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Effects of Aeration, Vegetation, and Iron Input on Total P Removal in a Lacustrine Wetland Receiving Agricultural Drainage

Yuanchun Zou [1], Linlin Zhang [1,2,3], Luying Wang [1,3], Sijian Zhang [1,3] and Xiaofei Yu [1,*]

1 Key Laboratory of Wetland Ecology and Environment & Jilin Provincial Joint Key Laboratory of Changbai Mountain Wetland and Ecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, China; zouyc@iga.ac.cn (Y.Z.); zhanglinlin@iga.ac.cn (L.Z.); wangluying@iga.ac.cn (L.W.); zhangsijian@iga.ac.cn (S.Z.)

2 The Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

3 University of Chinese Academy of Sciences, Beijing 100049, China

* Correspondence: yuxf@iga.ac.cn; Tel.: +86-431-8554-2274

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Abstract: Utilizing natural wetlands to remove phosphorus (P) from agricultural drainage is a feasible approach of protecting receiving waterways from eutrophication. However, few studies have been carried out about how these wetlands, which act as buffer zones of pollutant sinks, can be operated to achieve optimal pollutant removal and cost efficiency. In this study, cores of sediments and water were collected from a lacustrine wetland of Lake Xiaoxingkai region in Northeastern China, to produce a number of lab-scale wetland columns. Ex situ experiments, in a controlled environment, were conducted to study the effects of aeration, vegetation, and iron (Fe) input on the removal of total P (TP) and values of dissolved oxygen (DO) and pH of the water in these columns. The results demonstrated the links between Fe, P and DO levels. The planting of *Glyceria spiculosa* in the wetland columns was found to increase DO and pH values, whereas the Fe:P ratio was found to inversely correlate to the pH values. The TP removal was the highest in aerobic and planted columns. The pattern of temporal variation of TP removals matched first-order exponential growth model, except for under aerobic condition and with Fe:P ratio of 10:1. It was concluded that Fe introduced into a wetland by either surface runoff or agricultural drainage is beneficial for TP removal from the overlying water, especially during the growth season of wetland vegetation.

Keywords: agricultural runoff; phosphorus; iron; pollution control; treatment wetland; Lake Xingkai

1. Introduction

Phosphorus (P), as one of the most important biogenic elements in wetlands, is strongly controlled by the transformation of iron (Fe). The key roles of Fe have been increasingly valued by researchers [1–3]. Under anaerobic conditions in wetlands, microorganisms can obtain energy by oxidizing organic compounds via reducing Fe(III) as an electron acceptor [4,5], and Fe(II) will be re-oxidized by O_2 when the hydrological regime of wetlands shift from wet to unsaturated. During the rotation of this "ferrous–ferric redox wheel", the newly formed Fe(III) (hydr)oxides by the oxidation of O_2 can strongly bind to PO_4^{3-} and reduce the total phosphorus (TP) in the water [6–8], a process through which the wetland soils/sediments become P sinks. When the hydrological regime of wetlands shifts from unsaturated to wet, the Fe(III)-P complexes will be re-reduced by anaerobic microorganisms after the depletion of O_2 and NO_3^-, and the former PO_4^{3-} complex will be released again into the soil/sediment pore water and overlying water, a process through which sediments/soils become P sources [9].

Although ferrous or ferric solutions have been used in wastewater treatment to remove P for many years, there are not many studies on P demobilization in natural wetlands [10]. Some case studies in reservoirs and lakes confirmed the effectiveness of ferric solutions in removing P from overlying water [11–15]. A Fe:P ratio has also been used to predict the P adsorption capacity of soils/sediments due to the significant negative correlation between dissolved P and Fe:P [16,17]. Considering the risk of dissolved Fe-P complexes under anaerobic conditions over the long term, artificial aeration has been implemented to avoid this risk and increase the P removal efficiency [18]. In addition to artificial aeration, aeration through wetland vascular plants is also an important natural aeration mechanism [19–21].

Therefore, there is a coupled relationship between Fe, O, and P in wetlands, which affects the level of P in a wetland and further affects the eutrophication of water bodies; however, it is unclear how the exogenous Fe inputs from natural runoff and/or agricultural drainage affect the transport and transformation of P in wetlands when redox conditions change with artificial or natural aeration.

In this study, we compared the effects of aeration and rooting plants on dissolved O_2, pH, and TP removal under a lower Fe:P ratio of 5 and a higher ratio of 10 in the overlying water. The objectives of this study were (1) to test the temporal responses of O_2, pH, and TP in the overlying water to different redox conditions; and (2) to assess TP removal from the overlying water and test the effectiveness of Fe inputs on TP removal in the wetlands receiving agricultural drainage.

2. Materials and Methods

2.1. Sampling Site and Soil Core Sampling

Lake Xingkai is a transboundary lake shared by China and Russia. A narrow sandy ridge on the northern shore is separated from Lake Xingkai and forms a smaller lake named Xiaoxingkai that belongs to China. At Lake Xingkai, the annual mean precipitation is 561 mm, and the mean temperature is 3.5 °C [22]. The Fe concentration of the lake water can be as high as 0.62 mg·L^{-1} and is the highest concentration found among the waters in the Heilongjiang River system [23]. This high concentration is caused by both the high background levels of natural runoff and the agricultural drainage from upstream [24]. The sediment cores were collected from a lacustrine wetland covered by *Glyceria spiculosa* communities in Lake Xiaoxingkai (45°13′47″ N, 132°46′26″ E). *G. spiculosa* usually develops aerenchyma in its stems, leaves, rhizomes, and roots, which make this species adaptive to submerged conditions, and it has become one of the dominant species in lacustrine wetlands around Lake Xingkai. The sampled lacustrine wetland is mainly supplied by lake water and precipitation and has periodically received agricultural drainage.

Twenty-four intact wetland soil cores were collected randomly using polyvinyl chloride (PVC) tubes (30 cm length × 6.8 cm internal diameter). Each core included 15 cm of submerged soil and 6 cm of water and was sealed with plastic bungs, stored in a portable cold closet and then transported to the laboratory within 48 h. In addition, representative and homogeneous seedlings of *G. spiculosa* were collected and transferred to the laboratory as well.

2.2. Experimental Design and Chemical Analyses

The incubation was designed with three treatment factors and two levels (aeration × plant × Fe:P × replicates), and each treatment had three replicates. To not alter the integrity of the soil column, the overlying water of the 12 cores randomly selected from the 24 cores was siphoned out, and 48 seedlings (four seedlings per core) of *G. spiculosa* with the same height (approximately 7 cm) were selected for careful implantation in these cores using long forces. After three days of successful introductions and when all the plants survived, the overlying water siphoned out was siphoned back into the soil column. Six randomly selected cores with plants and six without plants were dosed and adjusted so that the overlying water contained 5 mg·L^{-1} Fe and 1 mg·L^{-1} P (Fe:P = 5) with $FeCl_2·4H_2O$ and 1 mg·L^{-1} $NaH_2PO_4·2H_2O$ solutions, respectively. The rest of the cores with or without plants were dosed and adjusted to contain 10 mg·L^{-1} Fe and 1 mg·L^{-1} P (Fe:P = 10). The aeration treatments were adjusted to establish high (>6 mg·L^{-1}, aerobic) and low (<2 mg·L^{-1}, anaerobic) DO concentrations by continuous gentle bubbling with O_2 or N_2, respectively.

Before incubation, the overlying water depth of each core was adjusted to 6 cm. The indoor incubation was completed at an ambient temperature of 23–25 °C for 25 days, with the necessary light compensation provided by a plant growth lamp. The overlying water was monitored and measured seven times on 0, 3, 5, 8, 12, 17, and 24 d. The pH and DO were determined in situ using the EXO2 Multiparameter Sonde (YSI Incorp., Yellow Springs, OH, USA). The TP was determined by extracting 15 mL of water with a syringe, and then this water was replaced with 15 mL of deionized water. The TP in the extracted water sample was measured using the ammonium molybdate spectrophotometric method (UV 2550, Shimadzu, Japan) after digestion with $HClO_4$-H_2SO_4 for 0.5 h [25].

2.3. Data and Statistical Analyses

TP removal percentages (R_{TP}) were calculated according to the formula

$$R_{TP} = \frac{C_0 - C_i}{C_0} \times 100\% \tag{1}$$

where C_0 and C_i are the TP (mg·L^{-1}) at the initial and the ith sampling, respectively, and i is the sampling frequency.

Two-way repeated measures analysis of variance (ANOVA) were performed to test the main and interaction effects of aeration and plant treatments using SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). All the means and standard errors were calculated using Origin Pro 8.0 (OriginLab Corp., Northampton, UK), and all the graphics were drawn using Origin Pro 8.0.

3. Results

The temporal changes of DO, pH, and TP removal varied with the different treatments. The two-way repeated measures ANOVA showed that the main and interaction effects of the aeration and plant treatments on the DO were all significant under the lower Fe:P ratio (Fe:P = 5), while only the main effects of the aeration and plant treatments on the pH were significant. For TP removal, the main effect of aeration was extremely significant and even caused the interaction effect to be significant as well (Table 1).

Table 1. Results of two-way repeated measures analysis of variance when Fe:P = 5. DO, dissolved oxygen; TP, total phosphorus.

Variable	Time		Time × Plant		Time × Aeration		Time × Plant × Aeration	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
DO	32.728	<0.0001	2.488	0.043	32.652	<0.0001	2.538	0.038
pH	21.401	<0.0001	2.658	0.026	7.001	<0.0001	1.920	0.097
TP removal	283.922	<0.0001	1.437	0.220	247.775	<0.0001	3.444	0.007

When more Fe was introduced (Fe:P = 10), the main effects of the aeration and plant treatments on the DO were both significant, while the interaction effect was non-significant. For pH, only the main effect of aeration was significant. For TP removal, neither the main effects nor the interaction effect were significant (Table 2).

Table 2. Results of two-way repeated measures analysis of variance when Fe:P = 10.

Variable	Time		Time × Plant		Time × Aeration		Time × Plant × Aeration	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
DO	48.639	<0.0001	4.412	0.001	34.669	<0.0001	1.377	0.243
pH	23.570	<0.0001	2.057	0.076	14.717	<0.0001	1.940	0.093
TP removal	6.309	<0.0001	2.154	0.064	1.751	0.130	2.191	0.060

3.1. DO Variation

The DO of the overlying water in the aerobic cores increased with the incubation time from approximately 6.0 to 9.0 mg·L^{-1}, while the DO in the anaerobic cores kept stable at approximately 1.0 mg·L^{-1}. The former was much higher than the latter, and the differences between these two values increased with the incubation time (Figure 1). In the first five days after beginning the incubation, the differences between the plant and non-plant cores were non-significant; however, significantly higher DO levels were observed in the plant cores than the non-plant cores except under anaerobic conditions with a higher Fe:P ratio after five days (Figure 1b).

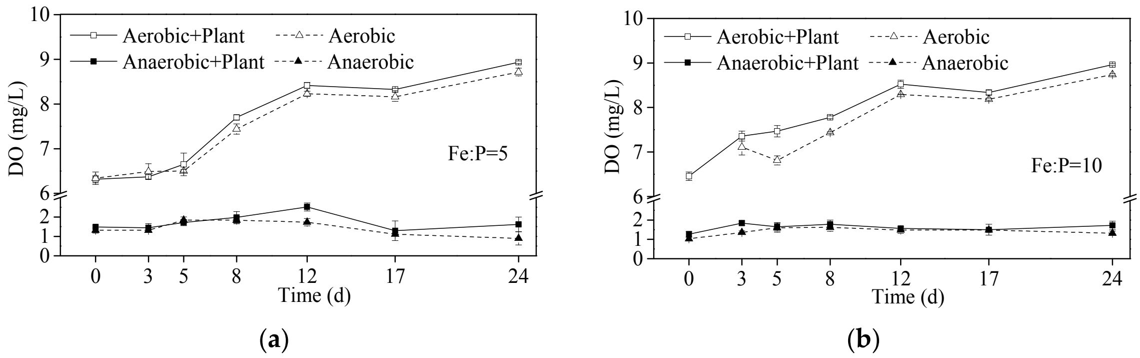


Figure 1. Variation of dissolved oxygen (DO) in the overlying water. (**a**) Iron: phosphorus ratio (Fe:P) = 5; (**b**) Fe:P = 10. Error bars represent the standard errors.

3.2. pH Variation

All the pH values of the overlying water in the different treatments fluctuated during the incubation. Compared with the other three treatments, the pH values of the aerobic and plant cores were the highest, for both Fe:P ratios. In the anaerobic cores, the pH values decreased compared with those in the beginning of the incubation. Compared with the pH values under two Fe:P ratios, the higher pH could be observed in the cores with the lower Fe:P ratio (Figure 2). The mean pH decreased from 6.93 to 6.92 for aerobic and plant cores, from 6.58 to 6.14 for aerobic cores, from 6.56 to 5.78 for anaerobic and plant cores, and from 6.43 to 5.95 for anaerobic cores. The mean pH values of the plant cores were greater than those of the non-plant cores, although this difference at higher Fe:P ratios was non-significant ($P = 0.76$).

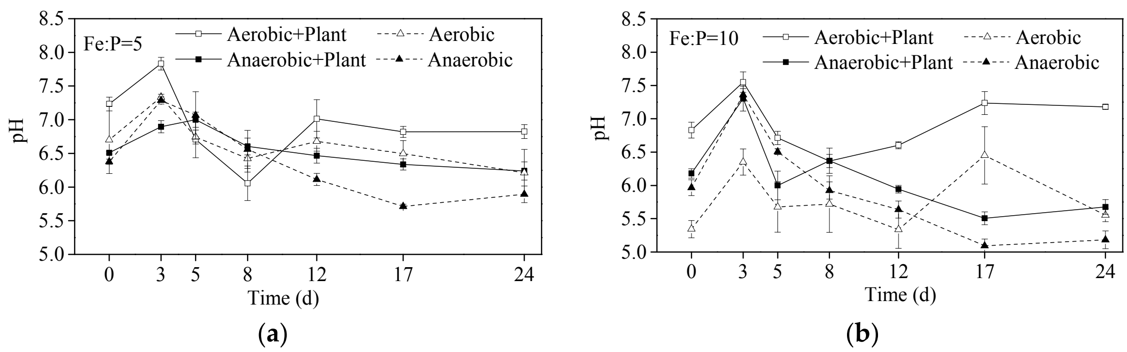
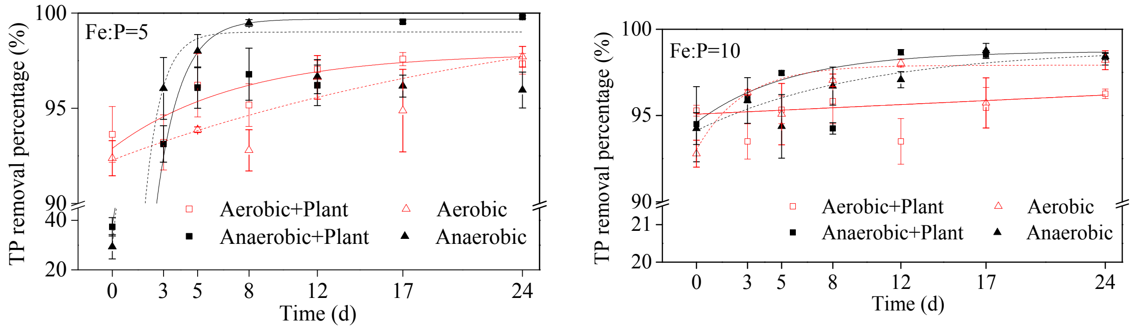


Figure 2. Variation of pH in the overlying water. (**a**) Fe:P = 5; (**b**) Fe:P = 10.

3.3. TP Removal Variation

Under the lower Fe:P ratio, two types of TP removal curves could be observed. For the aerobic cores, TP removal fluctuated at approximately the 95% level. For the anaerobic cores, TP removal increased exponentially. After three days of rapid growth, TP removal in the plant cores and non-plant cores rapidly increased by 2.5 times and 3.3 times more than the initial levels, respectively (Figure 3a). Under the higher Fe:P ratio, all TP removals fluctuated with the extension of incubation time but increased in comparison with the levels in the beginning (Figure 3b). Among the various treatments, TP removals were the highest in the aerobic and plant cores than in the other treatments. Except for the aerobic and plant cores under the higher Fe:P ratio, the TP removal curves could be fitted by first-order exponential growth equations (Tables 3 and 4).



(**a**) (**b**)

Figure 3. Removal percentage of total phosphorus (TP) in the overlying water. (**a**) Fe:P = 5; (**b**) Fe:P = 10.

Table 3. Fitting equations of TP removal when Fe:P = 5.

Treatment	Fitting Equation	R^2	*P*
Aerobic + Plant	y = −5.01 × exp(−x/7.37) + 97.90	0.75	<0.001
Aerobic	y = −9.16 × exp(−x/26.94) + 101.44	0.73	<0.001
Anaerobic + Plant	y = −61.76 × exp(−x/1.40) + 99.68	0.90	<0.001
Anaerobic	y = −69.73 × exp(−x/0.94) + 99.00	0.71	<0.001

Table 4. Fitting equations of TP removal when Fe:P = 10.

Treatment	Fitting Equation	R^2	*P*
Aerobic + Plant	y = 0.046x + 95.08 [1]	0.46	0.056
Aerobic	y = −4.91 × exp(−x/2.72) + 97.92	0.85	<0.001
Anaerobic + Plant	y = −4.13 × exp(−x/5.50) + 98.73	0.14	<0.001
Anaerobic	y = −4.76 × exp(−x/9.77) + 98.88	0.81	<0.001

Note: [1] First-order exponential equation was not applied for this treatment for the parameters were not reasonable and $R^2 < 0.5$.

4. Discussion

4.1. Interactions among Aeration, Plants, and Fe:P

Fe, P, O, and H are transformed and coupled to each other in wetland environments [5], and their interactions occur not only at an abiotic level but also a biotic level [4–7]. Regardless of bubbling with either O_2 or N_2, the DO in the plant cores was higher than that in the non-plant cores, indicating that the existence of wetland plants plays an important role in increasing DO concentrations in overlying water (Figure 1, Tables 1 and 2), and this result confirmed the process of internal aeration in wetland plants through aerenchyma [19,20]. Considering the *G. spiculosa* seedlings used in this study were newly planted, their roots were not well-developed, and the internal transport capacity of O_2 was restricted. However, when roots grow well and densely in wetland habitats in the field, natural aeration would be effective and create oxic conditions even though the soils are submerged during the growing season. When plant vitality is not strong and agricultural drainage flows into wetlands, artificial aeration is recommended. The effectiveness of aeration on P demobilization in wetland soils, however, depends on the Fe content [16,17] and the type of Fe-P complexes [18].

It is interesting that the pH values of the plant cores were greater than those of the non-plant cores, regardless of the O_2 levels, indicating that the presence of plants could increase the pH of water bodies (Figure 2). More O_2 released into the water by radial loss from roots would oxidize ferrous ions to ferric ions, and OH^- would be produced during this process. The newly formed ferric ions would be subject to hydrolysis. The higher Fe:P ratio could also decrease the pH (Figure 2), which could be attributed to the increased hydrolysis of Fe ions and the extra release of H^+, resulting in acidification [10,26]. Theoretically, the actual O_2, pH, and Fe:P in wetlands would be the balance among these coupled processes. Therefore, the Fe introduced by agricultural drainage and its transformation with P in soils should be further studied in the field to test the effectiveness of TP removal under different aeration conditions.

4.2. TP Removals under Different Fe:P Conditions and Management Recommendations

P is usually limited in most natural wetlands and other aquatic ecosystems; however, excessive P input has become one of the key control factors causing water eutrophication [1,3]. Among the exogenous P inputs, agricultural drainage is one of the most important sources in Lake Xiaoxingkai. Although there is no survey of how many fertilizers and pesticides have been used in the farms around the lake, the total amount of fertilizers and pesticides used in Heilongjiang Province where the lake is located, as well as the amount used per hectare, showed logistic growth from 1996 to 2015, and a considerable portion of the unused fertilizers and pesticides eventually enter and sink into the wetlands downstream [27]. For Lake Xiaoxingkai, over 0.5 billion m^3 of lake water were pumped for irrigation of the paddy fields in the state farms adjacent to Lake Xingkai, and large amounts of agricultural drainage return to the remaining natural lacustrine wetlands during the drainage return flow period. Consequently, P, Fe, and sediments have accumulated in these wetlands [24]. According to a recent survey, Lake Xiaoxingkai was experiencing moderate eutrophication, and P was identified as the restriction element [28]; therefore, the agricultural drainage that flows and accumulates in these wetlands with Fe:P should be considered as the key factor to controlling eutrophication internally.

TP removal under different Fe:P ratios gradually increased with incubation time (Tables 3 and 4), indicating that the input of Fe reduced TP to a certain extent. According to the fitting equations (Tables 3 and 4), although all the final TP removals would be more than 97.9% when the incubation time was long enough, the initial TP removals were 92.89, 92.28, 37.92, and 29.27% for the different treatments under the lower Fe:P ratio, and 95.08, 93.01, 94.6, and 94.12% for the higher Fe:P ratio. These results suggested that the higher Fe:P ratio could increase initial TP removal as in the field observations by Kleeberg et al. [15], especially under anaerobic conditions (increased TP removal by 56.68% for the anaerobic and plant cores and by 64.85% for the anaerobic cores).

In addition, when the Fe:P ratio is as high as 10 in the wetland, our results (Figure 3b) showed that the rapid growth period of TP removal was significantly shortened compared with the lower Fe:P ratio during the first three days (Figure 3a). This result indicated that more Fe could compensate for the disadvantage of low TP removal when the treatment time is too short, and there are a lack of aeration measures. Therefore, more Fe introduced by surface runoff, groundwater, or agricultural drainage could be beneficial to increasing TP removal from the overlying water, especially where wetland plants are growing well and are dense. For the practical use of Fe introduction method in natural water bodies' restoration, the Fe species and dosage should be further specified according to the local P and DO concentrations and an Fe:P ratio of 10 is recommended.

5. Conclusions

During indoor wetland incubation, the DO in the overlying water was affected both by the aeration and plant treatment and plant increased DO. The pH was affected by the aeration and plant treatment as well. TP removal was only affected by aeration under the lower Fe:P ratio. Introducing more Fe could increase initial TP removal and shorten the initial rapid growth period, especially under anaerobic conditions. We concluded that greater Fe introduction by agricultural drainage could be beneficial to increasing TP removal from the overlying water. This study contributes to highlighting the importance of further understanding the elemental coupled cycling involved in the water purification function of in wetland ecosystem and providing a scientific basis for the protection and management of Lake Xiaoxingkai. Considering the complicated interactions, more studies focusing on Fe and P fluxes introduced by agricultural drainage and their coupled transformation should be carried out in the field under different hydrological regimes and redox conditions, through which more scientifically reasonable and cost-effective management strategies and techniques could be developed.

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