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REGULAR ARTICLE

Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador:

Response of nutrient status to elevated Al concentrations

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Abstract
Aims We determined the reasons why in nutrient solution increasing Al concentrations > 300 μM inhibited shoot biomass production of Cedrela odorata L., Heliocarpus americanus L., and Tabebuia chrysantha (Jacq.) G. Nicholson while 300 μM Al stimulated root biomass production of Tabebuia chrysantha.
Methods Nutrient concentrations in plant tissue after a hydroponic growth experiment were determined.
Results Increasing Al concentrations significantly decreased Mg concentrations in leaves. Phosphorus concentrations in roots of C. odorata and T. chrysantha were significantly highest in the treatment with 300 μM Al and correlated significantly with root biomass.
Conclusions Shoot biomass production was likely inhibited by reduced Mg uptake, impairing photosynthesis. The stimulation of root growth at low Al concentrations can be possibly attributed to improved P uptake.

Keywords Aluminum toxicity · Tropical forest tree seedlings · Nutrient deficiency · Growth stimulation by phosphorus

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Introduction

The soils of the tropical montane rain forests in southern Ecuador have pH values < 5.5 resulting in high Al concentrations in soil solution. Therefore, Al phytotoxicity was discussed to be a major reason for limited aboveground biomass production and for a slow nutrient cycling (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). The currently observed soil acidification in tropical montane forests of southern Ecuador because of forest fire derived acids (Boy et al. 2008) and increasing NH_4^+ deposition with subsequent nitrification and NO_3^- leaching (Wilcke et al. 2013) might enhance Al toxicity in future.

A hydroponic Al-toxicity experiment with three selected native tree species from southern Ecuador

showed a negative impact of Al concentrations > 300 μM in nutrient solution on shoot biomass production, healthy leaf area and root properties of Cedrela odorata L., Heliocarpus americanus L., and Tabebuia chrysantha (Jacq.) G. Nicholson. However, these concentrations were higher than usual concentrations in organic layer leachate (< 60 μM) (Rehmus et al. 2014). Surprisingly, the root biomass production was not (C. odorata and T. chrysantha) or only marginally affected (H. americanus) by elevated Al concentrations, and therefore root to shoot biomass ratios increased in response to elevated Al concentrations. Increasing TOC concentrations with increasing Al concentrations in the nutrient solutions after the treatments nevertheless suggested a release of organic acids as a defence mechanism against Al toxicity. Because Ca:Al molar ratios in leaves and roots did not fall below reported threshold values for Al toxicity (< 12.5 in leaves and < 0.2 in fine roots, Cronan and Grigal 1995), the Ca-Al antagonism is unlikely to be a reason for the observed toxicity effects, pointing at the interaction between Al and other nutrients.

Aluminum was reported to reduce concentrations of P and K in Al-sensitive plants (Graham 2001; Osaki et al. 1997; Thornton et al. 1987). Reduced uptake and translocation of Mg was also observed in various studies (Graham 2001; Keltjens 1995; Kinraide 2003; Osaki et al. 1997; Thornton et al. 1987). As the central ion of the chlorophyll molecule, Mg has a crucial role in photosynthesis. Magnesium deficiency leads to depressed plant growth, the translocation of Mg from mature to young leaves and protein degradation, causing chlorosis in mature leaves (Marschner 2012). An impaired nutrient uptake and/or root-shoot translocation and resulting inhibition of physiological processes in the tree seedlings might therefore be the explanation for the reduction in shoot biomass production.

Moreover, in the experiment of Rehmus et al. (2014) enhanced root biomass production was observed for T. chrysantha in the treatment with 300 μM Al. Stimulation of plant growth induced by toxic metal ions at low concentrations is known as hormesis (Calabrese and Blain 2009; Poschenrieder et al. 2013). Poschenrieder et al. (2013) distinguish three main mechanisms behind growth stimulation, which are I) amelioration by substrate interactions, II) activation of stress specific defenses like lag-time dependent activation of tolerance mechanisms and priming or acclimation by preexposure to low concentrations of the toxic element, and III) activation of general defense mechanisms, for instance against oxidative and biotic stress. We suppose substrate interactions or amelioration of biotic or oxidative stress as reason for the observed root growth stimulation.

A stimulation of plant growth and especially of P but also N and K uptake by application of Al was observed for some Al-tolerant plant species by Osaki et al. (1997). Hajiboland et al. (2013a) investigated the mechanisms for Al-induced growth stimulation in an Al-tolerant tea plant (Camellia sinensis L. Kuntze) in a hydroponic experiment and attributed the growth stimulation to enhanced photosynthesis rates and a better protection of membranes against peroxidation by the activation of antioxidant defense enzymes.

Several studies investigated the P-Al interactions in the context of Al-toxicity resp. Al-tolerance (Jiang et al. 2009; Liao et al. 2006; Watanabe and Osaki 2001; Zheng et al. 2005), suggesting both, alleviation of Al toxicity by precipitation of Al-P compounds in the roots or on the root surface as well as stimulated extrusion of specific organic acids, which chelate the trivalent Al cation.

Our objectives were:

1. to test whether negative effects on shoot biomass production result from suppressed nutrient supply of shoots.
2. to identify the reason for enhanced root biomass production of T. chrysantha at 300 μM Al.

We hypothesize that

1. reduced shoot biomass production with increasing plant available Al concentrations can be explained by inhibited mineral nutrient supply, i.e. N, P, K, and/or Mg supply,
2. stimulated root growth of T. chrysantha at 300 μM Al was caused by elevated N and/or P uptake.

Materials & methods

Experimental design

We set up a hydroponic growth experiment with tree seedlings in a greenhouse at the research station

San Francisco (4° 00‘ S, 79° 05‘ W), located in the Reserva Biológica San Francisco on the eastern slope of the Cordillera Real, southern Ecuador. Tree seedlings selected for the experiment were C. odorata, H. americanus, and T. chrysantha, aged 7, 6, and 3 months, respectively. These tree species are currently tested as native alternative species for afforestation to replace the locally common exotic Pinus sp. and Eucalyptus sp. (Mosandl and Günter 2008). Tree seedlings were raised in a nursery from seeds collected from the local forest and germinated in a 50 % soil-sand mixture. At the start of the hydroponic experiment, roots were prewashed thoroughly with tap water to remove soil and rinsed with distilled water before placed in nutrient solution. Before addition of Al, tree seedlings were grown for two weeks in nutrient solution.

One tenth Hoagland solution (Hoagland and Arnon 1950) was used as the basis for the experiments because it resembles the nutrient composition of the organic layer leachate of the study area (Rehmus et al. 2014). However, an important dissimilarity to the organic layer leachate is that in our nutrient solution P concentrations were about 10 times higher than in organic layer leachate. This implies a better P supply of the plants grown in hydroponic solution than in the forest soil and possibly a related deterioration of the plant availability of Fe and Zn (Marschner 2012; Poschenrieder et al. 2013). Nine replicate seedlings per species were treated with 0, 300, 600, 1200, and 2400 µM Al, which was added as $AlCl_3$ to the nutrient solution. The pH was adjusted to 4 using NaOH and HCl.

Each tree seedling was treated with 0.5 L culture solution. Pots were placed at random and positions changed weekly, when culture solutions were replaced. Nutrient solutions were aerated for 15 minutes per hour to ensure aerobic conditions at all times. After six weeks, plants were harvested and washed thoroughly with distilled water. Leaves, stems, and roots were separated and dried in a drying oven at 55°C to constant weight. Shoot weight was determined for stems and leaves together. Of the 135 seedlings treated with Al and the control solution, only 4 plants died during the experiment (two seedlings of each of H. americanus and T. chrysantha, i.e. one seedling of each species in each of the 0 and 2400 µM treatments, respectively) and were removed without replacement.

Chemical analyses

Fifty mg of plant material (roots and leaves) were digested in a closed-vessel microwave system (MLS Ethos, Germany). To ensure dissolution of aluminosilicates a digestion with 1.6 mL 69 % HNO_3 , 0.6 mL 30 % H_2O_2, 0.1 mL 48 % HF, and 1 mL 5 % H_3BO_3 was chosen. Concentrations of P, Fe, Mn, Zn, Cu, Ni, Co, and Al were determined with ICP-MS (7700x Agilent Technologies, Germany). Potassium, Ca, and Mg concentrations were determined with AAS (Zeenit700P Analytik Jena, Germany). Carbon, N, and S concentrations were analyzed with a Vario EL Cube (Elementar Analysensysteme, Germany). If root quantity was not sufficient for both, digestion and CNS analysis, sample digestion was preferred.

The quality of digestions and analysis of K, Ca, Mg, P, Fe, Mn, Zn, Cu, and Al was controlled with the certified reference material (CRM) BCR-100 (beech leaves, IRMM, Geel, Belgium). The accuracy of P, K, Ca, Mn, and Al was within ± 5 %, while that of Mg, Fe, Zn, and Cu was within 10 % of the certified values. The precision of the method was tested by repeated digestion and analysis of samples and reference materials and was always within 10 %, except for Al and Cu, which ranged up to 11 and 13 % (RSD), respectively. No reference values are available for Ni and Co.

Statistical analyses

Differences in nutrient concentrations among treatments were tested using one-way ANOVA and post-hoc tests. When ANOVA residuals were normally distributed (Shapiro-Wilk normality test) and original data showed homogeneity of variances (Bartlett Test of Homogeneity of Variances), as post-hoc test Fisher’s least significant difference (LSD) test with Bonferroni correction was chosen for equal group n and Tukey’s honest significant difference (HSD) test for unequal group n. When normal distribution and homogeneity of variances could not be assumed, the Games-Howell test was used. Differences in P concentrations of roots and shoots were tested with Welch’s two sample t-test.

To test for correlations between selected variables a Pearson Product Moment Correlation was chosen for variables which showed normal distribution. For variables which did not show normal distribution, a

nonparametric Spearman's rank correlation (r_s) was conducted. Statistical analyses were carried out with R 3.0.2 (R Core Team 2013) and SPSS (SPSS 19 IBM Corp., United States).

Results

Concentrations of macronutrients in plant tissue

Nitrogen concentrations in roots and leaves of H. americanus increased significantly with increasing Al concentrations (Table 1). In leaves of C. odorata, N concentrations increased from the control to 1200 µM Al and dropped at 2400 µM Al to the control value. In roots of T. chrysantha treated with 300 µM Al, the mean N concentration was significantly lower than at 600 µM and 2400 µM Al. Mean N concentrations were not significantly different among the Al treatments in leaves of T. chrysantha and in roots of C. odorata.

The mean P concentrations in leaves of C. odorata and T. chrysantha were significantly highest in the control although P concentrations in leaves of T. chrysantha were not significantly different between the control and the 2400 µM Al treatment (Table 1). In H. americanus, P concentrations in leaves were higher in the control and at 600 and 2400 µM Al than at 300 and 1200 µM Al. In roots of all tree species, mean P concentrations were highest at 300 µM Al. In roots of C. odorata, the P concentrations decreased with further increase of Al concentration in nutrient solution. In H. americanus, the P concentration first decreased at 1200 µM Al and increased significantly at 2400 µM Al. The P concentrations in roots of T. chrysantha were significantly lowest at 600 µM Al and increased with further increasing Al concentration. In general, the P concentrations in the roots were significantly higher than in the leaves. This difference in P concentrations was highest at 300 µM Al, with P concentrations being up to three times higher in roots than in leaves.

Concentrations of K in leaves of all tree species increased significantly with increasing Al concentrations (Table 1). In roots of all tree species, K concentrations were significantly lowest at 2400 µM Al and not significantly different among the other treatments.

In leaves of C. odorata and H. americanus, the Ca concentrations decreased with increasing Al concentrations while T. chrysantha showed no clear effect (Table 1). In the roots of C. odorata, the Ca concentrations significantly decreased from control to 1200 µM Al and increased at 2400 µM Al, while H. americanus showed no effect. The Ca concentrations in roots of T. chrysantha were significantly highest in the control.

Magnesium concentrations in leaves of C. odorata and T. chrysantha were significantly higher in the control and at 300 µM Al than at 600, 1200, and 2400 µM Al (Table 1). In leaves of H. americanus, the significantly lowest Mg concentrations were observed at 1200 µM Al. In the roots, the Mg concentrations of all tree species decresased significantly from control to 2400 µM Al.

The mean S concentrations in leaves of C. odorata and H. americanus were not significantly different up to 1200 µM Al (Table 1). At 2400 µM Al, the mean S concentration in leaves of C. odorata was significantly lowest but significantly highest in leaves of H. americanus. In leaves of T. chrysantha, S concentrations significantly decreased from control to 2400 µM Al. There were no significant differences in S concentrations among the treatments in roots of C. odorata. In roots of H. americanus and T. chrysantha, the highest S concentrations occurred in the control, yet they increased from 300 to 2400 µM Al.

Concentrations of micronutrients, beneficial mineral elements, and Al in plant tissue

No consistent patterns which could be attributed to Al impact were found for Mn and Co concentration (data not shown) in leaf and root tissue, although in some cases differences among the treatments were significant.

Significant differences among the treatments were found in mean concentrations of Fe, Zn, Cu, and Ni, partly showing consistent increasing or decreasing patterns which could be attributed to Al exposure (Table 2).

No significant diferences in root Al concentrations were found for C. odorata and T. chrysantha growing at increasing Al concentrations, while for H. americanus, Al concentrations in roots increased significantly (Rehmus et al. 2014). Aluminum concentrations in the leaves of all three tree species increased significantly with increasing Al exposure (Table 2).

Table 1 Concentrations of macronutrients (mg g^{-1}) in leaf and root tissue of C. odorata, H. americanus and T. chrysantha after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al

Al concentration (μM)						
		0	300	600	1200	2400
N (mg g^{-1})						
C. odorata	leaves	32.2 ± 0.83 bc	36.6 ± 0.86 ab	36.6 ± 1.20 ab	38.4 ± 1.37 a	32.1 ± 1.03 c
	roots	31.9 ± 1.12	32.8 ± 1.05	34.8 ± 0.86	34.2 ± 1.04	35.0 ± 0.88
H. americanus	leaves	26.6 ± 1.3 c	27.8 ± 1.5 c	33.4 ± 0.82 (8) b	36.1 ± 0.81 ab	40.9 ± 1.59 a
	roots	27.9 ± 1.01 d	30.3 ± 1.02 cd	34.7 ± 1.45 (8) bc	38.9 ± 1.19 ab	40.9 ± 1.01 (7) a
T. chrysantha	leaves	35.3 ± 1.06	35.9 ± 0.73	36.9 ± 0.98	36.1 ± 1.19	35.5 ± 0.7
	roots	35.5 ± 1.75 (5) ab	33.9 ± 1.67 b	39.8 ± 1.46 a	40.5 ± 0.63 (5) ab	41.3 ± 1.30 a
P (mg g^{-1})						
C. odorata	leaves	3.92 ± 0.17 a	2.48 ± 0.20 b	2.51 ± 0.20 b	2.58 ± 0.28 b	2.28 ± 0.07 b
	roots	4.83 ± 0.22 b	8.21 ± 1.02 a	6.16 ± 0.41 ab	5.26 ± 0.27 b	5.45 ± 0.4 b
H. americanus	leaves	5.10 ± 0.40 a	1.88 ± 0.13 b	2.63 ± 0.86 ab	2.00 ± 0.09 b	4.21 ± 0.43 a
	roots	6.53 ± 0.47 ab	8.12 ± 0.59 a	6.50 ± 0.89 ab	5.58 ± 0.57 b	7.76 ± 0.27 a
T. chrysantha	leaves	3.17 ± 0.12 a	2.20 ± 0.15 b	2.43 ± 0.19 b	2.26 ± 0.12 b	2.74 ± 0.11 ab
	roots	4.02 ± 0.24 b	5.97 ± 0.24 a	2.90 ± 0.19 c	3.96 ± 0.29 b	4.82 ± 0.32 b
K (mg g^{-1})						
C. odorata	leaves	20.5 ± 0.49 c	25.0 ± 2.08 bc	31.5 ± 1.07 ab	31.3 ± 2.02 ab	33.6 ± 0.88 a
	roots	41.4 ± 1.87 (8) a	40.9 ± 1.81 a	35.9 ± 2.79 ab	36.5 ± 2.53 ab	29.2 ± 0.74 b
H. americanus	leaves	20.7 ± 1.02 c	21.6 ± 1.07 c	27.4 ± 2.73 bc	32.9 ± 1.54 b	47.5 ± 2.53 a
	roots	28.8 ± 1.49 a	29.8 ± 1.81 a	30.3 ± 2.15 a	27.2 ± 1.33 a	17.8 ± 0.77 b
T. chrysantha	leaves	22.3 ± 1.01 b	25.0 ± 1.80 ab	28.1 ± 1.68 ab	27.7 ± 0.98 a	28.2 ± 0.98 a
	roots	33.4 ± 1.09 ab	38.1 ± 1.98 a	38.3 ± 1.95 a	35.2 ± 1.92 a	26.2 ± 1.91 b
Ca (mg g^{-1})						
C. odorata	leaves	19 ± 0.4 a	11.3 ± 0.98 bc	13.6 ± 1.26 b	8.81 ± 0.8 c	7.49 ± 0.57 c
	roots	5.42 ± 0.26 a	4.56 ± 0.17 ab	4.04 ± 0.23 b	2.84 ± 0.12 c	3.97 ± 0.27 b
H. americanus	leaves	13.6 ± 0.47 a	7.84 ± 0.92 bc	11.3 ± 0.91 ab	5.45 ± 0.29 c	6.74 ± 0.68 c
	roots	4.4 ± 0.26	4.37 ± 0.37	4.65 ± 0.64	3.68 ± 0.21	4.79 ± 0.51
T. chrysantha	leaves	10.5 ± 0.45 a	13.7 ± 2.17 ab	6.96 ± 0.43 b	6.46 ± 0.32 b	8.99 ± 0.82 ab
	roots	5.42 ± 0.34 a	3.25 ± 0.08 b	2.99 ± 0.21 b	3.63 ± 0.25 b	3.51 ± 0.28 b
Mg (mg g^{-1})						
C. odorata	leaves	2.72 ± 0.13 a	2.17 ± 0.21 a	1.23 ± 0.04 b	1.15 ± 0.10 b	1.23 ± 0.11 b
	roots	1.79 ± 0.06 a	1.31 ± 0.04 b	0.83 ± 0.03 c	0.76 ± 0.04 c	0.75 ± 0.03 c
H. americanus	leaves	2.15 ± 0.09 a	2.09 ± 0.18 a	1.63 ± 0.16 a	1.06 ± 0.04 b	1.74 ± 0.12 a
	roots	2.57 ± 0.25 a	2.39 ± 0.14 a	1.41 ± 0.06 b	0.97 ± 0.02 c	0.88 ± 0.05 c
T. chrysantha	leaves	2.67 ± 0.14 (7) a	2.33 ± 0.10 a	1.51 ± 0.09 b	1.22 ± 0.07 b	1.36 ± 0.08 b
	roots	2.72 ± 0.09 a	2.22 ± 0.16 a	1.09 ± 0.04 b	1.00 ± 0.05 b	0.72 ± 0.04 c
S (mg g^{-1})						
C. odorata	leaves	2.74 ± 0.08 a	2.71 ± 0.08 a	2.46 ± 0.06 a	2.53 ± 0.09 a	2.14 ± 0.07 b
	roots	3.74 ± 0.33	3.10 ± 0.19	3.11 ± 0.10	3.30 ± 0.11	3.03 ± 0.04
H. americanus	leaves	1.86 ± 0.10 b	1.78 ± 0.06 b	1.88 ± 0.03 (8) b	1.91 ± 0.04 b	2.39 ± 0.12 a
	roots	4.97 ± 0.38 a	3.33 ± 0.20 c	3.76 ± 0.13 (8) bc	4.27 ± 0.19 abc	4.56 ± 0.25 (7) ab
T. chrysantha	leaves	2.70 ± 0.07 a	2.44 ± 0.04 ab	2.28 ± 0.08 bc	2.13 ± 0.07 c	2.21 ± 0.06 bc
	roots	5.09 ± 0.21 (5) a	2.53 ± 0.05 c	2.81 ± 0.08 bc	3.12 ± 0.05 (5) b	3.07 ± 0.13 b

Data refer to means of 9 replicates ± SE and 8 replicates ± SE for H. americanus and T. chrysantha in the treatments with 0 and 2400 μM Al. If we lost replicates because of insufficient sample quantity or eliminated outliers, numbers of replicates included in the statistical analysis are given in parentheses. Lower case letters depict significant differences among the treatments at $p < 0.05$

Table 2 Concentrations of micronutrients (μg g^{-1}) in leaf and root tissue and Al concentrations (mg g^{-1}) in leaf tissue of C. odorata, H. americanus and T. chrysantha after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al

Al concentration (μM)		0	300	600	1200	2400
Fe (μg g^{-1})						
C. odorata	leaves	112 ± 15.3 ab	105 ± 18.1 ab	84.4 ± 6.2 b	159 ± 17.9 a	138 ± 5.9 a
	roots	651 ± 100 b	1,517 ± 370 a	796 ± 133 ab	1,156 ± 154 ab	1,070 ± 149 ab
H. americanus	leaves	95.8 ± 14.8	56.4 ± 12.6	80.6 ± 13.9	77.7 ± 8.2	155 ± 62.2
	roots	91.9 ± 11.6	99.6 ± 13.8	211 ± 70.4	192 ± 48.9	179 ± 18.2
T. chrysantha	leaves	95.8 ± 17.8	94.3 ± 19.7	110 ± 8.29	108 ± 7.44	240 ± 71.0
	roots	381 ± 75	375 ± 145	235 ± 71.1	480 ± 137	221 ± 49.6
Zn (μg g^{-1})						
C. odorata	leaves	34.1 ± 1.83	38.3 ± 3.39	34.4 ± 2.38	37.7 ± 4.20	30.3 ± 1.87
	roots	86.7 ± 10.1 a	87.2 ± 11.4 ab	83.3 ± 8.22 a	72.6 ± 10.4 ab	50.8 ± 3.61 b
H. americanus	leaves	28.3 ± 2.45	31.7 ± 2.11	33.0 ± 7.17	23.0 ± 1.45	35.8 ± 4.17
	roots	21.4 ± 1.30 a	22.3 ± 1.64 a	22.4 ± 2.39 ab	15.2 ± 0.78 b	15.0 ± 0.89 b
T. chrysantha	leaves	52.1 ± 4.60 ab	58.2 ± 6.18 a	39.2 ± 2.36 b	38.4 ± 3.19 b	39.5 ± 1.67 b
	roots	58.4 ± 14.6 a	30.3 ± 3.31 b	26.7 ± 1.87 b	25.5 ± 1.97 b	22.9 ± 1.81 b
Cu (μg g^{-1})						
C. odorata	leaves	5.21 ± 0.82 b	8.50 ± 0.64 a	7.98 ± 0.89 ab	9.28 ± 1.60 ab	5.44 ± 0.52 b
	roots	8.55 ± 1.20 b	11.0 ± 1.03 ab	11.9 ± 0.84 ab	14.1 ± 0.82 a	14.4 ± 0.95 a
H. americanus	leaves	3.98 ± 0.28 b	5.06 ± 0.21 a	7.59 ± 1.59 ab	5.14 ± 0.17 a	5.08 ± 0.37 ab
	roots	6.42 ± 0.38 c	9.58 ± 0.47 b	14.1 ± 2.23 abc	11.9 ± 0.57 a	14.2 ± 1.14 a
T. chrysantha	leaves	6.13 ± 0.35 a	4.58 ± 0.25 bc	5.89 ± 0.51 ab	3.58 ± 0.25 c	3.55 ± 0.44 c
	roots	12.0 ± 1.13	11.0 ± 1.04	12.3 ± 0.54	14.5 ± 0.77	13.5 ± 0.82
Ni (μg g^{-1})						
C. odorata	leaves	3.41 ± 0.36 a	4.46 ± 0.23 a	3.75 ± 0.46 a	3.77 ± 0.30 a	1.33 ± 0.08 b
	roots	5.68 ± 0.70 ab	7.70 ± 0.76 a	5.88 ± 0.55 ab	4.21 ± 0.59 b	1.77 ± 0.12 c
H. americanus	leaves	3.97 ± 0.24 bc	5.49 ± 0.17 a	5.67 ± 0.98 abc	4.38 ± 0.08 b	3.41 ± 0.19 c
	roots	1.59 ± 0.15 c	5.00 ± 0.34 a	3.38 ± 0.20 b	1.10 ± 0.17 c	0.45 ± 0.06 d
T. chrysantha	leaves	3.29 ± 0.42 a	3.33 ± 0.40 a	2.68 ± 0.14 ab	1.47 ± 0.35 bc	0.63 ± 0.05 c
	roots	12.0 ± 0.74 a	11.3 ± 0.99 a	4.78 ± 0.56 b	3.31 ± 0.21 b	1.89 ± 0.21 c
Al (mg g^{-1})						
C. odorata	leaves	0.02 ± 0.01 c	0.09 ± 0.02 bc	0.12 ± 0.02 b	0.31 ± 0.05 a	0.41 ± 0.04 a
H. americanus	leaves	0.00 ± 0.0 d	0.00 ± 0.0 dc	0.06 ± 0.02 bc	0.11 ± 0.02 b	0.42 ± 0.08 a
T. chrysantha	leaves	0.01 ± 0.01 c	0.06 ± 0.02 c	0.18 ± 0.03 b	0.22 ± 0.03 b	0.63 ± 0.03 a

Data refer to means of 9 replicates ± SE and 8 replicates ± SE for H. americanus and T. chrysantha in the treatments with 0 and 2400 μM Al, as well as for roots of C. odorata in the 1200 μM Al treatment. Lower case letters depict significant differences among the treatments at $p < 0.05$. Al concentrations in roots of the studied tree species are given in Rehmus et al. (2014).

Relationship between nutrient status and biomass production

Calcium and Mg concentrations in leaves correlated positively with shoot biomass (Table 4, Fig. 1). Furthermore, P concentrations in roots correlated significantly with root biomass in T. chrysantha (Fig. 2) but not in the other two species.

Concentrations of Al and P in roots correlated significantly (Fig. 3). In the roots of the individual tree

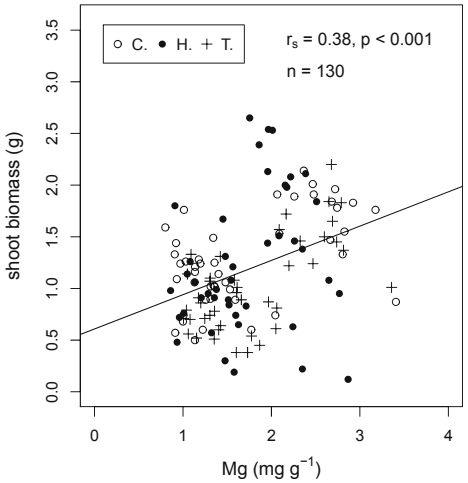

Fig. 1 Relationship between Mg concentrations in leaves (mg g^{-1}) and shoot biomass (g) of all tree species in all Al treatments. r_s = Spearman's rank correlation coefficient. C. is Cedrela odorata, H. is Helicarpus americanus and T. is Tabebuia chrysantha

species the correlation between Al and P was only significant for C. odorata (r_s = 0.50, p < 0.001, n = 45) and T. chrysantha (r = 0.43, p = 0.004, n = 43).

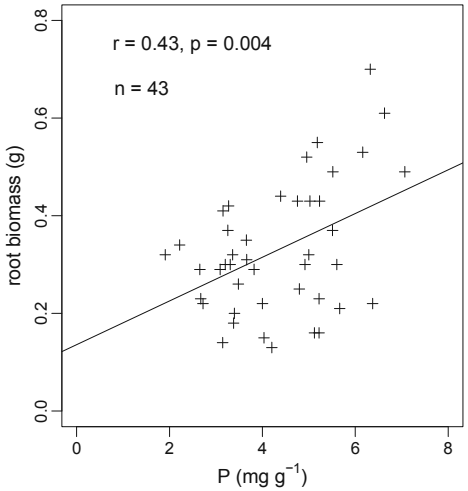

Fig. 2 Relationship between P concentrations in roots (mg g^{-1}) and root biomass (g) of T. chrysantha in all Al treatments

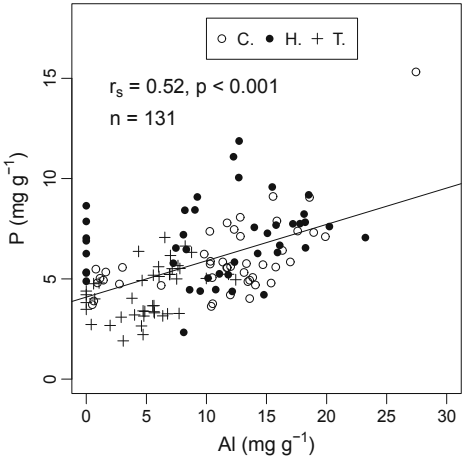

Fig. 3 Relationship between Al and P concentrations (mg g^{-1}) in roots of all species and in all treatments. r_s = Spearman's rank correlation coefficient. C. is Cedrela odorata, H. is Helicarpus americanus and T. is Tabebuia chrysantha

Discussion

Reduced shoot biomass

There is no published reference with respect to the specific nutrient demand of the investigated tree species. Hence, mineral concentrations in plant tissue can only be compared with general thresholds for optimum plant growth, usually derived for agricultural plants of the temperate zone, and to nutrient compositions of other woody plants and trees from tropical regions. We compared the mean nutrient concentrations in leaves and roots over all tree species and treatments to thresholds given by Amberger (1996) and Marschner (2012) for optimum growth of crop plants and to nutrient composition of tree leaves from a Brazilian Cerrado and from a tropical seasonal rain forest in southwest China (Table 3). Except for Mg concentrations, the nutrient concentrations were within ranges indicative for undisturbed nutrition.

Our finding that N concentrations increased while shoot biomass decreased with increasing Al concentration in nutrient solution clearly indicates that the Al effect on biomass production is not related with N supply. Decreasing biomass at consistently good N supply might have resulted in a concentration of N

Table 3 Range of selected mineral nutrient concentrations in plant tissue of crop plants required for optimal plant growth (Amberger 1996, Marschner 2012), in leaves of Brazilian Cerrado trees (Lilienfein et al. 2001), in tree leaves of a tropical seasonal rain forest in southwest China (Shanmughavel et al. 2001), and mean ± SE and range of respective nutrients in plant tissue (leaves and roots) of all tree species and all Al treatments in our study

	Demand for optimal growth of crops		Leaves Cerrado trees	Tree leaves southwest China	Concentrations present study	
	Amberger (1996)	Marschner (2012)	Lilienfein et al. (2001)	Shanmughavel et al. (2001)	mean ± SE	range
N (mg g^{-1})	5 – 50	10 – 50	9.7 – 28	7.4 – 10.5	35.0 ± 0.31	23.6 – 47.8
P (mg g^{-1})	1.5 – 8	3 – 5	0.48 – 1.6	0.8 – 1.0	4.27 ± 0.14	1.40 – 15.3
K (mg g^{-1})	5 – 50	20 – 50	1.4 – 5.9	7.5 – 10.6	30.4 ± 0.51	13.2 – 56.3
Ca (mg g^{-1})	< 10 – 50	1 – > 50	0.86 – 6.6	2.2 – 5.1	7.10 ± 0.27	1.81 – 21.0
Mg (mg g^{-1})	2 – 50	1.5 – 3.5	0.71 – 2.1	3.2 – 5.4	1.57 ± 0.04	0.51 – 3.55
S (mg g^{-1})	1 – 6	1 – 5	0.94 – 2.0	*	2.88 ± 0.06	1.57 – 7.26
Fe (μg g^{-1})	*	50 – 150	81 – 615	*	316 ± 28	20 – 3,856
Zn (μg g^{-1})	10 – 100	15 – 20	3.8 – 23	*	39.9 ± 1.55	12.2 – 155
Cu (μg g^{-1})	4 – 20	1 – 30	*	*	8.90 ± 0.28	1.78 – 31.0
Ni (μg g^{-1})	< 1	1 – > 10	*	*	4.05 ± 0.18	0.22 – 16.6

*no general range suggested/no data given

in the plants (Table 1). Aluminum is known to stimulate N uptake in plants like Camellia sinensis [L.] Kuntze which respond positively to increasing Al concentrations (Hajiboland et al. 2013a). Hajiboland et al. (2013a) attributed this observation to impaired protein synthesis by Al.

Concentrations of P in plant tissue were strongly affected by Al (Table 1). As the P concentrations in leaves of all tree species decreased in the Al treatments compared to the control, a deteriorated P supply in the leaves could have contributed to reduced shoot biomass production. Increasing P concentrations in leaves and roots of H. americanus and T. chrysantha in the highest Al treatments might result from a P concentration effect because of strongly reduced leaf biomass.

In our study, the K concentrations in the plant tissue increased while Ca and Mg concentrations decreased. This is inconsistent with other observations in the literature (Dogan et al. 2014). Thus, increasing Al concentrations seemed to inhibit Mg and Ca uptake by blocking specific channels (Rengel 1992), while K might have been unaffected by Al and even benefited from reduced ion competition.

Magnesium has a crucial function in the photosynthesis of green plants, because Mg is the central ion of the chlorophyll molecule (Marschner 2012). The Mg concentrations in leaves and roots treated with 600 μM Al and more were close to and even below the threshold for nutrient deficiency (1.5 mg g^{-1}, Marschner 2012) (Tables 1–3). The closest positive correlation occurred between Mg concentrations in leaves and shoot biomass (Fig. 1). Similarly, the correlation between Ca concentrations and shoot biomass production was highly significant yet not as close (Table 4). Thus, Mg deficiency might cause reduced photosynthesis and inhibited biomass production. Indeed, the symptoms on the leaf surface of C. odorata and H. americanus (Rehmus et al. 2014) were similar to those described by Amberger (1996) for Mg deficiency. Speckled patches of chlorophyll defects developed over time to necrosis. An Al-induced reduction in chlorophyll concentration and consequently reduced photosynthesis in different plant species has been reported in several studies (e.g. Ali et al. 2008; Silva et al. 2012; Zhang et al. 2007). A stronger decrease of the Mg concentrations in roots than in leaves (Table 1) might reflect the attempt of the plant to supply the leaves with Mg rather than the roots to maintain the photosynthesis functioning. However, it should be considered that nutrient demand of crop plants is higher than that of trees. Sun and Payn (1999)

Table 4 Correlations between macronutrient concentrations in leaves and roots with shoot and root biomass, respectively, of all tree species together

	Leaves			Roots		
	r	p	n	r	p	n
N	−0.73[a]	< 0.001	130	−0.56[a]	< 0.001	122
P	−0.15[b]	0.08	131	0.24[b]	0.006	131
K	−0.74[b]	< 0.001	131	0.08[b]	0.36	130
Ca	0.33[b]	< 0.001	131	−0.08[b]	0.31	131
Mg	0.38[b]	< 0.001	130	0.18[b]	< 0.001	131
S	−0.16[a]	0.06	130	−0.02[b]	0.87	122

[a] Pearson product-moment correlation coefficient

[b] Spearman's rank correlation coefficient

found the photosynthesis rate of Pinus radiata D. Don to be reduced at Mg concentrations in shoots below 0.6 mg g^{-1}. Laing et al. (2000) observed a strongly decreased photosynthesis in P. radiata at Mg concentrations in needles of 0.2 – 0.25 mg g^{-1}. The mean concentrations of Mg in our study were 1.57± 0.04 mg g^{-1}, which is higher than reported critical values of Sun and Payn (1999) and Laing et al. (2000). Thus, a suppressed Mg uptake might not be the only reason for negative effects on plant growth.

Concentrations of S were partly affected by increasing Al concentrations in nutrient solution. As even the lowest S concentrations were higher than thresholds suggested for optimum plant growth (Table 3), a considerable role of S in reduced shoot biomass production is unlikely.

Although micronutrients partly showed consistent responses to increased Al concentrations in nutrient solution (Table 2), their mean concentrations were in the range of thresholds for optimal plant growth, suggesting that micronutrient deficiencies are unlikely. Also toxic effects of micro elements like Fe are not likely as mean Fe concentrations in leaves (Table 2) were clearly below the suggested critical value for Fe toxicity which is 500 µg g^{-1} (Marschner 2012). High Fe concentrations in roots of C. odorata might be attributable to Fe binding or precipitation and detoxification on the root surface or in the roots, as translocation of Fe to the leaves was low (Table 2).

Energy-consuming defense mechanisms against Al toxicity like extrusion of Al-complexing organic acids might further weaken the plants (Cuenca et al. 1990; Ma et al. 2001). This observation is in line with our finding that TOC concentrations in the nutrient solution increased with increasing Al concentrations, probably attributable to organic acid exudation (Rehmus et al. 2014).

Stimulated root growth at 300 µM Al

Alleviation of latent Fe stress by exposure to 200 µM Al in nutrient solution and decrease of Fe concentrations in young leaves and roots as the driving mechanism for growth stimulation in tea plants (Camellia sinensis (L.) O. Kuntze) was suggested recently by Hajiboland et al. (2013b). In our study, exposure to 300 µM Al caused the inverse effect: Fe concentrations in roots of C. odorata increased significantly compared to the control. Alleviation of Fe toxicity by low Al concentrations can therefore be ruled out as mechanism supporting growth stimulation.

The P concentrations in roots treated with 300 µM Al were higher for C. odorata and T. chrysantha compared to the control (Table 1). This might indicate that improved P supply at low Al concentrations is the reason for stimulated root biomass production of T. chrysantha. In fact, we found a positive and significant correlation between root biomass and P concentrations in the root tissue of T. chrysantha (Fig. 2) supporting our assumption, that root biomass production is limited by P uptake. This effect would be in line with hormesis because of substrate amelioration according to Poschenrieder et al. (2013). Improved P supply by elevated Al concentrations below toxicity thresholds might even be more important in the P-poorer organic layer leachates.

The P translocation into the leaves seemed to be suppressed by Al as the P concentrations were significantly higher in roots than in the leaves at Al concentrations ≥ 300 μM. This suggests that Al-phosphates precipitated on the root surface or in the roots.

Correlations between P and Al concentrations in roots are highly significant for C. odorata and H. americanus but not for T. chrysantha. The relationship between P and Al concentrations is in line with findings by Jiang et al. (2009) that P can alleviate Al stress, probably by precipitation of Al-P compounds on the root surface and/or in the root tissue. Increased Al resistance has been reported to be attributable to detoxification by precipitation of Al phosphates in the roots (Zheng et al. 2005; Gaume et al. 2001). Alternatively, stimulated exudation of Al-chelating organic acids (Liao et al. 2006) and a shift from oxalate to citrate exudation, preventing the precipitation of Ca-oxalate in the root cytoplasm and xylem sap (Watanabe and Osaki 2001), has been reported to enhance Al tolerance and plant growth. However, Al-P precipitation in the roots and exudation of organic acids are mechanisms to counteract Al toxicity and do not explain an improved root biomass production compared to the Al free control. It has been shown that essential as well as non-essential metal ions, which can be even toxic to plants, can reduce biotic stress at low concentrations by counteracting pathogens and herbivorous damage, depending on the toxicity threshold of plant species and invaders (Poschenrieder et al. 2006, 2013). Thus, changes in P uptake and alleviation of biotic stress might be the reason for root growth stimulation at low Al concentrations.

Conclusions

1. Although the uptake of most crucial mineral nutrients, except for N, K, and the micronutrients, was impaired by specific Al concentrations, the element concentrations in plant tissue were mostly above tresholds for nutrient deficiency, except for Mg. Therefore, a disturbed Mg supply and consequently reduced photosynthesis is likely a major reason for low biomass production above 300 μM Al in hydroponic solution.
2. The stimulated P uptake in the treatment with 300 μM Al seems to promote root biomass production of T. chrysantha. The role of Al-induced biotic stress alleviation in the studied native montane forest trees still needs to be elucidated.

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