


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

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

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30 **Abstract**

31 Poplars accumulate high B concentrations and are thus used for the phytomanagement of
32 B contaminated soils. Here, we performed pot experiments in which *Populus nigra* ×
33 *euramericana* were grown on a substrate with B concentrations ranging from 13 to 280
34 mg kg⁻¹ as H₃BO₃. *Salix viminalis*, *Brassica juncea* and *Lupinus albus* were grown under
35 some growing conditions for comparison. Poplar growth was unaffected at soil B
36 treatment levels up to 93 mg kg⁻¹. Growth was progressively reduced at levels of 168 and
37 280 mg kg⁻¹. None of the other species survived at these substrate B levels. At leaf B
38 concentrations <900 mg kg⁻¹ only <10% of the poplar leaf area showed signs of toxicity.
39 Neutron radiography revealed that chlorotic leaf tissues had B concentrations of 1000-
40 2000 mg kg⁻¹, while necrotic tissues had >2000 mg kg⁻¹. Average B concentrations of up
41 to 3500 mg kg⁻¹ were found in leaves, while spots within leaves had concentrations >7000
42 mg kg⁻¹, showing that B accumulation in leaf tissue continued even after the onset of
43 necrosis. The B accumulation ability of *P. nigra* × *euramericana* is associated with B
44 hypertolerance in the living tissue and storage of B in dead leaf tissue.

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51 Introduction

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

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

70 Depending on growth conditions, poplar clone, B application form and salinity, B
71 accumulation in poplar leaves ranges between 500 and 1200 mg kg⁻¹, greatly exceeding
72 the B concentrations of the growing substrate and the poplar stems.^{10,12,13} In comparison

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6 73 to other species, the B accumulation of poplars was much higher in these studies. Apart
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9 74 from field surveys where B accumulation in poplars was found,¹⁴ there have been no
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11 75 studies following the original report by Bañuelos et al.¹², investigating the B
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13 76 accumulation of poplars in more detail, including bioaccumulation factors and B
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15 77 threshold concentrations compared to other species.

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18 78 Various *Salix* species have been shown to accumulate leaf B concentrations $>800 \text{ mg kg}^{-1}$
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20 79 ¹, exceeding those of poplars grown on the same fly ash disposal site, rendering also *Salix*
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22 80 interesting for the purpose of B extraction from contaminated soil.¹⁵ The phytoextraction
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24 81 efficiency of a plant species for a trace element depends on the respective accumulated
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26 82 concentration of the element and the amount of harvestable biomass.¹⁶ *Brassica juncea* is
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28 83 widely touted for use in phytoremediation and was reported to exhibit a high B
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30 84 tolerance.¹⁷ Despite its lower biomass production compared to poplars or willows, the
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32 85 phytoextraction efficiency of *B. juncea* may be similar if its B accumulation were higher.

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37 86 Boron accumulation varies widely among different parts of a plant, necessitating the
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39 87 analyses of all plant parts for their B concentration in order to elucidate the total B
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41 88 accumulation.¹⁸ The increase of leaf B concentration during the growing period makes it
42
43 89 difficult to determine toxicity thresholds for leaf B concentrations by foliar analysis, as B
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45 90 concentrations can vary considerably between old and young leaves. Moreover, when
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47 91 toxicity symptoms become visible in leaves, B concentrations can vary over several
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49 92 orders of magnitude even within single leaves.^{18,19} Therefore, the distribution of B not
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51 93 only among but also within leaves needs to be analyzed for the determination of B
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53 94 toxicity thresholds in leaf tissue. Various techniques have been applied to measure the
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55 95 spatial B concentration in leaves.¹⁹⁻²¹ However, these methods are either time-consuming,
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6 96 produce an incomplete picture of the B distribution within the leaves or their suitability
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9 97 for high B concentrations has not been shown. In this study, neutron radiography (NR)
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11 98 was applied for the first time to analyze the spatial distribution of ^{10}B in leaves. While the
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13 99 transfer of B from soil into the shoots of poplars is of great interest with respect to
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15 100 potential phytomanagement of contaminated sites, there is little knowledge on B
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17 101 accumulation by poplars. Therefore, the objectives of this study were to determine (1) the
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19 102 aboveground accumulation of B by *Populus nigra x euramericana* in comparison to *Salix*
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21 103 *viminalis*, *B. juncea* and *Lupinus albus* and their tolerance to B in soil under controlled
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23 104 growing conditions, (2) the accumulation of B in roots, shoots and leaves of poplars and
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25 105 (3) the distribution of B within individual poplar leaves in order to identify B threshold
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27 106 concentrations at which the tissue becomes chlorotic or necrotic.
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32 **Materials and Methods**

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36 108 **Plant growth.** *Populus nigra x euramericana*, (clone “Dorskamp”), *S. viminalis* (spp.),
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38 109 *B. juncea* (spp.) and *L. albus* (L.) plants were grown on a potting mix (PM) under
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40 110 greenhouse conditions with natural lighting at the Swiss Federal Research Institute, WSL
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42 111 (Birmensdorf, 47° 21' 16" N, 8° 26' 16" O), Switzerland. *Populus* was chosen because of
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44 112 its known B accumulation and phytoremediation potential of B contaminated sites.¹⁰
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46 113 *Salix viminalis* and *B. juncea* were chosen as alternative phytoremediation plants that are
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48 114 often used or proposed for the phytoremediation of contaminated sites,^{22,23} and *L. albus*
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50 115 was selected because of the phloem mobility of B in this species.²⁴ Apart from the control
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52 116 treatment with no added B, three soil B treatments were initially established by spiking
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54 117 the PM substrate with different amounts of ^{10}B -enriched H_3BO_3 (^{10}B >96%, EaglePicher
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56 118 Technologies, Quapaw, USA). The resulting HNO_3 - and CaCl_2 -extractable B
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6 119 concentrations of the substrates, which showed a linear relationship ($r^2 = 0.88$; $y = 0.50x -$
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8 120 13.1 ; $p < 0.001$), are given in Table S1 (Supporting Information (SI)). The chosen B
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10 121 treatments represent the range of soil B concentrations reported in previous studies on B
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12 122 uptake by poplars from contaminated soils.^{10,13,15} Nitric acid and CaCl_2 -extractable
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14 123 concentrations of macro- and micro-nutrients in the PM substrate are given in Table S2.
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16 124 The pH (CaCl_2 , substrate : 0.01 mol CaCl_2 ratio: 1 : 2.5) of the substrate was 5.0, the total
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18 125 carbon concentration was 270.6 g kg^{-1} and the nitrogen concentration was 6.78 g kg^{-1} .

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23 126 In April 2005, we prepared three replicate pots (5.5 L) for each treatment and plant
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25 127 species and planted 3 plants in each pot. Planting occurred immediately after the pots
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27 128 were filled with ca. 4 kg of substrate. *P. nigra* × *euramericana* and *S. viminalis* were
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29 129 planted as cuttings (20 cm in length and 1 cm diameter), *L. albus* and *B. juncea* as seeds.
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31 130 Two weeks after planting, all plants were thinned to one plant per pot. Because *S.*
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33 131 *viminalis*, *L. albus* and *B. juncea* did not grow at substrate B concentrations of 168 and
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35 132 280 mg kg^{-1} two intermediate treatments were set up on the same occasion with B
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37 133 concentrations of 22 and 45 mg kg^{-1} . *P. nigra* × *euramericana* was not planted in these
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39 134 two additional B treatments. The control treatment and the five B treatments are denoted
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41 135 as T_{13} , T_{22} , T_{45} , T_{93} , T_{168} and T_{280} according to the total initial B concentration of the
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43 136 respective substrate. Pots were irrigated with tap water 3-4 times per week to about field
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45 137 capacity, e.g. to the point where water started to drain into the trays. The leachates were
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47 138 collected and reapplied to the pots. All plants were harvested after four months of growth.
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49 139 The aboveground biomass was separated into leaves, stems, and in the case of *B. juncea*,
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51 140 also into pods. For *P. nigra* × *euramericana* and *S. viminalis*, only the new shoot growth
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53 141 and not the originally planted cuttings were used for analysis. Roots were separated from
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6 142 the substrate by washing with tap water, followed by rewashing with deionized water to
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9 143 remove small particles. Fine roots were collected using a 2 mm Nylon sieve. Plant
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11 144 biomass was dried until constant weight was obtained and the biomass was recorded. For
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13 145 *P. nigra* × *euramericana* we also recorded the position of the leaves in the sequence
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15 146 along the shoot starting with the 1st leaf at the bottom of the plant. ×

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18 147 **Neutron radiography.** We used ¹⁰B-enriched B to determine the areal distribution of
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20 148 accumulated B within leaves by means of neutron radiography.^{25,26} The neutron
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22 149 absorption cross section of ¹⁰B as determined at ICON (Instrument for Cold Neutron
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24 150 Radiography) is 8720 E⁻²⁴ cm⁻². This is several orders of magnitude higher than that of
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26 151 ¹¹B (11.5 E⁻²⁴ cm⁻²), enabling the visualization of ¹⁰B within leaf tissue. A preliminary
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28 152 test with NR revealed that only poplar, but none of the other plants accumulated
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30 153 sufficient ¹⁰B in their leaves for NR. Neutron radiographs of dried poplar leaves were
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32 154 taken at the ICON facility of the Paul-Scherrer-Institute (Villigen), Switzerland.²⁷ The
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34 155 NR data were calibrated against ICP-OES measurements of leaf B concentrations. After
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36 156 neutron imaging, the leaves were scanned using an office scanner (Agfa, SnapScan 1236)
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38 157 at 150 dpi. Colour images were analyzed using WinRhizoPro²⁸ to assess the ratio
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40 158 between healthy and chlorotic or necrotic leaf area ($R_{h/cn}$) for each leaf.
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47 159 **Chemical analysis.** For chemical analysis, aliquots of dried and ground plant samples
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49 160 were digested in a heating block at 130 °C in 15mL of a 65% HNO₃. The digests were
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51 161 analyzed for B and other elements by ICP-OES (Vista MPX, Varian, Australia). Samples
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53 162 of PM substrate were analyzed for B after nitric acid digestion in the same way. Certified
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55 163 plant reference material NCS DC-73350 (poplar leaves, China National Analysis Centre
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57 164 for Iron and Steel, Beijing, China) was used for quality control. The average recovery rate
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7 165 for B was $98.4 \pm 2\%$. To determine extractable concentrations of B and other elements in
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9 166 the PM substrate, 1:10 mixtures of substrate and 0.01 mol CaCl₂ were shaken for 16 h,
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11 167 centrifuged at $929.3\times g$ for 10 min, filtered through a 0.25- μm membrane filter and
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13 168 analyzed by ICP-OES. Carbon and nitrogen contents of the PM substrate were measured
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15 169 using an elemental analyzer (CNS-2000, Leco Corp., Saint Joseph, Michigan USA).

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19 170 **Statistics.** Mean whole-plant element concentrations were calculated as mass-weighted
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21 171 average of the respective element concentrations of individual plant parts. Kruskal-
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23 172 Wallis-ANOVA was performed to test for differences in biomass and element
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25 173 concentrations between B treatments, followed by the Mann–Whitney U-Test as post-hoc
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27 174 test to compare pair-wise differences between treatments. Values given for correlations
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29 175 between variables represent Pearsons' correlation coefficients. All statistical analyses
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31 176 were carried out using PASW Statistics (Release 17.0.2).

32 33 34 35 177 **Results and discussion**

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38 178 **Biomass.** All poplar saplings survived even at the highest B treatment levels, although
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40 179 they showed reduced growth in T₁₆₈ and severe growth reduction in T₂₈₀. Our results are
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42 180 consistent with the high B tolerance reported by Robinson et al.¹³ for poplars growing on
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44 181 B contaminated sites. Figure S1 (SI) shows the aboveground biomass of the harvested
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46 182 plants, excluding the part of the stem axis corresponding to the cutting originally planted
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48 183 in the case of *P. nigra* \times *euramericana* and *S. viminalis*. *L. albus* and *B. juncea* plants
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50 184 survived in the T₉₃ treatment without any reduction in growth, but failed to grow at higher
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52 185 B concentrations. *S. viminalis* only grew in the T₁₃ and the T₂₂ treatment and its biomass
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54 186 was significantly lower than that of *P. nigra* \times *euramericana* in T₁₃ and that of *B. juncea*
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6 187 in T₁₃ and T₂₂. Thus, *S. viminalis* was the least B tolerant of the four species tested, while
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8 188 poplar was the most tolerant. This was a surprising observation given that poplars and
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10 189 willows belong to the same family (*Salicaceae*). Plants that do not tolerate elevated soil B
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12 190 concentrations are obviously not suited to remediate B contaminated sites. However, both
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14 191 *Populus* and *Salix* exhibit considerable inter- and intra-specific genetic and phenotypic
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16 192 variability with respect to B uptake and tolerance.^{15,29} Therefore, other *Populus* and *Salix*
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18 193 species and genotypes may have different B tolerance characteristics.

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23 194 Figure 1 shows that the relative decrease in the biomass of the poplar plants was larger in
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25 195 the roots than in leaves and stems in the T₁₆₈ and T₂₈₀ treatments. The shoot: root biomass
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27 196 ratio increased from 6 in the control treatment to 25 and 57 in the T₁₆₈ and the T₂₈₀
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29 197 treatments, respectively. The fact that high soil B concentrations had a stronger negative
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31 198 effect on root than on shoot biomass in *P. nigra* × *euramericana* indicates a higher B
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33 199 sensitivity of the roots or a mode of biological protection to absorb less B. High
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35 200 concentrations of soil B are known to inhibit root growth relative to shoot growth.³⁰
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37 201 Reduced growth may be a general response of poplar roots towards contaminants as
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39 202 poplar roots were shown to react in the same way towards elevated soil Zn and Cd
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41 203 concentrations.³¹

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46 204 **Boron accumulation and allocation in the plants.** While in the control treatment shoot
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48 205 B concentrations did not differ among species, significant differences emerged at higher
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50 206 B treatment concentrations (Table 1). The bioconcentration factors (BCF) (plant/soil
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52 207 concentration quotients) ranged between 3.5-5 for all species and all treatments, except
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54 208 for *B. juncea* (BC: 1.5-2.7) in the B treatments. The highest BCF values were found for
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56 209 poplar in the T₁₆₈ and T₂₈₀. *Brassica juncea* was found to exclude B from entering its

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6 210 shoots. Shoot B concentrations in this species did not differ between T₁₃, T₂₂ and T₄₅ and
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8 211 were still less than half of the surviving *L. albus* plants in the T₉₃ treatment. The B
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10 212 concentrations found in *B. juncea* were in the same range as those reported by Bañuelos
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12 213 et al.³²

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16 214 If the B tolerance of *P. nigra* × *euramericana* was due to B exclusion from uptake by the
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18 215 roots, then we would expect non-tolerant plants to have higher shoot B concentrations
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20 216 than B-tolerant poplars grown on the same substrate. We did not find such a relationship
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22 217 between the plant species used in this study. The ability of the poplars to accumulate
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24 218 higher concentrations of B than the other species was apparently due to a greater B
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26 219 tolerance in their leaf tissues, demonstrating that this characteristic can be a useful
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28 220 strategy to deal with elevated soil B concentrations. The phloem mobility of B in *L. albus*
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30 221 did not increase its B tolerance in comparison to *P. nigra* × *euramericana*, *L. albus* and
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32 222 *S. viminalis*. Also, the lower B accumulation in *B. juncea* did not increase its B tolerance
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34 223 compared to the other species and was less successful under the conditions of our study.
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36 224 These results are consistent with findings that B can easily penetrate cell membranes,
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38 225 indicating that regulation of B entry into the symplast and further into the root xylem, by
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40 226 means of membrane transporters is ineffective.³³ Unlike other nutrient elements, B is
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42 227 taken up by plants as the neutral species H₃BO₃ which is dominant in soil solution at pH
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44 228 <9.24.³³ This species has a diameter of only 0.257 nm and thus may easily pass through
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46 229 cell membranes via aquaporins.³⁴

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54 230 Figure 2 shows that there were no significant differences between root and stem B
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56 231 concentrations, which both increased in the poplar plants with the B concentration of the
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58 232 substrate. In the T₁₆₈ and T₂₈₀ treatments, the average leaf B concentration exceeded 1000
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6 233 mg kg⁻¹. This is in agreement with the notion that B is primarily passively transported
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9 234 with the transpiration stream and deposited in the leaves upon evaporation of the water
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11 235 and is consistent with previous reports.^{10,13}
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14 236 Compared to the other tested species, *P. nigra* × *euramericana* has good potential for the
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16 237 phytomanagement of B contaminated sites. The total uptake of B into the aboveground
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18 238 biomass of *P. nigra* × *euramericana* during 4 months was 1 mg per plant in T₁₃ and 8 mg
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20 239 per plant in T₉₃, which represented about 2.1% of the total B initially present in the pots
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22 240 in T₉₃. In the T₁₆₈ treatment, the total uptake of B was 7.2 mg per plant. In T₁₆₈, the higher
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24 241 plant B concentration compensated the lower plant biomass in comparison to T₉₃.
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26 242 However, in T₁₆₈ the 7.2 mg B extracted were only 1% of the total B in the pot. This
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28 243 uptake was higher than found in *Gypsophila arrostil* and in the same range as reported for
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30 244 *Pucinnella distans*, two species considered as potential B hyperaccumulator plants.³⁵ The
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32 245 highest uptake found for one of the other species tested in this study was 0.7 mg B per
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34 246 plant in *B. juncea*. With an estimated annual leaf biomass production of 15 t ha⁻¹ a⁻¹ *P.*
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36 247 *nigra* × *euramericana* could extract 6.3 kg B ha⁻¹ a⁻¹ from contaminated topsoil
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38 248 containing 75 kg B ha⁻¹. To prevent the extracted B from returning to the soil via leaf fall,
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40 249 removal of the leaves from the site would be necessary. For that purpose poplars could be
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42 250 coppiced.¹³ The B rich leaves could be used as an organic fertilizer on B deficient sites or
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44 251 used as stock fodder.³⁶ Only leaves from T₁₃ and T₉₃ would be suitable as stock fodder, as
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46 252 B concentrations >800 mg kg⁻¹ may be toxic to livestock.³⁷ Leaves from the T₁₆₈ and T₂₈₀
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48 253 treatment could still be used as fodder if mixed with fodder produced on unpolluted soil.
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56 254 **Partitioning of B in *Populus nigra* × *euramericana* leaves.** In all treatments, B
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58 255 concentrations decreased exponentially with leaf number from the lower (older) to the
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6 256 upper (younger) leaves of the poplar saplings (Fig. 3). There was a more than tenfold
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9 257 difference in average B concentration between the oldest and the youngest leaves in all B
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11 258 treatments. The B concentration ranges from top to bottom leaves were 22-185 (T₁₃), 62-
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13 259 1725 (T₉₃), 190-3241 (T₁₆₈) and 298-3472 (T₂₈₀) for the respective treatments, with only
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15 260 small differences between the highest treatments T₁₆₈ and T₂₈₀. These results have
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17 261 implications for the interpretation of data for B accumulation in poplar trees sampled in
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19 262 the field.¹⁸ It is usually only possible to collect and analyze a small number of leaves
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21 263 from a tree. As our results show, B concentration data from leaf samples may vary by an
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23 264 order of magnitude depending on the position of the sampled leaves. Robinson et al.¹⁰
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25 265 found that leaf B concentrations also varied considerably with time over a growing
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27 266 season. Again, these findings are support that B accumulation in the leaves is primarily
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29 267 associated with the transpiration water flow and that there is little or no relocation of B in
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31 268 the phloem of poplars. The leaf B concentrations did not depend on the size of the leaves
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33 269 (data not shown). The leaves emerging in the middle of the growing season were larger
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35 270 than the leaves produced at the beginning and the end of the growing season, while the B
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37 271 concentration of the leaves that emerged in the middle of the growing season steadily
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39 272 increased with age.
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46 273 With increasing leaf B concentrations the fraction of chlorotic and necrotic areas on the
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48 274 sampled leaves increased (Fig. 4). At leaf B concentrations <900 mg kg⁻¹ R_{h/cn} was
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50 275 always <10%. The leaf B concentration range 900-1199 mg kg⁻¹ was a threshold across
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52 276 which R_{h/cn} jumped to values above 30%. At leaf B concentrations >1200 mg kg⁻¹ the
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54 277 value of R_{h/cn} increased linearly (r²= 0.98; y= 4.07x+27.21; p< 0.001), until a second
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56 278 threshold was reached at B concentrations >2100 mg kg⁻¹ where R_{h/cn} increased to >70%.
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6 279 Tripler et al.³⁸ found similar leaf necrosis effects associated with high leaf B
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8 280 concentrations in date palm. Increasing contaminant accumulation and leaf
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10 281 chlorosis/necrosis with leaf age is also known for Zn and Cd, although these metals were
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12 282 stored in different tissues.^{39,40}

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16 283 **The distribution of B within *Populus nigra* × *euramericana* leaves.** Comparison of the
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18 284 ICP-OES measurements and the NR results showed that local tissue ¹⁰B accumulation in
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20 285 leaves was detectable by NR if concentrations in leaves exceeded 300 mg kg⁻¹. The
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22 286 detection limit and the spatial resolution of neutron radiographs (130 μm) thus were
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24 287 sufficient for the determination of toxicity thresholds in *P. nigra* × *euramericana* leaf
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26 288 tissue. Boron concentrations in the leaves of *B. juncea*, *S. viminalis* and *L. albus* were
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28 289 below the detection limit. Here, laser ablation ICP-MS could be an alternative.²⁰

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33 290 Within individual leaves, the highest B concentrations occurred at the leaf margins and
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35 291 tips. The margins and tips were also the locations where chlorosis and necrosis occurred
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37 292 first and were strongest. At average leaf B concentrations greater than 1000 mg kg⁻¹ leaf
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39 293 margins and tips curled. At higher total leaf B concentrations necrotic spots occurred
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41 294 throughout the leaf. These spots contained >2000 mg B kg⁻¹. Leaf tissue containing
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43 295 between 1000 and 2000 mg B kg⁻¹ was chlorotic and tissue containing more than 2000
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45 296 mg kg⁻¹ was necrotic. The finding of B concentrations >7000 mg kg⁻¹ in some spots in
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47 297 necrotic leaf tissue indicates that B accumulation continued in leaf tissue even after the
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49 298 onset of necrosis and that necrotic tissue can still receive B via the transpiration flow.
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52 299 Similar findings were reported by Reid and Fitzpatrick¹⁹ for barley. Deposition of B at
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54 300 high concentrations in discrete patches may be a tolerance mechanism by which a small
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56 301 patch of photosynthetic tissue is sacrificed in order to prevent overloading of the
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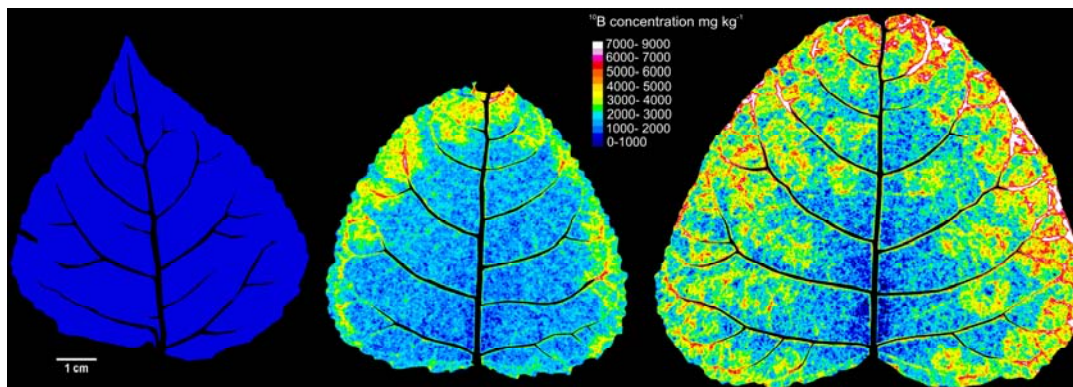
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6 302 surrounding tissues. The ability of *P. nigra* × *euramericana* to accumulate higher B
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8 303 concentrations in its aerial tissue than the other species tested can be attributed to the high
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10 304 B tolerance of the living leaf tissue and the storage of B in dead leaf tissue.

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13 305 The B accumulation characteristics of *P. nigra* × *euramericana* are consistent with the
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15 306 criteria compiled by Branquinho et al.⁴¹ for hyperaccumulation. The BCF as well as the
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17 307 shoot to root concentration ratio were >1 in *P. nigra* × *euramericana* and the
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19 308 aboveground B concentration in two (T₁₆₈ and T₂₈₀) of three B treatments was more than
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21 309 10-times higher than in the control (T₁₃). In contrast to many metals,⁴² there is no
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23 310 established shoot threshold B concentration above which a plant is considered to be a B
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25 311 hyperaccumulator. For example for Ni the threshold concentration used as criterion for
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27 312 hyperaccumulation is 1000 mg kg⁻¹,⁴³ which corresponds to 17.0 mmol kg⁻¹. The
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29 313 equivalent mass concentration of B is just 172 mg kg⁻¹ because of its 80% lower molar
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31 314 weight compared to Ni. This concentration was exceeded in some of the poplar leaves
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33 315 grown in the control treatment and in more than 85% of the leaves in the treatments with
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35 316 higher B concentrations. In addition, the accumulation of 1000 mg B kg⁻¹, a 20-times
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37 317 higher tissue concentration than the 50 mg kg⁻¹ that is generally considered to be toxic in
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39 318 tissues of most other plants, is an indicator of B hyperaccumulation in poplar⁴⁴. However,
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41 319 as the comparison with other species showed, B accumulation in poplars seems not to be
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43 320 active and they do not fulfil the criterion that hyperaccumulators should have at least 100-
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45 321 fold higher concentrations of the respective trace element than non-hyperaccumulators
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47 322 when grown in contaminated soil.⁴³ This indicates that B hyperaccumulation in poplars is
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49 323 not hyperaccumulation in the strictest sense, but rather B hypertolerance and thus
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51 324 comparable to the passive arsenic hyperaccumulation in aquatic macrophytes described
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6 325 by Robinson et al.⁴⁵. Our results indicate that poplar is better suited for phytomanagement
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8 326 of B contaminated soil than *S. viminalis* or *B. juncea*, which have been proposed for the
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10 327 phytoextraction of other trace elements.
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343 **Figures & Tables**

344 TOC Art



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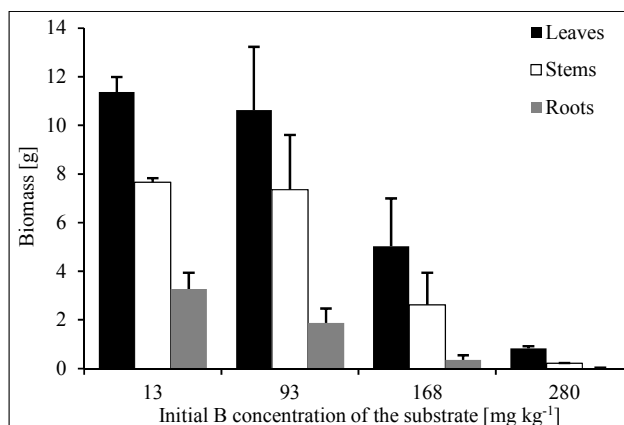
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358 Figure 1. Leaf, stem and root biomass of 5 month old *P. nigra* × *euramericana* saplings
359 grown on substrates with different B concentrations. The lowest B concentration (13 mg
360 kg⁻¹) is the control treatment. The mass of the cutting from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

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371 Table 1. Mean \pm S. E. B accumulation in the aboveground biomass of *L. albus*, *B. juncea*,
 372 *P. nigra* \times *euramericana* and *S. viminalis* grown on substrate with different B
 373 concentrations. T₁₃ is the control treatment.

Treatment	B concentration							
	<i>L. albus</i>		<i>B. juncea</i>		<i>P. nigra</i> \times <i>euramericana</i>		<i>S. viminalis</i>	
	[mg kg ⁻¹]							
T ₁₃	40.5 ^a	\pm 3.44	43.5 ^a	\pm 4.69	43.8 ^a	\pm 0.29	48.6 ^a	\pm 4.67
T ₂₂	114.2 ^{b1}	\pm 16.6	60.1 ^{aII}	\pm 4.37	N/A		118.3 ^{b1}	\pm 11.3
T ₄₅	174.6 ^{bc1}	\pm 27.2	68.1 ^{abII}	\pm 17.2	N/A		†	
T ₉₃	304.4 ^{c1}	\pm 20.7	136.4 ^{bII}	\pm 19.1	392.4 ^{b1}	\pm 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.

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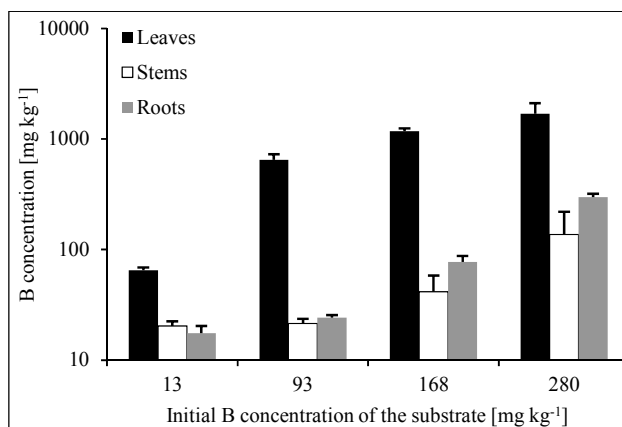
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385 Figure 2. Concentrations of B in roots, stems and leaves of 4 months old of *P. nigra* ×

386 *euramericana* plants. The lowest B concentration (13 mg kg⁻¹) is the control treatment.

387 Note that the B concentration is shown on logarithmic scale for better clarity. Error bars

388 represent standard errors (N=3).

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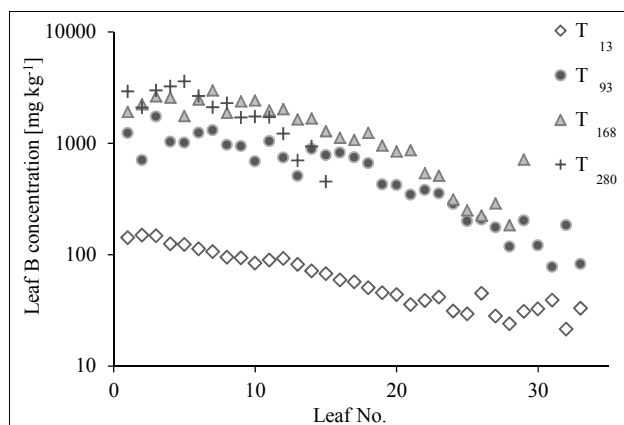
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399 Figure 3. Leaf B concentration as a function of leaf position, counting from bottom to top
400 along the stems of 4 months old poplars grown on substrate with different B
401 concentrations. Note that the B concentration is shown on logarithmic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

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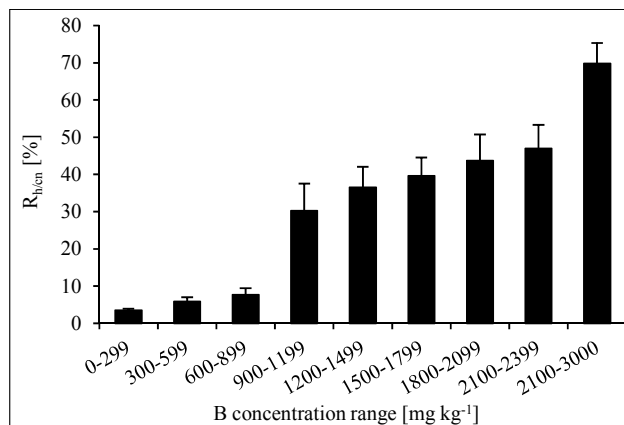
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413 Figure 4. Chlorotic and necrotic leaf area expressed as percentage of total leaf area ($R_{h/cn}$)
414 as a function of leaf B concentration. Note the large increase in chlorotic and necrotic leaf
415 area above 900 mg B kg⁻¹. Error bars represent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

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13
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18 435 **Supporting Information Available:**
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21 436 Details on the growing substrate and plant biomass. This information is available free of
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23 437 charge via the Internet at <http://pubs.acs.org/>
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