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Overall supply level, not the relative supply of nitrogen and phosphorus, affects the plant community composition of a supratidal wetland in the Yellow River Delta☆

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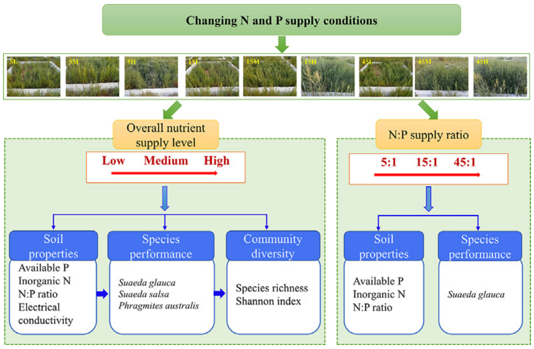
HIGHLIGHTS

- Effects of N and P supply on plant community composition studied in a supratidal wetland in 2015–2018.
- Soil properties affected by both overall nutrient supply level and N:P supply ratio.
- Plant community composition was only affected by overall nutrient supply level in 4 years.
- Variations of the community composition were dominated by the growth changes of *Suaeda glauca* caused by nutrient supply.

GRAPHICAL ABSTRACT



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ABSTRACT

Human activities have altered the environmental nitrogen (N) and phosphorus (P) supply from both aspects of overall supply level and relative supply ratio. However, the effects of the two aspects on plant community composition are still not clear. In this study, a field manipulation experiment combining 3 overall nutrient supply levels (Low, Medium and High) and 3 N:P supply ratios (5,1, 15:1 and 45:1) was conducted in a supratidal wetland in the Yellow River Delta from 2015 to 2018. The effects of the two aspects on soil properties, performance of dominant species and plant community diversity were examined. The results showed that the N:P supply ratio and overall supply level both affected the concentration of soil inorganic N and available P, and N:P ratio significantly, while only overall supply level exerted a significant effect on the importance value of the dominant species, species richness and Shannon diversity. There were big gaps in the N and P supply amounts among the treatments that having same overall supply level with different supply ratio, but the plant composition displayed no significant difference among these treatments, which suggested that P may be also very important in affecting plant community composition in the study area. The species richness and the Shannon diversity were negatively correlated with the importance value of *Suaeda glauca*. With the rise of overall supply level, *S. glauca* became increasingly dominant and suppressed other species. Compared with the control treatment, the species richness and the Shannon diversity declined significantly only at high supply level (minimum N supply amount of

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Abbreviations: P_{Sg}, the importance value of *Suaeda glauca*; P_{Pa}, the importance value of *Phragmites australis*; P_{Ss}, the importance value of *Suaeda salsa*.

☆ These authors contributed equally to the publication. GW and GH designed the research, XL, YR, DQ, BG and CH performed the research; and XL and GW wrote and revised the paper.

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$26.01\ g\ m^{-2}\ yr^{-1}$), indicated that the supratidal wetland had high resilience to nutrient enrichment. Our results revealed that the N:P supply ratio has little influence on plant composition, compared with overall supply, in relative short-term in the supratidal wetland.

1. Introduction

Due to massive fossil fuel burning and agricultural fertilization, global anthropogenic reactive nitrogen (N) inputs to the biosphere have been dramatically increasing from 15.3 Tg yr^{-1} in 1860 to 175–259 Tg yr^{-1} in 2011. Meanwhile, phosphorus (P) inputs have also increased from 0.3 Tg yr^{-1} to 14–16 Tg yr^{-1} (Penuelas et al., 2012, 2013). Human activities have significantly altered the environmental N and P supply from both aspects of overall supply level and relative supply ratio (Elser et al., 2009; Carnicer et al., 2015). Given the different resource use strategies among species (Lan and Bai, 2012; Luo et al., 2016), changes in N and P supply situation may alter the interspecific interactions (Ashton et al., 2010; Zhang et al., 2017), and further affect the structure and function of plant communities (Avolio et al., 2014; Zhao et al., 2019).

The impacts of N and P supply situation (either N, P enrichment alone or combined) on species richness and community structure have been intensively reported either in specific site empirical studies, gradient studies or through observational meta-analysis. At a global scale or across ecosystems, N or N plus P enrichment generally reduce plant species richness (Maskell et al., 2010; De Schrijver et al., 2011; Field et al., 2014; DeMalach et al., 2017), while the effect of P is not consistent. Some studies indicate that P enrichment may be as important as, or even more important than, N enrichment in reducing species richness (Wassen et al., 2005; Fujita et al., 2014), while a recent study (Soons et al., 2017) suggests the overall effect of P addition is neutral. To explain these findings, DeMalach (2018) suggested that the richness response to N and P addition need to be analyzed combined with the general biomass-dependent hypothesis (light asymmetry hypothesis, total competition hypothesis, and litter hypothesis) and biomass-independent mechanism (soil chemistry and stoichiometry, light acquisition and functional groups). Particularly, N:P supply ratio may be a key factor to affect the richness response to N and P addition (DeMalach, 2018), which could influence the performance of different species (Penuelas et al., 2013) and explain more of the variation in species composition than the availability of N and P separately (Roeling et al., 2018). Since the environmental N and P status had been changed from both overall supply level and supply ratio, the respective and interactive effects of the two aspects need to be discriminated. Nonetheless, for most nutrient addition studies were conducted for N or P alone, and the few experiments combined N and P often had correlated supply level, i.e., treatments with higher N additions also had higher P additions lacking variation of supply ratio (Soons et al., 2017), the respective effect of N:P supply ratio and overall supply level on plant community composition cannot be distinguished.

Considering the effects of N and P supply on plant growth are often multiplicative, Güsewell and Bollens (2003) set up an experimental design combining N:P supply ratio and overall supply level, in which overall nutrient supply was defined as the geometric mean of supplied amounts of N and P. This design enables the evaluation of N:P supply ratio effects independently from effects of differences in absolute nutrient supply and has been used in some pot experiments (Güsewell, 2005a, b; Fujita et al., 2010; Venterink and Güsewell, 2010). In these studies, both overall supply and N:P supply ratio had direct and interactive effects on plant growth or competition. Generally, plant growth depended more on overall supply than on N:P supply ratio in 12–15 weeks (Güsewell and Bollens, 2003; Güsewell, 2005b; Venterink and Güsewell, 2010), while the second-year growth was mainly influenced by N:P supply ratio for the consequent nutrient limitation with small effect of absolute supply (Güsewell, 2005a; Fujita et al., 2010). However, for these pot experiments were mostly concentrated on the effects on different species cultured alone or no >4 mixed species in short term (3–17 months), the effects of supply level and supply ratio on soil properties and plant composition in real ecosystems during relative long-time span are still not clear.

Owing to the differences in vegetation types, soil nutrients or climatic conditions, the responses of ecosystems to changes in N and P supply situation were not consistent (Bobbink et al., 2010; De Schrijver et al., 2011; Wang et al., 2015). Particularly, the size and the background of the species pools may determine the effect of N and P enrichment (DeMalach, 2018). However, most nutrition addition studies were concentrated on grassland ecosystems, while researches concerning wetland ecosystem were rather scarce (Lewandowska et al., 2016; Soons et al., 2017). More studies relative to wetlands are needed for further interpretation of the effects of N and P supply situation on plant community composition.

The Yellow River Delta wetland, the most extensive early successional wetland ecosystem in China, has significant ecological value due to its important overwintering stopover and breeding function for migrating birds (Li et al., 2019). In recent years, this area is undergoing increasing inputs of N and P caused by atmospheric deposition (Yu et al., 2014), water-sediment regulation (Li et al., 2017) and regional land use change (Yu et al., 2016). However, the response of the plant community to N and P supply situation variations remains unclear. In this study, an in situ experiment that simulated changes in N and P supply situation was conducted in the supratidal wetland of Yellow River Delta, in which 3 overall supply levels and 3 supply ratios were set according to the method of Güsewell and Bollens (2003). We hypothesized that: 1) both overall supply level and N:P supply ratio have direct and interactive effects on the soil properties, with soil N and P concentrations, soil electrical conductivity (EC) more affected by overall supply level and soil N:P ratio more influenced by N:P supply ratio (H_1); 2) the performance of dominant species is more affected by overall supply level in the first year, while the effect of N:P supply ratio increase gradually over time (H_2); 3) the community composition are more affected by overall supply level initially and then more influenced by N:P supply ratio gradually (H_3); 4) some present faster-growing species, such as *Suaeda salsa* (He et al., 2012), may benefit more from the variations of N and P supply situation, and the resulting inter-species competition would be the main driver of changes in community composition (H_4).

2. Materials and methods

2.1. Experimental site

The field experiment was conducted in Yellow River Delta Ecology Research Station of Coastal Wetland (37°45′52″ N, 118°58′52″ E), Chinese Academy of Sciences. The station is located on a supratidal wetland, experiencing a warm-temperate and continental monsoon climate with a mean annual air temperature of 12.9 °C. The mean annual precipitation is about 560 mm, nearly 70% occurring from June to September. For the shallow groundwater table with an average depth of 1.1 m, periodic surface ponding is often observed especially following heavy rainfall. According to the background investigation of the research station, the soil in the experimental site is a saline-alkali type, with a pH of 7.20–7.89 and conductivity of 1.64–3.15 ms cm^{-1}. The concentration of soil inorganic nitrogen (sum of $NH4^{+}$–N and $NO3^{-}$–N) and available phosphorus (inorganic P extracted with $NaHCO_3$ solution) were 7.67–11.4 mg kg^{-1} and 4.04–6.28 mg kg^{-1}, respectively. Most broadly distributed species in this area are *S. glauca*, *Suaeda salsa*, *Phragmites australis*, *Tamarix chinensis*, *Cynanchum chinense*, and *Tripolium vulgare* (Table S1).

2.2. Experimental design

In July 2014, a 40 m × 35 m location with fairly uniform vegetation composition was fenced within the research station, at which 42 3.5 m × 3.5 m plots were laid out separated by 2 m walkways. To prevent the horizontal flow of N and P fertilizer, the 0.3 m high hollow bricks wrapped with waterproof geotextile were used to enclose the sample plots, with those waterproof geotextiles buried 40 cm underground on both sides of the hollow brick. There were nine fertilization treatments combined with 3 overall nutrient supply levels (Low, Medium, High) and 3 N:P supply ratios (5:1, 15:1, 45:1) with four replicates. One control treatment was also set up with six replicates. All ten treatments were randomized to the 42 plots.

The overall nutrient supply was defined as the geometric mean of the supplied amount of N and P as the method of Güsewell and Bollens (2003). The N:P supply ratios 5:1, 15:1 and 45:1 corresponded to N-limited, basic N and P supply and P-limited conditions, respectively (Venterink and Güsewell, 2010). Particularly, the supply of N and P for each nutrient treatment were calculated as:

$$\mathrm{N}\ (\mathrm{g}) = L\ (\mathrm{g}) \cdot \sqrt{N:P} \quad (1)$$

$$\mathrm{P}\ (\mathrm{g}) = L\ (\mathrm{g}) / \sqrt{N:P} \quad (2)$$

where L (g) is the overall nutrient supply; N (g) and P (g) are the supply amount of N and P, respectively. This design enabled the evaluation of N:P supply ratio effects independently from the effects of differences in overall nutrient supply (Güsewell and Bollens, 2003; Güsewell, 2005a, b).

The Atmospheric nitrogen deposition of the study area is about 2.26 g m^{-2} in the growing seasons (Yu et al., 2014). Considering nitrogen deposition in non-growing seasons and the input of nitrogen from other sources, we defined 5 g m^{-2} y^{-1} as the annual supply of N for low supply level with basic N:P supply ratio (15:1 L). Thus, the annual supply of nitrogen and phosphorus for different supply situations were obtained (Table 1), and the minimum supply of N at 5:1 L treatment (2.89 g m^{-2}) was equivalent to the average annual atmospheric nitrogen deposition in North China (2.80 g m^{-2}, Zhang et al., 2006). N and P fertilization were initiated in 2015 and conducted twice annually with 50% of the supply in early April (germination stage) and 50% in late June (vigorous growth stage) of each year. N was supplied as urea and P as NaH_2PO_4 dissolved in 6 L water and applied to the plots on a rainy day with a portable sprayer. The control treatment received an equal amount of water. There was no >1 mm of water added in the plots every year. The precipitation data were obtained from a standard weather station which was installed 500 m away from the experimental site.

Table 1
Annual supply of nitrogen and phosphorus applied at the treatments (g per square meter).

N:P	Low supply level		Medium supply level		High supply level	
	N	P	N	P	N	P
5:1	2.89	0.58	8.67	1.73	26.01	5.19
15:1	5.00	0.33	15.00	1.00	45.00	3.00
45:1	8.67	0.19	26.01	0.58	78.03	1.73

2.3. Field sampling and measurement

Plant community investigation was performed each year for 2015–2018 in early August (peak biomass season). The importance value (P) was selected as the proxy of species performance and calculated as:

$$P_i = \left(h_i / \sum h_i + c_i / \sum c_i\right) / 2 \quad (3)$$

where h_i and c_i are the average height and coverage of species i, respectively. To avoid edge effects, the average height and coverage of each plant species were measured within 3 m × 3 m area (at least 0.25 m inside each plot).

Species richness (S) was recorded as the number of the species in each plot. The Shannon diversity (H) was calculated as:

$$H = -\sum\nolimits_{i=0}^{n} P_i \ \ln P_i \quad (4)$$

where P_i is the importance value of species i. According to the occurrence frequency and mean importance value in 2015 (Table S1), the dominant species (with occurrence frequency over 80% and importance value over 0.1) in the experiment site were *S. glauca*, *P. australis*, and *S. salsa*.

Soil sampling was performed concurrently with community investigation. Three soil cores with a diameter 3.8 cm and a depth of 10 cm were randomly collected in each plot and thoroughly mixed into one composite sample for laboratory analysis of soil ammonium (NH_4^+–N) and nitrate (NO_3^-–N) concentration. Subsamples were air-dried and then sieved through a 0.15-mm mesh to measure soil available P concentration and conductivity. For soil $NH4^+$–N and NO_3^-–N measurements, 5 g fresh soil samples were extracted with 25 ml 2 M KCl solution and then analyzed with AA3 continuous flow injection analyzer (SEAL Analytical, Germany). For soil available P analysis, 1.25 g of air-dried soil samples were extracted with 25 ml of 0.5 M $NaHCO_3$ (pH = 8.5) and measured using a T6 UV spectrophotometer (Beijing Purkinje General Instrument Co Ltd., China). The concentration of soil inorganic N was defined as the sum of the $NH4^+$–N and NO_3^-–N concentration, and soil N:P ratio was calculated the mass ratio of soil inorganic N and soil available P. The soil electrical conductivity was determined in water suspension (soil: water = 1: 5) by an EC meter.

2.4. Statistical analysis

Repeated measures ANOVA was employed to test the significance of soil properties (inorganic N, available P, N:P ratio and EC), importance values of dominant species, species richness, and Shannon diversity, using supply level, supply ratio, year and all interactions as fixed-effects. The significance of the above parameters in each year were analyzed by two-way ANOVA, using supply level, supply ratio, and their interaction as fixed-effects. These ANOVAS were followed by S-N-K post-hoc tests to determine difference level (if necessary). Redundancy analysis (RDA) and correlation analysis using the Pearson coefficient were performed to detect correlation among soil properties, importance values of dominant species, and diversity indexes. Redundancy analysis (RDA) was performed using the CANOCO 4.5 software. All the statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Significance level was set as 0.05.

3. Results

3.1. Effects on soil properties

Repeated measures ANOVA showed that the effects of supply level, supply ratio, year on the concentration of soil inorganic N and available P were highly significant, and that supply level × supply ratio interaction was also highly significant (Table 2). In general, both the

Table 2
Repeated measures analysis of variance for soil inorganic N concentration, available P concentration, N: P ratio and electrical conductivity (EC) using Supply Level, Supply Ratio, Year and all interactions as fixed-effects. Significant *P*-values marked in bold ($P < 0.05$).

	df	Inorganic N		Available P		N: P ratio		Soil EC	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Supply level (SL)	2	29.205	<**0.001**	117.631	<**0.001**	19.274	<**0.001**	11.792	<**0.001**
Supply ratio (SR)	2	15.068	<**0.001**	113.253	<**0.001**	100.797	<**0.001**	0.537	0.591
Year (Y)	3	61.830	<**0.001**	7.777	<**0.001**	18.947	<**0.001**	85.357	<**0.001**
SL × SR	4	6.707	<**0.001**	32.692	<**0.001**	11.279	<**0.001**	2.644	0.055
SL × Y	6	5.620	<**0.001**	1.484	0.194	4.111	**0.001**	3.820	**0.002**
SR × Y	6	3.111	**0.009**	3.517	**0.004**	6.791	<**0.001**	2.153	0.056
SL × SR × Y	12	1.442	0.165	3.469	<**0.001**	2.130	**0.024**	4.451	<**0.001**

concentration of soil inorganic N and available P tended to increase with the rise of supply level. With the rise of supply ratio, soil inorganic N also increased while soil available P declined (Fig. 1). Supply level affected soil inorganic N more than supply ratio, while it had a roughly equal effect on soil available P as supply ratio, which could be indicated by the *F*-value (Table 2). Correspondingly, soil inorganic N among 3 supply levels were all significantly different, while it only increased significantly at high supply ratio. Soil available P was significantly higher only at high supply level or low supply ratio. Compared to the control treatment, both soil inorganic N and available P tended to increase at all fertilizer treatments across 4 years (Fig. 1). However, soil inorganic N varied more than available P in different years. In general, soil inorganic N was highest in 2015 and lowest in 2017, while available P was only higher in 2016 with no significant difference among the other 3 years. Two-way ANOVA also showed that soil inorganic N responded significantly to supply situation just in the first 3 years (2015–2017), while available P responded significantly across all 4 years (Table S2).

Strong interannual variability was also observed in soil N:P ratio and soil EC. Variations in supply level and supply ratio had a remarkable influence on soil N:P ratio in each year (Table S2). However, soil N:P ratio was greatly influenced by supply ratio than supply level (Table 2), which was significantly increased with rising N:P supply ratio. As for the soil EC, it was only affected significantly by the supply level. Significant annual variation was observed during the experimental period: soil EC had no significant difference among 3 supply levels in 2017 and was highest at low supply level in the other 3 years (Fig. 1, Table S2).

3.2. Effects on importance value of dominant species

The importance values of the 3 dominant species showed significant variations over time (Fig. 2, Table 3). The importance values of *S. glauca* (P_{Sg}) and *S. salsa* (P_{Ss}) was higher in 2017 and 2016, respectively, with the other 2 years being relatively constant. However, the importance value of *P. australis* (P_{Pa}) tended to increase gradually compared with 2015.

Supply level had a significant effect on the importance value of all 3 dominant species, and had a significant interactive effect with year on P_{Sg} and P_{Ss}. On the contrary, supply ratio just had an interactive effect with year on P_{Sg}, which was highest at 45:1 supply ratio in 2016 and 2017 (Tables 3, S3). With the rise of overall supply level, P_{Sg} increased significantly in all 4 years, while P_{Pa} decreased significantly only at high supply level from the third year. P_{Ss} reduced significantly at both

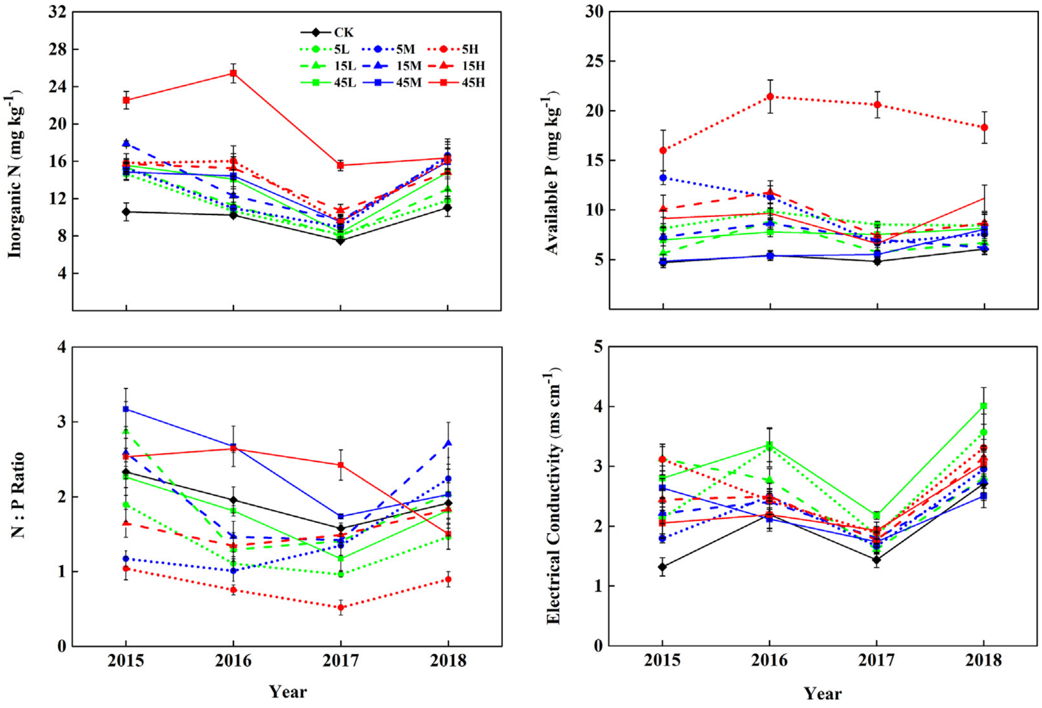

Fig. 1. Effects of experimental N and P addition on soil properties (inorganic N concentration, available P concentration, N: P ratio and electrical conductivity) from 2015 to 2018. CK, control; three overall nutrient supply levels: L = low, M = medium, H = high; three supply N: P supply ratios: 5 = 5:1, 15 = 15:1, 45 = 45:1. Different bars indicate mean value (± SE) for each treatment (N and P addition, n = 4; CK, n = 6).

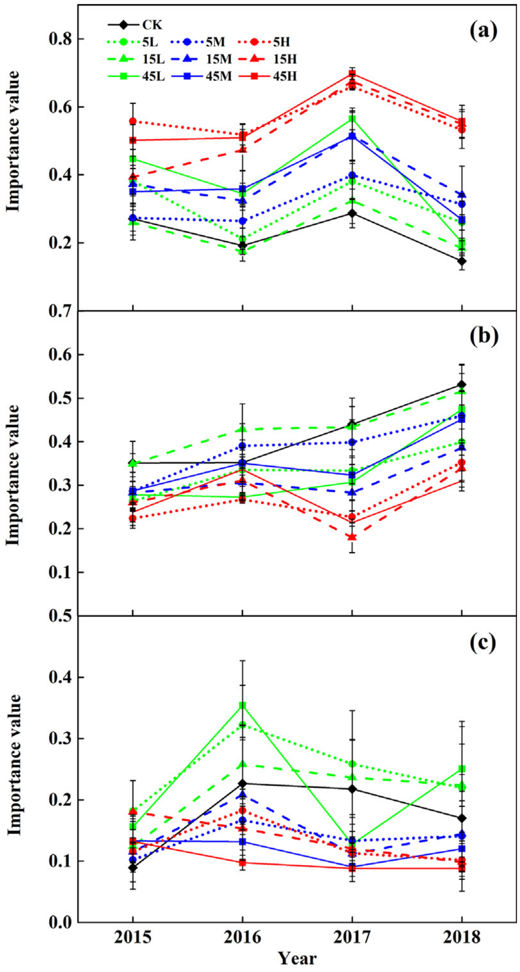

Fig. 2. Effects of experimental N and P addition on the importance value of each constructive species [*S. glauca* (a), *P. australis* (b) and *S. salsa* (c)] from 2015 to 2018. Different bars indicate mean value (±SE) for each treatment (N and P addition, n = 4; CK, n = 6). See Fig. 1 for treatment abbreviations.

medium and high supply level since the second year, but there was no difference between medium and high supply levels (Table S3).

Throughout the 4 years, on average, P_{Sg} and P_{Pa} were significantly higher than P_{Ss} (Fig. S1). Relative to P_{Pa}, P_{Sg} was lower at low supply level, nearly equal at the medium level, and higher at the high level. Compared with the control, P_{Sg} increased significantly between all gradual supply levels, while P_{Pa} decreased significantly just at the high supply level. P_{Ss} at all supply levels had no significant difference with the control, although it was highest at low supply level when compared among 3 supply levels (Fig. S1).

Table 3
Repeated measures analysis of variance for the importance value of each constructive species (*S. glauca*, *P. australis*, and *S. salsa*) using Supply Level, Supply Ratio, Year, and all interactions as fixed-effects. Significant *P*-values marked in bold (*P* < 0.05).

	df	*S. glauca*		*P. australis*		*S. salsa*	
		F	P	F	P	F	P
SL	2	31.747	**<0.001**	6.683	**0.004**	5.393	**0.011**
SR	2	1.976	0.158	0.247	0.783	0.228	0.798
Y	3	49.621	**<0.001**	26.659	**<0.001**	9.259	**<0.001**
SL × SR	4	1.664	0.187	1.829	0.152	0.121	0.974
SL × Y	6	4.958	**<0.001**	2.092	0.063	3.486	**0.004**
SR × Y	6	3.120	**0.008**	0.556	0.764	0.910	0.492
SL × SR × Y	12	1.319	0.224	0.838	0.612	1.280	0.247

3.3. Effects on community diversity

Among the total 16 plant species recorded in 2015 (Table S1), five occasional species disappeared in all experimental plots by 2018, including *Cirsium japonicum*, *Melilotus officinalis*, *Glycine soja*, *Cyperus rotundus*, and *Potentilla supina*. Meanwhile, two new species *Puccinellia tenuiflora* and *Metaplexis japonica*, were recorded.

Species richness and Shannon diversity changed substantially over time (Fig. 3, Table 4), which decreased gradually in the first 3 years and slightly rose in 2018. However, the effect of supply situation on community diversity was rather consistent across 4 years for no significant interactive effect between supply situation and year (Table 4). During the overall study period or in every particular year, supply level, rather than supply ratio, had a significant effect on species richness and Shannon diversity (all $P < 0.01$, Tables 4, S4). Among the three supply levels, the Shannon diversity decreased significantly at high supply level, while species richness showed a significant increase at medium supply level (Fig. S2). Furthermore, the species richness and the Shannon diversity at low and medium level had no significant difference with the control treatment, while they declined significantly at high supply level (Fig. S2).

3.4. Correlation among soil properties, importance values of dominant species and diversity indexes

The relationship among soil properties, importance values of the 3 dominant species, and diversity index in every particular year were illustrated in the ordination diagram (Fig. 4). Further analysis revealed that P_{Sg} was positively correlated with the concentration of soil inorganic N and available P significantly, while PPa and PSs had a negative

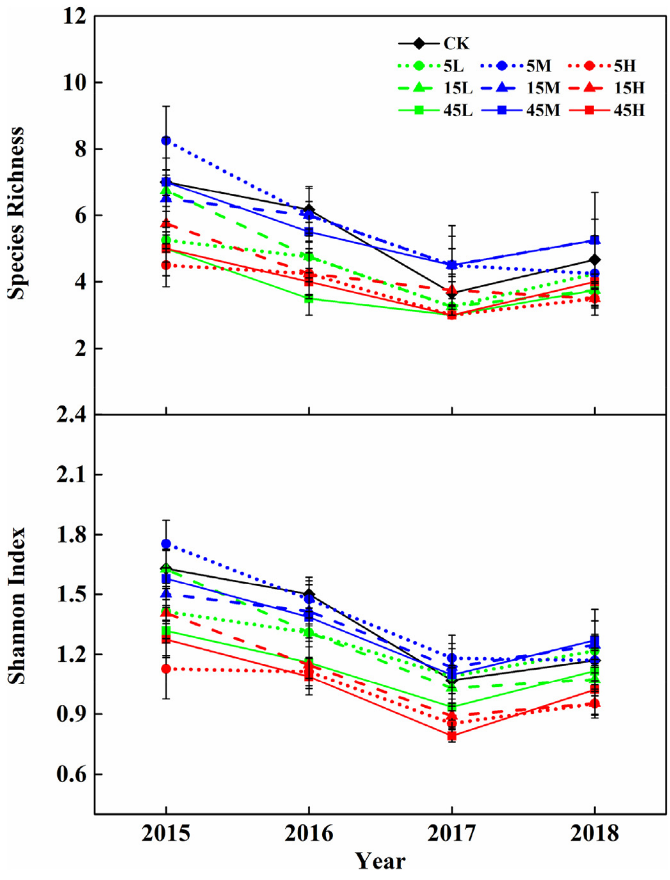

Fig. 3. Effects of experimental N and P addition on Species Richness and Shannon Index from 2015 to 2018. Different bars indicate mean value (±SE) for each treatment (N and P addition, n = 4; CK, n = 6). See Fig. 1 for treatment abbreviations.

Table 4
Repeated measures analysis of variance for Species Richness and Shannon Index using Supply Level, Supply Ratio, Year, and all interactions as fixed-effects. Significant *P*-values marked in bold ($P < 0.05$).

	df	Species richness		Shannon index	
		F	*P*	*F*	*P*
SL	2	7.360	**0.003**	9.447	<**0.001**
SR	2	0.353	0.706	0.426	0.657
Y	3	47.890	<**0.001**	80.274	<**0.001**
SL × SR	4	0.240	0.913	0.402	0.805
SL × Y	6	0.857	0.530	0.348	0.909
SR × Y	6	0.910	0.492	1.288	0.272
SL × SR × Y	12	1.645	0.096	1.625	0.101

correlation with them (though almost nonsignificant) (Table S5). PSg and PPa had no significant correlation with soil N:P ratio and soil EC across 4 years, while PSs was positively correlated significantly with soil N:P ratio in 2016, and with EC in 2016 and 2018 (Table S5). As for the relationships among the importance value of the 3 species, P_{Sg} had a significant negative correlation with P_{Pa} in all 4 years and with P_{Ss} began from the second year, while P_{Pa} and P_{Ss} had almost no significant correlation except in 2016 (significantly negative) (Table S6). When coming to the relationship between the diversity index and the importance value of 3 species, both species richness (except in 2018) and Shannon diversity were negatively correlated with P_{Sg} significantly across 4 years (Table S7). Otherwise, they had a weaker correlation with P_{Pa} and P_{Ss} (not significant in majority years).

4. Discussion

4.1. Effects of overall supply level and supply ratio on soil properties

The results correspond principally with our first hypothesis (H_1). The concentration of soil inorganic N and available P often increase with corresponding increasing N and P input amounts (Huang et al., 2018; Xing et al., 2019). For the input amounts of N are always several folds higher than P in our experiment (Table 1) as well as in the real world (Penuelas et al., 2012, 2013), the gap of N input among supply levels is much higher than among supply ratios, while the difference degree between the gap of P input among supply levels and the gap among supply ratios is not so much as N. Therefore, supply level affected soil inorganic N more than supply ratio but just had roughly equal effect intensity on soil available P. For soil N:P ratio was more correlated to available P ($r = -0.653$, $n = 144$, $P < 0.01$) than to inorganic N ($r = 0.563$, $n = 144$, $P < 0.01$) according to the Pearson correlation analysis across 4 years, correspondingly, supply ratio affected more on soil N:P than supply level, with higher supply ratio having higher soil N:P ratio. This result is in line with the study of Penuelas et al. (2013), which revealed that increase in N:P input substantially increased the N:P in the soils at a global scale. Additionally, N addition may affect ion exchange (Zhang et al., 2014; Tian et al., 2015) while such effect of P was seldom reported (DeMalach, 2018), suggesting that the response of soil EC was ultimately determined by N input amounts. Hence, the smaller gradient range of N amount among different supply ratios could partly explain that supply ratio had no significant effect on soil EC.

4.2. Effects of overall supply level and supply ratio on dominant species

Different dominant species responded to N and P supply divergently in our study. Contrast with our hypothesis (H_2), supply level was always the main factor affecting the performance of those dominant species, while supply ratio had no effect across 4 years. Generally, the different performances of species in the same community were caused by their distinct responses to soil properties and their interactions (Bobbink et al., 2010). Correlation analysis revealed that available N and P, soil EC (which were more influenced by supply level) affected the performance of 3 dominant species more than available N:P (which was overridingly influenced by supply ratio) (Fig. 4; Table S5). Taken together, it may explain the difference in the effect degree between supply level and supply ratio. The result is also inconsistent with those previous pot-culture studies which showed that supply level, supply ratio, and their interaction all had significant effects on the growth of the plant (Fujita et al., 2010; Venterink and Güsewell, 2010; Zhang et al., 2017). A possible reason could be that those pot-culture studies were conducted using sand as the substrate which contains no nutrition, therefore the N:P ratio in the substrate could be accurately controlled as same as the nutrient solution supplied. However, there always exist background interference of nutritional status under field condition (Bai et al., 2015). Though supply ratio did affect the soil N:P ratio significantly in our study, variations of soil N:P ratio are far slighter than the

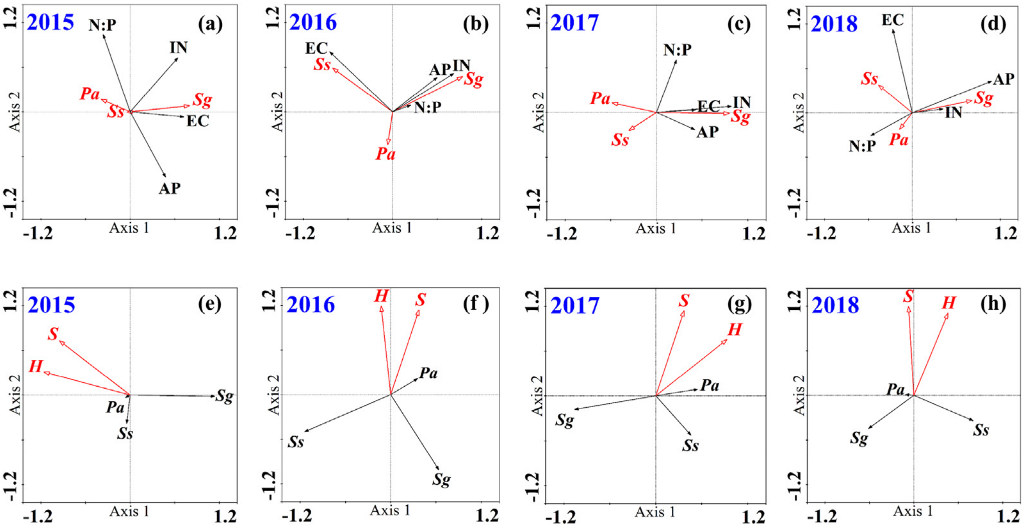

Fig. 4. Ordination diagram showing the correlation among soil properties, importance values of constructive species, and diversity indexes at nine N and P addition treatments from 2015 to 2018. (a)–(d), the results of redundancy analysis of soil properties and importance values of constructive species; (e)–(h), the results of redundancy analysis of community diversity and the importance values of three constructive species. IN, soil inorganic N concentration; AP, soil available P concentration; N: P, soil N: P ratio; EC, soil electrical conductivity. *Sg*, *Pa*, and *Ss* denote the importance value of *S. glauca*, *P. australis*, and *S. salsa*, respectively. *S*, species richness; *H*, Shannon index.

supply ratio, which may not reach the threshold to transfer nutrient limitation to influence the growth of these dominant species. Furthermore, even in the previous studies, the effect of high N:P supply ratio was only observed in the second year (Fujita et al., 2010; Güsewell, 2005a), which indicated the long-term need to test nutrient effects (Huang et al., 2018). Similar results were also found in the present study, which demonstrated that the supply ratio had a significant impact on P_{Sg} in the second and third year (Table S3). Therefore, the effect of the N:P supply ratio on plant community requires further study on a long-term scale.

Additionally, the underlying effect mechanisms of supply level on the 3 dominant species were different. P_{Sg} positively correlated with available nutrients significantly, indicating its improving performance along with supply level was mainly a direct outcome. This also could be verified by the result that P_{Sg} increased significantly at all supply levels compared with control. On the other hand, P_{Pa} and P_{SS} had a negative (though almost nonsignificant) correlation tendency with available nutrients. Analyzing together with the significant negative correlation of P_{Pa} and P_{Ss} with P_{Sg} (Table S6), it can be deduced that was caused by the increasing suppression of *S. gluaca*, for *S. gluaca* performed better at the higher supply level. That is to say, the negative effect of supply level on *P. australis and S. salsa* resulted mainly from an indirect manner, i.e., by the competition with *S. gluaca*.

4.3. Effects of overall supply level and supply ratio on community diversity

Nutrient supply ratio had been proposed as an important factor to control competition dynamics and species diversity (Tilman, 1982), which had been supported by some greenhouse experiments (Venterink and Güsewell, 2010; Yuan et al., 2013) and a meta-analysis study at large scale (Roeling et al., 2018). However, another meta-analysis study indicated that N:P ratio had no effect on diversity in grassland and salt marshes (Lewandowska et al., 2016). In the present study, the result also showed that supply level, rather than supply ratio, had a significant effect on the species richness and the Shannon diversity, which is inconsistent with our hypothesis (H_3). Specific to present study, except for the two above-discussed possible reasons, the background interference (Bai et al., 2015) and the long-term need for studying nutrient effects (Faust et al., 2012; Huang et al., 2018), the relative small species pool in our specific site may be another factor for explanation (DeMalach, 2018). A total 18 species were recorded in our study site across 4 years; additionally, only no >8 species on average occurred in either 3 m × 3 m treatment plot with other species having rather low importance value relative to the 3 dominant species. Such character is incomparable to a large scale that contains more species having more divergent niches, where N:P ratio could form a niche gradient to affect the community composition (Roeling et al., 2018). Hence, the response of dominant species, to a large extent, determined the variation in community diversity (Yang et al., 2011). Since the performance of the 3 dominant species were all significantly affected by supply level rather than supply ratio, it was a matter of course that the community diversity was chiefly controlled by supply level as well.

Statistical analyses on those N, P correlated enrichment experiments attributed the effect on species richness primarily to N (Soons et al., 2017). In our study, there were big gaps in the N supply amounts among the treatments having the same overall supply with different supply ratio (Table 1), however, the species richness and the Shannon diversity among these treatments had no significant difference (Tables 4, S4). This may most likely be a result of the complementary supply of P, for the lower the N amount the higher the P amount when the overall supply was the same. On the contrary, the significant difference of the species richness and the Shannon diversity existed between the treatments that having same N supply amount but different P amount thereby having different overall supply level (i.e., 45L vs 5M, and 45M vs 5H), which represented as the higher P amount the lower species richness and Shannon diversity. Therefore, the result indicated the role of P may be also very important on affecting plant community composition in our study area, which was coherent with previous studies at large scale (Fujita et al., 2014). A previous study conducted in a salt marsh demonstrated that the species-composition responses were measured only in the combined high N (25 g m^{-2} yr^{-1}) and high P (5 g m^{-2} yr^{-1}) treatments, compared with control situation (Van Wijnen and Bakker, 1999), which also verified the effect of N on species composition could not be separated from the effect of P. However, the synergistic effects of N and P on community composition need further study.

4.4. The effect mechanism of N and P supply on community composition

In our study, elevated nutrient supply level significantly stimulated the growth of *S. glauca*, thereby intensifying the interspecific competition for species (Xing et al., 2019; Zhao et al., 2019). Ultimately, *S. glauca* became more dominant at higher nutrient supply level and consequently affected the species richness and the Shannon diversity. That is to say, the variations of the community composition under different supply level were mainly driven by changes in the growth of *S. glauca* that were caused by nutrient addition, which was consistent with our hypothesis (H_4). The result was congruent with previous studies (Bai et al., 2004; Suding et al., 2005; Niu et al., 2018), which have shown that faster-growing species were more common and then inhibit the growth of other species follow fertilization.

Compared with the control treatment, the species and the Shannon diversity at low and medium supply level displayed no significant difference, and they declined remarkably only at the high supply level. The result was consistent with other studies (Achermann and Bobbink, 2003; Bobbink and Hettelingh, 2010), which reported that changes in community composition were often observed at high nutrient addition rates over a short period of time. Considering the minimum amount of N supply of these high supply level treatments in our study (26.01 g N m^{-2} yr^{-1}), it seemed that the supratidal wetland in the Yellow River Delta has high resilience to nutrient enrichment in plant composition relative to European saltmarshes, where the critical load of N addition that change community structure is only 2–3 g N m^{-2} yr^{-1} (Bobbink and Hettelingh, 2010). However, the N addition in our treatments was much higher than some other studies in European habitats (Phoenix et al., 2012), and it was applied with high solute concentrations. In such condition, ammonia volatilization from urea, denitrification and leaching may all lead to the higher N loss in our treatments (Zhang et al., 2015; Perron et al., 2019), and thus weaken the strength of N as a driver of changing community composition (Phoenix et al., 2012). More importantly, nutrient-induced plant community structure shift could have a lag phase >5 years (Faust et al., 2012). Generally, change seen with a high dose over a short time period often occurs at lower doses when the nitrogen is added for a longer time period, and short-term studies may underestimate the effects of low-level addition on the community composition changes (Clark and Tilman, 2008). Therefore, the current displayed high resilience presented in the 4-year study need longer time to verify, especially considering that the consequent variations of ecological function are still not clear.

5. Conclusion

In our 4-year experiment, the N:P supply ratio and overall supply level both had a significant effect on soil properties, however, the performance of the dominant species and plant diversity were only significantly affected by overall supply level. With the rise of overall supply level, P_{Sg} increased and consequently caused the decline of P_{Pa} and P_{Ss}. Variations of the community composition at different supply levels were mainly driven by changes in the growth of *S. glauca* that were caused by nutrient addition. The supratidal wetland in Yellow River Delta had high resilience to nutrient enrichment, and the role of P may be also very important on affecting plant community. The results can provide scientific references for ecological preservation of supratidal wetland ecosystems, but over the long term, the ecosystem may be more sensitive to changes in N and P supply situation than the current findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.133866.

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