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# Hydrological niche regulation induced by different resistance strategies facilitates coexistence of *P. longipes* and *L. communis* under drought stress

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## Abstract

Under global warming, the availability of water resources is one of the most important factors affecting trait evolution and plant species distribution across terrestrial ecosystems, and the relationships between drought resistance strategies and the hydrological niche characteristics of plants are worth studying. We continuously monitored physiological drought response parameters such as $g_s$, $T_r$, proline, soluble sugar, gene expression and activities of SOD, POD, and CAT to assess drought resistance strategies of *Platycarya longipes* and *Lindera communis*; determined plant soil hydrological niche separation by stable H and O isotope analysis; and analysed the effects of interspecific water competition by comparing the differences in morphological and physiological parameters between solo and mixed planting. Under drought stress, *L. communis* exhibited a drought avoidance strategy, and *P. longipes* exhibited a drought tolerance strategy. *L. communis* utilized the water within the shallow soil layer, while *P. longipes* mainly utilized the water in the deeper soil layer; there were fewer parameters with significant differences between the solo planting and the mixed planting of *L. communis* compared to *P. longipes*. Overall, *P. longipes* benefited from coexistence with *L. communis* under drought stress, which may be because *L. communis* employs a drought avoidance strategy, reducing soil water consumption in the drought environment. These results suggested that differences in functional traits or resistance strategies among species benefit species' coexistence in a community under drought stress.

**KEYWORDS**
Drought resistance strategy, Functional trait, Hydrological niche separation, Isotope, Species coexistence

## 1 | INTRODUCTION

The greenhouse effect caused by human activities will increase the duration, intensity, and frequency of drought worldwide in the foreseeable future (IPCC 2018; Cramer et al. 2018). The availability of water resources is one of the most important factors affecting trait evolution and plant species distribution across terrestrial ecosystems,

and hydrological niche separation and species coexistence mechanisms within a community have become important issues for ecologists (Silvertown et al. 2015; Brum et al. 2019; Veen & Sasidharan 2019; Callahan et al. 2022). It is widely believed that differences in functional traits among species are the basis for species coexistence in a community (McGill et al. 2006; Weiher et al. 2011; Shipley et al. 2016). Silvertown et al. (2015) proposed the hydrological niche

separation hypothesis (HNS), in which plants may exhibit different hydraulic traits to avoid or tolerate drought along water availability gradients to avoid competition within a community.

In addition to escaping arid environments temporally or geographically, plant drought resistance strategies are usually divided into drought avoidance strategies and drought tolerance strategies. Drought avoidance strategies refer to the ability of plants to minimize water loss and increase water absorption to avoid drought stress, while drought tolerance strategies refer to the ability of plants to reduce stress damage through various physiological and structural changes during drought stress (Anderegg and Hillerislambers 2016; Kramp et al. 2022). Differences in drought resistance strategies among plants can be distinguished by functional traits related to plant water use (Fonseca and Overton 2000; Niinemets 2001; Markesteijn and Poorter 2009), and these functional traits include both morphological and physiological traits. Currently, most research on plant drought resistance strategies is based on morphological traits, but there is little research based on physiological and biochemical traits. Compared to morphological traits, physiological and biochemical traits of plants, such as antioxidant enzyme activity (superoxide dismutase, SOD; peroxidase, POD; catalase, CAT), osmoregulation substance content (soluble sugar, SS; proline), malondialdehyde (MDA) content, stomatal conductance ($g_s$), transpiration rate ($T_r$) and water use efficiency (WUE), are more difficult to observe directly (Zhang et al. 2020). Furthermore, when environment changes, substantial changes in plant morphological traits require a relatively long process, but physiological and metabolic provide quicker and more accurate responses (Belluau & Shipley. 2017).

Different species can coexist through niche differentiation, and competition between species determines the interspecific or intraspecific distribution pattern of net available resources (Calama et al., 2019). Species with different hydrological niches often show different responses to drought stress. Ding et al. (2021) showed that species that utilize shallow soil moisture are more susceptible to injury or death during increasingly frequent and intense drought events in the karst region of southwestern China. A large number of studies have reported niche separation or water competition of mixed stands under water deficiency conditions, but the results are inconsistent (Grossiord 2019; Jourdan et al. 2019; Schwarz & Bauhus 2019). This may be because the results observed *in situ* experiments are usually determined by many ecological factors (Forrester & Bauhus 2016), so different experiments may produce contradictory and confusing results. The simulation experiment under controlled conditions is more conducive to clarify the internal mechanism of obtaining results. The hydrological niche will be determined by trade-offs of functional characteristics involved in the plant water economy (Belluau & Shipley 2017), and the diversity in plant water utilization is known to be beneficial for species coexistence and stable community function in communities under drought conditions, but the underlying mechanisms of hydrological niche separation and water utilization are still unclear (Lorts & Lasky 2020).

Stable isotope technology is an important tool in studying plant water sources and is widely used in ecological hydrological research (Tang et al. 2016; Grossiord et al. 2017). It is generally believed that water absorbed by roots does not cause isotopic fractionation, so the isotopic composition of water in the xylem can reflect the sources of soil water absorption by plant roots (Zimmermann et al. 1968; Dawson & Ehleringer 1993); furthermore, the enrichment of $^{18}O$ and $^2H$ near the soil surface due to evaporation and fractionation during the filling and release of water in the soil results in a vertical gradient of soil H and O isotopes (Grossiord et al. 2017).

The "mass ratio effect" theory suggests that ecosystem functions and services mainly depend on dominant species (Díaz et al. 2007). Different responses to light and water constraints during the establishment stage or the seedling stage of a species are considered key features in controlling community structure and dynamics; therefore, identifying seedling functional responses and implied trade-offs along resource gradients is important to understanding the internal mechanism of vegetation dynamics in the region and assessing the potential response of plant communities to global climate change (Sánchez-Gómez et al. 2006, Stark et al. 2015). *Platycarya longipes* and *Lindera communis* occur broadly across the karst region of subtropical China and often coexist in the same community. There are some differences in morphology and physiological characteristics between the two plants (Liu et al. 2009; Chen et al. 2019, Wang et al. 2021). Due to the shallow soil layer and porous bedrock, water brought into karst ecosystems by rainfall cannot be fully absorbed and utilized (Schwinning 2008, Zhang et al. 2022). Compared to non-karst regions, an increase in drought duration, intensity, and frequency may have a stronger impact on vegetation in karst regions. With the main objective of studying the drought resistance strategies and soil hydrological niche separation of *P. longipes* and *L. communis* under the combined effects of drought and competition, we used their seedlings to conduct a simulation experiment under laboratory control conditions and focused on the following specific questions:

1. Based on functional traits, what drought resistance strategies are shown by *P. longipes* and *L. communis*?
2. Are there differences in soil hydrological niche changes between *P. longipes* and *L. communis* under drought stress?
3. Do the characteristics of drought resistance strategies and hydrological niche separation facilitate the coexistence of *P. longipes* and *L. communis* under drought stress?

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material

*L. communis* and *P. longipes* were selected as experimental materials. *L. communis* is a small deciduous tree or shrub belonging to the family Lauraceae, while *P. orientalis*, of the Juglandaceae family, is an evergreen tree species. Seeds of both plants were grown in the greenhouse at 20°C with a 12 h photoperiod. After seed germination, vigorous seedlings were transplanted into soil from the karst region and grown in the greenhouse with a 12 h photoperiod, a day/night

temperature of 25/16°C, a photosynthetic photon flux density of 400 μmol m$^{-2}$ s$^{-1}$ and 60–65% relative humidity. 40 cm tall, uniform and vigorous seedlings were selected for this study.

## 2.2 | Experimental design and conditions

To study the changes in interspecific relationships between two plants under different treatments, we conducted indoor potted plant control experiments to grow these two plants with crossed treatments (for a total of six conditions): (1) well-watered [soil water content (SWC): ~40%], solo planting (i.e., a single plant in a 40 × 30 cm pot with soil 30 cm thick); (2) well-watered, mixed planting (planted the two species together in a 40 × 60 cm pot with soil 30 cm thick); (3) light drought (SWC: ~30%), solo planting; (4) light drought, mixed planting; (5) moderate drought (SWC: ~20%), solo planting; and (6) moderate drought, mixed planting. Figure 1 shows the planting modes and soil sampling points. Both plants in each treatment had 5 total replicates per treatment, and all morphological and physiological parameters were measured in 3 replicates. During the experiment, the water lost due to evapotranspiration was replenished daily by the weighing method to maintain the corresponding water content for each treatment.

To focus on studying the effects of drought on water competition between these two plants, we excluded light competition by maintaining sufficient row spacing to prevent mutual coverage of plant canopies. To limit nutrient competition, 1/4 of the Hoagland nutrient solution was used daily to replenish water and nutrients. The nutrient solution was prepared using distilled water (δ$^{18}$O = −9.162‰, δ$^2$H = −56.958‰) obtained from distilled local running water. Every five days after treatments, the fully expanded leaf in the middle of the tree crown was selected for measurement and sampling. The photosynthetic and morphological parameters were measured, and the leaves were sampled to determine the water content, antioxidant enzyme activity, antioxidant enzyme gene expression, osmotic regulating substance content and MDA content. To determine the water source of each treated plant, on the last day of the experimental treatment, a 5 cm long main stem was harvested for measuring the hydrogen and oxygen isotope composition of plant xylem water. Soil samples were obtained at depths of 5, 15, and 25 cm to determine hydrogen and oxygen isotope composition in each soil layer. Plant and soil samples used for isotope determination were wrapped in parafilm and stored at −80°C until analysis was performed.
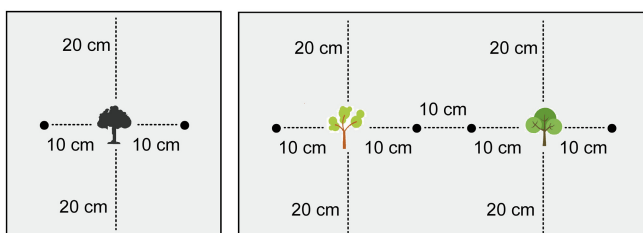


**FIGURE 1** Planting modes and soil sampling points. (a) Solo planting; (b) mix planting; • are the soil sampling points.

## 2.3 | Water content measurement

The leaf relative water content (LWC) was measured according to the method of Garnier and Laurent (1994). SWC was calculated by the following formula:

$$SWC = (SFM - SDM)/SDM \qquad (1)$$

where SDM and SFM are the dry mass and fresh mass of soils, respectively.

## 2.4 | Photosynthetic parameter measurement

The photosynthetic parameters were measured using a Li-6400 portable photosynthesis measurement system (Li-6400, Li-Cor). Briefly, the latest fully developed leaves in the middle layer of the canopy were selected for the determination from 10:00 to 11:00 am, the photosynthetic photon flux density was 400 μmol m$^{-2}$ s$^{-1}$, and the temperature was 25°C.

## 2.5 | Biochemical parameter measurements

SOD (SOD2Y, Comin), POD (POD2Y, Comin), CAT (CAT2W, Comin), SS (KT2Y, Comin), MDA (MDA2Y, Comin) and proline (PRO2Y, Comin) assay kits were used to determine these parameters, and these tests were carried out with a spectrophotometer (Bluestar, LabTech) according to the instructions. Briefly, enzyme extraction was performed from 0.1 g of leaves ground in liquid nitrogen, to which 1 mL of extraction buffer containing Na$_2$HPO$_4$·12H$_2$O was added. The homogenate was centrifuged at 8 000 $g$ for 10 min at 4°C. For SOD activity determination, 240 μL of reagent I, 6 μL of reagent II, 90 μL of sample extraction solution, 180 μL of reagent III and 510 μL of reagent IV were added to the sample tube; the same reagents were added to a control tube, with the extract replaced with the same volume of distilled water. The mixtures were homogenized, allowed to stand for 30 min at 25°C and then monitored at 560 nm. For POD activity determination, a POD activity assay buffer containing 2.6 mL reagent I, 1.5 μL reagent II, and 1 μL reagent III was prepared. 50 μL of extract were added to 950 μL of POD activity assay buffer, and the samples were monitored at 470 nm, with A$_1$ recorded at 1 min and A$_2$ at 2 min. For CAT activity determination, 90 μL reagent I and 150 μL sample extract were mixed and reacted for 10 min, and then 300 μL reagent II and 795 μL reagent III were added to the sample tube; the same reagents were added to a control tube, and the reactions were monitored at 405 nm. For MDA content determination, 200 μL of extract was added to 600 μL of reagent I and incubated in a water bath for 30 min at 95°C, cooled and then centrifuged at 10 000 $g$ for 10 min at 25 °C. The absorbance of the upper phase was measured at 532 and 600 nm. For Proline content determination, 0.5 mL reagent I, 0.5 mL reagent II and 0. 5 mL sample extraction

solution were combined in a tube with a stopper and incubated in a water bath for 30 min at 100°C; after the tube was cooled, 1 mL reagent III was added, and the tube was oscillated for 30 s. The absorbance of the upper phase was measured at 520 nm. For soluble sugar content determination, soluble sugar content assay buffer containing reagent I and 5 mL reagent II was mixed with 400 μL distilled water, 100 μL assay buffer and 1 mL $H_2SO_4$ in the control tube; 200 μL sample extraction solution, 200 μL distilled water, 100 μL assay buffer and 1 mL $H_2SO_4$ were added to the sample tube. The absorbance was monitored at 620 nm. The components of the reagents are listed in Table S1.

## 2.6 | Total RNA extraction and gene expression analysis

The total RNA was extracted, and *eSOD-Mn*, *eSOD-Fe*, *eSOD-Cu/Zn*, *ePOD*, and *eCAT* gene expression were measured according to the method of Yao and Wu (2016). Briefly, total RNA was extracted using Trizol (Takara) according to the supplier's instruction. The extracted RNA was treated with DNase to eliminate genomic DNA contamination. Total RNA was used to synthesize oligo(dT)-primed cDNA with a reverse transcription kit (Takara) according to the manufacturer's instructions. Semi-quantitative Real-Time PCR was performed using a StepOnePlus™ (Life Tech) using SYBR RT-PCR Kit (Takara) according to the manufacturer's instructions. PCR conditions consisted of denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, followed by annealing at 60°C for 34 s. Relative gene expression was calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method. Gene-specific primers that were used for amplification are listed in Table S2.

## 2.7 | Oxygen ($\delta^{18}O$) and hydrogen ($\delta^2H$) isotope composition and measurement

Plant tissue and soil water were extracted using the LI-2000 plant soil water vacuum extraction system (LI-2000, LICA). The water samples were stored in a sealed spiral-capped borosilicate glass bottle, wrapped with paraffin and stored at −20°C. The $\delta^{18}O$ and $\delta^2H$ were measured using a continuous-flow isotope ratio mass spectrometer (MAT 253; Thermo Fisher Scientific) according to the method of Yao et al. (2022).

## 2.8 | Calculation of plant soil moisture sources

Because only three different soil depths were sampled, it was assumed that plants had three sources of soil moisture. Therefore, after measuring the hydrogen and oxygen isotope composition of the xylem and three soil layers, the contribution of water sources in each soil layer to plant xylem moisture can be calculated through a three-variable linear equation.

## 2.9 | Statistical analysis

Key physiological traits, including osmoregulatory substance content, antioxidant enzyme activity, gene expression of antioxidant enzymes, stomatal conductance and transpiration rate, which are expected to influence drought responses, were entered into the principal component analysis (PCA) to assess the drought resistance strategies of plants using PCA v1. 50 of OriginPro 2021 (OriginLab Inc.). Pearson's correlations between soil water content and antioxidant enzyme activity, gene expression of antioxidant enzymes, SS, proline, MDA, height (H), crown width (CW), $P_n$, $g_s$, $T_r$ and leaf water content (LWC) were performed using Correlation Plot v1.31 of OriginPro 2021 (OriginLab Inc.). One-way ANOVA followed by Duncan's multiple range test was performed to explore the differences among the plant parameters under different treatments. A general linear model was used to explore the differences ($p < 0.05$) between sole and mixed planting modes, the planting pattern (sole or mixed) as fixed effects, and the plant parameters at every sampling time as dependent variables. ANOVA and a general linear model were performed using SPSS 25.0 (SPSS Inc.).

## 3 | RESULTS

### 3.1 | PCA for drought resistance strategies of *P. longipes* and *L. communis*

PCA was conducted based on the drought resistance and response parameters of *P. longipes* and *L. communis* to assess their differences in drought resistance strategies (Figure 2). PC1 and PC2 explained 38.8 and 21.3% of total variation, respectively. Based on drought resistance and response parameters, *P. longipes* and *L. communis* could be separated by PCs. In addition, the changing trajectory of their patterns significantly differed with the changes in drought intensity. $g_s$ and $T_r$ showed a strongly negative load, while POD has a positive load on PC1; genes related to antioxidant enzymes, such as *eSOD-Fe*, *eCAT*, and *eSOD-Mn*, and CAT activity loaded positively on PC2 (Figure 2B).

### 3.2 | WUE of P. longipes and L. communis

WUE was calculated by $P_n$ divided by $g_s$. For *L. communis*, there was no significant difference in WUE between solo planting and mixed planting, but for *P. longipes*, there was a significant difference in WUE at 15 d and 20 d between the two planting modes (Figure 3). The WUE of both species was sigificantly different between the control treatment and the MD treatment; in *L. communis* the difference in WUE was significant after 10 d, when comparing the control treatment and the LD treatment, the difference seen in *P. longipes* was significant after 15 d.
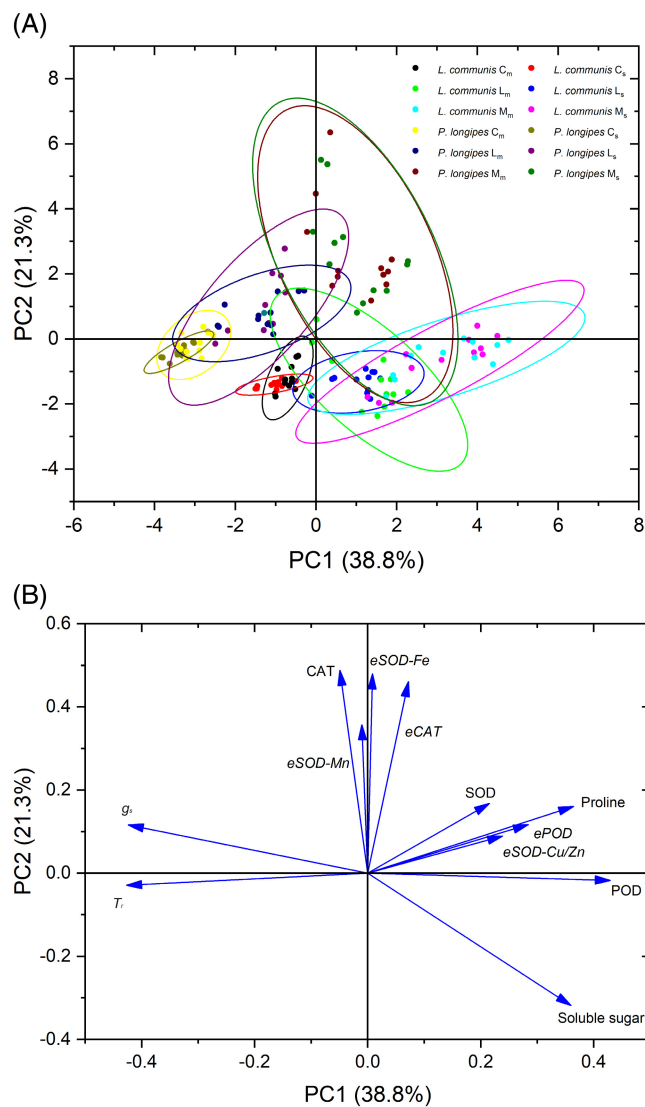
(A)



(B)



**FIGURE 2** PCA based on drought response physiological parameters of *P. longipes* and *L. communis* under different treatments and planting modes. (a) Score plot of parameters; (b) Loading plot of parameters. $C_s$, control × solo planting; $C_m$, control × mixed planting; $L_s$, light drought × no competition; $L_m$, light drought × mixed planting; $M_s$, moderate drought × solo planting; $M_m$, moderate drought × mixed planting; $g_s$, stomatal conductance; $T_r$, transpiration rate; SOD, activity of superoxide dismutase; POD, activity of peroxidase; CAT, catalase; *eSOD*, expression of genes encoding superoxide dismutase; *ePOD*, expression of genes encoding peroxidase; *eCAT*, expression of genes encoding catalase.

## 3.3 | Water source of *P. longipes* and *L. communis*

From the stable isotope composition of H and O in the plant xylem (Figure S1) and in each soil layer (Figures S2, S3), we can calculate the proportion of water from different soil layers in the plant xylem. As drought stress increasing, under solo planting, the proportion of the water from the 25 cm deep layer in the water source of *L. communis* was increased from 14% to 37%; and *P. longipes* increased water
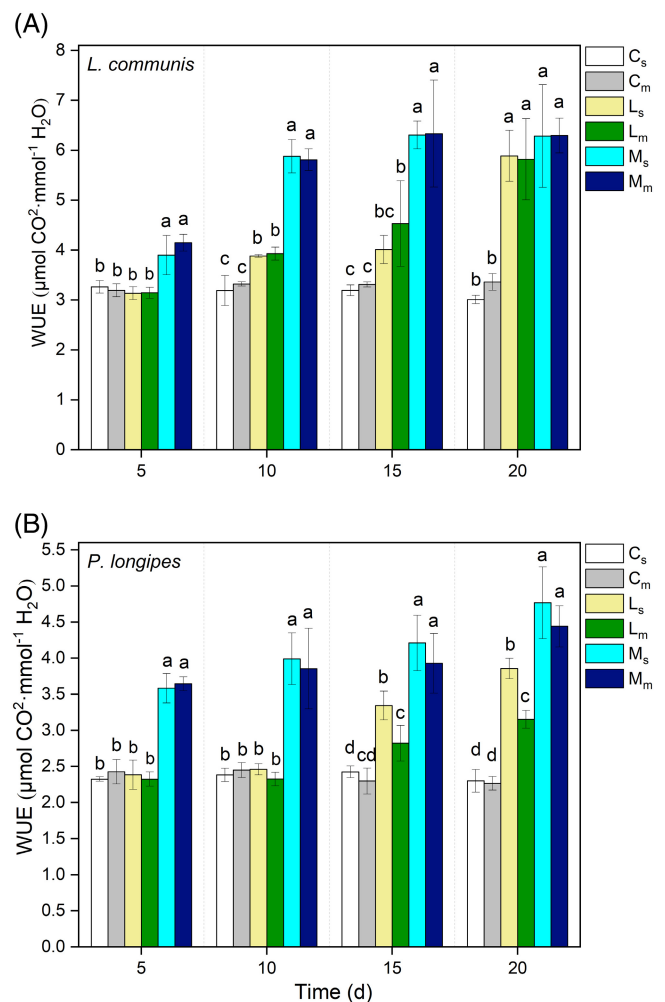
(A)



(B)



**FIGURE 3** WUE of *L. communis* (a) and *P. longipes* (b) under different treatments and planting modes. Mean ± standard deviation, n = 3. Different letters in a single site indicate significant difference at 0.05 level. $C_s$, control × solo planting; $C_m$, control × mixed planting; $L_s$, light drought × no competition; $L_m$, light drought × mixed planting; $M_s$, moderate drought × solo planting; $M_m$, moderate drought × mixed planting.

absorption from the 5 cm deep layer (from 17% to 28%). This result shows that both *L. communis* and *P. longipes* expand the soil water absorption range under drought stress. Under mixed planting, the proportion of the water from the 15 and 25 cm deep layers in the water source of *L. communis* decreased from 54% to 33%; and the proportion of *P. longipes* obtaining water from the 5 and 15 cm deep layers increased from 41% to 66%. This observation indicates *P. longipes* have a strong soil moisture competitive capacity compare to *L. communis* under drought stress (Figure 4).

## 3.4 | Correlation and difference analysis

Pearson's correlations were calculated between SWC and the different plant parameters. For *P. longipes*, there was a difference in the
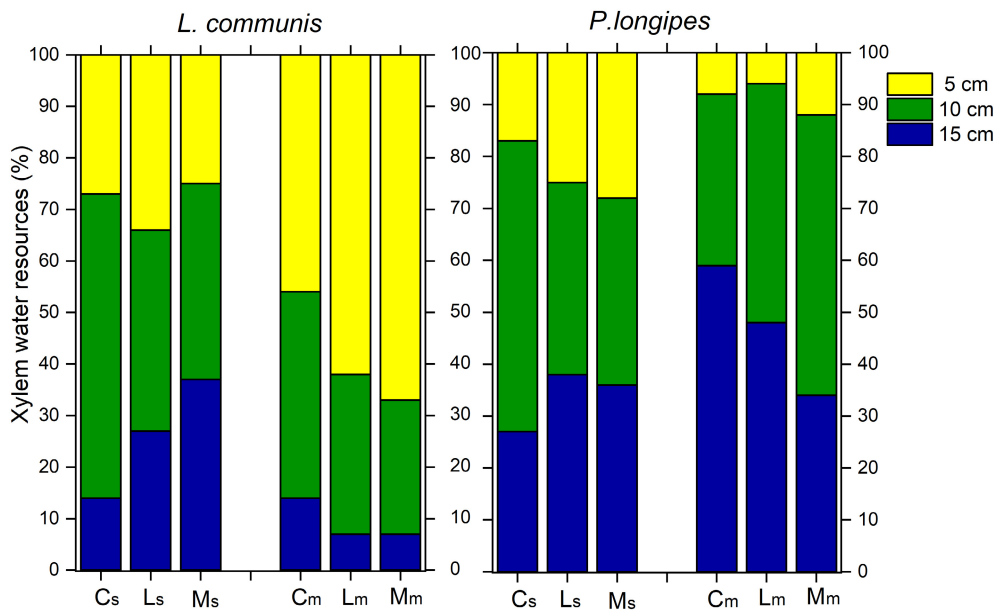
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**FIGURE 4** Xylem water sources of P. *longipes* and L. *communis*. $C_s$, control × solo planting; $C_m$, control × mixed planting; $L_s$, light drought × no competition; $L_m$, light drought × mixed planting; $M_s$, moderate drought × solo planting; $M_m$, moderate drought × mixed planting.

significance of the correlations of SOD, H and *eSOD-Mn* between the two planting modes (Figure 5). Different planting modes have changed the correlation significance level between SWC and some parameters. For *L. communis*, the correlation coefficients of SWC with CW, *eSOD-Fe* and *eSOD-Cu/Zn* under solo planting were all significant at 0. 001 level, but these correlation coefficients under mixed planting were all significant at 0. 01 level. Moreover, the correlation coefficient of SWC with H under solo planting was significant at 0. 01 level, but that of under mixed planting was significant at 0. 05 level. For *P. longipes*, the correlation coefficient of SWC with SOD under solo planting was significant at 0. 001 level, but that of under mixed planting was significant at 0. 05 level; the correlation coefficient of SWC with *eSOD-Mn* under solo planting was not significant, while that of under mixed planting was significant at 0. 05 level; the correlation coefficient of SWC with H under solo planting was significant at 0. 05 level, but that of under mixed planting was not significant. These results indicate that interspecific water competition alters the relationship between soil moisture and plant growth. Moreover, it was noticed that the mRNA level of SOD, POD and CAT were not always correlated with their enzymatic activities. This may have been due to the post-transcriptional and post-translational modifications, compartmentalization, metabolite stability, substrate availability, etc (Krasensky & Jonak, 2012).

As shown in Figure 6A, for *L. communis*, under the control treatment, a significant differences was seen for 11 parameters between the two planting modes at each sampling point. In the solo planting, two drought response parameters (15 d and 20 d SS), two photosynthetic parameters (10 d $P_n$ and $g_s$) and MDA (15d) were lower than those in mixed planting, and four drought response parameters (5 d and 10 d SOD; 10 d SOD, 10 *eSOD-Fe*)

and two photosynthetic parameters (5 d $g_s$ and 20 d $T_r$) were higher than those in mixed planting. Under the control treatment, 6 parameters (55%) were better under mixed planting than under solo planting. Under light drought treatment, there were significant differences in 14 parameters between the two planting modes at each sampling point. For solo planting, seven drought response parameters (5 d *eSOD-Cu/Zn*; 10 d SOD, *eSOD-Fe*, proline and SS; 15 d and 20 d *ePOD*) and 10 d MDA were lower than those in mixed planting, and five drought response parameters (5 d *eSOD-Cu/Zn*; 15 d *eSOD-Fe* and *eCAT*; 20 d proline and *eCAT*) and 10 d $g_s$ were higher than those in mixed planting. Under the light drought treatment, 5 parameters (38%) were better under mixed planting than under solo planting. Under moderate drought treatment, there were significant differences in 14 parameters between the two planting modes at each sampling point. For solo planting, four drought response parameters (5 d SS, proline and *eSOD-Fe*; 10 d *eSOD-Fe*) and 10 d $P_n$, 5 d LWC and 20 d MDA were lower than those in mixed planting, and four drought response parameters (5 d, 15 d and 20 d *eSOD-Cu/Zn*; 15 d *eSOD-Fe*), two photosynthetic parameters (20 d $P_n$ and $T_r$) and 15 d MDA were higher than those in mixed planting. Under moderate drought treatment, 7 parameters (50%) were better in mixed planting than under solo planting.

As shown in Figure 6B, for *P. longipes*, under the control treatment, there were significant differences in 15 parameters between the two planting modes at each sampling point. For solo planting, ten drought response parameters (5 d and 10 d SOD; 5 d, 15 d and 20 d proline; 10 d *eSOD-Cu/Zn*; 10 d, 15 d and 20 d SS), three photosynthetic parameters (5 d and 20 d $P_n$; 20 d $g_s$), 20 d MDA and CW were lower than those of mixed planting, and 15 d *ePOD* and $P_n$ were higher than those of mixed planting. Under light treatment,
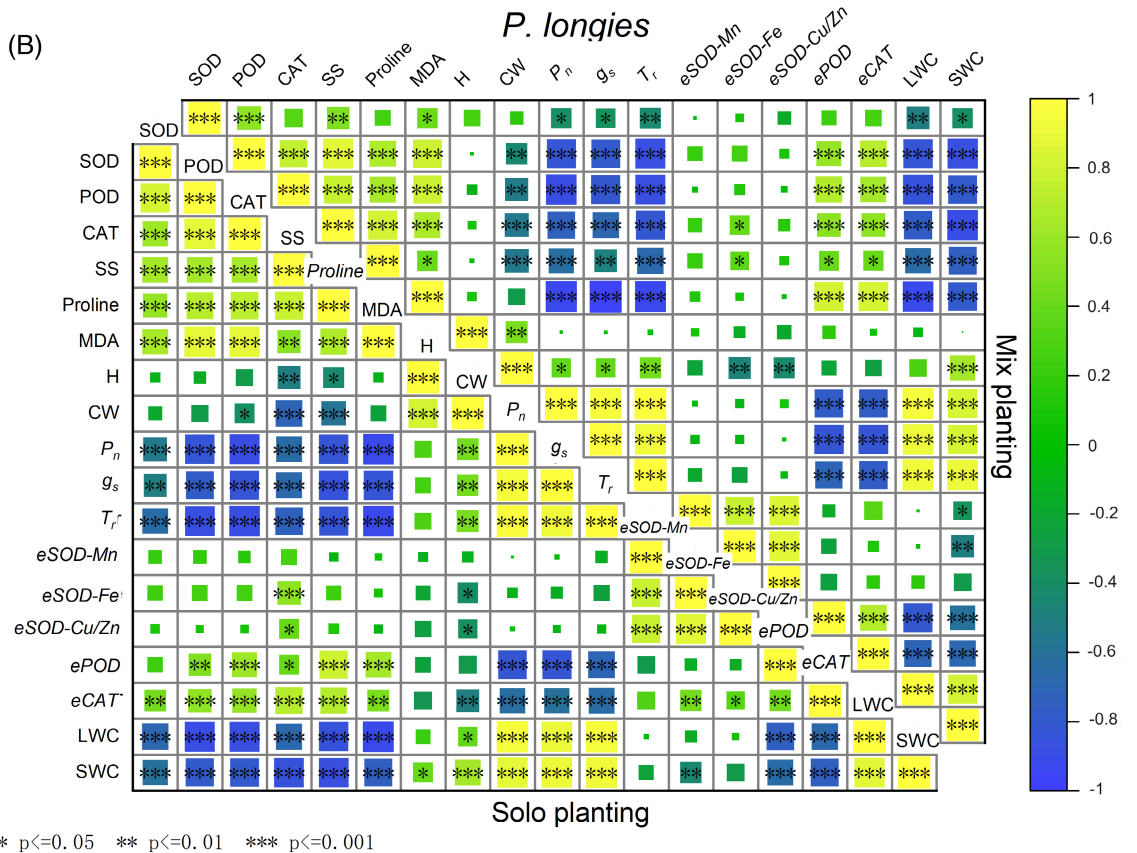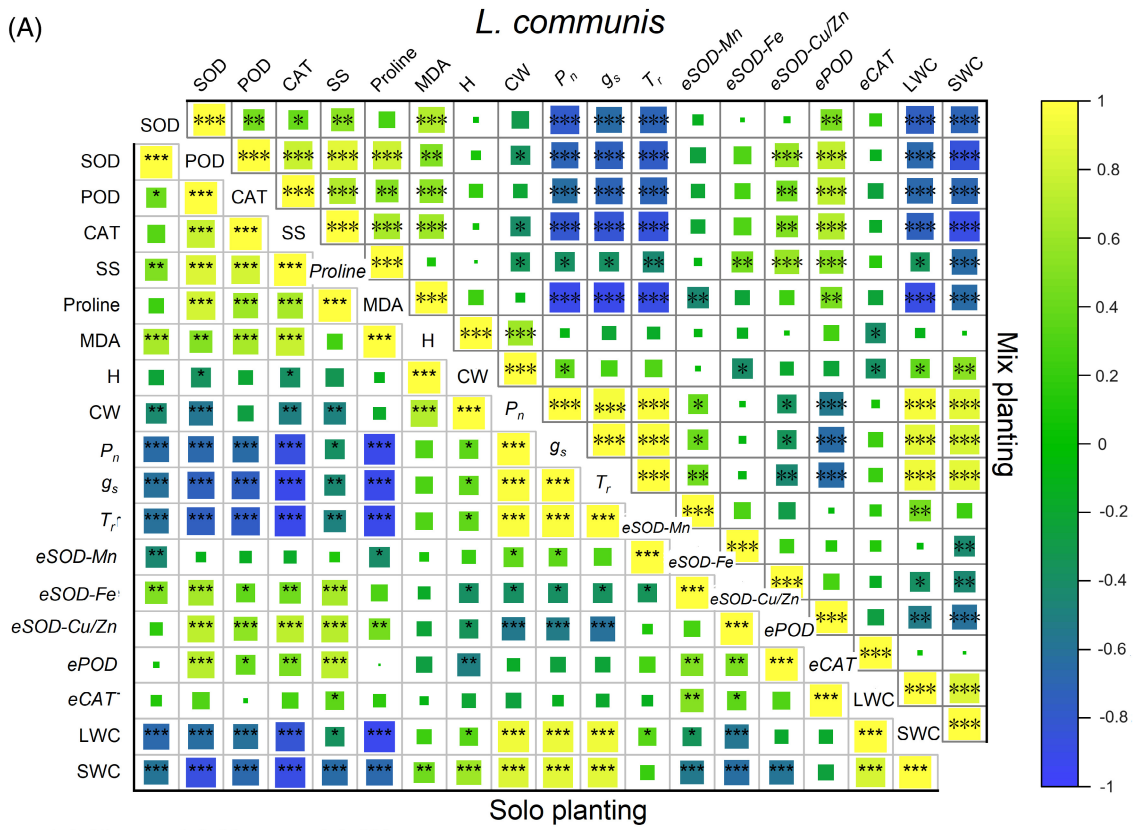
FIGURE 5    Legend on next page.

there were significant differences in 24 parameters between the two planting modes at each sampling point. For solo planting, nine drought response parameters (5 d and 15 d SOD; 5 d eSOD-Fe; 5 d eSOD-Cu/Zn; 10 d, 10 d, 15 d and 20 d SS; 15 d ePOD; 15 d eCAT), two photosynthetic parameters (15 d $P_n$ and $T_r$), and 20 d CW were lower than those in mixed planting, and six drought response parameters (5 d ePOD; 15 d and 20 d POD; 15 d proline, 15 eSOD-Mn; 20 CAT), three photosynthetic parameters (10 d and 20 d $P_n$; 20 d $T_r$), and 10 d, 15 d and 20 d MDA were higher than those in mixed planting. Under moderate treatment, there were significant differences in 23 parameters between the two planting modes at each sampling point. For solo planting, six drought response parameters (10 d SOD, proline and eSOD-Cu/Zn; 15 d and 20 d SS; 20 d SOD), seven photosynthetic parameters (5 d, 15 d and 20 d $P_n$, 15 d $g_s$ and 5 d, 15 d and 20 d $T_r$), 5 d and 10 d LWC, and 5 d and 20 d MDA were lower than those in mixed planting, and five drought response parameters (5 d ePOD; 10 d eCAT; 15 d proline, SOD and CAT) and 15 d MDA were higher than those in mixed planting.

## 4 | DISCUSSION

### 4.1 | Drought resistance strategies of *P. longipes* and *L. communis*

Plants will suffer drought stress when the water absorbed by roots is insufficient to supplement the water lost by evapotranspiration (Ozturk et al., 2021). Plants have different drought resistance strategies and abilities, related not only to species specificity but also to their habitats, interspecific competition and drought severity (Farrior et al., 2013). By analysing functional traits, we can better understand the adaptive mechanisms of plants under drought and other disturbances and further elucidate the mechanisms of species co-existence and spatial structure in communities (Wright et al., 2004; Reich, 2014). A large number of studies have shown that there is a significant correlation between plant functional traits and environmental water content, and there will be significant differences in functional traits between plants adopting drought avoidance strategies and plants adopting drought tolerance strategies (Fonseca
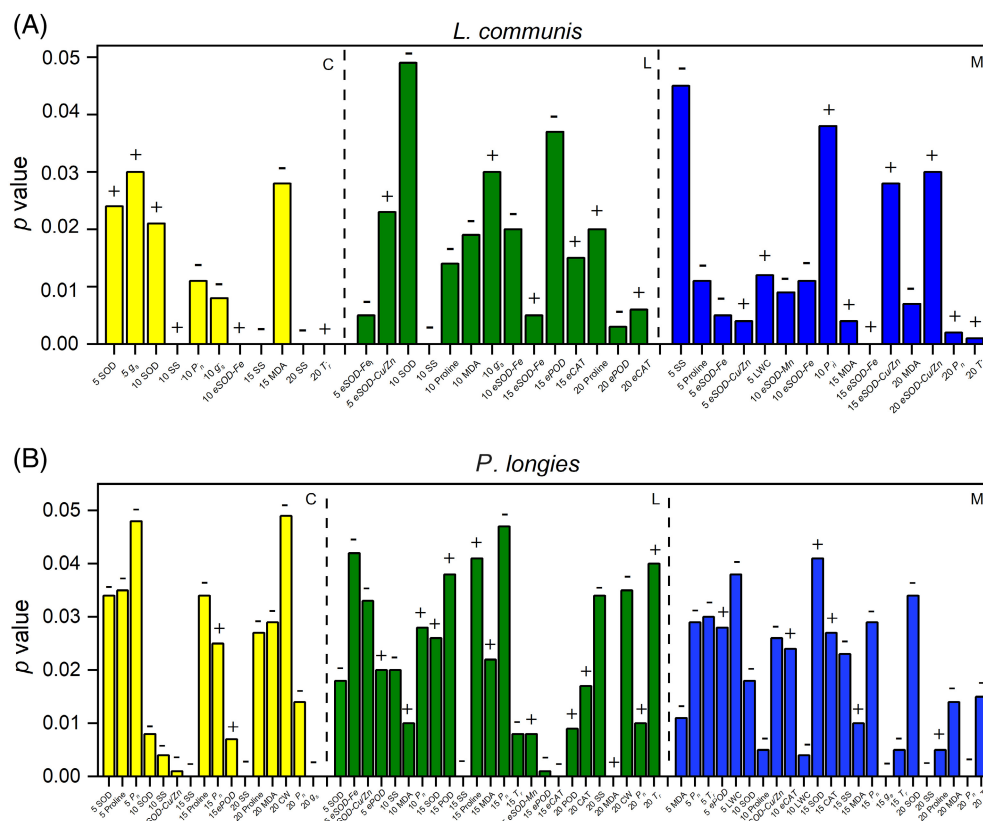


**FIGURE 6** The significant differences between solo and mixed planting in morphological and physiological parameters of *L. communis* (a) and *P. longipes* (b). The figures before the parameters are the time (day) of the treatments. The "+" or "-" above the parameters means this parameter in solo planting higher or lower than mixed planting. C, control; L, light drought; M, moderate drought. $P_n$, Net photosynthetic rate; $g_s$, stomatal conductance; $T_r$, transpiration rate; SOD, activity of superoxide dismutase; POD, activity of peroxidase; CAT, catalase; eSOD, expression of genes encoding superoxide dismutase; ePOD, expression of genes encoding peroxidase; eCAT, expression of genes encoding catalase; SS, soluble sugar; LWC, leaf water content; CW, crown diameter.

**FIGURE 5** *p*-value and Pearson's r between parameters of P. *longipes* and L. *communis*. *p*-value and Pearson's r were calculated with linear regression analysis. $P_n$, Net photosynthetic rate; $g_s$, stomatal conductance; $T_r$, transpiration rate; SOD, activity of superoxide dismutase; POD, activity of peroxidase; CAT, catalase; eSOD, expression of genes encoding su-peroxide dismutase; ePOD, expression of genes encoding peroxidase; eCAT, expression of genes encoding catalase; SS, soluble sugar; LWC, leaf water content; CW, crown diameter.

et al., 2000; Niinemets, 2001; Markesteijn & Poorter, 2009). To date, research on plant functional traits has mainly focused on morphological traits such as leaves and roots, while there are few reports on plant responses to disturbances through physiological traits. As the degree of drought intensified, *P. longipes* had an increasingly strong loading on PC2, while *L. communis* had increasingly strong loading on PC1 (Figure 2A). This shows that there is a significant difference in the drought resistance strategies of *P. longipes* and *L. communis*. The $g_s$ and $T_r$ have high loading on PC1, indicating that *L. communis* relies heavily on stomatal regulation to cope with drought stress (Figure 2b, Figure S4, S5). Reducing water dispersion during drought stress through stomatal closure is a typical characteristic of drought-avoidant plants. The strong loading on PC2 of various antioxidant enzyme genes indicates that to eliminate excessive antioxidants under drought stress, the upregulation of enzyme activity at the transcriptional level is necessary for *P. longipes*, although this requires more-material and energy consumption than the regulation of enzyme activity at the metabolic level. The upregulation of antioxidant enzyme activity and gene expression in arid environments prevents plant cells from being damaged by oxidative substances, and this drought adaptation strategy is in accordance with the basic characteristics of drought-tolerant plants. In the isohydric-anisohydric model, isohydric tree species adopt rigid stomatal regulation strategies to maintain constant leaf water potentials. In contrast, anisohydric tree species can maintain relatively high stomatal conductance to maintain carbon assimilation capacity, allowing leaf water potentials to track declining soil moisture (Tyree & Sperry, 1988; Ulrich & Grossiord, 2023). In the experiment, the $g_s$ of *P. longipes* was closer to that of isohydric plants, while that of *L. communis* was more similar to that of anisohydric plants. Many studies have shown that isohydric tree species often adopt drought avoidance strategies, while anisohydric tree species often adopt drought tolerance strategies under drought stress (Tardieu & Simonneau, 1998; Meinzer et al., 2014; Balekoglu et al., 2023). Plants that adopt drought avoidance strategies typically have a higher WUE (Franks., 2011). Under different treatments, the WUE of *L. communis* was significantly higher than that of *P. longipes* (Figure 3), indicating that *L. communis* adopts drought avoidance strategies under drought conditions.

## 4.2 | Changes in the hydrological niches of *P. longipes* and *L. communis* under different treatments

Distribution of soil water resources, water utilization features of species, and the interspecific and intraspecific water competitions are determinants of plant hydrological niche in a community (Bartelheimer et al., 2010). Brum et al. (2019) showed that the segregation in root water absorption of different species is the fundamental strategy for species coexistence within a community. Under solo planting, both *P. longipes* and *L. communis* tend to expand the width of the hydrological niche when drought stress occurs; that is, *P. longipes* increases the absorption of water from the deep soil, while *L. communis* increases the absorption of water from the surface soil, making the contribution

of water in each soil layer to the xylem water of both plants more at equilibrium. This indicates that there is substantial plasticity in the root water uptake of both plants and in the absence of interspecific competition, both plants can change their effective rooting depth to increase water absorption under drought stress (Doussan et al., 2006, Fan et al., 2017). This may be related to the fact that most plant species have similar optimal values in terms of environmental factors or resource gradients when growing in monoculture (Bartelheimer et al. 2010). Ellenberg (1953) proposed that interspecific competition often causes distributions to contract or to be displaced along a gradient from the fundamental to the realized niche, namely, that of 'Ellenberg-type differentiation'. Under the mixed planting and control treatments, the soil hydrological niches of *P. longipes* and *L. communis* had a significant separation; *P. longipes* mainly occupy the water of the deeper soil layer, while *L. communis* occupies the water of the shallow soil layer. This characteristic of hydrological niche separation between these dominant species in the community can be regarded as the result of the distribution of long-term hydraulic traits selected within the community (Connell 1980, Hillebrand et al., 2008). Brum et al. (2019) proposed that the strategy of competing for shallow soil water would seem to be advantageous when drought-stress avoidance strategies such as deciduous dormancy are employed; and to evergreen plants such as *P. longipes*, it seems more advantageous to compete for deeper soil water. This characteristic may be more prominent when plants face water-deficient environments, which also indicates that the ability of root water absorption has plasticity, which can change the effective rooting depth according to changes in soil moisture conditions (Brum et al., 2019). However, interestingly, under moderate drought, the proportion of water from shallow soil is the highest in *P. longipes* xylem compared with other treatments. This may indicate that the soil moisture competition ability of *P. longipes* is stronger than that of *L. communis*, and it may also be the result of *L. communis* adopting the drought avoidance strategy and closing stomata early, leading to the contraction of the soil hydrological niche. The ecological niche of plants is not only relates to the resource range of them, but also involves the resource amount of them (Bartelheimer et al. 2010). Due to the complexity of the source of transpiration water and limitations in measurement methods, it is difficult to accurately measure the amount of water consumption from different soil layers. However, Figures S4, S5 show that *L. communis* had a more intense decrease in $T_r$ than *P. longipes*, which implies that *L. communis* had a more intense contraction of the soil hydrological niche and reduced soil water absorption under the same soil moisture deficiency.

## 4.3 | *P. longipes* benefit from coexisting with *L. communis* under drought stress

The results of the correlation analysis indicate that most physiological and growth parameters of *P. longipes* and *L. communis* are significantly affected by soil moisture (Figure 5), which shows the strong effect of drought on plant growth and development. However, we noticed that

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the correlation between parameters (SOD, H, *eSOD-Mn* of *P. longipes*; and H, *eSOD-Fe* and *eSOD-Cu/Zn* of *L. communis*) and SWC decreased under mixed planting, which shows that interspecific water allocation affects the functional trait characteristics of plants under drought conditions. In the experiment, we found that there were fewer parameters with significant differences between the solo planting and mixed planting with *L. communis* compared with *P. longipes* (Figure 6), indicating that interspecific competition had a smaller effect on *L. communis* than on *P. longipes*, which may also reflect that *L. communis* has a more conservative strategy than *P. longipes*. Figure 6 shows that the environmental stress increases when *L. communis* coexists with *P. longipes* under light drought treatment, which indicates a disadvantage for *L. communis* in interspecific water competition with *P. longipes*. However, under moderate drought treatment, *L. communis* under different planting modes did not differ significantly in the number of parameters, which may reflect that the effect of moderate drought stress is much greater than the effect of interspecific water competition on *L. communis* and may also be related to the rapid and significant reduction in water absorption and consumption of *L. communis*, which adopted a drought avoidance strategy under this treatment. Under the control, light drought and moderate drought treatments, 13, 15 and 65% of the parameters were significantly different under mixed planting than under solo planting, respectively. This shows that *P. longipes* benefit significantly more from coexisting with *L. communis* with increasing drought stress. This increased benefit is likely related to the strong water competition ability of *P. longipes* and the initiative hydrological niche shrinkage of *L. communis*, which adopts a drought avoidance strategy under drought conditions.

Meanwhile, according to the results of Figures S4, S5, *L. communis* stomatal closure was faster than that of *P. longipes*. Stomatal regulation is an important trait that affects water transport through the hydraulic continuum of plants (Brodribb 2009). The diversity of plant hydraulic strategies and characteristics can buffer the ecosystem from the impact of drought stress, and some species reduce water consumption and latent heat flux by closing stomata before others (Anderegg et al., 2018). The smaller difference between the two planting modes and the earlier stomatal closure of *L. communis* suggests that the narrowing of the hydrological niche of this plant may be largely due to its drought resistance strategy (drought avoidance), which reduces its consumption of soil water under drought conditions, and plants such as *P. longipes* that coexist with it can benefit from this process (Figure S4).

# 5 | CONCLUSION

Based on the drought resistance and response parameters of *P. longipes* and *L. communis*, PCA was conducted to assess the differences in their drought resistance strategies. *L. communis* exhibited a drought avoidance strategy, and *P. longipes* exhibited a drought tolerance strategy. Both plants had a number of morphological and physiological parameters that were significantly different between solo planting and mixed planting, and the planting mode also affected the

hydrological niches of these two plants and the WUE of *P. longipes*. Considering the significant correlation between soil moisture and many plant parameters, it can be concluded that the difference in hydrological niche under different planting modes is the main reason for the differences in plant morphology and physiological parameters. Compared to *P. longipes*, fewer parameters of *L. communis* were significantly different between the two planting modes, which may be due to *L. communis* having a rigid stomatal regulation strategy and employing a drought avoidance strategy. Under the same resource conditions, *P. longipes* benefited from coexisting with *L. communis*, which suggested that differences in functional traits or differences in resistance strategies among species benefit species coexistence in a community under drought stress.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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