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Performance of Iron Plaque of Wetland Plants for Regulating Iron, Manganese, and Phosphorus from Agricultural Drainage Water

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Abstract: Agricultural drainage water continues to impact watersheds and their receiving water bodies. One approach to mitigate this problem is to use surrounding natural wetlands. Our objectives were to determine the effect of iron (Fe)-rich groundwater on phosphorus (P) removal and nutrient absorption by the utilization of the iron plaque on the root surface of *Glyceria spiculosa* (Fr. Schmidt.) Rosh. The experiment was comprised of two main factors with three regimes: Fe^{2+} (0, 1, 20, 100, 500 mg·L^{-1}) and P (0.01, 0.1, 0.5 mg·L^{-1}). The deposition and structure of iron plaque was examined through a scanning electron microscope and energy-dispersive X-ray analyzer. Iron could, however, also impose toxic effects on the biota. We therefore provide the scanning electron microscopy (SEM) on iron plaques, showing the essential elements were iron (Fe), oxygen (O), aluminum (Al), manganese (Mn), P, and sulphur (S). Results showed that (1) Iron plaque increased with increasing Fe^{2+} supply, and P-deficiency promoted its formation; (2) Depending on the amount of iron plaque on roots, nutrient uptake was enhanced at low levels, but at higher levels, it inhibited element accumulation and translocation; (3) The absorption of manganese was particularly affected by iron plague, which also enhanced phosphorus uptake until the external iron concentration exceeded 100 mg·L^{-1}. Therefore, the presence of iron plaque on the root surface would increase the uptake of P, which depends on the concentration of iron-rich groundwater.

Keywords: wetlands; agricultural drainage water; iron plague; phosphorus; manganese

1. Introduction

Agricultural activities are vital to the crop production and economy of terrestrial ecosystems; meanwhile, its practice continues to place environmental pressures on nearby lake and wetland ecosystems [1]. Agricultural drainage water originating from groundwater contains large amount of farm nutrients and high concentration of iron (Fe), which posing an environmental hazard to the surrounding ecosystems. In recent years, the area of rice cultivation has been increased to 4,000,000 ha of land in Heilongjiang Province, China. Such a large-scale agricultural production would inevitably bring great impacts on the surrounding ecosystems, especially for the sustainability and water security of the lake and wetlands.

Wetlands play important roles in nutrient recycling, increasing dissolved O_2 in the rhizosphere, recharging of aquifers, stabilization of water currents, improvement of water quality, and remediation of wastewater (including removal of excess iron, manganese, and other metals) [2–5]. Wetland plants have the capacity to improve water quality through mobilization and absorption of nutrients and contaminants [6]. O_2 diffuses from the roots to their surroundings and oxidizes Fe^{2+} in the rhizosphere into oxyhydroxide iron (Fe plaque) on the root surface of plants [7]. Iron plaque on roots absorbs metals onto its large surface area and co-precipitates with nutrients, such as P. Root activities influence plaque formation by releasing O_2 and exudates, and influencing the rates of enzyme and radial oxygen loss (ROL). Iron plaque formation occurs commonly on the surface of roots of wetland species, including *Typha latifolia* L., *Oryza sativa* L., and *Phragmites communis* Trin. [8].

Metal and nutrient enrichment and deposition in iron plaque are known to occur, but the effects of iron plaque on uptake and translocation of elements are still unclear [9–11]. Some studies reported that the formation of Fe plaque prevents the excessive uptake of Fe, Mn, Zn [12,13], and phosphorus (P) [14]. On the other hand, iron plaque acts as a buffer for P, Zn, Cu, Se, and As. While the plants may be lack nutrients, they are also at risk of metal toxicity [15,16]. The effects of Fe plaque on mineral nutrients and other elements may be related to the amount of deposition on the root. It has been reported that Fe plaque enhanced Zn and P uptake, and could be considered as a nutrient reservoir, but may also act as a barrier when excess Fe plaque forms on the root surface [7,17]. The presence of P is important in energy metabolism, the biosynthesis of nucleic acids, photosynthesis, and enzyme regulation [18], and also plays an important role in biogeochemical cycling with Fe and Mn [19,20]. The studies have demonstrated that P-deficiency may enhance the formation of iron plaque on the roots of plants [21,22]. At the same time, iron plaque on the root surface may affect the uptake and translocation of P, Mn, Zn, Cu, As, and Cd. Using reactive materials to immobilize P in soils or manure is considered a new way of managing P eutrophication [23].

In order to adapt to long-term iron-rich environmental changes, wetland plants have different growth strategies in different environments. Higher plants have developed two divided strategies to acquire slightly soluble iron from the rhizosphere: the chelation strategy of graminaceous plants and the reduction strategy of nongraminaceous plants [9]. The "exclusion" strategy is supported by studies, which showed that some species exclude phytotoxic metals rather than absorbing them. The presence of iron plaque on the root surface is another strategy, which is an adaptation to flooding. With the iron plaque strategy, the absorption and translocation of a large number of elements will be affected.

The aim of this study was to determine the effects of iron toxicity on plants when they are grown in the iron-rich groundwater and agricultural drainage water recharge marsh, such as the Xiaoxingkai Lake wetland in the south of the Sanjiang Plain, China. Xiaoxingkai Lake is a Chinese National Nature Reserve and a boundary lake between China and Russia. The experimental wetland plant was *Glyceria spiculosa* (*G. spiculosa*), a typical dominant but sensitive species with developed aerenchyma in the roots. Therefore, the variation of *G. spiculosa* has a certain indicative function on the change of the surrounding habitat. In the present investigation, our main objectives were to study the effects of: (a) P and Fe concentrations on the formation of iron plaque on root surfaces; (b) iron plaque on the uptake and translocation of Fe, Mn, and P; and (c) iron-rich groundwater as a reactive resource to manage P eutrophication water caused by the agricultural drainage water.

2. Materials and Methods

2.1. Greenhouse Experiment

To investigate the influence of various Fe^{2+} and P concentrations levels on the formation of iron plaque and its function on elements uptake and translocation, an experiment was performed with *G. spiculosa*. The experiment was carried out in a greenhouse at Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (43°59′53″ N and 125°23′48″ E) during July to

September, 2016. Average air temperatures during the day (7:00–19:00) were 25–28 °C, and during the night they were (19:00–7:00) 15–20 °C.

Before beginning the experiment, tillers training were pre-cultured for two weeks in the greenhouse. The plants were tiled on the plastic vessels ($100 \times 60 \times 5$ cm) containing 10 L half-strength Hoagland nutrient solution (Hoagland and Arnon 1950), and the solution was renewed every seven days. Tillers of uniform size (15–18 cm) were selected on the rhizomes of the *G. spiculosa*, cleaned with deionized water, and transplanted to plastic pots (12 cm diameter, 15 cm height) into quartz sand and half-strength Hoagland nutrient solution for one week. The Hoagland solution was composed of 0.5 mM $NH_4H_2PO_4$, 2 mM $Ca(NO_3)_2 \cdot 4H_2O$, 3 mM KNO_3, 1 mM $MgSO_4 \cdot 7\,H_2O$, 4.57 µM $MnCl_2 \cdot 4H_2O$, 23.13 µM H_3BO_3, 0.382 µM $ZnSO_4 \cdot 7H_2O$, 0.16 µM $CuSO_4 \cdot 5H_2O$, and 0.0695 µMMoO_3.

2.2. Experiment Design

The experiment was comprised of two main factors with three regimes: Fe^{2+} (0, 1, 20, 100, 500 mg·L^{-1}) and P (0.01, 0.1, 0.5 mg·L^{-1}) (Table 1). The first regime lasted three weeks, with ferrous sulfate in the half-strength Hoagland nutrient solution, which was designed to examine the influence of different Fe^{2+} concentrations and the resistance threshold of wetland plants to excessive Fe^{2+} treatment. The second regime lasted one week, with ferrous sulfate addition, but did not contain phosphorus in the solution, as it intended to flush out residual PO_4-P and prompt the iron plaque formation on the roots. The third regime lasted two weeks with ammonium dihydrogen phosphate addition, to determine the influence of iron plague in the asorption and translocation of Fe, Mn, and P. Each treatment consisted of five replications in a large rectangular trough ($67 \times 26 \times 22$ cm), with a total of 75 pots. The pots were waterlogged (2–3 cm) with the testing solution, and the bottom of each pot had five small holes. Each of the pots were wrapped with a black plastic bag. The nutrient solution was renewed every seven days, and the pH was adjusted to 5.5 using 0.2 mol·L^{-1} NaOH or HCl every two days.

2.3. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectrometry (EDS)

Root material was washed with tap water, then rinsed three times in deionized water. For the scanning electron microscopy analysis, 1 cm long lateral fresh root samples of *G. spiculosa* were prepared and fixed in formyl acetic alcohol (FAA). Samples were dehydrated in ethyl series and dried using liquid CO_2 (CPD 030 model, Bal-Tec Co., Balzers, Liechtenstein). The morphology of roots was observed using a scanning electron microscopy (SS-550, Shimadzu, Tokyo, Japan).

2.4. Chemical Analysis

At the end of the experiment, the plants were harvested and separated into roots, stems, and leaves, and washed with deionised water three times. Fresh root samples were weighted 1 g and then soaked by dithionite-citrate-bicarbonate (DCB) [24]. The DCB solution consisted of 40 mL of 0.3 M sodium dithionite and 5 mL of 1 M sodium citrate with 3.0 g sodium dithionite. The roots in the solution were shaken for 3 h for a complete extraction. The extrated roots were dried at 60 °C to constant weight. Fe concentrations in DCB were determined by atomic absorption (Optima 2000DV, Perkin Elmer, Waltham, MA, USA).

The other plant biomass comparments were dried at 55 °C for 72 h, used to measure iron, manganese, and phosphrous with perchloric ($HClO_4$) acids and nitric (HNO_3) (1:5, *v/v*). The concentrations of iron and manganese in plant tissues were assayed by atomic absorption spectrometry (Optima 2000DV, Perkin Elmer, Waltham, MA, USA), and phophrous was determined with Molybdenum antimony colorimetric method by Automatic chemistry analyzer (Smartchem 300, Advanced Monolithic Systems, Graz, Italy).

Table 1. Experimental treatment for a fully factorial design with three regimes and two main factors: Fe^{2+} (0, 1, 20,100, 500 mg·L^{-1} Fe) and P (0.01, 0.1, 0.5 mg·L^{-1} P).

Time (Weeks)	Treatments														
	Fe 0			**Fe 1**			**Fe 20**			**Fe 100**			**Fe 500**		
1	Fe 0 + *H*			Fe 1 + *H*			Fe 20 + *H*			Fe 100 + *H*			Fe 500 + *H*		
2	Fe 0 + *H*			Fe 1 + *H*			Fe 20 + *H*			Fe 100 + *H*			Fe 500 + *H*		
3	Fe 0 + *H*			Fe 1 + *H*			Fe 20 + *H*			Fe 100 + *H*			Fe 500 + *H*		
4	Fe 0+ *H(−P)*			Fe 1 + *H(−P)*			Fe 20+ *H(−P)*			Fe 100+ *H(−P)*			Fe 500+ *H(−P)*		
5	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*
6	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*	+0.01 P*H*	+0.1 P*H*	+0.5 P*H*

Notes: *H* = half-strength Hoagland nutrient solution; *H(−P)* = not contain phosphorus in *H*; 0.01 P*H* = 0.01 mg·L^{-1} phosphorus in the *H(−P)*; 0.1 P*H* = 0.1 mg·L^{-1} phosphorus in the *H(−P)*; 0.5 P*H* = 0.5 mg·L^{-1} phosphorus in the *H(−P)*.

2.5. Data Analysis and Statistical Analysis

Fe (Mn, P) content (g) = Fe (Mn, P) concentration × dry matter.

Concentration of Fe plaque (μg/g) = Fe plaque content/roots dry matter.

Translocation of Fe, P, Mn from roots to the stems and leaves, and element aquisition efficiency (FeAE, MnAE, PAE) were calculated as follows:

Translocation of Fe (Mn, P) in stems (%) = Fe (Mn, P) content in stems/total Fe (Mn, P) content in plants × 100.

Translocation of Fe (Mn, P) in leaves (%) = Fe (Mn, P) content in leaves/total Fe (Mn, P) content in plants × 100.

Fe acquisition efficiency (FeAE, $mg \cdot g^{-1}$) = total Fe content in plants/the root dry matter.

Mn acquisition efficiency (Mn AE, $mg \cdot g^{-1}$) = total Mn content in plants/the root dry matter.

P acquisition efficiency (PAE, $mg \cdot g^{-1}$) = total P content in plants/the root dry matter.

Statistical analyses were performed with SPSS Statistics 20.0. Two-way ANOVA tests were performed according to any interactions of the Fe and P treatments. The Kolmogorov-Smirnov test was used to test for normality. The data of iron plaque were square root transformed before parametric analysis. The Levene's test was performed to test for the homogeneity of variances. Significant differences between treatments in means were compared using Tukeys's with significance set at $p \leq 0.05$.

3. Result

3.1. Iron Plaque Formation on the Root

DCB-extractable Fe concentrations from the roots of *G. spiculosa* varied from 2.1–233 $μg \cdot g^{-1}$, with the highest value recorded at the Fe 500/P 0.01 treatment, and the lowest value at Fe 0/P 0.5 treatment (Figure 1). The iron plaque was visually present on the root surface of Fe 20–Fe 500. There was a sharp increase of iron plaque at the concentration of Fe 500, which was about seven times higher than that of Fe 100. In both Fe 0 and Fe 1 treatments, there were no statistically significant differences in the formation of iron plaque. With the increase of Fe^{2+} applied, the amount of iron plaque with P 0.01 were higher than P 0.1 and P 0.5 for 20–500 $mg \cdot L^{-1}$ Fe treatments.
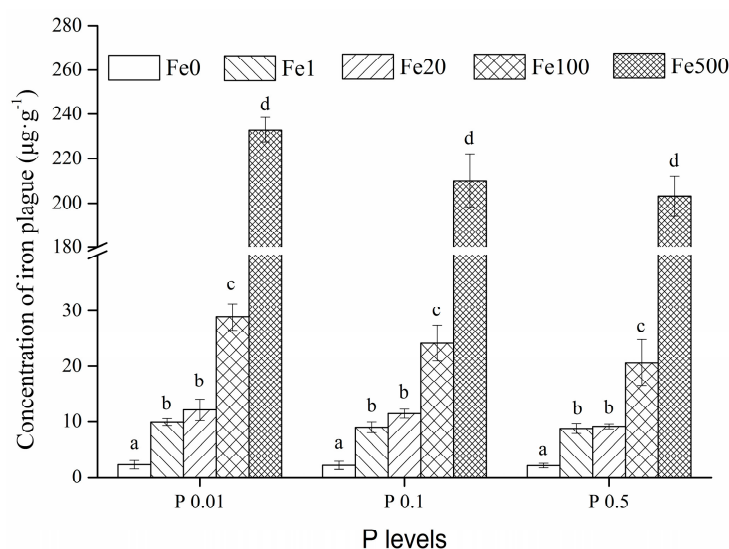
Figure 1. The amount of iron plaque on roots surface of *G. spiculosa* grown hydroponically with varying Fe^{2+} concentrations and P levels. Values (mean ± SE, *n* = 3) followed by different letters designate significant differences (*p* < 0.05) between Fe^{2+} concentrations for a given P level (a, b, c, d). The data were square root transformed before statistical analysis.

3.2. Dry Matter Accumulation

Dry matter of plants were significantly affected by Fe and P concentrations (Table 2). In plants exposed to Fe 20 and above, the dry matter of roots were decreased considerably, whereas the dry matter of stems and leaves showed similar response patterns, peaking at Fe 20 and subsequently decreasing with increasing Fe application, with stems ranging from 0.47 g (Fe 500/P 0.1) to 1.10 g (Fe 20/P 0.01) and leaves ranging from 0.15 g (Fe 500/P 0.1) to 0.93 g (Fe 20/P 0.01). The highest plant biomass at the three P levels was mostly observed at P 0.01 and a few appeared at P 0.5 treatment. The results showed that plants have different biomass distribution patterns for organs with varying Fe and P concentrations.

Table 2. Dry matter of *G. spiculosa* exposed to different concentrations of Fe and P (mean $\pm$ SE, *n* = 5), and the analysis of variance (two-way ANOVA) on dry matter.

Dry Matter (g)							
Treatment	Root	Stem	Leave	Treatment	Root	Stem	Leave
Fe 0/P 0.01	0.51 $\pm$ 0.03 b	0.59 $\pm$ 0.02 ab	0.52 $\pm$ 0.05 bc	Fe 1/P 0.01	0.54 $\pm$ 0.02 b	0.83 $\pm$ 0.04 c	0.69 $\pm$ 0.03 c
Fe 0/P 0.1	0.43 $\pm$ 0.04 b	0.64 $\pm$ 0.04 b	0.48 $\pm$ 0.03 b	Fe 1/P 0.1	0.39 $\pm$ 0.05 b	0.83 $\pm$ 0.05 c	0.72 $\pm$ 0.03 c
Fe 0/P 0.5	0.44 $\pm$ 0.03 bc	0.79 $\pm$ 0.06 b	0.56 $\pm$ 0.02 bc	Fe 1/P 0.5	0.33 $\pm$ 0.04 bc	0.86 $\pm$ 0.05 b	0.67 $\pm$ 0.05 cd
Fe 20/P 0.01	0.46 $\pm$ 0.03 b	1.10 $\pm$ 0.10 d	0.93 $\pm$ 0.08 d	Fe 100/P 0.01	0.43 $\pm$ 0.04 b	0.79 $\pm$ 0.08 bc	0.49 $\pm$ 0.02 b
Fe 20/P 0.1	0.34 $\pm$ 0.03 b	0.86 $\pm$ 0.07 c	0.80 $\pm$ 0.09 c	Fe 100/P 0.1	0.28 $\pm$ 0.04 b	0.54 $\pm$ 0.04 b	0.41 $\pm$ 0.05 b
Fe 20/P 0.5	0.46 $\pm$ 0.03 c	0.85 $\pm$ 0.06 b	0.76 $\pm$ 0.04 d	Fe 100/P 0.5	0.35 $\pm$ 0.03 b	0.67 $\pm$ 0.06 ab	0.42 $\pm$ 0.02 b
Fe 500/P 0.01	0.18 $\pm$ 0.02 a	0.55 $\pm$ 0.02 a	0.27 $\pm$ 0.02 a		Root	Stem	Leave
Fe 500/P 0.1	0.09 $\pm$ 0.01 a	0.47 $\pm$ 0.03 a	0.15 $\pm$ 0.02 a	Fe	<0.001	<0.001	<0.001
Fe 500/P 0.5	0.19 $\pm$ 0.02 a	0.50 $\pm$ 0.06 a	0.22 $\pm$ 0.03 a	P	<0.01	<0.05	NS
				Fe $\times$ P	NS	<0.01	NS

Note: Different letters designate significant differences ($p < 0.05$) between Fe^{2+} concentrations for a given P level (a, b, c, d).

3.3. SEM and EDS Analysis

The morphological analysis of roots by SEM showed that iron plaque clearly visible as an orange-brown deposition on the root surfaces. Figure 2A showed the root surface of Fe 0 treatment, which was the lowest deposition with plaque. The coating was extensively distributed across the root surface and consistently increased with increasing Fe^{2+} (Figure 2B–E,b–e). Microorganisms were also observed on the root surfaces of *G. spiculosa*, such as Figure 2a,b.

To confirm these visual observations, further examination was conducted by Energy Dispersive X-ray Spectrometry (EDS) scanning. Different colors were added to the image representing various elements. Figure 3 shows bright particles deposited on the root surfaces of Fe 500/P 0.1. In this image, Fe is represented by red, P by green, Mn by purple, and S by blue. The element of Fe was more dense than P and Mn.

3.4. Uptake and Accumulation of Fe, Mn, P

For each tissue type, the patterns of Fe content were similar for the same Fe and P treatments (Figure 4). Increasing Fe concentrations significantly increased the Fe content of roots, stems, and leaves. The highest Fe contents for Fe 0–Fe 20 treatments were found in the roots with 1.10–3.97 mg (stems with 0.33–2.11 mg and leaves with 0.10–0.46 mg); however, Fe 100 and Fe 500 were found in the stems with 9.63–27.87 mg (roots with 5.92–15.22 mg and leaves with 1.13–4.95 mg). At the three P levels in a given Fe concentration, the total Fe content of P 0.01 in Fe 0–Fe 20 treatment was higher than the other P levels, and higher in the Fe 100/P 0.5 and Fe 500/P 0.1 treatments.
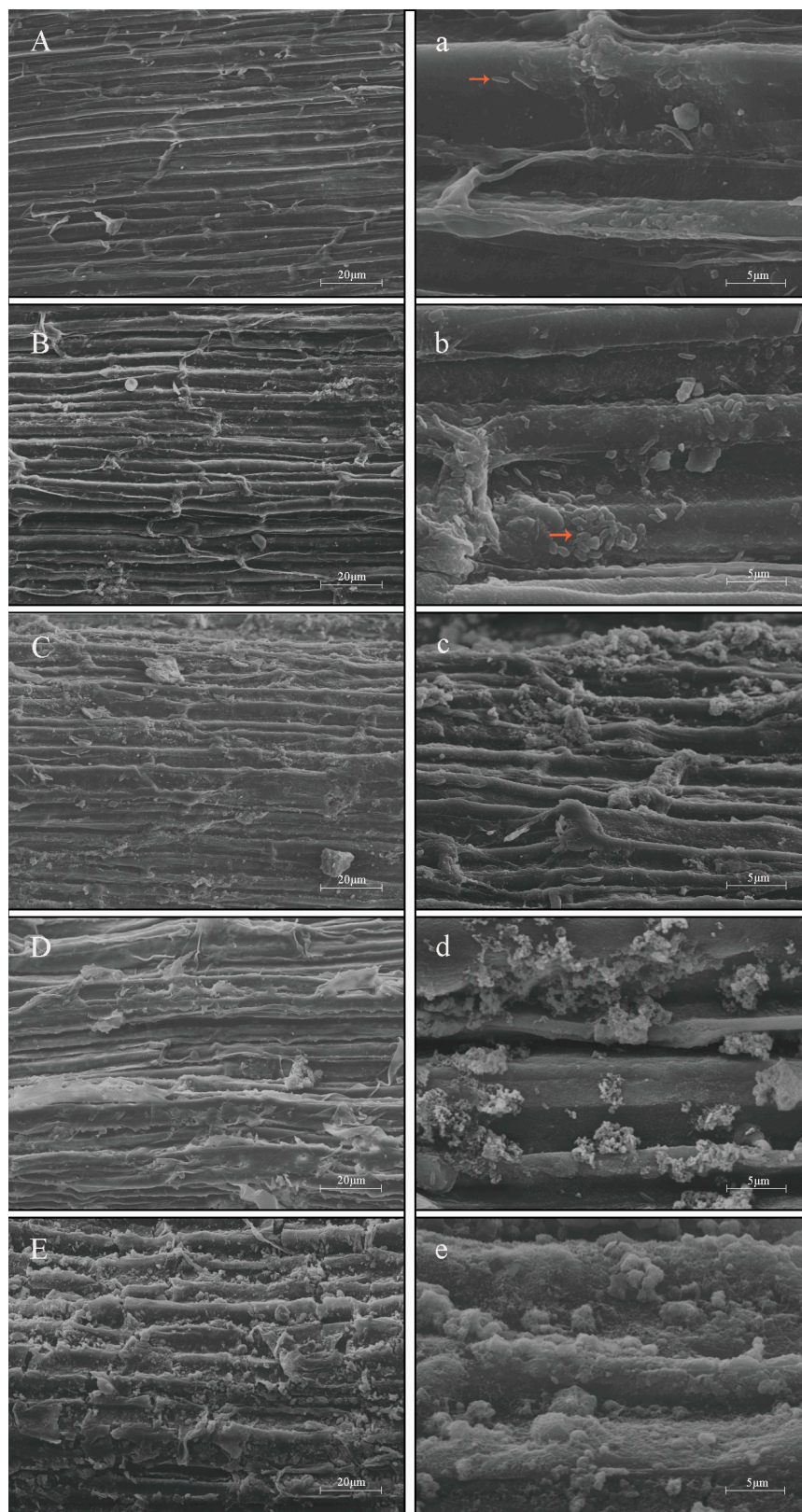
Figure 2. Different amounts of iron plaque formed on the roots of *G. spiculosa* observed by Scanning Electron Microscopy (SEM), accuracy of 20 μm: (**A–E**), accuracy of 40 μm: (**a–e**). Five treatments were represented: Fe 0/P 0.1, Fe 1/P 0.1, Fe 20/P 0.1, Fe 100/P 0.1, Fe 500/P 0.1. Red arrows indicated microorganisms exsiting on the roots surface.

(**a**) (**b**)
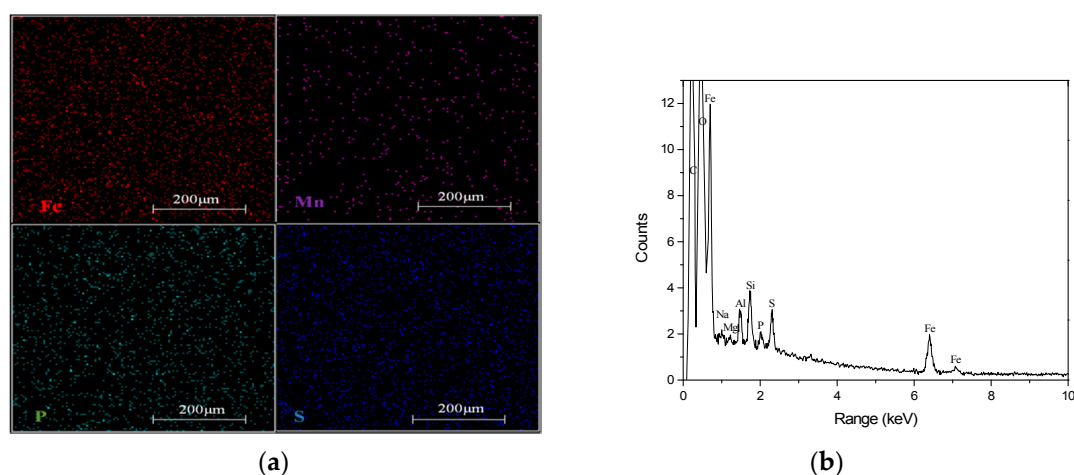
Figure 3. Energy Dispersive X-ray Spectrometry (EDS) on the root surface of *G. spiculosa*: distribution of Fe, Mn, P, and S (**a**), and a quantitative determination of elements composition (**b**).
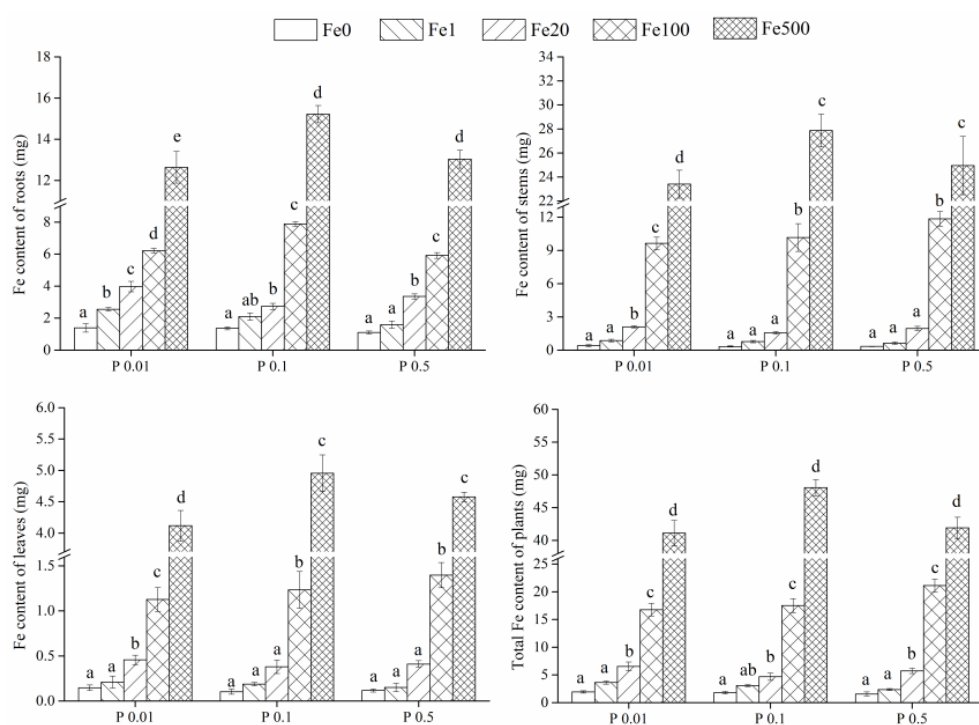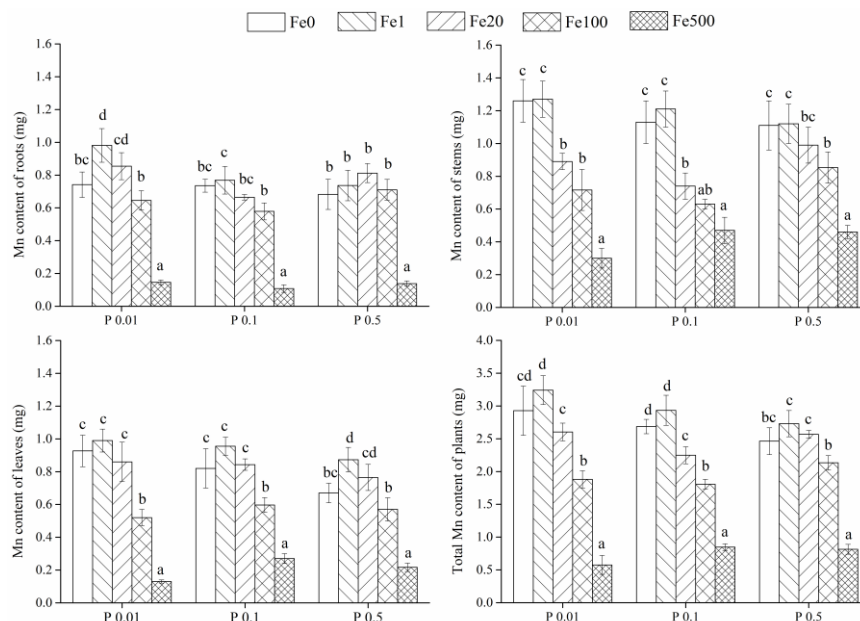

Figure 4. Effects of Fe^{2+} and P on roots, stems, leaves, and total Fe contents of *G. spiculosa*. Values (mean $\pm$ SE, $n = 3$) followed by different letters designate significant differences ($p < 0.05$) between Fe^{2+} concentrations for a given P level (a, b, c, d).

Mn content in stems was significantly higher than in roots (1.04–4.35 times) and leaves (1.03–2.31 times) (Figure 5). In contrast to Fe and P, total Mn content of Fe 0 and Fe 1 treatments at any given P level was greater than others. The total Mn content in P 0.01 of Fe 0–Fe 20 was higher than P 0.1 and P 0.5 treatments, and higher in the Fe 100/P 0.5 and Fe 500/P 0.1 treatments. Plants grown in the Fe 500 treatment had the lowest total Mn content (23.1%–41.1% of other treatments).

Stems (1.6–3.7 times) and leaves (1.4–2.9 times) had greater P content than roots for the same treatments (Figure 6). P content in each tissue type increased with Fe^{2+} addition until Fe 100 and Fe 500 treatments, which showed a downward trend. The total P content of P 0.01 treatment was higher than the other P levels except the Fe 0 treatment. Among five Fe concentrations at a given P level, total P content of Fe 1 was greater and greatest in the Fe 20 treatments.

Figure 5. Effects of Fe^{2+} and P on roots, stems, leaves, and total Mn contents of *G. spiculosa*. Values (mean $\pm$ SE, *n* = 3) followed by different letters designate significant differences (*p* < 0.05) between Fe^{2+} concentrations for a given P level (a, b, c, d).
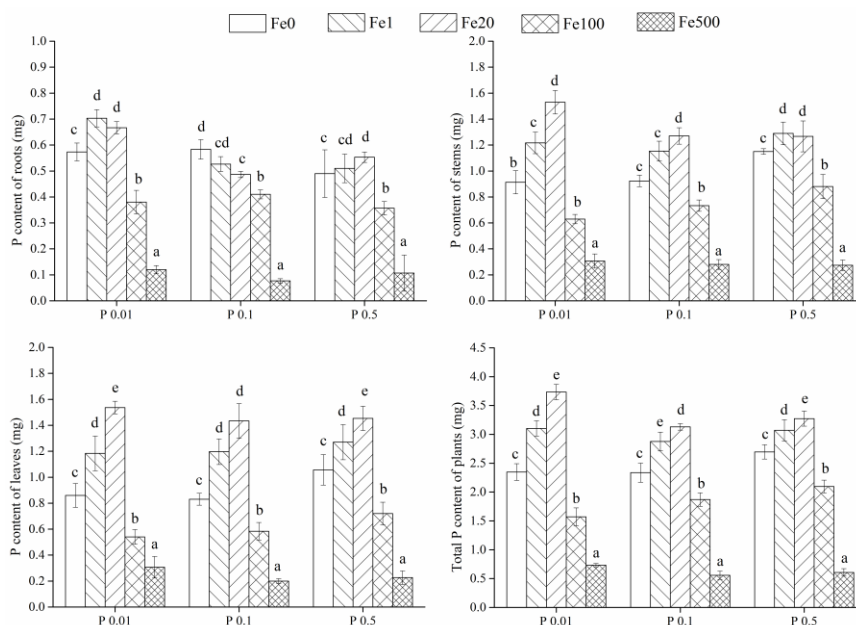
Figure 6. Effects of Fe^{2+} and P on roots, stems, leaves and total P contents of *G. spiculosa*. Values (mean $\pm$ SE, *n* = 3) followed by different letters designate significant differences (*p* < 0.05) between Fe^{2+} concentrations for a given P level (a, b, c, d).

There were close correlations between iron plaque and total Fe, Mn, P content in plants. For total Fe content, a positive correlation was observed with iron plaque (Figure 7a, r^2 = 0.86). Curviliner correlation between iron plaque and total Mn content (Figure 7c, r^2 = 0.89) was significant. The total P content in plants were also significantly correlated with iron plaque (Figure 7b, r^2 = 0.84).
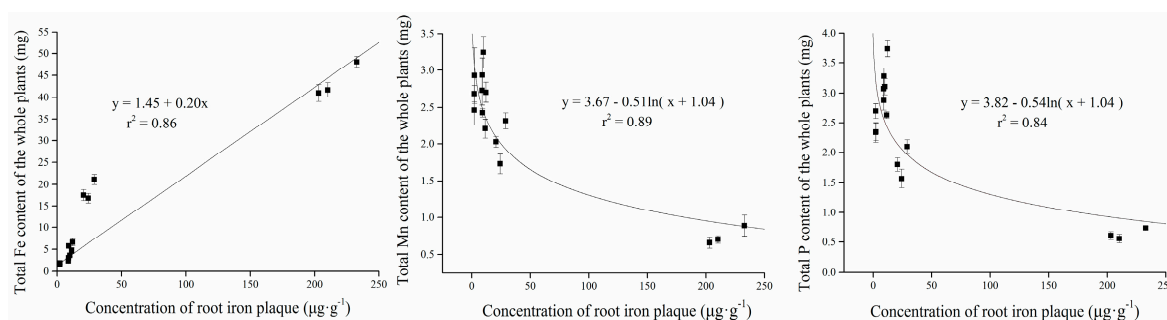
Figure 7. Relationship between the concentration of root iron plaque and total Fe content, total P content, and total Mn content of the whole plant. Data were means $\pm$ SE (*n* = 3).

3.5. Translocation and Acquisition Efficiency of Fe, Mn, and P

As expected, Fe translocation in stems was significantly increased with the increasing iron in the solution (Table 3). The distribution of Fe in leaves was obviously lower than stems. The P translocation in stems and leaves was not as significant as in Fe, but data of Fe 1 and Fe 20 concentrations were higher than other Fe treatments. The Mn translocation in stems and leaves was similar between those treatments, and the distribution of Mn (22.4%–37.5%) and P (30.0%–44.9%) in leaves was higher than that of Fe (5.7%–10.9%).

The responses of FeAE were similar to the Fe translocation between Fe treatments for a given P level, which showed a positive correlation with Fe applied. For the difference of FeAE between three P levels, higher values were found in P 0.1 treatment of Fe 0–Fe 500, except there was no difference found in Fe 20. The results of MnAE and PAE were similar in terms of translocation, with higher MnAE in Fe 1 and PAE in Fe 1–Fe 20 treatments. For three P levels, the difference between FeAE, MnAE and PAE were not significant.

Table 3. Effect of Fe and P on the translocation of Fe, Mn, and P from roots to the stems and leaves, and acquisition efficiency of Fe, Mn, and P (FeAE, MnAE, and PAE).

| Translocation (%) and Acquisition Efficiency of Fe, Mn, and P (FeAE, MnAE, and PAE, mg·g^{-1}) | | | | | | | | | |
| **Treatements** | | **Fe** | | | **Mn** | | | **P** | | |
Fe	**P**	**Stems**	**Leaves**	**FeAE**	**Stems**	**Leaves**	**MnAE**	**Stems**	**Leaves**	**PAE**
Fe 0	P 0.01	20.5 ± 3.3 a	7.6 ± 1.6 ab	3.8 ± 0.3 a	43.0 ± 6.7 a	31.6 ± 0.7 a	5.8 ± 0.2 bc	38.9 ± 7.9 a	36.6 ± 9.2 a	4.6 ± 0.3 ab
	P 0.1	18.5 ± 4.9 a	5.7 ± 0.8 a	4.1 ± 0.2 a	42.1 ± 9.4 ab	30.5 ± 0.9 ab	6.3 ± 0.4 ab	39.5 ± 4.4 a	35.5 ± 4.6 a	5.5 ± 0.5 ab
	P 0.5	21.8 ± 2.5 a	7.5 ± 0.7 a	3.7 ± 0.4 a	45.1 ± 6.7 a	27.2 ± 0.5 a	5.6 ± 0.4 b	42.6 ± 2.2 a	39.2 ± 9.2 a	6.2 ± 0.5 bc
Fe 1	P 0.01	23.9 ± 6.4 a	5.8 ± 0.7 a	6.8 ± 0.2 ab	39.2 ± 6.6 a	30.5 ± 1.2 a	7.6 ± 0.3 c	39.2 ± 8.3 a	38.1 ± 10.2 a	8.8 ± 0.2 b
	P 0.1	25.4 ± 7.2 a	6.1 ± 0.5 ab	7.9 ± 0.6 b	41.2 ± 8.0 ab	32.6 ± 3.5 ab	7.4 ± 0.8 b	40.1 ± 7.7 a	41.6 ± 9.9 ab	7.4 ± 0.9 bc
	P 0.5	26.6 ± 8.1 a	6.3 ± 0.7 a	7.2 ± 0.4 b	41.0 ± 5.1 a	32.0 ± 6.7 a	7.3 ± 0.7 c	42.0 ± 8.7 a	41.4 ± 4.7 a	8.3 ± 0.7 d
Fe 20	P 0.01	32.2 ± 6.3 a	7.0 ± 0.9 a	14.0 ± 0.7 b	34.2 ± 3.8 a	33.1 ± 5.1 a	5.9 ± 0.2 bc	41.0 ± 9.1 a	41.2 ± 4.8 a	8.1 ± 0.8 c
	P 0.1	33.6 ± 5.6 a	8.1 ± 1.1 bc	13.8 ± 0.8 c	32.9 ± 5.6 a	37.5 ± 6.5 b	6.6 ± 0.3 ab	39.8 ± 6.3 a	44.9 ± 6.9 b	7.8 ± 0.6 c
	P 0.5	34.2 ± 8.4 a	7.2 ± 0.7 a	12.5 ± 0.5 c	38.5 ± 3.5 a	29.8 ± 4.5 a	5.3 ± 0.2 b	38.7±9.4 a	44.4 ± 10.5 a	7.1 ± 0.5 c
Fe 100	P 0.01	57.4 ± 9.3 b	6.7 ± 0.4 a	49.0 ± 2 c	38.3 ± 9.5 a	27.7 ± 0.9 a	5.4 ± 0.5 b	44.5 ± 4.3 a	34.5 ± 9.3 a	4.9 ± 0.3 ab
	P 0.1	58.1 ± 7.2 b	7.0 ± 0.6 ab	61.2 ± 4.7 d	34.8 ± 3.0 a	33.1 ± 1.1 ab	6.3 ± 0.6 ab	40.1 ± 3.6 a	37.2 ± 5.7 a	5.7 ± 0.4 ab
	P 0.5	56.1 ± 6.5 b	6.6 ± 0.8 a	51.8 ± 1.8 d	39.9 ± 4.8 a	26.8 ± 3.8 a	5.8 ± 0.5 b	48.9 ± 9.3 a	30.0 ± 6.8 a	5.1 ± 0.2 b
Fe 500	P 0.01	56.9 ± 8.2 b	10.0 ± 1.3 b	265.7 ± 44.1 d	51.7 ± 4.0 a	22.4 ± 5.4 a	4.8 ± 0.1 a	41.8 ± 5.5 a	41.8±8.7 a	3.9 ± 0.6 a
	P 0.1	58.0 ± 7.9 b	10.3 ± 0.8 c	350.4 ± 18.9 e	55.3 ± 6.2 b	31.8 ± 0.4 a	5.3 ± 0.3 a	50.3 ± 3.8 a	35.9 ± 1.8 a	4.6 ± 0.3 a
	P 0.5	59.5 ± 5.7 b	10.9 ± 1.6 a	280.3 ± 16.6 e	56.1 ± 6.3 a	26.4 ± 2.5 a	3.5 ± 0.5 a	45.1 ± 4.1 a	37.4 ± 5.2 a	3.3 ± 0.2 a

Notes: The data of FeAE were square transformed before parametric analysis. Data are means ± SE (n = 3). For each parameter values followed by different letters designate significant differences ($p < 0.05$) between Fe^{2+} concentrations for a given P level (a, b, c, d).

4. Discussion

4.1. Iron Plaque Formation, SEM and EDS Analysis

Iron in the aquatic environment can not only directly participates in the physiological processes of wetland plants, but also forms iron plaque indirectly affecting the absorption and translocation of nutrients. Some research has shown that nutrient limitation may be the major factor to the growth of wetland plants [25]. In present study, the physico-chemical properties of iron plaque of *G. spiculosa* were characterized by SEM-EDX (Figures 2 and 3). The elements present in the plaque included essential plant nutrients, such as Fe, O, Mn, P, S, Mg, and Al. The amounts of iron plaque on the root surface were significantly affected by amounts of Fe supplied in the culture medium. Iron plaque formation on the roots increased with increasing levels of iron in the growth medium. Additionally, the root dry matter of *G. spiculosa* was declining with the increasing iron additon (Table 2), indicating that large amount of iron plaque harmed the growth of plant roots. Our results further showed that iron plaque formation was highest at the lower P treatments, which is comparable with data reported for rice [9,26]. Fu et al. [27] reported that P deficiency in the rhizosphere could increase the oxidizing capability of rice roots, which was associated with production of reactive oxygen species, increase in antioxidant enzyme activity, and the release of O_2 and oxidative substances from the root. The Fe^{2+} around the rhizosphere is oxidized by these oxidizing substances to Fe^{3+} that precipitates as orange iron plaque accumulation on the root surface of aquatic plants and on soil particles in the rhizosphere [28]. For wetland species, however, few studies focus on the iron plaque formation on the root surface under P-deficiency conditions [9,26].

4.2. Uptake, Translocation of Fe and FeAE

In wetland environments, Fe immobility is highly variable. Plants therefore need a tight regulation of Fe uptake, transport, and storage to ensure balanced development by avoiding both Fe deficiency and toxicity [29]. The total Fe contents of the plants significantly increased with the application of Fe. Meanwhile, our results indicated that wetland plants have high innate tolerance to iron in their habitats, which demonstrates the reasons for growing wetland plants in iron ore mining and other iron-contamined environments [30]. All plants were collected from areas that had not been exposed to high levels of Fe^{2+}. As a result, Fe tolerance would have to be innate. In response to Fe overload, plants express a series of Fe homeostasis-related genes, including those encoding for ferritin and a common protein for Fe storage [31]. The transporters are generally divided into low-affinity Fe transport systems and high-affinity Fe transport systems. Low-affinity transport systems take up sufficient Fe in high levels of Fe, meanwhile preventing Fe overload. In contrast, high-affinity Fe transport systems are activated by Fe deficiency conditions [32,33]. The formation of iron plaque under P-deficiency conditions had a large effect on Fe uptake of roots. Furthermore, the expression of ferritin genes in *Arabidopsis* plants can be effectively induced by P-deficiency conditions, such as encoding of NAS3 and ZIP5 for metal transport [21]. Moreover, the induction of genes involved in the transport and storage inside the plant in the forms of chelated iron, by metallothioneins, nicotianamine, and ferritin, suggesting a global and consistent plant response to P-deficiency [34].

Our results futher suggested the translocation was affected. The Fe translocation process involves various Fe transport components, such as those involved with long-distance transport within the vascular system (from root to shoot, cotyledons to young shoots), and short-distance transport within the cells (intracellularly in the symplast), to final destination targets within cells (compartment or biomolecules) [29]. These transport processes and strategies aim to protect and maintain plants' health. In this study, with increasing Fe supply, the content and translocation of Fe in the stems gradually increased, but Fe in leaves remained stable (5.7%–8.1%), except for the highest exposure (Fe 500: 10.0%–10.9%). Wetland plants seem to generally engage in an "exclusion" strategy, in which metals in aboveground parts are maintained at a constant low levels until a critical external concentration is reached [10]. In our experiment, leaf-wilting and desiccation were observed at the Fe 500 treatment,

and those are well-known symptoms, which may have caused unrestricted metal transport. Excessive absorption of Fe^{2+} by roots and subsequent translocation to leaves causes an elevated production of toxic oxygen radicals, which destroys cell structural components and damages physiological processes [35]. The critical Fe content depends on plant age. The color of leaves could be changed from orange to rusty brown with excessive Fe content in plant, especially in older leaves [36,37]. It is most likely that the result of damage to the root system, and the rapid reduction of root biomass was the best evidence. Consistent with our study, Batty et al. [38] found that the roots of aquatic plants were inhibited under conditions of excessive Fe supply, including root flaccidity and reduced root branching.

4.3. Uptake, Translocation of Mn and MnAE

Mn is essential for plant metabolism and develpment, and exists in oxidation states II, III, and IV inside about 35 enzymes of a plant cell [39]. Mn mainly achieves two functions in proteins: (1) it acts as a catalyzing active metal, or (2) it shows an activating role on enzymes [40]. The concentration of Mn in the nutrient solution was suitable for plant growth, so it can be used to detect the effects of Fe and P on the absorption of micronutrients. The Mn content was increased with the treatment of $1 \, mg{\cdot}L^{-1}$ Fe, which concentration was close to the natural habitat of the plants. The results indicated that large amount of agricultural drainage water containing iron-rich groundwater would promote the growth of wetland plants, due to the high iron tolerance of plants. The results confirmed that the Mn uptake was promoted by iron addition within the natural range, but significantly suppressed when iron concentration become excessively high. The mechanism by which plaque affects the precipitation and uptake of Mn, Pb, and Zn may involve the absorption of metals onto the large surface plaque area [12,41]. Inside the plants, an Fe transport protein (RcITP) binds preferentially Fe(III) over Fe(II), but it also forms compleses with Mn, Zn, and Cu, which also promote Mn absorption and migration at the same time [42]. Kruger et al. [42] further showed that a thick iron plaque may have limited capacity for the absorption of Mn and other metals, which was consistent with our study. Other results have also shown that the concentrations of Mn tends to be reduced under similar conditions, suggesting that uptake is particularly affected by large amounts of iron plaque [43]. Batty et al. [41] found that the Mn concentration in the absence of plaque was higher than when plaque was present. The EDX analysis in our study also showed that large amounts of Mn existed on the surface of the plaque, suggesting that abundant iron plaque acted as a barrier for micronutrients by forming a complex precipitation or adsorption on the plaque surface.

The absorption and translocation of micronutrients seemed to be sensitive to high concentrations of Fe application. Studies with Lynch and Clair [44], and Socha and Guerinot [45], have shown that Mn deficiency can be caused by multiple factors including high concentrations of other minerals in the soil, such as Fe, Mg, Ca, and P. The results of Ghasemi-Fasaei et al. [46] showed that foliar application of Fe on chickpea decreased Mn uptake owing to the antagonistic effect of Fe on translocation of Mn from root to shoot. Similar to Mn, the uptake and translocation of Zn was also inhibited by Fe in the solution [47,48]. Mn, Zn, or Cu deficiency may be induced following excessive Fe fertilization due to uptake of relatively large amounts of Fe [49]. Hayes et al. [48], showed a negative relationship between leaf Mn and total soil P concentration along a bay in western Australia. Soluble P in the rhizosphere can form coprecipitation with Fe^{3+} and Mn^{4+}, which can bind to amorphous oxyhydroxides in root plaques. The conclusion was consistent with our results: total Mn content in plants of P 0.5 was lower than the other P treatments. In general, plants receive higher MnAE by roots and transportation to aboveground with the Fe 1 treatment.

4.4. Uptake, Translocation of P and PAE

Excessive fertilization of agricultural land has resulted in large losses of P from paddy field to the aquatic environment. In recent years, interactions between Fe and P have already been utilized in technological applications. For example, studies have shown that Fe-coated sand as a liner in drainage systems could reduce P loading of surface water [50]. The formation of iron plaque on the root surface

of wetlands has a number of functions, such as nutrient and contaminant uptake. Khan et al. [5] reported that iron plaque has a high capacity to bind P in wetlands, and the sediments Eh, P, and Fe cycling have close links in lake ecosystems. Furthermore, Fe sludge and Fe-rich groundwater could be used as reactive materials to control the eutrophication water bodies, which could bound a large amount of P [23]. Apart from the potential role of iron plaque in changing the bioavailability of metals in the rhizosphere of wetland plants, iron plaque is believed to be important in plant P uptake and translocation. In our experiment, P 0.01 during the last two weeks was intended to simulate the P-deficient condition of *G. spiculosa* grown in solution. However, in retrospect, the plants may have obtained sufficient amounts of P from the nutrient solution in the initial three weeks, also aided by P adsorbed in the iron plaque. Similar results were found for common reed (*Phragmites australis* (Cav.) Trin ex. Steudel.), in which the amount of P adsorbing increased with the amount of plaque formation on the roots [8,38]. Even without P supplied in the solution, P concentrations in rice seedings were not deficient at the background levels of Fe applied [9].

Hu et al. [22] showed that P deficiency in habitats results in an increase in plant root length and porosity, thereby increasing aquatic plants ROL that subsequently would oxidize higher amounts of Fe^{2+} and form root plaque. Although previous studies indicated that root porosity, ROL, and Fe plaque are mainly controlled genetically [51], wastewater as an environmental stress or can change these properties. High strength wastewater, such as the agricultural drainage water from paddy fields, significantly decreased ROL and root porosity, but high level of iron promoted the formation of iron plaque, further enhancing the absorption of P from habitats. The success of iron addition in order to regulate P release can be calculated by using the Fe:P ratio in the sediment pore water, which ranging from 1–15 of pore water ratio [52,53]. The pore water Fe:P ratio provides a good prognosis for both the restoration of water quality and biodiversity after restoration measures. Therefore, these reactive materials can be used for trapping P in the soil solution by using iron rich groundwater for irrigation or adding the reactive materials in lakes or wetlands when agricultural drainage water exports into the surrounding ecosystems.

Apart from enhacing P absorption from habitats, iron plaque promoted P translocation to the aboveground. Unlike other elements, the P content of stems and leaves was nearly two times that of the roots for a given Fe and P treatment (Figure 6), and the PAE of Fe 1–Fe 20 showed much higher values. P plays a crucial role in plant cell division, growth, and biomass accumulation. Our results implied that iron-rich groundwater flowing into wetlands may be beneficial to P uptake and transfer to abovegound biomass in wetland plants. However, the P content was reduced significantly when the external Fe concentration was over 100 mg·L^{-1} in our study, partly because the roots of plants were harmed and large amounts of iron oxyhydroxides in the root plaque bound sequestrated P. Stimulation of development of iron plaque on the root surface has been used to reduce the toxic effects of As, Pb, and Cd, and the effect of P decreased metal desorption, solubility, and bioavailability in the rhizosphere; this even enhanced tolerance to heavy metals [20,54].

5. Conclusions

Our findings provided mechanistic insights into Fe biogeochemistry in the rhizosphere of *G. spiculosa* in relation to P uptake, Mn, and other micronutrients. The presence of excess Fe^{2+} in the aquatic environment induced the formation of iron plaque on the root surface. The amount of iron plaque increased with increasing Fe^{2+} supply, and P-deficiency promoted its production. Iron plaque on the root surface could function as a reservoir for nutrients, but inhibited element accumulation and translocation in the plants when present as a thick cover on the roots. In the presence of excessive iron plaque, the uptake and translocation of Fe in plants was decreased significantly. The absorption of Mn was particularly affected by iron plaque. Formation of iron plaque enhanced the P utilization until the external iron concentration exceeded 100 mg·L^{-1}.

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