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INDUSTRIAL AND URBAN ORGANIC WASTES INCREASE SOIL MICROBIAL ACTIVITY AND BIOMASS[1]

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SUMMARY

Microbial processes have been used as indicators of soil quality, due to the high sensitivity to small changes in management to evaluate, e.g., the impact of applying organic residues to the soil. In an experiment in a completely randomized factorial design 6 x 13 + 4, (pot without soil and residue or absolute control) the effect of following organic wastes was evaluated: pulp mill sludge, petrochemical complex sludge, municipal sewage sludge, dairy factory sewage sludge, waste from pulp industry and control (soil without organic waste) after 2, 4, 6, 12, 14, 20, 28, 36, 44, 60, 74, 86, and 98 days of incubation on some soil microbial properties, with four replications. The soil microbial activity was highly sensitive to the carbon/nitrogen ratio of the organic wastes. The amount of mineralized carbon was proportional to the quantity of soil-applied carbon. The average carbon dioxide emanating from the soil with pulp mill sludge, corresponding to soil basal respiration, was 0.141 mg $C\text{-}CO_2$ 100 g^{-1} soil h^{-1}. This value is 6.4 times higher than in the control, resulting in a significant increase in the metabolic quotient from 0.005 in the control to 0.025 mg $C\text{-}CO_2$ g^{-1} C_{mic} h^{-1} in the soil with pulp mill sludge. The metabolic quotient in the other treatments did not differ from the control ($p < 0.01$), demonstrating that these organic wastes cause no disturbance in the microbial community.

Index terms: microbial respiration, microbial carbon, microbial nitrogen, metabolic quotient, organic sludge.

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RESUMO: *RESÍDUOS ORGÂNICOS INDUSTRIAIS E URBANOS AUMENTAM A ATIVIDADE E A BIOMASSA MICROBIANA DO SOLO*

Processos microbianos têm sido utilizados como indicadores de qualidade do solo em razão da grande sensibilidade a pequenas mudanças no manejo - por exemplo, na avaliação do impacto da incorporação de resíduos orgânicos ao solo. Em experimento em delineamento inteiramente casualizado com esquema fatorial 6 x 13 + 4 (vaso sem solo e sem resíduo), foi avaliado o efeito dos seguintes resíduos orgânicos: lodo de fábrica de celulose, lodo de complexo petroquímico, lodo de esgoto urbano, lodo de esgoto de laticínio, resíduo da indústria de polpas de frutas e controle (solo sem resíduo orgânico), aos 2, 4, 6, 12, 14, 20, 28, 36, 44, 60, 74, 86 e 98 dias após a incubação, em alguns atributos microbianos do solo, com quatro repetições. O estímulo microbiano no solo foi altamente sensível à relação carbono / nitrogênio dos resíduos orgânicos. A quantidade de carbono mineralizado foi proporcional à quantidade de carbono adicionada ao solo. A quantidade de dióxido de carbono liberado do solo que recebeu o resíduo da fábrica de celulose, correspondendo à respiração basal do solo, foi de 0,141 mg C-CO_2 100 g^{-1} solo h^{-1}, sendo 6,4 vezes mais que o controle, resultando em aumento significativo no quociente metabólico de 0,005 no controle para 0,025 mg C-CO_2 g^{-1} C_{mic} h^{-1} no solo com resíduo de celulose. O quociente metabólico nos demais tratamentos não diferiu daquele do controle ($p < 0,01$), o que demonstra que esses resíduos orgânicos não causam distúrbios na comunidade microbiana.

Termos de indexação: respiração microbiana, carbono microbiano, nitrogênio microbiano, quociente metabólico, lodo orgânico.

INTRODUCTION

In Brazil, in 2008 and 2009, approximately 46,550.088 and 50,258.208 tons of municipal solid organic waste were removed from sewage systems (ABRELPE, 2009). Of these, 57 and 55 %, respectively, were disposed of appropriately (ABRELPE, 2009). The use of organic wastes from production processes as a source of plant nutrition in agricultural soils is one of the ways to employ these materials, as they can increase fertility and improve soil and physicochemical properties, as well as microbial activity (Brady & Weil, 2002).

When municipal organic waste is applied, the nutrients must be strictly monitored. The effect of organic wastes on the physicochemical characteristics of soil (De Maria et al., 2010; Araújo, 2011), their biological properties (Brady & Weil, 2002; Bueno et al., 2011) and crop yields (Nogueira et al., 2008; Araújo, 2011) has been investigated in numerous studies.

The complexity of anthropogenic substances and the nutrient quantity applied through organic wastes to soils calls for the constant monitoring of the agricultural ecosystems. Organic wastes used in quantities above the soil potential or crop needs can cause environmental problems associated with nitrogen (N) losses, pathogen increases and heavy metals in the soil (Silva et al., 2006) or with acidification (Boeira & Souza, 2007) or salinization of agricultural soils (Teixeira et al., 2006).

Microbiological parameters such as soil microbial biomass, soil basal respiration and metabolic quotient (qCO_2) have been used extensively as indicators of environmental impacts, because of their sensitivity to detect natural and anthropogenic sources of soil changes (Chaer & Tótola, 2007). Abiotic conditions such as soil moisture, temperature, aeration and fertility, among others, are directly related to soil microbial population and activity.

The microbial biomass carbon (C_{mic}) is related to several processes: the decomposition of organic composts, nutrient cycling, nutrient solubility, degradation of xenobiotic compounds and pollutants, soil structure and biological pathogen control and is therefore seen as an important component of soil quality and productivity, as it responds more promptly to environmental changes than any other agronomic parameters (Kaschuk et al., 2009).

Soil basal respiration is the total sum of all metabolic functions in which carbon dioxide (C-CO_2) is produced. The most widely used technique to quantify microbial activity is the evaluation of soil basal respiration, which was positively related to organic matter and microbial biomass content. The combined assessment of microbial biomass and soil respiration provides the amount of carbon dioxide developed per biomass unit, called metabolic quotient (qCO_2).

The purpose of this study was to evaluate the impact of different types of organic wastes on some microbial properties of an Oxisol Ustox.

MATERIAL AND METHODS

Soil and organic wastes

Chemical analysis data with assured quality standard of the soil and wastes were obtained from a laboratory of the Brazilian Agricultural Research

Corporation (Embrapa) in Cruz das Almas, Bahia, Brazil. Chemical analysis was performed according to Embrapa (1999) and total carbon (SOC), ammonium nitrogen (N-NH_4^+), nitrate nitrogen (N-NO_3^-), and total nitrogen (N) of all organic wastes analyzed by Tedesco et al. (1995).

The soil was classified as Oxisol Ustox (USDA, 1999) or "Latossolo Amarelo", according to the Brazilian System of Soil Classification (Embrapa, 2006). The analysis results (sampling layer 0–20 cm) are presented in table 1.

The following organic wastes were used in the experiment and the chemical properties of the organic wastes are presented in table 2:

1. Treated pulp mill sludge (PMS): the raw material for this industry consists of newspaper, books, bank and office wastes and other recyclable paper. The residues were treated with physical, chemical and biological processes and the activated sludge process to satisfy the biochemical oxygen demand and eliminate the resin concentration by liming (CaO) to eliminate pathogens and promote organic waste stabilization;

2. Petrochemical complex sludge (PS): residue from a sewage treatment plant of an industrial petrochemical complex. This industrial sludge had undergone an aerated biological treatment;

3. Treated municipal sewage sludge (MSS): organic waste from a sewage treatment plant where physical processes were used to remove coarse solids, biological activated sludge to eliminate the biochemical oxygen demand, and liming (CaO) to eliminate pathogens and stabilize wastes;

4. Treated dairy industry sludge (DIS): organic waste was generated by the dairy industry, in cheese, butter and milk production. The wastes were aerated in a pond to reduce the organic load and eliminate pathogens, and chemically stabilized by applying lime (CaO) in a sewage treatment plant in the industrial area;

5. Organic waste from the fruit pulp industry (FPW): organic waste consisting of fruit peel and seeds left over from the production of frozen fruit pulp. The organic wastes were stored in piles for three years for primary fermentation processes and partial decomposition of the material and stabilization of the organic loading.

The micronutrients and trace-elements in all residues studied were lower than the maximum permissible concentration (MPC) in biological sludge recommended for agricultural use (Conama, 2006; USEPA, 1993; Brasil, 2006) (Table 2).

Laboratory study

Prior to the experiment, about 100 kg of soil was sieved (Ø ≤ 4.75 mm), homogenized, moistened (70 % of field capacity) and stored in dark plastic bags at room temperature for 21 days for the recovery of the microbial communities.

The study was conducted in a laboratory using a BOD incubator without light, at controlled temperature (25 ± 0.20 °C) and humidity close to 70 % of field capacity. The moisture content was checked by weighing every two days and adjusted with deionized water.

The quantity of organic waste added to each treatment was calculated to provide a fixed N amount. The treatments were arranged in a factorial design 6 x 13 + 4 (pot without soil and residue or absolute control), i.e., five wastes were tested at application rates of 27.0 (PMS), 22.2 (PS), 3.0 (MSS), 5.2 (DIS) and 5.2 (FPW) Mg ha^{-1} on a dry matter basis (defined as the supply of 100 kg ha^{-1} N calculated based on N-Kjeldahl or total N), and soil without organic waste (control), evaluated 2, 4, 6, 12, 14, 20, 28, 36, 44, 60, 74, 86, and 98 days after incubation, in a completely randomized design, with four replications, plus four blank controls (bottles without soil and sludge), to eliminate the contamination effect of atmospheric carbon dioxide (CO_2) on the system.

Chemical analysis

The soil basal respiration (C-CO_2) was determined by incubating 100 g soil (dry weight) with the treatments in glass jars (height 18 cm, diameter 12 cm) sealed with plastic lids. The humidity was adjusted to 70 % of field capacity. A bottle containing 10 mL of 1 mol L^{-1} NaOH was placed on the soil surface of each jar to absorb CO_2. On the dates cited above, CO_2 was withdrawn from the bottle with a solution of NaOH and added to 5 mL of 4 mol L^{-1} $BaCl_2$ and 3 drops of phenolphthalein indicator at 1 %. The amount of CO_2 released from the soil was determined by titration of excess NaOH with 1 mol L^{-1} HCl solution.

Table 1. Chemical properties and particle-size distribution of the soil used in this study

Chemical property												Particle-size		
pH (H_2O)	P[(1)]	Ca^{2+}	Mg^{2+}	K^+	H+Al	CEC	N-NH_4^+	N-NO_3^-	SOC[(2)]	SOM[(3)]	N[(4)]	Clay	Silt	Sand
	mg dm^{-3}	———	———	$cmol_c$ dm^{-3}	———	———	———	mg kg^{-1}	———	———	———	———	g kg^{-1}	———
5.20	2.00	1.12	0.23	0.04	2.09	2.97	57.12	100.80	3.53	6.08	1.05	172	74	754

[(1)] P-Mehlich-1; [(2)]SOC: soil organic carbon; [(3)]SOM: soil organic matter (Embrapa, 1999). [(4)] Kjeldahl-Nitrogen.

Table 2. Chemical properties of organic wastes used in the experimental trials

Attribute	Organic waste[(1)]				
	PMS	PS	MSS	DIS	FPW
pH H_20 (1:2.5)	8.30	7.40	5.67	6.90	5.40
P-Mehlich-1 (g dm^{-3})	0.28	4.04	9.49	15.00	0.51
Ca^{2+} ($cmol_c$ dm^{-3})	190.23	116.80	59.86	198.20	28.20
Mg^{2+} ($cmol_c$ dm^{-3})	16.61	5.48	27.18	13.05	18.50
Organic carbon (g kg^{-1})	236.40	34.40	235.00	161.60	232.40
Organic matter (g kg^{-1})	407.55	59.31	405.14	278.60	400.60
N-NH_4^+ (mg kg^{-1})	263.20	750.12	8619.80	6182.40	460.60
N-NO_3^- (mg kg^{-1})	171.08	855.40	421.12	36.96	881.72
N-Kjeldahl (g kg^{-1})	3.72	4.49	32.63	19.20	19.50
Cu (mg kg^{-1})	162.20	373.20	334.02	141.79	101.72
Fe (mg kg^{-1})	3,240.86	7,640.86	7,364.59	11,752.69	701.08
Mn (mg kg^{-1})	50.34	83.58	113.39	292.28	68.43
Ni (mg kg^{-1})	0.70	1.10	0.77	1.13	0.27
Cd (mg kg^{-1})	0.11	0.12	0.12	0.12	0.10
Pb (mg kg^{-1})	37.61	32.48	8.55	44.44	3.42
Cr (mg kg^{-1})	6.22	4.22	3.82	6.83	0.60
C/N ratio	63.55	7.66	7.20	8.42	11.92

[(1)] PMS: Treated pulp mill sludge; PS: Petrochemical complex sludge; MSS: Treated municipal sewage sludge, DIS: Treated dairy industry sewage sludge; FPW: Organic waste from the fruit pulp industry. Values on a dry-matter basis.

The microbial biomass carbon and nitrogen were determined by the method described by Vance et al. (1987), using, instead of chloroform, a Philco microwave oven at a frequency of 2,450 MHz and power of 1.35 KW for 3 min to kill microorganisms and trigger the release of cellular components (Ferreira et al., 1999).

A solution of K_2SO_4 0.5 mol L^{-1} (soil:extractant = 1:4) was added to the radiated and non- radiated soils followed by horizontal circular shaking at 220 rpm for 30 min. The extracts were filtered through Whatman® n° 42 filter paper (diameter 7 cm). The microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) contents in extracts were determined by the wet combustion method and Kjeldahl-N (Tedesco et al., 1995).

Calculation of microbial properties

The soil basal respiration was measured by the sum of CO_2 released during the entire incubation period divided by the duration in hours. The C-CO_2 released per hour of incubation period was calculated by equation 1 and expressed in mg C-CO_2 100 g^{-1} soil h^{-1}:

$$SBR = (((Vb-Va)xMx6x\ 1000)/Ps)/T \quad (1)$$

where, SBR = carbon derived from soil basal respiration (mg C-CO_2 g^{-1} h^{-1}), Vb (mL) = volume of hydrochloric acid used for titration of the control solution (blank), Va (mL) = volume required in the sample; M = HCl molarity; 6 = equivalent weight of C-CO_2. According to the Richter Law, the equivalent weight of an element or substance is the mass of the substance corresponding to 8 g of oxygen (standard most commonly used). In the case of CO_2, it was found that the proportion of the elements C and O is 3:8 g; Ps (g) = dry soil mass; and T (h) = time.

The microbial biomass carbon (C_{mic}) was calculated by following equation 2 and expressed in mg 100 g^{-1} soil:

$$C_{mic} = E_C / K_C \quad (2)$$

where, E_C = (organic C extracted from radiated soil) - (organic C extracted from non-radiated soil) and K_C = conversion factor of 0.33 (Islam & Weil, 1998), for fumigation extraction or radiation extraction method, i.e., a weighting factor (C mineralization - a proportion of microbial C released as CO_2 during incubation).

The microbial biomass nitrogen (N_{mic}) was calculated by following equation 3 and expressed in mg 100 g^{-1} soil:

$$N_{mic} = E_N / K_{EN} \quad (3)$$

where, E_N = (total N extracted from radiated soil) - (total N extracted from non-radiated soil) and K_{EN} = is a constant representing the N rate of mineralized microbial biomass. The K_{EN} value used in this study was the factor 0.54, as suggested by Brookes et al. (1985).

The metabolic quotient (qCO_2) was calculated as the ratio between soil basal respiration rate and microbial biomass C and expressed as mg CO_2 g^{-1} C_{min} h^{-1} (Anderson & Domsh, 1993).

Statistical analysis

Data were subjected to analysis of variance (ANOVA). The Scott-Knott's test at a significance level of $p < 0.01$ was used to compare mean values for each variable studied. The program Statistical Analysis System was used to analyze the data (SAS, 2004).

RESULTS AND DISCUSSION

Soil basal respiration

The soil basal respiration ranged between 0.022 (control) and 0.141 (PMS) mg C-CO_2 g^{-1} soil h^{-1} (Table 3). The soil microbial stimulation was highly sensitive to the C/N ratio in the organic wastes. Thus basal respiration in the soil treated with PMS was 6.4 times higher than in the control. Reports from Cleveland et al. (2007) showed that the increased response in soil microbial activity was associated with readily available C sources in the soil.

The basal respiration from soil with DIS, PS and MSS was significantly different; however these wastes had a very similar C/N ratio (Table 2). The amounts

of organic waste added to the treatments provided a fixed amount of N (equivalent to 100 kg ha^{-1}). The treatments with organic wastes with a high C/N ratio, such as PMS (63.55) (Table 2), received a higher concentration of C compounds. The organic waste treatments with low C/N ratio such as MSS (7.20), PS (7.66) and DIS (8.42) were treated with a proportionately lower amount of soil-applied C, except for FPW, which had a higher C/N ratio than PS, MSS and DIS (Table 2), but lower basal respiration, not significantly different from the control. This was justified by the presence of recalcitrant C in the FPW, as a result of the stabilization process.

According to Smith & Bradford (2003), the low C/N ratio in waste accelerates the residue decomposition; however the opposite is also true, since a higher C/N ratio (63.55) and soil basal respiration rate were observed in pulp mill sludge. The reason is that when the C/N ratio is appropriate, microorganisms absorb C and N, regardless of whether the raw material to be degraded has a C/N ratio of 80/1 or of 8/1 (Kiehl, 2002). With the incorporation into the soil, the C/N ratio is adjusted, so that when the compound is humified, the C/N ratio will be around 10/1 (Kiehl, 2002).

Microbial biomass carbon

Microbial activity ranged from 4.63 (control) to 13.22 mg C_{mic} 100 g^{-1} in soil treated with petrochemical sludge. In the soils treated with residues, the resulting microbial activity was higher than in the control soil, except for the soil with pulp mill sludge, which was statistically similar to the control (Table 3). The behavior of soil C_{mic} from best to worst was: soil with PS > MSS > DIS = FPW > PMS. The different stimulation in the soil microbial population activity resulted from the C/N ratio of organic wastes, because the C and N content generally stimulate the development of the microbial soil community (Tables 2 and 3).

The PS applied to the soil increased microbial populations, when comparing the values for attribute C_{mic} with the other treatments (Table 3), although this increase was not observed for basal respiration (Table 3). Thus it was concluded that this organic waste caused less disturbance to these populations, allowing them to develop. A similar behavior was observed in soil with MSS, DIS and FPW; although they showed high values for this attribute, however lower than the soil with petrochemical sludge.

The increases in C_{mic} by PS, MSS, DIS and FPW application were accompanied by low rates of C-CO_2 emission, confirming that these wastes positively influenced the growth of native microbes, did not reduce the size of microbial communities and provided labile C (Table 3).

The differences in the substrate quantity and quality with addition of organic matter associated with specific nutrients may have been crucial to influence the soil microbial biomass development in this case (Feng et al., 2009; Vieira et al., 2011).

Moreover, it is interesting to emphasize that the C/N ratio of the wastes seems to affect the microbial biomass, since pulp mill sludge, in spite of the lower N content in its composition, was decomposed quickly and always sustained less microbial biomass than other wastes (Table 3).

Metabolic quotient

The metabolic quotient (qCO_2) of the soil is a sensitive indicator of the biological activity and substrate quality. The qCO_2 ranged from 0.002 (PS) to 0.025 mg CO_2 g^{-1} C_{mic} h^{-1} (PMS).

The qCO_2 of the soil treated with PMS was higher than in the other treatments, which did not differ significantly from each other (Table 3). Anderson & Domsch (1993) interpreted the quotient as a possible indicator of environmental stress that is directly related to the energy demand of maintenance of a

Table 3. Soil microbial properties after 98 days of incubation with industrial and urban organic wastes

Treatment[(1)]	N_{mic}	C_{mic}	Basal respiration	qCO_2
	mg 100 g^{-1} soil		mg C-CO_2 100 g^{-1} soil h^{-1}	mg C-CO_2 g^{-1} C_{mic} h^{-1}
Control	3.81 a	4.63 d	0.022 d	0.005 b
PMS	1.01 d	5.73 d	0.141 a	0.025 a
PS	4.58 a	13.22 a	0.029 d	0.002 b
MSS	3.06 b	10.42 b	0.039 c	0.004 b
DIS	2.30 c	7.62 c	0.046 b	0.006 b
FPW	3.10 b	8.29 c	0.027 d	0.004 b
CV (%)	20.05	18.18	8.06	26.17

(1) Control: soil without organic waste; PMS: pulp mill sludge, PS: Petrochemical sludge, MSS: municipal sewage sludge, DIS: dairy industry sewage sludge, FPW: waste from fruit pulp industry; C_{mic}: microbial biomass carbon; qCO_2: metabolic quotient; N_{mic}: microbial biomass nitrogen; CV: coefficient of variation. Means followed by the same letters do not differ by Scott-Knott test ($p < 0.01$).

microbial community (stress situation); this value increases to a maximum point, after which it decreases as the environment recovers over time.

Therefore, pulp mill sludge initially induces stress in the soil native microbial population, but after some time the population will tend to return to the previous stage. This process is known as soil resilience and is a component of stability and is defined as the recovery over time after a stress, while the other stability component is the immediate response to the effect of the stress on the soil defined as resistance (Seybold et al., 1999).

According to Insam & Domsch (1988), soil basal respiration per unit microbial biomass decreased in more stable systems. Based on this information it can be concluded that PS, MSS, DIS and FPW caused less stress to the microbial environment, while PMS stressed the microbial environment most, with low N concentration and high C/N ratio (Table 2).

Microbial biomass nitrogen

The microbial biomass nitrogen (N_{mic}) ranged from 1.01 (PMS) to 4.58 mg 100 g^{-1} soil (PS). The biomass N content were separated into four groups: the group of treatments with higher N content in biomass PS and control (4.58 and 3.81 mg 100 g^{-1}), the group with medium content, FPW and MSS (3.1 and 3.06 mg 100 g^{-1}), and the treatments groups with low contents as DIS (2.3 mg 100 g^{-1}) and very low as PMS (1.01 mg 100 g^{-1}) observed in table 3.

According to Gama-Rodrigues (1999), in environments with high concentration of N, the amount of N immobilized by soil microbial biomass would be lower, since this element would be sufficient to meet the metabolic activity of microorganisms and the process of organic matter decomposition. Possibly this may be the reason for the values found in the wastes with low C/N ratio, except the soil with PS.

In soil treated with PS, MSS, FPW and DIS, the attribute N_{mic} (Table 3) has the same behavior as C_{mic} (Table 3), which can be related to the growth of microbial populations stimulated by the incorporation of organic residue into the soil. It was therefore concluded that the stress induced in the microbiological systems should not be determined by a single parameter but by a relationship between several properties.

Anderson & Domsch (1980) observed that microorganisms differ far more in the N than in the C content, acccording to their growth stage. Therefore, small changes in the microbial biomass structure can result in major changes in microbial protein synthesis. In the short term, the addition of organic wastes to the soil probably affects a small, active part of the microbial biomass, whereas the long-term response will eventually affect the composition by selection.

The Nmic value was lowest for PMS-treated soil, probably due to the high C/N ratio of the material and low N content (Table 2), which negatively affected the N accumulation by microorganisms that had to use soil-available N to degrade the high concentration of labile organic C applied to the soil, confirming the stress on the microbial population.

Micronutrients and trace-element content

The micronutrients and trace-elements in all organic wastes studied did not reach the maximum permissible concentration (MPC) in biological sludge recommended for agricultural use (Conama, 2006; USEPA, 1993; Brasil, 2006) (Table 2). According to Araújo & Nascimento (2005), sludge application increased the available Zn content extracted by DTPA, although the available Zn content did not increase as a function of the incubation period.

The Zn mobility decreased in the sludge-incubated soils, since the organically bound Zn was mainly redistributed to residual fractions. The maximal annual application rate of Zn via sewage sludge suggested by the U.S. Environmental Protection Agency (USEPA, 1993) did not lead to phytotoxic Zn concentrations in soils (Araújo & Nascimento, 2005).

CONCLUSIONS

1. The petrochemical waste stands out from other wastes because it increases the soil microbial biomass nitrogen and soil microbial biomass associated with low loss of carbon in the carbon dioxide form.

2. The application of municipal sewage sludge followed by dairy industry sewage sludge and waste from fruit pulp industry had a positive impact on the analyzed soil microbial properties (metabolic quotient, microbial biomass carbon and nitrogen), with little loss of carbon dioxide followed by a growth of the microbial population.

3. The pulp mill sludge "in natura" has a high C/N ratio, which does not allow its use in view of the disturbances it would cause in the soil microbiota, such as a high metabolic quotient, high release of carbon dioxide and low growth of the microbial population.

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