

## Carbon sequestration and greenhouse gases emissions in soil under sewage sludge residual effects

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**ABSTRACT:** The large volume of sewage sludge (SS) generated with high carbon (C) and nutrient content suggests that its agricultural use may represent an important alternative to soil carbon sequestration and provides a potential substitute for synthetic fertilizers. However, emissions of CH<sub>4</sub> and N<sub>2</sub>O could neutralize benefits with increases in soil C or saving fertilizer production because these gases have a Global Warming Potential (GWP) 25 and 298 times greater than CO<sub>2</sub>, respectively. Thus, this study aimed to determine C and N content as well as greenhouse gases (GHG) fluxes from soils historically amended with SS. Sewage sludge was applied between 2001 and 2007, and maize (*Zea mays* L.) was sowed in every year between 2001 and 2009. We evaluated three treatments: Control (mineral fertilizer), 1SS (recommended rate) and 2SS (double rate). Carbon stocks (0-40 cm) were 58.8, 72.5 and 83.1 Mg ha<sup>-1</sup> in the Control, 1SS and 2SS, respectively, whereas N stocks after two years without SS treatment were 4.8, 5.8, and 6.8 Mg ha<sup>-1</sup>, respectively. Soil CO<sub>2</sub> flux was highly responsive to soil temperature in SS treatments, and soil water content greatly impacted gas flux in the Control. Soil N<sub>2</sub>O flux increased under the residual effects of SS, but in 1SS, the flux was similar to that found in moist tropical forests. Soil remained as a CH<sub>4</sub> sink. Large stores of carbon following historical SS application indicate that its use could be used as a method for carbon sequestration, even under tropical conditions.

**Keywords:** biosolid, nitrogen, carbon dioxide, methane, nitrous oxide

### Introduction

The use of organic wastes for agricultural fertilization is a growing practice worldwide following the guidelines of the modern concepts of waste management, where they should be viewed as byproducts (Wilson, 2007). Especially in developing countries, sewage sludge (SS) agricultural use is being increasingly adopted as a way of managing urban wastewater treatment chains and preventing the scarcity of water and landfill sites (Beecher, 2008; Wang, 2011). From the perspective of agricultural sustainability, SS use promotes the recycling of nutrients previously removed from the soil by crops (Elser, 2012), while reducing the use of synthetic fertilizers and their detrimental environmental effects (Childers et al., 2011). Also, SS may help prevent soil erosion (Galdos et al., 2009; Garcia-Orenes et al., 2005), increase enzymatic activity in soils (Singh and Agrawal, 2008) and decrease the incidence and viability of phytopathogenic organisms detrimental to crops (Bonanomi et al., 2010).

Despite the benefits, the use of SS in agriculture could also be associated with serious environmental, agricultural, and health risks if not properly planned, managed and implemented. Among the most frequently discussed issues are the high concentrations of organic pollutants and heavy metals in SS and their potential accumulation in soils (Nogueira et al., 2010; Smith, 2009). Contamination of soils, water, and crops with human pathogens has also been an important study subject (Gerba and Smith, 2005; Navarro et al., 2009). However, while climate change is an increasing global concern, the impact of SS use in agriculture on GHG emissions such as CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O has hardly been addressed.

Similar to the application of other organic fertilizers in soils, the use of SS in agriculture is prone to alter soil C and N dynamics and, consequently, change the rates of GHG emissions. For instance, part of the organic C present in SS might be stored more permanently in the soil and help mitigate the effects of agriculture on greenhouse emissions (Lal, 2008). On the other hand, much of the organic C and N in SS applied to soils can be used by the microbial community to fuel processes such as nitrification and denitrification, which will increase emissions of N<sub>2</sub>O (Davidson et al., 2000; Stein, 2011; Ward, 2008). Moreover, the application of SS, rich in organic matter and with high water holding capacity, may create anaerobic sites in soils and promote microbial methanogenic activity (Le Mer and Roger, 2001; Sey et al., 2008). In contrast, well-drained soils are recognized as CH<sub>4</sub> sinks (Conrad, 2009; Holmes et al., 1999).

We hypothesized that the application of SS to supply N for maize cropping increases N and C content in soil, as well as the emissions of greenhouse gases such as CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub>. This study aimed to: (i) quantify changes in soil carbon and nitrogen stocks after successive applications of SS; (ii) determine if greenhouse gas fluxes from soil increase with increases in soil organic C and N availability; and (iii) verify how soil physical-chemical parameters influence these fluxes.

### Materials and Methods

#### Site description

The experiment was conducted in Campinas, in the state of São Paulo, located in the southeast region

of Brazil (22°09' S, 47°01' W). The soil at the site was classified as Haplic Ferralsol according to the Food and Agriculture Organization system (FAO, 1998), with clay texture (58.3 % clay, 10.3 % silt, and 31.4 % sand). The climate is humid-tropical with rainy summers and dry winters, and is considered to be a Cwa type, according to the Köppen classification system (Alvares et al., 2013). The mean annual temperature and rainfall are 20.5 °C and 1,400 mm, respectively, and 76 % of the precipitation occurs between Oct and Mar (Galdos et al., 2009).

### Experimental design

The area of the experimental site was divided into twelve 4 × 25 m plots of uniform declivity (10 %) and separated by cemented borders and frames (2 m). The plots included three treatments (four replicates) on maize crops (*Zea mays* L.), including a control and two treatments with SS application (1SS and 2SS). In the Control, no SS was ever applied to the maize crops but synthetic fertilized N was added at the rate equivalent to 120 kg N ha<sup>-1</sup>; the 1SS plots were amended with the recommended amount of SS (1SS ≈ 10 Mg ha<sup>-1</sup> yr<sup>-1</sup> of SS on dry bases); while the 2SS plots were amended with twice the recommended amount (2SS ≈ 20 Mg ha<sup>-1</sup> yr<sup>-1</sup> of SS on dry bases). The average contents of C and N in SS were 279 and 30 kg Mg<sup>-1</sup>, respectively (Table 1).

Sewage sludge was applied in 1SS and 2SS for seven years (2001 to 2007), while the Control had no SS application but was fertilized with mineral N. Annual phosphorus (P) and potassium (K) fertilizers were also applied to the Control, and 1SS and 2SS received

supplementary fertilization with K only. Therefore, the GHG emissions and C and N stocks presented in this study represent the effects of residual SS fertilizer application on soils. Thus, it was expected that labile material would be readily degraded and organic matter would be in a more stabilized form as verified by Fernandez et al. (2007).

*Sewage sludge application* - The recommended sewage sludge rate was determined according to its chemical composition (Table 1) and the N fertilization rate recommended for maize crops (Rajj et al., 1997). Based on treatment adopted for wastewater treatment plant, the N mineralization rate used is equal to 30 % in accordance with Brazilian legislation (CONAMA, 2006), which adopts the same N mineralization rates established by USEPA (USEPA, 1994). The SS used in the experiments was generated at the Jundiá city water reclamation plant (23°08' S, 47°00' W), in the state of São Paulo, Brazil, where the water treatment process consists of a complete-mix aerated lagoon followed by decantation. At the plant, SS was centrifuged and subjected to poly-electrolyte, physical and sanitary conditioning over 60 to 90 days by revolving in an enclosure patio (Galdos et al., 2004). Sewage sludge was manually applied to the entire treatment area and then incorporated into the soil (0-10 cm) using mattock, while synthetic fertilizer was applied in rows. Maize was planted annually during the summer. During the intercrop period, the soil remained uncovered.

### Gas and soil sampling and analysis

On Sep 18, 2009, composite soil samples from

Table 1 – Physicochemical parameters of sewage sludges (SS) applied between the years 2001 and 2007.

pH	Water	Volatile solids	Organic carbon
	% (w/w)		g kg <sup>-1</sup>
6.6 ± 0.7 <sup>a</sup>	68 ± 3	57 ± 3	279 ± 43
Kjeldahl Nitrogen	NH <sub>4</sub> <sup>+</sup> -Nitrogen	NO <sub>3</sub> <sup>-</sup> , NO <sub>2</sub> <sup>-</sup> -Nitrogen	Fe
g kg <sup>-1</sup>	mg kg <sup>-1</sup>		g kg <sup>-1</sup>
29 ± 2	371 ± 133	48 ± 52	22 ± 3
P	Ca	Mg	S
g kg <sup>-1</sup>			
8.1 ± 2.7	15 ± 11	1.6 ± 0.2	20 ± 6
K	Na	Bo	Zn
mg kg <sup>-1</sup>			
2,538 ± 3,944 <sup>b</sup>	2,207 ± 2,613 <sup>b</sup>	24 ± 25	1,339 ± 279
Al	As	Ba	Cd
g kg <sup>-1</sup>	mg kg <sup>-1</sup>		
19 ± 2	< 0.5	347 ± 159 <sup>c</sup>	8.3 ± 3.6
Pb	Cu	Cr	Mn
mg kg <sup>-1</sup>			
170 ± 61	525 ± 267	168 ± 22	606 ± 116
Hg	Mo	Ni	Se
mg kg <sup>-1</sup>			
< 0.5	8.3 ± 2.7	59 ± 58	< 0.5

<sup>a</sup>± standard deviation; <sup>b</sup>in 2004, K and Na values were 11,470 mg kg<sup>-1</sup> and 8,102 mg kg<sup>-1</sup>, respectively; <sup>c</sup>means of 2006 and 2007 values because barium was not a parameter included in national legislation before that point.

each plot and the same soil depth were obtained from five randomly collected subsamples. These samples were taken at depths of 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm using hand augers. Soil samples were air-dried, grounded and sieved at 150  $\mu\text{m}$  for total C and N analysis using a CN analyzer. For bulk density determinations, undisturbed soil samples were collected using steel cylinders (5  $\times$  5 cm) in triplicate from each plot at different depths in the trenches. C and N stocks were calculated using the following equation:

$$\text{Stock} = C * W_s * h * 10,000 \quad (1)$$

where: *Stock* is C or N soil stock ( $\text{Mg ha}^{-1}$ ); *C* is the element concentration ( $\text{g g}^{-1}$ ); *W<sub>s</sub>* is the soil bulk density ( $\text{Mg m}^{-3}$ ); *h* is the soil depth (m); and 10,000 is the coefficient for converting  $\text{m}^2$  into hectare.

We determined soil  $\text{N}_2\text{O}$  and  $\text{CH}_4$  fluxes using a chamber-based method (Davidson et al., 2002) in which polyvinyl chloride chambers (30-cm diameter) were inserted 2-cm deep into the soil at randomly distributed points. After closing the chambers, 60 mL samples were collected using syringes at 1, 10, 20 and 30 min and stored under pressure in 20 mL evacuated penicillin flasks sealed with gas-impermeable butyl-rubber septa (Bellco Glass 2048). The samples were analyzed by gas chromatography with electron capture and flame ionization detectors for  $\text{N}_2\text{O}$  and  $\text{CH}_4$ , respectively (Shimadzu 2014). Each gas chamber flux was calculated from slope regressions between gas concentration and collection time. Measurements of atmospheric pressure, chamber height and air temperature were taken during gas sampling to determine the air chamber volume.

Soil  $\text{CO}_2$  fluxes were determined using a dynamic chamber as proposed by Davidson et al. (2002) and adapted by Carmo et al. (2006). For these measurements, we used the same polyvinyl chloride chambers (30 cm in diameter) used for  $\text{N}_2\text{O}$  and  $\text{CH}_4$  sampling. The chamber was coupled to a portable infrared gas analyzer that determined changes in  $\text{CO}_2$  concentration (ppmv) over time. Gas concentrations were measured in situ every 15 s for 5 min, and the data were stored using the Graph-Term Datastick software installed on a palmtop connected to the equipment. Subsequently, data were transferred to another computer where the flux for each chamber was calculated using Palm Flux software, which employs a regression slope to obtain the flux measurement in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

Soil temperature was measured at a depth of 0-10 cm using probe thermometers to assist in the interpretation of results. All chambers were installed in the between-row position. Thus, in the Control plots the fluxes do not represent direct emissions from fertilizer because it was applied parallel to the planting line. Gas samples were collected over 20 days between Oct 14, 2009 and Sep 29 2010. Precipitation and daily mean temperatures are shown in Figure 1.

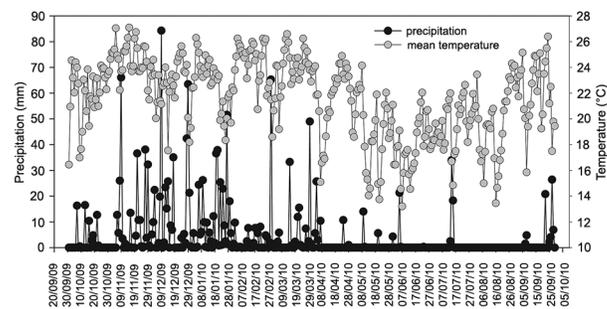


Figure 1 – Climate variables during the gas sampling period.

Gas emissions were estimated by weighting the data with seasonal fluxes (dry or wet period) to isolate conditions that support denitrification (water availability) and  $\text{CO}_2$  soil flux (temperature and water availability), because at the study site, lower temperatures coincide with lesser precipitation. The emissions representing the wet season were calculated based on the fluxes observed from Oct 1, 2009 to 31 Mar 31, 2010 (182 days). The emissions representing the dry season were calculated based on the fluxes from Apr to Sep (183 days). These periods are the same as the historically wetter and drier periods at the experimental site, respectively, according to the reported by Galdos et al. (2009). Seasonal  $\text{CO}_2$ ,  $\text{N}_2\text{O}$  and  $\text{CH}_4$  mean fluxes for these periods were estimated, as well as weighting the emissions to allow for seasonal fluxes (Table 3).

After collecting the gases, soil samples were taken from inside the chambers to determine N mineral concentration and soil moisture. Soil moisture was determined by gravimetry, and mineral N content was determined by colorimetry of soil extracts (2M KCl) using flow injection analysis based on the methods proposed by Kamphake et al. (1967) for  $\text{NO}_3^-$  and by Krom (1980) for  $\text{NH}_4^+$ . Soil water-filled pore space (WFPS) was calculated as follows:

$$\text{WFPS} = \text{Vol} / (1 - W_s / S_s) \quad (2)$$

where: *WFPS* is the water-filled pore space (%); *Vol* is the volumetric water content (%); *W<sub>s</sub>* is the soil bulk density ( $\text{Mg m}^{-3}$ ); and *S<sub>s</sub>* ( $2.65 \text{ Mg m}^{-3}$ ) is the particle density commonly used for tropical soils (ISO, 1998).

#### Statistical analysis

Differences in soil characteristics and in gas fluxes among treatments were determined using analysis of variance (ANOVA), where the historical SS rate, or amount of SS applied, was considered to be the variable factor (randomized set). The mean values for each treatment were compared using Tukey test ( $p < 0.05$  for chemical parameters;  $p < 0.10$  for soil density). To assist with the interpretation of gas fluxes, we used simple and multiple regressions in which *y* represented gas flux and *x* represented either temperature, or WFPS or both ac-

ording to the following equation:

$$x = (T_{soil}/T_{range}) + (WFPS/WFPD_{range}) \quad (3)$$

where:  $T_{soil}$  is the verified temperature at the respective point;  $T_{range}$  is the temperature range observed for the respective treatment; WFPS is the WFPS verified at the respective point; and  $WFPD_{range}$  is the WFPS range observed for the respective treatment.

### Results and Discussion

#### Soil carbon and nitrogen

In 1SS, soil C contents were 79 %, 56 % and 7 % higher than in the Control series at 0-5, 5-10 and 10-20 cm layers, respectively; whereas in 2SS, soil C contents were 163 %, 121 % and 22 % higher than in the same layers of the Control. In samples collected at a depth below 20 cm, there was no effect of SS on soil C content. According to soil C content data, the amount of SS ap-

plied explained the variance in the total soil C content in studied soil even two years after SS application stopped. However, variations tended to decrease with soil depth (Figure 2).

Soil N content was highly associated with C content ( $r = 0.996$ ;  $p < 0.0001$ ), as it changed accordingly with it (Figure 3). In 1SS, soil N contents were 68 % and 60 % higher than in the Control series at 0-5 and 5-10 cm layers, respectively; whereas in 2SS treatment, soil N contents were 174 % and 118 % higher than in same layers of the Control. Below 10 cm, soil N content was not statistically different across treatments.

Otherwise, soil C:N ratios were not different across all treatments. The mean ( $\pm$ SD) C:N ratios in soil samples from each treatment and all depths were 12.29 ( $\pm 0.31$ ), 12.58 ( $\pm 0.57$ ) and 12.23 ( $\pm 0.35$ ) in the Control, 1SS and 2SS, respectively. Lower soil bulk densities at 0-5 and 5-10 cm soil depths (Table 2) were observed according to sewage sludge application ( $p < 0.10$ ). Soil organic C content is positively related to soil density, since

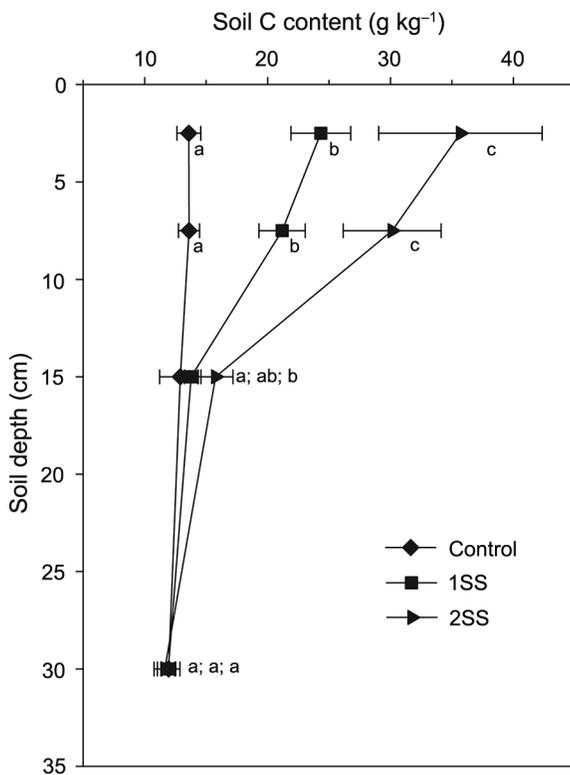


Figure 2 – A profile of carbon content distribution in soil. Different letters indicate differences between treatments ( $p < 0.05$ ). Bars indicate standard deviation. Control represents the treatment with no SS but N mineral used instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied.

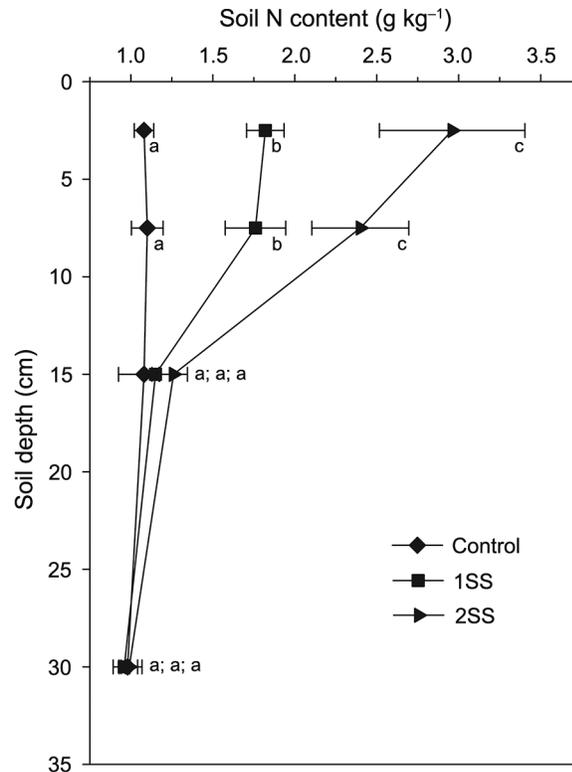


Figure 3 – A profile of nitrogen content distribution in soil. Different letters indicate differences between treatments ( $p < 0.05$ ). Bars indicate standard deviation. Control represents the treatment with no SS but N mineral used instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied.

Table 2 – Soil density across soil depth after two years with no sewage sludge application in treatments \*Control, 1SS and 2SS.

Treatment	Soil depth			
	0-5	5-10	10-20	20-40
	g cm <sup>-3</sup>			
Control	<sup>a</sup> 1.17 <sup>b</sup>	1.18 <sup>b</sup>	1.21 <sup>a</sup>	1.13 <sup>a</sup>
1SS	1.08 <sup>a</sup>	1.22 <sup>b</sup>	1.20 <sup>a</sup>	1.22 <sup>a</sup>
2SS	1.07 <sup>a</sup>	1.07 <sup>a</sup>	1.16 <sup>a</sup>	1.25 <sup>a</sup>
<sup>b</sup> CV (%)	5.04	4.10	7.74	5.78

\*Control represents the treatment with no SS but N mineral used instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied; <sup>a</sup>Values followed by different letters indicate differences between treatments ( $p < 0.10$ ); <sup>b</sup>coefficient of variation.

Table 3 – Mean CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes during wetter and drier periods after 2 year with no sewage sludge application in treatments \*Control, 1SS and 2SS.

Mean fluxes in wetter period			
Treatment	CO <sub>2</sub>	N <sub>2</sub> O	CH <sub>4</sub>
	μmol m <sup>-2</sup> h <sup>-1</sup>		
Control	<sup>a</sup> 2.37 <sup>a</sup>	0.11 <sup>a</sup>	-0.82 <sup>a</sup>
1SS	3.62 <sup>ab</sup>	0.31 <sup>ab</sup>	-0.37 <sup>a</sup>
2SS	4.30 <sup>b</sup>	0.70 <sup>b</sup>	-0.49 <sup>a</sup>
<sup>b</sup> CV (%)	51.02	187.98	-304.50
Mean fluxes in drier period			
Treatment	CO <sub>2</sub>	N <sub>2</sub> O	CH <sub>4</sub>
	μmol m <sup>-2</sup> h <sup>-1</sup>		
Control	0.21 <sup>a</sup>	0.01 <sup>a</sup>	-0.83 <sup>a</sup>
1SS	0.23 <sup>a</sup>	-0.01 <sup>a</sup>	-0.96 <sup>a</sup>
2SS	0.27 <sup>a</sup>	-0.02 <sup>a</sup>	-0.19 <sup>a</sup>
CV (%)	76.84	-1065.23	-114.64

\*Control represents the treatment with no SS but N mineral use instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied; <sup>a</sup>Values followed by different letters indicate differences between treatments ( $p < 0.05$ ); <sup>b</sup>coefficient of variation.

it improves soil porosity (Dexter et al., 2008). These layers were also the most affected by SS addition in terms of C content (Figure 2).

Although there was no difference between C and N soil content in the deepest layer, as well as soil density, we calculated the total increases in C and N stocks attributable to changes in management practices. The mean C stocks were 58.8 Mg ha<sup>-1</sup> in the Control, 72.5 and 83.1 Mg ha<sup>-1</sup> for 1SS and 2SS, respectively. The mean N stocks were 4.8, 5.8, and 6.8 Mg ha<sup>-1</sup> in the Control, 1SS and 2SS respectively.

Soil nitrate content (NO<sub>3</sub><sup>-</sup>-N) in the samples collected from inside each chamber after gas sampling ranged from 1.38 to 7.69 mg kg<sup>-1</sup> for the Controls, 2.97-15.47 mg kg<sup>-1</sup> for 1SS and 5.43-37.34 mg kg<sup>-1</sup> for 2SS. The mean nitrate values (NO<sub>3</sub><sup>-</sup>-N) were 3.62 mg kg<sup>-1</sup> for the Control, 8.20 for 1SS and 15.59 for 2SS. Soil ammonium (NH<sub>4</sub><sup>+</sup>-N) ranged from 0.04 to 4.08 mg kg<sup>-1</sup> for the Control, 0.27-11.09 mg kg<sup>-1</sup> for 1SS and 0.76-24.31 mg kg<sup>-1</sup> for 2SS. Mean ammonium values (NH<sub>4</sub><sup>+</sup>-N) were 1.50 mg kg<sup>-1</sup> for the Control, 3.85 for 1SS and 7.39 for 2SS.

#### Carbon and nitrogen relationship and elemental stocks

Considering mean C and N values of the annually applied SS (Table 2), we determined the total amount of

elements added to the soil after seven SS applications and compared it with respective stocks to assess the C and N dynamic. In 1SS, 19.5 and 2.0 Mg ha<sup>-1</sup> of C and N were added, respectively. In 2SS, 39.1 and 4.1 Mg ha<sup>-1</sup> of C and N were added, respectively. Thus, comparing these values with N increase we infer that 50 % of the N applied with SS remained in the soil both in 1SS and 2SS. Disregarding differences in residues from crop production and soil incorporation over years of different treatments, 70 % of C and 62 % of C applied to the 2SS explains the increases in soil C stocks at 1SS and 2SS, respectively. Because the C:N ratio settles around 12 and the fact that the same fraction of N from SS remained in both 1SS and 2SS suggest that N output is the primary variable that determines how much C is stored. Indeed, stoichiometry between C, N and P is a key factor that controls the C storage capacity in ecosystems (Hessen et al., 2004). Despite SS application having ceased 2 years before this research was conducted, it appears that plant uptake of N was greater in 1SS and 2SS than in the Control (data not shown).

Another important factor that might determine the amount of C stored in soil is the C:N ratio of the SS. Because the parameter that limits the amount of SS applied is generally N content, materials with different C:N ratios deposit the same amount of N and different amounts of

C. In addition, SS with lower C:N ratios might provide faster N mineralization, and thus element stocks stabilize at lower levels of N and C because the soil C:N ratio tends to stabilize at values close to those observed in Control treatments after successive SS applications as showed by Adani and Tambone (2005), Fernandez et al. (2009), Hallin et al. (2009), Lima et al. (2009) and in this study.

We can compare the data collected in the present study with those reported by Dias et al. (2007). The study sites for both sets of experiments have similar soil texture and classification, and were managed using the same practices and subjected to the same climatic conditions because they are in neighboring counties and at the same altitude. However, Dias et al. (2007) used SS with a mean C:N ratio of 7.3 and C content in soil 0-10 cm deep was estimated as 12 % greater than in N fertilized soil (Control) six years after treatment. In our study, the mean C:N ratio of SS was 9.6 and C content in 1SS-amended soil 0-10 cm deep remained 67 % higher than in N fertilized soil (Control) even after two years with no SS application. Adani and Tambone (2005) did not find any changes in C soil content (0-25 cm depth) between SS and Control treatments after 10 annual SS applications; thus, the C:N ratio was equal to 5.3. In this context, we attribute to the C:N ratio the high C storage achieved in this work, which reflects characteristics of recalcitrance. In this case, C accumulation overcomes those observed in Ferralsols after long term conversion from conventional tillage to no-tillage in subtropical climates in Brazil (Boddey et al., 2010).

Lower C:N ratios are found in less stable SS generated by the activated sludge process (Beecher, 2008). The activated sludge process is highly efficient in smaller areas and it is used in metropolitan regions abroad (Boon, 2003). Conversely, SS with a higher C:N ratio is produced by slower stabilization processes in water reclamation stations as the one used in this study. This slower process uses an aerated lagoon followed by decantation. In a region with the largest waste reclamation treatment plants in the world, SS is quickly generated by fast processing. However, it becomes biologically stabilized remaining for a relatively extended time period in patios (Tian et al., 2009). Thereafter, the soil supported high C storage after consecutive SS applications (Tian et al., 2009). Thus, an alternative for obtaining more stabilized SS could be implemented with secondary treatments.

## CO<sub>2</sub>

We found significant correlations between CO<sub>2</sub> fluxes and water availability (p-values in Control < 1SS < 2SS), temperature (p-values 2SS < 1SS < Control) and both variables together ( $p < 0.0001$ ). In 2SS alone, an exponential adjustment was included in the regression equation (Figure 4). For N<sub>2</sub>O and CH<sub>4</sub>, we found no significant correlations between these measurements or any other variables, including mineral N or WFPS. In addition, by curve inclination (Figure 4) and probability

levels obtained from regressions, we found that after SS treatment, CO<sub>2</sub> flux from soil is more dependent on temperature than the Control. The angular coefficients for the Control, 1SS and 2SS were 0.105, 0.3276 and 0.404  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  per °C change in temperature, respectively.

Temperature dependence of organic matter mineralization in soils is a function of organic matter availability (Davidson and Janssens, 2006). The more organic matter became available (i.e. as result of moisture status, less aggregation or adsorption capacity), the more intense the effects of temperature on C mineralization. On the other hand, Davidson and Janssens (2006) also highlighted the role of organic matter quality in temperature sensitivity. Although the added organic matter might in part be more available in the soil, it also presented intrinsic characteristics which led to C sequestration. Moreover, since N has been indicated as a primary parameter for C sequestration by regulating organic matter turnover, the higher C mineralization might be balanced by increases in crop residues' deposition (Kirschbaum, 2006), which corroborates the retention of the great amount of C observed. Although this feedback may contribute to retention of C in the system, it is not well established and is a gap in the precursor models developed to predict soil organic matter dynamics (Kirschbaum, 2006).

The simulations using these models have pointed to C accumulation as a result of global warming in tropical ecosystems due to this gap concerning N turnover (Kirschbaum, 2006). Recently, modules considering N use efficiency have been proposed to improve the models' performance (Mooshammer et al., 2014; Wieder et al., 2013) but it still needs to be validated under different scenarios. Considering the lack of response of maize respiration to temperature and the same autotrophic respiration rates in all treatments, we were able to estimate the effects of temperature on C release from the soil. According to our observations, C mineralization increased 0.2226  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  per °C from the Control to 1SS. Using stoichiometry (C:N = 12:1; m:m), it is equivalent to 0.0159  $\mu\text{mol of N m}^{-2} \text{ s}^{-1}$ . Following the same estimates, each 1 °C stimulates the mineralization of 840 kg of C and 70 kg of N ha<sup>-1</sup> yr<sup>-1</sup> in 1SS when compared with the Control. When comparing the Control and 2SS, each 1 °C stimulates the mineralization of 1131 kg of C and 94 kg of N ha<sup>-1</sup> yr<sup>-1</sup>. Since the C:N ratio tends to remain stable, the fraction of this N lost by leaching, denitrification or exported as grains was proportional to the C decay in the SS-amended soil as a result of increases in soil temperature. For maize, Vicca et al. (2010) verified that CO<sub>2</sub> emissions from plant respiration under water-sufficient conditions do not show sensitivity to temperature, unlike CO<sub>2</sub> emissions from organic matter in soil. However, other factors, such as water content, might also regulate CO<sub>2</sub> flux, which could act as a cofactor that regulates CO<sub>2</sub> flux from soils (Davidson et al., 1998; Molen et al., 2011). We obtained

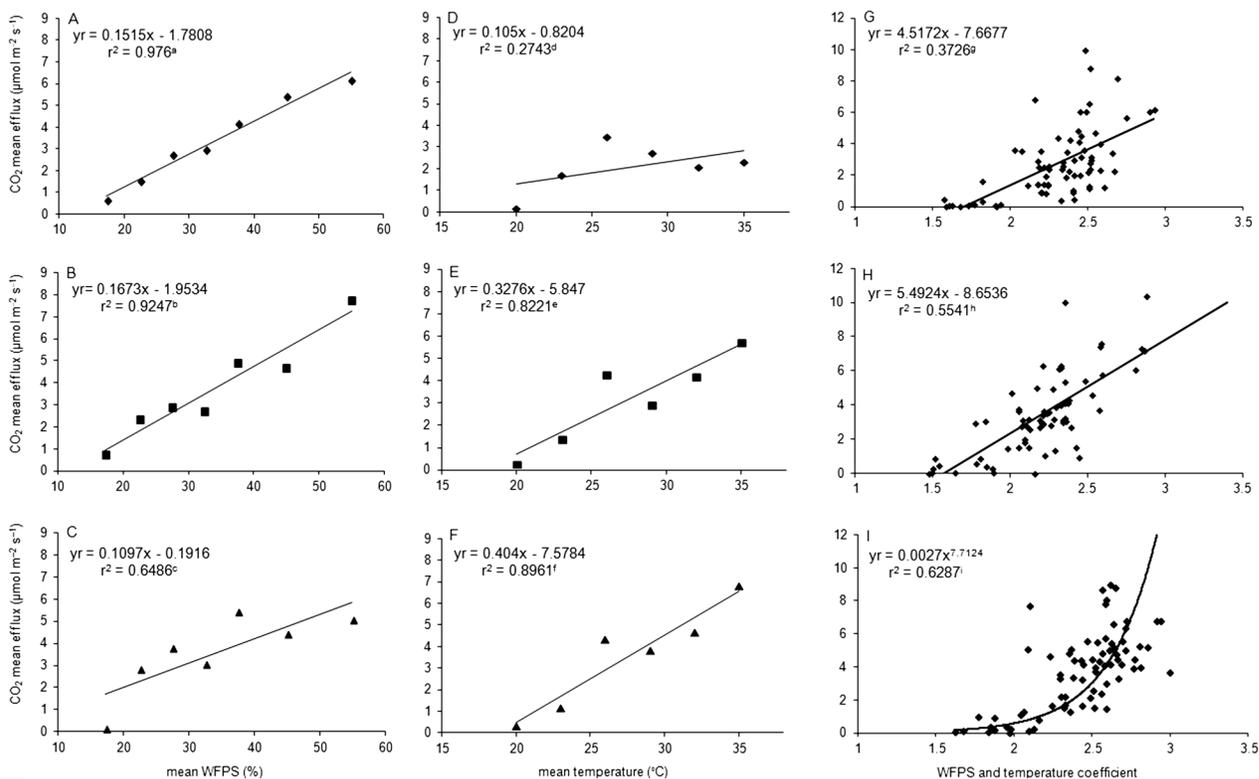


Figure 4 – Relationships between CO<sub>2</sub> flux, water-filled pore space (A, B and C), temperature (D, E and F) and multiple regression with both variables (G, H and I; see statistical methods) in the Control (A, D and G), 1SS (B, E and H) and 2SS (C, E and I) treatments. Control represents the treatment with no SS but N mineral used instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied. <sup>a</sup>*p* < 0.0001; <sup>b</sup>*p* = 0.0005; <sup>c</sup>*p* = 0.02882; <sup>d</sup>*p* = 0.28626; <sup>e</sup>*p* = 0.01265; <sup>f</sup>*p* = 0.0042; <sup>g</sup>*p* < 0.0001; <sup>h</sup>*p* < 0.0001; <sup>i</sup>*p* < 0.0001. In “I”, an exponential regression adjustment was applied.

high significance levels for multiple regressions between CO<sub>2</sub> flux and temperature associated with WFPS for all treatments, but in 2SS, an exponential adjustment was included in the regression equation.

Because heterotrophic and autotrophic metabolisms are significant sources of CO<sub>2</sub> from soil and the ratio between them is unknown (Kuzyakov, 2006), and we did not use plant productivity values, we were unable to predict the rate of C decay. However, emissions from 1SS and 2SS were 50 % and 78 % greater than from the Control, respectively (Table 4). Hence, we can use the same concepts as described for the effects of temperature to estimate the C lost related to the residual effects. In terms of N, these values represent 202 and 315 kg ha<sup>-1</sup> for 1SS and 2SS, respectively. The N exported from the system or lost by biogeochemical processes were proportional to the C lost. When we observed changes in soil management practices and C sequestration, it is assumed that this C could be returned to the atmosphere if the practices are reversed. This will follow as long as the system gets close to a new equilibrium in terms of C balance, which occurs in long term scales (Wutzler and Reichstein, 2007). As an example, Urzedo et al. (2013)

Table 4 – Weighting<sup>§</sup> seasonal fluxes of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O and estimated \*\*emissions in treatments \*Control, 1SS and 2SS.

Treatment	CO <sub>2</sub>		
	μmol m <sup>-2</sup> s <sup>-1</sup>	Mg C ha <sup>-1</sup> yr <sup>-1</sup>	Mg eqCO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup>
Control	1.29	4.88	17.90
1SS	1.93	7.30	26.78
2SS	2.29	8.67	31.78
Treatment	N <sub>2</sub> O		
	μmol m <sup>-2</sup> h <sup>-1</sup>	kg N ha <sup>-1</sup> yr <sup>-1</sup>	kg eqCO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup>
Control	0.06	0.15	68.54
1SS	0.15	0.37	172.84
2SS	0.34	0.83	390.28
Treatment	CH <sub>4</sub>		
	μmol m <sup>-2</sup> h <sup>-1</sup>	kg C ha <sup>-1</sup> yr <sup>-1</sup>	kg eqCO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup>
Control	-0.82	-0.86	-28.75
1SS	-0.66	-0.69	-23.00
2SS	-0.34	-0.36	-12.00

<sup>§</sup>Weighting accounts for wetter and drier seasonal fluxes; \*\*CO<sub>2</sub> equivalents according to IPCC (2007), where CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O GWPs are 1, 25 and 298, respectively; \*Control represents the treatment with no SS but N mineral use instead, 1SS represents the treatment in which the recommended SS rate was applied and 2SS represents the treatment in which two times the recommend SS rate was applied.

observed that in the first growing season after SS application less than 1 % of the added C was released as CO<sub>2</sub> in a forestry experiment.

### N<sub>2</sub>O and CH<sub>4</sub>

Because the GWP of N<sub>2</sub>O is 298 times greater than CO<sub>2</sub> (IPCC, 2007), emissions of this compound might reduce agriculture sustainability or compromise benefits arising from management changes. While IPCC (2006) recommends using a default value of 1 % for the applied N emitted as N<sub>2</sub>O, Crutzen et al. (2008) suggests that this amount ranges from 3 % to 5 %. Considering this range, ethanol from maize crops does not mitigate global warming. Briefly, Crutzen et al. (2008) obtained this range correlating increases in fertilizer use and N<sub>2</sub>O atmospheric concentrations. Thus, their estimate includes indirect emissions from agricultural N lost to the environment as those described by Galloway et al. (2004).

After SS use, indirect emissions would come from residual effects, in which C and N are still abundantly available to stimulate N<sub>2</sub>O emission. The residual SS effect increased N<sub>2</sub>O flux from soil, but our results in 1SS are similar to those found in non-fertilized moist tropical forests, as shown by Konda et al. (2010). In this report, the authors obtained a mean N<sub>2</sub>O flux of 0.39 μmol m<sup>-2</sup> h<sup>-1</sup> in the wet season. Souza-Neto et al. (2011) reported annual means ranging from 0.32 to 0.36 μmol m<sup>-2</sup> h<sup>-1</sup> in the Brazilian Atlantic forest. In 1SS, we found a mean flux of 0.31 μmol m<sup>-2</sup> h<sup>-1</sup> in the wetter season (Table 3).

No correlations between N<sub>2</sub>O flux and environmental variables were verified in any of the treatments. This might be explained by simultaneous processes involving production or consumption of N<sub>2</sub>O, like nitrification and denitrification, as demonstrated by Farquharson and Baldock (2008).

Between GHG emissions related to agriculture and land use changes, only CH<sub>4</sub> atmospheric concentration was decreased to levels comparable to those measured in the 1990's (Bousquet et al., 2006). Well-drained soils usually act as a sink for CH<sub>4</sub> (Conrad, 2009; Holmes et al., 1999), which was confirmed regardless of season either in the presence or absence of SS. For each treatment, no differences were observed between treatments or seasons, although gradual CH<sub>4</sub> uptake inhibition had been verified according to the SS rate.

### Conclusions

Carbon dioxide flux from soil under the residual effect of SS shows high temperature dependence, indicating that its organic matter is more available than in SS non-amended soil. However, large stores of carbon following historical SS application demonstrate that its use could be a method for carbon sequestration, even under tropical conditions. Sewage sludge properties (e.g. C:N ratio) contribute to the determination of carbon storage capacity. Although the residual effects of sewage sludge caused increases in N<sub>2</sub>O flux, after recommended rates

the fluxes were similar to those found in moist forest ecosystems and might not be considered an important indirect impact of SS residual effects.

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