

# Morphological and physiological responses and plasticity in *Robinia pseudoacacia* to the coupling of water, nitrogen and phosphorus

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## Abstract

**Background:** Under the background of drought and nitrogen deposition, global climate change is changing the supply of resources and environmental conditions that are crucial to plant growth, and plants respond to climate change by environmentally induced phenotypic changes.

**Aims:** The objective of this study was to assess the differences in the plasticity responses of the morphological, photosynthetic, fluorescence and antioxidant parameters of *Robinia pseudoacacia* seedlings under water, nitrogen and phosphorus interaction conditions.

**Methods:** We analyzed the above parameters of *R. pseudoacacia* seedlings using methods of morphological measurements, gas exchanges, fluorescence emission and antioxidant assays under two water levels (75% and 55% of field capacity), four nitrogen levels (no urea; 0.2 g kg<sup>-1</sup>, 0.4 g kg<sup>-1</sup>, 0.8 g kg<sup>-1</sup>), and two phosphorus levels (no superphosphate, 0.6 g kg<sup>-1</sup>).

**Results:** The results showed that drought stress significantly reduced the morphological parameters, actual photochemical efficiency of photosystem II ( $\Phi$ PSII), superoxide dismutase (SOD) activity, peroxidase (POD) activity and catalase (CAT) activity of *R. pseudoacacia* seedlings, and significantly increased nonphotochemical quenching (NPQ) and the malondialdehyde (MDA) content. Excessive application of nitrogen (N) fertilizer inhibited plant growth, and phosphorus (P) deficiency significantly weakened the positive effect of the high nitrogen treatment on morphological parameters under drought stress. The high nitrogen treatment effectively weakened the inhibitory effect of drought and phosphorus deficiency on chlorophyll formation. Proper N and P fertilizers increased the SOD, POD and CAT activities and decreased the MDA content under drought stress. The mean plasticity index of POD and CAT activities in response to water, nitrogen and phosphorus was the highest, which indicated that *R. pseudoacacia* seedlings can adapt to changes in water and nutrients mainly by adjusting their POD and CAT activities.

**Conclusions:** These results may explain the better survival of *R. pseudoacacia* under the condition of limited water and nutrient resources, which is helpful for understanding the response mechanism of *R. pseudoacacia* to changes in environmental factors in the future. The results also provide scientific data and a theoretical basis for vegetation restoration and sustainable development of the regional ecological environment.

**Key words:** climate change / drought stress / phenotypic plasticity / *Robinia pseudoacacia* / water and fertilizer coupling

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## 1 Introduction

In recent years, the impact of climate change on terrestrial ecosystems has become a hot issue in ecology, botany, and geography. Climate change is characterized by global warming and an increase in greenhouse gases in the atmosphere. Global warming will lead to changes in the global hydrological cycle and the redistribution of water resources in space and time (Stocker et al., 2013). The frequency of short-term drought will increase, and long-term drought will become increasingly common (Sheffield and Wood, 2008). Global climate change may cause increasingly arid and semi-arid areas in the world to be seriously affected by drought stress. Over the past century, global atmospheric nitrogen deposition

has increased as a result of fossil fuel combustion and the use of artificial nitrogen-containing fertilizers (Janssens et al., 2010). As the main part of the terrestrial ecosystem, forests provide various ecosystem services and play a crucial role in regulating global climate change. Therefore, under the background of increasing nitrogen deposition, it is urgent to study the physiological and ecological response of forests to nitrogen addition. Some studies have shown that slight nitrogen deposition can promote the growth of vegetation, but nitrogen saturation will occur when nitrogen deposition exceeds the available limit of the ecosystem, resulting in negative effects such as soil acidification, forest productivity reduction and bio-

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Supporting Information  
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diversity reduction (Aber and Magill, 2004; Stevens et al., 2004; Dise et al., 2009), which will then affect the structure and function of the ecosystem and the process of vegetation restoration. In addition, studies have shown that almost all ecosystems have phosphorus limitation due to nitrogen addition. The continuous increase in nitrogen deposition results in the acceleration of the phosphorus cycle in forest ecosystems, which further leads to low phosphorus stress as an important factor limiting plant growth (Elser et al., 2007; Vitousek et al., 2010).

The availability of nitrogen and phosphorus is closely related to the soil water status. The effects of water and nutrients on plant growth are interactive and have obvious coupling effects. Water and fertilizer coupling is a comprehensive effect of the interaction between water and nitrogen and phosphorus fertilizers on plant growth, physiological, biochemical and ecological characteristics and utilization efficiency (Guttieri et al., 2005). A large number of studies have shown that for plant growth, sufficient soil moisture can improve the movement ability of nitrogen and phosphorus elements in the soil and can affect the absorption, transportation and redistribution of nitrogen and phosphorus by plants. Conversely, with a decrease in soil moisture, the absorption of nitrogen and phosphorus by plants will decrease (Waraich et al., 2011; Sardans and Peñuelas, 2012; He and Dijkstra, 2014). However, a suitable supply of nitrogen and phosphorus can facilitate the absorption and utilization of soil water by plants to improve the drought tolerance of plants; in other words, fertilization can compensate for the lack of water in plants under drought stress (Graciano et al., 2005; Wu et al., 2018). However, because fertilization can significantly promote the growth of plants, drought stress may unexpectedly aggravate the water stress of plants. Therefore, increasing water and fertilization levels blindly not only wastes resources but may also inhibit plant growth (Faustino et al., 2013). Consequently, it is of great significance to study the physiology and ecology of the soil drought environment and nitrogen and phosphorus fertilizer application of pioneer tree species in vegetation restoration by carrying out simulation experiments, which is helpful for understanding the interaction between the climate, vegetation and soil and providing a scientific basis for the prediction of vegetation restoration under climatic environment change in the future.

Global climate change is changing the supply of resources and the environmental conditions that are critical to plant growth, and plants respond to climate change by environmentally induced phenotypic changes. Phenotypic plasticity is the response of a specific genotype to different environmental conditions, resulting in different phenotypic characteristics (Sultan, 2000), *i.e.*, the ability of organisms to adapt to environmental changes by changing their morphology, physiology, and behavior. Plants adapt to heterogeneous habitats and use phenotypic plasticity to reflect changes in the surrounding environment. Generally, species with strong plasticity have a wider ecological range and better tolerance, and can therefore occupy a broader geographical scope and a more diverse habitat (Sultan, 1995; Geng et al., 2004). Therefore, in the context of climate change, it is of great significance to

study the response of plant plasticity to understand the effect of climate change on forest ecosystems.

*R. pseudoacacia*, one of the most widely planted woody species in the world (Vitková et al., 2017), was selected for our study. *R. pseudoacacia* has become one of the main pioneer species in soil and water conservation and vegetation restoration because of its strong adaptability, drought tolerance, ability to tolerate bare land and fast growth, and plays an important role in vegetation restoration and ecological construction in ecologically fragile areas (Yan et al., 2017). The capacity of this species to face light stress by heliotropic movement is also an essential trait in enhancing the success of the species in natural ecosystems (Arena et al., 2008). It can also be used for erosion control and plays an important role in restoring degraded soil and improving soil properties and carbon sequestration (Nicolescu et al., 2018). The root system of *R. pseudoacacia* is well developed, and the root has nodules, which function in nitrogen fixation. However, dry and barren soil conditions are often not conducive to the survival and early growth of afforested seedlings.

*R. pseudoacacia* plays an important role in improving ecosystem services and its resistance to climate change deserves attention. Therefore, it is necessary to understand the response of *R. pseudoacacia* to the application of nitrogen and phosphorus fertilizers under drought stress to develop reasonable management and protection strategies for future climate change. The objectives of this study were the following: (1) to explore the changes in morphological parameters, photosynthetic parameters, fluorescence parameters, and antioxidant measurements of *R. pseudoacacia* seedlings under drought and N and P application and (2) to compare the differences in the plasticity responses of the above parameters of *R. pseudoacacia* seedlings under drought and N and P application.

## 2 Material and methods

### 2.1 Plant growth conditions

A pot experiment was performed in a removable rainproof greenhouse of the Institute of Soil and Water Conservation, Chinese Academy of Sciences. The study site is located on the Loess Plateau in Yangling, Shaanxi (34°17'56" N, 108°04'07" E) and is characterized by a temperate semi-humid climate, which has a mean annual temperature of 13°C and a mean annual precipitation of 632 mm, with approximately 70% of rainfall occurring from June to September.

### 2.2 Plant growth experiment

In late March 2019, one-year-old *R. pseudoacacia* seedlings were planted in cylindrical plastic pots with a diameter of 30 cm and a height of 30 cm. The bottom of the pot was lined with stones, and a PVC water pipe with an inner diameter of 2 cm was placed on the wall of the pot to minimize water loss by evaporation and to avoid surface soil hardening caused by surface irrigation. Each pot was filled with 15 kg dry soil or a mixture of soil and fertilizer; the soil was collected from the

local 0–20 cm soil layer. One *R. pseudoacacia* seedling was planted in each pot, and the three factors of water, nitrogen and phosphorus were completely randomized. Urea with 46% nitrogen was used as nitrogen fertilizer and was applied at four levels (N0, no urea; N1, 0.2 g kg<sup>-1</sup>; N2, 0.4 g kg<sup>-1</sup>; N3, 0.8 g kg<sup>-1</sup>), and N1, N2, and N3 levels were defined as low N (LN), medium N (MN), and high N (HN). Superphosphate with 16% phosphorus pentoxide was used as phosphorus fertilizer and was applied at two levels (P0, no superphosphate; P1, 0.6 g kg<sup>-1</sup>). Both nitrogen and phosphorus fertilizers were applied as base fertilizers at one time. In addition, two levels of a water treatment (75% of field capacity, NW, simulating normal water conditions and 55% of field capacity, DT, simulating drought conditions) were included in the experiment. A total of 16 treatments were tested and each treatment had six repetitions. Before the drought treatment was initiated, the soil moisture content was maintained at approximately the field water capacity to ensure the survival and early growth of the seedlings. Then, the soil moisture content was gradually adjusted to the designed level by weight at 18:00 every day.

## 2.3 Measurements

### 2.3.1 Morphological parameters

After three months of drought treatment, five individuals of *R. pseudoacacia* were selected to measure plant height (H) and stem diameter (D). The plant height (from the base of the plant to the top of the main stem) was measured with a centimeter ruler, and the stem diameter was measured with a Vernier caliper at the plant stem 20 cm above the soil surface. At the end of the experiment, the above- and belowground organs of the seedlings were collected separately and washed with deionized water. The roots, stems and leaves of the seedlings were heated at 105°C for 30 min and then dried at 75°C to a constant weight. The dry weights of the roots, stems and leaves of the seedlings were measured by an electronic balance to determine root biomass (RB), stem biomass (SB), leaf biomass (LB), and total biomass (TB).

### 2.3.2 Gas exchange and chlorophyll fluorescence parameters

The photosynthetic gas exchange parameters were measured using an LI-6400 open system (Lincoln, NE, USA) between 9:30 and 11:30 am on a sunny day. At least three fully expanded mature leaves from each of three replicate plants were selected to measure the net photosynthetic rate (P<sub>n</sub>, mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (g<sub>s</sub>, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and transpiration rate (Tr, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). Instantaneous water use efficiency (WUE, mol CO<sub>2</sub> m<sup>-2</sup> H<sub>2</sub>O) was calculated as P<sub>n</sub>/Tr. The environmental conditions in the leaf chamber during the measurement period were a saturating photosynthetic photon flux density of 1000 ± 5 μmol m<sup>-2</sup> s<sup>-1</sup>, air temperature of 32–37°C, CO<sub>2</sub> concentration of 380–400 μmol mol<sup>-1</sup>, and water vapor concentration of 20–26 mmol mol<sup>-1</sup>.

The chlorophyll fluorescence parameters were measured using an FMS 2.02 system (Hansatech, Norfolk, UK) from 9:30

to 11:30 am. Fv/Fm reflects the maximum light energy conversion efficiency of the PSII reaction center. ΦPSII refers to the proportion of light absorbed by PSII for the photochemical reaction. The higher the value, the stronger the electron transfer ability of the photosynthetic structure and the higher the proportion of absorbed light energy used for the photochemical reaction. NPQ reflects the proportion of light energy absorbed by the PSII antenna pigment that dissipates in the form of heat. The minimum fluorescence (F<sub>o</sub>) and the maximum fluorescence (F<sub>m</sub>) were measured after dark adaptation for 30 min, and the maximal fluorescence level (F<sub>m</sub>') and steady-state chlorophyll fluorescence (F<sub>s</sub>) were recorded under natural light adaptation. Then, the maximum quantum efficiency of photosystem II photochemistry (Fv/Fm), actual photochemical efficiency of photosystem II (ΦPSII), and non-photochemical quenching (NPQ) were calculated as follows (Baker and Rosenqvist, 2004):

$$Fv/Fm = (Fm - Fo)/Fm, \quad (1)$$

$$\Phi PSII = (Fm' - Fs)/Fm', \quad (2)$$

$$NPQ = (Fm - Fm')/Fm'. \quad (3)$$

The chlorophyll content of fully expanded leaves was measured in relative units (SPAD values) using a portable chlorophyll meter, SPAD-502 Plus (Konika-Minolta, Japan). The meter head was placed on the adaxial leaf surface to avoid a mid-vein. Five individual seedlings in each treatment group were selected, and five measurements were taken and averaged for each seedling.

### 2.3.3 Antioxidant measurements

Leaves were sampled from the upper shoot, frozen immediately in liquid nitrogen, and stored at -80°C until enzyme extraction. The enzymes were extracted from approximately 0.1 g of leaf samples with 1 mL of phosphoric acid buffer at 4°C. The extract was centrifuged at 8000 g for approximately 10 min. The supernatant was used to measure superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities and the malondialdehyde (MDA) content. SOD activity measurement was performed according to the WST-8 method (Ukeda et al., 1999). POD activity was measured according to the guaiacol method (Ekmekci and Terzioğlu, 2005). CAT activity was assayed by ammonium molybdate colorimetry (Goth, 1991). The MDA content was determined following the thiobarbituric acid method (Dhindsa and Matowe, 1981).

## 2.4 Phenotypic plasticity and statistical analysis

To compare the degree of plasticity among variables, the phenotypic plasticity index (P<sub>i</sub>) was used and calculated as (maximum mean values - minimum mean values) / maximum mean values for each variable, where the numerator is the difference among the mean values for the water, nitrogen and phosphorus treatments and the denominator is the mean value for the highest of the water, nitrogen and phosphorus treatments (Santiso and Retuerto, 2015). The phenotypic plasticity index ranges from zero to one; 0 indicates no plasti-

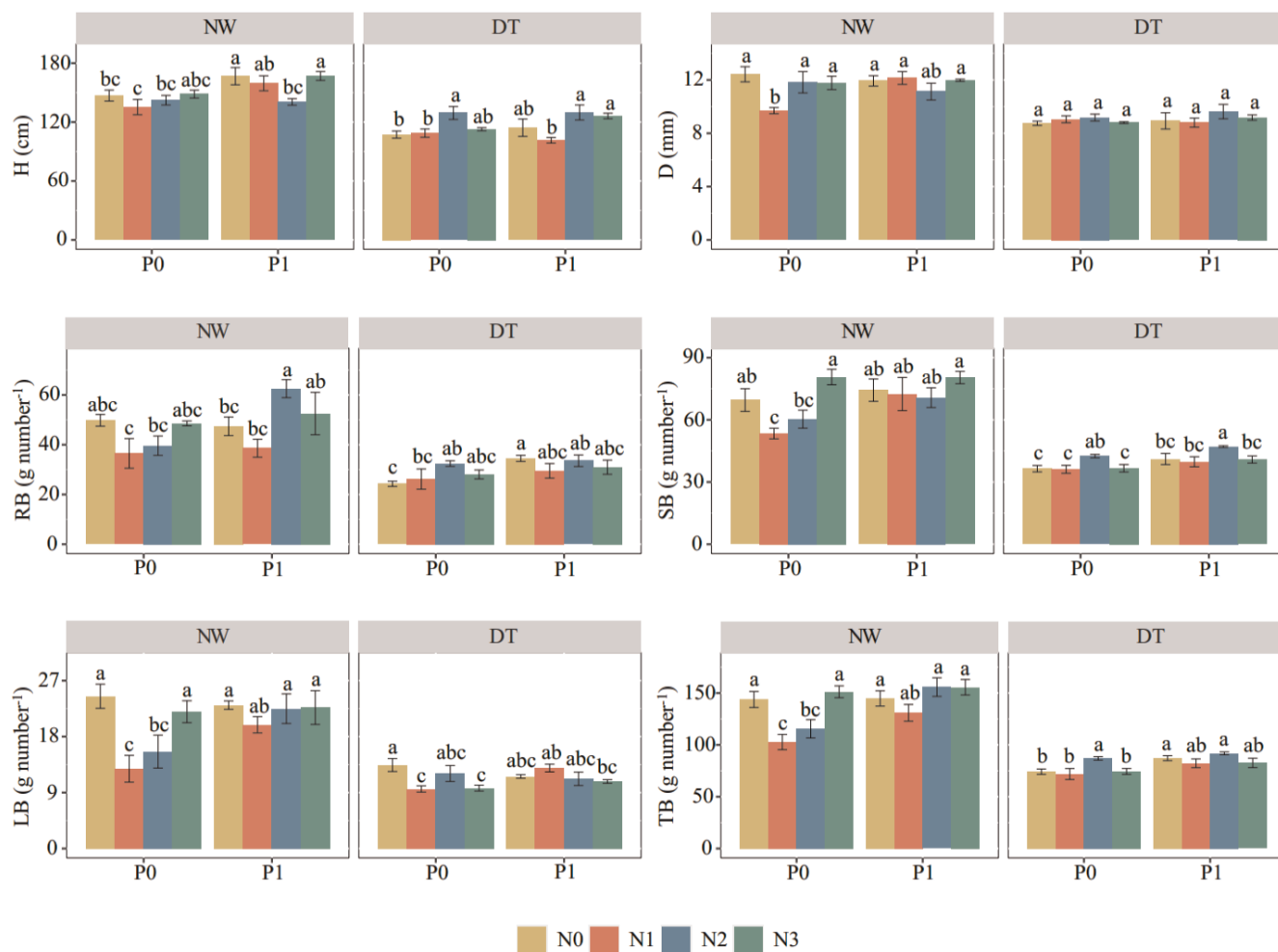
city and 1 indicates the highest plasticity. This index can be used to compare the plasticity of different dimensional characters. The mean phenotypic plasticity index ( $P_{lm}$ ) was calculated by averaging the index obtained for the morphological, photosynthetic, fluorescence and antioxidant parameters.  $P_{lm}$  (W),  $P_{lm}$  (N), and  $P_{lm}$  (P) corresponded to the mean phenotypic plasticity index to treatments of water, nitrogen and phosphorus, indicating the relative average morphological and physiological adaptability of *R. pseudoacacia* seedlings to water, nitrogen, and phosphorus.

All data are presented as the mean  $\pm$  standard error. Three-way ANOVAs were applied to evaluate the effects of water, N and P, and the interaction among the three factors. Least-significant differences (LSD) at the 0.05 level ( $p < 0.05$ ) were used to assess significant variations within the values among the treatments. Pearson's correlation analysis was used to test the relationships among morphological and physiological parameters. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc., USA), and figures were generated using the R software package (version 3.6.1).

### 3 Results

#### 3.1 Effects of water, nitrogen and phosphorus availability on *R. pseudoacacia* morphological traits

Drought treatment significantly reduced the plant height and stem diameter ( $p < 0.01$ ), and nitrogen and phosphorus treatment significantly affected only the plant height ( $p < 0.05$ ); the coupling effect of water and nitrogen and water and phosphorus significantly affected the plant height ( $p < 0.05$ ), while only the interaction effect of water, nitrogen and phosphorus significantly affected the stem diameter ( $p < 0.05$ ) (Tab. S1). Under NW conditions, compared with N0P0, the plant height increased by 13.44% and 13.61% under N0P1 and N3P1, respectively, but the stem diameter did not change significantly, except for N1P0. Under DT conditions, compared with N0P0, the plant height increased by 20.51%, 20.98%, and 17.72% under N2P0, N2P1, and N3P1 conditions, respectively, but the stem diameter did not change significantly (Fig. 1). The results showed that the MN and HN treatments could effectively



**Figure 1:** Effects of water, nitrogen, and phosphorus on plant height (H), stem diameter (D), root biomass (RB), stem biomass (SB), leaf biomass (LB), and total biomass (TB). NW and DT refer to soil water contents of 75 and 55% of field capacity, respectively. N0, N1, N2, and N3 refer to no-urea conditions and urea addition at 0.2, 0.4, and 0.8 g kg<sup>-1</sup>, respectively. P0 and P1 refer to no superphosphate and superphosphate addition at 0.6 g kg<sup>-1</sup>, respectively. The vertical bars in the figures represent the standard errors of the means. Bars with the same letter indicate no significant difference at the  $p < 0.05$  level.

slow the inhibitory effect of drought on plant height, while HN could not reduce the decrease in plant height and stem diameter caused by phosphorus deficiency under drought conditions.

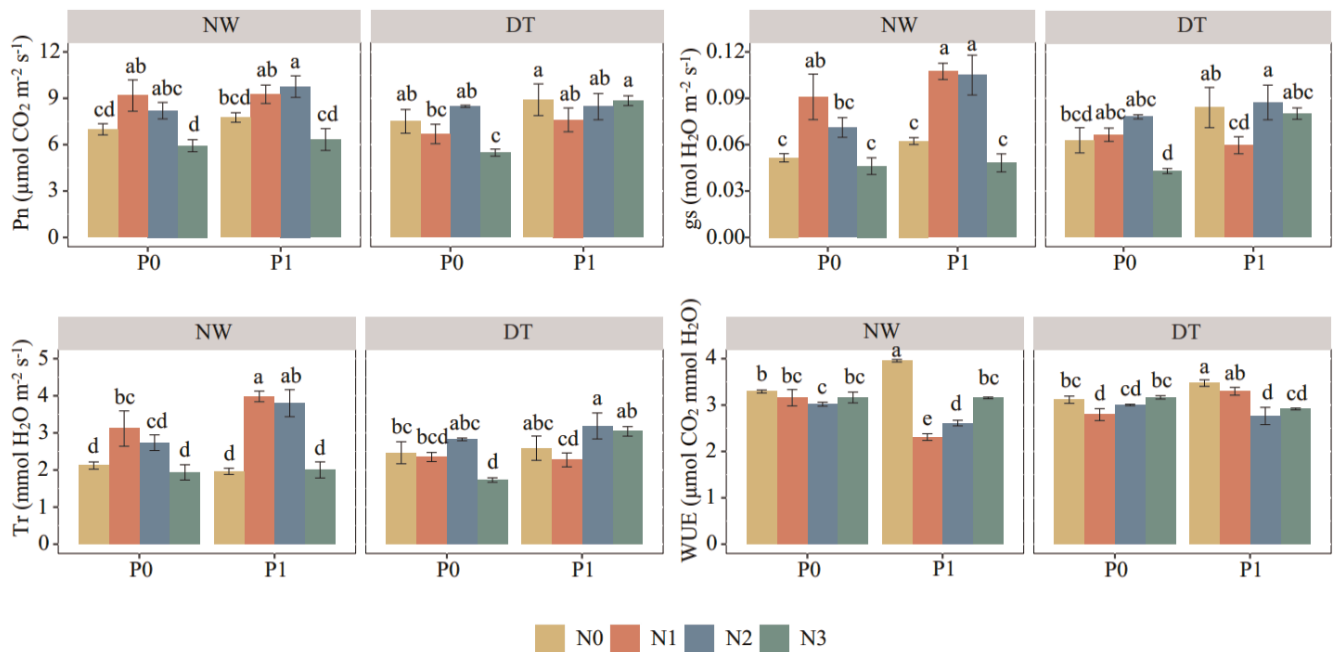
Drought treatment significantly reduced the RB, SB, LB, and TB ( $p < 0.01$ ), and nitrogen and phosphorus treatment significantly affected the RB, SB, LB, and TB ( $p < 0.05$ ); water and nitrogen coupling significantly affected the SB, LB, and TB ( $p < 0.01$ ), while nitrogen and phosphorus coupling only significantly affected the LB ( $p < 0.05$ ), and the interaction effect of water, nitrogen and phosphorus affected the RB and TB ( $p < 0.05$ ) (Tab. S1). Under NW conditions, there were no significant differences between N0P0 and other treatments in RB, SB, LB, and TB. Under DT conditions, compared with N0P0, the RB increased by 33.58%, 42.15%, and 38.28% under N2P0, N0P1, and N2P1, respectively; the TB increased by 17.32%, 17.59%, and 23.87% under N2P0, N0P1, and N2P1, respectively; and the SB increased by 16.60% and 29.19% under N2P0 and N2P1, respectively (Fig. 1). The results showed that the HN treatment could not effectively slow the inhibitory effect of drought on biomass; excessive application of nitrogen fertilizer can inhibit the growth of plants under DT conditions; and P deficiency significantly weakened the positive effect of HN on biomass under DT conditions.

### 3.2 Effects of water, nitrogen and phosphorus availability on the photosynthetic performance of *R. pseudoacacia*

Nitrogen treatment, phosphorus treatment and water and nitrogen coupling significantly affected the Pn, gs, and Tr

( $p < 0.05$ ); water, nitrogen and phosphorus coupling significantly affected the gs and Tr ( $p < 0.05$ ); and nitrogen treatment, water and nitrogen, nitrogen and phosphorus, and water, nitrogen and phosphorus coupling significantly affected the WUE ( $p < 0.01$ ) (Tab. S1). Under the NW condition, compared with N0P0, under the conditions of N1P0, N1P1, and N2P1, Pn increased by 31.19%, 32.47%, and 39.48%, gs by 76.50%, 108.54%, and 103.88%, and Tr by 47.02%, 87.89%, and 79.28%, respectively. WUE was the highest under N0P1, which was 20.15% higher than that under N0P0. Under DT conditions, compared with N0P0, the gs and Tr increased by 39.01% and 29.28%, respectively, under N2P1. Similarly, WUE was the highest under N0P1, which was 11.54% higher than that under N0P0 (Fig. 2). The results showed that Pn, gs, and Tr increased under NW conditions but were inhibited under the HN treatment. WUE was always the highest under the condition of P fertilizer alone, regardless of NW or DT conditions.

Drought treatment significantly affected the  $\Phi$ PSII and NPQ values ( $p < 0.05$ ), while nitrogen treatment significantly affected the Fv/Fm and SPAD values ( $p < 0.05$ ); the coupling effect of water and nitrogen significantly affected the SPAD value ( $p < 0.05$ ); the coupling effect of water and phosphorus significantly affected NPQ ( $p < 0.05$ ); and the coupling effect of nitrogen and phosphorus significantly affected the Fv/Fm value ( $p < 0.05$ ) (Tab. S1). Under NW conditions, compared with N0P0,  $\Phi$ PSII significantly increased by 6.51% under N3P1. Under DT conditions, NPQ in the N1P0 treatment was the highest, significantly higher than that in N1P1 and N3P1. The SPAD value of the HN treatment was significantly higher than that of the LN treatment for both the NW and DT condi-



**Figure 2:** Effects of water, nitrogen, and phosphorus on the net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Tr), and instantaneous water use efficiency (WUE). NW and DT refer to soil water contents of 75 and 55% of field capacity, respectively. N0, N1, N2, and N3 refer to no urea conditions and urea addition at 0.2, 0.4, and 0.8 g kg<sup>-1</sup>, respectively. P0 and P1 refer to no superphosphate and superphosphate addition at 0.6 g kg<sup>-1</sup>, respectively. The vertical bars in the figures represent the standard errors of the means. Bars with the same letter indicate no significant difference at the  $p < 0.05$  level.

tions (Fig. 3). The results showed that under drought conditions, the application of P decreased NPQ. The HN treatment effectively weakened the inhibitory effect of drought and phosphorus deficiency on the chlorophyll formation of the *R. pseudoacacia* seedlings.

### 3.3 Antioxidant response of *R. pseudoacacia* seedlings to water, nitrogen and phosphorus

There was no significant effect of phosphorus treatment on SOD or of water and phosphorus coupling on MDA, but other treatments had a significant effect on SOD, POD, CAT, and MDA ( $p < 0.01$ ) (Tab. S1). Under NW conditions, compared with N0P0, the SOD increased by 15.78%, 8.49%, and 2.93% under the N3P0, N2P1, and N3P1 treatments, respectively, while the POD and CAT values of N0P0 were the highest, which were significantly higher than those of the other treatments, except that there were no significant differences in CAT between the N0P0 and N3P0 treatments. The MDA content under N2P1 and N3P1 decreased by 4.80% and 12.11%, respectively, compared with N0P0. Under DT conditions, compared with N0P0, SOD increased by 4.74% and 8.08% under N0P1 and N2P1, respectively, while POD increased by 31.94%, 15.97%, and 13.10% and CAT increased by 19.12%, 10.64%, and 9.75% under N2P0, N3P0, and N2P1, respectively, and MDA decreased by 10.22%, 10.31%, and 15.07% under N2P0, N0P1, and N3P1, respectively (Fig. 4). The results showed that the POD and CAT activities in the leaves of the *R. pseudoacacia* seedlings decreased as a result of increased N and P fertilizers under NW conditions. Drought stress significantly reduced the SOD, POD, and CAT

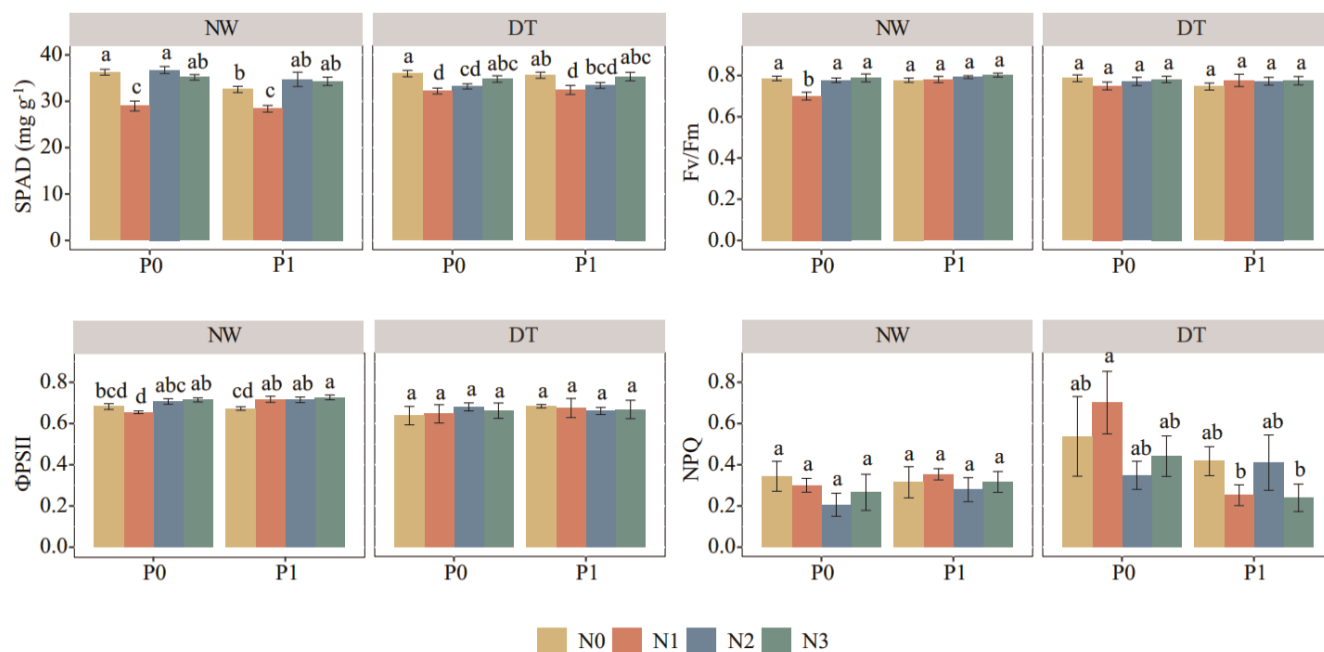
activities and significantly increased the MDA content. Under drought stress, proper N and P fertilizers could increase the SOD, POD and CAT activities and decrease the MDA content.

### 3.4 Relationships between morphological and physiological parameters

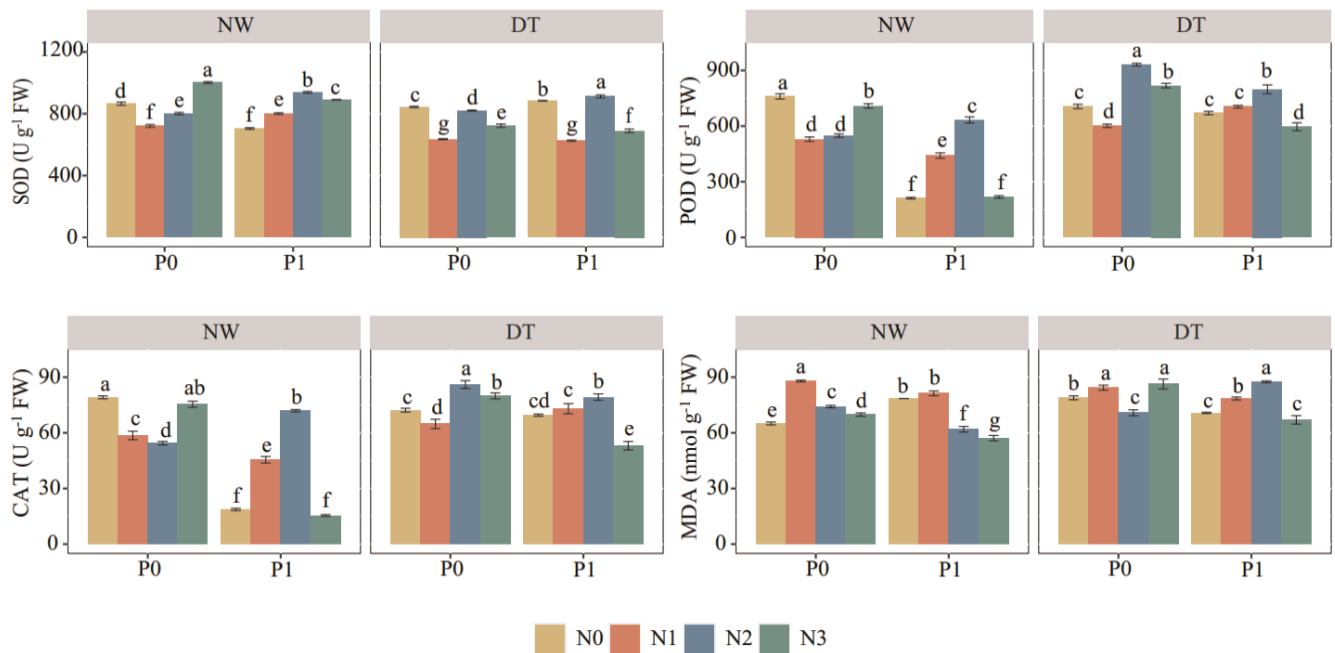
As shown in Fig. S1, SPAD had a significant negative correlation with Pn, gs, and Tr ( $p < 0.01$ ); RB, SB, LB, and TB had a significant positive correlation with  $\Phi$ PSII ( $p < 0.05$ ); POD and CAT were negatively correlated with H, D, TB, SB, and LB ( $p < 0.01$ ), and only POD was negatively correlated with RB ( $p < 0.05$ ); SOD was positively correlated with H and D ( $p < 0.05$ ) and positively correlated with TB, RB, SB, and LB ( $p < 0.01$ ); and MDA was the opposite, *i.e.*, negatively correlated with H and D ( $p < 0.05$ ) and negatively correlated with TB, RB, SB, and LB ( $p < 0.01$ ).

### 3.5 Plasticity indexes

Under different resources, the plasticity of different parameters is different (Tab. 1, Fig. 5). Regarding growth parameters, the plasticity indexes of TB, RB, SB, and LB were higher than those of H and D; regarding photosynthetic parameters, the plasticity indexes of gs and Tr were higher than those of Pn and WUE; regarding fluorescence parameters, the plasticity indexes of NPQ were much higher than those of Fv/Fm, PSII, and SPAD; and regarding antioxidant parameters, the plasticity indexes of POD and CAT were higher than those of SOD and MDA. The  $P_{lm}$  of antioxidant parameters was the highest,



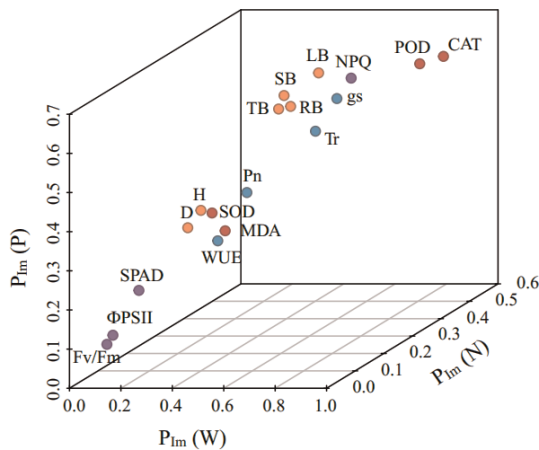
**Figure 3:** Effects of water, nitrogen, and phosphorus on the values of chlorophyll content in relative units (SPAD), the maximum quantum efficiency of photosystem II photochemistry (Fv/Fm), actual photochemical efficiency of photosystem II ( $\Phi$ PSII), and nonphotochemical quenching (NPQ). NW and DT refer to soil water contents of 75 and 55% of field capacity, respectively. N0, N1, N2, and N3 refer to no urea conditions and urea addition at 0.2, 0.4, and 0.8 g kg<sup>-1</sup>, respectively. P0 and P1 refer to no superphosphate and superphosphate addition at 0.6 g kg<sup>-1</sup>, respectively. The vertical bars in the figures represent the standard errors of the means. Bars with the same letter indicate no significant difference at the  $p < 0.05$  level.



**Figure 4:** Effects of water, nitrogen and phosphorus on superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and malondialdehyde (MDA). NW and DT refer to soil water contents of 75 and 55% of field capacity, respectively. N0, N1, N2, and N3 refer to no urea conditions and urea addition at 0.2, 0.4, and 0.8 g kg<sup>-1</sup>, respectively. P0 and P1 refer to no superphosphate and superphosphate addition at 0.6 g kg<sup>-1</sup>, respectively. The vertical bars in the figures represent the standard errors of the means. Bars with the same letter indicate no significant difference at the  $p < 0.05$  level.

while the  $P_{lm}$  of fluorescence parameters was the lowest. Moreover, the  $P_{lm}$  of POD and CAT were the highest regardless of drought or nitrogen or phosphorus treatments. The sensitivity of the  $P_1$  of each parameter to water, nitrogen and phosphorus was expressed by the coefficient of variation

(CV) (Tab. 1). The plasticity indexes of D, WUE, and Fv/Fm were the most sensitive to water, while the plasticity indexes of H, WUE, Fv/Fm, and MDA were the most sensitive to nitrogen, and the plasticity indexes of H, WUE, NPQ, and CAT were the most sensitive to phosphorus.



**Figure 5:** Plasticity of different parameters to water [ $P_{lm}(W)$ ], nitrogen [ $P_{lm}(N)$ ] and phosphorus [ $P_{lm}(P)$ ] levels. H, plant height; D, stem diameter; RB, root biomass; SB, stem biomass; LB, leaf biomass; TB, total biomass; Pn, net photosynthetic rate; gs, stomatal conductance; Tr, transpiration rate; WUE, instantaneous water use efficiency; SPAD, the values of chlorophyll content in relative units; Fv/Fm, the maximum quantum efficiency of photosystem II photochemistry;  $\Phi$ PSII, actual photochemical efficiency of photosystem II; NPQ, non-photochemical quenching; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase and MDA, malondialdehyde.

## 4 Discussion

### 4.1 Response of the morphological and physiological parameters of *R. pseudoacacia* seedlings to the coupling of water, nitrogen and phosphorus

The adaptation mechanism of plants to the specific living environment is first manifested in morphology. Many studies have shown that drought stress significantly inhibits plant growth, such as the decline of plant height and biomass (Zhang et al., 2018; Yang et al., 2019; Deng et al., 2020). Our study also reached a similar conclusion; specifically, under drought stress, the plant height, stem diameter, total biomass and biomass accumulation of roots, stems and leaves of *R. pseudoacacia* seedlings decreased, indicating the response of plants to drought stress. Other studies have shown that the inhibition of growth caused by drought stress can be compensated by increasing N application, which is shown to promote root development, enhance plant water absorption capacity, promote plant dry matter accumulation, and enhance plant tolerance to drought stress (Shi et al., 2017; Ding et al., 2018; Tariq et al., 2019). In our study, nitrogen treatment effectively slowed the inhibitory effect of drought on growth, and the morphological parameters of the MN treatment were higher than

**Table 1:** Phenotypic plasticity index of growth parameters, photosynthetic parameters, fluorescence parameters, and antioxidant parameters.

	Parameter	P <sub>1</sub> (NW)	P <sub>1</sub> (DT)	P <sub>1</sub> (N0)	P <sub>1</sub> (N1)	P <sub>1</sub> (N2)	P <sub>1</sub> (N3)	P <sub>1</sub> (P0)	P <sub>1</sub> (P1)	P <sub>1</sub>	P <sub>1m</sub> (W)	P <sub>1m</sub> (N)	P <sub>1m</sub> (P)	P <sub>1m</sub>	CV(W) (%)	CV(N) (%)	CV(P) (%)
Growth parameters	H	0.19	0.22	0.36	0.36	0.09	0.32	0.28	0.39	0.39	0.20	0.28	0.33	0.50	0.10	<b>0.46</b>	<b>0.24</b>
	D	0.22	0.09	0.30	0.28	0.22	0.27	0.30	0.28	0.30	0.16	0.27	0.29		<b>0.58</b>	0.11	0.05
	RB	0.42	0.30	0.51	0.32	0.48	0.47	0.51	0.53	0.61	0.36	0.45	0.52		0.24	0.19	0.02
	SB	0.34	0.23	0.51	0.50	0.40	0.55	0.55	0.51	0.55	0.29	0.49	0.53		0.26	0.13	0.06
	LB	0.48	0.29	0.53	0.52	0.50	0.57	0.61	0.53	0.61	0.38	0.53	0.57		0.35	0.06	0.10
	TB	0.34	0.22	0.49	0.45	0.44	0.52	0.52	0.47	0.54	0.28	0.48	0.50		0.31	0.08	0.07
Photosynthetic parameters	Pn	0.39	0.38	0.19	0.22	0.30	0.38	0.40	0.35	0.44	0.39	0.27	0.38	0.51	0.01	0.31	0.10
	gs	0.57	0.51	0.42	0.43	0.47	0.46	0.53	0.55	0.60	0.54	0.45	0.54		0.08	0.06	0.03
	Tr	0.51	0.46	0.38	0.48	0.39	0.43	0.45	0.50	0.57	0.49	0.42	0.47		0.08	0.11	0.08
	WUE	0.42	0.20	0.34	0.34	0.13	0.15	0.24	0.30	0.42	0.31	0.24	0.27		<b>0.48</b>	<b>0.48</b>	<b>0.15</b>
Fluorescence parameters	SPAD	0.23	0.10	0.10	0.13	0.10	0.03	0.21	0.20	0.23	0.17	0.09	0.21	0.30	0.52	0.47	0.03
	Fv/Fm	0.13	0.05	0.05	0.10	0.03	0.03	0.11	0.07	0.13	0.09	0.05	0.09		<b>0.60</b>	<b>0.64</b>	0.33
	ΦPSII	0.10	0.07	0.07	0.10	0.08	0.09	0.11	0.09	0.12	0.08	0.08	0.10		0.28	0.17	0.12
	NPQ	0.42	0.66	0.41	0.64	0.50	0.46	0.71	0.43	0.71	0.54	0.50	0.57		0.32	0.20	<b>0.35</b>
Antioxidant parameters	SOD	0.31	0.30	0.20	0.22	0.15	0.31	0.37	0.33	0.38	0.31	0.22	0.35	0.58	0.04	0.31	0.07
	POD	0.77	0.72	0.72	0.37	0.41	0.73	0.43	0.73	0.77	<b>0.74</b>	<b>0.56</b>	<b>0.58</b>		0.04	0.35	0.37
	CAT	0.82	0.80	0.76	0.38	0.37	0.81	0.37	0.81	0.82	<b>0.81</b>	<b>0.58</b>	<b>0.59</b>		0.01	0.41	<b>0.53</b>
	MDA	0.35	0.35	0.18	0.11	0.29	0.34	0.26	0.35	0.35	0.35	0.23	0.30		0.01	<b>0.46</b>	0.20

those of the HN treatment, which indicates that excessive application of N under drought conditions can inhibit the growth of plants, which is consistent with previous studies (Yin et al., 2010; Shi et al., 2020). This result may have occurred because excessive nitrogen under drought conditions inhibited RuBP carboxylase activity, which led to the decrease in gas exchange parameters and affected the growth of plants (Nakaji et al., 2002). Alternatively, because *R. pseudoacacia* functions in biological nitrogen fixation, it can make more effective use of low-level nitrogen, but excessive nitrogen may inhibit the nitrogen fixation ability of legumes and thus inhibit plant growth (Yamashita et al., 2019). In addition, under drought conditions, P deficiency obviously weakened the positive effect of the HN treatment on plant height, stem diameter and biomass, which may be because under the condition of soil water deficiency. P application improves the water status of the root system and thus promotes plant growth, reducing the inhibitory effect of the HN treatment on plant growth under drought conditions (Zhan et al., 2014).

Photosynthesis is one of the most important physiological processes in plants. Water, as an indispensable component of photosynthesis, plays an important role in plant growth and development. Our study shows that drought stress had no significant effect on the Pn, gs, Tr, and WUE of the *R. pseudoacacia* seedlings, which may have occurred because its leaves maintained a certain degree of activity under drought

stress due to the strong drought tolerance of the species, and the photosynthetic activity of the leaves reached a high level under the same light as the control. Under NW conditions, the photosynthetic capacity of the *R. pseudoacacia* seedlings was improved by the application of an appropriate amount of N fertilizer, which may be because N fertilizer improved the water condition of the leaves and increased the content of the photosynthetic pigments and the soluble protein in the plants, thus enhancing the photosynthetic activity of mesophyll cells and the photosynthetic rate of the leaves. However, the HN treatment inhibited the photosynthetic capacity of the *R. pseudoacacia* seedlings, which may be due to the limitation of electron transport and the decrease in RuBP carboxylase activity caused by excessive N application (Manter et al., 2005). These results are consistent with those from previous studies (Nakaji et al., 2002; Wu et al., 2008). The SPAD value could be used to indicate the photosynthetic ability of *R. pseudoacacia* seedlings, which also facilitates comparison of the differences in growth performance among *R. pseudoacacia* seedlings under different growth environments. In our study, the HN treatment effectively weakened the inhibitory effect of drought and phosphorus deficiency on the chlorophyll formation of the *R. pseudoacacia* seedlings, indicating that nitrogen promoted the accumulation of chlorophyll, which is consistent with previous research results (Borges et al., 2017; Mastalerczuk et al., 2017). The effect of HN treatment on SPAD and photosynthetic parameters may be used to ex-



plain why SPAD had a significant negative correlation with Pn, gs, and Tr. The response of the water status and photosynthesis of the *R. pseudoacacia* seedlings to water, nitrogen and phosphorus was ultimately expressed in WUE, which reflected the water use level and energy conversion efficiency of the *R. pseudoacacia* seedlings. The results showed that the WUE under P application was high under both NW and DT conditions, which may be a result of the improvement of water use efficiency due to the increase in P concentration by reducing the transpiration rate (Singh et al., 2000). In our study, drought treatment significantly reduced the  $\Phi$ PSII value and significantly increased the NPQ of the *R. pseudoacacia* seedlings, indicating that drought stress caused the absorption of more light energy by antenna pigments to dissipate in the form of heat energy, while the proportion of light energy used in photochemical reactions decreased, which may be a protective measure for the seedlings when encountering drought stress. Under drought condition, NPQ decreased with the application of P, but Fv/Fm and  $\Phi$ PSII did not change significantly, indicating that NPQ was more sensitive to P concentration under the drought condition, which may be due to the occurrence of oxidative stress caused by phosphorus stress, which caused the increase in NPQ.

Under normal growth conditions, the production and removal of active oxygen in plants are in a dynamic balance; however, under stress, oxygen metabolism is out of balance, and the production of active oxygen is accelerated. In this case, the activities of SOD, POD and CAT decrease and the removal rate decreases, leading to the accumulation of active oxygen, the induction of membrane lipid peroxidation, and the destruction of the structure and function of biomembranes. Our results showed that under NW conditions, SOD activity increased significantly; however, POD and CAT activities decreased significantly under the N2P1 and N3P1 treatments, indicating that excessive N fertilizer may reduce the ability of POD and CAT to scavenge active oxygen due to the reduction in the plant water status. In addition, drought stress significantly reduced the activities of SOD, POD and CAT, and significantly increased the MDA content. However, under drought stress, appropriate application of nitrogen and phosphorus improved the SOD, POD and CAT activities and reduced the MDA content, which is consistent with previous research (Saneoka et al., 2004; Song et al., 2017), indicating that appropriate application of nitrogen and phosphorus can reduce the damage caused by active oxygen to cell structure and function and can reduce the damage caused by drought stress to MDA. With the accumulation of biomass, the light energy conversion efficiency of the PSII reaction center of the *R. pseudoacacia* seedlings was enhanced, which also shows that the coupling of water, nitrogen and phosphorus would affect the accumulation of biomass by affecting the photosynthetic capacity of the PSII reaction center and would affect the growth of the plants by affecting the antioxidant system.

## 4.2 Comparison of the plasticity of morphological and physiological parameters of *R. pseudoacacia* seedlings to the coupling of water, nitrogen and phosphorus

Plasticity is key for ecologists to understand plant growth and development in the ecological environment (Valladares et al., 2006). Because of the heterogeneity of environmental conditions and the correlation with multifunctional changes, it is very complex to describe the plasticity of a species to changes in various resource supplies in different habitats. Studies have shown that biomass allocation characteristics are more suitable for evaluating plasticity than metamer traits because their response to different resources is constant (Navas and Garnier, 2002). Similar results were obtained in our study and showed that the plasticity index of RB, SB, LB, and TB was higher than that of H and D (Tab. 1, Fig. 5). Compared with the effect of plant size, leaf physiological characteristics are more affected by environmental change (Xu et al., 2015). In our study, the average plasticity index ( $P_{lm}$ ) of the antioxidant parameter was the highest, and the increase in these parameters is related to the occurrence of oxidative stress, which may be attributed to limited environmental variables (water and nutrients). Therefore, it is suggested that *R. pseudoacacia* seedlings adapt to changes in water and nutrients mainly by regulating their antioxidant properties. The  $P_{lm}$  of POD and CAT to water, nitrogen and phosphorus was the highest (Tab. 1, Fig. 5), which indicated that *R. pseudoacacia* seedlings adapt to changes in water and nutrients mainly by adjusting POD and CAT. Although the  $P_{lm}$  of the fluorescence parameters was the lowest, the  $P_1$  of NPQ was similar to that of CAT and POD (Tab. 1, Fig. 5). Therefore, NPQ plays an important role in the adaptation mechanism of *R. pseudoacacia* seedlings to different water and nutrient levels.

The sensitivity of the plasticity index of different parameters to water, nitrogen and phosphorus was different, which may be an adaptive strategy of *R. pseudoacacia* seedlings to the change in water and nutrients, indicating that it is necessary for plants to balance the effective acquisition of the necessary resources and the allocation of the available limited resources because available resources are limited in the process of plant growth and development (Wright et al., 2007; Ordoñez et al., 2009). When plants face changes in the soil water content, there is mainly a trade-off between D, WUE, and Fv/Fm; when plants face changes in the soil nitrogen content, there is mainly a trade-off between H, WUE, Fv/Fm, and MDA; and when plants face changes in the soil phosphorus content, there is mainly a trade-off between H, WUE, NPQ, and CAT.

## 5 Conclusions

In this study, the responses of the morphological, photosynthetic, fluorescence and antioxidant parameters of *R. pseudoacacia* seedlings under the conditions of drought and N and P application were investigated, revealing the adaptation mechanism of plants to different environment variables (water and nutrients) and reflecting the important role of trade-off relationships in the rational allocation of limited resources. Based on the above research, it is suggested that in the process of vegetation restoration, regular or dynamic plant

growth surveys should be conducted, and the soil nitrogen and phosphorus status should be changed according to the background level of soil nutrients to allow plant better adaptation to the arid environment as well as effective management and optimal resource utilization efficiency. The results are also helpful for understanding the physiological and ecological responses of *R. pseudoacacia* under different environmental conditions. In addition, plants have different responses to different environmental factors. It is very important to identify which traits may have important plasticity responses to specific environmental changes and help plants cope with changing environmental conditions. In future studies, the response of plants to plasticity should be taken into account when predicting the effect of global climate change on forest ecosystems.

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## Data Availability Statement

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

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