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The Alleviation of Nutrient Deficiency Symptoms in Changbai Larch (*Larix olgensis*) Seedlings by the Application of Exogenous Organic Acids

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Abstract: Exogenous organic acids are beneficial in protecting plants from the stress of heavy metal toxins (e.g., Pb) in soils. This work focuses on the potential role of organic acids in protecting Changbai larch (*Larix olgensis*) seedlings from the stress of growing in nutrient deficient soil. The seedlings were planted in a nutrient rich or deficient soil (A_1 horizon of a Haplic Cambisol without organic acid as the nutrient rich control, or fully-mixed A_1 + B horizons in a proportion of 1:2 as deficient) in pots in a greenhouse. In A_1 + B horizons the seedlings were treated daily with concentrations of oxalic or citric acid (OA or CA) at a rate approximately equivalent to 0, 0.04, 0.2, 1.0, or 2.0 mmol·kg^{-1} of soil for 10, 20, and 30 days. Nutrient deficiency stressed the seedlings as indicated by lipid peroxidation and malondialdehyde (MDA) content in leaves significantly increasing, and superoxide dismutase (SOD) activities, proline, photosynthetic pigment contents, and chlorophyll fluorescence (F_v/F_m) decreasing. The stress increased in controls over the application periods. When nutrient deficient plants were exposed to an organic acid (especially 5.0 or 10.0 mmol·L^{-1} for 20 days), the stress as indicated by the physiological parameters was reversed, and survival rate of seedlings, and biomass of root, stem, and leaf significantly increased; CA was more effective than OA. The results demonstrate that exogenous organic acids alleviate nutrient deficiency-induced oxidative injuries and improve the tolerance of *L. olgensis* seedlings to nutrient deficiency.

Keywords: nutrient deficiency; oxalic acid; citric acid; *Larix olgensis*; physiological and biochemical effects

1. Introduction

Soil nutrient availability and uptake play an important role in regulating plant growth, development, and biomass accumulation [1,2]. When plants suffer from nutrient deficiency, metabolic changes in the plants and a decrease in photosynthetic efficiency often can be found, and consequently reduce growth rate [3]. Understanding how plants adapt to the stress of nutrient deficiency is of interest. Some studies have demonstrated that organic acids play an important role in the mobilization, absorption, and transportation of nutrients in plants [4,5]. These acids are also involved in regulating physiological processes of plants, including decreasing lipid peroxidation, improving antioxidant enzyme activity, increasing contents of osmotic regulation substances, and consequently being beneficial to plant growth and biomass accumulation. As such, these acids increase the resistance of plants to a nutrient deficient environment [6,7].

The beneficial role of organic acids has been established in tobacco treated with malic acid, citric acid, tartaric acid, and lactic acid [8]. In general, these benefits are closely related to plant species, organic acid type and concentration, and application period [6–8]. Furthermore, nutrient deficiency has been shown to induce plant roots to excrete organic acids, particularly oxalic acid, citric acid, tartaric acid, malic acid, and succinic acid, and it is a common adaptive response with which plants counter a nutrient deficient environment [9,10]. It is generally acknowledged that there is a significant correlation between plant tolerance and organic acids secreted by roots under nutrient deficiency [11], and the different compounds and quantities of organic acids excreted from roots of plants are related to plant species, nutrient concentration, and time [12]. The focus of the above research, however, has been on agricultural row crops while research on plantation grown forest tree species is limited.

Changbai larch (*Larix olgensis*) is an important commercial forestry species in northeast China where areas of barren and uncultivated soils need to be reclaimed. Within this part of China, *L. olgensis* is widely planted due to rapid growth and its ability to survive in barren, low nutrient soils. Theoretically, these characteristics make it a good candidate for afforestation in these local forestry areas. However, under severe nutrient deficiencies, its survival and growth is restricted. Previous studies found that some kinds of organic acids could be leached from *L. olgensis* forest litter continuously, such as citric acid and oxalic acid, and their concentrations were considerable [13]. Here we hypothesize that exogenous organic acids may protect larch against nutrient deficiency. Therefore, the possible beneficial role of oxalic acid (OA) and citric acid (CA) on *L. olgensis* seedlings grown in nutrient deficient soil is evaluated by applying concentrations of OA and CA found in *L. olgensis* forest litters [13]. Specifically, changes in some physiological and biochemical parameters are investigated for 10, 20, and 30 days. These findings may help to guide future afforestation of nutrient deficient soils in northeast China with organic acid amendments directly or through mulch applications.

The specific objectives of this study were to: (1) investigate the physiological and growth effects of nutrient deficiency on *L. olgensis* seedlings; and (2) compare the effects of different concentrations and application periods of exogenous organic acids on *L. olgensis* biomass and nutrient accumulation, as well as on physiological and biochemical characteristics under nutrient deficient conditions. This study is the first to provide experimental evidence revealing the interrelations between organic acids and nutrient deficient stress to *L. olgensis* seedlings.

2. Materials and Methods

2.1. Plant Culture and Treatments

Experiments were conducted in the greenhouse of Maoershan Forest Research Station (127°30′–127°34′ E, 45°21′–45°25′ N) of Northeast Forestry University, in Harbin, Heilongjiang province, China, which has a continental temperate monsoon climate. The soil type is Typic Bori-Udic Cambosols [14], corresponding to Haplic Cambisols (Greyic, Dystric) [15] with high organic matter content [16].

After *L. olgensis* seed selection and disinfection, the seeds were vernalized and sown at the end of April in pots (the top of each pot was 42.5 cm × 19.0 cm, while the bottom was 33.0 cm × 10.0 cm, and the height was 18.0 cm). The soil matrix for potting comprised inclusion-free A_1 horizon from the Haplic Cambisols that had a uniform loam texture. After one month, 30 seedlings were transplanted in each pot, for a total of 300 pots with soil matrices of: (1) A_1 and B horizons mixed fully in proportion of 1:2 (270 pots); and (2) A_1 horizon only as a high nutrient control (Ck; 30 pots). The soil characters of A_1 and B horizons are reported in Table 1. The plants were subjected to unimpeded, ambient light and daily watering.

Table 1. Selected properties of A_1 and B horizon soils used in the experiment from a Haplic Cambisol collected in Maoershan Forest Research Station of Northeast Forestry University, Heilongjiang province, China in 2014.

Soil horizon	pH (H_2O)	CEC [a] ($cmol \cdot kg^{-1}$) [b]	Organic Matter ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$) [c]	Soil Texture	Clay (<2 μm) ($g \cdot kg^{-1}$)
A_1	5.36	40.5	108.0	6.41	2.10	46.1	loam [d]	134
B	5.10	21.0	12.2	1.10	1.32	10.3	loam [d]	191

[a] Cation exchange capacity; [b] Centimoles of cation charge per kg soil; [c] Extracted with 30 $mmol \cdot L^{-1}$ NH_4F + 25 $mmol \cdot L^{-1}$ HCl; [d] According to USDA (United States Department of Agriculture) soil classification.

One month after transplanting the seedlings, organic acid treatments were initiated. OA or CA at 0, 0.2, 1.0, 5.0, and 10.0 $mmol \cdot L^{-1}$ was produced from organic salt solutions. The pH of these solutions (~5.16) was modified with NaOH solution to match the average pH of local soil. There were three sampling and analysis times (i.e., 10, 20, and 30 days) after organic acid treatments. For each sampling time, there were 10 treatments, 10 pots per treatment, and 30 seedlings per pot or 900 seedlings total in each treatment. The experimental layout is described in Table 2. The 10 treatments sampled included Ck, organic acid at 0 $mmol \cdot L^{-1}$, OA at 0.2, 1.0, 5.0, and 10.0 $mmol \cdot L^{-1}$, and CA at 0.2, 1.0, 5.0, and 10.0 $mmol \cdot L^{-1}$. The Ck treatment was comprised of A_1 horizon only to serve as a high nutrient control (without organic acid) to compare to the effects of nutrient deficiency in the A_1 plus B horizon mixture. The A_1 and B horizons were mixed fully in a proportion of 1:2 and included a zero organic acid (0 $mmol \cdot L^{-1}$) treatment (T1). Thereafter, OA or CA was added in the concentrations shown above as the other eight treatments (T2–T9) to investigate the effects of different kinds and concentrations of organic acids on *L. olgensis* seedlings under nutrient deficiency. For T2 to T9, root irrigation was performed by saturating soils with the different concentrations of OA or CA solutions, and applying solution at a rate approximately equivalent to 0.04, 0.2, 1.0, and 2.0 $mmol \cdot kg^{-1}$ of soil for each acid concentration, respectively. In addition, due to the incomplete root development of the seedlings foliar applications of organic acid solutions were done to enhance organic acid uptake, a common approach used with foliar fertilization [17]. Organic acids were applied to the upper and lower surfaces of *L. olgensis* leaves using a sprinkling can until the leaf surfaces were uniformly wet, and liquid drops fell from the leaf surfaces. Application to upper and lower surfaces further enhances uptake as solutions mainly enter into the mesophyll cells of plant leaves through the pores and many more pores usually lie in the lower surface of leaves [18]. During the root irrigation and foliar spraying, Control and T1 were both treated with the same amount of daily organic acid-free watering. Organic acid treatments were applied once daily at 8:00 AM, seven days a week. At 10, 20, and 30 days after organic acid treatments, sampling and analysis of seedlings were conducted by harvesting 10 pots per treatment.

Table 2. The experimental layout. Each of the treatments below was sampled by complete harvest after 10, 20, or 30 days.

Treatment	Ck	T1	T2	T3	T4	T5	T6	T7	T8	T9
Soil	A_1 [a]	A_1:B [b]	A_1:B	A_1:B	A_1:B	A_1:B	A_1:B	A_1:B	A_1:B	A_1:B
Organic acid type	- [c]	-	OA	OA	OA	OA	CA	CA	CA	CA
Organic acid concentration ($mmol \cdot L^{-1}$)	0	0	0.2	1.0	5.0	10.0	0.2	1.0	5.0	10.0

[a] A_1 horizon; [b] A_1 and B soil mixture in proportion of 1:2; [c] without.

2.2. Measurements of Biomass

At 10, 20, and 30 days after organic acid treatments, seedling survival was measured in all pots. Thereafter, a total of 15 seedlings were carefully harvested with a shovel to avoid root damage from across the 10 pots within each treatment, washed thoroughly with running tap water, and divided into leaves, stems, and roots. The dry weights were measured after drying at 80 °C until a constant weight was reached.

2.3. Nutrient Element Analysis

Mature needles and fine roots (diameter less than 2 mm) were randomly collected from across the 10 pots within each treatment, washed with de-ionized water, dried, de-enzymed for 15 min at 105 °C, and dried to constant weight at 70 °C. The samples were then powdered and microwave digested prior to determination of Mg, K, and Fe concentrations by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, SCIEX ELAN 6000, Perkin Elmer, Waltham, MA, USA). All measurements were performed in triplicate.

2.4. Physiological Parameter Measurements

Chlorophyll fluorescence was measured on intact needles placed in the dark for 20 min [19] using the LI-6400 XT Portable Photosynthesis System (LI-COR, Lincoln, OR, USA). From each treatment, 10 seedlings total from across the 10 pots were randomly selected and measurements were made on needles in the middle of the seedling crown. Based on F_0 (initial fluorescence) and F_m (maximal fluorescence), F_v (variable fluorescence, $F_v = F_m - F_0$) and F_v/F_m (maximal photochemical efficiency of photosystemII (PSII) in the dark) were calculated.

In addition, from the 10 pots within each treatment, mature middle needles of seedlings were randomly collected and combined (about 3.0 g totally). The samples were frozen and ground in liquid N_2, and the following parameters were immediately determined. The level of lipid peroxidation was expressed as malondialdehyde (MDA, the final product of lipid peroxidation) content and was determined as 2-thiobarbituric acid (TBA) reactive metabolites, superoxide dismutase (SOD) activity was determined using a nitro blue tetrazolium (NBT) photochemical reduction, proline content was determined by the acidic ninhydrin reaction, and total chlorophyll and carotenoid contents were determined by 80% acetone extraction-spectrophotometry. All of these assays referred to the methods of Li et al. [20], and the details were the same as Song et al. [21]. The analyses were all replicated three times.

2.5. Data Analysis

Analysis of variance for a completely randomized design used the main effects of organic acid and concentration with a repeated measure with SPSS 18.0. Although there were ten pots per organic acid (acid) × concentration of organic acid (Conc) × length of application (or Day) not all measurements were made in all ten pots; sample sizes are listed explicitly in Table 3. Also, many leaf level metrics of stress were correlated, and although all attributes were analyzed correlations are noted.

Table 3. The p values of the main effects of application period (Day), organic acid (Acid), concentration of organic acid (Conc), and their interactions on the survival rate (Survival), biomass of leaf, stem, and root (Bio_{leaf}, Bio_{stem}, and Bio_{root}, respectively), contents of Mg, K, and Fe in roots and leaves (Mg_{root}, K_{root}, Fe_{root}, Mg_{leaf}, K_{leaf}, and Fe_{leaf}, respectively), malondialdehyde (MDA), superoxide dismutase (SOD), proline, carotenoid (Car), and total chlorophyll (Chl) contents and F_v/F_m in leaves.

Effect	*n*	Day	Acid	Conc	Day × Acid	Acid × Conc	Day × Conc
Survival	10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bio_{leaf}	10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bio_{stem}	10	<0.01	0.067	<0.01	<0.01	<0.01	<0.01
Bio_{root}	10	<0.01	<0.01	<0.01	0.035	<0.01	<0.01
Mg_{root}	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
K_{root}	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe_{root}	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mg_{leaf}	3	<0.01	<0.01	<0.01	0.155	0.165	<0.01
K_{leaf}	3	<0.01	0.239	<0.01	<0.01	<0.01	<0.01
Fe_{leaf}	3	<0.01	0.137	<0.01	<0.01	<0.01	<0.01
MDA	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SOD	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Proline	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Car	3	<0.01	0.110	<0.01	<0.01	<0.01	<0.01
Chl	3	<0.01	0.359	<0.01	<0.01	<0.01	<0.01
F_v/F_m	10	0.351	<0.01	<0.01	0.069	<0.01	<0.01

3. Results

3.1. Survival, Biomass, and Nutrient Element Contents of *L. olgensis* Seedlings

Organic acid (citric or oxalic) type, concentration, and application period (Day) all had an important influence on most of the parameters in this study, including survival rate, biomass of different parts of seedlings, and nutrient contents (Table 3). The survival rate of *L. olgensis* decreased over the 30 days in all treatments. In comparing the nutrient sufficient control (i.e., A_1 soil without organic acid inputs) with the T1 treatment (A_1:B soil without organic acid inputs) the survival rate decreased slightly and non-significantly ($p > 0.05$) by 0.76, 0.77, and 0.41% at 10, 20, and 30 days, respectively. Compared with the T1 treatment, the survival rate increased with organic acid application except in the 0.2 mmol·L^{-1} organic acid treatments at 10 and 20 days. The greatest increases in survival were at 5.0 mmol·L^{-1} organic acid (Figure 1; Table S1).

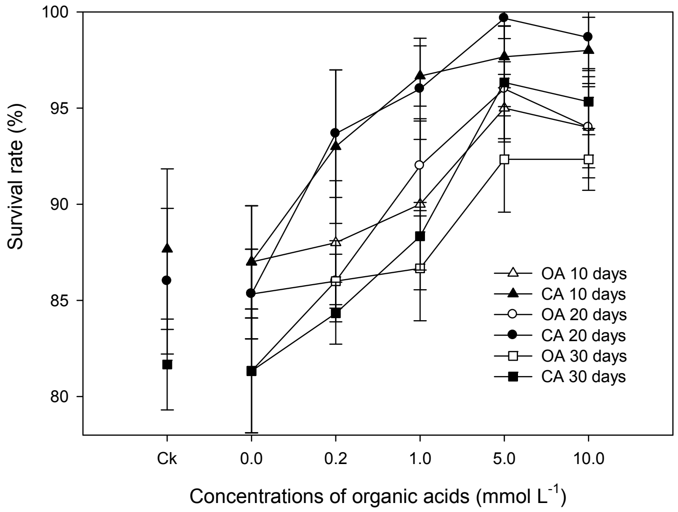

Figure 1. Survival rates of *L. olgensis* seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods. Ck is a check treatment of seedlings grown in nutrient rich soil without organic acid treatment. Values are means ± 1SD of 10 replications.

Compared with the nutrient sufficient control, the root, leaf, and stem dry biomasses decreased in T1. At 10 or 20 days the decrease was slight and non-significantly ($p > 0.05$), except for stem biomass that had declined at 20 days, but all declines were significant at 30 days. Under OA and CA treatments, most biomass components increased, although there were exceptions (e.g., stem and root biomass of 0.2 mmol·L^{-1} OA at 20 days or stem biomasses of 0.2, 1.0, and 5.0 mmol·L^{-1} CA at 30 days). The increases were greatest at 10.0 mmol·L^{-1} organic acid (Figure 2; Table S2). Compared with the T1 treatment, most of the organic acid treatments had significantly greater biomass and there was a significant acid × concentration interaction as the magnitude of differences varied with concentration. For example, the biomass increases in response to CA were generally greater than those of OA at most concentrations (Figure 2; Table S2).

Nutrient concentration also varied with treatment. Compared with the nutrient sufficient control (Ck: A_1 soil only) the A_1:B without acid treatment (T1) often had lower K concentrations for roots and leaves, while Fe contents were greater. The Mg contents in roots and leaves were not significantly different, though the values were often lower. With acid additions in the A_1:B soil mix, most root concentrations of Mg and K declined and Fe contents increased with effects of acid type and concentration being significant (except Fe and Mg concentrations of 0.2 mmol·L^{-1} CA at 20 days was not significant). In the case of leaves, the response in Mg concentrations was consistent with roots. For K and Fe concentrations, although patterns were similar, the effect of acid type on concentrations was not significant. Furthermore, although the effect of organic acid concentration on leaf K and Fe was significant, the impact on leaf Mg was not a strongly decreasing trend as observed in roots (Figure 3; Table S3).

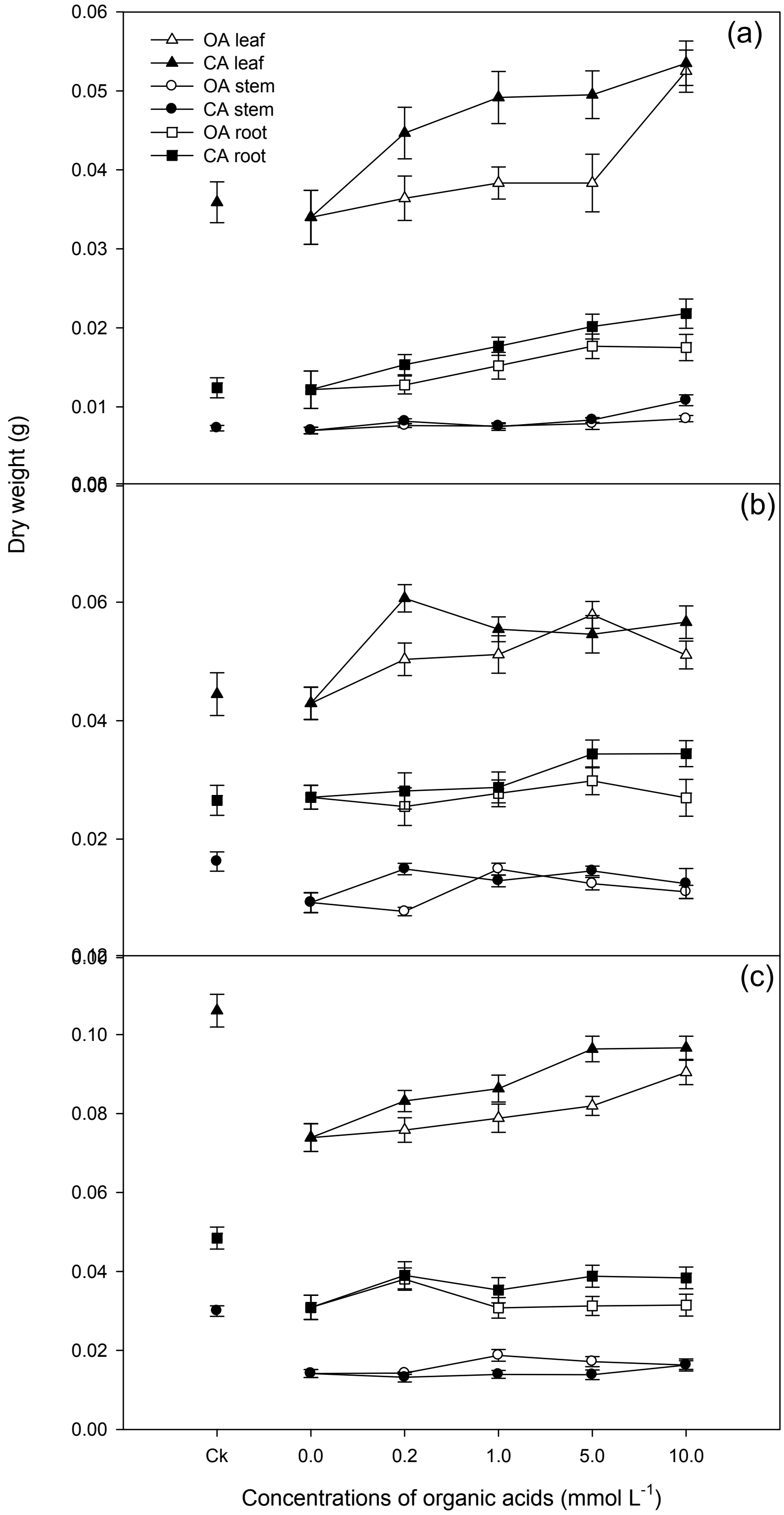

Figure 2. Dry weights of different parts of *L. olgensis* seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations at 10 days (**a**); 20 days (**b**) and 30 days (**c**). Ck is a check treatment of seedlings grown in nutrient rich soil without organic acid treatment. Values are means ± 1SD of 10 replications.

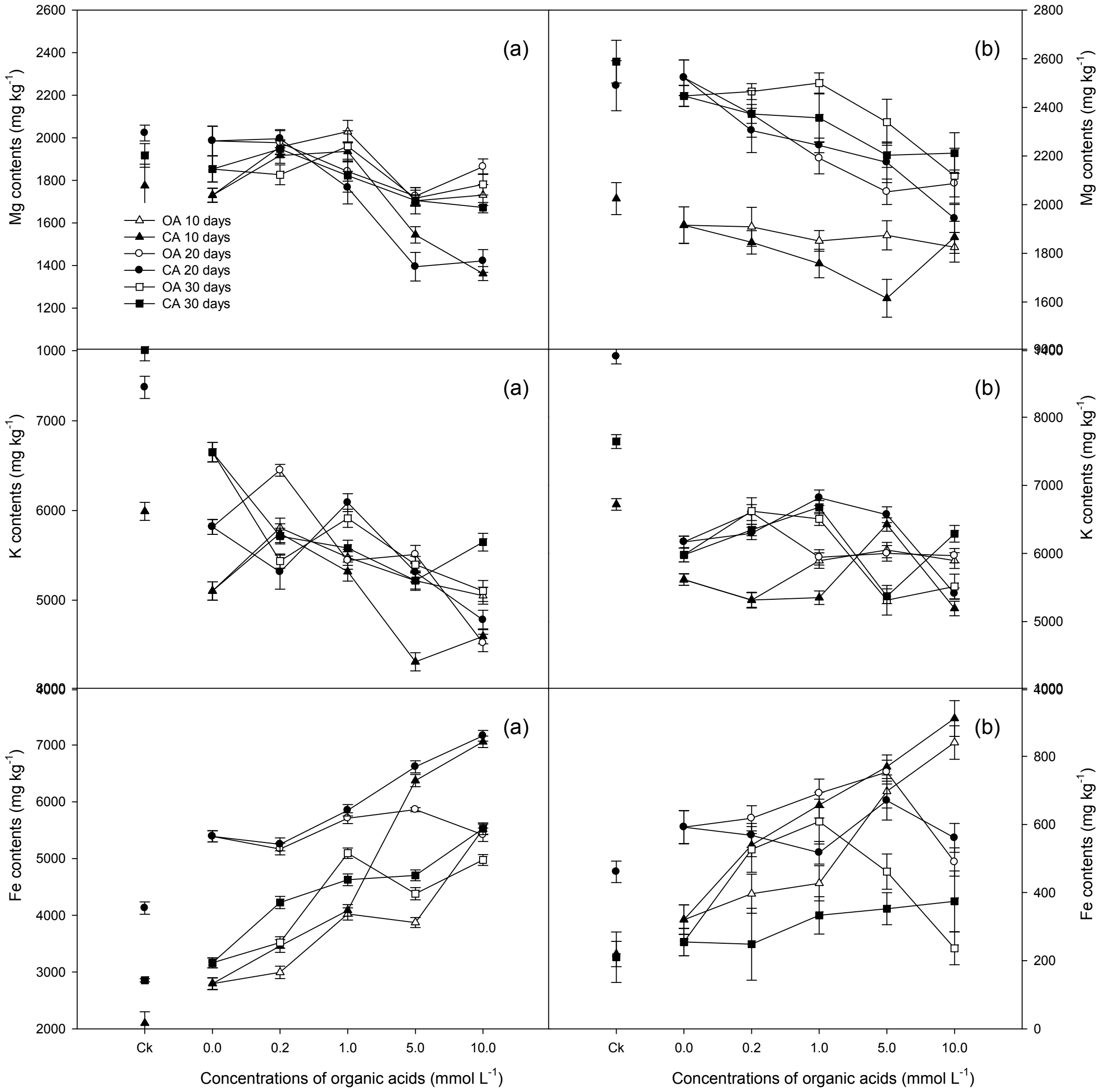

Figure 3. Mg, K, and Fe contents in roots (**a**) and leaves (**b**) of *L. olgensis* seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods. Ck is a check treatment of seedlings grown in nutrient rich soil without organic acid treatment. Values are mean ± 1SD of three replications.

3.2. Lipid Peroxidation

The MDA contents in nutrient deficient *L. olgensis* leaves (T1) were higher than those of the control (Ck), and the difference increased with Day. With organic acid treatments, the MDA concentration decreased, and most of the concentrations were below Ck. The most prominent effect was observed at 10.0 mmol·L^{-1} organic acid, the effects of CA were stronger than that of OA, and the order was 20 > 10 > 30 days. After organic acid treatments most MDA contents at 30 days were higher than 20 days, and much higher than 10 days (Figure 4; Table S4).

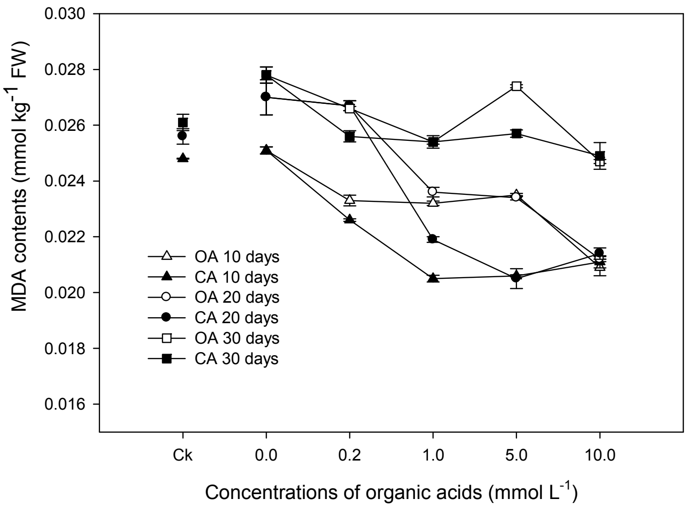

Figure 4. MDA contents of *L. olgensis* leaves for seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods. Values are mean ± 1SD of three replications.

3.3. Activities of SOD

Compared with Ck, the SOD activities in T1 decreased slightly, and the decrease was enhanced with application period. OA and CA treatments at 10, 20, and 30 days generally increased the SOD activities, and the biggest increases were found at 0.2, 10.0, and 1.0 mmol·L^{-1} OA or 5.0, 5.0, and 0.2 mmol·L^{-1} CA at 10, 20, and 30 days, respectively. There were exceptions to this general increase (e.g., 10.0 mmol·L^{-1} CA at 10 days, 5.0 mmol·L^{-1} OA and 10.0 mmol·L^{-1} CA at 20 days, 10.0 mmol·L^{-1} OA and 1.0 and 10.0 mmol·L^{-1} CA at 30 days). Among the treatments with increasing SOD activities, all except 1.0 mmol·L^{-1} OA at 10 days displayed significant increases. Although Day was significant, there was no obvious regular increase from day 10 to 20 or 30 days. Compared with the T1 treatment, CA and OA were both equally effective at most concentrations. After organic acid treatments all SOD activities at 30 days were higher than 20 days (Figure 5; Table S4).

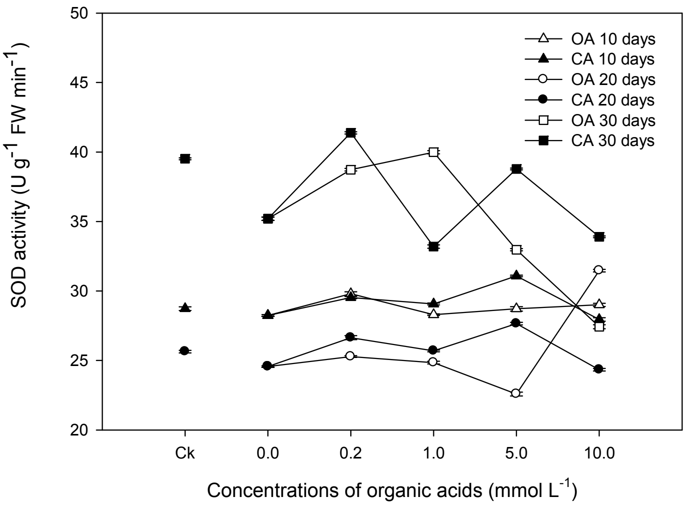

Figure 5. SOD activities of *L. olgensis* leaves for seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods. Values are mean ± 1SD of three replications.

3.4. Proline Contents

Compared with the control (Ck), the T1 treatment decreased the proline contents by 3.85, 4.15, and 5.34% at 10, 20, and 30 days, respectively. Compared with T1, the proline contents were higher in organic acid-treated leaves at 10, 20, and 30 days (Figure 6; Table S4). There were some exceptions with decreases at 10 days (5.0 mmol·L^{-1} OA), 20 days (1.0 mmol·L^{-1} OA), and 30 days (1.0 and 5.0 mmol·L^{-1} OA). Proline contents were greatest with 1.0, 10.0, and 10.0 mmol·L^{-1} OA or 5.0, 10.0, and 5.0 mmol·L^{-1} CA treatments at 10, 20, and 30 days, respectively. Among the treatments with increasing proline contents, the increases were significant; with OA increases were greatest at 20 > 10 > 30 days, and for CA the increases were greatest at 20 > 30 > 10 days (Figure 6; Table S4).

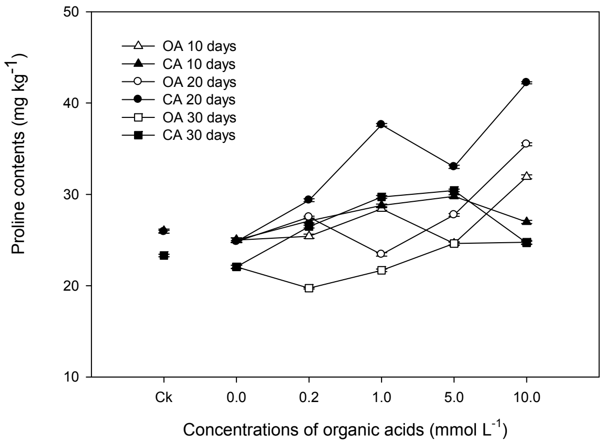

Figure 6. Proline contents of *L. olgensis* leaves for seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods. Values are mean ± 1SD of three replications.

3.5. Photosynthetic Pigment Contents and Chlorophyll Fluorescence

In comparing Ck to the T1 treatment, the total chlorophyll and carotenoid contents in *L. olgensis* leaves were not significantly different, but their values all decreased across all days (Table 4). Within the nutrient deficient soil (A_1:B) acid type did not significantly affect total chlorophyll or carotenoids. Acid concentration and day, however, were significant and the peak values were at 10.0, 5.0, and 10.0 mmol·L^{-1} organic acids at 10, 20, and 30 days, respectively. Nutrient deficiency also decreased F_v/F_m ratios in leaves, however, they increased significantly under organic acid treatments with effects of acid type and concentration being significant, and the peak values were generally found at 10.0 mmol·L^{-1} (Figure 7; Table S4).

Table 4. Changes in total chlorophyll and carotenoid contents (mg·g^{-1} FW) in *L. olgensis* leaves treated by root irrigation and foliar spraying with oxalic (OA) or citric (CA) acid of different concentrations and different application periods.

Treatment (mmol·L^{-1})	10 Days		20 Days		30 Days	
	Total Chl ^{a}	Car ^{b}	Total Chl	Car	Total Chl	Car
Ck	0.834 ± 0.033 ^{b}	0.145 ± 0.006 ^{abc}	1.017 ± 0.163 ^{ab}	0.168 ± 0.024 ^{bc}	1.192 ± 0.144 ^{f}	0.158 ± 0.023 ^{cd}
A_1:B + acid 0	0.798 ± 0.008 ^{a}	0.139 ± 0.008 ^{ab}	0.96 ± 0.188 ^{a}	0.157 ± 0.03 ^{ab}	1.085 ± 0.271 ^{d}	0.146 ± 0.04 ^{abc}
A_1:B + OA 0.2	0.904 ± 0.047 ^{cd}	0.14 ± 0.015 ^{ab}	0.962 ± 0.185 ^{a}	0.152 ± 0.034 ^{a}	1.038 ± 0.009 ^{c}	0.139 ± 0.003 ^{a}
A_1:B + OA 1.0	0.877 ± 0.228 ^{c}	0.152 ± 0.034 ^{bcd}	1.124 ± 0.131 ^{c}	0.166 ± 0.027 ^{b}	1.21 ± 0.165 ^{f}	0.161 ± 0.03 ^{de}
A_1:B + OA 5.0	0.803 ± 0.05 ^{a}	0.133 ± 0.01 ^{a}	1.112 ± 0.139 ^{c}	0.166 ± 0.019 ^{b}	1.166 ± 0.311 ^{e}	0.172 ± 0.047 ^{e}
A_1:B + OA 10.0	0.835 ± 0.037 ^{b}	0.148 ± 0.003 ^{bcd}	0.962 ± 0.046 ^{a}	0.147 ± 0.015 ^{a}	1.361 ± 0.335 ^{g}	0.206 ± 0.046 ^{f}
A_1:B + CA 0.2	0.922 ± 0.12 ^{d}	0.158 ± 0.019 ^{cd}	1.045 ± 0.201 ^{b}	0.179 ± 0.032 ^{cd}	0.924 ± 0.041 ^{a}	0.138 ± 0.002 ^{a}
A_1:B + CA 1.0	0.917 ± 0.074 ^{d}	0.161 ± 0.008 ^{d}	1.212 ± 0.148 ^{d}	0.191 ± 0.023 ^{d}	1.09 ± 0.133 ^{d}	0.155 ± 0.023 ^{bcd}
A_1:B + CA 5.0	0.88 ± 0.071 ^{c}	0.157 ± 0.011 ^{cd}	1.348 ± 0.18 ^{e}	0.184 ± 0.028 ^{d}	0.934 ± 0.232 ^{ab}	0.135 ± 0.038 ^{a}
A_1:B + CA 10.0	0.894 ± 0.062 ^{cd}	0.16 ± 0.009 ^{d}	1.109 ± 0.088 ^{c}	0.155 ± 0.017 ^{ab}	0.96 ± 0.136 ^{b}	0.143 ± 0.022 ^{ab}

[a] Chl: Chlorophyll; [b] Car: Carotenoid. Ck is a check treatment of seedlings grown in nutrient rich soil without organic acid treatment. Values followed by the same letter for the same parameter are not statistically different at $p < 0.05$ by ANOVA (DUNCAN).

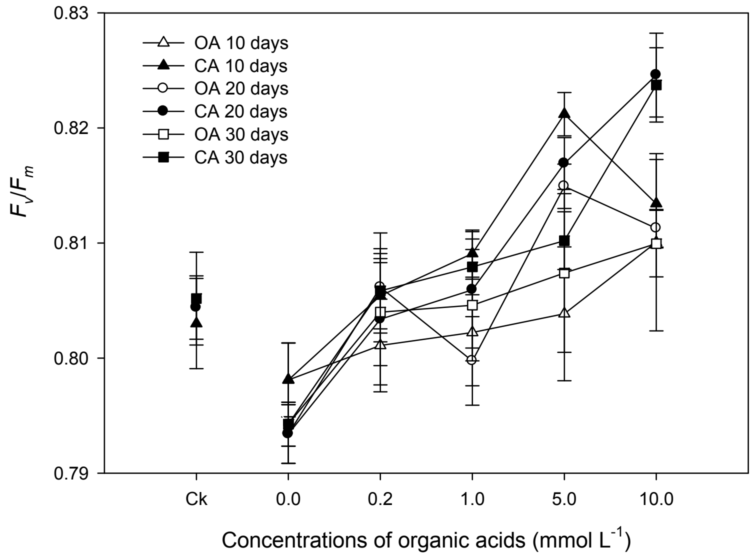

Figure 7. F_v/F_m ratios of *L. olgensis* leaves for seedlings grown in low nutrient soils treated by root irrigation and foliar spraying with different concentrations of organic acids and different application periods. Values are means ± 1SD of 10 replications.

4. Discussion

Tree growth under natural conditions can be usually restricted by limited availability of nutrients in soils and it is well-recognized that applying fertilizers (e.g., N or P) to soils can promote growth [22]. It is less well-known that applications of organic acids can also significantly promote seed germination, aboveground and belowground biomass accumulation, and plant growth and development. The beneficial role of organic acids for plants under natural conditions or under cold stress, water stress, or salinity stress has been established in *Picea abies*, cucumber, durum wheat, and cabbage treated with salicylic acid (SA) and other organic acids [6,23,24]. Plant height, leaf length, leaf width, and leaf area all increased in these studies, and the effects vary with the kind and concentration of organic acids, plant species, and plant stress. The current study builds on these previous organic acid studies but applies organic acids to *L. olgensis* seedlings, a critical tree crop for reforesting nutrient deficient soils in northeast China. Overall, *L. olgensis* seedling responses were consistent with previous results suggesting that seedling stress declined in the presence of organic acids. In this study, CA was more effective than OA in reducing stress under nutrient deficiency and higher concentrations (i.e., 5 to 10 mmol·L^{-1}) were most effective (Figures 1–7; Table 4).

MDA, SOD, and proline can be considered as major parameters of physiological plant stress, which is used to indicate the metabolic activities and resistance of plants to adverse environments. Lipid peroxidation (demonstrated by MDA content) is generally an indicator of oxidative injury in stressed plants [25,26]. MDA can crosslink with substances such as proteins, nucleic acids, or amino acids to form insoluble compounds (lipofuscin) that interfere in normal cell activities [27]. Various environmental stresses can induce MDA accumulation, and in severe cases this can lead to plant death. Previous research has shown increasing MDA concentrations in *Lupinus albus* with P deficiency [28]. This study, using low nutrient soil, found decreasing MDA concentrations of *L. olgensis* leaves (Figure 4). These lower MDA concentrations suggest exogenous organic acids are involved in protecting the cell membrane of *L. olgensis* seedlings against oxidative stress.

SOD is one of the most effective antioxidant enzymes scavenging oxygen radicals in plants [29] and can convert superoxide radicals into less toxic agents [30]. Furthermore, the concentration of SOD activity is directly related to the degree of cell damage and plant resistance [31]. SOD responses to soil stress have been observed. For example, SOD activities of *Avicennia marina* (a common mangrove species in South China) were decreased by Pb stress [32] and the activities of SOD decreased in Cd-treated *L. olgensis* leaves, possibly due to the binding of Cd to the thiol groups of the enzymes [21]. Previous studies have shown that spraying SA and other organic acids on tobacco [33], rice (*Oryza sativa*) [34], or perennial ryegrass [35] plants increases SOD activity. Similar responses in SOD activity were observed in maize leaves at six and then days after 1.0 mmol·L^{-1} SA treatment, where the copper couple superoxide dismutase (Sod4) transcription increased [36]. In this study the lower nutrient soil (A_1:B) resulted in a decrease of SOD activities that increased with application period. OA and CA treatments increased SOD, especially CA (Figure 5). This SOD increase likely contributed to the removal of reactive oxygen species such as MDA (Figure 4).

Proline is one of the most effective substances controlling osmotic adjustment [37] and also plays a protective role in protein stability. The use of proline as an osmolyte was first reported by Cavalieri and Huang [38] in *Juncus roemerianus*, and since has been shown to be a common compound accumulated by plants as a response to salt, water, or cold stress [39]. Under soil stress, the proline contents in plant tissues are greatly increased in order to improve plant resistance [29,40]. This study found that proline contents of *L. olgensis* leaves in the A_1:B soil were lower than those of Ck, which suggested that nutrient stress inhibited proline synthesis and disturbed their metabolisms. In A_1:B soil proline concentrations increased after most organic acid treatments (Figure 6). This increase suggests that organic acids may have induced proline synthesis and accumulation, thereby enhancing the ability of *L. olgensis* seedlings in osmotic regulation.

In contrast to the increases in proline contents and SOD activities noted above, there were a few concentrations of organic acids that resulted in contents or activities below the levels of the T1 control (Figures 5 and 6). These observations might suggest that low concentrations of organic acids can have beneficial effects while high concentrations may have some harmful effects [41]. A possible explanation is that the initial enhanced growth from high organic acid treatments leads to increased nutrient demand and stress, which eventually surpasses the benefits of the acid treatments in relieving stress. In other words, the beneficial effects of organic acids failed to compensate for the growth induced nutrient stress.

In addition to indicators of stress, photosynthetic pigments also can be viewed as an adaptive response to adverse environments. Chlorophyll is highly sensitive to stress and indicates the resistance of plants to stressed environments [41]. Environmental stress may harm the chloroplast structure of plants, inhibiting chlorophyll synthesis [35]. Carotenoid, a cell endogenous antioxidant, also plays an important role in scavenging reactive oxygen species and preventing membrane lipid peroxidation [40]. Studies have shown that organic acids can increase the pigment contents of plants growing in both normal and stressed environments. Pigment increases in normal environments have been reported for malic acid, CA, tartaric acid, lactic acid, and SA across many plant species, including cabbage, tobacco, and others [8]. Under adverse environments, one example of positive response is SA treatments for three and five days that increased total chlorophyll contents of maize seedlings under osmotic stress by 7.5 and 45.3%, respectively [42]. In the current study, results were similar with organic acid treatments increasing both pigments, although there was no difference between the acids (Table 4).

Chlorophyll fluorescence measures the absorption, transfer, consumption, and distribution of light energy by the optical system in the process of photosynthesis, and can be used to evaluate relative photosynthetic performance of plants [43]. F_v/F_m (described in the methods) is a sensitive metric of stress in plants and plant tolerance to environmental factors [44]. Decreases in F_v/F_m indicate photoinhibition or photooxidation, which can be caused by photodamage to PSII in response to environmental stresses [45]. Significant reductions in the F_v/F_m ratio have been found in Mn-treated 'Kothreiki' and 'FS-17' olive cultivars [46], and in *Caulerpa lentillifera* in response to high salinity [47]. In *C. lentillifera*, F_v/F_m was >0.80, indicating the plant was healthy (<0.80 indicated stress, and values <0.70 resulted in death) [47]. The reduction of foliar F_v/F_m also has been reported in strawberry [48], red spruce, and balsam fir [49], and Norway spruce [50] and radiata pine [45] in response to low nutrient supply or an imbalance in nutrients. In this study the F_v/F_m ratio declined in *L. olgensis* leaves in low nutrient soils but was increased with organic acid treatments (Figure 7). In this study, changes in F_v/F_m were caused by an increase of F_0 and at high acid concentrations some seedlings had ratios close to or higher than those of healthy leaves, indicating that the seedlings no longer suffered damages from nutrient deficiency.

There are several possible mechanisms for how organic acids in low nutrient soils may be reducing stress and promoting plant growth, although all cannot be definitively addressed in this study. (1) Organic acids could promote root growth or uptake activity, thus indirectly promoting aboveground plant growth [51]; (2) Organic acids could effectively enhance chlorophyll content and photosynthesis rates in plants, which would not only promote the accumulation of organic matter, but also facilitate the transportation of water and inorganic salts, and thus indirectly promote plant growth [52]; (3) Organic acid injection into soils could significantly improve the solubilization and availability of soil nutrients (e.g., P, Fe, K), and promote plant absorption and transportation of nutrients [53]. Root biomass of the *L. olgensis* seedlings did increase with acid treatment and was associated with greater aboveground growth. It is not clear, however, if root growth was the cause of aboveground growth or was itself a response to some other mechanism induced by the organic acids.

As to the mechanism of organic acids to increase chlorophyll contents, it is clear that the formation of chlorophyll is affected by soil nutrient status. Mg is an essential element composing chlorophyll while Fe and Zn may be catalysts to certain enzymes during chlorophyll formation. In this study, Mg concentrations in roots and leaves declined with acid concentration but Fe concentrations increased (Figure 3), as did chlorophyll contents (Table 4). This suggests that the changes in chlorophyll contents are more responsive to Fe concentration increases, which may reflect a greater percent increase (i.e., (sampling date − initial)/initial concentration) in Fe concentration (0.07%–1.52%, 0.01%–0.33%, and 0.11%–0.75% at 10, 20, and 30 days, respectively) being greater than the declining range of Mg concentrations (0.02%–0.21%, 0.00%–0.28%, and 0.01%–0.10% at 10, 20, and 30 days, respectively).

Finally, organic acids can significantly increase the availability of some mineral elements including Fe, Zn, or Mg in the soil by mechanisms of acidification, chelation, redox reactions, or inhibition of adsorption to minerals [54]. In the case of organic acids the release of nutrients from Haplic Cambisols is mainly by the mechanism of complexation. The complexation ability of CA is greater than that of OA [55], and the dissociation constant of CA (pKa 3.14) is also greater than that of OA (pKa 1.23). As such, CA should be able to release more nutrients from these soils [56] and, in fact, the response in stress indicators is generally greater to CA than OA.

In an earlier study, Song et al. [56] showed that OA and CA could mobilize P and Fe from Haplic Cambisols, especially the A_1 horizon used here, and promote their absorption and transportation by *L. olgensis* seedlings. P accumulation and uptake efficiency in *L. olgensis* fine roots and leaves were both increased [55]. In the current study, OA and CA increased Fe contents of *L. olgensis* roots and leaves (Figure 3). So from the perspective of plant nutrient supply, exogenous OA and CA could contribute to P and Fe absorption and accumulation in seedlings, and indirectly increase plant biomass. For improving nutrient release from the study soils in northeast China and increasing the subsequent P accumulation and P uptake efficiency in *L. olgensis* seedlings, CA > OA [55], a result consistent with the increase in biomass accumulation observed in this study (Figure 2).

Although the exact mechanism of organic acid treatment response is difficult to infer, this study clearly demonstrates the beneficial value of organic acids for reducing plant stress. Furthermore, since *L. olgensis* seedlings are planted in local forest soils rather than nutrient solution or hydroponic conditions, this study is closer to the plant growth media in an operational condition, and thus has practical application value for future nursery or reforestation undertakings in the region.

5. Conclusions

Soil nutrient deficiency had harmful effects on measures of plant stress in *L. olgensis* leaves. Survival rate and root, leaf, and stem biomass accumulation were all inhibited, MDA contents increased, and SOD activities, proline, total chlorophyll and carotenoid contents, and F_v/F_m ratios all decreased. These changes generally increased with the length of the application period. Nutrient deficiency decreased K concentrations in roots and leaves, while Fe contents were greater. The Mg contents in roots and leaves were not significantly different, although the values were often lower.

Compared with the nutrient deficiency treatment T1 (i.e., A_1:B soil with no organic acid inputs) the application of organic acids reversed the measures of plant stress in *L. olgensis* leaves. Organic acid (citric or oxalic) type, concentration, and application period all had an important influence on most of the parameters in this study. The most prominent effects were observed at 20 days under the 5.0 or 10.0 $mmol \cdot L^{-1}$ treatments, and CA was more effective than OA. Organic acids increase survival rate and biomass accumulation of root, leaf, and stem, and MDA, SOD, proline, chlorophyll, and carotenoid measures of plant condition all improve. Hence, exogenous organic acids alleviate the damage of nutrient deficiency to *L. olgensis* seedlings, and improve their resistance to nutrient deficiency.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/7/10/213/s1, Table S1: Statistical difference results of survival rates of *L. olgensis* seedlings induced by root irrigation and foliar spraying with different concentrations of organic acids and different application periods; Table S2: Statistical difference results of dry weights of different parts of *L. olgensis* seedlings induced by root irrigation and foliar spraying with different concentrations of organic acids and different application periods; Table S3: Statistical difference results of Mg, K and Fe contents in roots and leaves of *L. olgensis* seedlings induced by root irrigation and foliar spraying with different concentrations of organic acids and different application periods. Table S4: Statistical difference results of MDA contents, SOD activities, proline contents and *Fv/Fm* ratios of *L. olgensis* leaves for seedlings induced by root irrigation and foliar spraying with different concentrations of organic acids and different application periods.

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References

1. Li, Z.M.; Song, Z.L.; Parr, J.F.; Wang, H.L. Occluded C in rice phytoliths: Implications to biogeochemical carbon sequestration. *Plant Soil* **2013**, *370*, 615–623. [CrossRef]
2. Wu, J.S.; Xu, Q.F.; Jiang, P.K.; Cao, Z.H. Dynamics and distribution of nutrition elements in bamboos. *J. Plant Nutr.* **2009**, *32*, 489–501. [CrossRef]
3. Xie, J.S.; Shao, C.H.; Tang, X.Y.; Shi, Q.H. Proteomics analysis of nutrient stress effects on rice leaf senescence at grain filling stage. *Chin. J. Rice Sci.* **2011**, *25*, 143–149. (In Chinese)
4. Pizzeghello, D.; Zanella, A.; Carletti, P.; Nardi, S. Chemical and biological characterization of dissolved organic matter from silver fir and beech forest soils. *Chemosphere* **2006**, *65*, 190–200. [CrossRef] [PubMed]
5. Kpomblekou-A, K.; Tabatabai, M.A. Effect of low-molecular weight organic acids on phosphorus release and phytoavailabilty of phosphorus in phosphate rocks added to soils. *Agric. Ecosyst. Environ.* **2003**, *100*, 275–284. [CrossRef]
6. Kenji, M.; Yasuomi, T. Regulation of water, salinity, and cold stress responses by salicylic acid. *Front. Plant Sci.* **2014**. [CrossRef]
7. Mu, X.L.; Hu, X.L. Effects of salicylic acid on germination of cotton seeds and bud. *Cotton China* **2003**, *30*, 19–21. (In Chinese)
8. Wu, X.P.; Liu, G.S.; Zhu, K.; Yang, C.; Jing, Y.F. Effect of applying organic acids on tobacco physiology and the leaf chemical components. *Chin. Tob. Sci.* **2003**, *9*, 23–27. (In Chinese)
9. Shen, H.; Yan, X.L.; Zhao, M.; Zheng, S.L.; Wang, X.R. Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. *Environ. Exp. Bot.* **2001**, *48*, 1–9. [CrossRef]
10. Liu, Y.; Mi, G.H.; Chen, F.J.; Zhang, J.H.; Zhang, F.S. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Sci.* **2004**, *167*, 217–223. [CrossRef]
11. Ryan, P.R.; Elhaize, E.D.; Randall, P.J. Malate efflux from rot apices: Evidence for a general mechanism of Al-tolerance in wheat. *Aust. J. Plant Physiol.* **1995**, *22*, 531–536. [CrossRef]

12. Ashraf, M.; Iram, A. Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora* **2005**, *200*, 535–546. [CrossRef]
13. Song, J.F.; Cui, X.Y. Analysis of organic acids in selected forest litters of northeast China. *J. For. Res.* **2003**, *14*, 285–289.
14. Cooperative Research Group on Chinese Soil Taxonomy. In *Chinese Soil Taxonomy*; Science Press: Beijing, China; New York, NY, USA, 2001.
15. ISSS/ISRIC/FAO. *World Reference Base for Soil Resources*; World Soil Resource Reports 103; FAO: Rome, Italy, 2006.
16. Wang, Z.Q.; Guo, D.L.; Wang, X.R.; Gu, J.C.; Mei, L. Fine root architecture, morphology and biomass, of different branch orders of two Chinese temperate tree species. *Plant Soil* **2006**, *288*, 155–171. [CrossRef]
17. Pang, X.Q.; Cui, X.J.; Sun, Z.F.; Qi, Z.X. Application of Foliar fertilizer Potassium dihydrogen phosphate in culture of larch seedlings. *Pract. For. Technol.* **2010**, *3*, 22. (In Chinese)
18. Xiao, Y.; Tang, Y.K.; Cao, Y.P.; Wang, J.G. Application of surfactants in fertilizer for foliage dressing and its progress. *Phosphate Compd. Fertil.* **2003**, *18*, 14–16. (In Chinese)
19. Franco, A.C.; Herzog, B.; Hűbner, C.; De Mattos, E.A.; Scarano, F.R.; Ball, E.; Lűttge, U. Diurnal changes in chlorophyll a fluorescence, CO_2-exchange and organic acid decarboxylation in the tropical CAM tree *Clusia hilariana*. *Tree Physiol.* **1999**, *19*, 635–644. [CrossRef] [PubMed]
20. Li, H.S.; Sun, Q.; Zhao, S.J.; Zhang, W.H. *Principles and Techniques of Plant Physiological Biochemical Experiment*; Higher Education Press: Beijing, China, 2000; pp. 134–263. (In Chinese)
21. Song, J.F.; Ma, R.; Huang, W.B.; Yang, D.; Cui, X.Y. Exogenous organic acids protect Changbai Larch (*Larix olgensis*) seedlings against cadmium toxicity. *Fresenius Environ. Bull.* **2014**, *23*, 3459–3467.
22. Chris, K.L.; Fox, T.R.; Carlson, C.A. Foliage and litter chemistry, decomposition, and nutrient release in *Pinus taeda*. *Forests* **2013**, *4*, 595–612.
23. Panfili, F.; Schneider, A.; Vives, A.; Perrot, F.; Hubert, P.; Pellerin, S. Cadmium uptake by durum wheat in presence of citrate. *Plant Soil* **2009**, *316*, 299–309. [CrossRef]
24. Krajnc, A.U.; Kristl, J.; Ivancic, A. Application of salicylic acid induces antioxidant defense responses in the phloem of *Picea abies* and inhibits colonization by *Ips typographus*. *For. Ecol. Manag.* **2011**, *261*, 416–426. [CrossRef]
25. Sandalio, L.M.; Dalurzo, H.C.; Gomez, M.; Romero-Puertas, M.C.; DelRio, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **2001**, *52*, 2115–2126. [PubMed]
26. Belkhadi, A.; Hediji, H.; Abbes, Z.; Nouairi, I.; Barhoumi, Z.; Zarrouk, M.; Chaïbi, W.; Djebali, W. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1004–1011. [CrossRef] [PubMed]
27. Elastner, E.F. Oxygen activation and oxygen toxicity. *Ann. Rev. Plant Physiol.* **1982**, *33*, 73. [CrossRef]
28. Sas, L.; Rengel, Z.; Tang, C. Excess cation uptake, and extrusion of protons and organic acid anions by *Lupinus albus* under phosphorus deficiency. *Plant Sci.* **2001**, *160*, 1191–1198. [CrossRef]
29. Shakoor, M.B.; Ali, S.; Hameed, A.; Farid, M.; Hussain, S.; Yasmeen, T.; Najeeb, U.; Bharwana, S.A.; Abbasi, G.H. Citric acid improves lead (pb) phytoextraction in *Brassica napus* L. by mitigating pb-induced morphological and biochemical damages. *Ecotoxicol. Environ. Saf.* **2014**, *109*, 38–47. [CrossRef] [PubMed]
30. Fernández, R.; Bertrand, A.; Reis, R.; Mourato, M.P.; Martins, L.L.; González, A. Growth and physiological responses to cadmium stress of two populations of *Dittrichia viscosa* (L.) Greuter. *J. Hazard. Mater.* **2013**, *244*, 555–562. [CrossRef] [PubMed]
31. Qiao, X.Q.; Shi, G.X.; Jia, R.; Chen, L.; Tian, X.L.; Xu, J. Physiological and biochemical responses induced by lead stress in *Spirodela polyrhiza*. *Plant Growth Regul.* **2012**, *67*, 217–225. [CrossRef]
32. Zhong, Z.Y.; Lin, K.; Nora, F.Y.T. Lead stress in seedlings of *Avicennia marina*, a common mangrove species in South China, with and without cotyledons. *Aquat. Bot.* **2010**, *92*, 112–118.
33. Fodor, J.; Gullner, G.; Adam, A.L.; Barna, B.; Komives, T.; Kiraly, Z. Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid in tobacco. *Plant Physiol.* **1997**, *114*, 1443–1451. [PubMed]
34. Singh, I.; Shah, K. Evidences for suppression of cadmium induced oxidative stress in presence of sulphosalicylic acid in rice seedlings. *Plant Growth Regul.* **2015**, *76*, 99–110. [CrossRef]
35. Guo, H.; Hong, C.; Chen, X.; Xu, Y.; Liu, Y.; Jiang, D.; Zheng, B. Different growth and physiological responses to cadmium of the three *Miscanthus* species. *PLoS ONE* **2016**, *11*, e0153475. [CrossRef] [PubMed]

36. Scandalio, J.G. Molecular genetics of superoxide dismutases in plants. In *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*; Scandalios, J.G., Ed.; Cold Spring Harbor Laboratory Press: New York, NY, USA, 1997; pp. 545–546.
37. Xu, W.H.; Li, Y.R.; He, J.P.; Ma, Q.F.; Zhang, X.J.; Chen, G.Q.; Wang, H.X.; Zhang, H.B. Cd uptake in rice cultivars treated with organic acids and EDTA. *J. Environ. Sci.* **2010**, *22*, 441–447. [CrossRef]
38. Cavalieri, A.J.; Huang, A.H.C. Evaluation of proline accumulation in the adaptation of diverse species of marsh halophytes to the saline environment. *Am. J. Bot.* **1979**, *66*, 307–312. [CrossRef]
39. Boscaiu, M.; Lull, C.; Llinares, J.; Vicente, O.; Boira, H. Proline as a biochemical marker in relation to the ecology of two halophytic *Juncus* species. *J. Plant Ecol.* **2013**, *6*, 177–186. [CrossRef]
40. Li, H.Y.; Liu, Y.G.; Zeng, G.M.; Zhou, L.; Wang, X.; Wang, Y.Q.; Wang, C.L.; Hu, X.J.; Xu, W.H. Enhanced efficiency of cadmium removal by *Boehmeria nivea* (L.) Gaud in the presence of exogenous citric and oxalic acids. *J. Environ. Sci.* **2014**, *26*, 2508–2516. [CrossRef] [PubMed]
41. Fagbenro, J.A.; Agboola, A.A. Effect of different levels of humic acid on the growth and nutrient of teak seedlings. *J. Plant Nutr.* **1993**, *16*, 1465–1483. [CrossRef]
42. Han, Y.L.; Huang, S.Z.; Yuan, H.Y.; Zhao, J.Z.; Gu, J.G. Organic acids on the growth, anatomical structure, biochemical parameters and heavy metal accumulation of *Iris lactea* var. *chinensis* seedling growing in Pb mine tailings. *Ecotoxicology* **2013**, *22*, 1033–1042. [PubMed]
43. Yang, J.P.; Xu, H.M.; Wang, W.P.; Pan, J.B.; Xiao, N. The effects of SA and osmotic stress on physiological characteristics of maize seedlings. *J. Beijing Agric. Coll.* **2003**, *18*, 7–9. (In Chinese)
44. Madrid, E.N.; Armitage, A.R.; Quigg, A. The response of photosystem II to soil salinity and nutrients in wetland plant species of the northwestern gulf of Mexico. *J. Coast. Res.* **2012**, *28*, 1197–1207. [CrossRef]
45. Xue, J.M.; Clinton, P.W.; Davis, M.R.; Siddiqui, T.; Beets, P.N.; Leckie, A.C. Genotypic variation in foliar nutrient concentrations, $\delta^{13}C$, and chlorophyll fluorescence in relation to tree growth of radiata pine clones in a serpentine soil. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 724–733. [CrossRef]
46. Chatzistathis, T.A.; Papadakis, I.E.; Therios, I.N.; Giannakoula, A.; Dimassi, K. Is chlorophyll fluorescence technique a useful tool to assess manganese deficiency and toxicity stress in olive plants? *J. Plant Nutr.* **2011**, *34*, 98–114. [CrossRef]
47. Guo, H.; Yao, J.T.; Sun, Z.M.; Duan, D.L. Effects of salinity and nutrients on the growth and chlorophyll fluorescence of *Caulerpa lentillifera*. *Chin. J. Oceanol. Limnol.* **2015**, *33*, 410–418. [CrossRef]
48. Osório, J.; Osório, M.L.; Correia, P.J.; Varennes, A.D.; Pestana, M. Chlorophyll fluorescence imaging as a tool to understand the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants. *Sci. Hortic.* **2014**, *165*, 148–155. [CrossRef]
49. Boyce, R.L. Chlorophyll fluorescence response of red spruce and balsam fir to a watershed calcium fertilization experiment in New Hampshire. *Can. J. For. Res.* **2007**, *37*, 1518–1522. [CrossRef]
50. Strand, M. Effect of mineral nutrient content on oxygen exchange and chlorophyll *a* fluorescence in needles of Norway spruce. *Tree Physiol.* **1997**, *17*, 221–230. [CrossRef] [PubMed]
51. Hu, R.; Sun, K.; Su, X.; Pan, Y.X.; Zhang, Y.F.; Wang, X.P. Physiological responses and tolerance mechanisms to Pb in two xerophils: *Salsola passerina* Bunge and *Chenopodium album* L. *J. Hazard. Mater.* **2012**, *205–206*, 131–138. [CrossRef] [PubMed]
52. Chen, G.Y.; Ye, J.Y. Effects of oxaloacetate and malate on photosynthesis in leaves and in intact chloroplasts from spinach. *Acta Phytophystol. Sin.* **2001**, *27*, 478–482. (In Chinese)
53. Palomo, L.; Claassen, N.; Jones, D.L. Differential mobilization of P in the maize rhizosphere by citric acid and potassium citrate. *Soil Biol. Biochem.* **2006**, *38*, 683–692. [CrossRef]
54. Burckhard, S.R.; Schwab, A.P.; Banks, M.K. The effects of organic acids on the leaching of heavy metals from mine tailings. *J. Hazard. Mater.* **1995**, *41*, 135–145. [CrossRef]
55. Cui, F.X.; Song, J.F.; Yang, D. Effects of exogenous organic acids on phosphorus availability of dark brown forest soils and phosphorus absorption and accumulation of *Larix olgensis* seedlings with nutrient deficiency. *J. Soil Water Conserv.* **2012**, *26*, 116–121. (In Chinese)
56. Song, J.F.; Cui, X.Y.; Wang, Z.Q. Effects of low molecular-weight organic acids on P, Fe and K availability of dark brown forest soils and absorption of forest seedlings. *J. Soil Water Conserv.* **2011**, *25*, 123–127. (In Chinese)