



Arbuscular mycorrhizal fungus–induced decrease in phosphorus loss due to leaching in red soils under simulated heavy rainfall

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Abstract

Purpose Phosphorus (P) loss from the soil due to leaching is affected by plant roots and arbuscular mycorrhizal fungi (AMF). This paper aimed to (i) analyze the effects of plant roots and AMF on P concentrations in interflow and P loss from the soil due to leaching and (ii) explain whether there were interaction effects between plant roots and AMF on the loss of P from soils due to leaching.

Material and methods In this study, samples of red soil were collected from wasteland, farmland, and slopeland on the Yunnan Plateau. As a host plant, maize was cultivated in a dual-compartment cultivation system. There were mycorrhizal and hyphal compartments for the arbuscular mycorrhizal fungal inoculation treatment and root and soil compartments for the non-inoculation treatment. The maize biomass and P uptake, P concentrations in interflow within two soil layers (0–20 and 20–40 cm), and P losses due to leaching under simulated heavy rainfall (40 and 80 mm/h) were analyzed.

Results and discussion Arbuscular mycorrhizal fungal inoculation significantly enhanced the biomass and P uptake of maize, but had little effect on P concentration in the interflow under 40 mm/h rainfall. In addition, Arbuscular mycorrhizal fungal inoculation resulted in a decrease of 37–65% in P concentration in interflow under 80 mm/h rainfall, and a decrease of 21–39% in the P loss from soils due to leaching under heavy rainfall (40 and 80 mm/h). Moreover, significant or highly significant positive correlations were observed between P concentrations in the interflow and the loss of P due to leaching from the red soil. Two-way analysis of variance indicated that AMF significantly or highly significantly decreased P concentrations in the interflow and decreased P loss from red soils due to leaching, whereas the root effects were insignificant. In addition, there was no interaction between AMF and maize roots.

Conclusions Our study indicated that the arbuscular mycorrhizal fungus–induced decreases in the loss of P due to leaching from red soils were caused by decreased P concentrations in the interflow. The potential for AMF to reduce P leaching from soils is an important ecological function.

Keywords Arbuscular mycorrhizal fungi · Extraradical hyphae · Interflow · Phosphorus · Leaching loss

1 Introduction

Nutrients in farmland soils such as phosphorus (P) are depleted when leached with water flow during rainfall or irrigation, and these nutrients have become highly important sources of agricultural

non-point pollution (Sun et al. 2012; Zheng et al. 2014). Especially under a heavy rainfall condition, interflow in soils occurs after the soil water becomes saturated; moreover, P leaching from the soil is aggravated and becomes the main cause of water eutrophication and belowground water pollution (Ma et al. 2016).

As important components of the soil, plant roots and microorganisms have considerable influence on soil permeability and the P leaching (Jarvis, 2007). Plant roots improve the aggregate structure and porosity of the soil and enhance the permeability of soils through their interpenetration, network construction, and binding of soil particles (Czarnes et al., 2000). Under rainfall conditions, plant roots obviously promote rainwater infiltration and interflow production in soils and reduce the loss of P from soils due to leaching because the roots absorb P (Du et al. 2017).

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Arbuscular mycorrhizal fungi (AMF), which are important microorganisms in the soil, can form symbioses with most terrestrial plant species on Earth (Smith and Read 2008). Moreover, the arbuscular mycorrhizal fungal biomass of plant roots constitutes approximately 4% of the total biomass of soil microorganisms in the biosphere (Treseder and Cross 2006). In addition to colonizing plant roots, a large quantity of arbuscular mycorrhizal fungal hyphae extend into the soil (Drew et al. 2003). Moreover, the biomass of arbuscular mycorrhizal fungal extraradical hyphae is greater than that of arbuscular mycorrhizal fungal intraradical hyphae within roots (Olsson et al. 1999). Therefore, AMF form an enormous and dense belowground hyphal network in the soil, and the ecological effect of this network has garnered increased amounts of attention (Mohan et al. 2014).

AMF form mycorrhizae with plant roots and can substantially influence the loss of P due to leaching in the soil (Cavagnaro et al. 2015; van der Heijden et al. 2015). For instance, arbuscular mycorrhizal fungal inoculation was shown to reduce P concentrations significantly in leachates from test soils, such as those of grasslands, nursery containers and rice paddies, and reduce P leaching from soils (van der Heijden 2010; Asghari and Cavagnaro 2011; Corkidi et al. 2011; Zhang et al. 2016). Under moderate and heavy rainfall, AMF reduced P leaching by 50% from soils (Martínez-García et al. 2017). However, it was also shown that arbuscular mycorrhizal fungal inoculation had little effect on soil P leaching (Köhl and van der Heijden 2016). The potential of AMF to reduce P leaching from arable soils is low (Duffková et al., 2019). Hence, the effects of AMF on the loss of P due to leaching from soils are inconsistent. Moreover, whether there are interaction effects between plant roots and AMF on the loss of P due to leaching from farmland soils remains unknown.

Red soils are widely distributed across Yunnan Plateau (Lu et al. 2015). Moreover, 6.12 million hm^2 of farmland consists of 2.93 million hm^2 of slopeland ($> 15^\circ$) (Peng et al. 2009). In addition, there is an abundance of nonferrous metal sources on the Yunnan Plateau which has resulted in many wastelands due to mining (Li 2006). There is a large amount of surface runoff, and P losses from fields on red soil occur under heavy rainfall (Lu et al. 2012) and are influenced by slope, fertilization, mulching, and planting pattern (Lu et al. 2015; Zhong et al. 2018). However, the effects of AMF on P losses from red soils of the Yunnan Plateau have not yet been investigated.

Therefore, red soils from wasteland, farmland, and slopeland on the Yunnan Plateau were selected as test soils. A dual-compartment cultivation system was used. The system was divided into mycorrhizal and hyphal compartments for arbuscular mycorrhizal fungal inoculation treatments and into root and soil compartments for non-arbuscular mycorrhizal fungal inoculation treatments. Interflow and leachate in the four compartments were collected under simulated heavy rainfall conditions (40 and 80 mm/h for 30 min). P concentrations

and leaching were subsequently analyzed. The influence of AMF and roots on the P concentrations in the interflow and P leaching from the soils were investigated. We hypothesized that AMF decreased P concentrations in the interflow and reduced the loss of P due to leaching from the soils.

2 Materials and methods

2.1 Test soils and materials

Samples of red soil were collected from wasteland ($103^\circ 37' 17'' \text{ E}$, $26^\circ 34' 29'' \text{ N}$), farmland ($103^\circ 37' 46'' \text{ E}$, $26^\circ 34' 42'' \text{ N}$), and slopeland ($103^\circ 40' 16'' \text{ E}$, $26^\circ 36' 48'' \text{ N}$) from Huize County, Yunnan Province, Southwest China. The soil samples were collected at soil depths of 0–20 and 20–40 cm at an elevation of 2130 m above sea level. The basic physicochemical properties of the soils are listed in Table 1. The soils were dried indoors under a daytime temperature of 10–20 $^\circ\text{C}$, sieved through a mesh screen with a diameter of 2 mm, and autoclaved at 121 $^\circ\text{C}$ for 2 h.

The AMF used in the test consisted of soil-based and fine root-based segments of *Funneliformis mosseae* (BGC YN05, 1511C0001BGCAM0013), as well as their spores. The fungi were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry. The tested maize was a main locally cultivated variety (Huidan 4). Healthy maize seeds of the same size were selected before seed sowing. After surface sterilization of the seeds, they were cultured in the dark at a constant temperature of 28 $^\circ\text{C}$ for 3 days. Sterile seedlings displaying consistent growth were selected after the seeds germinated and were approximately 1 cm in length.

2.2 Experimental design

An improved dual-compartment cultivation system was used in this test. A 2-mm-thick organic glass plate was used to separate a rectangular plastic bucket (length \times width \times height = 40.5 cm \times 28.5 cm \times 50 cm) into two compartments. Holes 20 mm in diameter were punched uniformly on the organic glass plate, and two 30- μm nylon nets were affixed onto the two faces of the organic glass plate to form a 2-mm-thick gap. Because the diameter of the arbuscular mycorrhizal fungal hyphae is smaller than that of the plant roots, a nylon net with holes that are 30 μm in diameter was used to effectively isolate the plant roots from the arbuscular mycorrhizal fungal hyphae (Chen et al. 2001). Furthermore, adding a 2-mm-thick gap in the dual-compartment cultivation system effectively eliminated the direct diffusion of mineral elements between the compartments (Tanaka and Yano 2005). The use of the dual-compartment cultivation system verified that arbuscular mycorrhizal fungal extraradical hyphae have important functions

Table 1 Basic chemical properties of tested soils

Ecosystem	Soil depth (cm)	pH	Organic matter concentration (g/kg)	Cation exchange capacity (cmol/kg)	Nitrogen concentration (g/kg)	Phosphorus concentration (g/kg)
Wasteland	0–20	4.83	19.75	9.00	0.66	0.43
	20–40	5.00	12.88	12.75	0.63	0.45
Farmland	0–20	5.62	14.60	9.25	1.64	0.47
	20–40	6.54	11.16	10.00	1.58	0.50
Slopedland	0–20	5.45	16.75	26.00	0.44	0.49
	20–40	5.54	17.17	26.75	0.50	0.55

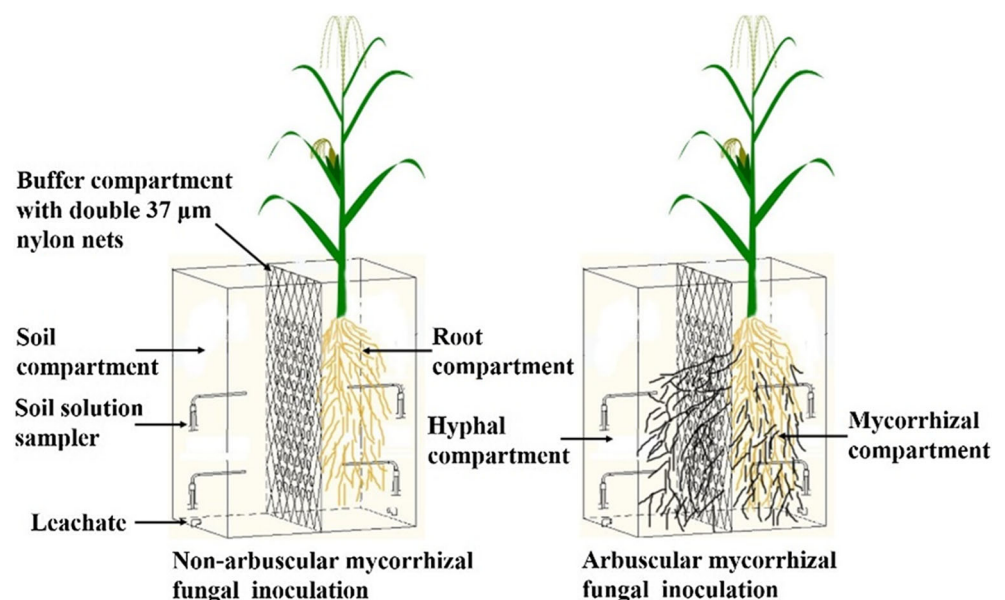
in absorbing and transporting P from the soil to plants (Behie and Bidochka 2014). Thus, this system provided a reliable method for studying the influence of arbuscular mycorrhizal fungal hyphae on the loss of P from soils due to leaching.

The dual-compartment cultivation system was divided into mycorrhizal and hyphal compartments for the arbuscular mycorrhizal fungal inoculation treatment and root and soil compartments for the non-arbuscular mycorrhizal fungal inoculation treatment (Fig. 1). Each compartment was filled with 15.0 kg of soil and sown with 2 germinated maize seeds inoculated with 300.0 g of the arbuscular mycorrhizal fungal inoculants at a depth of 20 cm. Soil solution samplers were installed at depths of 25 and 45 cm on both sides of the cultivation system; these samplers were used to collect the interflow in the soils. Drainage holes were added to the bottom to collect the leachate. Arbuscular mycorrhizal fungal inoculation and non-arbuscular mycorrhizal fungal inoculation treatments were applied in the test, and each treatment was repeated four times.

After the maize grew for 60 days, an artificial rainfall simulation system model (NLJY-09-4, Nanjing, China) was used to simulate rainfall. The rainfall source height, intensity, and duration were 10 m, 40 mm/h, and 30 min, respectively. The effective rainfall area was 32 m² (8 m × 4 m). The dual-compartment cultivation system containing the maize planted was placed below the rainfall simulation system. Both sides of the dual-compartment cultivation system received the same amount of water. On the 70th day, a second rainfall simulation was performed, in which the rainfall intensity and duration were 80 mm/h and 30 min, respectively.

2.3 Determination of arbuscular mycorrhizal fungal colonization

Maize roots were harvested after two rainfall simulations. Several fine and tender roots were selected and washed with water, cut into 1-cm-long root segments, placed in thermostatic water bath (90 °C), and dissociated using 10% (w/v) KOH

Fig. 1 Diagram of the dual-compartment cultivation system

solution for 1 h. A total of 50 segments were subsequently placed on a glass slide. Arbuscular mycorrhizal fungal infection was observed under a microscope after the specimens were dyed using acid fuchsin and decolorized using lactic acid and glycerine. The arbuscular mycorrhizal fungal colonization rate within roots was calculated using the cross-intersection method (McGonigle et al. 1990).

Soil samples from the 0–20 and 20–40-cm soil layers in the mycorrhizal and hyphal compartments and the root and soil compartments were collected. After the samples were air-dried at the room temperature, 20.0 g of soil samples was weighed. Arbuscular mycorrhizal fungal spores were separated using the wet sieve decantation saccharose centrifugation method, and the quantity of arbuscular mycorrhizal fungal spores was calculated under a stereoscopic microscope (Daniels and Skipper 1982).

2.4 Determination of maize biomass and phosphorus concentration

Maize plants were harvested after two rainfall simulations and divided into shoots and roots. The materials were cleaned using distilled water and then oven-dried at 75 °C for 48 h to determine their biomass. The dried maize shoots and roots in each pot were ground and filtered through a 0.5-mm sieve. Afterward, 0.25 g of plant tissue was digested by concentrated sulfuric acid and hydrogen peroxide ($\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$) and diluted with distilled water to a volume of 100 mL. The P concentration in the solution was measured by the vanadium-molybdenum colorimetric method and P concentration in maize was calculated according to a formula in which the P concentration was multiplied by solution volume and divided by the sample mass (Bao 2000).

2.5 Determination of phosphorus concentration and loss due to leaching

During rainfall simulations, interflow within the 20- and 40-cm depths in the mycorrhizal and hyphal compartments and the root and soil compartments was measured using a soil solution sampler (Rhizon MOM, Rhizosphere Research Products Co., Gelderland, Netherlands). The leachate was collected until no more water had dripped out after the rainfall finished, and its volume was measured with a graduated cylinder. The loss of P due to leaching was calculated by multiplying the leachate volume by its P concentration.

The P concentration in the solutions was determined using alkaline potassium persulfate digestion and ammonium molybdate spectrophotometry (Wei et al. 2002). A 10 mL water sample was placed in a 25-mL colorimetric tube, after which the water was added to a 5 mL alkaline potassium persulfate solution. The sample was subsequently placed in a high-pressure steam sterilization pot, maintained at 121 °C for 30

min, and then digested. The potassium persulfate–digested water sample and 1 mL of a 10% ascorbic acid solution were mixed together and blended, and 2 mL of molybdate solution was added, followed by sufficient blending for 30 s. After the solution was cooled at 20 °C for 15 min, the absorbance value was determined at a wavelength of 700 nm. Distilled water served as a blank control, and the P concentration was calculated according to a standard curve.

2.6 Data processing and analysis

The test data consisted of four repeated mean values expressed as the means \pm standard deviations. SPSS software (Version 22.0, IBM Corp., Chicago, IL, USA) and the Student-Newman-Keuls (SNK) test were used to test the significance of the data at a level of 0.05. Two-way analysis of variance (ANOVA) was used to determine the influence of roots and AMF on the P concentration in the interflow and P leaching from the soils, as well as the interaction effects between the roots and AMF.

3 Results

3.1 Arbuscular mycorrhizal fungal colonization

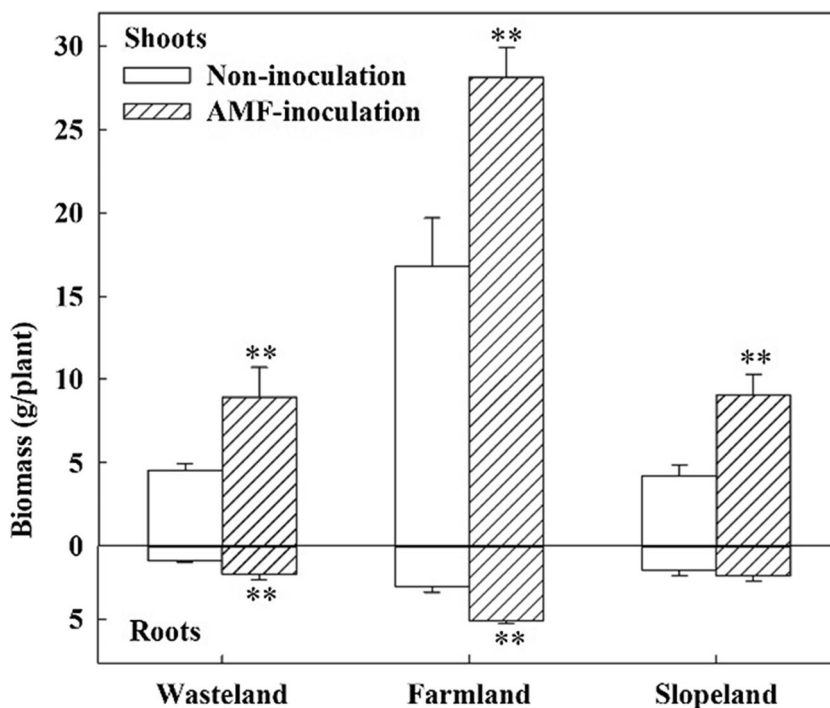
AMF successfully established a symbiotic relationship with maize. The arbuscular mycorrhizal fungal infection rate in the maize roots ranged from 41 to 65%, and the number of arbuscular mycorrhizal fungal spores in the soils ranged from 38 to 66/g dry soil. There were no significant differences in the arbuscular mycorrhizal fungal infection rate and spore number within the 0–20- and 20–40-cm soil layers among the wasteland, farmland, and slopeland soils.

3.2 Maize biomass and phosphorus uptake

Compared with no inoculation, arbuscular mycorrhizal fungal inoculation resulted in highly significant increases in both the root and shoot biomass of maize grown on wasteland and farmland soils and in the shoot biomass of maize grown on slopeland soil (Fig. 2).

Two-way ANOVA indicated that arbuscular mycorrhizal fungal inoculation significantly enhanced the P concentration only in the maize shoots grown on slopeland soil. However, due to the increase in maize biomass, arbuscular mycorrhizal fungal inoculation significantly increased the uptake of P into the roots and shoots of maize grown on wasteland and farmland soils and the uptake of P into the shoots of maize grown on slopeland soil (Fig. 3). Thus, arbuscular mycorrhizal fungal inoculation improved P uptake by maize plants.

Fig. 2 Effects of AMF on maize biomass. “***” means $p < 0.01$ according to SNK test



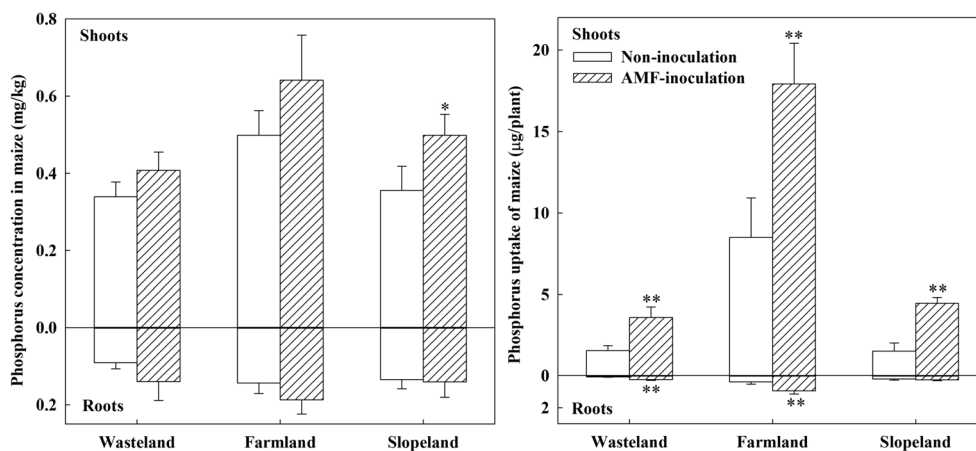
3.3 Phosphorus concentration in soil interflow

Under 40 mm/h simulated rainfall, with the exception of the P concentration in the interflow within the 20–40-cm soil layer in mycorrhizal and hyphal compartments being significantly lower than that in the soil compartment, the P concentration in the interflow of the four compartments was not significantly different among the wasteland, farmland, and slopeland soils. Under 80 mm/h simulated rainfall, the P concentration in the interflow within the 0–20-cm soil layer in the mycorrhizal and hyphal compartments of wasteland, farmland, and slopeland soils was significantly lower (by 37–65%) than that in the root and soil compartments. Within the 20–40-cm soil layer, the P concentration in the interflow in the mycorrhizal and hyphal

compartments was also lower than that in the root and soil compartments, with the exception that there were no significant differences between the hyphal (wasteland soil) and mycorrhizal (farmland soil) compartments with the soil compartment (Fig. 4).

Two-way ANOVA indicated that AMF had significant and highly significant influences on the P concentration in the interflow within the 0–20- and 20–40-cm soil layers, respectively, of the farmland soil and a significant influence on the P concentration in the interflow within the 20–40-cm soil layer of the slopeland soil, under 40 mm/h simulated rainfall. AMF had highly significant influences on the P concentration in the interflow within the 0–20- and 20–40-cm soil layers of the wasteland, farmland, and slopeland soils under 80 mm/h

Fig. 3 Effects of AMF on phosphorus concentration and uptake of maize. “*” and “***” mean $p < 0.05$ and $p < 0.01$ according to SNK test, respectively



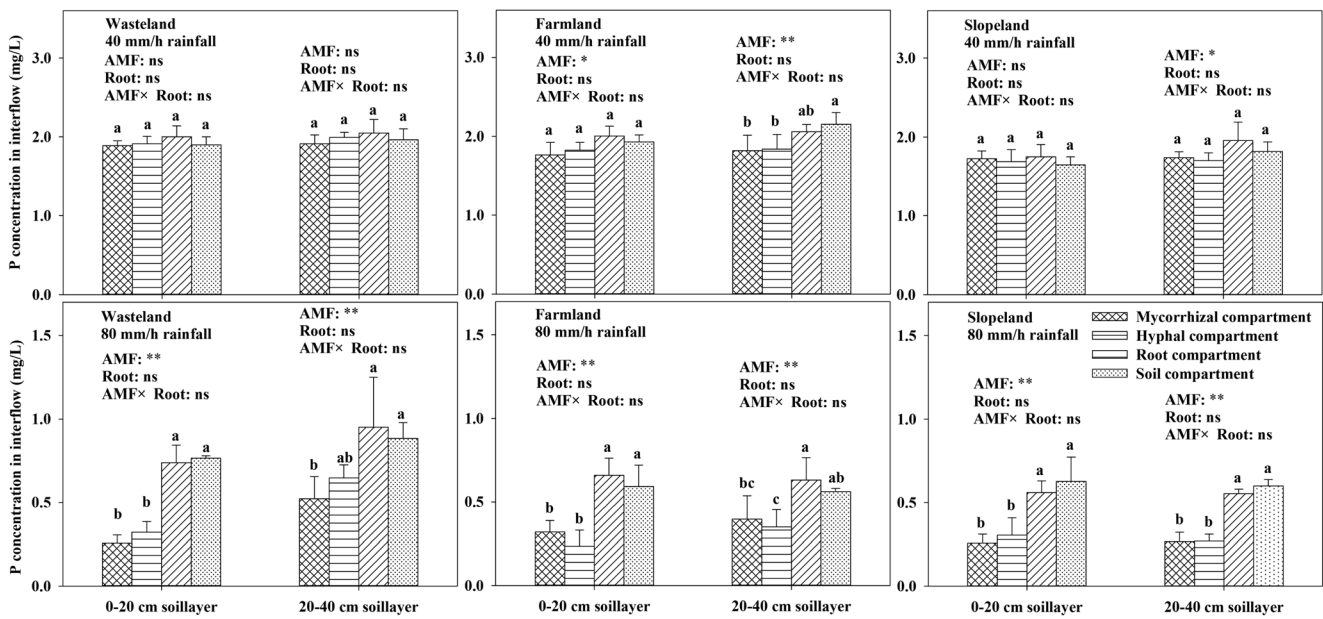


Fig. 4 Effects of AMF and maize root on phosphorus concentration in soil interflows. Different little letters refer to $p < 0.05$ according to SNK test. “ns”, “*”, and “**” mean no significance, $p < 0.05$ and $p < 0.01$ according to two-way ANOVA, respectively

simulated rainfall. Maize roots had an insignificant influence on the P concentration in the interflow, and the interaction effect between the AMF and roots was insignificant (Fig. 4). Thus, only the AMF contributed to the reduction in P concentration in soil interflow.

3.4 Loss of phosphorus from soils due to leaching

When the 40 and 80 mm/h rainfall simulation data were combined, the loss of P due to leaching from the mycorrhizal compartment of wasteland soil was significantly lower than that from the root compartment, the loss from the mycorrhizal and hyphal compartments of farmland soil was significantly lower than that from the soil compartment, and the loss from the mycorrhizal and hyphal compartments was significantly lower than that from the root compartment. The loss of P due

to leaching decreased by 30–39%. AMF had a significant or highly significant influence on the loss of P due to leaching, whereas the roots had no significant influence. The interaction effect between AMF and the roots was insignificant (Fig. 5). Thus, only AMF highly significantly reduced the loss of P from soils due to leaching.

3.5 Correlation analysis

Correlation analysis indicated highly significant positive correlations between P concentrations in the interflow within the 0–20- and 20–40-cm soil layers and the loss of P due to leaching under 40 mm/h simulated rainfall and a significant positive correlation between P concentration in interflow within the 20–40-cm soil layer with loss of P due to leaching under 80 mm/h simulated rainfall (Fig. 6).

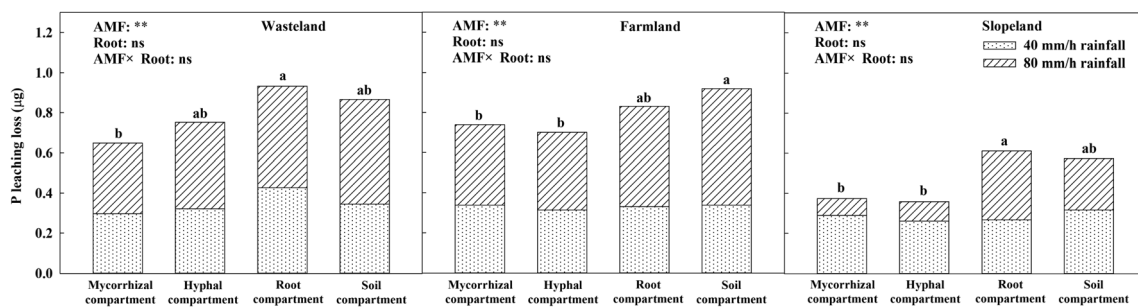


Fig. 5 Effects of AMF and maize root on phosphorus leaching loss from soils. Different little letters refer to $p < 0.05$ according to SNK test. “ns”, “*”, and “**” mean no significance, $p < 0.05$ and $p < 0.01$ according to two-way ANOVA, respectively

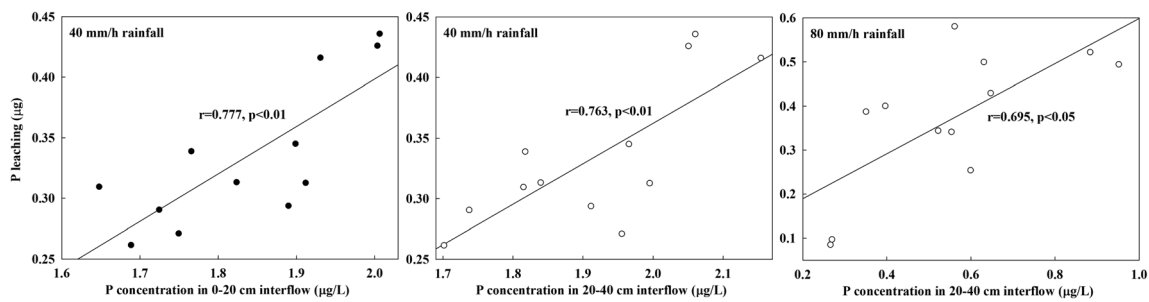


Fig. 6 Correlation analysis between phosphorus concentration in soil interflows with phosphorus leaching loss from soils

4 Discussion

In addition to horizontal nutrient loss caused by soil erosion and surface runoff, the downward infiltration of water caused by rainfall results in dissolution of mineral nutrients from the topsoil. The vertical movement of water with dissolved mineral nutrients in the soil profile causes a leaching, which is an important cause of P loss from soils (Huang et al. 2017). Especially in regions such as the Yunnan Plateau which has distinct wet and dry seasons and a typical monsoon climate, rainfall is concentrated during the summer and autumn, and heavy rainfall events cause large amounts of P loss from soils, which are the main cause of water eutrophication on the plateau (Zhang et al. 2004). Under heavy rainfall conditions, P loss from soil is mainly caused by surface runoff, but leaching is also an important way of soil P loss (Heathwaite and Dils 2000).

Nutrient leaching in farmland caused by rainfall is influenced by numerous factors, such as soil texture, nutrient levels, crop roots, and soil microorganisms (Cameron et al. 2013). Crop roots that directly absorb soil nutrients and water from the soil significantly affect both the preferential migration path of interflow in soils and nutrient leaching (Jiang et al. 2018). The growth of plant roots results in the formation of channels in the soil, which are soil macropores that serve as channels for the rapid migration of interflow, facilitating prior migration of soil nutrients. Moreover, relative to the living roots, the prior migration path of root channels formed by dead roots strongly contributes to the migration of interflow and nutrients (Ghestem et al. 2011). However, in this study, the maize roots showed no significant influence on P concentration in the interflow or loss of P due to leaching, which is probably related to the short experiment time, limited space for root growth, and viscous texture of the red soils in the pot experiment.

Through their own activities, soil microorganisms significantly influence soil physicochemical properties, interflow migration, and soil nutrient leaching (Morales et al. 2010). AMF, which have symbiotic relationships with plant roots, significantly influence mineral nutrient concentrations, such as P concentrations in interflow, and reduce soil nutrient losses due to leaching caused by rainfall (Cavagnaro et al. 2015). For example, arbuscular mycorrhizal fungal

inoculation of the grass *Trifolium subterraneum* reduced the available P content in the soil, P concentration in the leachate, and the loss of P due to leaching (Asghari et al. 2005), and arbuscular mycorrhizal fungal inoculation of a grassland reduced P losses in the soil by 60% (van der Heijden 2010). Arbuscular mycorrhizal fungal inoculation of a grassland reduced P loss from the soil by 50% under moderate and heavy rainfall conditions (Martínez-García et al. 2017). Similarly, AMF significantly reduced the P concentration in interflow and its loss due to leaching from wasteland, farmland, and sloped soils in this pot experiment.

Arbuscular mycorrhizal fungal extraradical hyphae form an enormous network in the soil and reduce nutrient concentrations in interflow by absorbing nutrients and improving soil structure. Arbuscular mycorrhizal fungal hyphae can absorb P and transport it to their host plants to increase the P uptake of the host plant (Smith and Smith 2011; Veresoglou et al. 2012), reducing the P concentration in soils and interflow and reducing P loss from soils (Asghari et al. 2005; Asghari and Cavagnaro 2011). In this study, the AMF reduced P concentrations in interflow within the 0–20- and 20–40-cm soil layers, and the P concentration in the interflow presented a significant or highly significant positive correlation with P loss due to leaching, thereby indicating that the arbuscular mycorrhizal fungus-induced reduction in P loss from soils due to leaching was closely related to the decreased P concentration in the interflow.

AMF reduce nutrient loss due to leaching by reducing the leachate volume. Arbuscular mycorrhizal fungal hyphae enwind and secrete various substances, such as glomalin-related soil proteins, into the soil, facilitate the mutual bonding of fine particles in the soil to form polymers, improve the stability and granular structure of soil aggregates (Rillig and Mummey 2006), and improve the water holding capacity of the soil to reduce nutrient loss due to leaching (Cavagnaro et al. 2015). Studies have shown that significantly negative correlations exist between the arbuscular mycorrhizal fungal colonization rate in ryegrass roots and phosphate loss due to leaching (Köhl and van der Heijden 2016) and between arbuscular mycorrhizal fungal hyphae density in the soil and loss of soil due to erosion (Mardhiah et al. 2016), thereby indicating that arbuscular mycorrhizal fungal hyphae have a direct effect on

reducing soil nutrient loss. Soil erosion can be partly explained through the combined effects of plant root biomass and arbuscular mycorrhizal fungal extraradical hyphae (Mardhiah et al. 2016). However, in this study, two-way ANOVA showed that the interaction between maize roots and arbuscular mycorrhizal fungal hyphae was not significant.

The role of AMF to reduce the loss of P from soils due to leaching may be important for ecosystems (Cavagnaro et al. 2015). However, most of the above research and reports on arbuscular mycorrhizal fungus-induced reduction in the loss of P from soils due to leaching involved indoor pot experiments and lack field data. The exact mechanisms employed by AMF for nutrient transformation in the soil and for reducing the loss of nutrient from soils due to leaching are still inconclusive (Parihar et al., 2019). Therefore, further studies on the influence and mechanisms of AMF on soil nutrient losses due to leaching under field conditions are needed.

5 Conclusions

There were significant or highly significant positive correlations between P concentrations in soil interflow and the loss of P from soils due to leaching. AMF significantly reduced the P concentration in interflow and the loss of P due to leaching from red soil on the Yunnan Plateau, whereas maize roots showed no significant influence, and the interaction between AMF and maize roots was not significant. The results indicated that AMF can ecologically function in reducing P nutrient losses from soils under heavy rainfall conditions.

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