soil systems

Article

Phytotoxic Effects of Al on Root Growth Are Confounded in the Presence of Fulvic and Humic Acids

Stephen Harper * and Neal Menzies

School of Agriculture and Food Sustainability, The University of Queensland, Brisbane, QLD 4072, Australia; n.menzies@uq.edu.au
* Correspondence: s.harper1@uq.edu.au

Abstract: Background and Aims: In acidic soils, aluminum (Al) toxicity remains a critical crop limitation that can be ameliorated by organic amendments through Al complexation with high-molecular-weight carbon compounds, particularly fulvic and humic acids (FA and HA) However, no research discriminates between the direct effects of FA and HA on plant growth and the indirect effect that occurs through ameliorating Al toxicity. This study delineates the direct and indirect effects of FA and HA on plant growth. Methods: Eucalyptus and Hay FA and HA, and Al effects on maize (*Zea mays*) root growth were investigated using dilute nutrient solution. Five Al concentrations (0–270 µM) were combined with four organic acid (OA) treatments, including Nil-OA, FA40, and HA40 (each at 40 mg C L^{-1}) and a combined treatment FA40HA40 (80 mg C L^{-1}). Results: Eucalyptus FA and HA stimulated root growth by ~20% compared with root growth in the Nil-OA (17.4 cm). In the absence of Al, Hay FA and HA inhibited root growth (by ~20%) compared with the Nil-OA but the addition of Al resulted in stimulation of root growth. In the presence of FA and HA, root growth was not inhibited by nominally toxic monomeric Al (Al^{3+}) concentrations (~20 µM Al). However, when expressed on a relative basis to remove the direct effect of the ligand, the response was consistent with Al toxicity. Conclusions: The effects of FA and HA were either inhibitory or stimulatory depending on the source while both sources of FA and HA mitigated Al toxicity through complexation. The study provides mechanistic data that highlights limitations of soil bioassays where the direct effects of organic ligands on root growth are confounded with the indirect effect of their reduction of Al toxicity. These two independent processes must be considered in evaluating the amelioration of Al by organic amendments.

Keywords: fulvic acid; humic acid; aluminum toxicity; root bioassay; eucalyptus

check for updates

Citation: Harper, S.; Menzies, N. Phytotoxic Effects of Al on Root Growth Are Confounded in the Presence of Fulvic and Humic Acids. *Soil Syst.* **2023**, *7*, 68. https://doi.org/10.3390/soilsystems7030068

Academic Editor: Ewald Schnug

Received: 26 April 2023
Revised: 27 June 2023
Accepted: 12 July 2023
Published: 27 July 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Acidic soils have been extensively cultivated throughout the world, but it has been difficult to sustain high agricultural productivity due to their propensity for fertility decline. Globally, acidic soil infertility affects about 4 billion ha of land [1] and is particularly prevalent in the tropical rainforest climes of developing countries. Rainforests represent the largest remaining potential area for further agricultural development but equally represent pristine landscapes that serve as highly diverse ecosystems and warrant protection. These systems maintain about 43% of the world's trees and have the highest rates of vegetation loss [2].

About 250 million ha of acidic soils that were historically cleared and cultivated, have subsequently been left deforested, degraded, and abandoned [3]. The loss of this productivity and the potential threat to existing forested systems means that agricultural development in the tropics should concentrate on improving the productivity of these severely degraded acidic soils [3]. Quintessential research by [4] at three sites in South America, tracked a chronosequence of soil fertility decline over seven years following the clearing of rainforest. Their study highlighted the many facets of acidic soil degradation, but ultimately, the expression of aluminum (Al) toxicity was the most serious manifestation of acidic soil infertility.

Aluminum toxicity remains the major limitation to crop growth in acidic soils where high soil solution concentrations of Al^{3+} (monomeric form) substantially reduce plant root growth [5–7] and crop yield [8]. Field amelioration of Al toxicity has been achieved through the application of inorganic and organic amendments. Though lime, the main inorganic amendment, is effective in ameliorating Al toxicity, the effect is transient [4] and its use in developing countries, where the need is great, may be limited by product availability, transport costs, processing infrastructure, and the rate required to remediate soil acidity [9].

Organic amendments, including manures, biochar, and crop residues, may represent more practical ameliorants for Al toxicity in such regions [8,10–12]. Conventional agricultural by-products, including hay and crop residues, can effectively ameliorate Al but require application at rates of 10 to 80 Mg ha^{-1} [10,13]. At these high rates, crop residue application is not feasible since at a crop harvest index of about 50% the estimated crop residue yield is only 1 to 2 Mg ha^{-1} [14,15].

Organic matter input to soils results in the production of a myriad of organic compounds ranging from low molecular weight aliphatic and aromatic organic acids (about 300 Da) [16] to high-molecular-weight organic acids, monosaccharides, and polysaccharides [17–19]. A considerable range of low molecular weight aliphatic acids are identified from soils [20] and have varying ability to complex Al rendering it innocuous to plant growth [21]. However, low molecular weight organic acids are also good sources for the metabolism of microorganisms, and hence their presence in soils is transient [21] providing only a short-term amelioration of Al toxicity.

In natural and agricultural systems high-molecular-weight FA and HA can strongly complex both plant essential and toxic elements [22–24]. The FA and HA have considerable structural variability consisting of diverse composites of smaller and larger molecular units of principally aliphatic and aromatic groups, oils, amino acids, phenols, phenolic acids, phenolic esters, fatty acids, alkanes, tannins, monosaccharides, and polysaccharides [17,19,25–28]. Various studies have shown that they can detoxify Al through complexation [21,24,29,30] due to negatively charged hydroxyl and carboxylic functional groups [31,32]. Guimarães et al. [33] showed that the combined FA and HA concentrations in forest soil systems (2.08 g kg^{-1}) were substantially higher than in agricultural systems (1.12–1.50 g kg^{-1}). Agroforestry is an alternative strategy that can increase soil organic carbon and mitigate the effects of soil acidity [34].

Given the severe impact of Al toxicity on root growth, diagnosis of Al toxicity is usually based on short-term (~4 days) root bioassays [35,36]. However, the bioassays fail to consider the direct effect of soluble organic components, including FA and HA, that can stimulate [37–41] or inhibit [42] root growth at low concentrations (<5 mg C L^{-1}). The FA and HA are present at low concentrations in soil solution, hence studies on FA and HA use harsh alkaline extractants to prepare sufficient quantities for studies e.g., [43,44]. Hence the derived FA and HA are not representative of the functioning soil system from which they were extracted. Therefore, studies that have considered the effects of extracted FA and HA on Al toxicity are compromised; first, by considering Al detoxification by complexation but not the direct effect of FA or HA on plant growth, and second, because the extracted FA and HA are not representative of organic matter in a functioning soil system.

This study investigates the direct effects of FA and HA on the root development of maize (*Zea mays*) and the indirect effects through the complexation of toxic Al under controlled solution culture. The study compares FA and HA from water extracts of two sources including one of agricultural origin (composted hay) and the other from leaves of *Eucalyptus camaldulensis* (Dehnh.) representing a tree species widely used in agroforestry systems [45]. The study evaluates the complex interaction between the direct effects of FA and HA on plant root growth and their indirect effect on growth mediated through complexing toxic Al. The results are considered in the context of current soil bioassay studies and their limitations.

2. Materials and Methods

Two solution culture experiments were conducted to evaluate the effects of combinations of FA and HA and Al concentrations on the root growth of maize. Clean polythene buckets were near-filled with 1.9 L of deionized water, covered with plastic lids, and placed in water baths at 27 °C. Stock nutrient solutions were added to achieve basal nutrient solution concentrations (μM): N 1350 (NO_3^- 1250 and NH_4^+ 100), Ca 500, K 250, Mg 200, S 200, Fe 10, B 3, P 1, Zn 1, Mn 0.5, Cu 0.1, Co 0.04 and Mo 0.02 [21]. Fulvic and humic acids were extracted from decomposed leaves of *E. camaldulensis* and from composted hay composed of a mixture of grass (*Sorghum halepense*) and lucerne (*Medicago sativa*) [46].

Briefly, the methodology was as follows. Leaf material of E. camaldulensis and composted hay were each placed in aerated aqueous solution at 30 °C. After 35 days of incubation, a water extract was taken and successively filtered through nylon screens to a minimum of 45 μm. The filtrate was centrifuged at 20,000× *g* relative centrifugal force for 20 min. The supernatant was decanted and acidified to pH 1.5 ± 2.0 to precipitate the HA component. The HA precipitate was purified by dialysis with deionized water. The supernatant containing the FA was passed through a glass column containing Amberlite XAD-7 polymeric resin conditioned to pH 2.0. The adsorbed FA was eluted with 0.1 M KOH and passed through a column containing Dowex MSC-1 cation exchange resin, to remove adsorbed cations. The extracted FA and HA were dried under vacuum at 55 °C and 5.3 kPa.

Aliquots of Eucalyptus FA and HA (E-FA, E-HA) (Experiment 1) and Hay FA and HA (H-FA, H-HA) (Experiment 2) solutions were added to appropriate buckets to establish a nominal organic carbon (OC) concentration of 40 mg C L^{-1} of each acid. This is consistent with the published humic substance concentrations in the range of 30–110 mg C L^{-1} [47].

Four organic acid (OA) treatments were imposed:

1. Control with no OA (Nil-OA)
2. HA at 40 mg C L^{-1} (HA40)
3. FA at 40 mg C L^{-1} (FA40)
4. Combined FA at 40 mg C L^{-1} and HA at 40 mg C L^{-1} (FA40HA40)

Twenty buckets of each organic acid treatment were prepared. Four pots of each set of 20 received aliquots of a 0.1 M $AlCl_3$ stock solution to give nominal total treatment Al concentrations of 0, 10, 30, 90, and 270 μM. Thus, each experiment consisted of 20 treatments (four OA treatments x five Al concentrations) replicated four times for a total of 80 pots. Solution pH in all pots was adjusted to 4.5 by drop-wise addition of either 0.1 M KOH or HNO_3 and maintained at pH 4.5 for the duration of the experiment. Aeration tubes were placed in each pot and solutions were equilibrated for 1 day.

Maize seeds were rinsed with tap water to remove fungicide and soaked in a 200 μM $CaSO_4$ plus 50 μM H_3BO_3 solution for 30 min. The seeds were rinsed three times with tap water and germinated at 28 °C using a rolled towel technique. When the average radicle length was 30–40 mm, a seedling was placed in each of five plastic cups supported in the lid of each pot; the transplanted seedlings were covered with black polythene beads. After 4 days, plants were harvested and the length of the longest root of each seedling was determined.

Immediately before planting, a 25 mL solution sample was taken from each pot, passed through a 0.22 μm filter (Millipore), and analyzed for OC concentration and total Al concentration using ICPAES, and for monomeric Al concentration (Al^{3+}) not complexed by organic matter using a colorimetric kinetics reaction [48]. Root growth was measured on an absolute basis as the length of the longest root and expressed on a relative root length (RRL) basis to allow comparison across experiments.

Root length data were analyzed using ANOVA and standard errors are presented for solution properties since the variance associated with these properties was not normally distributed. Appropriate regression functions were fitted to data sets and presented using SigmaPlot 14.0 (Systat Software Inc. Chicago, IL, USA).

3. Results

3.1. Changes in Solution Properties across FA, HA, and Al Treatments

In the Nil-OA treatments, measured total Al concentration increased progressively with increasing treatment Al concentration reaching an averaged maximum for the two experiments of 218 µM at a nominal treatment Al concentration of 270 µM (Figure 1). In the 0 µM Al treatments where FA and HA were added, low concentrations of Al were recorded in the FA and HA treatments due to residual Al in the source FA and HA, and in the 10 and 30 µM treatments total Al concentration was slightly higher than that nominally imposed. At 90 µM Al, the measured total Al concentration ranged from 20 to 100% of the nominal concentration across all treatments. However, at 270 µM Al, the measured Al concentrations in the presence of FA or HA were less than 50% of the nominal concentration in all treatments (Figure 1).

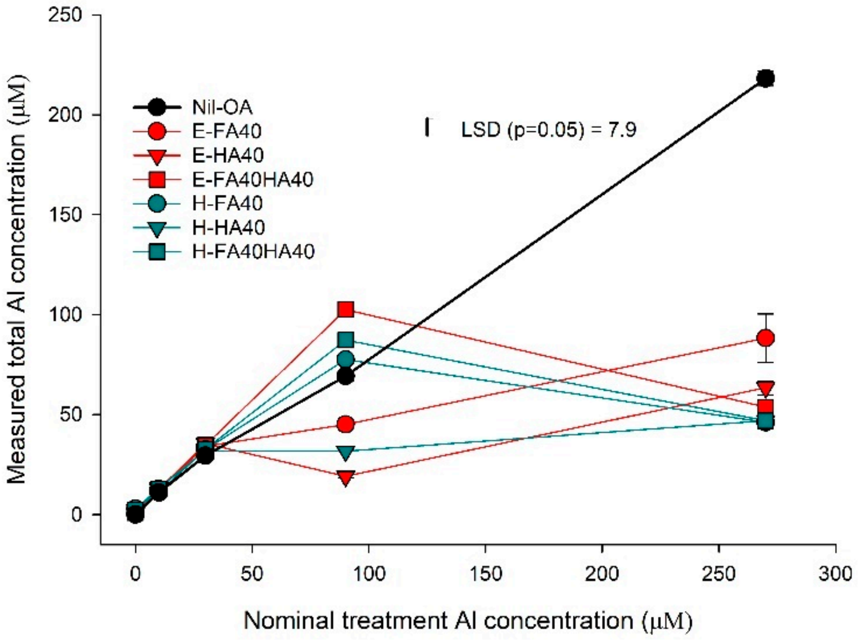

Figure 1. Relationship between measured total Al concentration and nominal treatment Al concentration (0, 10, 30, 90, and 270 µM) in nutrient solutions in which maize plants were grown for 4 days in the presence or absence of fulvic acid (FA) and humic acid (HA) extracted from Eucalyptus (E-FA and E-HA) and Hay (H-FA and H-HA), alone and in combination, at pH 4.5. Standard errors are presented unless obscured by symbols.

The measured OC concentration in the Nil-OA treatments was consistently 0 mg C L^{-1} (Figure 2). In the E-FA and E-HA treatments, OC concentration was about 10% higher than that nominally imposed at the treatment Al concentrations of 0–30 µM (Figure 2). The OC concentrations in all H-FA and H-HA treatments at 0–30 µM Al were consistent with the expected OC concentration and not affected by Al addition.

In the 90 µM Al treatment, the OC concentration in the E-FA40, E-HA40, H-FA40, and H-HA40 decreased relative to treatments with lower Al concentration (0–30 µM Al). However, the effect was variable; in the E-HA40 no OC was present, while in the E-FA40 and H-HA40, the OC concentration was substantially higher at about 13.6 mg C L^{-1}. In these latter treatments, the reduction in measured total Al concentration (Figure 1) was complemented by a similar reduction in OC concentration (Figure 2). In contrast to this, the H-FA40 treatment maintained a high OC concentration (37.4 mg C L^{-1}) at 90 µM Al and was reduced by only 10% compared with the 0 µM Al H-FA40 treatment. In both the E-FA40HA40 and H-FA40HA40 treatments, the OC concentration in the 90 µM Al treatment was the same as that in the comparable 0 µM Al treatments (Figure 2). However, with a further increase in Al concentration to 270 µM, the OC concentration in all FA and HA treatments dropped substantially and particularly in the E-HA and H-HA where OC concentration was 0 mg C L^{-1}. However, in the presence of 270 µM Al, detectable OC concentrations were measured in the E-FA40 (3.4 mg C L^{-1}) and E-FA40HA40 (5.4 mg C L^{-1}) while more substantial OC concentrations were recorded in the H-FA40 (13.5 mg C $^{L-1}$) and H-FA40HA40 (25.2 mg C L^{-1}) treatments; approximately 20–25% of C remained in solution.

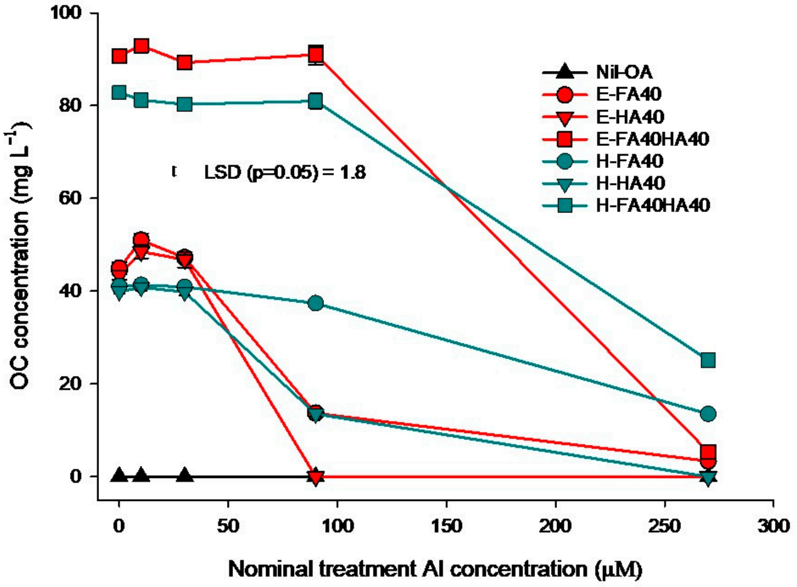

Figure 2. Relationship between organic carbon (OC) concentration and treatment Al concentration (0, 10, 30, 90, and 270 µM) in nutrient solutions in which maize plants were grown for 4 days in the presence or absence of fulvic acid (FA) and humic acid (HA) extracted from Eucalyptus (E-FA and E-HA) and Hay (H-FA and H-HA), alone and in combination, at pH 4.5. Standard errors are presented unless obscured by symbols.

At each treatment Al concentration, the highest Al^{3+} concentrations were maintained in the Nil-OA treatments (Table 1). In the presence of FA and HA in solution, Al^{3+} concentration was reduced compared with the Nil-OA treatments (Table 1). However, as the treatment Al concentration in the Nil-OA treatments increased, the proportion of Al present as Al^{3+} decreased (Figure 3). This was contrary to the general trend observed in the presence of OAs where the proportion of Al^{3+} increased with increasing treatment Al concentration (Figure 3).

Table 1. Concentrations of Al^{3+} (µM) in nutrient solutions in which maize seedlings were grown for 4 days at pH 4.5 in the presence of treatment Al concentrations from 0–270 µM and in the presence of Eucalyptus (E) and Hay (H) fulvic acid (FA) and humic acid (HA), each at 40 mg C L^{-1} alone or in combination.

Organic Acid Treatment	Treatment Al Concentration (µM)									
	0		10		30		90		270	
	Mean and SE		Mean and SE		Mean and SE		Mean and SE		Mean and SE	
Nil-OA	0.0	±0.0	8.4	±0.2	22.5	±2.3	47.4	±9.9	108.1	±11.5
E-FA40	0.0	±0.0	1.3	±0.1	6.5	±0.1	28.2	±1.0	57.4	±0.2
E-HA40	0.0	±0.0	1.4	±0.2	3.4	±0.1	11.9	±1.0	36.8	±4.5
E-FA40HA40	0.0	±0.0	1.6	±0.1	0.0	±0.0	23.1	±0.8	40.1	±4.7
H-FA40	0.0	±0.0	2.3	±0.1	9.7	±2.0	45.3	±1.2	30.5	±2.4
H-HA40	0.0	±0.0	0.2	±0.2	3.3	±0.3	18.8	±1.2	34.5	±2.2
H-FA40HA40	0.0	±0.0	0.0	±0.0	1.1	±0.6	31.9	±3.1	28.2	±1.7

SE denotes the standard error value. LSD (p = 0.05) = 8.2.

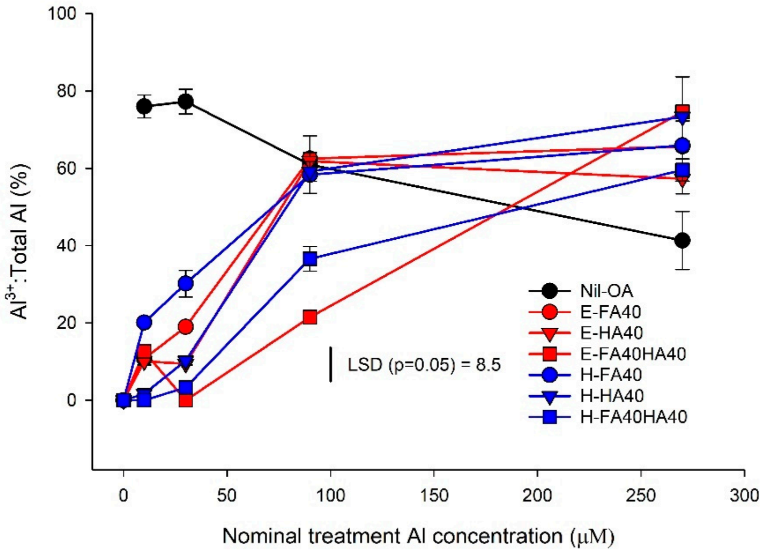

Figure 3. Relationship between Al^{3+} (as a proportion of measured total Al concentration) and treatment Al concentration, in nutrient solutions in which maize plants were grown for 4 days in the presence or absence of fulvic acids (FA) and humic acids (HA) extracted from Eucalyptus (E-FA and E–HA) and Hay (H-FA and H–HA), each at 40 mg C L^{-1} alone or in combination and at pH 4.5. Standard errors are presented unless obscured by symbols.

3.2. Effects of Al on Root Growth in the Absence of FA or HA

Root growth of maize seedlings in the absence of FA and HA was consistently greater in the presence of 10 μM Al compared with that in the 0 μM Al, indicating growth stimulation by low Al^{3+} concentrations (about 7–10 μM) (Figure 4). At higher Al concentrations (treatment Al concentrations of 30, 90, and 270 μM) root growth was progressively inhibited. A Gaussian function was fitted for plots of RRL against the Al^{3+} concentration for both the Eucalyptus and Hay Nil-OA treatments (0–270 μM Al). The Gaussian function accounted for the beneficial effect of Al at 10 μM Al where maximum root growth occurred while accounting for the subsequent reduction in root growth at higher Al concentrations.

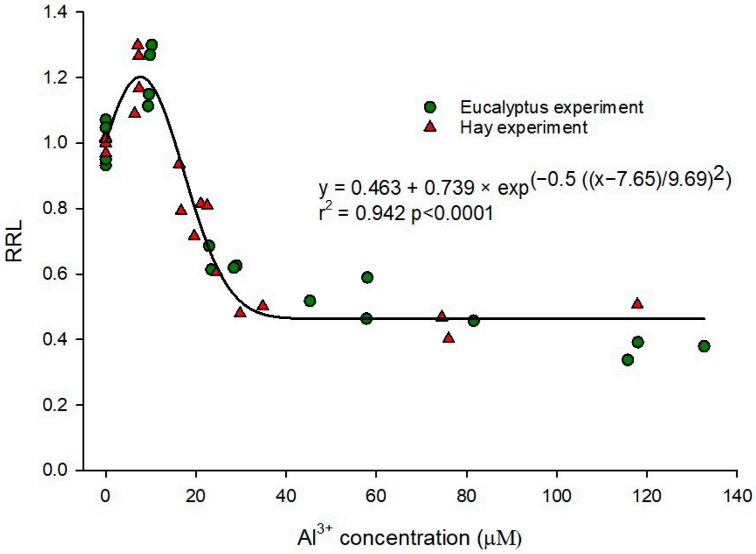

Figure 4. Relationship between relative root length (RRL) and Al^{3+} concentration in two experiments for maize plants grown in nutrient solutions for 4 days over a range of treatment Al concentrations (0–270 μM Al) at pH 4.5 in the absence of FA or HA. The solid line represents the fitted Gaussian model for the combined experiments. The root length in the 0 μM Al treatments was 16.6 cm (Eucalyptus experiment) and 18.1 cm (Hay experiment).

3.3. Direct Effects of FA and HA on Root Growth

In the absence of Al addition, root growth was stimulated in the E-FA40, E-HA40, and E-FA40HA40 treatments compared with that in the Nil-OA treatment. This beneficial effect was enhanced with the further addition of Al at treatment concentrations of 10 and 30 µM (Figure 5), and particularly in the E-HA40 treatment for which the RRL increased by about 20% compared with a 4% increase in the E-FA40. However, with the further addition of Al at concentrations greater than 30 µM, root growth in the presence of E-FA40 and E-HA40 decreased substantially. This effect was consistent with the observation that precipitation of the Eucalyptus FA and HA occurred when the treatment Al concentration increased to 90 and 270 µM Al.

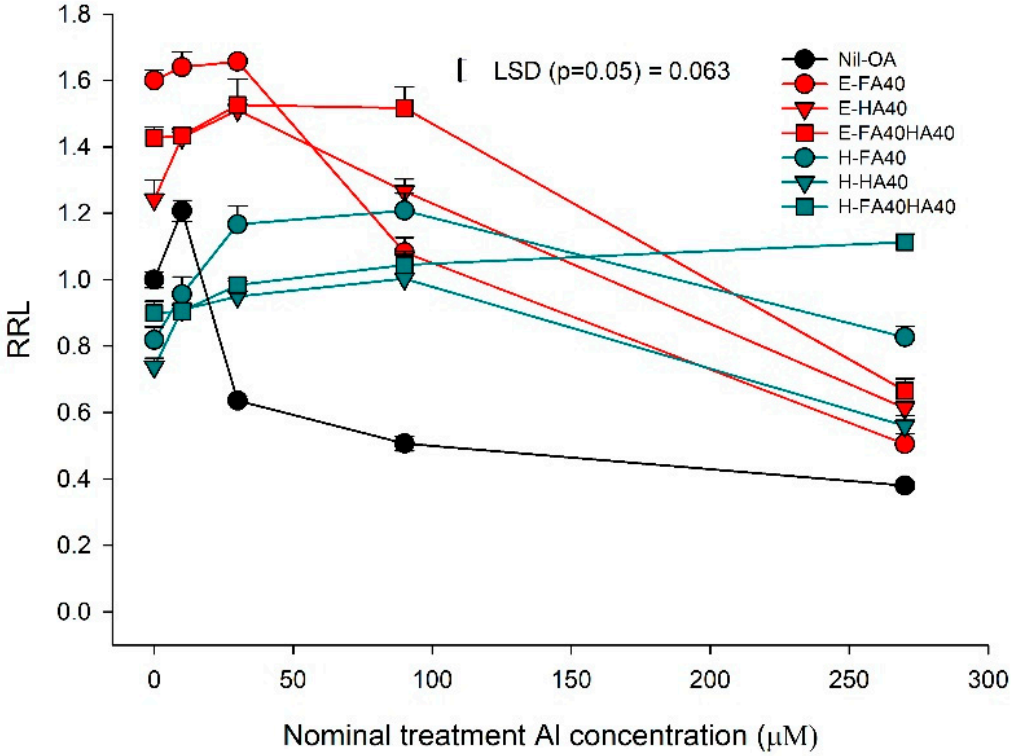

Figure 5. Relationship between relative root length (RRL) of maize plants grown in nutrient solutions for 4 days over a range of treatment Al concentrations (0–270 µM Al) at pH 4.5 and in the presence or absence of fulvic acids (FA) and humic acids (HA) extracted from Eucalyptus (E-FA and E-HA) and Hay (H-FA and H-HA), each at 40 mg C L^{-1} alone or in combination and at pH 4.5. Standard errors are presented unless obscured by symbols.

In contrast to the E-FA and E-HA, root growth was inhibited in all H-FA and H-HA treatments in the absence of added Al (Figure 5). Root growth in the H-FA40 was about 80% of that in the 0 µM Al Nil-OA treatment while in the H-HA40 treatment, it was only 74%. The inhibition of root growth in the H-HA40 at 0 µM Al (RRL = 0.774) was similar to the inhibition of root growth by Al in the 30 µM Nil-OA treatment in Experiment 2 (RRL = 0.737). With successive increases in treatment Al concentrations up to 90 µM, the inhibitory effect of both the Hay FA and HA was alleviated (Figure 5) and in the H-FA40 the inhibitory effect was superseded by a beneficial effect on root growth where the RRL in the H-FA40 in the presence of 30 µM Al (RRL = 1.21) was 20% greater than in the 0 µM Al Nil-OA control. Furthermore, root growth in the H-FA40HA40 was greater than that in either the H-FA40 or H-HA40 ($p < 0.05$) despite having the same concentration of both components. This highlights a strong direct effect of the OAs *per se* on root growth.

3.4. Interactive Effects of FA and HA with Al on Root Growth

The mean RRL in all E-FA and E-HA treatments was greater than that in the comparable Nil-OA treatments at all Al concentrations, highlighting the ability of these OAs to ameliorate the toxic effects of Al (Figure 5). In the presence of E-FA or E-HA, Al^{3+} did not inhibit root growth in the same way as it did in the Nil-OA treatments (Figure 6a). Root growth was not inhibited in the FA40 and FA40HA40 treatments (both in the presence of 90 μM treatment Al) despite the measurement of Al^{3+} concentrations of 28.2 and 23.1 μM, respectively, but was inhibited by an Al^{3+} concentration of 25.9 μM in the 30 μM Al Nil-OA treatment. However, in comparing these results the beneficial effects of the Eucalyptus FA and HA and the detrimental effects of Al^{3+} were confounded. To overcome this, the root length for each organic acid treatment (including the Nil-OA), at each treatment Al concentration, was expressed as the relative root length to that of the same organic acid treatment with no added Al RRL^{OA} (Figure 6b). Thus, the RRL^{OA} for the Nil-OA, and Eucalyptus and Hay FA40, HA40, and FA40HA40 treatments in the absence of Al were all 1.0. When expressed on this basis the relationship between Al^{3+} and root length in the presence of Eucalyptus FA and HA was similar to the response to Al^{3+} noted in the Nil-OA treatment (Figure 6b).

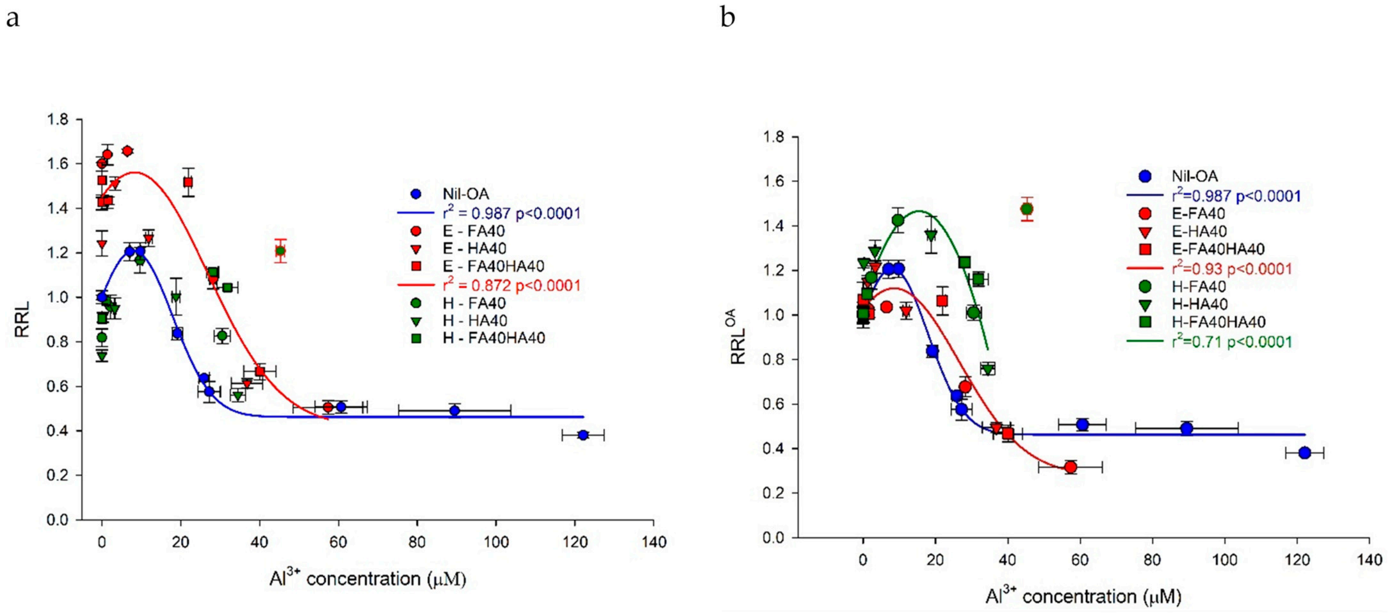

Figure 6. Relationship between relative root length of maize plants and solution Al^{3+} concentration (**a**) expressed relative to the Nil-OA 0 μM Al treatment and (**b**) expressed relative to the comparable FA and HA treatment with no added Al. Root length is expressed as a relative to the Nil-OA with no added Al treatment (RRL) and (**b**) expressed relative to the comparable FA and HA treatment with no added Al (RRL^{OA}). Plants were grown in nutrient solutions for 4 days over a range of treatment Al concentrations (0–270 μM Al) at pH 4.5 and in the presence and in the presence or absence of fulvic acids (FA) and humic acids (HA) extracted from Eucalyptus (E-FA and E-HA) and Hay (H-FA and H-HA), each at 40 mg C L^{-1} alone or in combination and at pH 4.5.

At 0 and 10 μM Al, root growth was considerably greater in the Nil-OA treatment than in either the H-FA40, H-HA40, or H-FA40HA40 treatments in the absence of Al (Figure 5). The H-FA and H-HA strongly inhibited root growth. However, at higher Al concentrations the relative root length in all Hay OA treatments was greater than that in the Nil-OA 0 μM Al treatment. This highlighted the effectiveness of the FA and HA in detoxifying Al. In the H-FA40 and H-FA40HA40 treatments, an Al^{3+} concentration greater than about 20 μM was substantially less inhibitory to root growth than was the same concentration of Al^{3+} in the Nil-OA treatment (Figure 6a). The data for the H-FA40 in the presence of 90 μM Al were inconsistent with other H-FA and H-HA treatments with increasing

Al where the RRL was 1.21 at an Al^{3+} concentration of 45.3 μM. This datum point may have been considered an outlier but has low SEs for both Al^{3+} and RRL. Nonetheless, the point was not included in the development of the relationship between RRL and Al^{3+}, due to its substantial displacement from the other points (Figure 6a,b). The displacement of the point likely represents the observed shift in the effect of H-FA on root growth from a negative to a positive role with increasing Al addition. Even with the addition of Al at a treatment concentration of 270 μM, root growth was not inhibited in the H-FA40HA40 treatment. Indeed, the root length in the H-FA40HA40, in the presence of 270 μM Al, was the same as that in the Nil-OA treatment, in the absence of Al, and had the highest root length of all treatments in the Hay experiment. Notwithstanding, despite the observation of increased root length in this treatment, lateral roots were shortened and stubby; symptoms consistent with that typically associated with Al toxicity. The presence of the Hay FA and HA appeared to only stimulate root growth of the main root while they had not afforded the same protection to lateral root development. This evidence suggests that root organs are differentially sensitive to the effects of toxic Al^{3+} and organic ligands.

Excluding the potential interaction between Al and the FA and HA components, the direct effect of Al toxicity was confounded with the direct effect of the FA and HA. To better understand this effect, root length was expressed relative to the same OA treatment (e.g., FA40-270 μM Al relative to FA40-0 μM Al) (Figure 6b). When expressed on this basis, the relationship between RRL^{OA} and Al^{3+} for all Eucalyptus OA treatments essentially matches the relationship observed between RRL and Al^{3+} in the absence of OA (Figure 6b). However, for the Hay FA and HA, the relationship was somewhat different from the Nil-OA and E-FA and E-HA treatments where RRL^{OA} tended to be higher (1.16–1.36) at Al^{3+} concentrations of about 15–30 μM and where >~10 mg C L^{-1} of FA or HA was in solution. Interestingly, for the H-HA40 treatment in the presence of 270 μM added Al, all the HA had precipitated from the solution and the RRL^{OA} (0.56) point at an Al^{3+} of 34.4 μM corresponded well to the Nil-OA response curve for RRL to Al^{3+} concentration (Figure 6b) (data point is obscured).

4. Discussion

4.1. Direct Effects of FA and HA on Root Growth

In the absence of added Al, root growth in the E-FA40 treatment was greater than that in the comparable Nil-OA and E-HA40 ($p < 0.05$) while root growth in the FA40HA40 was intermediary between the E-FA40 and E-HA40. Stimulation of plant growth by FA and HA is well documented [38–40]. In contrast to the E-FA and E-HA, the H-FA and H-HA inhibited root growth in the absence of Al. Effects of OA on plant growth are not consistently positive and Brunner et al. [42] found that the high-molecular-weight fractions (>10,000 Daltons) from an aqueous extract of chestnut (*Castanea sativa*) leaf litter inhibited barley (*Hordeum vulgare*) growth at concentrations of only 5 mg C L^{-1} yet the fraction below 1000 Daltons did not.

Furthermore, the co-addition of the H-FA with the H-HA (H-FA40HA40) was less inhibitory than was either component alone, suggesting the two components interacted in affecting root growth despite the OC in the H-FA40HA40 being twice that of either the H-FA40 or H-HA40. In contrast, there was no interaction between the E-FA and E-HA in their effects on root growth since root growth in the E-FA40HA40 was an intermediary of that in the E-FA40 and E-HA40 indicating independent effects of the E-FA and E-HA. Only limited research has been conducted on the interactions of FA and HA; however, Ernst et al. [49] found that combinations of FA and HA stimulated plant growth to a greater extent than FA or HA alone.

4.2. Confounding Effects of Al^{3+} and Organic Acids on Root Growth

In the Nil-OA treatments, the fitted Gaussian function for the relationship between root growth and Al^{3+} was significant ($r^2 = 0.942$ $p < 0.001$) (Figure 4) and root growth was enhanced at Al^{3+} concentrations up to 10 μM. Due to the stimulatory effect of Eucalyptus

OA on root growth, the Eucalyptus relationship of RRL to Al^{3+} on root growth in Figure 6a is displaced upward relative to the Nil-OA. The stimulatory effect of the E-FA had an overriding effect on Al toxicity effects since the RRL response, when expressed on an OA basis (Figure 6b), gave a response similar to that of Al^{3+} in the Nil-OA treatments. Where greater than about 10 mg C L^{-1} remained in solution in the E-FA treatments, root growth was stimulated and Al^{3+} concentration was not a good index for plant root growth response to Al toxicity. With the addition of Al at concentrations at 270 μM in the E-FA and E-HA treatments, substantial precipitation of the FA and HA occurred (Figure 2) so no stimulation of root growth due to FA or HA was recorded and root growth decreased substantially due to the overriding inhibitory effect of Al^{3+} (Figure 6a). The presence of only a small amount of un-precipitated FA or HA (<about 5 mg C L^{-1}) did not provide a stimulatory effect on root growth and the inhibitory effect of Al^{3+} on root growth prevailed.

In contrast, the Hay relationship of RRL to Al^{3+} on root growth (Figure 6a) is displaced downward relative to the Nil-OA due to the direct inhibitory effect of the Hay FA and HA. With increasing addition of Al to the H-FA and H-HA the effect on root growth shifted from inhibition to stimulation of root growth. In the Hay OA treatments, precipitation of FA and HA (Figure 2) resulted in the removal of the inhibitory effect of the OA, and the remaining inhibitory effect is attributed to Al toxicity.

In all OA treatments, the effect of Al^{3+} on root growth (initial stimulation up to 10 μM then toxicity) is confounded with the direct effect of the OA on root growth (stimulation for Eucalyptus and inhibition for Hay). As a means of resolving these two confounded effects, the RRLs were expressed relative to the relevant OA treatment at 0 μM Al (RRL^{OA}) (Figure 6b) as opposed to the Nil-OA at 0 μM Al (Figure 6a). For the Hay treatments addition of Al up to 30 μM reduced the inhibitory effect of the OA, producing an initial increase in RRL^{OA} compared to the Nil-OA treatment. Since the OC concentration of the solutions remained constant up to 30 μM Al, this effect is attributed to the complexation of toxic components of the H-FA and H-HA with Al; therefore, denaturing the toxic effect as opposed to the precipitation that occurs at higher Al concentrations. At Al concentrations of 30 μM and above, any positive growth effect of denaturing the inhibitory OA is outweighed by the toxic effect of increasing Al^{3+} concentrations.

Distinct differences can be observed in the organic components of the Eucalyptus and Hay FA and HA [46] where the ^{13}C NMR spectra for the Hay FA and HA show a strong peak at 147 ppm, which equates to aromatic diphenols, which are likely to cause phytotoxic effects. These groups would also be likely to complex with Al, and thus their inhibition of root growth would be expected to be altered as Al is added to the solution. Various authors have demonstrated the co-addition of toxic concentrations of soluble cations with toxic organic ligands results in no inhibition of plant growth [24,50].

A key finding in this study is that the direct effect of the FA and HA on root growth was independent of the effect of Al^{3+} toxicity, since in the presence of >~10 mg C L^{-1} of FA or HA otherwise toxic concentrations of Al^{3+} did not result in reduced root growth. This is likely related to the different effects of Al and FA or HA on root cell elongation. The primary site of Al toxicity is in the elongation zone of roots resulting in a substantial reduction in root cell elongation [51]. In contrast, the elongation of excised tomato roots in a nutrient solution containing FA over concentrations of 50–100 mg L^{-1} (~30–60 mg C L^{-1}) resulted in increased root cell extension compared with a nil FA control [38].

4.3. Implications of Findings for Acidic Soil Studies

In this study, the direct effect of the FA and HA (i.e., in the absence of added Al) was either positive as for Eucalyptus FA and HA or negative as for the Hay FA and HA. Indeed, the inhibitory effect on root growth of the Hay FA and HA per se was equivalent to that of Al toxicity at an Al^{3+} concentration of 20–25 μM. Increasing the solution Al concentration altered the direct effect of FA and HA on root growth and the magnitude of this effect varied depending on the source of FA and HA. For the Eucalyptus FA and HA, the enhancement was slight with the increase in Al, but for the Hay FA and HA further addition of Al resulted in a shift in the direct effect of OA on root growth from severe inhibition (0 μM Al treatment) to enhancement of root growth in the 30 and 90 μM Al treatments) (Figure 6b). In some treatments, the presence of toxic Al^{3+} concentrations (>20 μM) did not inhibit root growth to the anticipated extent. Furthermore, the study demonstrates that non-precipitated FA and HA can ameliorate Al toxicity through two independent mechanisms including complexation of Al or the direct beneficial effect of the OA on root growth. Hence, concentrations of Al^{3+} in the soil solution are not necessarily a good index of plant root response to Al toxicity.

In acidic soil studies the direct impact of soluble organic ligands on root growth and complexation of Al complicates the understanding of acidic soil infertility and particularly Al toxicity effects. Given the nature of Al toxicity inhibiting root growth, acidic soil studies utilize short-term (~4 days) plant root elongation experiments to diagnose and develop an understanding of Al toxicity impacts on plant growth e.g., [6,52–54]. Studies such as these often show poor coefficients of determination. Menzies et al. [53] in their study discriminated against the effects of non-toxic Al-organic complexes, which gave a better correlation. Various authors e.g., [55,56] have demonstrated that for soils low in soluble OC, plant growth is well correlated with measures of solution Al concentration, but for soils with higher solution OA concentrations, the effect of Al complexation and detoxification must be considered [31,53,57].

This controlled solution culture study highlights the complex role of high-molecular-weight OAs (FA and HA) in the expression of Al toxicity on plant root growth. In degraded acidic soils with low levels of organic matter and high soil solution Al concentrations, inorganic soil solution chemistry principles will prevail and most of the Al in soil solution will be present as Al^{3+}. In acidic soils, soluble FA and HA may be present in the soil solution at concentrations of about 30–110 mg C L^{-1} [47] while in the present study concentrations of FA or HA at only about 10 mg C L^{-1} had direct effects on plant growth; consistent with the finding of Ayuso et al. [58]. Therefore, it is likely that the effect of FA and HA, and other soluble organic compounds, on plant growth will be important in studies where bioassays are used as a means of determining the relative toxicity of Al. Further research is required to refine the diagnostic criteria for Al toxicity in acidic soils, taking into consideration the effect of the organic component per se on plant growth. This direct effect could be either inhibitory or stimulatory depending on the type of OAs present and the test plant species being grown.

4.4. Mechanisms for Reducing Solution Al^{3+}

At treatment Al concentrations of 10 and 30 μM, the concentration of OC and total Al were equivalent to that nominally imposed in the FA and HA (Eucalyptus or Hay) treatments. However, Al^{3+} concentrations were only 0–9.7 μM indicating complexation of Al was the principal mechanism by which Al^{3+} concentration was reduced. With an increase in treatment Al concentration to 90 μM Al, the total Al concentration in the E-FA40 (45.1 μM) and H-FA40 (77.6 μM), and E-FA40HA40 (102.0 μM), and H-FA40HA40 (87.1 μM) treatments were greater than that in the E-HA40 (19.2 μM) and H-HA40 (31.7 μM) indicating a greater capacity for the FA to complex Al at higher Al concentrations. No OC was present in the E-HA40 treatment and only 30% was present in solution in the H-HA40 (13.6 mg L^{-1}) indicating strong precipitation of Al with HA in these treatments. Across treatments at 90 μM added Al, (except for H-FA40) complexation and precipitation reactions, simultaneously reducing Al^{3+} concentration to varying degrees. In the H-FA40 in the presence of 90 μM Al, both total Al (Figure 2) and OC concentrations (Figure 4), though reduced, were similar to that nominally imposed. However, there was a considerable discrepancy between total Al and Al^{3+} concentrations (Figure 6) indicating complexation was the dominant mechanism for reducing Al^{3+}. The solubility of the H-FA at high Al concentrations was greater than that of the H-HA and the E-FA and E-HA. The ^{13}C NMR spectra for the Hay FA showed strong peaks at 172 ppm representing carboxylic functional groups and at 55 ppm for hydroxyl functional groups [46] the combination of which confers strong potential for metal ion complexation [59,60].

Schnitzer and Skinner [61] noted that, at a solution ratio of 1 M Al to 1 M of a high-molecular-weight organic extract (*viz.* FA or HA) (about 115 µM Al to 40 mg C L^{-1}), complexation was the most important reaction, and the complex was water-soluble (*viz.* it did not precipitate) while at higher ratios precipitation occurred. Consistent with this finding, the present study showed that as Al addition increased (to 90 and 270 µM) precipitation of Al occurred and complexation ceased to be the sole mechanism for reducing Al^{3+} concentration. In the E-FA40, E-HA40 and E-FA40HA40, and H-HA40 treatments in the presence of 270 µM Al, almost all the OA had precipitated from the solution, and in the H-FA40 and H-FA40HA40, the OC was substantially reduced indicating that at higher Al concentration, precipitation supersedes complexation as the most important mechanism in reducing Al. In the present experiments, a ratio of only 90 µM Al to 40 mg C L^{-1} resulted in substantial precipitation of organic C supplied as HA and to a lesser extent FA. Precipitation is demonstrated between other metal ions with FA and HA when the metal ion activity is high [62]. Hiradate et al. [44] identified that the mechanism for the formation of FA-Al precipitates includes a complexation reaction between Al and carboxylic functional groups on the FA.

Interestingly, with an increase in treatment Al concentration from 90 µM to 270 µM and despite all treatments that have 0 mg C L^{-1}, the increases in total solution Al concentration for the E-HA40 (44 µM increase) and H-HA40 (15.3 µM increase), were substantially less than the increase in the comparable Nil-OA treatment (~218 µM). This indicates that further complexation or precipitation of Al to the existing Al-HA precipitate occurred indicating a potential significant role for soil humic material in ameliorating Al toxicity.

The differences in complexation and precipitation reactions between the two sources of FA and HA relate to the considerable differences in their structure as shown by ^{13}C NMR and size exclusion chromatography spectra for these acids [46]. These findings demonstrate a fundamental broad difference in the reactivity of FA and HA with solution Al and further highlight a differential in solubility of the Al-OA complexes dependent on the source from which they were extracted. At low Al concentrations (10 and 30 µM) both sources of HA more effectively complexed Al than did FA and the Eucalyptus FA and HA formed stronger Al-complexes than the Hay FA and HA. However, at higher treatment Al concentrations the Hay FA and HA possessed a greater capacity for complexing Al than the Eucalyptus FA and HA for which precipitation was favored and represents an important mechanism for Al detoxification. The research shows a differential function of FA and HA based on the source from which they were extracted, and further research is required to understand the differential effects of source organic material on Al detoxification in soils.

5. Conclusions

This study shows a complex interaction between the effects of high-molecular-weight oAs and Al^{3+} on plant root growth. In the absence of Al, differential direct effects of FA and HA on root growth were observed with the Eucalyptus FA and HA stimulating root growth while the Hay FA and HA inhibited root growth. The detrimental effect of the Hay FA and HA was superseded by a beneficial effect when Al was added to solutions. A novel finding in this study is that the direct beneficial effect of the OA can override the toxic effect of Al which is not previously reported. In the presence of OA concentrations greater than about 10 mg C L^{-1}, Al^{3+} concentration was not a good index of plant response to Al toxicity since in the presence of an otherwise toxic Al^{3+} concentration, root growth was not inhibited. This study is the first that identifies multiple mechanisms interacting on root growth and Al detoxification including a direct positive or negative effect of OA on root growth, an interaction with Al causing detoxification, altered effects of OA with addition of Al, and the complexation and precipitation of Al.

The application of organic and waste-based products is an integral component of the move toward sustainable farming systems. In studies evaluating the ability of organic amendments to ameliorate the effects of toxic metal cation species, discrimination against the beneficial or detrimental effects of OAs per se must be considered.

Author Contributions: Conceptualization, S.H.; methodology, S.H. and N.M.; validation, S.H.; investigation, S.H.; writing—original draft preparation, S.H.; writing—review and editing, S.H. and N.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Australian Centre For International Agricultural Research (Grant numbers 8904 and SMCN/2012/029).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

FA Fulvic acid, HA Humic acid, ICPAES Inductively coupled plasma atomic emission spectrophotometer, OA Organic acid, OC Organic carbon, RRL Relative root length.

References

1. Noble, A.D.; Sumner, M.E. Soil acidification: The world story. In *Handbook of Soil Acidity*; CRC Press: Boca Raton, FL, USA, 2003.
2. Crowther, T.W.; Glick, H.B.; Covey, K.R.; Bettigole, C.; Maynard, D.S.; Thomas, S.M.; Smith, J.R.; Hintler, G.; Duguid, M.C.; Amatulli, G.; et al. Mapping tree density at a global scale. *Nature* **2015**, *525*, 201–205. [CrossRef]
3. von Uexküll, H.R.; Mutert, E. Global extent, development and economic impact of acid soils. *Plant Soil* **1995**, *171*, 1–15. [CrossRef]
4. Sanchez, P.; Villachica, J.; Bandy, D. Soil fertility dynamics after clearing a tropical rainforest in Peru. *Soil Sci. Soc. Am. J.* **1983**, *47*, 1171–1178. [CrossRef]
5. Blamey, F.P.C.; Nishizawa, N.K.; Yoshimura, E. Timing, magnitude, and location of initial soluble aluminum injuries to Mungbean roots. *Soil Sci. Plant Nutr.* **2004**, *50*, 67–76. [CrossRef]
6. Bruce, R.; Warrell, L.; Edwards, D.; Bell, L. Effects of aluminium and calcium in the soil solution of acid soils on root elongation of *Glycine max* cv. Forrest. *Aust. J. Agric. Res.* **1988**, *39*, 319–338. [CrossRef]
7. Comin, J.J.; Barloy, J.; Bourrié, G.; Trolard, F. Differential effects of monomeric and polymeric aluminium on the root growth and on the biomass production of root and shoot of corn in solution culture. *Eur. J. Agron.* **1999**, *11*, 115–122. [CrossRef]
8. Hale, S.E.; Nurida, N.L.; Jubaedah Mulder, J.; Sørmo, E.; Silvani, L.; Abiven, S.; Joseph, S.; Taherymoosavi, S.; Cornelissen, G. The effect of biochar, lime and ash on maize yield in a long-term field trial in a Ultisol in the humid tropics. *Sci. Total Environ.* **2020**, *719*, 137455. [CrossRef]
9. Gurmessa, B. Soil acidity challenges and the significance of liming and organic amendments in tropical agricultural lands with reference to Ethiopia. *Environ. Dev. Sustain.* **2020**, *23*, 77–99. [CrossRef]
10. Besho, T.; Bell, L. Soil and solution phase chargers and mung bean response during amelioration of aluminum toxicity using organic matter. *Plant Soil* **1992**, *140*, 183–196. [CrossRef]
11. De Bauw, P.; Shimamura, E.; Rakotoson, T.; Andriamananjara, A.; Verbeeck, M.; Merckx, R.; Smolders, E. Farm yard manure application mitigates aluminium toxicity and phosphorus deficiency for different upland rice genotypes. *J. Agron. Crop. Sci.* **2021**, *207*, 148–162. [CrossRef]
12. Shetty, R.; Vidya, C.S.-N.; Prakash, N.B.; Lux, A.; Vaculík, M. Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. *Sci. Total Environ.* **2020**, *765*, 142744. [CrossRef] [PubMed]
13. Hue, N.; Amien, I. Aluminum detoxification with green manures. *Commun. Soil Sci. Plant Anal.* **1989**, *20*, 1499–1511. [CrossRef]
14. Alva, A.; Edwards, D.; Asher, C. Effects of acid soil infertility factors on mineral composition of soybean and cowpea tops. *J. Plant Nutr.* **1991**, *14*, 187–203. [CrossRef]
15. Dolling, P.; Porter, W.; Robson, A. Effect of soil acidity on barley production in the south-west of Western Australia. 1. The interaction between lime and nutrient application. *Aust. J. Exp. Agric.* **1991**, *31*, 803–810. [CrossRef]
16. Piatt, J.J.; Brusseau, M.L. Rate-Limited Sorption of Hydrophobic Organic Compounds by Soils with Well-Characterized Organic Matter. *Environ. Sci. Technol.* **1998**, *32*, 1604–1608. [CrossRef]
17. Chernikov, V.; Raskatov, V.; Konchits, V. Composition and properties of fulvic acid fractions from chernozems. *Sov. Soil Sci.* **1991**, *23*, 61–72.
18. Kögel, I.; Hempfling, R.; Zech, W.; Hatcher, P.G.; Schulten, H.-R. Chemical composition of the organic matter in forest soils: 1. Forest litter. *Soil Sci.* **1988**, *146*, 124–136. [CrossRef]

19. Kononova, M.; Alexandrova, I. Formation of humic acids during plant residue humification and their nature. *Geoderma* **1973**, *9*, 157–164. [CrossRef]
20. Cieśliński, G.; Van Rees, K.C.J.; Szmigielska, A.M.; Krishnamurti, G.S.R.; Huang, P.M. Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. *Plant Soil* **1998**, *203*, 109–117. [CrossRef]
21. Kerven, G.; Asher, C.; Edwards, D.; Ostatek-Boczynski, Z. Sterile solution culture techniques for aluminum toxicity studies involving organic acids. *J. Plant Nutr.* **1991**, *14*, 975–985. [CrossRef]
22. Lakshman, S.; Mills, R.; Patterson, H.; Cronan, C. Apparent differences in binding site distributions and aluminum (III) complexation for three molecular weight fractions of a coniferous soil fulvic acid. *Anal. Chim. Acta* **1993**, *282*, 101–108. [CrossRef]
23. Lobartini, J.C.; Orioli, G.A. Absorption of iron Fe-humate in nutrient solutions by plants. *Plant Soil* **1988**, *106*, 153–157. [CrossRef]
24. Suthipradit, S.; Edwards, D.; Asher, C. Effects of aluminium on tap-root elongation of soybean (*Glycine max*), cowpea (*Vigna unguiculata*) and green gram (*Vigna radiata*) grown in the presence of organic acids. *Plant Soil* **1990**, *124*, 233–237. [CrossRef]
25. Almendros, G.; González-Vila, F. Degradative studies on a soil humin fraction—Sequential degradation of inherited humin. *Soil Biol. Biochem.* **1987**, *19*, 513–520. [CrossRef]
26. Baigorri, R.; Fuentes, M.; González-Gaitano, G.; García-Mina, J.M.; Almendros, G.; González-Vila, F.J. Complementary Multianalytical Approach To Study the Distinctive Structural Features of the Main Humic Fractions in Solution: Gray Humic Acid, Brown Humic Acid, and Fulvic Acid. *J. Agric. Food Chem.* **2009**, *57*, 3266–3272. [CrossRef] [PubMed]
27. Kuwatsuka, S.; Shindo, H. Behavior of phenolic substances in the decaying process of plants: I. Identification and quantitative determination of phenolic acids in rice straw and its decayed product by gas chromatography. *Soil Sci. Plant Nutr.* **1973**, *19*, 219–227. [CrossRef]
28. Malcolm, R.L.; MacCarthy, P. Limitations in the use of commercial humic acids in water and soil research. *Environ. Sci. Technol.* **1986**, *20*, 904–911. [CrossRef] [PubMed]
29. Diatloff, E.; Harper, S.; Asher, C.; Smith, F. Effects of humic and fulvic acids on the rhizotoxicity of lanthanum and aluminium to corn. *Soil Res.* **1998**, *36*, 913–920. [CrossRef]
30. Yang, Z.M.; Sivaguru, M.; Horst, W.J.; Matsumoto, H. Aluminium tolerance is achieved by exudation of citric acid from roots of soybean (*Glycine max*). *Physiol. Plant.* **2000**, *110*, 72–77. [CrossRef]
31. Hue, N.; Craddock, G.; Adams, F. Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.* **1986**, *50*, 28–34. [CrossRef]
32. McColl, J.; Pohlman, A. Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. In *Acidic Precipitation*; Springer: Berlin/Heidelberg, Germany, 1986.
33. Guimarães, D.V.; Gonzaga, M.I.S.; da Silva, T.O.; da Silva, T.L.; da Silva Dias, N.; Matias, M.I.S. Soil organic matter pools and carbon fractions in soil under different land uses. *Soil Tillage Res.* **2013**, *126*, 177–182. [CrossRef]
34. Vanlauwe, B.; Aihou, K.; Tossah, B.K.; Diels, J.; Sanginga, N.; Merckx, R. Senna siamea trees recycle Ca from a Ca-rich subsoil and increase the topsoil pH in agroforestry systems in the West African derived savanna zone. *Plant Soil* **2005**, *269*, 285–296. [CrossRef]
35. Ahlrichs, J.L.; Karr, M.C.; Baligar, V.C.; Wright, R.J. Rapid bioassay of aluminum toxicity in soil. *Plant Soil* **1990**, *122*, 279–285. [CrossRef]
36. Aitken, R.L.; Moody, P.W.; Compton, B.L. A simple bioassay for the diagnosis of aluminium toxicity in soils. *Commun. Soil Sci. Plant Anal.* **1990**, *21*, 511–529. [CrossRef]
37. Kumar Sootahar, M.; Zeng, X.; Su, S.; Wang, Y.; Bai, L.; Zhang, Y.; Li, T.; Zhang, X. The Effect of Fulvic Acids Derived from Different Materials on Changing Properties of Albic Black Soil in the Northeast Plain of China. *Molecules* **2019**, *24*, 1535. [CrossRef]
38. Linehan, D. Some effects of a fulvic acid component of soil organic matter on the growth of cultured excised tomato roots. *Soil Biol. Biochem.* **1976**, *8*, 511–517. [CrossRef]
39. Rauthan, B.; Schnitzer, M. Effects of a soil fulvic acid on the growth and nutrient content of cucumber (*Cucumis sativus*) plants. *Plant Soil* **1981**, *63*, 491–495. [CrossRef]
40. Schnitzer, M.; Poapst, P. Effects of a soil humic compound on root initiation. *Nature* **1967**, *213*, 598–599. [CrossRef]
41. Suh, H.Y.; Yoo, K.S.; Suh, S.G. Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). *Hortic. Environ. Biotechnol.* **2014**, *55*, 455–461. [CrossRef]
42. Brunner, I.; Luster, J.; Ochs, M.; Blaser, P. Phytotoxic effects of the high molecular weight fraction of an aqueous leaf litter extract on barley root development. *Plant Soil* **1996**, *178*, 83–93. [CrossRef]
43. Dobbss, L.B.; Medici, L.O.; Peres, L.E.P.; Pino-Nunes, L.E.; Rumjanek, V.M.; Façanha, A.R.; Canellas, L.P. Changes in root development of Arabidopsis promoted by organic matter from oxisols. *Ann. Appl. Biol.* **2007**, *151*, 199–211. [CrossRef]
44. Hiradate, S.; Yonezawa, T.; Takesako, H. Isolation and purification of hydrophilic fulvic acids by precipitation. *Geoderma* **2006**, *132*, 196–205. [CrossRef]
45. Alebachew, M.; Amare, T.; Wendie, M. Investigation of the Effects of *Eucalyptus camaldulensis* on Performance of Neighbouring Crop Productivity in Western Amhara, Ethiopia. *Open Access Libr. J.* **2015**, *2*, 1–10. [CrossRef]
46. Harper, S.; Kerven, G.; Edwards, D.; Ostatek-Boczynski, Z. Characterisation of fulvic and humic acids from leaves of *Eucalyptus camaldulensis* and from decomposed hay. *Soil Biol. Biochem.* **2000**, *32*, 1331–1336. [CrossRef]

47. Kerven, G.; Ostatek-Boczynski, Z.; Edwards, D.; Asher, C.; Oweczkin, J. Chromatographic techniques for the separation of Al and associated organic ligands present in soil solution. In *Plant-Soil Interactions at Low pH: Principles and Management*; Springer: Berlin/Heidelberg, Germany, 1995.
48. Kerven, G.; Edwards, D.; Asher, C.; Hallman, P.; Kokot, S. Aluminum determination in soil solution. 2. Short-term colorimetric procedures for the measurement of inorganic monomeric aluminum in the presence of organic acid ligands. *Soil Res.* **1989**, *27*, 91–102. [CrossRef]
49. Ernst, W.; Kraak, M.; Stoots, L. Growth and mineral nutrition of *Scrophularia nodosa* with various combinations of fulvic and humic acids. *J. Plant Physiol.* **1987**, *127*, 171–175. [CrossRef]
50. Tadano, T.; Pantanahiran, W.; Nilnond, C. Inhibitory effect of canal water drained from a tropical deep peat soil on the elongation of rice roots. *Soil Sci. Plant Nutr.* **1992**, *38*, 149–157. [CrossRef]
51. Kopittke, P.M.; Moore, K.L.; Lombi, E.; Gianoncelli, A.; Ferguson, B.J.; Blamey, F.P.; Menzies, N.W.; Nicholson, T.M.; McKenna, B.A.; Wang, P.; et al. Identification of the primary lesion of toxic aluminum in plant roots. *Plant Physiol.* **2015**, *167*, 1402–1411. [CrossRef]
52. Close, E.; Powell, H. Rapidly extracted (0.02 M $CaCl_2$-soluble) reactive aluminum as a measure of aluminum toxicity in soils. *Soil Res.* **1989**, *27*, 663–672. [CrossRef]
53. Menzies, N.; Edwards, D.; Bell, L. Effects of calcium and aluminum in the soil solution of acid, surface soils on root elongation of mungbean. *Soil Res.* **1994**, *32*, 721–737. [CrossRef]
54. Wright, R.; Baligar, V.; Ahlrichs, J. The influence of extractable and soil solution aluminum on root growth of wheat seedlings. *Soil Sci.* **1989**, *148*, 293–302. [CrossRef]
55. Adams, F.; Hathcock, P.J. Aluminum Toxicity and Calcium Deficiency in Acid Subsoil Horizons of Two Coastal Plains Soil Series. *Soil Sci. Soc. Am. J.* **1984**, *48*, 1305–1309. [CrossRef]
56. Evans, C.E.; Kamprath, E. Lime response as related to percent Al saturation, solution Al, and organic matter content. *Soil Sci. Soc. Am. J.* **1970**, *34*, 893–896. [CrossRef]
57. Zhao, W.-R.; Li, J.-Y.; Jiang, J.; Lu, H.-L.; Hong, Z.-N.; Qian, W.; Xu, R.-K.; Deng, K.-Y.; Guan, P. The mechanisms underlying the reduction in aluminum toxicity and improvements in the yield of sweet potato (*Ipomoea batatas* L.) After organic and inorganic amendment of an acidic ultisol. *Agric. Ecosyst. Environ.* **2020**, *288*, 106716. [CrossRef]
58. Ayuso, M.; Hernández, T.; Garcia, C.; Pascual, J.A. Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials. *Bioresour. Technol.* **1996**, *57*, 251–257. [CrossRef]
59. Saar, R.A.; Weber, J.H. Fulvic acid: Modifier of metal-ion chemistry. *Environ. Sci. Technol.* **1982**, *16*, 510A–517A. [CrossRef]
60. dos Santos, J.V.; Fregolente, L.G.; Mounier, S.; Hajjoul, H.; Ferreira, O.P.; Moreira, A.B.; Bisinoti, M.C. Fulvic acids from Amazonian anthropogenic soils: Insight into the molecular composition and copper binding properties using fluorescence techniques. *Ecotoxicol. Environ. Saf.* **2020**, *205*, 111173. [CrossRef]
61. Schnitzer, M.; Skinner, S. Organo-metallic interactions in soils: 1. Reactions between a number of metal ions and the organic matter of a podzol Bh horizon. *Soil Sci.* **1963**, *96*, 86–93. [CrossRef]
62. Chirenje, T.; Rivero, C.; Ma, L.Q. Leachability of Cu and Ni in wood ash-amended soil as impacted by humic and fulvic acid. *Geoderma* **2002**, *108*, 31–47. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.