ABSTRACT: Eutrophication and exotic species invasion are key drivers of the global loss of biodiversity and ecosystem functions in lakes. We selected two exotic plants (Alternanthera philoxeroides and Myriophyllum aquaticum) and two native plants (Myriophyllum spicatum and Vallisneria spinulosa) to elucidate the effect of eutrophication on exotic plant invasiveness. We found that (1) elevated nutrient favored invasion of exotic species and inhibited growth of native plants. Species combinations and plant densities of native plants had limited effects on the resistance to invasion of the exotics. (2) A. philoxeroides featured the tightest connectivity among traits, which is consistent with its high competitive ability. Although eutrophication caused physiological stress to A. philoxeroides, it could effectively regulate enzyme activity and alleviate the stress. (3) M. aquaticum possessed strong tolerance to habitat disturbance and was highly disruptive to the surrounding plants. Eutrophication will exacerbate the adverse effects of M. aquaticum on the littoral ecosystem. (4) Nutrient enrichment reduced the biomass and relative growth rates of V. spinulosa and lowered phenolics and starch contents of M. spicatum, thereby making them more susceptible to habitat fluctuations. Overall, our study highlights how eutrophication alters the invasiveness of exotic plants and the resistance of native plants in the littoral zone, which is of relevance in a world with intensified human activities.

KEYWORDS: aquatic plant, eutrophication, plant trait network, littoral zone

1. INTRODUCTION

Aquatic plants, being key main primary producers and ecosystem engineers of the littoral zone of lakes, create complex and diversified habitats and provide a variety of ecological functions. They provide spawning, incubation, and refuge sites for zooplankton, benthic animal, and fish and act as a trap and a purifier for pollutants flowing into the lake. Thus, the complex and highly structured environment of the littoral zone has important implications for lake biodiversity conservation. However, the vegetated area in this zone is shrinking, and the biodiversity is decreasing worldwide due to eutrophication, usually caused by agricultural pollution, extraordinary development of fishery culture, and over-exploitation by tourists. These changes alter the multiple ecological function of the littoral zone.

Aquatic plant invasions pose a widespread and ever-increasing threat to lake ecosystems, e.g., by causing decline in biodiversity, habitat homogenization, and degradation of ecological functions. In some cases, invasiveness of exotic species is dependent on the strength and type of stressor (e.g., nutrient enrichment and habitat destruction) as well as species composition and plant density in the invaded habitat. Eutrophication leads to reduced submerged plant diversity and habitat damage, and in this way, it may produce ecological niche vacancies that are open for aquatic plant invasion. In general, lake eutrophication leads to an increase in nitrogen concentration and a decrease in light availability in the water column, subsequently causing nitrogen accumulation and carbon shortage in plants. Under low light conditions, submerged plants usually elongate to reach the water surface in order to obtain more sunlight, while their biomass and ramet number usually show a decrease. Changes in plant traits may affect plant invasiveness or their resistance to invasion. Although studies on eutrophication promoting aquatic plant invasiveness have been widely reported, comprehensive consideration of the effects of eutrophication on plant invasiveness in the littoral zone based on numerous morphological and physiological traits is rare.

Most research on the effects of eutrophication on aquatic plants has focused on individual root, stem, and leaf tissues,
and there are comparatively few studies of plant performance responses to eutrophication from a whole-plant perspective. However, plant traits form a trait network with feedback regulation, and the connectivity among traits characterizes the trade-offs and resource redistribution of plant traits in response to the environment. Plant trait networks (PTNs) can be used to elucidate the complex relationships among traits and to calculate topological parameters of the network. If plant traits are considered as nodes and trait—trait relationships as edges, multiple traits can be integrated by the relationships used to form a PTN. Trait—trait relationships can be quantified by correlation analyses, and the overall plant resistance to changing environments can be evaluated by calculating the topological parameters of the network, e.g., average degree and modularity. “Degree” is the number of edges that connect a focal node trait to other nodes and can be used to quantify the relative importance of a trait for a given trait network. “Modularity” helps to identify the clustering tendency for responses to eutrophication from a whole-plant perspective. Environmental Science & Technology

In general, there is a trade-off between competition and tolerance in plants. Invasive plants are highly competitive, with high capacity for resource acquisition, uptake and assimilation, and rapid relative growth rates (RGR), allowing them to rapidly occupy vacant ecological niches. In contrast, environmentally tolerant plants have low photosynthetic and respiration rates, slow RGR, and large carbon reserves, and they have high tolerance to environmental stress. Thus, biomass and RGR respond to the plant growth rate, while the ramet number and plant height can reflect the ability of plants to expand spatially, and these four traits can be used to predict the competitive ability of plants. In addition, carbohydrate reserves, such as starch, are key traits for plant maintenance and growth in stressful environments. Phenolics give the plants the ability to withstand external animal feeding, cold weather, and drought events. Thus, starch and phenolics can be used to address the ability of plants to tolerate external disturbances. Although the high competitive ability of invasive plants is widely recognized, two issues remain unclear: (1) how the trade-off between competition and tolerance of invasive and native plants responds to eutrophication in the littoral zone, and (2) what the implications of this trade-off are for plant invasion of the littoral zone.

We tested the hypotheses that (1) there is a trade-off between competition and tolerance in invasive plants and that eutrophication changes this trade-off, which in turn affects plant invasiveness, and (2) compared to native plants, invasive plants have high trait connectivity and low modularity, corresponding to their high competitive ability and low tolerance. We selected two invasive plants (Alternanthera philoxeroides and Myriophyllum aquaticum) and two common native submerged plants (Myriophyllum spicatum and Vallisneria spinulosa), set different nutrient levels, and cultivated 2400 plants to study their invasiveness and resistance to invasion by measuring 24 morphological and physiological traits at different nutrient levels. We first clarified the effect of eutrophication on the growth performance of invasive and native plants. We then applied plant trait network analysis to elucidate the trait connectivity of each species and assessed the differences in trait connectivity between invasive and native plants. Finally, we calculated the competition and tolerance indices of the four species and analyzed the effects of eutrophication on competition and tolerance ability. Our study elucidated the invasion mechanism of plants in the eutrophic littoral zone based on the whole-plant-trait connectivity and the trade-off between competition and tolerance.

2. MATERIALS AND METHODS

2.1. Study Species. Two common invasive plants, Alternanthera philoxeroides (Mart.) Griseb. and Myriophyllum aquaticum (Vell.) Verdc., were selected to study the effect of eutrophication on their invasiveness. Both species are perennial herbs native to South America, preferring warm and humid environments, and are widely distributed throughout the world. A. philoxeroides has both aquatic and dry types, and the aquatic type was used. Both A. philoxeroides and M. aquaticum can adapt to semiaquatic and aquatic environments and can compete with native plants for light, water, nutrients, and growing space in lakes, ditches, and canals, inhibiting native plant growth. Previous studies have shown that A. philoxeroides grew actively at 0–1.0 m water depth, e.g., had new leaf sprouting and biomass accumulation. It adapts actively at 1.0–2.0 m water depth via fast stem elongation and extensive pith cavity development. M. aquaticum can maintain positive relative growth rates at up to 1.3 m water depths. Therefore, both invasive species could expand from humid conditions to shallow water depth, creating competitive pressure on the growth of native submerged plants. Compared to M. aquaticum, A. philoxeroides has a greater capacity for environmental adaptation and spatial expansion as well as a higher capacity for fragmentation and clonal reproduction, making it difficult to be effectively controlled and causing a great impact on the local ecology and economy.

Two dominant native submerged plants, Myriophyllum spicatum L. and Vallisneria spinulosa Yan, were chosen to study their resistance to invasive alien plants. Both plant species are widely distributed in China in various water bodies ranging from poor to eutrophic. They have different metabolic rates. M. spicatum has a high RGR and assimilation rate, as well as a high respiration rate, while V. spinulosa has a lower RGR. Furthermore, they represent different growth types, M. spicatum being the canopy type and V. spinulosa the rosette type. Moreover, the light compensation point and the saturation point of V. spinulosa are significantly lower than those of M. spicatum. Collectively, M. spicatum is considered as a resource-acquiring species with high competitive ability in suitable habitats, and V. spinulosa is a resource-conservative species with high tolerance in stressful environments.

2.2. Experimental Material and Design. This outdoor experiment was performed from 12 June to 23 August 2021 at Yunyuan Experimental Station (28°110 N, 113°040E), Hunan Agricultural University, China. The submerged plants were collected 2 years before the experiment from lakes in the middle and lower reaches of the Yangtze River in China and cultured in experimental buckets (diameter: 100 cm, height: 85 cm, water depth: 70 cm) for 2 years. The plants of the two
invasive species used for the experiment were collected from the littoral zone of several ponds at Hunan Agricultural University. These two invasive species were not collected for multiyear culture like the native ones, as they distribute largely in the pond around the experimental site and are easy to collect as experimental material. The collected two invasive and two native species were domesticated at similar conditions for 2 weeks in order to obtain similar conditions for the native and invasive plants at the start of the experiment. The selected plants were similar in size, healthy in appearance and without flowers. Apical shoots (20 cm length) of A. philoxeroides, M. aquaticum, and M. spicatum and intact plants (14 cm height) of V. spinulosa were collected and randomly planted in 320 plastic pots (diameter: 30 cm, height: 20 cm, sediment depth: 18 cm, 8 plants per pot), and then the pots were placed in 64 buckets (diameter: 100 cm, height: 85 cm, water depth: 70 cm, 5 pots per bucket). During the acclimation period, the water temperature was kept at 25–29 °C. Photosynthetic active irradiation (PAR) was approximately 620 μmol m⁻² s⁻¹ above the water surface at noon on sunny days. NH₄⁻N, NO₃⁻N, and PO₄³⁻P in the water column were 0.05 ± 0.01 mg L⁻¹, 0.10 ± 0.02 mg L⁻¹ and 0.027 ± 0.003 mg L⁻¹, respectively. After 2 weeks acclimation, A. philoxeroides, M. aquaticum and M. spicatum developed roots. The initial morphological traits of plants used in this experiment were measured with plant biomass and plant height being 1.6 ± 0.1 g and 25.9 ± 0.3 cm for A. philoxeroides, 2.3 ± 0.1 g and 26.3 ± 0.1 cm for M. aquaticum, 1.0 ± 0.05 g and 25.7 ± 0.2 cm for M. spicatum, and 2.0 g ± 0.2 g and 21.1 ± 0.8 cm for V. spinulosa, respectively.

We ran a three-factorial designed experiment with two nutrient levels (L: low nutrient, H: high nutrient), five planting patterns of different species combinations (A. philoxeroides vs V. spinulosa, A. philoxeroides vs M. spicatum, M. aquaticum vs V. spinulosa, M. aquaticum vs M. spicatum, and A. philoxeroides vs M. aquaticum) and five mixed planting ratios (8:0, 6:2, 4:4, 2:6, 0:8). There were six replicates of each treatment combination. As for nutrient control, two nutrient levels were established in the water column by adding, respectively, 5 and 30 g of the 3rd generation of controlled release fertilizer (Osmocote Plus 15 N-10P-12 K + 2MgO + TE, Everris) at the beginning of the experiment. The controlled release fertilizer was packed in a mesh bag and placed at the bottom of the water column. The macronutrients and likely also micronutrients in the fertilizer released slowly and continuously during the experiment.

A total of 2400 plants (720 of both A. philoxeroides and M. aquaticum, 480 of both M. spicatum and V. spinulosa) were selected after acclimation and transplanted evenly into 300 plastic pots (diameter: 30 cm, height: 20 cm, 8 plants per pot) according to the experimental design of species combination patterns and mixed planting ratios (Figure 1). Each plastic pot was filled with a layer of 18 cm clay sediment (TN: 1.60 mg g⁻¹, TP: 0.86 mg g⁻¹ and TOC: 19.95 mg g⁻¹ dry weight). Clay is the substrate in the ponds where the two invasive species grow around the experiment site and is a common substrate type for the two native species in lake ecosystem. The plant density in each pot was within the range of naturally growing population densities.19,20,39 The 300 pots were evenly placed in 60 buckets (diameter: 100 cm, height: 85 cm, water depth: 70 cm, 5 pots per bucket). Six buckets (replicates) were used for each treatment combination.

Each of them was filled with tap water, and additional water was added every week to compensate for the loss of water due to evaporation. During the experimental period, the water temperature was kept at 27–30 °C. The buckets were covered with a layer of black mesh nets to simulate reduced light availability due to eutrophication in water bodies at the littoral zone, with photosynthetic active irradiation (PAR) of approximately 640 μmol m⁻² s⁻¹ above the water surface at noon on sunny days. Nutrients in the water column, total nitrogen (TN), total phosphorous (TP), NH₄⁻N, NO₃⁻N and PO₄³⁻P were measured in each of the 60 buckets every 2 weeks. TN and NO₃⁻N were determined by a UV spectrophotometer.60 TP and PO₄³⁻P were analyzed using the molybdenum blue method.61 NH₄⁻N was measured by the phenolhypochlorite reaction.62 The concentrations in the low and high nutrient treatment were 0.77 ± 0.04 and 1.22 ± 0.07 mg L⁻¹ for TN, 0.13 ± 0.00 and 0.21 ± 0.01 mg L⁻¹ for TP, 0.06 ± 0.01 and 0.15 ± 0.02 mg L⁻¹ for NH₄⁻N, 0.06 ± 0.01 and 0.27 ± 0.04 mg L⁻¹ for NO₃⁻N and 0.032 ± 0.001, and 0.066 ± 0.005 mg L⁻¹ for PO₄³⁻P, respectively. Repeated measures analysis of variance (ANOVA) showed that these five water quality indices differed significantly between the two nutrient levels (TN: F = 18.5; TP: F = 49.7; NH₄⁻N: F = 14.1; NO₃⁻N: F = 25.1; F = 47.9; P < 0.05 and n = 30 for all these five indices). The low and high nutrient levels in the water column in this experiment correspond to mesotrophic and eutrophic water bodies at the natural littoral zones, respectively.55

2.3. Plant Trait Measurements. Plants were harvested and measured for morphological traits: plant biomass (g), ramet number, plant height (cm), root length (cm), root, stem and leaf biomass ratios, above-ground vs below-ground ratio (AB ratio) and RGR, and physiological traits: starch, free amino acid (FAA), soluble carbohydrate (SC), phenolics and proline contents (mg g⁻¹ dry weight) in roots, stems and leaves of A. philoxeroides, M. aquaticum and M. spicatum and in roots and leaves of V. spinulosa, only as the stems did not extend to the column of this species, as well as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA), and protein in the leaves of A. philoxeroides, M. spicatum, and V. spinulosa. Plant biomass refers to fresh weight of shoot, root, and stolon. The plant height was measured as the distance from the base of the shoot to the apex of the longest leaf. The root length was the longest distance from the initial growth of the root to the root tip. At the termination of the experiment, the maximum density of fresh biomass in the experimental system was 5.95, 2.07, 1.74, and 0.28 kg m⁻² for A. philoxeroides, M. aquaticum, M. spicatum, and V. spinulosa, respectively, which was lower than the density in the naturally distributed populations.20,63 All plants were morphologically
growing taller and larger throughout the experimental period, so we presume that they were in the vigorous growth phase. RGR of plant biomass for each of the four species was calculated as: $RGR = \frac{(\ln X_2 - \ln X_1)}{T}$, where $T$ is the experimental period, $X_1$ is the initial parameter value of the macrophytes, and $X_2$ is the parameter value at the end of the experiments.

FAA and SC are intermediate metabolites linking plant carbon and nitrogen metabolism, and they are susceptible to external nutrient variations. Proline is one of the components of plant proteins, and plants tend to accumulate large amounts of proline under stress conditions. Starch, as a form of energy storage, helps plants to cope with various adverse environments. Phenolics is usually used as an indicator of allelopathy and palatability of plants. SOD, POD, and CAT, as antioxidant enzymes, can respond to the stress intensity subjected by plant. MDA reflects the degree of damage to plant cell membranes and the resistance of plants to stress. After measuring the morphological traits, the tissues (leaves, stems, and roots) of plants were divided into two parts. One part was oven-dried at 80 °C to a constant weight to measure C/N metabolites, and the other part was stored in a −20 °C refrigerator for measuring enzyme activity and MDA content.

The dried tissues of the four species were ground to a fine powder. Approximately 50 mg of sample powder was extracted twice with 8 mL of 80% ethanol at 80 °C for 20 min followed by centrifugation at 10,000×g for 15 min. The supernatant was collected, decolorized with activated carbon, and filtered (microvoid membrane, 0.2 μm). The filtrate was used for the analysis of SC, FAA, phenolics and proline using glucose, alanine, tannic acid, and proline as standards, respectively. The residue after centrifugation was used for analysis of starch.

Plants for SOD, POD, and CAT analyses were ground into fine powder in liquid nitrogen with a mortar and a pestle. One gram of fine powder was extracted at 4 °C for 20 min in 5 mL of ice-cold buffer (50 mM potassium phosphate (pH 7.8) containing 1 mM EDTA, 1 mM PMSE, 1% (w/v) PVP, 0.5% (v/v) Triton X-100 and 1 mM Vc) and then centrifuged at 15,000×g for 10 min at 4 °C, and the supernatant was collected for analysis. SOD activity was assayed based on a method of Beyer and Fridovich, which measured the inhibition of nitroblue tetrazolium (NBT) photochemical reduction at 560 nm. One unit SOD activity was defined as 50% inhibition of the NBT reduction to blue formazan. POD and CAT activity was measured according to the methods of Kar and Mishra. The MDA content was determined by measuring the amount of trimethoprim, its reaction product with thiobarbituric acid. In addition, the fresh leaf samples of *M. aquaticum* were insufficient, so the three enzyme activities and MDA contents were not measured for this species.

2.4. Plant Trait Network Analysis. To decipher differences in trait interactions between two nutrient levels and four species, we performed a plant trait network analysis. The PTNs help to clarify complex relationships among traits and further enable calculation of metrics for the topology of trait coordination and how they shift across treatments. A plant trait network consists of a series of nodes and edges. In PTNs, plant traits are nodes, and trait–trait relationships are edges. A matrix of trait–trait relationships ($r$) was calculated after normalizing transformation for 23 plant traits of *A. philoxeroides*, *M. aquaticum*, and *M. spicatum* and 17 plant traits of *V. spinulosa*, and these relationships were quantified using significant Pearson correlations. A threshold of $|r| > 0.2$ indicated significant correlations among traits at $P < 0.05$, which was used to avoid spurious correlations among traits.

The following two parameters were calculated: (1) “Degree,” which is the number of edges that connect a focal node trait to other nodes. The degree value of a specific trait can be calculated according to the following equation:

$$k_i = \sum_{j \neq i} a_{ij}$$

where $k_i$ is the degree of the focal node trait $i$; $a_{ij}$ is the correlation between the focal node trait $i$ and node trait $j$ (adjacency matrix described above). Plant traits with a high degree can be considered as hub traits that play an important role in regulating the whole plant phenotype. (2) “Modularity” is defined as the fraction of connections among plant traits that falls within a given module minus the same fraction in a random model where trait–trait relationships are randomly distributed. High modularity means that plant traits within modules are closely connected with each other, while the connections between modules are weak, implying that the modules composed of specific traits perform specific functions. A higher modularity of the network (i.e., more modules or trait dimensions) may give plants more freedom and flexibility to respond to unfavorable environmental conditions while maintaining a normal performance.

2.5. Statistical Analysis. Before analysis, all data were tested for normality of distribution and variance of homogeneity using Shapiro–Wilks and Levene’s tests, respectively, with “log” or “square root” transformations when necessary. ANOVA analysis with Tukey’s test was used to compare the differences in the means of morphological and physiological traits among treatments. The explanation percentage of trait variation among treatment was calculated according to sum of square in ANOVA analysis. Pearson’s correlations between plant morphological and physiological traits were determined by the R package “ggcorr” (Csardi and Nepusz) in the R 4.1.0 software. PTNs were visualized using Cytoscape 3.9.1.

**2.5. Statistical Analysis.** Before analysis, all data were tested for normality of distribution and variance of homogeneity using Shapiro–Wilks and Levene’s tests, respectively, with “log” or “square root” transformations when necessary. ANOVA analysis with Tukey’s test was used to compare the differences in the means of morphological and physiological traits among treatments. The explanation percentage of trait variation among treatment was calculated according to sum of square in ANOVA analysis. Pearson’s correlations between plant morphological and physiological traits were determined by the R package “ggcorr” (Wickham).

After scale transformation of all trait data, principal component analysis (PCA) was performed to obtain loading coefficients of each trait on each principal component axis. The plant traits distributed on the first and second principal component axes with high loading coefficients corresponded to the tolerance index (i.e., starch and phenolics in roots, stems and leaves) and the competitive index (i.e., plant biomass, plant height, ramet number and RGR), respectively. These two types of traits were used to characterize the competitive and tolerant abilities of plants, respectively, after covariance diagnosis based on variance inflation factor (VIF) values. The abilities of competition/tolerance are calculated according to the following formula:

$$P_i = \sum_{j=1}^{n} A_{ij}W_j$$

where $P_i$ is the trait’s ability to compete/tolerate with $n$ factors $W_j$. $A_{ij}$ is the coefficient of the $i$th trait and $j$th factor.
where $P_i$ is the competition/tolerance ability of plant individual $i$, $A_{ij}$ is the value of the plant trait $j$ after scale transformation for plant individual $i$, $W_j$ represents the weight coefficient of plant trait $j$, and $n$ represents the number of plant traits evaluated (\(n = 6\) for tolerance ability, \(n = 4\) for competition ability). The weight coefficient matrix of each trait on each principal component axis was obtained by dividing the loading coefficients by square root of the corresponding eigenvalue and then the combined coefficient of each trait was obtained based on the variance explained percentage of each principal component axis. The weight of each trait was calculated by normalization of the combined coefficients.\(^{79,80}\)

The Mantel test focuses on the correlation between two metrics by comparing the correlation of two distance matrices. Relationships between competition and tolerance abilities and plant traits were determined by the Mantel test to evaluate the importance of network organization and interaction for the whole-plant-level performance. All trait data obtained from five species combination patterns at two light levels for four species were transformed to Euclidean distances before the Mantel test. These analyses were conducted using the package “vegan” (Oksanen et al.) in R 4.0.1 software.\(^81\)

| Table 1. Percentage of Explained Variance Based on Three-Way ANOVA for Plant Biomass and Ramet Number of Two Exotic and Two Native Species Sampled at the End of the Experiment Relative to Nutrient Levels (N), Species Combinations (V), and Plant Ratios (R)\(^\text{a}\) |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| nutrient (N) versus (V) ratio (R) N × V N × R V × R N × V × R |
| Plant biomass |
| A. philoxeroides 58.9(0.001) 25.9(0.001) 3.4(0.105) 6.3(0.017) 2.2(0.234) 1.9(0.279) 1.3(0.471) |
| M. aquaticum 11.2(0.209) 22.3(0.045) 3.3(0.628) 40.3(0.004) 0.4(0.951) 17.3(0.048) 5.3(0.553) |
| M. spicatum 1.2(0.712) 11.8(0.255) 0.9(0.902) 40.0(0.038) 29.6(0.042) 10.4(0.320) 6.1(0.508) |
| V. spinulosa 54.4(0.001) 18.6(0.001) 19.7(0.001) 0.0(0.821) 1.3(0.238) 5.9(0.002) 0.1(0.907) |
| Ramet number |
| A. philoxeroides 52.8(0.001) 24.2(0.001) 1.5(0.375) 14.0(0.001) 3.6(0.094) 2.0(0.250) 1.8(0.306) |
| M. aquaticum 15.8(0.136) 16.4(0.01) 6.1(0.423) 35.8(0.008) 5.7(0.448) 12.8(0.129) 7.5(0.373) |
| M. spicatum 0.0(0.930) 0.0(0.919) 5.3(0.234) 72.2(0.001) 8.6(0.096) 8.6(0.096) 5.2(0.240) |
| V. spinulosa 17.7(0.017) 16.2(0.022) 52.0(0.001) 0.2(0.784) 0.2(0.827) 11.7(0.023) 2.1(0.499) |

\(^a\)Bold indicates significance at $P < 0.05$ level.

Figure 2. Plant biomass (mean ± standard error, $n = 10–18$) of two exotic (A,B) and two native (C,D) species. (H: high nutrient, and L: low nutrient. vs_Ap, vs_Ma, vs_Ms, and vs_Vs represent the focal species co-cultured with A. philoxeroides, M. aquaticum, M. spicatum, and V. spinulosa, respectively. Letters indicate significant differences at $P < 0.05$.)
3. RESULTS

3.1. Responses of Plant Traits. Overall, there were marked effects of nutrient and species combinations on the morphological and physiological traits of the four species (Table 1 and S1–S8), while the effects of planting ratios were negligible (except for the morphological traits of *V. spinulosa*). Increased nutrient levels promoted an increased biomass and ramet number of *A. philoxeroides*, explaining 58.9 and 52.8% of the variation, respectively, followed by species combination with 25.9 and 24.2% of the variation, respectively (Table 1, Figure 2 and S1–S2). The biomass and ramet number of *V. spinulosa* were significantly influenced by nutrient levels, species combination and planting ratio (Table 1, Figure 2 and S1–S3). The biomass of *V. spinulosa* decreased significantly with increasing nutrient level but increased when planted together with *M. aquaticum* at low nutrient levels (Figure 2). Nutrient effects did not significantly affect the biomass and ramet number of *M. aquaticum* and *M. spicatum*, while these two traits were significantly affected by the...
interactions between nutrient and species combinations, explaining 35.8–72.2% of the variation (Table 1).

Compared with the other three species, *A. philoxeroides* can regulate enzyme activity more effectively and hence mitigate external abiotic and biotic disturbance of cell membranes. A nutrient level increase significantly enhanced the SOD content of all three species, while there was no significant effect on POD, CAT, and protein (Figure 3 and S4). For *A. philoxeroides*, SOD was highest in mixed cultures with *M. aquaticum*, followed by mixed cultures with *V. spinulosa* and *M. spicatum*. Being in mixed cultures with the other species had no significant effect on POD for *A. philoxeroides*, and CAT was high and MDA was low when the species was co-cultured with *M. aquaticum* (Figure 3 and S4). For both *M. spicatum* and *V. spinulosa*, SOD was higher when mixed-cultured with *M. aquaticum* than with *A. philoxeroides*. There was no significant effect of species combinations on POD, CAT, MDA, and protein contents (Figure 3 and S4).

### 3.2. Species Trait Network Characteristics.

For each species, the average degree of all traits corresponds to the strength of connectivity among traits from a whole plant perspective (Figure 4). *A. philoxeroides* had the highest average degree (i.e., the tightest connectivity among traits), while *V. spinulosa* had the lowest average degree. In addition, *A. philoxeroides* had the lowest modularity (i.e., the functional differentiation among traits was not clear), while *V. spinulosa* had the highest modularity of the four species. Furthermore, the degree of each plant trait responded to the number of edges that connect the focal trait to other traits. Overall, traits that respond to plant tolerance (e.g., starch and phenolics) and competitive ability (e.g., plant biomass, plant height) had high degrees. The degree of the same trait varied considerably among species (Figure 5). For *A. philoxeroides*, stem phenolics and root starch had the highest degree, followed by plant height and biomass. For *M. aquaticum*, root starch, leaf ratio, root ratio, leaf phenolics, root phenolics, plant height, plant biomass, and ramet number had the highest degree. For *V. spinulosa*, the traits with a higher degree were root length, root proline, root SC and root FAA.

### 3.3. Competition and Tolerance Trade-Offs.

Principal component analysis was performed for 24 plant traits, and five PCA axes with eigenvalues greater than 1 were extracted, with a total explanation of 73.2%, in which PCA1 and PCA2 explained 30.9 and 18.5%, respectively. On the PCA1 axis, phenolics and starch had large loading scores (Table S9), representing plant tolerance ability to external disturbances. On the PCA2 axis, plant biomass and RGR, plant height, and ramet number had high loading scores, representing the competitive ability of plants.

For the four species, *A. philoxeroides* had the highest competitive ability, followed by *M. aquaticum* and *V. spinulosa*, and *M. spicatum* had the lowest competitive ability (Figure 6). *M. aquaticum* was the most tolerant species, followed by *M. spicatum* and *V. spinulosa*, and *A. philoxeroides* had the highest degree of tolerance. For *V. spinulosa*, the traits with a higher degree were root length, root proline, root SC and root FAA.

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**Figure 5.** Alluvial plot of degree of each plant trait for the four species. The number in each node is the degree value of the corresponding plant trait. Each curve with different colors represents a plant trait, and the curvature reflects the variation of degree value among the four species. The greater the curvature, the greater the difference in degree value of trait among the four species. The curves connected by the same node indicate the same degree of the corresponding traits. The order of traits which are connected into the same node (top, middle, and bottom) is ranked according to the vertical coordinate labels on the left side of the figure. SC and FAA represent soluble carbohydrates and free amino acids, respectively. The AB_ratio represents above-below-ground biomass ratios.

**Figure 6.** Competition and tolerance of the four species planted at two nutrient levels (H: high nutrient; L: low nutrient). Letters indicate significant differences at $P < 0.05$. The values represent the standardized data.
spicatum, and A. philoxeroides and V. spinulosa had the lowest tolerance. Increased nutrient levels in the water column resulted in increased competitiveness of A. philoxeroides and reduced tolerance of M. aquaticum (Figure 6 and S5–S6).

Pearson correlation analysis showed that competition-related traits (plant biomass, plant height, ramet number and RGR) were negatively correlated with physiological traits (phenolics and proline in stems and leaves) for A. philoxeroides. Starch was positively correlated between roots, stems and leaves (Figure 7). For M. aquaticum, root starch was positively correlated with root phenolics, while leaf proline was positively correlated with leaf FAA and stem FAA (Figure S7).

Mantel analysis showed that competitive and tolerance abilities were related to different plant traits and that these relations were species-specific (Figure 7 and S7–S9). For A. philoxeroides, competition-related traits were plant biomass and height, root and stem ratio and RGR, while traits related to tolerance were root and leaf starch. The Mantel values for these relationships were higher than 0.5 (Figure 7). For M. aquaticum, traits related to competitive ability were plant biomass and height and RGR, and traits related to tolerance were root and stem starch. The Mantel values for these relationships were higher than 0.5 (Figure 7). For M. spicatum, competition correlated with plant height and RGR, while tolerance correlated with root starch and phenolics in roots, stems and leaves (Figure S8). For V. spinulosa, competition was correlated with plant biomass and height, followed by ramet number and RGR, while tolerance was correlated with root and leaf starch (Figure S9).

4. DISCUSSION

Eutrophication may alter the morphological and physiological traits of aquatic plants, affecting plant competition and tolerance, and subsequently plant invasiveness. Two invasive plants and two native plants were selected to elucidate the effects of a nutrient level increase in the water column on plant invasiveness. We found that (1) the effect of a nutrient level increase on plant invasiveness differed between species, while no effect of plant density on invasion and resistance to invasion was observed. (2) A. philoxeroides had the tightest connectivity among the traits studied and effective regulation of enzyme activity, which is consistent with its high competitive ability. (3) M. aquaticum had high tolerance and strong interaction with other plants, resulting in exacerbating adverse effects on the littoral ecosystem under eutrophication. (4) Eutrophication reduced the biomass accumulation and RGR of V. spinulosa, and diminished the contents of phenolics and starch of M. spicatum, making this species more vulnerable to environmental fluctuations. Overall, eutrophication in the littoral zone promoted exotic plant invasiveness and weakened the resistance of native plants to invasion.

4.1. Implications for Plant Invasion Control.

We found that the nutrient level had the greatest effect on plant morphological and physiological traits, followed by species combinations and planting ratios. Elevated nutrient levels significantly promoted the biomass accumulation of the invasive plant A. philoxeroides and inhibited that of the native plant V. spinulosa, which concurs with the results of previous studies. The combined effect of eutrophication on the inhibition of native plants and the promotion of invasive plants accelerate the propagation and spread of invasive plants. Therefore, controlling eutrophication in water bodies is particularly important to prevent the spread of A. philoxeroides. Furthermore, the native species combination in this study did not significantly affect the biomass, ramet number, and RGR of the invasive species A. philoxeroides and M. aquaticum and the effect of mixed species ratios on invasive plant traits was almost
negligible. Zhang et al.\textsuperscript{82} found no noticeable growth inhibition of \textit{A. philoxeroides} by the native plant \textit{Oenanthe javanica} (BL) DC. and \textit{Iris pseudacorus} L., indicating that the species composition and plant density of these two dominant native plants also had no significant effect on the resistance to invasion. Our results as well as those of Zhang et al. suggest that the effectiveness of exotic plant invasion prevention and control by regulating the biodiversity in the littoral zone is limited, and effective control of the arrival of invasive plants may be the most effective approach.\textsuperscript{6,83}

4.2. Plant Invasion and Littoral Eutrophication.

Eutrophication promoted the invasion of the resource-acquiring plant \textit{A. philoxeroides} and, in general, aquatic plant invasion is more likely to occur following intensified eutrophication of water bodies caused by human activities.\textsuperscript{84} This is because increased availability of resources induced by eutrophication enhances dominance of exotic species over native species as exotic species tend to have a high resource acquisition capacity.\textsuperscript{16,85} Our PTN analysis indicated that \textit{A. philoxeroides} had high connectivity between traits, supporting its high resource uptake and assimilation efficiency as well as its high RGR, which is consistent with the high invasiveness of this species. Furthermore, eutrophication led to a significant increase in leaf SOD activity for \textit{A. philoxeroides}, demonstrating that eutrophication caused physiological stress to the plants.\textsuperscript{86} Moreover, the value of MDA, an important trait that responds to damage of cell membranes, was positively related to the degree of impairment.\textsuperscript{86} However, the MDA content of \textit{A. philoxeroides} decreased significantly under eutrophication, likely reflecting its higher enzyme activity (e.g., POD) and resource turnover efficiency. This mitigating capacity to physiological stress on cell membranes under eutrophication ensures its higher invasiveness in eutrophic water bodies.

Eutrophication had stressful effects on the resource-tolerant invasive plant \textit{M. aquaticum}. Although eutrophication did not significantly inhibit the biomass, ramet number, and RGR of \textit{M. aquaticum}, its tolerance traits (e.g., phenolics and starch contents) were somewhat reduced. However, the negative effects of eutrophication were much greater on the native plants than on the invasive plants, such as a remarkable decrease in biomass and RGR of \textit{V. spinulosa} and in the starch and phenolics contents of \textit{M. spicatum}. Therefore, from a long-term perspective we speculate that the inhibitory effect of eutrophication on native plants will provide higher availability of resources and more space for the growth and reproduction of \textit{M. aquaticum}, which, together with its intrinsically larger RGR, promotes its spread. Since our experiment was maintained for only 2 months, the duration of eutrophication’s inhibitory effect on native plants was relatively short. A natural follow up would be long-term experiments.

Furthermore, plants usually accumulate phenolics and starch in their tissues as an adaptive response to unfavorable habitat conditions, and this plays a key role in their ability to adapt to various stresses such as high nutrient stress, pathogen infection, herbivores, high light, and low temperature.\textsuperscript{87,88} \textit{M. aquaticum} contained high amounts of phenolics (nearly 10 times that of \textit{V. spinulosa} and twice that of \textit{M. spicatum}) and starch (approximately 25 times that of \textit{V. spinulosa} and \textit{M. spicatum}), which improves its ability to resist environmental stresses and increases its capacity to interfere with the growth of other plants. We found that the SOD activity of native species was significantly higher when they were planted together with \textit{M. aquaticum} than with \textit{A. philoxeroides}, probably because \textit{M. aquaticum} releases large amounts of phenolics that interfere with the growth of the surrounding native plants.\textsuperscript{41} Previous studies have also found that phenolics released by \textit{M. aquaticum} adversely affect the growth and reproduction of the rotifer \textit{Platypus patulus}.\textsuperscript{81} Thus, the growth and spread of \textit{M. aquaticum} in eutrophic waters can cause degradation of species richness and notable alterations of the structural composition of species in the littoral zone.

4.3. Native Plant Resistance and Eutrophication.

Lakes that are biologically invaded become more similar in species composition.\textsuperscript{90,91} Invasion of exotic macrophytes has a strong detrimental impact on the abundance and diversity of the resident macrophytes, with common species becoming more widespread and rare species becoming rarer.\textsuperscript{92,93} A previous study showed invasion of \textit{A. philoxeroides} significantly reduced the number of species and seedling density in the soil seed bank of the reed community.\textsuperscript{91} However, we did not discover any obvious effects of invasive plants on the plant biomass, ramet number, and RGR of the native species, probably because the two species selected for this study had high environmental adaptability,\textsuperscript{94,95} resulting in a relatively weak influence of invasive plants on their morphological traits. Moreover, the experiment was of short-term duration. Our results, however, revealed a pronounced impact of invasive plants on the physiological traits of the native plants: under low nutrient conditions, \textit{A. philoxeroides} induced a decrease in the phenolics and starch contents of \textit{M. spicatum}, while \textit{M. aquaticum} led to increased SOD activity of native species. These stress effects on physiological traits could intensify the vulnerability of native submerged plants, especially that of rare species, to environmental changes. This would result in a more pronounced growth inhibition of native plants following habitat disturbances and would subsequently trigger changes in the composition of submerged vegetation. Thus, our physiological trait results indicated that invasion will lead to increased vulnerability of the native vegetation to environmental disturbance in the long term.

Furthermore, as a dominant species in lakes in China, \textit{V. spinulosa} features a low light saturation point, high tolerance to stressful conditions, and rosette characteristics, and it thus plays an essential role in purifying the water and preventing substrate suspension here.\textsuperscript{17,20} Eutrophication reduced the biomass of \textit{V. spinulosa} and thus its water purifying effect, which may further increase nutrient levels in water bodies and reduce the resistance of the littoral zone to plant invasion. Meanwhile, eutrophication caused an increase in plant SOD activity and a reduction of plant phenolics and starch contents in both native species, which reduced the ability of native species to resist invasion. Previous studies have shown that plant-invaded habitats have increased water turbidity and nitrogen and organic matter concentrations, which is associated with the ability of invaders to modify habitats and increase eutrophication.\textsuperscript{12,92} Moreover, invasive plant-habitat interactions, resulting in reduced habitat heterogeneity, may have a negative impact on plant community dynamics, which may then promote continued invasion.\textsuperscript{96,97} Therefore, eutrophication in the littoral zone has a suppressive effect on the morphology and physiology of native plants, causing habitat degradation, which may be followed by continued plant invasion, i.e., a vicious cycle that exacerbates the degradation of littoral habitats.

In conclusion, our study elucidated the impact of eutrophication on plant invasion and native plant resistance.
to invasion in the littoral zone as evidenced by numerous morphological and physiological traits of plants. Compared to native plants, exotic plants were highly adapted to eutrophication (A. philoxeroides with high competitive ability and M. aquaticum with high tolerance), while increased density and different combinations of native species did not considerably inhibit exotic plant growth. This demonstrates that controlling eutrophication in lakes is important to prevent rapid multiplication and spread of exotic plants and that the two dominant native species in the littoral zone did not effectively resist plant invasion. The high connectivity between traits and enzymatic activity in A. philoxeroides supports its rapid uptake and assimilation of resources under eutrophication, facilitating its rapid expansion to occupy ecological niches in the littoral zone. M. aquaticum has high tolerance and may occupy ecological niches by releasing substances that interfere with other creatures in the habitat. Under eutrophication, the high adaptability of exotic plants and the growth inhibition of native plants will intensify plant invasion, exacerbating the degradation of the littoral ecosystem. Our experiments provide morphological and physiological insight into how vegetation degradation in the littoral zone would be exacerbated by two common exotic species under eutrophication and clearly shows the value of analyzing both morphological and physiological traits.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c09486.

Variance for the morphological traits of A. philoxeroides; physiological traits of A. philoxeroides; morphological traits of M. aquaticum; physiological traits of M. aquaticum; morphological traits of M. spicatum; physiological traits of M. spicatum; morphological traits of V. spinulosa; physiological traits of V. spinulosa; loading contents of plant traits; ramet number of four species; relative growth rate of four species; plant biomass and ramet number of V. spinulosa sampled at five mixed planting ratios; POD, CAT, and protein of A. philoxeroides, M. spicatum, and V. spinulosa; starch contents of four species; phenolics contents of four species; correlation analysis for M. aquaticum; correlation analysis for M. spicatum; and correlation analysis for V. spinulosa (PDF).

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Author Contributions

G.Y. conceived and designed the research; L.S., P.G., J.X., and W.M. performed the experiments; G.Y. analyzed the data; H.F., Y.L., A.W., and B.R. provided reagents/materials/analysis tools and commented on the paper; G.Y. and E.J. wrote and edited the manuscript, respectively. All authors commented on and approved the manuscript.

Notes

The authors declare no competing financial interest. The data sets used or analyzed in the current study are available from the corresponding author upon reasonable request.

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