Plant Soil (2012) 358:403–415
DOI 10.1007/s11104-012-1183-x

REGULAR ARTICLE

# Response of *Populus tremula* to heterogeneous B distributions in soil

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Received: 16 November 2011 / Accepted: 10 February 2012 / Published online: 8 March 2012


**Abstract**
*Background* Poplars accumulate inordinate amounts of B in their leaves and are candidate plants for the remediation of B contaminated soil. We aimed to determine the effect of heterogeneous B distribution in soil by comparing the growth and B accumulation of young *Populus tremula* trees growing in soil with heterogeneous and homogeneous B distributions.
*Methods* The first of two experiments focused on the tolerance and B accumulation of *P. tremula* under heterogeneous soil B distributions, while the second was designed to study fine root growth under such conditions in detail.
*Results* Growth and B accumulation of *P. tremula* were unaffected by the spatial distribution of B. Root and shoot growth were both reduced simultaneously when leaf B concentrations increased above 800 mg $kg^{-1}$. In the heterogeneous soil B treatments, root growth was more reduced in spiked soil portions with B concentrations >20 mg $kg^{-1}$. Fine root length growth was stronger inhibited by B stress than secondary growth.
*Conclusions* The root growth responses of *P. tremula* to B are primarily a systemic effect induced by shoot B toxicity and local toxicity effects on roots become dominant only at rather high soil B concentrations. Local heterogeneity in soil B should have little influence on the phytoremediation of contaminated sites.

**Keywords** Boron · Heterogeneity · Local and systemic response · Root traits

**Abbreviations**
B Boron
NR Neutron radiography
FR Fine roots

Responsible Editor: Juan Barcelo.

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## Introduction

### Boron in soils

Boron (B) is an essential plant and animal micronutrient that becomes toxic at elevated concentrations (Mastromatteo and Sullivan 1994; Salisbury and Ross 1992; Xu et al. 2007). Boron deficiency symptoms

become evident in plants at tissue concentrations of 10–30 mg $kg^{-1}$ (Bell 1997), while tissue concentrations >100 mg $kg^{-1}$ are toxic for most plants. However, there is a wide range of toxicity thresholds in plants, sometimes even among varieties of the same species (Gupta et al. 1985; Nable et al. 1997).

Boron uptake by plants is closely related to soil B concentrations, which depend on geological parent material, soil type, soil pH, soil organic matter, natural and anthropogenic inputs and soil temperature (Goldberg 1997; Goldberg et al. 1993). High B concentrations occur naturally in many arid soils. Human activities leading to high soil B concentrations include the irrigation of agricultural fields with B laden waste or ground water, surface mining and the application of fly ash on agricultural fields (Nable et al. 1997; Parks and Edwards 2005).

Boron retention in soils generally increases with pH, organic matter and clay content (Goldberg 1997). The major form of B in the soil solution is boric acid, $H_3BO_3$ ($pK_a$: 9.24) (Power and Woods 1997). Since soils generally have a pH <9.24, the neutral species is predominant in the soil solution of most soils (Hu and Brown 1997). Soil organic matter has a higher capacity for B sorption than mineral soil constituents (Goldberg 1997). Sorbed B is released into soil solution when B-binding soil organic matter gets decomposed by microbial activity (Evans and Sparks 1983; Van et al. 2005). Boron adsorption on soil mineral surfaces takes place rapidly via ligand exchange (Goldberg 1997). It can usually be described by Langmuir and Freundlich isotherms (Communar et al. 2004; Goldberg et al. 1993).

## Phytomanagement of boron

Boron contamination should be well suited for phytoremediation, as B is very mobile in soils and readily taken up by plants. Boron is primarily transported by convection with the flow of soil water (Blevins and Lukaszewski 1998; Ishak et al. 2002; Kabata-Pendias and Pendias 2001). It is taken up by plants as undissociated $H_3BO_3$, which is small enough to pass through cell membranes via aquaporins (Tanaka and Fujiwara 2008). Passive uptake and translocation with the stream of transpiration water explains the results of Rees et al. (2011) who found the highest B concentrations in the oldest leaves of a hybrid poplar clone. In B-deficient conditions, active uptake may occur in some plant species (Miwa and Fujiwara 2010). Poplars are widely used for the phytomanagement of contaminated sites and are particularly suited for the revegetation of B contaminated sites because they are tolerant of high soil and leaf B concentrations (Bañuelos et al. 2010; Robinson et al. 2007, 2009). The species of *Populus* used in this experiment, *P. tremula* is known for its adaptability to a wide range of climate and soil conditions, is highly drought tolerant and may be the world's most widely distributed tree species (Worrell 1995). The B tolerance and accumulation potential of *P. tremula* has not been evaluated, however *P. tremula* is tolerant of elevated soil loadings of other trace elements such as Zn and Cd (Dos Santos Utmazian et al. 2007; Menon et al. 2007a).

## Heterogeneity and phytomanagement

While test trials in growth chambers or greenhouses provide useful information about the potential use of plants in phytomanagement, the performance of the same plants may be vastly different in the field. One important problem is the heterogeneous distribution of contaminants in field soils, which is usually not represented in pot experiments (Robinson et al. 2009; Wenzel 2009). This may affect contaminant tolerance and uptake, which are of critical importance in phytomanagement.

Soil B concentrations may change by an order of magnitude or more over just a few centimeters (Hall 1971; McLaren and Cameron 1996). Plant root growth can respond to soil patches with increased trace element concentrations in three different ways: reduction/avoidance (Breckle and Kahle 1992; Moradi et al. 2009b), indifference (Moradi et al. 2009b) or proliferation (Liu et al. 2010; Schwartz et al. 1999; Whiting et al. 2000). In contrast to macronutrient patchiness effects (Robinson 1994), little is known about the response of roots to B enriched soil patches. Menon et al. (2007b) found that roots of *Lupinus albus* L. did not grow into patches of high B concentrations in soil.

Poplars are plastic in their responses to variations in soil conditions (Friend et al. 1999; Mulia and Dupraz 2006). Rees et al. (2011) observed that the root:shoot ratio decreased in young poplar plants with increasing soil B concentration, indicating that root biomass responds more sensitively to B stress than the aboveground biomass in poplar. Adjusting the morphology of their fine roots (FR) to varying soil conditions

enables them to adapt to a wide range of stress conditions in soil (Pregitzer and Friend 1996). Fine root diameter and particularly relative FR diameter class length (ratio of FR length per diameter class/total FR length) are sensitive parameters often used to assess the response of FR to changing soil chemical conditions (Borken et al. 2007; Zobel 2008). However, there are no studies on the effect of patchy soil B distributions on poplar root growth.

Given the lack of knowledge on root responses to heterogeneous B distributions in soil and the potential implications of these responses for the phytomanagement of B-contaminated sites, the objectives of this study were to investigate (1) whether *P. tremula* tolerates higher average soil B concentrations when B is heterogeneously distributed than when it is homogeneously distributed, (2) how *P. tremula* roots respond to B-enriched soil patches, and (3) how B-uptake by *P. tremula* is affected by spatial heterogeneity in soil B. Root growth responses to soil heterogeneity are notoriously challenging to assess under field conditions, because it is usually difficult to determine the spatial distribution of all relevant factors around plant roots with sufficient accuracy or to control confounding factors. Therefore, we used pot experiments to assess growth and B uptake of *P. tremula* seedlings to well-defined constructed heterogeneities in the soil B distribution.

## Material & methods

### Experiment 1

Two experiments were performed. In the first experiment (Exp 1) we compared the growth and B uptake of *P. tremula* seedlings growing on soil with heterogeneous B distribution to that of *P. tremula* growing on soil with homogeneous B distribution. We used subsoil, an acidic loamy sand (87% sand, 8% silt, 5% clay) originating from a Haplic Alisol from Eiken, Switzerland. The B concentration of this soil was 2.1 mg $kg^{-1}$. Other physical and chemical soil properties have been described by Nowack et al. (2006) and Moradi et al. (2009a). This soil was chosen for both experiments because it allowed for easy root visualization by means of neutron radiography (NR) in Experiment 2 (Moradi et al. 2009a). To facilitate plant establishment and to avoid Al-toxicity, the soil pH was raised from 4.2 to 6.5 by addition of $CaCO_3$. A small portion of this soil was thoroughly mixed with $H_3BO_3$ powder giving a stock of soil with a total B concentration of 26.4 mg $kg^{-1}$. By means of stepwise dilution of the stock soil with unspiked soil, we produced soil batches with B concentrations of 2.7, 3.3, 4.3, 7.3 and 13.5 mg $kg^{-1}$. One set of Al containers (internal dimensions of 17 height×15 width×1.2 depth cm) was filled homogeneously with spiked soil from these batches and un-spiked soil for control. Containers of a second set of containers were each filled with unspiked soil on one and spiked soil on the other side. For this purpose they were divided into two compartments by a temporary divider during filling. After filling, the divider was removed, taking care that the two soils did not mix. Each of the resulting 2×6 heterogeneous and homogeneous treatments with B-spiked soil as well as the homogeneous treatment with unspiked soil (control) were prepared in triplicate. A 5 cm cutting of a *P. tremula* provenance *Birmensdorf* (Qin et al. 2007) clone was planted in the middle of each container. The plants were grown for 86 days in a climate chamber (16 h day (22°C)/7 h night (15°C) cycle, 0.5 h transition time, 75% relative humidity). The pots were irrigated every 2$^{nd}$ to 3$^{rd}$ day to keep the soil water content between 15 and 20%. Water and B-free Hoagland's solution (Hoagland and Arnon 1938) were alternately used for irrigation to meet the nutrient demands of the plants.

### Experiment 2

The second experiment (Exp 2) focused on FR growth responses to heterogeneous B distribution. Here, Al-containers with internal dimensions of 67 height×64 width×1.2 depth cm were used. The same soil and cuttings of the same *P. tremula* clone were used as in Exp 1. Again a control (unspiked soil), a homo- and a heterogeneous B-treatment were established in triplicate. In each container of the heterogeneous treatment, a lateral third of the total soil packing was spiked with the same total amount of $H_3BO_3$ as the whole packing in the homogeneous treatment. The resulting concentrations were 8.9 mg $kg^{-1}$ in the homogenous treatment and 22.5 mg $kg^{-1}$ in the spiked portion of the heterogeneous treatment. To avoid soil layering, the containers were laid down, the front panel was removed and the containers were filled from the open front side.

Before planting, the bare soil was irrigated for 2 weeks to allow the soil to settle. For the first 2 months, the plants were grown under greenhouse conditions with natural lighting at the Paul-Scherrer-Institute (PSI), Villigen, Switzerland. Then the containers were transferred for logistic reasons into the climate chamber at ETH, where the experiment was continued for another 4 months. As in Exp 1, the gravimetric water content of the soil was kept at 15–20%, which was a compromise avoiding water stress on one hand but still giving sufficient root-soil contrast in neutron radiography images on the other hand (Moradi et al. 2009a).

### Neutron radiography

Neutron imaging of root growth in Exp 2 was performed at the NEUTRA facility (Lehmann et al. 2001) of the PSI every 3–4 weeks over 6 months. Neutron radiography is a non-invasive and non-destructive technique that can be used to study the development of plant roots in soil (Luster et al. 2009; Menon et al. 2007b; Moradi et al. 2009a). Because hydrogen strongly attenuates neutrons, NR can image root structures non-destructively with high spatial resolution if there is sufficient contrast between the moisture content of the surrounding porous medium and the roots. As roots have a higher water content than soil, optimum contrast is achieved when the soil water content is low. The size of the scintillator used was 279×234 mm (1,048×879 pixel). The exposure time was 30 s and the scintillator object distance was 10 cm. The NR images were corrected using the method described by Moradi et al. (2009a). After correction, the 9 NR partial images of a given recording needed to cover a whole container were assembled to one single picture. Depth growth of the main root was measured manually by means of an image analysis program. To determine the minimum root diameter of roots visible in the neutron radiographs, the root length in the neutron radiographs were compared with root length measurements by size classes from WinRhizo (Regent Instruments, Inc. 2009).

### Analysis of plant samples

At the end of each experiment, the aboveground biomass of all seedlings was harvested, separated into stems and leaves and dried until no further weight loss occurred. After harvesting the plants, the containers were opened laterally and in the case of Exp 1 the soil of each container was sub-divided into two equal portions, representing the two halves of the container. In the case of Exp 2, the soil of each container was divided into 3×3 equal portions, representing the 3 soil depths layers and 3 vertical columns that had been imaged separately. The roots of each soil portion were separated from the soil by washing. In Exp 2, the roots were scanned in a water bath at 400 dpi with a backlighting scanner (Epson Expression 10000XL) prior to drying and further analysis. The maximum scanning density of 3 cm root lengths $cm^{-2}$ scanner surface recommended by Himmelbauer et al. (2004) was not exceeded. After scanning, root lengths and diameters were determined by means of image analysis software WinRhizo. The containers were stored at 4°C in a cooling chamber until scanning.

In both experiments, total root mass and FR biomass (<2 mm) were determined after drying at 40°C until a constant weight was obtained. For chemical analysis all plant samples (stems, leaves, roots) from both experiments were digested in a heating block at 130°C with 65% $HNO_3$ and analyzed for B and other elements using ICP-OES (Vista MPX, Varian, Australia). To minimize B memory effects the instrument was rinsed with a 2% mannitol solution after each sample.

### Statistics

Mean shoot B concentrations were calculated as mass-weighted averages of the B concentrations of the individual plant parts. Analysis of variance was performed to test for treatment effects on biomass and B concentrations, followed by Fisher-LSD post-hoc test to compare pair-wise differences between treatments. Values given for correlations between variables represent Pearson's correlation coefficients. All statistical analyses were carried out using Sigma Plot 11 (Systat Software 2008).

## Results

### Shoot and root growth

The soil B concentrations applied in Exp 1 resulted in a concentration series covering the entire range from deficiency to toxicity. The control treatment had the

lowest soil B concentration (2.1 mg $kg^{-1}$) and here the plants seemed to be marginal B deficient, as indicated by the significantly lower shoot growth compared to the homogeneous 2.7 mg $kg^{-1}$ and the heterogeneous 2.1|3.3 mg $kg^{-1}$ treatments. These two treatments had the same average soil B concentration and the largest shoot growth of all treatments (Fig. 1). Compared to the homogeneous 2.7 mg $kg^{-1}$ and the heterogeneous 2.1|3.3 mg $kg^{-1}$ treatment shoot growth was significantly reduced at soil B concentrations ≥7.3 mg $kg^{-1}$ and symptoms of B toxicity like burned leaf margins became visible. The plants did not survive at the highest homogeneous B treatment level (26.5 mg $kg^{-1}$) in contrast to the highest heterogeneous treatment level (2.1|26.5 mg $kg^{-1}$), at which the average B concentration was similar to the homogeneous 13.5 mg $kg^{-1}$ treatment. Although there was a trend for larger shoot growth in the heterogeneous treatments compared to the respective homogeneous treatments at average soil B concentrations ≥7.3 mg $kg^{-1}$, these differences were not significant. Compared to the homogeneous treatments the plants in the heterogeneous treatments did not benefit from the presence of half of their root system in low B soil.

While shoot biomass was always larger than root biomass, both were closely correlated to each other

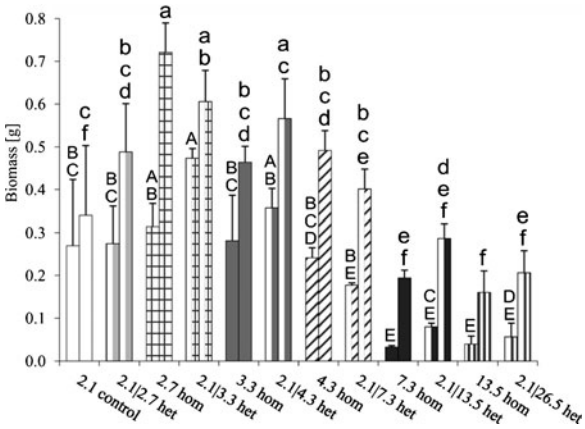


**Fig. 1** Mean fine root (*left bars*) and shoot (*right bars*) biomass of 3-month old poplar plants grown in B-spiked soil. The soil was spiked either homogeneously (hom) or heterogeneously (het). The numbers represent the soil B concentrations [mg $kg^{-1}$]. The two numbers given for the heterogeneous treatments (het) represent the unspiked and spiked compartment. Similar bar shades or fillings indicate similar average soil B concentrations of two treatments. Statistically significant differences ($p<0.05$) between treatments are indicated by characters. Error bars represent standard errors ($n=3$)

($y=1.11$ x+0.17; $r^2=0.78$; $p<0.001$). At low average soil B concentrations (2.1–2.7 mg $kg^{-1}$), shoot growth was more stimulated by B spiking than root growth. The root:shoot ratio was significantly lower in the homogeneous 2.7 mg $kg^{-1}$ treatment than in the control (data not shown). The maximum root:shoot ratio (0.8) was found in the heterogeneous treatment with an average soil B concentration of 2.7 mg $kg^{-1}$. At soil B concentrations ≥7.3 mg $kg^{-1}$ the root:shoot ratio significantly declined, indicating a stronger B toxicity in the roots than in the shoots.

In Exp 1 we found no significant effects of the heterogeneous distribution of soil B on FR biomass when we compared the spiked with the unspiked portions of the containers (Fig. 2). At the highest B level in the heterogeneous treatment, FR biomass was 4 times lower in the spiked than in the unspiked portion, but this difference was not significant, due to the high variability in FR biomass. In the heterogeneous treatments it became obvious that the significantly reduced total FR biomass (Fig. 1) at average soil B concentrations ≥7.3 mg $kg^{-1}$ was due to root growth reduction in the spiked and the unspiked soil portions.

Exp 2 showed that FR length was more affected by high B concentrations than FR biomass. The B treatments did not affect FR length in the uppermost third of the soil (0–21 cm) and total FR length. In contrast, we found significantly reduced FR lengths compared to the control in the heterogeneous B-treatment at soil depth >21 cm and in the homogeneous B treatment at soil depth >42 cm (Fig. 3). In two of the three

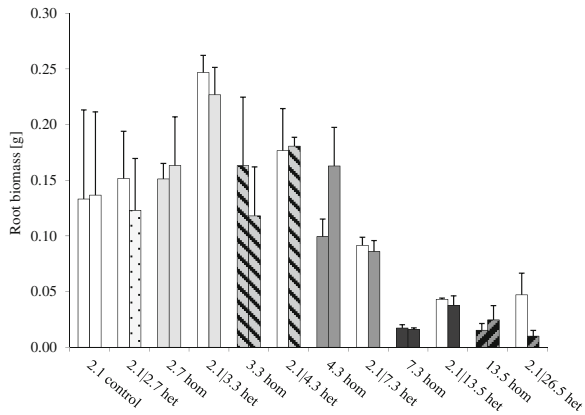


**Fig. 2** Mean fine root biomass in the unspiked (*left bars*) and the spiked (*right bars*) portions of the soil in Exp 1. Similar bar shades or fillings indicate similar average soil B concentrations of two treatments. Error bars represent standard errors ($n=3$)

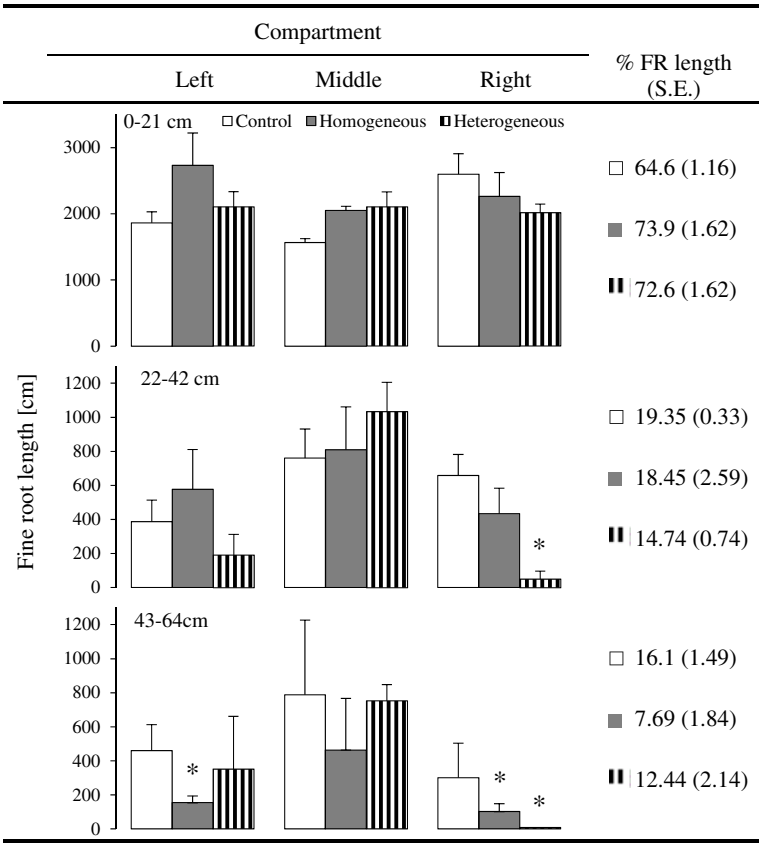


**Fig. 3** Mean fine root length after 6 months of growth in containers with no, homogeneous and heterogeneous soil B spiking. The right compartment is spiked in the heterogeneous treatment. The background B concentration of the unspiked soil was 2.1 mg $kg^{-1}$. In the homogeneous spiking the B concentration was 8.9 mg $kg^{-1}$, while in the heterogeneous spiking the spiking concentration was 22.5 mg $kg^{-1}$. Statistically significant differences ($p<0.05$, $n=3$) between the treatments and the control are indicated by asterisk. Error bars represent standard errors. The figures on the right give total poplar fine root length per depth as percentage of total FR length (S.E. = Standard Error)

replicates of the heterogeneous treatment we found no roots at all in the B-enriched parts of the lowest third (43–64 cm depth).

The highest root biomass and coarse root length was always found beneath the stem base, in the top middle compartment of the containers. Coarse root length in this compartment ranged from 16.5 cm in the heterogeneous, to 22.8 cm in the control and 23.9 cm in the homogeneous treatment, without showing significant differences. In all other compartments, the coarse root lengths were <1.5 cm. Coarse root lengths were unaffected by B patchiness in our experiment. All treatments with B spiking resulted in a greater proportion of FR length near the soil surface than in the control. At soil depth >21 cm FR lengths were always larger in the central than in the lateral compartments of the containers, independent of B treatments (Fig. 3).

Despite the large variability in root growth patterns, the neutron radiographs taken in Exp 2 revealed faster root system development in the control than in both B-treatments (Fig. 4). This effect was stronger in the homogeneous B treatment. After 4 months of growth (24.10.), NR-visible roots had already reached a soil depth of 40 cm in the control treatment, while in the B treatments NR-visible roots did not reach below 25 cm depth. The bottom of the containers was reached by the roots after 5 months of growth in the control

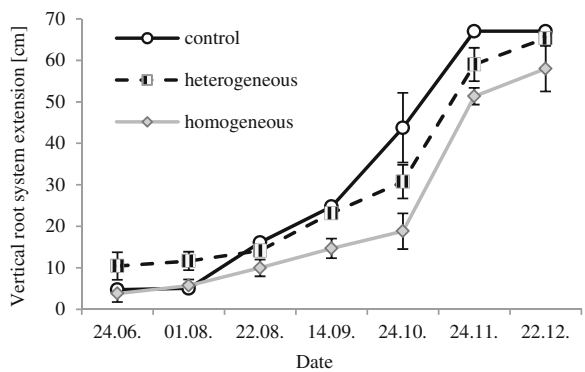


**Fig. 4** Mean vertical extension of the main root over time in Exp 2 as determined by repeated NR imaging. The maximum vertical extension of the roots determined by the size of the containers (64 cm) was reached in all control plants at the 24.11. Root length was assessed for roots >0.7 mm in diameter. Error bars represent standard errors ($n=3$)

treatment, while it was not reached during the course of the experiment in the homogeneous B treatment. These observations are in line with the greater fraction of FR in the uppermost soil in B-spiked than in unspiked soil mentioned before. Delayed root depth growth could make stand establishment difficult under field conditions, especially when factors such as water or nutrient deficiencies lead to additional stress.

The total length of the roots that were collected by soil washing was on average 15 times larger than the total root length determined from the NR images. The total length of roots determined by NR imaging was approximately equivalent to the length of roots with a diameter >0.7 mm obtained by soil washing. Thus, we considered the NR visible fraction as insufficient for a more detailed analysis of the root system. Nonetheless, there was a close relationship ($r^2$=0.77; $y$= 323.66 x+14.71; $p$<0.001) between total root biomass and NR-visible root length in the individual compartments, when we excluded the uppermost central compartment because of the extremely large root mass in this part of the container.

The lower two thirds of the B-treatments differed significantly in their root diameter distributions from the control treatment and the uppermost soil layer of the B-treatments. While in the control treatment and in the uppermost soil layer in the B-treatments, roots diameters of 0.1–0.3 mm were dominant, the peaks of the frequency distributions were broader and shifted to higher diameter classes in B-spiked soil (Fig. 5). These effects increased with soil depth and lateral distance from the stem.

## Influence of accumulated plant B on growth

Figure 6 shows high shoot biomass variability at shoot B concentrations <500 mg kg$^{-1}$. The maximum growth was found in plants with shoot B concentrations of 40–150 mg kg$^{-1}$. At shoot B concentrations above 500 mg kg$^{-1}$ shoot growth was reduced, indicating B toxicity. The leaf B concentrations found at average shoot B concentrations >500 mg kg$^{-1}$ were in the range 800–1,660 mg kg$^{-1}$, showing an onset of toxicity at similar leaf concentrations as found by Rees et al. (2011) in *Populus nigra x euramericana*.

Paralleling the larger variability in shoot biomass at shoot B concentrations <500 mg kg$^{-1}$, there was also a large variability in root biomass at root B concentrations <35 mg kg$^{-1}$, indicating that at lower B concentrations the accumulation of excessive B in the plants was not a growth-limiting factor. The maximum root biomass was significantly reduced when root B concentrations exceeded 35 mg kg$^{-1}$ in B-spiked soil (Fig. 7). This was the case at soil B concentrations of ≥4.3 mg kg$^{-1}$ in the homogeneous treatment (Fig. 8a). In the unspiked soil portions of the heterogeneous treatments, root growth tended to be reduced at root B concentrations >20 mg kg$^{-1}$, which occurred again at average soil B concentrations ≥4.3 mg kg$^{-1}$ in the heterogeneous treatments (B concentrations in the spiked portions: ≥7.3 mg kg$^{-1}$) (Fig. 8a).

Figure 8a shows that B accumulation by roots in the unspiked soil parts of the heterogeneous treatments increased proportionally to the B concentrations of the roots in the spiked parts of the soil, with a ratio of approximately 1:2 between the B concentrations in the roots of the unspiked and the spiked soil in a given treatment. The highest uptake of B into roots took place in the spiked soil at the two highest B levels in the heterogeneous treatments. In these cases, root B concentrations of up to 130 mg kg$^{-1}$ were found. The results from Exp 2 agreed with the findings of Exp 1 that root toxicity effects started to occur in B spiked soil when root B concentrations exceeded 35 mg kg$^{-1}$ (data not shown). Root B concentrations exceeded 35 mg kg$^{-1}$ in Exp 2 in the homogeneously spiked soil as well as in the spiked portion of the heterogeneous treatment and at the same time also FR length was reduced.

Leaf B concentrations increased with increasing average soil B concentrations up to 1,350 mg kg$^{-1}$ at average soil B concentrations of 13–14 mg kg$^{-1}$, irrespective of the B distribution. Between average soil B concentrations of 3 and 8 mg kg$^{-1}$ this increase was almost linear; then it leveled off (Fig. 8b). In Exp 2 leaf B concentrations ranged between 36.7 (control), 260 (homogeneous treatment) and 380 mg B kg$^{-1}$ (heterogeneous treatment). Significant differences were only found between the control and the two B treatments, but not among the homogeneous and the heterogeneous treatments. This was consistent with the result from Exp 1 that B uptake was only due to the average soil B concentration. In the B treatments of Exp 2 leaf B concentrations did not exceed the leaf toxicity threshold found in Exp 1 (800 mg kg$^{-1}$), which may explain the absence of significant effects on the shoot biomass (data not shown).

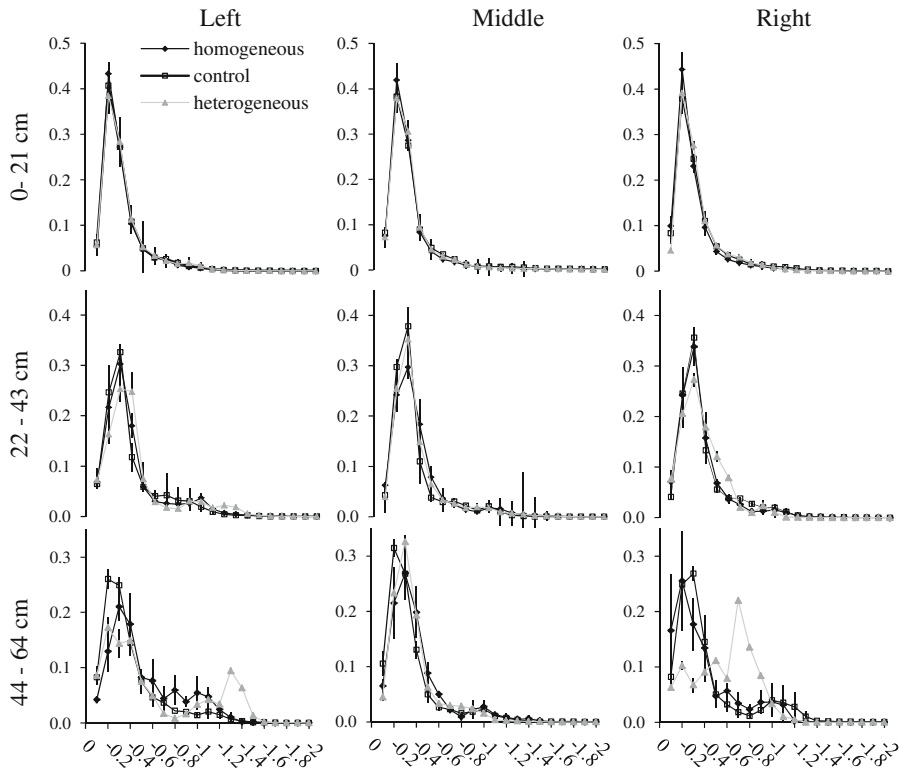


**Fig. 5** Mean root diameter distributions of fine roots in Exp 2. Root length per diameter class is given as fraction of total root lengths. Left, middle and right are the respective portions of the containers. Error bars represent standard errors ($n$=3)

The total uptake of B into the shoot biomass of *P. tremula* in Exp 1 ranged between 0.02 and 0.23 mg (Fig. 9). Significant difference in the accumulated B amounts emerged only between treatments with average soil B concentrations ≤3.3 mg kg$^{-1}$ and those with higher soil B concentrations. At similar average soil B concentrations no significant differences between heterogeneous and homogeneous treatments were found in Exp 1 and Exp 2. The maximum percentage of B initially present in the soil extracted was 11% (0.18 mg) in the homogeneous 4.3 mg kg$^{-1}$ treatment. At higher soil B concentrations the percentage of initially present soil B extracted decreased due to the greater total amounts of B in the soils.

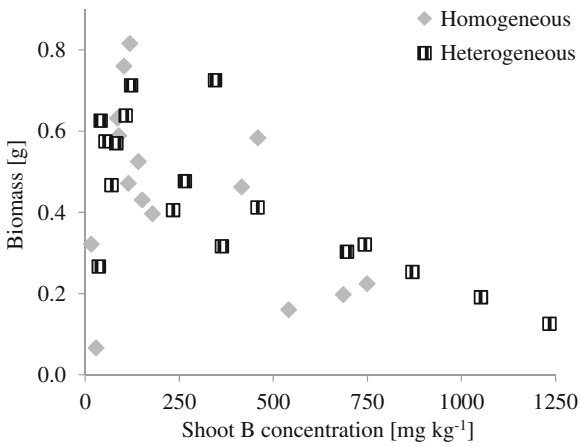


**Fig. 6** Shoot biomass as a function of shoot B concentration of poplars grown in Exp 1 in the homo- and in the heterogeneous treatment

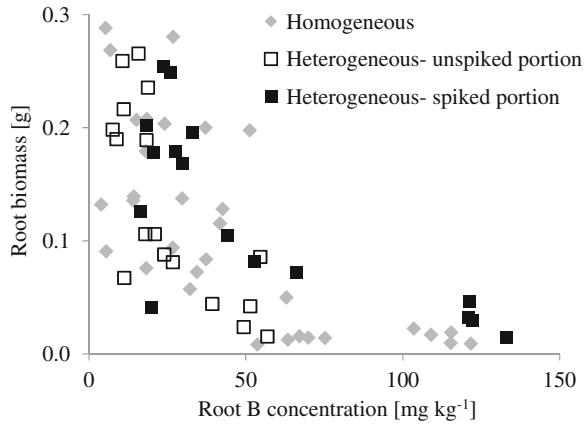


**Fig. 7** Root biomass as a function of root B concentration of roots grown in Exp 1. Each point represents one half of an either homogeneously or heterogeneously spiked container and the B concentration of the roots grown there

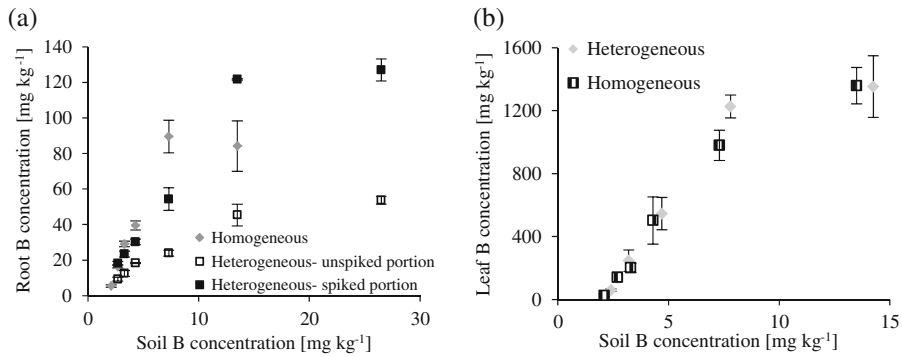


**Fig. 8** **a** Mean root B concentrations as function of soil B concentrations. For better clarity the B concentrations of roots from the unspiked soil portions of the heterogeneous treatments are plotted against the respective B concentrations of the spiked soil portions. **b** Mean leaf B concentration as function of the average B concentration in soil with heterogeneous or homogeneous B spiking in Exp 1. Error bars represent standard errors ($n$=3)

## Discussion

From a meta-analysis of published data, Audet and Charest (2008) concluded that plants tend to allocate relatively more biomass into roots with increasing soil metal concentrations. However, our data on the root: shoot ratio of *P. tremula* exposed to high soil B concentrations did not show this pattern, which is consistent with the findings of Reid et al. (2004) for wheat and Rees et al. (2011) for poplars. The fact that root biomass was also reduced in the unspiked soil portion in the heterogeneous treatments at average soil B concentrations ≥4.3 mg kg$^{-1}$ in Exp 1, indicates a systemic plant response. In contrast, the unilateral reduction in root growth at soil B concentrations >20 mg kg$^{-1}$ in the heterogeneous treatment of Exp 2 (and also in Exp 1, although not significant in the latter) indicates a local response. This local root growth response may have simply resulted from toxicity, resulting from direct exposure to the high soil B concentrations, but it may also represent an active response, reflecting a root growth strategy. Any growth strategy would need a signaling mechanism, with roots near or in the B patch communicating the location of the patch to the remainder of the root system. Such communication has been shown to occur via auxin-abscisic acid-ethylene cross-talks in poplars (Popko et al. 2010). However, recent findings indicate that auxin is not involved in B toxicity signaling in *Arabidopsis* and the main signaling mechanism for B toxicity seems to be abscisic acid, which triggers root growth inhibition via a hydric stress response (Aquea et al. 2011).

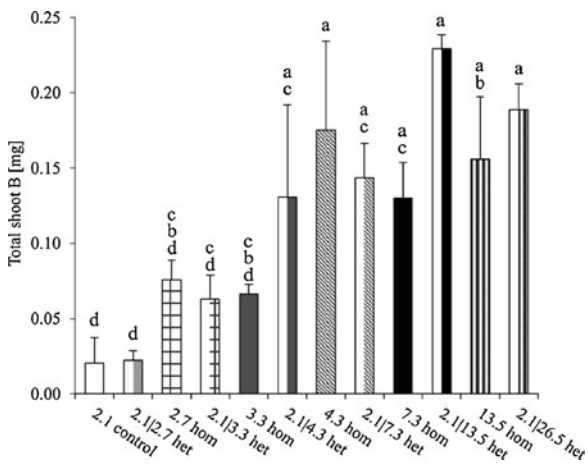


**Fig. 9** Mean amount of B accumulated by *P. tremula* during 86 days of growth in Exp 1. Similar bar shades or fillings indicate similar average soil B concentrations of two treatments. Statistically significant differences ($p$<0.05, $n$=3) between treatments are indicated by characters. Error bars represent standard errors

Although, NR has been applied for root growth investigations in various studies (Menon et al. 2007b; Moradi et al. 2009a), a quantitative comparison of the NR detectable root length and the real length of the root system was unavailable yet. The results of this comparison were disappointing, as only 6–8% of the total root system length was detectable with NR. However, future developments of the technique might further improve the proportion of the NR detectable root length and other plant species with higher root diameters should be better suited for working with this technique.

Changes in the root diameter distributions were also found in the unspiked soil of the heterogeneous B

treatment, indicating a systemic root system response. An explanation for these changes in diameter class distributions could be that *P. tremula* switches to an extensive rooting strategy under stress conditions, in which they invest more assimilates into exudate production and less into increasing specific root length [cm $g^{-1}$] (Lohmus et al. 2006; Ostonen et al. 2007). As far as the differences in root diameters reflect local toxicity effects, they could also have been due to a higher survival probability of thicker roots as well as to a stronger B toxicity effect on length than on diameter growth. The observed shift to higher FR diameters could be disadvantageous for nutrient and water acquisition because the fraction of soil pore space that can be accessed by roots decreases with root diameter. As coarse roots develop from FR by secondary thickening, coarse roots cannot establish in soil where FR cannot establish. Therefore, the inhibition of FR growth in a larger volume of soil will also impair the development of coarse roots and might thus reduce the stability of a tree as it grows and needs increasing anchorage in the soil.

The relationship between shoot biomass and shoot B accumulation was the same in heterogeneous and homogeneous treatments, suggesting that any B toxicity effect on shoot growth was only dependent on the shoot B concentrations. The leaf B toxicity threshold of 800 mg $kg^{-1}$ found in our experiment shows that *P. tremula* is highly B tolerant compared to other plant species and lies in the same range as found in hybrid poplars (Rees et al. 2011; Robinson et al. 2007). In *Quercus rubra* L., decreased growth was found at leaf B concentrations around 230 mg $kg^{-1}$ and some *Eucalyptus* species showed growth decline at B concentrations of only 75 mg $kg^{-1}$ (Crews and Dick 1998; Lehto et al. 2010). All plants with shoot B concentrations <40 mg $kg^{-1}$ produced less than 0.35 g biomass during the three months growth period of Exp 1. This was less than 40% of the maximum growth and the shoot biomass in the unspiked control treatment was significantly lower than in the homogeneous 2.7 mg $kg^{-1}$ and the heterogeneous 2.1|3.3 mg $kg^{-1}$ treatments. The corresponding leaf B concentrations of these plants were below 45 mg $kg^{-1}$ and the concentration in the top 3 leaves of one of those trees was only 15.1 mg $kg^{-1}$. While one of the plants with such a low B concentration was found in the heterogeneous treatment with the lowest level of B spiking, two others were obtained from the treatment with unspiked soil (control). Boron deficiency limits for tree foliage are in the range of 4 (*Picea Abies* (L.) H.Karst., *Pinus Sylvestris* L.) to 16 mg $kg^{-1}$ (*Eucalyptus globulus* (Labill)) in the youngest emerged leaf and are generally higher in broadleaf species than in conifers (Lehto et al. 2010). Our results indicate that *P. tremula* has a similarly sensitivity to low soil B supply as *E. globulus* and *P. tremula* has a higher B requirement than many other plant species (Bell 1997). This may be due to a lack of transporters for active xylem loading of B under deficiency conditions in *P. tremula* (Tuskan et al. 2006). The first symptom of B deficiency in plants usually is decreased root growth in relation to shoot growth (Dell and Huang 1997). Such an effect was not found here. We also found no enhanced proliferation of roots in B-enriched patches to compensate for low B supply in the heterogeneous treatments.

We showed roots grown in the unspiked soil of the heterogeneous treatment to be growth-reduced at root B concentrations of only >20 mg $kg^{-1}$. This growth reduction occurred at soil B concentrations >7.3 mg $kg^{-1}$ in the spiked compartments of the heterogeneous treatments, indicating that the root growth inhibition observed at moderately elevated B treatment levels was not a toxicity response to the accumulation of B in the roots or to soil B, but a systemic response to B in the shoots. This hypothesis also agrees with the observation that root biomass was more sensitive to the B treatments at moderately elevated B concentrations than shoot biomass.

The B accumulation of the roots in the unspiked soil parts of the heterogeneous treatment took place at a ratio of 1:2 compared to the accumulation in the spiked portions of the heterogeneous treatments. This ratio is much lower than one would expect from the B accumulation in the control soil. Therefore, there must have been substantial lateral B transfer from the spiked to the unspiked soil compartments. Such transfer could have occurred through roots extending from the unspiked into the spiked soil as well as through the soil solution. Even though transfer of B from spiked to unspiked soil portions via the soil solution was favored by the soil type, it was probably not the main transfer pathway of B, as the addition of $CaCO_3$ to the soil should have increased B retention (Goldberg 1997). Lateral B transfer should have mitigated the probably rather uncommon situation of B poor soil next to excess B soil in the heterogeneous treatments in Exp 1, as an increase in B concentration of only

0.6 mg $kg^{-1}$ would have led to an optimal soil B concentration. The addition of $CaCO_3$ might have also decreased the plant available fraction of soil B and thus its toxicity. Transfer of B within the root system could be another explanation for the high B concentrations in roots from unspiked soil portions (Bellaloui et al. 1999; Brown and Hu 1996).

The finding that the accumulation of B in the leaves only depended on the average concentration of B in the soil and not on its spatial distribution indicates that the spiked and the unspiked parts contributed equally to B uptake in the heterogeneous treatments. This is in agreement with the hypothesis that toxicity effects in the roots were primarily systemic plant responses to B accumulation in the shoots and thus similar in spiked and unspiked soil portions. In this regard B uptake appears to differ from the uptake of other trace elements with heterogeneous distribution in soil (Millis et al. 2004).

Our results on B tolerance and uptake from heterogeneously contaminated soils have implications for the phytomanagement of B contaminated sites. Long clean-up times are one of the major disadvantages of phytoremediation (Pulford and Watson 2003; Robinson et al. 2009). Thus, the finding that patchy soil B distribution did not lead to decreased B accumulation in the shoots, while it tended to result in larger growth compared to a homogenous distribution of the same amount of B is an encouraging result for further field applications of *P. tremula* in phytoremediation, as heterogeneous contamination is rather the rule than the exception under field conditions (Robinson et al. 2009). Breeding of poplars and their hybrids for B phytoremediation should focus on the B tolerance of poplars leaf tissue as it seems to govern the whole plant status.

## Conclusions

The tolerance of *P. tremula* to the average soil B concentrations applied in this study was the same, regardless if B was heterogeneously or homogeneously distributed in the soil. Furthermore, the heterogeneity of the soil B distribution had no effect on the B accumulation in the leaves of *P. tremula* and the total amount of B taken up into the shoots. Our results indicate that at low to medium soil B concentrations B toxicity on root growth is primarily a systemic response to B toxicity in the shoots, while only at high local soil B concentrations local effects on roots become dominant. We conclude from these results that local heterogeneity in soil B should have little influence on the phytoremediation of contaminated sites using *P. tremula*, as long as the contamination allows root growth.

**Acknowledgments** Funds for this study came from the Swiss National Science Foundation (SNSF). Funding for M.W.H. Evangelou came from the DFG (Deutsche Forschungsgemeinschaft). The technical assistance provided by S. Hartmann and P. Vontobel from PSI is gratefully acknowledged. We would also like to thank N. Grisel from the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) for the poplar cuttings. We would also like to thank H. M. Conesa for accepting to pre-review the manuscript and two anonymous reviewers for their very helpful review comments.

## References

Aquea F, Federici F, Moscoso C, Vega A, Jullian P, Haseloff JIM, Arce-Johnson P (2011) A molecular framework for the inhibition of *Arabidopsis* root growth in response to boron toxicity. Plant Cell Environ doi:10.1111/j.1365-3040.2011.02446.x

Audet P, Charest C (2008) Allocation plasticity and plant-metal partitioning: meta-analytical perspectives in phytoremediation. Environ Pollut 156:290–296

Bañuelos GS, LeDuc D, Johnson J (2010) Evaluating the tolerance of young hybrid poplar trees to recycled waters high in salinity and boron. Int J Phytoremediat 12:419–439

Bell RW (1997) Diagnosis and prediction of boron deficiency for plant production. Plant Soil 193:149–168

Bellaloui N, Brown PH, Dandekar AM (1999) Manipulation of in vivo sorbitol production alters boron uptake and transport in tobacco. Plant Physiol 119:735–741

Blevins DG, Lukaszewski KM (1998) Boron in plant structure and function. Annu Rev Plant Physiol Plant Mol Biol 49:481–500

Borken W, Kossmann G, Matzner E (2007) Biomass, morphology and nutrient contents of fine roots in four Norway spruce stands. Plant Soil 292:79–93

Breckle SW, Kahle H (1992) Effects of toxic heavy-metals (Cd, Pb) on growth and mineral-nutrition of beech (*Fagus-Sylvatica*). Vegetatio 101:43–53

Brown PH, Hu HN (1996) Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. Ann Bot 77:497–505

Communar G, Keren R, Li F (2004) Deriving boron adsorption isotherms from soil column displacement experiments. Soil Sci Soc Am J 68:481–488

Crews JT, Dick WA (1998) Liming acid forest soils with flue gas desulfurization by-product: growth of Northern red oak and leachate water quality. Environ Poll 103:55–61

Dell B, Huang LB (1997) Physiological response of plants to low boron. Plant Soil 193:103–120

Dos Santos Utmazian MN, Wieshammer G, Vega R, Wenzel WW (2007) Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. Environ Poll 148:155–165

Evans CM, Sparks DL (1983) On the chemistry and mineralogy of boron in pure and mixed systems—a review. Commun Soil Sci Plan 14:827–846

Friend AL, Mobley JA, Ryan EA, Bradshaw HD (1999) Root growth plasticity of hybrid poplar in response to soil nutrient gradients. J Sust For 10:133–140

Goldberg S (1997) Reactions of boron with soils. Plant Soil 193:35–48

Goldberg S, Forster HS, Heick EL (1993) Temperature effects on boron adsorption by reference minerals and soils. Soil Sci 156:316–321

Gupta UC, Jame YW, Campbell CA, Leyshon AJ, Nicholaichuk W (1985) Boron toxicity and deficiency—a review. Can J Soil Sci 65:381–409

Hall JB (1971) Pattern in a chalk grassland community. J Ecol 59:749–762

Himmelbauer ML, Loiskandl AW, Kastanek AF (2004) Estimating length, average diameter and surface area of roots using two different image analyses systems. Plant Soil 260:111–120

Hoagland DR, Arnon DI (1938) The water-culture method for growing plants without soil. Calif Agric Exp Stn Circ 357:1–39

Hu H, Brown PH (1997) Absorption of boron by plant roots. Plant Soil 193:49–58

Ishak CF, Seaman JC, Miller WP, Sumner M (2002) Contaminant mobility in soils amended with fly ash and flue-gas gypsum: intact soil cores and repacked columns. Water Air Soil Poll 134:285–303

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants. CRC Press, Boca Raton

Lehmann EH, Vontobel P, Wiezel L (2001) Properties of the radiography facility NEUTRA at SINQ and its potential for use as European reference facility. Nondest Test Eval 16:191–202

Lehto T, Ruuhola T, Dell B (2010) Boron in forest trees and forest ecosystems. For Ecol Manage 260:2053–2069

Liu F, Tang Y, Du R, Yang H, Wu Q, Qiu R (2010) Root foraging for zinc and cadmium requirement in the Zn/Cd hyperaccumulator plant *Sedum alfredii*. Plant Soil 327:365–375

Lohmus K, Truu J, Truu M, Kaar E, Ostonen I, Alma S, Kuznetsova T, Rosenvald K, Vares A, Uri V et al (2006) Black alder as a perspective deciduous species for reclaiming of oil shale mining areas. In: Brebbia CA, Mander Ü (eds) Brownfields III prevention, assessment, rehabilitation and development of Brownfield sites. WIT Press, Southampton, pp 87–97

Luster J, Gottlein A, Nowack B, Sarret G (2009) Sampling, defining, characterising and modeling the rhizosphere-the soil science tool box. Plant Soil 321:457–482

Mastromatteo E, Sullivan F (1994) Summary- International Symposium on the health-effects of boron and its compounds. Environ Health Perspect 102:139–141

McLaren RG, Cameron KC (1996) Soil science: sustainable production and environmental protection. Oxford University Press, Oxford

Menon M, Hermle S, Günthardt-Goerg M, Schulin R (2007a) Effects of heavy metal soil pollution and acid rain on growth and water use efficiency of a young model forest ecosystem. Plant Soil 297:171–183

Menon M, Robinson B, Oswald SE, Kaestner A, Abbaspour KC, Lehmann E, Schulin R (2007b) Visualization of root growth in heterogeneously contaminated soil using neutron radiography. Eur J Soil Sci 58:802–810

Millis PR, Ramsey MH, John EA (2004) Heterogeneity of cadmium concentration in soil as a source of uncertainty in plant uptake and its implications for human health risk assessment. Sci Total Environ 326:49–53

Miwa K, Fujiwara T (2010) Boron transport in plants: coordinated regulation of transporters. Ann Bot 105:1103–1108

Moradi AB, Conesa HM, Robinson B, Lehmann E, Kuehne G, Kaestner A, Oswald S, Schulin R (2009a) Neutron radiography as a tool for revealing root development in soil: capabilities and limitations. Plant Soil 318:243–255

Moradi AB, Conesa HM, Robinson BH, Lehmann E, Kaestner A, Schulin R (2009b) Root responses to soil Ni heterogeneity in a hyperaccumulator and a non-accumulator species. Environ Pollut 157:2189–2196

Mulia R, Dupraz C (2006) Unusual fine root distributions of two deciduous tree species in southern france: what consequences for modelling of tree root dynamics? Plant Soil 281:71–85

Nable RO, Banuelos GS, Paull JG (1997) Boron toxicity. Plant Soil 193:181–198

Nowack B, Rais D, Frey B, Menon M, Schulin R, Guenthardt-Goerg MS, Luster J (2006) Influence of metal contamination on soil parameters in a lysimeter experiment designed to evaluate phytostabilization by afforestation. For Snow Landsc Res 80:201–211

Ostonen I, Puttsepp U, Biel C, Alberton O, Bakker MR, Lohmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A et al (2007) Specific root length as an indicator of environmental change. Plant Biosyst 141:426–442

Parks JL, Edwards M (2005) Boron in the environment. Crit Rev Environ Sci Techn 35:81–114

Popko J, Hansch R, Mendel RR, Polle A, Teichmann T (2010) The role of abscisic acid and auxin in the response of poplar to abiotic stress. Plant Biol 12:242–258

Power PP, Woods WG (1997) The chemistry of boron and its speciation in plants. Plant Soil 193:1–13

Pregitzer KS, Friend AL (1996) The structure and function of *Populus* root systems. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckley TM (eds) Biology of Populus and its implications for management and conservation. National Research Council of Canada, Ottawa, pp 331–354

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540

Qin RJ, Hirano Y, Brunner I (2007) Exudation of organic acid anions from poplar roots after exposure to Al, Cu and Zn. Tree Physiol 27:313–320

Rees R, Robinson BH, Menon M, Lehmann E, Guenthardt-Goerg MS, Schulin R (2011) Boron accumulation and toxicity in hybrid poplar (*Populus nigra x euramericana*). Environ Sci Technol 45:10538–10543

Regent Instruments (2009) WinRhizo Pro. 2009c Regent Instruments

Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004) A critical analysis of the causes of boron toxicity in plants. Plant Cell Environ 27:1405–1414

Robinson D (1994) The responses of plants to non-uniform supplies of nutrients. New Phytol 127:635–674

Robinson BH, Green SR, Chancerel B, Mills TM, Clothier BE (2007) Poplar for the phytomanagement of boron contaminated sites. Environ Pollut 150:225–233

Robinson BH, Banuelos G, Conesa HM, Evangelou MWH, Schulin R (2009) The phytomanagement of trace elements in soil. Crit Rev Plant Sci 28:240–266

Salisbury FB, Ross CW (1992) Plant physiology. Wadsworth Publishing Co. Inc., Belmont

Schwartz C, Morel JL, Saumier S, Whiting SN, Baker AJM (1999) Root development of the Zinc-hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization in soil. Plant Soil 208:103–115

Systat Software (2008) Sigma Plot 11. Systat Software Inc.

Tanaka M, Fujiwara T (2008) Physiological roles and transport mechanisms of boron: perspectives from plants. Pflugers Archiv-Eur J Physiol 456:671–677

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A et al (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596–1604

Van TK, Kang Y, Sakurai K (2005) Boron fixation and its release in soils and bark compost. Soil Sci Plant Nutr 51:69–74

Wenzel W (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

Whiting SN, Leake JR, McGrath SP, Baker AJM (2000) Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. New Phytol 145:199–210

Worrell R (1995) European aspen (*Populus tremula L.*): a review with particular reference to Scotland I. Distribution, ecology and genetic variation. Forestry 68:93–105

Xu F, Goldbach HE, Brown PH, Bell RW, Fujiwara T, Hunt CD, Goldberg S, Shi L (eds) (2007) Advances in plant and animal boron nutrition. Springer, Dordrecht

Zobel RW (2008) Hardware and software efficacy in assessment of fine root diameter distributions. Comput Electron Agr 60:178–189