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CO_2 emissions from soil incubated with sugarcane straw and nitrogen fertilizer

Risely Ferraz de Almeida[1]*, Camila Haddad Silveira[2], Joseph Elias Rodrigues Mikhael[2], Fernando Oliveira Franco[1],Bruno Teixeira Ribeiro[2], Adão de Siqueira Ferreira[2], Eduardo de Sá Mendonça[3] and Beno Wendling[2]

[1]Universidade Estadual Paulista"Júlio de Mesquita Filho"-UNESP, Jaboticabal, Brazil.
[2]Universidade Federal de Uberlândia-UFU/Iciag, Uberlândia/MG, Brazil.
[3]Universidade Federal do Espírito Santo-UFES, Alegre/ES, Brazil.

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The decomposition/mineralization of organic material from crop residues constitutes an important nutrient reservoir for plants. This process produces CO_2 and is influenced by biophysical and environmental conditions such as temperature, oxygen availability and the chemical composition of the crop residue. We studied the effect of temperature and nitrogen fertilization on CO_2 emissions and the distinct contributions of C from sugarcane residue either left on the surface or incorporated into the red-yellow Oxisol. Incorporated sugarcane residue and N applications produce higher total organic carbon (TOC) mineralization rates when compared to application on the soil surface and without N. Nevertheless, there was no difference between TOC and C in the humin fraction (C-HU) 80 days after incubation. CO_2 emissions peaked at 5.45, 10.82, 14.00, 11.92 and 11.20, 14.47, 15.98,and 14.74 µg mol of CO_2 g^{-1} s^{-1} within the first four days of incubation for unincorporated and incorporated residues, respectively. After these first four days, emissions decreased until stabilizing at 40 days after incubation.

Key words: Greenhouse gases,organic matter, urea.

INTRODUCTION

Increasing atmospheric CO_2 will bring about serious global warming, which is expected to affect crop production (Fan et al., 2008). The soils are important reservoirs of active C and play an important role in the global C cycle. Decomposed and mineralized crop residue is an important source of available nutrients for sugarcane crops. Aerobic organotrophs decompose and mineralize organic matter, which produces energy for these organisms and liberates H_2O and CO_2 to the atmosphere (Brady and Weil, 2008).

Soil and vegetation management practices and the addition of organic residue (Wu et al., 2012) can affect CO_2 production from the soil. In addition, the chemical composition of the residue (C/N ratio and levels of lignin, cellulose, hemicellulose, various carbohydrates and polyphenols), temperature, soil moisture (Adachi et al.,

*Corresponding author. E-mail: rizely@gmail.com.

Table 1. Sugarcane residue attributes (chemical attributes) and red-yellow Oxisol (physical and chemical characteristics) used in the experiment.

Attribute	Soil	Attribute	Soil	Residue
Sand (g kg^{-1})	630	TN (g kg-^{1})	0.69	1.44
Silt (g kg^{-1})	140	TOC (g kg^{-1})	7.40	142.0
Clay (g kg^{-1})	230	C/N	-	99.0
pH (H_2O)	5.6	P (mg dm^{-3})	2.47	0.8
H+Al ($cmol_c$ dm^{-3})	15.8	K^+ (mg dm^{-3})	208.0	9.0
S-SO_4 (mg dm^{-3})	51.00	Mg^{2+} ($cmol_c$ dm^{-3})	0.56	1.3
CEC	5.53	Ca^{2+} ($cmol_c$ dm^{-3})	2.20	5.4

CEC, Cation exchange capacity.

2006; Fu et al., 2010) and oxygen availability (Herman et al., 1977) directly affect the decomposition/mineralization of organic material and CO_2 efflux, (Johnson et al., 2008). Among these the CO_2 emission is highly sensitive to changes in temperature because of its effects on almost all aspects of CO_2 emission processes (Townsend et al., 1992; Mikan et al., 2002; Luo and Zhou, 2006).

Additionally, physical and chemical characteristics of the soil, such as density, porosity, pore saturation and nutrient availability, influence CO_2 emissions (Brady and Weil, 2008). Accumulated soil residue is the main source of mineral N for plants and microorganisms. The immobilization or mineralization of N depends on the quality of the residue and can result in significant quantities during decomposition (Mengel and Schmeer, 1985).

Mechanized surgarcane harvesting in Brazil deposits crop residue on the soil surface (Panosso et al., 2009) at an average of 10 Mg ha^{-1} $year^{-1}$ and at a depth of 10 to 15 cm (Urquiaga et al., 1991). A 2002 federal law bans the burning of crop residue, which was a common practice for removing crop residue and facilitating sugarcane harvesting. Sugarcane residue in the soil and CO_2 emissions are linked to the various C sources in the soil. Understanding the proportion and dynamics of C is essential for understanding how different agricultural systems are sustained and the consequences for the C cycle (Liu et al., 2013).

The objective of this studywas to determine the effect of temperature and nitrogen fertilizer on CO_2 emissions and specific C contributions in soils managed with sugarcane residue in a red-yellow Oxisol.

MATERIALS AND METHODS

Soil sampling and analysis

The incubation experiment was carried out in lab conditions. The soil was collected in April, 2012 under sugarcane cropland (19°13'00,22″S latitude and 48º08'24,80″W longitude) and classified red yellow Latosol according to Brazilian System of Soil Classification (Embrapa, 2014). Before the experiment, the soil samples were air-dried, sieved (<2 mm) to obtain TFSA, and moistened to 60% of its water-holding capacity (WHC).

An aliquot of the resulting samples was used to characterize the chemical and physical attributes of the soil. The pipette method (Kilmer and Alexander, 1949) was used to determine soil texture. Total nitrogen (TN) was measured using Kjeldahl method (Black, 1965), available phosphorus (P) by Sommers and Nelson (1972) method and total organic carbon (TOC) according to Yeomans and Bremner (1988). The methodology recommended by Carter and Gregorich (2007) was used to determine potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}),sulphur (S-SO_4), potential acidity (H + Al), and in pH (water) (Table 1).

Soil incubation

700 g of soil sample were packed into a PVC column (15 cm height; 10.5 internal diameter; total volume of 1.298.2 cm^3) and incubated using a BOD incubator. The experiment was set up completely randomized, with three replicates (3×2×2 factorial) corresponding to: three temperatures (20, 25 and 30°C), two sugarcane straw conditions (surface and incorporated) and two nitrogen doses: 0 kg N ha^{-1} (control) and 120 kg N ha^{-1}, separated into two groups(Table 2).In addition, treatment controlswere incubated at 20, 25 and 30°C, without straw and fertilizer.

A recommended 120 Kg N ha^{-1} of granulated urea ($(NH_2)_2CO$) was added as solid and incorporated into the soil used in the treatments. Afterwards, 17 g of sugarcane straw (20 Mg of crop residue ha^{-1}) was either incorporated or added to the surface (depending on treatment).

For ratoon cane in Sao Paulo state, surface applications of 120 kg N $ha^{-1}$are recommended to achieve stalk productivity greater than 100 Mg ha^{-1} (Raij andCantarella, 1997). The temperatures used in these treatments are within the optimum range (20to40°C) for mesophilic microorganism growth and activity (Moreira and Siqueira, 2006). These temperatures also encompass soil temperatures found in the Triângulo Mineiro region of Brazil (average of 18°C in winter and 23°C in the hottest months) (Silva et al., 2003). The climate of this region is classified as Aw tropical hot and humid with cool and dry winters (Köppen).

After setting up the experiment, the samples were divided among the three different BOD temperatures. Soil moisture was maintained throughout the experiment at 60% of soil field capacity by measuring differences in sample weight. The soil water content and soil temperature are considered important abiotic factors regulating soil CO_2 efflux (Fu et al., 2010).

Measuring CO_2 emissions and organic carbon

An IRGA (Li-Cor 8100A) was used to measure CO_2 emissions from

Table 2. Treatment in experiment.

Temperature (°C)	Group 1 (surface sugarcane straw condition)	Group 2 (Incorporated sugarcane straw condition)
	Nitrogen fertilizer	Nitrogen fertilizer
20	0 kg N ha^{-1} (control)	0 kg N ha^{-1} (control)
25	0 kg N ha^{-1} (control)	0 kg N ha^{-1} (control)
30	0 kg N ha^{-1} (control)	0 kg N ha^{-1} (control)
20	120 kg N ha^{-1}	120 kg N ha^{-1}
25	120 kg N ha^{-1}	120 kg N ha^{-1}
30	120 kg N ha^{-1}	120 kg N ha^{-1}

the soil on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 13th, 16th, 19th, 22nd, 25th, 28th, 31st, 34th, 37th, 44th, 51st, 58th, 65th, 72nd, and 79th day after incubation (DAI) in the BODs. The IRGA has a closed internal volume of 854.20 and 83.70 cm² area of contact with the soil (Li-Cor Inc. Linclon, NE, USA). The device quantifies the concentration of CO_2 using optical absorption spectroscopy in the infrared range.

CO_2 emissions variables were used to calculate final accumulated respiration (Σ-CO_2 until the 79th DAI) and initial accumulated respiration (Σ-CO_2 until the 5th DAI).

Analytical procedures

At the 80th DAI, the incubated soils were sieved (2 mm opening) to remove the remaining crop residue from the soil. TOC (crop residue and soil) was determined by oxidation of potassium dichromate in an acid medium (Yeomans and Bremner, 1988). The same methodology was also used to characterize TOC in the soil and in the crop residue before incubation (Table 1).

Quantitative extraction and fractionation of C from the humic substances - HS (humin/C-HU, fulvic acid/C-FA and humic acid/C-HA) was determined by differential solubility, established by the International Society of Humic Substances (Swift, 1996).The same methodology was also used to characterize the soil before incubation (Table 1).

Microbial biomass C (Cmic) was determined with the method described by Vance et al. (1987), using a microwave oven to irradiate the samples (Islam and Weil, 1998). Labile C was determined according to Mendonça and Matos (2005) and N, P_2O_5, Ca^{+2} and Mg^{+2} levels according to the methodologies of Carter and Gregorich (2007) (Table 1).

Statistical analysis

The results were tested for normality of residuals (Shapiro-Wilk Test, SPSS Inc., USA) and homogeneity of variances (Bartlett Test, SPSS Inc., USA). Next, significant results (t-test) were then compared by a Tukey test at 5 % probability.

RESULTS AND DISCUSSION

CO_2emissions

In treatments with surface residue management, CO_2 emissions peaked within the first four days after incubation and were highest when incubated at 30°C with average daily emissions of 5.45, 10.82, 14.00, 11.92 and 9.66 µmol of CO_2 m^2 s^{-1} on the 1st, 2nd, 3rd, 4th and 5th DAI, respectively. Emissions decreased after the 6th DAI with small peaks until it reached stability after the 40th DAI at an average of 1.22 µmol de CO_2 m^2 s^{-1} (Figures 1A, C, E).The control treatment had the lowest CO2 emissions in all treatment (Figures 1A).

CO_2 emissions in treatments with incorporated crop residue also peaked within the first 5 DAI. However, daily average emissions were higher at 11.20, 14.47, 15.98, 14.74 and 11.42 µmol of CO2 m^2 s^{-1}. Emissions also decreased after the fifth DAI until reaching stability after the 40th DAI (Figure 1B, D and F). The tillage accelerates CO_2 emissions by increasing contact between soil and crop residue and speeding organic carbon decomposition (Gregorich et al., 2005; Bilen et al., 2010).

Similar CO_2 emissions rates were found with surface wheat residue in Luvisol with a peak emission at the 3rd DAI and an exponential decrease until the end of the experiment at 56 DAI (Guillou et al., 2011). Cayuela et al. (2009) found similar peaks in CO_2 emissions working with surface cotton residue on Regosol incubated for 25 days.

Fu et al. (2010) found that CO_2 emissions by soil temperature were described by multiple regression models. It can be seen from the regression equations that soil temperatures were more closely correlated with soil CO_2 efflux. The CO_2 emissions stabilized after the 40th DAI because the majority of labile C had been consumed. The remaining C is recalcitrant and associated with structural and more lignified crop residue (< 2 mm).

Oscillations in CO_2 emission peaks in the management of sugarcane residue, especially at 20°C (Figure 1A and B) are due to the succession and stability of soil micro-organism communities (Moreira andSiqueira, 2006). Opportunistic and colonizer organisms grow and reproduce rapidly in environments with abundant plant material.

High initial CO_2 emissions can be explained by the rapid mineralization of C-labile from vegetative tissue that quickly decomposes (Cayuela et al., 2009) over a few days or a few years (Brady and Weil, 2008). At 79 DAI, there was no correlation between C-labile with initial (r= - 0.12) and final (r= - 0.16) respiration. However, there was a positive correlation between the initial and final respiration (r=0.95), Table 3. Panosso et al. (2009) found decreases in C-labile with decreases in CO_2 in soils

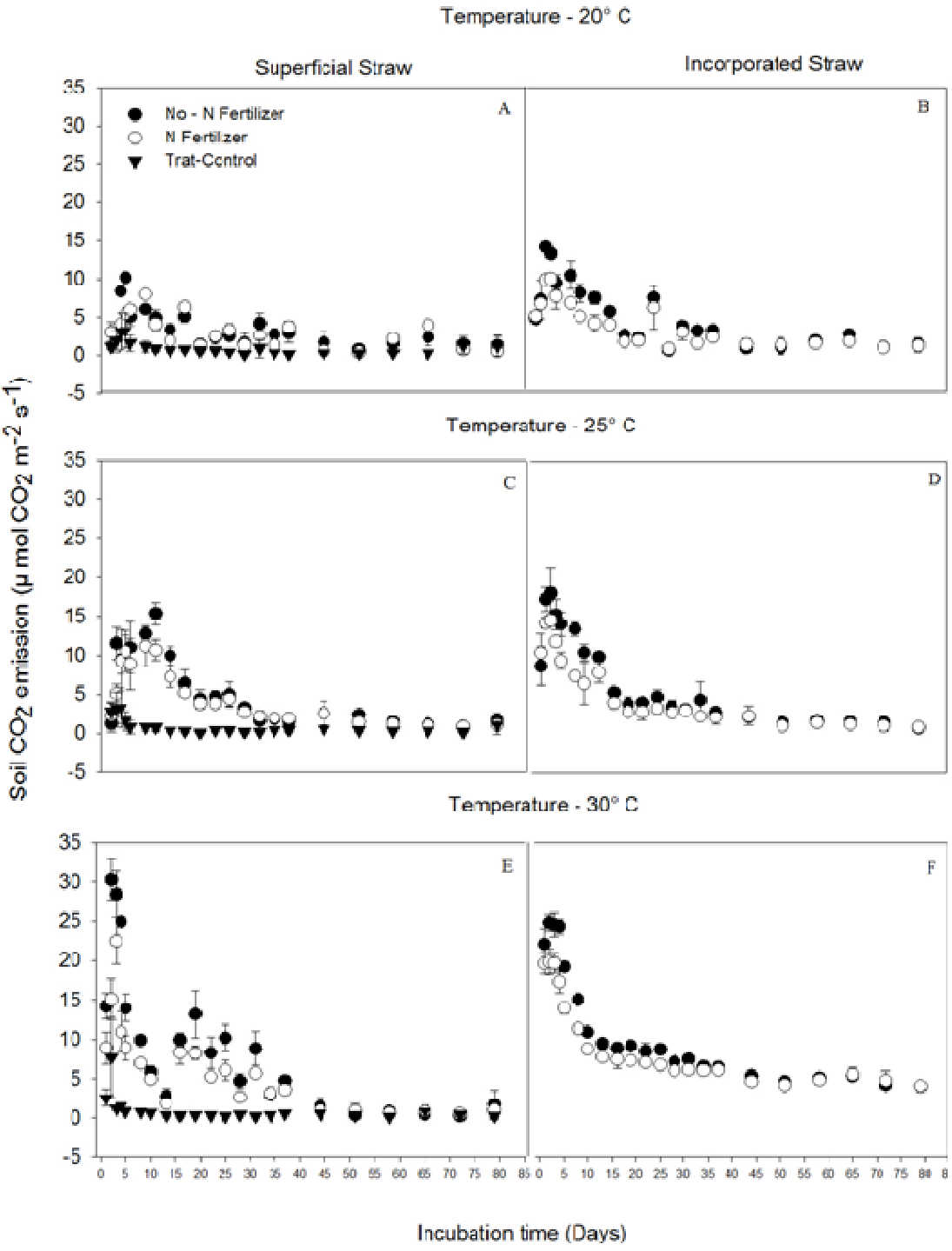

Figure 1. CO_2 emissions (microbial respiration) during incubation of soils with surface, Treatment control (Trat-Control) (Figures A, C, and E) and incorporated (Figures B, D and F) sugarcane residue, combined with nitrogen fertilization at 0 and 120 kg N ha^{-1} and incubated at 20, 25 and 30°C.

cultivated with sugarcane.

After incubation, Cmic, C-labile and Ca^{+2}, Mg^{+2} and P_2O_5 levels in the soil did not differ by management types (Table 4). However, C in the crop residue added to the soil decreased by 10.0 to 21.8 g Kg^{-1} of C (Table 4) with CO_2 emissions (Table 5). According to Brady and Wiel (2008), approximately 80% of total decomposed soil organic matter is emitted to the atmosphere as CO_2.

Table 3. Correlation coefficient and p value between variables: carbon in the humin fraction (C-HU), carbon in the humic acid fraction (C-HA) and fulvic acid (C-FA), total organic carbon (TOC), Labile carbon (C-Labile), HA/FA ratio (HA/FA), initial accumulated respiration (AR-I) and final accumulated respiration (AR-F).

Variable	C-HA	C-FA	TOC	C-labile	HA/FA	AR - F	AR - I
C-HU	0.35 (0.033)	0.16 (0.347)	0.39* (0.016)	-0.22 (0.185)	-0.06 (0.690)	-0.005 (0.97)	0.05 (0.767)
C-HA	-	0.37 (0.026)	0.11 (0.504)	-0.18 (0.294)	0.06 (0.693)	0.21 (0.210)	0.32 (0.051)
C-FA	-	-	0.09 (0.577)	0.11 (0.521)	0.29 (0.082)	-0.14 (0.405)	-0.07 (0.666)
TOC	-	-	-	-0.06 (0.690)	-0.50** (0.001)	0.02 (0.864)	-0.02 (0.885)
C-Labile	-	-	-	-	0.14 (0.407)	-0.12 (0.463)	-0.16 (0.327)
HA/FA	-	-	-	-	-	-0.46** (0.004)	-0.38** (0.021)
AR - F	-	-	-	-	-	-	0.95* ($7.84\ 10^{-19}$)

Variables are considered significant at $p < 0.050$. Correlation in bold: Positive correlation (*) and Negative (**). p value in table are in parentheses.

Table 4. Soil levels of microbial biomass carbon (Cmic), labile carbon (C-labile), nitrogen(N), carbon/nitrogen ratio (C/N), calcium (Ca^{+2}), magnesium (Mg^{+2}), phosphorus (P_2O_5) and crop residue carbon (RC) after 79 days of incubation.

Soil level	Cmic* (ug g^{-1} ha^{-1})	C-labile*	N*	RC	C/N	Ca^{2+}*	Mg^{2+}*	P_2O_5*
		----------g kg^{-1}----------				-----$cmol_c$ dcm^{-3}----		
Residue management								
Surface	238.30	5.05	0.81	132.0^{A}	9.6^{A}	1.94	0.88	3.00
Incorporated	289.07	4.77	0.87	118.1^{B}	10.2^{A}	2.05	0.94	2.94
Nitrogen fertilizer								
0Kg N ha^{-1}	245.22	5.11	0.83	128.9^{A}	9.7^{A}	2.05	0.94	3.0
120Kg N ha^{-1}	282.15	4.72	0.84	121.1^{A}	10.1^{A}	1.94	0.88	2.94
Temperature								
20º C	285.11	4.66	0.82	127.3^{A}	10.3^{A}	2.00	0.83	2.91
25º C	299.14	5.25	0.85	127.3^{A}	10.8^{A}	2.00	0.91	3.00
30º C	306.81	4.83	0.84	120.1^{A}	8.6^{B}	2.00	1.00	3.00

*Variables are not significantly different, whereas C/N and RC averages with distinct uppercase letters within the same column are significantly different (Turkey, P>0.05).

Soils incubated at 30°C, unlike soils incubated at the other temperatures, had greater CO_2 emissions (C losses) and thus significantly lower C/N ratios (Table 4). Soils with lower C/N ratios have fewer nutrients and greater competition among microorganisms for resources. This condition causes some microorganism deaths and lower CO_2 emissions to the atmosphere. Small microorganism populations survive by slowly digesting stable and resistant organic tissue (lignin and cellulose) in the soil (Brady andWiel, 2008).

Accumulated CO_2 emissions in the first five DAI represent 20.45% of total emissions from the soil under surface residue management and 25.53% from the incorporated management system. Rezende et al. (2004) achieved similar results (34 to 67% of total CO_2 emissions in the first 14 DAI) working with Alfisol, TypicKandiustalf and Oxisol, TypicHaplustox, which had been incubated with distillery yeast. During this period, average CO_2 emissions from incorporated residue incubated at 30°C increased 72.72% more than emissions from surface management incubated at 20°C. Emissions were 70.92% higher in a treatment incubated at 30°C with 120 Kg N ha^{-1} relative to a treatment without added N and incubated at 20°C (Table 5).

Temperature increases from 20 to 25°C and from 25 to 30°C raised CO_2 emissions by 40.00 % and 50.00% in treatments with surface crop residue. However, for treatments with incorporated residue, these same increases were lower, 37.50 and 27.27%, respectively (Table 5). This occurred because soil temperature is

Table 5. Accumulated CO_2 emissions from 5 to 79 DAI (days after incubation) in soils with surface and incorporated sugarcane residue with two nitrogen application rates (0 Kg N ha^{-1} and 120 Kg N ha^{-1}) and three incubation temperatures (20, 25 and 30° C).

Temperature (°C)*	Residue management[1]		Nitrogen fertilizer[2]	
	Surface	Incorporated	0 Kg N ha^{-1}	120 Kg N ha^{-1}
Accumulated emissions at 5 DAI				
20	24.1^{Bc}	42.8^{Ac}	29.0^{Bc}	38.0^{Ac}
25	42.1^{Bb}	67.1^{Ab}	48.3^{Bb}	61.0^{Ab}
30	89.1^{Aa}	93.6^{Aa}	73.1^{Ba}	109.6^{Aa}
Accumulated emissions at 79 DAI				
20	73.8^{Bc}	91.1^{Ac}	74.5^{Bc}	95.5^{Ac}
25	112.6^{Bb}	130.6^{Ab}	107.3^{Bb}	136.0^{Ab}
30	163.1^{Aa}	151.8^{Aa}	128.0^{Ba}	187.0^{Aa}

Accumulated Emissions at 5 DAI, CV: 9.6; [1]DMS, Residue management: 6.8; [2]DMS, Nitrogen fertilizer: 8.3. Accumulated Emissions at 79 DAI - CV: 7.9; [1]DMS - Residue management: 11.5; [2]DMS - Nitrogen application rates: 13.9. *CO_2 emissions in µmol CO_2 m^{-2} s^{-1}. Averages followed by distinct lowercase letters within a column and uppercase letters within a row are significantly different for temperature/residue management and temperature/nitrogen rate interactions (Tukey, P>0.05).

considered an important abiotic factor regulating soil CO_2 efflux (Fu et al., 2010).

According to Stanford et al. (1973), C mineralization increases in soils when incubation temperatures rise from 5 to 35°C. However, soil temperature and CO_2 emissions are not positively correlated under normal conditions for sugarcane cultivation in Brazil (Panosso et al., 2009). It is thought that soil temperatures in this environment remain near optimal levels for microbial activity without major fluctuations.

At 20 and 25°C, CO_2 emissions were higher with incorporated residue than with surface residue. However, at 30°C, there was no difference between management types. Incorporated residue produces greater emissions because it has greater contact area with microorganisms and reaches soil depths with higher microbial populations (Brady and Weil, 2008).

CO_2 emissions were 25.00% higher in treatments with N applications than in treatments without. Sugarcane residue is nitrogen deficient given that it contains 12.30% N and has a C/N ratio of 120g Kg^{-1} (Table 1). Microorganism development requires high levels of N for compounds such as enzymes and DNA. Therefore, applying N to the soil increases biological activity and consequently raises CO_2 emissions (Gifford et al., 2000). Management systems that add crop residue with high C/N ratios, such as sugarcane, must also add N to avoid N immobilization, which makes N unavailable for plants and soil microorganisms (Brady and Weil, 2008).

Total organic carbon (TOC) and humic substances (HS) in the soil

TOC levels varied erratically and thus it was not possible to identify positive interactions with management systems (Figure 2). This result is due to the low C/N ratio of sugarcane residue (Table 1) and the short observation period (79 days of incubation). There is a direct relationship between the C contribution to the soil and the addition rate of each type of organic residue. Deposition of vegetative material can significantly increase soil TOC over decades (Harrison et al., 1995).

C from humic substances (HS) represented 71.00% of the TOC concentration. More than 70.00% of TOC was in the form of more stable humic substances (Luo and Zhou, 2006; Stevenson, 1994).The humic substance with the highest average concentration was C-HU (7.0 g C kg^{-1}) followed by C-FA (1.63 g C kg^{-1}) and C-HA (1.36 g C kg^{-1}) (Figure 3). Other studies have also shown that in various classes of tropical soils with various types of management and usage, C-HU concentrations are higher than concentrations of other C-HS (Conteh and Blair, 1998). C-HU concentrations are higher because these substances are more insoluble (acid and base), more stable and more closely associated with the soil mineral fraction (Stevenson, 1994; Sparks, 2001). Additionally, the majority of TOC in sandy, low-fertility soils (Tables 1 and 3) is concentrated in the C-HU with greater C-HA and C-FA losses due to greater soil mobility (Andreux and Becerra, 1975).

Canellas et al. (2003) also found low C-HA concentrations in soils with surface management of sugarcane residue (raw cane). The predominance of C-FA over C-HA results from conditions that limit humification such as a low sum of bases and low Al^{3+} levels (Castillo and Wright, 2008).

C-HU did not vary by management type. However, there was a significant positive correlation between C-HU with soil TOC(r= 0.39) and C-HA (r= 0.35)(Table 3), C-

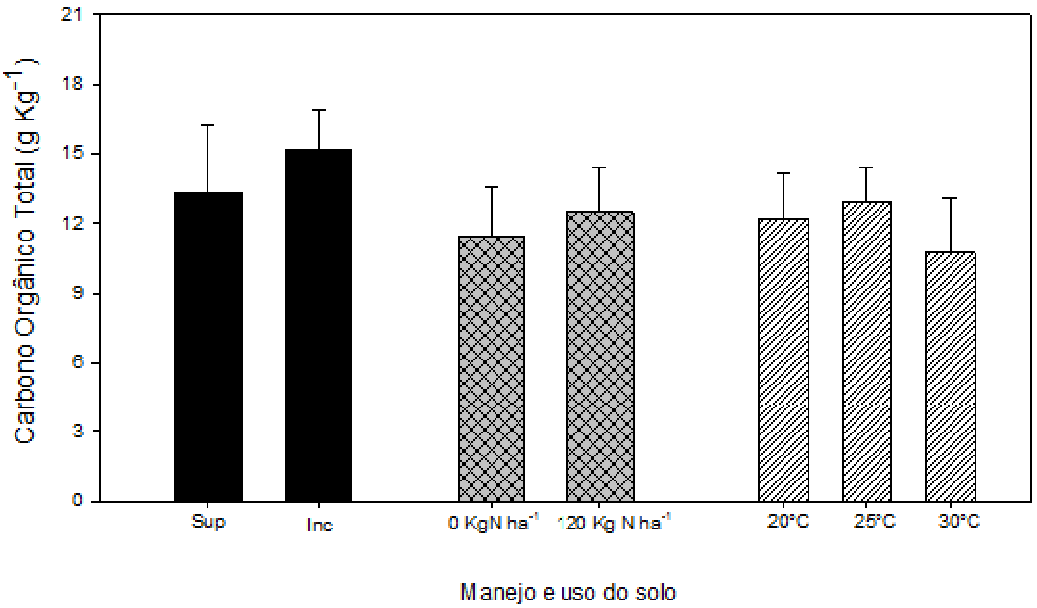

Figure 2. Total organic carbon in soils (gKg^{-1}) with surface (Sur) and incorporated (Inc) management of sugarcane residue and different nitrogen sources (0 and 120 Kg ha^{-1}), incubated for 79 days at three different temperatures (20, 25 and 30°C). Error bars represent the standard deviation from the mean for the averages (P>0.05).

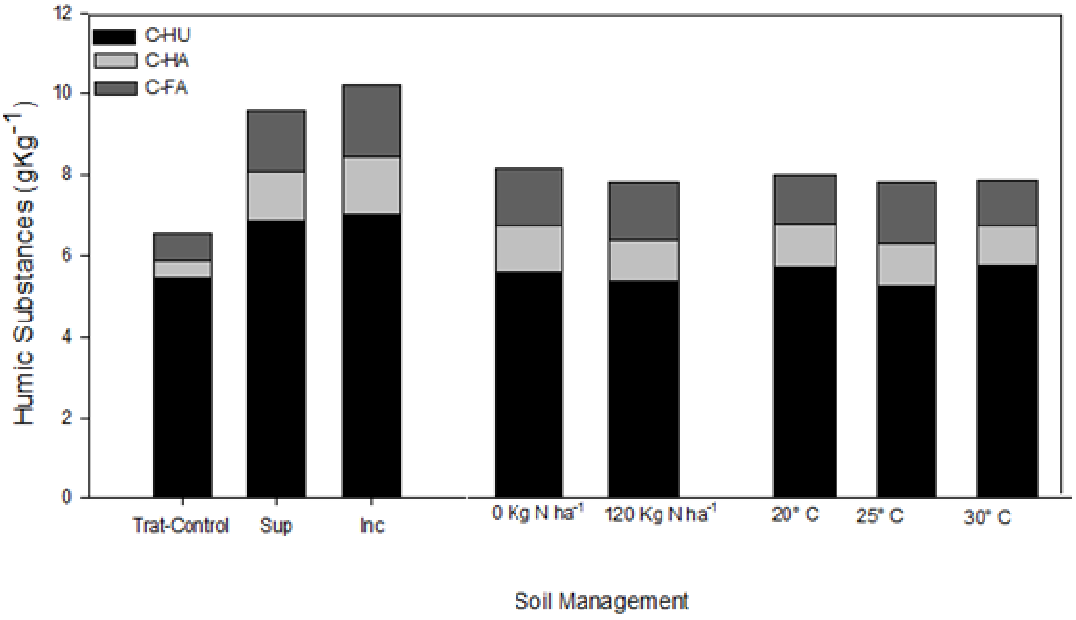

Figure 3. Carbon from humic substances (HS): humin(C-HU), fulvic acid (C-FA) and humic acid (C-HA) in the soil (g Kg^{-1}), with surface (Sur) and incorporated (Inc) sugarcane residue management, different nitrogen sources (0 and 120Kg N ha^{-1}) and Treatment control (Trat-Control) incubated for 79 days at three different temperatures (20, 25 and 30°C).

HU varying randomly and averaging 79.60% of soil TOC (Figure 3). Canellas et al. (2003) found the same positive correlation with C-HU concentrations ranging from 30 to 80% of soil TOC (Brady and Weil, 2008; Stevenson, 1994).

Higher levels of lignin in sugarcane residue form more

phenolic compounds during decomposition. This means that higher quantities of C remain in the soil, especially in the C-HU fraction, but over a longer time (Stevenson, 1994). Among the alkaline fractions (C-FA and C-HA), C-FA did not interact significantly with management type, but did vary randomly. However, C-HA was 6.15% greater with incorporated residue than with surface residue.

There was a positive correlation between the alkaline fractions (r= 0.37), negative interactions between the HA/FA ratio and initial (r= 0.38) and final respiration (r= 0.46) and a negative correlation between the HA/FA ratio and TOC (r= -0.50)(Table 3). This shows that HS plays an important role in the transfer of nutrients through ecological systems and the emission of CO_2 to the atmosphere. According to Fontaine et al. (2007), higher respiration is associated with reductions in the more stable fractions of SOM.These negative correlations probably result from the priming effect in which, according to Brady and Weil (2008), higher soil respiration with the addition of plant residue is stimulated by the breakdown of more resilient organic matter.

Conclusion

The CO_2 emission apex was present in the first 5 DAI and a subsequent decrease to acquire stability from 40 DAI. There was no correlation between the emissions of CO_2 to the C-Labile after 79 DAI but there was a negative correlation with HA/FA. The management with incorporated straw, nitrogen fertilization and increased of T°C contribute to higher CO_2 emissions. For TOC, C-HU, C-HA, there were no significant differences after 79 DAI and no correlation with the CO_2emission.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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