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Chemical and microbiological properties in soil cultivated with sugarcane (*Saccharum officinarum*)

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Abstract

The aim of this study was to evaluate the response of chemical parameters and microbiological processes related to the nitrogen (N) cycling in an area cultivated with sugarcane (SC), as compared to the native forest area (NF), considered as the reference. The pH value, the total C (C_{tot}), N (N_{tot}) contents, the P, K, Ca, Mg, Zn, Mn, B and Cu contents, the labile carbon (LC) content, cation exchange capacity (CEC), microbial biomass N (N_{mic}), potentially mineralizable nitrogen (PMN) and the urease activity (UA) were determined in soil samples taken at depths of 0-10 and 10-20 cm. Most of the chemical properties were higher in the NF soil at both depths, except for C_{tot}, N_{tot} and the total K content, which did not present significant differences between the areas at the deeper level. All microbiological processes were higher in the NF soil and showed positive correlations with the total Cu and B contents, demonstrating the importance of these nutrients in the biological N cycling. The higher values obtained for almost all parameters in the NF soil attest to the need for constant monitoring of areas cultivated with sugarcane in order to avoid the adverse effects of soil degradation. The results obtained between the areas, in relation to N cycling processes also demonstrated the suitability of using them as reliable indicators of soil quality.

Keywords: N cycling, urease activity, N immobilization, mineralizable nitrogen.

Introduction

Biofuels are considered to be one of the most promising alternatives to the use of fossil fuels, and in Brazil they are practically all sugarcane derivatives. Currently, Brazil is the world's largest producer of sugarcane. The cultivation area covers about 9.1 million hectares. Although Brazilian sugarcane production is significant, an additional of 6.4 million hectares would be required to meet the internal demand for ethanol projected for 2021. This is considered to be one of the main causes of land use changes in the south central region of Brazil (Goldemberg et al., 2014). However, the destruction of complex ecosystems to cultivate sugarcane can result in modifications of various soil properties, influencing the sustainability of the production systems (Walter et al., 2014).

It is thought that some biological properties of the soil are sensitive to changes when the soil is subjected to any type of anthropogenic activities. Quantifying the changes in those properties has been a key tool for monitoring soil quality (Neves et al., 2007). According to Marinari et al. (2006), microorganisms can show the changes caused by soil cultivation more quickly, since they are more susceptible to changes imposed by the environment. Of the microbiological parameters used to study the effects of agricultural soil use, the following stand out: microbial biomass since it represents the most active reservoir of organic matter (Roscoe et al., 2006), enzyme activities since they are essential for soil element cycles (Silva et al., 2012) and

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the potentially mineralizable nitrogen, which provides information about the ability of the soil to make N available to the cultures (Dilly et al., 2003).

The aim of this study was to evaluate the impact of long-term sugarcane cultivation on some chemical soil parameters and processes related to the nitrogen cycle.

Material and Methods

The study was carried out in a commercially cultivated sugarcane area (SC) and in a native forest (NF) area with no history of agricultural interference, which could therefore serve as the reference for the initial soil condition. Historically, the sugarcane area has been cultivated with sugarcane for more than 15 years. The two areas were located in adjacent positions in the municipality of Guaira, State of São Paulo, Brazil (latitude 20^{0}19'06" S, longitude 48^{0}18'38" W). The regional climate is of the Aw type according to the Köppen classification system. The minimum and maximum mean annual temperatures in the region are between 12 and 20^{0}C and between 28 and 33^{0}C, respectively. The rainfall varies from 12.3 mm to 267.3 mm (http://www.cpa.unicamp.br), concentrated in the hot months from November to March.

The soil samples were taken on October 24th 2012 from an area of approximately 2000 m^2 cultivated with sugarcane, subdivided into 500 m^2 quadrants from where six subsamples were randomly taken at depths of 0-10 cm and 10-20 cm. Homogenization of the subsamples resulted in four compound samples per depth. The samples were taken 20 cm from the plantation line, and those from the forest area were taken following the same system.

Table 1 shows the chemical attributes of the soils (Red Acriferric Latosol with 65% clay). The macronutrient (P, K, Ca e Mg) and micronutrient (Zn, Mn, B e Cu) contents and the cation exchange capacity (CEC) were determined according to Camargo et al. (2009). The pH was measured in an aqueous extract in a 1:2.5 soil:water proportion. The total C (C_{tot}) and N (N_{tot}) contents were determined using an N and C element analyzer (Truspec-Leco) and the labile carbon (LC) was extracted using 0.5 M K_2SO_4 and quantified in a total organic C analyzer (TOC – 500 Shimadzu). The microbial biomass N content (N_{mic}) was determined using the fumigation-extraction method described by Brookes et al. (1985) with a k_{EC} factor of 0.54.The total N content of the extracts was determined by the Kjeldahl digestion method (Bremner, 1996). The potentially mineralizable nitrogen (PMN) and urease activity were estimated using the anaerobic incubation method described by Kandeler (1996) and the method described by Tabatabai and Bremner (1972), respectively.

Table 1. Chemical characteristics of the soils

	Depths of soil sample collection			
	0-10 cm		10-20 cm	
	Soil Cultivated with sugarcane (SC)	Native Forest Soil (NF)	Soil Cultivated with sugarcane (SC)	Native Forest Soil (NF)
pH (H_2O)	6.11 a	4.55 b	5.38 a	4.25 b
Total N (N_{tot}), %	0.17 b	0.29 a	0.15 b	0.19 b
Total C (C_{tot}),%	2.10 b	3.50 a	1.91 b	2.13 b
Labile C (LC), µg g^{-1} soil	65.71 b	88.53 ab	44.07 c	118.80 a
C/N	12.00 a	12.00 a	13 a	11:00 AM
P, mg dm^{-3}	21.50 a	18.25 b	25.25 a	15.00 b
K, mmol$_c$dm^{-3}	8.58 a	5.03 b	5.40 b	5.40 b
Ca, mmol$_c$dm^{-3}	72.38 a	35.00 b	44.25 b	7.00 c
Mg, mmol$_c$dm^{-3}	19.88 a	18.75 a	9.00 b	4.50 c
CTC, mmol$_c$dm^{-3}	120.01 a	126.78 a	83,65 b	88.90 b
Mn, mg dm^{-3}	35.23 b	42.05 a	19.77 c	23.70 bc
Cu, mg dm^{-3}	4.50 b	5.78 a	3.85 bc	6.00 a
Zn, mg dm^{-3}	1.96 a	0.75 b	2.70 a	0.35 b
B, mg dm^{-3}	0.22 b	0.39 a	0.17 b	0.30 a

Means followed by the same letters in the same line are not significantly different (t test, $p \leq 0,05$)

All analyses were carried out with the natural soil moisture content and the results expressed per gram of dry soil. The data were discussed considering the results within each soil depth.

For the purposes of the statistical analysis, each quadrant was considered as a plot, used as the replicates during sampling and analysis. The dataset was subjected to a one-way ANOVA according to an entirely randomized design, followed by a means comparison using the t test ($p \leq 0.05$). A correlation matrix of the different properties was based on the Pearson correlation coefficients.

Results and Discussion

As compared to the NF soil, the pH of the soil in the SC area was higher at both depths (Table 1). These results corroborate those obtained by Corrêa et al. (2001). The lower pH values in the NF area could be due to a greater accumulation of humus as compared to the soil cultivated with sugarcane.

The C_{tot} content was 71% higher in the native forest soil at a depth of 0-10 cm, but at 10-20 cm there was no significant difference for this parameter between the two areas (Table 1). Marchiori Junior and Melo (2000) found smaller amounts of organic carbon in soil cultivated with sugarcane at depths of both 0-10 cm and 10-20 cm, as compared to those found in natural forest soil. The smaller value found for C_{tot} in the surface layer of SC soil could be related to the increase in the mineralization rate of the organic matter (OM), when NF is turned over to agriculture. In native forest soil the processes of adding and losing organic C are in a state of equilibrium, which can rapidly be undone when the forest is cut down for agricultural purposes. This occurs as a function of soil disturbance, which breaks up the aggregates that provide physical protection to the organic matter of the soil, thus exposing it to greater microbial degradation.

The results obtained for LC showed significant differences between the treatments at both depths, and were higher in the NF soil, the differences being 35% and 170% for the depths of 0-10 cm and 10-20 cm, respectively (Table 1). These results were to the contrary of those obtained for C_{tot}, showing that this last parameter is not always adequate to verify the sustainability of different types of soil management. This could also be observed from the lack of correlation between the C_{tot} and LC contents (Table 2).

Table 2. Pearson's correlation analysis between the variables measured

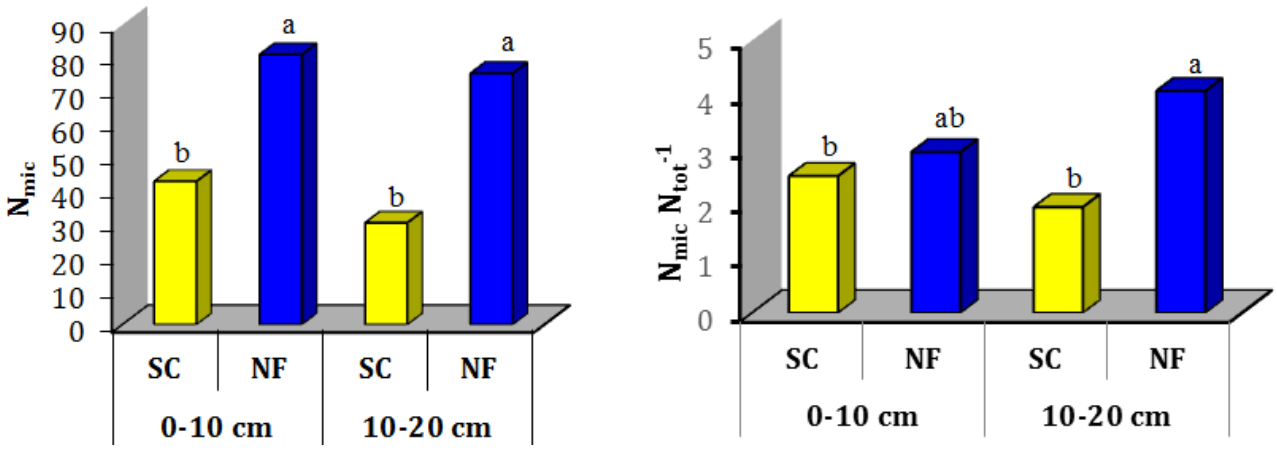
	C_{tot}	N_{tot}	LC	N_{mic}	MPN	UA
C_{tot}	1.00					
N_{tot}	0.94***	1.00				
LC	NS	NS	1.00			
N_{mic}	0.65**	0.56*	0.78**	1.00		
PMN	0.90***	0.97***	0.54*	0.70**	1.00	
UA	0.73**	0.77**	0.81*	0.88***	0.87***	1.00
pH	NS	NS	-0.73**	-0.75**	-0.57*	-0.79**
P	NS	NS	-0.74**	-0.71**	NS	-0.65**
Cu	0.55*	0.59**	0.75**	0.79**	0.69**	0.84***
Zn	NS	NS	-0.69**	-0.71**	-0.55*	-0.73**
B	0.84***	0.82***	0.64**	0.86***	0.89***	0.91***

$p \leq 0.001$***; $p \leq 0.01$**; $p \leq 0.05$*

The soil N_{tot} contents were higher in the NF soil, but only in the surface layer. Souza et al. (2012) verified higher N_{tot} contents in native forest soil at a depth of 0-20 cm, when compared to soil cultivated with sugarcane, harvested without burning. Despite the differences between the values obtained for C_{tot} and N_{tot}, the C/N ratios were similar for the two soils at the two depths, demonstrating differences in the compositions of degradable humic compounds between the two areas (Dilly et al., 2003). In the same way as that occurring with the C_{tot}, the N_{tot} did not present correlation with the contents of LC (Table 2).

The values for P, Ca and Zn were higher in the soil cultivated with sugarcane. Corrêa et al. (2001) also reported increases in the P and Ca contents in an area cultivated with sugarcane, as compared to a forest area. The Mg contents showed no differences between the two areas at a depth of 0-10 cm, but were higher in the NF soil at the depth of 10-20 cm. The soil K contents were higher in the area cultivated with sugarcane at the lower depth. The greater concentrations of some elements in the area cultivated with sugarcane could be related to the chemical fertilizations performed on this soil. For example, this fact could be observed from the absence of correlation between the P and Zn contents and the soil C_{tot} and N_{tot} contents (Table 2). The Mn contents showed tendencies to be higher in the NF soil at both depths, while the Cu and B contents were higher in the NF soil. No significant differences were found between the soils with respect to the CEC value.

The soil N_{mic} values were about 89% and 147% higher in the NF area, at the lower and greater depths, respectively (Figure 1a). These results demonstrate that the NF soil has a greater N immobilization capacity by the microorganisms, suggesting a reduced loss of this element to the environment. The microbial biomass is an important factor responsible for the liberation of nutrients during the turnover of organic matter, and reductions in this parameter have been associated with decreases in the transformation rates and availability of N in the soil (Tan et al., 2008). The positive correlations between the N_{mic} and the soil Cu ($r = 0.79$, $p \leq 0.01$) and B contents ($r = 0.86$, $p \leq 0.01$) demonstrate the importance of these elements in the incorporation of N by the soil microbiota (Table 2).



(a) (b)

Figure 1. (a) N_{mic}, N immobilized by microorganisms, (µg g^{-1} soil); (b) N_{mic} N_{tot}^{-1}, ratio between the N immobilized by microorganisms and the total N content of the soil, (%). SC, soil cultivated with sugarcane. NF, native forest soil. 0-10 and 10-20 cm, depths of soil sample collection. Means followed by the same letters are not significantly different (t test, $p \leq 0.05$)

The specific efficiency of the conversion of organic N into N_{mic} can be calculated from the N_{mic} N_{tot}^{-1} ratio and can be interpreted as the availability of substrate and the portion of total N immobilized on the microbial cells. There were no significant differences between the treatments for this parameter at a depth of 0-10 cm, but at the greater depth the ratio was 110% greater in the NF soil, despite there were no differences between treatments in relation to the soil N_{tot} (Figure 1b). This fact could be related to the greater amount of readily available organic N in the NF soil at the greater depth, or the occurrence of different microbial communities in the two soils

The PMN was greater in the NF soil at both depths (Figure 2), the percent differences being 217% and 248% at the lower and greater depths, respectively. This parameter refers to the soil´s ability to transform organic nitrogen compounds into ammonium/nitrate under optimal conditions of humidity and temperature, for a given period of time. Soil microorganisms are the primary agents responsible for the mineralization of organic N. They enzymatically degrade those compounds in simpler constituents such as amino acids, glucosamines and ammonium and immobilize them via cellular uptake to synthesize polymers used for growth (Paul and Clark, 1996). According to Dilly et al. (2003) the N mineralization rate is an indicator of the amount of biologically active N in the soil, and can provide indicatives concerning the potential of the soil to provide N to the cultures. The greater PMN values in the forest soil at both depths, suggests the occurrence of a greater organic matter turnover rate, and consequently a greater nutrient availability. This can be confirmed by the high correlation obtained between the PMN and the N_{tot} (r = 0.97, $p \leq 0.001$), C_{tot} (r = 0.90, $p \leq 0.001$) contents and the N_{mic} (r = 0.70, $p \leq 0.01$) (Table 2).

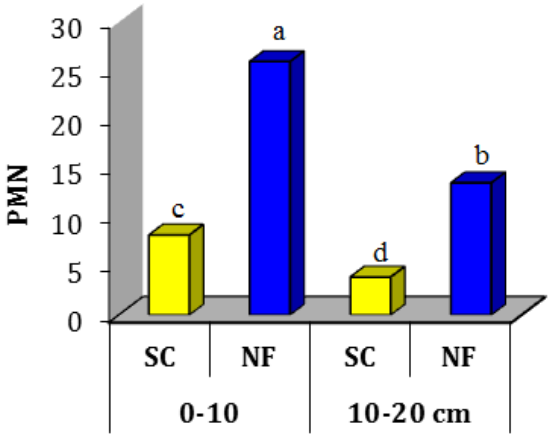


Figure 2. PMN, potentially mineralizable nitrogen, (µg NH_4^+ g^{-1} soil day^{-1}). SC, soil cultivated with sugarcane. NF, native forest soil. 0-10 and 10-20 cm, depths of soil sample collection. Means followed by the same letters are not significantly different (t test, $p \leq 0.05$)

The enzyme urease is one of the most frequently evaluated enzymes, since it has a great influence on the transformation and destiny of an important fertilizer that is the urea. This enzyme catalyzes the hydrolysis of organic N into inorganic forms, using substrates like urea. The UA values were different between the two soils at both depths (Figure 3). At the depths of 0-10 cm and 10-20 cm the values obtained in the forest soil were respectively 159% and 259% higher than those obtained in the soil cultivated with sugarcane. Kuwano et al. (2014) also found lower urease activity in an area cultivated with sugarcane as compared to the forest area. The lower activity in the soil cultivated with sugarcane could have been due to a greater number of final products containing compounds like urea, which repress synthesis of the enzyme (Tscherko et al., 2003), or to a smaller concentration of available substrates for urease. However, the positive correlations between urease activity (UA) and N_{tot} ($r = 0.77$, $p \leq 0.01$) and between UA and C_{tot} ($r = 0.73$, $p \leq 0.01$) suggest that this feedback may not have occurred. The negative correlations between UA, the soil pH ($r = -0.79$, $p \leq 0.01$), the P ($r = -0.65$, $p \leq 0.01$) and Zn ($r = -0.73$, $p \leq 0.01$) contents, demonstrate the importance of these factors in determining a greater or lesser activity of this enzyme. The strong positive correlation between N_{mic} and UA ($r = 0.88$, $p \leq 0.001$) supports the hypothesis that in the soil this enzyme is principally of microbial origin (Klose and Tabatabai, 2000). Roscoe et al. (2000) also obtained positive correlation between urease activity and N_{mic}. In this last study, the N microbial biomass accounted for 97% of the variance of soil urease activity. The positive correlation between UA and PMN ($r = 0.87$, $p \leq 0.001$) could suggest that the supply of N to the soil was amply regulated by this enzyme.

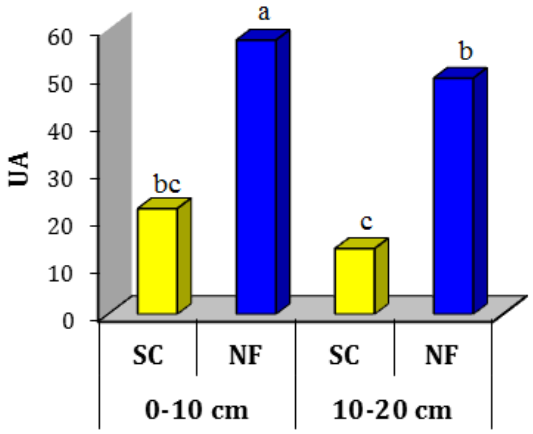


Figure 3. UA, urease activity, (μg NH_3 g^{-1} soil h^{-1}). SC, soil cultivated with sugarcane. NF, native forest soil. 0-10 and 10-20 cm, depths of soil sample collection. Means followed by the same letters are not significantly different (t test, $p \leq 0.05$)

Conclusion

The nitrogen cycling processes and chemical soil characteristics successfully discriminated the area cultivated with sugarcane from the native forest area. All the microbiological parameters evaluated were smaller in the soil cultivated with sugarcane demonstrating that is still possible to improve soil quality in comparison to the conditions of soil native forest. The results obtained with those parameters also showed that the soil C_{tot} and N_{tot} contents are not always adequate to show differences between areas with different types of soil management. The results also showed the need for constant monitoring of areas cultivated with sugarcane so as to prevent further soil degradation. Since the microbiological parameters vary with the climatic conditions, further studies should be carried out with soil samples taken at different times during the sugarcane growth cycle.

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