD is one of the most active regions of land–ocean interaction among the large river deltas in the world. It has been estimated that ~1300 ha territory land is formed here annually. As a newly formed estuarine delta, it has been naturally characterized by extensive coverage of primary salinization, which was mainly due to the presence of a shallow, saline water table and marine sediments (Han et al., 2012; Zhang et al., 2011). The region, which is rainy from June to August, is submitted to warm temperate continental monsoon climate. The average annual precipitation of the study region is 551.6 mm. The mean annual temperature of the region is ~12.1 °C and the frost-free period is ~196 d. The soil is typical saline alluvial soil (fluvisols, FAO).

2.2. Soil and plant collection

In order to examine the influence of anthropogenic cultivation on C, N and P stoichiometry in soil and plant of reed-dominated coastal wetlands in the YRD, three categories of wetlands, i.e. the new-born wetland (NW) located at the current estuary of Yellow River where a minor shift of the mouth channel occurred in 1996, farmland converted into wetland (FW) since 2009, and cotton field converted from natural wetland (CW) since 2002 in the study area were selected for sampling. Ten to twenty sample plots were taken in each category of wetland. Three replicate soil profile samples were collected randomly in each plot. Soil samples of 0–50 cm soil depth were collected using a stainless-steel slide hammer with an inner diameter of 3.5 cm on September 7th, 2012. Each collected sample in the soil profile was sectioned at 10 cm intervals in the field, and then stored in polyethylene plastic bags after air dried. Soil samples were ground using a mortar and pestle, and then sieved before laboratory analysis. At each category

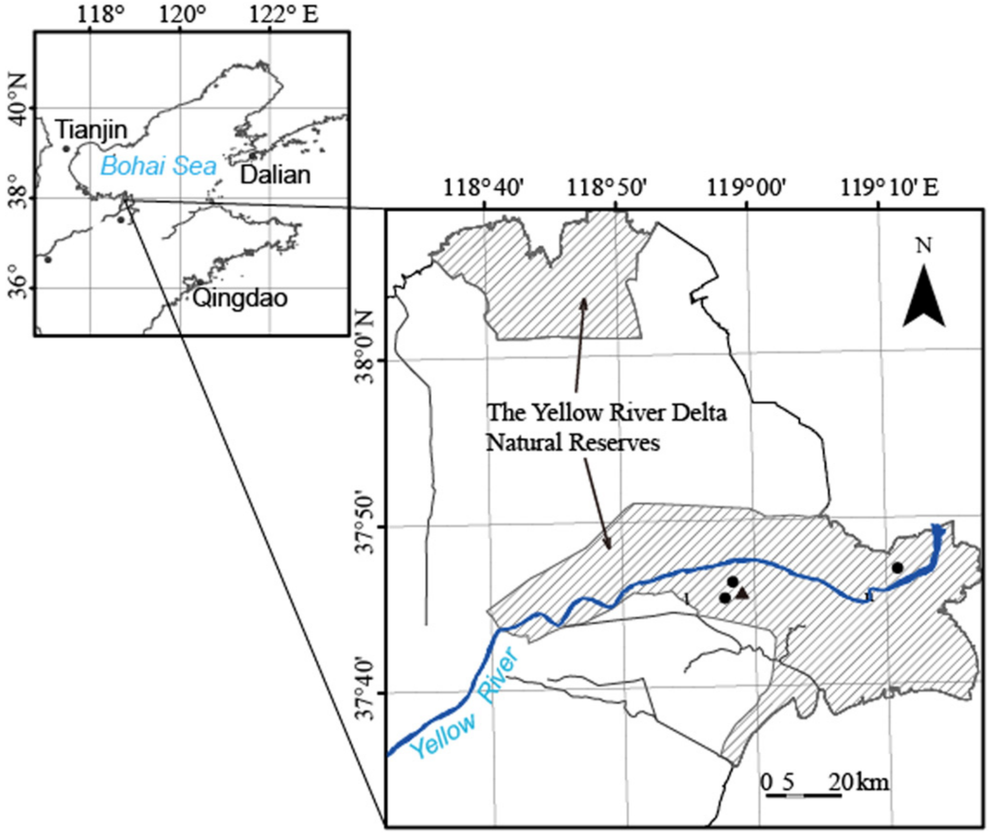

Fig. 1. Location of the sampling sites in the coastal wetland in the Yellow River Delta, China (▲: the Yellow River Delta Ecological Research Station of Coastal Wetland, Chinese Academy of Sciences, ●: sampling site).

Table 1
Basic soil characteristics of the wetland soils in the Yellow River Delta.

wetlands	pH	EC ($\mathrm{ms\ cm^{-1}}$)	Salinity (‰)	TC (%)	TN ($\mathrm{mg\ kg^{-1}}$)	TP ($\mathrm{mg\ kg^{-1}}$)
NW	8.56 ± 0.28	4.12 ± 0.98	6.10 ± 2.30	0.87 ± 0.32	385.2 ± 110.8	523.9 ± 24.5
FW	8.71 ± 0.31	5.36 ± 0.94	12.74 ± 2.22	1.47 ± 0.20	485.8 ± 210.0	531.5 ± 25.3
CW	8.12 ± 0.26	1.02 ± 0.58	2.52 ± 1.35	1.37 ± 0.24	495.4 ± 42.10	559.4 ± 42.1

Table 2
Correlation matrix of TC, TN and TP in reed-dominated wetland soils (n = 20, 25, 15) and plants (n = 12, 16, 9) in the YRD.

Wetlands	Bivariate variables	Soil	Plant
NW	TC & TN	0.679*	0.821
	TC & TP	0.654*	0.428
	TN & TP	0.782**	0.825
FW	TC & TN	0.940**	0.961*
	TC & TP	0.780**	0.971*
	TN & TP	0.788**	0.999**
CW	TC & TN	0.917**	0.964*
	TC & TP	0.430	0.701
	TN & TP	0.430	0.866
YRD	TC & TN	0.718**	0.902**
	TC & TP	0.476	0.824**
	TN & TP	0.580**	0.957**

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

of wetland, samples of reed and cotton plant tissues were collected for later analysis in the laboratory. Each intact plant (IP) was severed at the ground surface and the aboveground portions (AP) and belowground portions (BP) were collected. The fresh plant tissues were washed with tap water, rinsed with deionized water and then placed in open, clean paper bags, partially air-dried if possible, or kept in a cool environment during shipment to the laboratory.

2.3. Chemical and statistical analyses

The chemical analyses were performed at the Key Laboratory of Wetland Ecology and Environment, Yantai Institute of Coastal Research, Chinese Academy of Sciences. Measurements of pH and the electrical conductivity (EC) were performed using a 1:5 soil to deionized water ratio. The soils were mixed with the deionized water and stirred intermittently for 1 h before analysis. Soil pH was measured with a Beckman pH meter with combination electrode; EC/salinity was quantified with an Orion Conductivity/Salinity Model 140 m.

The majority of published estimates of soil and plant C and N had used the “cube” platform, which has been widely recognized as the benchmark for elemental analyzer design, operation and analytical performance. Total carbon (TC) and total nitrogen (TN) contents were measured with an elemental analyzer of vario MACRO cube (Germany, 2009). The methods which were developed by Murphy and Riley (1962) were used for the colorimetric determination of orthophosphate concentration in solutions. The perchloric acid ($HClO_4$) digestion method (Olsen and Sommers, 1982) was used to determine the total phosphorus (TP) in soil, while TP in the plant tissue digestion used the Thomas et al. (1967) method with H_2SO_4/H_2O_2 to digest a plant sample in an aluminum block. The soil total C, N and P concentrations ($\mathrm{mg\ kg^{-1}}$) were transformed to a unit of $\mathrm{mmol\ kg^{-1}}$, and C:N, C:P and N:P ratios for each type of soil were calculated as molar ratios (atomic ratio), rather than mass ratios.

The SAS v8.1 software (SAS Inc., Cary, NC) was used for all the statistical analyses and for the variance of analysis the NPAR1WAY procedure with Kruskal–Wallis test of significance to compare C, N and P concentrations and ratios within and across groups. The Pearson correlation coefficients were calculated by using SPSS 16.0 (SPSS Inc., 2010).

3. Results and discussion

3.1. Basic characteristics of the wetland soils

The high pH values of ~8.5 (classification: pH > 8.5 — strongly alkaline) were observed in the three categories of wetland soil profiles in the study (Table 1). Generally, the soil pH in natural wetlands generally ranged from around 6.5 to 7.5 (with a few exceptions) (Gambrell, 1994). Our results indicated that the unusually high pH values compared to those of wetlands could be caused by the regional geological, geochemical, and hydrologic conditions, as well as the land–ocean interaction in this region. The mean content of salinity was ranked as FW (mean, 12.74‰) > NW (mean, 6.10‰) > CW (mean, 2.52‰), primarily due to the influence of cultivation which reduced soil salt content. The land–ocean interaction led to the higher salinity of inland coastal wetland than the newly formed wetland. The soil TC, TN and TP in FW and CW soils averaged 14.7 $\mathrm{g\ kg^{-1}}$, 485.8 $\mathrm{mg\ kg^{-1}}$, 531.5 $\mathrm{mg\ kg^{-1}}$ and 13.7 $\mathrm{g\ kg^{-1}}$, 495.4 $\mathrm{mg\ kg^{-1}}$, 559.4 $\mathrm{mg\ kg^{-1}}$, respectively (Table 1), with no significant differences in each other. Though the mean content of TP was ranked as CW (mean, 559.4 $\mathrm{mg\ kg^{-1}}$) > FW (mean, 531.5 $\mathrm{mg\ kg^{-1}}$) > NW (mean, 523.9 $\mathrm{mg\ kg^{-1}}$), the differences of TP among NW, FW and CW were not significant, which provided a platform to do C:N:P stoichiometry. The mean content of TN in NW (mean 385.2 $\mathrm{mg\ kg^{-1}}$) was significantly lower than FW (mean 485.8 $\mathrm{mg\ kg^{-1}}$) and CW (mean 495.4 $\mathrm{mg\ kg^{-1}}$). There was a significant difference in TC ($p < 0.0001/p = 0.001$) and TN ($p = 0.040/p = 0.036$) between NW and FW/CW. This was mainly caused by cultivation during which people used a large amount of N (~150 $\mathrm{kg\ N\ ha^{-1}}$) but a relatively small amount of P (15–30 $\mathrm{kg\ P_2O_5\ ha^{-1}}$) fertilizers to increase the cotton yield. These results might reflect that the content of TP in the YRD mainly depended on the mineralogy of the parent material, while TC and TN in

Table 3
Comparison of total soil C, N and P ratios in the Yellow River Delta, national and global scales.

Cases of different scale	R_{CN}	R_{CP}	R_{NP}	Reference
New-born coastal wetland (YRD)	9.53[a]	17.64[a]	1.85	Yu et al. (2010)
Newly formed wetland (YRD)	26.52	42.55	1.61	This study
Farmland converted into wetland (YRD)	40.54	71.19	1.99	
Cotton wetland (YRD)	35.69	63.21	1.94	
Overall (China)	11.94 ± 0.4	61 ± 0.9	5.2 ± 0.1	Tian et al. (2010)
Grassland (global)	13.8 ± 0.4	166.0 ± 12.2	12.3 ± 0.7	Cleveland and Liptzin (2007)
Forest (global)	14.5 ± 1.2	211.7 ± 28.4	14.6 ± 1.8	
Overall (global)	14.3 ± 0.5	186.0 ± 12.9	13.1 ± 0.8	

[a] C in R_{CN} and R_{CP} ratio used total organic carbon.

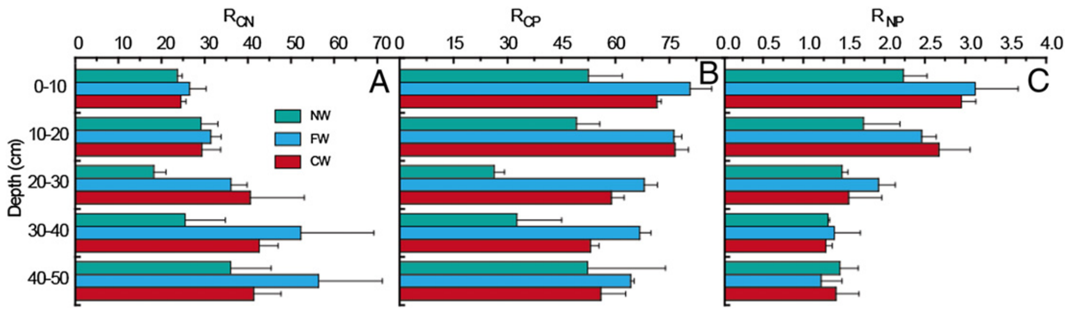

Fig. 2. The distribution of R_{CN} (A), R_{CP} (B) and R_{NP} (C) in soil profile in NW, FW and CW.

FW/CW were dramatically affected by agricultural fertilization and management.

3.2. Correlation analysis of C, N and P in wetland soil and plant

The biogeochemical cycles of C and N were tightly coupled in terrestrial and marine ecosystems (Cleveland and Liptzin, 2007). In particular, the photosynthetic requirement for nitrogen, which means that increases in primary production are dependent on the availability of N to fuel increased photosynthetic C acquisition, coupled with relatively low levels of available nitrogen in many terrestrial ecosystems, causes carbon uptake and storage on land tightly regulated by nitrogen cycle (Vitousek and Howarth, 1991). Correlation analysis suggested that there were significant positive correlations between TC, TN and TP in the reed-dominated wetland soils and plants in the YRD (Table 2). Meanwhile, correlation analysis suggested that there was a significant positive correlation between C:N:P in soils and C:N:P in plants ($p < 0.05$) in the reed-dominated wetlands in the YRD. In these three wetland types, there was a significant positive correlation between TC and TN for both soil and plant. However, correlations between TC and TP differed among wetland types, soils and plants. The correlations between TC and TP were similar to the correlations between TN and TP. TC/TN was significantly related to TP in soils and plants in FW. On the contrary, TC/TN was not significantly related to TP in soils and plants in CW. TC/TN was significantly related to TP in soils of NW, but not in plants. These differences might reflect the different sources of nutrients (N and P) that plants absorbed from the soil, as well as CO_2 from the air, and soil N was mainly affected by soil microorganisms and soil P variation depended on the soil parent material.

3.3. C, N and P stoichiometry in different wetland soils and plants

Redfield (1958) presented that marine plankton are composed of C, N, and P in a characteristic molar ratio of 106:16:1, which is similar to the ratio of C, N and P in marine water. Based on A. C. Redfield's classic paper, the method of elemental ratios has become widespread in marine and freshwater phytoplankton studies (Elser and Hassett, 1994) and also opened an avenue of exploration for terrestrial ecosystems (Albuquerque and Mozeto, 1997; Li et al., 2013; Reich and Oleksyn, 2004; Schaller et al., 2012; Taylor and Townsend, 2010; Tian et al., 2010).

Table 4
Summary of soil C, N and P stoichiometry in the Yellow River Delta.

Wetlands	Sample numbers	R_{CN}	R_{CP}	R_{NP}	R_{CNP}
NW	10	26.52 ± 7.83	42.55 ± 14.17	1.61 ± 0.40	42.6:1.6:1
FW	20	40.54 ± 15.46	71.19 ± 7.22	1.99 ± 0.79	71.2:2.0:1
CW	15	35.69 ± 9.57	63.21 ± 10.10	1.94 ± 0.76	63.2:1.9:1
Mean	45	35.81 ± 13.08	62.17 ± 14.97	1.89 ± 0.71	62.2:1.9:1

Cleveland and Liptzin (2007) found remarkably consistent C:N:P ratios in both total soil pools and the soil microbial biomass. Their analysis indicated that, similar to marine phytoplankton, element concentrations of individual phylogenetic groups within the soil microbial community might vary, but on average, atomic C:N:P ratios in both soil (186:13:1) and the soil microbial biomass (60:7:1) were well-constrained at the global scale (Table 3). Tian et al. (2010) explored

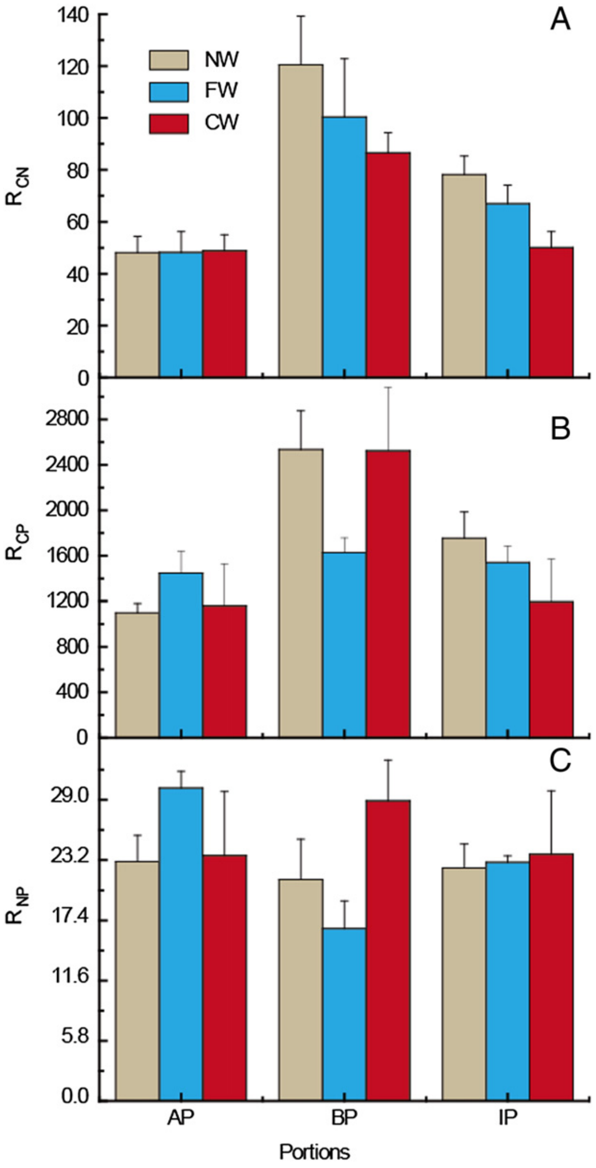

Fig. 3. The R_{CN} (A), R_{CP} (B) and R_{NP} (C) in plant tissue of NW, FW and CW (AP: the aboveground portions, BP: belowground portions, and IP: the intact plant).

general soil C:N:P ratio of 60:5:1 in China at the national scale, and for the 0–10 cm organic-rich soil which usually has the most active organism–environment interaction, they found a well-constrained C:N:P ratio of 134:9:1. Yu et al. (2010) studied the spatial distribution characteristics of soil nutrients in new-born coastal wetland in the Yellow River Delta and the results showed TOC:N:P ratio of 17.6:9.5:1.9 in an estuarine wetland scale. Related studies also found that element ratios in terrestrial systems appeared to be more variable than those in the ocean, but parallels between the nutrient abundance of organism and the environment seemed to exist in plant communities and forest ecosystems worldwide (Cleveland and Liptzin, 2007; Hedin, 2004; McGroddy et al., 2004; Reich and Oleksyn, 2004). Soil and plant C, N and P stoichiometry of reed-dominated coastal wetland in the YRD under the influences of anthropogenic cultivation are discussed in the following section.

R_{CN}, R_{CP} and R_{NP} distribution in soil profile varied in the reed-dominated wetland in the YRD with the influence of anthropogenic cultivation (Fig. 2A–C). In NW profile, R_{CN} and R_{CP} greatly changed with no obvious distribution patterns from the surface to the bottom. At 20–30 cm depth, R_{CN} and R_{CP} values were lower than those from other layers, while R_{NP} was higher in the top soil and decreased slowly with depth. In soil profiles of FW and CW, R_{CN} changed dramatically and increased with depth. R_{CP} and R_{NP}, which showed the same distribution trends, decreased with the depth, while there was no significant difference between the values at 30–40 and 40–50 cm. The average values of R_{CN}, R_{CP} and R_{NP} in the soil profiles were ranked as FW >CW > NW (Table 4). Significant differences existed between NW and CW ($F = 3.75$, $p = 0.047$ for R_{CN}; $F = 4.24$, $p = 0.007$ for R_{CP}; and $F = 3.97$, $p = 0.039$ for R_{NP}), but did not exist between NW and FW as well as FW and CW. Soil C:N:P ratios in NW, FW and CW were 42.6:1.6:1, 71.2:2.0:1 and 63.2:1.9:1, respectively.

The C:N ratios were higher than the average values of China national and global scales, while the N:P ratios were lower than those of them. Our data suggested that the soils of the study area were “N limitation”. Fixed soil C:N ratios across large geographical distances are consistent with the fact that plants are the major source of total soil C and N in terrestrial ecosystems, while the P content mainly depends on the mineral weathering and soil development of the parent material. Our results indicated the differences among the three categories of reed-dominated wetland under the effect of anthropogenic cultivation.

R_{CN}, R_{CP} and R_{NP} in the plant tissues of wetlands varied in YRD with the influence of anthropogenic cultivation (Fig. 3A–C). In different plant tissues of three categories of wetlands, there were no significant differences in the R_{CN} values of AP of reed tissues in NW, FW, and AP of cotton in CW. Meanwhile, there were no significant differences in the R_{CN} values of BP of reed and cotton tissues between NW and FW ($p = 0.149$), and between BP of reed tissues FW and BP of cotton in CW ($p = 0.289$). In contrast, R_{CN} values of BP of reed in NW were significantly ($p < 0.05$) higher than those of cotton in CW. The R_{CP} and R_{NP} values of AP of reed in FW tended to be higher than reed in NW and cotton in CW. The R_{CP} and R_{NP} values of BP of reed in FW were lower than BP of reed in NW and BP of cotton in CW. The values of R_{CP} in NW and CW were similar with significant differences ($p = 0.021$ and $p = 0.034$, respectively) from FW. For the value of R_{NP}, there was no significant difference ($p = 0.077$) between NW and CW, however, there were significant differences between NW and FW ($p = 0.008$) as well as FW and CW ($p = 0.034$), respectively. R_{CN}, R_{CP} and R_{NP} in the reed plants were averaged at 78.24, 1752.82 and 22.43 in plant community in NW, and 67.03, 1539.25 and 1.99 in FW, respectively (Table 5). Though the mean values of R_{CN}, R_{CP} and R_{NP} of plant community in FW were slightly higher than those in NW, there was no significant difference between them. Significant difference of R_{CN} ($p = 0.014$) existed between reed and cotton tissues (Table 5).

The elemental content of a plant is not a fixed entity, but varies from month to month, day to day, and even from hour to hour, as well as differing between the various parts of the plant itself (Kalra, 1998). Our data suggested that the value of plant N:P ratios was ~23 which was higher than a foliar N:P “breakpoint” between N limitation (N:P < 14) and P limitation (N:P > 16) (Aerts and Chapin, 2000; Reich and Oleksyn, 2004; Townsend et al., 2007), thus providing a direct evidence that the plants in the YRD were in “P limitation”; at least, the plants in the YRD were in a “P limitation” state at the sampling time in September 7th of 2012.

Table 5
Summary of plant C, N and P stoichiometry in the Yellow River Delta.

Wetlands	R_{CN}	R_{CP}	R_{NP}	R_{CNP}
NW	78.24 ± 7.14	1752.82 ± 231.12	22.43 ± 2.33	1753:22.4:1
FW	67.03 ± 7.12	1539.25 ± 144.12	22.99 ± 0.61	1539:23.0:1
CW	50.06 ± 6.23	1196.08 ± 372.18	23.76 ± 6.12	1196:23.8:1
Mean	66.48 ± 13.21	1523.32 ± 321.34	23.00 ± 3.09	1523:23.0:1

4. Conclusions

The Redfield ratio, per se, might be an inappropriate standard for defining the stoichiometric balance in coastal wetland ecosystems, however, our results confirmed that the predictable one, “Redfield-like” element ratios could indicate that both soil and plant may indeed be stoichiometrically balanced. Our results demonstrated that R_{CNP} in both soil and plant was not well-constrained as anthropogenic cultivation and had a serious impact on nutrient stoichiometry in soils and plants. Meanwhile, average atomic N:P ratios (R_{CN}) in both soil (~1.9:1) and plant (~23:1) were well-constrained in the reed-dominated wetland at the YRD scale, indicating that the soil was in a “N limitation” state and the plant was in a “P limitation” state at the sampling time. If so, spatial, temporal or site-specific differences in element ratios of soil and plant from the average soil and plant ratio could potentially provide insights into the coupling of biogeochemical cycles and nutrient limitation in coastal wetland ecosystems in the future research, as the Redfield ratio does in marine ecosystems.

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