RESEARCH ARTICLE

Water and nutrient use efficiency of three tree species in monoculture and mixed stands and potential drivers in the Loess Hilly Region, China

Senbao Lu · Yunming Chen · Jordi Sardans · Josep Peñuelas

Abstract

Background Mixed plantations utilize resources through specific strategies. However, the impact mechanism of mixed plantations on the water and nutrient use efficiency of different tree species remains unclear.

Methods Robinia pseudoacacia mixed (*R. pseudoacacia* and *Amygdalus davidiana*, RPAD; *R. pseudoacacia* and *Armeniaca sibirica*, RPAS) and monoculture stands (*R. pseudoacacia*, RP; *A. davidiana*, AD; and *A. sibirica*, AS) were used to determine mixing species relative to monoculture effects on leaf water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies and their potential drivers in the Loess Hilly Region.

Results Mixing *R. pseudoacacia* and *A. sibirica* is a good strategy to maximize soil nutrient resources. Compared to monocultures of all species, *R. pseudoacacia* and *A. davidiana* in RPAD and *A. sibirica* in RPAS had higher WUE and lower NUE and PUE; P limitation of *R. pseudoacacia* in mixed stands was lower than that in RP. Furthermore, *R. pseudoacacia* WUE was positively correlated with stand density and crown area, *A. davidiana* and *A. sibirica* were the opposite. Regarding all tree species, WUE was influenced by leaf dry matter content, leaf tissue density, and soil available P, while NUE, PUE, and N:P ratio were by average tree height and specific leaf area. Notably, irrespective of tree species, WUE exhibited

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a negative correlation with NUE and PUE, but a positive correlation with N:P ratio.

Conclusions Mixed plantations affect water and nutrient use efficiency by altering the above- and below-ground niche through tree structure, and there is a degree of trade-off between WUE, and NUE, PUE.

Keywords Mixed species · Water, nitrogen, and phosphorus use efficiencies · Carbon stable isotope · Nutrient limitation · Stand characteristics · Soil physicochemical properties

Introduction

The Chinese Loess Plateau (CLP) has an area of about 6.35×10^5 km², and the destruction of the original vegetation has resulted in one of the most severe water and soil erosion in the world (Fu et al. 2004). To improve the ecological environment of the CLP, the Grain for Green Project was launched in 1999 (Cao et al. 2011). The plantation coverage on the CLP increased from 1.42 Mha in 2000 to 7.13 Mha in 2021 (Meng et al. 2023). More importantly, the afforestation project changed the cycle of water and nutrients in the plant-soil system (Su and Shangguan 2021). In particular, plant growth is severely constrained by a deficient water supply and weak nutrient uptake (Wei et al. 2018). Previous studies used carbon stable isotope (δ^{13}C) analysis and chemical stoichiometry (C:N:P) in plant organs and soil at the stand or regional scale to study the water and nutrient status of plants on the CLP (Tanaka-Oda et al. 2010; Cao and Chen 2017). However, because of the use of various resource acquisition methods, plant growth in this region with different forms of expressions in water and nutrient use strategies (Su and Shangguan 2020; Wang et al. 2020).

Adaptation of different tree species to resource changes can be reflected through water and nutrient use efficiency (Luo et al. 2004; Liu et al. 2015). More specifically, plants responding to water and nutrient stress by changing their physiological activities, such as water (WUE), nitrogen (NUE), and phosphorus (PUE), use efficiencies (Forrester et al. 2005). Generally, plants indirectly reduce their WUE by opening leaf stomata to increase intercellular carbon dioxide (CO_2) concentration,

photosynthetic rate per unit nitrogen, and transpiration water consumption (Farquhar and Richards 1984; Garrish et al. 2010). With the increase in soil available nitrogen (AN), leaf nitrogen concentration also frequently increases while NUE decreases, increasing WUE (Ripullone et al. 2004). Guo et al. (2016) found that when soil AN was unchanged, interspecific competition significantly increased the leaf nitrogen concentration but decreased its WUE. Garrish et al. (2010) found that WUE of the tropical plant *Ficus insipida* Willd. (Moraceae) varied as a function of soil AN but not AP. Moreover, according to Dijkstra et al. (2016), WUE increased when the water supply was low while the nutrient supply was high, whereas NUE showed the absolutely opposite trend. Thus, plant physiological constraints generated a trade-off between WUE and NUE. Additionally, strong correlations have been observed between WUE and PUE that is determined by the plant genotype (Meier et al. 2022).

As previous studies focused on the changes in WUE and NUE of monoculture stands (Su and Shangguan 2021), it remains unclear whether mixed-species plantations can attenuate regional water and nutrient stress by increasing both WUE and nutrient use efficiency (i.e. NUE and PUE) of the overall community. A mixed-species plantation is a stand composed of at least two tree species, where the proportion of the main tree species is equal to or greater than 10% (Coll et al. 2018). Because of niche differentiation, positive interactions, or both, the mixed stand can improve its use efficiency of resources, such as light, water, and nutrients, compared with the monoculture stand (Anderegg et al. 2018). For instance, mixed stands containing N-fixing species can increase soil total nitrogen (TN) stocks by biological N fixation and improve the growth of neighboring species in N-limited ecosystems (Taylor et al. 2017). Moreover, the interaction of species with the same growth cycle or leaf phenology or belonging to the same general ecological niche may lead to increased competition for water and N and P among them (Drössler et al. 2018). However, coexisting species in a community can avoid direct interspecific competition, to some extent, when differences in root depth and density allow each species to exploit different underground niches, resulting in more efficient water and nutrient consumption (Dimitrakopoulos and Schmid 2004).

Robinia pseudoacacia L. is a representative N-fixing species in a wide range of habitats spanning from Europe to China (Tsunekawa et al. 2014). On the CLP, unreasonable afforestation measures can cause the ecological degradation of an *R. pseudoacacia* monoculture stand, frequently leading to dead branches and even death (Wei et al. 2018). To guide suitable management and conservation of *R. pseudoacacia* monoculture stands, numerous studies have explored their water use and nutrient strategies, as well as potential drivers. For example, Tanaka-Oda et al. (2010) found that WUE and leaf TN concentration of *R. pseudoacacia* decreased with increasing tree height, indicating that water and nutrients are crucial factors for its growth. Apart from water limitation (Wang et al. 2021), the growth of *R. pseudoacacia* is limited by the availability of P (Cao and Chen 2017). However, few studies have reported water and nutrient use strategies of *R. pseudoacacia* in mixed stands on the CLP. Two common native tree species in this area are *Amygdalus davidiana* (Carr.) C. de Vos ex Henry var. *davidiana* and *Armeniaca sibirica* (L.) Lam., and they are planted widely mixed with *R. pseudoacacia*. However, it is unclear whether these mixed patterns can improve the water and nutrient suitability of *R. pseudoacacia*, *A. davidiana*, and *A. sibirica*.

To address this knowledge gap, we analyzed water and nutrient use efficiencies of *R. pseudoacacia* and *A. davidiana* with *A. sibirica* in both monoculture and mixed stands and analyzed their correlations and potential drivers. We hypothesize that (1) the mixed stand can increase WUE, NUE, and PUE and alleviate nutrient restriction; (2) WUE, NUE, and PUE are affected by many factors, including stand characteristics and soil physicochemical properties; (3) there is a trade-off between WUE, and NUE, PUE, nutrient limitation for these tree species. The results offer valuable insights into tree mixtures and their management by elucidating the factors driving water and nutrient use efficiency in mixed stands, as well as the associated trade-offs.

Materials and methods

Study site

The study site was located in Ansai County (36°35′–36°36′ N, 109°13′′′–109°16′ E, 1195–1212 m a.s.l), Shaanxi Province, China. This region belongs to a typical forest-steppe ecotone with a warm temperate semi-humid continental monsoon climate. The mean annual temperature is 8.95 °C, and the mean annual precipitation is 500 mm, with most precipitation occurring from July to September. The soils are classified as Calcic Cambisols (FAO 2020). After 20 years of *R. pseudoacacia* afforestation in this region, the plant has become the leading tree species.

Experimental design

This study was conducted in five stand types with three replicates (15 plots): mixed stands of *R. pseudoacacia* with *A. davidiana* (RPAD) and with *A. sibirica* (RPAS), and monoculture stands of *R. pseudoacacia* (RP), *A. davidiana* (AD), and *A. sibirica* (AS). These plots share similar altitude, slope, soil condition, stand age (16–25 years), and climatic conditions (Table S1). For each stand type, three 20 m×20 m quadrats were used for the plot survey, which included calculating stand density (SD, trees·ha^{-1}), diameter at breast height (DBH, cm), average tree height (AH, m), and crown area (CA, m^2). To avoid edge effects, a 25 m buffer zone was established around each plot and the distance between two plots did not exceed 3 km.

Plot survey and sampling

In August 2021, green leaves from each plot were sampled from lower, middle, and upper canopies of five model trees with similar height and DBH and then divided into two parts. One part was placed in an ice box and brought back to the laboratory to analyze leaf functional traits. The other was heated at 105 °C for 15 min and then heated to a constant weight at 70 °C in an oven to analyze leaf chemical components. To analyze soil chemical properties, 0–20 cm soil was sampled using a soil drill (40 mm in diameter). Soil samples of mixed stands were composed of soil collected near *R. pseudoacacia* and *A. davidiana*, and *R. pseudoacacia* and *A. sibirica* in mixed stands. Near *R. pseudoacacia* and *A. davidiana* (RPAD), and near *R. pseudoacacia* and *A. sibirica* (RPAS), soils were collected and mixed into a soil sample, respectively. To analyze soil physical properties, undisturbed soil was sampled using a ring cutter (volume 100 cm^3) at 0–20 cm as above.

Sample measurement

Five to ten intact, disease-free leaves of the three tree species were sampled in monoculture and mixed stands to measure and calculate leaf functional traits. Specific leaf area (SLA, $cm^2 \cdot g^{-1}$), leaf dry matter content (LDMC, $g \cdot g^{-1}$), and leaf tissue density (LD, $g \cdot cm^{-3}$) were calculated by the following equation:

$$SLA = \frac{LA}{DW} \tag{1}$$

$$LDMC = \frac{DW}{FW} \tag{2}$$

$$LD = \frac{DW}{LT \times LA} \tag{3}$$

where fresh (FW) and dry (DW) leaf weights are determined using Electronic balance (accuracy 0.001 g), leaf thickness (LT, mm) is determined using a Digital vernier caliper, and leaf area (LA, cm^2) is determined using Epson Perfection V850 Pro Scanner, ImageJ software.

Soil pH was determined using an automatic acid–base titrator (PB-10 standard pH meter; Sartorius, Göttingen, Germany) with water:soil ratio of 2.5:1. Soil bulk density (BD, $g \cdot cm^{-3}$) was determined using the ring knife method (Bao 2000). Soil particle composition, e.g., soil sand content (SA, %), soil silt content (SI, %), and soil clay content (CL, %) were determined using a Mastersizer 2000 Laser Particle Size Analyzer (Malvern Panalytical, Malvern, UK).

Leaf total carbon (TC, $g \cdot kg^{-1}$) and soil organic carbon (SOC, $g \cdot kg^{-1}$) concentrations were determined using the potassium dichromate volumetric method (Nelson and Sommers 1982). Additionally, Leaves and soil total nitrogen (TN, $g \cdot kg^{-1}$) concentrations were determined using the Kjeldahl method (Bremner and Mulvaney 1982), while soil available nitrogen (AN, $mg \cdot kg^{-1}$) concentration was determined using the alkaline hydrolysis diffusion method (Bao 2000). Finally, both soil total phosphorus (TP, $g \cdot kg^{-1}$) and available phosphorus (AP, $mg \cdot kg^{-1}$) concentrations were determined using the molybdenum blue method (Murphy and Riley 1962).

The stable carbon isotope value $\delta^{13}C$ (‰) was determined by isotope mass spectrometry (MAT 253;

Thermo Fisher Scientific, Waltham, MA, USA) using the equation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}}\right) \times 1000 \tag{4}$$

where R_{sample} and $R_{standard}$ are the carbon isotope ratio ($^{13}C/^{12}C$) of the samples and the international carbon isotope standard (Pee Dee Belemnite (PDB), $R_{PDB} = 0.0112372$, $\delta^{13}C_{PDB} = 0‰$), respectively (Farquhar et al. 1989).

Calculation of water and nutrient use efficiency

There is a significant correlation between leaf $\delta^{13}C$ and the ratio of intercellular CO_2 concentration (C_i) to atmospheric CO_2 concentration (C_a, http://www.esrl.noaa.gov) (Farquhar et al. 1982), and the equation is as follows:

$$\Delta^{13}C = \delta^{13}C_a - \delta^{13}C = a + (b - a)\frac{C_i}{C_a} \tag{5}$$

where $\Delta^{13}C$ (‰) is the carbon isotope discrimination during photosynthesis, $\delta^{13}C_a$ ($-8‰$) is the ^{13}C abundance in the atmosphere, and a ($4.4‰$) and b ($27‰$) are the stable carbon isotope fractionation produced by the diffusion and carboxylation reactions, respectively (Farquhar and Richards 1984).

The leaf conductance to water vapor (g_{H_2O}) is 1.6 times the CO_2 conductance rate (g_{CO_2}), and the net photosynthetic rate (A) is significantly correlated with g_{H_2O} (Peñuelas et al. 2011). Thus, A can be calculated with the following equation:

$$A = g_{CO_2} \times (C_a - C_i) \tag{6}$$

$\Delta^{13}C$ can be related to A/g_{H_2O}, which is long-term water use efficiency (WUE, $\mu mol \cdot mmol^{-1}$) (Osmond et al. 1980):

$$WUE = \frac{A}{g_{H_2O}} = \frac{C_a - C_i}{1.6} = \frac{C_a(b - \Delta^{13}C)}{1.6(b - a)} \tag{7}$$

Leaf C:N and C:P ratios as a measure of nitrogen use efficiency (NUE, $gC \cdot g^{-1} \cdot N$) and phosphorus use efficiency (PUE, $gC \cdot g^{-1} \cdot P$), respectively (Vitousek 1982; Dijkstra et al. 2016). Leaf N:P ratio to determine plant nutrient limitation and leaf N:P ratios of < 10, $10–20$, and > 20 indicate N limitation, N and P co-limitation, and P limitation, respectively

(Güsewell 2004). Leaf C:N, C:P, and N:P mass ratios from leaf TC, TN, and TP concentrations.

Statistical analysis

The data were transformed to meet the assumptions of normality and homogeneity when needed. One-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test was used to analyze the differences in *R. pseudoacacia*, and *t*-test in *A. davidiana* and *A. sibirica*, for WUE, NUE, PUE, and N:P ratio, in different stand types (ST). A linear mixed-effects model (LMM) was performed to verify the interactive effects of ST and tree species (TS) on WUE, NUE, PUE, and N:P ratio. Pearson's correlation and partial correlation coefficients were used to quantify the correlations among WUE, NUE, PUE, N:P ratio, and their potential drivers. Principal component analysis (PCA) was performed to identify the primary axes of covariation among the potential drivers using Canoco 5.0 (ter Braak and Smilauer 2012). A linear regression analysis was performed to examine the relationship between WUE, and NUE, PUE, N:P ratio, respectively. Statistical analysis was performed using SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA) at a significance level of 0.05 ($^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$). Figures were created using Origin 2017 software (Originlab Inc., USA).

Results

Stand characteristics and soil physicochemical properties

As shown in Table 1, diameter at breast height (DBH), average tree height (AH), and specific leaf area (SLA) were lower for *R. pseudoacacia* growing in mixed stands than when growing in the monoculture stand ($P < 0.05$). Crown area (CA), and SLA were higher, and stand density (SD), leaf dry matter content (LDMC), and leaf tissue density (LD) were lower for *A. davidiana* growing in mixed stands than they were for the same species growing in monoculture stands ($P < 0.05$). AH, CA and SLA were higher, and SD, LDMC, and LD were lower for *A. sibirica*

growing in mixed stands than in the monoculture stand ($P < 0.05$).

The values of soil bulk density (BD), soil sand content (SA), and soil clay content (CL) were higher in the mixed stand of *R. pseudoacacia* and *A. davidiana* (RPAD) than in monoculture stands of *R. pseudoacacia* (RP) and *A. davidiana* (AD), whereas their values in the mixed stand of *R. pseudoacacia* and *A. sibirica* (RPAS) were between those in RP and monoculture stand of *A. sibirica* (AS) ($P > 0.05$). Soil pH was lower in the RPAD than in RP and AD, and it was lower in the RPAS than in RP and AS ($P > 0.05$). The soil total nitrogen (TN) value in the RPAD was between that in RP and AD, and it was between that in RP and AS in the RPAS ($P > 0.05$). The values of soil available nitrogen (AN) and available phosphorus (AP) were lower in the RPAS than in RP and AS, whereas their values in the RPAD were between those in RP and AD ($P > 0.05$) (Table 2).

Water and nutrient use efficiency

Compared to the RP, water use efficiency (WUE) of *R. pseudoacacia* was higher in the RPAD ($P < 0.05$) (Fig. 1a), and nitrogen use efficiency (NUE) of *R. pseudoacacia* was higher in the RPAS ($P < 0.05$) (Fig. 1b). WUE values of *A. davidiana* and *A. sibirica* in mixed stands were higher than they were in monoculture stands ($P < 0.05$) (Fig. 1a), while the values of NUE and phosphorus use efficiency (PUE) of *A. davidiana* and *A. sibirica* in monoculture stands were higher than in mixed stands ($P < 0.05$) (Fig. 1b-c). The N:P ratio of *R. pseudoacacia* was lower in mixed stands compared to monoculture stands, while that of *A. davidiana* and *A. sibirica* were higher in the mixed stands ($P > 0.05$) (Fig. 1d). Additionally, tree species (TS) and stand types (ST) had significant effects on WUE, NUE, and PUE ($P < 0.05$) (Fig. 1a-c); TS had significant effect on N:P ratio ($P < 0.001$) (Fig. 1d).

WUE of *R. pseudoacacia* and *A. davidiana* in the RPAD and that of *A. sibirica* in the RPAS was 19.20%, 19.74%, and 6.86% higher than that of tree species in the monoculture stand, respectively. NUE of *R. pseudoacacia* and *A. davidiana* in the RPAD and *A. sibirica* in the RPAS were 4.23%, 33.39%, and 29.56% lower, respectively. PUE of *R. pseudoacacia* and *A. davidiana* in the

Table 1 Stand characteristics of three tree species in monoculture and mixed stands

Stands	Tree species	Tree structures				Leaf functional traits		
		Stand density (trees·ha⁻¹)	Diameter at breast height (cm)	Average tree height (m)	Crown area (m²)	Specific leaf area (cm²·g⁻¹)	Leaf dry matter content (g·g⁻¹)	Leaf tissue density (g·cm⁻³)
RP	R.pseudoacacia	1083±42a	11.55±0.96a	9.54±0.41a	6.52±0.50b	335.17±5.91a	0.28±0.01c	0.32±0.01c
RPAD	R.pseudoacacia	1192±22a	9.30±0.22b	8.52±0.22b	4.71±0.38c	251.59±7.68bc	0.33±0.01c	0.37±0.02c
RPAS	R.pseudoacacia	1025±80a	9.21±0.24b	8.44±0.18b	6.24±0.35b	259.04±6.67b	0.32±0.00c	0.38±0.00c
AD	A. davidiana	733±33b	6.32±0.12c	3.58±0.12d	4.88±0.05c	114.83±6.37e	0.48±0.03a	0.53±0.02a
RPAD	A. davidiana	325±14c	5.16±0.15c	4.11±0.05d	6.19±0.08b	216.27±12.14 cd	0.30±0.00c	0.32±0.01c
AS	A. sibirica	700±58b	10.53±0.34ab	3.46±0.05d	4.26±0.21c	109.85±2.98e	0.39±0.00b	0.43±0.02 b
RPAS	A. sibirica	308±55c	9.75±0.12b	5.62±0.12c	7.42±0.07a	187.62±9.71d	0.33±0.02c	0.32±0.01c
One-way F-value		54.213	30.803	166.823	15.930	106.976	27.641	29.661
ANOVA P-value		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

RPAD R. pseudoacacia and *A. davidiana*; *RPAS R. pseudoacacia* and *A. sibirica*; *RP R. pseudoacacia*; *AD A. davidiana*; *AS A. sibirica*

Values are means ± SE (n=3)

Different lowercase letters (e.g., a–e) indicate significant differences among the same tree species for the different stands, and among different tree species for the same or different stands, and the F and P values are shown (*** P <0.001)

Table 2 Soil physicochemical properties of monoculture and mixed stands

Stand types	Stands	Soil physical properties					Soil chemical properties				
		pH	Bulk density (g·cm⁻³)	Sand content (%)	Silt content (%)	Clay content (%)	Organic C (g·kg⁻¹)	Total N (g·kg⁻¹)	Total P (g·kg⁻¹)	Available N (mg·kg⁻¹)	Available P (mg·kg⁻¹)
Monoculture	RP	8.80±0.01	1.48±0.07	30.16±0.57b	55.05±1.10a	14.79±0.56c	5.00±0.40	0.51±0.03	0.60±0.01ab	12.02±0.17	2.30±0.17ab
Stands	AD	8.85±0.06	1.49±0.01	30.28±0.32b	54.07±0.35ab	15.66±0.12bc	3.97±0.41	0.41±0.04	0.58±0.01ab	11.39±0.97	1.28±0.15c
	AS	8.88±0.03	1.52±0.07	33.91±0.19a	48.40±0.67c	17.69±0.49a	5.45±1.00	0.47±0.08	0.53±0.02c	10.23±1.14	1.63±0.11bc
Mixed stands	RPAD	8.64±0.16	1.51±0.03	31.40±1.06b	51.86±1.37b	16.73±0.43ab	5.80±0.96	0.57±0.08	0.57±0.00b	12.44±0.45	2.41±0.42a
	RPAS	8.66±0.04	1.53±0.04	30.37±0.54b	54.46±0.71ab	15.17±0.18c	5.65±0.55	0.57±0.05	0.61±0.00a	12.63±0.25	1.93±0.11abc
One-way	F	1.981	0.191	6.648	8.926	9.081	1.062	1.290	14.117	1.847	4.420
ANOVA	P	0.174	0.937	0.007**	0.002**	0.002**	4.24	0.337	0.000***	0.197	0.026*

RPAD R. pseudoacacia and *A. davidiana*; *RPAS R. pseudoacacia* and *A. sibirica*; *RP R. pseudoacacia*; *AD A. davidiana*; *AS A. sibirica*

Values are means ± SE (n=3)

Different lowercase letters (e.g., a–c) indicate significant differences among different stands for the same or different stand types, and the F and P values are shown (*** P <0.001; ** P <0.01; * P <0.05)
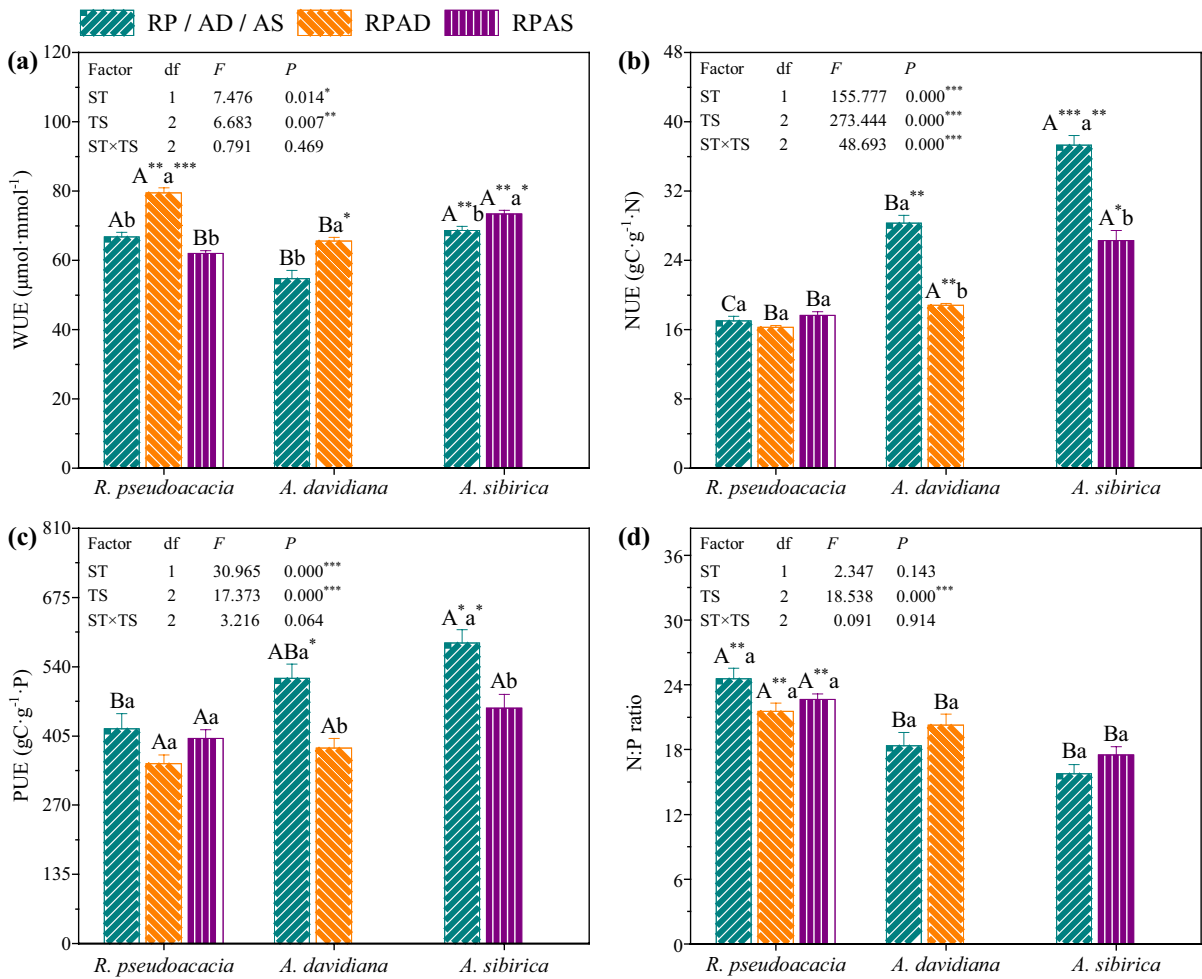
Fig. 1 Changes in water (WUE, **a**), nitrogen (NUE, **b**), and phosphorus (PUE, **c**) use efficiencies, and N:P ratios (**d**) of three tree species in monoculture and mixed stands, and as affected by stand types (ST), tree species (TS), and their interaction (ST×TS). RPAD, *R. pseudoacacia* and *A. davidiana*; RPAS, *R. pseudoacacia* and *A. sibirica*; RP, *R. pseudoacacia*; AD: *A. davidiana*; AS, *A. sibirica*. Values are means ± SE (n = 3). Different lowercase letters (e.g., a and b) above the bars indicate significant differences among the same tree species for the different stands, while different uppercase letters (e.g., A–C) indicate significant differences among different tree species for the same stands. The effects of TS and ST on nutrition use efficiency are shown with degree of freedom (df), *F*, and *P* values. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

RPAD and that of *R. pseudoacacia* and *A. sibirica* in the RPAS was 16.19%, 26.32%, 4.56%, 21.71% lower than that of tree species in the monoculture stands. The N:P ratio of *R. pseudoacacia* in RPAD and RPAS was 12.33% and 7.89% lower, while that of *A. davidiana* in RPAD and *A. sibirica* in RPAS was 10.38% and 10.99% higher than that of tree species in monoculture stands, respectively (Fig. 2).

Relationship between potential drivers and water and nutrient use efficiency

In both monoculture and mixed stands, WUE was positively correlated with SD for *R. pseudoacacia* ($P < 0.05$) (Fig. 3a), while it was the opposite for *A. davidiana* and *A. sibirica* ($P < 0.05$) (Fig. 3b-c); WUE was negatively correlated with CA for *R. pseudoacacia* ($P < 0.05$) (Fig. 3a), while it was the opposite for *A. davidiana* and *A. sibirica* ($P < 0.05$) (Fig. 3b-c). NUE and PUE were negatively correlated with SD

Fig. 2 Changes in water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies and N:P ratios of three tree species when transitioning from monoculture to mixed stands
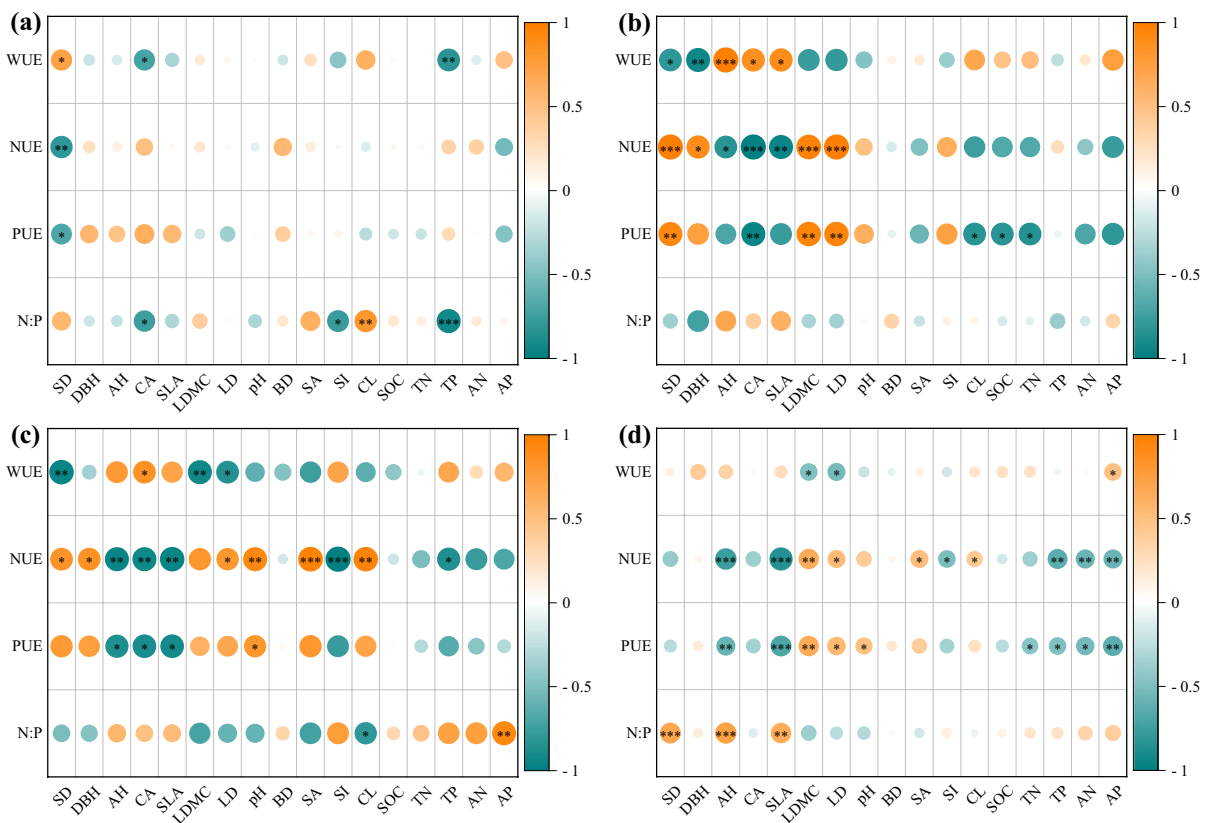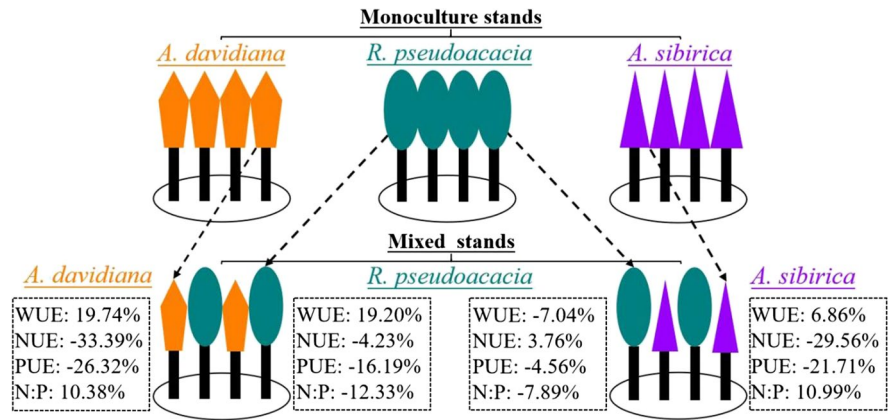
Fig. 3 Correlation matrix of water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies, and N:P ratios of (**a**) *R. pseudoacacia*, (**b**) *A. davidiana*, (**c**) *A. sibirica* and (**d**) all species, with stand characteristics and soil physicochemical properties in monoculture and mixed stands. SD, stand density; DBH, diameter at breast height; AH, average tree height;

CA, crown area; SLA, specific leaf area; LDMC, leaf dry matter content; LD, leaf tissue density; pH, soil pH; BD, soil bulk density; SA, soil sand content; SI, soil silt content; CL, soil clay content; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, soil available nitrogen; AP, soil available phosphorus. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

for *R. pseudoacacia* (*P* < 0.05) (Fig. 3a), while it was the opposite for *A. davidiana* (*P* < 0.05) (Fig. 3b) and *A. sibirica* (partly *P* < 0.05) (Fig. 3c); NUE and PUE were positively correlated with CA for *R. pseudoacacia* (*P* > 0.05) (Fig. 3a), while it was the opposite for *A. davidiana* and *A. sibirica* (*P* < 0.05) (Fig. 3b-c).

All in all, WUE was positively correlated with soil AP and negatively correlated with LDMC and LD (*P* < 0.05). NUE and PUE were positively correlated with LDMC and LD, and negatively correlated with AH, SLA, soil total phosphorus (TP), soil AN, and AP (*P* < 0.05). The N:P ratio was positively correlated with SD, AH, and SLA (*P* < 0.05) (Fig. 3d).

Combining the partial correlation coefficients of all potential drivers showed that AH had the greatest effect on WUE, NUE, PUE, and N:P ratio, SD and CA on WUE and N:P ratio, DBH on NUE, PUE, and N:P ratio. SLA had the biggest impact on NUE and N:P ratio. SA and CL had an important effect on WUE, NUE, and PUE, SI on WUE and PUE, and soil pH on NUE and PUE. Soil AP had the greatest influence on WUE, and SOC and soil TN on PUE (*P* < 0.05) (Table 3).

Potential drivers modulate water and nutrient use efficiency

Principal component analysis (PCA) results of potential drivers of water and nutrient use efficiency showed that PC1 and PC2 accounted for 37.02% and 22.17% of the total variance, respectively (Fig. 4). PC1 had loads mainly from AH (0.775), CA (0.693), SLA (0.844), LDMC (−0.788), LD (−0.733), CL (−0.625), TP (0.846), and AN (0.708), which could be identified as the "leaf functional trait component". PC2 could be defined as the "soil particle and chemical component" because it had loads from soil pH (−0.653), SI (−0.708), CL (0.714), SOC (0.862), and TN (0.77). PC3 was defined as the "tree structure component" because it had explained 13.60% of the

Table 3 Partial correlation coefficients of water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies and N:P ratios of three tree species with their potential drivers in monoculture and mixed stands

Variables	Factors	WUE		NUE		PUE		N:P ratio	
		Coef	Sig	Coef	Sig	Coef	Sig	Coef	Sig
Tree structures	SD	−0.633	0.005[**]	0.406	0.094	0.340	0.167	−0.479	0.044[*]
	DBH	0.355	0.149	0.938	0.000[***]	0.767	0.000[***]	−0.622	0.006[**]
	AH	0.642	0.004[**]	−0.889	0.000[***]	−0.678	0.002[**]	0.795	0.000[***]
	CA	−0.610	0.007[**]	0.180	0.475	0.211	0.401	−0.678	0.002[**]
Leaf functional traits	SLA	−0.142	0.561	−0.711	0.001[***]	−0.335	0.160	0.592	0.008[**]
	LDMC	−0.020	0.935	0.257	0.289	0.299	0.214	−0.021	0.931
	LD	−0.224	0.357	−0.296	0.218	−0.211	0.385	0.157	0.520
Soil physical properties	pH	−0.291	0.257	0.620	0.008[**]	0.654	0.004[**]	−0.332	0.193
	BD	−0.466	0.059	0.295	0.251	0.440	0.077	−0.148	0.571
	SA	−0.673	0.003[**]	0.537	0.026[*]	0.550	0.022[*]	−0.461	0.063
	SI	−0.673	0.003[**]	0.536	0.027	0.549	0.022[*]	−0.460	0.063
	CL	−0.672	0.003[**]	0.536	0.027[*]	0.549	0.023[*]	−0.459	0.064
Soil chemical properties	SOC	−0.012	0.964	0.433	0.082	0.608	0.010[**]	−0.295	0.250
	TN	0.065	0.806	−0.400	0.111	−0.606	0.010[**]	0.254	0.325
	TP	−0.097	0.712	0.089	0.733	0.378	0.135	−0.282	0.273
	AN	−0.128	0.624	−0.306	0.232	−0.291	0.258	0.306	0.232
	AP	0.483	0.050[*]	−0.437	0.079	−0.477	0.053	0.255	0.323

SD Stand density; *DBH* Diameter at breast height; AH, average tree height; *CA* Crown area; *SLA* Specific leaf area; *LDMC* Leaf dry matter content; *LD* Leaf tissue density. *pH* Soil pH; *BD* Soil bulk density; *SA* Soil sand content; *SI* Soil silt content; *CL* Soil clay content; *SOC* Soil organic carbon; *TN* Soil total nitrogen; *TP* Soil total phosphorus; *AN* Soil available nitrogen; *AP* Soil available phosphorus

[***]*P* < 0.001; [**]*P* < 0.01; [*]*P* < 0.05

Fig. 4 Principal component analysis of stand characteristics and soil physicochemical properties in monoculture and mixed stands. SD, stand density; DBH, diameter at breast height; AH, average tree height; CA, crown area; SLA, specific leaf area; LDMC, leaf dry matter content; LD, leaf tissue density; pH, soil pH; BD, soil bulk density; SA, soil sand content; SI, soil silt content; CL, soil clay content; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, soil available nitrogen; AP, soil available phosphorus
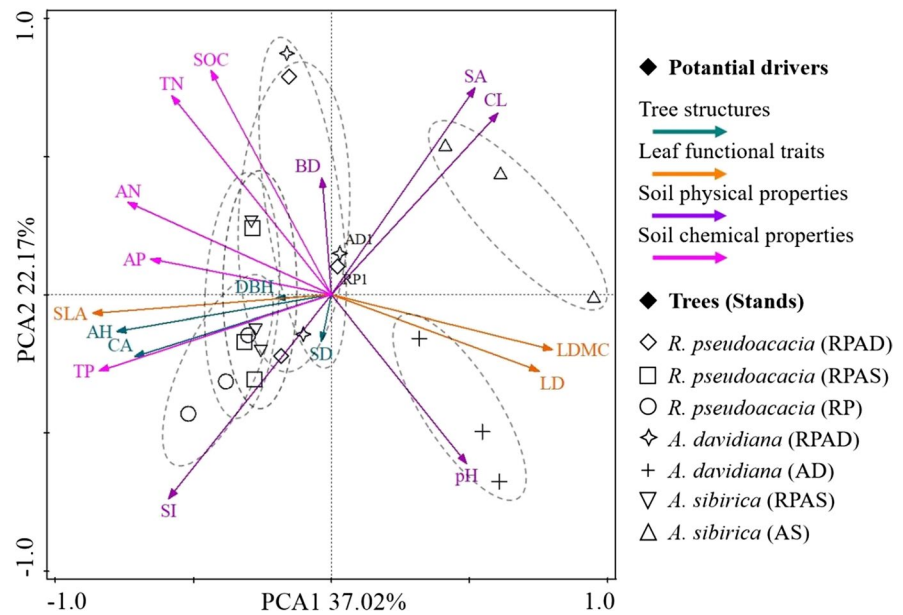
total variance and load mainly from SD (0.669) and DBH (0.703) (Table S2).

Additionally, PCA analysis results also showed that the dispersion within the same species depending on the community was lower for *R. pseudoacacia* than in the other two native non-N-fixing species (Fig. 4).

Further, a forward regression analysis was performed to determine the potential drivers of WUE, NUE, PUE, and N:P ratio in monoculture and mixed stands. WUE was mainly determined by LD; NUE was mainly determined by DBH, AH, SLA, soil pH, and AP; PUE was mainly determined by DBH, SLA, and soil pH; N:P ratio was mainly determined by SD, DBH, AH, and CA (Table 4).

Relationship between water and nutrient use efficiency

For individual trees or all species, there were negative correlations between WUE and NUE (Fig. 5a), and between WUE and PUE (Fig. 5b), while positive correlations between WUE and N:P ratio (Fig. 5c). Among them, WUE, was negatively correlated with NUE for *R. pseudoacacia* and *A. davidiana* ($P < 0.05$) (Fig. 5a), and with PUE for *R. pseudoacacia* ($P < 0.05$) (Fig. 5b); WUE and N:P ratio were positively correlated for *R. pseudoacacia* ($P < 0.05$) (Fig. 5c).

Table 4 Summary of stepwise regression models to detect the relationship between water (WUE), nitrogen (NUE), phosphorus (PUE) use efficiencies, and N:P ratios of three tree species and their potential drivers in monoculture and mixed stands

Factors	Models	R^2	Sig
WUE	y = 88.626 − 56.268LD	0.287	0.012[*]
NUE	y = −13.652 + 1.997DBH − 2.073AH − 0.035SLA + 4.849pH − 1.503AP	0.984	0.000[***]
PUE	y = −1042.363 + 14.253DBH − 0.804SLA + 175.273pH	0.755	0.000[***]
N:P ratio	y = 40.209 − 0.012SD − 0.976DBH + 3.972AH − 4.411CA	0.834	0.000[***]

SD Stand density; *DBH* Diameter at breast height; *AH* Average tree height; *CA* Crown area; *SLA* Specific leaf area; *LD* Leaf tissue density; *pH* Soil pH; *AP* Soil available phosphorus

[***]$P < 0.001$; [*]$P < 0.05$

Fig. 5 Regression analyses of water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies, and N:P ratios on specific and all tree species in monoculture and mixed stands; subfigures show the relationship between WUE, and NUE (**a**), PUE (**b**), N:P ratio (**c**), respectively. The R^2 and P values are shown. [*]$P < 0.05$
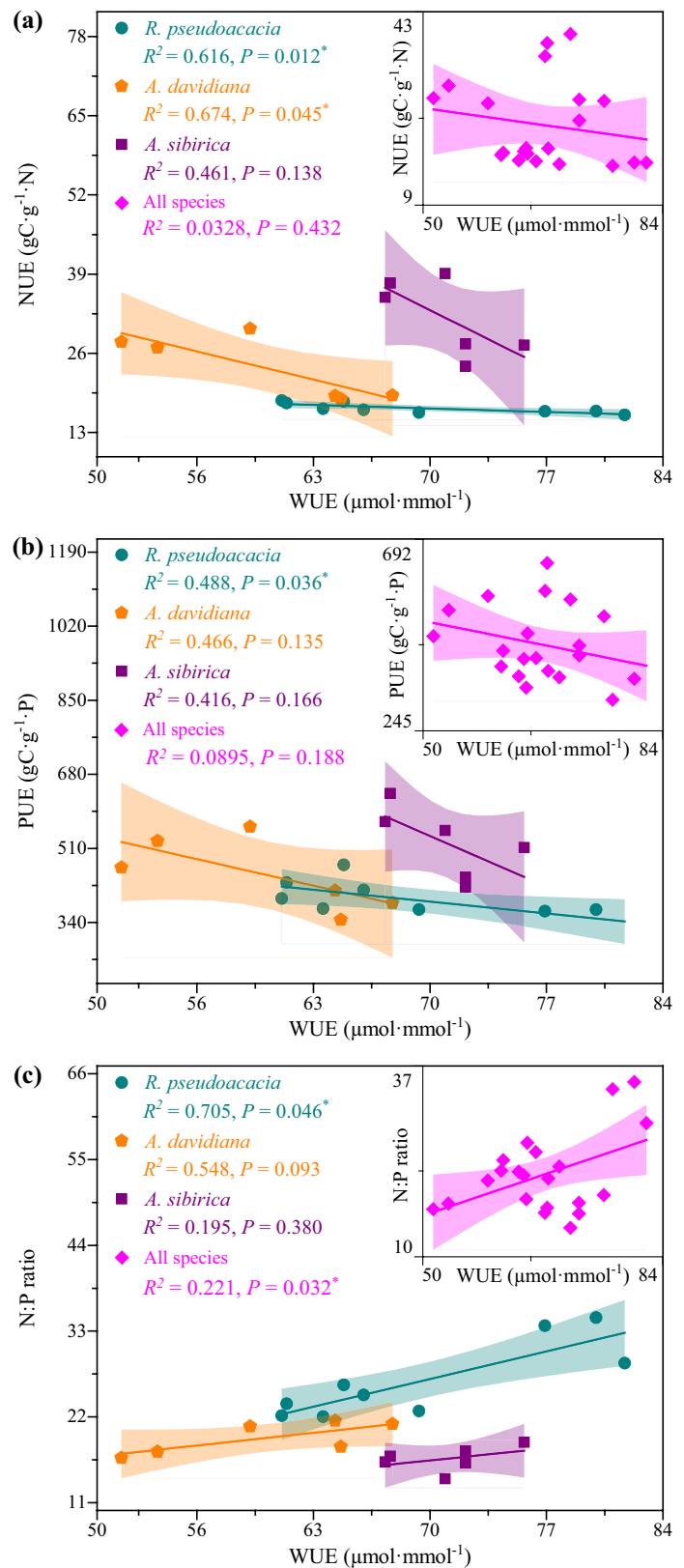
Discussion

Potential drivers of water and nutrient use efficiency

Generally, tree structure directly affects the morphological and physiological characteristics of the constituent species (Dawud et al. 2016). Compared to the monoculture stand, mixed stands can alter plants' water and nutrient use efficiencies (Fig. 1) by improving physiological functions and mitigating competition through niche segregation (Danescu et al. 2016; Coll et al. 2018). For instance, trees in mixed stands had lower DBH than those in monoculture stands (Table 1), indicating a reduction in productivity to adapt to the complex environment. Moreover, *A. davidiana* and *A. sibirica* exhibited higher AH and SLA in mixed stands compared to monoculture stands, while *R. pseudoacacia* was the opposite (Table 1). Thus, *A. davidiana* and *A. sibirica* in mixed stands tend to be more competitive, potentially decreasing the growth of *R. pseudoacacia*. However, the dispersion of *R. pseudoacacia* in terms of species composition, between monoculture and mixed-species communities, was relatively lower than that of *A. davidiana* and *A. sibirica* (Fig. 4). This suggests that *R. pseudoacacia* exhibits partial independence from biotic and abiotic factors and a competitive advantage over coexisting species.

In plantations, nutrients in plants and soil are mainly from rock weathering, litter decomposition, and N-fixing bacteria. Consistent with previous studies, soil TN, and AN concentrations were higher in mixed stands than in monoculture stands of *R. pseudoacacia* and non-N-fixing tree species (Table 2) (Manzoni et al. 2010; Coll et al. 2018). The potential explanations are as follows: 1) the microenvironment created by coexisting species in mixed stands accelerating litter decomposition and facilitating the rapid release of nutrients into the soil; 2) mixed stands containing *R. pseudoacacia* could enhance rhizobium N fixation capacity, resulting in higher soil AN concentrations (Forrester et al. 2006; Cao and Chen 2017); 3) coexisting species with varying root depths secrete extracellular enzymes that promote rock weathering (Dimitrakopoulos and Schmid 2004; Stubbs and Wilson 2004).

Water use efficiency and its correlation with potential drivers

Effect of mixed stand on water use efficiency

Plants growing in comparable environments tend to adopt similar resource utilization strategies, regardless of their structural features, species composition, and functional traits. For example, water supply plays a crucial role in plants' adaptation to drought stress, making high WUE an important trait for plant growth in semi-arid regions (Gong et al. 2011). Additionally, increased N supply did not affect the diurnal pattern of photosynthetic parameters in response to soil water content, but enhanced plant WUE by reducing transpiration rates. For these reasons, *R. pseudoacacia* exhibited a high WUE in response to water stress and increased soil TN (Fig. 1a, Table 2). However, *A. sibirica* demonstrated a higher WUE than *R. pseudoacacia* and *A. davidiana*, in monoculture stands, indicating that tree species affect plant-level WUE (Fig. 1a) (Wang et al. 2020). Thus, *A. sibirica* was more negatively affected by drought stress, showing higher sensitivity to stomatal conductance under water-deficit conditions (Du et al. 2011; Wei et al. 2018). Edwards et al. (2012) and Meier et al. (2022) reported that plant genotypes with high WUE are less susceptible to drought stress than those with low WUE. Consequently, *A. davidiana* exhibited the lowest WUE in monoculture stands (Fig. 1a), indicating a profligate water use strategy in response to drought stress (Garrish et al. 2010; Su and Shangguan 2020).

However, the WUE of *R. pseudoacacia* and *A. davidiana* in the RPAD were higher than in RP and AD (Figs. 1a and 2), which may be attributed to various factors (Wang et al. 2020). First, trees tend to develop superior morphological structures and physiological functions to adapt to drought stress. For instance, *A. davidiana* growing below the main canopy of *R. pseudoacacia* experiences less coupling with the atmosphere, resulting in a higher ratio of boundary layer conductance to canopy stomatal conductance (Wullschleger et al. 2000; Forrester et al. 2006). This leads to less CO_2 (lower $\delta^{13}C$) assimilated within the canopy and closure of leaf stomata, thereby reducing mesophyll conductance (g_m) while increasing *A. davidiana* WUE (Fig. 1a). Additionally, coexisting species with lateral and deep roots adopt different strategies for water uptake, potentially

leading to high WUE (Hooper and Dukes 2004). Similar results were found in mixtures of *Eucalyptus globulus* Labill. and *Acacia mearnsii* De Wilde compared to the respective monocultures (Forrester et al. 2010). Second, increased soil TN from N-fixing species can alleviate N restriction for coexisting species, stimulate N accumulation in leaves, and improve photosynthesis, thereby enhancing WUE (Batterman et al. 2018). Thus, increased WUE of *A. davidiana* when mixed with *R. pseudoacacia* may be related to enhanced leaf photosynthesis caused by increased soil TN concentration (Jennings et al. 2016; Battipaglia et al. 2017).

What is more, mixed planting decreased the WUE of *R. pseudoacacia*, while the WUE of *A. sibirica* increased, compared to their respective monocultures (Figs. 1a and 2). This suggests that coexisting species exhibit different sensitivities to changing soil water reserves. Jian et al. (2015) reported that *A. sibirica* has a wider lateral root distribution than *R. pseudoacacia*. In the mixed stands, *A. sibirica* obtained a greater percentage of water from the topsoil (60.96%) than *R. pseudoacacia* (43.72%) during the growing season (Wang et al. 2020). Hence, there exists species-specific niche segregation in ecophysiology between *R. pseudoacacia* and *A. sibirica* within the RPAD community (Moreno-Gutiérrez et al. 2012). Moreover, topsoil did not meet the water requirements of *A. sibirica*, while that of deeper soil layers partially alleviated drought stress for *R. pseudoacacia*.

Influence of potential drivers on water use efficiency

In arid and semi-arid areas, when the air temperature rises or soil water content decreases, increased plant WUE can be directly attributed to reduced stomatal conductance (Jia et al. 2022). For example, in response to the gradual depletion of soil water reserves (Ouyang et al. 2022), *R. pseudoacacia* enhances WUE through tight stomatal control and limited transpiration rates (Fu et al. 2020). Tanaka-Oda et al. (2010) reported that *R. pseudoacacia* plantations with high SD tend to consume more soil water stores than the same stand with low SD. Moreover, the larger the SD, the smaller the CA of individual tree species. As a result, *R. pseudoacacia* WUE was positively correlated with SD, but negatively correlated with CA (Fig. 3a) (Brookshire et al. 2020). In contrast, small-sized trees growing in mixed stands

with high SD and large canopy closures may receive less photosynthetically active radiation (PAR), resulting in lower air temperatures within the stand than in open ground (Forrester et al. 2006; Khanna et al. 2008; Nygren and Leblanc 2015). Meanwhile, high CA induced the leaves to close the stoma and reduce transpiration rates, thus increasing WUE. Hence, *A. davidiana* and *A. sibirica* WUE were negatively correlated with SD and positively correlated with CA (Fig. 3b-c), indicating the negative association of low temperature with WUE of understory tree species. Therefore, soil water content and air temperature jointly regulate leaf stomatal conductance and further regulate the WUE of understory coexisting species.

When soil nutrient availability increases, plants tend to accumulate nutrients in their leaves, while reducing nutrient utilization efficiency, resulting in an elevated WUE (Ripullone et al. 2004), partly consistent with our results (Table 2, Fig. 1a). However, *R. pseudoacacia* in RPAS shows an opposite trend than expected compared to the RP, as higher soil TN, TP, and AN concentrations imply lower rather than higher WUE (Table 2, Figs. 1a and 2). One possible explanation is that soil particle composition greatly impacts the N-fixing capacity of rhizobia (Table 2), leading to increased N supply for *R. pseudoacacia*, causing fine roots to uptake more water to absorb N, and synchronize absorb large amounts of soil P. To reduce leaf transpiration and adapt to low soil water supplies, *R. pseudoacacia* tends to maintain WUE by strictly controlling stomatal closure. As a result, *R. pseudoacacia* WUE was negatively correlated with soil TP concentration (Fig. 3a). Thus, *R. pseudoacacia* WUE was not dependent on soil TN concentration, while closely correlated with the N-fixing capacity of rhizobia. Similarly, WUE of *A. davidiana* and *A. sibirica* showed no significant correlations with soil TN concentrations (Fig. 3b-c), consistent with previous studies, such as *Eucalyptus saligna* Sm. (Hubbard et al. 2004), *Picea abies* (L.) H. Karst. and *Eucalyptus grandis* W. Hill ex Maiden stands (Phillips et al. 2001; du Toit and Dovey 2005). In brief, the impact of altered soil nutrients and their interactions with other factors on coexisting species (i.e. *A. davidiana*, and *A. sibirica*) WUE vary by tree species and site-specific.

Nutrient use efficiency and its correlation with potential drivers

The NUE of different tree species was affected by various factors. For instance, *R. pseudoacacia* NUE was the lowest in RP compared to AD and AS (Fig. 1b), while soil AN concentration in RP was the highest (Table 2), suggesting that *R. pseudoacacia* NUE was closely correlated with soil AN (Dijkstra et al. 2016). However, in addition to *R. pseudoacacia*, the NUE of *A. davidiana* and *A. sibirica* were less associated with soil AN (Fig. 3a-b). The reason may be that litter decomposition of both tree species releases N primarily into the topsoil (Voigtlaender et al. 2012); *R. pseudoacacia* growth also absorbed N fixed by rhizobia, resulting in *R. pseudoacacia* NUE being partially correlated with soil AN (Fig. 3a) (Turner and Lambert 2014; Nygren and Leblanc 2015). Relatively, NUE of all species was negatively correlated with soil AN (Fig. 3c-d), indicating that *R. pseudoacacia* in mixed stands facilitates N cycling in the plant-soil system (Forrester et al. 2006; Su and Shangguan 2020). Additionally, NUE of *A. sibirica* and all tree species were negatively correlated with soil TP (Fig. 3c-d); soil TP concentrations were higher in RP than in AD and AS, and they were higher in RPAS than in RP and AS (Table 2). This suggests that leaf functional traits of *A. sibirica* may promote P cycling, particularly in RPAS. Thus, lower *A.sibirica* NUE in RPAS than in AS (Figs. 1b and 2) is likely associated with plants high water demand (Wang et al. 2020) promoting root uptake of water and nutrients (Du et al. 2011; Sardans and Peñuelas 2012). Moreover, plantation stands with high density may exhibit rich roots, which benefits rhizobia in fixing N; increased soil AN concentration leads to higher levels of leaf TN concentration. Hence, *R. pseudoacacia* NUE was negatively correlated with SD (Fig. 3a). In contrast, NUE of *A. davidiana* and *A.sibirica* were positively correlated with SD (Fig. 3b-c), possibly due to high-density stands consuming huge soil water, and thus reducing access of non-N-fixing species to soil N (Tanaka-Oda et al. 2010; Dijkstra et al. 2016).

Factors affecting the PUE of different tree species are more complex. For example, the PUE of *R. pseudoacacia* was negatively correlated with SD (Fig. 3a), while that of *A. davidiana* and *A. sibirica* were the opposite (Fig. 3b-c). This is because high-density stands exhibit abundant root systems, which

benefits rhizobia in fixing N, and promotes plants that are limited by soil P to allocate more resources to synthesize phosphatase (Treseder and Vitousek 2001). Meanwhile, high-density stands consume huge soil water, thus reducing access of non-N-fixing species root to soil P. Moreover, the PUE of *A. davidiana* and *A. sibirica* was negatively correlated with CA (Fig. 3b), suggesting that large CA may limit P transport to leaves (Johnson 2010). Additionally, low light leads to a large SLA (Xu et al. 2022). Thus, *A. davidiana* PUE was positively correlated with PAR; lower *A. davidiana* PUE in RPAD compared to AS (Figs. 1c and 2) may be attributed to the canopy shade of *R. pseudoacacia*. In comparison, *A. sibirica* PUE in RPAS was less affected by the canopy shade of *R. pseudoacacia*, but increased chlorophyll to improve the rate of leaf photosynthesis. Interestingly, mixed planting of *A. sibirica* and *R. pseudoacacia* enhanced soil P mineralization and leaching processes (Table 2). Consequently, *A. sibirica* PUE was negatively correlated with soil TP (Fig. 3c) and lower in RPAS than in AS (Figs. 1c and 2) (Vitousek et al. 2010; Turner and Lambert 2014). This was consistent with previous studies that mixed species with comparable growth cycles or leaf phenology typically exhibit intense competition for resources (Feller et al. 1999; de-Dios-García et al. 2018). Hence, *A. sibirica* in the RPAS compared to AS may show greater competition for soil AP.

The N:P ratio of all tree species was > 20 (20.67, Fig. 1d) (Güsewell 2004), suggesting that the growth of all species was limited by soil P (Cao and Chen 2017). Moreover, tree species with high SD, AH, and SLA tend to be more susceptible to soil P constraints, due to the positive correlations between the N:P ratio and SD, AH, and SLA (Fig. 3d). Specifically, large tree structures indicate rich root systems, which benefit N fixation by rhizobia but lead to increased soil P restriction (Treseder and Vitousek 2001). Another study found that high-density stands with sufficient soil N sources tend to allocate more P to synthesize biomass in plant crowns, roots, and mycorrhizas, thereby reducing P transport to leaves (Johnson 2010). Additionally, SLA was larger for *R. pseudoacacia* growing in mixed stands than in monoculture stands, and *A. davidiana* and *A. sibirica* were the opposite (Table 1). Based on a previous study, low PAR leads to large SLA and leaf photosynthetic intensity was positively correlated with LDMC (Xu

et al. 2022). Therefore, *R. pseudoacacia* growth was less restricted by soil P, in mixed stands compared to monoculture stands, while that of which was more for *A. davidiana* and *A. sibirica*. Predictably, mixed species, especially these large sizes, need to assimilate amounts of soil P to support physiological and biochemical reactions.

Trade-off between water, and nutrient use efficiency

Water and nutrients are vital resources for plant growth, and their efficient utilization significantly influences plant functional traits (Huang et al. 2015). Inefficient WUE may indirectly lead to higher transpiration rates, favoring the uptake of more soluble P and overall N. Consequently, there may be an inverse relationship between WUE, and NUE, PUE (Patterson et al. 1997; Dijkstra et al. 2016). Thus, WUE was negatively correlated with NUE, for individual trees or all species (Fig. 5a) (Gong et al. 2011; Dijkstra et al. 2016; Su and Shangguan 2020). Additionally, *R. pseudoacacia* exhibited a greater AH and CA than *A. sibirica* and *A. davidiana* (Table 1), indicating a higher water demand and WUE for *R. pseudoacacia* than *A. sibirica* and *A. davidiana*. Furthermore, achieving high WUE is only possible if the photosynthetic machinery and energy transfer capacity are robust, which necessitates higher N concentrations or a preference for low NUE (Dijkstra et al. 2016). Accordingly, the decreasing order of the adaptability of these three trees (R^2) to water stress is as follows: *A. davidiana* (0.674), *R. pseudoacacia* (0.616), and *A. sibirica* (0.461) (Fig. 5a). Furthermore, understory tree species are often influenced by lower air temperatures and reduced PAR, resulting in lower transpiration rates but increased WUE (Garrish et al. 2010). Thus, a trade-off between WUE and NUE is expected to be more pronounced for *A. davidiana* than *A. sibirica,* in mixed stands compared to monoculture stands.

Few studies have confirmed a stable trade-off between WUE and PUE, as overall P uptake depends not only on soil water migration and transpiration, but also on root structure, distribution, and its exudates (e.g., extracellular enzyme) (Brown et al. 2011; Huang et al. 2015). For N-fixing species, the process of water uptake and nutrient absorption is less connected, because rhizobia can independently fix N and mobilize more soil P to synthesize phosphatase, thereby enhancing protein production capacity (Cregger et al. 2014). Therefore, *R. pseudoacacia* WUE showed a significant negative correlation with PUE ($P < 0.05$) (Fig. 5b), probably due to the effect of microbial regulation on P supply leading to a decrease in PUE and an increase in WUE (Ripullone et al. 2004; Cleveland and Liptzin 2007). Additionally, plants may not have access to larger amounts of soil AP, especially under water stress conditions where the mobility of soil P is strongly reduced (Lambers et al. 2008; Dijkstra et al. 2016). Thus, the trade-off between WUE and PUE was lower for *A. davidiana* and *A. sibirica* than for *R. pseudoacacia* (Fig. 5b).

There was a positive correlation between WUE and N:P ratios for individual trees or all species (Fig. 5c), consistent with various shrubs and trees on the CLP (Yan et al. 2016). A closer examination reveals that WUE increases with higher N concentrations in plant biomass, all other variables being equal (Cernusak et al. 2007). Additionally, increased soil AP or alleviation of P deficiency tends to improve WUE (Raven et al. 2004). This correlation is likely widespread because it links the N:P ratio of plants to transpiration, thereby integrating nutrient and hydrological cycles (Dijkstra et al. 2016; Su and Shangguan 2020). Furthermore, the correlation between WUE and leaves TN and TP concentrations may differ depending on restricted soil nutrients. For instance, under N-limited conditions, WUE showed a positive correlation with the N:P ratio and a negative correlation with P concentration, suggesting that WUE increased in response to higher leaf TN concentrations (Cernusak et al. 2007). However, when soil P is limited, leaf TN concentrations may increase significantly compared to soil N shortages, and excessive N leads to reduced transpiration rate and then decreases soil water availability and P uptake through reduced soil solution mass flow (Wilkinson et al. 2007; Cernusak et al. 2010). As such, the correlation between WUE and N:P ratio is particularly pronounced for *R. pseudoacacia* compared to other tree species (Fig. 5c). This implies that *R. pseudoacacia* exhibits elevated leaf TN concentrations when faced with limited soil P. Finally, it is worth noting that in this water-limited region, mixing different tree species can alleviate nutrient limitations, resulting in improved WUE but potentially reduced NUE and PUE. These can be

advantageous for plant growth in environments where water availability is severely limited.

Conclusions

Compared to the respective monocultures, mixing *R. pseudoacacia* and *A. sibirica* was a good strategy to maximize soil nutrient resources.

R. pseudoacacia and *A. davidiana* showed higher WUE in RPAD than in RP and AD, which were attributed to leaf photosynthesis influenced by canopy shade and lateral and deep roots. Mixed planting decreased *R. pseudoacacia* WUE while *A. sibirica* WUE increased in RPAS, compared to monoculture stands, possibly due to water niche segregation caused by different root distributions. *R. pseudoacacia* WUE was closely correlated with rhizobia N fixation capacity, while soil nutrient effects and interactions with other factors on WUE of *A. davidiana* and *A. sibirica* are species and site-specific.

A trade-off between WUE and NUE is expected to be more pronounced for *A. davidiana* than *A. sibirica*, in mixed stands compared to monoculture stands, as smaller trees adapt to low temperature and less PAR with reduced transpiration rates but increased WUE. A trade-off between WUE and PUE was weak in *A. davidian*a and *A. sibirica*, possibly because soil P mobility was reduced under water-scared conditions. The correlation between WUE and N:P ratio suggests that *R. pseudoacacia* exhibits elevated leaf TN concentration when faced with limited soil P.

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