JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Short-Term Effects of Deinking Paper Sludge on the Dynamics of Soil Carbon, Nitrogen, and Phenolic Compounds

Y. MACHRAFI, F.-P. CHALIFOUR, J. WAMEGNI, AND C. J. BEAUCHAMP*

Département de Phytologie, Faculté des Sciences de l'Agriculture et de l'Alimentation, Pavillon Paul-Comtois, 2425 rue de l'Agriculture, Université Laval, Québec, QC, Canada G1V 0A6

Applications of deinking paper sludge (DPS) decreased the establishment of some crops, indicating that it may have inhibiting effects. The effects of soil-applied DPS on total carbon (C), nitrogen (N), C:N ratio, and nitrate, ammonium, and phenolic compounds were studied for 2 years. The phytotoxicity of simulated phenolic solutions of raw DPS and DPS-amended soil was investigated. Twelve phenolic compounds were quantified in raw DPS. Vanillin and 3-hydroxy-4-methoxycinnamic acids increased with DPS applications in amended soil for both years. Total soil C and the C:N ratio increased with DPS applications, while nitrate soil content decreased. Germination indices were affected differently by the phenolic compound solution that simulated DPS. This study highlights the lack of availability of nitrate as the main factor involved in the inhibiting effect of DPS. However, other inhibiting effects of phenolic compounds cannot be ruled out since they are known to inhibit nitrification and to trap nitrate into organic N compounds.

KEYWORDS: Phytotoxicity; paper waste; phenolic acids; nitrate; ammonium; *Lepidium sativum*; *Glycine max*; *Zea mays*

INTRODUCTION

The deinking process produces a waste byproduct, deinking paper sludge (DPS), which contains mainly paper fibers (lignin, cellulose, etc.), clay particles, and inks (*1*). Several million tons of pulp and paper waste are generated each year in North America and are available for soil amendments. DPS is low in toxic products (i.e., dioxins and furans) and trace elements (*2*) and meets Quebec quality standards for the beneficial use of fertilizing residues (*3*). However, the high carbon (C):nitrogen (N) ratio of DPS (ca. 300; *2*) can limit its use as compared with combined paper sludge, which has a lower C:N ratio. DPS often requires an exogenous N source to decrease its C:N ratio for good plant growth (*4, 5*). In a field study that evaluated the effects of DPS application on dinitrogen (N_2)-fixing legume crops (*6*), establishment differences were noted for alfalfa (*Medigago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) as compared with sweetclover (*Melilotus officinalis* L.) and red clover (*Trifolium pratense* L.); the latter two species were unaffected. Also, initial establishment of bromegrass (*Bromus inermis* L.) was suppressed with high levels of DPS applications (*6*). In the present study, we observed that weed growth appeared to be suppressed during establishment of forage species. The reasons for these differences are not clear. One hypothesis is that DPS, with its high C:N ratio, caused N immobilization and suppressed the growth of some plant species. However, differences among the legume crops suggest that DPS may have inhibiting effects on plants.

In Canada, wood is the main raw material used in paper manufacturing. Previous studies have identified water extractive compounds, including phenolic compounds, in wood and their wastes (*7, 8*). In wood-derived wastes used in soil amendments, we can assume the presence of some phenolic compounds and degraded byproducts; these substances are derived, in part, from the degradation of lignin, which is the second most abundant compound of wood (*8*−*10*). This biodegradation occurs when wood waste comes in contact with microorganisms present in the environment. Many studies report that phenolic compounds affect plant growth by altering the uptake and transport of ions, thereby reducing chlorophyll content, protein synthesis, enzymatic activity, respiration, and the water ratio (*8*−*11*).

For the most part, differences in legume establishment and the growth suppression of some plant species with DPS application are not well-understood. The objectives of this study were to (1) study the short-term dynamics of C, N, C:N ratio, nitrate, and ammonium in soil following three rates of applied DPS [0 (control), 8, or 16 Mg C ha^{-1}] without additional N; (2) characterize the phenolic compound profile of DPS and the dynamics during decomposition of DPS byproduct in DPS-amended soil; and (3) investigate the toxicity of a simulated solution of phenolic compounds contained in DPS, and in DPS-amended soils, on the germination index of several crops.

* To whom correspondence should be addressed. Tel: 418-656-2131ext. 7349. Fax: 418-656-7856. E-mail: Chantal.Beauchamp@fsaa.ulaval.ca.

10.1021/jf801443a CCC: $40.75
Published on Web 11/13/2008

MATERIALS AND METHODS

Site Management and Soil Sampling. The experiment was conducted in 1999 and 2000 at two different field locations previously not amended with DPS. Both sites were at St.-Augustin-de-Desmaures agronomic research station of Laval University (Quebec City, Quebec, Canada) (46° 38′ 09″ N, 71° 48′ 56″ W). The soil was well-drained, and its texture was classified as Tilly sandy loam (52 ± 5% sand, 30 ± 4% silt, 14 ± 2% clay, 4.1% organic matter, and pH 5.9). Because no crop was established, no fertilization was applied. The experimental design was a split-plot with three replicated blocks assigning DPS applications factor to main plots and sampling dates to subplots. Each year, DPS (Papiers White Birch Division Stadacona, Quebec City, Quebec, Canada) was spread uniformly on the soil surface of new plots and incorporated within the upper 0−15 cm of soil, applied at 0 (control), 8, or 16 Mg C ha^{-1}. The main plot size was 4.5 m × 6 m. A buffer zone of 0.5 m at the edge of the main plot was created before the main plot was divided in eight subplots of 1.25 m × 1.75 m. Sampling started immediately after the soil amendment was applied (week 0), at 1 week intervals for the next 4 weeks (weeks 1, 2, 3, and 4), and at 2 week intervals until week 8. Sampling started on June 17 in 1999, and 630 crop heat units (CHU) had already been accumulated. In 2000, sampling started on June 5, and 250 CHU had already been accumulated. Soil cores were collected to a depth of 15 cm using metal cores (8 cm diameter × 10 cm long). Soil samples were sieved to pass through a 2 mm screen, stored in plastic bags, and frozen prior to laboratory processing and analyses.

Soil Carbon and Nitrogen. The soil samples were dried at 65 °C for 24 h and ground at 0.12 mm (Retsch Ultracentrifugal Mill, Brinkmann Instruments Canada, Ltd., Rexdale, Ontario). Subsequently, total organic C and total N were determined by dry combustion (CNS-1000, Leco; St. Joseph, Michigan). To extract nitrate and ammonium, 5 g of soil was added to 50 mL of 2 M KCl and agitated for 30 min on a reciprocating shaker (Eberbach Corp., Ann Arbor, Michigan). Subsequently, the solutions were filtered through Whatman #42 paper filter. The nitrate extracts were diluted 10 times prior to high-performance liquid chromatography (HPLC) quantification. The ion chromatograph used was a Dionex DX 500 (Dionex Corp., Sunnyvale, CA). Chromatography was carried out using an ionpac cation-exchange CS5 (10−32, 4 mm) column and CG5 (10−32, 4 mm) guard column. Nitrate was isocratically eluted with a 35 mM KCl solution at 1 mL min^{-1} flow rate and characterized by its UV absorbance at 215 nm. Nitrate was quantified by Peaknet software Release 4.30, using KNO_3 as a standard, and reported as mg NO_3^- kg^{-1} soil. Ammonium nitrogen was quantified by a colorimetric method (*12*). Briefly, 1.5 mL of buffer reagent (14.25 g of Na_2HPO_4, 50.7 g of $KNaC_4H_4O_6 \cdot 4H_2O$, 54 g of NaOH in 1000 mL of deionized water) and 0.4 mL of sodium salicylate nitroprusside reagent were added to 0.5 mL of each filtered solution. Mixtures were kept in a water bath at 25 °C for 10 min, mixed with 0.2 mL of 1.2% sodium hypochlorite, and incubated for another 30 min at 25 °C. Finally, the absorbance was measured with a spectrophotometer (Pharmacia Biotech Ultrospec 3000 UV−visible spectrophotometer, Cambridge, United Kingdom) at 645 nm.

Phenolic Compound Extraction and Characterization. The extraction method was described in Machrafi et al. (*8*). Briefly, for raw DPS, 20 g of DPS [wet basis (w.b.)] was suspended in 100 mL of 0.1 M NaOH and agitated for 16 h at room temperature on a reciprocal shaker (Eberbach Corp., Ann Arbor, Michigan). For DPS-amended soil, 50 g of soil (w.b.) was used. All suspensions were centrifuged, and the supernatants were filtered. The filtrates were acidified overnight at 4 °C with 1 M HCl to pH 2.5, centrifuged, and filtrated. The supernatants were washed three times with 10 mL of ethyl acetate for 5 min, and the resulting 30 mL pooled solution was evaporated to dryness. Solids were dissolved in 2 mL of 50% methanol and kept in darkness at −4 °C until undergoing chromatography.

Phenolic compounds were identified by injecting a 10 μL aliquot into a Dionex DX 500 chromatograph equipped with an AS40 Automated Sampler, a 10 μL valve loop injector, a Dionex GP40 Gradient Pump, and an AD20 absorbance detector. Chromatography was carried out using a DuPont Zorbax ODS (4.6 mm × 25 cm) column and a Zorbax guard column. Peaknet software Release 4.30 was used for data acquisition and processing. The chromatographic conditions were similar to the ones described in Machrafi et al. (*8*).

On the basis of a literature review (*9*) and previous observations on wood residues (*8*), 15 phenolic compounds were selected as standards to calibrate the HPLC. However, four unknown peaks were observed. Two major peaks were isolated and identified by gas chromatography/mass spectrometry (GC/MS) analyses as terephthalic and isophtalic acids, but two minor peaks remained unknown since there was not enough material to isolate and identify them. The standard curve was determined with high-purity grade standards (Sigma Co., St. Louis, MO) and included gallic acid, protocatechuic acid, catechol, hydroxybenzoic acid, vanillic acid, caffeic acid, terephthalic acid, vanillin, isophtalic acid, *p*-coumaric acid, ferulic acid, 3-hydroxy-4methoxycinnamic acid (thereafter hydroxycinnamic acid), benzoic acid, salicylic acid, and *trans*-cinnamic acid. Quantification was based on peak areas as determined by Peaknet software Release 4.30 using external standards. These values, in μg g^{-1}, were transformed using the following formula:

$$\text{value } (\mu\text{mol L}^{-1}) = [\text{value } (\text{mg kg}^{-1}) \times \text{sample dry bulk density } (\text{g cm}^{-3}) \times 10^3]/\text{molecular weight}$$

where the dry bulk densities of soil and DPS were 0.81 and 0.21 g cm^{-3}, respectively. The mean and standard error of each phenolic compound were calculated; the total phenolic compound content represents the sum of the mean quantity of the measured phenolic compounds.

Phytotoxicity Tests. The germination index is the most widely used phytotoxicity test because of its simplicity. It involves seed germination and seedling growth, mainly due to their ecological relevance and involvement in several physiological processes. In laboratory bioassays, we assessed the phenolic compounds found in DPS and from soil amended with the highest level of DPS application (16 Mg C ha^{-1}) for their inhibiting effects on seed germination and the rootlet length of four dicotyledonous and three monocotyledonous species. Our simulated solutions contained the mean of each individual phenolic compound found in DPS and DPS-amended soil at 16 Mg C ha^{-1} in 1999 (**Table 1**). The bioassay test seeds were cress (*Lepidium sativum*), a reference species (*8*) for non-N_2-fixing dicotyledonous species; alfalfa, birdsfoot trefoil, and soybean (*Glycine max* [L.] Merr.) for N_2-fixing dicotyledonous species; and bromegrass, corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.) for monocotyledonous species.

For each species, 25 seeds were placed on three sterile filter papers at the bottom of a Petri dish and impregnated with 6 mL of phenol compound solution (pH 7) or distilled water (control; pH 7). The selected pH for this phytotoxicity test was based on the soil field pH following DPS amendment. Five replicates of each treatment were incubated in a dark germination chamber at 27 °C; 48 h for bromegrass, 36 h for birdsfoot trefoil, and 24 h for the other species. The response effects to water, soil:DPS, and DPS-simulated solutions was determined by counting the number of germinated seeds and measuring the rootlet length. The germination index was calculated by multiplying the percentage of germination by the average rootlet length (*8*).

Statistical Analyses. For the field soil experiment with three replicates, a split-plot analysis was followed. The main treatment was DPS application, and the split factor was sampling date. Bartlett's test assessed the homogeneity of the variances (*13*). Because all variances were homogeneous, analyses of variances were carried out using the general linear models (GLM) procedure in SAS Release 8.02 (*14*), and the treatment effects were partitioned into single degree of freedom contrasts. The interpretation of results was mainly based on linear and quadratic components of DPS applications, when interactions over time (i.e., sampling date) were not significant, and the linear and quadratic components of the times when the interaction over DPS rates was not significant (i.e., total C, total N, C:N ratio, nitrate in 2000, total phenolic compounds, and individual phenolic compounds). However, when the joint interaction was significant for DPS application and sampling date, the interactive contrasts were discussed (i.e., nitrate in 1999).

Table 1. Phenolic Compounds Present in DPS and Soil Amended at Three Application Rates of DPS

	concentration (mg kg^{-1} dry DPS) ± standard error of mean[a]							
	1999				2000			
		applied DPS				applied DPS		
phenolic compound	DPS	0 Mg C ha^{-1}	8 Mg C ha^{-1}	16 Mg C ha^{-1}	DPS	0 Mg C ha^{-1}	8 Mg C ha^{-1}	16 Mg C ha^{-1}
gallic acid	ND	ND	ND	ND	ND	ND	ND	ND
protocatechuic acid	ND	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	ND	0.08 ± 0.01	0.09 ± 0.01	0.14 ± 0.01
catechol	ND	1.62 ± 0.37	1.69 ± 0.23	1.60 ± 0.33	ND	ND	ND	ND
hydroxybenzoic acid	15.2 ± 1.7	0.21 ± 0.03	0.26 ± 0.03	0.23 ± 0.03	6.3 ± 0.6	0.31 ± 0.04	0.37 ± 0.06	0.39 ± 0.06
vanillic acid	15.4 ± 0.6	0.31 ± 0.05	0.43 ± 0.07	0.31 ± 0.04	10.2 ± 1.7	0.47 ± 0.08	0.60 ± 0.10	0.70 ± 0.11
caffeic acid	3.2 ± 0.8	0.41 ± 0.15	0.41 ± 0.09	0.59 ± 0.10	2.7 ± 0.3	0.04 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
terephthalic acid	29.0 ± 7.9	ND	ND	ND	49.6 ± 1.3	ND	ND	ND
vanillin	34.8 ± 9.3	0.40 ± 0.06	0.90 ± 0.08	1.17 ± 0.11	24.6 ± 2.8	0.28 ± 0.06	0.33 ± 0.07	0.47 ± 0.09
isophthalic acid	89.4 ± 19.7	ND	ND	ND	100.7 ± 15.7	ND	ND	ND
p-coumaric acid	16.8 ± 6.1	0.62 ± 0.07	0.85 ± 0.08	0.95 ± 0.07	6.7 ± 1.2	0.40 ± 0.10	0.58 ± 0.12	0.69 ± 0.14
ferulic acid	5.7 ± 0.7	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	2.2 ± 0.7	0.15 ± 0.04	0.22 ± 0.05	0.25 ± 0.06
hydroxycinnamic acid	1.6 ± 0.3	0.01 ± 0.00	0.03 ± 0.01	0.05 ± 0.01	1.6 ± 0.5	0.02 ± 0.00	0.04 ± 0.01	0.06 ± 0.02
benzoic acid	28.0 ± 10.2	0.19 ± 0.02	0.27 ± 0.03	0.32 ± 0.05	39.2 ± 4.8	0.33 ± 0.04	0.42 ± 0.05	0.41 ± 0.05
salicylic acid	14.5 ± 13.9	0.42 ± 0.04	0.42 ± 0.03	0.35 ± 0.05	6.9 ± 8.1	0.60 ± 0.15	0.56 ± 0.12	0.69 ± 0.17
trans-cinnamic acid	0.6 ± 0.2	0.03 ± 0.00	0.04 ± 0.01	0.16 ± 0.10	1.1 ± 0.5	0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.00
total phenolic compounds	254.2 ± 72.7	4.47 ± 0.54	5.56 ± 0.38	5.99 ± 0.42	251.8 ± 38.2	2.79 ± 0.34	3.31 ± 0.45	3.87 ± 0.56

[a] Mean of three replicates.

For the phytotoxicity tests, the analysis followed a randomized design with three treatments [water control, simulated solution of phenolic compounds found in soil amended at the highest DPS application (16 Mg C ha^{-1}), and simulated solution of phenolic compounds found in DPS] and four replicates. The analyses of variances were carried out using the GLM procedure in SAS Release 8.02 (*14*), followed by LSD tests to compare treatments.

Statistical significance was assumed at $P = 0.05$. Effects significant at this level are declared without the term "significant". However, this term is used to say the effects are "not significantly different" whenever appropriate.

RESULTS

Soil Carbon and Nitrogen. In 1999 and 2000, total soil C was affected by DPS application; no significant effects of application × sampling date or sampling date alone were noted. Total soil C increased linearly with DPS application ($P < 0.01$; **Figure 1**). The means of total soil C averaged over sampling dates were 1.86 ± 0.10% at 0 Mg C ha^{-1} (i.e., control) to 4.61 ± 0.8% at 16 Mg C ha^{-1} in 1999 and 2.16 ± 0.26% at 0 Mg C ha^{-1} to 4.24 ± 1.54% at 16 Mg C ha^{-1} in 2000.

For both years, total soil N was not affected by DPS applications, sampling date, or their interaction. On average, total soil N was 0.24 ± 0.02 and 0.20 ± 0.02% in 1999 and 2000, respectively. The C:N ratio increased linearly with DPS application ($P < 0.01$; **Figure 1**), but there were no significant effects from DPS × sampling date or sampling date alone. The mean soil C:N ratios averaged over sampling dates were 8 ± 1 and 11 ± 1% at 0 Mg C ha^{-1} and 20 ± 4 and 22 ± 6% at 16 Mg C ha^{-1}, in 1999 and 2000, respectively.

In 1999, soil nitrate was affected by DPS application, sampling date, and their interaction, whereas in 2000, soil nitrate was only affected by DPS application. Generally, soil nitrate decreased linearly with DPS applications; at the highest level of application, soil nitrate content decreased by 50 and 80% for the first and second years, respectively (**Figure 1**). Thus, nitrate means averaged over sampling dates were 66 ± 7 and 120 ± 7 mg kg^{-1} soil (d.w.) at 0 Mg C ha^{-1} and 31 ± 6 and 26 ± 3 mg kg^{-1} soil (d.w.) at 16 Mg C ha^{-1} in 1999 and 2000, respectively. In 1999, however, the magnitude of soil nitrate content differed over DPS application and sampling date (linear DPS application × linear sampling date ($P < 0.01$). At week 0, regardless of DPS application, soil nitrate content was approximately 56 ± 5 mg kg^{-1} soil (d.w.). However, at week 8, soil nitrate content increased to 125 ± 18 mg kg^{-1} soil (d.w.) at 0 Mg C ha^{-1} but decreased in amended plots to 28 ± 13 mg kg^{-1} soil (d.w.).

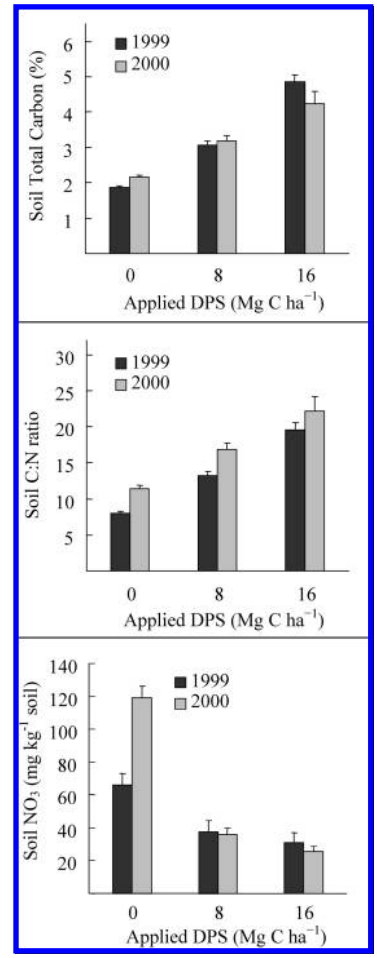
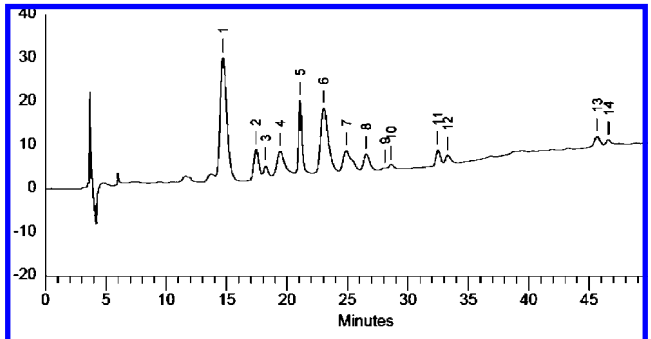


Figure 1. Total carbon, carbon:nitrogen ratio, and nitrate content in control soil (0 Mg C ha^{-1}) and soils amended with DPS (8 or 16 Mg C ha^{-1}) in 1999 and 2000. Means of three replicates ± standard errors of the mean.



Peak n°	Name
1	Hydroxybenzoic acid
2	Vanillic acid
3	Caffeic acid
4	Terephthalic acid
5	Vanillin
6	Isophtalic acid
7	*p*-Coumaric acid
8	Ferulic acid
9	Hydroxycinnamic acid
10	Benzoic acid
11	Unknown
12	Salicylic acid
13	Unknown
14	*trans*-Cinnamic acid

Figure 2. Chromatogram of DPS phenolic compounds.

The soil ammonium content was not affected by DPS application, sampling date, or their interaction. In 1999, the ammonium content was 74 ± 7 mg kg^{-1} soil (d.w.), whereas in 2000, it was 71 ± 7 mg kg^{-1} soil (d.w.).

DPS Phenolic Compound Characterization. Twelve phenolic compounds were identified in DPS while two minor peaks were not identified (**Figure 2** and **Table 1**). On a concentration basis, the most important phenolic compounds identified were terephthalic acid, isophtalic acid, vanillin, and benzoic acid. The content of these phenolic compounds ranged from 28 to 89 mg kg^{-1} dry DPS in 1999 and from 25 to 101 mg kg^{-1} dry DPS in 2000. Vanillic, *p*-coumaric, salicylic, and hydroxybenzoic acids were present at intermediate levels, from 15 to 16 mg kg^{-1} dry DPS in 1999 and from 6 to 10 mg kg^{-1} dry DPS in 2000. *trans*-Cinnamic, hydroxycinnamic, caffeic, and ferulic acids were found in DPS at less than 5 mg kg^{-1} dry DPS in both years. Total phenolic compounds, estimated as the sum of individual identified phenolic compounds, averaged about 250 mg kg^{-1} dry DPS in both years.

Soil Phenolic Compounds. Up to 12 phenolic compounds were quantified after DPS soil amendments in 1999 and 2000. In general, for both years, gallic, terephthalic, and isophthalic acids were not detected, and cathecol was detected only in 1999. No peak remained unknown (**Table 1**). The fluctuation in the concentration of phenolic compounds was mainly due to DPS application and sampling date. No interaction was found between these two factors, and the following results are the means of one main factor (DPS) averaged over the second factor (sampling date).

For DPS applications averaged over sampling date for both years, total phenolic compounds increased linearly with increasing levels of DPS application. In 1999, the overall mean phenolic compounds content averaged over sampling dates were 4.47 ± 0.54 mg kg^{-1} soil (d.w.) for the control soil, 5.56 ± 0.38 mg kg^{-1} soil (d.w.) for the soil amended with 8 Mg C ha^{-1}, and 5.99 ± 0.42 mg kg^{-1} soil (d.w.) for the soil amended with 16 Mg C ha^{-1} (**Table 1**). Similarly in 2000, overall mean phenolic compounds averaged over sampling dates were 2.79 ± 0.34 mg kg^{-1} soil (d.w.) for the control soil, 3.31 ± 0.45 mg kg^{-1} soil (d.w.) for the soil amended with 8 Mg C ha^{-1}, and 3.87 ± 0.56 mg kg^{-1} soil (d.w.) for the soil amended at 16 Mg C ha^{-1}. Among the 12 phenolic compounds identified, vanillin (both years), hydroxycinnamic acid (both years), benzoic acid (both years), *p*-coumaric acid (1999), vanillic acid (2000), and ferulic acid (2000) increased linearly with DPS application, whereas salicylic acid (1999) decreased (**Table 1**).

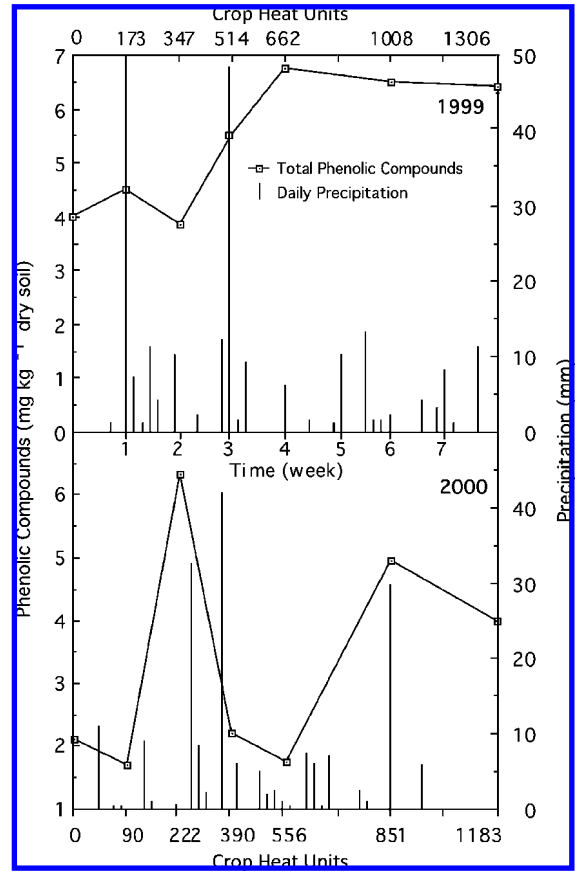


Figure 3. Precipitation and evolution of total phenolic compounds in the soil over time and cumulated crop heat units in 1999 and 2000.

For sampling dates averaged over DPS application, total phenolic compounds increased linearly over time in 1999 and 2000. Rainy conditions and lower crop heat units (CHU) (i.e., cool weather) explain sporadic decreases in total phenolic compounds in the spring, whereas warm weather conditions explain increases in the summer (**Figure 3**). In 1999, overall means of total phenolic compounds content averaged over DPS application were 3.41 ± 1.06 mg kg^{-1} soil (d.w.) at week 0 and increased to 5.71 ± 2.18 mg kg^{-1} soil (d.w.) at week 8. In 2000, they were 2.10 ± 0.34 mg kg^{-1} soil (d.w.) at week 0 and increased to 4.00 ± 0.39 mg kg^{-1} soil (d.w.) at week 8.

For both years, several individual phenolic compounds increased linearly over time. In 1999, protocatechuic acid, catechol, vanillic acid, vanillin, *p*-coumaric acid, and ferulic acid contents were, respectively, 0.03 ± 0.00, 0.66 ± 0.29, 0.29 ± 0.11, 1.00 ± 0.16, 0.50 ± 0.04, and 0.13 ± 0.01 mg kg^{-1} soil (d.w.) at week 0 and increased to 0.06 ± 0.01, 1.75 ± 0.49, 0.36 ± 0.05, 1.20 ± 0.25, 0.87 ± 0.13, and 0.25 ± 0.02 mg kg^{-1} soil (d.w.) at week 8.

Similarly in 2000, protocatechuic acid, vanillic acid, vanillin, *p*-coumaric acid, and ferulic acid contents were, respectively, 0.02 ± 0.00, 0.31 ± 0.04, 0.24 ± 0.05, 0.28 ± 0.07, and 0.08 ± 0.02 mg kg^{-1} soil (d.w.) at week 0 and increased to 0.17 ± 0.02, 0.93 ± 0.08, 0.45 ± 0.05, 0.80 ± 0.09, and 0.26 ± 0.03 mg kg^{-1} soil (d.w.) at week 8. In addition, in 2000, the hydroxybenzoic and hydroxycinnamic acid contents in soil

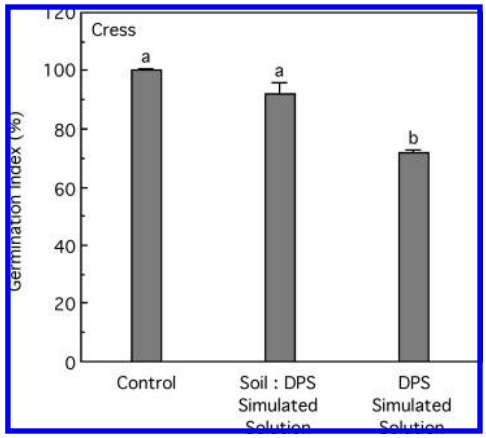


Figure 4. Germination index of cress. Means of four replicates ± standard errors of the mean. Means followed by a common letter are not significantly different at the 5% level by LSD test.

increased linearly over sampling date from 0.24 ± 0.02 and 0.01 ± 0.00 mg kg^{-1} soil (d.w.) at week 0 to 0.50 ± 0.03 and 0.06 ± 0.03 mg kg^{-1} soil (d.w.) at week 8, respectively.

Phytotoxicity Tests. For the non-N_2-fixing dicotyledonous species, cress was negatively affected by the phenolic compound solution that simulated DPS but not significantly affected by the solution that simulated DPS-amended soil (**Figure 4**). The germination index of cress decreased by about 30% as compared with the control.

For the N_2-fixing dicotyledonous species, the germination index of alfalfa was not significantly affected by the phenolic compounds solution that simulated DPS nor by the solution that simulated DPS-amended soil. The germination index of birdsfoot trefoil and soybean was negatively affected only by the phenolic compounds solution that simulated DPS (**Figure 5**). The germination indices for these species decreased by about 45 and 35%, respectively, as compared with the control.

For the non-N_2-fixing monocotyledonous species, the germination index of bromegrass was not significantly affected by the phenolic compounds solution that simulated DPS and by the solution that simulated DPS-amended soil (**Figure 6**). For corn and wheat, the germination index decreased by about 60% as compared with the control in the presence of the phenolic compounds solution that simulated DPS but was not significantly affected by the solution that simulated DPS-amended soil.

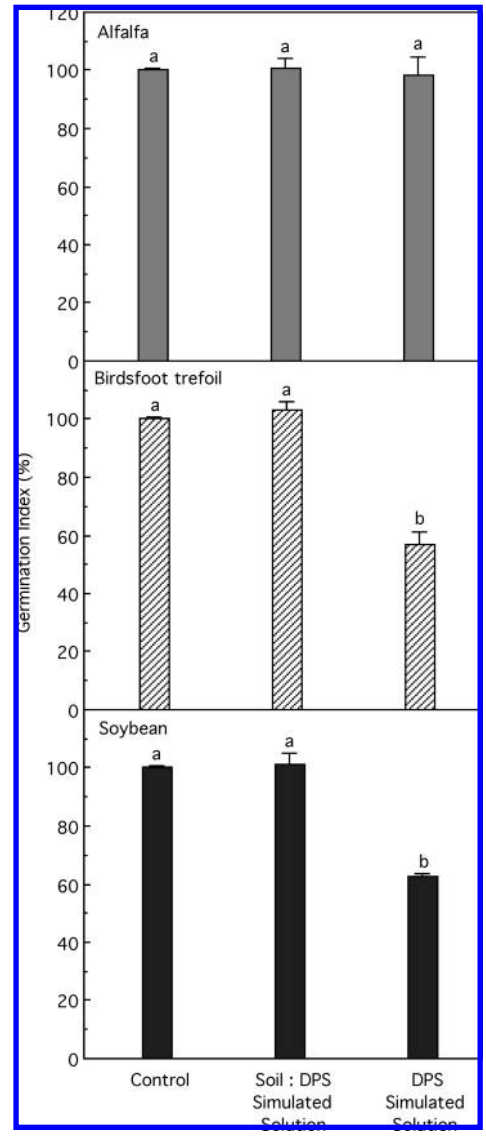


Figure 5. Germination index of three dicotyledonous species that received a water control treatment, a simulated solution of phenolic compounds found in soil amended with the highest level of DPS application (16 Mg C ha^{-1}), and a simulated solution of phenolic compounds found in DPS. Means of four replicates ± standard errors of the mean. Means followed by a common letter are not significantly different at the 5% level by LSD test.

DISCUSSION

While the impact of DPS has been studied for soil properties and crop performance, there is limited research on how DPS affects crop establishment by reducing or repressing some plant species (e.g., weeds). The present study indicates that a reduction in soil nitrate availability is the main factor responsible for reduced plant growth. This result is based on the low concentrations of the phenolic compounds present in DPS and DPS-amended soil and the general lack of phytotoxicity of a simulated solution of DPS-amended soil on germination index.

Soil Carbon and Nitrogen. In this study, the N cycle was affected by DPS application; soil nitrate decreased 2−3-fold while total N and ammonium levels remained unchanged. This indicates an increase in the proportion of organic N relative to inorganic N. Soil microbes multiply from bioavailable C in DPS and immobilize N (*15*). In the current study, we propose that the nitrification process was impaired by the immobilization of ammonium or nitrate by soil microbes, nitrosation, nitration, or by the fixation of N into the phenolic compounds of DPS and humus compounds (*9, 16*). In all cases, part of the inorganic N presumably became organically bound.

The DPS used in this study had a high C:N ratio, and in the absence of crop growth and fertilization, soil C and C:N ratio increased linearly with increasing DPS applications. The C:N ratio of DPS-amended soil was above the value of ca. 10 for soil used in agriculture. Nonetheless, soil C:N ratios in excess of 10 have been reported for other soil amendments with a high C:N ratio, including paper sludge (*17*), barks and wood wastes (*18*), and tree leaf or crop residues (*19*). Also, the C:N ratio was similar within one treatment for at least 8 weeks after soil amendment; this occurrence is common in other wastes that are rich in lignin and have a high initial C:N ratio (*20*). These results corroborate those of Zibilske (*20*) and Kirchmann and Bergström (*17*) who reported very low levels of soil inorganic N (nitrate + ammonium) after soil amendment with various paper sludge treatments. Nitrogen immobilization appears underestimated when determining plant growth inhibiting effects of soil amendments; Inderjit (*21*) shows that the phytotoxicity

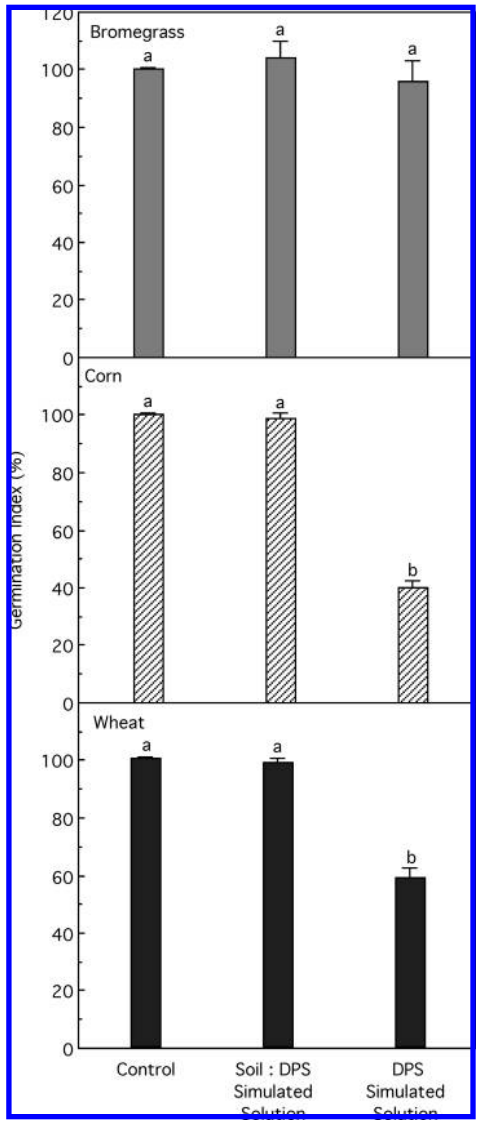


Figure 6. Germination index of the three monocotyledonous species that received a water control treatment, a simulated solution of phenolic compounds found in soil amended with the highest level of DPS application (16 Mg C ha^{-1}), and a simulated solution of phenolic compounds found in DPS. Means of four replicates ± standard errors of the mean. Means followed by a common letter are not significantly different at the 5% level by LSD test.

of leaf leachate was eliminated after crop N fertilization. Follow-up studies should evaluate how different mineral N applications can be used to prevent yield reductions in nitrate-demanding crops and determine the nitrification inhibitors of DPS.

DPS Phenolic Compounds. The sums of the mean quantity of the measured phenolic compounds gave totals that were similar from year to year. This study is the first to detect the presence of isophthalic and terephthalic acids in DPS. The presence of *m*- and *p*-xylene has been reported previously (*2*), and these molecules are used to make isophthalic acid, terephthalic acid, and dimethyl terephthalate (*22*). The presence of these solvents in DPS is likely related to printing inks; isophthalic and terephthalic acids are used in the industrial synthesis of colored polyester resins of ink. Most phthalates are also used as plasticizers, and some could originate from resin containers used to store liquids (*23*). Both molecules have no or low toxicity in aquatic and terrestrial systems; therefore, their study is a low research priority for intergovernmental organizations concerned with chemical safety (*24, 25*).

Most other phenolic compounds detected in DPS, that is, vanillin, caffeic, vanillic, hydroxybenzoic, *p*-coumaric, ferulic, hydroxycinnamic, and *trans*-cinnamic acids, have been identified in gymnosperm wood and decayed plant materials (*10*). In a previous study, our team (*8*) determined that fresh bark residue contained about 24 $\mu g\ g^{-1}$ hydroxybenzoic acid, 31 $\mu g\ g^{-1}$ vanillic acid, 7 $\mu g\ g^{-1}$ caffeic acid, 39 $\mu g\ g^{-1}$ vanillin, 80 $\mu g\ g^{-1}$ *p*-coumaric acid, 91 $\mu g\ g^{-1}$ ferulic acid, 14 $\mu g\ g^{-1}$ hydroxycinnamic acid, and 12 $\mu g\ g^{-1}$ *trans*-cinnamic acid. Similarities between the two studies regarding the composition of individual phenolic compounds may be due to the use of spruce to produce paper in Quebec. The lower phenolic compound concentration in DPS may be attributed to the paper floating and being washed during the recycling process.

Soil Phenolic Compounds. The present study provides evidence that increased phenolic compound content in soil is associated with increasing levels of DPS application. However, although DPS contained phenolic compounds, their incorporation into the soil only slightly increased soil phenolic compounds. Chou and Patrick (*26*) also report an increase in total phenolic compound content with increasing levels of corn residue incorporated into the soil. Some specific phenolic compounds that were abundant in DPS, that is, isophthalic and terephthalic acids, could not be detected in DPS-amended soil. Isophthalic acid was presumably photolyzed and biodegraded at the soil surface as it is highly mobile in soil (*24*). Terephthalic acid can be biodegraded in soil or adsorbed to soil particles where it has medium mobility (*25*). The main reason for not detecting these two phthalic acids is likely their sorption to soil particles, but photo-oxidation, volatilization, and biodegradation may also play a role. Soil sorption may also lower the recovery of other phenolic compounds since strong sorption of hydroxybenzoic and vanillic acids to soil particles has been reported (*10*).

When DPS is applied to soil, it decomposes into simple molecules or polymerizes with microbial compounds into humic substances. Changes in soil phenolic compound concentrations can be expected from the microbial decomposition action on DPS. The detection of ferulic, *p*-coumaric, and hydroxycinnamic acids can be related to DPS application since these acids are present in cell wall complexes forming cross-linkages between lignin and polysaccharides (*27*). When cell walls undergo microbial breakdown, these pools are refreshed (*28*). For example, when ferulic acid undergoes microbial decomposition the vanillin pool is refreshed. Both vanillin and benzoic acids were abundant in DPS. The microbial pathway of vanillin decomposition involves its transformation to vanillic acid to protocatechuic acid before ring fission, whereas benzoic acid decomposition involves its transformation to hydroxybenzoic acid and to cathecol before ring fission (*28*). These molecules were possibly degraded by soil microorganisms, and their pools were exhausted rapidly, explaining the small increase in total phenolic compounds.

In addition, variation of phenolic compounds content from one sampling date to another may be explained by weather conditions. Blum et al. (*29*) report decreased phenolic compound content after rain; in this study, cumulative precipitation of about 80−90 mm of rain, within 1 week, combined with cool weather decreased the total amount of phenolic compounds. In contrast, when rainfall was combined with warm weather (about 300 CHU within a 2 week period), microbial DPS decomposition appeared optimal and the phenolic compound pool was refreshed. The lower CHU in 2000 could explain the lower total

phenolic compounds that year. Rice (*10*) reports that nonmicrobial decomposition may relate to photo-oxidation (*9*) of phenolic compounds or volatilization.

Phytotoxicity Test. Although DPS contained phenolic compounds, after soil amendment, their content in simulated phenolic compound solution was low, with a mean of 5×10^{-6} M, well under the minimal phytotoxicity threshold for most crops. The phenolic compound solution that simulated DPS phenolic compounds, with a mean of 3.5×10^{-4} M, had a phytotoxic effect on several crops, but sensitivity varied. Germination tests showed the typical phytotoxic behavior observed in the test plant species, that is, inhibition at high concentrations and no effect at low concentrations.

In the dicotyledonous group, germination indices were affected differently by the solution that simulated DPS extract but not by the solution that simulated DPS-amended soil. Indeed, cress was negatively affected by the simulated DPS extract, a response similar to one obtained with a simulated phenolic compound solution for fresh barks (*8*). Similarly, for the dicotyledonous legumes group, the solution that simulated the phenolic compounds in DPS had a negative effect on the germination index of birdsfoot trefoil and soybean. Alfalfa appeared to be more tolerant as compared with the other crops. However, under field conditions, our team (*6*) reported reduced alfalfa establishment with high levels of DPS application; cold environmental conditions in May could have decreased DPS decomposition and increased the half-life of phenolic compounds.

Birdsfoot trefoil was the most sensitive species studied in the dicotyledonous legumes group. Phytotoxicity was observed at 3.5×10^{-4} M, which is higher than in a previous report for bark extracted with hot water and 0.1 M NaOH. (*8*). This difference could be due to variation in the concentration of each individual phenolic compound, but a more likely explanation is that the solutions in the current study were adjusted to a pH of about 7. Establishment, dry matter yield, and N uptake of birdsfoot trefoil have reportedly been reduced by high levels of DPS application (*6*). Some phenolic compounds may be partly responsible for these results considering that environmental conditions may alter the composition of phenol compounds after DPS soil amendment.

Finally, for soybean, our results agree with those of Baziramakenga et al. (*11*); shoot and root biomass of soybean seedlings were reduced following the application of phenolic compounds at 10^{-4} M. Phenolic compounds may interfere with root meristimatic processes, resulting in impaired cell division. Phenolic compounds may also affect plant growth through alteration of hormonal balance, membrane permeability, wall extension, and enzymatic activity (*10*).

For the monocotyledonous group, the germination index of corn and wheat decreased with the phenolic compound solution that simulated DPS while the solution that simulated DPS-amended soil had no effect. The results for corn agree with Janovicek et al. (*30*); vanillic, *p*-coumaric, ferulic, and *p*-hydroxybenzoic acids inhibited corn seedling radicle elongation in bioassay studies, especially at concentrations exceeding 10^{-4} M. In contrast, the germination index of bromegrass was not affected by either solution. This is in contrast with the alfalfa results (*6*), and here, phenolic compounds may not be responsible for bromegrass growth repression reported previously. Under field conditions, the lack of nitrate is likely the main factor involved, but the possibility exists that DPS remaining on the soil surface after application may block light to planted seeds, decreasing plant establishment.

ACKNOWLEDGMENT

We gratefully thank Gilles Leroux and his field team for their comments, input, and assistance throughout this study.

LITERATURE CITED

(1) NCASI (National Council of the Paper Industry for Air and Stream Improvement). Solid Waste Management and Disposal Practices in the U.S. Paper Industry; Technical Bulletin No. 641; NCASI: New York, NY, 1992.

(2) Beauchamp, C. J.; Charest, M.-H.; Gosselin, A. Examination of environmental quality of raw and composting de-inking paper sludge. *Chemosphere* **2002**, *46*, 887–895.

(3) Hébert, M. *Guidelines for the Beneficial Use of Fertilizing Residuals.* Reference Criteria and Regulatory Standards. Environdoq ENV/2004-0020 http://www.mddep.gouv.qc.ca/matieres/mat_res-en/fertilisantes/critere/guide-mrf.pdf, 2004.

(4) Fierro, A.; Angers, D. A.; Beauchamp, C. J. Restoration of ecosystem function in an abandoned sandpit: Plant and soil responses to paper de-inking sludge. *J. Appl. Ecol.* **1999**, *3*, 244–253.

(5) Filiatrault, P.; Norrie, J.; Camiré, C.; Beauchamp, C. J. Effects of de-inking paper sludge on growth and nutritional status of alder and aspen. *Res. Conserv. Recycl.* **2006**, *48*, 209–226.

(6) Allahdadi, I.; Beauchamp, C. J.; Chalifour, F.-P. Symbiotic dinitrogen fixation in forage legumes amended with high rates of de-inking paper sludge. *Agron. J.* **2004**, *96*, 956–965.

(7) U.S. Department of Agriculture. Forest Service. Bark and its Possible Uses; Research Note, FPL-091; U.S. Department of Agriculture Forest Service: Madison, Wisconsin, 1971.

(8) Machrafi, Y.; Prévost, D.; Beauchamp, C. J. Toxicity of phenolic compounds extracted from bark residues of different ages. *J. Chem. Ecol.* **2006**, *32*, 2595–2615.

(9) Siqueira, J. O.; Nair, M. G.; Hammerschmidt, R.; Safir, G. R. Significance of phenolic compounds in plant-soil-microbial systems. *Crit. Rev. Plant Sci.* **1991**, *10*, 63–121.

(10) Rice, E. L. *Alellopathy*; Academic Press: Orlando, FL, 1984; p 422.

(11) Baziramakenga, R.; Simard, R. R.; Leroux, G. D. Effects of benzoic and cinnamic acids on growth, mineral composition, and chlorophyll content of soybean. *J. Chem. Ecol.* **1994**, *20*, 2821–2833.

(12) Nkonge, C.; Ballance, G. M. A sensitive colorimetric procedure for nitrogen determination in micro Kjeldahl digests. *J. Agric. Food Chem.* **1982**, *30*, 416–424.

(13) Gomez, K. A.; Gomez, A. A. *Statistical Procedure for Agricultural Research*, 2nd ed.; John Wiley and Sons: New York, NY, 1984.

(14) SAS (Statistical Analysis System Institute). *The SAS System for Windows, Release 8.02*; SAS Institute: Cary, NC, 2001.

(15) Chantigny, M. H.; Angers, D. A.; Beauchamp, C. J. Decomposition of de-inking paper sludge in agricultural soils as characterized by carbohydrate analysis. *Soil Biol. Biochem.* **2000**, *32*, 1561–1570.

(16) Subbarao, G. V.; Ito, O.; Sahrawat, K. L.; Berry, W. L.; Nakahara, K.; Ishikawa, T.; Watanabe, T.; Suenaga, K.; Rondon, M.; Rao, I. M. Scope and strategies for regulation of nitrification in agricultural systems—Challenges and opportunities. *Crit. Rev. Plant Sci.* **2006**, *25*, 1–33.

(17) Kirchmann, H.; Bergström, L. Use of paper-mill wastes on agricultural soils: Is this a way to reduce nitrate leaching. *Soil Plant Sci.* **2003**, *53*, 56–63.

(18) Duryea, M. L.; English, R. J.; Hermansen, L. A. A Comparison of landscape mulches: Chemical, allelopathic, and decomposition properties. *J. Arboricult.* **1999**, *25*, 88–97.

(19) Heckman, J. R.; Kluchincki, D. Soybean nodulation and nitrogen fixation on soil amended with plant residues. *Biol. Fertil. Soils* **1995**, *20*, 284–288.

(20) Zibilske, L. M. Dynamics of nitrogen and carbon in soil during papermill sludge decomposition. *Soil Sci.* **1987**, *143*, 26–33.

(21) Inderjit. Experimental complexities in evaluating the allelopathic activities in laboratory bioassays: A case study. *Soil Biol. Biochem.* **2006**, *38*, 256−262.

(22) Ullmann, F. *Ullmann's Encyclopedia of Industrial Chemistry*; John Wiley & Sons: New York, 2006.
(23) Thiele, O. W.; Trüper, H. G. Esters of phthalic acid as artifacts in bacterial lipids. *Arch. Mikrobiol.* **1972**, *82*, 91–94.
(24) HSDB (Hazardous Substances Data Bank). Isophthalic acid. National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov, 2001.
(25) HSDB. Terephthalic Acid. National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov, 2005.
(26) Chou, C.; Patrick, Z. Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. *J. Chem. Ecol.* **1976**, *2*, 369–387.
(27) Yu, P.; McKinnon, J. J.; Christensen, D. A. Hydroxycinnamic acids and ferulic acid esterase in relation to biodegradation of complex plant cell walls. *Can. J. Anim. Sci.* **2005**, *85*, 255–267.
(28) Peng, X.; Misawa, N.; Harayama, S. Isolation and characterization of thermophilic bacilli degrading cinnamic, 4-coumaric and ferulic acids. *Appl. Environ. Microbiol.* **2003**, *69*, 1417–1427.
(29) Blum, U.; Wentworth, T. R.; Klien, K.; Worsham, A. D.; King, L. D.; Gerig, T. M.; Lyu, S.-W. Phenolic compound content of soils from wheat-no till, wheat-conventional till, and fallow-conventional till soybean cropping systems. *J. Chem. Ecol.* **1991**, *17*, 1045–1068.
(30) Janovick, K. J.; Vyn, T. J.; Voroney, R. P.; Allen, O. B. Early corn seedling growth response to phenolic compounds. *Can. J. Plant Sci.* **1997**, *77*, 391–393.

Received for review May 8, 2008. Revised manuscript received October 14, 2008. Accepted October 15, 2008. We thank the "Conseil des recherches en pêche et en agro-alimentaire du Québec (CORPAQ)" for their financial support to F.-P.C., Gilles Leroux, C.J.B., and Gilles Tremblay. This study was also supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to F.-P.C.

JF801443A