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REGULAR ARTICLE

# Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of biomass and plant morphology to elevated Al concentrations

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**Abstract** *Aims* In acid tropical forest soils (pH <5.5) increased mobility of aluminum might limit aboveground productivity. Therefore, we evaluated Al phytotoxicity of three native tree species of tropical montane forests in southern Ecuador.
*Methods* An hydroponic dose-response experiment was conducted. Seedlings of *Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson were treated with 0, 300, 600, 1200, and 2400 $\mu M$ Al and an organic layer leachate. Dose-response curves were generated for root and shoot morphologic properties to determine effective concentrations (EC).
*Results* Shoot biomass and healthy leaf area decreased by 44 % to 83 % at 2400 $\mu M$ Al, root biomass did not respond (*C. odorata*), declined by 51 % (*H. americanus*), or was stimulated at low Al concentrations of 300 $\mu M$ (*T. chrysantha*). EC10 (i.e. reduction by 10 %) values of Al for total biomass were 315 $\mu M$ (*C. odorata*), 219 $\mu M$ (*H. americanus*), and 368 $\mu M$ (*T. chrysantha*). *Helicarpus americanus*, a fast growing pioneer tree species, was most sensitive to Al toxicity. Negative effects were strongest if plants grew in organic layer leachate, indicating limitation of plant growth by nutrient scarcity rather than Al toxicity.
*Conclusions* Al toxicity occurred at Al concentrations far above those in native organic layer leachate.

**Keywords** Aluminum toxicity · Tropical forest tree seedlings · Dose-response curves · Organic layer leachate

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## Introduction

Aluminum phytotoxicity is known to occur in acid soils (Alleoni et al. 2010; Delhaize and Ryan 1995; Kochian et al. 2004; Schaedle et al. 1989) and is discussed to be one of the major reasons for limited aboveground biomass productivity and low nutrient-cycling rates in tropical montane forests (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). At soil pH values < 5.5, Al is plant-available and phytotoxic as $Al^{3+}$, $AlOH^{2+}$, or $AlOH_2^+$ and in various inorganic complexes (Kabata-Pendias and Pendias 2001; Macdonald and Martin 1988). For economic reasons, many studies on Al toxicity have been conducted on crop plants (Kochian et al. 2004; Ryan et al. 2011) and tree species

used for afforestations (Kinraide 2003; Schaedle et al. 1989). However, only few tropical forest tree species have been tested for their susceptibility to Al toxicity (Cuenca et al. 1990; Watanabe et al. 1998).

Negative effects of high Al availability include, for instance, inhibition of water and nutrient uptake and plant growth, at which particularly root biomass production is negatively affected (Delhaize et al. 2012; Kochian 1995; Rout et al. 2001; Schaedle et al. 1989; Thornton et al. 1987). Inhibition of root elongation is suggested to be a result of Al stress to the root meristem (Ryan et al. 1993). Additionally, under limiting conditions such as root damage or nutrient stress, the plants assign carbohydrates to roots rather than to plant shoots (Harris 1992; Leuschner et al. 2007). Furthermore, exudation of chelating compounds to counteract Al toxicity is energy consuming and happens at the cost of shoot growth (Cuenca et al. 1990). In contrast to these findings, enhancement of root growth (Hajiboland et al. 2013) or plant growth on the whole (Watanabe et al. 1998) has been reported for some tree species at low to moderate Al concentrations in soil solution.

At our study site in southern Ecuador, a wide distribution of Al-accumulating plant species has been observed (Homeier 2008), which according to Chenery (1948) have Al concentrations $>$ 1000 mg $kg^{-1}$ in leaves. Predominantly, the Al accumulators appertain to the families Rubiaceae and Melastomataceae (Homeier 2008). Aluminum accumulation is usually interpreted as adaptation to high plant-available Al concentrations (Jansen et al. 2002) and thus Al toxicity.

Processes that increase soil acidification in Ecuadorian montane rain forest, for example fire-derived acid deposition (Boy et al. 2008) and nitrogen input in the form of $NH_4^+$ (Galloway et al. 2004; Wilcke et al. 2013), might increase Al availability in the future. The increased Al availability may expose native tree species to a greater risk of Al stress. It can furthermore be expected that the fast growing short-lived pioneer tree species are more susceptible to Al toxicity than the more slowly growing long-lived pioneer tree species.

Currently the pH value in the leachate solution below the thick organic layers of the Ecuadorian montane rain forest ranges between 4.6 and 5.2, below the critical value of 5.5 for Al mobilization. Total Al concentrations in these solutions are low ($<60$ $\mu M$) and $>97$ % of Al in the leachate occurs in nontoxic organo-complexes (Wilcke et al. 2001; Wullaert et al. 2013).

Common and economically important tree species in the montane rain forests of southern Ecuador are *Cedrela sp* (Meliaceae) and *Tabebuia chrysantha* (Jacq.) G. Nicholson (Bignoniaceae), which are long-lived pioneers with slow growth rates (Homeier 2008). They belong to the mid-successional tree species and are typically present in primary old-growth forests (Mosandl and Günter 2008). A representative of early-successional species is *Heliocarpus americanus* L. (Tiliaceae), a light demanding fast-growing short-lived pioneer tree (Homeier 2008). These three tree species are not Al accumulators.

In order to test Al toxicity to non-accumulating tropical montane rain forest trees in southern Ecuador, a hydroponic experiment with seedlings of *C. odorata*, *H. americanus*, and *T. chrysantha* was conducted. Our objectives were to:

1. investigate the response of biomass and morphology of native tree species to Al stress,
2. estimate the sensitivity of the seedlings of native tree species to Al toxicity using dose-response curves, thereby assessing whether fast-growing pioneer tree species or old-growth forest tree species are more susceptible to Al toxicity and
3. compare growth of tree seedlings under nutrient-optimized conditions to growth in native organic layer leachate.

We hypothesize that:

1. Native tree species in the tropical montane rain forest in southern Ecuador respond negatively to in-vitro elevated Al concentrations and root morphological properties are more strongly affected than shoot morphological properties.
2. Highly productive pioneer tree species are more susceptible to Al toxicity than slow-growing tree species.
3. Critical Al concentrations for toxicity in native tree species are above plant-available Al concentrations in organic layer leachate.

## 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 (Table 1). Nine replicate seedlings per species were treated with 0, 300, 600, 1200, and 2400 $\mu M$ Al, which was added as $AlCl_3$ to the nutrient solution. The pH was adjusted to 4 using NaOH and HCl. In addition, to simulate plant growth in natural organic layer leachate, one set of replicates of each tree species was treated with an organic layer leachate, without addition of Al. The organic layer leachate used in the experiment was prepared by irrigating a homogenized fresh sample of the whole organic layer from the local forest with distilled water (Table 1). 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 and sampled (mixed sample of the nine replicates per species and treatment). Nutrient solutions were aerated for 15 minutes per hour to ensure aerobic conditions at all times. After six weeks, plants were harvested, washed thoroughly with distilled water and leaves, stem, and roots were separated. Leaves were scanned immediately after separation and dried in a drying oven at 55°C to constant weight. Roots were

**Table 1** Nutrient composition of made organic layer leachate and Hoagland nutrient solution

| | organic layer leachate | Hoagland nutrient solution |
|---|---|---|
| Macronutrients ($\mu M$) | | |
| N | 281± 58.5 | 1934 ± 29.7 |
| P | 10.4 ± 2.8 | 115 ± 12.1 |
| K | 258 ± 56.9 | 601 ± 3.8 |
| Ca | 36.9 ± 6.6 | 391 ± 2.7 |
| Mg | 98.3 ± 21.7 | 102 ± 0.9 |
| Micronutrients ($nM$) | | |
| Fe | 1486 ± 572 | 857 ± 15.1 |
| Mn | 666 ± 200 | 198 ± 1.07 |
| Ni | 29.6 ± 5.1 | 60.1 ± 2.0 |
| Cu | 122 ± 10.6 | 94.9 ± 3.6 |
| Zn | 133 ± 19.5 | 252 ± 7.3 |
| Mo | 0.36 ± 0.21 | 51.9 ± 0.75 |
| TOC ($nM$) | 2825 ± 451 | 24.7 ± 1.2 |
| Al ($\mu M$) | 44.0 ± 11.3 | 0 – 2400 |

Values are means ± SE of solutions before weekly replacement, except for Al in nutrient solution where the range of the treatment is given

**Table 2** Principal component (PC) loadings of plant biomass and morphological properties

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| healthy leaf area | **0.91** | 0.11 | −0.25 |
| diseased leaf area | −0.03 | 0.39 | **0.63** |
| shoot biomass | **0.89** | 0.36 | −0.16 |
| root biomass | **0.88** | 0.21 | 0.34 |
| root-to-shoot biomass ratio | 0.07 | −0.13 | **0.88** |
| total root length | 0.52 | **0.82** | 0.08 |
| root diameter | 0.46 | **−0.73** | 0.40 |
| root volume | **0.86** | −0.02 | 0.40 |
| root tips | 0.18 | 0.95 | 0.13 |
| root forks | 0.26 | **0.93** | 0.07 |
| explained variance | 0.37 | 0.33 | 0.17 |

The data was varimax rotated and only PCs with Eigenvalues > 1 were accepted. Loadings > 0.6 are bold

**Table 3** Net total plant growth (g, fresh weight), shoot and root biomass (g, dry weight), root-to-shoot biomass ratio, healthy leaf area ($cm^2$), deseased leaf area (% of total leaf area), total root length (cm), root diameter (mm), root volume ($cm^3$), forks, and tips for *C. odorata*, *H. americanus*, *T. chrysantha* after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 $\mu M$ Al and organic layer leachate

| Al concentration ($\mu M$) | | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 300 | 600 | 1200 | 2400 | organic layer leachate |
| Net plant growth (g) | | | | | | |
| *C. odorata* | 11.3 ± 1.00 a | 10.5 ± 1.20 ab | 7.80 ± 0.57 abc | 7.16 ± 0.71 bc | 5.80 ± 0.39 c | 1.24 ± 0.06 |
| *H. americanus* | 14.2 ± 0.87 a | 13.8 ± 1.02 a | 7.10 ± 1.03 b | 6.99 ± 0.72 b | 3.60 ± 0.78 b | 2.62 ± 0.11 |
| *T. chrysantha* | 8.10 ± 0.66 ab | 9.02 ± 0.82 a | 6.21 ± 0.41 bc | 6.07 ± 0.48 bc | 4.37 ± 0.40 c | 0.68 ± 0.14 |
| Shoot biomass (g) | | | | | | |
| *C. odorata* | 1.71 ± 0.12 a | 1.48 ± 0.16 ab | 1.17 ± 0.11 bc | 1.07 ± 0.12 bc | 0.96 ± 0.07 c | 0.30 ± 0.04 |
| *H. americanus* | 2.11 ± 0.16 a | 1.67 ± 0.16 a | 1.01 ± 0.14 b | 0.88 ± 0.09 b | 0.56 ± 0.10 b | 0.31 ± 0.03 |
| *T. chrysantha* | 1.40 ± 0.10 b | 1.40 ± 0.10 a | 0.87 ± 0.10 c | 0.89 ± 0.09 bc | 0.61 ± 0.06 c | 0.22 ± 0.03 |
| Root biomass (g) | | | | | | |
| *C. odorata* | 0.61 ± 0.06 | 0.68 ± 0.08 | 0.49 ± 0.07 | 0.54 ± 0.07 | 0.46 ± 0.04 | 0.13 ± 0.02 |
| *H. americanus* | 0.65 ± 0.07 ab | 0.70 ± 0.06 a | 0.39 ± 0.06 bc | 0.40 ± 0.06 bc | 0.32 ± 0.08 c | 0.12 ± 0.01 |
| *T. chrysantha* | 0.28 ± 0.04 b | 0.47 ± 0.05 a | 0.31 ± 0.02 ab | 0.34 ± 0.04 ab | 0.24 ± 0.03 b | 0.06 ± 0.01 |
| Root-to-shoot biomass ratio | | | | | | |
| *C. odorata* | 0.35 ± 0.02 b | 0.45 ± 0.02 a | 0.42 ± 0.03 ab | 0.51 ± 0.03 a | 0.47 ± 0.01 a | 0.41 ± 0.04 |
| *H. americanus* | 0.30 ± 0.02 | 0.42 ± 0.02 | 0.40 ± 0.04 | 0.48 ± 0.08 | 0.52 ± 0.07 | 0.41 ± 0.06 |
| *T. chrysantha* | 0.20 ± 0.01 b | 0.32 ± 0.01 a | 0.38 ± 0.02 ab | 0.38 ± 0.02 a | 0.39 ± 0.02 a | 0.32 ± 0.08 |
| Healthy leaf area ($cm^2$) | | | | | | |
| *C. odorata* | 1207 ± 119 a | 985 ± 110 ab | 794 ± 70 b | 705 ± 82 b | 597 ± 45 b | 144 ± 10 |
| *H. americanus* | 1075 ± 63 a | 924 ± 59 a | 605 ± 98 b | 528 ± 56 b | 187 ± 39 b | 155 ± 4.9 |
| *T. chrysantha* | 866 ± 77 a | 848 ± 88 a | 603 ± 49 ab | 548 ± 50 b | 410 ± 46 b | 79 ± 11 |
| Diseased leaf area (% of total) | | | | | | |
| *C. odorata* | 10 ± 4.0 | 8.2 ± 2.3 | 12 ± 1.7 | 12 ± 2.7 | 12 ± 3.0 | 11 ± 1.5 |
| *H. americanus* | 11 ± 1.2 bc | 8.9 ± 1.2 c | 12 ± 1.1 bc | 18 ± 2.3 b | 54 ± 4.5 a | 15 ± 1.3 |
| *T. chrysantha* | 2.6 ± 0.24 b | 2.9 ± 0.34 b | 6.7 ± 2.0 ab | 8.3 ± 1.4 a | 13 ± 2.1 a | 19 ± 1.7 |
| Total root length (cm) | | | | | | |
| *C. odorata* | 1033 ± 74 a | 1086 ± 110 a | 756 ± 52 ab | 795 ± 58 ab | 638 ± 39 b | 421 ± 24 |
| *H. americanus* | 1967 ± 118 a | 2065 ± 108 a | 1319 ± 158 b | 1202 ± 93 b | 840 ± 139 b | 950 ± 38 |
| *T. chrysantha* | 960 ± 80 a | 946 ± 76 a | 737 ± 31 ab | 795 ± 72 ab | 644 ± 50 b | 203 ± 39 |
| Root diameter (mm) | | | | | | |
| *C. odorata* | 0.68 ± 0.02 ab | 0.67 ± 0.01 b | 0.72 ± 0.02 ab | 0.71 ± 0.02 ab | 0.76 ± 0.02 a | 0.51 ± 0.01 |
| *H. americanus* | 0.42 ± 0.02 b | 0.43 ± 0.02 b | 0.48 ± 0.03 ab | 0.54 ± 0.02 a | 0.51 ± 0.03 ab | 0.32 ± 0.01 |
| *T. chrysantha* | 0.48 ± 0.02 b | 0.55 ± 0.02 a | 0.56 ± 0.01 a | 0.54 ± 0.02 a | 0.48 ± 0.02 b | 0.53 ± 0.02 |
| Root volume ($cm^3$) | | | | | | |
| *C. odorata* | 3.83 ± 0.34 | 3.87 ± 0.46 | 3.11 ± 0.37 | 3.24 ± 0.36 | 2.88 ± 0.23 | 0.86 ± 0.06 |
| *H. americanus* | 2.75 ± 0.33 | 3.17 ± 0.41 | 2.65 ± 0.42 | 2.87 ± 0.39 | 1.88 ± 0.42 | 0.80 ± 0.08 |

**Table 3** (continued)

| Al concentration ($\mu M$) | | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 300 | 600 | 1200 | 2400 | organic layer leachate |
| Root forks | | | | | | |
| *T. chrysantha* | 1.76 ± 0.24 ab | 2.32 ± 0.31 a | 1.80 ± 0.07 a | 1.80 ± 0.13 a | 1.19 ± 0.14 b | 0.43 ± 0.07 |
| *C. odorata* | 1340 ± 171 ab | 1527 ± 235 a | 1011 ± 114 ab | 847 ± 115 ab | 722 ± 77 b | 405 ± 81 |
| *H. americanus* | 8505 ± 784 a | 8801 ± 1039 a | 4098 ± 470 b | 3548 ± 247 b | 2532 ± 488 b | 2430 ± 115 |
| *T. chrysantha* | 1956 ± 175 a | 1784 ± 235 ab | 1275 ± 96 b | 1464 ± 197 ab | 1255 ± 168 ab | 284 ± 51 |
| Root tips | | | | | | |
| *C. odorata* | 459 ± 43 | 614 ± 76 | 445 ± 37 | 519 ± 48 | 464 ± 50 | 250 ± 24 |
| *H. americanus* | 3466 ± 224 a | 3114 ± 275 a | 1792 ± 194 b | 1674 ± 140 b | 1467 ± 231 b | 1203 ± 72 |
| *T. chrysantha* | 587 ± 32 | 723 ± 65 | 575 ± 43 | 808 ± 81 | 646 ± 94 | 202 ± 28 |

Data refer to means of 9 replicates (8 replicates for *H. americanus* and *T. chrysantha* for the treatments with 0 and 2400 $\mu M$ Al) ± SE
Lower case letters depict significant differences among treatments with Hoagland nutrient solution at $p < 0.05$

stored cool until scanning with a root scanner and dried at 55°C to constant weight immediately after scanning. In addition to the dry weight of roots and shoots (stem and leaves), the fresh weight of the total plants before and after the experiment (growth rate) was determined. Of the 162 seedlings in total, 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 $\mu M$ treatments, respectively) and were removed without replacement. Leaves were scanned at 300 dpi (24 bit) in color with a Canon scanner (CanoScan LiDE 100). Roots were scanned with an Epson Expression 10000 XL at 600 dpi (8 bit) grey scale. The scanner was equipped with additional lighting system in the lid to avoid distortion by shadows. Analysis of healthy and diseased leaf area, total root length, average root diameter, root volume, root tips, and root forks was carried out with WinRhizo 2009 (Regent Instruments Inc., Canada).

## Chemical analysis

Roots, stems, and leaves were separated and 50 mg plant material were digested in a microwave oven (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. Aluminum concentrations in digests of plant tissue were determined with ICP-MS 7700X (Agilent Technologies, Germany). Calcium concentrations were determined with AAS Zeenit 700P (Analytik Jena, Germany). Total nitrogen and total organic carbon (TOC) in nutrient solution were analyzed with a Vario TOC Cube (Elementar Analysensysteme, Germany). $NH_4^+$-N, $NO_3^-$-N, and $PO_4^{3-}$-P concentrations in organic layer leachate before and after weekly treatment were analyzed with a Continuous Flow Analyzer (CFA AutoAnalyzer 3 HR , SEAL Analytical, Germany).

## Statistical analysis

A Principal Component Analysis (PCA, R, package psych, Revelle 2013) with varimax rotation was applied in order to reduce the number of variables and to extract interrelations among the root and shoot parameters. The PCA was conducted for the three tree species combined and included the variables healthy and diseased leaf area, shoot and root biomass (dry weight), root-to-shoot biomass ratio, total root length, root diameter, root volume, root tips, and root forks. As an important morphological property which is considered indicative of toxicity effects, the root-to-shoot biomass ratio was included in addition to root and shoot biomass (Graham 2001).

Differences among treatments were tested using one-way ANOVA and post-hoc tests. When ANOVA residuals were normally distributed and showed homogeneity of variances, as post-hoc test Fisher's

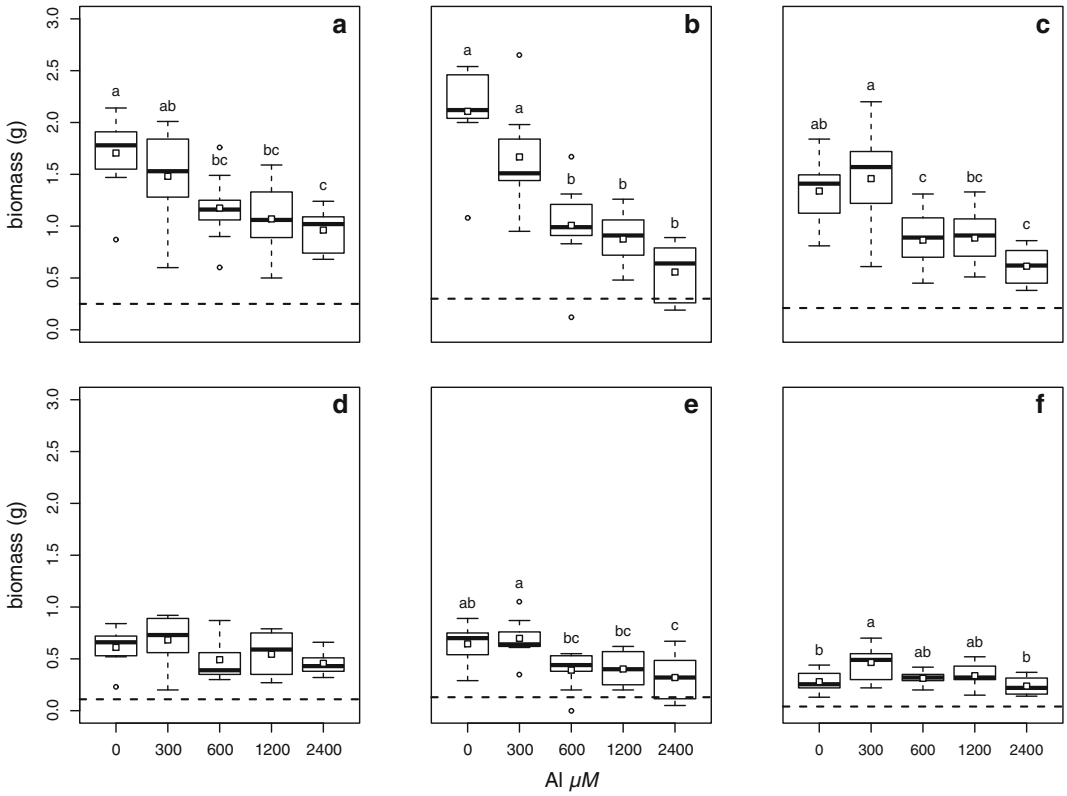


**Fig. 1** Boxplots of aboveground (*upper row*) and root (*lower row*) biomass for *C. odorata* (**a**, **d**), *H. americanus* (**b**, **e**), and *T. chrysantha* (**c**, **f**) by treatment (0, 300, 600, 1200, and 2400 $\mu M$ Al). *Black bars* represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as *white squares*. *Lower case letters* above the boxplots depict significant differences among the treatments at $p < 0.05$. The *dashed line* shows the median in treatment with organic layer leachate

least significant difference (LSD) test with Bonferroni correction was chosen for *C. odorata* and Tukey's honest significant difference (HSD) test for unequal N for *H. americanus* and *T. chrysantha*, because of the loss of two replicates of each of these species. When normal distribution and homogeneity of variances could not be assumed, the Games-Howell test was used. Differences in concentrations of $NH_4^+$-N, $NO_3^-$-N, and $PO_4^{3-}$-P in organic layer leachate before and after weekly treatment were tested with the Mann-Whitney U test. Significance was set at $p < 0.05$ unless otherwise indicated; ** denotes $p < 0.01$ and *** $p < 0.001$.

Dose-response curves (DRC) were fitted using log(x+1)-transformed Al concentrations in nutrient solutions vs. total biomass (dry weight), healthy leaf area, number of root tips, root diameter, root-to-shoot biomass ratio and diseased leaf area (in % of total leaf area). For the number of root tips a function could only be fitted for *H. americanus*. Fitting failed for root diameter of *T. chrysantha*, for root-to-shoot biomass ratio of *H. americanus*, and for diseased leaf area of *C. odorata* (Fig. 5).

Effective concentrations (EC) were calculated for 10, 20, and 50% reduction or enhancement, respectively, compared to control. The treatment with organic layer leachate was considered as a supplemental experiment and was excluded from ANOVA, PCA, and DRC fitting, because strong differences in chemical composition of the solutions complicated a comparison. Statistical analyses were carried out mainly with R 2.13.1 for Windows GUI front-end (R Foundation, Austria). For the Games-Howell test SPSS 19 (IBM Corp., United States) was used. Dose-response curves were fitted with Origin 8.5 for Windows (OriginLab Corporation, USA).

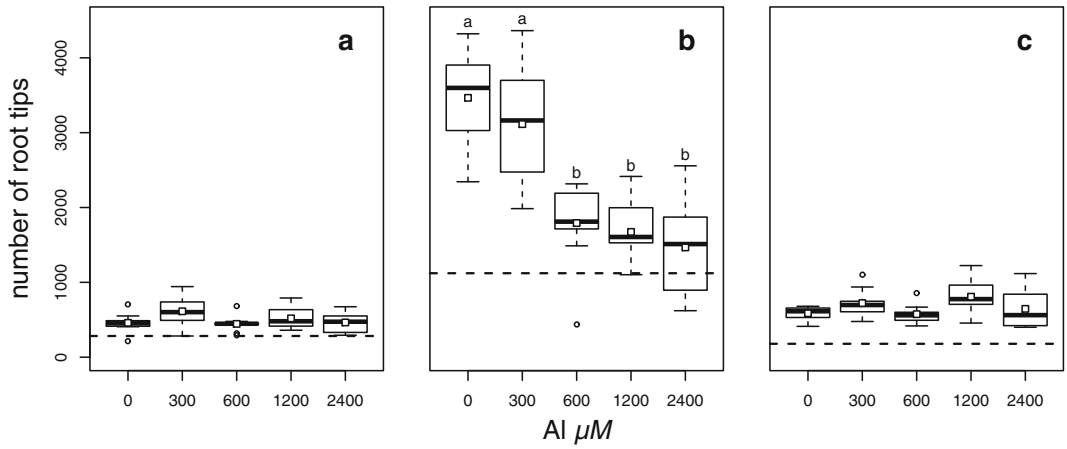


**Fig. 2** Boxplots of the number of root tips for *C. odorata* (**a**), *H. americanus* (**b**), and *T. chrysantha* (**c**) by treatment (0, 300, 600, 1200, and 2400 $\mu M$ Al). *Black bars* represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as *white squares*. *Lower case letters* above the boxplots depict significant differences among the treatments at $p < 0.05$. The *dashed line* shows the median in treatment with organic layer leachate

## Results

### Principal component analysis of plant properties

Three principal components (PC) were extracted. The first PC was highly loaded by properties characteristic for biomass (Table 2). The second PC was highly loaded by properties related to root morphology. Root diameter was negatively related to all other root parameters. The third PC was highly loaded by plant properties which are known for their particular susceptibility to Al toxocity, i.e. diseased leaf area and root-to-shoot biomass ratio. All plant properties were significantly affected by increasing Al concentrations with few exceptions (Table 3).

*Biomass related plant properties* Net total plant growth (difference in fresh weight of the whole plant between start and harvest, not included in the PCA) decreased with increasing Al concentrations by 49 %, 77 %, 46 % for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively, and was smallest in the

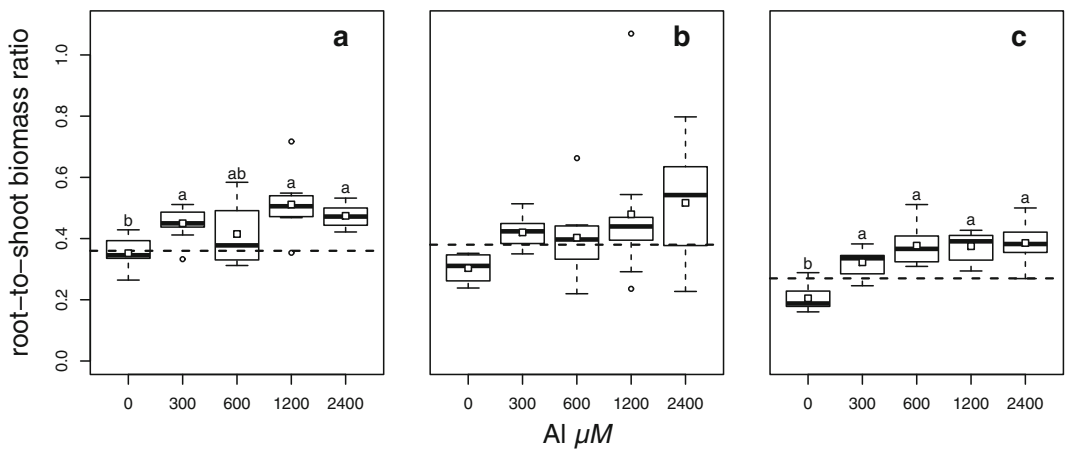


**Fig. 3** Boxplots of root-to-shoot biomass ratio for *C. odorata* (**a**), *H. americanus* (**b**), and *T. chrysantha* (**c**) by treatment (0, 300, 600, 1200, and 2400 $\mu M$ Al). *Black bars* represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as *white squares*. *Lower case letters* above the boxplots depict significant differences among the treatments at $p < 0.05$. The *dashed line* shows the median in treatment with organic layer leachate

treatment with organic layer leachate. Shoot biomass decreased as Al concentrations increased to 2400 $\mu M$ by 44 %, 73 %, 56 % for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively (Fig. 1, Table 3). *H. americanus* showed the most distinct decrease of shoot biomass, reflected in the slope of the regressions ($\beta$) of shoot biomass on Al concentrations: $\beta = -2.8 \times 10^{-4}$, r= −0.54***; $\beta = -5.8 \times 10^{-4}$, r = −0.73***; $\beta = -3.2 \times 10^{-4}$, r = −0.60*** for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively.

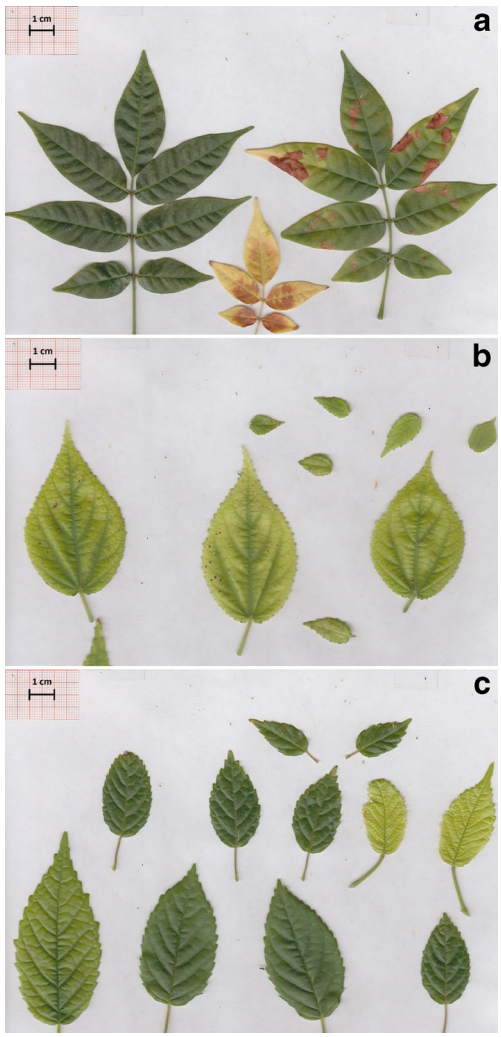


**Fig. 4** Diseased leaves of *C. odorata* (**a**), *H. americanus* (**b**), and *T. chrysantha* (**c**) in the 2400 $\mu M$ Al treatment. The picture gives a qualitative example of the pale green, yellow, and brown colors of diseased spots

Root biomass was not significantly different among the treatments for *C. odorata*, and decreased significantly by 51 % for *H. americanus* as Al concentrations increased (Fig. 1, Table 3). For *T. chrysantha* root biomass was significantly higher by 68 % at 300 $\mu M$ Al compared to control, and decreased to near the control value at 2400 $\mu M$ Al. This pattern suggests a stimulation in root biomass production of *T. chrysantha* at low Al concentrations.

Similar to shoot biomass, healthy leaf area decreased for all tree species with increasing Al concentration to 2400 $\mu M$ by 51 %, 83 %, 53 %, for

**Table 4** EC10, EC20, and EC50 values (in $\mu M$ Al, i.e. effective Al concentrations, at which 10, 20, and 50% reduction or enhancement compared to control occurs) for total biomass, healthy leaf area, number of root tips, root diameter, root-to-shoot biomass ratio, and diseased leaf area (% of total) of *C. odorata*, *H. americanus*, *T. chrysantha*

| Parameter | *C. odorata* | *H. americanus* | *T. chrysantha* |
|---|---|---|---|
| Total biomass | | | |
| EC10 | 315 | 219 | 368 |
| EC20 | 467 | 315 | 461 |
| EC50 | – | 733 | – |
| Healthy leaf area | | | |
| EC10 | 150 | 163 | 241 |
| EC20 | 299 | 350 | 440 |
| EC50 | 2271 | 1093 | 2001 |
| Number of root tips | | | |
| EC10 | – | 299 | – |
| EC20 | – | 359 | – |
| EC50 | – | 657 | – |
| Root diameter | | | |
| EC10 | 1736 | 473 | – |
| EC20 | – | 693 | – |
| EC50 | – | – | – |
| Root-to-shoot biomass ratio | | | |
| EC10 | 5.48 | – | 182 |
| EC20 | 38.3 | – | 212 |
| EC50 | – | – | 281 |
| Diseased leaf area (% of total) | | | |
| EC10 | – | 563 | 229 |
| EC20 | – | 726 | 311 |
| EC50 | – | 1017 | 475 |

The hyphen (–) indicates that no EC values could be determined

*C. odorata*, *H. americanus*, and *T. chrysantha*, respectively (Table 3). Again, a regression of leaf area on Al concentrations showed the most distinct gradient in decrease of healthy leaf area for *H. americanus* (slope of regression: $\beta = -0.22$, r = −0.57***; $\beta = -0.35$, r = −0.81***; $\beta = -0.19$, r= −0.64*** for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively).

*Root morphology* Morphological root properties of *H. americanus* responded most markedly to increasing Al concentrations (Fig. 2). The number of root tips was not significantly different among the Al treatments for *C. odorata* and *T. chrysantha*, yet in *H. americanus* it decreased significantly by 58 % at 2400 $\mu M$ Al. The root diameter of *C. odorata* and *H. americanus* increased with increasing Al concentration (by 12 % and 21 % at 2400 $\mu M$, respectively). Root diameter of *T. chrysantha* increased by 17 % at 600 $\mu M$ Al and decreased at further increasing Al concentrations to similar values like in the control (Table 3).

*Indications of Al toxicity* There was a significant negative correlation between number of root tips and root diameter for all tree species (r = −0.82***) and individually for *H. americanus* (r = −0.93**). Root-to-shoot biomass ratio increased significantly with Al concentration for *C. odorata* and *T. chrysantha* by 34 % and 95 % at 2400 $\mu M$ Al, resulting from a more pronounced decrease in shoot biomass compared to root biomass (Fig. 3, Table 3).

We observed diseased areas of pale green, yellow, and brown colors (Fig. 4). The diseased leaf area in % of total leaf area was not significantly different among the Al treatments for *C. odorata*. However, the fraction of the deseased leaf area increased from 11±1.2 % and 2.6±0.24 % in the control treatment to 54±4.5 % and 13±2.1 % in the treatment with 2400 $\mu M$ Al in *H. americanus* and *T. chrysantha*, respectively (Table 3).

## Dose-response curves

Best fits for all tree species were achieved for the relationship between Al concentrations and total biomass and healthy leaf area, respectively. The EC10 values, i.e. the effective concentration of Al at which the respective plant property was affected by 10 % relative to the control, for all tree species and all fitted plant properties (total biomass, healthy leaf area, number of root tips, root diameter, and diseased leaf area in % of

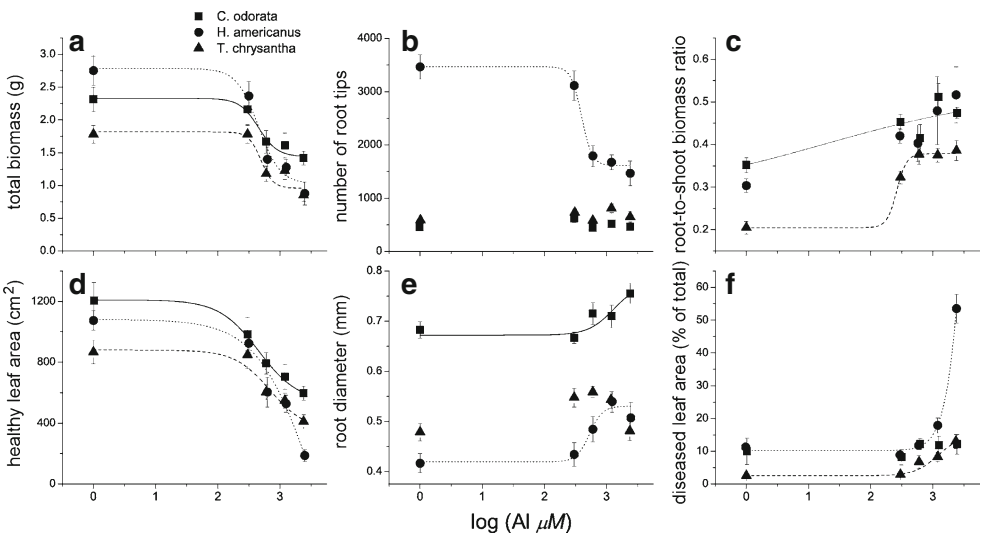


**Fig. 5** Dose-response curves of plant properties to Al concentrations for total biomass (dry weight) (**a**), number of root tips (**b**), root-to-shoot biomass ratio (**c**), healthy leaf area ($cm^2$) (**d**), root diameter (mm) (**e**), and diseased leaf area (% of total) (**f**), for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively. *Error bars* represent SE of means. *Lines* are fitted sigmoid growth functions

total) ranged from 150 to 1736 $\mu M$, EC20 from 299 to 726 $\mu M$, and EC50 from 475 to 2271 $\mu M$. The EC10 values of root-to-shoot biomass ratio ranged from 5.48 to 182 $\mu M$, the EC20 values from 38.3 to 212 $\mu M$, and the EC50 value of *T. chrysantha* was 281 $\mu M$. The root-to-shoot biomass ratio appeared to be the most Al-sensitive plant property, with *C. odorata* responding far more sensitively than *T. chrysantha* (Table 4). With respect to total biomass reduction at increasing Al concentrations, *H. americanus* responded most sensitively, while *C. odorata* and *T. chrysantha* had similar EC values. Ten and 20 % reduction of healthy leaf area occurred first in *C. odorata*, followed by *H. americanus* and *T. chrysantha*. With respect to root diameter enhancement, *H. americanus* responded more sensitively to increasing Al concentrations than *C. odorata*. Except for the response of diseased leaf area in *C. odorata*, either *H. americanus* or *C. odorata* responded most sensitively to increasing Al concentrations (Fig. 5, Table 4).

## Chemical plant properties

Aluminum concentrations in roots were significantly higher in the treatments with Al than in the control treatments (means±SE for *C. odorata*, *H. americanus*, and *T. chrysantha*: 1.42±0.30, 0.00±0.00, and 0.35±0.14 mg $g^{-1}$). Among the treatments with 300 to 2400 $\mu M$ Al no significant differences were observed for *C. odorata* and *T. chrysantha* (mean Al concentrations ±SE over all Al treatments: 14±0.6

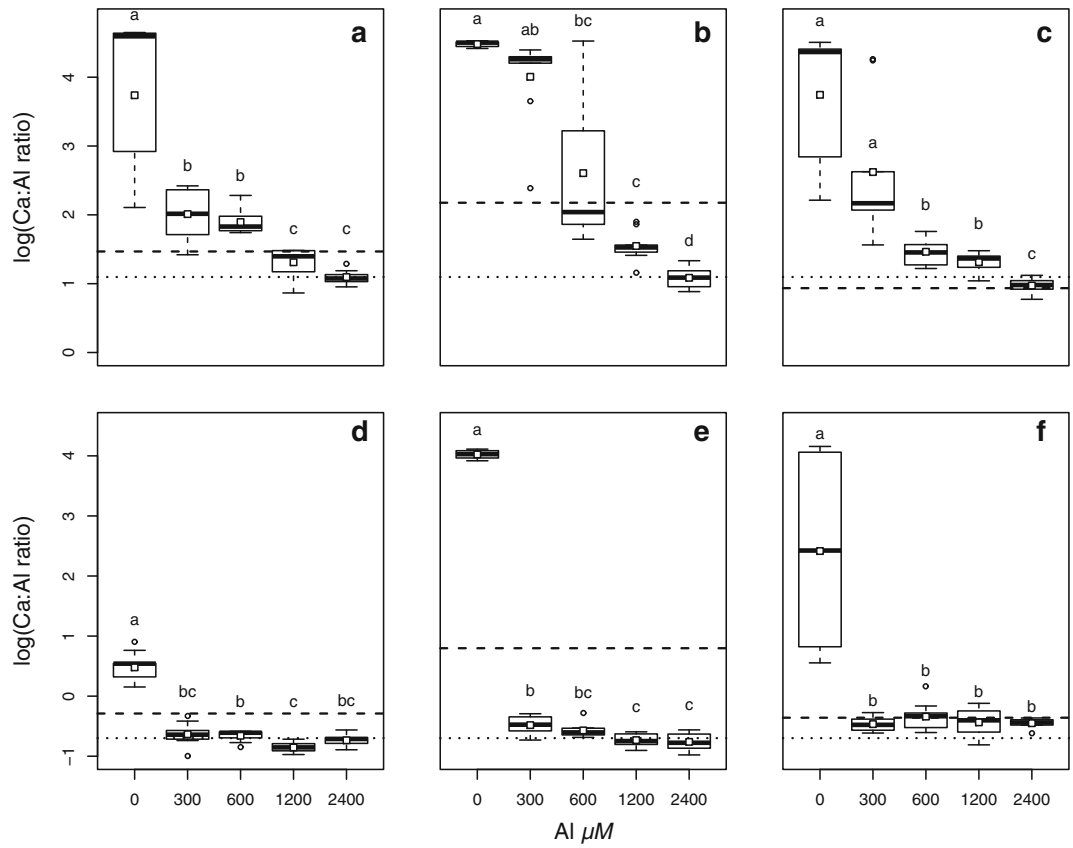


**Fig. 6** Boxplots of log-transformed Ca:Al molar ratios in leaf tissue (*upper row*) and root tissue (*lower row*) of *C. odorata* (**a**, **d**), *H. americanus* (**b**, **e**), and *T. chrysantha* (**c**, **f**) by treatment (0, 300, 600, 1200, and 2400 $\mu M$ Al). *Black bars* represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as *white squares*. *Lower case letters* above the boxplots depict significant differences among the treatments at $p < 0.05$. The *dashed line* shows the median in treatment with organic layer leachate. The *dotted line* shows critical Ca:Al molar ratios as Al-stress indicators for leaves and roots, respectively (Cronan and Grigal 1995)

and 6.2±0.4 mg $g^{-1}$, respectively). Only for *H. americanus*, Al concentrations in roots increased significantly from 9.2±0.7 mg $g^{-1}$ at 300 $\mu M$ Al to 19±0.8 mg $g^{-1}$ at 2400 $\mu M$ Al.

The Ca concentrations in leaves (mean±SE for *C. odorata*, *H. americanus*, and *T. chrysantha* in control/2400 $\mu M$ Al, respectively: 19±0.4/7.5±0.6, 14±0.5/6.7±0.7, 11±0.5/9±0.8 mg $g^{-1}$) were significantly different among the treatments. In *C. odorata* and *H. americanus* they first decreased from control to treatment with 300 $\mu M$ Al, increased at 600 $\mu M$ Al and decreased again with further increasing Al treatment. In *T. chrysantha* the mean Ca range was high in leaves treated with 300 $\mu M$ Al and then decreased in treatments with 600 and 1200 $\mu M$ Al and increased again in the highest Al treatment.

The Ca:Al molar ratios in leaf tissue decreased significantly from control to treatment with 2400 $\mu M$ Al in all tree species (Fig. 6). In root tissue, Ca:Al molar ratios of all plants were distinctly higher in control than in all other treatments (Fig. 6).

The TOC concentrations in nutrient solution after weekly treatment increased significantly in the treatments above 300 $\mu M$ Al (Fig. 7).

## Organic layer leachate experiment

The mean of shoot and root biomass (Fig. 1), healthy leaf area, and number of root tips (Fig. 2) for all tree species was lower in the treatment with organic layer leachate than in control and all Al treatments (Table 3). The mean root diameter of *C. odorata* and *H. americanus* was lower in the organic layer leachate than in control and all Al treatments. The mean of root-to-shoot biomass ratios of the seedlings of all species grown in organic layer leachate was higher than that of the control but lower than the mean of all Al treatments (Table 3). The mean diseased leaf area (in % of total leaf area) of plants grown in organic layer leachate exceeded that of all other treatments for *T. chrysantha* and the majority of the other treatments for *C. odorata* and *H. americanus* (Table 3).

Mean Ca concentrations in leaves of plants treated with organic layer leachate were lower than in plants of the control treatment and ranged among the Al treatments.

The TOC concentrations in organic layer leachate after weekly treatment were with 32.8±6.4, 32.4±5.4, and 30.6±5.5 mg $L^{-1}$ (mean±SE for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively) almost 20 times higher than in Hoagland nutrient solution. The concentrations of $NH_4^+$-N in organic layer leachate decreased during the weekly treatments significantly for *C. odorata* and *H. americanus* from 1.65±0.3 mg $L^{-1}$ to 0.6±0.2 and 0.4±0.1 mg $L^{-1}$, respectively. The concentrations of $NO_3^-$-N and $PO_4^{3-}$-P in organic layer leachate decreased significantly in the course of the experiment, illustrating substantial nutrient depletion by plant uptake for *H. americanus* only (from 1.1±0.5 and 0.2±0.08 mg $L^{-1}$ to

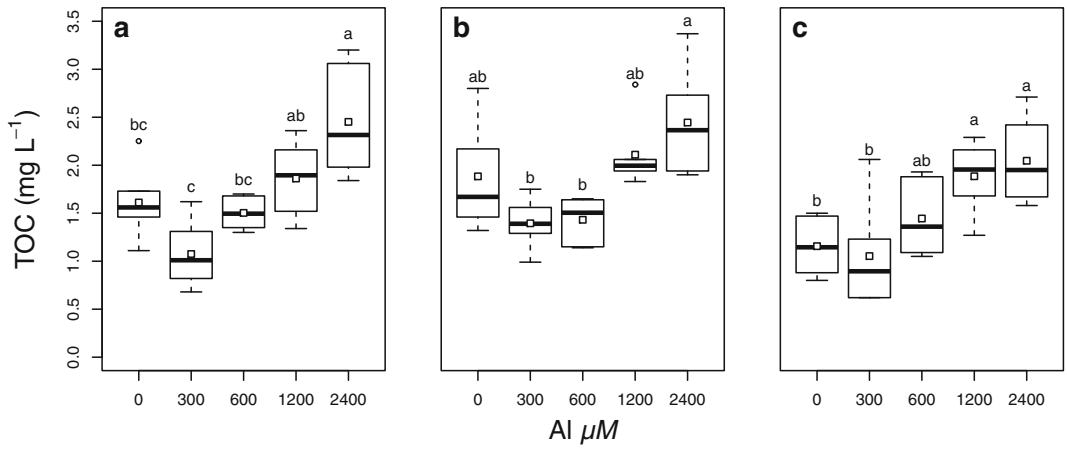


**Fig. 7** Boxplots of TOC concentrations (mg $L^{-1}$) in nutrient solution after weekly treatment of *C. odorata* (**a**), *H. americanus* (**b**), and *T. chrysantha* (**c**) by treatment (0, 300, 600, 1200, and 2400 $\mu M$ Al). N=6, *black bars* represent the median, *whiskers* represent the minimum-maximum range of the group data. Group means are given as *white squares*. *Lower case* letters above the boxplots depict significant differences among the treatments at $p < 0.05$

0.1±0.08 and 0.03±0.01 mg $L^{-1}$, respectively), but not for the other two species.

## Discussion

### Aluminum effects on plant biomass and morphology

Several plant properties were negatively affected by increased dissolved Al concentrations. For most properties, there was no significant difference between control and treatment with 300 $\mu M$ Al, as well as among treatments with 600, 1200, and 2400 $\mu M$ Al, suggesting that Al concentrations of 300 $\mu M$ must be exceeded to induce significant negative effects on biomass and morphology (Figs. 1 and 2). Significant reduction of plant biomass at Al concentrations of 250 $\mu M$ was reported for red spruce (*Picea rubens* Sarg.) seedlings by Thornton et al. (1987). The reason for lacking plant responses to low Al concentrations may be related with efficient defense mechanisms against Al effects. Some of the mechanisms counteracting Al stress are release of chelating agents like citrate, malate, and oxalate from the root apex which form non-toxic Al-complexes, or phosphate, that increases pH of the rhizosphere and precipitates insoluble compounds which cannot be taken up by plants (Brunner and Sperisen 2013; Hafkenscheid 2000; Pellet et al. 1997; Pellet et al. 1995). The increasing TOC concentrations in nutrient solutions of the high Al treatments suggest that the plants responded to Al stress by the release of chelating compounds (Fig. 7).

Surprisingly, aboveground plant properties (shoot biomass and leaf area) responded more sensitively to Al stress than root properties (Fig. 1 and 2). While shoot biomass decreased by 44 to 73 % compared to control, root biomass either remained unchanged across the treatments (*C. odorata*), declined (*H. americanus*) or was even stimulated al low Al concentrations of 300 $\mu M$ and then decreased again at higher Al concentrations (*T. chrysantha*). This pattern shows that Al was not interacting directly with the root apex but possibly disturbed nutrient uptake.

The decrease of Ca concentrations in leaf tissue might result from an inhibited long distance transport of Ca, induced by Al blocking Ca channels (Huang et al. 1992; Rengel 1992), which contributes to a negative shoot biomass response to elevated Al concentrations. In *T. chrysantha* the Ca decrease was less pronounced than in the other two species, indicating less blocking of Ca transport, which is in line with the less negative response of aboveground biomass production compared to the other two tree species. Compared to other tree species from the same study site, however, even the lowest Ca concentration in leaves was high. Wilcke et al. (2008) reported for *Graffenrieda emarginata* (Ruiz & Pav.), an Al-accumulating plant species, Ca concentrations in leaves of 1.2-3.7 mg $g^{-1}$. In a Ca fertilizing experiment in the Ecuadorian montane forest (Wullaert et al. 2013) trees from control plots had Ca concentrations in leaves ranging from 0.82±0.3 mg $g^{-1}$ (*Myrcia* sp.nov.) up to 3.25±1.05 mg $g^{-1}$ (*Alchornea lojaensis* Secco). Our results demonstrate that the increasing Al concentrations in the solution indeed reduced Ca concentrations in leaves suggesting that reduced Ca translocation in the plant contributed to decreased biomass production. However, even at the highest Al concentration in solution, Ca concentrations in leaves did not drop below those found in the forest.

According to Cronan and Grigal (1995) Ca:Al molar ratios of $< 12.5$ and $< 0.2$ in leaf and root tissue, respectively, can be used as threshold values indicating Al stress to forest trees. In our experiment, Ca:Al molar ratios in leaves of all three plant species only approached the threshold value of 12.5 in the highest Al treatment but did not reach a value below this threshold. However, in the treatment with organic layer leachate, *T. chrysantha* showed a molar Ca:Al ratio below 12.5 suggesting Al stress (Fig. 6). In root tissue of *C. odorata* and *H. americanus* the Ca:Al molar ratios in all Al treatments were close to the threshold value for roots. In *T. chrysantha* Ca:Al molar ratios in roots were in all treatments above the threshold. Wullaert et al. (2013) reported Ca:Al molar ratios in leaves of tree species native to the Ecuadorian montane forest, ranging from 0.31±0.07 in the Al-accumulating *Graffenrieda emarginata* (Ruiz & Pav.) to 71 in *Hieronyma fendleri* Briq. We attribute the high Ca:Al molar ratios to the growth of the seedlings in a particularly Ca-rich substrate in the tree nursery.

Root-to-shoot biomass ratios increased (Fig. 3), contrasting some results in the literature. Thornton et al. (1987) found decreasing root-to-shoot ratios for red spruce seedlings, treated with Al concentrations as high as 2000 $\mu M$. Other studies, however, are more consistent with our findings. Graham (2001) reported

a negative effect of 1000 $\mu M$ Al on peach seedlings (*Prunus persica* (L.) Batsch) in sand culture, reducing number and length of plant lateral shoots, total shoot growth, leaf number, and leaf area, but not root, stem, or leaf dry weight. Kidd and Proctor (2000) investigated the Al tolerance of birch populations (*Betula pendula* Roth) from different ecological sites in a culture solution experiment. In some populations Kidd and Proctor (2000) discovered an enhancement in plant growth at 74 – 185 $\mu M$ Al, followed by growth inhibition at Al concentrations > 370 $\mu M$. Growth of an Al-sensitive population from a calcareous soil was inhibited at all Al concentrations in solution (74 − 1300 $\mu M$). In contrast, growth of Al-tolerant populations increased with increasing Al concentrations up to 926 $\mu M$ in solution.

Furthermore, a recent study by Hajiboland et al. (2013) revealed stimulation of root growth in a tea plant (*Camellia sinensis* (L.) Kuntze) at 300 $\mu M$ Al. Beneficial effects in culture solution for both, root and shoot biomass, were found for pine (*Pinus radiata* D.Don and eucalypt (*Eucalyptus mannifera* Mudie subsp. *mannifera*) seedlings, whereas strongest plant growth of eucalypt occurred at 2222 $\mu M$ Al and of pine at 370 $\mu M$ Al (Huang and Bachelard 1993). The beneficial effects of Al on plant growth are explained by alleviation of $H^+$ toxicity at low pH values, which is ascribed to promoted $H^+$ extrusion and increase of cell membrane electrical polarity (Kinraide 1993). The resulting electrochemical gradient induces a stimulation of nutrient uptake (Osaki et al. 1997).

In our experiment, the number of root tips was unaffected in *C. odorata* and *T. chrysantha*, but reduced by 60 % in *H. americanus*. This pattern is in line with the Al concentrations in roots, which were not significantly different among the Al treatments for *C. odorata* and *T. chrysantha* but increased for *H. americanus* with increasing Al cocentrations in the nutrient solution. The higher sensitivity of *H. americanus* can be explained by the fact that the number of root tips in the control was 6 to 7 times higher than of the other two species, illustrating a highly dispersed root architecture, which might be particularly vulnerable to Al stress (Table 3).

## Sensitivity to Al exposure

Schaedle et al. (1989) classified sensitive species with growth effects at Al concentrations below 150 $\mu M$, which include honeylocust (*Gleditsia triacanthos* L.), coffee (*Coffea arabica* L.), white spruce (*Picea glauca* Voss), and peach (*Prunus persica* (L.) Batsch), intermediately sensitive species responding between 150 and 800 $\mu M$ Al which include sugar maple (*Acer saccharum* Marsch.), red (*Picea rubens* Sarg.) and black spruce (*Picea mariana* Mill.), European beech (*Fagus sylvatica* L.) and loblolly pine (*Pinus taeda* L.), and resistant species which only respond to Al concentrations above 800 $\mu M$. To directly compare the response of our studied tree species with those in the review of Schaedle et al. (1989), we calculated the EC10 values for aboveground biomass, as aboveground biomass was highly susceptible to Al toxicity. According to the classification by Schaedle et al. (1989) under in-vitro conditions, the light-demanding fast-growing short-lived pioneer species *H. americanus* (EC10 126 $\mu M$) can be classified as sensitive species, and the long-lived pioneers *C. odorata* (EC10 238 $\mu M$) and *T. chrysantha* (EC10 376 $\mu M$) as intermediately sensitive species.

## Hoagland nutrient solution versus organic layer leachate

Plants grew generally worse and showed more damages when grown in organic layer leachate than in treatments with Hoagland nutrient solution, irrespective of Al concentration. Accordingly, the highly complex matrix of the organic layer leachate, i.e. nutrient scarcity and possibly complexation of essential micronutrients by organic compounds, must pose problems influencing plant growth more than dissolved Al.

Although pH values in the organic layer leachate were below 5.5, mean total Al concentrations in solution were low (< 44±11 $\mu M$, Table 1) and > 97 % were generally bound in organo-Al-complexes (Wullaert et al. 2013), which are known to mask and detoxify Al. At present, our results indicate limitation of biomass production of *C. odorata*, *H. americanus*, and *T. chrysantha* by other factors than Al phytotoxicity in the organic layer leachate. The most likely reason are the low concentrations of nutrients (Table 1). This explanation would be in line with findings of Homeier et al. (2012), who recently reported limitation of aboveground productivity in the studied area by simultaneous N and P scarcity. Accordingly, plant-available N and P concentrations in the organic

layer leachate tended to decrease during the treatment. However, the reduction of $NH_4^+$-N was only significant for *C. odorata* and *H. americanus*. The reduction of $NO_3^-$-N and $PO_4^{3-}$-P was only significant for *H. americanus*, indicating that either N and P uptake is complicated by other factors for *C. odorata* and *H. americanus* or that other nutrients which we did not consider (e.g. B) could also play a role in limitation of aboveground biomass productivity of the tropical montane forests in southern Ecuador.

## Conclusions

1. Aluminum stress caused negative effects on root and shoot morphology of *C. odorata*, *H. americanus*, and *T. chrysantha* above 150 $\mu M$ of dissolved Al. Yet, shoot properties were stronger affected than root properties.
2. The short-lived pioneer *H. americanus* was most sensitive to Al toxicity confirming that highly productive pioneer tree species could be considered as more vulnerable to Al stress than old-growth forest tree species.
3. Based on the Al concentrations in organic layer leachate which fall below critical Al concentrations detected in our experiment, there are no indications for an important role of Al toxicity at our study site.

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