EFFECTS OF DIFFERENT MIXED PLANTING PATTERNS OF CHINESE FIR (CUNNINGHAMIA LANCEOLATA) ON CHEMICAL FORMS OF ALUMINUM IN SOIL

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Abstract. Aluminum (Al) toxicity is an important cause of forest degradation. Al phytotoxicity is closely related to its chemical forms in soils. In this study, the effects of different mixed planting patterns of Chinese fir (*Cunninghamia lanceolata*) on four chemical forms of Al in soils were examined. One-year-old seedlings of *C. lanceolata*, *Michelia macclurei* and *Schima superba* were used. The results showed that the content of exchangeable Al and monomer hydroxy Al decreased by 2.3–8.1% and 2.6–14.2% in soil of mixed plantation of *C. lanceolata* with *M. macclurei*, whereas they decreased by 6.6–6.9% and 7.2–7.3% in bulk soil of *C. lanceolata* mixed with *S. superba*. The content of acid-soluble inorganic Al in soil of mixed plantation of *C. lanceolata* also indicated a decreasing tendency, whereas the content of humic acid Al indicated an increasing tendency. Meanwhile, soil pH indicated an increasing tendency and had a negative correlation with all chemical forms of Al in soil. These results indicated that mixed planting patterns of *C. lanceolata* could reduce active Al in soil and would be an alternative alleviation method to control the harmful effects of Al on *C. lanceolata* forest.

Keywords: Cunninghamia lanceolata, exchangeable Al, monomer hydroxy Al, acid-soluble inorganic Al, humic acid Al

Introduction

Aluminum (Al) in soil is one of the toxic metals to plants, especially for plants growth in acid soil (Kochian et al., 2015). Al can be absorbed by plants, although it is not a nutrient element (Arunakumara et al., 2013). Al in plant cells would be combined with cell pectin, membrane components, proteins, and nucleic acids (Matsumoto, 1988; Jones and Kochian, 1997; Tabuchi et al., 2001; Arunakumara et al., 2013; Shahnawaz et al., 2016), and thus it can influence the growth, cell membrane integrity, photosynthesis, nitrogen metabolism, nutrient uptake, and deoxyribonucleic acid (DNA) fragmentation (Prabagar et al., 2011; Ribeiro et al., 2013; Cruz et al., 2014; Yang et al., 2015; Anjum et al., 2016; Vardar et al., 2016; Awasthi et al., 2017). The potential toxicity of active Al in the terrestrial ecosystem was a major factor leading to the forest degradation overspreading across Central Europe in the 1970s (Ulrich et al., 1980).

However, phytotoxicity of soil Al does not depend on the total content of Al in soil but on its chemical forms (Shahnawaz et al., 2016). In fact, Al is the most abundant metal present in the Earth's crust (Arunakumara et al., 2013). It exists in many different forms in soil such as Al³⁺, Al(OH)²⁺, Al(OH)₂+, Al(OH)₃, Al-OH polymer, Al-F complex, Al-SO₄ complex, phosphate, organic species, carbonate species, and oxide species (Wang et al., 2007; Zhou et al., 2011; Shahnawaz et al., 2016). Al toxicity to plants varies according to different chemical forms. Al in insoluble form is nonphytotoxic whereas Al³⁺, Al(OH)²⁺, and Al(OH)₂+ are considered as the most toxic forms in soil and named

active Al (Zhou et al., 2011; Shahnawaz et al., 2016). The distribution of Al forms in the soil can be influenced by many factors including soil pH, soil types, predominant clay mineral, organic matter, organic acid, and plant species (Xu and Zhang, 2017). Therefore, many alleviating methods for Al toxicity are associated with the above-mentioned factors. Controlling soil acidity is one of the most common methods to alleviate Al toxicity, because Al forms are found to be dependent on soil pH. Under acidic conditions, Al can be solubilized into more toxic forms. The addition of alkali materials such as calcium carbonate, magnesium limestone, and calcium silicate could increase soil pH and reduce the stress effects of Al on plants (Cristancho et al., 2011; Elisa et al., 2016). Addition of organic materials and mineral ions are also alternative options (Muhrizal et al., 2003; Yang et al., 2009; Iqbal, 2014; Liu et al., 2014). For forest plants, a mixed species plantation might be an effective ecological method to alleviate Al toxicity. However, only a few cases have been reported until now (Li et al., 2008; Lei et al., 2014).

Chinese fir (*Cunninghamia lanceolata*) is one of the most important timber tree species in Southern China where the soil is acidic and ferrallitic. Increasing soil acidification caused by acid rain and the continuous planting of *C. lanceolata* will inevitably lead to Al toxicity in *C. lanceolata* (Liu et al., 2014), which may be closely related to its chemical forms in soil. A mixed plantation of *C. lanceolata* with other forest species has been confirmed to have promoting effects on phosphorus nutrition, microbial community, chemical properties, and carbon storages in soil (Zou et al., 1995; Liu et al., 2010; Richards et al., 2010; Wang et al., 2010). However, little is known about the changes in Al chemical forms. The aim of this study is to examine the effects of different mixed planting patterns of *C. lanceolata* on the chemical forms of Al in soils including exchangeable Al, monomer hydroxy Al, acid-soluble inorganic Al, and humic acid Al in both the rhizosphere and bulk soils. One-year-old seedlings of *C. lanceolata* were used, and the mixed plantation of *C. lanceolata* with both *Michelia macclurei* and *Schima superba* in high planting density was carried out.

Materials and methods

Study area

The experiment was performed in the Experimental Station of Forest located at a hillside of the campus of Fujian Agriculture and Forestry University, in Fujian Province, China (119°14′ N and 26°05′ E) where has subtropical monsoon climate with an annual average temperature of 15-20°C and an average annual rainfall of 900-2100 mm. The experiment started on May 8, 2014 when it was spring.

Soil for this study was a kind of acid red soil (based on Chinese Soil Taxonomy). The clay mineral composition was characterized by kaolinite with gibbsite and ferric oxide. The soil was taken from the same site of the Experimental Station of Forest. There were no trees except some natural herbs in this chosen area. Only the soil down to a depth of 1-2 m was collected in order to avoid any previous plant residues. Soil properties were examined before the onset of our experiment. The results are presented in *Table 1*.

Table 1. Properties of the soil used in this experiment

pН	Total N	Total P	Total K	Available N	Available P	Available K	Organic
	(%)	(%)	(%)	(mg/kg ⁻¹)	(mg/kg ⁻¹)	(mg/kg ⁻¹)	matter (%)
4.29	0.048	0.017	1.48	58.9	1.45	42.3	0.7

Mixed plantation

One-year-old seedlings of C. lanceolata, M. Macclurei, and S. superba were used as plant materials. Seedlings were planted in pots (length \times width \times height = 1 m \times 1 m \times 0.8 m) (Fig. 1). The pots were made of concrete and filled with soils mentioned in the above "Soil" section. Five different planting patterns including pure C. lanceolata, pure M. macclurei, pure S. superba, C. lanceolata in mixture with M. macclurei (1:1) and C. lanceolata in mixture with S. superba (1:1) were compared. For each planting patterns, there were four replicates (one pot per replicate). For each replicate, there were 16 seedlings for pure planting patterns and 8:8 seedlings for mixed planting patterns, respectively (Table 2). Seedling to seedling distance was 20 cm. All seedlings was watered regularly using tap water to avoid the pot soil too dry. The watering requency differed in different seasons (once per day in spring and autumn, once two days in winter, twice per day in summer). Four seedlings (2:2 for mixed planting patterns) were uprooted together with surrounding soil to the depth of 50 cm by using a plastic tube (15 cm in diameter for 6 months seedlings or 20 cm in diameter for 18 months seedlings) with sharp edge after 6 and 18 months, respectively. The uprooted soils were separated into rhizosphere and bulk soil. The rhizosphere soil was collected by gently shaking the plant roots and carefully removing the soil adhering to them. The rest soil was considered as bulk soil. Both rhizosphere and bulk soils were air-dried, passed through 2-mm sieve after removing visible plant material for further analyses.



Figure 1. Soil culture experiment

Table 2. Design of experiment

N.	Dlau4:	Plants/pot				
No	Planting patterns	C. lanceolata	M. macclurei	S. superba		
1	Pure C. lanceolata	16				
2	Pure M. macclurei		16			
3	Pure S. superba			16		
4	C. lanceolata/ M. macclurei	8	8			
5	C. lanceolata/S. superba	8		8		

Determination of Al chemical forms and pH in soil

Chemical forms of Al in soils were extracted using continuous extraction methods (Huang and Qu, 1996; Zhou et al., 2011). Al in each fraction was determined by an auto discrete analyzer (SmartChem 200, WestCo Scientific Instruments Inc., and Italy).

Exchangeable Al: 1 mol L⁻¹ KCl was used to extract exchangeable Al (mainly Al³⁺).

Monomer hydroxy Al: 1 mol L^{-1} CH₃COONH₄ was used to extract monomer hydroxy Al (mainly Al(OH)²⁺ and Al(OH)²⁺).

Acid-soluble inorganic Al: 1 mol L⁻¹ HCl was used to extract acid-soluble inorganic Al (mainly Al(OH)₃).

Humic acid Al: 0.5 mol L⁻¹ NaOH was used to extract humic acid Al (mainly humic acid bound Al).

Soil pH was measured in 1 mol L⁻¹ KCl (1:2.5, w:v) using an PHS—3C pH meter (Shanghai INESA Scientific Instrument Co., Ltd, China).

Statistical analysis

Initial data were analyzed using Microsoft Excel. Values were represented as the means of four replicates (Mean \pm SD) for each treatment. The relationship between soil pH and chemical forms of Al in soil was also analyzed using linear regression in Microsoft Excel. Differences between different treatments were statistically calculated using method of Tukey for analysis of variance (ANOVA) in SPSS 13.0. The data were considered to be significantly different at P < 0.05.

Results

Content of exchangeable Al

As presented in Fig. 2, the content of exchangeable Al deceased by 2.3-3.0% in rhizosphere soil and 7.3-8.1% (P < 0.01 for 6 months and P < 0.05 for 18 months) in bulk soil of mixed plantation of *C. lanceolata* with *M. macclurei* when compared to that of pure *C. lanceolata* plantation. The content of exchangeable Al increased by 2.1-5.4% in rhizosphere soil whereas it decreased by 6.6-6.9% (P < 0.01 for 6 months) in bulk soil of mixed plantation of *C. lanceolata* with *S. superba*.

As showed in *Table 3*, the content of exchangeable Al increased in rhizosphere soil but decreased in bulk soils of *M. macclurei* after mixed plantation. The soil exchangeable Al reduced in both rhizosphere and buck soils of *S. superba*, and the changes were significant for the 6-month treatment.

Table 3. Changes in content of exchangeable Al in soils of M. macclurei and S. superba after mixed plantation with C. lanceolata (Mean + SD, n = 4 for all the treatments)

Dianting nottowns	Rhizosphere	soil (mg/kg)	Bulk soil (mg/kg)	
Planting patterns	6 months	18 months	6 months	18 months
M. macclurei	190.9±22.3a	194.4±9.2b	224.3±14.6a	221.9±12.1a
C. lanceolata/M. macclurei	209.5±19.5a	215.9±10.7a	220.4±3.6a	219.9±8.5a
S. superba	189.5±5.1a	197.1±7.8a	238.1±3.0a	226.7±9.1a
C. lanceolata/S. superba	179.7±4.4a	191.1±6.6a	224.1±3.4b	217.8±4.37a

Different letters indicate significant differences at P< 0.05 between pure plantation and its mixed plantation as determined by the method of Tukey test

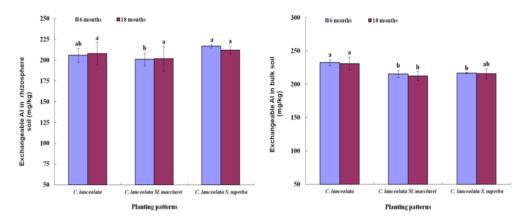


Figure 2. Effect of the mixed plantation of C. lanceolata on the content of exchangeable Al in both rhizosphere and bulk soils. Error bars are standard errors of the mean (n = 4 for all the treatments). Significant differences (P < 0.05) between pure C. lanceolata and its mixed plantation are marked by different letters

Content of monomer hydroxy Al

Content of monomer hydroxy Al (Fig. 3) in rhizosphere soil of mixed plantation of C. lanceolata with M. macclurei was 13.1-14.2% (p < 0.01 for 18 months) less than that of pure C. lanceolata plantation whereas the value in bulk soil was 2.6-12.4% (P < 0.01 for 18 months). The content of monomer hydroxy Al in bulk soil of mixed plantation of C. lanceolata with S. superba was 7.2-7.3% (P < 0.05 for 18 months) less than that of pure C. lanceolata plantation whereas its changing trend in the rhizosphere soil was inconsistent between 6 and 18 months treatments.

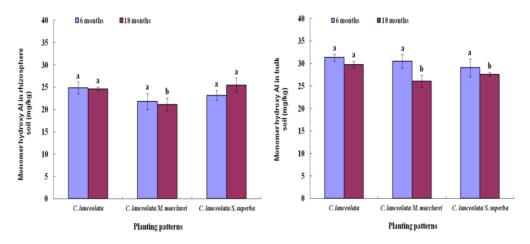


Figure 3. Effect of the mixed plantation on the content of monomer hydroxy Al in both rhizosphere and bulk soils. Error bars are standard errors of the mean (n = 4 for all the treatments). Significant differences between pure C. lanceolata and its mixed plantation are marked by different letters

The data of the effects of mixed plantation on *M. macclurei* and *S. superba* soils showed that the content of monomer hydroxy Al in both rhizosphere and bulk soils (*Table 4*) of *M. macclurei* in mixture with *C. lanceolata* and *S. superba* in mixture with

C. lanceolata was less than that of their pure plantations. The difference in monomer hydroxy Al content in bulk soil between mixed plantation and pure plantation was sinificant after a 6-month mixed plantation treatment of both species, and a 18-month mixed plantation treatment of S. superba.

Table 4. Changes in content of monomer hydroxy Al in soils of M. macclurei and S. superba after mixed plantation with C. lanceolata (Mean + SD, n = 4 for all the treatments)

Diontinonottomo	Rhizosphere soil (mg/kg)		Bulk soil (mg/kg)	
Planting patterns	6 months	18 months	6 months	18 months
M. macclurei	22.6±4.5a	30.5±2.7a	36.2±5.3a	32.7±3.5a
C. lanceolata/M. macclurei	21.3±1.6a	29.5±0.4a	28.7±2.3b	30.1±1.6b
S. superba	28.2±4.4a	31.2±2.2a	30.6±0.7a	29.8±0.2a
C. lanceolata/S. superba	23.8±3.1a	30.2±1.6a	26.4±1.7b	28.5±2.0a

Different letters indicate significant differences at P < 0.05 between pure plantation and its mixed plantation as determined by the method of Tukey test

Content of acid-soluble inorganic Al

Content of acid-soluble inorganic Al (*Fig. 4*) in rhizosphere soil of mixed plantation of *C. lanceolata* with *M. macclurei* was 6.5–9.9% less than that of pure *C. lanceolata* plantation whereas it increased slightly in the bulk soil. The content of acid-soluble inorganic Al in rhizosphere soil of mixed plantation of *C. lanceolata* with *S. superba* was 6.2–9.6% less than that of pure *C. lanceolata* plantation whereas the value in bulk soil was 2.5–7.0%. The statistic analysis did not show that all of the above changes in the content of acid-soluble inorganic Al after mixed plantation were significant.

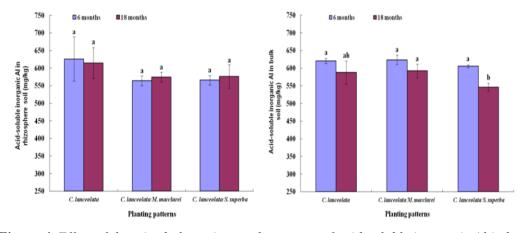


Figure 4. Effect of the mixed plantation on the content of acid-soluble inorganic Al in both rhizosphere and bulk soils. Error bars were standard errors of the mean (n = 4 for all the treatments). Significant differences between pure C. lanceolata and its mixed plantation are marked by different letters

Content of acid-soluble inorganic Al in rhizosphere soil of both *M. macclurei* and *S. superba* increased after mixed plantation (*Table 5*). But the changes were not significant. In contrast, the content of acid-soluble inorganic Al in bulk soil was significantly reduced after a 6-month treatment.

Table 5. Changes in content of acid-soluble inorganic Al in soils of M. macclurei and S. superba after mixed plantation with C. lanceolata (Mean + SD, n = 4 for all the treatments)

Diouting nottoms	Rhizosphere	soil (mg/kg)	Bulk soil (mg/kg)	
Planting patterns	6 months	18 months	6 months	18 months
M. macclurei	598.6±30.7a	613.6±36.1a	619.7±18.9a	591.6±57.2a
C. lanceolata/M. macclurei	607.1±10.6a	628.2±10.3a	588.6±9.3b	583.2±13.8a
S. superba	635.2±15.5a	645.0±32.2a	624.4±11.7a	596.9±17.1a
C. lanceolata/S. superba	656.8±12.2a	667.9±7.3a	592.9±13.7b	631.9±29.6a

Different letters indicate significant differences at P< 0.05 between pure plantation and its mixed plantation as determined by the method of Tukey test

Content of humic acid Al

Content of humic acid Al (Fig. 5) in rhizosphere and bulk soils of mixed plantation of C. lanceolata with S. superba increased by 5.4–9.9% and 5.4–6.2% (P < 0.01 for bulk soil after 6 months) when compared to that of pure C. lanceolata plantation. For mixed plantation of C. lanceolata with M. macclurei, the content of humic acid Al had an increase of 0.8–3.4% in rhizosphere soil and a decrease of 0.7–3.5% in bulk soil, but the above changes were not significant.

For both *M. macclurei* and *S. superba*, the content of humic acid Al increased after mixed plantation (*Table 6*). The changes were significante in rhizosphere soil except 18 month treatment of *M. macclurei*.

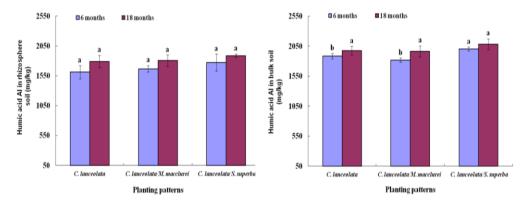


Figure 5. Effect of the mixed plantation on the content of humic acid Al in both rhizosphere and bulk soils. Error bars are standard errors of the mean (n = 4 for all the treatments). Significant differences between pure C. lanceolata and its mixed plantation are marked by different letters

Table 6. Changes in content of humic acid Al in soils of M. macclurei and S. superba after mixed plantation with C. lanceolata (Mean + SD, n = 4 for all the treatments)

Planting nottowns	Rhizosphere	sphere soil (mg/kg) Bulk soil (mg/kg)		(mg/kg)
Planting patterns	6 months	18 months	6 months	18 months
M. macclurei	1856.1±223.1b	1874.4±232.7a	2084.4±280.6a	2060.5±271.2a
C. lanceolata/M. macclurei	2051.3±101.7a	1983.4±81.5a	2145.6±52.5a	2073.0±117.9a
S. superba	1907.0±20.7b	1925.9±38.8b	2055.0±42.8b	2076.9±93.2a
C. lanceolata/S. superba	2197.1±145.9a	2160.4±95.8a	2135.9±17.9a	2130.1±96.1a

Different letters indicate significant differences at P< 0.05 between pure plantation and its mixed plantation as determined by the method of Tukey test

Soil pH

The changed in soil pH was also observed after mixed plantation (Fig.~6). There was an increase in pH in both rhizosphere (P < 0.05) and bulk soils (P < 0.05 after 6 months) of the mixed plantation of C. lanceolata with M. macclurei when compared to that of pure C. lanceolata plantation. For mixed plantation of C. lanceolata with S. superba, only bulk soil pH showed a significant increase (P < 0.05). Table 7 shows the relationship between soil pH and four chemical forms of Al in soil of C. lanceolata. We found that soil pH had a negative correlation with exchangeable Al and monomer hydroxy Al, but the correlation was not significant. There was also a negative correlation between soil pH and both exchangeable Al and humic acid Al in soils of M. macclurei and S. superba plantation which were used as mixed species with C. lanceolata (Table~8). The the correlation between soil pH and exchangeable Al in soil was significant at 0.01 level.

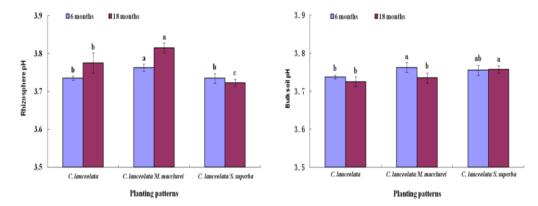


Figure 6. Effect of different mixed planting patterns of C. lanceolata on soil pH. (a) Rhizosphere soil; (b) Bulk soil. Error bars were standard errors of the mean (n=4 for all the treatments). Significant differences between pure C. lanceolata and its mixed plantation are marked by different letters

Table 7. Relationship between soil pH and chemical forms of Al in soil of C. lanceolata (n=12)

	Chemical forms	Correlation coefficient
Soil pH	Exchangeable Al	-0.5562
	monomer hydroxy Al	-0.4238
	Acid-soluble inorganic Al	-0.0960
	Humic acid Al	-0.1589

Table 8. Relationship between soil pH and chemical forms of Al in soil of M. macclurei and S. superba (n=16)

	Chemical forms	Correlation coefficient
Soil pH	Exchangeable Al	-0.6955**
	monomer hydroxy Al	0.0173
	Acid-soluble inorganic Al	0.5411*
	Humic acid Al	-0.4577

^{*}P< 0.05, **P< 0.01

Discussion

Our results indicated that there were differences in chemical forms of soil Al between mixed and pure plantations of C. lanceolata. The mixed plantation of C. lanceolata with both M. macclurei and S. superba could reduce the exchangeable Al and monomer hydroxy Al content in all soils except in the rhizosphere soil of mixed plantation of C. lanceolata with S. superba (Figs. 2 and 3). This decreasing tendency was consistent with the result of Li et al. (2010) on the mixed plantation of Pinus massoniana with Cinnamomum camphora. Our finding could be explained through some research results related to mixed plantation in the literatures. It has been documented that mixed plantation could increase plant biomass, soil pH, organic carbon, organic matter, phosphorus nutrition, soil N availability, and microbial biomass, as well as an improvement in microbial community diversities and enzyme activities (Li et al., 2010; Richards et al., 2010; Singh et al., 2012; Forrester et al., 2013; Dutta and Hossain, 2017; Tchichelle et al., 2017). Then such changes may be attributed to the changes in Al chemical forms because the distribution of Al chemical forms in soil could be affected by some of the above-mentioned factors such as soil pH, organic carbon, organic matter and phosphorus nutrition (Godsey et al., 2007; Fang et al., 2014; Hagvall et al., 2015). In our experiement, C. lanceolata is an important coniferous species whereas M. macclurei and S. superba are broadleaved tree species. Broadleaf litter generally has higher nutrient concentrations and lower lignin and polyphenol concentrations than needle litter and has faster litter decomposition rate (Prescott et al., 2004). Moreover, coniferous species may create more acid soils (Götmark et al., 2005). Therefore, introducing broadleaved species in C. lanceolata plantation would be very useful for the improvement of soil properties. Our results showed that the soil pH in C. lanceolata had an increasing tendency after mixed plantation. This results is in accordance with Cremer et al. (2017), who reported that a mixed plantation of Douglas fir (Pseudotsuga menziesii) and European beech (Fagus sylvatica) had a higher forest floor and mineral soil pH than pure conifer stands. Similar report came from Berger et al. (2004), who documented that soil pHs under mixed species were higher than soil pHs under pure spruce. Statistic analysis further showed that there was a negative correlation between soil pH in C. lanceolata and Al chemicla forms in soil, indicating that the change in soil pH may be one of the reasons for the changes in Al chemical forms after mixed plantation. However, further studies on the effects of the mixed plantation of C. lanceolata with both species on other soil properties and their relationships with Al chemical forms are needed in the future.

Our results also showed that the content of acid-soluble inorganic Al in soils of *C. lanceolata* decreased after mixed plantation except that in the rhizosphere soil of mixed *C. lanceolata* and *M. macclurei* plantation (*Fig. 4*), whereas content of humic acid Al increased in all soils except in the rhizosphere soil of mixed *C. lanceolata* and *M. macclurei* plantation (*Fig. 5*). Yang had found that there was a significant increase in humic acid in a the mixed forest of *C. lanceolata* when compared to its pure stand (Yang et al., 2002). Therefore, the increase of humic acid Al in soil in our experiment may be because of the increase of humic acid after mixed plantation of *C. lanceolata*. As mentioned in the introduction section, different Al forms in soil differed in their phytotoxicity (Zhou et al., 2011; Shahnawaz et al., 2016). The toxicity potential was ranked in decreasing order as exchangeable Al, monomer hydroxy Al, acid-soluble inorganic Al, and humic acid Al. Therefore, the decrease in exchangeable Al, monomer hydroxy Al, and acid-soluble inorganic Al contents and the increase in humic acid Al

content indicated that such a mixed plantation could decrease the toxic Al forms, and consequently, Al phytotoxicity would be reduced.

We observed that chemical forms of Al in soil of *M. macclurei* and *S. superba* plantation also changed to different extent after mixed plantation with *C. lanceolata*. The exchangeable Al and monomer hydroxy Al content showed a decreasing trend as that of *C. lanceolata*. These results suggested that the mixed plantation is not only useful for *C. lanceolata*, but is also good for the two mixed species as for soil aluminum toxicity.

Conclusions

The experimental results indicated that mixed plantation of *C. lanceolata* could change the distribution of Al speciation in the soil. However, the changes varied among different chemical forms. There was a decreasing tendency in exchangeable Al in both mixed planting patterns. Similar results were also observed in the content of monomer hydroxy Al and acid-soluble inorganic Al. These results indicated that there was a general decrease tendency in the content of chemical forms with higher phytotoxicity including two most active chemical forms of Al in soils (i.e., exchangeable Al and monomer hydroxy Al). Therefore, we suggested that mixed planting patterns could be an alternative alleviation method to control the harmful Al effects on *C. lanceolata* forest although further studies are still needed.

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