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Boron accumulation and tolerance of hybrid poplars grown on a B-laden mixed paper mill waste landfill

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ABSTRACT

Paper mill wastes are a mixture of by-products from pulp production and on-site energy production, consisting of paper mill sludge, ash and cinders. Landfilling of these highly boron (B) and heavy metal laden waste products carries environmental risks. Poplars have been successfully employed in the phytomanagement and hydraulic control of B contaminated sites. Here, we assess the performance of hybrid poplars on a paper-mill waste landfill, investigate the accumulation of B by the trees and explore the relationship between local-scale root growth and substrate properties. Leaf and root tissue samples were collected on three plots and analyzed for their chemical properties and root traits. Additionally, we sampled four soil cores in the vicinity of each of the trees and determined chemical and physical properties. Using a principal component analysis followed by a cluster analysis, we identified three substrate types. This method delineated the soil effects on tree survival and growth, although correlations with individual soil element concentrations were weak. Despite signs of B toxicity in some leaves, B was not the key limiting factor for poplar growth. Instead, Ca deficiency caused by a Mg:Ca imbalance was the primary reason for the poor performance of some trees. Root growth was not limited by toxicity effects of soil contaminants. Our results show that hybrid poplars perform well under the harsh growing conditions on a multi-contaminated, B-laden substrate in a hemiboreal climate. Exploiting the differences in the performance of the four clones in relation to the soil types, could increase the success of revegetation on this and other landfills.

1. Introduction

Paper mill sludge is, by volume, the most important by-product of paper production. It is used as raw material in other industries, spread on agricultural land as fertilizer, incinerated for energy production in paper mills or otherwise landfilled (Lteif et al., 2007; Monte et al., 2009). Sludge production was found to vary widely among 104 mills (U.S.EPA, 1988); ranging from 14 to 140 kg per 10^3 kg of pulp with a total generation of 2.5×10^9 kg a^{-1}. Other solid wastes from paper mills include various kinds of ash and cinders, originating from on-site energy production by incineration of wood wastes and paper mill sludge. Fly and other ashes contain high concentrations of boron (B), besides other potentially toxic elements such as Cu and Ni (Dellantonio et al., 2008). Landfilling and agricultural application of paper mill wastes, including ash, are the major source of B in the environment (Nable et al., 1997; Pavlović et al., 2004). Boron is an essential micronutrient for plants and animals at low concentrations (Mastromatteo and Sullivan, 1994; Salisbury and Ross, 1992), but like other trace elements it becomes toxic at higher concentrations. Boron is mobile in the environment and significant quantities can leach from landfills into ground or surface waters if not contained properly. In contrast to many other trace elements, B transport in the soil solution and plant uptake occurs as neutral H_3BO_3 over a wide pH range (pK_a: 9.24) (Goldberg, 1997). Thus, landfilling paper mill sludge or their incineration products require particular attention.

Poplars are used extensively for soil stabilization and erosion control (McIvor et al., 2009; Wilkinson, 1999), for phytomanagement and hydraulic control of contaminated sites (Robinson et al., 2007; Zalesny et al., 2009). Being passive B hyperaccumulators, they are of particular interest for the treatment of B contaminated soils. No growth reduction was found in a *Populus x canadensis* Moench clone at average leaf B concentrations of 800 mg kg^{-1} (Rees et al., 2011). By extracting large amounts of water for transpiration and simultaneously taking up B from the soil into their aerial parts, poplars can reduce B leaching from contaminated soils into receiving waters (Robinson et al., 2003). The accumulated B then can be removed

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from the site by harvesting the aboveground biomass. Complete clean-up of a B-contaminated site is limited in theory only by the depth to which the contamination is accessible by root water uptake and by the tolerance of the tissues to the accumulated contaminant. Our previous pot experiments have shown that poplars are promising as phytoremediation plants on B-rich soils.

However, in phytoremediation and phytomanagement research there is often a discrepancy between results obtained under well-controlled experimental conditions and in real-world field situations. A major problem in the design of phytomanagement systems, under field situations, is the large spatial variability in soil properties and contaminant distribution (Dickinson et al., 2009), which can strongly affect the development and activity of the root system and thus the success and efficiency of the operation (Keller et al., 2003; Wenzel, 2009). Reports on root reactions to trace element hot spots provide contrasting information and root responses can therefore not be predicted (Breckle and Kahle, 1992; Whiting et al., 2000). Soil is a heterogeneous medium in general. Not only trace elements but also other soil factors influencing root traits and morphology, such as water, air or macronutrients, often show quite heterogeneous distribution patterns (Hodge, 2010). Under well-defined conditions, poplar root growth was shown to be inhibited in soil patches with B concentrations >20 mg kg^{-1} (Rees et al., 2012), but there are no studies on the response of poplar roots to a spatially heterogeneous distribution of soil B under field conditions.

Here, we report the results of a study on the relationship between root development, tree growth and the concentrations of soil B and other contaminants in landfilled paper mill wastes that had been revegetated with various *P. x canadensis* Moench clones. The aims of this study were: (1) to assess the growth and survival of the different poplar clones (a) in response to soil conditions and (b) in relation to the concentrations of B and other potentially toxic or deficient elements in the tissues of the trees; (2) to investigate the accumulation of B by the trees in relation to the extent of soil contamination and (3) to determine the relationship between local-scale root growth, B contamination and other soil factors.

2. Material and methods

2.1. Site description

The study was carried out at "Pine Lake Landfill", Rhinelander, WI, U.S. (Fig. 1(a)). The average annual mean temperature at Rhinelander is 4.9 °C, with a maximum of 19.6 °C in July and a minimum of −11.9 °C in January (NCDC normal 1971–2000). The annual precipitation averages 810 mm. The landfill has an area of about 4 ha. Paper mill sludge, fly ash and cinders had been deposited on the site until 1995. The dumped truck loads created a pattern of patches with an average patch size of approximately 40 m^2. A bottom liner and a leachate collection system were installed. The leachates are collected in tanks, from where they are brought to a wastewater treatment facility. A 2-ha area of the landfill had been covered only provisionally with an approximately 10 cm thick layer of subsoil (Podzol, loamy sand,) and a geotextile (Fig. 1(a)). This part of the landfill had a slight slope facing southwards. The sub-soil layer was partially eroded due to runoff below the geotextile.

In July 2006, a pilot project was started to investigate whether a poplar stand could be established and provide an alternative cover to the weakened geotextile for erosion and leachate control. Three 10×10 m test plots (A, B and C, Fig. 1(a), (b)) were established with 4 commercially available, non-transgenic poplar clones of *P. x canadensis* (DN-2, DN-34, DN-182, OP-367). On each plot 36 poplar cuttings were planted in a 6×6 grid with a spacing of 1.7 m (9 cuttings of each clone). While DN-2 was planted as rooted stock, DN-34, DN-182 and OP-367 were planted as 25 cm cuttings. Holes were made into the geotextile, allowing the planting of the cuttings into the substrate. The trees were neither irrigated nor fertilized; weeds were pulled once in summer '07.

2.2. Sampling and sample analyses

The survival and height of the trees were recorded for up to 39 months in July '07, July '08, October '08 and September '09. In September '09 also the diameter at breast height (DBH) was determined. Woody biomass (stem + branches) of the trees was estimated using the equation of Riemenschneider et al. (2001):

$$\text{Woody biomass}\,[\text{kg}] = 6.16-(2.23\times \text{DBH}) + \left(0.353\times \text{DBH}^2\right). \quad (1)$$

In September 2009, soil and leaf samples were taken over a 10 day period. Ten leaves each were taken from each of the lower and the upper half of the trees. The leaf samples were dried at 60 °C until constant weight and then ground, digested in a heating block (130 °C, 65% HNO_3) and analyzed by means of ICP-OES (Vista MPX, Varian, Australia). Carbon and N were determined by elemental analyzer (CNS-2000, Leco Corp., Saint Joseph, Michigan USA). Soils including roots were sampled by taking 4 cores (5 cm length, 5 cm diameter), one by one to a total depth of 20 cm. Four samples from 15 to 20 cm depth could not be taken for mechanical reasons. The direction of the sampling point for each tree was randomly selected at a distance of 70 cm (Fig. 1(c)). For the geostatistical analysis the coordinates of the sampling locations were calculated in relation to the coordinate system origin, which was defined as the south-west corner of plot B. The samples were stored in a cool box until they were further processed in the lab. Substrate subsamples, from each collected sample, were taken for chemical and physical analyses. Roots were separated from the substrate by washing in tab water using a 1 mm sieve and then were scanned in a water bath at 400 dpi with a backlighting scanner. The morphology of the roots was analyzed by image analysis software (Regent Instruments. Inc., WinRhizo 2009c). Specific root length (SRL, cm fine root (FR) length g^{-1} FR dry weight) and root length density (RLD, cm FR length cm^{-3} soil) were calculated. After scanning, FRs was dried, biomasses were recorded and then digested in the same way as the leaf samples. Only FR samples >50 mg were analyzed chemically. This criterion was met by about half of the samples.

Soil B and other element concentrations were analyzed in a HNO_3/H_2O_2 solution (microwave digestion, 0.1 g sample, 180 °C, 1 h, 5 ml 69% HNO_3:2 ml 30% H_2O_2) to obtain quasi-total concentrations (denoted "$_{tot}$"). Extraction with a $CaCl_2$ – solution was used to characterize the availability of soil B and other elements (denoted: "$_{CaCl2}$") (1 g sample, 10 ml 0.01 mol $CaCl_2$, 16 h shaken, centrifuged at 2500 rpm, 0.25 μm filter). Soil bulk density (ρ [g cm^{-3}]) was calculated, gravimetric water content (H_2O_{grav}) and pH (soil:H_2O ratio: 1:2.5) were also determined. Carbon and N concentrations of soil samples were determined. Digested FRs and digests and extracts of soil samples were analyzed by ICP-OES as described above. Certified reference material used for quality control for plant samples was: NCS DC-73350 (China National Analysis Centre for Iron and Steel, Beijing, China) and for soil samples we used samples from WEPAL (Wageningen, Netherlands).

2.3. Statistics

Based on varimax-rotated principal component analysis (PCA) of the standardized and normalized physical and chemical soil parameters (Reimann et al., 2008), the soil samples were classified into 3 substrate types (ST) using hierarchical clustering (Ward, 1963). To find the best cluster model for our data, an algorithm was applied to determine the best model according to the average silhouette width (3 clusters, average silhouette width: 0.7). Non-detects were treated according to the method proposed by Farnham et al. (2002).

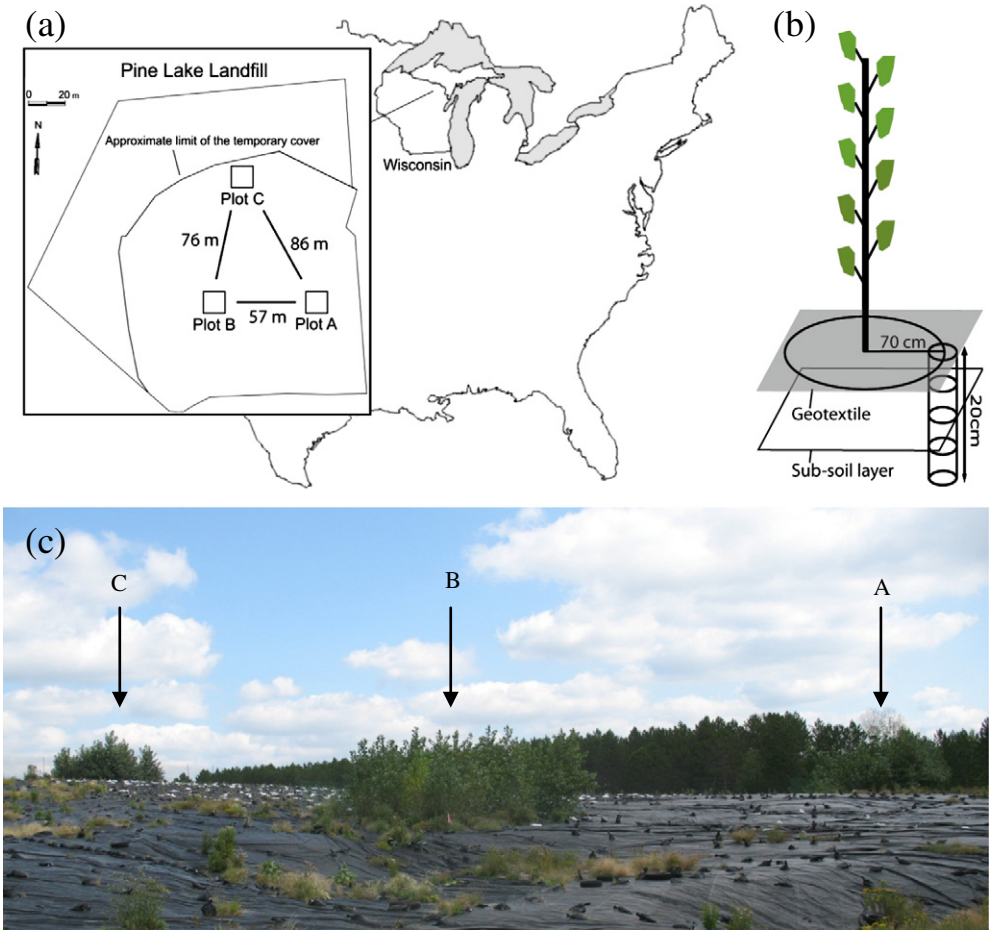

Fig. 1. (a) Map of Pine Lack Landfill within USA and WI, in particular; localization of pots A, B and C in the landfill. (b) View of Pine Lake Landfill from south-west showing the three test plots. The black geotextile is visible. The trees on the plots were on average around 5 m high. (c) Sampling scheme for soil, root and leaf samples. Soil and root samples were taken with a soil auger at 70 cm distance from each trunk.

Table 1
Factor loadings of the analyzed soil parameters on the first three principal components of the varimax-rotated PCA. Values between −0.1 and 0.1 are not shown. The highest (lowest) loadings on each component are highlighted.

Parameter	Principal component		
	PC 1	PC 2	PC 3
	Factor loadings [−]		
B_{CaCl2}	0.78	0.47	0.22
B_{tot}	**0.92**	0.29	–
C_{tot}	0.77	**0.56**	−0.10
Cu_{tot}	0.86	0.22	−0.20
Fe_{tot}	0.85	0.41	–
H_2O	0.84	0.43	–
K_{CaCl2}	0.71	0.29	0.22
K_{tot}	0.81	−0.45	–
Mg_{CaCl2}	0.80	0.32	0.30
Mg_{tot}	0.79	0.13	**0.39**
Mn_{CaCl2}	–	–	**−0.79**
Mn_{tot}	–	**−0.81**	–
N_{tot}	0.63	0.56	−0.24
Na_{CaCl2}	0.74	0.36	0.33
Na_{tot}	0.86	0.23	0.13
Ni_{tot}	0.46	**0.82**	–
Pb_{tot}	0.49	**0.82**	–
P_{CaCl2}	0.42	0.40	−0.43
P_{tot}	**0.92**	–	−0.11
pH	0.14	–	**0.78**
Zn_{tot}	**0.89**	0.35	–

To describe the spatial auto-correlation structure of the substrate, semi-variograms were computed for the PC scores of the samples and FR length, using the "modulus" estimator proposed by Cressie and Hawkins (1980). The dependence of tree growth, element uptake, and root morphology on soil properties was analyzed by means of multiple analysis of variance and regression analysis. Each tree was assigned to the ST of the soil core taken next to it. For the regression analysis of tree growth and element accumulation, average values of the core segments were used. For the leaf samples, a 2-way factorial ANOVA was conducted using substrate and clone as independent factors. For the root samples, a 2-way factorial ANOVA was conducted for each clone × depth and depth × substrate combination. Significant differences between groups were post-hoc tested using the Holm–

Table 2
Association of surviving trees with the 3 substrate types (ST) for each plot (number and percentage per plot).

	Plot						
	A		B		C		
	Number	%	Number	%	Number	%	Sum
ST1	24	80.0	2	7.1	0	0.0	26
ST2	6	20.0	20	71.4	7	41.2	33
ST3	0	0.0	6	21.4	10	58.8	16
Sum	30		28		17		75

Table 3
Physical and chemical characteristics of the 3 substrate types.

Parameter		Substrate type								
		1			2			3		
		Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
pH_{H2O}	[−]	7.66	±	0.43	7.73	±	0.42	7.54	±	0.50
H_2O	[g kg^{-1}]	11.06	±	10.03	37.52	±	10.82	31.14	±	11.18
Bulk density [ρ]	[g cm^{-3}]	1.13	±	0.27	0.58	±	0.13	0.53	±	0.11
C	[g kg^{-1}]	45.93	±	82.77	283.84	±	78.13	293.16	±	69.77
N	[g kg^{-1}]	1.44	±	2.52	7.56	±	2.31	8.59	±	3.01
B_{tot}	[mg kg^{-1}]	61.14	±	79.57	304.9	±	101.9	173.0	±	79.95
B_{CaCl}	[mg kg^{-1}]	6.09	±	6.69	23.16	±	10.26	20.49	±	9.89
Ca_{tot}	[g kg^{-1}]	4.69	±	6.06	16.50	±	7.58	11.23	±	4.01
Ca_{CaCl}	[g kg^{-1}]	N/A			N/A			N/A		
Cu_{tot}	[mg kg^{-1}]	129.9	±	288.7	496.7	±	212.1	316.1	±	271.4
Cu_{CaCl}	[mg kg^{-1}]	0.09	±	0.05	0.13	±	0.11	0.22	±	0.10
Fe_{tot}	[g kg^{-1}]	12.80	±	8.16	35.56	±	8.12	30.54	±	7.50
Fe_{CaCl}	[mg kg^{-1}]	1.32	±	2.71	7.43	±	16.43	5.56	±	14.35
K_{tot}	[g kg^{-1}]	2.67	±	1.14	4.96	±	1.26	1.29	±	1.16
K_{CaCl}	[mg kg^{-1}]	72.96	±	64.71	196.6	±	110.6	160.0	±	119.3
Mg_{tot}	[g kg^{-1}]	1.94	±	0.84	3.79	±	1.35	2.62	±	1.21
Mg_{CaCl}	[mg kg^{-1}]	161.6	±	137.9	603.2	±	308.7	470.9	±	303.7
Mn_{tot}	[mg kg^{-1}]	126.9	±	72.16	151.8	±	66.11	535.2	±	419.1
Mn_{CaCl}	[mg kg^{-1}]	1.46	±	1.86	2.71	±	3.83	4.20	±	6.01
Na_{tot}	[g kg^{-1}]	0.83	±	0.47	1.88	±	0.46	1.43	±	0.55
Na_{CaCl}	[mg kg^{-1}]	50.64	±	51.99	187.4	±	93.34	154.1	±	105.1
Ni_{tot}	[mg kg^{-1}]	35.39	±	37.10	102.6	±	42.50	204.9	±	79.87
Ni_{CaCl}	[mg kg^{-1}]	0.51	±	0.64	0.38	±	0.64	0.84	±	1.63
P_{tot}	[g kg^{-1}]	0.60	±	0.82	2.63	±	0.85	1.23	±	0.80
P_{CaCl}	[mg kg^{-1}]	0.46	±	0.46	0.81	±	0.47	0.95	±	0.87
Pb_{tot}	[mg kg^{-1}]	138.0	±	119.5	307.9	±	89.31	606.1	±	252.5
Pb_{CaCl}	[mg kg^{-1}]	N/A			N/A			N/A		
Zn_{tot}	[mg kg^{-1}]	60.30	±	79.20	294.0	±	92.41	227.1	±	77.35
Zn_{CaCl}	[mg kg^{-1}]	N/A			N/A			N/A		

S.D. = standard deviation; N/A = not applicable.

Sidak test (Holm, 1979). All data were tested for normality prior to statistical analysis and transformed if necessary.

To analyze root diameter distributions, a log-normal distribution was fitted to the cumulative frequency distribution of the measured root radii of each sample. From μ_{ln} and σ_{ln} parameter values of the best-fit distribution, the back-transformed distribution mean and variance were calculated for each distribution (Scanlan and Hinz, 2010).

3. Results and discussion

3.1. Substrate properties and spatial structure

The data classification procedure applied worked well for our data set as the results of the PCA and the clustering showed. The 1st principal component (PC1) explained 50.3% of the total variance of the soil parameters, PC2 20.0% and PC3 9.6%. The highest loadings on PC1 were obtained from B_{tot}, P_{tot} and Zn_{tot} while PC2 was dominated by Pb_{tot} and Ni_{tot} (Table 1). PC3 was characterized by a high negative score for Mn_{CaCl2} and a high positive score for pH. The three STs derived by hierarchical grouping were primarily determined by PC1 and PC2. The ST1 was defined by high negative scores on PC1 and PC2, while ST2 had a high positive score on PC1 only. ST3 had a high positive score on PC2 and a less pronounced negative score on PC1. Table 2 gives an overview of the association of the STs with the surviving trees. Of the 296 soil samples taken, 100 were classified as ST1, 135 as ST2, and 61 as ST3. Other authors have used this method, i.e. a combination of principal component and cluster analysis, to identify sources of contaminants (Micó et al., 2006). Our motivation for using this approach was to reduce the complexity of the dataset and to classify our substrate samples based on the a priori knowledge that 4 substrates with distinct properties had been deposited on the landfill. Using this technique to classify substrates with less distinct boundaries between different types would probably have been less successful, especially at the relatively high spatial resolution used in our study. Some mixing of the four initial substrates certainly occurred, creating transition forms

Table 4
Tree survival rates by clones and plots, presented as [%] of living trees relative to the total number of trees initially planted per plot.

Survival rate					
Plot	Clone				Average
	DN-182	DN-2	DN-34	OP-367	
A	100.0	33.3	100.0	100.0	83.3
B	55.6	77.8	77.8	100.0	77.8
C	66.7	66.7	11.1	44.4	47.2
Average	74.1	59.3	63.0	81.5	69.4

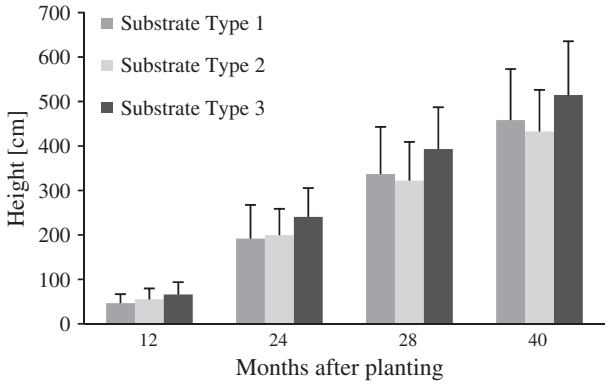

Fig. 2. Average tree height (all poplar clones pooled) after 12, 24, 28 and 40 months of growth on the 3 STs identified (± standard deviation).

Table 5
Woody biomass [$10^3 \times$ kg ha^{-1}] of the surviving trees on the three plots. The standing woody biomass was calculated on a per hectare basis and extrapolated for a complete tree survival.

Woody biomass					
Plot	Clone				Average
	DN-182	DN-2	DN-34	OP-367	
A	20.6	3.5	11.0	15.4	12.6
B	5.4	8.0	8.4	12.5	8.6
C	13.2	13.0	1.9	10.2	9.6
Average	12.7	8.2	7.1	12.7	10.3

between the substrates. Even though the classification quality was slightly lower for mixed samples, no samples were misclassified as indicated by the positive silhouette widths of all samples (data not shown). However, mixed samples probably caused the relatively high variation of some substrate properties.

The semivariograms showed that the substrate samples were a good representation of the soil conditions around the trees, showing spatial autocorrelation of the PCA scores of substrate samples over lag distance ranges of 5–7 m (data not shown). There was still considerable short range variability, as the nugget value was still about half of the sill variance.

ST1 was characterized by lower C and N concentrations, a lower H_2O-content and higher bulk density than STs2 and 3 (Table 3). The concentrations of B, P and metal elements were lowest in ST1, except for K. Most of the elements including B_{tot}, Ca_{tot}, Cu_{tot}, Fe_{tot}, Ni_{tot}, P_{tot}, Pb_{tot} and Zn_{tot} were on average at least twice as concentrated in STs2 and 3 as in ST1. ST1 probably consisted to a large extent of soil from the adjacent forest used as cover, as it was rarely found covered by other materials and it was the substrate with the lowest trace element concentrations. B_{tot}, Cu_{tot}, K_{tot} and P_{tot} were on average highest in ST2, while Mn_{tot}, Ni_{tot} and Pb_{tot} were most abundant in ST3. The trace element concentrations were all in ranges of values that are frequently found in contaminated soils and fly ash disposal sites (Domínguez et al., 2008; Fässler et al., 2010; Pavlović et al., 2004). The main source of B on the site was probably fly ash. The B concentrations were higher than usually found in contaminated soils, particularly in ST2, where B_{tot} averaged 305 mg kg^{-1}.

The solubility of the investigated trace elements was low, which is consistent with the high pH of the soils (7.5–7.7). Only 0.3–0.7% of Cu_{tot}, 0.8–1.8% of Mn_{tot} and 0.4–1.4% of Ni_{tot} were extractable with $CaCl_2$. The proportion of $CaCl_2$-extractable B was higher in comparison (7.6–11.8% of B_{tot}).

3.2. Tree survival and growth of the surviving trees

Table 4 gives a comparison of tree survival rates, among clones and plots. It was not possible to calculate survival rates for STs, because we have no data on the substrates associated with the non-surviving trees. Among the surviving trees, there was no significant correlation between clones and associated STs. No specimen of DN-34 clone was found associated with ST3. Mortality was highest on plot C, where more than 50% of the trees died and only one specimen of DN-34 clone survived. The survival rate of DN-2 clone and DN-34 clone was lower than that of OP-367

Table 6
Concentrations of macro- and micro-nutrients in the leaves of the different poplar clones planted on the experimental site (±standard error).

Clone	Element concentration					
	C	N	Ca	K	Mg	P
	[g kg^{-1}]					
DN-182	449.3 (28.37)	30.76 (3.37)	13.32^{a} (3.66)	26.24^{a} (3.36)	3.77^{bc} (1.32)	2.82 (0.45)
DN-2	451.5 (13.59)	33.45 (2.38)	11.25^{ab} (5.48)	25.68^{ab} (3.84)	4.59^{a} (1.33)	2.59 (0.43)
DN-34	460.6 (21.19)	31.24 (4.67)	9.47^{b} (2.01)	26.66^{a} (2.86)	3.55^{b} (0.62)	2.91 (0.48)
OP-367	454.2 (19.72)	32.30 (3.32)	11.29^{ab} (2.96)	24.00^{b} (2.34)	4.35^{ac} (1.25)	2.59 (0.39)
	B	Cu	Fe	Mn	Na	Zn
	[mg kg^{-1}]					
DN-182	636.9^{a} (143.9)	25.19 (6.13)	67.01 (14.93)	127.1^{a} (48.87)	39.74^{b} (7.19)	239.5^{ab} (104.2)
DN-2	830.5^{b} (220.1)	29.91 (7.58)	69.56 (27.28)	85.73^{ab} (51.05)	74.00^{b} (56.31)	313.6^{a} (124.4)
DN-34	636.3^{a} (99.43)	28.60 (6.26)	63.26 (11.70)	72.38^{b} (27.24)	87.88^{b} (75.72)	181.2^{b} (67.59)
OP-367	1017.8^{c} (180.9)	27.17 (8.04)	65.89 (15.54)	103.8^{ab} (46.27)	193.5^{a} (130.1)	253.2^{ab} (81.38)

Different letters indicate statistically significant differences in leaf element concentrations between clones at the $p<0.05$ level (Holm–Sidak-Test).

Table 7
Linear correlations (Pearson's coefficients) between the average scores of the first 3 principal components (PC) of the soil cores taken next to each tree, tree growth parameters (height and biomass after 40 months), leaf nutrient concentrations and the Mg:Ca ratio. Only coefficients of significant correlations are given.

	Height	Biomass	N	B	Ca	Cu	Fe	K	Mn	P
PC 1	−0.40***	−0.35**								
PC 2	0.25*	–								
PC 3	−0.24*	−0.44***								
Mg:Ca ratio	−0.58***	−0.50***								
B	–	–	0.38**							
Ca	0.63***	0.60***	–	–						
Cu	–	–	0.36**	0.35**	0.23*					
Fe	–	–	0.36**	–	0.45***	0.24*				
K	–	–	–	–	–	0.33**	0.35**			
Mg	−0.37**	−0.36**	–	0.60***	–	0.32**	–	–		
Mn	0.65***	0.44***	–	–	0.79***	0.31**	–	–		
Na	–	–	–	0.60***	–	–	–	–	–	
P	–	–	0.36**	–	0.28	0.47***	0.25*	0.42***	0.31**	
Zn	0.29*	0.31**	–	–	0.61***	0.28*	0.27*	–	0.50***	0.28**

Significance levels are indicated by asterisks: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; – = non-significant.

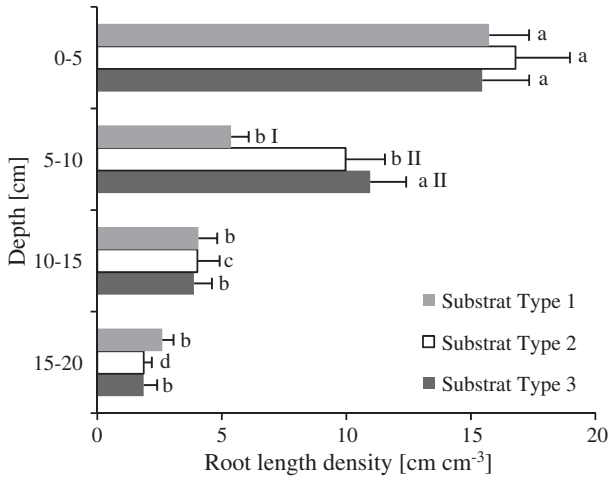

Fig. 3. Average root length density in the 3 substrate types after 40 month growth (±standard error). Statistically significant differences within substrates, between depths are indicated by characters ($p<0.05$) and between substrates for a given depth by Roman numbers.

clone and DN-182 clone. Mortality was highest in the first year (88%). This could be probably due to the late planting in the growing season, which left the trees approximately 2–3 months to establish before the first frosts. A second planting of 200 cuttings in 2007 failed completely due to early frost. Another factor that may have increased the first year mortality was incidental covering of cuttings by the geotextile. Furthermore, single trees tipped over and died because of insufficient mechanical stability of the rather soft paper mill sludge substrate. However, the mortality rates found are not unusual for poplar stands, on both contaminated and uncontaminated sites (Hansen, 1992; Laureysens et al., 2005).

On average, surviving trees associated with ST3 grew higher than trees associated with STs1 and 2 (Fig. 2). There was a wide range in biomass production among clones and plots, with a minimum of 1.9 Mg ha^{-1} and a maximum of 20.6 Mg ha^{-1} (Table 5), showing a similar pattern to the survival rates. Thus, OP-367 clone and DN-182 clone, the clones with the lower mortality also had the higher biomass. On the other hand, trees on plot C produced more biomass than on plot B, even though mortality on C was 30% higher. The higher biomass production could be attributed to the wider spacing, resulting in reduced competition and improved nutrient and water acquisition of the trees on plot C. While a dense spacing (1 × 1 m) was found to be adequate for poplar coppice cultures (Armstrong et al., 1999), it can cause density dependent mortality in single-stem plantations (Karacic et al., 2003). According to Green et al. (2001) DN-34 does not perform well under strong competition for light, which may be a major reason for its poorer growth compared to the other clones. The mean annual biomass production of all clones and on all plots was 3.4 Mg ha^{-1} a^{-1}, exceeding the averages of respective values given by Hansen (1990) for DN-34 clone and DN-182 clone, 2.5 and 3.3-fold, respectively.

3.3. Element concentrations of leaves

The concentrations of macro and micro-nutrients in the leaf samples of the four poplar clones are given in Table 6. Leaf element concentrations differed more strongly among clones than among STs. Significant substrate-related differences in leaf element concentrations were only found for Mn and Zn (data not shown). The foliar concentrations of N, K, Mg and P were in the same range as reported for fertilized poplar cultures (Jug et al., 1999; van den Driessche et al., 2008) and always above the respective sufficiency levels (van den Burg, 1985, 1990). Foliar Ca levels were below the recommended concentration of 7 mg g^{-1} in 7 trees, the other trees were well supplied with Ca. However, Ca deficiency threshold concentrations for foliage vary widely and are not well established (Jug et al., 1999). The Ca concentration of 22 g kg^{-1}, which was proposed for poplars as optimum level by Côté and Camiré (1987), was not reached in any leaf sample.

The concentrations of B were extraordinarily high in the leaves of all clones. DN-34 clone (636 mg kg^{-1}) and DN-182 clone (637 mg kg^{-1}) had a similar leaf B concentration, differing significantly from DN-2 clone (831 mg kg^{-1}) and OP-367 clone (1018 mg kg^{-1}). In some leaves,

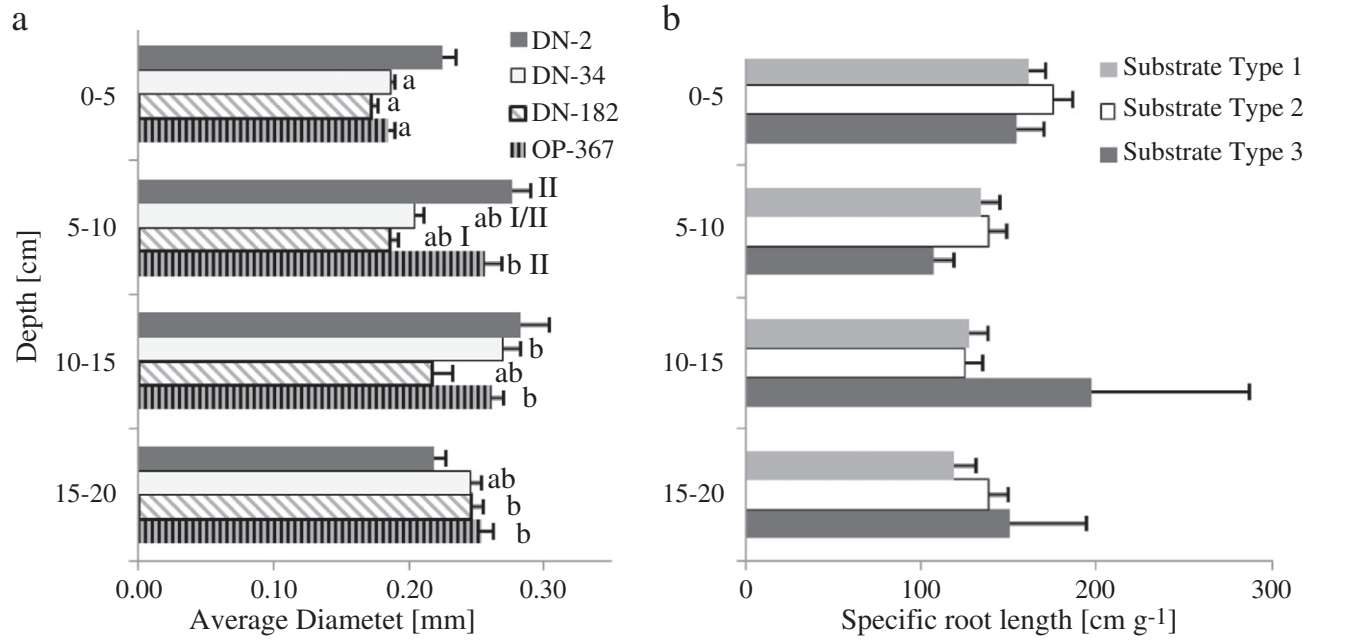

Fig. 4. Depth distribution of (a) average root diameters for the 4 poplar clones and (b) specific fine root length found in the 3 substrate types associated with the trees after 40 months of growth (±standard error). Bars within a given clone with the same letter are not significantly different. Likewise, bars within a given depth do not differ significantly if they are marked with the same Roman number.

Table 8
Mean concentrations of elements in poplar fine roots for the 3 substrate types (±standard error).

Substrate	Element concentration											
	Ca	Fe	K	Mg	Na	P						
	[g kg^{-1}]											
Type 1	14.81^{a}	(1.20)	13.24	(1.40)	5.71	(0.54)	2.65^{a}	(0.14)	2.31^{a}	(0.28)	1.82^{a}	(0.09)
Type 2	19.13^{ab}	(1.73)	15.32	(1.22)	4.28	(0.38)	2.50^{a}	(0.12)	1.66^{ab}	(0.13)	2.47^{b}	(0.11)
Type 3	21.85^{b}	(1.97)	17.13	(1.77)	4.47	(0.41)	1.77^{b}	(0.12)	1.29^{b}	(0.18)	2.31^{b}	(0.11)
	B	Cu	Mn	Ni	Pb	Zn						
	[mg kg^{-1}]											
Type 1	134.4^{a}	(9.49)	393.6^{a}	(36.61)	383.4^{a}	(53.35)	28.99	(2.78)	141.4^{a}	(19.01)	149.0^{a}	(16.45)
Type 2	186.5^{b}	(8.59)	569.9^{b}	(31.95)	367.9^{a}	(48.18)	39.21	(2.48)	221.0^{b}	(16.13)	222.5^{b}	(11.90)
Type 3	150.1^{a}	(8.56)	533.2^{ab}	(50.83)	208.6^{b}	(33.01)	69.72	(12.82)	132.4^{a}	(9.23)	214.5^{b}	(17.20)

Different letters indicate statistically significant differences in leaf element concentrations ($p<0.05$).

B toxicity symptoms such as necrotic leaf margins were observed. Leaf B concentrations were only weakly correlated to the soil B_{CaCl2} concentrations of the associated cores ($r^2=0.061$; $y=5.21x+702.14$; $p<0.05$). The leaf B concentrations measured here are comparable in magnitude to those found by Bañuelos et al. (1999) and Robinson et al. (2003). The findings of Bañuelos et al. (1999) also confirm the higher B accumulation of clone OP-367 compared to DN-34. The capacity of poplars to accumulate high B concentrations in their leaves indicates that they have special mechanisms for B detoxification and storage. There is limited information on B detoxification in plants. The binding of B to alcohol sugars in some *Rosaceae* species and the complexation of B with *cis*-diol groups in Halophytes are detoxification mechanisms that work at tissue concentrations of up to 300 mg kg^{-1} (Brown and Shelp, 1997; Rozema et al., 1992). However, this is well below the toxicity threshold level in poplar leaves (800 mg kg^{-1}). Leaf B concentrations were found to decrease with leaf position along the axis of young pot-grown poplar shoots from base to top (Rees et al., 2011). Here we found no such relationship between leaf position and B concentration, which might have been due to early senescence and fall of older leaves. Some of the trees were strongly defoliated at the time of sampling, especially in their lower parts.

Other trace elements, such as Zn, were also present in the leaves at elevated concentrations (Vandecasteele et al., 2003). This was especially true for clone DN-2, in which leaf Zn concentrations averaged 314 mg kg^{-1} and reached a maximum concentration of 508 mg kg^{-1}. Such concentrations are not uncommon in poplars and not known to be phytotoxic in poplar leaves (Dos Santos Utmazian et al., 2007; Hermle et al., 2007; Robinson et al., 2005). The leaf Cu concentrations varied between 25.2 and 30.0 mg kg^{-1}, which is within the range where Cu toxicity can occur (Kramer, 2010). The concentrations of Cd, Pb and Ni were well below toxicity levels for poplars and other plants (Kramer, 2010; Vandecasteele et al., 2003).

3.4. Regression analysis of tree growth and leaf element concentrations

There were significant correlations between the average PC scores of the soil cores and tree growth parameters (Table 7). PC1 and PC3 were found to be negatively correlated with tree height and biomass, while PC2 showed a positive correlation with tree height. This agrees with the finding of a positive influence of ST3 on tree growth, as this ST had a high positive score on PC2, and the rather negative influence on tree growth associated with ST2, which scored high on PC1.

Tree height and biomass showed strong correlations with the concentrations of some elements in leaves, in particular Ca, Mn and to a lesser degree Mg and Zn (Table 6). The strongest positive correlation between nutrient element concentration in foliage and tree growth parameters was found for Ca and the strongest negative correlation was found for Mg. Calcium and B play an important role in the formation of pectic polysaccharides in cell wall constituents and Ca is especially important for wood formation in poplars (Lautner et al., 2007; Matoh and Kobayashi, 1998). Teasdale et al. (1986) showed that at a high Mg:Ca ratio, Mg is able to displace Ca from the binding sites of an acceptor molecule that is only activated when both, Ca and B, are bound to it. At high Mg:Ca ratios, high B concentrations were found to promote growth in cell cultures. The Mg:Ca ratio was as high as 1 in some of our leaf samples. Under these conditions, the high B concentrations found in the leaves might have partially compensated growth reduction induced by low Ca supply to the leaves. However, this was not expressed by a positive correlation of tree growth with leaf B accumulation as B was present in excess in all leaf samples.

Low foliage Ca concentrations were not related to low soil Ca concentrations, as substrate Ca concentrations were lowest in ST1, whereas most of the trees (5 out of 7) with foliage Ca concentrations <7 g kg^{-1} were associated with ST2. Leaf Zn and Mn concentrations were far above deficiency levels and their positive correlation with tree growth might have been co-incidental due to their correlation with leaf Ca.

3.5. Fine root traits

All three substrates were intensively colonized by roots (Fig. 3). The semivariograms for fine root (FR) biomass and FR length showed a pure nugget effect, meaning that these parameters were spatially independent (data not shown). Fine root length densities were found to vary between 1.9 and 16.8 cm cm^{-3}. These values are 2–6 times higher than those found by Al Afas et al. (2008) in a poplar coppice culture. No significant differences in RLD were found among clones and STs when all soil samples were pooled. However, pair-wise comparisons revealed differences in RLD among STs collected at 5–10 cm depth. Trees associated with ST1 developed fewer roots at this depth than in association with STs2 and 3. For all STs and clones, RLD decreased significantly with depth, in contrast to the FR diameter which increased (Fig. 4(a)). The FR diameter differed significantly between clones. Specific root length decreased with depth and was higher in ST3 than in STs1 and 2 (Fig. 4(b)). The SRL values found here are twice as high as those reported by Ostonen et al. (2007) and Brunner et al. (2008), but in the same order of magnitude as those reported by Pregitzer et al. (2002) for *Populus balsamifera* L.. Also our FR diameter values agreed well with those of Pregitzer et al. (2002).

The cumulative frequency distributions of the FR diameters were well described by log-normal distributions. The average r^2 of all samples was 0.993 for all models. The back-transformed model distribution parameters agreed well with the respective parameters compiled by Scanlan and Hinz (2010). Differences between sample average diameters determined by model-fitting were significant for all three factors: substrate, clone and depth. The sample variances in root diameter were independent of the clone, but showed the same trends as the distribution mean otherwise. Pair-wise comparison

Table 9
Linear correlations between root traits and root element concentrations, between root traits and principal component (PC) scores and between root traits and soil parameters of the substrate samples. Arrows indicate the soil parameters with the highest scores on the respective PC. Only coefficients of significant correlations are given.

		FR Biomass	RLD	SRL	Avg. Diam.[1]	Dist. Mean[2]	Dist. Variance[2]
Root element concentration	B	0.22*	0.29**	0.30***	–	0.20*	–
	Ca	–	–	–0.19*	–	–	–
	Cu	–	–	0.31***	–0.22*	–	–
	K	–	–	–0.20*	–	–	–
	Mg	–	0.27**	0.23*	–	–	–
	Na	–	0.19*	0.19*	–	–	–
	Pb	–	–	–	–	0.37***	0.35**
	Zn	–	–	0.23*	–	–	–
Principal components	PC 1	–	–	–	0.17**	0.20***	0.15*
	PC 2	–	–	–	–	–0.13*	–0.17**
	PC 3	0.25***	0.39***	0.31***	–0.13*	–	–
Soil parameters PC 1	P_{tot}	–	–	–	0.15*	0.13*	–
	B_{tot}	–	–	–	0.17**	0.15**	–
	Zn_{tot}	–	–	–	0.17**	0.13*	–
	Na_{tot}	–	–	–	0.18**	0.17**	–
	H_2O	–	–	–	0.17**	0.23***	0.14*
	K_{tot}	–	–	–	0.14**	0.21***	0.18**
	K_{CaCl2}	–	–	–	0.17**	0.19**	–
	C	–	–	–	0.12*	–	–
Soil parameters PC 2	Ni_{tot}	–	–	–	–	–	–
	Pb_{tot}	–	–	–	0.12*	–	–
	Mn_{tot}	–	–	–	–	–0.15*	–0.14*
Soil parameters PC 3	pH	0.19**	0.29***	0.115*	–	–	–
	Mn_{CaCl2}	–0.16**	–0.26***	–0.15***	–	–	–
	Na_{CaCl2}	-	0.11*	–	–	–	–
	B_{CaCl2}	0.14*	0.22***	–	–	–	–
	Ca_{tot}	–	–	–	–	0.13**	–
	Cu_{CaCl2}	–	–	–0.12*	–	–	–
	Ni_{CaCl2}	–	–0.13*	–	–0.15*	0.13*	–
	P_{CaCl2}	–	–	–	–	–	–

Significance levels are indicated by asterisks: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; – = non-significant.
[1]Average FR diameter directly determined by means of WinRhizo for each sample.
[2]Mean and variance of the best-fit log-normal distribution of sample FR diameters.

showed that the uppermost substrate samples (0–5 cm) significantly differed in variance from the deeper samples. The latter had the highest distribution variance.

A higher SRL is associated with a higher root surface area for a given biomass and thus with an improved capacity for nutrient and water uptake. SRL is known to decrease under abiotic stresses

from elevated trace element concentrations, because roots thicken under such conditions (Ostonen et al., 2007). Under abiotic stress conditions black alder FRs were found to avoid direct soil contact and invest more into exudation than into root growth (Lohmus et al., 2006). Poplars were found to react to high concentrations of heavy metals in nutrient solutions by exuding organic acids (Qin et al., 2007). The composition of the exudates differed for different trace elements. Metals bound to organic acids are less reactive than free metal ions. The combination of such morphological and chemical responses might enable poplar FR to deal flexibly with different stress conditions in soils.

3.6. Element concentrations of fine roots

Table 8 shows that there were significant differences among STs in the concentrations of various elements in the FR. Significant differences between clones were only found for Na, where a significant difference between DN-34 clone and DN-182 clone emerged. Boron concentrations in roots are typically in the same magnitude as soil and below leaf B concentrations (Rees et al., 2011), which was the case here as well. Fine-root Ca concentrations were higher than soil and leaf Ca, suggesting that the low leaf Ca concentrations were not due to insufficient supply of soil Ca to the roots.

Zinc and Mn FR concentrations were in the same order of magnitude as reported by Brunner et al. (2008) from uncontaminated soil. The FR Fe concentrations, however were similar to the substrate Fe concentrations and far above the concentrations reported for poplar roots (Zalesny et al., 2008). These extraordinarily high root Fe concentrations might have been due to temporarily anaerobic conditions in the Fe-rich substrates, indicated by high soil water contents during sampling and precipitated Fe-oxides on root surfaces. Changes in redox conditions would have also affected redox state and thereby the mobility of Mn and possibly Cu. The Cu concentrations of the FR were indeed high. They ranged between 400 and 570 mg kg^{-1} and exceeded soil Cu concentrations. In roots, Cu is primarily bound in epidermis cell walls to pectins and thus hindered to be translocated into the shoots (Brunner et al., 2008). Copper concentrations of 3 mg l^{-1} were shown to be toxic to roots of *Populus tremula* L. in a 2×10^{-4} mol l^{-1} $CaCl_2$-solution (Qin et al., 2007). In our study, $CaCl_2$-extractable Cu concentrations were always more than an order of magnitude lower than 3 mg l^{-1} and should therefore not be toxic to poplar roots. Lead concentrations in roots were high. Like Cu, Pb is known to accumulate in tree roots and not to be translocated into stems and leaves readily (Marmiroli et al., 2005; Pulford and Watson, 2003).

3.7. Regression analysis of fine root traits

Various mineral element concentrations of the sampled FR were significantly correlated with SRL (Table. 9). While the macro-nutrient elements K and Ca were negatively correlated to SRL, Na and Mg and the micronutrients B, Cu and Zn showed a positive correlation with SRL. Copper was the only mineral element that was significantly correlated to the average root diameter determined directly by WinRhizo. In contrast to expectation, the correlation was negative, meaning that diameters decreased with increasing root Cu concentration. This finding was confirmed by the negative correlation between root Cu and root diameter, as thinner roots lead to a higher SRL. SRL also showed positive correlations with root B, Mg, Na and Zn, although all these elements were not significantly correlated with root diameter, suggesting that these relationships to SRL were of an indirect nature. Only root B concentrations were found to be positively correlated to FR biomass. The finding that SRL increased with the concentrations of some trace elements in the roots is in contrast to the opposite conclusion of a negative metal influence on SRL that Ostonen et al. (2007) drew from a meta-analysis of published root data. Root B, Mg and Na concentrations were also positively correlated with RLD.

There was a good agreement between the correlations of PC scores and root traits and the correlations of these traits with those soil parameters showing high negative or positive loadings on the respective PCs: PC1 scores correlated positively to the average root diameters of the samples and so were all soil parameters with a high score on PC1; PC2 scores showed only a weak negative correlation to the parameters of the back-transformed model; and PC3 showed a weak negative correlation to the WinRhizo measured average root diameters, but no correlation with mean diameter of the best-fit model distribution. PC3 was also strongly correlated with FR biomass, SRL and RLD, as were the two main factors loading on PC3, i.e. pH and Mn_{CaCl2}. The correlation coefficients between root traits and soil chemical parameters were generally low, ranging from −0.26 to 0.3, suggesting that in our experimental field soil root growth was not simply governed by a few dominant factors, but by rather complex interactions among a large variety of factors.

4. Conclusions

Substrate type classification based on PC analysis proved to be an efficient method to detect soil effects on poplar survival and growth, despite of the complexity of the site conditions on the investigated landfill. Despite signs of B toxicity in the leaves and elevated concentrations also of some other trace elements in the substrates and the leaves, survival and growth of the poplars trees were in the range of normal performance. Nonetheless, taking into account that tree growth was clearly affected by the associated substrates could greatly enhance the chances of a successful establishment of poplars on the remainder of the site or on other sites with similar conditions. Our results show that poplars are well suited for establishing vegetation on B-laden paper mill sludge and similar landfill sites in order to stabilize the soil and control trace element leaching.

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