

Aluminum Accumulation and Release and the Alleviating Effects of Biochar and Lime as Soil Amendments in *Camellia oleifera* Leaves

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[Summary]

Camellia oleifera is known as an aluminum (Al) hyper-accumulator, and the Al mainly accumulates in its leaves. However, little is known regarding the accumulation of Al, the decomposition of fallen leaves, and its effect on soil exchangeable Al contents, or ways to reduce Al contents in *C. oleifera* leaves. In this study, litter bag and pot experiments were carried out to investigate Al accumulation and decomposition of *C. oleifera* leaves, and the effects of lime and biochar applications as soil amendments on leaf Al contents. Results showed that higher Al contents were observed in older leaves. The highest Al content of fallen leaves was 15,748.62 mg kg⁻¹. In the first 4 months, 28.73% of the total mass of fallen leaves had decomposed, while 35.64% of the Al was lost in the first month, followed by 7.57% in the second and 4.15% in the third month, and leaf decomposition significantly affected the soil exchangeable Al contents. The content of total non-crystalline Al was highest, followed in descending order by organically bound Al, exchangeable Al, and water-soluble Al in treated soils. The addition of biochar and lime as soil amendments had synergic effects on reducing the Al contents of *C. oleifera* leaves, and they interactively influenced the exchangeable Al and organically bound Al. These results indicate that the Al fixed in leaves that then falls onto the soil is one of the important ways that *C. oleifera* alleviates Al toxicity, which can be further improved by applying lime and biochar as soil amendments.

Key words: tea oil camellia, Al accumulation, soil Al fractions, nutrient release, reduction.

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研究報告

油茶葉片鋁累積、釋放以及活性炭和鈣 對其含量的消滅研究

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摘要

油茶是鋁超累積樹種，鋁主要累積在其葉片中。然而，目前對油茶葉片鋁累積、凋落葉的降解、鋁釋放及其對土壤可交換鋁的影響，以及如何消滅鋁含量還未見報導。本研究通過田間採樣、分解袋法和盆栽試驗等研究了油茶葉片鋁累積、凋落葉降解過程中鋁釋放以及石灰和生物炭對油茶葉片鋁含量的影響。結果表明，隨著葉齡的增加，葉片鋁含量增加，其中落葉中鋁含量達 $15,748.62 \text{ mg kg}^{-1}$ 。在降解的第1個月，落葉降解了總幹重的28.73%，第1、2和3個月凋落葉鋁減少量為35.64、7.57和4.15%。落葉顯著影響土壤的可交換鋁含量。土壤中不同鋁分級含量順序為非晶質鋁 > 有機絡合鋁 > 可交換鋁 > 水溶態鋁。在土壤中施用石灰和生物炭均能減少油茶葉片鋁含量，二者耦合影響土壤可交換鋁和有機絡合鋁的含量。該研究表明鋁在油茶葉片中固定並及時凋落是油茶緩解鋁毒的重要方式，在土壤中施用石灰和生物炭均能有效緩解鋁毒。

關鍵詞：油茶、鋁累積、鋁分級、養分釋放、消滅。

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INTRODUCTION

Aluminum (Al) is the most abundant metal in the earth's crust and generally exists as non-phytotoxic solid forms in soils (Zeng et al. 2011). However, when soils are acidic (pH < 5.5), soluble Al species, such as Al^{3+} and $\text{Al}(\text{OH})^{2+}$, and their mobility and toxicity to plants quickly increase (Kochian et al. 2004). Among them, Al^{3+} is considered to be a major limiting factor to plant growth in acidic soils (Zheng 2010). Previous studies found that Al can inhibit root growth (Zakir Hossain et al. 2006), cause excessive accumulation of reactive oxygen species, and destroy the ultrastructure of root cells (Bartels and Sunkar 2005). The abundance of Al^{3+} in soil solutions depends on the solution pH and the existence of components that are able to adsorb Al. Thus, the stability of Al forms in the soil solid

phase is one of the key factors in avoiding solubilization of this element (Álvarez et al. 2012). Many researchers are searching for methods to alleviate Al toxicity. Organic compost amendments can alleviate the potential toxicity of Al in acidic soils by increasing the soil pH and converting exchangeable Al to organically bound and other noncrystalline fractions (Vieira et al. 2008). Meanwhile, many plant species have evolved mechanisms to improve their survival in acidic soils, such as those that can likely exclude Al^{3+} from roots and those that enable plants to safely accommodate Al^{3+} once it enters the symplast (Ryan et al. 2011). For example, buckwheat and *Camellia sinensis* can accumulate as much as 10 g kg^{-1} Al in their leaves (Shen et al. 2006, Chen et al. 2008).

Camellia oleifera (tea oil camellia) is an important woody species, the edible oil of which has been used for more than 2300 years in southern China (Yuan et al. 2017), and it is mainly cultivated in acidic soils, which comprise approximately 21% of the total arable land area in China (Qian et al. 2014). Tea oil camellia trees are typically grown in red acidic soils with high concentration of active Al (Yuan et al. 2017). Chen et al. (2008) showed that *C. oleifera* accumulated more than 13.5 g kg⁻¹ Al in its old leaves and was tolerant to Al toxicity. Huang et al. (2017) reported that Al at low concentrations (0.5~2.0 mM) enhanced the growth of *C. oleifera*. Therefore, Al fixed in leaves is beneficial to Al tolerance in tea oil camellia, but to date, little information is available about Al accumulation in leaves at different ages, the decomposition of fallen leaves, and its effect on soil exchangeable Al contents or ways to reduce Al contents in *C. oleifera* leaves. The objectives of this study were to understand Al accumulation and decomposition of tea oil camellia leaves, and the effects of the addition of lime and biochar as soil amendments on soil Al fractions and contents in leaves of *C. oleifera* grown in acidic soils.

MATERIALS AND METHODS

Leaf samples. Leaves were collected from tea oil camellia orchards in Dongcheng Town, Changsha City (Hunan Province, China). Thirty trees (of 18-years old) of each variety (*C. oleifera* ‘Huashuo’, ‘Huaxin’, and ‘Huajin’) were selected as sample trees. New leaves sprouting as shoots from 4 directions of the trees were labeled on 4 April 2017. Leaves on labeled shoots were collected 1 and 8 mo later, as young and old leaves, respectively. Meanwhile, freshly fallen leaves

(litter leaves) were collected from the same varieties on 4 April 2017. Al concentrations of leaves were determined according to Yuan et al. (2017). All measurements were carried out in triplicate.

Litter decomposition experiment. Decomposition rates of litter leaves were determined using a litter bag method (Zhao et al 2013). Collected fallen leaves of the 3 varieties were cut into pieces without veins and were well mixed to obtain a uniform composition. Then, the leaf mixture was oven-dried at 60°C, and 10.0 g of dried leaf litter was placed in a polyvinyl screen mesh bag (10×10 cm, with a mesh size of 0.5 mm). Fifty treatment bags with leaves were evenly distributed on the soil surface under the tea oil camellia trees, on 20 April 2017, and bags without leaves were used as a control. Five treatment bags were collected every month and oven-dried, and then the mass loss was calculated, and Al contents of the litter leaves were determined (Yuan et al. 2017). Meanwhile, soils under the treatment bags and control bags were collected, and soil exchangeable Al was measured (Yuan et al. 2017).

Pot experiment. One-year-old grafted seedlings with uniform growth were transplanted to 2.5-L plastic pots (12 cm in diameter and 18 cm tall) containing 4 kg of red acidic soil on 27 April 2017. The soil was collected from the same orchards described above, and the air-dried soil was sieved to pass a 2-mm sieve before potting. The soil was quaternary red clay, the pH (H₂O) was 4.30, the content of exchangeable Al was 398.3 ± 25.7 mg kg⁻¹, and organic matter was 8.5 g kg⁻¹. The potted seedlings were grown outdoors and protected from rainfall by a plastic canopy. Sixteen treatments (completely randomized experiment) of different combinations of lime and biochar concentrations were created by adding Ca(OH)₂ (AR) or

biochar (from Acticarbon Changsha Co., Ltd., Changsha, China), pH 6.0, and the carbonized temperature was 600°C) to soils. The levels of lime were 0, 2.0, 4.0, and 8.0 g kg⁻¹, while biochar levels were 0, 8.0, 16.0, and 32.0 g kg⁻¹. Plants were irrigated with 200 ml of distilled water every 3 d starting on 30 April 2017 and fertilized with 5.0 g NH₄NO₃ in each pot on 20 June 2017. There were 4 replicates in each treatment, and pots were arranged in a completely randomized design. Seedlings were harvested on 30 August 2017 and oven-dried at 65°C to a constant weight. Then the Al contents of stems, leaves, and roots were determined using the same methods described above. Rhizosphere soil samples were taken by shaking off the soil adhering to the roots. Non-rhizosphere soil samples were taken from the remaining bulk of the soil. Soils collected from different replicates were pooled within each treatment. All soil samples were air-dried and manually ground to determine the Al fractions.

Fractionation of soil Al. Various forms of Al after treatment with lime and biochar were extracted by a single extraction procedure with different reagents (Dai et al. 2011, Álvarez et al. 2012): for water-soluble Al (Al_w), 1.0 g of soil was mixed with 10 mL of distilled water and shaken for 2 h; for exchangeable Al (Al_e), 1.0 g of soil was mixed with 10 mL of 1 mol L⁻¹ KCl and then shaken for 2 h; for organically bound Al (Al_p), 5.0 g of soil was mixed with 50 mL of 0.1 mol L⁻¹ sodium pyrophosphate (Na₄P₂O₇·10H₂O) and then shaken for 16 h; and for total non-crystalline Al (Al_o), 5.0 g of soil was mixed with 50 mL of 0.2 mol L⁻¹ acid ammonium oxalate (C₂H₁₀N₂O₅) and then shaken for 4 h. Shaking was done at 25°C. Al contents of all of the above samples were determined following the same method described above.

Statistical analysis. Data from all sample

sites were pooled because no site differences were found. Results are reported as the mean ± stand deviation (SD) in each treatment, and all data were analyzed for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test for mean separation at the $p < 0.05$ level. Statistical analysis was performed using SPSS 22.0 (IBM, Chicago, IL, USA), and all graphs were made by Origin 8.5 (OriginLab, Northampton, MA, USA).

RESULTS

Al contents in new, old, and fallen leaves. In general, Al content in leaves of the 3 varieties increased with age (Fig. 1), and the highest Al content (mean of 3 varieties was 15,748.62 mg kg⁻¹) was found in fallen leaves, followed by old leaves and new leaves. In new leaves, the Al content of *C. oleifera* ‘Huaxin’ leaves was significantly higher than those of the other 2 cultivars. However, no significant difference was found in Al contents of fallen leaves among the 3 cultivars.

Litter decomposition. Decomposition of tea oil camellia fallen leaves appeared to be faster in the early stage, and the mass remaining gradually stabilized in the later stage (Fig. 2). In the first 4 mo of decomposition, 28.73% of the total mass of leaves had decomposed, and the residual mass of leaves was 66.2% after 8 mo. Meanwhile, Al contents of *C. oleifera* leaves had the same trend as the mass loss in decomposition. Al concentrations of leaves decreased from 27,755.52 to 19,404.90 mg kg⁻¹, and Al contents dropped from 277.56 to 130.19 mg per bag (Fig. 2). The loss of Al in the first month was up to 35.64%, followed by 7.57% in the second and 4.15% in the third month.

Exchangeable Al contents in the soil covered by the mesh bags containing *C. ole-*

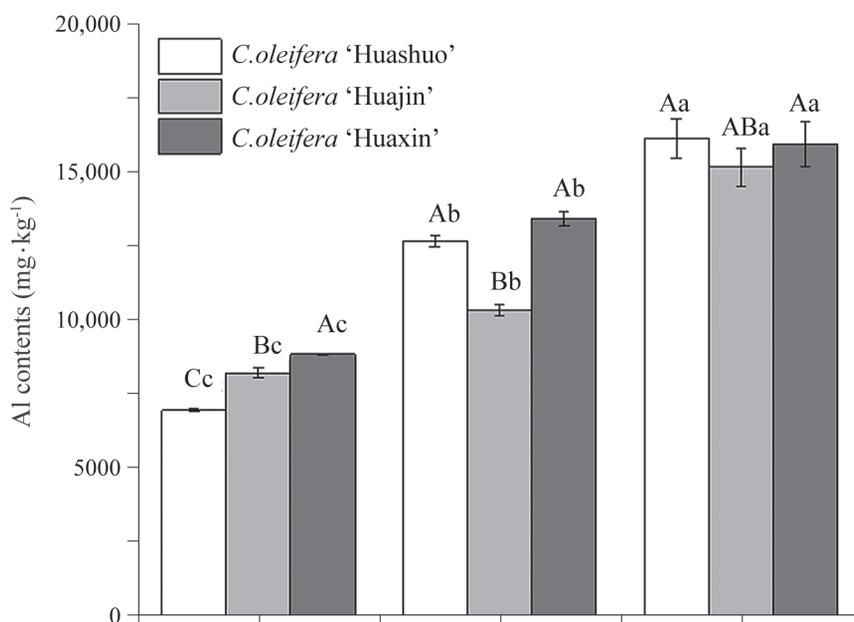


Fig. 1. Aluminum (Al) contents in tissue samples of 3 *Camellia oleifera* varieties sampled from a plantation. Data points with different capital and lowercase letters respectively indicate a significant difference at $p \leq 0.05$ in different varieties at the same age and different ages of the same variety.

ifera leaves. The exchangeable Al content in the soil gradually decreased in the first 5 mo in both treatments, i.e., covered by mesh bags containing *C. oleifera* leaves or without leaves, and then it increased in the next 2 mo (Fig. 3). A paired *t*-test showed that the exchangeable Al covered by mesh bags containing *C. oleifera* leaves was significantly higher than that of soil covered by empty bags ($p < 0.01$), while the maximum increase (difference) ($330.00 \text{ mg kg}^{-1}$) in the soil Al concentration was found in July between the 2 treatments.

Soil Al fractions after lime and biochar treatments. Among the 4 different soil fractions, contents of total non-crystalline Al (Alo) was the highest, followed in descending order by organically bound Al (Alp), exchangeable Al (Ale), and water-soluble Al (Alw). Lime

and biochar addition to the soil significantly affected the soil Al fractions (Table 1). Water-soluble Al (Alw) was significantly affected by the interaction between biochar and lime. Biochar, lime, and their interaction influenced organically bound Al (Alp) and exchangeable Al (Ale), and biochar and lime interactively affected the total non-crystalline Al.

Al contents of *C. oleifera* under lime and biochar treatments. Al contents in leaves, roots, and stems were affected by biochar and lime addition (Table 2). Al contents in leaves were significantly higher than those in roots and stems, regardless of the treatment. Al contents in leaves, stems, and roots were significantly affected by biochar, lime, and their interaction, despite biochar addition having no effect on stem contents.

Relationships between plant Al contents

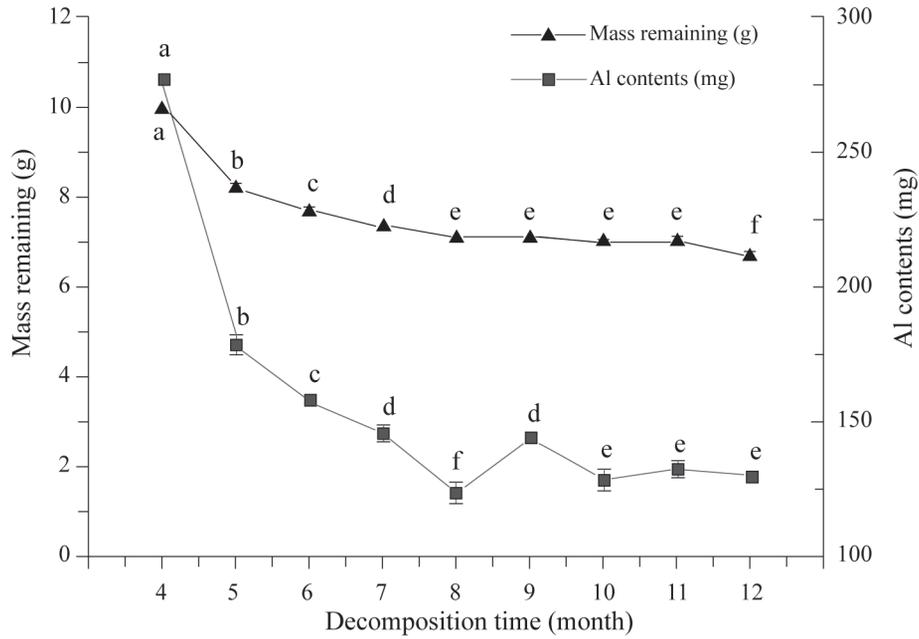


Fig. 2. Mass remaining and aluminum (Al) contents of *Camellia oleifera* leaves during the decomposition process. Data points with different letters indicate a significant difference at $p \leq 0.05$. Error bars represent the standard deviation.

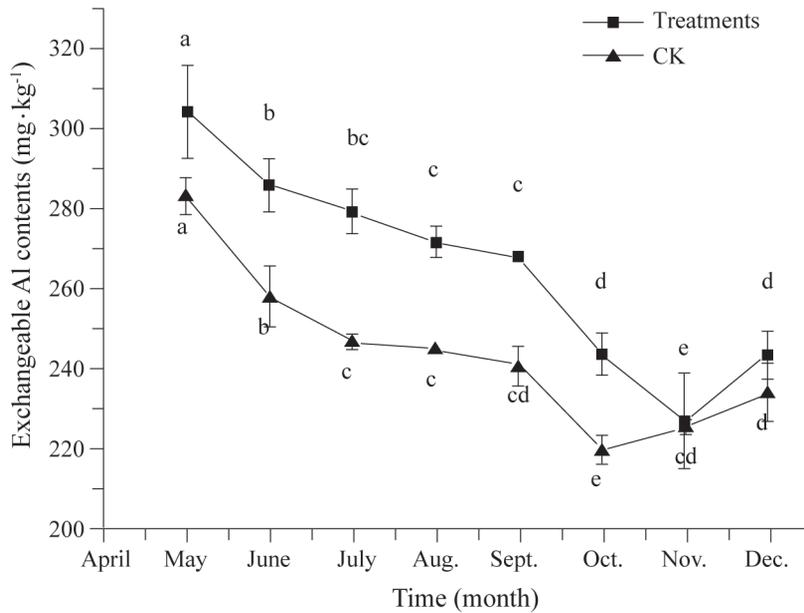


Fig. 3. Exchangeable aluminum (Al) contents at different times in soil covered by mesh bags containing *Camellia oleifera* leaves and control bags (CK). Data points with different letters indicate a significant difference at $p \leq 0.05$. Error bars represent standard errors.

Table 1. Soil aluminum fractions treated by lime and biochar as soil amendments

Lime (g kg ⁻¹)	Biochar (g kg ⁻¹)	Water-soluble Al (Alw) (mg kg ⁻¹)	Exchangeable Al (Ale) (mg kg ⁻¹)	Organically bound Al (Alp) (mg kg ⁻¹)	Total noncrystalline Al (Alo) (mg kg ⁻¹)
0.00	0.00	11.96±0.89 Da	402.05±10.97 Cd	1177.22±21.83 Babcd	3669.29±325.93 Aa
0.00	8.00	10.15±0.95 Db	520.99±31.51 Cbc	939.05±52.20 Bf	2877.86±314.63 Abcd
0.00	16.00	7.67±0.16 Def	526.51±18.34 Cabc	1110.59±18.14 Bde	2858.34±251.56 Abcd
0.00	32.00	7.47±0.04 Df	533.46±18.34 Cabc	1148.86±23.40 Bcde	2675.66±211.76 Acd
2.00	0.00	7.43±0.29 Df	564.66±7.23 Cabc	1310.97±25.39 Ba	2858.23±333.91 Abcd
2.00	8.00	8.30±0.21 Ddef	538.04±14.74 Cabc	1294.01±63.29 Bab	3267.54±311.11 Aab
2.00	16.00	8.33±0.60 Ddef	593.44±32.43 Cab	1248.51±66.64 Babcd	2901.62±136.46 Abcd
2.00	32.00	8.95±0.76 Dcd	531.36±65.89 Cabc	1244.22±43.35 Babcd	2636.72±88.13 Ad
4.00	0.00	8.46±0.54 Ddef	586.37±22.28 Cab	1202.27±32.71 Babcd	2726.45±387.85 Acd
4.00	8.00	9.64±0.12 Dbc	610.70±68.6 Ca	1284.34±91.68 Babc	3285.80±245.79 Aab
4.00	16.00	8.59±0.42 Dde	546.65±30.10 Cabc	1159.54±30.69 Bbcde	2886.03±166.54 Abcd
4.00	32.00	9.09±0.66 Dcd	506.05±30.48 Cbc	1107.53±13.36 Bde	2901.61±313.19 Abcd
8.00	0.00	7.74±0.36 Defg	545.58±83.86 Cabc	1129.76±56.31 Bde	3137.40±337.75 Abc
8.00	8.00	7.89±0.40 Def	494.42±26.03 Cc	1034.71±93.77 Bef	2606.00±118.34 Ad
8.00	16.00	8.34±0.57 Ddef	570.43±51.90 Cabc	1188.35±204.65 Babcd	2901.38±51.31 Abcd
8.00	32.00	8.12±0.50 Ddef	372.02±91.20 Cd	910.61±36.90 Bf	2633.39±171.06 Ad
ANOVA					
Lime		NS	*	*	NS
Biochar		NS	*	*	*
Lime×Biochar		*	*	*	*

Note: Means with different lowercase letters in the same column and different capital letters in the same line significantly differ as tested by least significant difference multiple comparison at $p \leq 0.05$. NS, no significant difference; * $p \leq 0.05$.

and soil Al fractions. Correlation analyses showed that there were significant correlations of Al root contents with water-soluble Al (Alw), organically bound Al (Alp), and total non-crystalline Al (Alo), while Al contents in stems had a significant relationship with water-soluble Al (Alw) and highly significant relationships with organically bound Al (Alp) and total non-crystalline Al (Alo). Al contents of leaves were not significantly correlated with soil Al fractions.

DISCUSSION

Al toxicity is considered the most important growth-limiting factor for plants in acidic soils, and many plants have evolved different

resistance mechanisms. The most important mechanisms are to facilitate Al exclusion from the root apex (external tolerance mechanisms) and to tolerate Al in the plant symplasm (Brunner and Sperisen 2013). As an Al hyper-accumulator, *C. oleifera* accumulates more than 10,000 mg kg⁻¹ Al in leaves, demonstrating high tolerance to Al (Zeng et al. 2011). But to date, little is known about the tolerance mechanism to Al of *C. oleifera*. Zeng et al. (2012) found that Al was redistributed among leaves, but this research found that Al contents continually increased as leaves aged. Therefore, in order to prevent the translocation of Al to new leaves, it is important for leaves to fall “in time”. Our research data showed that the Al content of fallen leaves was up to 15,748.62

Table 2. Aluminum contents in different organs of *Camellia oleifera* treated with lime and biochar

Lime (g kg ⁻¹)	Biochar (g kg ⁻¹)	Al contents in roots (mg kg ⁻¹)	Al contents in stems (mg kg ⁻¹)	Al contents in leaves (mg kg ⁻¹)
0.00	0.00	8120.07 ± 469.75 Ba	5374.41 ± 335.30 Ca	11271.31 ± 1676.55 Aa
0.00	8.00	5486.95 ± 555.97 Bd	3282.64 ± 323.25 Ce	11700.47 ± 850.50 Aa
0.00	16.00	6448.70 ± 730.18 Bbc	4383.33 ± 132.71 Cb	7669.80 ± 447.95 Abcde
0.00	32.00	6910.03 ± 371.06 Bb	3810.52 ± 220.10 Cbcde	11004.97 ± 1044.91 Aa
2.00	0.00	6412.62 ± 442.58 Bbc	3589.20 ± 485.37 Ccde	11759.90 ± 1285.65 Aa
2.00	8.00	6907.92 ± 15.67 Bb	5264.46 ± 496.26 Ca	8756.04 ± 523.97 Abc
2.00	16.00	6674.04 ± 131.12 Bbc	4258.59 ± 381.17 Cbc	11122.24 ± 747.04 Aa
2.00	32.00	6324.61 ± 815.76 Bbc	4298.67 ± 351.50 Cbc	11404.94 ± 1377.77 Aa
4.00	0.00	5861.46 ± 273.92 Bcd	3953.07 ± 476.72 Cbcde	8566.07 ± 840.21A bcd
4.00	8.00	5970.70 ± 573.69 Bcd	4344.28 ± 479.35 Cbc	9017.75 ± 306.98 Ab
4.00	16.00	6442.59 ± 370.68 Bbc	3982.73 ± 489.21 Cbcde	7685.37 ± 532.74 Abcde
4.00	32.00	6502.22 ± 362.20 Bbc	4190.88 ± 298.97 Cbcd	7348.18 ± 374.72 Acde
8.00	0.00	6622.90 ± 299.72 Bbc	3480.24 ± 161.14 Cde	7921.08 ± 752.15 Abcde
8.00	8.00	6375.32 ± 216.41 Bbc	3563.35 ± 662.87 Ccde	7722.57 ± 271.66 Abcde
8.00	16.00	6353.55 ± 148.80 Bbc	3387.47 ± 436.64 Ce	7003.20 ± 273.17 Ade
8.00	32.00	5315.37 ± 169.38 Bd	3280.51 ± 387.90 Ce	6791.26 ± 183.50 Ae
ANOVA				
Lime		*	*	*
Biochar		*	NS	*
Lime × Biochar		*	*	*

Note: Means with different lowercase letters in the same column and different capital letters in the same line significantly differ as tested by least significant difference multiple comparison at $p \leq 0.05$. NS, no significant difference; * $p \leq 0.05$.

g kg⁻¹, and leaves fell at about 12 mo of age, which indicates that the return of Al to the soil through litter is a key Al tolerance mechanism of *C. oleifera*.

Litter production and nutrient cycling in terrestrial ecosystems play important roles in turnover of nutrients and maintenance of soil fertility and productivity (Saha et al. 2016). The mass of fallen leaves is quickly lost in the first few months. Similar results were reported in various plant species (Cornelissen 1996). Al concentrations and contents in leaves also decreased at the same time, and the rate of decomposition and Al release to the soil gradually decreased, because of the slow breakdown of lignin (Saha et al. 2016). The

release of Al from fallen leaves increased the exchangeable Al in the soil in the first 5 mo, but the effect did not last, indicating the short-term influence of litter on soil exchangeable Al contents in *C. oleifera* forest systems.

The toxicity of Al cations depends on the distribution of Al species in the soil solution, and Al³⁺, AlOH²⁺, and Al(OH)₂⁺ were found to be essential for evaluating Al toxicity (Matuš et al. 2006). The 4 fractions of Al in acidic red soil indicated that active Al mainly existed as total noncrystalline Al (Al_o), and the Al_e and Al_p fractions were affected by biochar, lime, and their interaction, suggesting that lime and biochar can reduce Al toxicity and play important roles in amending acidic

Table 3. Correlations (*r* values) between aluminum (Al) contents of *Camellia oleifera* plant tissues and soil Al fractions

Tissue	Water-soluble Al (Alw) (mg kg ⁻¹)	Exchangeable Al (Ale) (mg kg ⁻¹)	Organically bound Al (Alp) (mg kg ⁻¹)	Total noncrystalline Al (Alo) (mg kg ⁻¹)
Roots	0.302*	-0.111	0.367*	0.318*
Stems	0.327*	-0.0971	0.437**	0.410**
Leaves	0.193	0.118	0.232	0.08

Note: * $p \leq 0.05$; ** $p \leq 0.01$.

soils. The addition of amendments favors the formation of highly stable organo-Al complexes (Álvarez et al. 2012), and also prevents the evolution toward crystalline forms of Al. The significant interaction of lime and biochar on the 4 Al fractions indicates that lime or biochar alone cannot reduce Al toxicity as effectively as the addition of both biochar and lime. The fact that lime and biochar amendment affected Al contents of *C. oleifera* plants but not plant growth, indicates that some Al fractions are not toxic to plant growth (Menzies et al. 1994); on the contrary, they may have benefited plant growth (Huang et al. 2017). Al contents of leaves have positive relationships with soil Al fractions, but their relationship was not significant, indicating that Al accumulation can reduce available Al fractions but is more greatly influenced by the Al absorption characteristics of *C. oleifera*.

CONCLUSIONS

In summary, high Al contents in fallen leaves and slow decomposition rates indicated that the return of Al to the soil through litter is a key Al tolerance mechanism of *C. oleifera*. The addition of lime and biochar to the soil reduced the Al contents in leaves, which was influenced by the Al absorption characteristics of *C. oleifera* in acidic soils. Therefore, the Al absorption characteristics and the Al accumulation ability of *C. oleifera* need to be identified in future studies.

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