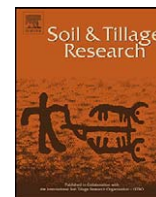




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Soil nitrous oxide emissions in long-term cover crops-based rotations under subtropical climate

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ABSTRACT

It has been shown that cover crops can enhance soil nitrous oxide (N₂O) emissions, but the magnitude of increase depends on the quantity and quality of the crop residues. Therefore, this study aimed to evaluate the effect of long-term (19 and 21 years) no-till maize crop rotations including grass [black oat (*Avena strigosa* Schreb)] and legume cover crops [vetch (*Vigna sativa* L.), cowpea (*Vigna unguiculata* L. Walp), pigeon pea (*Cajanus cajan* L. Millsp.) and lablab (*Dolichos lablab*)] on annual soil N₂O emissions in a subtropical Acrisol in Southern Brazil. Greater soil N₂O emissions were observed in the first 45 days after the cover crop residue management in all crop rotations, varying from -20.2 ± 1.9 to $163.9 \pm 24.3 \mu\text{g N m}^{-2} \text{h}^{-1}$. Legume-based crop rotations had the largest cumulative emissions in this period, which were directly related to the quantity of N ($r^2 = 0.60$, $p = 0.13$) and inversely related to the lignin:N ratio ($r^2 = 0.89$, $p = 0.01$) of the cover crop residues. After this period, the mean fluxes were smaller and were closely related to the total soil N stocks ($r^2 = 0.96$, $p = 0.002$). The annual soil N₂O emission represented 0.39–0.75% of the total N added by the legume cover crops. Management-controlled soil variables such as mineral N (NO₃⁻ and NH₄⁺) and dissolved organic C influenced more the N₂O fluxes than environmental-related variables as water-filled pore space and air and soil temperature. Consequently, the synchronization between N mineralization and N uptake by plants seems to be the main challenge to reduce N₂O emissions while maintaining the environmental and agronomic services provided by legume cover crops in agricultural systems.

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1. Introduction

Nitrous oxide (N₂O) is a major greenhouse gas (GHG). Although emissions of N₂O are numerically smaller than those for the other GHGs, they are a major contributor (about 15%) to the current increase in global warming (Isermann, 1994), mostly because the global warming potential (GWP) of N₂O is about 296 times higher than that from carbon dioxide (CO₂). Arable soils are responsible for about 57% of the annual N₂O emissions in the world (Mosier et al., 1998). In Brazil, agriculture contributes about 94% to the total N₂O emissions (EMBRAPA, 2006). According to estimations based on the emission factors currently assumed by the Inter-

governmental Panel on Climate Change (IPCC), 478 Gg N₂O are annually emitted from arable soils in Brazil.

In Southern Brazil, approximately 43% of total N₂O emissions (107 Gg) come from agricultural soils and constitute the greatest single source of N₂O to the atmosphere (EMBRAPA, 2006). However, most GHG studies have been conducted in the North Amazonia region (Neill et al., 2005; Carmo et al., 2007), while only few studies have attempted to evaluate the influence of different soil management practices on N₂O emissions and to identify the main soil and environmental driving factors determining N₂O emissions in subtropical agricultural systems of Southern Brazil (Pavei, 2005; Giacomini et al., 2006; Jantalia et al., 2008). In Southern Brazil, 8 Mha are cultivated with annual crops and the use of cover crops-based cropping systems has substantially increased in the last years as a consequence of the adoption of conservation practices, mainly no-tillage (Mielniczuk et al., 2003).

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Often, short-term peaks in N₂O emissions have been observed after crop residues are returned to the soil (Baggs et al., 2000; Millar et al., 2004). The magnitude of the emissions, however, is dependent on the chemical composition (Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007), and the quantity of plant residues added to the soil (Aulakh et al., 2001). The contents of N and lignin in the plant residues are important variables determining the N mineralization kinetics in the soil (Constantinides and Fownes, 1994) and thus can also affect soil N₂O emissions. Soil N₂O emissions tend to be greater when the added crop residues have a low C:N ratio (Huang et al., 2004), as well as a low lignin:N ratio (Millar and Baggs, 2004). In this context, the addition of legume cover crop residues—residues characterized by a high N concentration and a low C:N ratio—to the soil is expected to increase N₂O emission in comparison to emissions observed in grass-based cropping systems (Baggs et al., 2001). Garcia-Ruiz and Baggs (2007) have reported N₂O emissions up to three times higher in a soil with a legume residue input than in a non-amended soil. However, the magnitude of response to legume residue addition is expected to be dependent on environmental and soil conditions (Rochette et al., 2004). Namely soil N₂O emissions are affected by water content, soil temperature, and soil compaction (Ball et al., 1999) due to their impact on oxygen supply and substrate availability (labile organic C and mineral N) (Beauchamp, 1997). The complexity of interactions among these driving factors controlling soil N₂O emissions has been largely reported (Kaiser et al., 1998; Baggs et al., 2003). However, few studies have assessed these interactions under subtropical climatic conditions of Southern Brazil (Jantalia et al., 2008).

This study aimed to evaluate the long-term (>19 years) effect of no-till cover crop based rotations on the annual soil N₂O emissions from a subtropical Acrisol in Southern Brazil, as well as to identify environmental and soil factors controlling soil N₂O emissions in these systems.

2. Material and methods

2.1. Local climate and soil characteristics

Soil N₂O emissions were evaluated in two long-term field experiments (19 and 21 years) established at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, located in Eldorado do Sul county (30°06'S, 51°41'W; about 45 m altitude), in Rio Grande do Sul State, Southern Brazil. The local climate is subtropical humid (Cfa type according to Köppen classification), with a mean annual temperature of 19.4 °C and a mean annual rainfall of 1440 mm. The soil is classified as an Aluic Acrisol by the FAO classification system and as a Typic Paleudult by the USA classification system. Soil particle size distribution is 540 g sand kg⁻¹ soil, 240 g silt kg⁻¹ soil, and 220 g clay kg⁻¹ soil, with the clay fraction dominated by kaolinite (720 g kg⁻¹) and iron oxides (109 g kg⁻¹ of Fe₂O₃).

2.2. Long-term experiments

Two experiments were adjacently established in 1983 and 1985, both in a randomized block design and with three replicates. At that time, the soil showed visible signs of physical degradation (e.g. like rill erosion, low water infiltration, and compaction) caused by conventional ploughing and disking implemented for the previous two decades. The crop rotations (winter/summer crop) selected for this study were pigeon pea (*Cajanus cajan* L. Millsp.) + maize (*Zea mays* L.) (P + M), lablab (*Dolichos lablab*) + maize (L + M), and black oat (*Avena strigosa* Schreb) + vetch (*Vigna sativa* L.)/maize + cowpea (*Vigna unguiculata* L. Walp) (O + V/M + C) from the oldest experiment (21 years), and black oat/maize (O/M) and vetch/maize (V/M) from the 19-year-old experiment. Vetch is a subtropical legume, while pigeon pea, lablab, and cowpea are tropical legume species. All crop rotations have been managed under no-till and there was no N fertilizer applied since the establishment of the experiments.

After the management of winter and summer cover crops by glyphosate-based herbicide and roller-cutter in October 30, 2003, a 2 m × 2 m area was delimited in one plot of each treatment, where two aluminum-made bases for gas sampling were placed. Shoot residues of the cover crops were uniformly distributed across the surface of the soil, at amounts equivalent to 5 and 4 Mg ha⁻¹ of dry matter (DM) for the single oat and vetch, respectively, and a mix of 4:2.5 Mg DM ha⁻¹ (oat:vetch) for the O + V/M + C crop rotation (Table 1). For pigeon pea, cowpea and lablab, historical shoot biomass production of 12.6, 3.2 and 7.7 Mg DM ha⁻¹, respectively, were assumed. In the 2 m × 2 m area, spontaneous weeds were managed with glyphosate when necessary. In April 2005, after the maize grain harvest, shoot residues of maize were uniformly distributed across the soil surface in amounts equivalent to the DM input measured in each respective treatment (Table 1).

2.3. Air sampling and N₂O analysis

The sampling for soil N₂O emission analysis was performed for about a year, with weekly sampling in the post-management period that corresponded to the first 45 days after cover crop management (seven sampling events in this period). In the following period, sampling had intervals from 15 to 60 days (seven sampling events). The sampling schedule is shown in Fig. 1.

Air samples were manually taken from closed flux chambers (0.25 m diameter × 0.2 m height) per treatment composed of a PVC-cylinder with the top border hermetically closed. At the time of the gas measurements this chamber was fitted on to an aluminum base (0.0346 m²) inserted 5 cm into the soil. The aluminum base was equipped at the top with a circular water channel (diameter of 0.21 and 0.28 m of inner and outer ring, respectively, and height of 0.05 m) to ensure a good seal between the base and the PVC chamber. The aluminum bases were only

Table 1

Dry matter (DM) and N content of plant biomass from winter and summer cover crops plus maize added annually to a subtropical Acrisol under no-till crop rotations in Southern Brazil.

Crop rotation ^a	Winter		Summer			
	Cover crops		Cover crops		Maize	
	DM (Mg ha ⁻¹)	N (kg ha ⁻¹)	DM (Mg ha ⁻¹)	N (kg ha ⁻¹)	DM (Mg ha ⁻¹)	N (kg ha ⁻¹)
O/M	5.0	61	–	–	6.2	37.3
V/M	4.0	115	–	–	8.4	29.8
O + V/M + C	6.5	121	3.2	76	8.5	34.7
P + M ^b	–	–	12.6	327	7.0	40.6
L + M ^b	–	–	7.7	128	8.0	39.5

^a O, oat; V, vetch; C, cowpea; P, pigeon pea; L, lab-lab; M, maize.

^b Dry matter and N and C additions estimated from historical data of the experiment.

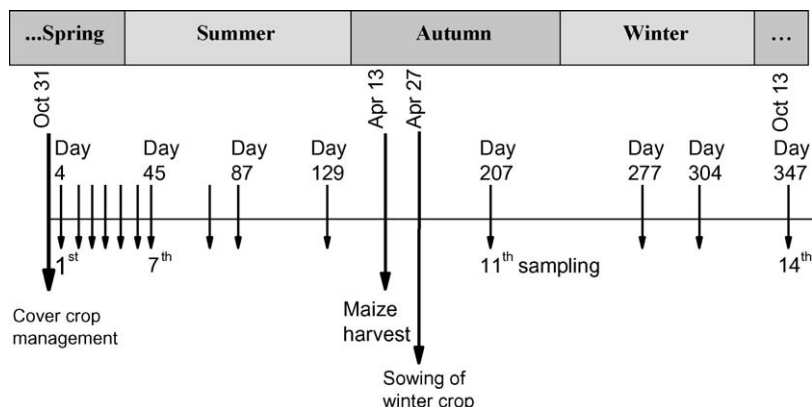


Fig. 1. Schedule of air sampling and agricultural practices across the seasons.

removed from the field at the time of sowing and harvest. The chambers had a thermometer with outside display for monitoring the temperature of the inside the chamber, and an internal fan for homogenizing the chamber atmosphere before sampling. In the top, the chambers were equipped with a rubber septum to take air samples.

Air samples were taken simultaneously in the treatments, beginning at 9 a.m. and taking samples at 0, 15, 30, and 45 min after closing the chamber. The syringes (polypropylene, 20 mL) were immediately disposed in a cooler box, where they were kept at low temperature, and dispatched by express mail to the lab (i.e., the Environmental Biogeochemical Lab, Nuclear Energy Centre, University of Sao Paulo) for analysis of N₂O concentrations by gas chromatography (GC–Shimadzu 14A); samples were analyzed within 24 h of the sampling. The chromatograph was equipped with Porapak-Q column set at 30 °C temperature, N₂ as carrier gas in flow of 30 mL min⁻¹, injector temperature was 50 °C, and ECD detector was at a temperature of 320 °C.

Gas molar volume (V_m) was corrected for headspace chamber air temperature measured at the sampling time, and the N₂O fluxes (f) were calculated taking into account the change in N₂O concentration in the chamber during the chamber closure time ($\Delta C/\Delta t$), the volume of the chamber (V , 0.00982 m³), the area of the soil covered by the chamber (A , 0.0346 m²), and the molecular weight of N₂O (m), according to the following equation (Jantalia et al., 2008):

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{V_m}$$

Mean N₂O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$) and standard deviations were calculated from the fluxes measured in the two chambers per treatment. Daily N₂O fluxes were estimated by linear interpolation assuming that the 9:00–10:00 a.m. sampling period provided a valid estimation of average daily $f_{\text{N}_2\text{O}}$. This assumption is fully supported by the results reported by Jantalia et al. (2008) working in the same region. These authors verified that air sampling at 10:00 a.m. resulted in N₂O fluxes only 11% higher than the mean daily N₂O fluxes, and air sampling in the middle of the morning was, therefore, considered the most optimal sampling time to estimate mean daily N₂O flux (Jantalia et al., 2008). Cumulative fluxes for the post-management period (45 days) and for the one-year period were calculated by integration of the daily N₂O emissions.

2.4. Soil and environment analyses

The soil N stock was evaluated through a soil sampling at 0–2.5, 2.5–7.5, 7.5–17.5, and 17.5–30.0 cm soil layers in October 2002, before the cover crop management, in three field replicates of each

treatment. Soil sub-samples were oven-dried (50 °C), ground, and the total N concentration was determined by digestion followed by Kjeldahl distillation according to procedures described by Bremner (1960). Soil N stocks (0–30 cm) were calculated using the equivalent soil mass approach (Ellert and Bettany, 1995), taking the soil mass from adjacent native grassland as reference. Soil bulk densities used for the equivalent soil mass calculations are reported by Zanatta et al. (2007) and Vieira et al. (2008).

The first 11 sampling events for N₂O analyses were complemented by evaluations of soil gravimetric moisture, dissolved organic C (DOC), and NO₃⁻ and NH₄⁺ contents in composite soil samples (4 sub-samples) of 0–0.3 m depth, and of soil temperature at 0.05 m depth, within the 2 m × 2 m area. The gravimetric moisture content was obtained by drying the soil at 105 °C for 24 h, and geo-thermometers were used to measure soil temperature. Samples for DOC analysis were air dried, ground, and sieved through a 2 mm sieve. The DOC was extracted by horizontally shaking a suspension of 5 g soil and 50 mL distilled water (1:10) for 10 h. The suspension was centrifuged and the supernatant filtered through regenerated cellulose membrane filters of 0.45 μm pore diameter (Chantigny, 2003). The DOC contents were determined by dry combustion using a TOC-VCSH Analyzer Shimadzu®. Soil mineral N was extracted with 1 M KCl and determined by the Kjeldahl distillation method according to procedures described by Bremner (1960).

The water-filled pore space (% WFPS) was calculated based on the results of gravimetric water content determined for each sampling date and the soil bulk density values reported by Vieira et al. (2008, 21-year-old exp.) and Zanatta et al. (2007, 19-year-old exp.). We assumed that the main factor affecting WFPS in this well structured no-tilled soil during the short sampling period (1 year) was the variation of soil water content (range of 5.8–26.1 g kg⁻¹) due to rainfall events. This approach is coherent with the small temporal variation of soil bulk density verified in previous studies on these experiments. For example, soil bulk density (mean for the 0–0.3 m soil layer) in no-tillage soil varied from 1.58 g cm⁻³ in 1990 to 1.62 g cm⁻³ in 2001 (Zanatta et al., 2007), while the range of soil bulk density in the was 1.49–1.62 g cm⁻³ for the different no-till crop rotations in the 19th year of the experiment (Vieira et al., 2008).

Local data of air temperature and rainfall (Fig. 2) for the evaluated period were obtained from the Automatic Meteorological Station from the Department of Forage and Meteorology (UFRGS), located at about 1 km from the experimental area.

2.5. Quality of crop residues

Samples of cover crops were taken in their flowering stage and analyzed for total C and N contents using dry combustion (TOC-VCSH Analyzer Shimadzu®) and digestion followed by Kjeldahl

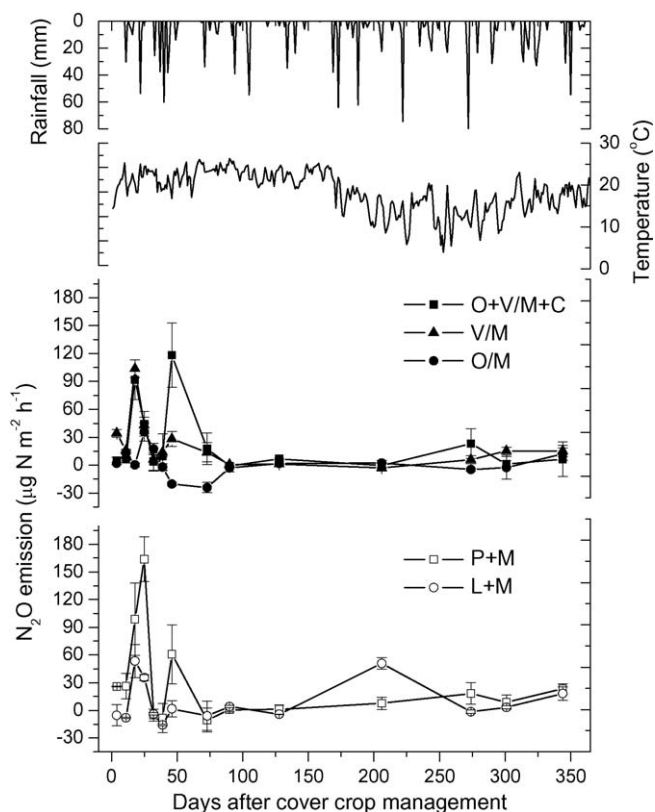


Fig. 2. Rainfall (mm), air temperature (°C) and soil N₂O emissions (μg N m⁻² h⁻¹) from an Acrisol under no-till crop rotations in Southern Brazil. O, oat; V, vetch; C, cowpea; P, pigeon pea; L, lablab; M, maize. Vertical bars represent the standard deviation (n = 2).

Table 2
Mean chemical composition (±standard deviation, n = 3) of cover crop residues added to a subtropical Acrisol under no-till crop rotations in Southern Brazil.

Crop	N (%)	C:N ratio (g kg ⁻¹)	Lignin (g kg ⁻¹)	Lignin:N (g kg ⁻¹)
Oat	1.22 ± 0.15	33 ± 3	7.2 ± 0.3	5.9 ± 0.2
Vetch	2.88 ± 0.22	14 ± 1	9.6 ± 0.2	3.3 ± 0.1
Oat + vetch	1.86 ± 0.10	22 ± 2	8.3 ± 0.2	4.5 ± 0.2
Cowpea	2.37 ± 0.18	17 ± 2	9.8 ± 0.3	4.1 ± 0.2
Pigeon pea	2.59 ± 0.19	15 ± 1	6.6 ± 0.1	2.6 ± 0.1
Lablab	1.66 ± 0.09	24 ± 2	10.1 ± 0.3	6.1 ± 0.2

distillation, respectively (Table 1). Lignin contents (Table 2) were determined by the van Soest method (van Soest and Wine, 1968). All analyses were performed in triplicate.

2.6. Statistical analysis

Considering that the measurements of N₂O were accomplished with two chambers in the same plot and, thus, the evaluations are not based on field replicates, a non-descriptive statistical analysis was performed and the mean standard deviation values were used to discriminate the crop rotations. Relationship between variables was evaluated by means of determination coefficient (r²) of polynomial regressions. Results of soil N₂O emissions, soil mineral N, DOC, WFPS, soil temperature and air temperature were subjected to multivariate analysis (Principal Component Analysis, PCA) in order to evaluate the importance of each driving factor on the soil N₂O emission, using the software MULTIV (Pillar, 1997). The significance of ordination axes and clusters were evaluated by using bootstrap re-sampling. Correlation coefficients (r) larger than |0.60| were considered for the significance between variables and ordination axes.

3. Results

3.1. Soil N₂O emissions

The crop rotations had the greatest soil N₂O emission in the first 45 days after management of cover crop residues (herein called post-management period), which ranged from -20.2 ± 1.9 μg N m⁻² h⁻¹ in the O/M to 163.9 ± 24.3 μg N m⁻² h⁻¹ in P + M systems (Fig. 2). The greatest soil N₂O emissions were found in the legume-based crop rotations, with a mean of 31.6 ± 3.7 μg N m⁻² h⁻¹ for the post-management period, while the O/M exhibited the lowest mean emission (8.6 ± 2.2 μg N m⁻² h⁻¹). The largest fluxes were observed at the 18th and 25th day, which coincided with the greatest contents of mineral N and DOC, and a WFPS larger than 70% (Fig. 3). The V/M crop rotation had its maximum soil N₂O emission earlier in the season than the other crop rotations, with 103.5 ± 9.4 μg N m⁻² h⁻¹ at the 18th day. On the other hand, the O + V/M + C system had its greatest emission rate (118.2 ± 34.6 μg N m⁻² h⁻¹) only at the 46th day after crop residue management.

Among the legume-based crop rotations, the L + M had the smallest soil N₂O emission at all sampling events of the post-management period (mean of 9.2 ± 3.2 μg N m⁻² h⁻¹), while the mean emission for P + M was about five times higher (49.5 ± 6.7 μg N m⁻² h⁻¹). Consequently, the cumulative emission for the post-management period was ranked in the following order: P + M (0.535 ± 0.072 kg N ha⁻¹) > O + V/M + C (0.374 ± 0.005 kg N ha⁻¹) > V/M (0.358 ± 0.047 kg N ha⁻¹) > L + M (0.103 ± 0.035 g N ha⁻¹); these emissions were equivalent to 43%, 29%, 44%, and 9% of the cumulative annual emissions, respectively. The

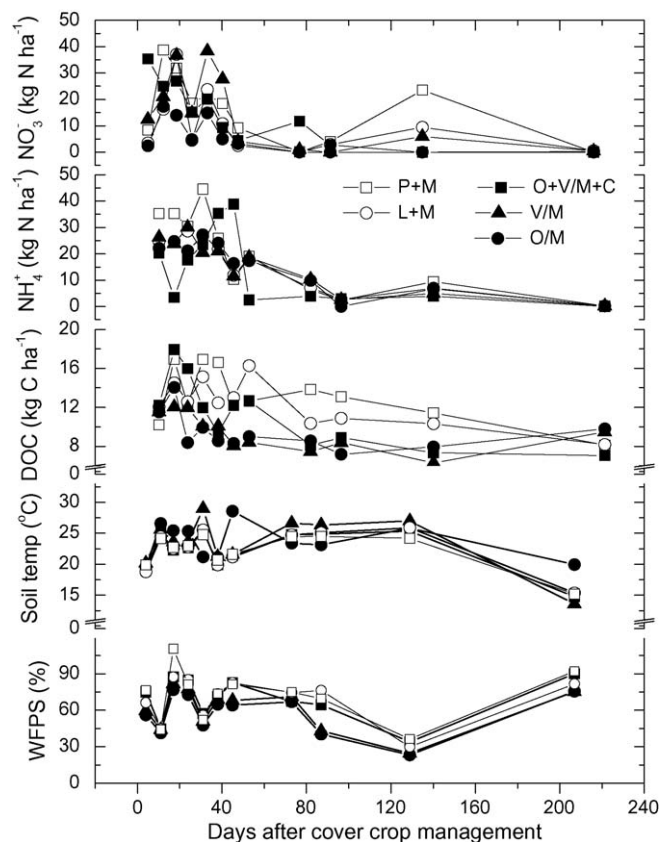


Fig. 3. Temporal variation in mineral N (NO₃⁻-N, NH₄⁺-N), dissolved organic carbon (DOC), and water-filled pore space (WFPS) at 0–0.3 m layer and soil temperature (°C) at 0–0.05 m layer of an Acrisol under no-till crop rotations in Southern Brazil. O, oat; V, vetch; C, cowpea; P, pigeon pea; L, lablab; M, maize. Mean values of a composite soil sample from four sub-samples.

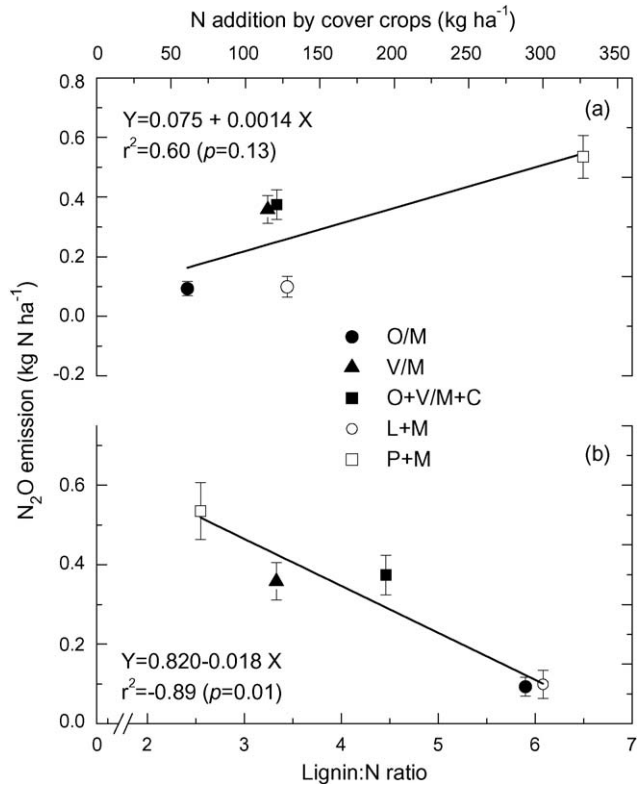


Fig. 4. Relationship between accumulated soil N₂O emissions in the first 45 days of evaluation (post-management period) and plant biomass attributes [N addition (a) and lignin:N ratio (b)] for an Acrisol under no-till crop rotations in Southern Brazil. O, oat; V, vetch; M, maize; C, cowpea; P, pigeon pea; L, lablab. Vertical bars represent the standard deviation ($n = 2$).

cumulative soil N₂O emission for the post-management period was directly related to the added N ($r^2 = 0.60$, $p = 0.13$; Fig. 4a) and it was negatively related to the lignin:N ratio of the added cover crop residues ($r^2 = 0.89$, $p = 0.01$; Fig. 4b).

After the post-management period, the mean soil N₂O emissions of all crop rotations decreased from $27.0 \pm 3.4 \mu\text{g N m}^{-2} \text{h}^{-1}$ (0th to 45th day) to $7.9 \pm 1.5 \mu\text{g N m}^{-2} \text{h}^{-1}$ (46th to 344th), and the negative fluxes were eventually observed in most crop rotations reaching the greatest uptake rate of $-24.0 \pm 5.4 \mu\text{g N m}^{-2} \text{h}^{-1}$ in the O/M system (Fig. 2). In spite of the decrease in magnitude of values, the crop rotations L + M, O + V/M + C, and P + M, which included the tropical legume species, sustained higher emissions than the other crop rotations, similarly to what was observed for the first 45 days period. While the soil N₂O emissions in the post-management period were related to the N addition by cover crop biomass, the emissions in the subsequent period were closely related to the total soil N stocks ($r^2 = 0.96$, $p = 0.002$; Fig. 5).

The largest annual soil N₂O emissions were observed in tropical legume-based crop rotations (1.116 ± 0.143 , 1.316 ± 0.103 , and $1.323 \pm 0.221 \text{ kg N ha}^{-1}$ for L + M, O + V/M + C, and P + M, respectively; Fig. 6). On the other hand, 30% less N₂O ($0.805 \pm 0.072 \text{ kg N ha}^{-1}$) was emitted from the soil under the V/M than the L + M crop rotation, and the O/M rotation resulted in a net annual uptake of $-0.074 \pm 0.056 \text{ kg N ha}^{-1}$. Assuming that the total amount of soil N₂O emitted from the crop rotations in the year was derived from the N input by crop residues (Table 1), it would imply that about 0.39–0.75% of the added N were lost as N₂O.

3.2. Mineral nitrogen, dissolved organic C and water-filled pore space

Mineral N and DOC contents in the 0–30 cm soil layer substantially increased after the management of the cover crop

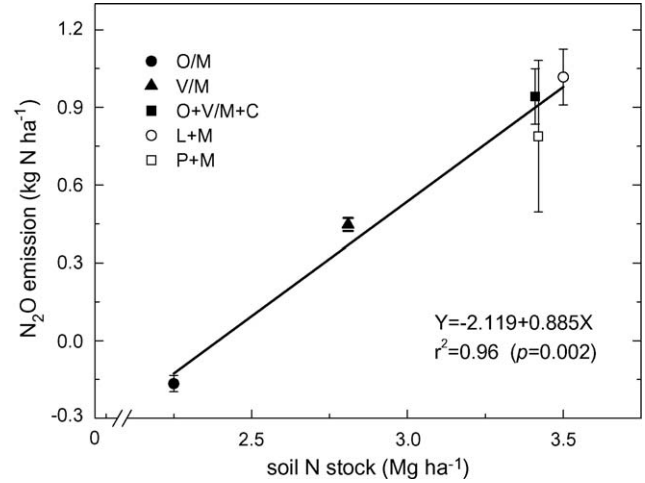


Fig. 5. Relationship between cumulative soil N₂O emissions from the period after the post-management period (from 45th to 344th day period) and the total soil N stock (0–0.3 m) of an Acrisol under no-till crop rotations in Southern Brazil. O, oat; V, vetch; M, maize; C, cowpea; P, pigeon pea; L, lablab. Vertical bars represent the standard deviation ($n = 2$).

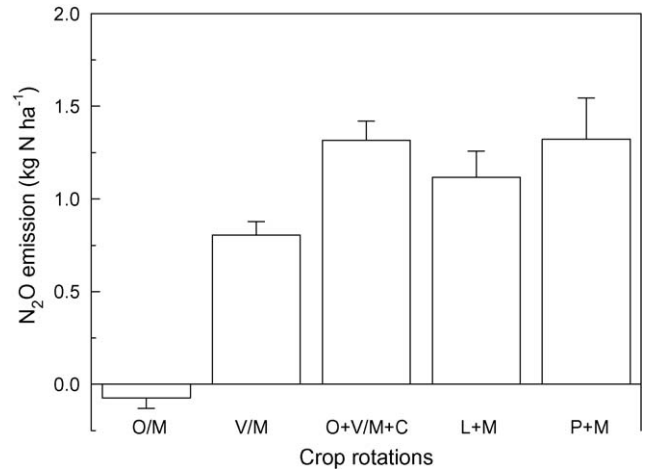


Fig. 6. Annual soil N₂O emissions from an Acrisol under no-till crop rotations in Southern Brazil. O, oat; V, vetch; M, maize; C, cowpea; P, pigeon pea; L, lablab. Vertical bars represent the standard deviation ($n = 2$).

residue, promoting the highest values in the post-management period (Fig. 3). In the following period, however, their contents were generally low. The sum of NO₃⁻ and NH₄⁺ contents exceeded 70 kg ha^{-1} for the P + M, V/M, and L + M in some sampling events (Fig. 3). The highest DOC contents were observed in the soil under P + M and L + M, especially in sampling events immediately after rainfall events (Fig. 3). There was no large difference in the annual mean contents of DOC among the crop rotations. However, the soil under P + M and L + M systems tended to have higher contents (mean of 13.1 and 12.4 kg ha^{-1} , respectively) than the O/M (mean of 9.4 kg ha^{-1}) and the other legume-based crop rotations (mean of 9.4 and 11.2 kg ha^{-1} for V/M and O + V/M + C, respectively).

The WFPS in the soil ranged from about 20% up to 100%, with most values in the 60–80% range (Fig. 3). The crop rotations had negligible effect on the WFPS, which was basically dependent on the rainfall.

3.3. Multivariate analysis

The first two ordination axes represented 66% of the variability in the factors that affect soil N₂O emissions (Fig. 7). The principal

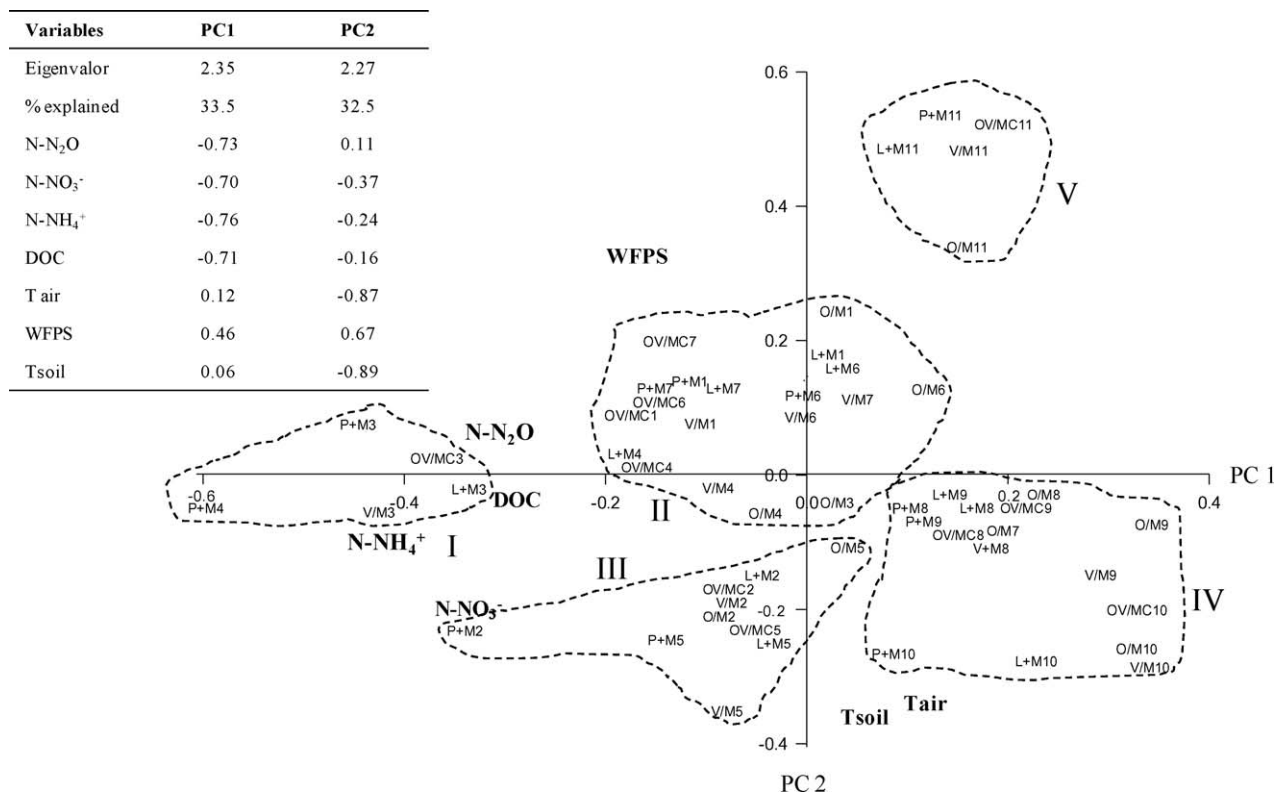


Fig. 7. Principal component analysis (PCA) for soil and environment attributes [NO₃⁻, NH₄⁺, dissolved organic carbon (DOC), air and soil temperature, and water-filled pore space (WFPS) and soil N₂O emissions]. O, oat; V, vetch; C, cowpea; P, pigeon pea; L, lablab; M, maize. Number after crop rotation refer to air sampling event (1st to 11th).

component 1 (PC1) had higher correlation with soil N₂O emissions and contents of NO₃⁻, NH₄⁺, and DOC, all of them with relatively similar correlation (*r*) coefficient (N₂O: -0.73, NO₃⁻: -0.70, NH₄⁺: -0.76, DOC: -0.71). The principal component 2 (PC2), in turn, represented better the variability of WFPS and soil and air temperature, with the axis having a positive relation with WFPS (0.67) and a negative relation to air and soil temperature (-0.87 and -0.89, respectively). Therefore, PC1 signifies more the variables connected to the effect of crop rotation, while PC2 indicates more the influence of the environmental conditions. The clusters of the crop rotations and corresponding sampling events in the representation of principal component analysis (Fig. 7) allowed assorting the results in five groups. Group I contained the observations with the greatest N₂O emissions and NO₃⁻, NH₄⁺ and DOC contents in soil. Groups II and III contained the observations with smaller values of these variables in comparison to group I, and they were distinguished by observations made under higher WFPS conditions (group II) or higher soil temperature conditions (group III). The lowest N₂O emission observations were grouped in groups IV and V.

4. Discussion

4.1. Effect of legume residues on soil N₂O fluxes

The implementation of contrasting crop rotations with no-till management for about two decades clearly affected soil N₂O emissions in two main ways. First, the effect of crop rotations manifested immediately after the addition of the fresh residues of different quality to the soil by promoting the more intense and short-lived increases in soil N₂O emissions (Figs. 2 and 4). Second, after the fresh residue effect, the emissions returned to background rates, which were then mainly influenced by the legacy effect of

the different crop rotations on long-term soil attributes, especially total N stocks (Fig. 5).

Although the intensity of the soil N₂O fluxes were a little greater in our study under subtropical climate, similar fast effects of residue addition was observed by Baggs et al. (2006) in a tropical no-tilled soil from Western Kenya. The addition of residues (i.e., *Tephrosia* and *Crotalaria*) resulted in short-lived fluxes of N₂O in comparison with a natural fallow system, and the highest peaks of N₂O emission occurred on the 20th day after crop residue addition. At the 20th day, the mean soil N₂O flux from the soil under natural fallow system (control) was about 12 μg N m⁻² h⁻¹, while the residues increased emission up to 110 μg N m⁻² h⁻¹. Other authors have reported similar effects of crop residues on soil N₂O emissions under field conditions (Rochette et al., 2004; Gregorich et al., 2005).

Legume cover crop-based crop rotations increased soil N₂O emissions, mainly the tropical legume species, in comparison to the O/M system, in which a net annual uptake was observed (Figs. 2 and 6). Increases in soil N₂O emission after legume residue input to the soil were also documented by other authors (Baggs et al., 2000, 2006; Millar et al., 2004; Millar and Baggs, 2005). Due to the high quality of biomass i.e., low C:N and lignin:N ratio (Constantinides and Fownes, 1994; Baggs et al., 2001), the addition of legume residues to the soil stimulates the microbial activity and, thereby, enhances N₂O emissions for two complementary reasons. First, the fast mineralization of N from legume residues and the resulting increase in soil mineral N (NH₄⁺ and NO₃⁻) contents provides substrate for N₂O production. Second, the increased consumption of available oxygen during legume decomposition increases the occurrence of anaerobic microsites and, therefore, the N₂O production by denitrification, which is more effective in producing N₂O than the nitrification process (Khalil and Baggs, 2005). In addition, the deposition and maintenance of crop residues on the soil surface in no-till systems lead to conditions suitable for

denitrification due to the enhancement of water storage and C supply (Linn and Doran, 1984; Baggs et al. 2003). Both factors can amplify the N₂O production in legume-cultivated no-till soils (Baggs et al., 2003; Rochette et al., 2004).

Due to all the factors cited above, the greatest soil N₂O emissions were observed in the first 45 days after the crop residue management (Fig. 2) and they were directly related to the N input by biomass addition and their quality—mainly expressed by the C:N and lignin:N ratio (Baggs et al., 2001, 2006; Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007). The L + M system, however, had only 9% of the total annual emission in the first 45 days after crop management, which is probably attributed to the fact that the leaves of lablab fall in the winter. Therefore, a substantial proportion of N from lablab residues had probably mineralized before the post-management period.

It is interesting to note that the presence of black oat residues in addition to vetch in the O + V/M + C system delayed the increase in soil N₂O emissions in the post residue management period in comparison to the other legume-based crop rotations. This is likely due to the greater C:N and lignin:N ratios (Table 2) of the oat residues, promoting greater N immobilization during residue decomposition (Garcia-Ruiz and Baggs, 2007). The delay in N mineralization would help to keep the mineral N levels low in the soil, potentially enhancing the synchronism between N mineralization and plant uptake (Amado et al., 1998; Baggs et al., 2001) and, thereby, decreasing soil N₂O emissions. Moreover, the use of vetch plus oat as mixed cover crops is a viable option for reducing the chemical N fertilizer requirements by the cash crop because of the biological N fixation by the vetch (Amado et al., 1998).

Soil N₂O uptake was eventually observed in almost all crop rotations after the first 45 days of evaluation, and the O/M system had the highest annual net uptake of -0.074 ± 0.056 kg N ha⁻¹ year⁻¹. Soil N₂O uptake has been reported under different soil and climate conditions at rates up to -484 μg N m⁻² h⁻¹ (Chapuis-Lardy et al., 2007). The N₂O uptake in arable soils has been attributed to the conversion of N₂O to N₂ in the denitrification process, when NO₃⁻ concentration is low and WFPS is high (Blackmer and Bremner, 1978; Chapuis-Lardy et al., 2007). However, additional research is required for a more complete understanding, especially considering the potential of GHG mitigation this N₂O uptake can entail in agricultural soils.

The larger total soil N stocks under the tropical legume-based rotations than in the V/M and O/M systems led to greater soil N₂O fluxes in the period following the post-management period. The greater input to the soil of biomass and N through biological N fixation promoted a gradual raise in total N stocks in the soils cultivated with legume species (Amado et al., 1998; Diekow et al., 2005). The greater N stocks enhance N mineralization and consequently induce the potential of soil N₂O emissions. Under the conditions of this study, the N input by crop residues was, therefore, more important in controlling soil N₂O emissions in the short-term (i.e., the first 45 days after cover crop residue management), while the total soil N stock was more important in the subsequent period. These results illustrate that the long-term historical soil use and management influence soil N₂O emissions, and not only the short-term effects of treatments, is of importance in regulating N₂O emissions.

The annual soil N₂O emissions were a relatively low percentage of the total N added to the soil (0.39–0.75%) when compared to the mean losses of $1.25 \pm 1.0\%$ globally ascribed for N inputs to arable soils (IPCC, 2006). The IPCC emission factors have been criticized by many researchers, especially in relation to concerns about their generalized adoption in GHG inventories (Rochette et al., 2004; Gregorich et al., 2005). In a recent study carried out in Southern Brazil, Jantalia et al. (2008) verified the same tendency of overestimation of N₂O emission by using IPCC emission factors. The use of IPCC factors

resulted in estimates of 23–250% greater than field N₂O measurements (Jantalia et al., 2008).

The maximum annual emission in the present work under subtropical climate was about 1.323 ± 0.221 kg N ha⁻¹ year⁻¹, which falls within the range of 0.16–3.70 kg N ha⁻¹ year⁻¹ reported by Gregorich et al. (2005) for Canadian arable soils, and is proportionally similar to the cumulative emissions (99 days) for several agroforestry residues in Kenya; they ranged from about 0.10 to 0.60 kg N₂O-N ha⁻¹ (Baggs et al., 2006). However, our results are less than the mean annual emission rates of 2.70 kg N ha⁻¹ year⁻¹ for European arable soils (Melillo et al., 2001).

4.2. Variables driving soil N₂O emissions

In addition to the crop residue effect, soil N₂O emissions were impacted by soil physical conditions (i.e., WFPS), which were likely influencing oxygen availability (Beauchamp, 1997; Neill et al., 2005). Similar to the studies of Marinho et al. (2004) and Du et al. (2006) the greatest daily soil N₂O emissions were observed immediately after rainfall events, mainly at the 18th and 25th day after crop residues management, when WFPS was indeed high and consequently oxygen availability in the soil must have been low. Taking into account that the mineral N content, especially NO₃⁻ content, in the post-management period was not limiting, the increases in WFPS probably generated anaerobic sites where denitrification of NO₃⁻ was intensified (Dobbie and Smith, 2003; Neill et al., 2005). In addition, greater NO₃⁻ concentrations in soil may have inhibited the conversion of N₂O to N₂ because of the preference for NO₃⁻ instead of N₂O as final electron acceptor (Blackmer and Bremner, 1978; Chapuis-Lardy et al., 2007). It has been suggested that ≥ 10 mg NO₃⁻-N kg⁻¹ soil is the threshold value for such inhibition. However, we observed a maximum mean concentration of 8 mg NO₃⁻-N kg⁻¹ for the 0–0.3 m (approximately 38 kg ha⁻¹ in the 0–0.3 cm layer, Fig. 3). Probably, the greater depth of soil sampling diluted the mean NO₃⁻ content, as N mineralization from crop residues in no-till mostly occurs in the surface soil layers. In the same experiment as in the present study, Dieckow et al. (data not published) found frequently NO₃⁻-N contents higher than 15 mg N kg⁻¹ in the 0–0.05 m soil layer under legume cover crops-based rotations. Therefore, NO₃⁻-N concentrations were probably high enough to inhibit the reduction of N₂O to N₂ in certain parts of the 0–0.3 m soil layer.

The results of the multivariate analysis pointed to an interaction among the driving factors controlling soil N₂O emissions in the present study. The most important factors were mineral N (NH₄⁺, NO₃⁻) and DOC contents, and they interacted with soil WFPS, as observed in the grouping interpretation (Fig. 7). Group I contained the observations with greatest N₂O emissions (mean of 102.5 μg N-N₂O m⁻² h⁻¹), was composed mainly by legume-based cropping systems, and the emissions were directly related to the largest soil mineral N and DOC contents, but no distinctive importance was observed between these factors as correlation coefficients of these factors and PC1 axis were very similar. On the other hand, the air temperature and WFPS seemed to have secondary importance, demonstrating that the soil management effect was more important for determining the greatest N₂O emission. This affirmation is supported by the fact that N₂O emission was better related to PC1 axis, similarly to the NH₄⁺, NO₃⁻, and DOC contents, while air temperature and WFPS were better related to PC2.

Observations classified in groups II and III had lower soil N₂O emissions (mean 27.1 and 7.1 μg N m⁻² h⁻¹, respectively) than those from group I, which was related to the decrease in mineral N and DOC and/or in WFPS. However, between groups II and III, the management-controlled parameters (mineral N and DOC) were similar (Fig. 3) and therefore the greater mean soil N₂O emission

from group II than from group III was due to greater soil WFPS (Fig. 3), reducing the oxygen availability and enhancing denitrification (Beauchamp, 1997). Several authors have indicated the predominance of denitrification as the source for N_2O production in soils when WFPS values are larger than 70% (Bateman and Baggs, 2005; Khalil and Baggs, 2005; Ciarlo et al., 2007). Similar to the study of Marinho et al. (2004), the greatest daily fluxes were observed immediately after rainfall events (Fig. 2), mainly at the 18th and 25th day after crop residue management, when WFPS was high (Fig. 3). Assuming that the mineral N content in the post-management period was not limiting, especially NO_3^- , the increases in WFPS generated anaerobic sites where denitrification was intensified (Dobbie and Smith, 2003). In addition, greater NO_3^- concentrations in soil may have inhibited the conversion of N_2O to N_2 , as previously discussed.

Group IV consists of flux observations ranging from very low N_2O emissions to even uptake of N_2O , with a mean of $-1.33 \mu\text{g N m}^{-2} \text{h}^{-1}$. In this group, both the low DOC and N availability and the low WFPS led to low N_2O emissions and even an uptake of N_2O . Group V, however, had similar DOC and N availability as in group IV, but the WFPS was greater in group V than in group IV, leading to the greater emissions (mean of $11.5 \mu\text{g N m}^{-2} \text{h}^{-1}$). However, the flux rates were still modest when compared to those observed in group I, probably because of the limited available of C and mineral N.

In summary, among all the variables, those related to differences in management practices affected more soil N_2O fluxes. The environmental variables were important only when C and N availability were not limiting, and even when they were not limiting, soil N_2O emissions were more responsive to increases in N availability and DOC contents than to increases in WFPS. Similarly, by using a multivariate approach, Lee et al. (2006) have reported that the labile C and N availability were the main driving variables controlling N_2O fluxes from no-tillage and standard tillage soils.

In this context, controlling the synchronization between N mineralization/availability and its uptake by plants seems to be the best option to mitigate N_2O emissions from legume residue-amended soils. It can be inferred, therefore, that management practices aiming at decreasing soil N_2O emissions should maximize plant growth and avoid fallow periods. Even though cover crops legumes contribute considerably to the soil N_2O emissions, a global analysis of the environmental services of legume cover crops in agricultural systems should be performed for evaluating their effect on net global warming potential by considering also the increased carbon sequestration in soil, the fluxes of methane and the reduction of carbon costs from reduced N fertilizer use (Meyer-Aurich et al., 2006), in addition to their agronomic benefits and positive effects on soil quality (Reeves, 1997; Six et al., 2004).

5. Conclusions

Legume cover crops increase soil N_2O emissions to the atmosphere. Greater soil N_2O emissions are observed in the short period (45 days in this study) after cover crop management and are directly related to the quantity of N and inversely related to the quality (lignin:N ratio) of the cover crop biomass. After this initial short period, N stocks drive soil N_2O emission in long-term no-till crop rotations. These effects of soil conditions and cover crops on N_2O emissions are mainly related to the N availability and labile C supply. Management-controlled soil variables such as mineral N (NO_3^- and NH_4^+) and DOC contents are more related to soil N_2O emissions than environmental-related variables as water-filled pore space and air and soil temperature. Thus, managing the synchronism between N mineralization/availability and its uptake by plants is the most pertinent to control N_2O emissions from legume residue-amended soils.

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