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Mixtures of papermill biosolids and pig slurry improve soil quality and growth of hybrid poplar

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Abstract

Hybrid poplar plantations in Quebec, Canada, are generally established on marginal agricultural lands characterized by low pH and low inherent soil fertility. Here, we tested the hypothesis that two potential organic fertilizer (OF) sources, papermill biosolids (PBs) and liquid pig slurry (LPS), would improve soil quality and the growth performance of hybrid poplars (*Populus trichocarpa* × *Populus deltoides*), especially if applied in mixtures rather than separately. The fertilizer treatments included an unfertilized control, inorganic fertilizer (IF) (calcium ammonium nitrate and triple superphosphate) and OFs (PBs alone, LPS alone and two combinations of PBs and LPS) applied at two rates. Fertilizers were broadcast within 1 m of tree trunks and unincorporated, to prevent damage to tree roots. Hybrid poplar growth was the greatest in plots fertilized with a combination of PBs and LPS, suggesting that the two OFs complemented themselves and/or interacted to improve soil nutritional quality. PBs were the most efficient at raising soil pH, providing plant-available Ca and increasing nitrification rates over the long term, whereas LPS provided more readily available NO_3-N, P and K. Applied together, PBs and LPS interacted to provide more extractable P and mineralizable NH_4-N than when applied separately. OFs increased soil biological activity, notably basal respiration, microbial biomass, metabolic quotient and mineral N production rates. Community-level catabolic profiles of the extractable soil microflora in plots with OFs differed significantly from the control and IF treatments. This implies that surface-applied OFs may induce fundamental changes to the diversity and composition of microbial communities in the underlying rooting zone. Although this study has shown beneficial effects of OF mixtures on soil quality and hybrid poplar growth, further research should focus on their possible environmental impacts.

Keywords: Hybrid poplar, organic fertilizers, soil nutrients, soil microbial dynamics, microbial community-level catabolic profiles

Introduction

Over the past 60 years, total world wood harvest has increased at a near steady annual rate of 100 million m^3 year $^{-1}$, whereas the total forested land area has fallen at an annual rate of 0.2%. To meet increasing wood demand with a dwindling forested land base, there has been a progressive interest in developing productive short rotation tree plantations, even in countries such as Canada where forestry has historically been extensive with low management implications. Accordingly, hybrid poplar (*Populus spp.*) plantations in Canada had risen to 14.3×10^3 ha by the year 2002 (Food and Agriculture Organization, FAO, 2004). These trees, resulting from the combination of various poplar species into a hybrid, are among the fastest growing in North America given the proper growing conditions (US Environmental Protection Agency, 1999).

Many hybrid poplar plantations in Québec were established on marginal or degraded soils, rather than on prime agricultural land. These marginal lands are characterized by low organic matter content and feature low inherent soil fertility and other production constraints (e.g. low pH). Adding fertilizer and lime to provide N, P and K, and to adjust soil pH during stand establishment is routinely used to achieve high biomass production in hybrid poplar plantations (e.g. Dickmann *et al.*, 2001; DesRochers *et al.*, 2006). By contrast, organic fertilizers (OFs), which generally contain all of the essential plant nutrients, are seldom used. As a perennial crop, hybrid poplars may respond well to OFs, because these

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may increase both the short- and long-term supply of soil nutrients as well as improve other aspects of soil quality such as soil pH and microbial biomass.

In Québec, Canada, two million tons of papermill biosolids (PBs) are produced each year during treatment of wastewater from the pulping and papermaking processes (Désilets, 2000). From a nutritional standpoint, PBs not only contain N–P–K, but are especially rich in Ca, an element that is in particularly high demand by poplars (Alban, 1982; Lamarche *et al.*, 2004). Increasing soil Ca may also improve soil pH in degraded agricultural soils. Due to its high fibre content, the application of PBs may improve soil structure as well as increase soil organic matter content and soil CEC (Chantigny *et al.*, 1999; Camberato *et al.*, 2006). One potential downside of PBs is that these are expected to immobilize soil mineral N when initially applied, and to liberate no more than 25% of total N during the first year after field application (Hébert & Gagné, 2003). A second potential OF for hybrid poplar is liquid pig slurry (LPS), of which nine million m^3 are spread annually on Québec agricultural lands (Chantigny *et al.*, 2004). While LPS is rich in mineral N and contains substantial quantities of other macronutrients, its low C:N ratio (3.6:1) (Sánchez & González., 2005) results in rapid N mineralization and subsequent loss via NO_3^- leaching, NH_3 volatilization and denitrification (Sieling *et al.*, 1998; Rochette *et al.*, 2001; Centre de référence en agriculture et agroalimentaire du Québec, CRAAQ, 2003) thus diminishing the fertilizer value of the slurry.

The major difficulty in using OFs in hybrid poplar plantations is that they cannot be incorporated by tillage, due to the presence of tree roots. Leaving LPS on the soil surface is expected to exacerbate N loss via NH_3 volatilization (Rochette *et al.*, 2001), whereas unincorporated PBs may decompose more slowly than on conventionally tilled soils (Entry *et al.*, 1996). Given the complementary characteristics of these two organic amendments, namely the high fibre and Ca content of the first, and the readily mineralizable N of the second, we hypothesized that a combination of PBs and LPS would have better fertilizer value for hybrid poplar than applying these individually. PBs could potentially immobilize excess N mineralized from LPS, and the combination of the two could have a more balanced nutritional value as well as increase other aspects of soil quality. We conducted, therefore, an experiment during two growing seasons in a young hybrid poplar plantation established on marginal agricultural land in southern Québec, Canada. Our goal was to evaluate the fertilizer value of PBs and LPS, applied singly and in mixtures compared with mineral fertilizers. We also measured other indices of soil quality such as soil pH, soil nutrient concentrations, microbial biomass and mineralizable N. Finally, we assessed whether OFs also induced changes in the underlying structure and functional diversity of soil microbial communities compared with mineral fertilizers.

Materials and methods

Site description

The study site, located near the Town of Ste-Camille (45°40′36″N, 71°44′13″W), is classified as marginal agricultural land, and was used as an unimproved hayfield for more than 10 years before our experiment began. The soil was a 'Magog Stony Loam' of the Orthic Gleysol subgroup (Soil Classification Working Group, 1998), imperfectly drained and occurring on a gentle slope (< 1% slope). The soil had a clayey loam texture, 30 g organic C kg^{-1} and a pH_{water} of 5.6 in the 0–15 cm depth.

In May 2001, the field had been tilled to a 30-cm depth with a chisel plough along a north–south axis. One-year-old bare root hybrid poplar (*Populus trichocarpa* × *Populus deltoides*) seedlings were hand planted at 3-m intervals in the ploughed furrows with 3-m spacing between rows. Vegetation growing between rows was controlled in June and July 2001 with the herbicide glyphosate (2.4 kg a.i. ha^{-1}). In subsequent years, the vegetation between rows was controlled by mowing twice each summer.

Experimental design

The field experiment was laid out in a randomized complete block design (four blocks), with 10 plots per block, for a total of 40 experimental plots. The plots were arranged in staggered rows and each 100-m^2 plot contained 16 trees. The four trees in the middle of each plot received an equal amount of fertilizer and their growth was followed throughout the study. Fertilizers were applied during the fourth (2004) and fifth year (2005) after plantation establishment.

Soil analyses performed in 2003, 1 year before treatments were applied, indicated low Mehlich-III extractable K concentrations (45 mg kg^{-1}). All plots received, therefore, KCl (0–0–60) at a rate of 30 kg K_2O ha^{-1} in May 2004. Ten fertilizer treatments were assigned randomly to plots in each block. These included a control (no fertilizer), an inorganic fertilizer (IF) treatment [calcium ammonium nitrate (27.5–0–0) + triple superphosphate (0–46–0)] applied at a base rate of 35 kg N ha^{-1} (referred to as 1×) and 30 kg P_2O_5 ha^{-1}, four OF treatments applied at the 1× rate, and four OF treatments applied at the 2× rate, (i.e. targeted to provide 70 kg N ha^1). The 1× rate of 35 kg N ha^{-1} comes from the Conseil des production végétales du Québec (CPVQ) (2000) fertilizer recommendations manual for field grown deciduous trees. The four OF treatments within each of the 1× and 2× rates were PBs alone, LPS alone and two mixtures (33%:66% and 66%:33%) of PBs:LPS. The mass of PBs and LPS applied to deliver the target N input of 35 or 70 kg N ha^{-1} $year^{-1}$ (Table 1) were based on the inorganic N fertilizer equivalency and expected N losses following surface application without incorporation (CPVQ, 2000;

Table 1 Target N input and mass (fresh wt) of papermill biosolids (PBs) and liquid pig slurry (LPS) applied in May 2004 and May 2005

Treatment	Target N input[a] (kg N ha^{-1} $year^{-1}$)	Abbreviations	PBs mass (t ha^{-1})	LPS mass (t ha^{-1})
Control	0	C (0×)	0	0
Inorganic fertilizer	35	IF (1×)	0	0
100% PBs	35	B100 (1×)	39	0
66%:33% = PBs:LPS	35	B66P33 (1×)	26	6.8
33%:66% = PBs:LPS	35	B33P66 (1×)	12	13.8
100% LPS	35	P100 (1×)	0	20.7
100% PBs	70	B100 (2×)	78	0
66%:33% = PBs:LPS	70	B66P33 (2×)	52	13.6
33%:66% = PBs:LPS	70	B33P66 (2×)	24	27.5
100% LPS	70	P100 (2×)	0	41.4

[a]Based on an estimated dry matter content of 30 and 3.5% for PBs and LPS, and on the estimate that these, respectively, contained 30 and 60% plant-available N over the study period (Conseil des production végétales du Québec, CPVQ, 2000; Centre de référence en agriculture et agroalimentaire du Québec, CRAAQ, 2003).

CRAAQ, 2003). The chemical composition of PBs and LPS applied in this study are given in Table 2. All fertilizer treatments were surface applied in a circle (~1 m radius) around the trunk of each tree.

In 2004, precipitation between May and October (685 mm) was near the 30-year average (Environment Canada, 2006) and a heavy rainfall event (15 mm) occurred on the day we fertilized. In 2005, there was 7% more precipitation between May and October (726 mm) than the 30-year average and a moderate rainfall event (< 5 mm) occurred the day following fertilizer application. During both growing seasons, average monthly temperatures were warmer than the 30-year average.

Tree biomass increment and nitrogen use efficiency

The 160 trees were measured at the end of the growing season for height (H) and diameter at breast height (DBH at 1.3 m height) 1 year before the experiment began (Sept-03) and in the two subsequent years (Sept-04 and Sept-05). Height was measured with a pole digital measuring rod and DBH with a measuring tape. The leafless aboveground biomass (LAB) of each tree was estimated following the general hybrid poplar biomass equation (Boysen and Strobl, 1991):

$$\mathrm{LAB} = 0.202 \times \mathrm{DBH}^{(1.326+0.19\times\ln(H))} \times \mathrm{H}^{0.175}. \qquad (1)$$

The total biomass increment (t ha^{-1}) in each plot was calculated as the increase in LAB between Sept-03 and Sept-05, and this value was scaled up assuming a stand density of 1600 trees ha^{-1}. Nitrogen use efficiency (NUE) in each plot was calculated (kg DW kg^{-1} N) from total biomass increment in trees receiving fertilizer and the cumulative total N input from fertilizer added in both years (Van Miegroet *et al*., 1994; Sardans *et al*., 2005):

$$\mathrm{NUE} = \frac{\text{Biomass Increment}}{\text{Cumulative N input}}. \qquad (2)$$

Table 2 Moisture and nutrient contents of papermill biosolids (PBs) and liquid pig slurry (LPS) used in this study[a]

Parameter	PBs	LPS
Moisture content (%)	78.0 ± 1.9	99.3 ± 0.03
Organic C (%)[b]	413 ± 3.1	334 ± 7.1
Total N (g kg^{-1})[c]	26.1 ± 3.7	462 ± 119
C/N ratio	15.9 ± 0.8	0.72 ± 0.06
P_2O_5 (g kg^{-1})[c]	2.5 ± 0.2	28.8 ± 0.08
K_2O (g kg^{-1})[c,d]	3.2 ± 0.2	302 ± 0.7

Carbon and nutrient contents are expressed on a dry weight basis. [a]Values are the means ± standard errors of nine PBs and 16 LPS samples. [b]Analysed using a Flash EA 1112 NC soils analyser (Carlo-Erba, Milan, Italy). [c]H_2O_2/H_2SO_4 acid digests subsequently analysed using a Quick-Chem AE autoanalyser (Lachat Instruments, Milwaukee, WI, USA). [d]Analysed by atomic absorption spectrometry (Perkin-Elmer Corp., Norwalk, CT, USA).

Soil sampling

Soil samples were collected 1 month after fertilizer application, in June 2004 (June-04) and 2005 (June-05). We also collected soils before leaf senescence, in September 2004 (Sept-04) and 2005 (Sept-05). After removing surface plant material, two soil cores (7.5 cm dia.) from the 0- to 15-cm mineral soil layer were collected from random locations within a 1-m radius of each experimental tree in each plot. The eight cores from each plot were mixed and sieved (5-mm mesh) in the field to create a composite sample, and transported on ice and stored at 4 °C until analysed within 1 week of sampling. We also collected a composite soil sample from the 15- to 30-cm depth of each plot on June-04 and June-05 using the same procedure. Field moist soils were sieved (5-mm mesh) and gravimetric moisture content

was calculated based on mass loss after drying subsamples at 105 °C for 48 h.

Soil pH and extractable nutrients

Soil subsamples collected in June-04 and June-05 were air dried and ground to pass through a 2-mm sieve. Soil pH was measured in a soil:water (1:2) suspension. Available P, K, Ca and Mg were extracted using Mehlich III solution and analysed colorimetrically or by atomic absorption spectrometry as described in Table 2. Mineral-N (NH_4^+-N and NO_3^-N) was extracted in 0.5 M K_2SO_4 (soil:extractant = 1:10) solution and analysed colorimetrically as described in Table 2.

The potential for surface mineral soil to supply mineral N to plants following the second treatment year (Sept-05) was assessed using aerobic laboratory incubations (Fyles *et al.*, 1990). Fresh subsamples of the 0–15 cm soil layer (15 g dry mass equiv.) were transferred to 500 mL Mason jars, covered with a polyethylene film to prevent desiccation and allow gas exchange, and left to incubate 30 days in the dark at 20 °C. The subsamples were then extracted in 100 mL of 1 N KCl solution, and extracts were analysed for mineral N as previously described. Net ammonification and nitrification rates were calculated from the difference between pre- and post-incubation mineral N concentrations.

Soil respirometry

Basal respiration, an index of instantaneous C availability (Bradley & Fyles, 1995a), was determined by weighing fresh subsamples (10 g dry wt equiv.) into 57-mL gas sampling jars, allowing 1 week for soils to condition to room temperature, flushing the headspace with ambient air for 5 min, sealing jars with air-tight lids equipped with rubber septa, and sampling aliquots of air in the headspace with a needle and syringe after 24 h. Air samples were analysed for CO_2 concentrations using a model CP-2002P Micro-GC (Chrompack, Middelburg) equipped with a TCD and He as carrier gas. Room temperature and ambient CO_2 concentration were measured several times daily. For each measurement, ambient CO_2 concentration was subtracted from sampled CO_2 concentration and the difference was adjusted according to Ideal Gas Laws and centred at 22 °C using $Q_{10} = 2$.

Microbial biomass, an index of historical C availability (Bradley & Fyles, 1995a), was determined by substrate-induced respirometry (SIR) (Anderson & Domsch, 1978). Fresh subsamples (10.0 g dry wt equiv.) were weighed into 500-mL plastic containers and amended with ground and sieved (65 μm) glucose (1000 μg C g^{-1} soil). The amendments were applied as 250-mg mixtures with talc and dispersed using a kitchen handmixer with one beater. Amended subsamples were transferred into 115-mL gas sampling jars and left uncovered for 100 min to reach optimum SIR rates. Subsamples were then flushed for 5 min with ambient air, sealed for 30 min, and headspace air was analysed for CO_2 concentration using a GC (as described above). SIR rates were converted to microbial biomass using equations derived by Anderson & Domsch (1978).

Microbial metabolic quotient (qCO_2) in soil from each plot was calculated as the quotient of basal respiration and microbial biomass.

Microbial community-level catabolic profiles

Community-level catabolic profiles (CLCPs) of the extractable microflora from each bulked sample (0–15 cm layer) collected on Sept-05 were characterized using BIOLOG EcoPlates (BIOLOG Inc., Hayward, CA, USA). A 15-g (dry wt equiv.) soil subsample was added to 150-mL of sterile 0.1% Na-pyrophosphate solution (pH 7.0) to which 15–20 glass beads (3 mm) were added. The mixture was shaken 20 min and centrifuged ($500 \times \boldsymbol{g}$, 10 min, 4 °C) to obtain a microbial suspension. A 1-mL aliquot of supernatant was diluted into 99 mL of sterile saline solution (0.85% NaCl). Inoculum density was not standardized as total microbial numbers were considered an intrinsic characteristic of microbial diversity in each sample. A 150-μL aliquot of the diluted solution was pipetted into each of 96 BIOLOG EcoPlate wells. These comprised a triplicate set of 31 wells containing a unique C-source plus redox-sensitive tetrazolium dye, and one control well containing dye only. Plates were incubated at 25 °C and colour formation in each well was monitored as monochromatic light (595 nm) absorbance, using a Bio-Tek FL600 automated plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). Measurements were made three times daily until average well colour development (AWCD) exceeded a value of 0.50 standardized absorbance units (i.e. 3–5 day). At each reading, the absorbance value of control wells were subtracted from absorbance values of the 31 associated wells containing C substrates.

Statistical analyses

Prior to analyses, the data were Ln-transformed when required to adjust for normality and stabilize the variance. At each sampling date, data were analysed with the SAS statistical software (SAS Institute Inc., 2003) by a one-way ANOVA (10 treatments), and by a two-way ANOVA (excluding the control and IF treatments), and significantly different means were separated using a Tukey multiple comparisons test. Pre-planned comparisons of various linear treatment combinations were evaluated with single degree of freedom orthogonal contrasts. The significance level for each test was set at $P < 0.05$.

Average well colour development of each replicate 32-well set in each BIOLOG EcoPlate was calculated at each reading interval to determine the incubation time ($T_{0.50}$) corresponding to AWCD = 0.50 absorbance units. Absorbance values

of each well at $T_{0.50}$ were centred and normalized [i.e. (Abs. − AWCD)/σ], and principal component analysis (PCA) was performed on transformed data to explore effects of treatments on C source utilization patterns by microbial communities. PCA biplots were produced for visual assessments of treatment effects on microbial functional diversity. Because the segregation of treatments along the first two principal components refers to only a portion of the total variation in the data set, the EcoPlate data were also analysed with a more robust non-parametric multivariate analysis of variance, implemented as permutation tests, using the PERMANOVA software (Anderson, 2001; McArdle & Anderson, 2001). PERMANOVA uses all of the information and has, therefore, greater statistical power to detect differences than PCA. Under PERMANOVA, the full 32-variable data set was permuted 4999 times for one-way and two-way analyses to compute sums-of-squares among and within treatments, and to generate an ANOVA-type table that could be interpreted in the conventional manner. Subsequent single degree of freedom contrasts were performed using the DISTLM v.5 statistical program (Anderson, 2004).

For each OF source, simple linear regressions were fitted between the fertilizer N input and the mineral N pool in the 0- to 30-cm soil layer.

Results

Biomass accumulation and nitrogen use efficiency

Leafless aboveground biomass increment was greater in plots that received OFs than in control plots (Table 3). Single d.f. orthogonal contrasts showed that biomass increment in the four OF treatments was greater than in the IF treatment, at both the low and the high OF application rates (Table 4). At both application rates, biomass increment was greater in OF mixtures compared with PBs and LPS applied separately (Table 4). The fertilizer NUE was greater in IF than in OF treatments. Among OF treatments the NUE was consistently higher at the low N rate than at the high N rate and consistently lower in B100 (within each rate) than in the other treatments.

Table 4 Orthogonal contrasts comparing biomass increment of organic and inorganic fertilizer treatments, as well as the biomass increment of organic fertilizers applied in mixtures as opposed to separately

Contrast	d.f.	*F*-value	$P > F$
Sept-04			
IF < OF (1×)	1	4.41	0.044
IF < OF (2×)	1	12.61	0.001
(B100 + P100) (1×) < (B66P33 + B33P66) (1×)	1	6.10	0.019
(B100 + P100) (2×) < (B66P33 + B33P66) (2×)	1	10.23	0.003
Sept-05			
IF < OF (1×)	1	4.20	0.049
IF < OF (2×)	1	16.54	< 0.001
(B100 + P100) (1×) < (B66P33 + B33P66) (1×)	1	5.47	0.026
(B100 + P100) (2×) < (B66P33 + B33P66) (2×)	1	16.00	< 0.001

Abbreviations are explained in Table 1.

Fertilizer effects on soil pH and extractable nutrients

Initial (Sept-03) average soil pH on the site was 5.6. Soil pH remained between 5.6 and 5.8 in the control plots over the course of the study (Figure 1). Soil pH in IF and P100 plots remained below pH 6.0 and were not significantly different

Table 3 Biomass increment and nitrogen use efficiency (NUE) after 2 years of fertilizer application in a hybrid poplar plantation

Treatment	Total N input[a] (kg N ha^{-1} year^{-1})	Biomass increment (t DW ha^{-1})	NUE (kg DW kg^{-1} N)
C (0×)	0	2.05 ± 0.76d	–
IF (1×)	35	3.20 ± 0.72cd	45.7 ± 10.2a
B100 (1×)	216	4.18 ± 0.83bcd	9.68 ± 1.92c
B66P33 (1×)	182	6.00 ± 1.20ab	16.5 ± 3.30bc
B33P66 (1×)	144	5.46 ± 1.10bc	19.0 ± 3.80b
P100 (1×)	116	3.84 ± 0.77bcd	16.5 ± 3.33bc
B100 (2×)	432	4.81 ± 0.70bc	5.56 ± 0.81d
B66P33 (2×)	365	7.53 ± 1.47a	10.3 ± 2.01bcd
B33P66 (2×)	287	7.93 ± 1.480a	13.8 ± 2.60bc
P100 (2×)	233	5.20 ± 1.46bc	11.2 ± 3.14bcd

Values are the mean ± SE (n = 4). Within a column, significantly different treatment means are indicated by different lowercase letters ($P < 0.05$, Tukey test). Abbreviations are explained in Table 1. [a]The total N input was based on the measured N in biosolids and pig slurry. Biosolids contained 5.54 kg N t^{-1} and pig slurry contained 5.62 kg N t^{-1} (2004/2005 samples).

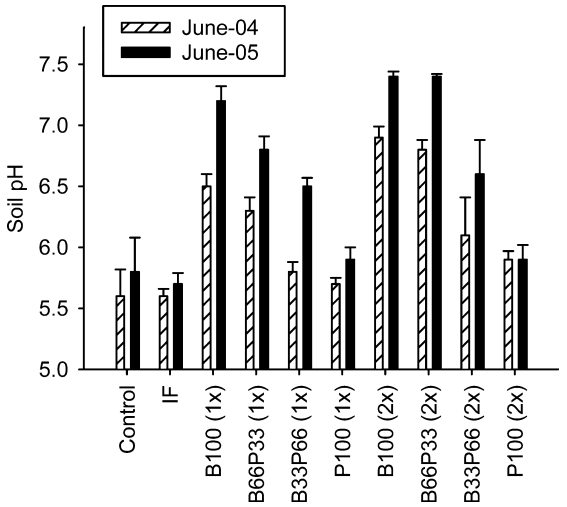

Figure 1 Average soil pH (0- to 15-cm layer) of each treatment on June-04 and June-05 sampling dates. Vertical lines represent 1 SE. Abbreviations for fertilizer treatments are found in Table 1.

from control plots. With one exception, there was a significant increase of 0.7–1.6 pH units in B66P33 and B100 plots. Average soil pH was significantly higher in 2005 than in 2004.

On June-04, one-way ANOVA revealed a significant treatment effect on extractable P and K (data not shown). Subsequent contrast analyses (Table 5) revealed higher extractable P in IF than in OF plots, and higher extractable P where OFs were applied as mixtures rather than singly. Control plots had less extractable K than OF plots.

Table 5 Selected orthogonal contrasts testing the effects of various linear combinations of fertilizer treatments on extractable soil nutrients measured in June-04 and June-05

Variable	Contrast	d.f.	*F*-value	$P > F$
June-04				
Extractable P	OF (1×) < IF	1	13.89	< 0.001
Extractable P	(B100 + P100) < (B66P33 + B33P66)	1	9.00	0.006
Extractable K	Control < OF	1	13.59	0.010
June-05				
Extractable NH_4	Control < OF	1	0.72	0.004
Extractable NO_3	B100 < P100	1	12.8	0.001
Extractable P	Control < OF	1	15.51	0.003
Extractable P	OF (1×) < IF	1	10.35	0.010
Extractable P	B100 < P100	1	4.13	0.052
Extractable K	B100 < P100	1	11.03	0.003
Extractable Ca	Control < OF	1	0.09	0.002
Extractable Ca	IF < OF (1×)	1	6.22	0.019
Extractable Ca	P100 < B100	1	19.95	< 0.001
Extractable Mg	IF < OF (1×)	1	4.63	0.040

OF = organic fertilizers; IF = inorganic fertilizer; (1×) = low rate (35 kg N ha^{-1} $year^{-1}$); (2×) = high rate (70 kg N ha^{-1} $year^{-1}$). For B100, P100, B66P33 and B33P66, see Table 1.

Extractable nutrient concentrations were higher in June-05 than in June-04 (data not shown). In June-05, one-way ANOVA revealed a significant effect of treatments on the six extractable nutrients (Figure 2). Contrast analyses (Table 5) revealed that: (i) extractable NH_4, P and Ca were lower in the control than in the OF treatments; (ii) extractable NO_3, P and K were lower in B100 than in P100 treatments; (iii) extractable P was lower with OFs than with IF; (iv) extractable Ca and Mg were lower with IF than with OFs; and (v) extractable Ca was lower in P100 than in B100 treatments.

Nitrogen and microbial and dynamics

One-way ANOVA revealed significant ($P < 0.001$) effects of treatments on net nitrification rates, and near-to-significant effects of treatments on basal respiration ($P = 0.075$), microbial biomass ($P < 0.067$) and net ammonification ($P < 0.077$). Orthogonal contrasts showed net nitrification was significantly ($P < 0.001$) higher in plots amended with OFs than in control or IF plots (Table 6). Basal respiration, microbial biomass, net ammonification were also higher in organically fertilized than in control or IF plots, but the significance level of these contrasts was generally weaker ($P < 0.10$; not shown). One weaker contrast ($P = 0.08$) worth mentioning is the higher net ammonification rates measured where OFs were applied in mixtures (6.3 ± 3.1 mg kg^{-1}) rather than singly (2.6 ± 0.7 mg kg^{-1}). Results of two-way ANOVA (excluding control and IF treatments) revealed no significant effect of OF application rate, but significant effects of OF mixtures on basal respiration ($P = 0.037$), metabolic quotient ($P = 0.050$) and net nitrification rates ($P = 0.027$). Tukey tests showed the value of these variables to be significantly higher in the B100 than in the P100 treatments (Figure 3).

Results from PCA revealed that the first two ordination axes explained 41% of the total variation in C source utilization patterns by microbial communities (Figure 4). The four organic mixtures were segregated from the control and IF treatments along the first principal component. The cluster of organic mixtures was separated along the second principal component with a clear segregation between the B100 and P100 treatments. The IF and control treatments were also segregated along the second principal component. The five substrates that most discriminated treatments were D-galactonic acid γ-lactone, D-xylose, Tween 80, glycogen and D-glucosaminic acid. Results from one-way PERMANOVA confirmed significant treatment effects on C source utilization patterns and pairwise contrasts confirmed a significant difference between OF treatments and both the IF and control treatments, but they failed to find a significant difference between the control and IF treatments (Table 7a). Results from two-way PERMANOVA confirmed significant effects of organic mixtures on C source utilization patterns

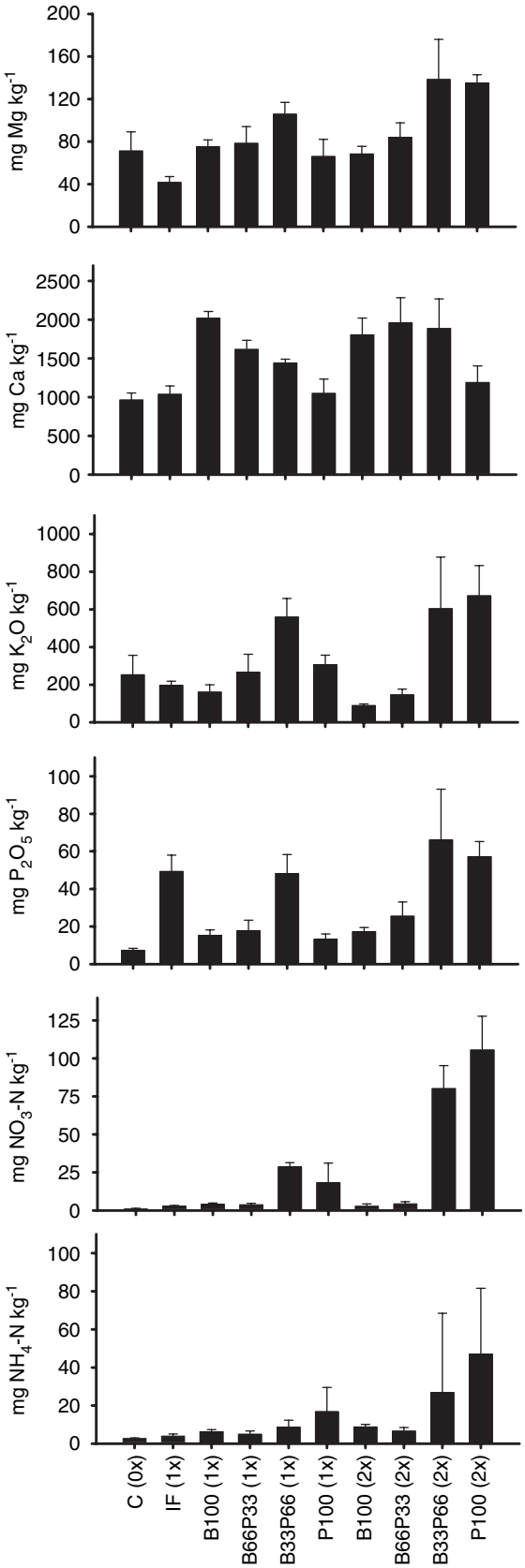

Figure 2 Average soil extractable nutrient concentrations of all 10 treatments on the June-05 sampling date. Vertical lines represent 1 SE Significant orthogonal contrasts are presented in Table 5. Abbreviations for fertilizer treatments are found in Table 1.

Table 6 Orthogonal contrasts testing the effects of organic fertilizers on net nitrification rates measured on samples collected in Sept-05

Contrast	d.f.	*F*-value	$P > F$
Control < OF (1×)	1	13.90	< 0.001
Control < OF (2×)	1	20.00	< 0.001
IF < OF (1×)	1	21.47	< 0.001
IF < OF (2×)	1	28.91	< 0.001

Abbreviations are the same as in Table 1.

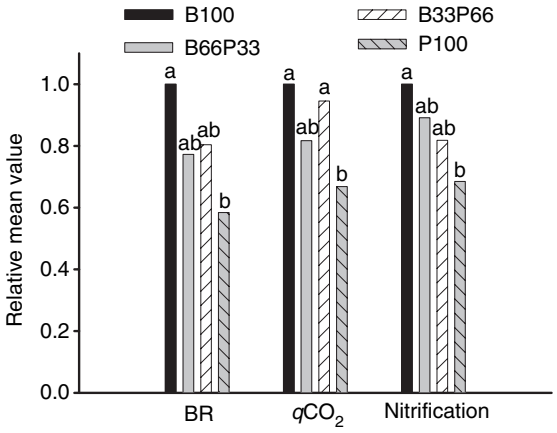

Figure 3 Effects of organic fertilizer treatments, pooled across both N rates, on soil basal respiration (BR), microbial metabolic quotient (qCO2) and net nitrification rate. Mean values for each treatment are reported relative to the maximum mean value measured in plots that received 100% biosolids (BR = 4.18 μg CO_2-C g^{-1} h^{-1}; qCO_2 = 0.202 μg CO_2-C mg^{-1} C_{mic} h^{-1}; net nitrification = 43.7 μg NO_3-N g^{-1}). Bars within the same cluster designated with different lowercase letters differ significantly ($P < 0.05$) according to Tukey tests.

(Table 7b). Pairwise contrasts confirmed that the greatest difference occurred between the B100 and P100 treatments, but there were also significant differences in three of the remaining five possible pairwise comparisons (Table 7b).

Discussion

Our study showed that OFs may improve hybrid poplar yield, and may even outperform the IF treatment. Our soil analyses suggest that this improved biomass increment over the IF treatment could be due to more plant-available Ca

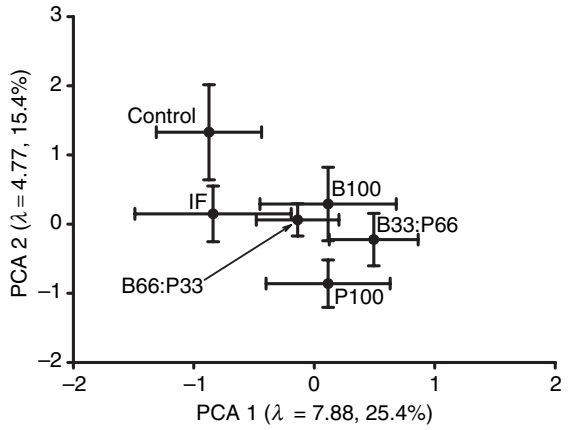

Figure 4 Principal component analysis ordination of plots that received the four organic fertilizer mixtures (pooled across both application rates), and those that received inorganic fertilizer (IF) or no fertilizer at all (control). Values represent the mean (n = 4) component scores along the first two axes ±1 SE.

and Mg, and higher potential nitrification rates. Plant-available P remained, however, higher with IF. To compare the fertilizer value of OFs relative to IF is, however, tenuous, because OFs carried far more total nutrients than IF and we simply estimated the proportion that would be mineralized over the study period. The NUE of IF was, of course, greater than that of OFs, given that IF is 100% water soluble and thus 100% plant-available. The IF treatment was included in our experimental design essentially to evaluate whether an increase in poplar yield with OFs applied at our experimental rates would be comparable with a yield increase using IF rates recommended for hybrid poplar. Our study has shown that this is clearly the case.

The salient result of our study was showing that hybrid poplar yield can be higher with PBs and LPS applied in mixtures rather than applied singly. In other words, our study showed that the two OF sources can interact to improve the soil's nutritional quality. A clear sign of an interaction occurring between the two OF sources is reflected by the NUE values of the four OF treatments within each application rate. Based on the NUE values of PBs and LPS applied separately, we found that mixing LPS to PBs resulted in NUE values that were larger than the expected intermediate values. Our study provided some insights as to how PBs and LPS applied in mixtures may complement themselves or interact to improve soil nutritional quality. Hybrid poplars grow best in soil pH ranges between 6.0 and 7.0 (Timmer, 1985) and PBs were most efficient at raising soil pH within this range as well as providing plant-available Ca which would otherwise be leached in acidic soils. Thus, including PBs in the OF mixtures may eliminate the need for liming when hybrid poplar plantations are established on acidic soils. On the other hand, LPS provided more NO_3-N, P and K early in the growing season. Applied together, PBs and LPS interacted to provide more extractable P and mineralizable NH_4-N than when applied separately.

Contrary to IF-amended soil, OFs improved soil nutritional quality with attendant changes in the biological activity of the soil. For example, we witnessed higher basal respiration and higher microbial biomass in OF-amended soil

Table 7 Results of PERMANOVA and selected single degree of freedom pairwise contrasts testing for significantly different C-source utilization patterns among treatment combinations

Source	Contrast	d.f.	Mean squares	Pseudo-F	P-value
(a) One-way PERMANOVA including all 10 treatments					
Block		3	98.0	2.17	0.005
Treatment		9	112.8	2.50	<0.001
	Control vs. IF	1	65.1	1.44	ns
	Control vs. OF (1×)	1	231.3	5.13	<0.001
	IF vs. OF (1×)	1	187.8	4.16	0.001
	Control vs. OF	1	249.9	5.54	<0.001
	IF vs. OF	1	191.2	4.24	0.001
Error		27	45.1		
(b) Two-way PERMANOVA excluding control and IF treatments					
Rate		1	41.9	41.94	ns
Mixture		3	150.8	3.99	<0.001
	B100 vs. P100	1	362.4	9.59	<0.001
	B100 vs. B33P66	1	80.1	2.11	0.045
	B100 vs. B66P33	1	44.0	1.16	ns
	B66P33 vs. P100	1	214.8	5.69	<0.001
	B66P33 vs. B33P66	1	43.7	1.16	ns
	B33P66 vs. P100	1	159.6	4.23	<0.001
Rate × mixture		3	37.1	0.98	ns
Error		21	793.3		

Abbreviations are explained in Tables 1 and 3.

which reflects more reduced C substrates to drive the metabolism and growth of heterotrophic soil micro-organisms. Higher microbial metabolism is expected to result in higher rates of nutrient turnover and release (Bradley & Fyles, 1995b), which is consistent with the higher potential ammonification and nitrification rates that we observed in OF-amended plots. Among the two OF sources, PBs proved to be the main contributor to this increase in microbial metabolism, as we witnessed higher basal respiration, metabolic quotient (qCO_2) and potential nitrification rates in plots treated with PBs than those treated with LPS. Although the organic C content of PBs was 24% higher than that of LPS, the difference in microbial activity between the two could also be ascribed to differences in their respective chemical qualities. A greater proportion of the organic C found in LPS is expected to be low molecular weight and labile, whereas a greater proportion of the organic C found in PBs is expected to contain moieties of lignin that are complex and degrade more slowly. The former is expected to boost microbial activity soon after being applied to soil, while the latter is expected to sustain microbial activity later in the growing season. This is corroborated by the fact that available NO_3-N was higher in the LPS treatment in June and potential nitrification rates higher in PBs treatment in September. We acknowledge that this last conjecture is dubious because NO_3-N concentrations and potential nitrification rates are not equivalent variables, and the higher nitrification rates in soils treated with PBs may also have resulted from the higher pH in these plots (e.g. Paavolainen & Smolander, 1998).

The BIOLOG assay is a cultivation-based method that considers only a fraction of soil microbial species (Muyzer, 1998) and preferentially selects fast-growing micro-organisms that develop at high substrate concentrations (Smalla *et al*., 1998). To correct for these shortcomings, Degens & Harris (1997) proposed multiple substrate-induced respirometric assays to be performed on whole soil samples to describe CLCPs. While it is time consuming, new technologies such as the MicroResp System (Campbell *et al*., 2003) will foreseeably allow this approach to be adopted more extensively in future. The other caveat associated to the BIOLOG assay is the poor extraction efficiency of fungal hyphae (Zak *et al*., 1994). Underestimating soil fungal activity in our particular study was an important omission as various biosolids have been shown to either increase (Barbarick *et al*., 2004) or decrease (Sullivan *et al*., 2006) mycorrhizal densities. Despite these limitations, CLCPs of the extractable soil microflora are commonly used as approximate indicators of microbial functional diversity (Bradley *et al*., 2006). Although CLCPs do not identify specific taxonomic groups, differences in the metabolic capacities of different microbial sub-communities imply differences in their underlying composition. Contrary to IF-amended soils, CLCPs of OF-amended soils were distinct from the control treatment. Among OF treatments, both PCA and PERMANOVA showed that microbial sub-communities in plots amended with either 100% PBs or 100% LPS differed the most from each other, a reflection of the differences in their respective chemical qualities. It is important to note that CLCPs were assessed in the rooting zone of the mineral soil, whereas OFs were surface applied. Our study suggests, therefore, that surface-applied OFs not only increased biological activity and nutrient turnover in the underlying rooting zone, but also induced fundamental changes to the structure and diversity of the soil microbiota. This could be confirmed with molecular techniques such as T-RFLP, DGGE or PLFA that describe the genetic and taxonomic diversities of soil microbial communities. Future research should strive to determine whether OF-induced changes in microbial community structure confer other ecological advantages such as a greater stability of dynamic soil processes. Indices of microbial resistance and resilience to environmental stress, such as those developed by Orwin & Wardle (2004), could be used for this purpose.

In evaluating the economic benefits of OF mixtures to improve soil quality and growth of hybrid poplar within a 'green economy', it will be important to address a wide range of environmental concerns. On the one hand, hybrid poplar may be used as a bio-energy crop that would offset CO_2 emissions from fossil fuels. Converting agricultural fields to tree plantations may also increase soil C sequestration thereby reducing atmospheric CO_2 (Lal, 2004; Adler *et al*., 2007). However, the use of OF mixtures in hybrid poplar plantations cannot be considered an environmental panacea. In the province of Quebec, manure application guidelines are based on surface soil P saturation levels which determine the eutrophication potential of waterways subjected to surface run-off from fertilized fields (CRAAQ, 2003). High LPS application can also result in leaching losses of NO_3-N to groundwater systems which is hazardous for human consumption. A third concern is the expected increase in denitrification following the application of PBs and LPS (Rochette *et al*., 2001). Our forthcoming paper will specifically address this latter point.

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References

Adler, P.R., Del Grosso, S.J. & Parton, W.J. 2007. Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. *Ecological Applications*, **17,** 675–691.

Alban, D.H. 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. *Soil Science Society of America Journal*, **46,** 853–861.

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26,** 32–46.

Anderson, M.J. 2004. *DISTLM v.5: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model*. Department of Statistics, University of Auckland, New Zealand

Anderson, J.P.E. & Domsch, K.H. 1978. A physiological method for the quantitive measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, **10,** 215–221.

Barbarick, K.A., Doxtader, K.G., Redente, E.F. & Brobst, R.B. 2004. Biosolids effects on microbial activity in shrubland and grassland soils. *Soil Science*, **169,** 176–187.

Boysen, B. & Strobl, S. 1991. *A grower's guide to hybrid poplar*. Ontario Ministry of Natural Resources, Toronto, Canada.

Bradley, R.L. & Fyles, J.W. 1995a. A kinetic parameter describing soil available carbon and its relationship to rate increase in C mineralization. *Soil Biology & Biochemistry*, **27,** 167–172.

Bradley, R.L. & Fyles, J.W. 1995b. Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil nutrients. *Soil Biology & Biochemistry*, **27,** 1565–1571.

Bradley, R.L., Shipley, B. & Beaulieu, C. 2006. Refining numerical approaches for analyzing soil microbial community catabolic profiles based on carbon source utilization patterns. *Soil Biology & Biochemistry*, **38,** 629–632.

Camberato, J.J., Gagnon, B., Angers, D.A., Chantigny, M.H. & Pan, W.L. 2006. Pulp and papermill by-products as soil amendments and plant nutrient sources. *Canadian Journal of Soil Science*, **86,** 641–653.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. & Potts, J.M. 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology*, **69,** 3593–3599.

Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ). 2003. *Guide de référence en fertilisation*, 1st edn. Centre de référence en agriculture et agroalimentaire du Québec, Ste Foy, QC.

Chantigny, M.H., Angers, D.A. & Beauchamp, C.J. 1999. Aggregation and organic matter decomposition in soils amended with deinking paper sludge. *Soil Science Society of America Journal*, **63,** 1214–1221.

Chantigny, M.H., Rochette, P., Angers, D.A., Massé, D. & Côté, D. 2004. Ammonia volatilization and selected soil characteristics following application of anaerobically digested pig slurry. *Soil Science Society of America Journal*, **68,** 306–312.

Conseil des production végétales du Québec (CPVQ). 2000. Fertilizer recommendations. *Conseil des productions végétales du Québec*. AGDEX 540, Québec, Canada.

Degens, B.P. & Harris, J.A. 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology & Biochemistry*, **29,** 1309–320.

Désilets, L. 2000. Promotion of paper-mill residues: report on the situation and outlook. In: *Proceedings of a Conference on Biosolids ''Biosolids: enriching the soil'' held in Montebello, Québec, Canada*, pp. 7–19. Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ), Ste Foy, QC.

DesRochers, A., van den Driessche, R. & Thomas, B.R. 2006. NPK fertilization at planting of three hybrid poplar clones in the boreal region of Alberta. *Forest Ecology and Management*, **232,** 216–225.

Dickmann, D.I., Isebrands, J.G., Blake, T.J., Kosola, K. & Kort, J. 2001. Physiological ecology of poplars. In: *Poplar culture in North America* (eds D.I. Dickmann, J.G. Isebrands, J.E. Eckenwalder & J. Richardson), pp. 77–118. NRC Research Press, Ottawa, ON.

Entry, J.A., Reeves, D.W., Backman, C.B. & Raper, R.L. 1996. Influence of wheel traffic and tillage on microbial biomass, residue decomposition and extractable nutrients in a coastal plain soil. *Plant and Soil*, **180,** 129–137.

Environment Canada. 2006. Climate data online. *Environment Canada's World Wide [Online]*. Available at: http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricDataStations_e.html, accessed 10/07/2007.

Food and Agriculture Organization (FAO). 2004. *The contribution of poplars and willows to sustainable forestry and rural development*. Santiago, Chile, 29 November–2 December, 2004. IPC, Forest Resources Division, FAO, Rome. Available at: http://www.fao.org/forestry/ipc2004, accessed 10/07/2007.

Fyles, J.W., Fyles, I.H. & Feller, M.C. 1990. Comparison of nitrogen mineralization in forest floor materials using aerobic and anaerobic incubation and bioassay techniques. *Canadian Journal of Soil Science*, **70,** 73–81.

Hébert, M. & Gagné, G. 2003. *Guide de référence en fertilisation*, 1st edn. Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ), Ste Foy, QC.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science*, **304,** 1623–1627.

Lamarche, J., Bradley, R.L., Pare, D., Legare, S. & Bergeron, Y. 2004. Soil parent material may control forest floor properties more than stand type or stand age in mixedwood boreal forests. *Écoscience*, **11,** 228–237.

McArdle, B.H. & Anderson, M.J. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82,** 290–297.

Muyzer, G. 1998. Structure, function and dynamics of microbial communities: the molecular biological approach. *Advances in Molecular Ecology*, **306,** 87–117.

Orwin, K.H. & Wardle, D.A. 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. *Soil Biology & Biochemistry*, **36,** 1907–1912.

Paavolainen, L. & Smolander, A. 1998. Nitrification and denitrification in soil from a clear-cut Norway Spruce (Picea abies) stand. *Soil Biology & Biochemistry*, **30,** 775–781.

Rochette, P., Chantigny, M.H., Angers, D.A., Bertrand, N. & Côté, D. 2001. Ammonia volatilization and soil nitrogen dynamics following fall application of pig slurry on canola crop residues. *Canadian Journal of Soil Science*, **81,** 515–523.

Sánchez, M. & González., J.L. 2005. The fertilizer value of pig slurry. I. Values depending on the type of operation. *Bioresource Technology*, **96,** 1117–1123.

Sardans, S., Peñuelas, J. & Rodà, F. 2005. Changes in nutrient use efficiency, status and retranslocation in young post-fire regeneration *Pinus halepensis* in response to sudden N and P

input, irrigation and removal of competing vegetation. *Trees*, **19,** 233–250.

SAS Institute Inc. 2003. *SAS 9.1 – The power to know*. SAS Institute Inc., Cary, NC.

Sieling, K., Schroder, H., Finck, M. & Hanus, H. 1998. Yield, N uptake, and apparent N-use efficiency of winter wheat and winter barley grown in different cropping systems. *Journal of Agricultural Science*, **131,** 375–387.

Smalla, K., Wachtendorf, U., Heuer, H., Liu, W. & Forney, L. 1998. Analysis of BIOLOG GN substrate utilization patterns by microbial communities. *Applied and Environmental Microbiology*, **64,** 1220–1225.

Soil Classification Working Group. 1998. *The Canadian system of soil classification*, 3rd edn. Agriculture and Agri-food Canada, Publication 1646, Ottawa, Canada.

Sullivan, T.S., Stromberger, M.E. & Paschke, M.W. 2006. Parallel shifts in plant and soil microbial communities in response to biosolids in a semi-arid grassland. *Soil Biology & Biochemistry*, **38,** 449–459.

Timmer, V.R. 1985. Response of a hybrid poplar clone to soil acidification and liming. *Canadian Journal of Soil Science*, **65,** 727–736.

US Environmental Protection Agency. 1999. *Biological aspects of hybrid poplar cultivation on floodplains in western North America – a review*. Prepared by J.H. Braatne. US EPA Document No. 910-R-99-002. US Environmental Protection Agency, Seattle, WA.

Van Miegroet, H., Norby, R.J. & Tschaplinski, T.J. 1994. Nitrogen fertilization strategies in a short-rotation sycamore plantation. *Forest Ecology and Management*, **64,** 13–24.

Zak, J.C., Willig, M.R., Moorhead, D.L. & Wildman, H.G. 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biology & Biochemistry*, **26,** 1101–1108.