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The responses of soil microbial community and enzyme activities of *Phoebe zhennan* cultivated under different soil moisture conditions to phosphorus addition

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The importance of conservation and ecological restoration of the rare and economically important tree *Phoebe zhennan* is increasingly recognized. To this purpose, phosphorus (P) addition has been proposed to improve soil biological attributes and face the anticipated drought under climate change, though few studies have investigated its effect on the interaction between the soil microorganisms and plant host, as well as on ecosystem productivity. We investigated the effect of P addition on soil chemical properties, microbial communities, and enzyme activities in a soil planted with *P. zhennan* under two levels of water treatments (optimum water and drought treatments). P additions had no significant effect on microbial communities, dissolved organic nitrogen (DON), pH and soil moisture (SM), though the available P (aP) increased. Compared with no P treatment, alkaline phosphate and β-fructofuranosidase activities increased with P additions in the drought treatment. Drought decreased the total phospholipid-derived fatty acids (PLFAs), arbuscular mycorrhiza fungi (AMF), and fungi PLFAs compared to the well-watered. These findings indicated that P additions does not ameliorate the impact of drought on soil microbial communities and enzyme activities, except alkaline phosphate and β-fructofuranosidase, and P may not be responsible for regulating biochemical processes essential for maintaining the fertility of soil planted with *P. zhennan* under drought conditions. It is hypothesized that the lack of effects of P addition on the majority of the microbial properties could be due to the soil mechanism employed by *P. zhennan* to tolerate harsh conditions.

Keywords: Alkaline Phosphatase, Biomass, Drought, Enzymes, Microbial, *Phoebe zhennan*

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Introduction

Phoebe zhennan S. Lee (Family: Lauraceae; common name: Golden Phoebe), also known as Nanmu, is a well-known and rare tree species valued for its high timber quality and medicinal values (Hu et al. 2015). *P. zhennan* is listed as a threatened species by the International Union for Conservation of Nature (IUCN), and it is protected nationwide in China (Gao et al. 2016a). In the past few years, there has been a decline in the acreage of *P. zhennan* (Cao et al. 2016). At the moment, cultivation of *P. zhennan* has gained attention for use in tree resource conservation and ecological restoration projects. A program focusing on the protection and development of rare and precious tree species resources is currently being undertaken in Sichuan province (Hu et al. 2015). However, the increased aridity due to climate change may have implications for *P. zhennan,* either direct or indirect. To face this problem, it is therefore necessary to develop improved soil management practices such as phosphorus (P) application. Indeed, our previous study suggested that P addition could improve drought tolerance of *P. zhennan* seedlings through physio-biochemical adjustments (Tariq et al. 2017), though the impact of P on soil chemical properties, microbial communities, and enzyme activities has not been investigated yet. Moreover, P in soil is subject to extensive physicochemical and biological reactions, with only a small component of total soil P being in a form directly available for plant or microbial uptake (Gao et al. 2016b). The seedling stage in forest restoration is critical for successful establishment. The application of P might increase the tolerance of soil microbes to soil water deficit (Nielsen et al. 2015, Huang et al. 2016) and subsequently improve seedling performance after transplantation in the field.

Soil microorganisms are the critical link between the composition of above ground plant species and ecosystem functioning (You et al. 2014). Recent studies reported a close two-way interaction between microbial communities and plant host as a vital determinant of plant health and productivity (Sardans & Penuelas 2012, Rivest et al. 2015, Gunina et al. 2017). While plants can affect soil microbial communities *via* their biomass, nutrient demand and water use efficiency, plant development can be strongly influenced by soil microbial communities (Sayer et al. 2016). This reciprocal exchange and interactions between plants

and soil microbial communities underpins forest soil health, ecosystem function, and the ecosystem resilience after disturbance, which is fundamental in response to global change (Sayer et al. 2016). Although several studies have reported that the coupling between plant and soil microorganisms is vital for plant performance and maintenance of soil health (Wei et al. 2017), both plants and soil microorganisms can be affected by change in soil water content and nutrient availability, such as P (Sanaullah et al. 2011, Cavagnaro 2016, Sun et al. 2018). Moreover, the shifts in soil chemical properties and biological parameters can provide an initial indication of soil quality because of their sensitivity, rapid response to short-term disturbances, and capacity to integrate many environmental factors (Richardson et al. 2011, Temesgen et al. 2016).

Changes in soil water content and nutrient availability can affect soil microbial communities directly by altering their activity, but also indirectly through their influence on aboveground tree species (Huang et al. 2016, Sayer et al. 2016, Olatunji et al. 2018). Several studies have reported that drought can reduce the water potential of the physical soil matrix, and worsen nutrient deficiency by reducing its acquisition, mobility, and accessibility in the soil for plants and soil biota (Cavagnaro 2016, Sun et al. 2018). Therefore, it is anticipated that improved practices such as P application could buffer soil conditions, halting and even reversing the adverse effects of P deficiency in soils and increasing the stress-tolerating ability of soil microbes (Nielsen et al. 2015, Huang et al. 2016, Olatunji et al. 2018). The influence of P on aboveground plant communities is well understood; however, much less is known about the impact of P addition on belowground microbial communities. For instance, a limited response of soil microbial biomass and some enzyme activities to P application have been reported (Yang & Zhu 2015, Gao et al. 2016a). Such feedback may be context-dependent and could vary depending on the influence of host plant species, soil water content, and other variables (Wei et al. 2017, Sun et al. 2018). To our knowledge, even though the necessity for conservation and ecological restorations of *P. zhennan* is well recognized, there is limited information about the effect of P addition as an improved management practice and its interaction with soil water content on *P. zhennan* chemical properties, microbial communities, and enzyme activities. Hence, understanding the effects of P addition on belowground microbial communities associated with *P. zhennan* seedlings under various levels of water treatment may be beneficial to improve cultivation strategies and may also provide better insights into effective management practices to maintain soil health under varying climate conditions.

In this study, we conducted a P addition experiment with two levels of water treatment (optimum water addition and drought) in a soil planted with *P. zhennan*, and measured the response of soil chemical properties, microbial communities, and enzyme activities. Our aim was to determine if P addition affects the soil chemical parameters, microbial community, and enzyme activities of soil planted with *P. zhennan* under changes in soil water content, as well as to ascertain if improved practices such as P application could buffer the soil environment against drought conditions.

Materials and methods

Experimental design

This study was conducted at the Center for Ecological Studies, Chinese Academy of Science, Sichuan Province, China (104° 04′ E, 30° 37′ N). The study area has a mild subtropical monsoon climate with a mean annual temperature of 16.7 °C and precipitation of 945.6 mm. Soils used in this study were collected at 15 cm depth and thoroughly mixed. The chemical properties of the soil samples were 0.89 g kg^{-1} total phosphorus (TP), 27.6 mg kg^{-1} available phosphorus (aP), 1.9 g kg^{-1} total nitrogen (TN), 26.7 g kg^{-1} total carbon (TC), and pH of 7.3.

A complete randomized experimental design was established consisting of two levels of water treatment (well-watered and drought) and two levels of P application (with and without P). The *P. zhennan* seedlings used in this study were grown for approximately two years, after which healthy and uniform seedlings were transplanted into 30 (10 L) pots filled with approximately 4000 g of a homogenized soil sample. Then, the plants were grown for another two months to allow adequate acclimation to the new environment before treatments were applied.

After 2 months growth, 20 healthy plants were selected and subjected to five replicates of four treatments: water with P-addition (WP), water only (W), drought with P-addition (DP), and drought only (D), for 3 months. Phosphorus fertilizer was supplied as sodium dihydrogen phosphate (NaH_2PO_4, 25.5% P) at the rate of 129.3 mg P per pot. The fertilizer was mixed with 200 ml of water per pot and supplied every 30 days for three months.

The soil-relative water content (SRWC) was determined according to Xu et al. (2009) and categorized into two levels: optimum water addition (80%-85%) and severe drought (30%-35%). The SRWC of each pot was maintained by replacing the amount of water transpired and evaporated. A distance of 40 cm was maintained between pots to prevent shading by neighboring plants.

Determination of soil parameters

The soil obtained after removing plants from the pots was thoroughly homogenized and transferred to the laboratory in a sealed plastic bag. Soil moisture content was determined gravimetrically from mass loss after oven drying 10 g of moist soil to a constant weight at 105 °C for 24 h. The pH was measured using a pH meter (PHS-25®, INESA Instruments, China) at a ratio of 25 ml deionized water to 10 g soil. Ammonia (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 50 ml of 2.0 M KCl and determined on a flow injection auto-analyzer (AutoAnalyzer3®, Bran-Luebbe, Germany). The aP was extracted with 0.5 M $NaHCO_3$ (pH 8.2) according to Olsen & Sommers (1982) and measured colorimetrically by the molybdate-ascorbic acid method (Murphy & Riley 1962). Dissolved organic carbon (DOC) and nitrogen (DON) were extracted from fresh moist soils with an addition of 2 M KCl at 20 °C and measured using a TOC/ TN analyzer (Multi N/C 2100(S)®, Analytik Jena AG, Germany).

Estimation of microbial communities

Using phospholipid-derived fatty acids (PLFAs – Bossio & Scow 1998), soil microbial communities were assessed. Microbial lipids were extracted from frozen soil samples corresponding to 8 g dry mass of soil, placed in 50 ml centrifuge tubes, and then extracted with a mixture of chloroform ($CHCl_3$), methanol, and phosphate buffer (1:2:0.8). The fatty acids in the lower chloroform phase were saponified and transformed into fatty acid methyl esters using a strong acid methylation. PLFAs methyl esters were separated and identified using gas chromatography (model 6890N®, Agilent, USA) coupled with a flame ionization detector (model 19091B-102®, Agilent, USA) and an HP-5 capillary column. The concentration of each PLFAs was quantified according to the 19:0 internal standards (nonadecanoic acid methyl ester). The abundance of individual fatty acids in each sample was expressed as nmol per g dry soil. Individual fatty acids were used to indicate broad functional groups of microorganisms according to existing PLFAs biomarker data (Frostegard et al. 2011, Kaiser et al. 2010). Actinomycetes and fungi were expressed by the PLFAs 10me 18:0, 10me 17:0, 10me 16:0, 10me 19:0 and 18:1ω9c, 18:2ω6.9c, 16:1ω5c, respectively. PLFAs 16:1ω5c was used as the maker for arbuscular mycorrhiza fungi (AMF). The sum PLFAs i15:0, i16:0, a17:0, i14:0, a15:0, i17:0, i13:0, a16:0, i18:0 were chosen to represent the Gram-positive (G^+) bacteria, while Gram-negative (G^-) bacteria were express by the sum of 16:1ω7c, 15:1ω6c, 16:1ω11c, 18:1ω7c, cy17:0, 17:1ω8c, 16:1 2OH, 11Me18:1ω7c, 18:1ω5c, cy19:0ω8c. The sum of all extracted PLFAs was used as the estimate of total microbial biomass (totPLFAs).

Enzyme activities assay

Activities of soil enzymes, including, β-glucosidase, alkaline phosphatase (AP), amidohydrolase (urease), and β-fructofuranosidase (invertase) were measured colorimetrically using the procedure of Tabatabai & Bremner (1969), Tabatabai (1982), and Torres et al. (2016). Alkaline phos-

phatase activities were determined by the release of p-nitrophenol (PNP) after cleavage of the enzyme-specific synthetic substrate. One gram of soil was incubated for 1 h at 37 °C with a mixture of 4 ml modified universal buffer (pH 6.5), 0.2 ml toluene, and 1 ml appropriate substrate solution. One ml 0.5 M NaOH and 4 ml 0.5 M $CaCl_2$ were used to stop the reaction and allow for color development. The soil solution was then filtered and analyzed colorimetrically at 660 nm. β-glucosidase activity was measured by adding 2 ml of MUB pH 6.0 and 0.5 ml of 0.025 mol l^{-1} *p*-nitrophenyl β-D-glucopyranoside to 1 g soil sample and determined colorimetrically at 400 nm after incubating the mixtures at 37 °C. A control test was performed for each sample and the control (without soil), with the substrate being added after the reactions were stopped. For the determination of β-fructofuranosidase activities, 2 g air-dried soil sample was extracted with 0.2 ml toluene, 5 ml phosphate buffer solution, and 5 ml 10% sucrose solution. The mixture was incubated at 37 °C for 2 h. The resultant mixture was treated with a 3 ml of 3,5-dinitrosalicylic acid solution and determined colorimetrically at 508 nm. The urease activities were determined by incubating 2 g soil samples with an aqueous urea solution at 37 °C for 2 h. NH_4^+ released from the soil was extracted with 1 M KCl solution and measured by a modified indophenol-blue colorimetric reaction.

Data analysis

For all the data obtained, homogeneity of variance was tested and log-transformation was applied when necessary (applicable to soil enzyme activities). Data obtained for chemical properties, enzyme activities, and microbial communities were analyzed by analysis of variance (two way-ANOVA) using SPSS® ver. 18.0 (SPSS Inc., Chicago, IL, USA). The treatment means were compared using Duncan's multiple range tests at the 0.05 probability level. Figures were prepared using Origin pro ver. 8.5 (OriginLab Corp., Northampton, MS, USA).

Results

Effects of P addition and water deficit on soil chemical properties

P addition had no significant effects on soil NH_4^+-N, DON, pH, and soil moisture contents (Tab. S1 in Supplementary material). Soil aP significantly increased ($p < 0.05$) in soils receiving P addition irrespective of the level of water treatments compared to those without P addition (Tab. 1). The aP of the drought treatments was higher (+P and -P: 105.33 and 48.66 mg kg^{-1} dry soil, respectively) than that of the well-watered (+P and -P: 71.33 and 44.66 mg kg^{-1}, respectively) treatments. P addition significantly increased ($p < 0.05$) NO_3^--N in well-watered treatments (8.23 mg kg^{-1}) compared to no P (5.52 mg kg^{-1}), but no significant differences were observed in NO_3^--N between the P and no P additions in drought treatments. The DOC contents significantly decreased ($p < 0.05$) in soil receiving P addition irrespective of the level of water treatments (Tab. 1). Generally, NO_3^--N, DON and aP in drought treated soils was higher than those of the well-watered soil. The result of the ANOVA showed that the interactions of water and P treatments had no significant effects on the majority of the soil chemical properties, except NO_3^-- N and aP (Tab. S1 in Supplementary material).

Tab. 1 - Effects of P additions under the two level of water treatments on soil chemical properties. Values are means ± SE. Means with different letters represent significant differences ($p<0.05$) among the four treatments after Duncan's test. (SM): soil moisture (%); (pH): soil pH; (DOC): dissolved organic carbon; (DON): dissolved organic nitrogen; (NH_4^+-N): soil ammonium nitrogen (mg kg^{-1}); (NO_3^--N): soil nitrate nitrogen (mg kg^{-1}); (aP): available phosphorus.

Parameters	Well-watered		Drought	
	-P	+P	-P	+P
NO_3^--N (mg kg^{-1})	5.52 ± 0.05 ^{c}	8.23 ± 0.36 ^{b}	9.73 ± 0.48 ^{a}	9.19 ± 0.57 ^{a}
NH_4^+N (mg kg^{-1})	0.16 ± 0.04 ^{b}	0.17 ± 0.01 ^{ab}	0.14 ± 0.01 ^{b}	0.24 ± 0.02 ^{a}
DOC (mg kg^{-1})	351.54 ± 32 ^{a}	259.73 ± 5.95 ^{b}	305.45 ± 34 ^{a}	255.18 ± 11.32 ^{b}
DON (mg kg^{-1})	103.29 ± 1.38 ^{a}	117.83 ± 6.84 ^{a}	114.27 ± 7.73 ^{a}	128.85 ± 13.18 ^{a}
aP (mg kg^{-1})	44.66 ± 3.30 ^{c}	71.33 ± 0.66 ^{b}	48.66 ± 3.52 ^{c}	105.33 ± 3.33 ^{a}
pH	7.52 ± 0.09 ^{a}	7.71 ± 0.11 ^{a}	7.50 ± 0.08 ^{a}	7.58 ± 0.03 ^{a}
SM	15.51 ± 0.86 ^{a}	16.28 ± 0.70 ^{a}	9.21 ± 0.41 ^{b}	9.05 ± 0.56 ^{b}

Phosphorus addition and water deficit impacts on soil microbial community and enzyme activities

Soil microbial communities, including total PLFAs, AMF, fungal PLFAs, G^- bacteria, total bacteria, and actinomycete PLFAs in P addition treatments were not significantly different from the no P treatments in well-watered or drought treated soils (Fig. 1, Fig. 2b, Fig. 2c). Compared with the no P addition treatments, P addition significantly increased ($p < 0.05$) G^+ bacteria in well-watered treatments (Fig. 2a).

A significant effect of water treatments was observed on total PLFAs, AMF, fungi, G^+ bacteria, and total bacteria PLFAs (Tab. S2). Drought decreased the total PLFAs, AMF, fungi, and total bacteria compared to well-watered treatments (Fig. 1a, Fig. 1b, Fig. 1c, Fig. 2b). There were no significant differences in G^- bacteria and actinomycete PLFAs in the well-watered and drought treatments (Fig. 1d, Fig. 2c). The results of

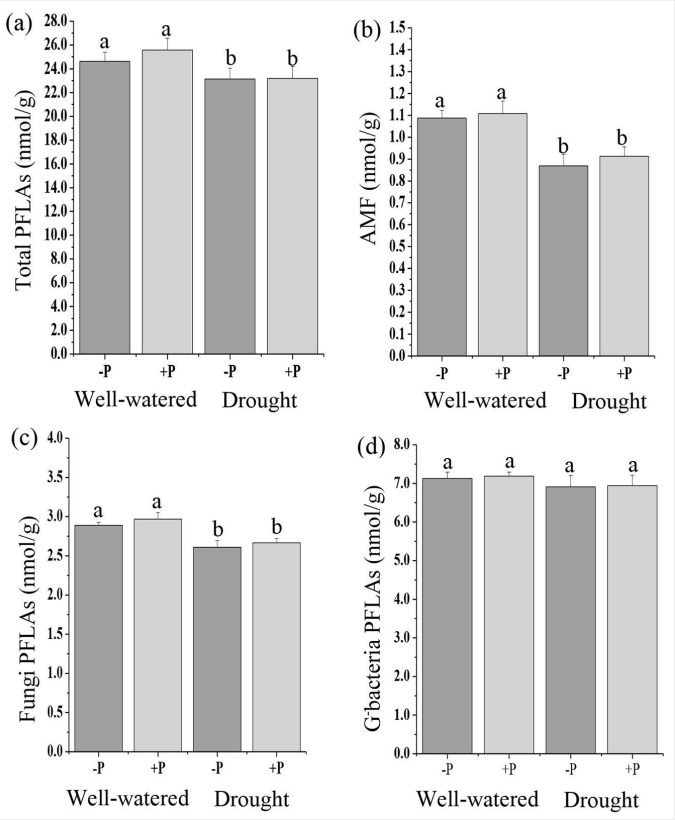

Fig. 1 - Response of soil microbial community, (a) total PLFAs, (b) AMF, (c) fungi PLFAs, (d) G^- bacterial PLFAs to phosphorus (P) application in well-watered and drought treatments. Different lowercase letters indicate significant differences ($p<0.05$) among the treatments. The error bar represents standard error (SE).

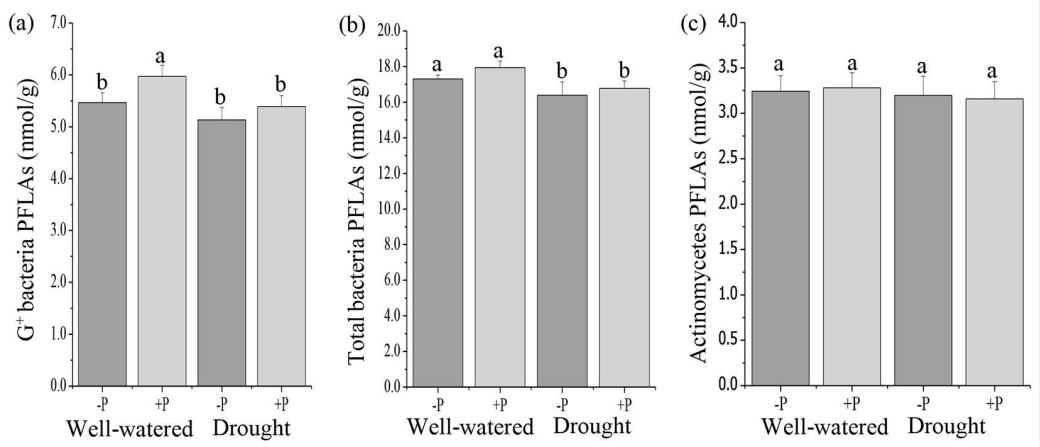

Fig. 2 - Response of soil microbial community, (a) G^+ bacterial PLFAs, (b) total bacterial PLFAs, and (c) actinomycetes PLFAs to phosphorus (P) application in well-watered and drought treatments. Different lowercase letters indicate significant differences ($p<0.05$) among the treatments. The error bar represents standard error (SE).

the ANOVA indicated that there were no significant interactive effects of P addition and water treatments on all the microbial communities (Tab. S2 in Supplementary material).

The effects of P addition on soil enzyme activities varied depending on the level of water treatments. P addition significantly increased ($p < 0.05$) alkaline phosphate and β-fructofuranosidase activities in drought treatments but had no significant effects in well-watered treatments (Fig. 3a, Fig. 3b). β-glucosidase activity was lower with P addition, irrespective of the level of water treatments, although there were no significant differences compared to their no P counterparts (Fig. 3c).

The result of the ANOVA showed that P addition and its interactions with water treatment had no significant effects on any enzyme activity (Tab. S3 in Supplementary material). However, independent factors of water treatments alone significantly affected alkaline phosphate, β-glucosidase, and urease activities (Tab. S3). Urease and β-glucosidase activities were higher in well-watered treatments than in the drought treatments (Fig. 3c, Fig. 3d).

Discussion

A comprehensive understanding of the interactive influence of climate and biotic factors on soil microbial communities and enzyme activities is vital for our understanding of changes in soil health (You et al. 2014), particularly as the aridity of the soil environment limits P availability for plant growth in many soils (Richardson et al. 2011). In this study, we determined the effects of P addition on soil chemical properties, microbial communities, and enzyme activities in soil planted with *P. zhennan* at two levels of water treatments. Consistent with previous studies (Sanaullah et al. 2011, Sayer et al. 2016), it was observed that the majority of the microbial communities and enzymes activities were partitioned along the water levels. The decrease in the total PLFAs, AMF, fungi, and total bacteria in drought treatments compared to well-watered treatments indicated that microbial communities were under physiological or nutritional stress, most probably because of competition between plant and soil microrganisms for limited accessible nutrients under drought conditions (Huang et al. 2016, Cavagnaro 2016, Olatunji et al. 2018). Studies have shown that decreases in soil moisture also reduce N and/or P uptake by plants (Cramer et al. 2009, Sardans & Penuelas 2012). Likewise, the higher NO_3^--N and DON in drought treatments compared to the well-watered treatments suggested a reduction in nutrient uptake by *P. zhennan* during drought conditions. The negative feedback of less water and lower nutrient uptake could negatively affect the production capacity and fitness of plants when drought persists or becomes more severe (Sanaullah et al. 2011, Cavagnaro 2016, Sayer et al. 2016). Nevertheless, the abundance of actinomycetes, a heterogeneous group of bacteria noted for their filamentous and branching growth pattern, which may confer resistance to desiccation, were similar for both well-watered and drought treatments. These results suggested that *P. zhennan* may adapt to drought conditions by enhanced hydraulic conductance of the root system (Tariq et al. 2017) and increased production of biochemical compounds, which in turn increases actinomycete abundance (Koyama et al. 2017).

Contrary to the hypothesis that improved management practices such as P applica-

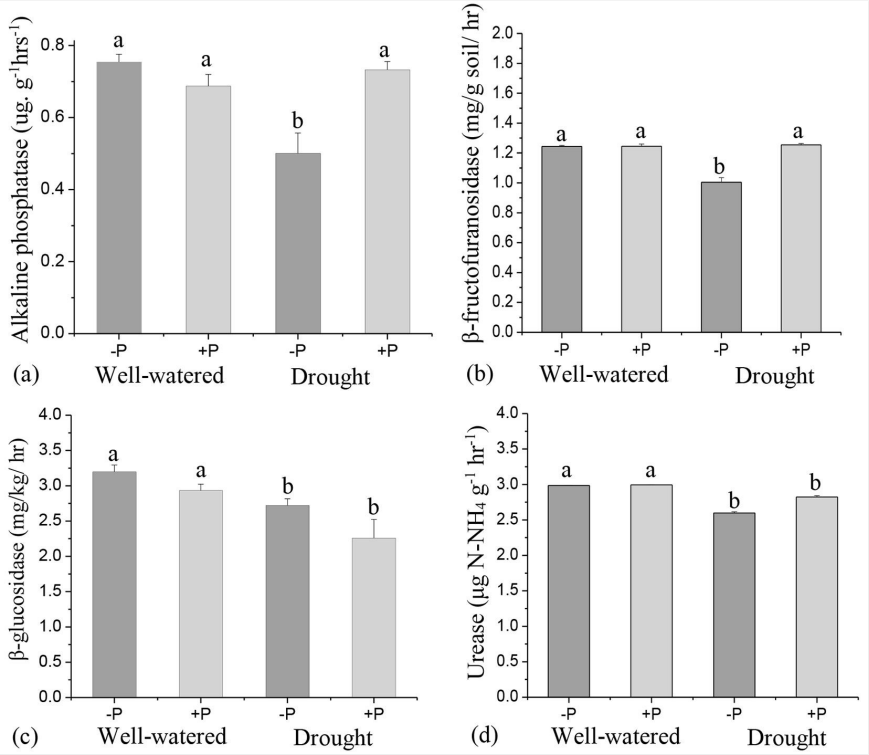

Fig. 3 - Response of soil enzyme activities, (a) alkaline phosphate, (b) β-fructofuranosidase, (c) β-glucosidase and urease to phosphorus (P) application in well-watered and drought treatments. Different lowercase letters indicate significant differences ($p<0.05$) among the treatments. The error bar represents standard error (SE).

tion could buffer soil conditions and increase the drought-tolerating ability of soil microbes (Nielsen et al. 2015, Huang et al. 2016), we found that P addition had no significant impact on the total PLFAs, AMF, fungi PLFAs, G^- bacteria, total bacteria, and actinomycete PLFAs of *P. zhennan*. Although the root biomass of *P. zhennan* was enhanced with P addition, as reported by Tariq et al. (2017), the DOC, which represents the labile soil carbon and acts as the energy source for soil microbes (Huang et al. 2016), decreased with P addition. This may be the reason for our results, which showed no significant effects of P addition on microbial communities. Moreover, the leaf dry matter, which can be used as a proxy for the proportion of vascular tissue, cellulose, insoluble sugar, and leaf lignin, and thus an additional carbon source for microbial communities, was lower with P addition (Pei et al. 2016, Tariq et al. 2017). Leaf nitrogen content is an indicator of plant growth and resource uptake, and it has been recognized that N-rich leaves could lead to bacterial dominated soil microbial communities (Pei et al. 2016). Corresponding to the lower leaf nitrogen content reported by Tariq et al. (2017) in drought-treated soil regardless of P addition, we observed that bacterial communities were lower in drought-treated soil. This further suggests that the application of P as an improved management strategy could buffer the soil environment of *P. zhennan* against drought conditions. Studies have reported substantial increases in phosphatase activity in the rhizosphere of plants experiencing depletion of soil organic P (Richardson et al. 2011). However, we observed that increased aP corresponded to an increase in alkaline phosphate in P treated dry soil. The mechanism underpinning this observation may be that even in the presence of high aP, P uptake by *P. zhennan* was constrained, hence hampering the effective utilization of P by *P. zhennan* under drought stress (Richardson et al. 2011, Sardans & Penuelas 2012). Taken together, the observed differences in the microbial communities appears to be related more to other abiotic variables or the growing nature of *P. zhennan* rather than to the availability of P due to P addition. Moreover, *P. zhennan* is a slow-growing forest tree species with a low mineral absorption rate and a slow growth strategy (Chapin 1980). Examining the impact of P addition on the drought tolerance of *P. zhennan*, Tariq et al. (2017) demonstrated that, although drought stress severely affected the growth and metabolism, the species employs a range of strategies to tolerate harsh conditions. The strategies include limited stomatal conductance and transpiration rate, increased antioxidative activities, and accumulation of osmoprotectors. Although *P. zhennan* exhibits some inherent strategy which allowed it to lower the critical P requirement for growth and allowed its soil environment to operate at lower accessible levels of soil P (Richardson et al. 2011), we suggest that the negative impact of less water and lower nutrient uptake could impact on the production capacity and fitness of *P. zhennan* when drought persists or becomes more severe. Our ongoing work is investigating other management strategies that could maintain soil health for the economically important but threatened *P. zhennan* under adverse environmental conditions.

Conclusions

Our results indicates that P addition does not have an impact on the composition of soil microbial communities or enzyme activities, except alkaline phosphate and β-fructofuranosidase, which are sensitive indicators of soil quality. In this study, the composition of microbial communities tended to be more sensitive to the level of water treatment than to P addition. Drought decreased the soil total PLFAs, AMF, fungal PLFAs, and G^+ bacteria compared to well-watered treatments, irrespective of P addition. These results suggest that as P application may not be able to sustain the microbial properties of soil planted with *P. zhennan*, the negative impact of less water and lower nutrient uptake could impact the production capacity and fitness of *P. zhennan* when drought persists. The present findings highlight the need to further investigate a wide range of management strategies to maintain soil health for the economically important but threatened *P. zhennan* under changes in environmental conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary Material

Tab. S1 - ANOVA results of the effects of phosphorus addition, water treatment, and their interaction on soil chemical properties.

Tab. S2 - ANOVA results of the effects of phosphorus addition, water treatment, and their interaction on soil microbial PLFAs.

Tab. S3 - ANOVA results of the effects of phosphorus addition, water treatment, and their interaction on the enzyme activities.

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