

Article

Responses of *Phragmites australis* to Nitrogen Addition along Salinity Gradients in Coastal Saline–Alkali Soil

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Abstract: Soil salinization and nitrogen (N) enrichment in saline–alkali soils resulting from human activities cause potential environmental pressure on *Phragmites australis*. However, the response of *P. australis* to N addition under different salt conditions remains unknown. This study examined the changes in soil properties and growth indices as well as their relationship to N addition through an in situ field experiment using three soil salinity levels with *P. australis* in the Yellow River Delta. The study showed that soil salinity levels significantly affected the effects of N addition on soil pH and water contents. N addition increased the soil NO₃[−] contents and decreased soil available phosphorus (Avail. P) contents; however, soil salinity levels did not impact the effects of N addition on soil NO₃[−] and Avail. P contents. N addition decreased the biomass of *P. australis*, since the decrease in the competitiveness for N sources changed the vegetation diversity. The results suggest that the biomass, plant height, and leaf soil plant analysis development (SPAD) values of *P. australis* increased with increasing soil Avail. P contents rather than soil NO₃[−] contents. Therefore, we suggest the important role of Avail. P addition in N enrichment conditions in saline–alkali wasteland and estuarine wetland ecosystems.

Keywords: N addition; soil properties; growth indices; salinity gradients; Yellow River Delta



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1. Introduction

Phragmites australis is one of the most extensively distributed emergent plant species throughout the world [1,2]. *P. australis* provides ecosystem services such as nitrogen removal, water purification, and maintaining biodiversity for saline–alkali wasteland and estuarine wetland ecosystems [3,4]. Human activities, including reclamation, aquaculture, and pollutant emissions, aggravate soil salinization and water eutrophication, which can cause irreversible changes to the *P. australis* community in saline–alkali wasteland and estuarine wetland ecosystems [5–7].

Nitrogen (N) is one of the key elements limiting the growth of salt marsh plants [8,9]. The increased N input resulting from human activities has many deleterious effects on ecological function in saline–alkali wasteland and estuarine wetland ecosystems [10,11]. N enrichment has affected plant morphological traits, thus changing plant N uptake strategies [12,13]. For example, the excessive N input has increased the stem production rates of *P. australis* [14,15] and decreased the flexural strength of *P. australis* by reducing sclerenchyma [16]. However, when N is no longer a limiting resource, the competitive advantage for N in plant communities changes [17]. Therefore, *P. australis* regulates

morphological traits for superior performance, resulting in a competitive advantage for N resources [18,19].

The Yellow River Delta is located at the junction of the Bohai Sea and Yellow River and has a unique ecosystem and important ecological functions [20,21]. In recent decades, coastal agriculture, fertilization, irrigation, tillage, and other management practices in the Yellow River Delta have added complexity to soil water and salt transport processes and intensified soil salinization and degradation [22]. Primary and secondary salinization and soil degradation cause potential environmental pressure on *P. australis* [23,24]. Meanwhile, the increasing N deposition caused by agricultural N inputs in the Yellow River Delta seriously affects the structure and function of the saline–alkali wasteland and estuarine wetland ecosystems [25,26]. However, the response of *P. australis* to N addition under different salt conditions remains unknown.

In this study, we examined differences in the response of *P. australis* to N addition among soil salinity gradients as well as the links between *P. australis* growth and changes in soil characteristics. To achieve our objectives, we conducted a field experiment in which N was not added or was added to the soil of *P. australis* at each of three sites exclusively dominated by *P. australis* and differing in soil salinity levels. Specifically, we hypothesized that (1) N addition would affect the performance of *P. australis* and alter soil characteristics and (2) the impacts of N addition would vary with different soil salinity levels.

2. Materials and Methods

2.1. Site Description

The study sites were located in the Yellow River Delta adjacent to the muddy coastal zone (118°59'26" E, 37°39'54" N; Figure 1). Sites were characterized by a temperate semi-arid climate, which is representative of agriculturally intensive areas of North China, with a mean annual temperature of 12.6 °C and a mean precipitation of 580 mm. Approximately 70% of the annual precipitation occurs in June–September. The soil is classified as a coastal saline soil derived from alluvial loess parent materials [27].

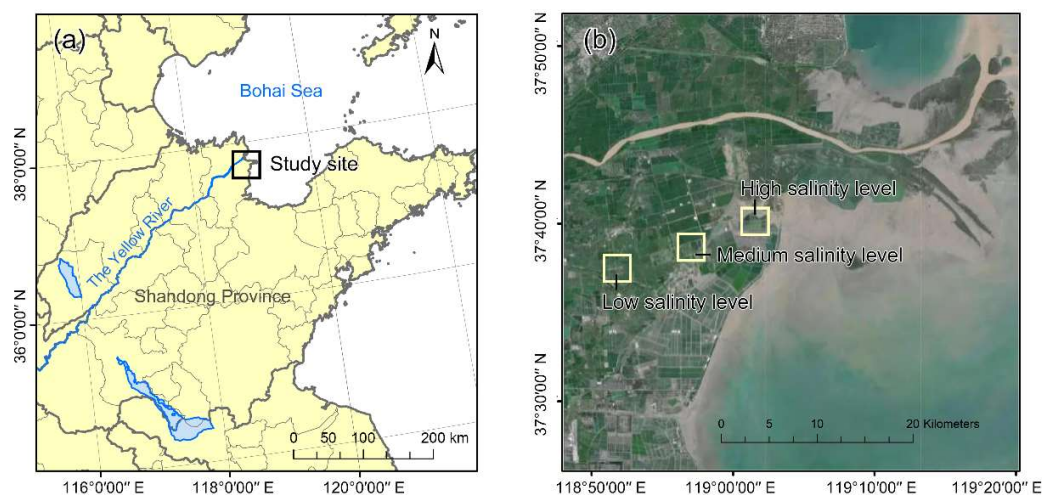


Figure 1. Location of the study area (a) and overview of the experimental site (b).

2.2. Experimental Design

We selected three soil salinity levels with *P. australis* at the study sites based on a soil investigation in June: (1) high salinity level (soil salt contents $\geq 8 \text{ g kg}^{-1}$), (2) medium salinity level (soil salt contents ranged between 4 and 8 g kg^{-1}), and (3) low salinity level (soil salt contents $\leq 4 \text{ g kg}^{-1}$). To keep microclimatic conditions similar, the distance between any two adjacent stands of the three soil salinity levels was 5 m. The details of the soil physicochemical characteristics at the three salinity levels are shown in Table 1.

Table 1. Soil physicochemical characteristics of 20 cm topsoil samples at three salinity levels.

Salinity Level	Salt Contents (g kg ⁻¹)	EC (ds m ⁻¹)	pH	Organic Matter (g kg ⁻¹)	Total N (g kg ⁻¹)	Avail. P (mg kg ⁻¹)	Avail. N (mg kg ⁻¹)
High							
Range	13.06–8.54	2.23–3.39	8.28–7.97	8–4.19	0.53–0.25	13.4–12.3	43.9–18.9
Mean	11.03	2.87	8.07	5.92	0.39	12.7	26.6
S.E.s	1.71	0.47	0.11	1.30	0.11	0.4	9.2
Medium							
Range	6.94–4.19	1.11–1.82	8.49–8.13	12.16–2.8	0.86–0.47	12.3–11.2	49.1–26.7
Mean	5.43	1.42	8.29	6.97	0.55	11.7	40.0
S.E.s	0.95	0.27	0.12	3.66	0.14	0.4	9.0
Low							
Range	3.97–2.02	0.55–1.05	8.65–8.24	11.5–5.72	0.76–0.43	12.4–11.1	82.7–20.7
Mean	3.20	0.85	8.49	7.92	0.60	11.7	49.1
S.E.s	0.75	0.22	0.15	1.90	0.10	0.5	21.9

The experimental site with N addition was set up on 10 July 2020 and was then followed by high (H), medium (M), and low (L) salinity levels without N addition and three soil salinity levels with N addition (HN, MN, and LN, respectively), with three replicates for each treatment. Within each salinity level, 6 plots (2 m × 2 m), including three N addition plots and three control plots, were randomly established. The distance between any two adjacent plots within a stand was 5 m. To achieve the N addition, N was added to the plots in the form of urea by dissolution in deionized water with application, for a total of 20 g N m⁻² [28]. The N addition rates were in accordance with the N deposition rates in the Yellow River Delta. Before the N addition experiment, the living plants were removed inside the plots to ensure that the initial state of the test was consistent.

2.3. Sampling and Analyses

The growth indices of *P. australis* in each plot were measured at 20, 30, 40, 50, 60, and 70 d after N addition, including plant height and leaf soil plant analysis development (SPAD) values. The leaf SPAD values indicated the level of chlorophyll and were measured with a SPAD-502 (Minolta Camera Co. Ltd., Osaka, Japan) portable chlorophyll meter. Ten healthy and fully expanded leaves were randomly measured at the middle layer leaf and averaged to a single SPAD value for each experimental plot [29].

To measure aboveground biomass, *P. australis* and other plant stems in each plot were cut at the soil level 70 d after N addition, the period of maximum biomass of *P. australis*. The aboveground biomass was separated by species. The aboveground stems of *P. australis* were dried at 70 °C for 48 h and weighed. Vegetation diversity in each plot was calculated using Shannon–Weiner diversity [30].

Soil was collected at 30, 50, and 70 d after N addition. Five soil cores were taken at two depths in each plot and combined into one mixed sample. The soil samples were immediately transported to the laboratory and sieved through a 2 mm mesh to determine the physicochemical properties.

2.4. Soil Properties

The soil water content (SWC) was measured by the oven-drying method [31]. The soil electrical conductivity (EC) and pH were measured using a 1:5 soil:water solution (*w/v*). The amount of 0.5 M NaHCO₃ was used to extract soil available phosphorus (P) contents (Avail. P) using an ultraviolet spectrometer (UV2600, Shimadzu, Kyoto, Japan). Fresh soils were extracted with 2 M KCl, and the extracts were used to determine soil NO₃⁻ using an autoanalyzer (AA3, Bran-Luebbe, Norderstedt, Germany).

The mean values of soil properties at 0–20 and 20–40 cm represented the soil properties in the upper layer. The mean values of soil properties at 40–60, 60–80, and 80–100 cm represented the soil properties in the deeper layer.

2.5. Data Analysis

One-way ANOVA with least significant difference (LSD) multiple comparisons was performed to explore the differences in growth indices of *P. australis* and soil physicochemical properties. Two-way ANOVA was applied to examine the main and interactive effects of N addition and salinity levels on *P. australis* biomass, dry matter, plant moisture and vegetation diversity. Multiple-factor repeated-measures ANOVA was used to assess the effects of N addition, salinity levels, growth stage, and soil depth as fixed factors on plant growth indices and soil physicochemical properties.

We used linear mixed models to evaluate the effect of soil characteristics in the upper and deeper layers on the dependent variables with growth indices of *P. australis* developed in the “lme4” and “lmerTest” packages [32]. This allowed the potential differences in variance among mesocosms to be considered when evaluating the coefficients of the models and their confidence intervals.

All statistical analyses were performed by using R 3.6.2.

3. Results

3.1. Soil pH and Water Contents in Different Growth Stages

The mean values of soil pH under high, medium, and low salinity levels were 8.1, 8.2, and 8.7, respectively (Figure 2a). The soil pH increased with the decrease in soil salinity levels. N addition increased soil pH under medium and low salinity levels ($p < 0.05$) during the growth periods and had no significant effect on soil pH under high salinity levels. Meanwhile, soil pH was also significantly affected by the soil depth and growth stages of *P. australis* (Table 2).

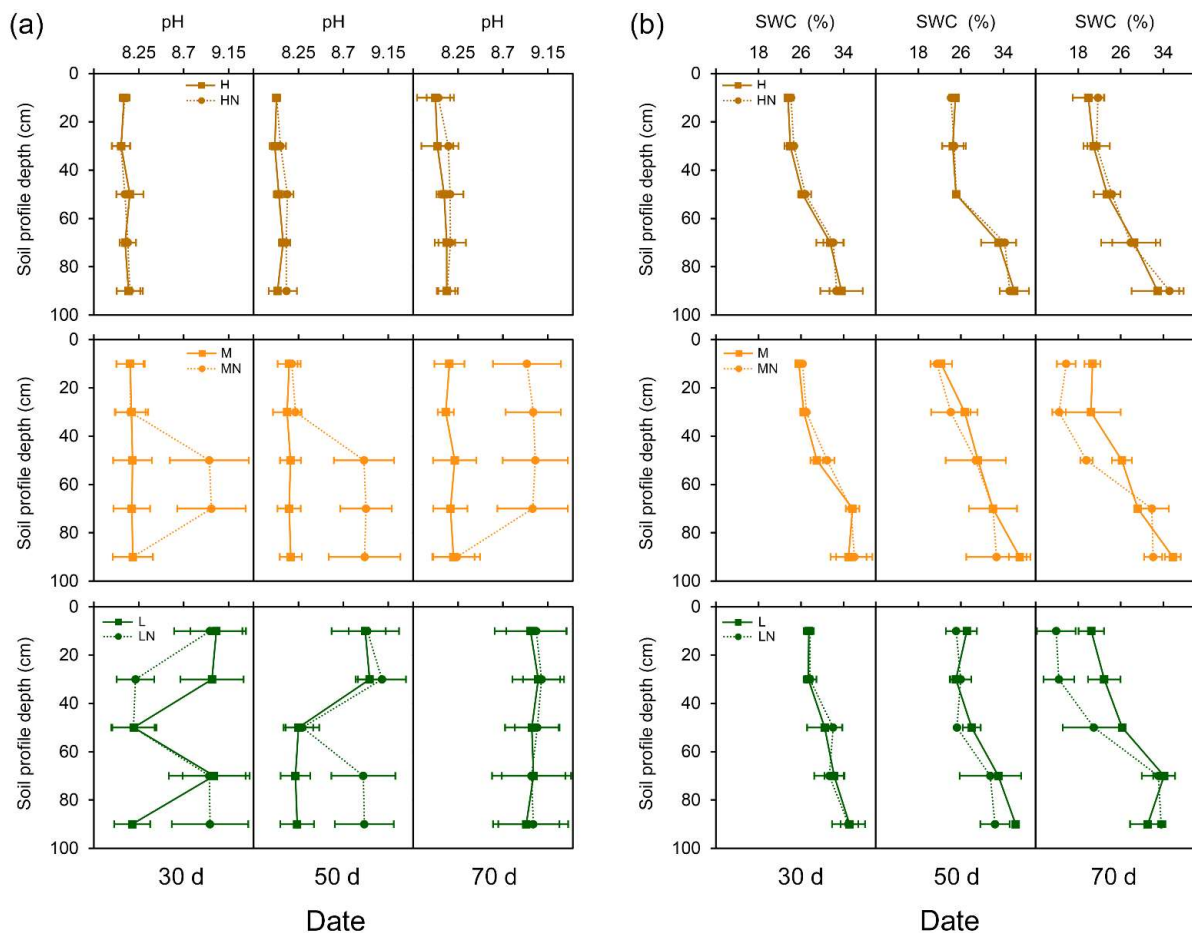


Figure 2. Soil pH (a) and water contents (SWC) (b) in the soil profile (0–100 cm) during the experimental periods.

Table 2. Results of multiple-way ANOVA on the effects of salinity (S), N addition (N), growth stage (G), and soil depth (D) and their interactions on soil pH and water contents.

Soil Properties	Salinity (S)	N Addition (N)	Growth Stage (G)	Soil Depth (D)	S × N
pH	***	***	***	*	***
SWC	*	**	***	***	**

The multiple-way ANOVA analysis results are also shown for indicating the significance of main and interaction effects: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

The soil salinity levels significantly changed the SWC in the upper layer (0–40 cm) at 30 d (Figure 2b). Compared with the SWC under the high salinity level, the SWC under the low salinity level increased by 14.2% and 16.6% at 0–20 and 20–40 cm, respectively, at 30 d ($p < 0.05$). However, the soil salinity level had no significant effect on the SWC in the deeper layer (40–100 cm). Compared with the effects of soil salinity and N addition, the soil depth had more significant effects on the SWC (Table 2), which increased with increasing soil depth.

3.2. Soil Salt and Nutrient Characteristics in Different Growth Stages

The soil EC increased with the increase in soil salinity levels and was also not significantly affected by N addition (Figure 3a). The soil EC increased with the growth of *P. australis* in the soil depth profiles and decreased with increasing soil depth ($p < 0.001$, Table 3).

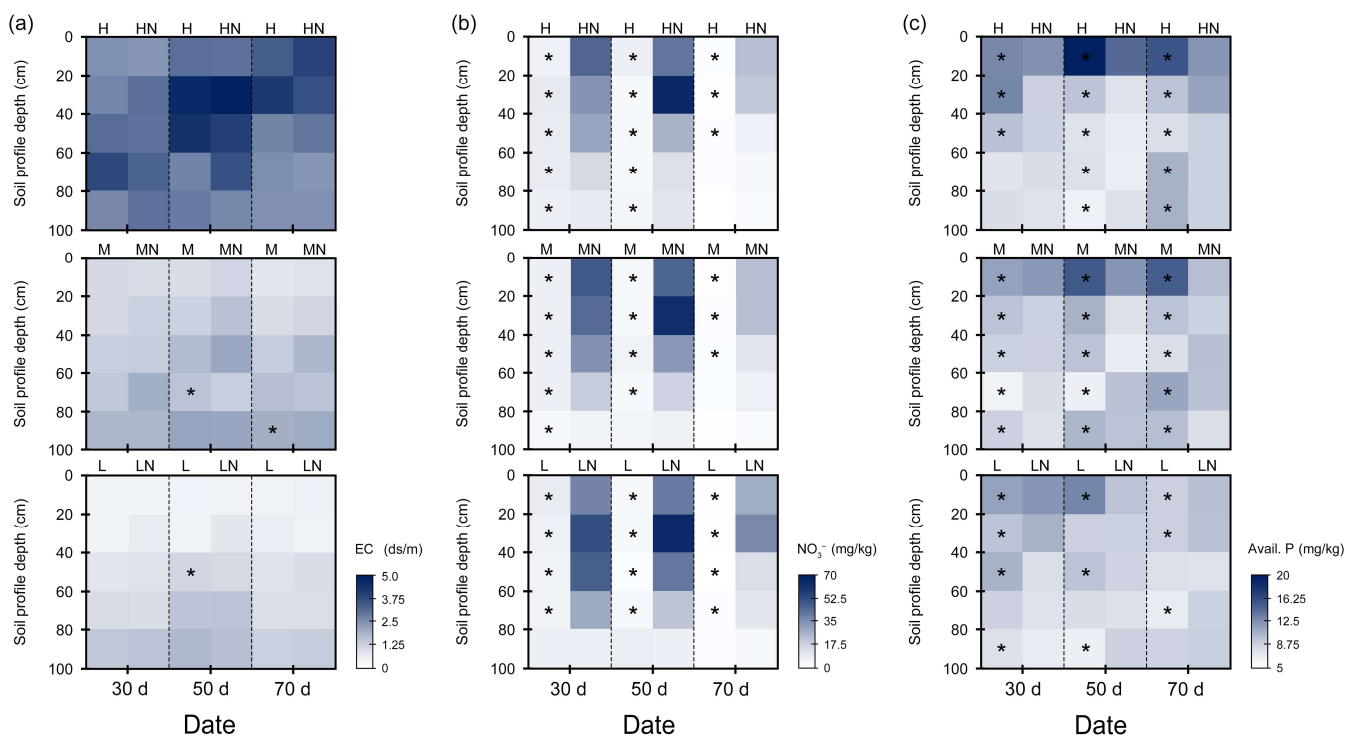
**Figure 3.** Soil EC (a), NO_3^- contents (b), and Avail. P (c) in the soil profile (0–100 cm) during the experimental periods. * Indicates a significant difference between the plots without N addition and with N addition in terms of soil characteristics under the same soil salinity level in the same determination period ($p < 0.05$).

Table 3. Results of multiple-way ANOVA on the effects of salinity (S), N addition (N), growth stage (G), soil depth (D) and their interactions on soil salt and nutrient characteristics.

Soil Properties	Salinity (S)	N Addition (N)	Growth Stage (G)	Soil Depth (D)	S × N
EC	***	*	***	***	—
NO ₃ [−]	*	***	***	***	***
Avail. P	***	***	—	***	***

The multiple-way ANOVA analysis results are also shown for indicating the significance of main and interaction effects: ***, $p < 0.001$; *, $p < 0.05$; —, not significant.

Soil NO₃[−] contents were not significantly affected by soil salinity but were significantly changed by N addition (Figure 3b). Under the three soil salinity levels at 30d, the soil NO₃[−] contents increased from 5.6 mg kg^{−1} to 45.8 mg kg^{−1} ($p < 0.05$) in the upper layer (0–40 cm) and increased from 4.9 mg kg^{−1} to 20.5 mg kg^{−1} ($p < 0.05$) in the deeper layer (40–100 cm). With the growth of *P. australis*, N addition still affected soil NO₃[−] contents in the upper layer but had no significant changes in the deeper layer. The soil NO₃[−] contents decreased with the growth of *P. australis* and the increase in the soil depth ($p < 0.001$, Table 3).

Soil salinity levels and N addition had significant effects on the soil Avail. P (Figure 3c, Table 3). During the three growth stages, the soil Avail. P in the upper layer under the high salinity level was 14.5% and 55.9% greater than that under the medium and low salinity levels, respectively ($p < 0.05$). However, the soil salinity levels did not significantly change the soil Avail. P in the deeper layer. Under high and medium salinity levels, N addition decreased the soil Avail. P during experimental periods at the 0–40 cm depth by 5.6–41.3% ($p < 0.05$). The soil Avail. P with N addition at the 40–100 cm depth was also lower than that without N addition ($p < 0.05$). The soil Avail. P decreased with increasing soil depth ($p < 0.001$) but was not affected by the growth of *P. australis* (Table 3).

3.3. Growth Indices of *P. australis*

The soil salinity levels had significant effects on the growth indices of *P. australis* (Figure 4a). With increasing soil salinity levels, the plant height increased during the growth periods. For the influence of N addition, the plant height with N addition was greater than that without N addition during 20 d–30 d; in particular, a significant difference in plant height between the treatments with and without N addition existed under high and medium soil salinity levels ($p < 0.05$). With the growth of *P. australis*, the plant height without N addition was greater than that with N addition. During 50 d–70 d, the plant height with N addition decreased by 44.8–85.6% ($p < 0.05$) under the high soil salinity level, decreased by 8.7–11.9% under the medium soil salinity level, and decreased by 8.8–45.6% ($p < 0.05$) under the low soil salinity level compared with those without N addition.

Consistent with the effect of soil salinity levels on the plant height of *P. australis*, leaf SPAD values also increased with the soil salinity levels. Under the high salinity level, the leaf SPAD values with N addition were 11.6% and 24.1% greater than those without N addition at 20 d and 30 d, respectively. During 50 d–70 d, N addition decreased leaf SPAD values by 8.6–12% ($p < 0.05$) under the high salinity level. Under medium and low salinity levels, N addition had no significant effect on leaf SPAD values. Therefore, the soil salinity significantly affected the leaf SPAD values ($p < 0.001$), which were not significantly affected by N addition (Table 4).

The effects of soil salinity levels on the biomass and dry matter of *P. australis* were significant. The biomass and dry matter of *P. australis* under the high salinity level were significantly greater than those under the medium and low salinity levels ($p < 0.05$, Table 5). N addition decreased the biomass and dry matter of *P. australis* by 35.4–39.1% ($p < 0.05$) and 36.2–40.3% ($p < 0.05$), respectively, compared with those without N addition under the three soil salinity levels ($p < 0.05$). The plant moisture under the high soil salinity level was greater than that under the medium and low salinity levels. Compared with the effects of N addition, the soil salinity levels had more significant effects on the plant moisture ($p < 0.001$). For the vegetation diversity, the treatment with the medium salinity level had

the greatest values among the six treatments, which was significantly greater than the vegetation diversity under the high salinity level ($p < 0.05$). Under the three salinity levels, N addition increased the vegetation diversity in the plots ($p < 0.05$).

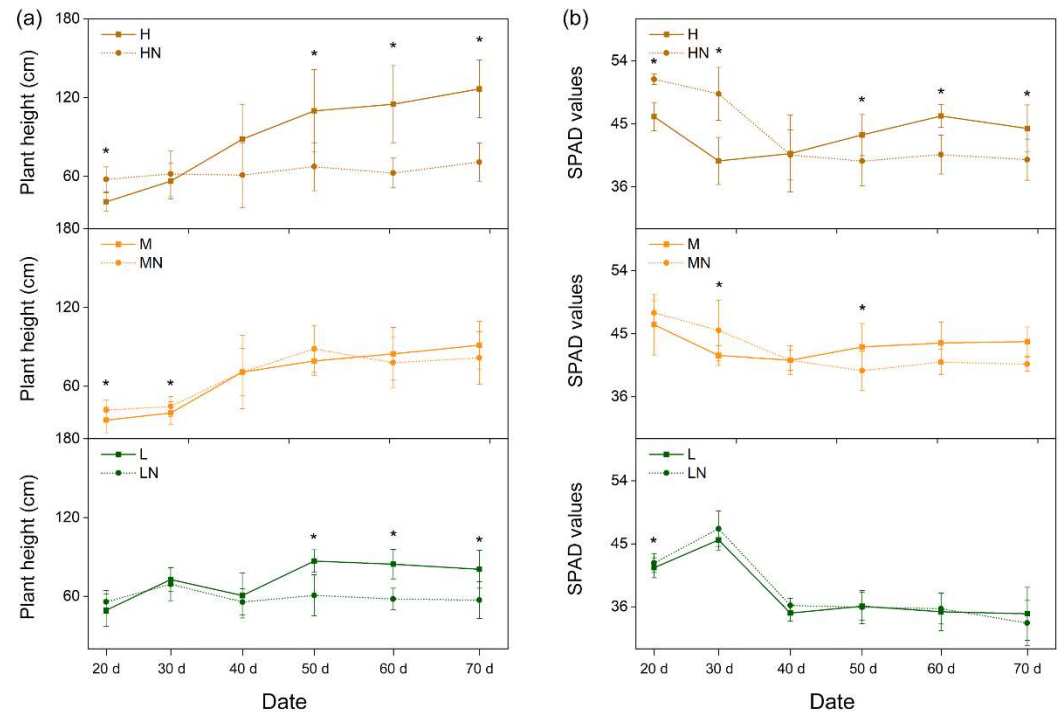


Figure 4. Plant height (a) and leaf SPAD values (b) of *P. australis* during experimental periods. * Indicates a significant difference between the plots without N addition and with N addition in terms of soil characteristics under the same soil salinity level in the same determination period ($p < 0.05$).

Table 4. Results of multiple-way ANOVA on the effects of salinity (S), N addition (N), growth stage (G), and soil depth (D) and their interactions on growth indices of *P. australis*.

Growth Indices	Salinity (S)	N Addition (N)	Growth Stage (G)	S × N	S × N × G
Plant height	***	***	***	***	***
SPAD	***	—	***	—	*

The multiple-way ANOVA analysis results are also shown for indicating the significance of main and interaction effects: ***, $p < 0.001$; *, $p < 0.05$; —, not significant.

Table 5. Mean values and results of multiple-way ANOVA on the effects of salinity (S), N addition (N), and their interactions (S × N) on aboveground biomass and other growth indices of *P. australis*.

Treatments	Biomass (t ha ⁻¹ F.W.)	Dry Matter (t ha ⁻¹ D.W.)	Plant Moisture (%)	Vegetation Diversity
H	33.5 ± 1.2 a	18.1 ± 0.8 a	53.8 ± 0.6 a	0.50 ± 0.01 c
HN	21.7 ± 0.5 c	11.5 ± 0.3 c	53.2 ± 0.3 ab	0.75 ± 0.01 b
M	28.5 ± 1.1 b	14.7 ± 0.6 b	51.5 ± 0.6 b	0.82 ± 0.02 b
MN	18 ± 0.4 d	8.8 ± 0.2 d	48.6 ± 0.3 c	1.36 ± 0.02 a
L	12.5 ± 0.5 e	5.9 ± 0.3 e	47 ± 0.5 c	0.44 ± 0.01 c
LN	7.6 ± 1.2 f	3.6 ± 0.5 f	46.9 ± 1 c	0.74 ± 0.06 b
S	***	***	***	***
N	***	***	*	***
S × N	**	**	—	***

Mean values ± S.E.s (n = 3) are displayed. Significant differences are denoted by letters ($p < 0.05$). The two-way ANOVA analysis results are also shown for indicating the significance of main (S, salinity levels; N, N addition) and interaction effects: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; —, not significant.

3.4. The Relationship between *P. australis* Growth and Soil Characteristics

For growth indices of *P. australis*, we estimated the fixed effects of the soil characteristics at different soil depths (SWC and Avail. P in the upper and deeper layers, NO_3^- , pH and EC) at the three growth stages of *P. australis* with linear mixed models (Figure 5). We found significant positive effects of soil Avail. P in the upper layer on plant height (estimate = 4.35, $p < 0.01$) and leaf SPAD values (estimate = 0.922, $p < 0.01$). In particular, an increase in the biomass of *P. australis* with increasing soil Avail. P contents was also observed (Figure 5c). For vegetation diversity in the plots, significant positive effects of soil Avail. P in the deeper layer (estimate = 0.09, $p < 0.05$) occurred (Figure 5d). The soil pH had significant negative effects on the biomass of *P. australis* (estimate = -4.8 , $p < 0.001$) and vegetation diversity (estimate = -0.28 , $p < 0.05$).

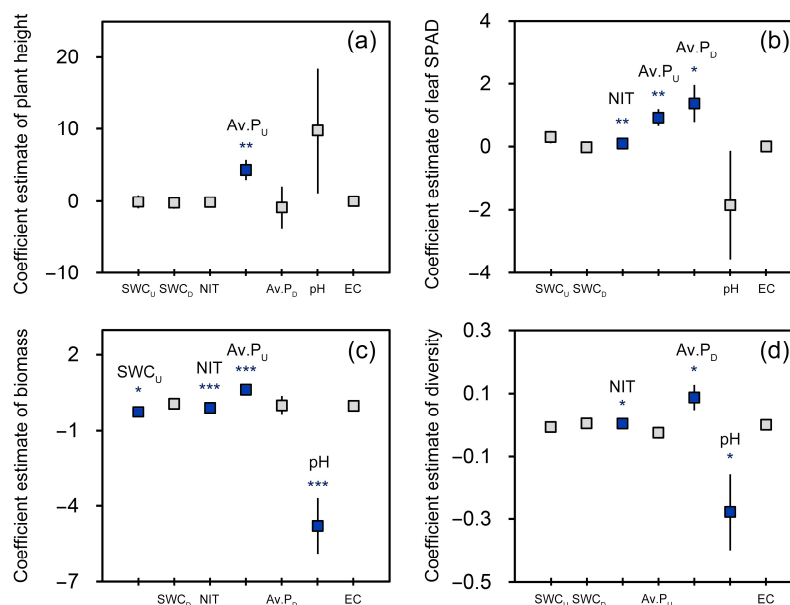


Figure 5. Relationship between growth indices of *P. australis* and soil properties. The coefficient estimate of plant height (a, AIC = 475.2, $R^2 = 0.56$), leaf SPAD value (b, AIC = 328.2, $R^2 = 0.69$), biomass (c, AIC = 285.8, $R^2 = 0.63$) and vegetation diversity (d, AIC = 81.79, $R^2 = 0.64$) effect sizes with $\pm 95\%$ confidence intervals, while accounting for the random effect of soil properties tested using the linear mixed-effects model. Mean coefficient estimates are significant if their 95% confidence intervals did not contain 0. Negative or positive trend values indicate temporal decrease or increase, respectively. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$. SWC_U, soil water content in the upper layer (0–40 cm). SWC_D, soil water content in the deeper layer (40–100 cm). NIT, soil NO_3^- contents. Av. P_U, soil Avail. P contents in the upper layer. Av. P_D, soil Avail. P contents in the deeper layer.

4. Discussion

4.1. Responses of Soil Characteristics at Salinity Levels to N Addition

N inputs directly changed the soil salinity, soil pH, inorganic N pools, and other soil chemical characteristics [33,34]. The high background values of soil salinity in saline–alkali soils mitigated the effects of N addition on soil salinity [35,36]. Soil electrical conductivity (EC) is a measurement that correlates with soil properties, including soil water-soluble base cations and cation exchange capacity [37]. Therefore, we reported that N addition did not change the soil EC values (Figure 3a). N addition significantly improved soil nitrification rates, resulting in more released H^+ [38,39]. Therefore, N addition decreased soil pH and intensified soil acidification [40]. The complicated ion constitution in saline–alkali soils reduced the impact of short-term N inputs on soil pH [41]. In this study, N addition increased soil pH under high and medium salinity levels in the upper layer (Figure 2a), caused by NH_4^+ leaching into the upper layer. Moreover, the increased soil salt contents by N inputs directly decreased the soil water potential and mitigated soil water evaporation

rates; therefore, N addition could maintain moisture in the soil [42]. Our results also suggested that N addition increased the soil water contents under medium and low salinity levels at 30 d (Figure 2b). Therefore, soil salinity levels significantly affected the effects of N addition on soil pH and water contents.

Urea, as an N source, directly increased soil inorganic N pools and soil nitrification rates, resulting in increased soil NO_3^- contents. Due to leaching, sedimentation and nitrification, the effects of N addition on soil NO_3^- contents were reduced with increased soil depth [43]. We also reported that N addition increased the soil NO_3^- contents in the upper layer at the three salinity levels (Figure 3b). With the growth of *P. australis*, N addition increased soil NO_3^- contents in the deeper layer, caused by NO_3^- leaching along the soil profile. Under the wetting–redrying alternating environment in the saline–alkali wasteland and estuarine wetland ecosystems, the stable soil Avail. P contents caused by the slow decomposition of soil organic matter and plant litter did not increase microbial P limitation [44]. Previous studies [36,45] have suggested that the effects of N addition on soil total and available P contents were not significant. However, N addition improved the soil phosphatase activities and increased the soil P availability, enhancing the acquisition of P by plants [46]. The increased plant P uptake decreased the soil Avail. P contents. Our results also indicated that N addition decreased soil Avail. P contents (Figure 3c). Therefore, N addition increased the soil NO_3^- contents and decreased soil Avail. P contents, but soil salinity levels did not change the effects of N addition on soil NO_3^- and Avail. P contents.

4.2. Effects of N Addition on Growth Indices of *P. australis* at Different Salinity Levels

High plant height and leaf chlorophyll improve access to light and increase the photosynthetic carbon-assimilating pathway, resulting in greater biomass and promoting the growth of *P. australis* [11,47,48]. Under salt stress, *P. australis* adapts to saline conditions by adjusting the distribution of photosynthate [49]. Therefore, the biomass of *P. australis* increases with increasing soil salt content, which is basically consistent with the results of the effects of salinity levels on the biomass and dry matter of *P. australis* in this study (Table 5). Sufficient available N stimulates the dry matter production of *P. australis*, whereas superfluous N supply results in the death of the plants [14,15]. In this study, N addition increased the plant height of *P. australis* during 20 d–30 d, especially under high and medium salinity levels (Figure 4a). N addition supported available N for plant growth, resulting in an increase in tolerance to salt stress in *P. australis* [50].

As limiting factors of chlorophyll biosynthesis, N inputs directly regulate the chlorophyll content and SPAD values of salt marsh plants [51]. Our results suggested that N addition increased leaf SPAD values from 20 d to 30 d (Figure 4b). Along with the phenological development of *P. australis*, N addition decreased the plant height and leaf SPAD values (Figure 4). Previous studies have suggested that superfluous N supply caused a lack of mineral elements related to chlorophyll biosynthesis (e.g., Ca^{2+} , Mg^{2+}), decreasing the leaf chlorophyll contents [52,53]. However, we suggest that N addition changes the competitive relationship for N sources among plant communities under high salinity levels, resulting in the loss of competitiveness of *P. australis* [54,55]. Therefore, more available N is taken up by other superior plants, limiting the N uptake and chlorophyll biosynthesis of *P. australis*. We also report that N addition increased the vegetation diversity and decreased the biomass of *P. australis* (Table 5), which proved that N addition affected the N uptake of *P. australis* by changing the plant communities.

4.3. Relationship between *P. australis* Growth and Soil Characteristics

In response to N addition, the differentiation of soil characteristics affected plant communities. The changes in soil characteristics and plant communities both affected *P. australis* growth. N sources are essential for *P. australis* growth and development, whereas N addition changes the competitiveness for N sources of *P. australis* by improving vegetation diversity, resulting in decreased *P. australis* growth. Therefore, soil available N contents under salt conditions may not affect or limit the growth of *P. australis*. The organic acid

secreted from the rhizosphere of *P. australis* solubilizes soil sediment P [56]. Therefore, *P. australis* with greater biomass and developed roots might be related to an increase in soil Avail. P contents. Our results of linear mixed models suggested an increase in biomass, plant height, and leaf SPAD values of *P. australis* with increasing soil Avail. P contents instead of soil NO_3^- contents (Figure 5). This phenomenon is most likely attributed to the coordinated effects of N and P on plant growth [57]. Therefore, we suggest the important role of available P addition in N enrichment conditions in saline–alkali wasteland and estuarine wetland ecosystems.

5. Conclusions

Our study demonstrates that soil salinity levels significantly affected the effects of N addition on soil pH and water contents. N addition increased the soil NO_3^- contents and decreased soil Avail. P contents, but soil salinity levels did not change the effects of N addition on soil NO_3^- and Avail. P contents. N addition increased the plant height and leaf SPAD values of *Phragmites australis* during the jointing stage under high and medium salinity levels. With the growth of *P. australis*, N addition decreased plant height, leaf SPAD values, and biomass, since the decrease in the competitiveness for N sources of *P. australis* changed the vegetation diversity. The results of linear mixed models suggested an increase in biomass, plant height, and leaf SPAD values of *P. australis* with increasing soil Avail. P contents instead of soil NO_3^- contents. Therefore, we suggest the important role of available P addition in N enrichment conditions in saline–alkali wasteland and estuarine wetland ecosystems.

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References

1. Li, L.; Zerbe, S.; Han, W.; Thevs, N.; Li, W.; He, P.; Schmitt, A.O.; Liu, Y.; Ji, C. Nitrogen and phosphorus stoichiometry of common reed (*Phragmites australis*) and its relationship to nutrient availability in northern China. *Aquat. Bot.* **2014**, *112*, 84–90. [\[CrossRef\]](#)
2. Rezaei, S.; Park, J.; Rupani, P.F.; Darajeh, N.; Xu, X.; Shahrokhishahraki, R. Phytoremediation potential and control of *Phragmites australis* as a green phytomass: An overview. *Environ. Sci. Pollut. Res.* **2019**, *26*, 7428–7441. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Minkina, T.; Fedorenko, G.; Nevidomskaya, D.; Fedorenko, A.; Chaplygin, V.; Mandzhieva, S. Morphological and anatomical changes of *Phragmites australis* Cav. due to the uptake and accumulation of heavy metals from polluted soils. *Sci. Total Environ.* **2018**, *636*, 392–401. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Liu, X.; Zhang, Y.; Li, X.; Fu, C.; Shi, T.; Yan, P. Effects of influent nitrogen loads on nitrogen and COD removal in horizontal subsurface flow constructed wetlands during different growth periods of *Phragmites australis*. *Sci. Total Environ.* **2018**, *635*, 1360–1366. [\[CrossRef\]](#)
5. Wang, J.; Chen, G.; Fu, Z.; Qiao, H.; Liu, F. Assessing wetland nitrogen removal and reed (*Phragmites australis*) nutrient responses for the selection of optimal harvest time. *J. Environ. Manag.* **2021**, *280*, 111783. [\[CrossRef\]](#)

6. Feng, H.; Zhao, H.; Xia, L.; Yang, W.; Zhao, Y.; Jeelani, N.; An, S. Nitrogen cycling in plant and soil subsystems is driven by changes in soil salinity following coastal embankment in typical coastal saltmarsh ecosystems of Eastern China. *Ecol. Eng.* **2022**, *174*, 106467. [\[CrossRef\]](#)
7. Hazelton, E.L.; Mozdzer, T.J.; Burdick, D.M.; Kettenring, K.M.; Whigham, D.F. *Phragmites australis* management in the United States: 40 years of methods and outcomes. *AoB Plants* **2014**, *6*, plu001. [\[CrossRef\]](#)
8. Leghari, S.J.; Wahocho, N.A.; Laghari, G.M.; HafeezLaghari, A.; MustafaBhabhan, G.; HussainTalpur, K.; Bhutto, T.A.; Wahocho, S.A.; Lashari, A.A. Role of nitrogen for plant growth and development: A review. *Adv. Environ. Biol.* **2016**, *10*, 209–219.
9. Luo, L.; Zhang, Y.; Xu, G. How does nitrogen shape plant architecture? *J. Exp. Bot.* **2020**, *71*, 4415–4427. [\[CrossRef\]](#)
10. Zhang, X.; Davidson, E.A.; Mauzerall, D.L.; Searchinger, T.D.; Dumas, P.; Shen, Y. Managing nitrogen for sustainable development. *Nature* **2015**, *528*, 51–59. [\[CrossRef\]](#)
11. Jordan, S.J.; Stoffer, J.; Nestlerode, J.A. Wetlands as sinks for reactive nitrogen at continental and global scales: A meta-analysis. *Ecosystems* **2011**, *14*, 144–155. [\[CrossRef\]](#)
12. Zhou, M.; Yan, G.; Xing, Y.; Chen, F.; Zhang, X.; Wang, J.; Zhang, J.; Dai, G.; Zheng, X.; Sun, W. Nitrogen deposition and decreased precipitation does not change total nitrogen uptake in a temperate forest. *Sci. Total Environ.* **2019**, *651*, 32–41. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Seghatoleslami, M.J.; Bradaran, R.; Ansarinia, E.; Mousavi, S.G. Effect of irrigation and nitrogen level on yield, yield components and some morphological traits of sunflower. *Pak. J. Bot.* **2012**, *44*, 1551–1555.
14. Ravit, B.; Ehrenfeld, J.G.; Häggblom, M.M.; Bartels, M. The effects of drainage and nitrogen enrichment on *Phragmites australis*, *Spartina alterniflora*, and their root-associated microbial communities. *Wetlands* **2007**, *27*, 915–927. [\[CrossRef\]](#)
15. Clevering, O.A. An investigation into the effects of nitrogen on growth and morphology of stable and die-back populations of *Phragmites australis*. *Aquat. Bot.* **1998**, *60*, 11–25. [\[CrossRef\]](#)
16. Koppitz, H.; Dewender, M.; Ostendorp, W.; Schmieder, K. Amino acids as indicators of physiological stress in common reed *Phragmites australis* affected by an extreme flood. *Aquat. Bot.* **2004**, *79*, 277–294. [\[CrossRef\]](#)
17. Brooks, M.L. Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. *J. Appl. Ecol.* **2003**, *40*, 344–353. [\[CrossRef\]](#)
18. Jiang, L.; Luo, Y.; Chen, J.; Li, B. Ecophysiological characteristics of invasive *Spartina alterniflora* and native species in salt marshes of Yangtze River estuary, China. *Estuar. Coast. Shelf Sci.* **2009**, *81*, 74–82. [\[CrossRef\]](#)
19. Qing, H.; Cai, Y.; Xiao, Y.; Yao, Y.; An, S. Nitrogen uptake and use efficiency of invasive *Spartina alterniflora* and native *Phragmites australis*: Effect of nitrogen supply. *Clean Soil Air Water* **2015**, *43*, 305–311. [\[CrossRef\]](#)
20. Mao, W.; Kang, S.; Wan, Y.; Sun, Y.; Li, X.; Wang, Y. Yellow river sediment as a soil amendment for amelioration of saline land in the Yellow River Delta. *Land Degrad. Dev.* **2016**, *27*, 1595–1602. [\[CrossRef\]](#)
21. Zhang, H.; Sun, Y.; Zhang, W.; Song, Z.; Ding, Z.; Zhang, X. Comprehensive evaluation of the eco-environmental vulnerability in the Yellow River Delta wetland. *Ecol. Indic.* **2021**, *125*, 107514. [\[CrossRef\]](#)
22. Xia, J.; Ren, J.; Zhang, S.; Wang, Y.; Fang, Y. Forest and grass composite patterns improve the soil quality in the coastal saline-alkali land of the Yellow River Delta, China. *Geoderma* **2019**, *349*, 25–35. [\[CrossRef\]](#)
23. Sun, X.-S.; Chen, Y.-H.; Zhuo, N.; Cui, Y.; Luo, F.-L.; Zhang, M.-X. Effects of salinity and concomitant species on growth of *Phragmites australis* populations at different levels of genetic diversity. *Sci. Total Environ.* **2021**, *780*, 146516. [\[CrossRef\]](#)
24. Munzarova, E.; Lorenzen, B.; Brix, H.; Vojtiskova, L.; Votrubova, O. Effect of $\text{NH}_4^+/\text{NO}_3^-$ availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*. *Environ. Exp. Bot.* **2006**, *55*, 49–60. [\[CrossRef\]](#)
25. Lu, G.; Xie, B.; Cagle, G.A.; Wang, X.; Han, G.; Wang, X.; Hou, A.; Guan, B. Effects of simulated nitrogen deposition on soil microbial community diversity in coastal wetland of the Yellow River Delta. *Sci. Total Environ.* **2021**, *757*, 143825. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Zhang, G.; Bai, J.; Zhao, Q.; Jia, J.; Wang, W.; Wang, X. Bacterial succession in salt marsh soils along a short-term invasion chronosequence of *Spartina alterniflora* in the Yellow River Estuary, China. *Microb. Ecol.* **2020**, *79*, 644–661. [\[CrossRef\]](#)
27. Zhu, W.; Rezaei, E.E.; Nouri, H.; Yang, T.; Li, B.; Gong, H.; Lyu, Y.; Peng, J.; Sun, Z. Quick detection of field-scale soil comprehensive attributes via the integration of UAV and sentinel-2B remote sensing data. *Remote Sens.* **2021**, *13*, 4716. [\[CrossRef\]](#)
28. Yu, G.; Jia, Y.; He, N.; Zhu, J.; Chen, Z.; Wang, Q.; Piao, S.; Liu, X.; He, H.; Guo, X. Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nat. Geosci.* **2019**, *12*, 424–429. [\[CrossRef\]](#)
29. Nasso, N.; Roncucci, N.; Bonari, E. Seasonal dynamics of aboveground and belowground biomass and nutrient accumulation and remobilization in giant reed (*Arundo donax* L.): A three-year study on marginal land. *BioEnergy Res.* **2013**, *6*, 725–736. [\[CrossRef\]](#)
30. Spellerberg, I.F.; Fedor, P.J. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the ‘Shannon–Wiener’ Index. *Glob. Ecol. Biogeogr.* **2003**, *12*, 177–179. [\[CrossRef\]](#)
31. Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H. *Methods of Soil Analysis, Part 3: Chemical Methods*; John Wiley & Sons: New York, NY, USA, 2020; Volume 14.
32. Luke, S.G. Evaluating significance in linear mixed-effects models in R. *Behav. Res. Methods* **2017**, *49*, 1494–1502. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Lu, M.; Yang, Y.; Luo, Y.; Fang, C.; Zhou, X.; Chen, J.; Yang, X.; Li, B. Responses of ecosystem nitrogen cycle to nitrogen addition: A meta-analysis. *New Phytol.* **2011**, *189*, 1040–1050. [\[CrossRef\]](#) [\[PubMed\]](#)

34. Midolo, G.; Alkemade, R.; Schipper, A.M.; Benítez-López, A.; Perring, M.P.; De Vries, W. Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. *Glob. Ecol. Biogeogr.* **2019**, *28*, 398–413. [\[CrossRef\]](#)
35. Wang, J.; Xu, T.; Feng, X.; Zhu, W.; Zhang, L.; Pan, D.; Akram, N.A.; Ma, Q.; Zhong, Z.; Mahroof, S. Simulated grazing and nitrogen addition facilitate spatial expansion of *Leymus chinensis* clones into saline-alkali soil patches: Implications for Songnen grassland restoration in northeast China. *Land Degrad. Dev.* **2022**, *33*, 710–722. [\[CrossRef\]](#)
36. Yin, Z.; Yu, X.; Zou, Y.; Ding, S.; Zhang, J. Nitrogen Addition Effects on Wetland Soils Depend on Environmental Factors and Nitrogen Addition Methods: A Meta-Analysis. *Water* **2022**, *14*, 1748. [\[CrossRef\]](#)
37. Grisso, R.D.; Alley, M.M.; Holshouser, D.L.; Thomason, W.E. *Precision Farming Tools. Soil Electrical Conductivity*; Virginia State University: Ettrick, VA, USA, 2005.
38. Šimek, M.; Cooper, J. The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years. *Eur. J. Soil Sci.* **2002**, *53*, 345–354. [\[CrossRef\]](#)
39. Kuypers, M.M.; Marchant, H.K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276. [\[CrossRef\]](#)
40. Tian, D.; Niu, S. A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.* **2015**, *10*, 024019. [\[CrossRef\]](#)
41. Moseman-Valtierra, S.; Gonzalez, R.; Kroeger, K.D.; Tang, J.; Chao, W.C.; Crusius, J.; Bratton, J.; Green, A.; Shelton, J. Short-term nitrogen additions can shift a coastal wetland from a sink to a source of N₂O. *Atmos. Environ.* **2011**, *45*, 4390–4397. [\[CrossRef\]](#)
42. Liu, C.; Yao, M.; Stegen, J.C.; Rui, J.; Li, J.; Li, X. Long-term nitrogen addition affects the phylogenetic turnover of soil microbial community responding to moisture pulse. *Sci. Rep.* **2017**, *7*, 17492. [\[CrossRef\]](#)
43. Long, G.; Li, L.; Wang, D.; Zhao, P.; Tang, L.; Zhou, Y.; Yin, X. Nitrogen levels regulate intercropping-related mitigation of potential nitrate leaching. *Agric. Ecosyst. Environ.* **2021**, *319*, 107540. [\[CrossRef\]](#)
44. Bostic, E.; White, J. Soil phosphorus and vegetation influence on wetland phosphorus release after simulated drought. *Soil Sci. Soc. Am. J.* **2007**, *71*, 238–244. [\[CrossRef\]](#)
45. Whigham, D.F.; Verhoeven, J.T.; Samarkin, V.; Megonigal, P.J. Responses of *Avicennia germinans* (black mangrove) and the soil microbial community to nitrogen addition in a hypersaline wetland. *Estuaries Coasts* **2009**, *32*, 926–936. [\[CrossRef\]](#)
46. Li, Y.; Niu, S.; Yu, G. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: A meta-analysis. *Glob. Change Biol.* **2016**, *22*, 934–943. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Falster, D.S.; Westoby, M. Plant height and evolutionary games. *Trends Ecol. Evol.* **2003**, *18*, 337–343. [\[CrossRef\]](#)
48. Liang, X.; Zhang, T.; Lu, X.; Ellsworth, D.S.; BassiriRad, H.; You, C.; Wang, D.; He, P.; Deng, Q.; Liu, H.; et al. Global response patterns of plant photosynthesis to nitrogen addition: A meta-analysis. *Glob. Change Biol.* **2020**, *26*, 3585–3600. [\[CrossRef\]](#)
49. Lissner, J.; Schierup, H.-H. Effects of salinity on the growth of *Phragmites australis*. *Aquat. Bot.* **1997**, *55*, 247–260. [\[CrossRef\]](#)
50. Abd-Alla, M.H.; Nafady, N.A.; Bashandy, S.R.; Hassan, A.A. Mitigation of effect of salt stress on the nodulation, nitrogen fixation and growth of chickpea (*Cicer arietinum* L.) by triple microbial inoculation. *Rhizosphere* **2019**, *10*, 100148. [\[CrossRef\]](#)
51. Hassan, M.S.; Khair, A.; Haque, M.M.; Azad, A.K.; Hamid, A. Genotypic variation in traditional rice varieties for chlorophyll content, SPAD value and nitrogen use efficiency. *Bangladesh J. Agric. Res.* **2009**, *34*, 505–515. [\[CrossRef\]](#)
52. Zhao, D.; Raja Reddy, K.; Kakani, V.; Read, J.; Carter, G. Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. *Plant Soil* **2003**, *257*, 205–218. [\[CrossRef\]](#)
53. Weese, D.J.; Heath, K.D.; Dentinger, B.T.; Lau, J.A. Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution* **2015**, *69*, 631–642. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Rickey, M.A.; Anderson, R.C. Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. *J. Appl. Ecol.* **2004**, *41*, 888–896. [\[CrossRef\]](#)
55. Hirtreiter, J.; Potts, D. Canopy structure, photosynthetic capacity and nitrogen distribution in adjacent mixed and monospecific stands of *Phragmites australis* and *Typha latifolia*. *Plant Ecol.* **2012**, *213*, 821–829. [\[CrossRef\]](#)
56. Yu, J.; Zhong, J.; Chen, Q.; Huang, W.; Hu, L.; Zhang, Y.; Fan, C. An investigation of the effects of capping on internal phosphorus release from sediments under rooted macrophytes (*Phragmites australis*) revegetation. *Environ. Sci. Pollut. Res.* **2018**, *25*, 24682–24694. [\[CrossRef\]](#)
57. Jiang, J.; Wang, Y.-P.; Yang, Y.; Yu, M.; Wang, C.; Yan, J. Interactive effects of nitrogen and phosphorus additions on plant growth vary with ecosystem type. *Plant Soil* **2019**, *440*, 523–537. [\[CrossRef\]](#)