eLife

Reviewed Preprint
v1 • August 13, 2026
Not revised

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Competing interests: No competing interests declared

Funding: See page 17

Reviewing editor: Sergio Rasmann, University of Neuchâtel, Switzerland

Genetic canalization of nutrient resorption: evidence from a widespread grass under effective salt stress

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eLife Assessment

This **important** study demonstrates that nutrient resorption efficiency in the widespread wetland grass Phragmites australis is largely canalized by phylogeographic lineage, ecotype and geographic origin, rather than responding plastically to short-term salt stress. The findings have implications beyond plant ecophysiology because they suggest that predictions of wetland nutrient cycling under increasing salinization should account for the genetic composition of plant populations. The evidence supporting the central conclusions is **compelling**, based on a well-designed common-garden experiment involving 110 genotypes, paired control and salinity treatments, and convergent metabolomic, ionomic and whole-plant evidence confirming that the treatment imposed substantial physiological stress. The interpretation is nevertheless limited to one growing season and a single, moderate salinity treatment, leaving open whether chronic, more severe or multigenerational exposure might induce plastic or transgenerational responses; further clarification of the relationships among phylogeographic lineage, ecotype and latitude, and a more cautious interpretation of the variance explained by latitude, would strengthen the manuscript.

https://doi.org/10.7554/eLife.111792.1.sa3

Abstract

Nutrient resorption is a key plant strategy for nutrient conservation, yet whether it responds plastically to non-nutrient stressors such as salinity remains unresolved. Using a common garden experiment with 110 genotypes of the cosmopolitan grass *Phragmites australis*, we first established that the imposed salinity treatment induced strong multi-level stress responses: above-ground biomass declined by >60%, sodium accumulated 3- to 5-fold across all leaf stages, and 484 metabolites showed significant differential accumulation, including canonical markers of osmotic and oxidative stress. Against this backdrop of confirmed stress, we found that nutrient resorption efficiency (NuRE) remained largely unaffected by salinity. Instead, NuRE was strongly correlated with phylogeographic lineage, ecotype, and latitude of origin, demonstrating evolutionary canalization rather than short-term acclimation. Element-specific regulatory patterns were also evident: while phosphorus resorption followed concentration-dependent control regardless of stress, nitrogen control was disrupted under salinity, and potassium resorption showed no such dependence. Our findings reveal that intraspecific variation in nutrient resorption is

predominantly shaped by historical adaptation and geographic context, not by plasticity to salinity. This genetic canalization of a key functional trait implies that predictions of nutrient cycling under global change must account for the phylogeographic composition of plant populations.

1. Introduction

Nutrient resorption, the process by which plants translocate nutrients from senescing leaves to perennial tissues, is a pivotal conservation strategy that reduces dependence on soil nutrients and enhances nutrient use efficiency (Drenovsky et al., 2019; Jiang et al., 2023; Killingbeck, 1996). This mechanism is especially critical in nutrient-poor or stressful environments, influencing plant fitness, competitive ability, and ecosystem nutrient cycling (Yang et al., 2025). As a key functional trait, nutrient resorption efficiency (NRE) reflects adaptive responses to environmental conditions. A suite of drivers, operating across environmental and plant-intrinsic levels, governs nutrient resorption. Environmentally, soil nutrient availability, temperature, and water stress are established abiotic modulators (Chen et al., 2021). More profoundly, plant-intrinsic control is conceptualized through several non-exclusive physiological hypotheses (Kobe et al., 2005; Sun et al., 2023): the nutrient concentration control posits that resorption efficiency declines as green leaf nutrient concentration rises; the stoichiometric control hypothesis suggests nutrients are resorbed proportionally to maintain internal balance; and the nutrient limitation control predicts plants preferentially resorb the nutrient that most limits their growth. Understanding how these mechanisms operate within species across diverse populations remains an important objective in functional ecology.

Intraspecific variation may arise from phenotypic plasticity in response to immediate stressors (Ramirez-Parada et al., 2024; Zhang et al., 2022), or from inherent genetic differences among populations shaped by evolutionary history and local adaptation (Ferrer Obiol et al., 2025; Radersma et al., 2020). Disentangling their contribution to NRE is essential for predicting adaptive potential under environmental change (Zhang et al., 2022). However, most research on nutrient resorption has focused on its adaptation to nutrient limitation. Critically, its response to non-nutrient stresses, which disrupt plants through fundamentally different mechanisms like ionic toxicity and osmotic stress, remains a significant knowledge gap (Luo et al., 2021). Testing this gap is urgent because salinity stress is becoming a pervasive global change factor, albeit driven by distinct processes in different ecosystems (Zuo et al., 2024).

In coastal zones, salinity intrusion is primarily accelerated by sea-level rise and storm surges, threatening foundational wetland species and their biogeochemical functions (Seibert et al., 2024). In parallel, across arid and semi-arid inland regions, secondary soil salinization due to irrigation and groundwater over-exploitation is a major driver of land degradation and ecosystem alteration (Hassani et al., 2021). Despite these divergent drivers, the resultant physiological challenge to plants, ionic imbalance and water deficit, is convergent. This convergence provides a unique opportunity to investigate whether plants employ general or context-specific nutrient conservation strategies when facing salt stress, irrespective of its geographic origin.

Critically, predicting the direction of nutrient resorption responses to salinity remains challenging, rooted in two competing physiological and evolutionary perspectives (Luo et al., 2021; Zuo et al., 2024). On one hand, salinity imposes severe ionic and osmotic stress, disrupting membrane integrity, enzyme activity, and energy metabolism (Zuo et al., 2024). This could force a re-allocation of resources and potentially increase nutrient resorption efficiency as a compensatory conservation strategy to mitigate internal nutrient imbalance caused by uptake inhibition, consistent with the principles of nutrient limitation control (Kobe et al., 2005; Sun et al., 2023). Alternatively, nutrient resorption, particularly its stoichiometric relationships (e.g., N:P), may be under strong internal homeostasis control or subject to evolutionary canalization as a core nutrient conservation trait (Alseekh et al., 2025; Nicotra et al., 2010; Huang et al., 2022). If this is the case, resorption patterns may exhibit conservatism, remaining unaltered by short-term abiotic stressors like salinity, with variation being primarily dictated by inherent genetic differences among populations shaped by long-term adaptation (Ren et al., 2020; Liu et al., 2025). Disentangling these possibilities (plastic acclimation versus inherent conservatism) is

fundamental (Drenovsky et al., 2019; Prieto & Querejeta, 2020). It addresses whether this key functional trait (Killingbeck, 1996) can provide short-term flexibility to individual plants facing rapid environmental change, or whether ecosystem-level nutrient cycling responses will be constrained and predictable primarily from the genetic composition of plant populations.

These competing predictions can be tested by examining trait variation across natural geographic gradients. Geographic patterns, particularly across latitudinal and saline habitat gradients, offer a powerful lens to decipher the drivers of NRE (Ren et al., 2020; Liu et al., 2025). In plant ecology, latitudinal clines in functional traits often reflect adaptive responses to overarching climatic filters such as temperature, growing season length, and nutrient availability (Li & Prentice, 2024). For nutrient resorption, broad-scale biogeographic studies have frequently documented increased efficiency at higher latitudes, a pattern interpreted as a conservative strategy to cope with shorter growing seasons and more constrained nutrient mineralization (Sun et al., 2016; Vergutz et al., 2012). However, in the context of salinization, an additional layer of complexity emerges: adaptive traits may be filtered not only by climate but also by historically saline conditions. Populations from coastal and inland salt marshes, despite their vast geographic separation, may have undergone convergent evolution for salt tolerance, potentially shaping similar nutrient management strategies. Disentangling whether observed trait variation aligns more strongly with broad-scale climatic gradients or with local adaptive pressures from saline habitats is therefore critical.

The common reed (*Phragmites australis*) is a uniquely powerful model to explore such intraspecific variation (Liu et al., 2025; Ren et al., 2020). It exhibits extensive geographic distribution and occupies the full spectrum of habitats relevant to this question, from freshwater wetlands to coastal saltmarshes and inland plateau saltmarshes, with distinct ecotypes and phylogenetic lineages demonstrating significant phenotypic divergence (Meyerson et al., 2025). By including populations from both coastal and inland saline origins, our experimental design explicitly captures the potential for convergent adaptation to salt stress, while also controlling for broad phylogeographic history. This allows us to separate the effects of evolutionary lineage, local habitat adaptation (salinity), and macro-climatic (latitudinal) gradients on a key functional trait.

In this study, we used a common garden experiment with multiple populations of *P. australis* spanning major phylogeographic groups and ecotypes (coastal vs. inland saline vs. freshwater) to: (1) first verify that the salinity treatment induces multi-level physiological stress (via biomass, Na^+ accumulation, and metabolomics), a prerequisite for any meaningful genotype-by-environment analysis; (2) test whether the classic resorption control hypotheses are maintained or disrupted under salt stress; (3) quantify the relative contributions of phenotypic plasticity (to salinity) and population origin (phylogeography and ecotype) to NRE variation, thereby directly assessing the 'plastic acclimation versus inherent conservatism' paradigm; and (4) decipher whether geographic variation in NRE is primarily aligned with broad-scale climatic (latitudinal) gradients. We hypothesize that NRE is a canalized trait largely unresponsive to short-term salt stress, and that its intraspecific variation is predominantly shaped by genetic origin and historical adaptation. By clarifying the mechanistic basis of intraspecific variation, this work seeks to scale from plant nutrient-use strategies to their potential impacts on ecosystem nutrient dynamics, improving predictions for ecological outcomes of salinization.

2. Materials and Methods

2.1 Common garden design

The common garden experiment was conducted in Changle County, Weifang City, Shandong Province, China (36°22' N, 118°53' E). On 5th April 2024, we collected 110 individuals (genotypes) of *P. australis* rhizomes from the common garden for cultivation. To disentangle the effects of deep evolutionary history from local adaptation, we employed a dual classification of our plant materials. The 110 individuals were assigned to six phylogeographic groups (AU, CN_N, CN_E, EU, EU_NA, NA; Table S1) based on genetic evidence from chloroplast haplotypes, nuclear microsatellites, and genomic analyses (Liu et al., 2022; Saltonstall, 2002; Wang et al., 2024).

Concurrently, the populations from China were categorized into three ecotypes reflecting their source habitats: freshwater wetland, coastal saltmarsh, and inland plateau saltmarsh (Table S1). Ecotype classification was not applied to non-Chinese populations due to insufficient habitat documentation at the time of collection.

The plants were planted in barrels (total volume 25 L; top diameter 32.5 cm, bottom diameter 28.4 cm, height 38.5 cm) filled with 20 L of a soil substrate mixed in a 2 (soil):1 (peat moss):1 (river sand) ratio (Figure S1). The salinity treatment was applied as a 1% NaCl solution (10 ppt), calculated based on the estimated porewater volume of the 20 L soil system. Each individual was planted in two barrels: one for the salt treatment and one for the control. This concentration falls within the range encountered by *P. australis* in saline wetlands, and represents an ecologically recurrent stress that is moderate for most lineages of *P. australis* but severe for specific genotypes (e.g., those native to North America) (Eller et al., 2017; Meyerson et al., 2025), thus capturing the natural variation in salinity tolerance across its global distribution. This was achieved by adding 100 g of NaCl dissolved in water to each barrel on June 2 and 9, for a total of 200 g. Green leaves and senesced litter were sampled for elemental analysis at two critical phenological stages: one month after salt addition (July 11, 2024; coinciding with active growth) and six months later (December 4, 2024; coinciding with seasonal senescence), respectively.

At each sampling, we collected fully expanded, healthy green leaves from the top of the shoots, typically the 2nd to 4th leaf from the apex. Senesced litter was identified as leaves that had completely yellowed, which typically corresponded to the same 2nd to 4th positional nodes prior to senescence. This standardized protocol ensured the comparison of equivalent physiological tissues across all individuals and treatments.

2.2 Elemental and metabolomic profiling

The plant leaves and litter were dried at 45 °C to constant weight, cut with scissors, placed in a 2ml centrifuge tube and placed in two small steel balls, and ground with a grinder (Shanghai Jingxin Industrial Development Co., Ltd., Shanghai, China) at 55 Hz for 5 min to powder. Total carbon (C) and nitrogen (N) of leaves was measured using an element analyzer (UNICUBE, Germany) after weighing 2 mg (accurate to three decimal places) of plant samples in tin paper. A total of 0.1 g of the sample was weighed and placed in a polytetrafluoroethylene digestion tube, to which nitric acid was added, and then digested by microwave to obtain the digestive solution. The concentrations of K and P in the digest were determined by ICP-OES (iCAP Pro, Thermo Scientific, Germany).

Besides C, N and P, other eight elements (K, Cu, Zn, Fe, Mn, Mg, Si, Na) were quantified in leaf tissues. Concentrations of Na, Si, Fe, Mn, and Mg were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP Pro, Thermo Scientific, Germany), while Cu and Zn were measured by inductively coupled plasma mass spectrometry (ICP-MS; NexION 1000G, PerkinElmer, USA).

For untargeted metabolomic profiling, approximately 50 mg of fresh leaf tissue was extracted with 1,000 µL of ice-cold extraction solvent (methanol:acetonitrile:water = 2:2:1, v/v/v) containing 20 mg/L of 2-chloro-L-phenylalanine as an internal standard. The mixture was homogenized with steel beads (45 Hz, 10 min), sonicated in an ice-water bath (10 min), and incubated at –20°C for 1 h. After centrifugation (12,000 rpm, 15 min, 4°C), the supernatant was vacuum-dried, and the residue was reconstituted in 160 µL of acetonitrile:water (1:1, v/v). Chromatographic separation was performed on an Acquity UPLC HSS T3 column (1.8 µm, 2.1 × 100 mm) using a Waters Acquity I-Class PLUS UHPLC system coupled to a Waters Xevo G2-XS QTOF mass spectrometer. The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 400 µL/min with the following gradient: 0–0.5 min, 5% B; 0.5–5.5 min, 5–50% B; 5.5–9.0 min, 50–95% B; 9.0–10.5 min, 95% B; 10.5–12.0 min, 95–5% B. The injection volume was 2 µL. Mass spectrometry was operated in both positive and negative ion modes with an electrospray ionization source; capillary voltage was set to 2500 V (positive) or –2000 V (negative), cone voltage to 30 V, ion source temperature to 100°C, desolvation gas temperature to 500°C, cone gas flow to 50 L/h, and desolvation gas flow to 800 L/h. Data were acquired in MSe mode over a mass range of

m/z 50–1200, alternating between low (off) and high (10–40 V) collision energies. Raw data were processed using Progenesis QI software for peak extraction, alignment, and annotation against the online METLIN database, public databases, and an in-house library.

For statistical analysis, raw metabolomic data were $\log_2$-transformed, missing values were imputed with half of the minimum positive value, and constant metabolites were removed. Data were autoscaled (mean-centered and scaled to unit variance) prior to principal component analysis (PCA). Permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations was performed on Euclidean distances to test the effect of salinity while accounting for genotype as a covariate. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to extract variable importance in projection (VIP) scores. Differentially accumulated metabolites between salt and control groups were identified using limma with empirical Bayes moderation; significance was defined as $|\log_2 \text{fold change}| > 1$, false discovery rate (FDR) < 0.05, and VIP > 1.

2.3 Nutrient resorption calculation

The continuous growth strategy and substantial leaf mass heterogeneity in *P. australis* preclude reliable mass loss correction factor (MLCF) determination. Uncontrolled physical losses during asynchronous senescence (notably a high rate of leaf tip abscission) decouple dry mass reduction from physiological nutrient resorption. Therefore, to ensure equitable comparisons, nutrient concentration in senesced leaves were standardized for carbon-basis shifts using the fractional change in the measurement basis (FCMB) protocol. Specifically, litter nutrient concentrations were multiplied by the ratio of green leaf carbon content to litter carbon content (Cgreen / Clitter) (Prieto & Querejeta, 2020). The Cgreen/Clitter ratios used for standardization varied among samples, with a mean of 1.06; a one-sample t-test confirmed this mean was significantly greater than 1 ($p < 0.001$), justifying the correction for a systematic shift in the carbon measurement basis. This approach directly quantifies nutrient withdrawal per structural carbon unit, neutralizing mass heterogeneity through carbon-standardization, bypassing fragmentation artifacts, and remaining valid across continuous growth gradients. Finally, nutrient resorption efficiency (NuRE) for N, P, and K was calculated as the percentage of nutrient recovered from senescing leaves: NuRE = (Green nutrient concentration - Litter nutrient concentration) / Green nutrient concentration × 100.

2.4 Data analysis

A principal component analysis (PCA) was conducted to visualise the overall structure of multi-element composition (N, P, K) across leaf types and salinity treatments, using the *rda* function in the *vegan* package after standardising the data.

Prior to multivariate analysis, all eleven elemental concentrations were standardized to zero mean and unit variance. A global principal component analysis (PCA) was performed using the *FactoMineR* package in R. To evaluate the overall effect of salinity on elemental composition, a multivariate analysis of variance (MANOVA) was conducted on PC1 and PC2 scores using Pillai's trace test. Pairwise comparisons between control and salt treatments were performed using Welch's *t*-test for PC1 and PC2 separately. To assess whether salinity effects differed across genetic lineages and developmental stages, MANOVA was repeated for each lineage × stage combination.

We analyzed differences in nutrient resorption efficiency among phylogeographical groups and ecotypes under salinity treatments using linear mixed-effects models fitted with the *lme4* package in R, with phylogenetic group (or ecotype) and salinity treatment included as fixed effects and genotype incorporated as a random effect to account for the paired experimental design in which each genotype was exposed to both control and salt conditions. When significant effects were detected ($p < 0.05$), post-hoc pairwise comparisons were performed using Tukey's HSD test via the *emmeans* package, with additional sensitivity analyses conducted on the within-genotype difference (Δ) between salt and control treatments.

The three fundamental control strategies over leaf nutrient resorption were assessed following established conceptual frameworks. To assess the nutrient concentration control strategy, we analyzed the relationships between log10-transformed nutrient concentrations in green leaves and senesced litter. A slope (β) significantly greater than 1 was interpreted as evidence of nutrient concentration control, indicating that resorption efficiency decreases as green leaf nutrient concentration increases. Conversely, a slope not significantly different from or less than 1 suggested the absence of this control strategy. To evaluate the stoichiometric control strategy, we examined the relationships between N and P resorption efficiencies, as well as between the resorbed N:P ratio and the N:P ratio in green leaves (Yang et al., 2025). Consistent positive correlations in both relationships were taken as support for stoichiometric control, reflecting proportional resorption aligned with initial nutrient ratios in green tissues. Non-significant or negative correlations indicated a deviation from this strategy. For the nutrient limitation control strategy, we tested the relationship between log10-transformed ratios of resorbed nutrients (e.g., N:P) and log10-transformed ratios in green leaves (Kobe et al., 2005). A slope significantly less than 1 was considered evidence of nutrient limitation control, implying that the resorption efficiency ratio between elements shifts in response to their relative limitation. A slope ≥ 1 suggested the 'inverted' nutrient limitation control (Sun et al., 2023).

To rigorously estimate the functional (structural) slopes between bivariate nutrient traits (e.g., green leaf vs. senesced litter concentrations), we employed Standardized Major Axis (SMA) regression implemented via the *smatr* package in R (Warton et al., 2012). SMA regression was selected as the primary analytical method because it is a Model II regression approach that appropriately accounts for measurement and biological variation in both variables—a critical consideration given the observational nature of our data where both nutrient concentrations in green leaves and senesced litter are measured with error. This method provides unbiased estimates of the true structural relationship between variables, unlike Ordinary Least Squares (OLS) regression which assumes the predictor variable is error-free and systematically underestimates slope magnitudes under these conditions (Warton et al., 2012).

For each bivariate relationship, we performed SMA regression analyses separately for each salinity level. The *smatr* package was used to estimate the SMA slope (β) along with its 95% confidence interval and to calculate the coefficient of determination (R^2). Crucially, we specifically tested whether each estimated SMA slope significantly deviated from theoretical values, particularly from 1. This slope comparison against 1 was conducted using the built-in permutation tests in the *smatr* package, which generated *p*-values indicating the statistical significance of the deviation. For comparative purposes and to maintain consistency with the methodological approaches employed in foundational studies of nutrient resorption (e.g., Kobe et al., 2005; Sun et al., 2023) that relied on OLS regression, we also calculated and report OLS slope estimates in supplementary materials. However, all formal hypothesis testing and interpretation regarding slope values, particularly tests of deviation from 1:1 relationships, are based on the more appropriate SMA regression results, which represent robust, unbiased estimates of the functional relationships between nutrient traits.

The influence of geographical origin (absolute latitude) on NuRE was analysed using linear mixed-effects models (LMMs) fitted with the *lmer* function in R package *lme4* (Bates et al., 2015), where phylogenetic group was included as a random intercept term to account for non-independence among individuals from the same source. Model assumptions of normality and homoscedasticity were verified by inspecting residual plots, and NuRE values were arcsine-square-root transformed when necessary to meet these assumptions. To quantify the individual contributions of the fixed factors (latitude, leaf nutrient concentration, and stoichiometry) to the variance in NuRE, we performed a variation partitioning analysis flowing the LMMs with phylogenetic group as a random intercept. This variation partitioning was conducted using the *glmm.hp* package in R (Lai et al., 2022), which implements hierarchical partitioning to calculate the marginal R^2 for each predictor within the fitted linear mixed-effects model framework. This approach allowed us to

estimate the unique proportion of variance in NuRE attributable to each fixed factor, independent of the random effect of phylogeographic group. All figures were generated using ggplot2 and assembled with the patchwork library. All statistical analyses were performed in R version 4.3.0.

3. Results

3.1 Salt stress induces multi-level physiological responses

To establish that the salinity treatment imposed a physiologically meaningful stress before evaluating NuRE responses, we first examined multi-level indicators of salt stress

Principal component analysis (PCA) of the metabolome revealed a clear separation between salt-treated and control plants (Figure 1a), with the first two principal components explaining 16.51% and 7.81% of the total variance, respectively; orthogonal partial least squares discriminant analysis yielded a Q^2(cum) of 0.233, indicating that the treatment effect is detectable albeit accompanied by substantial genotypic variation. A total of 484 metabolites showed significant differential accumulation between treatments ($|\log_2$ fold change$| > 1$, $FDR < 0.05$, $VIP > 1$) (Figure 1b). Among the metabolites, canonical stress markers were prominently altered: N,N-dimethylglycine (a glycine betaine precursor) and sucrose 6-phosphate were significantly upregulated ($\log_2$FC = +1.04 and +2.93, respectively), while glutathione ($\log_2$FC = –0.27) and N-caffeoylputrescine ($\log_2$FC = –1.34) were significantly downregulated. Collectively, these results demonstrate that the salinity treatment induced widespread metabolic reprogramming consistent with osmotic and oxidative stress responses.

Salt treatment drastically reduced above-ground biomass by more than 60% (control: 835.7 ± 36.4 g, salt: 310.4 ± 15.0 g; Welch's $t = 13.26$, $p < 0.001$; Figure 2a). Sodium (Na) accumulation, a direct indicator of salt uptake, increased dramatically across all leaf developmental stages. In green leaves, Na concentrations rose from 475 to 1929 mg kg^{-1} $p < 0.001$), in yellowing leaves from 508 to 2027 mg kg^{-1} ($p < 0.001$), and in litter leaves from 1379 to 3722 mg kg^{-1} ($p < 0.001$; Figure 2b).

The elemental composition of leaves was also significantly altered by salinity. Principal component analysis of 11 elements revealed that PC1 (41.9% variance) separated nutrient-related elements (C, N, P, K, Cu, Zn) from stress-related elements (Si, Mn, Na), while PC2 (14.3%) was primarily driven by Mn, Zn, Cu, Fe, and Si (Figure S2a). Salinity significantly increased PC2 scores ($p < 0.001$, MANOVA), indicating a shift in elemental balance (Figure S2bc). Notably, the effect of salinity on elemental composition was stage- and lineage-dependent: during the Green stage, all lineages showed significant responses; during the Yellowing stage, only CN_N and EU lineages were affected; and by the Litter stage, no significant salinity effect was detected in any lineage (Figure S2d-f).

3.2 Nutrient resorption efficiency is genetically canalized under effective salt stress

The PCA of leaf N, P, and K concentrations showed a clear separation between green leaves and senesced litter along the first principal component (PC1, Figure S3a). This separation was driven by the strong positive loadings of all three nutrient concentrations on PC1 (Figure S3b). This pattern indicates that the dominant trend in the data is the distinction between nutrient-rich green leaves and nutrient-poor senesced litter, a pattern that directly results from substantial nutrient resorption during senescence.

The results of the linear mixed-effects models indicated a significant effect of phylogenetic group on the resorption efficiency of N, P, and K ($p < 0.001$); however, neither salinity nor the interaction between salinity and phylogenetic group had a significant effect (Figure 3; Figure S4). For instance, the CN_N group exhibited the lowest N and P resorption efficiencies among all groups, whereas both the CN_N and CN_E groups showed high K resorption efficiency. Meanwhile, the results revealed a significant effect of ecotype on the resorption efficiency of N and P ($p < 0.001$), but not on K ($p = 0.694$) (Figure 4; Figure S5). A significant interaction between salinity and ecotype was observed for N resorption efficiency ($p = 0.038$), indicating that salinity treatment

Figure 1. Metabolomic responses of *Phragmites australis* to salinity stress.

(a) Principal component analysis (PCA) score plot of the metabolome (3,413 metabolites) under control (blue) and salt (red) treatments. Ellipses represent 95% confidence intervals for each group. PERMANOVA (Euclidean distance, 9,999 permutations) with genotype as covariate revealed a significant effect of salinity ($p < 0.001$). (b) Volcano plot showing differential accumulation of metabolites between salt and control groups. Red points indicate metabolites that are significantly different ($|\log_2$ fold change$| > 1$, false discovery rate < 0.05, variable importance in projection > 1). Selected stress-responsive metabolites (e.g., N,N-dimethylglycine, sucrose 6-phosphate, glutathione) are labeled.

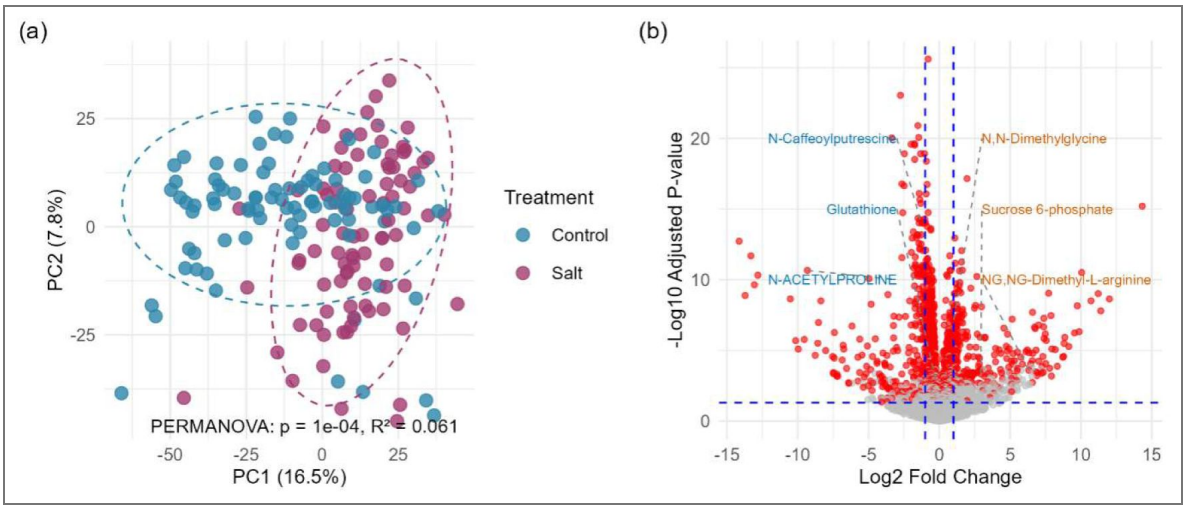
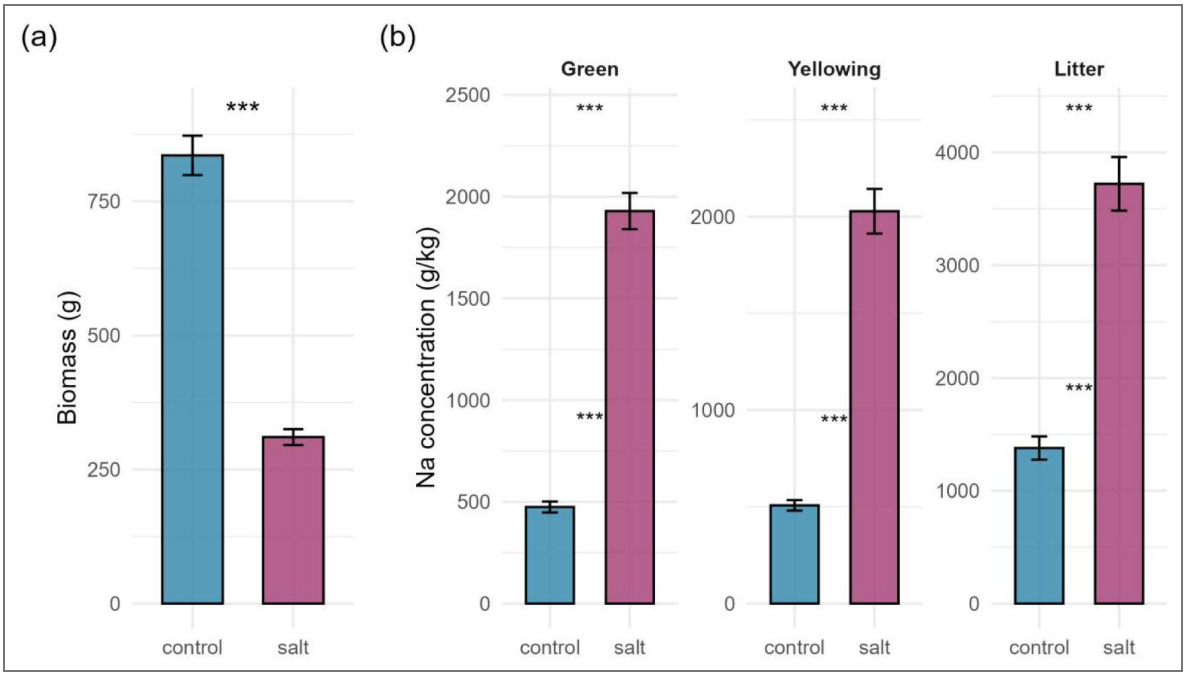


Figure 2. Effects of salinity on biomass and sodium accumulation in *Phragmites australis*.

(a) Biomass of control and salt-treated plants (harvest at the final stage). Data are means ± SE. *** $p < 0.001$ (Welch's *t*-test, $n = 106$ per group). (b) Sodium (Na) concentrations in green, yellow, and brown leaves under control and salt conditions. Data are means ± SE. *** $p < 0.001$ (Welch's t-test for each stage, $n = 105–106$ per group per stage).



enhanced N resorption in the saltmarsh ecotype relative to the freshwater ecotype. Among the ecotypes, coastal saltmarsh populations displayed the highest N and P resorption efficiencies, while populations from inland plateau saltmarsh had the lowest.

3.3 Element-specific regulatory strategies govern nutrient resorption

A significant positive association was observed between N concentrations in green leaves and litter under control conditions ($\beta > 1$, $p < 0.05$; Figure 5a), indicating the presence of nutrient concentration control over leaf N resorption in *P. australis* under non-saline conditions. However, this relationship disappeared under salt treatment ($p > 0.05$; Figure 5a), suggesting that salinity disrupted the N concentration control strategy. For P, a significant positive association was maintained under both control and salt conditions ($\beta > 1$, $p < 0.05$; Figure 5b), indicating robust nutrient concentration control over leaf P resorption in *P. australis* regardless of salinity. In contrast, correlations for K were non-significant under both conditions ($p > 0.05$; Figure 5c), suggesting the absence of nutrient concentration control for this element.

Significant positive relationships were detected between N and P resorption efficiencies (Figure 5d), as well as between the resorbed N:P ratio and the green leaf N:P ratio under both control and salt conditions (Figure 5e), supporting the presence of stoichiometric control over P resorption. Similarly, N and K resorption efficiencies were positively correlated (Figure 5g), and the resorbed N:K ratio was significantly related to the green leaf N:K ratio under both conditions (Figure 5h), indicating stoichiometric control also regulates K resorption.

Additionally, significant linear relationships were found between the $\log_{10}$-transformed resorbed ratio and $\log_{10}$-transformed green leaf ratio for N:P and N:K, with slopes greater than 1 (Figure 5f, i), demonstrating an 'inverted' nutrient limitation control for P and K relative to N. The OLS slope estimates (Figure S6) generally aligned with these resorption control strategies inferred from the SMA regressions.

3.4 Latitudinal variation reflects macro-scale adaptation

The linear mixed-effects models revealed that NuREs of N, P, and K were significantly positively correlated with the absolute latitude of population origin (Figure 6). Furthermore, variation partitioning analysis indicated that latitude served as a stronger predictor of NuRE than both nutrient concentrations and stoichiometry in green leaves across all three elements (Table 1).

4. Discussion

4.1 Effective salt stress meets conservative NuRE: reconciling the paradox

We first confirmed that the salinity treatment imposed a strong and sustained physiological stress at multiple biological levels. Metabolomic profiling revealed clear separation between control and salt-treated plants, with 484 metabolites differentially accumulated, including canonical markers of osmotic and oxidative stress such as N,N-dimethylglycine (a glycine betaine precursor) and sucrose 6-phosphate (Figure 1). Above-ground biomass declined by more than 60% (Figure 2a), and leaf Na concentrations increased 3- to 5-fold across all developmental stages (Figure 2b). Elemental composition also shifted significantly, with salinity increasing PC2 scores (mainly driven by Mn, Zn, Cu, Fe, Si) in a stage- and lineage-dependent manner (Figure S2). Collectively, these results confirm that the treatment was physiologically effective, providing a robust foundation to evaluate NuRE responses. Against this backdrop of confirmed stress, the intraspecific variation in nutrient resorption efficiency (NuRE) we observed in *P. australis* reflects divergent genetic origins, not phenotypic plasticity. Our results demonstrate that phylogeographic group and ecotype are primary determinants of NuRE, while salt stress elicited no overall plastic response in NuRE. This indicates that NuRE is a conservative trait under this non-nutrient stress condition.

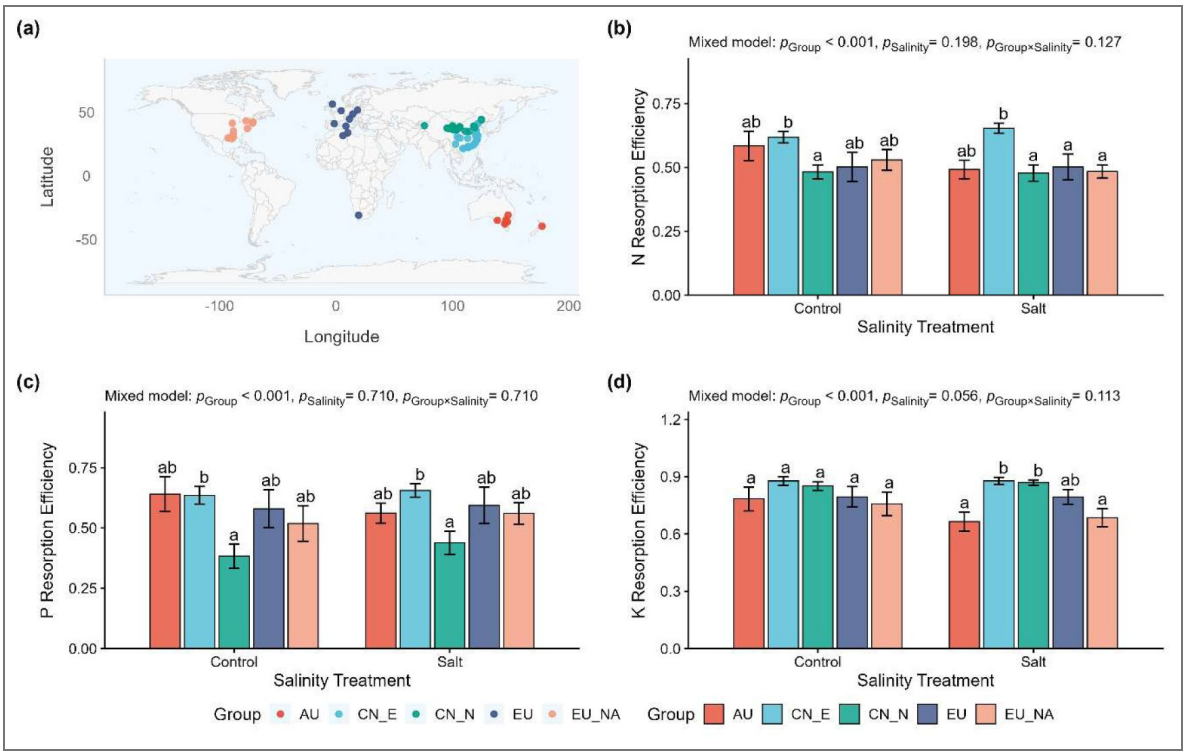


Figure 3. Geographical distribution (a) of the phylogeographical groups of *Phragmites australis* and leaf nutrient resorption efficiency (b–d) for nitrogen (N), phosphorus (P), and potassium (K).

Phylogeographic groups reflect deep evolutionary lineages defined by genetic markers (e.g., chloroplast haplotypes). Statistical significance was assessed using linear mixed-effects models that included genotype as a random effect to accommodate the paired experimental design. The *p*-values shown in panels b–d refer to the fixed effects of group, salinity and their interaction. When significant group effects were detected, post-hoc pairwise comparisons were performed separately within each salinity treatment using Tukey's HSD test; different letters denote statistically significant differences (α = 0.05).

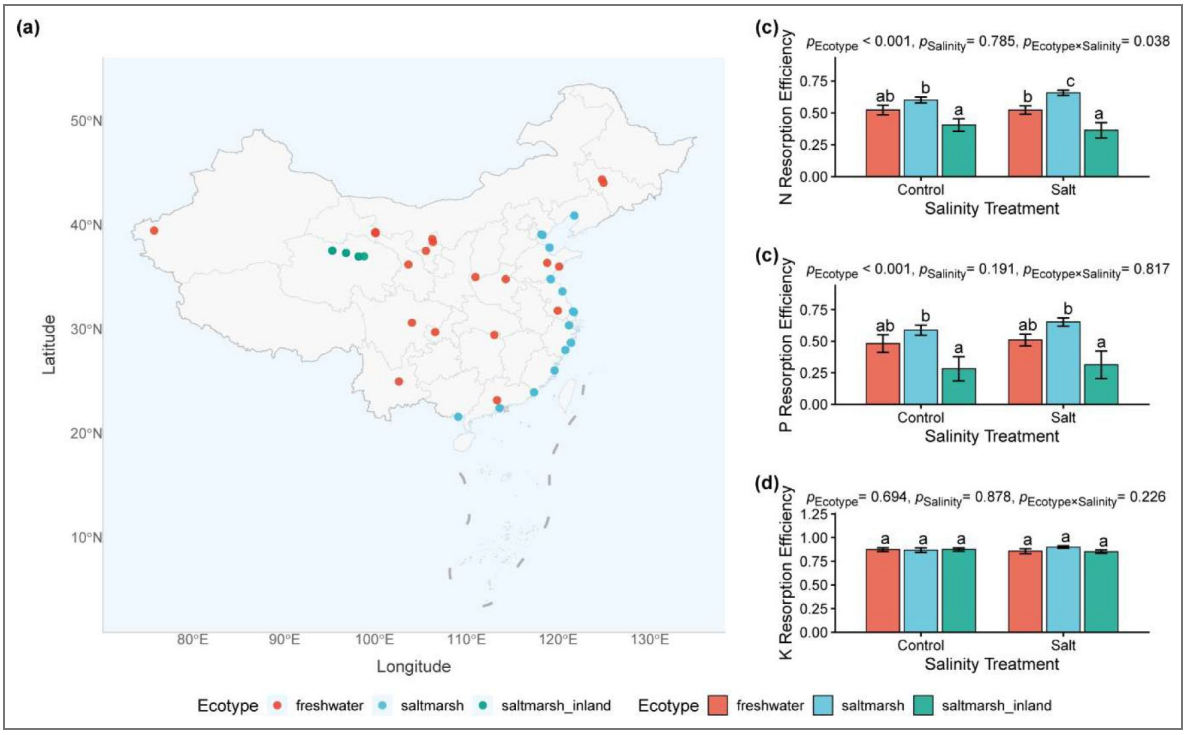


Figure 4. Geographical distribution (a) of *Phragmites australis* ecotypes and leaf nutrient resorption efficiency (b–d) for nitrogen (N), phosphorus (P), and potassium (K).

The colour scheme represents habitat-based adaptations and is independent of that used in Figure 3. Ecotypes categorize populations according to adaptive phenotypes to specific local habitats (freshwater wetlands, coastal saltmarsh, inland saltmarsh). Significance was tested with linear mixed-effects models incorporating genotype as a random effect to account for paired measurements. The reported *p*-values correspond to the fixed effects of ecotype, salinity and their interaction. Where ecotype effects were significant, post-hoc Tukey's HSD comparisons were conducted within each salinity level; different letters indicate statistically significant differences (α = 0.05).

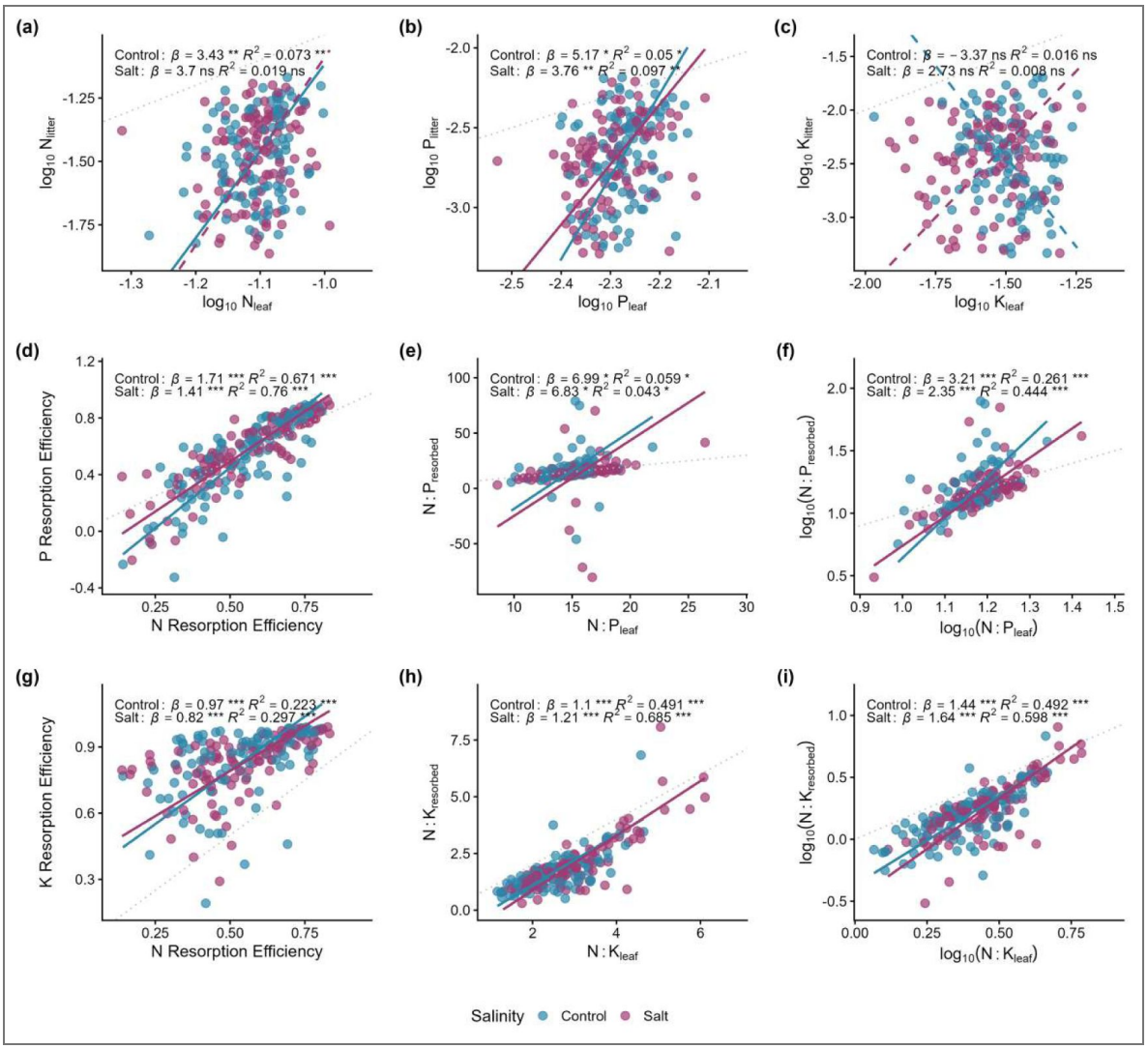


Figure 5. Assessment of three control strategies for nutrient resorption in *Phragmites australis* under varying salinity levels.

(a–c) Relationship between nutrient concentrations (N, P, K) in green leaves and senesced litter. (d, g) Association between phosphorus and nitrogen resorption efficiencies. (e, h) Linkage between resorbed N:P(K) ratios and green leaf N:P(K) ratios. (f, i) Correlation between $\log_{10}$-transformed resorbed N:P(K) and $\log_{10}$-transformed green leaf N:P(K) ratios. The gray dashed line in each panel indicates the 1:1 reference. Solid lines represent significant relationships ($p < 0.05$ for the SMA regression model), while dashed lines indicate non-significant relationships. Statistical annotations show results from standardized major axis (SMA) regression for each salinity level: β (with asterisks) is the SMA slope, where asterisks after β indicate significant deviation from 1 (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), and asterisks after R^2 indicate significance of the SMA regression model.

Figure 6. Influence of latitude on leaf nitrogen (N), phosphorus (P), and potassium (K) resorption efficiencies of *Phragmites australis*.

The analysis was performed using linear mixed-effects models, treating phylogeographical groups as a random effect.

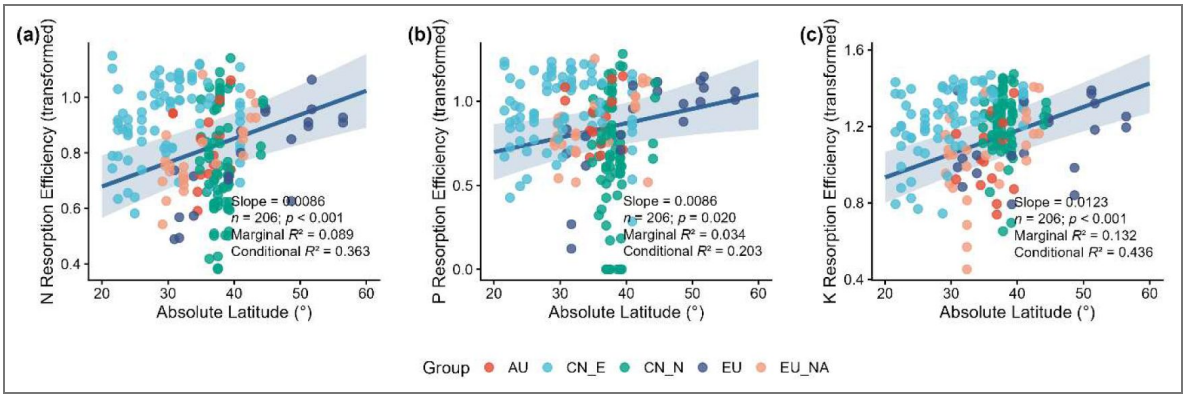


Table 1. Individual effects (R^2) of original latitude, nutrient concentration, and nutrient stoichiometry on leaf N, P, and K resorption according to linear mixed-effects models with phylogeographical groups as a random factor and decomposition marginal R^2 by the approach average shared variance

	Latitude	Concentration	Stoichiometry
N	0.092	0.044	-
P	0.057	0.006	< 0.001
K	0.094	0.043	0.078

Nutrient resorption is established as a key plant functional trait governing nutrient conservation strategies and ecosystem processes (Killingbeck, 1996; Kobe et al., 2005). Therefore, deciphering the drivers of its intraspecific variation (genetic adaptation versus plasticity) is central to functional ecology (Zhang et al., 2022; Liu et al., 2025). Our finding that this variation is shaped by long-term adaptive evolution rather than a general short-term acclimation capacity underscores that macroecological patterns can arise primarily from genetic differentiation. Such trait conservatism, also observed in desert plants under saline stress (Luo et al., 2021), suggests evolutionary optimization under consistent selective pressures, which may constrain phenotypic adjustments to sudden environmental shifts. The predominance of genetic over plastic controls highlights the necessity of incorporating intraspecific diversity into future models of nutrient cycling.

The lack of a plastic increase in NuRE under salinity presents a physiological puzzle. Theoretically, if salt stress inhibits root nutrient uptake through ionic competition or root damage, enhancing resorption from senescing leaves could serve as a critical internal compensation mechanism to maintain minimum nutrient levels for growth and metabolism. This aligns with ecological principles of optimal allocation and stress adaptation, where optimizing internal recycling is a predicted strategy under resource limitation (Nicotra et al., 2010). However, our data did not support this expected plastic increase, a phenomenon also noted in some desert plants under saline stress (Luo et al., 2021). This discrepancy suggests the existence of substantive physiological constraints that override this optimal strategy. Salt stress may disrupt the finely tuned cellular senescence program necessary for efficient nutrient remobilization, possibly through the accumulation of incompatible solutes or oxidative damage that affect element stability (Zuo et al., 2024), or through a preferential reallocation of energy towards immediate survival mechanisms (e.g., osmotic adjustment and ion exclusion). Therefore, the canalization of NuRE may reflect an evolutionary 'optimum' under stress (Huang et al., 2022), where the metabolic and logistical costs of enhancing resorption outweigh its potential benefit, or where the core resorption machinery is intrinsically vulnerable to ionic interference (Alseekh et al., 2025).

Our findings appear to contrast with studies investigating other abiotic stressors. For example, a five-year field experiment simulating climate change, specifically warming and reduced rainfall, reported significant declines in resorption efficiency for multiple nutrients across several shrub species (Prieto & Querejeta, 2020). This discrepancy may stem from differences in the type and duration of stress applied: chronic, multi-year climate manipulations may trigger adaptive responses not seen in our short-term salt stress experiments. Alternatively, nutrient resorption may exhibit stressor-specific plasticity, being more responsive to soil nutrient availability and climate-related shifts than to ionic stress such as salinity (Drenovsky et al., 2019; Luo et al., 2021; Yang et al., 2025; Yuan & Chen, 2015). Moreover, the contrast underscores the potential importance of experimental duration (long-term gradual stress versus short-term shock) in shaping plant physiological responses. Such comparisons highlight the need for integrative approaches across stress types and time scales to fully understand the plasticity and evolution of nutrient conservation strategies.

4.2 Divergent strategies control the resorption of N, P, and K

Our study revealed distinct regulatory mechanisms governing the resorption of N, P, and K in *P. australis*. Phosphorus resorption was consistently influenced by initial green tissue P concentration under both control and saline conditions, supporting the nutrient concentration control hypothesis. In contrast, N resorption followed concentration control only under non-saline conditions, with this relationship disappearing under salinity. Potassium resorption showed no concentration dependence. Notably, N and K resorption exhibited tight stoichiometric coordination, as reflected in stable N:P and N:K ratios across treatments. The consistent "inverted" limitation pattern, indicating higher relative recovery of P and K compared to N, further underscores the role of stoichiometric control in maintaining nutrient balance under varying environmental conditions.

These element-specific strategies align with broader patterns documented across biomes and taxa. For instance, the prevalence of stoichiometric control for multiple nutrients, including N and K, has been demonstrated at a global scale (Sun et al., 2023), suggesting a universal mechanism that complements nutrient limitation effects. Notably, K resorption did not follow a concentration-dependent pattern, despite this element typically exhibiting high resorption efficiencies globally (often >70%; Vergutz et al., 2012). This suggests that high K recovery may be driven by factors other than green leaf K concentration. Furthermore, the preferential resorption of P under certain conditions echoes patterns found in nutrient-limited systems such as tropical forests and alpine permafrost ecosystems, where P resorption can surpass N resorption due to strong local constraints (Yang et al., 2025). This suggests that the strong concentration-dependent control of P resorption we observed may be a widespread adaptation to P limitation, which can override other biogeographic expectations. The stability of these stoichiometric relationships under salt stress implies that internal nutrient regulation is a conservative trait, potentially shaped by evolutionary history more than immediate abiotic context. Such stoichiometric flexibility may be especially critical in environments subject to periodic nutrient imbalances, enabling plants to maintain functional homeostasis.

It is important to acknowledge that our experimental design did not include a nutrient addition treatment, which represents the most direct approach to test the nutrient limitation control hypothesis (Chen et al., 2021). Future studies incorporating nutrient manipulations would provide more definitive evidence. However, our use of resorbed nutrient ratios (e.g., Resorbed N:P) as a proxy for relative nutrient limitation is a well-established methodology in resorption ecology (Kobe et al., 2005; Sun et al., 2023), and has been effectively applied in other recent studies (e.g., Yang et al., 2025). The significant intraspecific variation across our studied populations inherently created a broad gradient of nutrient status and demand. Within this context, the consistent observation of an 'inverted' nutrient limitation pattern (i.e., a slope significantly >1 for the relationship between log Resorbed N:P and log Green N:P) provides robust, albeit indirect, evidence for the operation of nutrient limitation control at the intraspecific level in *P. australis*.

4.3 Latitudinal gradient in resorption reflects macro-Scale adaptation

Latitude consistently predicted nutrient resorption efficiency (NuRE) in our study, with populations from higher latitudes exhibiting greater resorption for all nutrients. This macroecological pattern likely reflects adaptive responses to environmental constraints such as shorter growing seasons, lower temperatures, and reduced nutrient availability, which collectively favor more conservative nutrient use strategies. The dominance of latitudinal trends over local soil conditions underscores the overarching role of broad-scale climatic drivers in shaping functional trait variation.

This gradient aligns with biogeographic patterns observed in other widely distributed species. For instance, increasing nutrient resorption efficiency with latitude has been documented in temperate trees such as *Quercus variabilis* across China, where N, P, K, and S resorption all increased significantly with latitude, paralleled by declining nutrient concentrations in senesced leaves (Sun et al., 2016). Such convergent evolution of nutrient conservation strategies across distinct lineages and biomes suggests a general principle in plant adaptation to high-latitude environments. However, the relative contribution of genetic differentiation versus phenotypic plasticity to these latitudinal clines may vary by species and ecosystem. For example, in the grass *Stipa breviflora*, phenotypic plasticity to soil nutrient availability was the dominant factor explaining latitudinal variation in resorption, with genetic effects playing a modest role (Zhang et al., 2022). This contrasts with our findings in *P. australis*, wherein geographic origin (reflecting genetic or deep-time adaptive processes) outweighed plastic responses to salinity. Therefore, while latitudinal clines in resorption traits are common, our study demonstrates that they can arise predominantly from genetic differentiation rather than phenotypic plasticity.

4.4 Ecological and evolutionary implications of genetic canalization

Our findings demonstrate that NuRE in *P. australis* is a genetically conservative trait with limited phenotypic plasticity to salt stress. This conclusion is reinforced by the clear evidence that the salinity treatment was effective at multiple levels (metabolic reprogramming, biomass reduction, sodium accumulation, and altered elemental balance) yet NuRE remained unchanged. Such a decoupling of a core functional trait from an effective environmental stress challenges the prevailing assumption of broad trait plasticity to environmental change (Nicotra et al., 2010) and underscores the role of evolutionary constraints, such as stabilizing selection, in canalizing this trait (Luo et al., 2021). Crucially, we show that while salinity can disrupt specific regulatory patterns (e.g., concentration control for N), the overall efficiency of nutrient recovery remains fixed and is predominantly governed by geographic origin (phylogeographic group and ecotype).

This conservatism reframes how we predict ecosystem function under global change. Since trait variation is phylogenetically anchored, the pace and direction of ecosystem nutrient cycling changes will be forecast primarily by the pre-existing genetic composition of plant populations. In rapidly salinizing habitats, adaptive potential therefore relies on standing genetic variation rather than short-term acclimation, making the conservation of intraspecific genetic diversity critical for ecosystem resilience (Liu et al., 2021). To accurately project biogeochemical feedbacks, models must integrate intraspecific variation and phylogeographic history alongside environmental drivers (Vahsen et al., 2023).

These principles have direct management and biosecurity relevance. *P. australis* is widely introduced for wetland restoration and can be invasive (Meyerson et al., 2025). Our study shows that key functional traits like NuRE are strongly conserved within lineages. For instance, the invasive European lineage in North America (EU_NA) and its native progenitor (EU) showed no significant divergence in NuRE (Figure 3), a functional stasis consistent with observations of genetic and epigenetic stability during invasion (Liu et al., 2018). Consequently, the transfer or expansion of distinct genotypes (e.g., high-NuRE types) could establish populations with inherent, non-plastic nutrient cycling signatures in new ecosystems. Therefore, the functional traits of source populations must be a key criterion in restoration projects, and monitoring of invasive genotypes should account for their potential to alter local nutrient dynamics.

By evaluating multiple nutrients across diverse genetic lineages, our study reveals that classical resorption hypotheses operate as conditional and hierarchical regulatory strategies (Alseekh et al., 2025; Chen et al., 2021). Their influence is element-specific: concentration control firmly governs P resorption (Kobe et al., 2005) but is disrupted for N under salinity, while stoichiometric control persists throughout (Sun et al., 2023). This integrative framework moves beyond single hypotheses and offers a more realistic basis for predicting plant responses to complex stress. To advance this framework, future studies should: (a) combine nutrient additions with salt stress to test interactive effects on resorption control; (b) apply transcriptomic or metabolomic tools to elucidate the regulatory pathways underlying these canalized traits (Huang et al., 2022); and (c) examine whether conservative resorption is common observed across other foundation species. These steps are vital for assessing the adaptive capacity of plant communities in a changing world.

5. Conclusions

This study demonstrates that intraspecific variation in nutrient resorption efficiency in *P. australis* is primarily determined by genetic origin and geographic context, particularly latitude, rather than by plastic responses to non-nutrient stressors. Despite clear evidence of effective salt stress, including substantial biomass reduction, pronounced sodium accumulation, and widespread metabolic reprogramming, NuRE showed no overall plasticity. Instead, we identified distinct, element-specific control strategies: under control conditions, N resorption followed concentration control, a pattern disrupted by salt stress; P resorption was consistently regulated by initial tissue concentration across treatments; and both N and K resorption were under strong stoichiometric control, with evidence of inverted nutrient limitation for P and K relative to N. These patterns

reflect evolutionary adaptations to local nutrient conditions and are likely conserved across widely distributed plant species. The limited phenotypic plasticity of NuRE under salt stress suggests that ecosystem resilience to rapid non-nutrient environmental change may depend more on standing genetic variation than on short-term acclimation. Consequently, integrating phylogenetic and macroecological perspectives, particularly the phylogeographic composition of plant populations, is essential for accurately forecasting future nutrient cycling dynamics in a changing world.

Data availability

The complete dataset and associated analysis scripts are permanently hosted on Zenodo under the DOI 10.5281/zenodo.18321077 and can be accessed directly via *https://doi.org/10.5281/zenodo.18321077*.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (No. 32470388; U22A20558), and Shandong Provincial Natural Science Foundation (ZR2024QC197), and fundamental research funds of CAF (Grant No. CAFYBB2023ZA004). Lele Liu is supported by the Cyrus Tang Foundation through the Tang Scholar Program.

Additional information

Author contributions

L.Liu. and W.S. planned and designed the research, performed the investigation and data analysis, created visualizations, and wrote the original draft. H.S., C.W., N.L., L.Lin, Y.G., and N.D. contributed to the investigation and manuscript review. W.G. conceived and supervised the research, acquired funding and resources, and reviewed & edited the manuscript. L.Liu. and W.S. contributed equally. All authors critically revised the manuscript and approved its submission.

Funding

Funder	Grant reference number	Author
MOST | National Natural Science Foundation of China (NSFC)	32470388	Lele Liu
MOST | National Natural Science Foundation of China (NSFC)	U22A20558	Weihua Guo
Natural Science Foundation of Shandong Province	ZR2024QC197	Lele Liu
Fundamental Research Funds of CAF	CAFYBB2023ZA004	Lele Lin
Cyrus Tang Foundation through the Tang Scholar Program		Lele Liu

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Additional files

Supplementary file 1.

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Peer reviews

Reviewer #1 (Public review):

Summary:

This study demonstrates that nutrient resorption efficiency (NuRE) in Phragmites australis is genetically canalized rather than plastic to salt stress. Using 110 genotypes in a common garden, the authors show that intraspecific variation in NuRE is explained by phylogeographic lineage, ecotype, and latitude, not by effective salinity. Element-specific regulatory strategies further reveal how N, P, and K resorption are differentially controlled. At the population level, this is an important study that fundamentally advances our understanding of plant functional trait evolution and its implications for ecosystem nutrient dynamics under global change.

Strengths:

This study is the first to demonstrate genetic determination of a key nutrient conservation trait under effective salt stress in a widespread macrophyte, directly testing the 'plastic acclimation versus inherent conservatism' paradigm in a non-nutrient stress context. The experimental design is rigorous: each genotype was paired across control and salt treatments, and multilevel stress effectiveness (metabolomics, biomass, Na accumulation) was confirmed before evaluating NuRE. The large sample size of a macrophyte and dual classification (phylogeography + ecotype) allow robust disentangling of genetic versus plastic sources of variation.

The analysis comprehensively tests three resorption control hypotheses using appropriate SMA regression, revealing element-specific and condition-dependent patterns. The latitudinal gradient and variation partitioning provide strong evidence that genetic origin and geographic context outweigh short-term plasticity, with important implications for predicting ecosystem nutrient cycling under global change. This study provides a clear empirical demonstration that a key nutrient conservation trait can remain homeostatic under non-nutrient stress, and that intraspecific variation is primarily a product of population differentiation rather than short-term plasticity.

Weaknesses:

First, the salinity treatment spanned only one growing season. The conclusion of genetic canalization therefore specifically refers to the absence of plasticity to an acute salt shock. Whether long-term, multigenerational chronic salinity could act as a selective agent or induce transgenerational plasticity remains an open and interesting question for further research. Likewise, the physiological mechanisms underlying the observed lack of plastic increase in NuRE (for example, phloem loading or senescence gene expression) are not directly resolved,

leaving some inference about trade-offs versus true unresponsiveness. These points do not weaken the study's main conclusion. Instead, they suggest productive future directions, such as longer-term field manipulations and targeted molecular investigations.

Second, the test of nutrient limitation control relies on resorbed N:P and N:K ratios as proxies, an established but indirect approach. Direct nutrient addition experiments would provide stronger causal evidence. Also, the metabolomic analysis is used primarily to validate stress effectiveness; deeper integration of specific metabolites with NuRE variation across genotypes could have offered mechanistic insights but was not pursued. Additionally, the potential collinearity between ecotype and phylogeographic lineage among Chinese populations is not quantitatively addressed. None of these considerations undermines the main finding, which is supported by a robust experimental design and widely accepted analytical approaches.

https://doi.org/10.7554/eLife.111792.1.sa2

Reviewer #2 (Public review):

Summary:

The study finds that nutrient resorption efficiency in Phragmites australis shows no plastic response to salinity stress but is canalized by phylogeographic lineage, ecotype, and latitude. In a common garden with 110 genotypes, salinity induced stress, yet no plastic change occurred for N, P, or K resorption. The authors conclude that intraspecific variation is historical and geographic; thus, predictions of wetland nutrient cycling need to account for phylogeographic composition.

Strengths:

The core finding that NuRE shows no plastic response to salinity, but is instead evolutionarily canalized by lineage and latitude, challenges a key assumption of broad trait plasticity. This conclusion is firmly supported by a robust common garden design with 110 genotypes, rigorous multi-level stress validation, and element-specific resorption analyses. The work provides compelling evidence that intraspecific variation in this critical nutrient cycling trait is shaped by phylogeographic history rather than short-term acclimation. The implications for predicting wetland responses to salinization are significant, as ecosystem-level nutrient dynamics may be constrained by the genetic composition of plant populations.

Weaknesses:

The experiment covers only one growing season, with salinity applied in June and measurements in December. While the stress is clearly effective, longer-term or multi-year stress might reveal acclimation or epigenetic effects that are not captured. Given the author team's expertise in parental and transgenerational effects in clonal plants, this limitation is particularly relevant and warrants more thorough discussion in the manuscript.

The salinity treatment uses a single moderate level of 10 ppt, which does not allow assessment of whether more extreme stress might trigger a plastic response. A dose-response design across a gradient would have provided stronger inference about the threshold at which NuRE canalization might be overcome. Additionally, the ecotype analysis in Figure 4 applies only to Chinese populations, as classification was not available for non-Chinese populations, which should be stated more explicitly in the Results.

The variation partitioning shows latitude as a significant predictor, but the R^2 values are relatively low, indicating that much variance remains unexplained. The manuscript should avoid overinterpreting latitude's explanatory power and more openly acknowledge the role of unmeasured factors. The interpretation of slopes greater than 1 for the resorbed N:P

versus green N:P relationship, labeled as "inverted limitation", also needs further explanation regarding its functional significance.

https://doi.org/10.7554/eLife.111792.1.sa1

Author response:

We sincerely thank the editors and reviewers for the positive assessment of our work and for the constructive and insightful feedback. We are grateful that the experimental design and the evidence for genetically canalized nutrient resorption efficiency (NuRE) were recognized as compelling and important, with clear implications for predicting wetland nutrient cycling under salinization. We fully agree with the major points raised in the public reviews and outline below our planned revisions to address them, with particular attention to the weaknesses noted.

Reviewer #1 raised two important concerns regarding the scope and generality of our conclusions. First, the salinity treatment spanned only one growing season, so the genetic canalization we document specifically refers to the absence of a plastic response to an acute salt shock; whether long-term, chronic or multigenerational salinity could act as a selective agent or induce transgenerational plasticity remains an open and interesting question. Second, the test of nutrient limitation control relies on resorbed N:P and N: K ratios as proxies rather than direct nutrient manipulation, and the metabolomic analysis is used primarily to validate stress effectiveness. We accept these criticisms and will address them as follows.

Regarding the temporal scope of the treatment, we will explicitly state in the Discussion that our conclusion of canalization pertains to short-term acclimation to an acute salt shock, and we will discuss the scenarios under which chronic, more severe or multigenerational exposure could trigger plastic, acclimatory or transgenerational responses. Given our team's prior work on parental and transgenerational effects in clonal plants, we will frame these as testable hypotheses for future research. We will also acknowledge that the physiological mechanisms underlying the lack of a plastic increase in NuRE (for example, phloem loading or senescence-associated gene expression) are not directly resolved in the present study and will propose targeted molecular investigations as a natural next step.

Regarding the nutrient limitation tests, we will clearly acknowledge in the Discussion that the resorbed N:P and N:K ratios provide an established but indirect proxy for nutrient limitation, and we will discuss how direct nutrient addition experiments could provide stronger causal evidence, while deepening the integration of the metabolomic profiles with genotype-level NuRE variation where feasible. We will also quantitatively assess the potential collinearity between ecotype and phylogeographic lineage among the Chinese populations.

Reviewer #2 raised three substantive issues regarding the interpretation and presentation of our results. First, although latitude emerged as a significant predictor in the variation partitioning, the relatively low R^2 values indicate that much variance remains unexplained, and our interpretation should be more cautious; in addition, the functional significance of the "inverted" nutrient limitation (slopes greater than 1 for resorbed versus green N:P) needs further explanation. Second, the single moderate salinity level (10 ppt) does not allow assessment of whether more extreme stress might trigger a plastic response. Third, the ecotype analysis applies only to the Chinese populations, as ecotype classification was not available for non-Chinese populations, and this should be stated explicitly in the Results. We fully agree with these points and will revise accordingly.

To address the concern about latitude, we will temper the language in the Results and Discussion, explicitly noting the limited proportion of variance explained by latitude and acknowledging the role of unmeasured factors, while retaining the study's central message

that genetic and phylogeographic origin outweigh short-term plasticity in shaping NuRE. We will also expand the Discussion to explain the functional significance of the “inverted” nutrient limitation, that is, why P and K are resorbed more completely relative to N, whether as a strategy to maintain optimal N:P:K ratios or a reflection of the higher costs and lower availability of N.

To address the salinity gradient concern, we will acknowledge in the Discussion that the single moderate salinity level may not have been severe enough to trigger a plastic response and will justify future dose-response experiments to identify the threshold at which canalization of NuRE might be overcome.

To address the ecotype limitation, we will explicitly state in the Results that the ecotype analysis in Figure 4 is based only on the Chinese populations, for which ecotype classification was available.

https://doi.org/10.7554/eLife.111792.1.sa0