

# Inter-species root interaction can shift feedback effect of arbuscular mycorrhizal fungi from neutral to positive or negative

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## Research Article

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# Abstract

## *Aims*

The growth-improvement of *Robinia pseudoacacia* in the presence of *Platycladus orientalis* is well documented, but the effects of arbuscular mycorrhizal (AM) fungi and roots on the growth of these trees remain largely unexplored. Here, we show different effects of changed AM fungi under distinct belowground interaction pathways.

## *Methods*

An AM fungal community native to grassland and a remodeled AM community from an adjacent *R. pseudoacacia* and *P. orientalis* mixed stand were collected and used in a pot experiment to estimate the role of AM fungi in the co-existence of conspecific and heterospecific neighbor trees. Using pots compartmented with mesh of different pore sizes, we also investigated how the feedback effects of remodeled AM fungi on *R. pseudoacacia* and *P. orientalis* growth were affected by different belowground interaction pathways between plants.

## *Results*

The biomass of *P. orientalis* coexisting with conspecific plants decreased under the remodeled AM treatment while that of *R. pseudoacacia* increased. The contrasting response of these tree species is attributable to different tree-tree interaction patterns. *P. orientalis* and *R. pseudoacacia* also experienced different AM-induced plant-soil feedback responses (APSF) in the presence of interspecific root interaction. Furthermore, we found the mechanisms of how AM colonization, photosynthesis and root morphology affected APSF in presence of different intra- and interspecific interaction pathways.

## *Conclusion*

There is a critical influence of neighbor tree identity and of tree-tree interaction pathways in the feedback effects induced by AM fungi on *R. pseudoacacia* and *P. orientalis*. The use of high proportion of *P. orientalis* is not advisable in *R. pseudoacacia* × *P. orientalis* mixed stands.

# 1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbioses with about 80% of terrestrial plant species (Smith and Read, 2008). The AM symbiosis is a mutually beneficial association between AM fungi and host plant roots in which AM fungi provides plants with nutrients and protection against antagonists in exchange for carbohydrates (Smith and Read, 2008; van der Heijden et al., 2007). The structure of the AM symbiosis is related to many plant characteristics, such as photosynthesis and root morphology (Bergmann et al., 2020; Callaway, 1998; Mikkelsen et al., 2008). The mycorrhizal symbiosis is a many-to-many relationship (Lang et al., 2011), and despite most mycorrhizal fungi are broad host generalists different AM fungal species can have different host preference (Klironomos, 2003; Naylor et al., 2017;

Raju et al., 1990). Consequently, the AM fungal community is seen as a determinant of the composition and function of its associated plant community (van der Heijden et al., 1998).

The partners in AM symbiosis live in a dynamic equilibrium. The composition of AM fungal communities influences plant community composition and, in turn, host plants as well as non-host plants influence the composition of the AM fungal community establishing in host plant rhizosphere (Hawkes and Hausmann, 2009; Shah et al., 2009; Veselkin and Prokina, 2016). These plants-induced changes may affect the benefits host plants derive from the symbiosis since most of these benefits, like better plant establishment or stress mitigation, largely depend on the composition of the AM fungal community engaged in the symbiosis (Bever, 2002; Gillespie and Allen, 2006; Spatafora et al., 2016). Thus, understanding the mechanism regulating plants and AM fungal communities is of primary importance in the management of terrestrial ecosystems.

Forests play a key role in producing timber, fuel and pulp wood, and in providing ecosystem services (Lang et al., 2014). Afforestation, an effective way to improve degraded areas, could reshape the resident AM fungal community in afforested zones since environmental factors such as soil cover, soil microclimatic conditions and physico-chemical properties are greatly modified by artificial plants (Antoninka et al., 2011; Su and Guo, 2007; Yang et al., 2013; Zheng et al., 2014). In a feedback loop, the biotic and abiotic changes occurring in response to afforestation influence the performance of the tree species introduced. Biotic factors such as soil pathogens and AM fungi, commonly generate feedback effects on plant growth, species composition and plant diversity (Mangan et al., 2010; Lin et al., 2015). If the AM fungi are disturbed by a change in plant cover, as in afforestation, the proportion of resident AM fungal species may be modified according to preferential associations with the new host plants. Since different AM fungi have different properties and functionalities, the functionality of the AM fungal community may change with plant cover. Therefore, such reshaping of the AM fungal community may change the effects of the AM fungal community on tree plantations.

Plant–plant interactions are among the principal factors regulating the dynamics of plant populations (Morris, 2003) and the structure of plant communities (Callaway, 1995). Two adjacent plants (conspecific or heterospecific) can simultaneously take part in competition and facilitation processes (Callaway and Walker, 1997). The plant-plant relationships within communities can be separated as aboveground and belowground interactions. Belowground interactions between plants are regulated by soil solution, mycorrhizal hyphae, and root factors (Schenk 2006). For example, the AM mycelia enmeshing the soil matrix access soil nutrients that plants cannot reach. These AM mycelia influence the distribution of mineral nutrients among plants in plant communities (Smith and Read 2008; Ren et al. 2013) and a recent study even reports the transfer of chemical signal between connected individuals (Song et al., 2010). Root competition can potentially extirpate certain plants in plant communities (Levine and D'Antonio, 1999). Previous study also showed that root interaction largely regulates the competitive ability of species in mixed stands (Ren et al., 2017). Considering plant photosynthetic efficiency, root morphology, and AM root colonization play important roles in plant growth and productivity (Bergmann et al., 2020; Eissenstat, 1992; Nouvellon et al., 2010; Shipley, 2006), we would like to know how different

levels of belowground interaction would affect plant traits and growth? What's more, it is acknowledged that relationships among plants in mixed communities may be totally renewed in a changed ecosystem due to changed microbiomes (Casper and Castelli, 2007; Peay et al., 2016; Pendergast et al., 2013). It is still of great importance of learning how changed AM fungi affected plant growth.

The Loess Plateau, located in western China, is a soil erosion-prone area (Shi and Shao, 2000; Sun et al., 2019; Tsunekawa et al., 2014). Its vegetation cover has undergone degradation due to its geographic landscape and long history (over 5000 years) of anthropogenic disturbance (Zhang, 2000). Afforestation to improve ecological conditions in the Loess Plateau, often involves *Robinia pseudoacacia* monoculture, *Platycladus orientalis* monoculture and the mixture of these trees (Sheng et al., 2017; Zhang, 2010). *P. orientalis* is an evergreen tree of the family Cupressaceae endemic to China and *R. pseudoacacia* is an introduced deciduous tree of the legume family Fabaceae. This species native to eastern North America is the broadleaved tree species most planted worldwide after *Eucalyptus* spp (Li et al., 2021). Both species form arbuscular mycorrhizal symbiosis. These mixed stands of *P. orientalis* and *R. pseudiacacia* create microclimates and soil conditions helping their growth (Zhai et al., 1991). This mixed-stand afforestation strategy has remodeled the community of rhizosphere-inhabiting AM fungi on the Loess Plateau according to our previous study (Chen et al., 2018a, Chen et al., 2018b). The AM fungal community in native grasslands strongly differed from those in afforested zones (Fig. S1). It is still largely unknown how remodeled AM communities in afforestation areas affect tree growth and tree-tree interactions. An integrated experiment considering all these factors would determine if and how an afforestation-remodeled AM fungal community affects tree-tree relationships in the ecosystem of diversified tree plantations.

In this study, we used soil containing the AM fungal community present in nearby native grasslands (Native-AM) and afforested soil containing the AM fungal community remodeled by *R. pseudoacacia* × *P. orientalis* mixtures (Remodeled-AM) as inocula to compare the effects of these communities on tree growth. We tested if and how the afforestation-remodeled AM fungal community may affect the relationships among these trees. The following questions are addressed in our study. Are there differences in the tree growth enhancing effect of AM fungi from native and remodeled AM communities? Do feedback effects from the remodeled AM community on a tree differ in conspecific and heterospecific neighbor tree pairs? And if so, how and by which pathways of interaction do an heterospecific neighbor influence tree production, through the soil solution, AM hyphae, root or through aboveground interaction only?

## 2. Materials and methods

### 2.1 Experimental design

The experiment was conducted in compartmented pots between October 2017 and July 2018. This experiment was arranged in a complete randomized design with four repetitions, in the greenhouse. The twenty-four experimental treatments were factorial combinations of three factors: (1) AM type had two

levels (Native-AM and Remodeled-AM), (2) neighbor tree had three levels: conspecific *R. pseudoacacia* monoculture, conspecific *P. orientalis* monoculture and heterospecific (*R. pseudoacacia* × *P. orientalis* mixture), and (3) tree-tree belowground interaction had four belowground interaction levels (BIL): no belowground interaction, belowground interaction through the soil solution, through soil solution + hyphae (no-root), and through the soil solution + hyphae + roots (full interaction). These BIL were created by walls of different pore sizes separating the trees planted in 3-compartment pots, as shown in Fig. 1.

## 2.2 Pots

Pots, were made of tempered glass by hand. The A and C compartments (13 cm × 13 cm × 13 cm), which received trees, were larger than the B compartment (6 cm × 13 cm × 13 cm), which was a interaction zone (Fig. 1). A 9-cm-diameter hole was cut in the walls separating the A and the B-compartment and the wall separating the C and the B compartments, and one of four kinds of separators (plastic film, 0.45 μm nylon mesh, 38 μm nylon mesh and 380 μm nylon mesh) were used to separate the interaction compartment from tree compartments, as prescribed by BIL treatments. Nylon mesh with a pore size of 38 μm excluded fine roots, but allows fungal hyphae to cross, while the 0.45 μm mesh exclude both fine roots and hyphae, with only the soil solution possibly carrying soil bacteria and archaea passing through. Four 3-compartment pots (repetitions) per treatment were used for a total of 96 pots, which were randomized on the greenhouse bench.

All the pots used in this study, were filled with the sterilized sand/soil mixture described below.

## 2.3 Tree and soil

The culture substrate in this study was top soil (0–20 cm layer) taken from the Forestry College nursery, Northwest A&F University in Yangling, Shaanxi Province, China. The soil (pH 7.6, soil/water ratio of 1:2.5 w/v) contained 20 g/kg organic matter, 37 mg/kg available nitrogen, 12 mg/kg available phosphorus, and 207 mg/kg available potassium, as measured with the method used by Bago (2000). This soil was ground, passed through a 2-mm sieve and mixed with fine sand (sand/soil, 1:2 v/v). The mixture was autoclaved at 121°C for 2.5 h before use.

Both *R. pseudoacacia* and *P. orientalis* seeds were surface sterilized with 5% (w/v) calcium hypochlorite for 10 min and rinsed thoroughly 5 times with sterile deionized water. Surface-sterilized seeds were then transferred to Petri dishes containing three layers of sterilized moist filter paper and allowed to germinate in a growth chamber at 28°C. Pre-germinated seeds were sown into seedling nursing containers in sterilized vermiculite. For each species, 30-days old seedlings of similar size were chosen to carry out the experiments.

## 2.4 AM inoculation

In 2017, mycorrhizal inocula consisting of soil, spores, mycelia were taken from an experimental site at the Changwu Research Station for Soil and Water Conservation of the Chinese Academy of Sciences in the Wangdonggou watershed, Shaanxi Province, northwest China (longitude 107°42'E, latitude 35°12'N;

elevation 1206 m, details can be seen in supplemental methods). And we got two AM inocula from native grassland (Native-AM) and mixture-stands of *R. pseudoacacia* and *P. orientalis* (Remodeled-AM).

Both tree compartments (A and C) of each 3-compartment pot were inoculated with 20 g live inoculum of one of the two AM type treatments (Remodeled-AM or Native-AM). The live inoculum was placed 1–2 cm below the seedlings at the time of planting. For conspecific neighbors, two seedlings of the same species were transplanted into the A and C compartments of pots, whereas for heterospecific neighbors, different species were transplanted into the A and C compartments.

Trees were grown for 10 months under ambient light in the greenhouse (25/18 ± 5°C, day/night) and watered as needed with tap water.

## 2.4 Determination of tree growth

After 10 months of growth, tree shoots and roots were collected. The roots of *R. pseudoacacia* and *P. orientalis* in the interaction zone were also collected and sorted out based on their contrasting morphology. Tree shoots were cut at the soil surface, and roots gently removed from pots and washed. Subsamples of fresh roots (2 g) were stored in a 4°C refrigerator for measuring mycorrhizal colonization. The dry biomasses of remaining tree roots and shoots were determined by oven drying at 65°C until constant weight and then weighed for tree biomass determination. The roots used to measure AM colonization were processed as described below, and their mass was mathematically included in total root biomasses.

To determine whether Remodeled-AM affected tree growth at community level, we quantified the pairwise AM-induced feedback effect (Pairwise APSF) values as suggested by Bever et al. (1997):

$$\text{Pairwise APSF} = ((A_{\text{Remodeled}} - A_{\text{Native}}) + (B_{\text{Remodeled}} - B_{\text{Native}})) / (A_{\text{Native}} + B_{\text{Native}})$$

where  $A_{\text{Remodeled}}$  /  $B_{\text{Remodeled}}$  refers to biomass production of species A or B inoculated with the Remodeled-AM community, and  $A_{\text{Native}}$  /  $B_{\text{Native}}$  describes biomass production of species A or B in Native-AM inoculum. Pairwise APSF values were calculated for the trees in mixture.

To facilitate the comparison of our results with that of others, individual APSF values between Native-AM and Remodeled-AM for each tree species were defined as:  $(\text{Biomass}_{\text{Remodeled}} - \text{Biomass}_{\text{Native}}) / \text{Biomass}_{\text{Native}}$ . APSF values > 0, = 0, or < 0 indicated positive, neutral, and negative feedback, respectively.

## 2.5 AM fungal colonization.

Collected root subsamples were chopped into 1 cm segments after clearing, then roots were treated with 10% KOH solution at 95°C for 1.5 h, acidified with 3% HCl for 5 min at room temperature and staining in a 0.05% solution of Trypan blue for 20 min. Afterwards, stained roots were left in a de-staining solution (50% glycerol) to remove colorations from empty root cells overnight. Finally, arbuscular (Col\_Arb), hyphal

(Col\_Hyp) and vesicular (Col\_Ves) colonization levels were assessed under light microscope in about 100 root segments, using the gridline intersect method (Giovannetti and Mosse, 1980).

Spores of AM fungi were extracted from 10 g air-dried substrate from each tree compartment using a wet sieving and decanting method (Daniels and Skipper 1982). Samples were sieved through 150  $\mu\text{m}$ , and then, 53  $\mu\text{m}$ . The number of spores retained on the sieves was counted and spore density (D\_Spore) was calculated. Hyphal length density (D\_Hyphae) was estimated by the modified procedure of Sylvia (1992). 30g soil samples were suspended in a mixture of 100 mL of deionized water and 15 mL of a solution of sodium hexametaphosphate (5%) for 10 min and subsequently shaken vigorously to pass through 150  $\mu\text{m}$  and then 53  $\mu\text{m}$  meshes. The sample was collected on a 45  $\mu\text{m}$  sieve and transferred to a beaker with 200 mL water. A 10 mL aliquot of these hyphal suspension was transferred on a 0.8  $\mu\text{m}$  membrane filter and vacuum-filtered, and hyphae were stained with 2 drops of trypan blue solution (0.05%) for 5 min. D\_Hyphae was estimated under the light microscope (Table S1, Table S2).

## 2.6 Photosynthetic efficiency

A portable photosynthesis system (model 6400; Li-Cor, Lincoln, NE) was used to measure net photosynthetic rate (Photo), transpiration rate (Trmmol), intercellular  $\text{CO}_2$  concentration (Ci), and stomatal conductance (Cond), prior to harvest. Measurements were done at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  light level and an air rate of  $0.5 \text{ dm}^3 \text{ min}^{-1}$ , on the fifth fully expanded leaf from the top of trees. Measurements were recorded after having been steady for two minutes.

Chlorophyll content (Leaf\_G) was measured by SPAD (SPAD-502, Minolta, Tokyo, Japan) at the same day as photosynthetic rate. Chlorophyll fluorescence parameters were recorded using a portable fluorometer (Opti-sciences OSI 30p) for leaves acclimated to darkness for 30 min using leaf clips before measurements were taken. Initial ( $F_0$ ), maximum ( $F_m$ ), and the fluorescence emission in steady state ( $F_s$ ) were evaluated. These basic parameters were used to calculate maximum efficiency of PSII ( $F_v/F_m$ ), the fraction of PSII centers that are open ( $q_L$ ), photochemical quenching ( $q_P$ ) and non-photochemical quenching coefficient (NPQ), non-photochemical quenching of chlorophyll fluorescence ( $q_N$ ), quantum efficiency of photosystem II (PSII) and electron transport rate (ETR). Five replicate measurements were made on each leaf.

## 2.7 Root Morphology

We processed digital images of the intact root system of each tree using a desktop scanner, and root surface area (Area), average root diameter (Aver\_dia), root volume (Volume), and number of tips and forks were quantified using WinRHIZO 2007d (Regent Instruments Inc., Quebec, Canada).

## 2.8 Statistical analyses

Statistical analyses were conducted using R 4.0.0, unless otherwise mentioned. With the R-package vegan, we produced six standardized and centered principal component analysis (PCA) to explore the effect of factors on AM fungal colonization, photosynthesis and root morphology related variables for

each tree species. The significance of the effect of AM type, neighbor tree and BIL on the dissimilarity of *R. pseudoacacia* and *P. orientalis* - related AM fungal colonization, photosynthesis and root morphology were tested with PERMANOVA using the *adonis()* function. We also extracted the site scores of the first two axes of each PCA to represent the characteristics of these dataset in further analyses. Hereafter, the data about AM fungal colonization, photosynthesis and root morphology were presented as APC1 and APC2, PPC1 and PPC2, and RPC1 and RPC2, respectively (Table S3, Table S4). For both trees, APC1 mainly correlated with hyphal and vesicular formation in roots, and higher APC1 can be seen as higher energetic cost for symbiosis. APC2 mainly correlated with root arbuscular formation, soil hyphae/spore density, and it can be seen as the probability that nutrients exchange through AM symbiosis. For both *R. pseudoacacia* and *P. orientalis*, PPC1 correlated with the efficiency of PSII photochemistry, PPC2 correlated with the gas exchange. High PPC1 or PPC2 would imply trees have efficient photosynthesis. RPC1 correlated with root length, tips, forks and surface area, and higher RPC1 means tree can get the soil nutrients mainly by itself. The second axis, RPC2, mainly correlated with root diameter, and higher RPC2 means plant had weak ability to uptake nutrients by itself (Fig S5).

To assess the significance of the effects of AM type, neighbor trees and BIL on tree biomass, photosynthesis and root morphology and colonization of AM fungi, three-way ANOVA was performed using *aov()* function of the 'base' packages. Comparisons of AM type treatments were performed using independent one sample t-tests at  $p < 0.05$  using *t.test()* function of the 'base' packages. And any non-normal data were transformed to meet the requirements of the test.

Based on the above results, we found that Remodeled-AM and Native-AM had different effects on tree growth. To clarify the different effects of AM type treatments on tree growth under different neighbors and BIL treatments, AM-induced plant-soil feedback effects were calculated. The Student's t test was used to assess the difference in APSF at levels of individual species and communities of mixture of *R. pseudoacacia* and *P. orientalis*, while the difference between conspecific and heterospecific neighbor trees and the difference between the BIL treatments (i.e. no pore, 0.45- $\mu\text{m}$ , 38- $\mu\text{m}$ , and 380- $\mu\text{m}$  nylon meshes) were assessed by t-tests. Only two BIL treatments - i.e. those created by the nylon mesh sizes 38- and 380  $\mu\text{m}$ , allowing interaction through the soil solution + AM hyphae and allowing full interaction, were kept for further analysis because only these treatments had significantly different feedback effects (Figs. 3 and S5).

Multiple linear regression analysis was conducted to assess the effects of APC1, APC2, PPC1, PPC2, RPC1, RPC2, neighbor trees and BIL on APSF. The relative importance of each predictor was determined using the Lindeman-Merenda-Gold relative importance method with the 'boot.relimp' function of the 'relaimpo' package. The 95% confidence interval for the relative importance of each predictor was estimated with 1000 bootstrap iterations.

Structural equation modelling (SEM) was used to assess direct and indirect effects of neighbor trees, BIL, APC1, APC2, PPC1, PPC2, RPC1, RPC2 on feedback effects under different neighbor trees and BIL treatments. SEM was performed using the 'sem' function of the 'lavaan' package.

### 3. Results

#### 3.1 Effects of AM fungi, neighbor tree, and pore size on plant biomass

The main effect of AM type was significant on the biomass of *P. orientalis* only. AM type influenced *R. pseudoacacia* biomass only through interactions with neighbor tree and BIL (Table 1). AM type had different effects on *R. pseudoacacia* and *P. orientalis* biomasses. Overall, *R. pseudoacacia* tended to accumulate more total biomass (about 9%) with Remodeled-AM, while *P. orientalis* accumulated significantly less biomass (25% reduction) (Fig. S2).

**Table 1.** *F* values expressing the significance level of the effects of AM-type, neighbor tree, belowground interaction level (BIL) and the interactions of these factors on tree total biomass (TB), aboveground biomass (AB) and belowground biomass (BB) of *R. pseudoacacia* and *P. orientalis*.

|                        | <i>R. pseudoacacia</i> |                |               | <i>P. orientalis</i> |                  |                  |
|------------------------|------------------------|----------------|---------------|----------------------|------------------|------------------|
|                        | TB                     | AB             | BB            | TB                   | AB               | BB               |
| AM type(A)             | 0.442                  | 0.043          | 1.647         | <b>9.502**</b>       | <b>6.558*</b>    | <b>8.478*</b>    |
| Neighbor(N)            | <b>5.239*</b>          | 3.618          | <b>4.291*</b> | <b>35.038***</b>     | <b>26.825***</b> | <b>22.077***</b> |
| Interaction level(BIL) | <b>6.22**</b>          | <b>5.286**</b> | <b>4.081*</b> | 0.451                | 0.21             | 1.477            |
| A×N                    | 0.099                  | 0.47           | 0.303         | <b>5.588*</b>        | <b>6.794*</b>    | 0.002            |
| A×BIL                  | 2.405                  | 2.109          | 1.210         | 0.892                | 1.111            | 0.942            |
| N×BIL                  | <b>3.248*</b>          | <b>2.948*</b>  | 2.030         | 0.382                | 0.683            | 0.558            |
| A×N×BIL                | <b>4.358**</b>         | <b>3.945*</b>  | 2.388         | 0.43                 | 0.647            | 0.839            |

\*, \*\*, \*\*\* and *F* values in bold face indicate significant effects and their level of significance (*p* values <0.05, <0.01 and <0.001, respectively).

The effects of BIL and neighbor tree were the strongest effects determining *R. pseudoacacia* total biomass, while *P. orientalis* biomass was only influenced by AM type and neighbor tree (Table 1, Fig. 2). For *R. pseudoacacia* total and aboveground biomasses, 3-way interaction reflected that the highest biomass was found when this tree was grown in full BIL with a heterospecific neighbor and remodeled AM type (Fig. 2, S3). For *P. orientalis*, the significant interaction AM type x neighbor tree reflected that the inhibition of *P. orientalis* total biomass production by remodeled AM was relieved by a *R. pseudoacacia* neighbor (Fig. 2, S4).

#### 3.2 Effects of AM type, neighbor tree, and BIL on plant photosynthetic, root morphology, and colonization

To explore the effects of AM type, neighbor tree and BIL on plant photosynthetic efficiency, root morphology and colonization, we conducted principal component analysis (Figs. S5, Figs. S6). For

*R. pseudoacacia*, the largest effect on all traits was exerted by BIL (photosynthesis:  $R^2=0.16^{**}$ ; root:  $R^2=0.13^{***}$ ; colonization:  $R^2=0.13^{***}$ ). For *P. orientalis*, the interaction of AM type and BIL was the dominant effect (photosynthetic:  $R^2=0.12^{***}$ ; root:  $R^2=0.17^{**}$ ; colonization:  $R^2=0.09^{**}$ ).

As described in the statistical methods, we extracted the site scores of the first two axes to simplify further analysis. APC1, which was associated with AM fungi colonization, was affected by AM type in both tree species, according to three-way ANOVAs (Table S3, S4). In *R. pseudoacacia*, BIL was the main factors affecting PPC1, PPC2, RPC1, RPC2. The highest PPC1, PPC2 and RPC2 and lowest RPC1 score values appeared when pore size of 380  $\mu\text{m}$  allowed full BIL and these score values were different from those associated with a pore size of 38  $\mu\text{m}$  only allowing interactions through the soil solution and hyphae ( $p < 0.05$ ). Other factors and interaction seldom had effects on score values in *R. pseudoacacia*. *P. orientalis* RPC1 was affected by AM type, neighbor trees and the interaction of these factors, and the lowest values appeared with conspecific neighbor trees and remodeled AM type, similarly to total biomass (Table S3, S4). For *P. orientalis*, AM type would reverse the trend that APC1, APC2, PPC1, RPC1, RPC2 changing with wall propensity (Fig. 3). RPC1 was affected by AM type, neighbor trees and their interaction (Fig. S6).

### 3.3 Feedback effects of AM fungi as affected by neighbor tree and BIL

ANOVA results revealed significant effects of the interaction between neighbor trees and BIL on the feedback effect of remodeled AM fungi on *R. pseudoacacia*. Individual APSF was neutral regardless of neighbor tree and BIL treatments, except at full BIL where *R. pseudoacacia* biomass was much larger in presence of *P. orientalis* than with a *R. pseudoacacia* neighbor (Fig. 3). The feedback effect of AM fungi on *P. orientalis* depended on the neighbor tree. *P. orientalis* experienced negative to neutral feedback in the presence of a conspecific tree, but feedback was usually neutral in the presence of *R. pseudoacacia* (Fig. 3).

Pairwise APSF of remodeled AM inoculum, which was calculated only for pots with heterospecific neighbor trees, showed that reciprocal feedback values differed with different BILs ( $p < 0.05$ , Fig. S7). ANOVA results revealed mean pairwise APSF were significantly higher than zero when pore size was 380  $\mu\text{m}$ . i.e. under conditions of full belowground interaction. This result could be attributed to greater positive individual APSF values for *R. pseudoacacia* (Fig. S7, Fig. 3).

### 3.4 Plant-root-derived feedback effects and controlling factors

For both species, the BIL treatments allowing hyphae but no roots to interact (38  $\mu\text{m}$ ) had a different influence on individual APSF than the BIL treatment allowing hyphae and roots (380  $\mu\text{m}$ ) contact between trees (Fig. 3). Thus, we further explored these treatments. Results of multiple linear regression showed that APC1, APC2, neighbor tree and PPC1 together explained 55% of the variations in the individual APSF on *R. pseudoacacia*. In contrast, 54% of the variations in the feedback effect of the individual APSF on *P. orientalis* was explained by RPC1, BIL, APC1 and APC2 together. Individual APSF was best predicted by APC2 in *R. pseudoacacia* and by RPC1 in *P. orientalis*, respectively (Fig. 4).

The SEM results showed that neighbor tree and BIL affected feedback effects on *R. pseudoacacia* and *P. orientalis* growth through totally different mechanisms. Coexistence with *P. orientalis* was related with a direct positive influence of AM fungi on *R. pseudoacacia* biomass. In *P. orientalis*, AM root interaction was strongly and directly related to a negative feedback effect of AM fungi on the biomass of this evergreen. Greater negative feedback effects on *P. orientalis* biomass were mainly associated with high number of root tips and forks. Both neighbor trees and AM root interaction were indirectly associated with feedback effect through APC1 or APC2. However, contrary to *P. orientalis*, the feedback effect of AM fungi on *R. pseudoacacia* was positively related to high AM colonization (APC1) and soil hyphae density (APC2) (Fig. 5).

## 4. Discussion

Afforestation in the Loess Plateau using the legume tree *R. pseudoacacia* and the evergreen tree *P. orientalis* - both mycorrhizal hosts - had changed the community of resident AM fungi (Chen et al., 2018b). Here, we further show that such a change in plant cover can modify the influence of the AM symbiosis on the growth of trees and shed light on the mechanisms involved in feedback effects. Using compartmented pots with different mesh pore sizes to create levels of belowground interaction between conspecific and heterospecific tree pairs, and AM fungi from afforested and non-afforested soil, we showed that AM root contact is a crucial condition for the creation of feedback effects in co-cultured *R. pseudoacacia* and *P. orientalis*.

### 4.1 AM type x neighbor trees affecting plant growth

Soil microbes can influence the relationship among coexisting trees in mixed stands and, hence, tree community structure (Bever et al., 1997, 2015). There is evidence that arbuscular mycorrhizal (AM) trees diversity can benefit seedlings survival, especially in sites dominated by conspecific adults (Liang et al., 2020). Our results showed a similar tendency. The biomass of both *R. pseudoacacia* and *P. orientalis* was affected by the interaction of the AM community composition and neighbor tree identity.

How conspecific and heterospecific neighbors influence the growth of individual plants in a community has raised attention and became central in the field of plant community development and health (Niering et al., 1963; Callaway et al., 2002; Callaway, 2007; Fajardo and McIntire, 2011). It was discovered that *R. pseudoacacia* as a neighbor could promote the growth of *P. orientalis* through nitrogen fixation and reduction of vesicular colonization (Chen et al 2018a).

In our study, the effect of neighbor trees was AM fungi-dependent (Table 1, Fig. 2). The biomass of *P. orientalis* decreased with the remodeled AM community (Fig. 2, S4). Previous research reported that the different influence of AM fungi on different plant-plant interaction, was related with the distribution of nutrients among plant species (Casper and Castelli 2007; Zhou et al 2018). Therefore, it was assumed that AM fungi remodeled by the influence of *R. pseudoacacia* / *P. orientalis* mixed stands reduced the access of *P. orientalis* to soil resources when growing with conspecific neighbor trees, but not with *R. pseudoacacia* neighbors. Which species of AM fungi are responsible for this effect remains to be found.

## 4.2 Feedback on tree growth depends on neighbor identity and interaction intensity

Plant-soil feedbacks are plant-induced changes in abiotic and biotic soil properties that in turn influence the performance of the same or other plant species (Ehrenfeld et al., 2005; van der Putten et al., 2013). The plant-soil feedbacks play a crucial role in driving species composition and plant diversity, community succession, and plant invasions (Bever et al., 2015; Castle et al., 2016; van der Putten et al., 2010). The involvement of AM fungi in soil feedback on plants and ultimately on the structure of plant communities, was demonstrated and widely discussed (García-Parisi and Omacini, 2017).

Our results indicate the feedback effects on a tree can be modulated by the influence of a neighbor tree on the pathways of interaction between these trees. Using *R. pseudoacacia* and *P. orientalis*, we found that conspecific neighbors can exert a different influence on AM fungi-induced feedback effects on tree production. A plant can be affected by its neighbors because of both aboveground and belowground interactions (Cahill 1999). Few previous studies have focused on belowground processes (Lin et al., 2017; Stampe and Daehler, 2003). However, it is of great importance to understand the factors determining how the plant species used in afforestation influence their soil environment, and may create feedback effects on tree stand productivity through their selective effect on AM fungi (Chen et al., 2008; Tateno et al., 2007).

Mycorrhizal fungi have positive effects on their host in promoting nutrients uptake and protecting them from biotic and abiotic stress (Smith and Read, 2008; van der Heijden et al., 2007). These benefits are derived from the ability of arbuscular mycorrhizas to explore thoroughly the soil (Barto et al., 2012). Root interactions, including biological, physical and chemical interactions, occurring between plants, are an important component of plant interaction (Schenk, 2006).

We failed to detect significant effects of AM hyphal networks on feedback effects on any tree pair; 38  $\mu\text{m}$  pore size allowing hyphal connection through dividing walls did not influence tree growth differently than did non-porous walls. This is consistent with the result from Ren et al. (2017). However, we show that AM root interaction is involved in the soil feedback effects in mixed tree stands.

## 4.3 Factors controlling arbuscular mycorrhiza-related feedback on trees

In this experiment, we observed different feedback effects of afforestation on *R. pseudoacacia* and *P. orientalis*. The neighborhood of *P. orientalis* was the necessary condition for a feedback effect on *R. pseudoacacia*. Feedback effects were significantly different in presence and absence of inter-species root interaction. Our results further showed that the abundance of arbuscules and spore and hyphae density in the soil (APC2), contributed 42% of the feedback effects on *R. pseudoacacia*. That means *R. pseudoacacia* would have stronger ability to exchange nutrients through AM symbiosis in presence of inter-species root interaction, thus, experiencing positive APSF. While it was the number of root tips and forks and to surface area (RPC1) that contributed most to variation in the feedback effects on *P. orientalis*. So, inoculated with remodeled AM fungi, *P. orientalis* tended to grow with a “fast” belowground resource acquisition strategy and weak ability to outsource resource acquisition via AM fungi (Bergmann

et al., 2020). The higher AM colonization would lead to lower APSF (Fig. 5). But the reason why it experienced changing APSF from neutral to negative with or without inter-species AM root interaction is still unclear.

Its expansive root system, long lifespan (Rice et al., 2004; Tatenos et al., 2007; Yang et al., 2014), and high affinity for the AM symbiosis should make *R. pseudoacacia* a better host than *P. orientalis*, for reintroducing local AM fungi into restoration area, especially at the initial stage of restoration (Noyd et al., 1995). And *R. pseudoacacia* not only persists, but is an invasive species in the Loess Plateau that commonly experiences positive feedback from resident AM fungi (Ehrenfeld et al., 2005; Meisner et al., 2014; Reynolds et al., 2003). Furthermore, *R. pseudoacacia* was shown as the strongest influence reshaping the AM community in mixed stands, and AM community change was shown to benefit this legume tree (Wolfe and Klironomos 2005; Mummey and Rillig 2006; Niu et al. 2007; Zhang et al. 2010).

Here, the feedback effects on *P. orientalis* were negative across all interaction pathways. This was in line with the works of Bennett et al. (2017) and Liang et al. (2020). Our SEM results further demonstrated that the variation in feedback effects on *R. pseudoacacia* and *P. orientalis* was explained, at least partly, by AM root colonization, photosynthesis, and root morphology. However, direct effects of neighbor tree and BIL on feedback effects suggest that competition between trees was another important factor of variation. Competition for soil nutrients between these trees and the higher competitive ability of *R. pseudoacacia* over *P. orientalis* are known (Chen et al., 2018b).

In mixed stands, the mixture ratio of species has direct effect on their biomass (Huang et al., 2022). Here, the inhibition of *P. orientalis* growth by remodeled AM fungi in monoculture suggests that this tree species should not be planted at high density in mixed stands of *R. pseudoacacia* and *P. orientalis*. Field experiments are needed to determine the ration of *R. pseudoacacia* and *P. orientalis* most appropriate for afforestation in the Loess Plateau and elsewhere.

## 5. Conclusion

The data presented here provided empirical evidence that the AM fungal community remodeled by afforestation of the Loess Plateau with mixed stands of *R. pseudoacacia* and *P. orientalis* can increase tree stand productivity. The remodeled AM community acted mainly on regulating the ability to outsource resource acquisition and increased the competitive ability of *R. pseudoacacia* over *P. orientalis*. Our findings provide evidence that high density of *P. orientalis* is not recommendable in mixed stands of *P. orientalis* and *R. pseudoacacia*. By linking the framework of feedback effects with plant interactions, we revealed potential mechanisms of regulation by remodeled AM communities on plant performance during coexistence. It appears that physical AM root interaction among plants may be crucial for species coexistence, especially at the early stage of plant growth. This is important in afforestation of degraded sites and subsequent site management.

## Declarations

## Author contributions

RJ, ML, SY collected the samples and XL, WD, QL, KL performed the data analysis and XL and MS wrote the manuscript.

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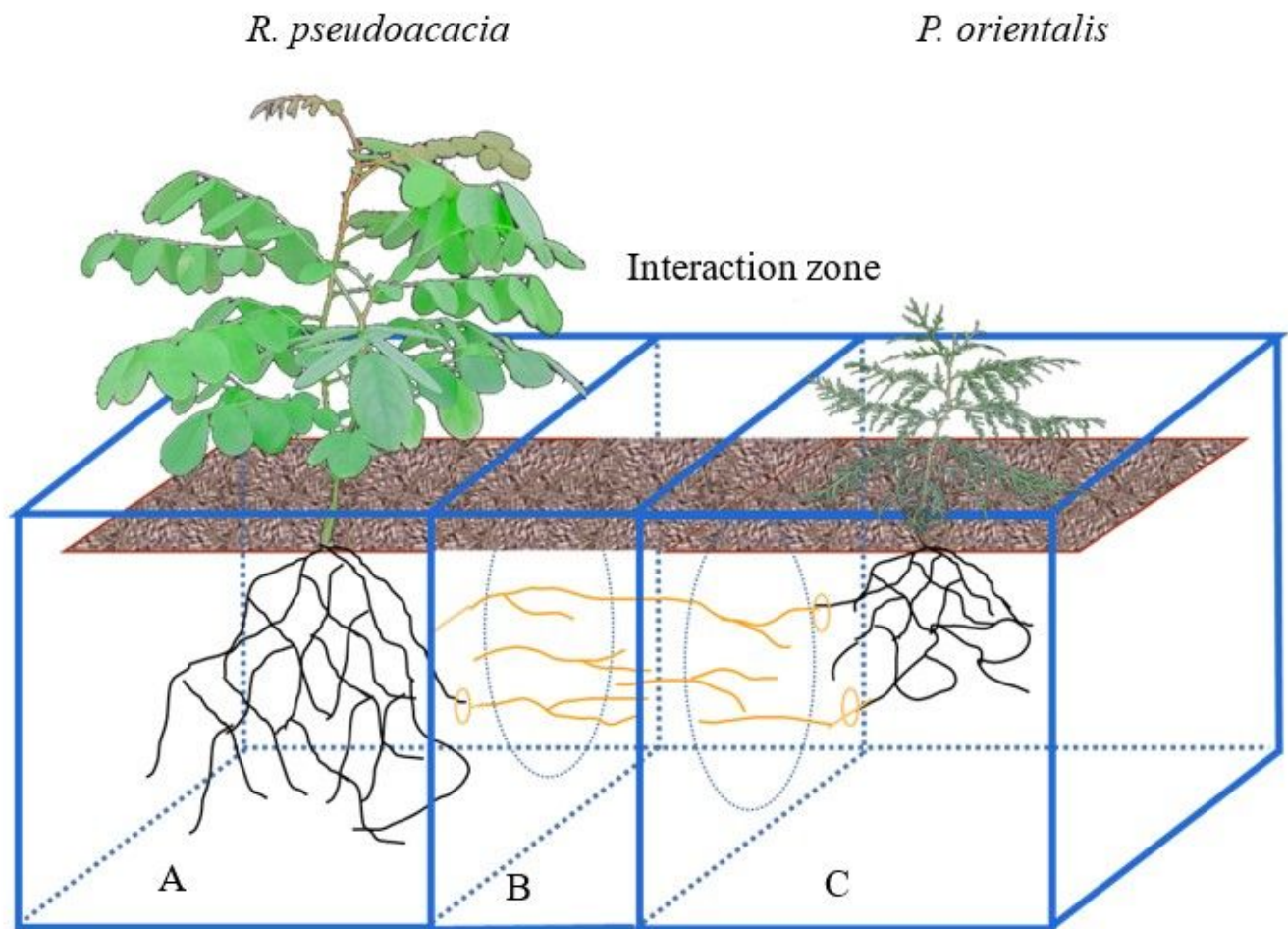
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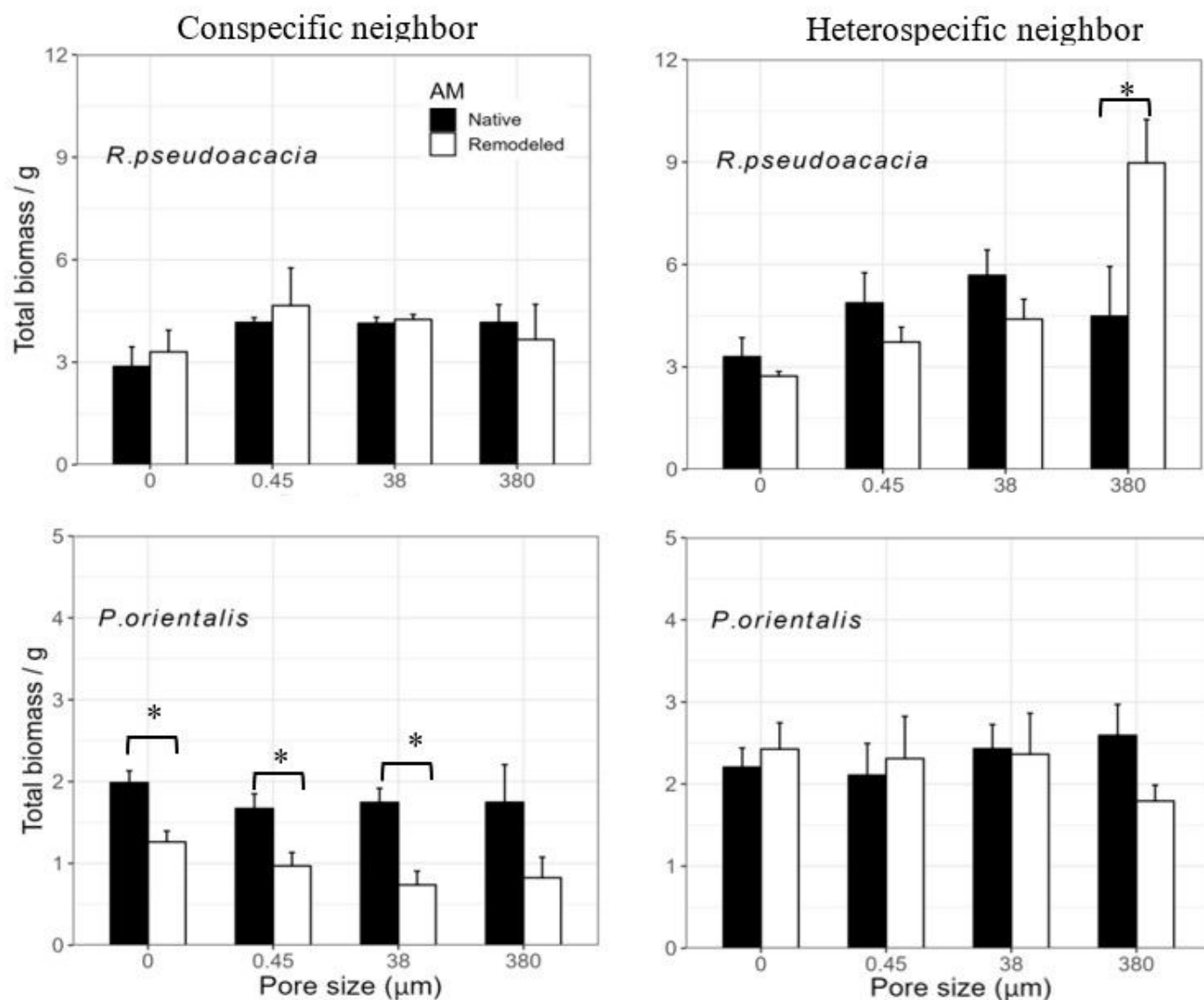
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## Figures



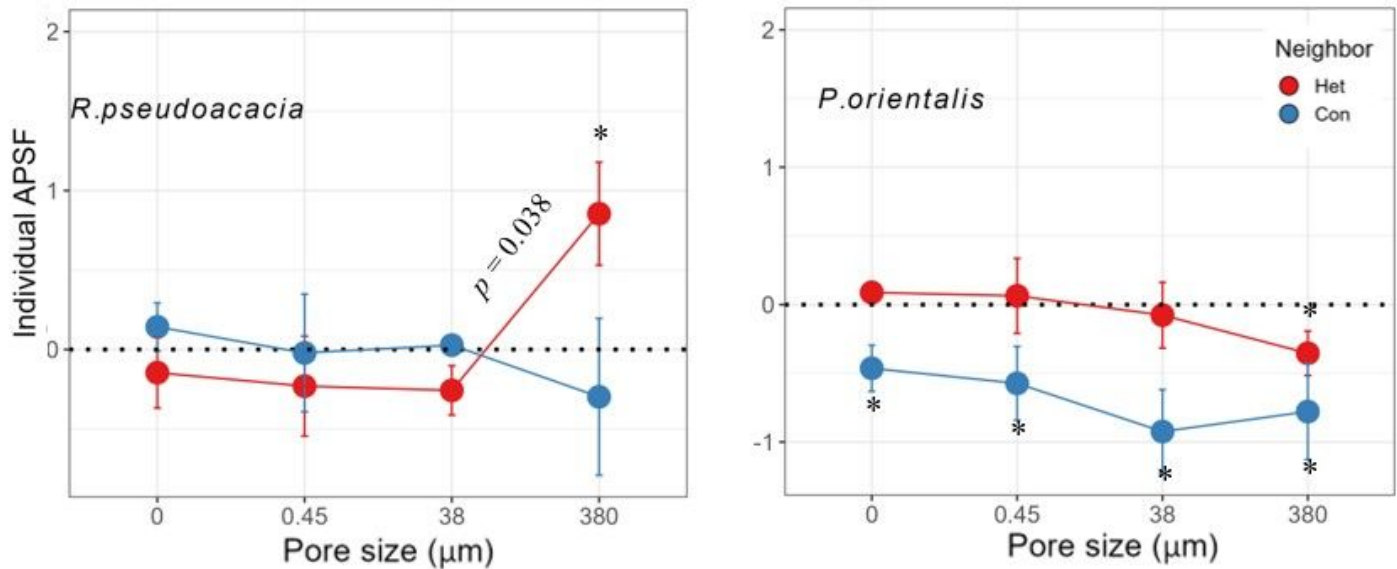
**Figure 1**

Experimental pot design. The A and C compartment received a tree. Compartment B is a interaction zone between compartments A and C. The circular holes at the interface of compartments A and B and C and B were covered with material of different porosity to create four levels of belowground interaction between trees: (1) a plastic film allowing no belowground interaction, (2) a nylon mesh with a pore size of 380  $\mu\text{m}$  allowing full belowground interaction (3) a 38- $\mu\text{m}$  nylon mesh excluding fine roots, but allowing fungal hyphae to cross, and (4) a 0.45- $\mu\text{m}$  nylon mesh excluding both roots and hyphae, giving the way only to the soil solution possibly carrying soil bacteria and archaea. The orange line represents the hyphae.



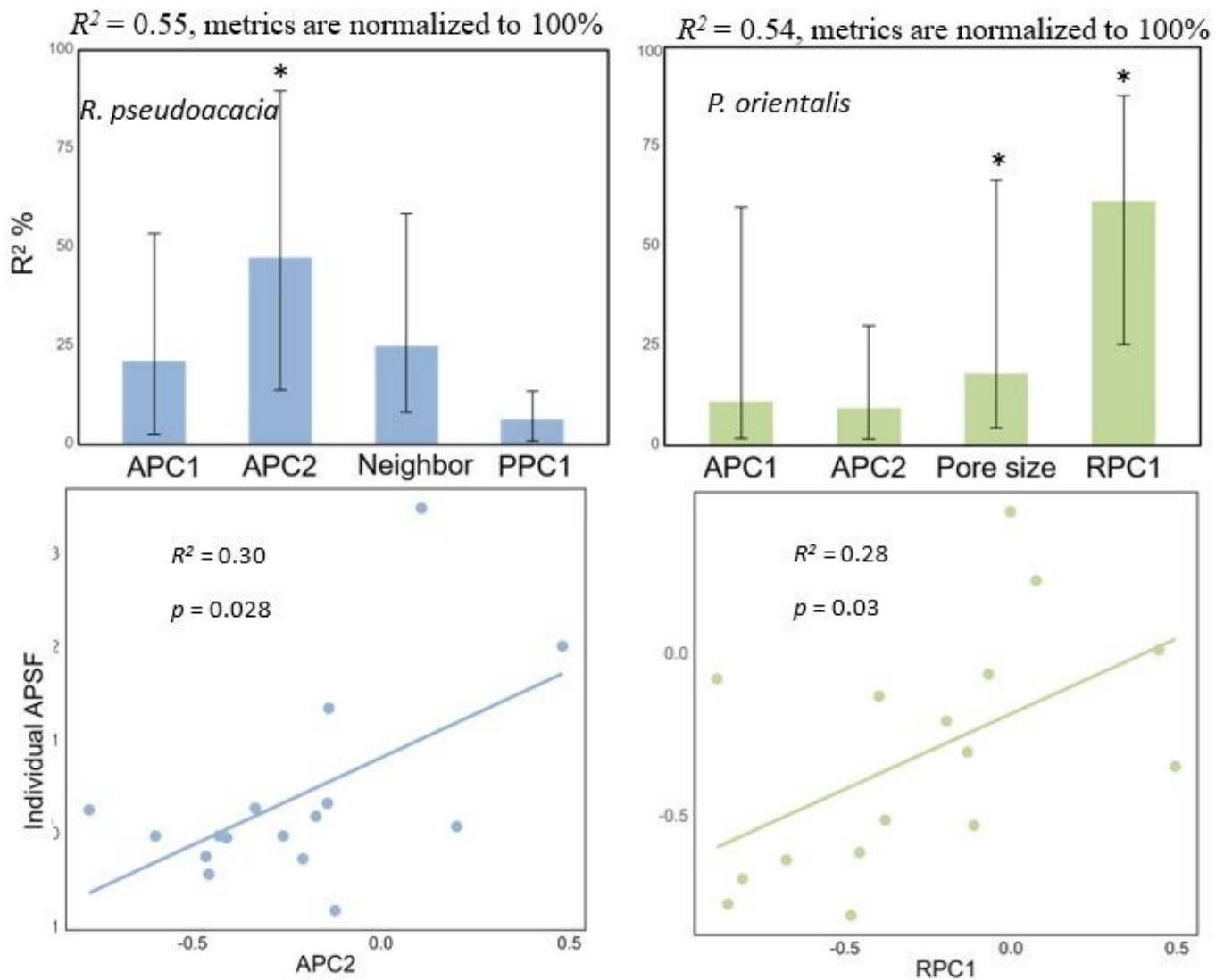
**Figure 2**

Total biomass (mean  $\pm$  se) of *R. pseudoacacia* and *P. orientalis* trees as affected by a heterospecific or conspecific neighbor, a native or remodeled AM community, and the level of belowground interaction between trees (BIL), as created by different pot wall porosities: (1) no belowground interaction in absence of pores, (2) pore size of 380  $\mu$ m allowing full belowground interaction (3) 38- $\mu$ m pores excluding fine roots, but allowing fungal hyphae to cross, and (4) 0.45- $\mu$ m pores excluding both fine roots and hyphae and giving the way only to the soil solution possibly carrying soil bacteria and archaea. Asterisks indicate significant ( $p < 0.05$ ) effects of AM type within a belowground interaction level.



**Figure 3**

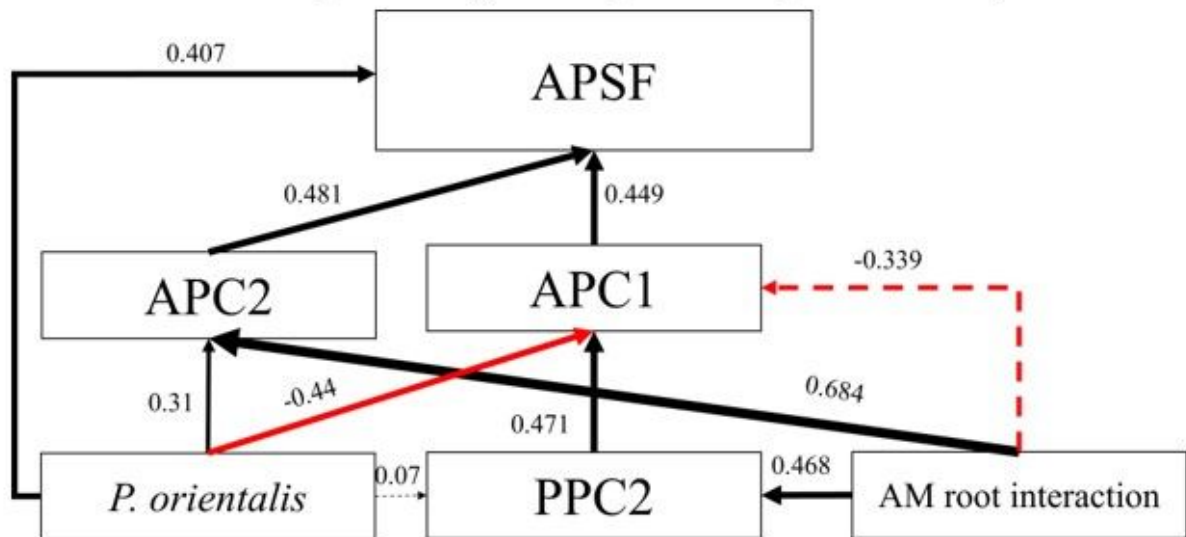
The individual APSF of *R. pseudoacacia* and *P. orientalis* planted with a conspecific (Con) or heterospecific (Het) neighbor tree under different BIL treatments: pore size 0, no belowground interaction; 380 μm, full belowground interaction; 38-μm, interaction through the soil solution and AM hyphae; 0.45-μm, interaction through the soil solution only. *P*value adjacent to lines represent significant differences in individual APSF between two different BIL treatments with same neighbor trees. Asterisks adjacent to dots represent significant differences in individual APSF when plants were grown with native vs. remodeled AM type based on one-sample t-test.



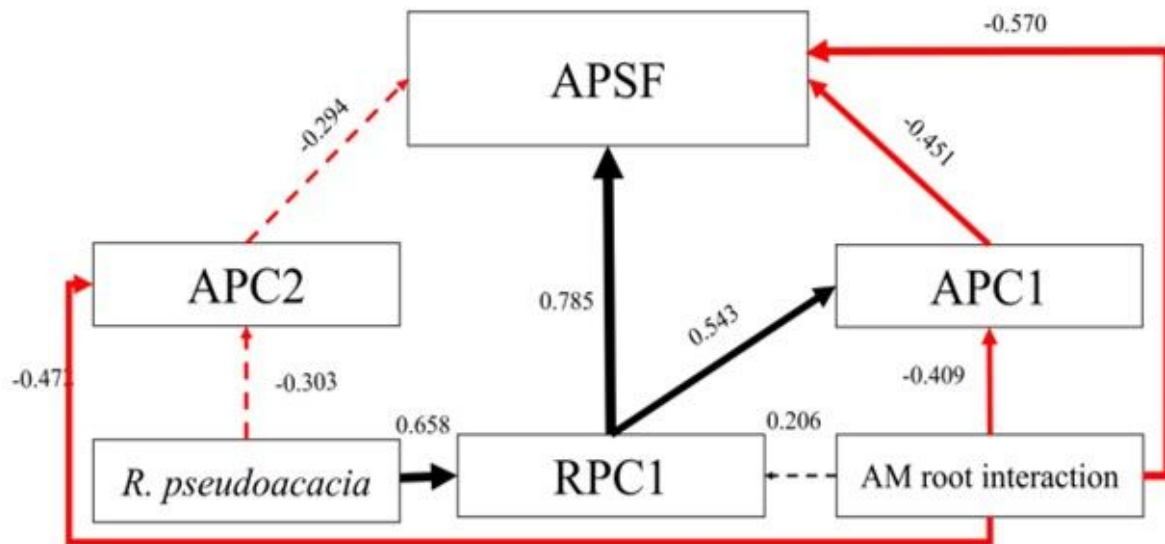
**Figure 4**

The relative importance the metrics of APC1, APC2, PPC1 and neighbor tree as predictors of individual APSF on *R. pseudoacacia* (top left), or APC1, APC2, RPC1, BIL for *P. orientalis* (top right), respectively, and relationships of individual APSF with APC2 and RPC1 for *R. pseudoacacia* (bottom left) and *P. orientalis*, respectively (bottom right). The error bars show 95% confidence intervals. The asterisks indicate the coefficient of the factor in multiple linear regression is significant.

*R. pseudoacacia*  $R^2 = 0.48, X^2 = 3.26, p = 0.521, CFI = 1.00, SRMR = 0.061, RSMEA = 0.000$



*P. orientalis*  $R^2 = 0.556, X^2 = 0.61, p = 0.960, CFI = 1.00, SRMR = 0.029, RSMEA = 0.000$



**Figure 5**

A schematic representation of the structural equation modeling (SEM) analysis used to identify how neighbor trees and AM root interaction control the feedback effects induced by remodeled AM community on *R. pseudoacacia* (top) and *P. orientalis* (bottom). Path coefficients and coefficients of determination ( $R^2$ ) were calculated after 1000 bootstraps. Solid and dash arrows denote significant and insignificant relationships, respectively. The arrow width represents the strength of significant standardized path coefficients which are adjacent to the arrows. Black and red arrows represent positive and negative relationship, respectively.

## Supplementary Files

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- [Supplementalfiles.docx](#)