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IRRIGATING POPLAR ENERGY CROPS WITH LANDFILL LEACHATE NEGATIVELY AFFECTS SOIL MICRO- AND MESO-FAUNA

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Increased municipal solid waste generated worldwide combined with substantial demand for renewable energy has prompted testing and deployment of woody feedstock production systems that reuse and recycle wastewaters as irrigation and fertilization.* Populus *selections are ideal for such systems given their fast growth, extensive root systems, and high water usage rates. Maintaining ecological sustainability (i.e., the capacity for an ecosystem to maintain its function and retain its biodiversity over time) during tree establishment and development is an important component of plantation success, especially for belowground faunal populations. To determine the impact of solid waste leachate on soil micro- and meso-fauna, we compared soil from eight different* Populus *clones receiving municipal solid waste landfill leachate irrigation with clones receiving fertilized (N, P, K) well water irrigation. Microfauna (i.e., nematodes) communities were more diverse in control soils. Mesofauna (i.e., insects) were associated with all clones; however, they were four times more abundant around trees found within the control plot than those that received leachate treatments. Nematode and insect abundance varied among* Populus *clones yet insect diversity was greater in the leachate-treated soils. Phytotechnologies must allow for soil faunal sustainability, as upsetting this balance could lead to great reductions in phytotechnology efficacy.

KEY WORDS insects, nematodes, phytotechnologies, *Populus*, waste management, wastewater reuse

INTRODUCTION

Poplars (*Populus* species and hybrids) have been extensively studied in short rotation woody biomass production (SRWBP) systems for multiple uses such as fiber, fuel, and environmental benefits (Hasselgren 1998; Zalesny and Zalesny 2009). Exemplary traits that have contributed to the success of such uses include ease of rooting, quick establishment, high rates of photosynthesis and transpiration, and fast growth (Zalesny et al. 2006, 2007b, 2009a). Broad genetic diversity among poplar genomic groups and selection of specific genotypes within such groups can increase the potential establishment area and growth

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rates of poplar crops for various uses across heterogeneous sites (Rajora and Zsuffa 1990; Zalesny et al. 2009b).

While much information exists regarding the use of poplars for SRWBP systems, there are relatively fewer reports about using these trees for phytotechnologies. Additional information focused on phytotechnologies will help increase the success of using poplars for remedial benefits, especially with ecologically-damaging contaminants such as those found in wastewaters, including landfill leachate (Shrive et al. 1994; Erdman and Christenson 2000; Kjeldsen et al. 2002). Overall, the use of SRWBP systems for remediation supports improved environmental quality and secondary benefits such as carbon sequestration, harvestable products, aesthetic improvements, and erosion control (Isebrands and Karnosky 2001; Licht and Isebrands 2005; Mirck et al. 2005). Maintaining ecological sustainability, i.e., the maintenance of soil health, biodiversity, and integrity, during SRWBP system establishment is an important component of success, especially for belowground faunal populations.

For phytoremediation to truly be effective and socially accepted, more knowledge is needed regarding the interactions between plants, chemicals, and organisms (Vangronsveld et al. 2009). Despite the immense impact soil-dwelling organisms have on soil physical properties, water infiltration, plant community dynamics, biodiversity, and microbial communities, there is a disproportionately smaller amount of research conducted on belowground fauna compared to aboveground fauna. For example, Gremion et al. (2004) showed that microbe communities can be affected by phytoremediation of heavy metals, and it has been demonstrated that the effects of phytoremediation can be observed throughout several trophic levels (Vickerman et al. 2004). Additionally, certain soil faunal groups can be used as bioindicators of the recovery progress of remediated land (Adl 2008). Overall, the importance of soil fauna cannot be understated, as they have a role in decomposition, nutrient cycling, and soil structure.

We have a unique study in that we are merging SRWBP with phytotechnology. While research on the importance and impact of SRWBP has been ongoing for several decades, relatively little effort has been put toward understanding interactions with belowground fauna. SRWBP can improve soil health and faunal abundance compared to agricultural land (Makeschin 1994), but not when compared to natural forest ecosystems (Johnston and Crossley 2002). Given the importance of soil fauna on ecosystem health, the overall objective of this study was to test the effects of leachate irrigation on soil micro- and meso-fauna populations after irrigating eight *Populus* genotypes with fertilized (N, P, K) well water (control) or municipal solid waste landfill leachate for two growing seasons at the Oneida County Landfill in northern Wisconsin, USA. We tested the hypotheses that leachate irrigation would negatively affect fauna diversity and abundance, and that the magnitude of the effects would vary among the genotypes. We also examined the relationship between tree root mass and faunal abundance, as roots serve as food for many belowground organisms. We expected greater faunal abundance with a larger root mass. This information is useful to SRWBP system managers for environmental benefits, because fauna play a crucial role in long term sustainability.

MATERIALS AND METHODS

Site Description

The study was conducted at the Oneida County Landfill located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Temperature, precipitation, and

growing degree days across the experimental period were described previously (Zalesny et al. 2007b). The soils surrounding the landfill are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0 to 6 percent slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash. One factor in choosing this site was its relatively consistent soils across the entire study area.

Clone Selection

Eight *Populus* clones were selected from 25 original genotypes, based on aboveground and belowground traits, after being irrigated with leachate in a series of greenhouse experiments that constituted three phyto-recurrent selection cycles (Zalesny et al. 2007a). The clones and their parentages (i.e., genomic groups) were: NC13460, NC14018 [(*P. trichocarpa* Torr. & Gray × *P. deltoides* Bartr. ex Marsh) × *P. deltoides* 'BC1']; NC14104, NC14106, DM115 (*P. deltoides* × *P. maximowiczii* A. Henry 'DM'); DN5 (*P. deltoides* × *P. nigra* L. 'DN'); and NM2, NM6 (*P. nigra* × *P. maximowiczii* 'NM'). In this paper we use the *Populus* section names as specified by Eckenwalder (1996), but we have retained the species nomenclature for *P. maximowiczii* (Japanese poplar) now classified as a subspecies of *P. suaveolens* Fischer (Eckenwalder 1996; Dickmann 2001).

Tree Establishment and Experimental Design

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, with cuts made to position at least one primary bud not more than 2.54 cm from the top of each cutting. Cuttings were stored at 5°C and soaked in water to a height of 15 cm for 3 d before planting on 14 June 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted at a spacing of 1.2 × 2.4 m (i.e., 3472 trees ha^{-1}) in a split plot design with eight blocks (i.e., replications, rep size = 23.04 m^2), two irrigation treatments (whole plots, plot size = 184.32 m^2), and eight clones (sub plots, sub plot size = 2.88 m^2). Clones were planted in single tree plots arranged in a randomized complete block design to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen 1981; Zavitkovski 1981). Mechanical and hand weeding were performed weekly throughout the study to ensure maximum tree survival. Electric fencing was used to prevent deer browse and injury to the trees. Polyvinylchloride tubing, 15.24 cm in diameter, was installed after leaf senescence in November 2005 on each tree to protect the trunk from damage by rodents during the winter.

Treatment Application

Water (control) from a non-impacted well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 d. Following establishment, trees were hand irrigated with either fertilized water or municipal solid waste landfill leachate that was collected weekly, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects of macronutrients. The 2005 weekly application rate was 3.8 L $tree^{-1}$ (23.1 mm ha^{-1} assuming an irrigated soil surface area of 0.16 m^2 per tree). Given eight applications, a total of

1.9 kL of each treatment was applied across the 2005 growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006 was increased to 22.7 L tree^{-1} (34.6 mm ha^{-1} assuming an irrigated soil surface area of 0.66 m^2 per tree) because of tree growth and development and increased water demand. A total of 17.4 kL irrigation was applied in twelve applications throughout the 2006 growing season. To prevent substantial leaching from experimental plots, treatment applications were adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 d prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.

Water Chemical Properties

Well water and municipal solid waste landfill leachate from the same source as the irrigation treatments were sampled from the Oneida County Landfill during April and October of 2005 and 2006. Water and leachate chemistry was analyzed using approved United States Environmental Protection Agency methods (Northern Lake Service, Inc., Crandon, Wisconsin, USA). The primary toxicity concern of the leachate was chloride and sodium. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development. Water and leachate characteristics were described previously (Zalesny et al. 2008a) and are shown in Table 1. Salt was the major contaminant in this study.

Soil Chemical Properties

Using a 5-cm diameter hand auger, nine soil samples at a depth of 0 to 30 cm were collected from the control plot and the leachate treatment plot one day before planting (13 June 2005) and harvesting (17 August 2006). For each date by treatment combination, soil from three sampling points was bulked, and the three bulked samples were sent to the University of Wisconsin Soil & Plant Analysis Laboratory (Madison, Wisconsin, USA) and Iowa State University Soil & Plant Analysis Laboratory (Ames, Iowa, USA) for analyses. Soil characteristics were described previously (Zalesny et al. 2008b) and are shown in Table 2.

Table 1 Composition of well water (control) and landfill leachate from the Oneida County landfill during 2005 and 2006

	2005		2006	
Component	Control	Leachate	Control	Leachate
pH	6.2 ± 0.1	8.8 ± 0.0	6.3 ± 0.2	8.4 ± 0.2
N (mg L^{-1})	480	598 ± 86	660	685 ± 25
P (mg L^{-1})	1.5	1.9 ± 0.1	3.7	3.0 ± 0.7
K (mg L^{-1})	400	450 ± 24	420	450 ± 30
Na^+ (mg L^{-1})	na[a]	690 ± 10	2.4[c]	1200 ± 0
Cl^- (mg L^{-1})	nd[b]	1093 ± 178	1.8 ± 1.8	1250 ± 50

Data are means ± one standard error (n = 2), except N, P, and K for the control treatment in both years (n = 1).
[a]Not available.
[b]Not detectable.
[c]One sample collected at harvest.
Data from Zalesny et al. 2008a.

Table 2 Concentration (mean ± SE) of soil elements over the course of the study period from the Oneida County landfill near Rhinelander, Wisconsin, USA. Means within an element sharing a letter are not significantly different at $\alpha = 0.05$

		Postharvest (17 August 2006)	
Element[a]	Preplant (13 June 2005)	Control	Leachate
N	1.44 ± 0.34b	1.37 ± 0.59b	3.45 ± 0.22a
P	3.55 ± 0.23a	0.30 ± 0.01b	0.35 ± 0.01b
K	0.83 ± 0.01a	0.08 ± 0.00b	0.10 ± 0.01b
Ca	4.81 ± 0.24a	1.49 ± 0.36c	2.88 ± 0.13b
Mg	1.99 ± 0.00a	1.38 ± 0.08c	1.73 ± 0.06b
S	1.36 ± 0.09a	0.01 ± 0.00b	0.01 ± 0.00b
Zn	48.00 ± 4.04a	2.55 ± 0.09b	5.30 ± 0.00b
B	8.00 ± 0.00a	1.00 ± 0.00c	2.15 ± 0.03b
Mn	0.20 ± 0.01a	0.10 ± 0.01b	0.19 ± 0.02a
Fe	10.98 ± 0.36a	5.41± 0.41c	7.43 ± 0.36b
Cu	16.00 ± 1.15a	11.03 ± 1.56b	15.33 ± 0.66a
Al	16.61 ± 0.70a	6.12 ± 1.12c	10.08 ± 0.52b
Pb	3.66 ± 0.04a	1.86 ± 0.59b	0.80 ± 0.10b

[a]N, P, K, Ca, Mg, S, Mn, Fe, and Al (g kg^{-1}); Zn, B, Cu, and Pb (mg kg^{-1}).
Data from Zalesny et al. 2008b.

Destructive Tree Harvests

All surviving trees (n = 100) were destructively harvested on 18 August 2006. Growth, biomass, and uptake of micro- and macro-nutrients into aboveground tree tissues were described previously (Zalesny et al. 2007b, 2008a, 2008b). Root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (0.28 m^3) surrounding each tree's root system (Zalesny et al. 2009a). Root systems were washed, and only roots connected to the main stump were retained. Root systems were separated into fine root (<2 mm diameter), lateral and basal root (each divided into 2–5 mm diameter and >5 mm diameter size classes), and stump components. Root component tissues were bulked according to root type, dried to a constant weight, and weighed.

Soil Fauna Sampling

Prior to the destructive harvests, three soil samples to a depth of 30 cm were collected with a 5-cm diameter soil corer from near each harvested tree in both treatment plots (total samples = 300). The first two samples were taken from the southwest and southeast corners of each 2.9-m^2 area per tree, and the third core was randomly sampled from either of the northern corners. Soil samples were bagged, brought to the greenhouse at the Institute for Applied Ecosystem Studies, and individually placed in Berlese funnels, which were constructed out of 15.2-cm diameter polyvinyl chloride material. Funnel tops were left open, and lights were left on in the collection room for 7-d. Inside and at the top of the funnels was screen with 25-mm^2 holes onto which soil samples were placed. This size mesh allowed both micro and mesofauna to pass through. Soil fauna were collected in vials containing 70% ethyl alcohol. Abundance of morphospecies was recorded by microscopic evaluation of each 1 × 1 cm grid in 9-cm diameter Petri dishes. Identification to species is the most effective method when assessing arthropod bioindicators, but morphospecies

can be a useful and valid surrogate when species identification is either impractical or impossible (Oliver and Beattie 1996; Derraik et al. 2002; Nahmani et al. 2006).

One limitation to our study is that the dry extraction method we used is suitable for arthropods, but is not optimal for nematodes. Typically, a wet method is employed for nematode extraction (Viglierchio and Schmitt 1983). We acknowledge that the most efficient method was not used for nematode extraction, but we feel that all nematode species were sampled with equal efficacy, and therefore the relative differences among samples was not altered.

Data Analysis

The number of each morphospecies present for each sample was divided by the soil corer volume of 589 cm^3 (i.e., $\pi \times r^2 \times h = \pi \times 2.5\ cm^2 \times 30\ cm$) to acquire the abundance per unit of soil volume. The Shannon-Wiener Diversity Index (H') was calculated for all nematode and insect morphospecies in each treatment and clone using the equation:

$$H' = -\sum_{i=1}^{S} (p_i \cdot \ln p_i)$$

where S is the total number of morphospecies, and p_i is the relative abundance of each morphospecies i, calculated as the proportion of individuals of a given species to the total number of individuals in the community.

Abundance of each morphospecies was analyzed using analyses of variance (PROC MIXED; SAS Institute, Inc., Cary, NC, USA) assuming a split plot design with a random block (i.e., replication) effect and fixed main effects for irrigation treatment (whole plot) and clone (sub plot). Given the fixed main effects, means were evaluated rather than variances. The Satterthwaite approximation was used to estimate degrees of freedom, and means were considered different at probability levels of $\alpha < 0.05$.

Regression analyses (PROC REG; SAS Institute, Inc., Cary, NC, USA) were conducted using all sample trees ($n = 100$), by treatment ($n = 50$ per treatment), and by clone (n varies depending on number of surviving trees) to examine the relationship between dry root mass and faunal abundance. Samples within the growing space of a single tree were pooled for all regression analyses. The root extraction method we employed may not account for fine roots that become detached from the root ball. However, since fine root biomass is proportional to total root mass (Coyle and Coleman 2005), we used total root mass as a surrogate for the quantity of fine root mass on which soil fauna would have had access to and likely fed upon.

RESULTS

Soil Fauna Biodiversity

Seven unique nematode morphospecies, five beetle species, and one true bug species were captured (Table 3). Nematode fauna in leachate-treated soil ($H' = 0.98$) was less biologically diverse than the community in control soil ($H' = 1.27$). Total insect diversity was nearly 50% greater in leachate-treated soil ($H' = 0.67$) compared to control soil ($H' = 0.45$), although this result was most likely driven by a high number of morphospecies I1. Among clones, nematode diversity (H') ranged from 0.21 in clone NC13460 to 0.34 in clone NC14104, with a mean of 0.25. Insect diversity ranged from 0.18 in clone NC14106

Table 3 Descriptions of morphospecies collected on 17 August 2006 from soil at the Oneida County Landfill near Rhinelander, Wisconsin, USA

Morphospecies[a]	Physical description
N1	Relatively short (<1 mm), fat, white, probable plant feeder
N2	Relatively short (<1 mm), skinny, white, probable predator
N3	Relatively long (>1 mm), hairlike, white, probable predator
N4	Relatively long (>1 mm), skinny, translucent, probable predator
N5	Relatively short (<1 mm), brown, probable plant feeder
N6	Relatively short (<1 mm), white, probable plant feeder
N7	Relatively long (>1 mm), skinny, red, probable predator
I1	Curculionidae larvae, most likely invasive species (Coyle et al. 2008), herbivores
I2	Purplish ground beetle (Carabidae), probable predator
I3	Tan rove beetle (Staphylinidae), probable predator
I4	Dark beetle with enlarged abdomen, probable Tenebrionidae, probable herbivore
I5	Striped ground beetle (Carabidae), probable predator
I6	Clear true bug nymph (Hemiptera), probable herbivore

[a]N denotes nematode, I denotes insect.

to 0.31 in clone NM6, with a mean of 0.25. Diversity was lower overall in each treatment × clone combination (Figure 1). Nematode diversity was greatest in control NC14104 and lowest in leachate-treated NC14106. Only clone NC14018 had a greater nematode diversity in leachate-treated areas. Insect diversity was comparable to nematode diversity, and was greatest in control NM6 soil. No insects were captured in leachate-treated NM2 soil, but where insects were captured, diversity was lowest in leachate-treated NC14106 soil. All clones except NC14014 and DM115 had a greater diversity in control soils compared to leachate-treated areas. The greatest difference between control and leachate-treated soil for both nematode and insect diversity occurred in clone NC14106 (Figure 1).

Soil Fauna Abundance

Leachate application and clone significantly affected abundance of several fauna groups (Table 4). Abundance of one nematode morphospecies and total nematode abundance was lower in leachate treated soils (Figure 2). Abundance of one insect morphospecies and total insect abundance was lower in leachate treated soils, but one insect morphospecies had a greater abundance in leachate treated soils (Figure 2). Abundance of one insect morphospecies (I6) was greater in soils planted with clone NC14104 compared with all other clones.

Relationship Between Root Mass and Faunal Abundance

Insect abundance was positively affected by total root mass more often than nematode abundance (Table 5). In several cases, such as when both treatments were pooled, insect abundance appeared to drive the positive relationship between total faunal abundance and total root dry mass. Insect abundance was positively correlated with increased total root mass in control trees, but there were no such relationships in leachate-treated trees. Clones NC14106, NC14018, and DN5 showed the most positive relationships between total root mass and soil fauna abundance (Table 5). In no case was total root mass negatively associated with increased faunal abundance.

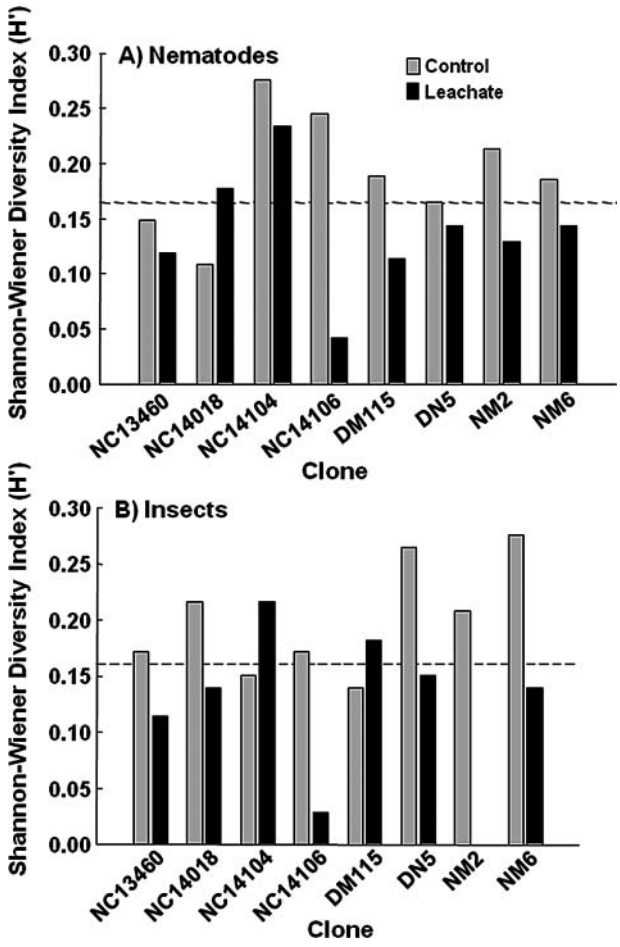

Figure 1 Soil nematode (a) and insect (b) diversity on eight *Populus* clones irrigated with fertilized well water (control) or landfill leachate for two growing seasons at the Oneida County Landfill near Rhinelander, Wisconsin, USA. The dashed line represents the overall mean.

DISCUSSION

Phytotechnologies that merge intensive forestry strategies with waste management practices are suitable to many regions of the world, including the north central United States. However, a thorough understanding of the ecological ramifications of such treatments is essential if the use of phytotechnologies is to become cosmopolitan. Soil-dwelling invertebrate biodiversity can be used as a bioindicator of polluted sites, and also to determine the effect of pollutants on the organisms themselves (Chen et al. 2009). Thus, the effects of pollutants on the health and biodiversity of the local flora and fauna is crucial information when determining whether a particular phytotechnology holds potential for use.

Nematode diversity was lower in leachate-treated soils in our study. Leachate-treated soils had much higher salinity and other elemental concentrations than control soils. Salinity is known to negatively affect nematode diversity (Adão et al. 2009). However, the negative effect leachate appeared to have can be site-specific, as other studies have reported

Table 4 Effect of irrigation treatment (well water versus leachate), *Populus* clone, and their interaction on abundance of nematode (N) and insect (I) morphospecies after two years at the Oneida County Landfill near Rhinelander, Wisconsin, USA

Morphospecies	Effect	F	df	*P*
N1	Treatment	0.00	1,284	0.9773
	Clone	0.77	7,284	0.6140
	Trt × Clone	0.75	7,284	0.6337
N2	Treatment	0.23	1,284	0.6306
	Clone	0.66	7,284	0.7100
	Trt × Clone	0.40	7,284	0.9015
N3	Treatment	2.65	1,284	0.1047
	Clone	0.93	7,284	0.4836
	Trt × Clone	0.65	7,284	0.7124
N4	Treatment	0.73	1,284	0.3943
	Clone	1.54	7,284	0.1534
	Trt × Clone	1.85	7,284	0.0783
N5	Treatment	1.82	1,284	0.1781
	Clone	1.80	7,284	0.0872
	Trt × Clone	0.35	7,284	0.9303
N6	Treatment	1.49	1,284	0.2226
	Clone	0.36	7,284	0.9249
	Trt × Clone	1.64	7,284	0.1238
N7	Treatment	7.65	1,284	**0.0060**
	Clone	0.23	7,284	0.9766
	Trt × Clone	0.67	7,284	0.6959
Nematodes	Treatment	5.44	1,284	**0.0204**
	Clone	1.21	7,284	0.2987
	Trt × Clone	0.40	7,284	0.9038
I1	Treatment	6.95	1,284	**0.0089**
	Clone	0.59	7,284	0.7622
	Trt × Clone	0.85	7,284	0.5438
I2	Treatment	3.99	1,284	**0.0466**
	Clone	1.80	7,284	0.0864
	Trt × Clone	1.80	7,284	0.0864
I3	Treatment	1.82	1,284	0.1783
	Clone	0.61	7,284	0.7499
	Trt × Clone	0.91	7,284	0.4986
I4	Treatment	0.55	1,284	0.4590
	Clone	0.70	7,284	0.6751
	Trt × Clone	0.70	7,284	0.6751
I5	Treatment	3.15	1,284	0.0772
	Clone	1.38	7,284	0.2115
	Trt × Clone	1.38	7,284	0.2115
I6	Treatment	0.94	1,284	0.3327
	Clone	2.94	7,284	**0.0054**
	Trt × Clone	0.50	7,284	0.8326
Insects	Treatment	5.37	1,284	**0.0212**
	Clone	0.63	7,284	0.7372
	Trt × Clone	0.87	7,284	0.5307

Significant *P*-values at $\alpha = 0.05$ are indicated by boldface type.

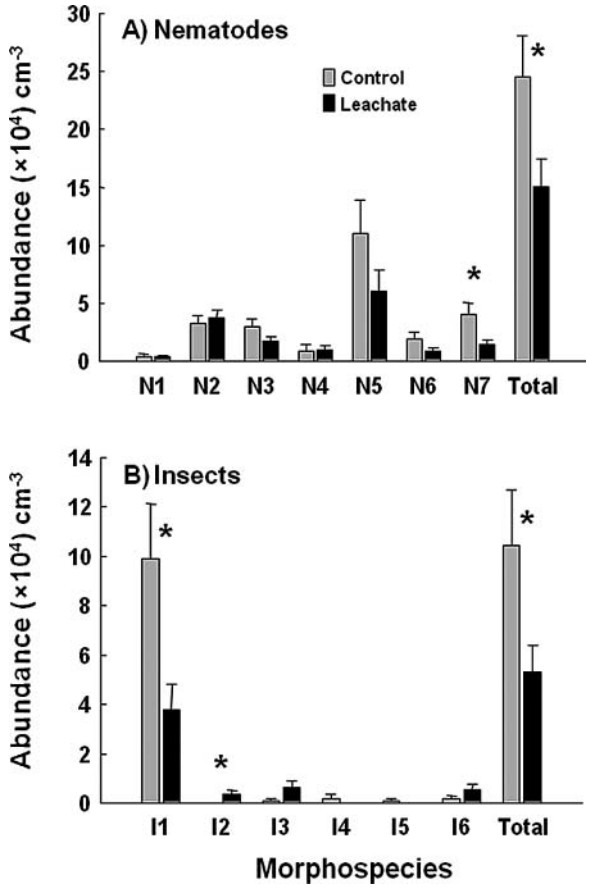

Figure 2 Soil nematode (a) and insect (b) abundance on eight *Populus* clones irrigated with fertilized well water (control) or landfill leachate for two growing seasons at the Oneida County Landfill near Rhinelander, Wisconsin, USA. Treatment comparisons with an asterisk within a morphospecies and their total were different at $\alpha < 0.05$.

inconsistent effects of pollution on nematode diversity. Nematode diversity was lower as a result of lead pollution in some study sites in China, but there was no negative effect of pollution on overall nematode abundance (Shao et al. 2008). In Spain, heavy metal (copper, nickel, lead, and zinc) pollution had significant negative impacts on nematode diversity, but these effects were not apparent at all their sampling sites (Sánchez-Moreno et al. 2006). Chen et al. (2009) sampled five sites polluted with heavy metals and polycyclic aromatic hydrocarbons along the Yellow River in China, and found different nematode species composition among the sampling sites, although in general pollution negatively affected nematode diversity. In our study, the insect community was more diverse in leachate-treated soils. However, this was driven by a single morphospecies, root-feeding weevil larvae. Greater diversity in soil arthropod communities in polluted compared to non-polluted soils has also been recorded in heavy metal-contaminated soils (Chan et al. 1997; Nahmani and Lavelle 2002; Migliorini et al. 2004).

Nematode and insect abundance were lower in leachate-treated, high salinity soils in our study. Salinity is well-documented as having a negative effect on nematode performance (Thurston et al. 1994; Finnegan et al. 1999; Moens and Vincx 2000; Nkem et al. 2006). Soil macrofauna density was negatively affected by heavy metals in France (Nahmani and

Table 5 Effect of dry root mass on the abundance of nematode and insect morphospecies after two years at the Oneida County Landfill near Rhinelander, Wisconsin, USA

	Effect	F	df	*P*	R^2
All trees	Nematodes	0.15	1,98	0.6790	0.0016
	Insects	17.32	1,98	**<0.0001**	0.1502
	All Fauna	4.37	1,98	**0.0391**	0.0427
Treatment	Effect	F	df	*P*	R^2
Leachate	Nematodes	0.48	1,48	0.4913	0.0099
	Insects	3.61	1,48	0.0635	0.0699
	All Fauna	2.29	1,48	0.1366	0.0456
Control	Nematodes	0.04	1,48	0.8382	0.0009
	Insects	10.85	1,48	**0.0019**	0.1844
	All Fauna	1.37	1,48	0.2468	0.0278
Clone	Effect	F	df	*P*	R^2
NC13460	Nematodes	0.67	1,5	0.4503	0.1182
	Insects	0.79	1,5	0.4146	0.1366
	All Fauna	0.13	1,5	0.7325	0.0255
NC14018	Nematodes	5.16	1,10	**0.0464**	0.3404
	Insects	53.40	1,10	**<0.0001**	0.8423
	All Fauna	38.83	1,10	**<0.0001**	0.7952
NC14104	Nematodes	0.89	1,13	0.3615	0.0644
	Insects	0.63	1,13	0.4416	0.0462
	All Fauna	0.56	1,13	0.4681	0.0412
NC14106	Nematodes	17.38	1,10	**0.0019**	0.6347
	Insects	3.73	1,10	0.0822	0.2718
	All Fauna	13.93	1,10	**0.0039**	0.5821
DM115	Nematodes	0.09	1,9	0.7718	0.0098
	Insects	0.39	1,9	0.5503	0.0410
	All Fauna	0.79	1,9	0.3984	0.0803
DN5	Nematodes	0.64	1,12	0.4384	0.0508
	Insects	7.77	1,12	**0.0164**	0.3930
	All Fauna	7.18	1,12	**0.0200**	0.3744
NM2	Nematodes	0.01	1,12	0.9312	0.0006
	Insects	2.58	1,12	0.1344	0.1768
	All Fauna	0.84	1,12	0.3774	0.0654
NM6	Nematodes	3.39	1,13	0.0885	0.2068
	Insects	0.03	1,13	0.8691	0.0022
	All Fauna	1.87	1,13	0.1947	0.1257

Significant *P*-values at $\alpha = 0.05$ are indicated by boldface type.

Lavelle 2002) and Italy (Migliorini et al. 2004). Abundance of only one nematode and one insect morphospecies was lower in leachate-treated soils. This suggests that the effects of pollutants may be species-specific, a finding corroborated by other studies (Chan et al. 1997; Nahmani and Lavelle 2002; Migliorini et al. 2004; Sánchez-Moreno et al. 2006; Chen et al. 2009).

Insect soil fauna in our study was dominated by larvae of a complex of invasive weevils (Coyle et al. 2008). These weevils are rhizophagous as larvae, and we observed several instances where increased insect abundance was associated with larger total (and presumably fine) root mass. This relationship is expected and common (Prins et al. 1992; Parmelee et al. 1993), as most soil-dwelling arthropods are herbivores. *Populus* traits are, in general, very clone specific, and this is the pattern we saw with our data—some clones

maintained a positive relationship between root mass and faunal abundance, while other clones did not. Further examination of this complex interaction is required to elucidate the exact reason for these relationships.

Clones NC14018, NC14104, and DM115 had diversity patterns different from all other clones; namely, there was greater diversity in leachate-treated soil instead of the control soil. This suggests that, like many traits in *Populus* selections, the ability to support biological diversity under stressful conditions, especially high salinity soils, may be clone-specific (Chen and Polle 2010). This trait could potentially be selected for and used in breeding programs to create new selections for use in phytoremediation situations.

We observed few diversity or abundance differences among individual clones, but there were several differences between treatments within a clone. A larger sampling area may have increased the number of clones in which differences between treated and untreated soils occurred, however, we believe that the soil volume sampled represented the soil community accurately. Soil fauna, in general, are not particularly mobile, and this soil volume would have provided ample space for the micro-and meso-fauna we examined in our study.

Leachate application can be a viable remediation tactic. In this system, we saw minimal effects on tree growth (Zalesny et al. 2007b, 2009a), but did see negative effects on soil fauna, which are known to be essential in healthy ecosystem functioning (Postma-Blaauw et al. 2010). Our observed results agree with many previous studies in that the effects of leachate application on soils can be very heterogenous, and the effects can vary spatially and regionally. Leachate application negatively affected diversity of nematodes, but not insects, and negatively affected abundance of most morphospecies. Additional studies should investigate the effect of leachate on soil food webs and higher organisms to fully assess the impact of leachate application as a remediation technique.

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