Applied Soil Ecology 61 (2012) 92–99

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effects of tannery sludge application on physiological and fatty acid profiles of the soil microbial community

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ARTICLE INFO

Article history:
Received 24 April 2011
Received in revised form 27 April 2012
Accepted 8 May 2012

Keywords:
Biolog
Carbon source utilization
Nitrogen
pH
PLFA
Sludge land utilization

ABSTRACT

The impact of tannery sludge application on soil microbial community and diversity is poorly understood. We studied the microbial community in an agricultural soil following two applications (2006 and 2007) of tannery sludge with annual application rates of 0.0, 2.3 and 22.6 Mg ha^{-1}. The soil was sampled 12 and 271 days after the second (2007) application. Community structure was assessed via a phospholipid fatty acid analysis, and the physiological profile of the soil microbial community via the Biolog method. Tannery sludge application changed soil chemical properties, increasing the soil pH and electrical conductivity as well as available P and mineral N concentrations. The higher sludge application rate changed the community structure and the physiological profile of the microbial community at both sampling dates. However, there is no clear link between community structure and carbon substrate utilization. According to the Distance Based Linear Models Analysis, the fatty acids 16:0 and *i*17:0 together contributed 84% to the observed PLFA patterns, whereas the chemical properties available P, mineral N, and Ca, and pH together contributed 54%. At 12 days, tannery sludge application increased the average well color development from 0.46 to 0.87 after 48 h, and reduced the time elapsed before reaching the midpoint carbon substrate utilization (*s*) from 71 to 44 h, an effect still apparent nine months after application of the higher sludge application rate. The dominant signature fatty acids and kinetic parameters (*r* and *s*) were correlated to the concentrations of available P, Ca, mineral N, pH and EC.

1. Introduction

The tanning industry generates great quantities of waste during leather processing (Pacheco, 2005). Producing "wet blue" leather in particular requires a broad array of chemical compounds (Martines et al., 2010) and generates a nutrient-rich and high pH waste sludge. Thus, this sludge, if carefully managed, could be recycled as fertilizer and acidity-neutralizer, making them a potentially useful tool in agriculture and for the amelioration of degraded soils (Kray et al., 2008). In recent years, tanneries have improved the tanning process to reduce the amount of chromium in the waste; hence chromium pollution is no longer the leading concern regarding the disposal of tannery waste. However, there is still concern about the high nitrogen and sodium concentrations in the sludge (Nakatani et al., 2011a).

The soil microbial community plays a pivotal role in nutrient cycling by mineralizing organic matter and transforming nutrients. Because it can respond quickly to environmental change, it is considered a sensitive indicator of soil health and of anthropogenic disturbance (Bending et al., 2004). However, little is known about the effect of the application of tannery sludge on soil biological properties. Trasar-Cepeda et al. (2000) reported lower microbial biomass and enzyme activities in areas polluted with tannery effluent, compared to unpolluted control areas in Spain. On the other hand, the microbial biomass increased in soils treated with tannery waste in Mexico (Alvarez-Bernal et al., 2006). Barajas-Aceves et al. (2007) also observed tannery sludge stimulated soil microbial activity in Mexico. The microbial community structure based on PLFA profiling changed from gram-positive bacteria to gram-negative bacteria in heavily tannery waste-polluted soils in Australia (Kamaludeen et al., 2003). These contrasting results suggest that more studies need to be conducted to assess the effects of tannery sludge of different origins on microbial communities under different soil and climatic conditions.

Biotic and abiotic factors modulate metabolic diversity and biological activity (Marschner et al., 2003), and consequently the microbial community structure (Zak et al., 1994). Therefore, the

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http://dx.doi.org/10.1016/j.apsoil.2012.05.003

assessment of the functional diversity is as important as assessment of the microbial species diversity (Tótola and Chaer, 2002).

The aim of this study was to assess the metabolic profile and microbial community structure of soils treated with tannery sludge. Our hypothesis was that the PLFA analysis and carbon substrate consumption profile of the soil microbial community are sensitive indicators of the effects of tannery sludge application.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in an agricultural area in the municipality of Rolândia in Paraná state (23°17′S, 51°29′W, 650 m), Brazil. The climate at the site is classified as Cfa under the Köppen system, with hot summers, undefined dry season, and average temperature of 21 °C (means ranging from 16 to 27 °C), with mean annual rainfall of 1600 mm falling mostly between September and March. The study area has been managed for more than 10 years under a no-till system with rotating crops (soybean/corn in summer and wheat/oats in winter). The soil has a high clay content (74%) and is classified as Rhodic Kandiudult (US Soil Taxonomy). Sludge was first applied at the site in 2006 and reapplied in 2007. The experiment had a completely randomized block design with four replications (15 m × 6 m plots) that had the following treatments: zero, 2.3 and 22.6 Mg ha^{-1} of tannery sludge (dry weight basis) annually, corresponding to zero (no sludge), 120 and 1200 kg ha^{-1} of total N. These relatively high rates are commonly used in Brazil due to lack of federal laws that regulate the limits to be applied to the soil. The tannery sludge was incorporated into the soil by plowing to 0–20 cm depth 89 days after the first application. Corn was then planted in summer receiving 48 kg ha^{-1} of P (triple superphosphate) and 42 kg ha^{-1} of K (KCl), and black oats in winter, both without irrigation. After oat cultivation, sludge was applied again 393 days after the first application and the sludge was kept on the soil surface for 87 days, after which it was plowed and planted with corn.

For this study, soil samples were collected 12 and 271 days after the second application of tannery sludge. Nine subsamples were collected at 0–10 cm of soil depth in each experimental plot and pooled. Soil samples were stored at 4 °C and sent to the laboratory for analysis.

2.2. Tannery sludge and soil chemical analyses

The tannery sludge used in the experiment was obtained from the Curtume Vanzella (Rolândia, Paraná, Brazil) and was composed of equal parts of liming sludge and primary waste treatment (WTP) sludge. Table 1 provides details of the sludge used in both applications and results were expressed in dry basis, after drying at 65 °C for 48 h. The concentrations of N-NH_4^+ and N-NO_3^- + N-NO_2 were determined by steam distillation (Mulvaney, 1996); pH and electrical conductivity (EC) were read directly in samples, and total and volatile solids were obtained by drying at 65 °C and 500 °C, respectively (APHA, 2005). Total organic carbon was analyzed by oxidation with dichromate under external heating (Nelson and Sommers, 1996). Total N was determined by the Kjeldahl method after sulfuric digestion (Bremner, 1996); neutralization power via alkalimetry (Brazil, 2007); total Ca, Mg, K, P, S, Na, Mn, Fe, B, Zn, Cu, Mo, Al, As, Cd, Pb, Hg, Ni, Se, Cr concentrations were determined by ICP-AES (model Vista MPX, Varian, Mulgrave, Australia) after nitric digestion in a microwave oven (USEPA, 1986); K and Na in the digest were determined by flame photometer. The tannery sludge used in this study had a relatively low Cr concentration compared to sludges used in other studies (Table 1).

Table 1
Physical and chemical attributes of the tannery sludge used in the experiment.

Variable	1st application	2nd application	Range[c]
pH[a]	12.7	9.7	7.7–11.8
EC (dS m^{-1})[a]	29.5	16.6	–
Total solids, at 65 °C[e]	53.3	55.4	120.0–390.0
Volatile solids[e]	442.0	554.0	–
Neutralization power ($CaCO_3$ eq.)[e]	262.0	361.0	160.0–315.0
Organic C[e]	308.0	321.0	65.0–257.8
Total N[e]	35.7	53.2	9.8–53.4
NH_4^+-N[e]	20.4	21.9	–
NO_3^--N[e]	0.2	0.2	–
C/N ratio	8.7	6.0	4.1–13.8
Ca[e]	78.9	88.0	20.0–210.0
Mg[e]	0.7	1.0	0.2–7.5
K[e]	0.1	3.3	0.1–1.7
P[e]	3.9	3.8	1.1–7.5
S[e]	36.1	43.0	13.0–15.0
Na[e]	10.0	66.9	8.1–59.9
Mn[f]	2858.0	3340.0	128.0–6350.0
Fe[f]	408.0	1249.0	–
B[f]	4.5	5.6	–
Zn[f]	43.3	73.0	48.0–176.0
Cu[f]	4.5	16.0	14.0–81.0
Mo[f]	3.3	<0.5[b]	–
Al[f]	2257.0	13,440.0	–
As[f]	<1.0[b]	<0.5[b]	–
Cd[f]	<1.0[b]	<0.5[b]	0.1–4.0
Pb[f]	<1.0[b]	9.3	15.0–35.0
Hg[f]	<1.0[b]	<0.5[b]	–
Ni[f]	3.0	7.8	1.3–20.0
Se[f]	<1.0[b]	<0.5[b]	–
Total Cr[f,d]	1613.0	580.0	798.0–22,200.0

[a] Measured in *in natura* samples.
[b] Concentrations below the detection limit.
[c] Values obtained from Kray et al. (2008), Alcântara et al. (2007), Barajas-Aceves et al. (2007), Martines et al. (2006) and Barajas-Aceves and Dendooven (2001).
[d] EU upper limit value of chromium allowed for a sludge to be incorporated in agricultural soil according to Directive 86/278/EEC = 1000 mg kg^{-1} (European Community, 1986).
[e] g kg^{-1}.
[f] mg kg^{-1}.

Soil chemical properties were determined as described in Nakatani et al. (2011a). Briefly, the pH and electrical conductivity (EC) were determined in $CaCl_2$ (0.01 mol l^{-1}) and water, respectively. Total organic carbon (Corg) was determined by oxidation with dichromate. Ca, Mg, K, Na, and P were extracted with ion exchange resins. Ca and Mg were determined by atomic absorption spectrometry with flame atomization, Na and K by atomic flame photometry, and P by atomic absorption spectrophotometry. Ammonium and nitrate were extracted with KCl (2 mol l^{-1}) extracts at a 1:10 ratio (m:v). N-NH_4^+ was determined in a continuous flow injection analysis system with spectrophotometric reading at 605 nm (Kamogawa and Teixeira, 2009), while N-NO_3^- was determined at 220 nm and 275 nm (APHA, 2005).

In a previous study we showed that tannery sludge increased soil pH and electrical conductivity and concentrations of available P and mineral N with the strongest increase shortly after application (12 days) (Nakatani et al., 2011b).

2.3. Phospholipid fatty acid (PLFA) analysis

The PLFA were extracted from 4 g of soil (dry weight) using a procedure based on Frostegard et al. (1993) and Bardgett et al. (1996). Lipids were extracted with a monophasic chloroform–methanol-buffer citrate solvent, and the PLFA fraction separated using silicic acid columns before transesterification with mild alkali and final capture in dichloromethane. Methyl-nonadecanoate (C19:0) was added to each sample as an internal standard. Fatty