Laccaria bicolor Mobilizes both Labile Aluminum and Inorganic Phosphate in Rhizosphere Soil of *Pinus massoniana* Seedlings Field Grown in a Yellow Acidic Soil

Xirong Gu,^a Jie Li,^a Xiaohu Wang,^a Xinhua He,^{a,b} Yao Cui^a

^aCentre of Excellence for Soil Biology, College of Resources and Environment, Southwest University, Beibei, Chongqing, China

^bSchool of Biological Sciences, University of Western Australia, Perth, Western Australia, Australia

ABSTRACT Plant growth is often limited by highly activated aluminum (Al) and low available phosphorus (P) in acidic soil. Ectomycorrhizal (ECM) fungi can improve their host plants' Al tolerance by increasing P availability while decreasing Al activity *in vitro* or in hydroponic or sand culture systems. However, the effect of ECM fungi on inorganic P (IP) and labile Al in acidic soil in the field, particularly in conjunction with Al treatment, remains poorly understood. The present study aimed to determine the influence of ECM fungal association on the mobilization of IP and labile Al in rhizosphere soil of host plants grown in the field with external Al treatment and the underlying nutritional mechanism in plant Al tolerance. To do so, 4-week-old *Pinus massoniana* seedlings were inoculated with three ECM isolates (*Laccaria bicolor* 270, *L. bicolor* S238A, and *L. bicolor* S238N) and grown in a Haplic Alisol field with or without Al treatment for 12 weeks. Results showed that *L. bicolor* association enhanced the available P depletion and facilitated the mobilization of IP and labile Al, in turn improving the capacity of host plant to use Al-bound P, Ca-bound P, and occluded P, particularly when *P. massoniana* seedlings were inoculated with *L. bicolor* S238A. Inoculation with *L. bicolor* isolates also enhanced the solubility of labile Al and facilitated the conversion of acid-soluble Al into exchangeable Al. Our findings suggested that ECM inoculation could enhance plant Al tolerance in the field by mobilizing IP to improve the P bioavailability but not by decreasing Al activity.

IMPORTANCE Here, we reveal the underlying nutritional mechanism in plant Al tolerance conferred by ectomycorrhizal (ECM)-fungus inoculation in the field and report the screening of a promising ECM isolate to assist phytoremediation and afforestation using *Pinus massoniana* in acidic soil in southern China. This study advances our understanding of the contribution of ECM fungi to plant–ECM-fungus symbiosis and highlights the vital role of ECM-fungus inoculation in plant Al tolerance. In addition, the results described in the present study confirm the importance of carrying out studies in the field rather than only *in vitro* studies. Our findings strengthen our understanding of the role of ECM-fungus association in detecting, utilizing, and transporting unavailable nutrients in the soil to enhance host plant growth and adaptability in response to adverse habitats.

KEYWORDS ectomycorrhizae, aluminum activation, aluminum tolerance, phosphorus availability, plant nutrition

Aluminum is the most abundant metal in soil, and Al toxicity is a primary limiting factor for plants grown in acidic soil, which accounts for ~40% of the world's arable land (1). In general, Al can be released from minerals and become toxic when the soil gets acidic (2). Increased nitrogen deposition and mineralization *in situ* resulting from climate warming, a global issue with impacts extending beyond industrialized

Citation Gu X, Li J, Wang X, He X, Cui Y. 2020. *Laccaria bicolor* mobilizes both labile aluminum and inorganic phosphate in rhizosphere soil of *Pinus massoniana* seedlings field grown in a yellow acidic soil. Appl Environ Microbiol 86:e03015-19. <https://doi.org/10.1128/AEM.03015-19>.

Editor Isaac Cann, University of Illinois at Urbana-Champaign

Copyright © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Xirong Gu, gxr0956@163.com, or Xinhua He, xinhua.he@uwa.edu.au.

Received 3 January 2020

Accepted 5 February 2020

Accepted manuscript posted online 14 February 2020

Published 1 April 2020

FIG 1 Conceptual diagram displaying Al toxicity from acidic soil (a) and potential plant Al tolerance mechanisms involved in IP mobilization and labile-Al immobilization by ectomycorrhizal (ECM) fungus *in vitro* (b) or ECM inoculation in the field (c).

regions, exacerbate soil acidification and Al mineral dissolution (3). Al toxicity limits plant growth, accompanied by a decrease in nutrient uptake (Fig. 1a), mainly by inhibiting root elongation (4) and subsequently reducing the area of root contact with soil and nutrients (5). In addition, the activated Al fractions, which are often considered exchangeable Al (Al^{3+}) and hydroxyl Al [$Al(OH)^{2+}$ and $Al(OH)_2^+$] (6), may precipitate further with phosphate, leading to a decrease in phosphorus availability (7–9). This reaction exacerbates P deficiency in acidic soil, where P is easily adsorbed onto soil mineral surfaces and hardly mobilized after precipitation by iron and Al oxide minerals (9–12), although total P (TP) is adequate for plant growth and development. Consequently, both highly activated Al and low available P result in a decline of plant biomass production in acidic soil (13, 14). Therefore, Al toxicity has been presumed to be a critical factor in “new-type forest decline” in central Europe and North America (15). Several studies in China also reported that Al toxicity decreased the productivity of forests, including Masson pine (*Pinus massoniana* Lamb.) and Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook.] grown in acidic soil in southern China (16–21).

Studies have demonstrated that plants can establish symbiotic associations with ectomycorrhizal (ECM) fungi to facilitate Al tolerance by increasing available P concentration while decreasing activated Al concentration *in vitro* or in hydroponic and sand culture systems (Fig. 1b). For example, *Laccaria bicolor* 1283 could detoxify Al^{3+} *in vitro* through the formation of a chelate complex with Al^{3+} and the release of P from $AlPO_4$ in a modified Melin-Norkrans agar medium (22). Similarly, *Pisolithus tinctorius* cultured *in vitro* on pond ash facilitated tolerance to Al and its detoxification through the exudation of low-molecular-weight organic acids that chelate Al^{3+} (23). In addition, *Lactarius deliciosus* and *Suillus luteus* cultured *in vitro* on Pachlewski liquid medium could reduce active Al^{3+} concentrations while increasing P availability in response to Al stress (24). Compared with their non-ECM-inoculated counterparts, both biomass production and P uptake were significantly higher in *L. bicolor*-inoculated *P. massoniana* grown in acid yellow soil (25) and *Pisolithus tinctorius*-inoculated *Pinus densiflora* cultured on perlite containing Al^{3+} (26). In a neutral purple soil (pH 6.8), Al-bound P (Al-P) and Ca-bound P (Ca-P) decreased significantly when the soil was incubated *in vitro* with *Boletus* sp. 7 or *Cenococcum geophilum* 4, compared with non-ECM controls (27). In addition, *Paxillus involutus*-inoculated *Pinus sylvestris* showed increased levels of P release from apatite (28).

Numerous studies also found that Al uptake was increased with external Al addition

by ECM fungi or ECM seedlings. For example, the uptake of Al by *Laccaria bicolor*, *L. deliciosus*, *Lactarius rufus*, *Hebeloma crustuliniforme*, *S. luteus*, and others was increased with an increase in Al concentration *in vitro* (18, 29, 30). Meanwhile, Wang et al. (24) showed that ECM fungi, including *L. deliciosus* and *S. luteus*, produce oxalates, which could decrease active Al^{3+} concentrations via formation of chelates. The uptake of Al was also enhanced in *L. bicolor*-inoculated *P. massoniana* (25), *P. tinctorius*-inoculated *Pinus strobus* seedlings (31), and *Hebeloma crustuliniforme*-inoculated *Picea abies* (32). In addition, studies have reported that the acidification of rhizosphere soil could facilitate the dissolution of labile Al (33). Scholl et al. (34) and Zhou et al. (35) also found that exchangeable Al concentrations in soil solution were increased when ECM fungi dissolved soil mineral particles to release P. Similarly, a previous *in vitro* study showed that *L. bicolor* S238A and *L. bicolor* S238N increased the mycelial uptake of Al after addition of Al (30). Accumulations of Al in an acidic soil (pH 4.64) were significantly higher in three ECM isolate-inoculated *P. massoniana* seedlings than in non-ECM seedlings (25).

At present, the role of ECM inoculation with regard to inorganic P (IP) availability after Al treatment in acidic soil remains poorly understood. In particular, information regarding whether ECM fungi play a role in the immobilization of activated Al in the field is limited. Based on the increased uptake of P and Al in ECM fungi (30) or ECM seedlings (25) in previous studies, we hypothesized that ECM fungal association can improve the availability of P and Al in the field (Fig. 1c). Therefore, the present study aimed to address the possible influence of ECM fungal association on the mobilization of IP and labile Al in the rhizosphere soil of host plants grown in the field after external Al treatment, with additional investigation into nutritional mechanisms in plant Al tolerance. In doing so, we designed a field study using *P. massoniana*, a major tree species widely growing in acidic soil in south China and severely suffering from Al toxicity, to address the idea that ECM-fungus inoculation could (i) influence the availability of P and Al, (ii) facilitate the mobilization of both IP and labile Al, and (iii) enhance host plant Al tolerance.

RESULTS

pH, available P, and IP in rhizosphere soil of *P. massoniana* seedlings. Compared to the non-ECM (–ECM) inoculation, the pH in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving 1.0 mM Al^{3+} (+Al) treatment was significantly decreased in both the *L. bicolor* S238A and *L. bicolor* S238N inoculations, being decreased more in the latter than the former. In contrast, the pH in soil with no- Al^{3+} (–Al) treatment was decreased significantly in the *L. bicolor* 270 inoculation but was increased significantly in the *L. bicolor* S238A inoculation ($P \leq 0.05$) (Fig. 2a). In addition, soil pH was significantly lower under +Al than under –Al conditions for both the *L. bicolor* S238A- and *L. bicolor* S238N-inoculated seedlings, but the pHs under both conditions for the *L. bicolor* 270-inoculated seedlings were similar.

The level of available P in rhizosphere soil with +Al treatment was significantly lower in the 16-week-old *L. bicolor*-inoculated (+ECM) *P. massoniana* seedlings than in their –ECM counterparts, with the order among the ECM seedlings being *L. bicolor* S238N > *L. bicolor* 270 > *L. bicolor* S238A. In contrast, the level of available P with –Al treatment was significantly lower in both the *L. bicolor* S238N- and *L. bicolor* 270-inoculated seedlings but was significantly higher in *L. bicolor* S238A-inoculated seedlings than the –ECM seedlings ($P \leq 0.05$) (Fig. 2b). In addition, the level of available P with +Al treatment was significantly higher than that with –Al treatment for the *L. bicolor* S238N-inoculated seedlings but was significantly lower than the –Al treatment level for the *L. bicolor* S238A-inoculated seedlings, and the P levels were similar for the *L. bicolor* 270-inoculated seedlings under the two treatments.

No significant differences were observed in rhizosphere soil TP among the 16-week-old *P. massoniana* seedlings based on ECM inoculations or Al treatments, except for a significantly lower level of rhizosphere soil TP with –Al treatment in *L. bicolor* S238A-inoculated seedlings than –ECM seedlings ($P \leq 0.05$) (Fig. 2c).

FIG 2 Effects of three *L. bicolor* isolates on pH (a), available P (b), total P (c), inorganic P (d), Al-P (e), Fe-P (f), Ca-P (g), and O-P (h) in rhizosphere soil of 16-week-old *Pinus massoniana* seedlings grown in an acidic yellow soil with Al treatment. “-Al” and “+Al” indicate that seedlings were watered once weekly with Hoagland solution (pH 3.8) containing 0.0 mM Al³⁺ or 1.0 mM Al³⁺, respectively. Data are means $\pm$ SD ($n = 3$); different uppercase or lowercase letters indicate significant differences between ectomycorrhizal treatments or between Al treatments, respectively, at a P value of ≤ 0.05 .

The level of IP in rhizosphere soil with +Al treatment was significantly lower in 16-week-old +ECM *P. massoniana* seedlings than in their -ECM counterparts ($P \leq 0.05$) (Fig. 2d), with no significant differences among the +ECM seedlings. Conversely, the level of IP with -Al treatment was significantly lower in the *L. bicolor* 270-inoculated seedlings but was significantly higher in the *L. bicolor* S238A-inoculated seedlings than in the -ECM seedlings. In addition, the level of IP with +Al treatment was significantly lower than that with -Al treatment for the *L. bicolor* S238A-inoculated seedlings, but the IP levels under the two treatments were similar for both the *L. bicolor* S238N- and *L. bicolor* 270-inoculated seedlings.

IP fractions in rhizosphere soil of *P. massoniana* seedlings. The Al-P in rhizosphere soil of the 16-week-old *P. massoniana* seedlings was significantly increased in

the *L. bicolor* 270 inoculation but was decreased significantly in the *L. bicolor* S238A inoculation, compared to the –ECM inoculation ($P \leq 0.05$) (Fig. 2e). It is worth noting that the *L. bicolor* S238A inoculation decreased Al-P more with +Al (–38%) than –Al treatment (–11%). There were no significant differences in Al-P between Al treatments with the same ECM inoculation, except for *L. bicolor* S238A-inoculated seedlings, in which the rhizosphere soil Al-P was significantly lower (40%) with +Al than –Al treatment.

Compared to the –ECM inoculation, the *L. bicolor* 270 inoculation significantly decreased the Fe-P in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving –Al treatment ($P \leq 0.05$) (Fig. 2f). In contrast, the *L. bicolor* S238N inoculation significantly increased the Fe-P with +Al treatment. There were no significant differences in rhizosphere soil Fe-P between Al treatments when the same ECM inoculation was used.

The Ca-P in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving +Al treatment was significantly decreased with ECM compared to without ECM, with no significant differences among the ECM inoculations ($P \leq 0.05$) (Fig. 2g). In contrast, the Ca-P with –Al treatment was significantly decreased in the *L. bicolor* 270 inoculation but was significantly increased in the *L. bicolor* S238A inoculation. In addition, Ca-P levels were similar between the two Al treatments with the same ECM inoculation, except that the Ca-P level with +Al treatment was significantly lower than that with the –Al treatment for the *L. bicolor* S238A-inoculated seedlings.

The occluded P (O-P) in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving +Al treatment was significantly decreased with +ECM compared to –ECM inoculation ($P \leq 0.05$) (Fig. 2h), with no significant differences among the ECM inoculations. In contrast, the O-P levels in rhizosphere soil with –Al treatment were similar between +ECM- and –ECM-inoculated seedlings, although significantly higher rhizosphere soil O-P was observed in the *L. bicolor* S238A- than in the *L. bicolor* 270-inoculated seedlings. In addition, the O-P was significantly higher with +Al than –Al treatment for the *L. bicolor* 270-inoculated seedlings, but these levels for both the *L. bicolor* S238A- and *L. bicolor* S238N-inoculated seedlings were similar under the two treatments.

TAI and labile Al in rhizosphere soil of *P. massoniana* seedlings. Compared to total Al (TAI) in the –ECM inoculation, TAI in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving +Al treatment was significantly decreased in both the *L. bicolor* S238N and *L. bicolor* S238A inoculations ($P \leq 0.05$) (Fig. 3a), with similar effects between the two inoculations. The TAI with –Al treatment was significantly decreased in all the ECM inoculations, with the order *L. bicolor* 270 > *L. bicolor* S238N > *L. bicolor* S238A. In addition, TAI was significantly higher with +Al than –Al treatment for the seedlings inoculated with the same ECM fungus.

The labile Al in rhizosphere soil of the 16-week-old *P. massoniana* seedlings receiving +Al treatment was significantly decreased in the ECM inoculation, with the order *L. bicolor* 270 > *L. bicolor* S238N $\approx$ *L. bicolor* S238A, compared to the –ECM inoculation ($P \leq 0.05$) (Fig. 3b). In contrast, the labile Al with –Al treatment was significantly increased in the *L. bicolor* 270 inoculation but was significantly decreased in the *L. bicolor* S238A inoculation. In addition, the level of labile Al was significantly higher with +Al than –Al treatment in the case of the *L. bicolor* S238A-inoculated seedlings.

Labile Al fractions in rhizosphere soil of *P. massoniana* seedlings. Compared to the –ECM inoculation, the levels of exchangeable Al in rhizosphere soil of the 16-week-old *P. massoniana* ECM-inoculated seedlings were significantly increased with +Al treatment but were significantly decreased with –Al treatment, with the order *L. bicolor* S238A (–78%) > *L. bicolor* S238N $\approx$ *L. bicolor* 270 (–11%) ($P \leq 0.05$) (Fig. 3c). In addition, the level of exchangeable Al was significantly higher with +Al than –Al treatment for all three *L. bicolor* inoculations.

The level of hydroxyl Al in rhizosphere soil of the 16-week-old +ECM *P. massoniana* seedlings was similar to that in the –ECM seedlings receiving +Al treatment, and these

FIG 3 Effects of three *L. bicolor* isolates on total Al (a), labile Al (b), exchangeable Al (c), hydroxyl Al (d), acid-soluble Al (e), and organic-complexed Al (f) in rhizosphere soil of 16-week-old *Pinus massoniana* seedlings grown in an acidic yellow soil with Al treatment. “-Al” and “+Al” indicate that seedlings were watered once weekly with Hoagland solution (pH 3.8) containing 0.0 mM Al³⁺ or 1.0 mM Al³⁺, respectively. Data are means $\pm$ SD ($n = 3$); different uppercase or lowercase letters indicate significant differences between ectomycorrhizal treatments or between Al treatments, respectively, at a P value of ≤ 0.05 .

levels were also similar among the seedlings with the three ECM inoculations. In contrast, both the *L. bicolor* S238N and *L. bicolor* 270 inoculation with -Al treatment significantly increased the hydroxyl Al levels compared to the -ECM inoculation, with the *L. bicolor* S238N inoculation having a greater increase (50%) than the *L. bicolor* 270 inoculation (34%) ($P \leq 0.05$) (Fig. 3d). In addition, levels of hydroxyl Al were significantly higher with +Al than -Al treatment for the same ECM inoculation.

The level of acid-soluble Al in rhizosphere soil of the 16-week-old +ECM *P. massoniana* seedlings receiving +Al treatment was significantly lower than that in the -ECM counterparts ($P \leq 0.05$) (Fig. 3e), with similar acid-soluble Al levels among seedlings with the three ECM inoculations. Conversely, the acid-soluble Al levels in rhizosphere soil with -Al treatment were similar in +ECM seedlings and -ECM seedlings, with the order *L. bicolor* 270 $\approx$ *L. bicolor* S238N $>$ *L. bicolor* S238A among the +ECM seedlings. There were no significant differences in levels of acid-soluble Al in rhizosphere soil between Al treatments with the same ECM inoculation.

In the +Al treatment, the level of organic-complexed Al in rhizosphere soil of the +ECM 16-week-old *P. massoniana* seedlings was similar to that of the -ECM seedlings, with no obvious differences among the three ECM inoculations. In the -Al treatment,

TABLE 1 Correlations between accumulations of P and Al and biomass production as well as rhizosphere soil IP and Al fractions in 16-week-old *Pinus massoniana* seedlings grown in an acidic yellow soil^a

Dependent variable	Model	B	β	t (24)	P	r ²	F	P
Biomass production	Constant	210.78		7.89	0.00	0.92	52.47*	0.00
	Aboveground P	556.23	0.70*	7.38	0.00			
	Belowground P	-2,310.17	-0.36*	-4.57	0.00			
	Aboveground Al	122.35	0.08	0.73	0.48			
	Belowground Al	638.62	0.36*	4.50	0.00			
P accumulation	Constant	1.25		5.28	0.00	0.55	5.92*	0.00
	Al-P	-0.02	-0.58*	-3.71	0.00			
	Fe-P	-0.00	-0.12	-0.75	0.46			
	O-P	-0.00	-0.10	-0.53	0.60			
	Ca-P	-0.02	-0.48*	-2.72	0.01			
Al accumulation	Constant	0.03		0.15	0.89	0.56	6.03*	0.00
	Exchangeable Al	-0.02	-0.79*	-3.55	0.00			
	Hydroxyl Al	0.03	0.97*	4.50	0.00			
	Acid-soluble Al	0.00	0.01	0.07	0.95			
	Organic-complexed Al	0.00	0.08	0.41	0.69			

^an = 24. *, significant difference at a P value of ≤ 0.05 ; B, unstandardized coefficients; β , standardized coefficients.

the *L. bicolor* S238A inoculation significantly decreased the organic-complexed Al compared to the -ECM inoculation, with the order *L. bicolor* 270 > *L. bicolor* S238N > *L. bicolor* S238A among the ECM-inoculated seedlings ($P \leq 0.05$) (Fig. 3f). In addition, the level of organic-complexed Al in rhizosphere soil was significantly higher with +Al than -Al treatment for the *L. bicolor* S238A-inoculated seedlings, but these levels were similar for the *L. bicolor* 270- and *L. bicolor* S238N-inoculated seedlings under the two conditions.

Correlations between biomass production and accumulations of P and Al and between accumulations and rhizosphere soil fractions in *P. massoniana* seedlings.

The biomass production significantly correlated with the accumulations of P and Al in 16-week-old *P. massoniana* seedlings ($r^2 = 0.92$; $P \leq 0.05$) (Table 1). With levels of aboveground P ($\beta = 0.70$) being higher than levels of belowground Al ($\beta = 0.36$), both the accumulation of P aboveground and that of Al belowground were beneficial to seedling growth. Conversely, the accumulation of P belowground adversely influenced biomass production ($\beta = -0.36$). The aboveground Al had no obvious effects on biomass production ($P > 0.05$).

Rhizosphere soil IP fractions significantly negatively correlated with the P accumulation in 16-week-old *P. massoniana* seedlings ($r^2 = 0.56$; $P \leq 0.05$) (Table 1). With the order Al-P ($\beta = -0.58$) > Ca-P ($\beta = -0.48$) > Fe-P ($\beta = -0.12$) > O-P ($\beta = -0.10$), both the rhizosphere soil Al-P and Ca-P were significantly negative for P accumulation ($P < 0.05$), but the rhizosphere soil Fe-P and O-P had no obvious effects on P accumulation ($P > 0.05$).

The labile Al fractions in rhizosphere soil significantly correlated with the Al accumulation in 16-week-old *P. massoniana* seedlings ($r^2 = 0.56$, $P \leq 0.05$) (Table 1). The rhizosphere soil exchangeable Al had a significantly negative ($\beta = -0.79$), but the hydroxyl Al had a significantly positive ($\beta = 0.97$) contribution to Al accumulation ($P \leq 0.05$). Both acid-soluble Al and organic-complexed Al in rhizosphere soil had no obvious effects on Al accumulation ($P > 0.05$).

DISCUSSION

Plant Al tolerance by ECM inoculation. In the present study, all tested ECM isolates significantly increased the growth and Al tolerance in *P. massoniana* seedlings grown in an acidic yellow soil, even with +Al treatment (see Fig. S1a in the supplemental material). Furthermore, the ECM inoculation facilitated aboveground biomass production and P accumulation (see Fig. S1a and b), and the seedlings' growth benefited from the accumulation of P aboveground and Al belowground (Table 1). Growth inhibition is an index used extensively for the assessment of Al toxicity (5, 36). Numerous studies

have demonstrated that various ECM fungi, including *L. bicolor* (25), *P. tinctorius* (26, 31, 37), and *P. involutus* (38), could enhance plant growth and Al tolerance in acidic culture media. *P. tinctorius* inoculation also enhanced *P. strobus* shoot but not root growth with +Al treatment (31). Moreover, the Al tolerance conferred by the ECM inoculation varied among the three isolates tested (see Fig. S1a). The *L. bicolor* S238A-inoculated seedlings were Al tolerant, but both the *L. bicolor* 270- and *L. bicolor* S238N-inoculated seedlings were Al sensitive. However, Gu and Huang (30) reported that these three ECM isolates *in vitro* were sensitive to 1.0 mM Al treatment and that *L. bicolor* S238A was the most vulnerable. Thompson and Medve (39) observed that the responses of three *P. tinctorius* isolates *in vitro* to Al were not consistent with that in the field. The above-mentioned results suggested that diverse factors, such as ECM-fungus species or isolate, host species, and growth conditions, could influence plant Al tolerance following ECM inoculation. Bucking et al. (40) suggested that mycorrhizae altered the host and fungus function, while Costa et al. (41) observed that *Pisolithus* inoculation enhanced the growth of *Eucalyptus urophylla*. Overall, among the three tested ECM isolates, *L. bicolor* S238A was the most promising isolate, considering its capacity to confer Al tolerance in *P. massoniana* seedlings grown in an acidic yellow soil.

Mobilization of IP by ECM inoculation. The present study confirmed that ECM inoculation enhanced plant P uptake from soil whether or not the host plant was grown with +Al treatment (see Fig. S1b in the supplemental material). Similar results had previously indicated that ECM fungi, such as *Hebeloma cylindrosporum*, *L. bicolor*, and *Pisolithus* spp., could increase plant P uptake from culture media (37, 42, 43). In addition, the increased P was mainly allocated to the aboveground location in ECM seedlings, particularly with +Al treatment (see Fig. S1b), and the P translocation contributed to plant Al tolerance (Table 1). There were significant differences among the three ECM inoculations with +Al treatment, and *L. bicolor* S238A-inoculated seedlings had the highest rate of P accumulation and translocation to the aboveground level (see Fig. S1b). These results suggested that Al-tolerant ECM seedlings had higher P uptake from soil and also higher P transportation upward than Al-sensitive ECM seedlings receiving +Al treatment. Becquer et al. (44) and Cairney (45) had suggested that P uptake by ECM fungi was mediated by specific P transporters. However, the mechanisms of P translocation in plants remain poorly understood.

The available P and IP in rhizosphere soil were significantly decreased following ECM inoculations (Fig. 2b and d), in addition to the increased aboveground P accumulation by ECM-inoculated seedlings receiving +Al treatment (see Fig. S1b). *L. bicolor* S238A-inoculated seedlings used more available P and Al-P than both the *L. bicolor* 270 and *L. bicolor* S238N-inoculated seedlings receiving +Al treatment (Fig. 2b and e), which indicated that the Al-tolerant ECM seedlings increased the depletion rate of available P and the release rate of Al-P from the rhizosphere soil, compared to the Al-sensitive ECM seedlings. Both Desai et al. (42) and Plassard et al. (46) observed similar results indicating that ECM could mobilize IP to maintain host plant growth under P limitation conditions.

Moreover, the IP fractions mobilized by ECM inoculation varied with ECM fungus species or strain, host plant, soil type, and growth conditions. In the present study, the three tested ECM isolates mobilized both Ca-P and O-P when the seedlings were grown with +Al treatment (Fig. 2g and h). In addition, *L. bicolor* S238A also mobilized Al-P (Fig. 2e). In contrast, *L. bicolor* 270 mobilized both Fe-P and Ca-P (Fig. 2f and g), while *L. bicolor* S238A mobilized Al-P (Fig. 2e) when the seedlings were grown with -Al treatment. In addition, the P accumulation in seedlings was attributed to the mobilization of both Al-P and Ca-P (Table 1). The decreased pH in rhizosphere soil (Fig. 2a) indicated that increased H⁺ secretion due to ECM inoculation could facilitate phosphate release from IP. In addition, Gu and Huang (30) had observed that organic acids exudation by ECM fungi *in vitro* benefited to the IP dissolution. Shi et al. (47) also suggested that the secretion of H⁺ and organic acids by ECM fungi played a vital role in dissolving IP. It is noteworthy that the mobilized Al-P accounted for the major

difference between the Al-tolerant ECM-inoculated and Al-sensitive ECM-inoculated seedlings receiving +Al treatment (Fig. 2e). These results were in accordance with the observation that Al-P was the most effective IP fraction for the improvement of wheat yield (48). In addition, Zhang et al. (27) suggested that the ECM fungi *Boletus* sp. 7 and *Cenococcum geophilum* 4 mobilized Al-P and Ca-P in a purple soil (pH 6.8) *in vitro*. Huang and Lapeyrie (49) observed that *L. bicolor* S238N mobilized more Al-P in rhizosphere soil of *Pseudotsuga menziesii* seedlings than was seen in soil of –ECM seedlings receiving –Al treatment. Several studies have shown that both Al-P and Ca-P in acidic soil are the active IP fractions that can be used by plants, but the O-P fraction is an inactive fraction that is hardly used by plants (9, 48, 50). In contrast, the present study confirmed that *L. bicolor* mobilized not only the active Ca-P and/or Al-P fractions but also the inactive IP fraction (O-P) in yellow acidic soil with +Al treatment (Fig. 2e, g, and h); further studies are needed to understand the underlying mechanisms.

Activation of labile Al by ECM inoculation. ECM fungi can improve plant Al tolerance, partly because they can prevent the host plant from absorbing Al in the soil (37, 38, 43), which is partly consistent with the results of the present study. Both the *L. bicolor* S238N and *L. bicolor* 270 inoculations decreased but the *L. bicolor* S238A inoculation increased Al accumulation in seedlings receiving +Al treatment. However, all three ECM fungi tested enhanced Al accumulation after –Al treatment (see Fig. S1c in the supplemental material). These results suggested that *L. bicolor* S238N or *L. bicolor* 270 inoculation could decrease Al uptake and, in turn, improve the host Al tolerance in response to +Al treatment. Notably, the Al-tolerant ECM (*L. bicolor* S238A)-inoculated seedlings accumulated and translocated more Al to the aboveground location than the Al-sensitive ECM seedlings receiving +Al treatment (see Fig. S1c). According to Schier and McQuattie (31), *P. tinctorius*-inoculated *Pinus strobus* could ameliorate Al toxicity, which mainly resulted from enhanced nutrient uptake, particularly P, as opposed to a decrease in P uptake. Considering the increased accumulation of P, Ca, and Mg in seedlings (19, 25) and P accumulation aboveground, which benefit seedling growth (Table 1), the *L. bicolor* S238A inoculation facilitated such mechanisms through the enhancement of nutrient uptake and transportation upward but not by decreasing Al uptake, which would enhance Al tolerance in *P. massoniana*.

Studies *in vitro* have suggested that ECM could reduce activated-Al concentrations through the formation of chelate complexes with organic acids released from ECM to improve host Al tolerance (22, 24, 26, 27, 51, 52). The results in the field confirmed such an Al decrease, with ECM inoculation decreasing the concentrations of labile Al in rhizosphere soil when the seedlings were grown with +Al treatment (Fig. 3b). However, the decrease was observed only in the acid-soluble Al $[\text{Al}(\text{OH})_3^0]$ fraction (Fig. 3e). Conversely, the exchangeable Al (Al^{3+}) fraction was increased with ECM inoculation (Fig. 3c). Both the hydroxyl Al and organically complexed Al fractions showed no obvious alterations in response to +Al treatment (Fig. 3d and f). Among the four labile Al fractions, both the acid-soluble Al and organically complexed Al were often considered low-activity Al, while the exchangeable Al and hydroxyl Al were high-activity Al (5, 53). In acidic soil, acid-soluble Al could be transformed into exchangeable Al with a decline in pH (17, 33). The rhizosphere soil pH was decreased following ECM inoculation when the seedlings were grown with +Al treatment (Fig. 2a), which indicated that ECM fungi could stimulate proton exudation from ECM seedlings, in turn facilitating the dissolution of acid-soluble Al and its transformation into exchangeable Al. Organically complexed Al did not significantly change following ECM inoculation (Fig. 3f), although numerous studies had suggested that ECM fungi could transform high-activity Al into low-activity Al, since ECM-fungus-colonized plants were able to excrete low-molecular-weight organic acids, such as oxalic acid, citric acid, malic acid, and tartaric acid, to chelate Al and then form organically complexed Al (22–24, 26, 27, 52). Rajkumar et al. (54) suggested that organic acids secreted by plant-associated microbes might mobilize mineral nutrients and solubilize heavy metals in rhizosphere soil. For instance, *Pseudomonas* sp. Lk9 enhanced the availability of Fe and P in soil and the accumulation of

Zn, Cu, and Cd in *Solanum nigrum* by improving organic acid secretion (55). These results indicated that the organic acid secretion in ECM fungi stimulated by +Al treatment (30) might act similarly to plant Al tolerance by mobilizing IP and solubilizing Al. These results suggested that the activated Al might not inhibit the growth of ECM seedlings grown in acidic soil with +Al treatment, or that inoculation with ECM fungus could enhance the threshold of active Al that a host could tolerate (56). In addition, *P. massoniana* is an Al hyperaccumulator (16). Therefore, *L. bicolor* S238A might enhance the capacity of *P. massoniana* to absorb large amounts of Al from soil without adverse effects on its own growth and the growth of the host plant (52).

Conclusion. Our results demonstrated that inoculation with either *L. bicolor* 270, *L. bicolor* S238A, or *L. bicolor* S238N enhanced Al tolerance of 16-week-old *P. massoniana* seedlings that were field grown in an acidic yellow soil, particularly in the case of *L. bicolor* S238A with +Al treatment. Furthermore, the ECM inoculation may have stimulated proton exudation from *P. massoniana* roots colonized by ECM fungi, enhanced the depletion of available P, and facilitated the simultaneous mobilization of IP and labile Al, which in turn could have improved the plants' capacity to exploit O-P, Ca-P, and Al-P, particularly when the roots of the pine seedlings were colonized by *L. bicolor* S238A. Ectomycorrhization with *L. bicolor* isolates enhanced the solubility of labile Al and facilitated the conversion of labile Al from acid-soluble Al to exchangeable Al, although such conversions did not inhibit plant growth. Results from the present study could facilitate further field studies on plant Al tolerance conferred by ectomycorrhization (Fig. 1c).

MATERIALS AND METHODS

Study site. The experimental site is located at the Southwest University Farm (29°48'39" N, 106°24'54" E; 255 m above sea level), 28 km north of Chongqing, China. The region has a subtropical humid monsoon climate with an annual average temperature of 18.9°C, an annual rainfall of 1,152 mm, and monthly mean warmest and coldest temperatures of 28.6°C (July and August) and 8.7°C (January and February). The yellow soil (Haplic Alisols; pH 4.64) under a *P. massoniana* stand on Mount Jigong (29°48'54" N, 106°26'36" E; 534 m above sea level), Chongqing, China, developed from sandstone of the Jurassic Period. At a 0- to 20-cm depth, the soil contains 0.99 g total N kg⁻¹, 0.35 g TP kg⁻¹, 21.68 g total K kg⁻¹, 72.95 mg available N kg⁻¹, 3.93 mg available P kg⁻¹, 62.71 mg available K kg⁻¹, 46.82 g TAI kg⁻¹, and 1.23 g labile Al kg⁻¹. Soil was sterilized with 2% formaldehyde before the sowing of germinated seeds and then loaded on concrete chambers (2.0 by 6.0 by 0.5 m [length by width by depth]) at the experimental field site.

Fungal isolates and growth of ECM-inoculated plant seedlings. Three *L. bicolor* isolates (*L. bicolor* 270, *L. bicolor* S238A, and *L. bicolor* S238N) were used in the experiment. These ECM isolates can be obtained from the Microbiology Laboratory, Southwest University, Chongqing, China. The *L. bicolor* 270 isolate was obtained from a *Pseudotsuga menziesii* forest in Canada, while both *L. bicolor* S238A and *L. bicolor* S238N were originally from a *Pinus taeda* stand in France. Four mycelial agar discs (3 mm in diameter) for inoculation were subcultured in separate 250-ml flasks with 50 ml Pachlewski liquid medium (pH 5.5) for 21 days at 25°C under dark conditions in the Silviculture Lab at Southwest University, Chongqing, China.

After 21 days of subculture and the removal of growth medium, the fungal mycelia were mixed using a blender (Philips HR 2024; Philips, Inc., Tokyo, Japan) for 1 min. A total of 200 germinating *P. massoniana* seeds (obtained from the Chongqing Tree Seeds and Seedlings Service, Chongqing, China) inoculated with five flasks of mixed fungal mycelia were sowed on 1.0 m² sterilized soil (0.5-cm depth) to develop ECM associations (+ECM). The control (−ECM) seedlings were inoculated with sterilized fungal mycelia. Prior to the ECM inoculations, uniform seeds were sterilized with 0.3% formaldehyde for 30 min, rinsed with sterilized water, and then germinated on moistened paper towels at 25°C in the dark.

Al treatment. After 4 weeks, the seedlings were watered weekly with Hoagland's solution (pH 3.8), which contained 5 mM Ca(NO₃)₂·4H₂O, 5 mM KNO₃, 2 mM MgSO₄·7H₂O, 1 mM KH₂PO₄, and 0.02 mM FeC₄H₄O₆, without Al³⁺ (−Al) or with 1.0 mM Al³⁺ (+Al) as an Al₂(SO₄)₃·18H₂O solution, for 12 weeks. The total amount of Al added to soil was 81 mg kg of soil⁻¹. Seedlings with or without ECM inoculation or Al treatment in a 1.0-m² plot were randomly arranged, with three replicates. There were a total of 24 1.0-m² plots (four ECM inoculations × two Al treatments × three replicates). The seedlings were grown in the field for 16 weeks, from 20 May to 8 September 2016.

Sampling and analyses. After 12 weeks of Al treatment, 25 16-week-old seedlings were selectively harvested from each 1.0-m² plot, and the proportions of roots colonized by mycorrhizae were examined using a dissecting microscope (XSZ-Hs7; Chongqing Photoelectric Instrument Co., Ltd., Chongqing, China). All the *P. massoniana* seedlings grew normally through the growing seasons. Root ECM colonization ranged from 95% to 100% in ECM seedlings, but no obvious root colonization was observed in −ECM seedlings. Aboveground and belowground plant parts were separately harvested, oven dried at 70°C for 48 h, weighed, and then ground using a grinder (BJ-150; Baijie, Inc., Zhejiang, China) at 25,000

rpm for 1 min, and digested in a nitric acid-perchloric acid solution ($\text{HNO}_3\text{-HClO}_4$; 5:1, vol/vol) for P and Al analyses (57). The accumulation of P or Al was calculated as the concentration of P or Al multiplied by the biomass of the respective plant parts.

All the following extraction procedures were performed in triplicate, and the concentrations of soil P and Al were measured spectrometrically using the Mo-Sb and aluminon methods (58), respectively. First, the rhizosphere soil, collected by shaking the roots, was ground using a wooden rolling pin and sieved (using a 1.0-mm sieve for pH and available-P analyses and a 0.25-mm sieve for TP, TAl, and IP fraction and labile-Al-fraction analyses) after litter and debris removal and oven dried at 110°C. According to the method of Bao (57), soil pH (1:2.5, soil to water) was measured using a pH meter (pH-3C; Leici Co., Shanghai, China). The concentrations of TP and TAl in soil were measured after extraction with $\text{H}_2\text{SO}_4\text{-HClO}_4$ (10:1, vol/vol). The levels of available P in soil were determined after extraction with 0.05 M HCl and 0.025 M H_2SO_4 .

Fractions of IP were sequentially extracted from the soil as described by Chang and Jackson (58). Briefly, 1.0 g of soil and 50 ml of 1.0 M NH_4Cl were added to a 100-ml centrifuge tube, shaken for 30 min, and centrifuged (Sorvall ST 16R; Thermo Fisher, Dreieich, Germany) at 3,500 rpm for 8 min; the supernatant was decanted, and 50 ml of 0.5 M NH_4F (pH 8.2) was added to the residue. The mixture was shaken again for 1 h and centrifuged (3,500 rpm) again for 8 min, and the supernatant was decanted for Al-P analyses. The residue was washed twice with 25 ml of saturated NaCl and centrifuged at 3,500 rpm for 10 min. Subsequently, 50 ml of 0.1 M NaOH was added to the residue, and the solution was shaken for 2 h and again for 2 h after 16 h and centrifuged (4,500 rpm) for 10 min, and the supernatant was decanted for iron-bound P (Fe-P) analyses. Subsequently, the residue was washed twice with 25 ml saturated NaCl, centrifuged (3,500 rpm) for 10 min, and washed with 40 ml of 0.3 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$; 1.0 g $\text{Na}_2\text{S}_2\text{O}_4$ was added, and the suspension was heated in a water bath at 80 to 90°C for 15 min. Afterward, 10 ml of 0.5 M NaOH was added to the suspension, and the mixture was stirred for 10 min and centrifuged (4,500 rpm) for 10 min after cooling; the supernatant was then decanted for occluded-P (O-P) analyses. Subsequently, the residue was washed twice with saturated NaCl and centrifuged again, 50 ml of 0.25 M H_2SO_4 was added, and the mixture was shaken for 1 h and centrifuged (4,500 rpm) for 10 min; the supernatant was then decanted for Ca-P analyses. Soil IP was calculated as the sum of the four P fractions.

The labile-Al fractions were extracted sequentially from the soil as described by Huang and Qu (59). Briefly, 1.0 g soil and 50 ml 1.0 M KCl were placed in a 100-ml centrifuge tube, shaken for 30 min, and centrifuged (5,000 rpm) for 15 min, and the supernatant was decanted for exchangeable-Al (Al^{3+}) determination. The residue was washed with deionized water and centrifuged (5,000 rpm) for 15 min, 50 ml of 1.0 M NH_4Ac was added, the mixture was shaken for 30 min and centrifuged (5,000 rpm) for 15 min, and the supernatant was decanted for hydroxyl Al [$\text{Al}(\text{OH})_2^{2+}$ and $\text{Al}(\text{OH})_2^{+}$] analyses. The residue was then washed with deionized water and centrifuged (5,000 rpm), 50 ml of 1.0 M HCl was added, the mixture was shaken for 30 min and centrifuged (5,000 rpm) for 15 min, and the supernatant was decanted for acid-soluble-Al [$\text{Al}(\text{OH})_3^0$] determination. The residue was washed with deionized water and centrifuged (5,000 rpm), 50 ml of 0.5 M NaOH was added, the mixture was shaken for 15 min and centrifuged (5,000 rpm) for 15 min, and the supernatant was decanted for measurement of organically complexed Al. The amount of labile Al was calculated as the sum of the four Al fractions.

Statistical analyses. Data (means $\pm$ standard deviations [SD], $n = 3$) were analyzed using a two-way analysis of variance to test the effects of the treatments (ECM inoculation, Al treatment, and ECM inoculation plus Al treatment) on examined variables. Then, significant differences between treatments were determined using Fisher's least significant difference *post hoc* test at a P value of ≤ 0.05 . Regression analyses were used to assess the correlations between biomass production and accumulations of P and Al in above- and belowground parts and between P accumulation and IP fractions in rhizosphere soil as well as between Al accumulation and labile Al fractions in rhizosphere soil. All statistical analyses were performed with SPSS v.21.0 software (IBM Corp., Armonk, NY). Graphs were produced using GraphPad Prism version 5.01 (GraphPad Software, Inc., San Diego, CA).

Data availability. All raw data described in this study are deposited in the figshare database (<https://doi.org/10.6084/m9.figshare.11821767>).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31570599 and 31100464).

We thank Jianguo Huang and Ling Yuan for supplying the ECM isolates.

REFERENCES

- Moustaka J, Ouzounidou G, Baycu G, Moustakas M. 2016. Aluminum resistance in wheat involves maintenance of leaf Ca^{2+} and Mg^{2+} content, decreased lipid peroxidation and Al accumulation, and low photosystem II excitation pressure. *Biometals* 29:611–623. <https://doi.org/10.1007/s10534-016-9938-0>.
- Cai Z, Wang B, Xu M, Zhang H, He X, Zhang L, Gao S. 2015. Intensified soil

- acidification from chemical N fertilization and prevention by manure in an 18-year field experiment in the red soil of southern China. *J Soils Sediments* 15:260–270. <https://doi.org/10.1007/s11368-014-0989-y>.
3. Mayor JR, Mack MC, Schuur E. 2015. Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions. *Soil Biol Biochem* 88:247–256. <https://doi.org/10.1016/j.soilbio.2015.05.028>.
 4. Hirano Y, Isomura A, Kaneko S. 2003. Root morphology and nutritional status of Japanese red cedar saplings subjected to in situ levels of aluminum in forest soil solution. *J Forest Res* 8:209–214. <https://doi.org/10.1007/s10310-003-0027-1>.
 5. Poschenrieder C, Gunsé B, Corrales I, Barceló J. 2008. A glance into aluminum toxicity and resistance in plants. *Sci Total Environ* 400:356–368. <https://doi.org/10.1016/j.scitotenv.2008.06.003>.
 6. Smits MM, Hoffland E. 2009. Possible role of ectomycorrhizal fungi in cycling of aluminum in podzols. *Soil Biol Biochem* 41:491–497. <https://doi.org/10.1016/j.soilbio.2008.11.023>.
 7. Jongbloed RH, Tosserams MWA, Borst-Pauwels GWFH. 1992. The effect of aluminium on phosphate uptake by three isolated ectomycorrhizal fungi. *Plant Soil* 140:167–174. <https://doi.org/10.1007/BF00010594>.
 8. Lee G, Ro H, Lee S, Lee S. 2006. Electrokinetically enhanced transport of organic and inorganic phosphorus in a low permeability soil. *Geosci J* 10:85–89. <https://doi.org/10.1007/BF02910335>.
 9. Ranatunga TD, Taylor RW, Bhat KN, Reddy SS, Senwo ZN, Jackson B. 2009. Inorganic phosphorus forms in Soufrière Hills volcanic ash and volcanic ash-derived soil. *Soil Sci* 174:430–438. <https://doi.org/10.1097/S5.0b013e3181b6deab>.
 10. Olander LP, Vitousek PM. 2005. Short-term controls over inorganic phosphorus during soil and ecosystem development. *Soil Biol Biochem* 37:651–659. <https://doi.org/10.1016/j.soilbio.2004.08.022>.
 11. Ma B, Zhou ZY, Zhang CP, Zhang G, Hu YJ. 2009. Inorganic phosphorus fractions in the rhizosphere of xerophytic shrubs in the Alxa Desert. *J Arid Environ* 73:55–61. <https://doi.org/10.1016/j.jaridenv.2008.08.006>.
 12. Achat DL, Pousse N, Nicolas M, Brédoire F, Augusto L. 2016. Soil properties controlling inorganic phosphorus availability: general results from a national forest network and a global compilation of the literature. *Biogeochemistry* 127:255–272. <https://doi.org/10.1007/s10533-015-0178-0>.
 13. Opala PA, Okalebo JR, Othieno CO, Kisinyo P. 2010. Effect of organic and inorganic phosphorus sources on maize yields in an acid soil in western Kenya. *Nutr Cycl Agroecosyst* 86:317–329. <https://doi.org/10.1007/s10705-009-9294-3>.
 14. Mimmo T, Ghizzi M, Cesco S, Tomasi N, Pinton R, Puschenreiter M. 2013. Aluminium-phosphate interactions in the rhizosphere of two bean species: *Phaseolus lunatus* L. and *Phaseolus vulgaris* L. *J Sci Food Agric* 93:3891–3896. <https://doi.org/10.1002/jsfa.6392>.
 15. Jentschke G, Godbold DL. 2000. Metal toxicity and ectomycorrhizas. *Physiol Plant* 109:107–116. <https://doi.org/10.1034/j.1399-3054.2000.100201.x>.
 16. Wu R, Zhuang J, Huang J, Chen W. 2009. Response and resistance mechanism of *Pinus massoniana* under the stresses of simulated acid rain and aluminum. *Sci Silvae Sin* 45:22–29.
 17. Ren J, Zhang Y, Qian C, He Q, Xiang X, Guo J. 2011. Fraction distribution and release mechanism of aluminum in acidic forest soils of Southern China. *Environ Chem* 30:1131–1135.
 18. Wu X, Zhou Q, Gu X, Wan Y, Mao W, Song Y, Zhou Q. 2015. Resistance and nutritional response to aluminum toxicity of three ectomycorrhizal fungi from forest soils in Southwest China. *J Southwest Univ (Natural Sci Ed)* 37:13–19. <https://doi.org/10.13718/j.cnki.xdzk.2015.08.003>.
 19. Gu X, Ni Y, Jiang Y, Jia H. 2018. Effects of *Laccaria bicolor* on growth, uptake and distribution of nutrients and aluminum of *Pinus massoniana* seedlings under acid aluminum exposure. *Sci Silvae Sin* 54:170–178. <https://doi.org/10.11707/j.1001-7488.20180220>.
 20. Yu J, Xia L, Yin D, Zhou C. 2018. Effects of phosphorus on aluminum tolerance of Chinese fir seedlings. *Sci Silvae Sin* 54:36–47.
 21. Jiang Y, Gu X, Zhou Q, Jia H. 2019. Organic acid secretion and aluminum absorption kinetics by *Lactarius deliciosus* and *Pisolithus tinctorius* under aluminum stress. *Chinese J Ecol* 38:3441–3449.
 22. Ozasa T. 2012. A comprehensive analysis of the changes in organic acid metabolism of ectomycorrhizal fungus *Laccaria bicolor* in response to aluminum salts. *Sustain Humanosph* 8:33.
 23. Ray P, Adholeya A. 2009. Correlation between organic acid exudation and metal uptake by ectomycorrhizal fungi grown on pond ash in vitro. *Biometals* 22:275–281. <https://doi.org/10.1007/s10534-008-9163-6>.
 24. Wang M, Yuan L, Zhou Z, Yang H, Huang J. 2012. Efflux of oxalate and uptake of nitrogen, phosphorus and potassium by ectomycorrhizal fungal isolates *in vitro* in response to aluminum stress. *Sci Silvae Sin* 48:82–88.
 25. Gu X, Wang X, Li J, He X. 2019. Accumulation and translocation of phosphorus, calcium, magnesium, and aluminum in *Pinus massoniana* Lamb. seedlings inoculated with *Laccaria bicolor* growing in an acidic yellow soil. *Forests* 10:1153–1066. <https://doi.org/10.3390/f10121153>.
 26. Tahara K, Norisada M, Tange T, Yagi H, Kojima K. 2005. Ectomycorrhizal association enhances Al tolerance by inducing citrate secretion in *Pinus densiflora*. *Soil Sci Plant Nutr* 51:397–403. <https://doi.org/10.1111/j.1747-0765.2005.tb00045.x>.
 27. Zhang L, Wang M, Li H, Yuan L, Huang J, Penfold C. 2014. Mobilization of inorganic phosphorus from soils by ectomycorrhizal fungi. *Pedosphere* 24:683–689. [https://doi.org/10.1016/S1002-0160\(14\)60054-0](https://doi.org/10.1016/S1002-0160(14)60054-0).
 28. Smits MM, Bonneville S, Benning LG, Banwart SA, Leake JR. 2012. Plant-driven weathering of apatite—the role of an ectomycorrhizal fungus. *Geobiology* 10:445–456. <https://doi.org/10.1111/j.1472-4669.2012.00331.x>.
 29. Browning MHR, Hutchinson TC. 1991. The effects of aluminum and calcium on the growth and nutrition of selected ectomycorrhizal fungi of jack pine. *Can J Bot* 69:1691–1699. <https://doi.org/10.1139/b91-214>.
 30. Gu X, Huang J. 2010. Effect of aluminum on growth, oxalate exudation, and uptake of aluminum, phosphorus and potassium by ectomycorrhizal fungi *in vitro*. *Acta Ecol Sin* 30:357–363.
 31. Schier GA, McQuattie CJ. 1995. Effect of aluminum on the growth, anatomy, and nutrient content of ectomycorrhizal and nonmycorrhizal eastern white pine seedlings. *Can J For Res* 25:1252–1262. <https://doi.org/10.1139/x95-138>.
 32. Heim A, Luster J, Brunner I, Fray B, Frossard E. 2003. Aluminum effects on *Picea abies* at low solution concentrations. *Soil Sci Soc Am J* 67:895–898. <https://doi.org/10.2136/sssaj2003.0895>.
 33. Lv H, Wang K, Xie X. 2007. Character of soil aluminum forms under different land use and slope position. *J Soil Water Conserv* 21:172–175.
 34. Scholl L, Kuyper TW, Smits MM, Landeweert R, Hoffland E, Breemen N. 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil* 303:35–47. <https://doi.org/10.1007/s11104-007-9513-0>.
 35. Zhou C, Lin J, Li Y, Liu A. 2016. Effects of glyphosate on inorganic phosphorus transformation in soil. *J Northwest Univ* 31:71–77.
 36. Yang WQ, Goulart BL. 1997. Aluminum and phosphorus interactions in mycorrhizal and nonmycorrhizal highbush blueberry plantlets. *J Am Soc Horticultural Sci* 122:24–30. <https://doi.org/10.21273/JASHS.122.1.24>.
 37. Egerton-Warburton L. 2015. Aluminum-tolerant *Pisolithus* ectomycorrhizas confer increased growth, mineral nutrition, and metal tolerance to *Eucalyptus* in acidic mine spoil. *Appl Environ Soil Sci* 2015:1–10. <https://doi.org/10.1155/2015/803821>.
 38. Khosla B, Kaur H, Reddy MS. 2009. Influence of ectomycorrhizal colonization on the growth and mineral nutrition of *Populus deltoides* under aluminum toxicity. *J Plant Interact* 4:93–99. <https://doi.org/10.1080/17429140802382953>.
 39. Thompson GW, Medve RJ. 1984. Effects of aluminum and manganese on the growth of ectomycorrhizal fungi. *Appl Environ Microbiol* 48:556–560. <https://doi.org/10.1128/AEM.48.3.556-560.1984>.
 40. Bucking H, Beckmann S, Heyser W, Kottke I. 1998. Elemental contents in vacuolar granules of ectomycorrhizal fungi measured by EELS and EDXS. A comparison of different methods and preparation techniques. *Micron* 29:53–61. [https://doi.org/10.1016/S0968-4328\(97\)00059-0](https://doi.org/10.1016/S0968-4328(97)00059-0).
 41. Costa LS, Graziottini PH, Graziottini D, Silva AC, Rossi MJ, de Silva E, da Costa VHD, Gomes A. 2015. *In vitro* evaluation of *Eucalyptus* ectomycorrhizae on substrate with phosphorus dose for fungal pre-selection. *Rev Arvore* 39:127–136. <https://doi.org/10.1590/0100-67622015000100012>.
 42. Desai S, Naik D, Cumming JR. 2014. The influence of phosphorus availability and *Laccaria bicolor* symbiosis on phosphate acquisition, antioxidant enzyme activity, and rhizospheric carbon flux in *Populus tremuloides*. *Mycorrhiza* 24:369–382. <https://doi.org/10.1007/s00572-013-0548-1>.
 43. Kayama M, Qu L, Koike T. 2015. Elements and ectomycorrhizal symbiosis affecting the growth of Japanese larch seedlings regenerated on slopes of an active volcano in northern Japan. *Trees Struct Funct* 29:1567–1579. <https://doi.org/10.1007/s00468-015-1238-8>.
 44. Becquer A, Trap J, Irshad U, Ali MA, Claude P. 2014. From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. *Front Plant Sci* 5:548–547. <https://doi.org/10.3389/fpls.2014.00548>.
 45. Cairney J. 2011. Ectomycorrhizal fungi: the symbiotic route to the root

- for phosphorus in forest soils. *Plant Soil* 344:51–71. <https://doi.org/10.1007/s11104-011-0731-0>.
46. Plassard C, Louche J, Ali MA, Duchemin M, Legname E, Cloutier-Hurteau B. 2011. Diversity in phosphorus mobilisation and uptake in ectomycorrhizal fungi. *Ann For Sci* 68:33–43. <https://doi.org/10.1007/s13595-010-0005-7>.
 47. Shi L, Xue J, Liu B, Dong P, Wen Z, Shen Z, Chen Y. 2018. Hydrogen ions and organic acids secreted by ectomycorrhizal fungi, *Pisolithus* sp1, are involved in the efficient removal of hexavalent chromium from waste water. *Ecotoxicol Environ Saf* 161:430–436. <https://doi.org/10.1016/j.ecoenv.2018.06.004>.
 48. Mandal K, Mukhopadhyay D. 2015. Effects of inorganic phosphorus fractions under different tillage practices on wheat (*Triticum aestivum*) in an acid soil of west Bengal (India). *Trop Agric Res Ext* 18:102–106. <https://doi.org/10.4038/tare.v18i3.5332>.
 49. Huang J, Lapeyrie F. 1996. Ability of ectomycorrhizal fungus *Laccaria bicolor* S238N to increase the growth of Douglas fir seedlings and their phosphorus and potassium uptake. *Pedosphere* 6:217–224.
 50. Zhang L, Liu Y, Gu L, Yue X, Gu K, Zi G, Xia Y. 2016. Effect of phosphorus addition and different AMF on inorganic phosphorus forms in red soil under intercropping maize plants. *Soil Fertil Sci China* 1:26–33.
 51. Li H, Huang J, Yuan L. 2013. Influence of aluminum and manganese on the growth, nutrient uptake and the efflux by ectomycorrhizal fungi. *Environ Sci* 34:315–320.
 52. Adeleke R, Nwangburuka C, Oboirien B. 2017. Origins, roles and fate of organic acids in soils: a review. *S Afr J Bot* 108:393–406. <https://doi.org/10.1016/j.sajb.2016.09.002>.
 53. Zhu X, Lv B, Shang X, Wang J, Li M, Yu X. 2019. The immobilization effects on Pb, Cd and Cu by the inoculation of organic phosphorus-degrading bacteria (OPDB) with rapeseed dregs in acidic soil. *Geoderma* 350:1–10. <https://doi.org/10.1016/j.geoderma.2019.04.015>.
 54. Rajkumar M, Sandhya S, Prasad MNV, Freitas H. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnol Adv* 30:1562–1574. <https://doi.org/10.1016/j.biotechadv.2012.04.011>.
 55. Chen L, Luo SL, Li XJ, Wan Y, Chen JL, Liu CB. 2014. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. *Soil Biol Biochem* 68:300–308. <https://doi.org/10.1016/j.soilbio.2013.10.021>.
 56. Moyer-Henry K, Silva I, Macfall J, Johannes E, Allen N, Goldfarb B, Rufty T. 2005. Accumulation and localization of aluminium in root tips of loblolly pine seedlings and the associated ectomycorrhiza *Pisolithus tinctorius*. *Plant Cell Environ* 28:111–120. <https://doi.org/10.1111/j.1365-3040.2004.01240.x>.
 57. Bao SD. 2000. Soil and agricultural chemistry analysis, 3rd ed. China Agriculture Press, Beijing, China. <https://doi.org/10.1070/PU2000v043n09ABEH000782>.
 58. Chang SC, Jackson ML. 1957. Fractionation of soil phosphorus. *Soil Sci* 84:133–144. <https://doi.org/10.1097/00010694-195708000-00005>.
 59. Huang Y, Qu C. 1996. Studies on the leaching and species of aluminum in soil. *Chin J Env Sci* 17:57–59.