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Effects of Liming on the Morphologies and Nutrients of Different Functional Fine Roots of *Cunninghamia lanceolata* Seedlings

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Abstract: Soil acidification is an important cause of the productivity decline of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook)—one of the most important timber species in China. Although liming is an effective measure for reversing the effects of soil acidification, the effects on the morphologies and nutrients of different functional roots remain ambiguous. Thus, this study aimed to investigate the effects of liming on fine root traits of Chinese fir seedlings between two root function types (absorptive roots (AR) and transport roots (TR)). Chinese fir seedlings with equal performance were planted in each pot with two acidification soils (pH 3.6 and pH 4.3) and three levels of liming (0, 1000, and 4000 kg CaO ha^{-1}). Our data showed that liming had no effect on the root biomass (RB) of AR and TR in mildly acidified soil, but it decreased the RB in severely acidified soil. Specific root length (SRL) of AR and TR were significantly increased by 24% and 27% with a high liming dose in mildly acidified soil, respectively. The specific root areas (SRA) of AR and TR were significantly increased by 10% and 22% with a high liming dose in mildly acidified soil, respectively. Furthermore, root N concentrations were significantly increased by 26% and 30% in AR and TR with a high liming dose in mildly acidified soil, respectively. Root P concentration of AR was significantly increased by 21% with a high liming dose in mildly acidified soil while root Ca concentration was significantly increased with all treatments. A similar trend was also observed in the Ca/Al ratio of roots. Both low and high doses of liming decreased the root Al concentration of AR by 26% and 31% in mildly acidified soil, respectively; however, there was no significant effect on TR in both soils. Our findings indicated that liming could alleviate Al toxicity to fine roots and increase root investment efficiency and absorption capacity. Liming also had coordinate effects on SRL, SRA, Root tissue density (RTD), N, P, Ca and Ca/Al between AR and TR. Our study suggested that to gain a comprehensive understanding of plant growth strategy, researchers in future studies must consider different functional roots rather than just the absorption part. Our results also revealed that the root system became more "acquisitive" due to the remediation of Al toxicity, which may be an important mechanism underlying the increment of the productivity of Chinese fir plantations undergoing liming.

Keywords: liming; Chinese fir; absorptive root; specific root length; Ca/Al ratio

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1. Introduction

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is one of the most widely planted tree species in South China, covering more than 1.10 × 10^7 ha [1]. Due to the rapid growth and the excellent timber quality of Chinese fir, its timber production account for a quarter of the national commercial timber [2]. Given the massive extent of their planted areas, Chinese fir plantations also have considerable ecological benefits, such as carbon sequestration [3]. However, due to the increasing intensity of acid deposition in Southern China, some ecological problems have been reported successively since the 1980s, including soil acidification (decreasing of the pH value), soil cation depletion, and soil degradation [1,4]. It has also been reported that the productivity of Chinese fir plantations has been damaged by soil acidification caused by acid rain in parts of Southern Chinese provinces [5,6]. Meanwhile, liming is considered an effective measure to alleviate soil acidification [7,8]. Previous studies have proven that liming increases soil pH and decreases the concentration of dissolved inorganic Al [9,10], thus increasing forest productivity [8]. Although the positive effects of liming on Chinese fir growth have already been proven [11], the mechanism by which liming improves its productivity remains unclear, especially how the root system specifically responds to liming.

Root systems have shown an enormous diversity of properties and forms among other plant parts [12]. The adjustment of root phenotypic traits in response to variable environmental conditions is critical for plant growth [13,14]. For example, specific root length (SRL) is a common morphological parameter, which represents the exploiting area per unit root mass invested and can further be used as an indicator of root uptake efficiency [13,15]. Usually, plants prefer lower SRL and specific root areas (SRA) in acidic conditions [16], which enable roots to yield good performance because they provide better protection against Al toxicity hazards [17]. Liming can increase soil pH value and alleviate soil acidity, thus increasing theoretically the SRL of plants [13]. However, no effect of liming or pH on SRL has also been reported [18,19]. This uncertainty of the effect of liming on SRL may be ascribed to the unsuitable definition of fine roots, which include whole roots with sizes that are less than an arbitrary diameter (e.g., 2 mm).

Fine roots make up a multi-hierarchical structure that contains both absorptive roots (AR) and transport roots (TR) according to branching hierarchy [14,20]. Generally, the AR occupies the lowest branch orders (1–2 order) with good water and nutrient uptake ability, while TR is located in higher branching orders (3–5 order), playing transportation and structural roles [21,22]. AR is generally characterized by a small diameter, high SRL, or low root tissue density (RTD), while TR possesses the opposite traits [14]. Researchers have shown that SRL in the lower-order roots is almost 32 times bigger than that in the higher-order roots [23]. Considering these gigantic discrepancies, defining fine roots as an entirety according to an arbitrary diameter could lead to an erroneous result. Furthermore, compared with TR, AR has more frequent and intense interactions with soil and less defensive capacity, which allows for sensitivity to environmental changes [24,25]. For example, Zhou, et al. [26] found that N application significantly reduced the root length of the first- and second-order roots, but it did not affect the higher-order roots of *Pinus koraiensis*. Therefore, investigating how plant root morphologies adapt to the alleviation of soil acidification requires understanding the potentially contrasting responses of different functional roots to liming.

Root nutrients have a close relationship with plant functioning and are more sensitive than root morphology in responding to environmental changes [12,27]. For example, the calcium-aluminum (Ca/Al) ratio of roots has been found to be an important indicator reflecting whether the roots are restricted by Al toxicity [28,29]. This ratio has also been positively correlated with the length and biomass of roots [30]. Root N concentrations are often measured for their role in determining root activity [31] and, by extension, may be assumed to have a positive correlation with the root uptake ability [32]. However, the current understanding of liming-induced changes in root N and P remains vague [33,34]. Similar to root morphology, contrasting root N concentration between AR and TR may be a possible factor. Moreover, the nutrients of different functional roots also have various responses to environmental changes. For example, N addition enhanced the N concentration of AR, but did not affect the N concentration of TR [35]. Zhang, et al. [36] also showed that acid rain treatment increased the N concentration of AR, but it did not affect the N concentration of

TR. However, whether the nutrients of different functional roots have various responses to liming has yet to be fully investigated.

In this study, we conducted a pot experiment involving the liming treatment of Chinese fir seedlings using two types of soils with different degrees of acidification. This study aimed (1) to investigate the effects of liming on the morphologies and nutrients of fine roots, and (2) to investigate the differences in liming effects between various functional roots (AR and TR) of Chinese fir seedlings. As described above, liming could alleviate root Al toxicity and AR can be more sensitive to environmental changes compared to TR. Hence, we hypothesize that liming would have positive effects on the morphology and nutrients of AR but will not affect those of TR. Our results could deepen our understanding of the root economic spectrum and provide theoretical guidance for sustainable planted forest management under soil acidification.

2. Materials and Methods

2.1. Site Description

This experiment was conducted at the Huitong National Research Station of Forest Ecosystem in Hunan Province, South China (hereafter referred to as "Huitong Station", 26°40′ to 27°09′ N and 109°26′ to 110°08′ E) with mean annual temperature of 16.5 °C and mean annual precipitation of 1400 mm. A severely acidified soil sample was collected from an acidic treatment from the simulated acid rain experiment platform on Chinese fir plantation. This treatment plot was treated with simulated acid rain solution using a mixing solution of 1 mmol L^{-1} HNO_3 and 1 mmol L^{-1} H_2SO_4 at a molar ratio of 1:5. The solution pH was adjusted to pH 2.5 and sprayed two times a month since 2015. A mildly acidified soil sample that was used in this pot experiment was collected from the Chinese fir plantation. The soil samples' chemical characteristics are shown in Table 1.

Table 1. Chemical properties of the soil sample used in the experiment.

Soil Acidification	pH	C (%)	N (%)	Available P (mg/kg)	Exchangeable Al (mg/kg)	Exchangeable Ca (mg/kg)	Ca/Al Molar Ratio
Mild	4.28 ± 0.05	1.47 ± 0.02	0.13 ± 0.00	1.56 ± 0.34	44.35 ± 1.11	125.62 ± 12.89	1.93 ± 0.23
Severe	3.61 ± 0.02	1.41 ± 0.03	0.13 ± 0.01	0.86 ± 0.05	48.52 ± 0.99	19.50 ± 4.46	0.27 ± 0.06

2.2. Pot Experiment

A pot experiment was conducted from March 2017 to October 2018. The pot experiment design included two factors: soil acidification degree (the pH values of the soil sample were 4.3 and the 3.6, respectively) and liming dose, which included three levels: the control (CK), 0 kg CaO ha^{-1}; low level (L), 1000 kg CaO ha^{-1}; and the high level (H), 4000 kg CaO ha^{-1}. One healthy seedling was planted in a pot (depth 40 cm, diameter 30 cm) containing approximately 27 kg of soil. All selected seedlings were approximately equal performances (height, 21 cm; ground diameter, 4.8 mm). After survival, $CaCO_3$ powder was added to each pot. Each treatment involved eight replicates for a total of 48 pots in this pot experiment. All pots were placed in an open greenhouse with a transparent plastic film top. All pots were watered three times a week, using tap water (pH~6.9). All pots were randomly placed at first, and then were rearranged every 2 weeks to avoid side effects. The seedlings were harvested after 20 months of transplanting and were cut along the base of the stem. Complete root systems were harvested by carefully rinsing the soil with deionized water.

2.3. Root Trait Analysis

Different orders of roots were separated according to the methods in a previous work [14]. Roots with 1–5 orders were scanned using a 400 dpi Epson root scanner (Expression1600 pro, Model EU-35; Epson, Tokyo, Japan). The Win-RHIZO system (WINRHIZO PRO2004B software, v.5.0, Regent Instruments Inc., Quebec, QC, Canada) was used to analyze the total length, surface area, and volume of roots. Then, all the roots were dried for 72 h at 65 °C and subsequently weighed to determine the SRL, SRA, and RTD. Here, 1–2 order roots were considered as AR and 3–5 order roots were TR [14].

The N concentrations of roots were determined using an element analyzer (Model CN, Elementar Vario Macro cube, Elementar Analysensysteme GmbH, Hanau, Germany). The Ca and Al concentrations of roots were gauged using an atomic absorption spectrometer analyzer (novAA 350, Analytik Jena AG, Jena, Germany). Root P concentration was calorimetrically measured using the phosphomolybdic blue color method.

2.4. Data Analysis

The data were tested for homogeneity of variances (Brown and Forsythe's variation of Levene's test) before statistical analysis. Tukey's HSD method was used when one-way ANOVA showed that the respective liming treatment effects on the root morphologies and nutrients of AR and TR were significant under a soil type (mild or severe). Three-way ANOVA using the General Linear Model procedure was performed to test the significance of soil acidification degree, liming level, root type, and their interactive effects on root morphologies and nutrients, these processes were performed in SPSS 19.0. (SPSS Inc., Chicago, IL, USA). Principal components analysis was performed to visualize the root morphology and nutrient coordination between two functional roots under liming using the 'PCA' function in the R package FactoMineR and Pearson's correlation analysis was performed to assess the relationships between all root traits in AR and TR using the 'corrplot' function in R package corrplot.

3. Results

3.1. Effects of Liming on Root Morphology

As shown in Table 2, we found significant interactive effects of soil acidification degree and liming on root biomass (RB). The RB of TR with two liming levels was significantly lower than that with CK treatment in severely acidified soil. However, no significant differences in RB were found among different liming doses in mildly acidified soil (Figure 1). Furthermore, in mildly acidified soil, SRL increased as liming increased. Compared to CK, SRL of AR significantly increased by 24% and that of TR increased by 27%, with a high liming dose in mildly acidified soil. Although SRL also increased in severely acidified soil with liming treatment, only that of AR with a low liming dose was statistically significant. A similar trend was also observed in SRA: increasing SRA with increasing liming was found in mildly acidified soil. Furthermore, SRA significantly increased by 10% in AR and by 22% in TR under high liming treatment in mild soil. SRA also increased under liming treatment in severely acidified soil; although, there was no significant difference. There was also no significant difference in RTD among the different liming treatments, except for TR with high liming dose treatment in severe soil. There were significant differences observed in RB, SRL, SRA, and RTD of Chinese fir seedlings between different functional roots (Table 2). In particular, SRL, SRA, and RTD of AR were 5.9, 2.9, and 1.1 times higher than that of TR, respectively, but RB of AR was 1.9 times lower than that of TR.

Table 2. Three-way ANOVA analysis for fine-root morphologies and nutrient concentrations among different soil acidification degrees (Acid), liming levels ($CaCO_3$), and fine root functional group (Root).

Treatment	RB	SRL	SRA	RTD	N	P	Al	Ca	Ca/Al
$CaCO_3$	ns	<0.01	<0.001	ns	<0.001	<0.001	ns	<0.001	<0.001
Root	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
Acid	<0.001	ns	ns	ns	ns	<0.05	<0.001	ns	ns
Ca × R	ns	<0.05	ns	ns	ns	ns	<0.05	ns	ns
Ca × A	<0.01	ns	ns	<0.05	ns	ns	ns	ns	<0.05
R × A	ns	ns	ns	ns	ns	ns	<0.001	ns	ns
Ca × R × A	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: RB, root biomass; SRL, specific root length; SRA, specific root area; RTD, root tissue density; N, root nitrogen concentration; P, root phosphorous concentration; Ca, root calcium concentration; Al, root aluminum concentration; Ca/Al, root calcium/aluminum ratio; ns, $p > 0.05$.

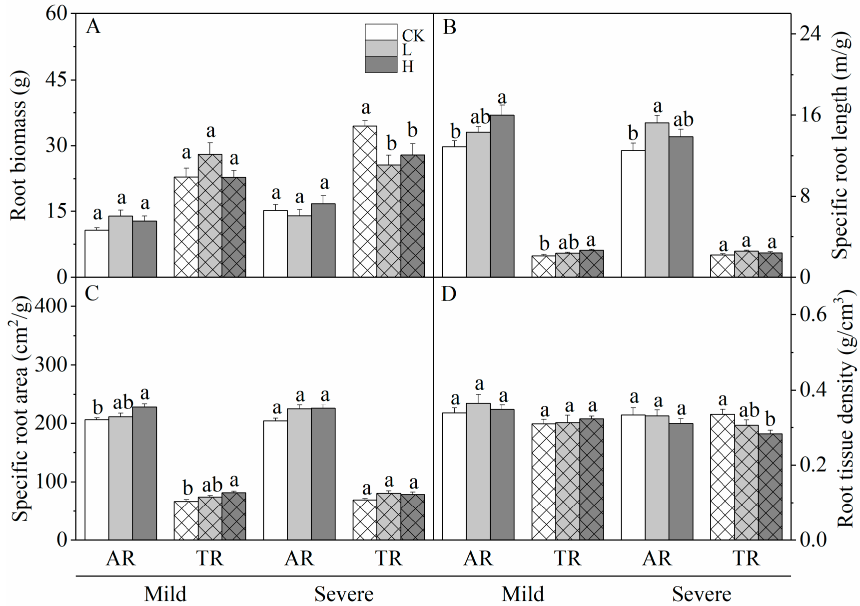

Figure 1. Fine-root biomass and morphologies of two functional roots among three treatments (CK, control; L, low $CaCO_3$ addition; H, high $CaCO_3$ addition) and two acidification degree soil samples (Mild, mildly acidified soil; Severe, severely acidified soil). Data shown are mean + SE (N = 8). Bars without grid represent absorptive fine roots (AR), and bars with grid represent transportation fine roots (TR). Different lower-case letters indicate significant differences at $p < 0.05$ level among different liming treatments in the same functional root (AR or TR) within the same soil type (Mild or Severe), as evaluated by one-way ANOVA; (**A**) root biomass; (**B**) SRL or specific root length; (**C**) SRA o specific root area; (**D**) RTD or root tissue density.

3.2. Effects of Liming on Root Nutrient Concentrations

Liming significantly affected the root nutrient concentrations of Chinese fir seedlings (Table 2; Figure 2). Root N, Ca, and Ca/Al all increased with increasing liming dose. In particular, root N concentrations increased significantly by 26% and 30% with high liming dose in AR and TR in mildly acidified soil, respectively, and by 25% in AR in severely acidified soil. Root Ca concentrations significantly increased under all treatments. The maximum value occurs in a high liming dose. High liming dose significantly increased root Ca concentrations by 217% and 172% of AR and 285% and 224% of TR in mildly acidified soil and severely acidified soil, respectively. Similar trends were also observed in the Ca/Al ratio. In mildly acidified soil, root P concentration of AR increased significantly by 21% with a high liming dose, and a similar trend was observed in TR, but it was not significant. Root P concentration increased with increasing liming dose; although, it did not show statistical significance in severely acidified soil.

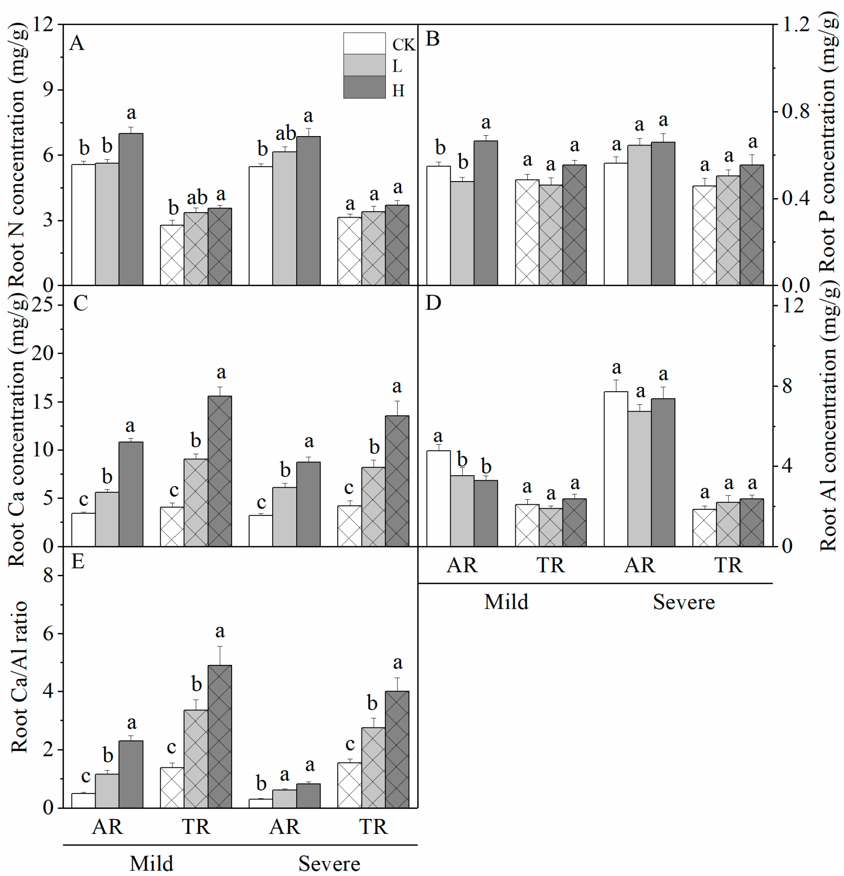

Figure 2. Fine root nutrient concentrations of two functional roots among three treatments (C, control; L, low $CaCO_3$ addition; H, high $CaCO_3$ addition) and two acidification degree soils (Mild, mildly acidified soil; Severe, severely acidified soil). Data shown are mean + SE (N = 8). Bars without grid represent Absorptive fine roots (AR), and bars with grid represent transportation fine roots (TR). Different lower-case letters indicate significant differences at $p < 0.05$ level among different liming treatments in the same functional root (AR or TR) within the same soil (Mild or Severe), as evaluated by one-way ANOVA; (**A**) root N concentration; (**B**) root P concentration; (**C**) root Ca concentration; (**D**) Root Al concentration; (**E**) Ca/Al ratio in the root system.

As shown in Table 2, we found significant interactive effects of liming treatment and root types on root Al concentrations. Low and high doses of liming decreased the root Al concentration of AR by 26% and 31% in mildly acidified soil, respectively; however, there was no significant effect on TR by liming in both two soils. The interaction of root types and soil acidification degree significantly affected root Al concentrations. Specifically, soil acidification degree varied the root Al concentration of AR such that in severely acidified soil root Al concentration was 1.9 times more than that in mildly acidified soil. By contrast, soil acidification degree had no significant effect on the root Al concentration of TR.

Root functional types significantly affected the root N, P, Al, and Ca concentrations and Ca/Al ratio (Table 2). In particular, the root N and P concentrations of AR were 1.8 and 1.2 times higher than that of TR, respectively (Figure 2). The root Al concentrations of AR were 1.8 and 3.5 times higher than that of TR in mildly and severely acidified soil, respectively. However, the root Ca concentration and Ca/Al ratio of AR were 1.4 and 3.7 times lower than that of TR.

3.3. Relationships among Root Traits under Liming

The PCA with two main components explained 54.8% and 52.6% of the total variations in root traits in mildly and severely acidified soil samples, respectively, across liming treatment (Figure 3a,c). There were two leading dimensions of the root trait: the two orthogonal trait axes, RB and N-Ca/Al-SRL or SRA. In mildly acidified soil, root traits treated by CK, L, and H were separated along the first PCA axis. The first axis explained 40.4% of the variance. As shown in Figure 3b, the Al concentration of AR has a greater relevance with the first axis, while the Al concentration of TR has a greater relevance with the fourth axis. The results indicate that liming has an inconsonant effect on root Al concentrations between AR and TR in mildly acidified soil. Similarly, in severely acidified soil, root traits treated by CK, L, and H were separated along the first PCA axis as well. The first axis explained 37% of the variance. As shown in Figure 3d, the RB of AR has a greater relevance with the fourth axis, while that of TR has a greater relevance with the first axis. The results indicate that liming has an inconsonant effect on RB between AR and TR in severely acidified soil.

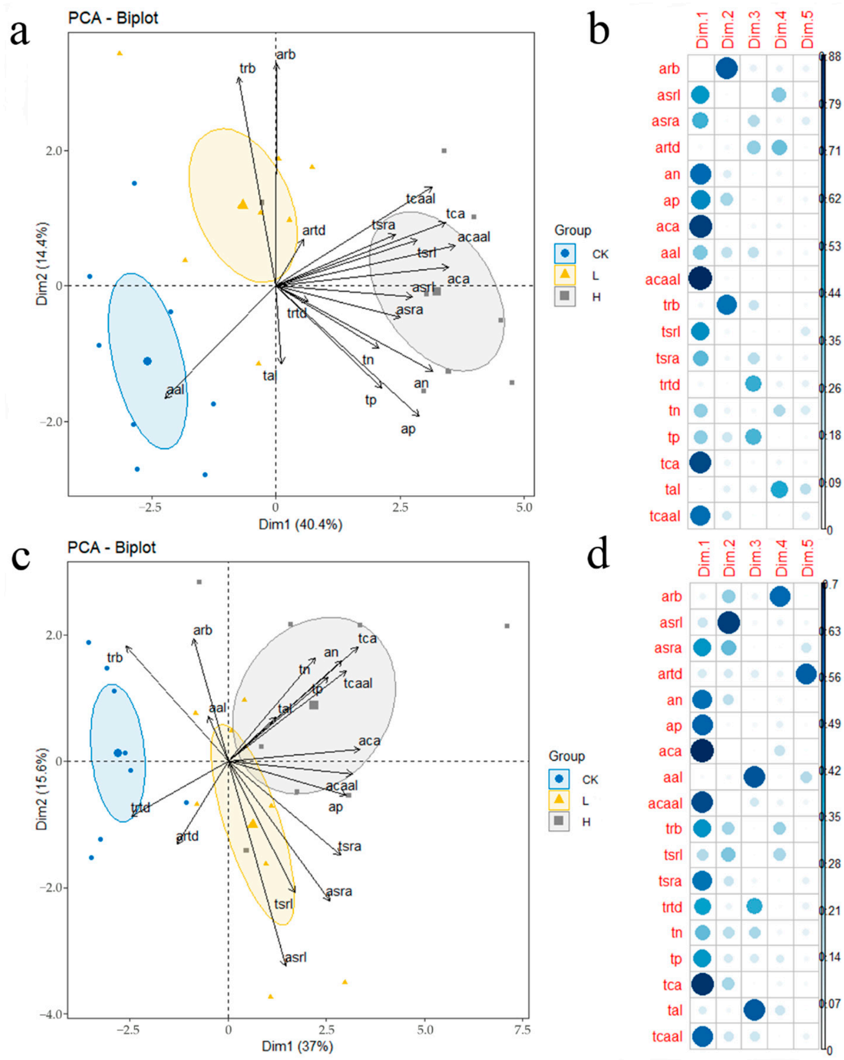

Figure 3. Principal component analysis for root traits of Chinese fir in mildly and severely acidified soil. CK, L, and H respectively represent three treatments (CK, control; L, low $CaCO_3$ addition; H, high $CaCO_3$ addition) in mildly (**a**) and severely (**c**) acidified soil samples; Figure (**b**,**d**) show the cos2 (quality of representation of the variables on factor map) of variables on all the dimensions of Figure (**a**,**c**), respectively. A high cos2 value indicates a good representation of the variable on the principal component. The size of circles and the shades of color is proportional to the value of cos2. arb/trb, root biomass of AR/TR; asrl/tsrl, root SRL of AR/TR; asra/tsra, root SRA of AR/TR; atrd/trtd, root RTD of AR/TR; aca/tca, root Ca concentration of AR/TR; aal/tal, root Al concentration of AR/TR; an/tn, root N concentration of AR/TR; ap/tp, root P concentration of AR/TR; acaal/tcaal, root Ca/Al ratio of AR/TR.

4. Discussion

Our study revealed that liming increased SRL, SRA, root N and P concentrations and root Ca/Al. These indicate that plants explore soil and uptake nutrients more efficiently with the root Al toxicity alleviation. Furthermore, our study revealed a transformation of plant growth strategy, that is, from a "conservative" strategy to an "acquisitive" strategy. Interestingly, we found that SRL, SRA, RTD, Ca/Al ratios, and root N, Ca, and P concentrations showed coordinated responses between AR and TR to liming. However, root biomass and root Al concentration showed disparate reactions to liming in severely and mildly acidified soil, respectively. Overall, the whole root system became more acquisitive due to the remediation of Al toxicity, which may be an important mechanism underlying the increase in production of *C. lanceolate* with liming.

4.1. Effect of Liming on Root Traits

SRL and SRA characterize the economic aspects of the root system construction costs and represent the system's investment efficiency in relation to soil exploration and water and nutrient acquisition [13,37]. In our experiment, liming increased the SRL and SRA of Chinese fir seedlings, indicating that root investment efficiency was enhanced by liming (Figure 1). This is in accordance with the results reported by Bakker [38], who suggested that liming could improve the root uptake performance by the increase in the SRL of sessile oak (*Quercus petraea*). A previous study has demonstrated that the SRL decreased by 13% due to Al toxicity [13]. Whereas liming is considered an effective measure for the alleviation of Al toxicity and enhancement of fine roots performance. On the one hand, liming could improve soil pH and suppress exchangeable Al content [39]. On the other hand, Ca^{2+} is an essential element in alleviating Al-induced growth inhibition because of its role in maintaining the integrity of the cell wall [40]. It can also adjust organic acid metabolism, which can chelate toxic Al^{3+} in the rhizosphere [41]. In our results, liming reduced the root Al concentration of AR in mildly acidified soil, and that the increased root Ca/Al ratio and SRL or SRA were positively correlated with the root Ca/Al ratio (Figure S1). Usually, the root Ca/Al ratio is considered an effective indicator of Al toxicity and acidity stress [30,42]. These results, therefore, indicate that liming alleviated Al toxicity and can help enhance root investment efficiency of Chinese fir seedlings.

With the alleviation of Al toxicity, root nutrient uptake capacity could be enhanced [32,43]. This is supported by our results, which revealed that liming enhanced root N and P concentrations and that they all positively correlated with root Ca/Al (Figure 2 and Figure S1). Moreover, another important reason could be that liming enhanced soil nutrient availability by increasing the soil pH. Heyburn, et al. [44] found that liming increased the mineralization of N and, thus, increased soil N availability. DeForest and Snell [45] also showed that liming led to the desorption of P combined with soil and increased the P mineralization, thereby increasing the effectiveness of the P in the soil. However, another study also reported that soil P availability was reduced with liming due to the binding of Ca with P [46]. Hence, future studies should further explore how the availabilities of soil N and P change and how plants utilize them after liming.

The PCA results showed that the root trait has a multidimensional economics space under liming (Figure 3). Root Ca/Al, SRL or SRA and root N were mainly loaded on PC1, and RB was mainly loaded on PC2 in mild and severe soil. These two nearly orthogonal trait axes reflected two different strategies of root under liming. Combined with our results, liming increased the SRL or SRA, root Ca/Al and root N, but it did not affect the RB of AR. This indicated that the plant did not increase its root C investment but was transformed from a short and thick root to a long and thin root with high root activity, which facilitated efficient soil exploration and resource acquisition [13,37]. This strategy was deemed to be "acquisitive". However, a thin root with high root N concentration usually correlated with a high turnover rate [12]. Future long-term experiments are warranted to better enable an estimation to determine the real root C investment with liming.

4.2. Responses of Different Functional Roots to Liming

Previous studies that investigated the response of root traits under liming showed inconsistent results [11,33,47], and typically neglected the role of root order as an important cause. Hence, in our experiment, we tried to divide the roots into AR and TR to evaluate whether these two parts of roots have different responses under liming. However, strangely, we found that SRL, SRA, RTD, root Ca/Al ratio, and root N, P, and Ca concentrations showed coordinated response to liming (Figures 1–3). It has been suggested that lower-order roots are more sensitive to the change in environmental factors [24]. However, in our study, the response of TR morphology to liming was the same as that of AR morphology. One possible explanation may be the synergistic effect produced by the adjustment of the root system to environmental change. Hodge [15] pointed out that the different functional roots are usually closely related to one another and that there is a coordinated response among the systems of this root. In addition, we speculated that tree age could be an important reason because the root showed increased secondary growth with the increase in tree age [14], and roots with higher secondary growth had better defense against environmental changes. However, our experimental sample was just a sapling and it probably did not have enough secondary development. Our results imply that studies on the root economics spectrum being mainly based on AR could hinder a more comprehensive understanding of plant growth strategy.

Interestingly, we found that liming had a different effect on the root biomass between the AR and TR in severely acidified soil, but a similar effect in mildly acidified soil (Figure 1). Plants usually allocate more biomass to belowground parts for acquiring nutrients under adverse conditions; however, increasing soil nutrient availability by liming reversed the plant allocation pattern from belowground to aboveground [48]. This could explain a reduction in root mass in our result. Interestingly, biomass reduction was mainly at the expense of TR but not AR. Seemingly, the plant "wisely" adjusted the biomass allocation for preserving root uptake capacity efficiently. One possible explanation for it is that the TR is a major storage organ [14]; thus, it would be inefficient to maintain a larger TR biomass when liming alleviates adverse conditions and increases soil available nutrients. Furthermore, conflicts of results between mildly and severely acidified soil samples might be related to the differences in soil initial pH. In fact, previous research has demonstrated that higher soil pH is related to better soil quality and that liming does not cause adverse effects on fine roots on more fertile sites [49,50].

Apart from root biomass, the result of Al concentration also showed a discrepancy between AR and TR in mildly acidified soil (Figures 2 and 3). In particular, liming decreased Al concentration in AR but it did not have an effect on TR. Actually, root Al concentration in TR was not affected by both liming and soil acidification degree, which may indicate that TR was not affected by Al toxicity in the current acidification degree. Previous research demonstrated that part of Al tolerant plants usually entrap more Al in roots and have less Al in aboveground parts [34]. Our subjects may fit this pattern. This has also been proven in a previous study [51], which demonstrated that Al usually accumulated on the cell wall of the Chinese fir root system, thus preventing the transportation of active Al^{3+} to the aboveground by roots. Furthermore, our results demonstrated that AR was a major organ in entrapping Al but not a whole root system, because TR was not affected by soil acidification degree. Meanwhile, the root Al concentration of AR in severely acidified soil was higher than that in mildly acidified soil, and root Al concentration of AR was not decreased by liming in severely acidified soil (Figure 2). These results demonstrate that the process of liming alleviates Al toxicity in severely acidified soil and may be needed for a longer time. This phenomenon also warns us that as soil acidification is aggravated, plant roots may suffer irreversible damage.

Admittedly, our experiment had some limitations, which call for further improvement. First, our results were based on a pot experiment in an open greenhouse, which may not reflect actual wild conditions, especially considering the effect of intraspecific and interspecific competition. Second, in addition to root morphology adjustment, arbuscular mycorrhizal fungi (AMF) forming a symbiotic relationship with the roots is the most common strategy, which improves soil resource acquisition through increased soil exploration. To understand plant belowground acquisition strategies and root response to environmental changes, future studies should not neglect mycorrhizal fungi. Therefore, future experiments must be performed on a wild environment and include mycorrhizal fungi and other physiological indices.

5. Conclusions

In this study, we investigated the root system response to liming of two functional roots through a two-growing season pot experiment. We found that liming enhanced the root carbon investment efficiency and root activity by alleviating root Al toxicity. Liming also transformed the plant growth strategy from "conservative" to "acquisitive". In addition, liming showed a consistent influence on root morphologies and nutrients between the AR and TR apart from root biomass and root Al concentration. These indicated that the adjustments in root response to environment changes had a coordinated process. Thus, toward a more comprehensive understanding of plant growth strategy, we should consider different functional roots rather than just the absorption part of the root system in future studies. Our experiments also showed that liming was an effective measure to promote the root absorption efficiency and capacity of Chinese fir under acidic soil. Finally, given that Al toxicity alleviation in severely acidified soil requires a longer time, liming is urgently needed for promoting Chinese fir production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13060822/s1, Figure S1. Pearson's correlation analysis for root traits of the Chinese fir seedlings treated with liming.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Yu, Y.; Yang, J.; Zeng, S.; Wu, D.; Jacobs, D.F.; Sloan, J.L. Soil pH, organic matter, and nutrient content change with the continuous cropping of *Cunninghamia lanceolata* plantations in South China. *J. Soils Sediments* **2017**, *17*, 2230–2238. [CrossRef]
2. Farooq, T.H.; Yan, W.; Rashid, M.H.U.; Tigabu, M.; Gilani, M.M.; Zou, X.H.; Wu, P.F. Chinese fir (*Cunninghamia lanceolata*) a green gold of China with continues decline in its productivity over the successive rotations: A review. *Appl. Ecol. Environ. Res.* **2019**, *17*, 11055–11067. [CrossRef]
3. Tian, D.; Xiang, W.; Chen, X.; Yan, W.; Fang, X.; Kang, W.; Dan, X.; Peng, C.; Peng, Y. A long-term evaluation of biomass production in first and second rotations of Chinese fir plantations at the same site. *Forestry* **2011**, *84*, 411–418. [CrossRef]
4. Bohan, L.; Larssen, T.; Seip, H.M. Response of five Chinese forest soils to acidic inputs: Batch experiment. *Geoderma* **1998**, *86*, 295–316. [CrossRef]
5. Liu, X.; Fu, Z.; Zhang, B.; Zhai, L.; Meng, M.; Lin, J.; Zhuang, J.; Wang, G.G.; Zhang, J. Effects of sulfuric, nitric, and mixed acid rain on Chinese fir sapling growth in Southern China. *Ecotoxicol. Environ. Saf.* **2018**, *160*, 154–161. [CrossRef]
6. Fan, H. A review of research on interactions between acid rain and forest decline. *J. Fujian Coll. For.* **2003**, *23*, 88–92.
7. Leys, B.A.; Likens, G.E.; Johnson, C.E.; Craine, J.M.; Lacroix, B.; McLauchlan, K.K. Natural and anthropogenic drivers of calcium depletion in a northern forest during the last millennium. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 6934–6938. [CrossRef]
8. Battles, J.J.; Fahey, T.J.; Driscoll, C.T.; Blum, J.D.; Johnson, C.E. Restoring soil calcium reverses forest decline. *Environ. Sci. Technol. Lett.* **2014**, *1*, 15–19. [CrossRef]

9. Rosi-Marshall, E.J.; Bernhardt, E.S.; Buso, D.C.; Driscoll, C.T.; Likens, G.E. Acid rain mitigation experiment shifts a forested watershed from a net sink to a net source of nitrogen. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 7580–7583. [CrossRef]
10. Cho, Y.; Driscoll, C.T.; Johnson, C.E.; Siccama, T.G. Chemical changes in soil and soil solution after calcium silicate addition to a northern hardwood forest. *Biogeochemistry* **2010**, *100*, 3–20. [CrossRef]
11. Liu, T.-W.; Wu, F.-H.; Wang, W.-H.; Chen, J.; Li, Z.-J.; Dong, X.-J.; Patton, J.; Pei, Z.-M.; Zheng, H.-L. Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain. *Tree Physiol.* **2011**, *31*, 402–413. [CrossRef] [PubMed]
12. Freschet, G.T.; Roumet, C.; Comas, L.H.; Weemstra, M.; Bengough, A.G.; Rewald, B.; Bardgett, R.D.; De Deyn, G.B.; Johnson, D.; Klimesova, J.; et al. Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytol.* **2021**, *232*, 1123–1158. [CrossRef] [PubMed]
13. Ostonen, I.; Püttsepp, Ü.; Biel, C.; Alberton, O.; Bakker, M.R.; Lõhmus, K.; Majdi, H.; Metcalfe, D.; Olsthoorn, A.F.M.; Pronk, A.; et al. Specific root length as an indicator of environmental change. *Plant Biosyst.* **2007**, *141*, 426–442. [CrossRef]
14. McCormack, M.L.; Dickie, I.A.; Eissenstat, D.M.; Fahey, T.J.; Fernandez, C.W.; Guo, D.; Helmisaari, H.S.; Hobbie, E.A.; Iversen, C.M.; Jackson, R.B.; et al. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* **2015**, *207*, 505–518. [CrossRef] [PubMed]
15. Hodge, A. Root decisions. *Plant Cell Environ.* **2009**, *32*, 628–640. [CrossRef]
16. Eissenstat, D.M.; Wells, C.E.; Yanai, R.D.; Whitbeck, J.L. Building roots in a changing environment: Implications for root longevity. *New Phytol.* **2000**, *147*, 33–42. [CrossRef]
17. Kochian, L.V.; Pineros, M.A.; Liu, J.; Magalhaes, J.V. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annu. Rev. Plant Biol.* **2015**, *66*, 571–598. [CrossRef]
18. Bakker, M.R.; Kerisit, R.; Verbist, K.; Nys, C. Effects of liming on rhizosphere chemistry and growth of fine roots and of shoots of sessile oak (*Quercus petraea*). *Plant Soil* **1999**, *217*, 243–255. [CrossRef]
19. Miyatani, K.; Mizusawa, Y.; Okada, K.; Tanikawa, T.; Makita, N.; Hirano, Y. Fine root traits in *Chamaecyparis obtusa* forest soils with different acid buffering capacities. *Trees* **2015**, *30*, 415–429. [CrossRef]
20. Guo, D.; Xia, M.; Wei, X.; Chang, W.; Liu, Y.; Wang, Z. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytol.* **2008**, *180*, 673–683. [CrossRef]
21. Pregitzer, K.S. Tree root architecture—Form and function. *New Phytol.* **2008**, *180*, 562–564. [CrossRef] [PubMed]
22. Zadworny, M.; McCormack, M.L.; Zytkowiak, R.; Karolewski, P.; Mucha, J.; Oleksyn, J. Patterns of structural and defense investments in fine roots of Scots pine (*Pinus sylvestris* L.) across a strong temperature and latitudinal gradient in Europe. *Glob. Chang. Biol.* **2017**, *23*, 1218–1231. [CrossRef] [PubMed]
23. Valenzuela-Estrada, L.R.; Vera-Caraballo, V.; Ruth, L.E.; Eissenstat, D.M. Root anatomy, morphology, and longevity among root orders in *Vaccinium corymbosum* (ericaceae). *Am. J. Bot.* **2008**, *95*, 1506–1514. [CrossRef] [PubMed]
24. Pu, X.; Yin, C.; Xiao, Q.; Qiao, M.; Liu, Q. Fine roots branch orders of *Abies faxoniana* respond differentially to warming in a subalpine coniferous forest ecosystem. *Agrofor. Syst.* **2016**, *91*, 955–966. [CrossRef]
25. Guo, D.L.; Mitchell, R.J.; Hendricks, J.J. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* **2004**, *140*, 450–457. [CrossRef] [PubMed]
26. Zhou, M.; Yan, G.; Xing, Y.; Chen, F.; Zhang, X.; Wang, J.; Zhang, J.; Dai, G.; Zheng, X.; Sun, W.; et al. Nitrogen deposition and decreased precipitation does not change total nitrogen uptake in a temperate forest. *Sci. Total Environ.* **2019**, *651*, 32–41. [CrossRef]
27. Kramer-Walter, K.R.; Laughlin, D.C. Root nutrient concentration and biomass allocation are more plastic than morphological traits in response to nutrient limitation. *Plant Soil* **2017**, *416*, 539–550. [CrossRef]
28. Haling, R.E.; Simpson, R.J.; Delhaize, E.; Hocking, P.J.; Richardson, A.E. Effect of lime on root growth, morphology and the rhizosheath of cereal seedlings growing in an acid soil. *Plant Soil* **2010**, *327*, 199–212. [CrossRef]
29. Halman, J.M.; Schaberg, P.G.; Hawley, G.J.; Pardo, L.H.; Fahey, T.J. Calcium and aluminum impacts on sugar maple physiology in a northern hardwood forest. *Tree Physiol.* **2013**, *33*, 1242–1251. [CrossRef]
30. Vanguelova, E.I.; Hirano, Y.; Eldhuset, T.D.; Sas-Paszt, L.; Bakker, M.R.; Puttsepp, U.; Brunner, I.; Lohmus, K.; Godbold, D. Tree fine root Ca/Al molar ratio—Indicator of Al and acidity stress. *Plant Biosyst.* **2007**, *141*, 460–480. [CrossRef]
31. Reich, P.B.; Tjoelker, M.G.; Pregitzer, K.S.; Wright, I.J.; Oleksyn, J.; Machado, J.L. Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecol. Lett.* **2008**, *11*, 793–801. [CrossRef] [PubMed]
32. Comas, L.; Bouma, T.; Eissenstat, D. Linking root traits to potential growth rate in six temperate tree species. *Oecologia* **2002**, *132*, 34–43. [CrossRef] [PubMed]
33. Hwang, J.; Son, Y.; Kim, C.; Yi, M.-J.; Kim, Z.-S.; Lee, W.-K.; Hong, S.-K. Fine root dynamics in thinned and limed pitch pine and Japanese larch plantations. *J. Plant Nutr.* **2007**, *30*, 1821–1839. [CrossRef]
34. Hettiarachchi, L.S.K.; Sinclair, A.H. Effects of addition of magnesium and calcium supplied in liming and non-liming materials on the growth of *Camellia japonica* in an acid soil, and its soil ph changes, nutrient uptake, and availability. *Commun. Soil Sci. Plant Anal.* **2002**, *33*, 2965–2988. [CrossRef]
35. Chen, G.T.; Zheng, J.; Peng, T.C.; Li, S.; Qiu, X.R.; Chen, Y.Q.; Ma, H.Y.; Tu, L.H. Fine root morphology and chemistry characteristics in different branch orders of *Castanopsis platyacantha* and their responses to nitrogen addition. *Chin. J. Appl. Ecol.* **2017**, *28*, 3461–3468. (In Chinese) [CrossRef]

36. Zhang, Y.F.; Fang, X.M.; Chen, F.S.; Zong, Y.Y.; Gu, H.J.; Hu, X.F. Influence of simulated acid rain on nitrogen and phosphorus contents and their stoichiometric ratios of tea organs in a red soil region, China. *Chin. J. Appl. Ecol.* **2017**, *28*, 1309–1316. (In Chinese) [CrossRef]
37. Lambers, H.; Shane, M.W.; Cramer, M.D.; Pearse, S.J.; Veneklaas, E.J. Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Ann. Bot.* **2006**, *98*, 693–713. [CrossRef]
38. Bakker, M.R. The effect of lime and gypsum applications on a sessile oak (*Quercus petraea* (M.) Liebl.) stand at La Croix-Scaille (French Ardennes) II. Fine root dynamics. *Plant Soil* **1999**, *206*, 109–121. [CrossRef]
39. Rengel, Z.; Zhang, W.H. Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. *New Phytol.* **2003**, *159*, 295–314. [CrossRef]
40. Hepler, P.K. Calcium: A central regulator of plant growth and development. *Plant Cell* **2005**, *17*, 2142–2155. [CrossRef]
41. Ma, J.F.; Chen, Z.C.; Shen, R.F. Molecular mechanisms of Al tolerance in gramineous plants. *Plant Soil* **2014**, *381*, 1–12. [CrossRef]
42. Li, Z.; Wang, Y.; Liu, Y.; Guo, H.; Li, T.; Li, Z.-H.; Shi, G. Long-term effects of liming on health and growth of a Masson pine stand damaged by soil acidification in Chongqing, China. *PLoS ONE* **2014**, *9*, e94230. [CrossRef]
43. Burton, A.J.; Jarvey, J.C.; Jarvi, M.P.; Zak, D.R.; Pregitzer, K.S. Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Glob. Chang. Biol.* **2012**, *18*, 258–266. [CrossRef]
44. Heyburn, J.; McKenzie, P.; Crawley, M.J.; Fornara, D.A. Long-term belowground effects of grassland management: The key role of liming. *Ecol. Appl.* **2017**, *27*, 2001–2012. [CrossRef] [PubMed]
45. DeForest, J.L.; Snell, R.S. Tree growth response to shifting soil nutrient economy depends on mycorrhizal associations. *New Phytol.* **2020**, *225*, 2557–2566. [CrossRef] [PubMed]
46. Burke, M.K.; Raynal, D.J. Liming influences growth and nutrient balances in sugar maple (*Acer saccharum*) seedlings on an acidic forest soil. *Environ. Exp. Bot.* **1998**, *39*, 105–116. [CrossRef]
47. Jiang, J.; Sheng, X.; Shao, J.; Lin, Y.; He, Q.; Han, W. Responses of the major functional traits of *Pinus massoniana* and Schima superba seedlings to nitrogen, phosphorus, and lime addition. *Chin. J. Appl. Environ. Biol.* **2020**, *26*, 410–416.
48. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytol.* **2012**, *193*, 30–50. [CrossRef] [PubMed]
49. Helmisaari, H.S.; Hallbacken, L. Fine-root biomass and necromass in limed and fertilized Norway spruce (*Picea abies* (L.) Karst.) stands. *For. Ecol. Manag.* **1999**, *119*, 99–110. [CrossRef]
50. Clivot, H.; Pagnout, C.; Aran, D.; Devin, S.; Bauda, P.; Poupin, P.; Guerold, F. Changes in soil bacterial communities following liming of acidified forests. *Appl. Soil Ecol.* **2012**, *59*, 116–123. [CrossRef]
51. Yu, J.; Xia, L.; Yin, D.; Zhou, C. Effects of phosphorus on aluminum tolerance of Chinese fir seedlings. *Sci. Silvae Sin.* **2018**, *54*, 36–47. (In Chinese)