

Changes of Biogenic Elements in *Phragmites australis* and *Suaeda salsa* from Salt Marshes in Yellow River Delta, China

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Abstract: Little information is available on biogenic elements (carbon, nitrogen, phosphorus and sulfur) and the ecological stoichiometric characteristics of plants in coastal wetlands. To investigate the contents of carbon, nitrogen, phosphorus and sulfur of plants, and their ecological stoichiometric characteristics in the Yellow (Huanghe) River Delta, plant samples were collected from two typical salt marshes (*Suaeda salsa* and *Phragmites australis* wetlands) during the period of from August to October in 2007, and the ratios of C/N, C/P, N/P, C/N/P and C/N/P/S were calculated. Results showed that during the studying period, plant C, N and P were lower than the global average values, and plant N and P were lower than the China's average values. Leaf C and S in *Suaeda salsa* were significantly lower than those in *Phragmites australis* ($P < 0.05$), and leaf N and P in *Suaeda salsa* and *Phragmites australis* showed no significant differences ($P > 0.05$). Average C/N ratios were 23.75 in leaf, 73.36 in stem, 65.67 in root of *Suaeda salsa*, and 33.77 in leaf, 121.68 in stem, 97.13 in root of *Phragmites australis*. Average C/N ratios of *Suaeda salsa* and *Phragmites australis* were all great than 25, indicating the salt marsh in the Yellow River Delta is an N limitation system. Average C/P ratios were 276.78 in leaf, 709.28 in stem and 1031.32 in root of *Suaeda salsa*, and 536.94 in leaf, 768.13 in stem and 875.22 in root of *Phragmites australis*. The average N/P ratios of *Suaeda salsa* were 12.92 in leaf, 10.77 in stem and 10.91 in root, and the average N/P ratios of *Phragmites australis* were 16.40 in leaf, 7.40 in stem and 6.92 in root, indicating the *Suaeda salsa* wetlands were N limited and *Phragmites australis* wetlands were N limited in August and P limited in October in 2007. The average C/N, C/P and C/N/P ratios in *Suaeda salsa* and *Phragmites australis* were higher than the global average values, indicating the lower quality of organic matter provided by wetland plants in the Yellow River delta.

Keywords: biogenic elements; *Phragmites australis*; *Suaeda salsa*; salt marsh; Yellow River Delta.

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1 Introduction

Coastal wetlands are the transitional zones between the terrestrial and marine ecosystems, which are characterized by high biodiversity, productivity and susceptibility (Mitsch and Gosselink, 2015), and the 'sinks', 'sources' and 'transformations' of chemical elements (i.e., nitrogen and phosphorous) (Flynn, 2008). The Yellow River

Delta is one of the most extensive and youngest regions, and it is also the fastest growing wetland among large river deltas worldwide (Wang et al., 2016). It is an important transit point, wintering habitat and breeding grounds for bird migration in northeast Asia and the west Pacific and has been included in the list of wetlands of international importance, and joined the Ramsar Convention. Although most studies have focused on the

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grassland, forest, lakes and rivers, little information is available on the composition of biogenic elements and the ecological stoichiometric characteristics in the Yellow River Delta of China (Reich and Oleksyn, 2004; Wright et al., 2004; Kerkhoff et al., 2005; Elser et al., 2010). Especially, the changes of biogenic elements of different plant communities and tissues in coastal wetlands are still less known. However, wetlands are becoming one of the most degraded ecosystems on earth (Amezaga et al., 2002), and more than 50% of global wetlands have already been destroyed as a result of various human and natural causes (Cui et al., 2012). Therefore, it is very necessary to investigate the composition of biogenic elements and ecological stoichiometric characteristics in coastal wetland plants, which can contribute to providing a basis for the restoration and conservation of coastal wetlands.

Carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) are the most important biogenic elements for wetland plants since their contents are closely related to the biogeochemical cycles in soil and consequently the ecological structure, processes, and functions (Zedler, 2000; Michaels et al., 2001; Hansson et al., 2005). However, it is difficult to clarify the links between nutrient biogeochemical circulations and ecological processes only through the contents of biogenic elements (Zelder and Kercher, 2005; Hall et al., 2011). Ecological stoichiometrical theories have integrated the first law of thermodynamics, natural selection during biological evolutions, and the central dogma of molecular biology (Elser et al., 2000). In terrestrial ecosystems, nitrogen often acts as a limiting nutrient (Mitsch and Gosselink, 2015). However, in the Yellow River Delta, whether nitrogen is always a limiting nutrient in different wetlands or seasons is little known. Therefore, the study of C, N, P and S contents and their stoichiometrical characteristics in coastal wetland plants can contribute to better understanding of the elementary composition of wetland plants and their ecological significance in coastal wetlands, and comprehending the changes of limiting elements in different seasons and vegetation in coastal wetlands.

Most studies have widely focused on the C, N, P and S in aquatic ecosystems (Elser and Hassett, 1994; Frigstad et al., 2011). In terrestrial ecosystem, the main concerns on plant litters in forest land and grassland are given (Vitousek 1984; McGroddy et al., 2004). How-

ever, litter information is available on the biogenic elements and stoichiometry in coastal wetland plants. Coastal wetlands are the transitional zones between the terrestrial and marine ecosystems, which have high productivity and sensibility (Mitsch and Gosselink, 2015). Biogenic elements in plants can provide some important traits for ecosystems. For example, N is one of the most limiting elements for terrestrial vegetation and leaf N is a key foliar trait, has drawn great attention (Aerts and Chapin III, 2000). Leaf P can be an indicator of vegetation composition, functioning and nutrient limitation at the community level (Güsewell, 2004). C/N and C/P ratios can reflect the carbon storage capacity of plants (Donohue and Brann, 1984) and N/P ratios can reflect the limitation conditions in ecosystem (Koerselman and Meuleman, 1996). C/N ratios can contribute to understanding the quality of organics provided by plants and the nutritional status of the soil. N/P ratios can provide the fundamental insights into trophic dynamics, biodiversity and biochemistry cycling in eco in ecosystems and is a key to bridge between far separated subjects in ecology, individual fitness and biogeochemical cycling in ecosystems (Elser et al., 1996). The combination of contents and stoichiometrical values of biogenic elements can supply more significant information for the plant-soil system in coastal wetlands. Therefore, the primary objective of the study was to investigate the dynamics of C, N, P and S and their stoichiometric ratios in *Phragmites australis* and *Suaeda salsa* from salt marshes in the Yellow River Delta, China.

2 Materials and Methods

2.1 Site description

The Yellow River Delta (37°35'N–38°12'N, 118°33'E–119°20'E) is located in the northeastern Dongying City of Shandong Province, China (Fig. 1). Its total area is 8100 km², in which wetland area occupies 4500 km². It has warm temperate continental monsoon climate, with distinctive seasons and rainy summers. The average annual temperature is 12.5°C, and the frost-free period is 196 days. The average annual precipitation is 660 mm, with about 70% occurring between June and August; and the average annual evaporation is 1900 mm. The dominant soil types are intrazonal tide soil and salt soil. *Phragmites australis* and *Suaeda salsa* are the pre-

dominant vegetative species. *Suaeda salsa* is a succulent halophytic herb (Wang et al., 2004), which is always prone to surviving in salty soil environment and has two phenotypes according to the color of the leaf, including green phenotype (living in lower salty soil environment) and purple phenotype (living in higher salty soil environment) (Yue et al., 2008). *Phragmites australis* is an economic plant distributed in many countries and different habitats in China, which mainly included two species such as halophyte and non-halophyte (Waisel, 1972). Comparatively, *Suaeda salsa* is located closer to the sea compared with *Phragmites australis*, and is subjected to more intense tidal flooding.

2.2 Sample collection and analysis

Based on the effects of vegetation types, two dominant wetlands (*Suaeda salsa* and *Phragmites australis* wetlands) were chosen as the study area, and plant samples were collected in two sampling periods (August and October in 2007). The sampling area included 28 *Suaeda salsa* sampling sites and 15 *Phragmites australis* sampling sites in August, and 21 *Suaeda salsa* sampling sites and 14 *Phragmites australis* sampling sites in salt marshes in October, and they all mainly distributed in the tidal flat wetlands (Fig. 1). *Suaeda salsa* was collected in the whole plant by excavation in the quadrat of 1 m × 1 m, and packed in plastic bag and then taken to the laboratory. Due to the well-developed root system in *Phragmites australis*, the aboveground and underground parts were separately collected in the quadrat of 1 m × 1 m. The aboveground part was col-

lected by harvesting method, and the underground part was collected by excavating to 0.5 m or 1 m depth, and the soil was packed into the 70 mesh nylon mesh and then rinse with water to get the roots. After washing with deionized water, plants were dried in an oven at 105°C for two hours and then dried at 70°C to constant weight. After drying, the aboveground and underground parts of plant samples were sorted respectively, in which the aboveground part of plants was divided into leaves and stems, and then all the samples were grounded though a 2 mm sieve for the determination of biogenic elements. The contents of C and N in plants were determined on a CHNOS Elemental Analyzer (Vario EL, German). The contents of P and S in plants were determined by Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES) (Spectro, American), and the recoveries of plant P and S were 83.03 % and 93.89 %, respectively.

One-way variance analysis (ANOVA) was conducted to identify the significance of the effects of the types of wetlands, vegetation and sampling time on the C, N, P and S contents and C/N, C/P, N/P, C/N/P and C/N/P/S in plants. Statistical analysis was carried out using SPSS 16.0 software package. The difference was considered to be significant if $P < 0.05$.

3 Results and Discussion

3.1 Dynamics of C, N, P and S contents of *Suaeda salsa* and *Phragmites australis*

Plant carbon contents ranged from 14.47% to 55.50%

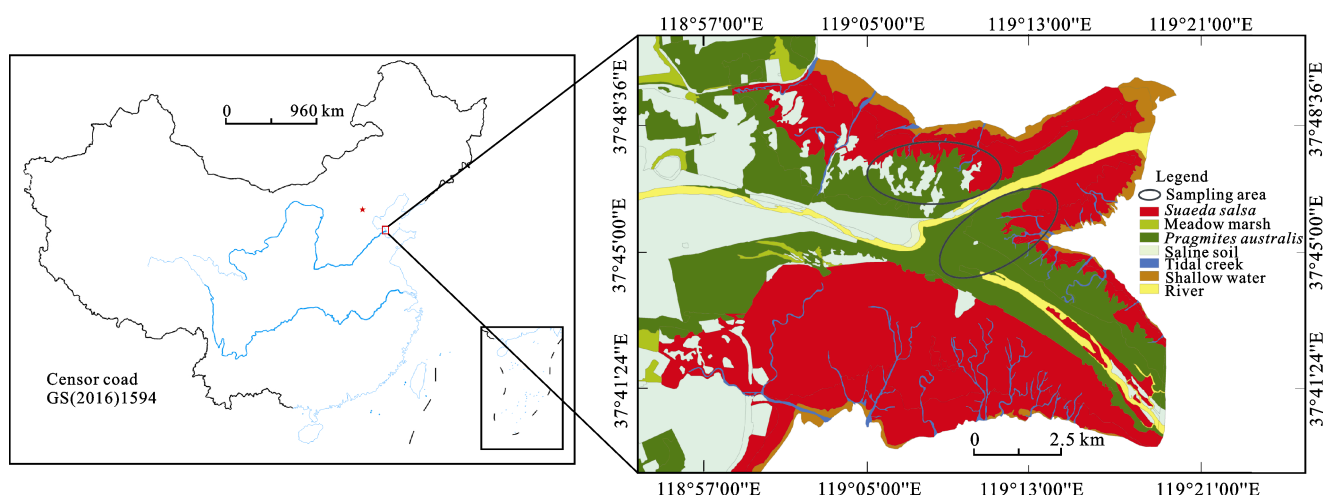


Fig. 1 Location map of study area and sampling area

for leaves, from 33.27% to 42.74% for stems, and from 23.37% to 43.12% for roots of *Suaeda salsa* during the period from August to October (Fig. 2A), and varied from 20.83% to 44.22% for leaves, from 39.60% to 44.42% for stems, and from 35.19% to 44.83% for roots of *Phragmites australis* (Fig. 2B), respectively. *Suaeda salsa* leaves contained 0.40%–2.02% of N, 0.02%–0.36% of P and 0.10%–1.68% of S contents (Fig. 2). The stems of *Suaeda salsa* had 0.15%–1.14% of N, 0.02%–0.14% of P and 0.04%–1.11% of S contents (Fig. 2). There were 0.15%–1.12% of N, 0.02%–0.16% of P and 0.04%–0.98% of S in the roots of *Suaeda salsa* (Fig. 2). Comparatively, approximately 0.69%–2.09% of N, 0.03%–0.15% of P and 0.06%–0.15% of S contents were observed in *Phragmites australis* leaves (Fig. 2). The *Phragmites australis* stems contained 0.21%–0.99% of N, 0.03%–0.17% of P and 0.03%–0.22% of S contents (Fig. 2). The contents of N, P and S were 0.11%–0.87%, 0.02%–0.22% and 0.03%–0.38% in the roots of *Phragmites australis*, respectively (Fig. 2). Generally, the contents of biogenic elements followed the order in *Suaeda salsa* and *Phragmites australis*: C > N > S > P. The C, N and P contents of both plants were lower than the global average value (46.60% for C, 2.06% for N, 0.199% for P) (Elser et al., 2000; Reich and Oleksyn, 2004), and N and P were lower than the China's average value (2.02% for N, 0.15% for P) (Liu, 2013). Lower soil N and P contents in the Yellow River Delta caused by the frequent flooding might be the possible explanation due to the intimate relationship between soil nutrients and plants (Aerts and Chapin, 2000).

The contents of C, N, P and S between *Suaeda salsa* and *Phragmites australis* exhibited some differences (Fig. 2). The leaf C of *Suaeda salsa* were significantly lower than those in *Phragmites australis* ($P < 0.05$), indicating the higher C fixation capacity of *Phragmites australis* leaves. This was consistent with the result of Liu et al. (2014), who presented the similar trend and the C contents showed an increase along the stage of succession (from *Suaeda salsa* to *Phragmites australis*), indicating the higher C accumulation capacity along the succession. Zhang (2016) studied the plant photosynthesis of *Suaeda salsa* and *Phragmites australis* and found *Suaeda salsa* had lower plant photosynthesis than *Phragmites australis* since the chlorophyll *a* content of *Suaeda salsa* (0.0546 mg/g for purple phenotype;

0.2321 mg/g for green phenotype (Duan et al., 2008)) is considerably lower than that of *Phragmites australis* (approximately 1.8200 mg/g (Gu et al., 2009)). In addition, the higher cellulose content of *Phragmites australis* (41.49%, fresh weight (Zhang et al., 2015)) than that of *Suaeda salsa* (only 0.78% for purple phenotype and 1.58% for green phenotype, fresh weight (Duan et al., 2008)) was also observed. These might be the reason for the lower carbon contents of *Suaeda salsa* due to the fact that the organic matter of plants mainly derived from photosynthesis. However, Li (2015) reported that plant C and N were (17.65 ± 6.39)% and (0.59 ± 4.04)% in *Suaeda salsa*, (11.44 ± 1.06)% and (0.29 ± 0.08)% in *Phragmites australis*, respectively, which was associated with spatial heterogeneity of soil nutrients and plant growth in different landscape positions. As for P and S, there were no significant differences between *Suaeda salsa* and *Phragmites australis* except for leaf P in October, and leaf S in August and October. This could be contributed to the terrestrial input of P and S through Yellow River or tidal inputs (Yu et al., 2010), and the living environment of *Suaeda salsa* was more fluently flooded by tides. Soil P mainly comes from the Yellow River (Benitez-Nelson, 2000) and seawater, however, soil S in the Yellow River Delta mainly derives from the seawater, therefore, the higher S in *Suaeda salsa* could be contributed to the closer distance from sea, and higher soil S contents were also found in *Suaeda salsa* wetlands (unpublished data). Miu (2014) and Yu et al. (2010) also found the soil P in *Suaeda salsa* wetlands was higher than that in *Phragmites australis* wetlands.

The contents of C, N, P and S showed the similar distributions in leaves, stems and roots in *Suaeda salsa* and *Phragmites australis* except for leaf C (Fig. 2). C contents in stems and roots of *Suaeda salsa* were higher than leaf C ($P < 0.05$), but no significant differences were observed for C contents in leaves, stems and roots of *Phragmites australis* ($P > 0.05$). Similarly, Yong et al. (2016) presented lower leaf C of four typical halophytes in Xinjiang of China, which might because leaves are the main part of photosynthesis in plants. C contents in *Phragmites australis* is evenly distributed in leaves, stems and roots ($P > 0.05$). In contrast, leaf N contents of *Suaeda salsa* and *Phragmites australis* were significantly higher than N contents in stems and roots ($P < 0.05$). Leaf P of *Suaeda salsa* in October was significantly higher than stem and root P of both *Suaeda salsa* ($P < 0.05$) and

Phragmites australis ($P < 0.05$). Leaf S of *Suaeda salsa* were significantly higher than stem S and root S levels of *Suaeda salsa* ($P < 0.05$), and higher than the S levels in leaves, stems and roots of *Phragmites australis* ($P < 0.05$). These results indicate the higher leaf P and S contents of *Suaeda salsa*. Comparatively, no significant differences in S were observed among the leaves, stems and roots of *Phragmites australis* ($P > 0.05$).

As for nitrogen, Hu et al. (2014) presented the N contents of various tissues of main vegetation in Chinese wetlands followed the order leaf N (16.07 mg/g) > aboveground N (13.54 mg/g) > root N (7.86 mg/g). N contents in leaves of *Suaeda salsa* and *Phragmites australis* were significantly higher than that in stems and roots ($P < 0.05$). This implies that the leaves of *Suaeda salsa* and *Phragmites australis* had higher capacity of storing nitrogen. Liu et al. (2014) also observed higher average leaf N contents (29.83 g/kg) than stem N contents (11.23 g/kg) of *Phragmites australis*. This is in agreement with the result of Yong et al. (2016), who presented that four typical halophytes in Xinjiang had

much higher leaf N contents, which can be attributed to the higher fibrotic in stems. In addition, N status of terrestrial ecosystem showed strong and regional signals due to their acquisition of mineral nutrients mainly via weathering and microbial decomposition at local site (Chapin III, 1980; Chadwick et al., 1999). For example, an increase in leaf N at high altitudes reflects adaptive mechanisms for offsetting temperature-induced reductions in reaction rates or for enhancing cold hardness (Woods et al., 2003). Therefore, the high contents of leaf N in both *Suaeda salsa* and *Phragmites australis* salt marshes might be ascribed to the adaptive mechanisms for flooding and saline environment. As for P and S, there were no significant difference among the leaves, stems and roots of *Phragmites australis* ($P > 0.05$), but P and S contents of *Suaeda salsa* leaves were significantly higher than those of stems and roots ($P < 0.05$). P contents in China's wetland plants generally followed the order: leaf (1.85 mg/g) > aboveground parts (1.72 mg/g) > stem (1.71 mg/g) (Hu et al., 2014), attributing to the active leaf activities (Li, 2015).

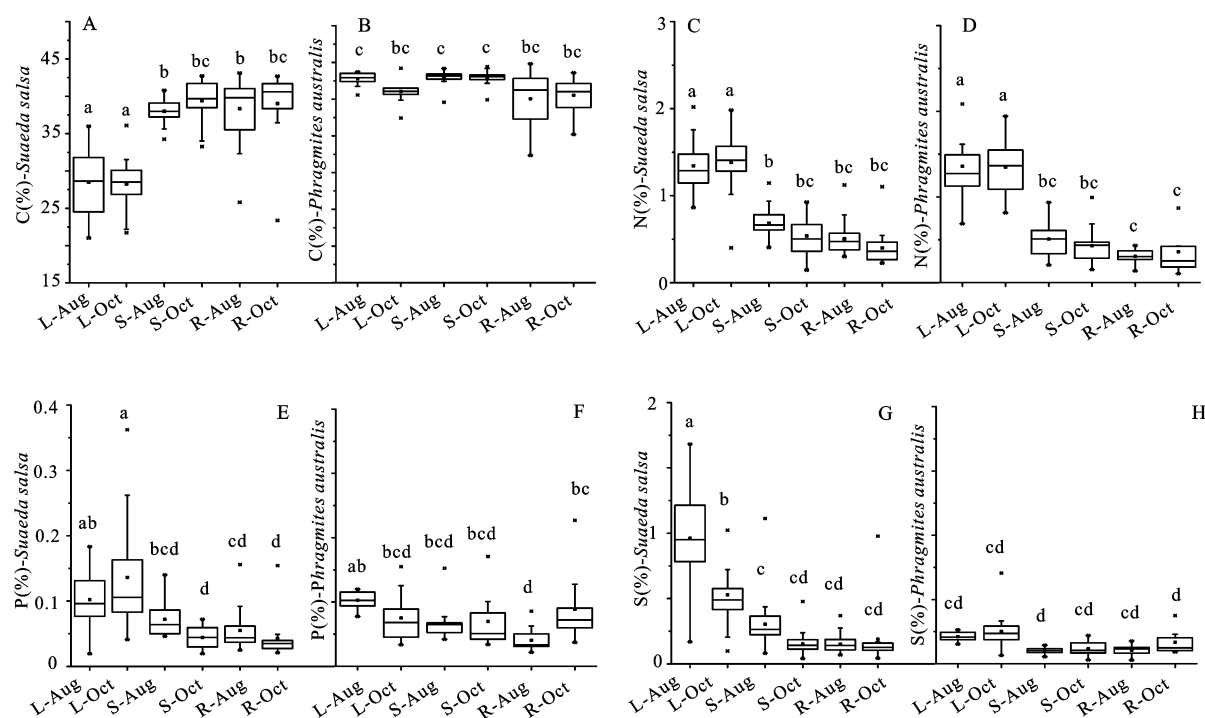


Fig. 2 Contents of C, N, P and S in leaves, stems and roots of *Suaeda salsa* and *Phragmites australis* in salt marshes in the Yellow River Delta. L-Aug: content for leaf in August; L-Oct: content for leaf in October; S-Aug: content for stem in August; S-Oct: content for stem in October; R-Aug: content for root in August; R-Oct: content for root in October; a, b, c and d represent the differences in leaf, stem and root contents for same biogenic element of both *Suaeda salsa* and *Phragmites australis*

3.2 Dynamics of C/N, C/P, N/P, C/N/P and C/N/P/S ratios in *Suaeda salsa* and *Phragmites australis*

C/N and C/P ratios can reflect the carbon storage capacity of plants, and have big differences among different plant tissues (Donohue and Brann, 1984). As shown in Fig. 3A and Fig. 3B, higher C/N ratios of leaf (33.77), stem (121.68), and root (97.13) of *Phragmites australis* were observed compared with *Suaeda salsa* (23.75 for leaf, 73.36 for stem, and 65.67 for root). Average C/N ratios of *Suaeda salsa* and *Phragmites australis* were all in excess of 25 in August and October (Reddy and Delaune, 2008), indicating the salt marshes in the Yellow River Delta is N-limited system. The C/N ratios in wetland plants in this region were higher than the global average level (22.5) (Elser et al., 2000) (Table 1), the average foliar C/N ratio for all species across China's grassland (17.9) (He et al., 2006), and the marine system (5.7) (Redfield, 1958), indicating the lower quality of organic matter provided by wetland plants. Additionally, the leaf average C/N ratios of *Suaeda salsa* and *Phragmites australis* were significantly lower than the average C/N ratios of stems and roots ($P < 0.05$). This is consistent with the results of Wang and Yang (2011), and Yong et al. (2016), who presented lower leaf average C/N in plants due to the higher cellulose in stems and roots. The C/N ratios reflect traits influencing plants' performance in response to the abiotic characteristics of their immediate surroundings (He et al., 2006). The lower leaf C/N ratios were caused by high N contents in leaf in response to flooding and salinity environment. The average C/P ratios were 276.78 for leaves, 709.28 for stems and 1031.32 for roots of *Suaeda salsa*, and 536.94 for leaves, 768.13 for stems and 875.22 for roots of *Phragmites australis* (Fig. 3C and Fig. 3D). The C/P ratios of *Suaeda salsa* and *Phragmites australis* were higher than the global average level (232) (Elser et al., 2000) (Table 1), indicating the lower growing ability (Li et al., 2012) and lower quality of organic matter provided by wetland plants. In addition, *Suaeda salsa* and *Phragmites australis* showed lower leaf C/P ratios than stem and root C/P ratios due to the higher activities of leaf during the period of plant growth.

During the August-October period, the average N/P ratios for stems (10.77) and roots (10.91) of *Suaeda salsa* were higher than those of *Phragmites australis* (7.40 for stems and 6.92 for roots), except for the average lower leaf N/P ratios of *Suaeda salsa* (12.92) and

Phragmites australis (16.40) (Fig. 3E and Fig. 3F). And they were lower than the global average values (Elser et al., 2000; Reich and Oleksyn, 2004) (Table 1). Generally, N/P ratios of leaves have been used to assess nutrient limitation in wetland ecosystems and indicate nitrogen saturation (Tessier and Raynal, 2003). Published N/P ratio thresholds of N limitation ranged from 6.7 to 16, while those for P limitation ranged from 12.5 to 26.3 (Tessier and Raynal, 2003). Koerselman and Meuleman (1996) analyzed 45 fertilization studies carried out in European herbaceous wetlands and found that sites with plant N/P < 14 were N limited, sites with N/P > 16 were P limited, and sites with N/P between 14 and 16 were co-limited by N and P. Hu et al. (2014) studied the C/P ratios in Chinese wetlands and reported wetlands were limited by N when N/P < 14. According to the standard mentioned above, *Suaeda salsa* wetlands were N limited and *Phragmites australis* wetlands were N limited in August and P limited in October in 2007 in the Yellow River Delta.

The average C/N/P ratios of *Suaeda salsa* (3443.1: 10: 1 for leaves, 134 472: 10: 1 for stems, and 213 809: 10: 1 for roots) were considerably lower than those of *Phragmites australis* (4385.7: 10: 1 for leaves, 21731.0: 10: 1 for stems, and 34 080.2: 10: 1 for roots) (Fig. 3G and Fig. 3H). Similarly, the average C/N/P/S ratios in *Suaeda salsa* (21 866.0: 10: 1: 1 for leaves, 16 534.3: 10: 1: 1 for stems, 23 586.3: 10: 1: 1 for roots) were much lower than those of *Phragmites australis* (24 380.9: 10: 1: 1 for leaves, 228 162.0: 10: 1: 1 for stems, and 395 462.1: 10: 1: 1 for roots) (Fig. 3I and Fig. 3J). The leaf average C/N/P and C/N/P/S ratios were significantly lower than stem and root average C/N/P and C/N/P/S ratios ($P < 0.05$). The C/N/P ratios were higher than the global average value (160: 10: 1 or 133: 7: 1) (Nielsen et al., 1996; Elser et al., 2000) (Table 1) and the value (265: 15: 1) in grassland in the west part of China (He et al., 2006; 2008), contributing to the lower N and P contents of plants in the Yellow River Delta resulting from the frequent flooding, which could increase the leaching of NO_3^- (Bai et al., 2006) and the release of N_2O (unpublished data).

4 Conclusions

Dynamics of biogenic elements (C, N, P and S) and ecological stoichiometric characteristics of *Phragmites*

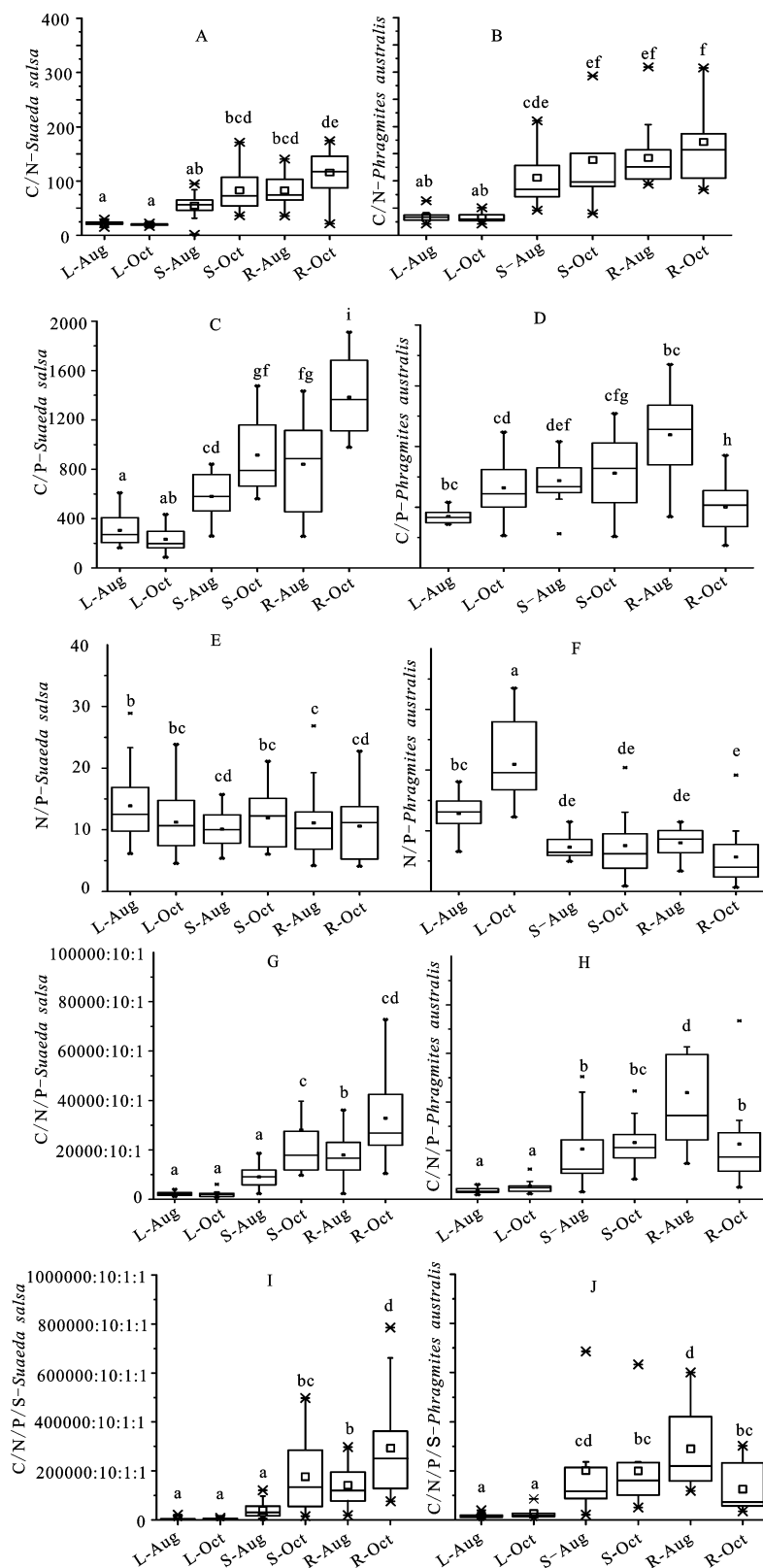


Fig. 3 Plant C/N (A and B), C/P (C and D), N/P (E and F), C/N/P (G and H) and C/N/P/S (I and J) ratios of leaves, stems and roots of *Suaeda salsa* and *Phragmites australis* in salt marshes in the Yellow River Delta (L-Aug: ratios for leaf in August; L-Oct: ratios for leaf in October; S-Aug: ratios for stem in August; S-Oct: ratios for stem in October; R-Aug: ratios for root in August; R-Oct: ratios for root in October; abcdefghi represent the differences in same ratios for leaf, stem and root in both *Suaeda salsa* and *Phragmites australis*)

Table 1 Global average values of C/N, C/P, N/P and C/N/P

Stoichiometric ratio	Global average value	References
C/N	22.5	Elser et al., 2000
C/P	232	Elser et al., 2000
N/P	13.8 or 12.9	Elser et al., 2000; Reich and Oleksyn, 2004
C/N/P	160 : 10 : 1 or 133 : 7 : 1	Nielsen et al., 1996; Elser et al., 2000

australis and *Suaeda salsa* were studied in coastal wetlands in the Yellow River Delta in China. Leaf C and S of *Suaeda salsa* were lower than that of *Phragmite australis*, and leaf N and P of *Suaeda salsa* and *Phragmite australis* showed no significant difference. The C/N ratios of *Suaeda salsa* and *Phragmite australis* all exceed 25, indicating the salt marsh in Yellow River Delta is a N limitation system. The average N/P ratios of *Suaeda salsa* and *Phragmite australis* indicate the *Suaeda salsa* wetlands were N limited and *Phragmite australis* wetlands were N limited in August and P limited in October in 2007, which would contribute to the restoration and development of coastal wetlands.

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