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This document is the accepted manuscript version of the following article:
Rees, R., Robinson, B. H., Menon, M., Lehmann, E., Günthardt-Goerg, M. S., & Schulin, R. (2011). Boron accumulation and toxicity in hybrid poplar (*Populus nigra* x *euramericana*). *Environmental Science and Technology*, 45, 10538-10543. http://doi.org/10.1021/es201100b

Boron accumulation and toxicity in hybrid poplar (*Populus nigra* × *euramericana*)

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Abstract

Poplars accumulate high B concentrations and are thus used for the phytomanagement of B contaminated soils. Here, we performed pot experiments in which *Populus nigra* × *euramericana* were grown on a substrate with B concentrations ranging from 13 to 280 mg kg^{-1} as H_3BO_3. *Salix viminalis, Brassica juncea* and *Lupinus albus* were grown under some growing conditions for comparison. Poplar growth was unaffected at soil B treatment levels up to 93 mg kg^{-1}. Growth was progressively reduced at levels of 168 and 280 mg kg^{-1}. None of the other species survived at these substrate B levels. At leaf B concentrations <900 mg kg^{-1} only <10% of the poplar leaf area showed signs of toxicity. Neutron radiography revealed that chlorotic leaf tissues had B concentrations of 1000-2000 mg kg^{-1}, while necrotic tissues had >2000 mg kg^{-1}. Average B concentrations of up to 3500 mg kg^{-1} were found in leaves, while spots within leaves had concentrations >7000 mg kg^{-1}, showing that B accumulation in leaf tissue continued even after the onset of necrosis. The B accumulation ability of *P. nigra* × *euramericana* is associated with B hypertolerance in the living tissue and storage of B in dead leaf tissue.

Introduction

At low concentrations, boron (B) is an essential plant and animal micronutrient.[1] Recent studies suggest that B is also essential for humans.[2] Boron deficiencies in plants have been reported in over 80 countries for a total of 132 crops.[3] Like other trace elements, B becomes toxic for plants at elevated concentrations. The concentration range between B deficiency and toxicity is smaller than for any other nutrient element.[4] Boron is transported from soil into roots and thence into stems and leaves primarily by convection, with the stream of transpiration water.[5] However, active metabolic-driven uptake has been shown to occur under B deficiency conditions.[6] High levels of B occur naturally in many soils of arid regions.[7] In addition, human activities can lead to high soil B concentrations, such as the irrigation of agricultural fields with B-laden water, coal mining or fly ash deposition onto agricultural land.[7,8]

Poplars (*Populus* spp.) are used for wood production, supplementary stock fodder during times of drought, and for the phytomanagement of contaminated sites.[9,10] Due to their high transpiration rates and B accumulation, poplars have been employed in B phytoremediation to reduce B leaching from contaminated sites into receiving waters.[10] Removal of B from contaminated sites can be achieved by harvesting the aboveground biomass.[10] Boron-enriched poplar twigs and leaves from contaminated sites could be used as livestock forage, providing a supplementary source of this essential trace element.[11]

Depending on growth conditions, poplar clone, B application form and salinity, B accumulation in poplar leaves ranges between 500 and 1200 mg kg^{-1}, greatly exceeding the B concentrations of the growing substrate and the poplar stems.[10,12,13] In comparison to other species, the B accumulation of poplars was much higher in these studies. Apart from field surveys where B accumulation in poplars was found,[14] there have been no studies following the original report by Bañuelos et al.[12], investigating the B accumulation of poplars in more detail, including bioaccumulation factors and B threshold concentrations compared to other species.

Various *Salix* species have been shown to accumulate leaf B concentrations >800 mg kg^{-1}, exceeding those of poplars grown on the same fly ash disposal site, rendering also *Salix* interesting for the purpose of B extraction from contaminated soil.[15] The phytoextraction efficiency of a plant species for a trace element depends on the respective accumulated concentration of the element and the amount of harvestable biomass.[16] *Brassica juncea* is widely touted for use in phytoremediation and was reported to exhibit a high B tolerance.[17] Despite its lower biomass production compared to poplars or willows, the phytoextraction efficiency of *B. juncea* may be similar if its B accumulation were higher.

Boron accumulation varies widely among different parts of a plant, necessitating the analyses of all plant parts for their B concentration in order to elucidate the total B accumulation.[18] The increase of leaf B concentration during the growing period makes it difficult to determine toxicity thresholds for leaf B concentrations by foliar analysis, as B concentrations can vary considerably between old and young leaves. Moreover, when toxicity symptoms become visible in leaves, B concentrations can vary over several orders of magnitude even within single leaves.[18,19] Therefore, the distribution of B not only among but also within leaves needs to be analyzed for the determination of B toxicity thresholds in leaf tissue. Various techniques have been applied to measure the spatial B concentration in leaves.[19-21] However, these methods are either time-consuming, produce an incomplete picture of the B distribution within the leaves or their suitability for high B concentrations has not been shown. In this study, neutron radiography (NR) was applied for the first time to analyze the spatial distribution of ^{10}B in leaves. While the transfer of B from soil into the shoots of poplars is of great interest with respect to potential phytomanagement of contaminated sites, there is little knowledge on B accumulation by poplars. Therefore, the objectives of this study were to determine (1) the aboveground accumulation of B by *Populus nigra x euramericana* in comparison to *Salix viminalis*, *B. juncea* and *Lupinus albus* and their tolerance to B in soil under controlled growing conditions, (2) the accumulation of B in roots, shoots and leaves of poplars and (3) the distribution of B within individual poplar leaves in order to identify B threshold concentrations at which the tissue becomes chlorotic or necrotic.

Materials and Methods

Plant growth. *Populus nigra x euramericana*, (clone "Dorskamp"), *S. viminalis* (spp.), *B. juncea* (spp.) and *L. albus* (L.) plants were grown on a potting mix (PM) under greenhouse conditions with natural lighting at the Swiss Federal Research Institute, WSL (Birmensdorf, 47° 21′ 16″ N, 8° 26′ 16″ O), Switzerland. *Populus* was chosen because of its known B accumulation and phytoremediation potential of B contaminated sites.[10] *Salix viminalis* and *B. juncea* were chosen as alternative phytoremediation plants that are often used or proposed for the phytoremediation of contaminated sites,[22,23] and *L. albus* was selected because of the phloem mobility of B in this species.[24] Apart from the control treatment with no added B, three soil B treatments were initially established by spiking the PM substrate with different amounts of ^{10}B-enriched H_3BO_3 (^{10}B >96%, EaglePicher Technologies, Quapaw, USA). The resulting HNO_3- and $CaCl_2$-extractable B concentrations of the substrates, which showed a linear relationship (r^2= 0.88; y= 0.50x-13.1; p<0.001), are given in Table S1 (Supporting Information (SI)). The chosen B treatments represent the range of soil B concentrations reported in previous studies on B uptake by poplars from contaminated soils.[10,13,15] Nitric acid and $CaCl_2$-extractable concentrations of macro- and micro-nutrients in the PM substrate are given in Table S2. The pH ($CaCl_2$, substrate : 0.01 mol $CaCl_2$ ratio: 1 : 2.5) of the substrate was 5.0, the total carbon concentration was 270.6 g kg^{-1} and the nitrogen concentration was 6.78 g kg^{-1}.

In April 2005, we prepared three replicate pots (5.5 L) for each treatment and plant species and planted 3 plants in each pot. Planting occurred immediately after the pots were filled with ca. 4 kg of substrate. *P. nigra* × *euramericana* and *S. viminalis* were planted as cuttings (20 cm in length and 1 cm diameter), *L. albus* and *B. juncea* as seeds. Two weeks after planting, all plants were thinned to one plant per pot. Because *S. viminalis*, *L. albus* and *B. juncea* did not grow at substrate B concentrations of 168 and 280 mg kg^{-1} two intermediate treatments were set up on the same occasion with B concentrations of 22 and 45 mg kg^{-1}. *P. nigra* × *euramericana* was not planted in these two additional B treatments. The control treatment and the five B treatments are denoted as T_{13}, T_{22}, T_{45}, T_{93}, T_{168} and T_{280} according to the total initial B concentration of the respective substrate. Pots were irrigated with tap water 3-4 times per week to about field capacity, e.g. to the point where water started to drain into the trays. The leachates were collected and reapplied to the pots. All plants were harvested after four months of growth. The aboveground biomass was separated into leaves, stems, and in the case of *B. juncea,* also into pods. For *P. nigra* × *euramericana* and *S. viminalis*, only the new shoot growth and not the originally planted cuttings were used for analysis. Roots were separated from the substrate by washing with tap water, followed by rewashing with deionized water to remove small particles. Fine roots were collected using a 2 mm Nylon sieve. Plant biomass was dried until constant weight was obtained and the biomass was recorded. For *P. nigra* × *euramericana* we also recorded the position of the leaves in the sequence along the shoot starting with the 1st leaf at the bottom of the plant. ×

Neutron radiography. We used ^{10}B-enriched B to determine the areal distribution of accumulated B within leaves by means of neutron radiography.[25,26] The neutron absorption cross section of ^{10}B as determined at ICON (Instrument for Cold Neutron Radiography) is 8720 E^{-24} cm^{-2}. This is several orders of magnitude higher than that of ^{11}B (11.5 E^{-24} cm^{-2}), enabling the visualization of ^{10}B within leaf tissue. A preliminary test with NR revealed that only poplar, but none of the other plants accumulated sufficient ^{10}B in their leaves for NR. Neutron radiographs of dried poplar leaves were taken at the ICON facility of the Paul-Scherrer-Institute (Villigen), Switzerland.[27] The NR data were calibrated against ICP-OES measurements of leaf B concentrations. After neutron imaging, the leaves were scanned using an office scanner (Agfa, SnapScan 1236) at 150 dpi. Colour images were analyzed using WinRhizoPro[28] to assess the ratio between healthy and chlorotic or necrotic leaf area ($R_{h/cn}$) for each leaf.

Chemical analysis. For chemical analysis, aliquots of dried and ground plant samples were digested in a heating block at 130 °C in 15mL of a 65% HNO_3. The digests were analyzed for B and other elements by ICP-OES (Vista MPX, Varian, Australia). Samples of PM substrate were analyzed for B after nitric acid digestion in the same way. Certified plant reference material NCS DC-73350 (poplar leaves, China National Analysis Centre for Iron and Steel, Beijing, China) was used for quality control. The average recovery rate for B was 98.4 ± 2%. To determine extractable concentrations of B and other elements in the PM substrate, 1:10 mixtures of substrate and 0.01 mol $CaCl_2$ were shaken for 16 h, centrifuged at 929.3×g for 10 min, filtered through a 0.25-µm membrane filter and analyzed by ICP-OES. Carbon and nitrogen contents of the PM substrate were measured using an elemental analyzer (CNS-2000, Leco Corp., Saint Joseph, Michigan USA).

Statistics. Mean whole-plant element concentrations were calculated as mass-weighted average of the respective element concentrations of individual plant parts. Kruskal-Wallis-ANOVA was performed to test for differences in biomass and element concentrations between B treatments, followed by the Mann–Whitney U-Test as post-hoc test to compare pair-wise differences between treatments. Values given for correlations between variables represent Pearsons' correlation coefficients. All statistical analyses were carried out using PASW Statistics (Release 17.0.2).

Results and discussion

Biomass. All poplar saplings survived even at the highest B treatment levels, although they showed reduced growth in T_{168} and severe growth reduction in T_{280}. Our results are consistent with the high B tolerance reported by Robinson et al.[13] for poplars growing on B contaminated sites. Figure S1 (SI) shows the aboveground biomass of the harvested plants, excluding the part of the stem axis corresponding to the cutting originally planted in the case of *P. nigra* × *euramericana* and *S. viminalis*. *L. albus* and *B. juncea* plants survived in the T_{93} treatment without any reduction in growth, but failed to grow at higher B concentrations. *S. viminalis* only grew in the T_{13} and the T_{22} treatment and its biomass was significantly lower than that of *P. nigra* × *euramericana* in T_{13} and that of *B. juncea* in T_{13} and T_{22}. Thus, *S. viminalis* was the least B tolerant of the four species tested, while poplar was the most tolerant. This was a surprising observation given that poplars and willows belong to the same family (*Salicaceae*). Plants that do not tolerate elevated soil B concentrations are obviously not suited to remediate B contaminated sites. However, both *Populus* and *Salix* exhibit considerable inter- and intra-specific genetic and phenotypic variability with respect to B uptake and tolerance.[15,29] Therefore, other *Populus* and *Salix* species and genotypes may have different B tolerance characteristics.

Figure 1 shows that the relative decrease in the biomass of the poplar plants was larger in the roots than in leaves and stems in the T_{168} and T_{280} treatments. The shoot: root biomass ratio increased from 6 in the control treatment to 25 and 57 in the T_{168} and the T_{280} treatments, respectively. The fact that high soil B concentrations had a stronger negative effect on root than on shoot biomass in *P. nigra* × *euramericana* indicates a higher B sensitivity of the roots or a mode of biological protection to absorb less B. High concentrations of soil B are known to inhibit root growth relative to shoot growth.[30] Reduced growth may be a general response of poplar roots towards contaminants as poplar roots were shown to react in the same way towards elevated soil Zn and Cd concentrations.[31]

Boron accumulation and allocation in the plants. While in the control treatment shoot B concentrations did not differ among species, significant differences emerged at higher B treatment concentrations (Table 1). The bioconcentration factors (BCF) (plant/soil concentration quotients) ranged between 3.5-5 for all species and all treatments, except for *B. juncea* (BC: 1.5-2.7) in the B treatments. The highest BCF values were found for poplar in the T_{168} and T_{280}. *Brassica juncea* was found to exclude B from entering its shoots. Shoot B concentrations in this species did not differ between T_{13}, T_{22} and T_{45} and were still less than half of the surviving *L. albus* plants in the T_{93} treatment. The B concentrations found in *B. juncea* were in the same range as those reported by Bañuelos et al..[32]

If the B tolerance of *P. nigra* × *euramericana* was due to B exclusion from uptake by the roots, then we would expect non-tolerant plants to have higher shoot B concentrations than B-tolerant poplars grown on the same substrate. We did not find such a relationship between the plant species used in this study. The ability of the poplars to accumulate higher concentrations of B than the other species was apparently due to a greater B tolerance in their leaf tissues, demonstrating that this characteristic can be a useful strategy to deal with elevated soil B concentrations. The phloem mobility of B in *L. albus* did not increase its B tolerance in comparison to *P. nigra* × *euramericana*, *L. albus* and *S. viminalis*. Also, the lower B accumulation in *B. juncea* did not increase its B tolerance compared to the other species and was less successful under the conditions of our study. These results are consistent with findings that B can easily penetrate cell membranes, indicating that regulation of B entry into the symplast and further into the root xylem, by means of membrane transporters is ineffective.[33] Unlike other nutrient elements, B is taken up by plants as the neutral species H_3BO_3 which is dominant in soil solution at pH <9.24.[33] This species has a diameter of only 0.257 nm and thus may easily pass through cell membranes via aquaporins.[34]

Figure 2 shows that there were no significant differences between root and stem B concentrations, which both increased in the poplar plants with the B concentration of the substrate. In the T_{168} and T_{280} treatments, the average leaf B concentration exceeded 1000 mg kg^{-1}. This is in agreement with the notion that B is primarily passively transported with the transpiration stream and deposited in the leaves upon evaporation of the water and is consistent with previous reports.[10,13]

Compared to the other tested species, *P. nigra* × *euramericana* has good potential for the phytomanagement of B contaminated sites. The total uptake of B into the aboveground biomass of *P. nigra* × *euramericana* during 4 months was 1 mg per plant in T_{13} and 8 mg per plant in T_{93}, which represented about 2.1% of the total B initially present in the pots in T_{93}. In the T_{168} treatment, the total uptake of B was 7.2 mg per plant. In T_{168}, the higher plant B concentration compensated the lower plant biomass in comparison to T_{93}. However, in T_{168} the 7.2 mg B extracted were only 1% of the total B in the pot. This uptake was higher than found in *Gypsophila arrostil* and in the same range as reported for *Pucinella distans*, two species considered as potential B hyperaccumulator plants.[35] The highest uptake found for one of the other species tested in this study was 0.7 mg B per plant in *B. juncea*. With an estimated annual leaf biomass production of 15 t ha^{-1} a^{-1} *P. nigra* × *euramericana* could extract 6.3 kg B ha^{-1} a^{-1} from contaminated topsoil containing 75 kg B ha^{-1}. To prevent the extracted B from returning to the soil via leaf fall, removal of the leaves from the site would be necessary. For that purpose poplars could be coppiced.[13] The B rich leaves could be used as an organic fertilizer on B deficient sites or used as stock fodder.[36] Only leaves from T_{13} and T_{93} would be suitable as stock fodder, as B concentrations >800 mg kg^{-1} may be toxic to livestock.[37] Leaves from the T_{168} and T_{280} treatment could still be used as fodder if mixed with fodder produced on unpolluted soil.

Partitioning of B in *Populus nigra* × *euramericana* leaves. In all treatments, B concentrations decreased exponentially with leaf number from the lower (older) to the upper (younger) leaves of the poplar saplings (Fig. 3). There was a more than tenfold difference in average B concentration between the oldest and the youngest leaves in all B treatments. The B concentration ranges from top to bottom leaves were 22-185 (T_{13}), 62-1725 (T_{93}), 190-3241 (T_{168}) and 298-3472 (T_{280}) for the respective treatments, with only small differences between the highest treatments T_{168} and T_{280}. These results have implications for the interpretation of data for B accumulation in poplar trees sampled in the field.[18] It is usually only possible to collect and analyze a small number of leaves from a tree. As our results show, B concentration data from leaf samples may vary by an order of magnitude depending on the position of the sampled leaves. Robinson et al.[10] found that leaf B concentrations also varied considerably with time over a growing season. Again, these findings are support that B accumulation in the leaves is primarily associated with the transpiration water flow and that there is little or no relocation of B in the phloem of poplars. The leaf B concentrations did not depend on the size of the leaves (data not shown). The leaves emerging in the middle of the growing season were larger than the leaves produced at the beginning and the end of the growing season, while the B concentration of the leaves that emerged in the middle of the growing season steadily increased with age.

With increasing leaf B concentrations the fraction of chlorotic and necrotic areas on the sampled leaves increased (Fig. 4). At leaf B concentrations <900 mg kg^{-1} $R_{h/cn}$ was always <10%. The leaf B concentration range 900-1199 mg kg^{-1} was a threshold across which $R_{h/cn}$ jumped to values above 30%. At leaf B concentrations >1200 mg kg^{-1} the value of $R_{h/cn}$ increased linearly (r^2= 0.98; y= 4.07x+27.21; p< 0.001), until a second threshold was reached at B concentrations >2100 mg kg^{-1} where $R_{h/cn}$ increased to >70%.

Tripler et al.[38] found similar leaf necrosis effects associated with high leaf B concentrations in date palm. Increasing contaminant accumulation and leaf chlorosis/necrosis with leaf age is also known for Zn and Cd, although these metals were stored in different tissues.[39,40]

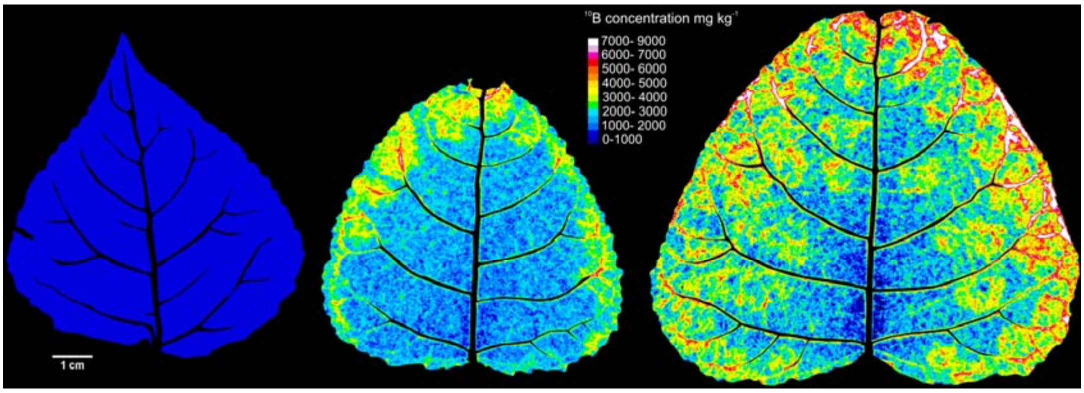
The distribution of B within *Populus nigra* × *euramericana* leaves. Comparison of the ICP-OES measurements and the NR results showed that local tissue ^{10}B accumulation in leaves was detectable by NR if concentrations in leaves exceeded 300 mg kg^{-1}. The detection limit and the spatial resolution of neutron radiographs (130 µm) thus were sufficient for the determination of toxicity thresholds in *P. nigra* × *euramericana* leaf tissue. Boron concentrations in the leaves of *B. juncea*, *S. viminalis* and *L. albus* were below the detection limit. Here, laser ablation ICP-MS could be an alternative.[20]

Within individual leaves, the highest B concentrations occurred at the leaf margins and tips. The margins and tips were also the locations where chlorosis and necrosis occurred first and were strongest. At average leaf B concentrations greater than 1000 mg kg^{-1} leaf margins and tips curled. At higher total leaf B concentrations necrotic spots occurred throughout the leaf. These spots contained >2000 mg B kg^{-1}. Leaf tissue containing between 1000 and 2000 mg B kg^{-1} was chlorotic and tissue containing more than 2000 mg kg^{-1} was necrotic. The finding of B concentrations >7000 mg kg^{-1} in some spots in necrotic leaf tissue indicates that B accumulation continued in leaf tissue even after the onset of necrosis and that necrotic tissue can still receive B via the transpiration flow. Similar findings were reported by Reid and Fitzpatrick[19] for barley. Deposition of B at high concentrations in discrete patches may be a tolerance mechanism by which a small patch of photosynthetic tissue is sacrificed in order to prevent overloading of the surrounding tissues. The ability of *P. nigra* × *euramericana* to accumulate higher B concentrations in its aerial tissue than the other species tested can be attributed to the high B tolerance of the living leaf tissue and the storage of B in dead leaf tissue.

The B accumulation characteristics of *P. nigra* × *euramericana* are consistent with the criteria compiled by Branquinho et al.[41] for hyperaccumulation. The BCF as well as the shoot to root concentration ratio were >1 in *P. nigra* × *euramericana* and the aboveground B concentration in two (T_{168} and T_{280}) of three B treatments was more than 10-times higher than in the control (T_{13}). In contrast to many metals,[42] there is no established shoot threshold B concentration above which a plant is considered to be a B hyperaccumulator. For example for Ni the threshold concentration used as criterion for hyperaccumulation is 1000 mg kg^{-1},[43] which corresponds to 17.0 mmol kg^{-1}. The equivalent mass concentration of B is just 172 mg kg^{-1} because of its 80% lower molar weight compared to Ni. This concentration was exceeded in some of the poplar leaves grown in the control treatment and in more than 85% of the leaves in the treatments with higher B concentrations. In addition, the accumulation of 1000 mg B kg^{-1}, a 20-times higher tissue concentration than the 50 mg kg^{-1} that is generally considered to be toxic in tissues of most other plants, is an indicator of B hyperaccumulation in poplar[44]. However, as the comparison with other species showed, B accumulation in poplars seems not to be active and they do not fulfil the criterion that hyperaccumulators should have at least 100-fold higher concentrations of the respective trace element than non-hyperaccumulators when grown in contaminated soil.[43] This indicates that B hyperaccumulation in poplars is not hyperaccumulation in the strictest sense, but rather B hypertolerance and thus comparable to the passive arsenic hyperaccumulation in aquatic macrophytes described by Robinson et al.[45]. Our results indicate that poplar is better suited for phytomanagement of B contaminated soil than *S. viminalis* or *B. juncea,* which have been proposed for the phytoextraction of other trace elements.

Figures & Tables

TOC Art



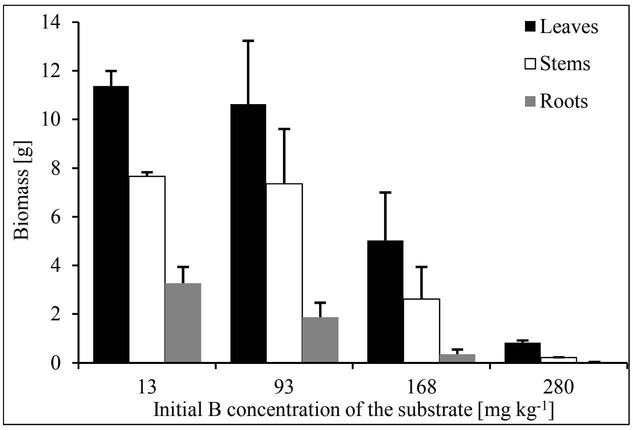


Figure 1. Leaf, stem and root biomass of 5 month old *P. nigra* × *euramericana* saplings grown on substrates with different B concentrations. The lowest B concentration (13 mg kg^{-1}) is the control treatment. The mass of the cutting from which the saplings were grown is not included. Error bars represent standard errors (N=3).

Table 1. Mean ± S. E. B accumulation in the aboveground biomass of *L. albus*, *B. juncea*, *P. nigra* × *euramericana* and *S. viminalis* grown on substrate with different B concentrations. T_{13} is the control treatment.

B concentration								
Treatment	*L. albus*		*B. juncea*		*P. nigra* × *euramericana*		*S. viminalis*	
	[mg kg^{-1}]							
T_{13}	40.5^{a}	± 3.44	43.5^{a}	± 4.69	43.8^{a}	± 0.29	48.6^{a}	± 4.67
T_{22}	$114.2^{b\ I}$	± 16.6	$60.1^{a\ II}$	± 4.37	N/A		$118.3^{b\ I}$	± 11.3
T_{45}	$174.6^{bc\ I}$	± 27.2	$68.1^{ab\ II}$	± 17.2	N/A		†	
T_{93}	$304.4^{c\ I}$	± 20.7	$136.4^{b\ II}$	± 19.1	$392.4^{b\ I}$	± 28.7	†	

Statistically significant differences between treatments are indicated by characters and differences between plant species within the same treatment by roman numerals (Mann-Whitney U-test, $p<0.05$, N=3). N/A: not applicable. †: plant died.

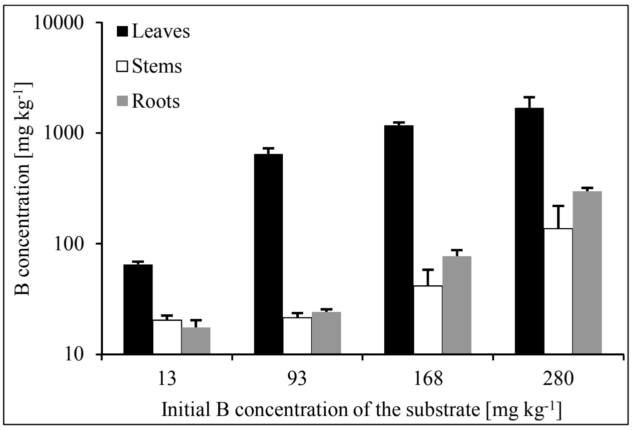


Figure 2. Concentrations of B in roots, stems and leaves of 4 months old of *P. nigra* × *euramericana* plants. The lowest B concentration (13 mg kg^{-1}) is the control treatment. Note that the B concentration is shown on logarithmic scale for better clarity. Error bars represent standard errors (N=3).

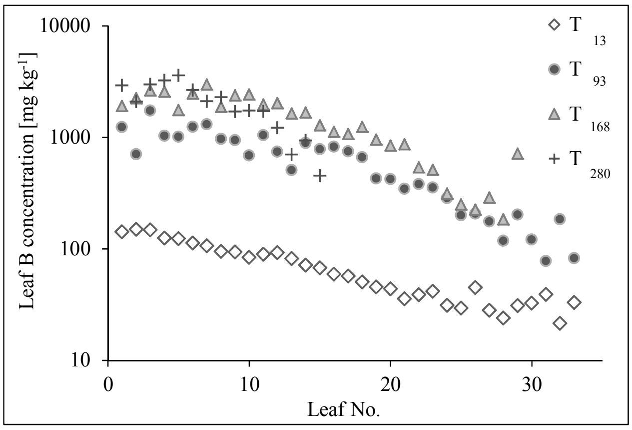


Figure 3. Leaf B concentration as a function of leaf position, counting from bottom to top along the stems of 4 months old poplars grown on substrate with different B concentrations. Note that the B concentration is shown on logarithmic scale for better clarity.

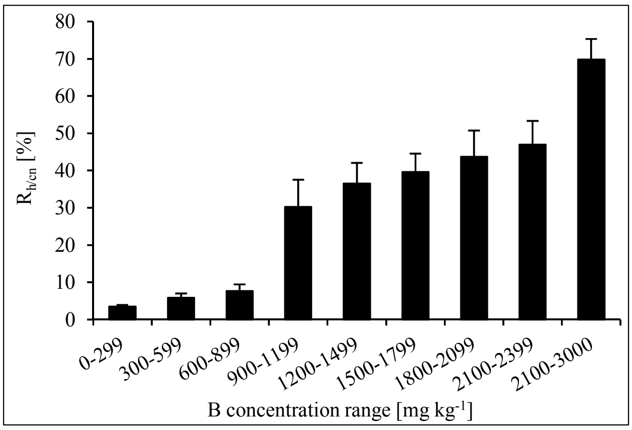


Figure 4. Chlorotic and necrotic leaf area expressed as percentage of total leaf area ($R_{h/cn}$) as a function of leaf B concentration. Note the large increase in chlorotic and necrotic leaf area above 900 mg B kg^{-1}. Error bars represent standard errors.

Acknowledgements:

Funds for this study came from the Swiss National Science Foundation (SNSF). We would also like to thank Rene Saladin from the Soil Protection lab at ETH, Lidija Josic from PSI for help with the NR analysis and providing the ^{10}B cross section data and Anton Burkart and his team at WSL for the cuttings and tending the plants.

Supporting Information Available:

Details on the growing substrate and plant biomass. This information is available free of charge via the Internet at http://pubs.acs.org/

References

(1) Salisbury, F. B.; Ross, C. W., *Plant Physiology* 4th Edition.; Wadsworth Publishing Co., Inc.: Belmont, California, USA., 1992.
(2) Hunt, C. D., Dietary boron: Evidence for essentiality and homeostatic control in humans and animals. In *Advances in Plant and Animal Boron Nutrition*, Springer: 2007; pp 251.
(3) Shorrocks, V. M. The occurrence and correction of boron deficiency. *Plant and Soil* **1997,** *193* (1-2), 121-48.
(4) Goldberg, S. Reactions of boron with soils. *Plant and Soil* **1997,** *193* (1-2), 35-48.
(5) Schulin, R., et al., Trace element deficient soils. In *Trace Elements in Soils*, Hooda, P., Ed. Wiley-Blackwell Publishing: Chichester, U.K, 2010; pp 175.
(6) Takano, J.; Noguchi, K.; Yasumori, M.; Kobayashi, M.; Gajdos, Z.; Miwa, K.; Hayashi, H.; Yoneyama, T.; Fujiwara, T. Arabidopsis boron transporter for xylem loading. *Nature* **2002,** *420* (6913), 337-40.
(7) Nable, R. O.; Banuelos, G. S.; Paull, J. G. Boron toxicity. *Plant and Soil* **1997,** *193* (1-2), 181-98.
(8) Parks, J. L.; Edwards, M. Boron in the environment. *Critical Reviews in Environmental Science and Technology* **2005,** *35* (2), 81-114.
(9) Hathaway, R. Short-rotation coppiced willows for sheep fodder in New Zealand. *New Zealand Agriculture Science* **1986,** *20* (3), 140-42.
(10) Robinson, B. H.; Green, S. R.; Chancerel, B.; Mills, T. M.; Clothier, B. E. Poplar for the phytomanagement of boron contaminated sites. *Environmental Pollution* **2007,** *150* (2), 225-33.
(11) Mastromatteo, E.; Sullivan, F. Summary- International Symposium on the health-effects of boron and its compounds. *Environ. Health Perspect.* **1994,** *102*, 139-41.
(12) Bañuelos, G. S.; Shannon, M. C.; Ajwa, H.; Draper, J. H.; Jordahl, J.; Licht, J. Phytoextraction and Accumulation of Boron and Selenium by Poplar (*Populus*) Hybrid Clones. *International Journal of Phytoremediation* **1999,** *1* (1), 81- 6. DOI 10.1080/15226519908500006
(13) Robinson, B.; Green, S.; Mills, T.; Clothier, B.; Velde, M. v. d.; Laplane, R.; Fung, L.; Deurer, M.; Hurst, S.; Thayalakumaran, T.; Dijssel, C. v. d. Phytoremediation: using plants as biopumps to improve degraded environments. *Australian Journal of Soil Research* **2003,** *41* (3), 599-11.
(14) Dellantonio, A.; Fitz, W. J.; Repmann, F.; Wenzel, W. W. Disposal of Coal Combustion Residues in Terrestrial Systems: Contamination and Risk Management. *Journal of Environmental Quality* **2010,** *39* (3), 761-775. 10.2134/jeq2009.0068
(15) Dellantonio, A.; Fitz, W. J.; Custovic, H.; Repmann, F.; Schneider, B. U.; Grunewald, H.; Gruber, V.; Zgorelec, Z.; Zerem, N.; Carter, C.; Markovic, M.; Puschenreiter, M.; Wenzel, W. W. Environmental risks of farmed and barren alkaline coal ash landfills in Tuzla, Bosnia and Herzegovina. *Environmental Pollution* **2008,** *153* (3), 677-86.
(16) Pulford, I. D.; Watson, C. Phytoremediation of heavy metal-contaminated land by trees-a review. *Environment International* **2003,** *29*, 529-40.
(17) Bañuelos, G. S.; Cardon, G. E.; Phene, C. J.; Wu, L.; Akohoue, S.; Zambrzuski, S. Soil boron and selenium removal by three plant species. *Plant and Soil* **1993,** *148* (2), 253-63.
(18) Oertli, J. J. Nonhomogeneity of boron distribution in plants and consequences for foliar diagnosis. *Commun. Soil Sci. Plant Anal.* **1994,** *25* (7-8), 1133-47.
(19) Reid, R.; Fitzpatrick, K. Influence of Leaf Tolerance Mechanisms and Rain on Boron Toxicity in Barley and Wheat. *Plant Physiol.* **2009,** *151* (1), 413-20.
(20) Wu, B.; Zoriy, M.; Chen, Y.; Becker, J. S. Imaging of nutrient elements in the leaves of *Elsholtzia splendens* by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *Talanta* **2009,** *78* (1), 132-37.

(21) Loria, L. G.; Jimenez, R.; Badilla, M.; Lhuissier, F.; Goldbach, H.; Thellier, M. Neutron-capture-radiography study of the distribution of boron in the leaves of coffee plants grown in the field. *J. Trace Microprobe Tech.* **1999,** *17* (1), 91-99.
(22) Dickinson, N. M.; Pulford, I. D. Cadmium phytoextraction using short-rotation coppice Salix: the evidence trail. *Environment International* **2005,** *31* (4), 609-13.
(23) Vamerali, T.; Bandiera, M.; Mosca, G. Field crops for phytoremediation of metal-contaminated land. A review. *Environmental Chemistry Letters* **2010,** *8* (1), 1-17.
(24) Huang, L.; Bell, R. W.; Dell, B. Evidence of phloem boron transport in response to interrupted boron supply in white lupin (Lupinus albus L. cv. Kiev Mutant) at the reproductive stage. *Journal of Experimental Botany* **2008,** *59* (3), 575-583. 10.1093/jxb/erm336
(25) Zawisky, M.; Basturk, M.; Derntl, R.; Dubus, F.; Lehmann, E.; Vontobel, P. Non-destructive B-10 analysis in neutron transmission experiments. *Applied Radiation and Isotopes* **2004,** *61* (4), 517-23.
(26) Menon, M.; Robinson, B.; Oswald, S. E.; Kaestner, A.; Abbaspour, K. C.; Lehmann, E.; Schulin, R. Visualization of root growth in heterogeneously contaminated soil using neutron radiography. *European Journal of Soil Science* **2007,** *58* (3), 802-10.
(27) Kuhne, G.; Frei, G.; Lehmann, E.; Vontobel, P. CNR - the new beamline for cold neutron imaging at the Swiss spallation neutron source SINQ. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.* **2005,** *542* (1-3), 264-70.
(28) Regent Instruments: *WinRhizo Pro*, c; 2009.
(29) Bañuelos, G. S.; LeDuc, D.; Johnson, J. Evaluating the Tolerance of Young Hybrid Poplar Trees to Recycled Waters High in Salinity and Boron. *International Journal of Phytoremediation* **2010,** *12* (5), 419-39.
(30) Reid, R. J.; Hayes, J. E.; Post, A.; Stangoulis, J. C. R.; Graham, R. D. A critical analysis of the causes of boron toxicity in plants. *Plant, Cell and Environment* **2004,** *27* (11), 1405-14.
(31) Dos Santos Utmazian, M. N.; Wieshammer, G.; Vega, R.; Wenzel, W. W. Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. *Environmental Pollution* **2007,** *148* (1), 155-165. DOI: 10.1016/j.envpol.2006.10.045
(32) Bañuelos, G. S.; Cardon, G. E.; Mackey, B.; Benasher, J.; Wu, L.; Beuselinck, P.; Akohoue, S.; Zambruzski, S. Boron and selenium removal in boron-laden soils by 4 sprinkler irrigated plant-species. *Journal of environmental quality* **1993,** *22* (4), 786-92.
(33) Hu, H.; Brown, P. H. Absorption of boron by plant roots. *Plant and Soil* **1997,** *193* (1), 49-58.
(34) Tanaka, M.; Fujiwara, T. Physiological roles and transport mechanisms of boron: perspectives from plants. *Pflugers Archiv-European Journal of Physiology* **2008,** *456* (4), 671-77.
(35) Stiles, A. R.; Bautista, D.; Atalay, E.; Babaoğlu, M.; Terry, N. Mechanisms of Boron Tolerance and Accumulation in Plants: A Physiological Comparison of the Extremely Boron-Tolerant Plant Species, Puccinellia distans, with the Moderately Boron-Tolerant Gypsophila arrostil. *Environmental Science & Technology* **2010,** *44* (18), 7089-95.
(36) Robinson, B.; Mills, T.; Green, S.; Chancerel, B.; Clothier, B.; Fung, L.; Hurst, S.; McIvor, I. Trace element accumulation by poplars and willows used for stock fodder. *N. Z. J. Agric. Res.* **2005,** *48* (4), 489-97.
(37) Underwood, E. J.; Suttle, N. F., *The mineral nutrition of livestock*. CAB International Publishing: Wallingford, UK, 1999.
(38) Tripler, E.; Ben-Gal, A.; Shani, U. Consequence of salinity and excess boron on growth, evapotranspiration and ion uptake in date palm (*Phoenix dactylifera* L., cv. Medjool). *Plant and Soil* **2007,** *297* (1-2), 147-55.

(39) Vollenweider, P.; Menard, T.; Günthardt-Goerg, M. S. Compartmentation of metals in foliage of Populus tremula grown on soils with mixed contamination. I. From the tree crown to leaf cell level. *Environmental Pollution* **2011,** *159* (1), 324-36.
(40) Vollenweider, P.; Cosio, C.; Günthardt-Goerg, M. S.; Keller, C. Localization and effects of cadmium in leaves of a cadmium-tolerant willow (Salix viminalis L.): Part II Microlocalization and cellular effects of cadmium. *Environmental and Experimental Botany* **2006,** *58* (1-3), 25-40.
(41) Branquinho, C.; Serrano, H. C.; Pinto, M. J.; Martins-Loução, M. A. Revisiting the plant hyperaccumulation criteria to rare plants and earth abundant elements. *Environmental Pollution* **2007,** *146* (2), 437-443. 10.1016/j.envpol.2006.06.034
(42) Kramer, U., Metal Hyperaccumulation in Plants. In *Annual Review of Plant Biology, Vol 61*, Annual Reviews: Palo Alto, 2010; Vol. 61, pp 517.
(43) Brooks, R. R.; Lee, J.; Reeves, R. D.; Jaffre, T. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration* **1977,** *7*, 49-57.
(44) Jones, J. B. J., Plant tissue analysis in micronutrients. In *Micronutrients in Agriculture*, Mortvedt, J. J.; Cox, F. R.; Shuman, L. M.; Welch, R. M., Eds; SSSA: Madison, WI, U.S., 1991; Vol. 2nd ed. , pp 477.
(45) Robinson, B.; Kim, N.; Marchetti, M.; Moni, C.; Schroeter, L.; van den Dijssel, C.; Milne, G.; Clothier, B. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. *Environmental and Experimental Botany* **2006,** *58* (1-3), 206-215. 10.1016/j.envexpbot.2005.08.004