# Soil Aggregation and Biochemical Properties following the Application of Fresh and Composted Organic Amendments

**M. Bipfubusa**
**D. A. Angers***
Soils and Crops Research and Development Centre
Agriculture and Agri-Food Canada
2560 Hochelaga Blvd, Québec
QC, G1V 2J3 Canada

**A. N'Dayegamiye**
Institut de Recherche et de Développement en Agroenvironnement (IRDA)
Complexe Scientifique
2700 rue Einstein, Québec
QC, G1P 3W8 Canada

**H. Antoun**
Département des Sols et de Génie Agro-alimentaire
Faculté des Sciences de l'Agriculture et de l'Alimentation
Université Laval, Québec
QC, G1K 7P4 Canada

The aim of this study was to evaluate the effects of fresh and composted paper sludge on macroaggregate stability of a silt loam under field conditions, and to assess the possible role of carbohydrate fractions and humic substances. The treatments included fresh paper mill sludge (PMS) and its compost (CPMS) applied at a rate of 40 Mg ha$^{-1}$ with or without a mineral N fertilizer (120 kg N ha$^{-1}$), N fertilizer only (recommended rate of 160 kg N ha$^{-1}$), and an unamended control. Measurements of total and amino sugars and humic substances were made on slaking-resistant aggregates 2 yr after the last of three successive annual applications of the treatments. Compared with the treatments that received no organic amendment, the PMS and CPMS applications increased macroaggregate stability by an average of 45%. The effects of fresh vs. composted amendments on soil macroaggregates and their organic C contents were similar but differences in C composition were observed. Humic acid content of aggregates >2 mm was significantly higher (50%) with CPMS than PMS, although part of this effect could be attributed to the slightly greater C application rate with CPMS. Conversely, glucosamine content, an indicator of fungi abundance, was significantly greater following PMS than CPMS application. We concluded that microorganisms, in particular fungi, were a more important factor of stable macroaggregation in the soil amended with fresh sludge, while humic substances played a greater role in compost-amended soil. These effects were long lasting in the field since they were still noticeable 2 yr after the last application.

Abbreviations: $C_{FA}$, organic carbon of fulvic acids; $C_{HA}$, organic carbon of humic acids; $C_{UHF}$, organic carbon of unhumified fraction; CPMS, composted paper mill sludge; MNF, mineral nitrogen fertilizer; PMS, fresh mixed paper mill sludge; UHF, unhumified fraction of organic matter.

Adding organic matter, such as crop residues and other organic substrates, to soil generally results in an increase in soil aggregate stability. Soil aggregate stability is not only influenced by the quantity but also by the quality of organic matter inputs (Martin et al., 1955; Abiven et al., 2007). The potential of organic residues to improve soil aggregation is often attributed to their degree of susceptibility to microbial decomposition and, consequently, to their capacity to stimulate soil microbial growth and activity (Lynch, 1984). Soil microorganisms, particularly fungi, have a critical role in the formation and stabilization of soil aggregates by enmeshing soil particles (Tisdall and Oades, 1982). Moreover, they excrete glucidic, proteinic, and lipidic compounds (Lynch and Bragg, 1985) that can bind soil particles together. The role of bacteria is more associated with the production of extracellular polysaccharides (EPS) and their adhesion to soil particles (Lynch and Bragg, 1985).

Fungal hyphae and EPS may not persist for a long time in soil, and their effects on aggregate stability are often considered temporary (Tisdall and Oades, 1982). Consequently, it has been suggested that the improvement and maintenance of soil aggregate stability depends on the capacity of organic amendments to produce humic substances (Martens, 2000). Studies have shown significant correlations between aggregate stability and the soil content of various forms of humic substances (Chaney and Swift, 1984; Fortun et al., 1989). Piccolo and Mbagwu (1989) found that aggregate stability increased after the addition of humic substances to soil. In addition, Chaney and Swift (1984, 1986) showed that humic acids could stabilize soil aggregates under conditions where EPS were ineffective and that their binding effect was persistent.

The interactions between organic matter and soil structure are very complex, and the mechanisms that govern them are still being debated (Kay and Angers, 1999; Six et al., 2004). In a recent study, we showed the positive effects of applying mixed fresh paper mill sludge (PMS) and composted paper mill sludge (CPMS) on the formation and stabilization of soil macroaggregates (Bipfubusa et al., 2005). To gain information on the biochemical factors that control aggregate stabilization under these treatments, in this study we examined the organic

Soil Sci. Soc. Am. J. 72:160-166
doi:10.2136/sssaj2007.0055
Received 8 Feb. 2007.
*Corresponding author (angersd@agr.gc.ca).

C, total sugar, amino sugar, and humic substance contents fractions of different water-stable aggregate size classes.

## MATERIALS AND METHODS

### Site Characteristics and Experimental Design

This research was initiated in 2000 at the Research Institute for the Agri-Environment (IRDA) Experiment Station of Saint-Lambert-de-Lauzon (46°34' N, 71°13' W), near Québec City. The soil is a Le Bras loam containing 20% clay, 47% silt, and 33% sand. This soil had an initial pH of 6.2, 16.5 g C $kg^{-1}$, and 1.4 g total N $kg^{-1}$. The soil was rich in cations, with 103, 1195, and 98 mg $kg^{-1}$ of Mehlich-III extractable K, Ca, and Mg, respectively, but poor in P (50 mg $kg^{-1}$). Cropping history for the previous 3 yr was continuous silage corn (*Zea mays* L.).

The experimental design was described by Bipfubusa et al. (2005). Briefly, a complete block design with six treatments and four replicates was used. The treatments included PMS and CPMS at a rate of 40 Mg $ha^{-1}$ (wet basis) with or without a reduced rate of MNF(120 kg N $ha^{-1}$), complete MNF (160 kg N $ha^{-1}$), and an unamended, unfertilzed control. Detailed composition of the sludge is given in Bipfubusa et al. (2005). Briefly, the fresh sludge (PMS) contained 280 g dry matter $kg^{-1}$, 334 g C $kg^{-1}$ dry matter, and 11 g N $kg^{-1}$ dry matter, while the CPMS contained 400 g dry matter $kg^{-1}$, 281 g C $kg^{-1}$ dry matter, and 8 g N $kg^{-1}$ dry matter. Therefore the C application rates were 3.74 Mg C $ha^{-1}$ for the PMS and 4.49 Mg C $ha^{-1}$ for the CPMS. The PMS and CPMS were spread by hand in the spring and autumn of 2000 and in the autumn of 2001. All plots received 26 kg P $ha^{-1}$ and 83 kg K $ha^{-1}$. Inorganic mineral N ($NH_4NO_3$), superphosphate, and KCl were broadcast. Afterward, the organic amendments and mineral fertilizers were incorporated into the top 10 cm by disking. No organic amendment was added after 2002 so that the residual effect on subsequent crops could be investigated; however, all plots were treated with 120 kg N, 26 kg P, and 83 kg K $ha^{-1}$. The plots were 3 by 10 m and separated by 1-m buffer zone. The plots included four rows of silage corn at 75-cm spacing.

### Soil Sampling and Aggregate Preparation

Composite soil samples were obtained from five cores (5-cm diam.) collected from the topsoil layer (0–20 cm) of each experimental plot in October 2003, 2 yr after the last treatments. A portion was air dried and sieved to pass a 2-mm sieve, then ground to pass through a 0.15-mm sieve for organic C analysis. The other portion was prepared for aggregate analysis by separating 5- to 8-mm aggregates by dry sieving.

### Aggregate Stability

The stability of macroaggregates was determined by wet sieving (Angers et al., 2008). Briefly, 50 g of 5- to 8-mm air-dried aggregates was put on the top of a series of sieves with decreasing openings (2 and 0.25 mm). The sieves were placed in a wet-sieving apparatus and sieved in deionized water for 10 min at 30 cycles $min^{-1}$. Three water-stable aggregate size classes were obtained: large macroaggregates (2–8 mm), small macroaggregates (0.25–2 mm), and microaggregates (<0.25 mm). The microaggregates were recovered by centrifugation of the water used for sieving for 10 min at 3000 × *g*. The three fractions of aggregates were collected in a beaker and oven dried at 50°C to constant weight. The aggregate weight was corrected for the presence of sand. The stability of macroaggregates was estimated by the mean weight diameter of the water-stable aggregates, which was the sum of the mass of soil remaining in each sieve multiplied by the mean aperture of the adjacent meshes. The results were the means of two replicates. A part of the soil of each aggregate fraction was crushed to pass through a 0.15-mm sieve, and analyzed for organic C, total sugar, amino sugar, and humic substance contents.

### Organic Carbon Contents

Total C contents of the whole soil and the three water-stable aggregate size classes were determined by dry combustion with a LECO CNS-1000 (LECO Corp., St. Joseph, MI.). Total C content was considered to represent organic C because this soil does not contain carbonates.

### Humic Substance Analysis

Humic substances were extracted according to the method described by Schnitzer et al. (1981). A 10-g sample of aggregates crushed to pass through 0.15-mm sieve was placed in a 250-mL centrifuge tube containing 100 mL of 0.1 mol $L^{-1}$ NaOH and 0.1 mol $L^{-1}$ $Na_4P_2O_7 \cdot 10H_2O$. The tubes were shaken for 24 h on a reciprocating shaker, and centrifuged for 20 min at 3000 × *g*. The supernatant was decanted and centrifuged for 15 min at 15,000 × *g* again. A 25-mL aliquot of the supernatant was acidified to pH 2 with 50% $H_2SO_4$, and the humic acids (HA) were allowed to precipitate for 24 h at 4°C. The precipitated HA were separated from fulvic acids (FA) through centrifugation for 15 min at 15000 × *g*. The precipitated HA were oven dried at 45°C and redissolved in 25 mL of 0.5 mol $L^{-1}$ NaOH.

The FA were separated from unhumified organic matter fractions (UHF) by adsorption of FA onto polyvinylpyrolidone resin (Sequi et al., 1986). A 25-mL aliquot of FA extract was passed through a column containing 12 g of polyvinylpyrolidone resin (Sigma-Aldrich, Munich, Germany), previously purified and equilibrated by 0.5 mol $L^{-1}$ NaOH and 0.005 mol $L^{-1}$ $H_2SO_4$, respectively. The column was rinsed with 0.005 mol $L^{-1}$ $H_2SO_4$ to remove all UHF, and sorbed FA were eluted with 0.5 mol $L^{-1}$ NaOH.

The three fractions of humic substances (UHF, FA, and HA) were stored at 4°C until analysis. Organic C in the FA ($C_{FA}$) and HA ($C_{HA}$) was determined by the $KMnO_4$ oxidation method (Nelson and Sommers, 1982), whereas UHF organic C ($C_{UHF}$) was determined on a Technicon autoanalyzer (Technicon Instruments, Tarrytown, NY). The humification index ($C_{UHF}/(C_{HA}+C_{FA})$) and polymerization index ($C_{HA}/C_{FA}$) were calculated as suggested by Sequi et al. (1986) and Orlov (1995), respectively.

### Total Sugar Analysis

Total sugar content for each aggregate size class was determined following Angers et al. (1988). In short, a 2-g sample of aggregate crushed to pass through a 0.15-mm sieve was placed in a sealed 50-mL tube, and 8 mL of 12 mol $L^{-1}$ $H_2SO_4$ was added. The suspension was slightly mixed and incubated for 2 h at room temperature. At the end of the incubation period, the solution was transferred to a 250-mL centrifuge tube with 192 mL of deionized water and incubated at 85°C for 24 h. After cooling, soil extracts were centrifuged for 20 min at 16,000 × *g*, filtered (Whatman no. 42), and stored at −20°C until analysis.

The concentration of total sugars was determined by the anthrone method according to Brink et al. (1960). A 5-mL aliquot of the soil hydrolysates was placed in a tube and 10 mL of 0.2% anthrone in 95% $H_2SO_4$ were added. After 15 min of color development, absorbance of the resulting solution was read at 625 nm using a Hitachi U-2010 spectrophotometer (Hitachi High Technologies, Schaumburg, IL). The standard curve was established with 50, 100, 150, and 200 mg $L^{-1}$ of glucose.

**Table 1. Effects of fresh and composted mixed paper mill sludge applications on aggregate size distribution and mean weight diameter of water-stable aggregates (MWD).**

| Treatment† | >2 mm | 0.25–2 mm | <0.25 mm | MWD |
|---|---|---|---|---|
| | % | | | mm |
| Control | 8.3 | 34.2 | 56.0 | 1.86 |
| MNF | 5.2 | 29.5 | 63.9 | 1.42 |
| PMS | 13.3 | 37.5 | 47.6 | 2.40 |
| CPMS | 14.6 | 32.6 | 51.1 | 2.43 |
| PMSN | 12.0 | 37.1 | 49.5 | 2.40 |
| CPMSN | 13.7 | 34.8 | 50.0 | 2.30 |
| | | *F* value | | |
| Treatments | 5.5* | 1.7 | 3.5* | 4.7** |
| Contrasts | | | | |
| Control vs. others | 4.1* | 0.0 | 1.0 | 2.6 |
| MNF vs. PMS + CPMS | 22.1** | 5.5* | 16.0** | 20.7** |
| PMS + PMSN vs. CPMS + CPMSN | 1.0 | 2.4 | 0.4 | 0.1 |
| PMS vs. PMSN | 0.4 | 0.0 | 0.2 | 0.2 |
| CPMS vs. CPMSN | 0.2 | 0.5 | 0.0 | 0.0 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N ha$^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg ha$^{-1}$); CPMS, composted paper mill sludge (40 Mg ha$^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N ha$^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N ha$^{-1}$.

## Amino Sugar Analysis

Amino sugar analysis was performed according to the method of Chantigny et al. (1997) with minor modifications. A 1-g sample of aggregate crushed to pass through 0.15-mm sieve was placed in a 250-mL polypropylene copolymer centrifuge tube and hydrolyzed with 20 mL of 6 mol L$^{-1}$ HCl in an oven at 105°C for 6 h. The hydrolysate was cooled on ice to room temperature and centrifuged for 10 min at 15,000 × *g*. The supernatant was decanted into a plastic vial and stored at −20°C until analysis.

A 1-mL aliquot of the supernatant was dried completely by a rotary evaporator at 45 to 50°C under vacuum. The residue was redissolved in 980 μL of 99% *o*-phthaldialdehyde and 20 μL of mercaptoethanol. The precipitate was removed by centrifugation (3 min at 15,000 × *g*). The amino sugars (glucosamine, galactosamine, mannosamine, and muramic acid) were quantified by high performance liquid chromatography on a Waters HPLC (Waters Associates, Milford, MA) equipped with an autoinjector, a Waters analytical column no. 8NVC18 4μ, a column oven at 40°C, a Xenon lamp, and a fluorometric detector (Waters 474). The fluorometric detector was set at 338- and 425-nm wavelengths for excitation and emission, respectively. A 25-μL sample was injected manually and the flow rate was 1.0 mL min$^{-1}$. The mobile phase (pH 5.3) consisted of 0.05 mol L$^{-1}$ sodium citrate/0.05 mol L$^{-1}$ NaOAc, 95% methanol (HPLC grade), and tetrahydrofuran at a ratio of 90:8.5:5. The column was cleaned with a 65% methanol solution and then reconditioned with the mobile phase.

## Statistical Analysis

Data obtained were subjected to the GLM procedure of SAS (SAS Institute, 1985) using a randomized complete block design model. Single degree-of-freedom orthogonal contrasts were used to make preplanned comparisons among means for groups of treatments.

# RESULTS

## Aggregate Stability

Two years after the last application of the treatments, the mean weight diameter of water-stable aggregates was, on average, 2.4 mm for the PMS and CPMS treatments, while it was 1.9 and 1.4 mm in the control and MNF plots, respectively (Table 1). Similar trends were noticed in 2001, the year of the last application of PMS and CPMS (Bipfubusa et al., 2005). The effects of the organic residues were mostly apparent in the large macroaggregates (>2 mm). In contrast to PMS and CPMS, MNF application resulted in a lower level of macroaggregate stability compared with the control. We hypothesize that MNF increased the mineralization of the organic binding agents that form stable macroaggregates (Lynch and Bragg, 1985).

## Carbon in Whole Soil and Aggregates

Two years after the last application of the treatments, the organic C content of the whole soil was still 15% higher in the PMS and CPMS treatments than in the control treatment (Table 2), as previously reported by Bipfubusa et al. (2005). Organic C contents were also significantly greater in macroaggregates than in microaggregates (Table 2). There were also significant differences in aggregate organic C contents among treatments (Table 2). Organic C concentrations of >2-mm macroaggregates were greatly increased by both PMS and CPMS, but there were no differences in the effects of the two types of amendment. Organic amendments also increased the C content of small macroaggregates of 0.25- to 2-mm size, but to a much lower extent than larger aggregates. There was no significant difference in organic C content between treatments for the microaggregates.

In the present study, organic C contents in the whole soil and aggregates were not affected

**Table 2. Organic C concentrations in whole soil and aggregate size fractions as influenced by fresh and composted mixed paper mill sludge applications.**

| Treatment† | Organic C | | | |
|---|---|---|---|---|
| | Whole soil | >2 mm | 0.25–2 mm | <0.25 mm |
| | g C kg$^{-1}$ soil | g C kg$^{-1}$ aggregate | | |
| Control | 15.8 | 18.5 | 15.9 | 14.9 |
| MNF | 15.8 | 18.7 | 15.2 | 14.3 |
| PMS | 18.7 | 27.3 | 21.9 | 16.0 |
| CPMS | 18.2 | 28.3 | 23.1 | 16.0 |
| PMSN | 17.1 | 26.4 | 20.3 | 14.9 |
| CPMSN | 18.9 | 28.6 | 24.0 | 16.6 |
| | | *F* value | | |
| Treatments | 2.6* | 6.6** | 7.6** | 0.7 |
| Contrasts | | | | |
| Control vs. others | 4.3* | 13.1** | 11.3** | 0.3 |
| MNF vs. PMS + CPMS | 6.3* | 18.9** | 22.5** | 1.9 |
| PMS + PMSN vs. CPMS + CPMSN | 0.6 | 0.7 | 3.3* | 0.7 |
| PMS vs. PMSN | 1.6 | 0.1 | 0.7 | 0.5 |
| CPMS vs. CPMSN | 0.3 | 0.0 | 0.2 | 0.2 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N ha$^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg ha$^{-1}$); CPMS, composted paper mill sludge (40 Mg ha$^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N ha$^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N ha$^{-1}$.

by the MNF treatment compared with the control (Table 2). There are reports, however, that mineral N fertilization generally improves crop yields and crop residue input to the soil, which in turn may result in increased soil organic matter levels (Haynes and Naidu, 1998). Our result may be explained by the fact that silage corn is a low-residue-producing crop, with only roots and crown being returned to the soil. Clapp et al. (2000) found that 13 yr of continuous silage corn resulted in a loss of soil organic matter.

## Humic Substances

### Fulvic and Humic Acids

The soil aggregate $C_{FA}$ content varied from 0.90 to 3.10 g C kg$^{-1}$ aggregate, while the $C_{HA}$ content varied from 2.03 to 4.08 g C kg$^{-1}$ aggregate (Table 3). The highest values of $C_{HA}$ content were observed for the >2-mm macroaggregates under the CPMS and the CPMS plus 120 kg N ha$^{-1}$ treatments (average of 3.78 g C kg$^{-1}$), which were significantly higher than those of the PMS and the PMS plus 120 kg N ha$^{-1}$ treatments (average of 2.54 g C kg$^{-1}$). Since the C application rate was slightly greater (20%) with the composted than with the fresh residue, we calculated a ratio of the $C_{HA}$ content to the amount of C added. The results still show a higher value for the composted sludge (0.84) than the fresh sludge (0.66), which suggests that the effect of the composts on $C_{HA}$ was not due only to the higher C loading rate.

The combined application of PMS with MNF reduced the $C_{FA}$ content of the stable >2-mm macroaggregates by 30% without increasing their HA contents compared with the PMS applied alone (Table 3). These results corroborate previous studies that showed that the addition of mineral N fertilizer during the decomposition of organic residues reduced the production of humic substances (Fog, 1988). In contrast to PMS, the combination of the CPMS with MNF did not decrease the $C_{FA}$ content of the aggregates in comparison with CPMS applied alone (Table 3).

The applications of PMS and CPMS did not affect the $C_{HA}$ of the small macroaggregates (0.25–2 mm) but significantly increased their $C_{FA}$ content (Table 3). In this fraction, the highest $C_{FA}$ content was observed with the MNF treatment, which was significantly higher than those of the PMS and CPMS (Table 3). No relationships could be established, however, between these effects and aggregate stability.

### Unhumified Fraction

The soil aggregate $C_{UHF}$ content varied from 1.56 to 2.13 g C kg$^{-1}$ aggregate (Table 3). The application of PMS and CPMS had a positive effect on the $C_{UHF}$ content of the stable macroaggregate fractions compared with the control (Table 3). On the other hand, MNF treatment had no significant effect on the $C_{UHF}$ content. In comparison with CPMS, and despite a lower C loading rate, the application of PMS increased the UHF content of the macroaggregates >2 and 0.25 to 2 mm by 18 and 7%, respectively (Table 3). According to N'Dayegamiye and Watt (2000), this positive effect of PMS on $C_{UHF}$ would be related to their high content of easily degradable C, which would support microbial growth in the soil. Indeed, Stout et al. (1981) suggested that the UHF is partly composed of plant residues modified by the microorganisms, microbial bodies, or compounds newly synthesized by the microorganisms such as metabolites. The decrease in the content of $C_{UHF}$ of the >2-mm macroaggregates following the combined application of PMS and MNF (Table 3) confirms that this fraction consists of easily mineralizable organic compounds.

### Humification Indices

The index of humification, $C_{UHF}/(C_{FA} + C_{HA})$, varied between 0.3 and 0.5 (Table 4), which is close to an average value of 0.5 proposed by Gigliotti et al. (2001). For both macroaggregate fractions, the results suggest that the organic matter of the soil amended with the CPMS was more humified. Indeed, contrary to the CPMS addition, the application of PMS resulted in an enrichment of the soil mostly in $C_{FA}$ and not in $C_{HA}$ (Table 3).

**Table 3. Organic C contents in the unhumified fraction ($C_{UHF}$) and fulvic ($C_{FA}$) and humic acids ($C_{HA}$) of aggregate size fractions as influenced by fresh and composted paper mill sludge applications.**

| Treatment† | >2 mm | | | 0.25–2 mm | | | <0.25 mm | | |
|---|---|---|---|---|---|---|---|---|---|
| | $C_{UHF}$ | $C_{FA}$ | $C_{HA}$ | $C_{UHF}$ | $C_{FA}$ | $C_{HA}$ | $C_{UHF}$ | $C_{FA}$ | $C_{HA}$ |
| | g C kg$^{-1}$ aggregate | | | | | | | | |
| Control | 1.56 | 1.80 | 2.18 | 1.50 | 0.95 | 2.10 | 1.55 | 0.90 | 2.33 |
| MNF | 1.70 | 1.63 | 2.95 | 1.59 | 3.10 | 2.03 | 1.57 | 0.98 | 2.35 |
| PMS | 2.13 | 2.70 | 2.63 | 1.77 | 1.60 | 2.60 | 1.61 | 1.00 | 2.13 |
| CPMS | 1.80 | 1.95 | 4.08 | 1.65 | 2.38 | 2.68 | 1.64 | 0.95 | 2.30 |
| PMSN | 1.91 | 1.83 | 2.45 | 1.74 | 1.23 | 2.20 | 1.67 | 1.00 | 2.38 |
| CPMSN | 1.74 | 1.95 | 3.48 | 1.64 | 2.13 | 2.60 | 1.54 | 0.98 | 2.63 |
| | *F* value | | | | | | | | |
| Treatments | 8.7** | 4.6** | 9.8** | 3.5* | 12.9** | 2.1 | 2.5 | 0.5 | 0.5 |
| Contrasts | | | | | | | | | |
| Control vs. others | 16.3** | 1.2 | 14.4** | 9.8** | 21.9** | 2.1 | 2.0 | 1.8 | 0.0 |
| MNF vs. PMS + CPMS | 6.7* | 6.1* | 0.7 | 3.5 | 26.3** | 4.8* | 1.5 | 0.0 | 0.0 |
| PMS + PMSN vs. CPMS + CPMSN | 14.6** | 3.2 | 30.0** | 4.2 | 14.3** | 1.4 | 2.5 | 0.5 | 0.8 |
| PMS vs. PMSN | 5.5* | 12.5** | 0.3 | 0.1 | 1.4 | 2.0 | 1.6 | 0.0 | 0.6 |
| CPMS vs. CPMSN | 0.4 | 0.0 | 3.5 | 0.0 | 0.6 | 0.1 | 5.0* | 0.1 | 0.9 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N ha$^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg ha$^{-1}$); CPMS, composted paper mill sludge (40 Mg ha$^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N ha$^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N ha$^{-1}$.

Table 4. Humification indices based on the organic C contents in the unhumified fraction ($C_{UHF}$) and fulvic ($C_{FA}$) and humic acids ($C_{HA}$) in aggregate size fractions as influenced by fresh and composted paper mill sludge applications.

| Treatment† | >2 mm | | 0.25–2 mm | | <0.25 mm | |
|---|---|---|---|---|---|---|
| | $C_{HA}/C_{FA}$ | $C_{UHF}/(C_{HA}+C_{FA})$ | $C_{HA}/C_{FA}$ | $C_{UHF}/(C_{HA}+C_{FA})$ | $C_{HA}/C_{FA}$ | $C_{UHF}/(C_{HA}+C_{FA})$ |
| Control | 1.25 | 0.40 | 2.15 | 0.51 | 2.53 | 0.51 |
| MNF | 1.88 | 0.36 | 0.65 | 0.30 | 2.43 | 0.47 |
| PMS | 1.05 | 0.40 | 1.63 | 0.43 | 2.13 | 0.51 |
| CPMS | 2.10 | 0.30 | 1.28 | 0.33 | 2.43 | 0.49 |
| PMSN | 1.35 | 0.47 | 1.93 | 0.51 | 2.33 | 0.49 |
| CPMSN | 1.83 | 0.28 | 1.23 | 0.35 | 2.23 | 0.45 |
| | | | | *F* value | | |
| Treatments | 7.2** | 3.1* | 7.8** | 9.4** | 0.8 | 0.6 |
| Contrasts | | | | | | |
| Control vs. others | 5.3* | 0.7 | 14.6** | 13.9** | 1.5 | 0.8 |
| MNF vs. PMS + CPMS | 2.9 | 0.0 | 15.9** | 9.8** | 0.7 | 0.2 |
| PMS + PMSN vs. CPMS + CPMSN | 24.3** | 13.0** | 7.4* | 19.0** | 0.4 | 1.1 |
| PMS vs. PMSN | 1.9 | 1.6 | 1.2 | 4.2* | 0.7 | 0.2 |
| CPMS vs. CPMSN | 1.6 | 0.1 | 0.0 | 0.1 | 0.7 | 0.8 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N $ha^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg $ha^{-1}$); CPMS, composted paper mill sludge (40 Mg $ha^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N $ha^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N $ha^{-1}$.

The $C_{HA}/C_{FA}$ ratio of the microaggregates was generally >2, while its value was <2 for the macroaggregates (Table 4). This reflects the higher degree of humification of the organic matter associated with microaggregates, and agrees with the concept of younger and less processed organic matter associated with stable macroaggregates than with microaggregates (Puget et al., 1995; Angers and Giroux, 1996). Furthermore, this index varied significantly with treatment for the >2-mm macroaggregates, with much higher values in the compost-amended soil than in the other treatments, reflecting the enrichment of the compost-amended soil aggregates in humified organic matter.

## Total Sugars

Total sugar content varied from 3.7 to 7.7 g C $kg^{-1}$ aggregate (Table 5), which is in the range of values found in Québec soils (Angers et al., 1988). Sugar contents generally increased with aggregate size (Table 5), which agrees with a previous study also performed on slaking-resistant aggregates (Puget et al., 1999). The application of PMS and CPMS significantly increased the total sugar content of the stable macroaggregates >2 mm by an average of 29% compared with the control, but there were no significant differences between the effect of composted and fresh sludge. Considering that carbohydrates determined by the anthrone reagent method are mainly hexoses and deoxyhexoses (Brink et al., 1960), which are predominantly of microbial origin (Cheshire, 1979), our results suggest that the application of PMS and CPMS has stimulated microbial activity in stable macroaggregates >2 mm. There were greater increases of total sugar contents in macroaggregates >2 mm, however, when PMS was applied in combination with MNF (Table 5), suggesting that the application of PMS along with inorganic N fertilizer led to increased microbial activity, and subsequently to an increase in microbial polysaccharide production. The small effect of adding mineral N fertilizer with CPMS on the sugar content of aggregates (Table 5) might be partly due to the low availability of labile C in these amendments.

Table 5. Total sugar concentration in aggregate size fraction as influenced by fresh and composted paper mill sludge applications.

| Treatments† | Total sugar concentration >2 mm | 0.25–2 mm | <0.25 mm |
|---|---|---|---|
| | | g C $kg^{-1}$ aggregate | |
| Control | 5.01 | 5.10 | 4.35 |
| MNF | 5.35 | 4.35 | 3.73 |
| PMS | 6.50 | 5.34 | 4.02 |
| CPMS | 6.46 | 5.61 | 4.37 |
| PMSN | 7.70 | 5.52 | 4.36 |
| CPMSN | 6.97 | 4.98 | 4.39 |
| | | *F* value | |
| Treatments | 8.6** | 4.0* | 3.8* |
| Contrasts | | | |
| Control vs. others | 19.6** | 0.1 | 2.0 |
| MNF vs. PMS + CPMS | 17.5** | 18.2** | 17.1** |
| PMS + PMSN vs. CPMS + CPMSN | 1.3 | 0.4 | 3.2 |
| PMS vs. PMSN | 6.2* | 0.3 | 3.1 |
| CPMS vs. CPMSN | 1.0 | 3.7 | 0.0 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N $ha^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg $ha^{-1}$); CPMS, composted paper mill sludge (40 Mg $ha^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N $ha^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N $ha^{-1}$.

Contrary to the organic amendments, the application of MNF alone did not significantly affect the sugar content of >2-mm macroaggregates but decreased that of smaller aggregates 0.25 to 2 and <0.25 mm (Table 5). These effects of inorganic N fertilizer suggest a reduced microbial activity due to low organic matter inputs in the soil receiving only inorganic fertilizers (Table 2). Based on long-term studies, Černý et al. (2003) indicated the negative effect of mineral N fertilization on the soil microbial biomass under silage corn production.

## Amino Sugars

The amino sugar contents of the aggregate fractions ranged from 396 to 563 mg C $kg^{-1}$ aggregate (Table 6) and represented 1.8 to 3.2% of their total organic C. The sum of glucosamine and galactosamine accounted for 90 to 93% of total amino sugar, and mannosamine and muramic acid accounted for 7 to 10%. Generally, the amino sugar content was greater in the macro- than in the microaggregates (Table 6).

The application of organic amendments generally increased the amino sugar contents of the aggregates compared with the control and MNF treatments (Table 6). Overall, the applications of PMS and CPMS had similar effects on the various amino sugars except for glucosamine, for which significantly higher values were recorded in macroaggregates >2 mm from the soils that received fresh sludge (average of 242 mg C $kg^{-1}$ for PMS and PMS plus 120 kg N $ha^{-1}$) compared with composted sludge (average of 191 mg C $kg^{-1}$ for CPMS and CPMS plus 120 kg N $ha^{-1}$) (Table 6). Glucosamine has been suggested as an indicator of fungal biomass (Zelles, 1988) and has been successfully used to study the role of fungi on soil macroaggregation (Chantigny et al., 1997; Guggenberger et al., 1999). Thus, compared with the compost and the unamended control, the addition of fresh PMS appears to have stimulated fungal growth, which was reflected by higher glucosamine content in the aggregates >2 mm.

## DISCUSSION

Three successive applications of PMS and CPMS significantly increased soil aggregate stability and most soil C fractions associated with the >2-mm macroaggregates. This effect was still noticeable 2 yr after the last application of the treatments. In a previous study, Bipfubusa et al. (2005) showed that the effect of these residues on aggregate stability occurred rapidly. The rapid and relatively long-lasting effect of residues derived from the paper industry on soil macroaggregate formation and stability was also clearly shown by Chantigny et al. (1999) and N'Dayegamiye (2006). These residues contain lignocellulosic materials, which provide a mixture of rapidly and slowly decomposing compounds (Chantigny et al., 2000). Overall, the effects of mineral N, applied alone or with organic residues, on aggregate stability and C fractions were relatively small and not consistent.

The >2-mm size fraction was the most influenced by the application of the organic residues. The millimeter-size aggregate fraction has been found to be highly responsive to organic residue addition in this soil (N'Dayegamiye et al., 1997; Aoyama et al., 1999) but also in other soils and under other management practices in Québec (Angers, 1998). The large macroaggregates (>2 mm) were also generally enriched in the various organic matter fractions. Isotopic studies have shown that newly added organic matter is preferentially incorporated in the stable macroaggregates (Puget et al., 1995; Angers and Giroux, 1996). This enrichment supports the hypothesis that organic matter from PMS and CPMS favored binding of microaggregates into macroaggregates, as proposed by the hierarchical soil aggregation model (Tisdall and Oades, 1982).

The HA contents of the aggregates >2 mm was greater in the soil amended with composted residue than with the fresh material and, conversely, their glucosamine content was greater with the application of fresh residues. We hypothesize that the effects of the fresh sludge are attributable to their greater lability and, consequently, to their capacity to stimulate the soil microflora, in particular fungi, as quantified in this study by the glucosamine content. Fungi have been shown to play a significant role in macroaggregate stabilization (Tisdall and Oades, 1982). Several mechanisms have been invoked, such as a direct effect on cohesion by physical enmeshment by the hyphae or by the production of extracellular products that can increase cohesion or reduce wettability.

Contrary to fresh PMS, the application of the CPMS resulted in HA enrichment of the >2-mm macroaggregates. The addition of humic substances contributes to binding soil particles and increases soil aggregate stability (Piccolo and Mbagwu, 1989). In an incubation study, Annabi et al. (2007) showed a rapid effect of composted organic matter on aggregates, which they attributed to the diffusion of humic material within the aggregate, and a consequent increase in aggregate cohesion.

Our results illustrate the beneficial effects of both fresh and composted organic residues on aggregate stability under field conditions. The effects of three consecutive applications lasted for at least 2 yr after the last application. The effects were

**Table 6. Amino sugar contents in aggregate size fractions as influenced by fresh and composted paper mill sludge applications.**

| Treatment† | Amino acid sugar content‡ >2 mm | | | | | 0.25–2 mm | | | | | <0.25 mm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mur | MaN | GalN | GclN | Total | Mur | MaN | GalN | GclN | Total | Mur | MaN | GalN | GclN | Total |
| | mg C $kg^{-1}$ aggregate | | | | | | | | | | | | | | |
| Control | 19 | 18 | 237 | 183 | 457 | 20 | 19 | 239 | 213 | 482 | 16 | 13 | 209 | 196 | 437 |
| MNF | 22 | 14 | 235 | 235 | 506 | 18 | 18 | 233 | 232 | 560 | 15 | 16 | 180 | 200 | 409 |
| PMS | 25 | 19 | 277 | 241 | 563 | 21 | 32 | 275 | 229 | 538 | 16 | 18 | 210 | 191 | 432 |
| CPMS | 19 | 16 | 274 | 190 | 499 | 22 | 20 | 267 | 257 | 542 | 17 | 16 | 200 | 163 | 397 |
| PMSN | 26 | 25 | 233 | 242 | 526 | 23 | 26 | 235 | 221 | 489 | 18 | 20 | 178 | 183 | 396 |
| CPMSN | 25 | 29 | 283 | 191 | 526 | 25 | 25 | 220 | 221 | 489 | 18 | 20 | 204 | 185 | 407 |
| | *F* value | | | | | | | | | | | | | | |
| Treatments | 2.3 | 3.3* | 1.3 | 3.9* | 1.49 | 3.4* | 5.1** | 2.6 | 2 | 2 | 1.7 | 0.8 | 1.2 | 5.7* | 1.4 |
| Contrasts | | | | | | | | | | | | | | | |
| Control vs. others | 3.9 | 0.6 | 1 | 5.5* | 4.6* | 2.9 | 4.6* | 0.2 | 1.1 | 1.2 | 1.2 | 0.3 | 0.9 | 3.8 | 3.1 |
| MNF vs. PMS + CPMS | 0.3 | 6.1* | 1.7 | 1.4 | 0.5 | 9.4** | 8.1* | 1.2 | 3.1 | 3.7 | 0.4 | 1.4 | 1.4 | 10.0** | 0 |
| PMS + PMSN vs. PMS + CPMSN | 5* | 0 | 1.2 | 13.0** | 1.2 | 0.3 | 7.4* | 0.7 | 2.9 | 2.6 | 0.7 | 1.3 | 0.3 | 5.6* | 0.7 |
| PMS vs. PMSN | 0.5 | 1.6 | 2.2 | 0 | 0.8 | 4.3 | 3.8 | 4.7* | 2.6 | 0.3 | 2.4 | 0 | 3 | 1.3 | 3 |

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MNF, mineral N fertilizer (160 kg N $ha^{-1}$); PMS, fresh mixed paper mill sludge (40 Mg $ha^{-1}$); CPMS, composted paper mill sludge (40 Mg $ha^{-1}$); PMSN, fresh paper mill sludge plus 120 kg N $ha^{-1}$; CPMSN, composted paper mill sludge plus 120 kg N $ha^{-1}$.

‡ Mur, muramic acid; MaN, mannosamine; GalN, galactosamine; GlcN, glucosamine; Total, Mur + MaN + GalN + GlcN.

quantitatively similar for both residues but the mechanisms involved appear to be different. Microorganisms, in particular fungi, were a more important factor of stable macroaggregation in the soil amended with fresh sludge, while humic substances played a greater role in the compost-amended soil.


## ACKNOWLEDGMENTS

This study was supported by the Research Institute for the Agri-Environment (IRDA), Les Composts du Québec Inc., and Agriculture and Agri-Food Canada. The assistance of Patrice Jolicoeur, Anne Drapeau, Nicole Bissonnette, and Lucette Chouinard for the aggregate separation and humic substance and sugar analyses is gratefully acknowledged.


## REFERENCES


Abiven, S., S. Menasserri, D.A. Angers, and P. Leterme. 2007. Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. Eur. J. Soil Sci. 58:239–247.

Angers, D.A., M.S. Bullock, and G.R. Mehuys. 2008. Aggregate stability to water. p. 811–820. *In* M.R. Carter and E.G. Gregorich (ed.) Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL.

Angers, D.A. 1998. Water-stable aggregation of Québec silty clay soils: Some factors controlling its dynamics. Soil Tillage Res. 47:91–96.

Angers, D.A., and M. Giroux. 1996. Recently deposited organic matter in soil water-stable aggregates. Soil Sci. Soc. Am. J. 60:1547–1551.

Angers, D.A., P. Nadeau, and G.R. Mehuys. 1988. Determination of carbohydrate composition of soil hydrolysates by high-performance liquid chromatography. J. Chromatogr. 45:444–449.

Annabi, M., S. Houot, C. Francou, and Y. Le Bissonnais. 2007. Soil aggregate stability improvement with urban composts of different maturities. Soil Sci. Soc. Am. J. 71:413–423.

Aoyama, M., D.A. Angers, A. N'Dayegamiye, and N. Bissonnette. 1999. Protected organic matter in water-stable aggregates as affected by mineral fertilizer and manure applications. Can. J. Soil Sci. 79:419–425.

Bipfubusa, M., A. N'Dayegamiye, and H. Antoun. 2005. Effets des boues mixtes de papetières fraîches et compostées sur l'agrégation du sol, l'inclusion et la minéralisation du carbone dans les macro-agrégats stables à l'eau. Can. J. Soil Sci. 85:47–55.

Brink, R.H., P. Dubach, and D.L. Lynch. 1960. Measurement of carbohydrates in soil hydrolysates with anthrone. Soil Sci. 89:157–166.

Černý, J., J. Balík, D. Pavlíková, M. Zitková, and K. Sýkora. 2003. The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long-term experiments with maize. Plant Soil Environ. 49:560–564.

Chaney, K., and R.S. Swift. 1984. The influence of organic matter on aggregate stability in some British soils. J. Soil Sci. 35:223–230.

Chaney, K., and R.S. Swift. 1986. Studies on aggregate stability: II. The effect of humic substances on the stability of re-formed soil aggregates. J. Soil Sci. 37:337–343.

Chantigny, M.H., D.A. Angers, D. Prévost, L.P. Vézina, and F.P. Chalifour. 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Sci. Soc. Am. J. 61:262–267.

Chantigny, M.H., D.A. Angers, and C.J. Beauchamp. 1999. Aggregation and organic matter decomposition in soils amended with de-inking paper sludge. Soil Sci. Soc. Am. J. 63:1214–1221.

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

Cheshire, M.V. 1979. Nature and origin of carbohydrates in soils. Academic Press, London.

Clapp, C.E., R.R. Allmaras, M.F. Layese, D.R. Linden, and R.H. Dowdy. 2000. Soil organic carbon and $^{13}C$ abundance as related to tillage, crop residue and nitrogen fertilization under continuous management in Minnesota. Soil Tillage Res. 55:127–142.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. Cambridge Philos. Soc. 63:432–462.

Fortun, A., C. Fortun, and C. Ortega. 1989. Effect of farmyard manure and its humic fractions on the aggregate stability of a sandy loam soil. J. Soil Sci. 40:293–298.

Gigliotti, G., P.L. Giusquiani, and D. Businelli. 2001. A long-term chemical and infrared spectroscopy study on a soil amended with municipal sewage sludge. Agronomie 21:169–178.

Guggenberger, G., S.D. Frey, J. Six, K. Paustian, and E.T. Elliott. 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J. 63:1188–1198.

Haynes, R.J., and R. Naidu. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. Nutr. Cycling Agroecosyst. 51:123–137.

Kay, B.D., and D.A. Angers. 1999. Soil structure. p. 229–276. *In* M.E. Summer (ed.) Handbook of soil science. CRC Press, Boca Raton, FL.

Lynch, J.M. 1984. Interactions between biological processes, cultivation and soil structure. Plant Soil 76:307–318.

Lynch, J.M., and E. Bragg. 1985. Microorganisms and soil aggregate stability. Adv. Soil Sci. 2:133–171.

Martens, D.A. 2000. Management and crop residue influence soil aggregate stability. J. Environ. Qual. 29:723–727.

Martin, J.P., W.P. Martin, J.B. Page, W.A. Raney, and J.D. De Ment. 1955. Soil aggregation. Adv. Agron. 7:1–37.

N'Dayegamiye, A. 2006. Mixed paper mill sludge effects on corn yield, nitrogen efficiency, and soil properties. Agron. J. 98:1471–1478.

N'Dayegamiye, A., M. Goulet, and M.R. Laverdière. 1997. Effet à long terme d'apports d'engrais minéraux et de fumier sur les teneurs en C et en N des fractions densimétriques et des agrégats du loam limoneux Le Bras. Can. J. Soil Sci. 77:351–358.

N'Dayegamiye, A., and S. Watt. 2000. Changes in soil organic matter and humic substances after paper mill sludges application to corn. p. 1009–1013. *In* Entering the third millenium with a common approach to humic substances and organic matter in water, soil and sediments: Proc. Int. Meet. Int. Humic Subst. Soc., 10th, Toulouse, France. 24–28 July 2000. Vol. 2. IHSS, St. Paul, MN.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 961–1010. *In* D.L. Sparks et al. (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.

Orlov, D.S. 1995. Humic substances of soils and general theory of humification. p. 235–286. *In* D. Orlov-Grishina (ed.) Humic substances of soils and general theory of humification. Balkema Publ., Rotterdam, the Netherlands.

Piccolo, A., and J.S.C. Mbagwu. 1989. Effects of humic substances and surfactants on the stability of soil aggregates. Soil Sci. 147:47–54.

Puget, P., D.A. Angers, and C. Chenu. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. Soil Biol. Biochem. 31:55–63.

Puget, P., C. Chenu, and J. Balesdent. 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. Eur. J. Soil Sci. 46:449–459.

SAS Institute. 1985. SAS user's guide: Statistics. Version 5 ed. SAS Inst., Cary, NC.

Schnitzer, M., L.E. Lowe, J.F. Dormaar, and V. Martel. 1981. Chemical parameters for the characterization of soil organic matter. Can. J. Soil Sci. 61:517–519.

Sequi, P., M. de Nobili, L. Leita, and G. Cercignani. 1986. A new index of humification. Agrochimica 30:175–179.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79:7–31.

Stout, J.D., K.M. Goh, and T.A. Rafter. 1981. Chemistry and turnover of naturally occurring resistant organic compounds in soil. p. 1–73. *In* E.A. Paul and J.N. Ladd (ed.) Soil biochemistry. Vol. 5. Marcel Dekker, New York.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141–163.

Zelles, L. 1988. The simultaneous determination of muramic acid and glucosamine in soil by high-performance chromatography with precolumn fluorescence derivatization. Biol.Fertil. Soil 6:125–130.