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REGULAR ARTICLE

Effects of heavy metal soil pollution and acid rain on growth and water use efficiency of a young model forest ecosystem

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Abstract In a 4-year lysimeter experiment, we investigated the effects of topsoil heavy metal pollution (3,000 mg kg^{-1} Zn, 640 mg kg^{-1} Cu, 90 mg kg^{-1} Pb and 10 mg kg^{-1} Cd) and (synthetic) acid rain (pH 3.5) on tree growth and water use efficiency of young forest ecosystems consisting of Norway spruce (*Picea abies*), willow (*Salix viminalis*), poplar (*Populus tremula*) and birch (*Betula pendula*) trees and a variety of understorey plants. The treatments were applied in a Latin square factorial design (contaminated vs uncontaminated topsoil, acidified rain vs ambient rain) to 16 open-top chambers, with 4 replicates each. Each open-top chamber contained two lysimeters, one with a calcareous, and the other with acidic subsoil. The four tree species responded quite differently to heavy metal pollution and type of subsoil. The fine root mass, which was only sampled at the end of the experiment in 2003, was significantly reduced by heavy metal pollution in *P. abies*, *P. tremula* and *B. pendula*, but not in *S. viminalis*. The metal treatment responses of above-ground biomass and leaf area varied between years. In 2002, the heavy metal treatment reduced above-ground biomass and leaf area in *P. tremula*, but not in the other species. In 2003, metals did not reduce above-ground growth in *P. tremula,* but did so in the other species. It appears that the responses in above-ground biomass and leaf area, which paralleled each other, were related to changes in the relative competitive strength of the various species in the two experimental years. *S. viminalis* gained relative to *P. tremula* in absence of metal stress, in particular on calcareous subsoil, while *P. abies* showed the largest increases in growth rates in all treatments. Above- and below-ground growth was strongly inhibited by acidic subsoil in *S. viminalis* and to a lesser degree also in *P. abies*. In *P. abies*, this subsoil effect was enhanced by metal stress. Acid rain was not found to have any substantial effect. Whole-system water use efficiency was reduced by metal stress and higher on calcareous than on acidic subsoil.

Keywords Acid rain · Acidic subsoil · Biomass · Calcareous subsoil · Forest ecosystem · Heavy metals · Lysimeters · Soil pollution · Water use efficiency

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Introduction

Apart from large areas of agricultural land, there are also many sites at which forest soils have been polluted by various heavy metals (Hüttl and Schneider 1998; Bergkvist et al. 1989), in particular through atmospheric deposition from industrial and traffic emissions (Steinnes and Friedland 2006; Spellerberg 1998; Kahle 1993), shooting activities (Bennett et al. 2007; Cao et al. 2003; Darling and Thomas 2003) or waste disposal including landfills (Toribio and Romanya 2006; Lim and Chu 2006). Heavy metal pollution has been found to decrease forest productivity (Pukacki and Kaminska-Rozek 2002), vitality, biodiversity and stand structure (Koptsik et al. 2004; Chernenkova and Kuperman 1999). The risks from heavy metal pollution depend on the types and concentrations of metals, soil properties such as pH, CEC, redox potential, soil texture, clay and organic matter content etc (Prasad 1997). Soil pH is a key factor, which determines metal availability to and toxicity for plants (Krebs et al. 1998). The upper horizons of many forest soils are quite acidic, resulting in much higher risks of metal leaching and uptake by plants than on agricultural soils, which are usually maintained at close-to-neutral pH conditions (Kahle 1993). Decrease of soil pH due to acid rain (Watmough et al. 1999; Walker and Mclaughlin 1993) has increased forest soil acidity in many parts of Europe and North America in the past and aggravated the problem of metal leaching and toxicity (Lapenis et al. 2004; Barton et al. 2002; Kahle 1993; Guo et al. 2005).

Elevated concentrations of heavy metals have been found to damage root growth and functions (Rautio et al. 2005; Arduini et al. 1994, 1995; Ewais 1997; Helmisaari et al. 1999; Karolewski and Giertych 1994; Oberlander and Roth 1978), impairing the water relations of affected plants (Poschenrieder and Barceló 1999). Metal effects on trees were mostly studied at the seedling stage in pot experiments or hydroponic cultures. While these studies provided valuable information about physiological responses to metal stress under well-defined conditions, they are not representative for field conditions, where roots are not confined to a small volume of usually uniform soil substrate and where the plants in general have to compete with other plants for above- and below-ground resources. Furthermore, the duration of pot experiments is comparatively short (several days or weeks). On the other hand, studies comparing the responses of plants grown on metal-contaminated soils under field conditions with plants grown on uncontaminated or less-contaminated reference sites (Keller 2005) face the problem to separate metal effects from the effects of other factors, in particular soil factors, given the often extreme spatial variability of soils in the field.

In order to avoid the problems of these two approaches, a model ecosystem experiment was performed at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland, in which mixed coniferous-deciduous young forest stands were replicated in lysimeters with soil and exposed to different combinations of well-defined treatments in a factorial design. The goal was to study the effects of a mixed heavy metal (HM) contamination of the topsoil (mimicking the deposition of metal-contaminated dust from a metal smelter) under the influence of different acidities of (synthetic) rain water and two types of uncontaminated subsoil (calcareous vs acidic) on various physical, chemical and biological processes and functions from the sub-cellular to the ecosystem scale (Hermle et al. 2006, 2007; Matyssek et al. 2006; Nowack et al. 2006). The motivation behind the experiment was to explore the potential of commercial trees to be used for the phytostabilization of metal-contaminated sites (Robinson et al. 2006). The heavy metal treatment significantly reduced root growth and evapotranspiration (ET), increasing soil wetness and drainage discharge (Menon et al. 2005). After the vegetation had been established in the year 2000, the type of subsoil at first had the strongest influence on ET in the two subsequent years 2001 and 2002, being substantially higher on calcareous than acidic subsoil. In the last experimental year (2003), this difference had disappeared. In contrast, there was no significant influence of acidified irrigation on the water regime, although it increased dissolved Zn and Cd concentrations in the topsoil (Rais 2005).

The study presented here was performed in the framework of the model-ecosystem experiment outlined before. The objective was to investigate the treatment effects on the growth of the young trees and the overall water use efficiency of the stands. At the end of the experiment in fall 2003, all trees were harvested and analyzed biometrically, allowing us to compare growth responses between the various tree species and to relate them to the water balances of the lysimeters. Based on the analysis of twig and leaf samples collected in previous years (Hermle et al. 2006), our hypothesis was that the responses to the metal treatments would vary strongly among the different tree species, and that they would depend strongly on subsoil, but little on irrigation acidity. Independently of such variations, we expected that metal stress would reduce water use efficiency on the whole-system level.

Materials and methods

Lysimeters and soils

The experiment was carried out in the open-top chamber (OTC) facility of the Swiss Federal Institute of Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. Each of the 16 hexagonal and 3 m high OTCs was surrounded by glass walls. These were kept partially open in order to increase ventilation. The glass roofs of the OTC were set to close automatically upon the onset of rain. Below ground, each OTC was divided into two lysimeters of 1.5 m depth and 3 m^2 surface area each. Each lysimeter was filled in summer 1999 from bottom to top with a 0.5 m thick quartz sand drainage packing, a 0.8 m subsoil layer and a 0.15 m topsoil layer. The drainage layer consisted of three sub-layers with increasingly coarse grain sizes (0.7–1.2, 1.5–2.2 and 5–8 mm grain diameters, respectively) towards the concrete bottom of the lysimeters. In each OTC, the subsoil of one lysimeter was a calcareous sandy loam (pH 7.4) originating from a Calcaric Fluvisol along the river Aare, while the subsoil of the other lysimeter was an acidic loamy sand (pH 4.2) originating from a Haplic Alisol along the river Rhine, both from sites in the vicinity of Zurich. The topsoil was the same in all lysimeters. It was non-calcareous, loamy and slightly acidic (pH 6.4–6.7), taken from an arable field in the vicinity of Zurich. Physico-chemical properties of the three soil materials are given in Table 1.

Table 1 Physical and chemical properties of the soil materials used in the experiment

	Topsoil	Acidic subsoil	Calcareous subsoil
Texture (% sand; silt; clay)	36; 49; 15	87; 8; 5	74; 16; 10
pH (0.01 M $CaCl_2$)	6.55±0.12	4.2	7.4
$C_{inorganic}$ ($g.kg^{-1}$)	<1	<1	21
$C_{organic}$ ($g.kg^{-1}$)	15.1	3.2	11.2
N_{total} ($g.kg^{-1}$)	1.5	<0.3	0.6
$C_{organic}/N_{total}$	10	n.d.	18
$P_{extractable}$ ($g.kg^{-1}$)	1160	298	296
$P_{organic}$ ($g.kg^{-1}$)	862	84	54
$P_{available}$ ($g.kg^{-1}$)	49±5	18	11
$K_{exchageable}$ ($g.kg^{-1}$)	283	23	21
Cation exchange capacity ($mmol_c.kg^{-1}$)	55	12	67
Base saturation (%)	99.9	35.9	99.9
Cu_{total} ($g.kg^{-1}$)	28	7.4	14
Zn_{total} ($g.kg^{-1}$)	97	39	58
Cd_{total} ($g.kg^{-1}$)	0.1	<0.2	0.2
Pb_{total} ($g.kg^{-1}$)	37	13	19

Plantation

In spring 2000 the same selection of trees and understorey plants was planted in each lysimeter, consisting of one rooted cutting of birch (*Betula pendula* Roth), one rooted cutting of willow (*Salix viminalis* L.), two poplar cuttings without roots (*Populus tremula* L.) and three 3-year-old trees of Norway spruce (*P. abies* (L.) *Karst.*). All cuttings were six months old and planted before bud break. The spruce trees represented three different Swiss provenances: Bremgarten, Murg and Conters, the poplar trees two provenances: Birmensdorf and Orvin. The understorey was composed of wood sedge (*Carex sylvatica* Hudson), ramsons (*Allium ursinum* L.), tansy (*Tanacetum vulgare* L) and various small tree seedlings. The birches were harvested at the end of the first growing season and replaced by new plants in the subsequent spring. The plants were arranged in such a way that a mixed distribution was obtained and that the ground was covered as evenly as possible. Positions were fixed with respect to groups of plants, i.e. for deciduous trees, conifers or understorey plants, but random within each group. More details on the plantation are given by Hermle et al. (2006).

Treatments

In eight chambers, the topsoil was artificially contaminated with filter dust from a non-ferrous metal smelter containing approximately 654 g kg^{-1} Zn, 65 g kg^{-1} Cu, 12 g kg^{-1} Pb and 270 mg kg^{-1} Cd. The metals were present in the dust in the form of oxides (predominantly zincite (around 85%)), brass (around 10% $Cu_{0.6}Zn_{0.4}$) and minor amounts of other minerals (<1%). Further cadmium was added in the form of cadmium oxide to raise its Cd content by approximately a factor of ten. The dust was mixed into the topsoil by using a small harrow. The final total metal concentrations of the contaminated topsoil were 3000 mg kg^{-1} Zn, 640 mg kg^{-1} Cu, 90 mg kg^{-1} Pb and 10 mg kg^{-1} Cd (Nowack et al. 2006), as compared to total concentrations of 97 mg kg^{-1} Zn, 28 mg kg^{-1} Cu, 37 mg kg^{-1} Pb and 0.1 mg kg^{-1} Cd in the uncontaminated topsoil (Table 1). Soluble metal concentrations (determined by extraction with 1 M NH_4NO_3) were 683 mg kg^{-1} Zn, 43 mg kg^{-1} Cu, 1.0 mg kg^{-1} Pb and 3.0 mg kg^{-1} Cd in the contaminated topsoil at the end of the experiment, while they were 1.3 mg kg^{-1} Zn, <0.1 mg kg^{-1} Cu, <0.1 mg kg^{-1} Pb and 0.1 mg kg^{-1} Cd in the uncontaminated topsoil.

Irrigation was applied by means of sprinklers (six per soil compartment) with computer-controlled flow meters. The irrigation was scheduled, based on tensiometer measurements, so as to keep the soil moisture content around field capacity. The ion composition of the irrigation water mimicked the 30-year mean ambient rain composition of the site while the pH was adjusted to a value of pH of 5.5 (mean ambient) in eight OTC, and 3.5 in the other eight OTC by addition of HCl. HCl was selected instead of HNO_3 or H_2SO_4 in order to avoid differences in added nutrients between the irrigation treatments.

The metal (contaminated vs uncontaminated topsoil) and acid ‘rain’ (acidified vs ambient ‘rain’ composition) treatments were assigned to the 16 OTC according to a Latin-square design with four replicates for each of the 2×2 combinations. Combined with the variation of the subsoil (acidic vs calcareous) in each OTC this gave a fully balanced factorial design with four replications. The following abbreviations are used to denote the treatments: HM=heavy metal contaminated topsoil, AR=acidified ‘rain’, HMAR=combination of heavy metal contaminated topsoil and acidified ‘rain’, CO=control (uncontaminated topsoil, ambient ‘rain’). The experiment was started with the establishment of the plantation in early spring of the year 2000 and ended with the final harvest in the autumn of 2003.

Soil water regime

Evapotranspiration (ET) was calculated during the growing season for each lysimeter from the water balance equation:

$$\mathrm{ET} = I - D - \Delta S \qquad (1)$$

where ET is evapotranspiration, I is amount of irrigation, D is amount of drainage, and ΔS is the change in soil water storage. Drainage water was collected in plastic containers at the bottom of an access tube in the middle between the two lysimeters. The containers were emptied at weekly or biweekly intervals by means of a pump. In order to obtain ΔS, profile soil water contents were measured periodically by Time Domain Reflectometry (TDR), using a Tektronix 1502B instrument. TDR probes of 25 cm length were installed vertically at 0–25, 25–50, 50–75 and 75–100 cm depth. The recorded TDR signals were calibrated and analysed using the procedure of Roth et al. (1989). WUE (water use efficiency) was calculated for each lysimeter by dividing the yearly above-ground tree biomass production by ET.

Above- and below-ground biomass

Prior to the final harvest, the entire above-ground biomass of poplars and willows was harvested by coppicing at the end of each growing season, while only twig samples were taken from spruce and sampling from birch was restricted to the collection of foliage in 2002 because of the small size of the trees. In autumn 2003, all trees were harvested completely, including roots. Foliage, wood and root biomass were determined separately for each tree. Roots were separated in coarse (>2 mm diameter) and fine (<2 mm diameter) root biomass. Spruce trees were portioned by annual growth increments. Values of the needle and wood mass produced by spruce in 2002 were calculated for each tree from yearly measured stem diameters using the following linear regression equation:

$$\begin{aligned}&\text{Mass produced in current year}\\&= A + B \times \text{Stem diameter}\end{aligned} \qquad (2)$$

where A (intercept) and B (slope) are regression parameters. The equation was parameterized separately for needle and wood mass by linear regression from the respective biometric data obtained from the final harvest in 2003 (R=0.9 for both regression equations).

Leaf area

In each year, ten leaves were selected on each deciduous tree, distributed over the entire plant, and their areas determined using a leaf area meter. In the case of *P. abies*, we used an aliquot of 20 current year needles and determined their projected needle area and dry mass. Total leaf areas were then calculated by multiplying the specific leaf area (leaf area divided by leaf dry mass) of the selected leaves with the dry mass of the total foliage for each tree species and treatment. Understorey plants were not taken into account.

Statistical analysis

The statistical significance of treatment effects was determined by analysis of variance (ANOVA, SAS Institute Inc. Cary NC). According to the 4×4 Latin square arrangement of the treatments between open-top chambers, metal and acid rain effects were tested against 'chamber mean squares'. As subsoils were nested within chambers, their effects and interactions with metal and acid rain effects were tested against 'subplot mean squares' [error term=interaction metal contamination×acid rain × subsoil × block (position of the chamber in the 4×4 arrangement)]. Error bars in figures represent standard errors. The provenances showed the same treatment effects. So data from different provenances were pooled.

Results

Data on aboveground growth and water use are analyzed in the following only for the two experimental years 2002 and 2003, when tree canopies had closed and understorey vegetation had become negligible. Roots were harvested only at the end of experiment in 2003 in order to limit disturbance.

Fine root biomass

The fine root biomass harvested at the end of the experiment was in general significantly reduced in metal treatments, except in the case of *S. viminalis*, where it showed large variations within the treatments (Fig. 1; Table 3). Despite this variation, *S. viminalis* showed the largest subsoil effect on roots: Except for the metal treatment in absence of acid rain, the root mass of *S. viminalis* was much less in the lysimeters with acidic than in those with calcareous subsoil. Acidic subsoil also reduced the

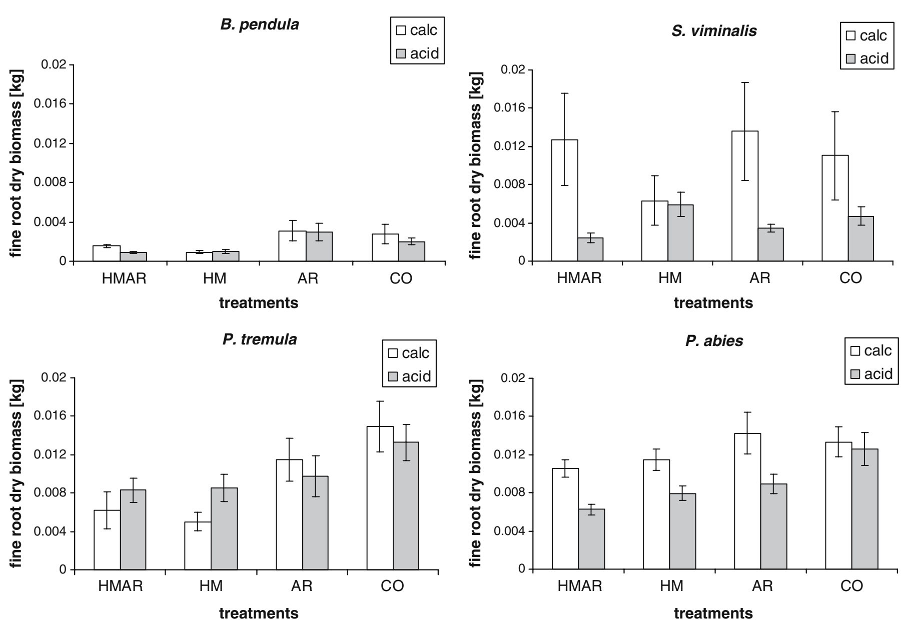

Fig. 1 Fine root dry biomass per tree at the final harvest in autumn 2003 (mean values with standard error bars). Treatments: *CO* control, *AR* acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain. Subsoils: *calc* calcareous, *acid* acidic

Table 2 ANOVA results for the different treatments (level of significance: n.s.=not significant for $P>0.05$); from other species (2002)

2002		HM	AR	HM*AR	Subsoil	HM* Subsoil	AR* Subsoil	Significant difference from another species
Above-ground biomass	B. pendula[a] Bp (n=64)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–
	S. viminalis Sv (n=64)	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	Pt; Pa
	P. tremula Pt (n=128)	0.024	n.s.	n.s.	n.s.	n.s.	n.s.	Sv;
	P. abies Pa (n=192)	n.s.	n.s.	n.s.	0.044	n.s.	n.s.	Pt;
Leaf Area	*S. viminalis* (n=64)	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	Pt; Pa
	P. tremula (n=128)	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	Sv; Pa
	P. abies (n=192)	n.s.	n.s.	n.s.	n.s.	0.043	n.s.	Pt; Sv;
Evapotranspiration	(n=24)	0.001	n.s.	n.s.	0.002	n.s.	n.s.	–
Water use efficiency	(n=24)	0.002	n.s.	n.s.	0.000	n.s.	n.s.	–

[a] Only leaf biomass

fine root growth in *P. abies*, while *P. tremula* showed the opposite subsoil effect on root growth in metal treatments. Acid rain had no significant effect on fine root growth, but showed a tendency to decrease it on acidic subsoil in *S. viminalis* and *P. abies*.

Above ground biomass and leaf area

Overall, the pattern of treatment effects on above-ground growth was similar to that on fine root biomass (Tables 2 and 3). In both experimental years analyzed here, above-ground growth was significantly less on acidic than on calcareous subsoil in *S. viminalis* and *P. abies* (Fig. 2). Above-ground biomass was two to four times as large in *S. viminalis* on calcareous than on acidic subsoil. In *P. abies* the subsoil effect was again particularly strong in combination with heavy metal stress, like the subsoil effect on fine-root biomass. Acidic rain had no significant effect on above-ground growth, except that it favored above-ground growth in *P. abies* on calcareous subsoil in comparison to acidic subsoil in 2003.

The responses of the above-ground growth to the metal treatment contrasted strikingly between the two experimental years (Fig. 2). In 2002, the metal treatment inhibited above-ground growth in *P. tremula*, but not in *S. viminalis* and *P. abies*. In 2003 the opposite was observed, i.e. the metal contamination reduced growth in *S. viminalis* and *P. abies*, but not in *P. tremula*. A comparison of the growth in the two years shows interesting differences between these three species: The growth of *S. viminalis* was smaller in the metal treatments in 2003 than in 2002 (in particular on calcareous subsoil), that of *P. tremula* was reduced in 2003 in the non-metal treatments (except for the acid rain treatment on acidic subsoil), whereas the above-ground growth of *P. abies* was increased in all treatments. This growth increase in *P. abies* was least in the metal treatments on acidic subsoil with less than 30%, but it more than doubled from 2002 to 2003 in all treatments on calcareous subsoil and also in the non-metal treatments on acidic subsoil. As a result, metals had a stronger effect on above-ground biomass of *P. abies* on acidic than on calcareous subsoil.

The treatment responses of leaf area paralleled those of above-ground biomass (Tables 2 and 3, Fig. 3). Also the changes from 2002 to 2003 were very similar, although somewhat less pronounced.

Evapotranspiration and water use efficiency

Figure 4 shows the treatment effects on the overall evapotranspiration of the lysimeters in the two experimental years 2002 and 2003. Metal contamination reduced evapotranspiration on both subsoils in both years. The effect was weak, but significant. In 2002, evapotranspiration was on average 11.3% higher on calcareous than on acidic subsoil. Then it increased more on the acidic than on the calcareous subsoil, so that the difference almost disappeared in 2003. Contrary to our expectation acidic rain did not enhance the metal effect. The whole-system evapotranspiration was closely correlated with the total above-ground biomass and total leaf area of the tree vegetations (Fig. 5).

Table 3 ANOVA results for the different treatments (level of significance: n.s.=not significant for $P>0.05$); from other species and between the years (2003)

2003		HM	AR	HM*AR	Subsoil	HM*Subsoil	AR*Subsoil	Overall difference from another species	Difference to 2002 and interactions
Fine root mass	*B. pendula* (n=64)	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	Sv; Pt; Pa	–
	S. viminalis (n=64)	n.s.	n.s.	n.s.	0.034	n.s.	n.s.	Bp; Pa	–
	P. tremula (n=128)	0.005	n.s.	n.s	n.s	0.007	n.s.	Bp	–
	P. abies (n=192)	0.048	n.s.	n.s.	0.001	n.s	n.s	Bp; Sv	–
Above-ground biomass	*B. pendula* (n=64)	0.010	n.s.	n.s.	n.s.	n.s.	n.s.	Pt; Sv	–
	S. viminalis (n=64)	0.000	n.s.	n.s.	0.000	0.002	n.s.	Bp; Pt	0.019; year*HM 0.032
	P. tremula (n=128)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	Bp; Sv; Pa	0.000; year*HM 0.000
	P. abies (n=192)	0.000	n.s.	n.s.	0.000	0.000	0.002	Pt	0.000; year*HM 0.027
Leaf Area	*B. pendula* (n=64)	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	Pt; Pa; Sv	–
	S. viminalis (n=64)	0.001	n.s.	n.s.	0.000	0.003	n.s.	Bp; Pt; Pa	n.s.
	P. tremula (n=128)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	Bp; Sv; Pa	n.s.
	P. abies (n=192)	0.003	n.s.	n.s.	0.000	0.000	0.001	Bp; Pt; Sv;	0.000; year*HM 0.002
Evapotranspiration	(n=24)	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	–	–
Water use efficiency	(n=24)	0.005	n.s.	n.s.	0.000	n.s	n.s	–	–

In both experimental years 2002 and 2003 the whole-system water use efficiency (WUE), i.e. the above-ground biomass produced per unit amount of evapotranspiration at the whole-system level, was higher on calcareous than on acidic subsoil and considerably reduced on both subsoils in the metal treatments (Fig. 6, Tables 2 and 3). Acid rain did not affect WUE. The treatment effects primarily reflected the corresponding effects (as described above) on biomass, as evapotranspiration only showed very small effects in comparison.

Discussion

The results show that each of the four tree species responded differently to the applied metal and subsoil treatments, and partially the responses were even opposite in their directions. To some extent the effects also varied between the two years of observation. Subsoil effects can be attributed to differences in acidity, but also in nutrient contents (Table 1). Effects on above- and below-ground growth were closely related to each other, but there were also interesting differences.

S. viminalis did not show a metal effect on final root biomass and no metal effect on above-ground biomass in 2002, but in 2003 the above-ground biomass was strongly reduced in metal treatments on calcareous subsoil. The latter response was rather surprising in light of the facts that *S. viminalis* grew much better on the calcareous subsoil, that no metal leaching was found in the calcareous subsoil (Nowack et al. 2006) and that metal stress in the topsoil was the same above both types of subsoil (Rais 2005). In contrast, slightly increased subsoil metal concentrations below metal-contaminated topsoil were observed, as to be expected, only for Zn in the acidic subsoil. It was plausible, therefore, to find enhanced metal effects on acidic subsoil as was the case in *P. abies*. This species showed little growth response to the metal treatments on calcareous subsoil, but reduced growth on contaminated topsoil in combination with acidic subsoil. In the absence of metal stress, *P. abies* was less dependent on the type of subsoil than *S. viminalis*. These findings suggest that *S. viminalis* was so much inhibited by other stress factors on the acidic subsoil that the additional metal stress did not play a role. Such stress may have been due to Al toxicity in the acidic subsoil.

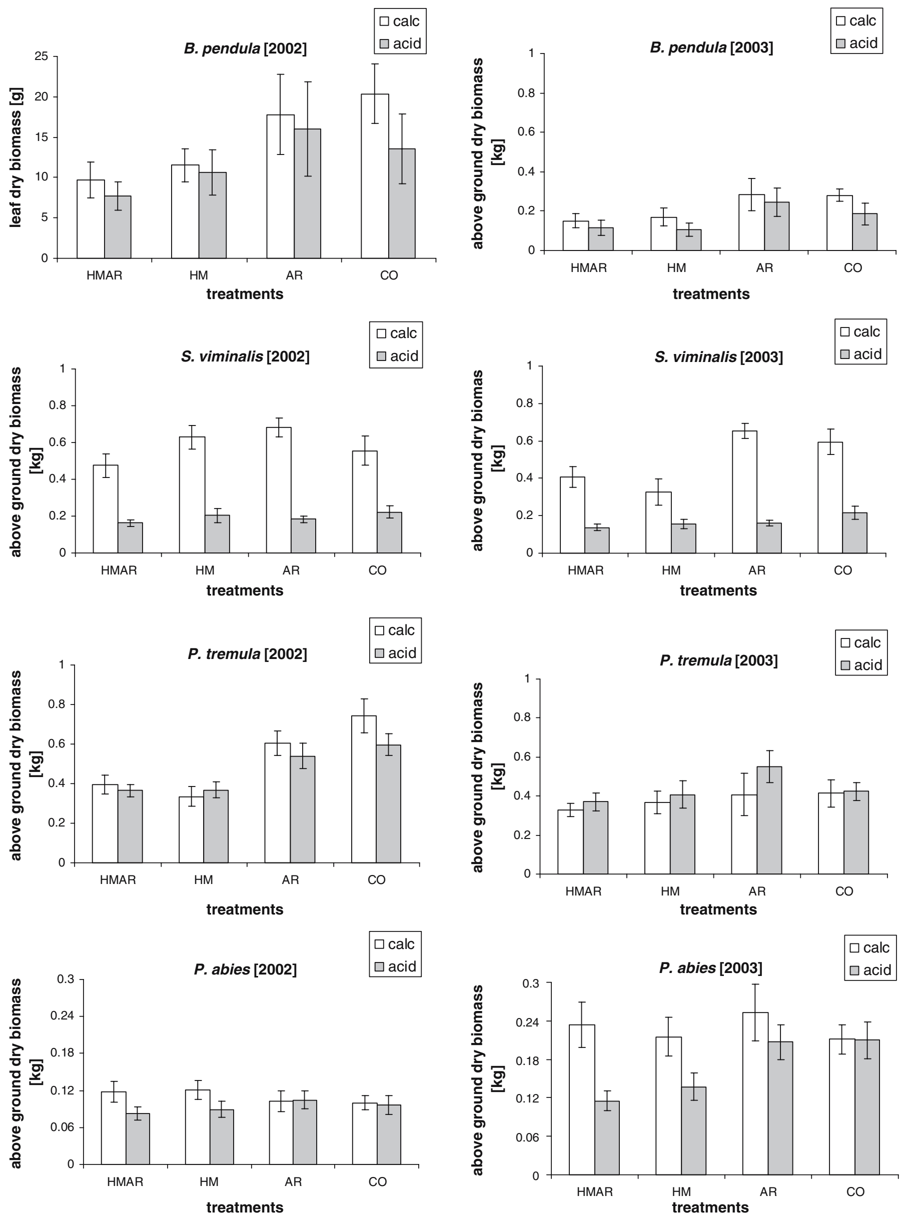

Fig. 2 Above-ground dry biomass produced per tree in the experimental years 2002 and 2003 (mean values with standard error bars). Treatments: *CO* control, *AR* acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain. Subsoils: *calc* calcareous, *acid* acidic

Aluminium was found to decrease fine root growth of rooted cuttings of *S. viminalis* and to reduce the uptake of N, Ca^{2+}, Mg^{2+} and P (Gobran et al. 1993).

In *P. abies*, the sensitivity to the metal contamination became clearly pronounced only in the last experimental year. The lack of a strong effect in the preceding year may have been related to the fact that the trees were still too small to compete efficiently with the willows and poplars, so that the metals could not cause much additional growth retardation. The clear metal effect in the following season agrees with the metal-sensitivity that has been ascribed to this species also in other studies. For example, Godbold et al. (1987) found that the elongation of *P. abies* roots was significantly reduced compared to controls if they were grown in culture solutions with 30 and 60 μM Zn or Cd. In another solution culture study, application of 65 μM Zn or 15 μM Cd reduced root biomass production of spruce seedlings to 68% and 59% of the control treatments, respectively (Godbold et al. 1985).

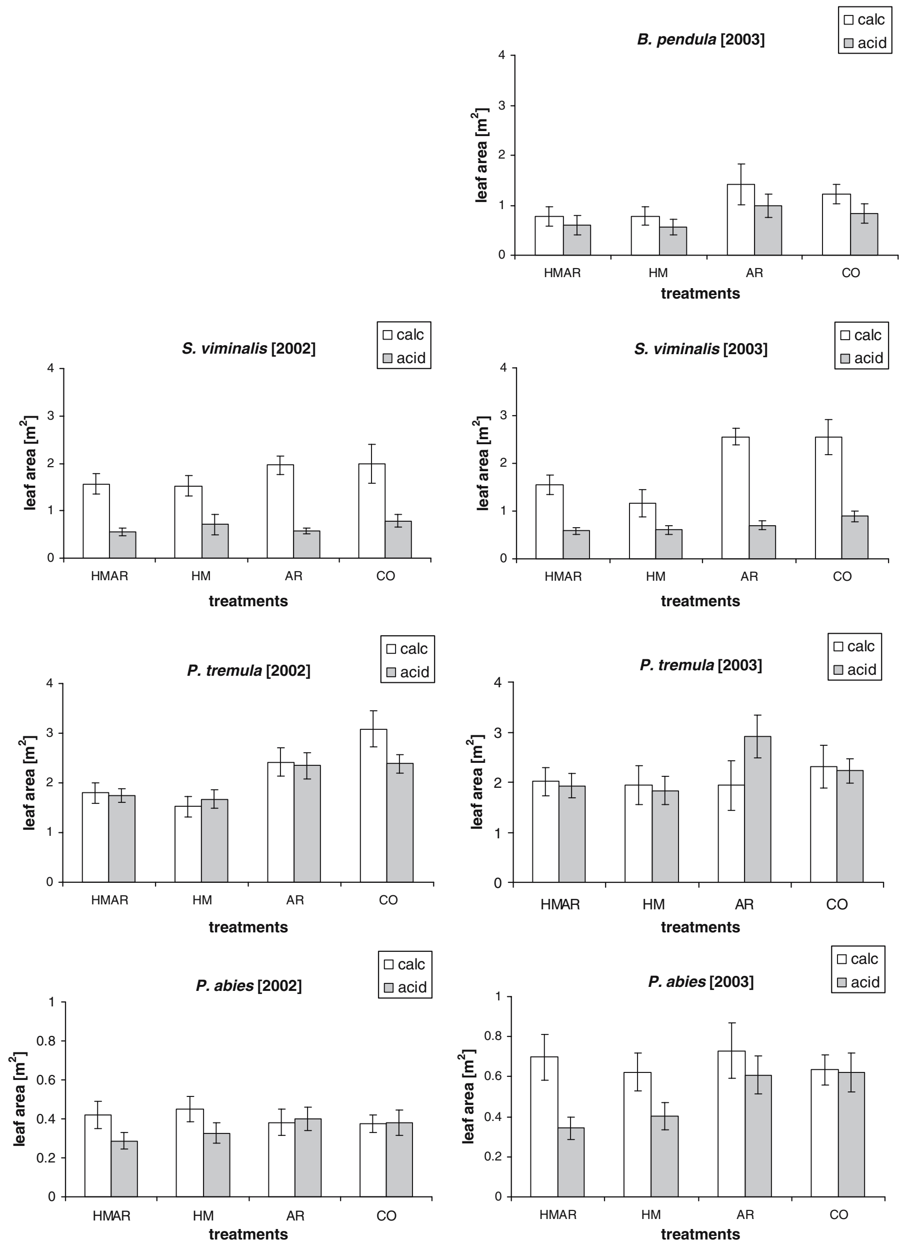

Fig. 3 Leaf area per tree in 2002 (*left*) and 2003 (*right*; mean values with standard error bars). Note differences in scales. No leaf areas were determined for birch in 2002. Treatments: *CO* control, *AR* acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain. Subsoils: *calc* calcareous, *acid* acidic

Also *S. viminalis* has not been portrayed as a particularly metal-resistant species in the literature, at least in comparison to other species of the genus *Salix*. For example, Vandecasteele et al. (2005) compared the metal response of *Salix fragilis* 'Belgisch Rood' and *Salix viminalis* 'Aage' in a greenhouse pot experiment using six field-contaminated soils with Cd levels between 0.9 and 41.4 mg kg^{-1} and found that root biomass and shoot length were significantly smaller in *S. viminalis* than in *S. fragilis* at all Cd levels. Watson et al. (2003) found that *S. viminalis* clones and *S. triandra* produced less biomass under soil metal stress than e.g. *S. burjatica* 'Germany', *S.x dasyclados*, *S. candida* and *S. spaethii*. Hermle (2004) found that the root biomass of *S. viminalis* was significantly reduced and *P. abies* failed to grow at all when seedlings of the same willow clones and spruce provenances as used in the lysimeter experiment here were grown in pots filled with the same soil that was used for the topsoil of the lysimeter experiments and exposed also to the same total metal concentrations as applied in the latter experiment. The only difference in

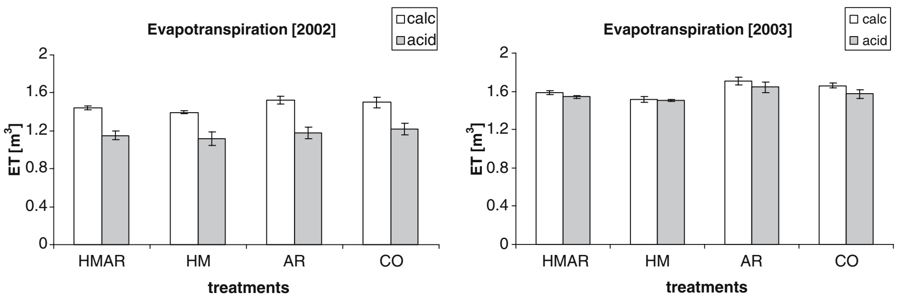

Fig. 4 Whole-system evapotranspiration (*ET*) in the years 2002 and 2003 (mean values with standard error bars). Treatments: *CO* control, AR acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain. Subsoils: *calc* calcareous, *acid* acidic

the contamination was that the metals were applied in dissolved form to the soil in the pot experiment of Hermle (2004), whereas the contamination was mixed into the topsoil of the lysimeters in the form of smelter dust. This suggests that the apparently high metal tolerances of the trees on the lysimeters may have been due to a lower solubility of the metals applied with the smelter dust. Another reason may also have been that the roots had no possibility to evade the contamination in the pots by growing into uncontaminated subsoil. In the lysimeters this possibility existed. Rais (2005) found no significant effect of the metal treatment on the Cu concentrations in the sampled subsoil solutions and only a small increase in dissolved Zn concentrations in acidic subsoil, in no case exceeding the range of dissolved Zn concentrations of the uncontaminated topsoil.

The differences in treatment effects between the two experimental years 2002 and 2003 may to some extent also be due to the different climate conditions. The summer of 2003 was exceptionally hot and dry. Despite the irrigation, moisture stress evolved in late summer (Menon et al. 2005). It is possible that *S. viminalis* was less capable to cope with metal stress than the other tree species, when soil water availability became more and more a limiting factor. As growth was already severely restricted on acidic subsoil, the moisture stress showed little additional effect in willows growing on this type of subsoil, whereas it became effective in combination with metal stress on calcareous subsoil. The different response of *P. abies* suggests that this species was much less sensitive to subsoil and metal stress conditions than *S. viminalis* and that the combination of moisture and metal stress, enhanced by the acidic subsoil, was required to inhibit growth.

Birch was metal-sensitive independent of subsoil type. The absence of any subsoil effect may have been due also to the weak development of the seedlings, limiting the growth of their roots into the subsoil. We found no data in the literature that are directly comparable to our results. Utriainen et al. (1998) found that different clones of *B. pendula* varied considerably in their responses to Zn and Cu. Osteras et al. (2000) observed a high Cd tolerance of birch roots.

Like *B. pendula*, also *P. tremula* differed from *S. viminalis* and *P. abies* by being indifferent to the subsoil. Furthermore, *P. tremula* contrasted *S. viminalis* and *P. abies* in the year-to-year variation in metal effects. Whereas significant metal effects on the growth of the latter only emerged in the last experimental year, the growth reduction observed in *P. tremula* under

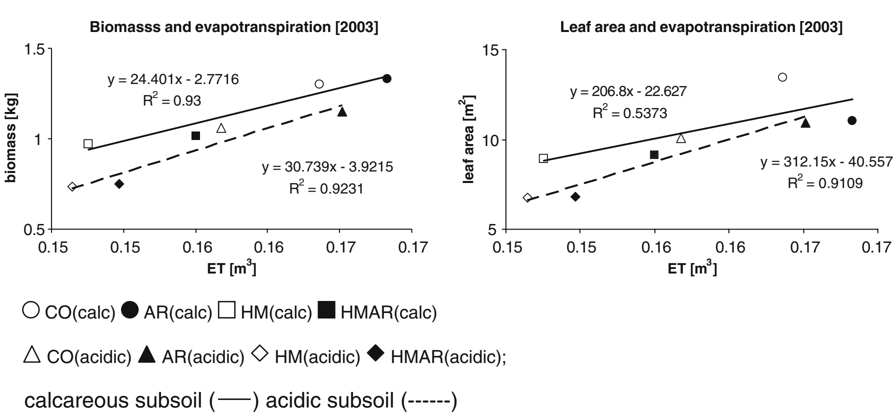

Fig. 5 Relationship between whole-system evapotranspiration (*ET*) and total aboveground biomass (*left*) and between ET and total leaf area of the tree vegetations (*right*) in 2003. Treatments: *CO* control, *AR* acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain

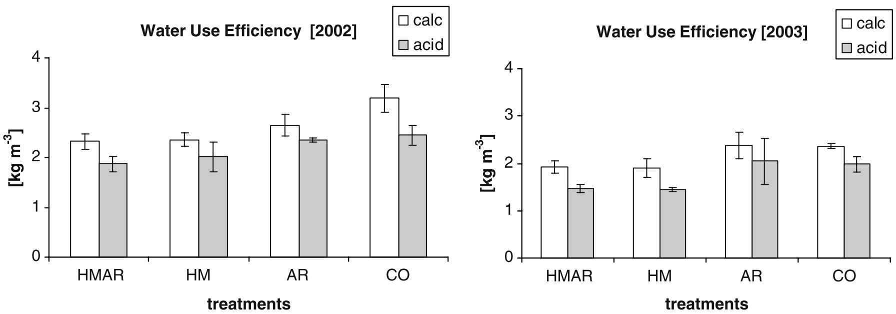

Fig. 6 Whole-system water use efficiency in the years 2002 and 2003 (mean values with standard error bars). Treatments: *CO* control, *AR* acidic rain, *HM* heavy metals in topsoil, *HMAR* heavy metals in topsoil with acidic rain. Subsoils: *calc* calcareous, *acid* acidic

metal stress in the previous year vanished in the last year of the study. As the data reveal, this disappearance was primarily due to a growth reduction in the non-metal treatments on calcareous subsoil and not to a growth increase in the metal treatments. Comparing the growth of the three dominating species *P. tremula*, *P. abies* and *S. viminalis* in the two experimental years, it appears that the expansion of *P. abies* from 2002 to 2003 went on cost of *P. tremula* in the non-metal treatments and primarily on cost of *S. viminalis* in the metal treatments on calcareous subsoil. Thus, the apparent change in metal sensitivity may have been at least partially a result of changes in relative competitive strength of these species, as canopies and root systems became denser and as *P. abies* caught up with *S. viminalis* and *P. tremula*.

Interestingly, there was no substantial acid rain effect on growth and water consumption. It is well known that increased proton activity solubilizes metal cations in soil and thus can enhance metal toxicity. Rais (2005) indeed found that Zn and Cd concentrations were increased by roughly a factor of two in the solutions of contaminated topsoils in the acid rain treatments. The fact that this increase did not translate into negative growth effects means that the input of acidity had other effects overriding any enhancement of metal toxicity effects, such as for example a concomitant mobilization of nutrients like phosphate or potassium.

The observed treatment effects on evapotranspiration represent the summed treatment responses of the individual tree species. Although the available data do not allow us to quantify the individual contribution of each species, the disappearance of the subsoil effect on evapotranspiration in 2003 may have been related to the fact that *P. abies* biomass increased more on the acidic subsoil than on the calcareous subsoil in proportion to the other species. Water use efficiency decreased in the metal treatments as expected and in agreement with findings of other studies. Maustakas et al. (1997) found that wheat plants grown on Cu-rich soil exhibited considerable less water use efficiency than plants grown on fertile soil. A copper-induced decrease of water use efficiency was also observed in solution-cultured pea plants (Angelov et al. 1993). On the other hand, it has also been reported that soil metal stress did not influence WUE although reducing growth. For example in the study of Maustakas et al. (1997), aluminium toxicity reduced transpiration and net CO_2 assimilation to a similar extent in *Thiopyrum bessarabicum*. Becerril et al. (1989) found that different metals may have different effects on transpiration and growth in the same plant: At similar leaf metal concentrations, Pb caused a drastic reduction of water use efficiency, while Cd inhibited transpiration and carbon assimilation to a similar degree and thus did not change WUE. Our study shows, to our best knowledge for the first time, that also the type of subsoil can have a strong effect on WUE, and interestingly the difference in WUE remained, while the difference in ET between lysimeters with different subsoils disappeared in the last experimental year.

In conclusion, this study shows how differently young trees of different species can respond to heavy metal soil contamination under near-natural conditions, how much such responses may depend on subsoil properties and how much they may vary from year to year in developing stands under conditions of evolving competition.

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