


Effects of elevated CO₂ concentration and nitrogen addition on foliar phosphorus fractions of *Mikania micranatha* and *Chromolaena odorata* under low phosphorus availability

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Abstract

Invasive plants rapidly spread in habitats with low soil phosphorus (P) availability and have triggered a sharp decline in the diversity of native species. However, no studies have explored how widespread invasive species acclimate to low soil P availability via changing foliar P fractions, especially under elevated atmospheric CO₂ concentrations ([CO₂]) and nitrogen (N) deposition. Here, an open-top chamber experiment was conducted to explore the effect of nutrient addition and elevated [CO₂] on leaf traits and foliar functional P fractions (i.e., Pi, metabolite P, lipid P, nucleic acid P, and residual P) of two aggressive invasive species (*Mikania micranatha* and *Chromolaena odorata*). We found that foliar N/P ratios were more than 20, and P addition significantly increased plant biomass. Both results indicated P-limited plant growth at our studied site. Elevated [CO₂], N and N + P addition greatly increased plant biomass, photosynthetic rates, and photosynthetic P-use efficiency (PPUE) in invasive species, but PPUE decreased with increasing P addition. Nitrogen addition slightly decreased the concentration of leaf total P, decreased foliar residual P, but increased metabolite P concentrations in invasive species. Similar changes in foliar P fractions were found under N + P addition. Phosphorus addition increased foliar P concentrations, which was strongly correlated with an increase in metabolite P concentrations in invasive species. Elevated [CO₂] alleviated these effects and increased PPUE. The present results suggest that future elevated [CO₂] and N deposition allow the invasive species to acclimate to low soil P availability and support their successful invasion by greatly reducing P allocation to non-metabolite foliar P fractions (i.e., nucleic acid P and residual P) to meet their demand of metabolite P for photosynthesis and exhibit a high PPUE.

1 | INTRODUCTION

Phosphorus (P) is an essential plant nutrient and often limits net primary productivity (Hidaka & Kitayama 2013; Mo et al. 2019). In subtropical forests, plant productivity is generally limited by low availability of soil P, rather than by low availability of nitrogen (N) due to the long-term weathering of bedrock and the gradual loss of P (Jonard et al. 2015; Mo et al. 2019; Walker & Syers 1976). While P is often limiting, N is increasingly available in subtropical forests because of atmospheric N deposition, which has increased to ~30–50 kg N ha yr⁻¹ in subtropical forests in China (Luo et al. 2019; Mo et al. 2006). Another factor that may greatly affect plant growth is the atmospheric concentration of CO₂, which has increased from ~280 μmol mol⁻¹ in 1840 to ~410 μmol mol⁻¹ in 2021 (IPCC, 2013; <https://www.co2.earth/>). Under such environmental conditions, invasive species exhibit rapid invasion in P-impooverished habitats (Dukes & Mooney 1999) and can potentially alter the functioning and structure of terrestrial ecosystems (Li and Xie, 2002; Tang et al. 2006; Song et al. 2009; Sage, 2019). However, the effects of increases in atmospheric [CO₂] and N deposition on the strategies that plants have evolved to use P efficiently in P-impooverished forests have rarely been documented in invasive species (Campbell & Sage 2006; Lewis et al. 2010; Tissue & Lewis 2010; Wang et al. 2016). Therefore, it is important to identify the strategies that invasive species have evolved to use P efficiently at low soil P availability under elevated [CO₂] and N deposition to provide new insight to predict and perhaps control plant invasions.

Regulating P allocation in leaves is a vital strategy in plants to acclimate to extreme soil conditions (e.g., low soil P availability; Zhang et al. 2018; Wang et al. 2019) and environmental change (e.g., elevated [CO₂] and N deposition; Tissue & Lewis 2010). Foliar P can be fractionated into inorganic phosphate (Pi) and organic P fractions (metabolite P, lipid P, nucleic acid P and residual P; Hidaka & Kitayama 2013). Pi represents a significant fraction of leaf P and is generally stored in the vacuole when a plant acquires more Pi than it needs (Veneklaas et al. 2012). The foliar metabolite P fraction consists mainly of intermediates of carbon metabolism, such as bioactive-molecular compounds (e.g., phosphorylated sugars, ADP and ATP). Nucleic acid P is the major organic P fraction (≥50% of the foliar organic P pool) and rRNA, essential for protein synthesis, consists of up to 85% of nucleic acid P (Matzek & Vitousek 2009). Lipid P comprises phospholipids, most of which are components of the plasmalemma and organelle membranes (Veneklaas et al. 2012). Finally, the uncharacterized residual fraction may include phosphorylated proteins, some of which regulate cellular processes (Mo et al. 2019).

Under P deficiency, photosynthesis is generally reduced due to feedback inhibition resulting from reduced leaf growth (Zhang et al. 2016) or the limitation of orthophosphate (Pi) in the cytosol (Mo et al. 2019). These decreases in photosynthetic activity might increase photosynthetic N-use efficiency (PNUE) and photosynthetic P-use efficiency (PPUE), and may also decrease the leaf mass per unit leaf area (LMA; Ghannoum et al. 2010). Plant growing at low soil P availability can reduce their overall need for foliar P by decreasing non-metabolite P fractions and buffer the direct Pi-restriction of photosynthesis (Hidaka & Kitayama, 2011; Warren 2011). Moreover, the replacement of phospholipids (lipid P) in membranes by sulfolipids and

galactolipids allows plants to maintain foliar metabolite P concentrations in P-deficient soil (Lambers et al. 2012; Veneklaas et al. 2012). Despite studies on the allocation of leaf P fractions following N or P addition (Mo et al. 2019) in different soil conditions (e.g., soil age; Yan et al. 2019), it remains unclear how plants acclimate to low soil P availability under elevated [CO₂] and N addition (Song et al. 2009; Tissue & Lewis 2010; Zhang et al. 2016).

Since their invasion in southern China in the 1980s, *Mikania micrantha* and *Chromolaena odorata* have caused serious damage to secondary forests and crops (Li & Xie 2002; Song et al. 2009). The rapid spread of both invasive plants has triggered a serious decline in the diversity of native species in terrestrial ecosystems (Bradley et al., 2010). The photosynthetic rate is faster in invasive species than in co-occurring native species (Baruch & Goldstein 1999; Deng et al. 2004; Song et al. 2009). Relative to native species, invasive species generally have greater phenotypic plasticity and are more tolerant to environmental changes such as elevated [CO₂], N deposition, or low soil P availability (Alpert et al. 2000; Feng et al. 2007; Geng et al. 2006; Tissue & Lewis 2010). The objectives of this study were to determine how, under elevated [CO₂] as well as N and P additions, the invasive plants *M. micrantha* and *C. odorata* respond to low P availability (in terms of P allocation to leaves and related foliar traits) to maintain photosynthetic rates and growth. To accomplish these objectives, we conducted an open-top field chamber experiment with N and P addition under elevated [CO₂]. We hypothesized that (1) foliar traits (i.e., LMA and N and P concentrations) and photosynthetic capacity of the invasive species would increase with increasing P-application rate, and that these increases would be greater with elevated [CO₂] and N addition; (2) the increase in photosynthetic capacity in response to P and N addition under elevated [CO₂] would be more pronounced in invasive species than in a native species; and (3) elevated [CO₂] and N addition would change the allocation of P to different foliar P fractions, thus maintaining photosynthetic capacity and achieving a high PPUE, which allows the invasive plants to sustain growth in a soil with low P availability.

2 | MATERIALS AND METHODS

2.1 | Site description

The open-top field chamber experiment was conducted at the South China Botanical Garden (23°08'N, 113°17'E), Guangzhou Province, China. The region has a subtropical monsoon climate (Luo et al. 2019; Zhang et al. 2016) with a mean annual precipitation of 1750 mm, a mean annual temperature of 21.5°C, and mean relative air humidity of 77% (Zhang et al. 2016).

2.2 | Experimental design

The experiment included two widespread invasive species, that is, *M. micrantha* and *C. odorata*. For comparison, the experiment also included co-occurring native species (i.e., *Paederia scandens* and

Eupatorium chinense) that have similar morphologies as the invasive species. We selected *P. scandens* instead of *Mikania cordata* for the similar morphology with *M. micrantha*, because *M. cordata* is uncommon in subtropical region. *M. micrantha*, *C. odorata* and *P. scandens* were collected in South China Botanical Garden, *Eoanthidium chinense* was collected in Zhejiang Province (Figure S1).

Seedlings were initially grown under suitable soil water and light conditions in a nursery. Seedlings of similar size (about 100 mm tall) were then transplanted into pots (one seedling per pot) 280 mm tall and 320 mm in diameter containing 20 kg of soil. The soil had been collected at 0–400 mm depth from a primary broadleaf forest in South China Botanical Garden; the soil was mixed before it was transferred to pots. The soil chemical properties (means \pm SE) before treatments were: pH = 5.0 ± 0.05 ; organic C = $16.1 \pm 0.6 \text{ mg g}^{-1}$; total N = $1.9 \pm 0.04 \text{ mg g}^{-1}$; total P = $0.35 \pm 0.02 \text{ mg g}^{-1}$; available P (extracted with Bray-1 method) = $6.7 \pm 1.6 \text{ mg kg}^{-1}$; $\text{NH}_4\text{-N}$ = $30 \pm 3.1 \text{ mg kg}^{-1}$; and $\text{NO}_3\text{-N}$ = $8.1 \pm 0.2 \text{ mg kg}^{-1}$.

The experiment used 12 open-top chambers. Six of the chambers were “new” (i.e., they were constructed in January 2016) and cylindrical (3.5 m in height and 5.0 m in diameter). The other six open-top chambers were “old” (i.e., they were constructed in 2012) and smaller (3.5 m in height and 3.4 m in diameter). The above-ground section of the chamber was wrapped with transparent and impermeable plastic sheets, and the top was totally open. The light intensity in the chambers was 97% of full light. Air temperature and humidity were recorded by thermometers and humidity meters inside and outside the chambers, and no significant difference was found between inside and outside of the chambers. The elevated $[\text{CO}_2]$ treatment was achieved by supplying additional CO_2 from a tank until the CO_2 concentration of the chamber was about $700 \mu\text{mol mol}^{-1}$. In every chamber, a transparent pipe was hung 1.5 m from the ground, and a fan was connected at the end. The pipe contained pinholes, and the distance between pinholes was 10 cm. The concentration of elevated CO_2 from the tank was controlled by a flowmeter, and CO_2 concentrations on four planes (1.0, 1.5, 2.5, and 3.0 m in height) in the chambers were weekly monitored using a Licor-6400 (LI-COR Inc.; Figure S1).

On June 18, 2016, 2-month-old uniform and healthy seedlings of each species were selected and assigned to each chamber; each chamber contained 24 pots of each species. On July 2, 2016, two old and four new open-top chambers were exposed to elevated $[\text{CO}_2]$ ($700 \pm 50 \mu\text{mol mol}^{-1}$), and the other six chambers were exposed to ambient $[\text{CO}_2]$ ($400 \pm 50 \mu\text{mol mol}^{-1}$). We detected no significant difference in the light, temperature, or moisture conditions between the old and new chambers. For each species in each chamber, with randomized complete block design, four pots were not treated with P or N and were used as controls; four pots were treated with N ($6.25 \text{ g N m}^{-2} \text{ yr}^{-1}$); four pots each were treated with P fertilizer at rates of 0.75 (1/2P), 1.5 (1P), or 3.0 (2P) $\text{g P m}^{-2} \text{ yr}^{-1}$; and four pots were treated with both N and P ($6.25 \text{ g of N m}^{-2} \text{ yr}^{-1} + 1.5 \text{ g of P m}^{-2} \text{ yr}^{-1}$). The amount of N and P fertilizer was set according to the effect of N and P addition on plant biomass in our previous study (Zhang et al. 2016). In total, there were 12 conditions: three levels of

P addition, one level of N addition, one level of N + P addition, the control, and two levels of $[\text{CO}_2]$ for each of the previous six conditions, and there are 12 pots for each species each treatment. Chemically pure NH_4NO_3 was used as the N source, and chemically pure $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ was used as the P source (Guangzhou Chemical Reagent Factory). The solutions of N, P, or NP were sprayed on the soil surface of each pot.

2.3 | Measurement of foliar gas exchange

Healthy sun-exposed mature leaves were chosen for foliar gas exchange measurement from 9:00 to 12:00 h during 11 days in October 2016. For each gas exchange coefficient, at least six individuals for each combination of species and treatment were measured. Following the order of photosynthetic photon flux density (PPFD) 1200, 1000, 800, 500, 300, 200, 120, 50, 20, and $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$, photosynthetic light-response curves were made. When the measurements were conducted, the vapor pressure deficit was set at $2.0 \pm 0.5 \text{ kPa}$, and leaf temperature at $30 \pm 1^\circ\text{C}$. The non-rectangular hyperbola model of Thornley (1976) was used to calculate the maximum light-saturated photosynthetic rate.

2.4 | Measurement of foliar structural traits

After leaf gas exchange was measured, the leaf area of each projected leaf was determined using a leaf area meter (LI-3100C; LI-COR Biosciences); these leaves were then collected and oven-dried at 65°C to a constant weight for calculating the leaf mass per area (LMA). The remaining mature leaves on the sampled branch of each plant were freeze-dried and ground (after main veins and petioles were removed) to determine their foliar N and P concentrations, and the concentrations of foliar P fractions. Foliar N and P concentrations were measured by a colorimetric assay after sulfuric acid (H_2SO_4) digestion (Carter & Gregorich 2007; Liu et al. 1996; Sommers et al. 1970).

PPUE and PNUE were calculated as the ratio of the maximum photosynthetic rates per unit P or N. Photosynthetic capacity was expressed on a leaf area and dry mass basis. Finally, the plants were harvested and divided into roots, stems and leaves, then dried and weighed to calculate the biomass (g plant^{-1}).

2.5 | Measurement of leaf P fractions

Foliar P is generally divided into inorganic P (Pi) and organic P (metabolite P, lipid P, nucleic acid P, and residual P). Organic P fractions were sequentially extracted (Hidaka & Kitayama 2013) following the methods of Kedrowski (1983) and Close and Beadle (2004). Foliar Pi was extracted by the acetic-acid extraction method (Yan et al. 2019) and determined using a molybdenum blue-based method (Ames 1966). To determine the four fractions of organic P, 0.5 g subsample of a freeze-dried and ground foliar sample was homogenized

TABLE 1 Effects of species (S), phosphorus (P) addition, elevated [CO₂], nitrogen (N) addition, and their interaction on the foliar traits of invasive and native species as determined by multi-way ANOVA (F values are shown in the table)

Fixed effect	A _{area}	A _{mass}	LMA	PNUE	PPUE	[N]	[P]	N: P ratios	Phosphate	Metabolite P	Nucleic acid P	Lipid P	Residual P	Biomass
S	39.5	134	97.5	36.4	2.8	128	343	16.3	212.9	57.8	190	157	32.9	61.4
P	0.9	1.5	9.1	14.6	32.8	2.4	55.4	55.0	134	14.8	10.3	57.3	3.9	14.4
CO ₂	147	41.1	5.6	36.4	2.8	1.6	3.7	11.2	0.3	8.5	15.4	1.4	6.6	27.6
N	48.0	12.1	0.1	1.4	26.0	27.4	3.4	20.4	0.6	2.7	0.02	0.4	2.5	37.0
S × P	0.6	1.1	4.4	1.4	4.5	3.8	3.7	7.6	1.6	4.5	1.3	3.9	2.9	1.7
S × [CO ₂]	3.2	0.6	5.8	8.8	19.4	5.6	11.6	5.7	24.4	0.8	3.8	0.8	6.5	0.9
[CO ₂] × P	1.3	1.5	0.8	6.0	9.9	0.5	5.0	6.8	2.2	2.9	2.4	5.9	2.0	2.4
S × P × [CO ₂]	1.4	1.0	4.6	2.1	2.5	0.6	0.7	1.6	1.1	1.6	1.5	1.7	4.8	0.3
S × P × [CO ₂] × N	4.1	2.5	4.5	11.9	15.6	1.0	0.3	0.4	0.2	1.1	0.8	3.8	7.6	1.2

Note: A_{area} is photosynthetic rates per unit area; A_{mass} is photosynthetic rate per unit mass; LMA is leaf mass per unit area; PNUE is photosynthetic N-use efficiency and PPUE is photosynthetic P-use efficiency; [N] is N concentration, [P] is P concentration. Significant F values (p < 0.05) are in bold.

with 15 ml of 12:6:1 CMF (chloroform, methanol, and formic acid, v/v/v) in a 50 ml centrifuge tube (first tube). The liquid was extracted twice with a total of 19 ml of 1:2:0.8 CMW (chloroform, methanol, water, v/v/v), and then in those extract, 9.5 ml of chloroform-washed water was added. The final solvent was 1:1:0.9 CMW (v/v/v), which caused the extract to separate into a sugar-and nutrient-rich upper layer and a lipid-rich organic bottom layer. The upper layer in the second tube was transferred to a new tube (the third tube), and the bottom layer was used to determine lipid P.

A 5 ml volume of 85:100 methanol (v/v) was added to the material in the third tube, which was then placed in a vacuum dryer for 48 h to remove dissolved chloroform and methanol. The aqueous layer was refrigerated (4°C) for 1 h, and 5:100 (w/v) trichloroacetic acid (TCA) solution was made by adding 1 ml 100% (w/v) TCA. A 10-ml volume of cold 5% (w/v) TCA was then added to the tube. After 1 h, the material in the tube was shaken for 1 h and then centrifuged at 3000g for 10 min. The supernatant was prepared for the determination of the sum of Pi and metabolite P. We subtracted Pi from the sum to obtain the metabolite P.

Finally, the remaining residue after extraction of the cold TCA was mixed with 35 ml 2.5% TCA (w/v), and extracted for 1 h at 95°C in a hot water bath. Aliquots were centrifuged at 3000g for 10 min, and taken for analysis of nucleic acid P. The residue remaining from the hot TCA final extraction was the residual P fraction. The determination method of all foliar P fractions was similar to that of foliar total P, and the quantity of the fractions was expressed on a dry mass basis. Proportions of P fractions were expressed as the ratio of the concentration of P fractions to the total foliar P concentration in every treatment and each species.

2.6 | Data analyses

The foliar N to total P ratio or the foliar N to P fraction ratios were calculated based on mass. The effects of species, N addition, P addition, elevated [CO₂] and their interactions on foliar P fractions and foliar traits were assessed by multi-way ANOVA with species, N and P addition, and elevated [CO₂] as fixed factors. One-way analyses of variance (ANOVAs) were used to compare the effects of treatments on LMA, PPUE, PNUE, N/P ratios, and the concentrations of N, P, and P fractions. Relationship between plant biomass and concentration of foliar P fractions or total P was conducted with Regression linear analysis. Means were compared with LSD test at a significance level of 0.05. SPSS 19.0 (SPSS, Inc.) was used for statistical analyses, and figures were drawn with Origin 2015 (Origin Lab, Inc.).

3 | RESULTS

3.1 | Foliar traits and plant biomass

P addition did not significantly affect photosynthetic capacity (A_{area} and A_{mass}), while it positively affected plant biomass in invasive and

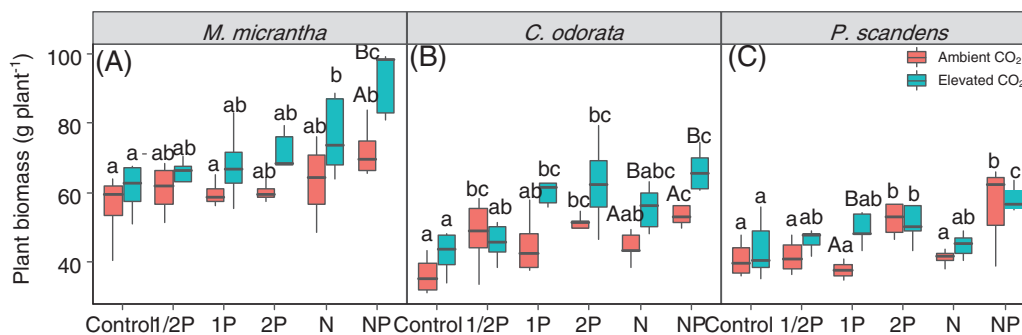


FIGURE 1 Plant biomass for the invasive species (*Mikania micrantha* and *Chromolaena odorata*) and the native species (*Paederia scandens*) as affected by P addition rate and the combined addition of P and N under ambient [CO₂] (400 ± 50 μmol mol⁻¹) or elevated [CO₂] (700 ± 50 μmol mol⁻¹). The six treatments listed along X-axis are as follows: Control (neither P nor N added); 1/2P, 1P, and 2P (0.75, 1.5, and 3 g P m⁻² yr⁻¹, respectively); N (6.25 g N m⁻² yr⁻¹); NP (6.25 g N m⁻² yr⁻¹ + 1P). For each species and each [CO₂] treatment, means with different uppercase letters are significantly different at *p* < 0.05. For each species and within each P or N addition treatment, means with different lowercase letters are significantly different at *p* < 0.05. In all cases, the absence of lowercase or uppercase letters indicates the absence of statistical significance

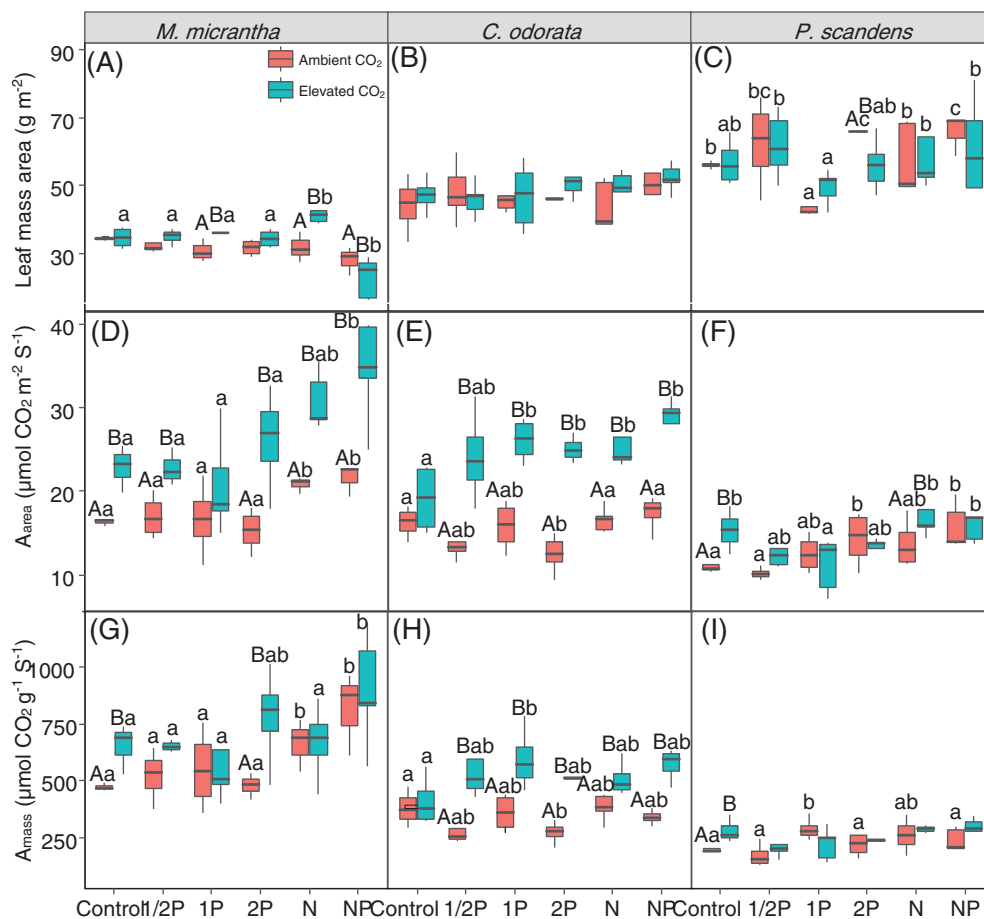
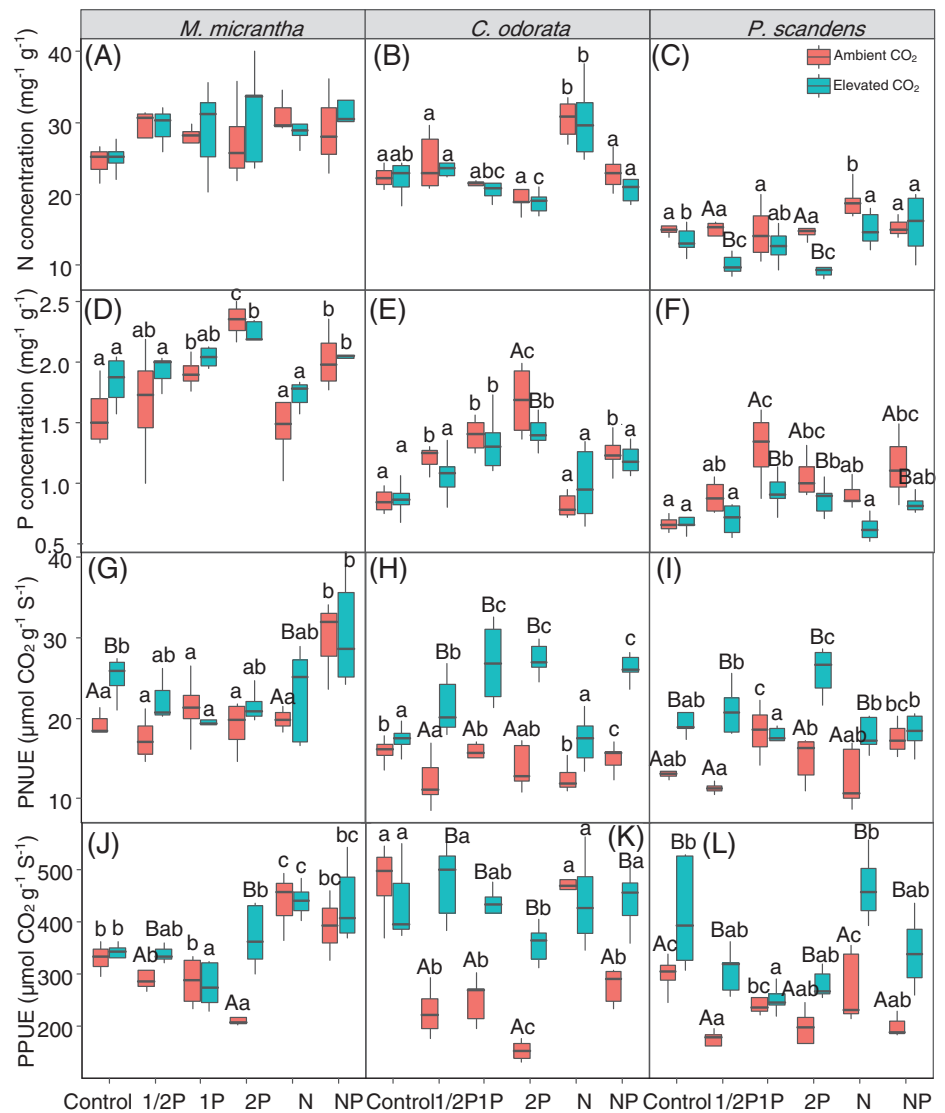


FIGURE 2 Leaf mass per unit area (LMA), photosynthetic rates per unit area (A_{area}), and photosynthetic rates per unit mass (A_{mass}) for the invasive species (*M. micrantha* and *C. odorata*) and the native species (*P. scandens*) as affected by P addition rate and the combined addition of P and N under ambient or elevated [CO₂]. Treatments and statistical comparisons are described in Figure 1

native species (Table 1). 2P addition significantly increased plant biomass of *M. micrantha*, *C. odorata*, and *P. scandens* by 8%, 43%, and 28%, respectively, whereas N addition increased their biomass by 13%, 28%, and 2%, respectively (Figure 1). Elevated [CO₂] significantly

increased A_{area} and A_{mass} in the invasive species (*M. micrantha* and *C. odorata*) by 17–101% and 5.5–97%, respectively (Figure 2D,E,G,H); however, elevated [CO₂] did not significantly affect A_{area} or A_{mass} in the native species (*P. scandens*; Figure 2F,I). Combined with elevated

FIGURE 3 Foliar nitrogen (N) and phosphorus (P) concentrations, and photosynthetic nutrient-use efficiency for nitrogen (PNUE) and phosphorus (PPUE) for invasive species *M. micrantha* and *C. odorata* and the native species *P. scandens* as affected by P addition rate and the combined addition of P and N under ambient or elevated $[CO_2]$. Treatments and statistical comparisons are described in Figure 1



$[CO_2]$, N addition significantly increased A_{area} and A_{mass} in *M. micrantha* and *C. odorata* by 35–38% and 2.8–41%, respectively, (Figure 2D,E,G,H), but also did not significantly affect A_{area} or A_{mass} in *P. scandens* (Figure 2F,I). Elevated $[CO_2]$, N addition, and P addition did not significantly affect LMA in *M. micrantha* or *C. odorata*, while 2P addition increased LMA significantly more than 1P addition in *P. scandens* (Table 1 and Figure 2A–C).

3.2 | Foliar P and N, and PPUE and PNUE

The interaction among species, elevated $[CO_2]$, N and P addition significantly affected foliar P and N concentrations (Table 1). Nitrogen addition significantly increased the foliar N concentration in all species but slightly decreased the foliar P concentration in invasive species (Figure 3A–F). Under elevated $[CO_2]$, 2P addition significantly decreased N concentrations by 15% in *C. odorata* and by 13% in *P. scandens* (Figure 3B,C). Foliar P concentrations significantly increased with increased P added in *M. micrantha* and *C. odorata*, and the increase was greater for the

invasive species than for *P. scandens*; this effect of added P was slightly reduced by elevated $[CO_2]$. 2P addition significantly increased foliar P concentrations by 50% and 96% in *M. micrantha* and *C. odorata*, respectively, and by 58.3% in *P. scandens* (Figure 3D–F).

The interaction of species, elevated $[CO_2]$, N addition, and P addition significantly affected PNUE and PPUE (Table 1). On average, elevated $[CO_2]$ increased PNUE and PPUE by 59% and 43%, respectively, in *C. odorata*, and by 38% and 47%, respectively, in *P. scandens* (Figure 3H,I,K,L). When combined with 2P addition, elevated $[CO_2]$ significantly increased PPUE in *M. micrantha* by 73% (Figure 3J). Nitrogen addition did not significantly affect PNUE in invasive species but increased PPUE in *M. micrantha* (Figure 3G,H,J,K). Under elevated $[CO_2]$, 1/2P, 1P, and 2P addition significantly increased PNUE by 24%, 55%, and 57%, respectively, in *C. odorata*, and 2P addition significantly increased PNUE by 34% in *P. scandens* (Figure 3G,H,I). However, PPUE significantly decreased in all species as the quantity of P added increased further. 2P addition significantly decreased PPUE by 25% in *C. odorata* and by 32% in *P. scandens* under elevated $[CO_2]$ (Figure 3J,K,L).

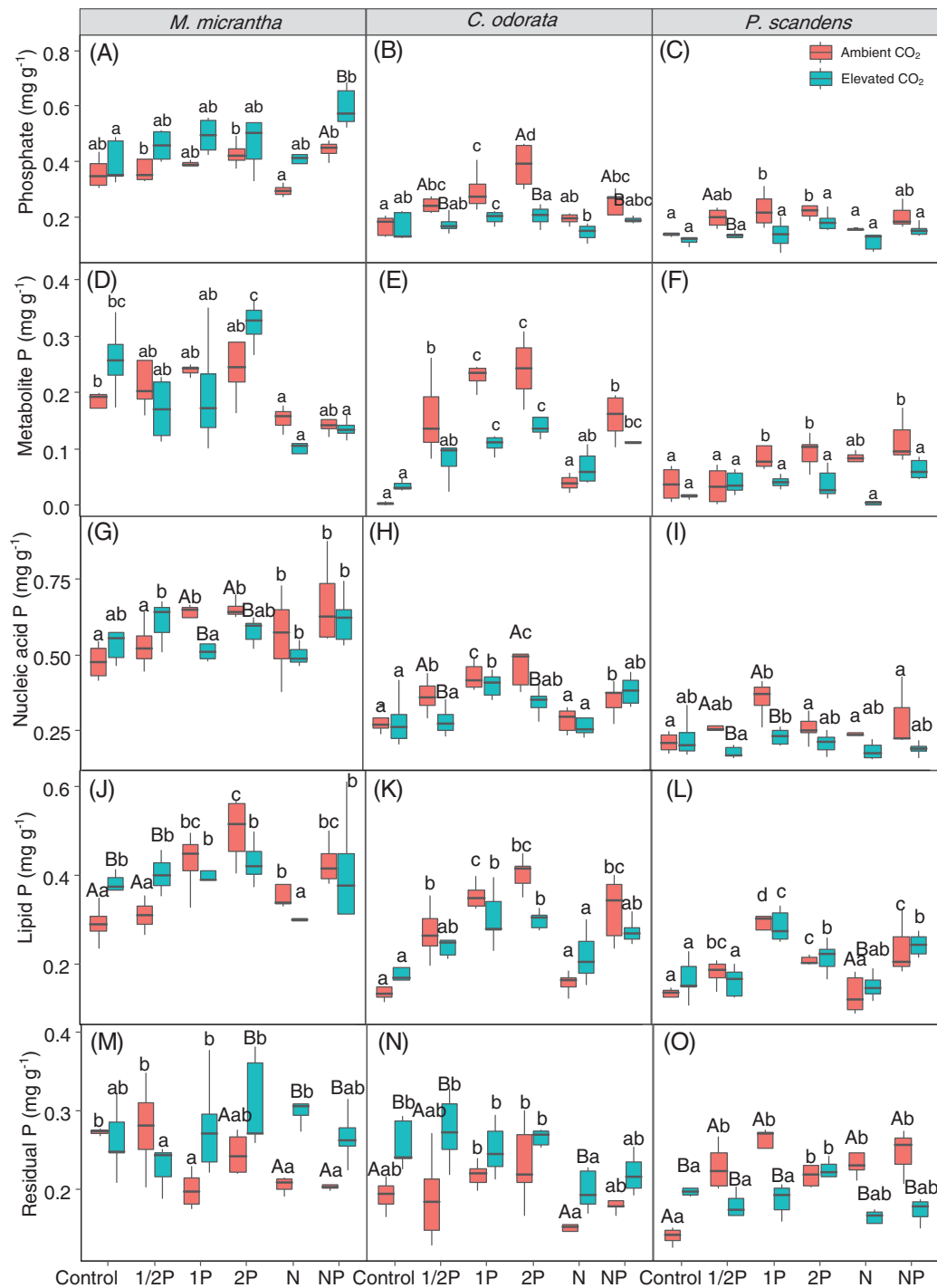


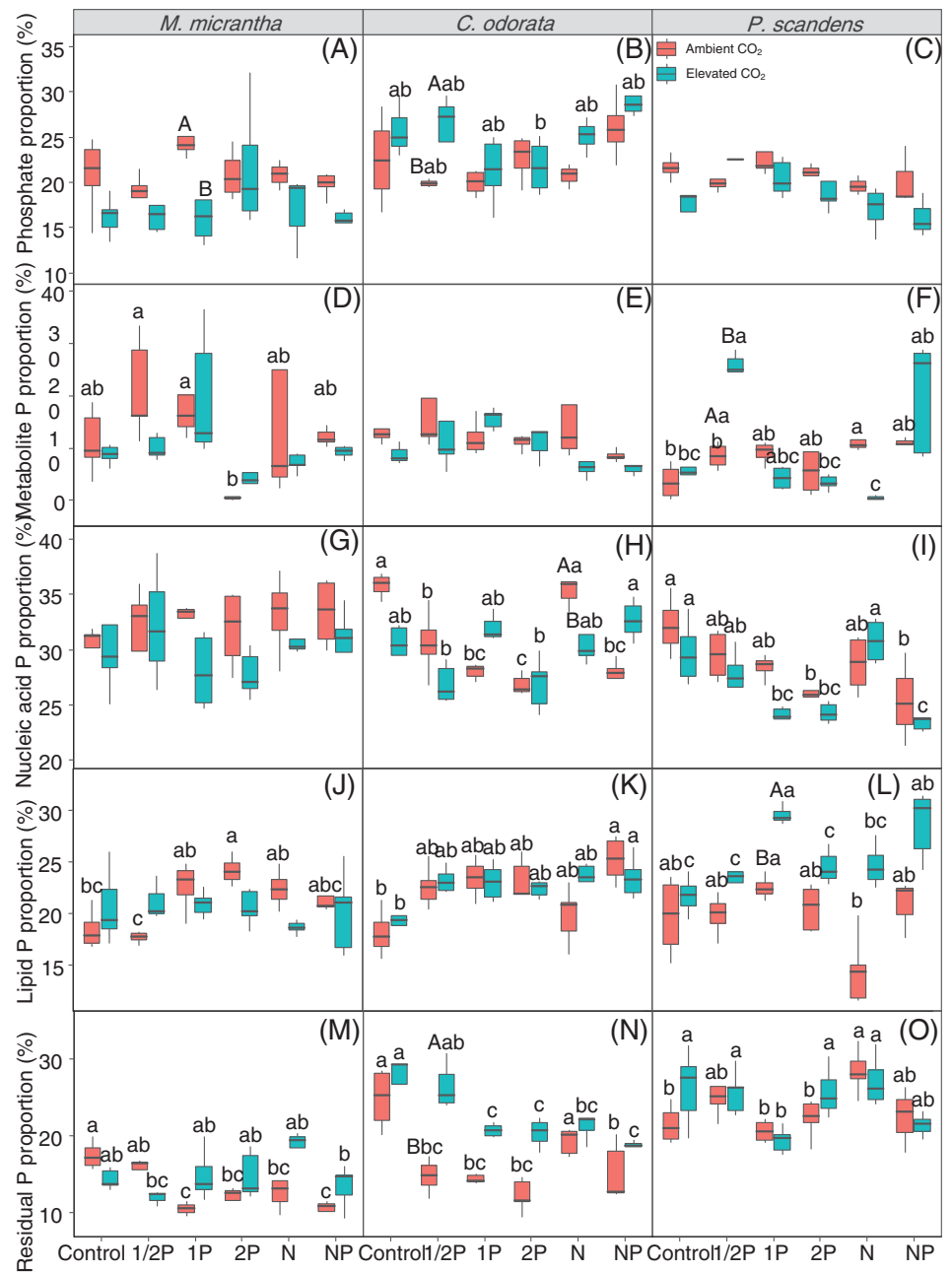
FIGURE 4 Concentrations of foliar inorganic P (phosphate, Pi) and organic P (metabolite P, nucleic acid P, structural P, and residual P) of the invasive species *M. micrantha* and *C. odorata* and the native species *P. scandens* as affected by P addition rate and the combined addition of P and N under ambient or elevated [CO₂]. Treatments and statistical comparisons are described in Figure 1

3.3 | Foliar P fractions and N/P ratios

Overall, both species and P addition significantly affected foliar P fractions, that is, Pi, metabolite P, nucleic acid P, lipid P, and residual P (Table 1). Nitrogen addition decreased the residual P concentration but increased the metabolite P concentration in invasive species

(Figure 4). In *M. micrantha* and *C. odorata*, Pi (Figure 4A,B), metabolite P (Figure 4D,E), nucleic acid P (Figure 4G,H) and lipid P (Figure 4J,K) were significantly increased by 2P addition with an average of 53%, 754%, 38%, and 82%, respectively, but residual P was not significantly increased by P addition (Figure 4M,N). In *P. scandens*, foliar P fractions significantly increased with the amount of P added from 1/2P to 1P,

FIGURE 5 Foliar phosphorus (P) fractions (%). Treatments and statistical comparisons are described in Figure 1



but tended to decrease with 2P addition (Figure 4F,I,L,O). In response to P addition, the metabolite P concentration increased the most, followed by those of lipid P, Pi, and nucleic acid P in *C. odorata*, while the lipid P concentration increased the most in *M. micrantha*, followed by that of metabolite P; however, residual P did not respond in a consistent pattern to P addition. Elevated [CO₂] weakened the enhancing effect of P addition on foliar P in nucleic acids and the lipid P fraction in invasive species (Figure 4). As P addition increased, the proportion of foliar P in lipid increased significantly in *M. micrantha* and *C. odorata*, and the proportion of foliar P in nucleic acids and residual fractions decreased slightly, but foliar P in Pi and metabolites did not respond in the same manner to P addition (Figure 5). Under P addition, elevated CO₂ increased the proportion of foliar P in nucleic acids

and the residual fraction, while it decreased the proportion of foliar P in nucleic acids and lipid. In contrast, the proportion of foliar P fractions did not show a clear response to N addition (Figure 5). The concentration of foliar Pi, metabolite P, nucleic acid P, lipid P and residual P were all significantly and positively correlated with plant biomass, suggesting that plant biomass increased with the increase of the concentration of foliar P fractions (Figure 6).

As the amount of P added was increased, the foliar N/P ratios, foliar N/phosphate ratios, foliar N/metabolite P ratios and foliar N/lipid P ratios decreased more in *M. micrantha* and *C. odorata* than in *P. scandens* under ambient [CO₂] (Table S1). Under ambient [CO₂], however, the foliar N/residual P ratio did not significantly differ among 1/2P, 1P, and 2P treatments in any species, except between 1/2P and 1P in *C. odorata*

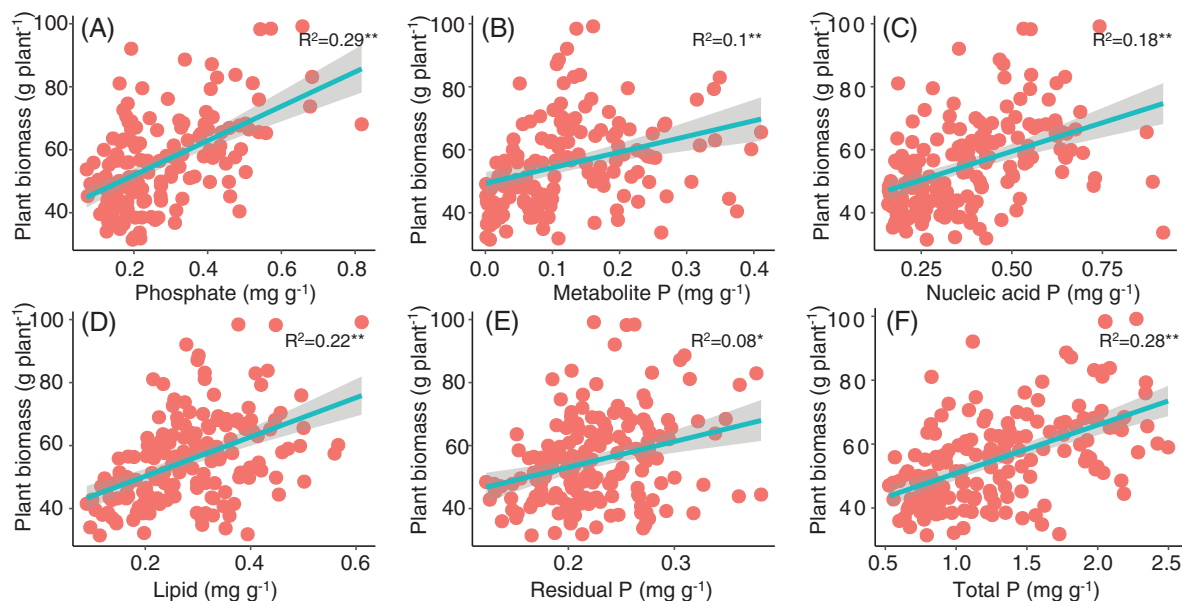


FIGURE 6 Correlation between foliar phosphorus (P) fractions concentration and plant biomass. The data from three target species and six treatments. R^2 values for linear trend lines are shown on each plot. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively

TABLE 2 Correlations between foliar phosphorus (P) fractions and foliar traits in the two invasive species

P fraction	A_{area}	A_{mass}	LMA	PNUE	PPUE	[N]	[P]	N: P ratios
Phosphate	0.33**	0.58**	-0.55**	0.24**	0.01	0.47**	0.80**	-0.36**
Metabolite P	0.12	0.32*	-0.42**	0.03	-0.13	0.39**	0.70**	-0.32**
Nucleic acid P	0.35**	0.61**	-0.61**	0.26**	-0.05	0.66**	0.88**	-0.24**
Lipid P	0.29**	0.53**	-0.59**	0.30**	-0.20*	0.49**	0.89**	-0.43**
Residual P	0.36*	0.39**	-0.20*	0.28**	-0.04	0.29**	0.54**	-0.30**

Note: A_{area} is photosynthetic rates per unit area; A_{mass} is photosynthetic rates per unit mass; LMA is leaf mass per unit area; PNUE is photosynthetic N-use efficiency; PPUE is photosynthetic P-use efficiency; [N] is N concentration, [P] is P concentration. Values are correlation coefficients. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

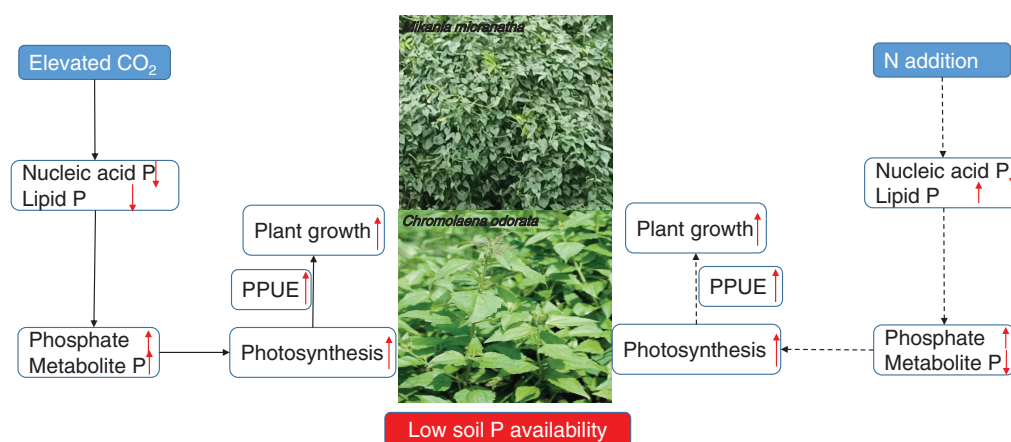


FIGURE 7 A conceptual framework of a pathway to sustain P demand of invasive species under elevated $[CO_2]$ and N addition in soil with a low P availability. Solid and dashed lines indicate significant and insignificant effects, respectively

(Table S1). Elevated $[CO_2]$ significantly decreased the foliar N/Pi ratio, foliar N/lipid P ratio, and foliar N/residual P ratio in *C. odorata* (Table S1). In the invasive species, the concentrations of foliar P fractions (phosphate,

metabolite P, nucleic acid P, lipid P and residual P) were positively correlated with A_{mass} , N concentration, and P concentration, but negatively with LMA, PPUE, and N/P ratios (Table 2).

4 | DISCUSSION

Phosphorus addition significantly increased the plant biomass of *M. micrantha* and *C. odorata*, consistent with previous studies on P limitation of plant growth in subtropical forest ecosystems (Hidaka & Kitayama 2013; Hou et al. 2020). Recent studies, however, show that the invasive species *M. micrantha* and *C. odorata* grown in a soil with low P availability under ambient conditions of [CO₂] and N deposition maintain high plant biomass and show rapid invasion in a forest ecosystem (Song et al. 2009; Tang et al. 2006; Zhang et al. 2016). In the present study, we found that elevated [CO₂] and N addition changed the allocation of foliar P fractions under P addition, which may help explain how invasive species maintained their photosynthetic capacity and, therefore, possibly their invasiveness in soils with low P availability under conditions of rising atmospheric [CO₂] and N deposition.

4.1 | Foliar P fractions in responses to N and P addition under ambient [CO₂]

Nitrogen addition alone did not significantly affect the total leaf P concentration in the invasive species, thus leading to rejection of our hypothesis. This result was consistent with a previous study that N addition did not affect soil P supply because N addition did not affect soil P mineralization (Dong et al. 2015). Nitrogen addition generally increases leaf N concentrations and may promote plants to absorb more P to maintain plant nutrient balance (Wang et al. 2019). However, we detected an increase in foliar N concentration, but not a corresponding increase in foliar P concentration, suggesting that N addition did not alleviate P limitation in invasive species by increasing foliar total P concentration. Nitrogen addition reduced the residual P concentration but increased the metabolite P concentration, and increased the photosynthetic rates of invasive species, which led to the invasive species exhibiting a higher photosynthetic P-use efficiency and faster growth. Nitrogen addition slightly increased the foliar total P concentration in native species, which was accompanied by a significant increase of the residual P concentration and a slight increase of the metabolite P concentration. The slight change in P fractions did not increase the photosynthetic rate and PPUE of native species, and did not significantly increase the growth of native species under N addition. Phosphorus addition or combined N and P addition increased the foliar concentration of total P and P fractions, which was consistent with previous study showing that P addition or combined N and P addition both increased plant-available soil P, and stimulated plant P uptake (Zhang et al. 2016).

Plants growing with a low soil P availability can enlarge P acquisition through increasing carboxylate exudation or phosphatase release (Lambers et al. 2015). The present result shows that the low P availability in tropical forests did not necessarily affect rates of photosynthesis, and P addition significantly decreased PPUE. These results suggest that the unfertilized invasive species have efficient P-use strategies by increasing PPUE and maintained rates of photosynthesis to adapt to P-impoverished soils (Mo et al. 2019). Plants may also

regulate the balance between the levels of phosphorylated intermediates and inorganic P to maintain photosynthetic rates and plant biomass when soil P availability is low (Wang et al. 2019).

In our study, P addition and combined N and P addition significantly increased foliar Pi concentrations in *M. micrantha* and *C. odorata*. Among foliar P fractions, the increase was not the greatest for leaf Pi because Pi in the leaf was generally diverted to other P fractions for photosynthesis (Ostertag 2010). We also found that the concentration of the metabolite P fraction in all species was low but increased more than that of the other P fractions in response to N and P addition. The largest increase in metabolite P indicated that plant metabolic intermediates increased in response to P addition or combined N and P addition, which is associated with increased carbon metabolism in photosynthesis (Ostertag 2010; Veneklaas et al. 2012). In our study, we observed that P addition and combined N and P addition increased the concentrations of all foliar P fraction, and the increases were greatest for metabolite P. These results further indicate that invasive plants may alter the balance between foliar metabolite P and other fractions (lipid P and nucleic acid P) to maintain a high photosynthetic capacity in soils with low P availability.

As expected, our results show that foliar N/P or foliar N/P fraction ratios were more than 20, and decreased with increased foliar P concentration due to P addition or combined N and P addition. Phosphorus addition or combined N and P addition increased foliar P concentration and plant biomass in our study, indicating that the growth of invasive species was limited by P (Güsewell 2004; Mo et al. 2019; Tang et al. 2006). Phosphorus addition or combined N and P addition increased plant biomass by increasing foliar P fractions (Pi, metabolite P, lipid P and nucleic acid P), and decreased foliar N/P ratios, which might promote photosynthesis and growth of invasive species by increasing the amount of foliar P available for the synthesis of rRNA and membrane phospholipids (Reef et al. 2010). Changes in the foliar N/P have previously been associated with physiological growth strategies in both invasive and native species (Hidaka & Kitayama 2011). We found that foliar N/P ratios were significantly related to Pi, metabolite P, lipid P and nucleic acid P in this study, which suggests that nutrient addition might alter foliar N/P ratios to meet the requirement for rRNA and membrane phospholipids synthesis (Reef et al. 2010).

4.2 | Foliar P fractions in responses to N and P addition under elevated [CO₂]

As hypothesized, elevated [CO₂] significantly increased A_{area} and A_{mass} in both invasive species. This result was consistent with a previous study showing that increased [CO₂] generally stimulates plant photosynthesis (Campbell & Sage 2006) and accelerates plant growth (Zhang et al. 2016). Elevated [CO₂] did not significantly affect LMA or concentrations of foliar N, P (except 2P addition in *C. odorata*) or N/P ratio in invasive species, in accordance with a previous study showing that plants experiencing increased A_{area} and A_{mass} under elevated [CO₂] may not use the newly fixed carbohydrates for new growth or plant nutrient (Prescott et al. 2020).

In P-deficient soils, elevated $[\text{CO}_2]$ increases foliar PNUE and PPUE (Mo et al. 2019; Zhang et al. 2016). Under P addition or combined N and P addition, elevated $[\text{CO}_2]$ increased PPUE in invasive species more than in native species, suggesting that elevated $[\text{CO}_2]$ increased plant P-use efficiency and maintained functions at low soil P availability, and that elevated $[\text{CO}_2]$ affected PPUE more in invasive species than in native species. PPUE was higher in invasive than in native species despite significant differences in foliar P concentration in treatments that differed in quantities of P added under elevated $[\text{CO}_2]$, probably due to the higher photosynthetic capacity (A_{area} and A_{mass}) of invasive species than that of native species.

Under elevated $[\text{CO}_2]$, the shifts in the foliar P fractions also provide clues to the underlying adaptive mechanisms that explain the success of invasive species (Hidaka & Kitayama 2011). Previous studies found that the status of Pi in the cytosol in Proteaceae strongly affected photosynthetic rates (Lambers et al. 2015), and that elevated $[\text{CO}_2]$ supported stable photosynthetic rates by maintaining Pi and metabolite P in invasive species in P-poor soils (Song et al. 2007). In our study, under N and P addition, elevated $[\text{CO}_2]$ weakened the positive effect of P addition or combined N and P addition on concentrations of foliar lipid P and nucleic acid P in invasive species. These results indicate that to maintain photosynthesis when soil P availability was low, elevated $[\text{CO}_2]$ accelerated plants to use P from lipids or nucleic acids (i.e., membrane lipid and nucleic acid P remodeling) to maintain foliar P in the form of Pi and metabolite P in the cytosol for photosynthesis (Mo et al. 2019; Ostertag 2010; Prodhan et al. 2019).

The results in this study were used to develop a conceptual framework for the mechanism of P maintenance in low-P soil under further increased $[\text{CO}_2]$ and N deposition (Figure 7). Elevated $[\text{CO}_2]$ and N addition alleviated the P demand of invasive species by reducing the transformation of Pi and metabolite P to nucleic acid P and lipid P to maintain the Pi and metabolite P for photosynthesis. Therefore, under elevated $[\text{CO}_2]$ and N addition, the transformation of non-metabolite P (lipid P and nucleic acid P) to metabolite P and phosphate was enhanced. This pathway was essential to meet the increased P requirement for the growth of invasive species under low soil P availability and elevated $[\text{CO}_2]$ and N deposition in the subtropical forest ecosystem.

5 | CONCLUSIONS

In the current study, elevated $[\text{CO}_2]$ and N addition allowed invasive plants to adjust their foliar traits and thus acclimate to low soil P availability; the acclimation was substantially greater in the two invasive species than in the native species. Plant biomass significantly increased under P addition, and the foliar N:P ratio >20 of the invasive species indicated P limitation of their growth. Rather than decreasing their LMA, the invasive species acclimated to low soil P availability under elevated $[\text{CO}_2]$ by greatly reducing their allocation of P to non-metabolite foliar P fractions (nucleic acid P and lipid P). Conversely, the concentrations of metabolite P and Pi were

not reduced, and this may have allowed maintenance of a high photosynthetic capacity and high PPUE. The total foliar P concentrations decreased slightly under N addition, accompanied by a decrease of residual P, while metabolite P concentration increased in invasive species, which allowed high photosynthetic rates and PPUE. These adaptive responses help explain the success of invasive plants under conditions of rising atmospheric $[\text{CO}_2]$ and N deposition in soil with low P availability.

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AUTHOR CONTRIBUTIONS

Lingling Zhang and Dazhi Wen conceived and designed the research; Lingling Zhang, Guihua Zhang, Nan Liu, Xiaowei Zang and Meijuan Xiao performed the research; Lingling Zhang and Xianzhen Luo analyzed and interpreted the data; Lingling Zhang and Xianzhen Luo wrote the paper, Hans Lambers revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available on request from the corresponding author (Dazhi Wen).

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