

Soil nitrous oxide emissions from a soybean-wheat succession under different tillage systems in Southern Brazil

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ABSTRACT: No-tillage (NT) has been largely adopted in Brazil as a strategy for soil conservation, but for the last decade, there have been governmental incentives for its adoption arising from its potential for soil C accumulation. Notwithstanding, the soil mulch formed from crop residues favors the maintenance of soil moisture and nutrients in the upper soil layers, which stimulates soil microbial activity and may increase the potential for nitrous oxide (N₂O) emissions. In addition, double-cropping systems in the same year are typical in Brazil and the impact on the fraction of fertilizer N lost as N₂O needs to be evaluated. This study aimed to assess the influence of soil tillage and N fertilization on N₂O emissions in a wheat-soybean succession system as commonly practiced in southern Brazil. The experiment was carried out at Embrapa Soybean research station located in Southern Brazil. Treatments were conventional tillage (CT) and no-tillage (NT), with and without nitrogen fertilization for the wheat and no N fertilizer for the soybean. Closed-static chambers were used to monitor N₂O fluxes for two consecutive years. Together with gas monitoring, soil samples were also taken and analyzed for mineral N, soil moisture and labile carbon. Soybean yields were higher under NT, which seemed to be the result of a higher soil water availability that helped to overcome extended periods without rainfall. Soil N₂O emissions were similar between CT and NT, with just a tendency for higher emissions under NT. The highest emissions occurred from the soybean crop. In the second year under NT, the emissions from the soybean crop were higher when preceded by N-fertilized wheat, but the converse was true under CT. None of the soil variables consistently correlated with N₂O emissions, with mineral-N as the best predictor in the second wheat cycle and soil moisture in the first soybean cycle. Calculated emission factors were not statistically different between CT and NT and consistently lower than the IPCC default of 1 %. The calculated N₂O emission intensity by relating N₂O emission to grain yield showed an environmental advantage of NT compared to CT by presenting a 44 % reduction in soybean and similar values for fertilized wheat.

Keywords: greenhouse gas, N₂O, soil mineral N, soil moisture, *Glycine max*, *Triticum aestivum*.

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INTRODUCTION

In areas with more than 20 years under a constant annual cropping system, soil carbon stocks will have attained a steady state (IPCC, 2006) and hence nitrous oxide (N₂O) becomes the most important greenhouse gas (GHG) emitted. Conventional tillage (CT) systems using soil plowing and chiseling have been shown to provoke soil erosion and fertility loss in tropical regions, especially in sloped areas (Labrière et al., 2015). As an alternative, no-tillage (NT) systems gained significant importance as a conservative strategy with economic advantages and capable of preserving the soil resource and contributing to GHG mitigation by storing atmospheric CO₂ in the soil profile as organic matter (Bernoux et al., 2009).

In Brazil, such potential has been stimulated by the so called Low-Carbon Agriculture Plan, which provides subsidized credits for financing mitigation actions in agriculture through the ABC program (Sá et al., 2016). However, given the importance of N₂O as a gas with 273 times higher global warming potential than CO₂ (Smith et al., 2021), there have been concerns about the impact of NT adoption in the final GHG balance of cropping areas (Bernoux et al., 2009).

Cropped areas under NT usually present higher microbial activity in the upper soil layers due to a concentration of labile organic matter and nutrients, compared to CT (Zhang et al., 2018). In addition, soil moisture tends to be preserved by the mulch formed from harvest residues and without plowing, soils may become compacted. Such a combination of factors may increment hotspots of N₂O production in NT areas (Mei et al., 2018).

However, field monitoring of GHG does not always confirm NT as a greater inducer of N₂O emissions than CT (Rochette et al., 2008; Feng et al., 2018). In Brazil, where mechanized crop production occupies approximately over 60 million hectares (Mha), of which more than half is dedicated to soybean (*Glycine max*) (IBGE-LSPA, 2021) growing on well drained Oxisols, results are also inconclusive. Jantalia et al. (2008) monitored different crop rotations under CT and NT and no contrasting differences between tillage systems were detected. On the other hand, Escobar et al. (2010) observed higher N₂O emissions induced during soybean crop in NT areas compared to CT, but the reverse was observed in the following corn crop. In a similar experiment, Bayer et al. (2015) observed a significant increase in N₂O emissions under NT, where legume cover-crop was practiced, indicating NT induces higher N₂O emissions than CT where N availability is high. However, this study was on an Acrisol with restricted drainage. Campanha et al. (2019) studied a Ferralsol in the Cerrado region and found that N fertilization of corn rendered higher N₂O emission in CT than in NT areas.

Apart from the variable effect of NT and CT on N₂O emissions, there is also lack of data on emission factors for fertilizers where two crops are carried out in the same year. In the southern region of Brazil, the soybean crop is most often followed by wheat (*Triticum aestivum*), the former occupying the rainy and warm season (October to March) and the latter the drier and cold season, both in the same year. In this region, the wheat-soybean succession is responsible for 87 and 33 % of the national production of these two grains, respectively (IBGE-LSPA, 2021).

Following the recommendation of measuring the fertilization effect on soil N₂O emissions for a whole year, the emission factor should include both crops, even though there is evidence that the fertilization effect practically ceases after two to three months (de Moraes et al., 2013). The possibility of carry-over effects between crops should be investigated on fertilizer emission factors and also on potential differences between emissions under NT and CT owing to soil variables when the two crops are carried out in the same year, which was the objective of this study.

MATERIALS AND METHODS

The experiment was conducted over two consecutive years at the experimental station of the National Soybean Research Center (Embrapa Soja), in the municipality of Londrina, Paraná (23° 12' S, 51° 11' W, 585 m a.s.l.). The climate of the region is classified as subtropical humid, Cfa, according to the classification system of Köppen, with dry winter and humid summer with an annual mean temperature of 21°C (Peel et al., 2007). Mean annual rainfall is 1626 mm, with greater incidence between October and March. The soil in the area is classified as *Latossolo Vermelho Distroférrico* by the Brazilian Soil Classification System or by the FAO system as a Rhodic Ferralsol (Rhodic Eutradox, USDA Soil Taxonomy), presenting a high (low-activity) clay content (787 g kg⁻¹ clay). In the 1981/1982 season, a long-term experiment was established with different tillage systems in a completely randomized block design, with six replications. The treatments utilized in this study were a conventional tillage (CT) system with a heavy disk plough (66 cm diameter discs) followed by a light disc harrow with 22 cm soil depth operation, and a no-tillage treatment (NT). Each experimental plot had dimensions of 8 × 50 m, with a total area of 400 m².

From the start of the experiment in 1981, the plots selected were planted with soybean in summer and wheat in winter. Dolomitic lime was applied every four years starting in 1980 previously to plots set up and with the last application carried out after the wither harvest of 2012. Liming rate was 2 Mg ha⁻¹, which resulted in a mean pH(H₂O) between 5 and 6 in the 0.00-0.20 m soil layer. In the NT treatment, the lime was applied to the soil surface and not incorporated mechanically, while in the CT, it was distributed and then incorporated with the heavy disc plough.

The study started in the winter of 2013, and the chemical analysis of soil samples were performed in the soil layer of 0.00-0.10 and 0.10-0.20 m just before planting (Table 1). For the two years of this study, the wheat cultivar Pardel was used, and the cultivar for soybean was BRS 360RR. The plots under NT and CT were divided in half; one half received N fertilizer and the other no N to serve as the control. Wheat was first sown on May 03, 2013 with a row spacing of 0.17 m. For the treatments with N fertilization, compound fertilizer 8-20-20 was added at 260 kg ha⁻¹ in the furrow by the planting machine, and for the control treatment, the same quantity of 0-20-20 was applied. Seventeen days after sowing, 90 kg ha⁻¹ of urea (41 kg N ha⁻¹) was applied on the surface only in the N treatments. The harvest of the wheat was performed on September 20, 2013. The summer planting of soybean began on October 10, 2013, with tillage of the soil in the area under CT and the seeds were sown on 19 October with row spacing of 0.45 m. A stand of 16 plants per linear meter was the target. Fertilizer applied to all plots was 270 kg ha⁻¹ of the 0-20-20. The seeds received a liquid inoculant based on *Bradyrhizobium* spp. (5 × 10⁹ CFU mL⁻¹) at the dose of 100 mL per 50 kg of seeds. The crop was harvested on February 19, 2014.

Table 1. Chemical analyses of soil samples from the layers of 0.00-0.10 and 0.10-0.20 m in a wheat-soybean succession under no-tillage (NT) and conventional tillage (CT)

Soil layer	pH(H ₂ O)	Al ³⁺	Ca ²⁺	Mg ²⁺	K	P	CEC	C
		cmol _c kg ⁻¹			mg kg ⁻¹		cmol _c kg ⁻¹	g kg ⁻¹
No-tillage								
0.00-0.10 m	5.91	0.00	5.63	1.86	203	82	8.01	17.2
0.10-0.20 m	5.26	0.00	2.88	1.22	117	13	4.39	9.1
Conventional tillage								
0.00-0.10 m	5.67	0.00	3.97	1.17	183	33	9.52	12.3
0.10-0.20 m	5.60	0.00	3.65	1.08	148	23	8.91	11.1

pH (1:2.5, soil:water); P (Mehlich-1); C (Walkley-Black).

The 2014/2015 sequence began on April 10, 2014, with the tillage of the plots under CT as before. On April 30, wheat was sown with a row spacing of 0.17 m, and 300 kg ha⁻¹ of 08-28-16 fertilizer was applied in the furrow in all treatments. On May 21, urea fertilizer (46 kg ha⁻¹) was broadcast only to the +N treatments at tillering. The wheat was harvested on September 15, 2014. The summer planting started on this same date with soil tillage using CT and the soybean seeds inoculated as before were seeded on November 07 at a row spacing of 0.50 m and fertilized in the furrow with 300 kg ha⁻¹ of the 0-20-20 formulation for all treatments. The soybean was harvested on March 13, 2015. During all the sequence of crops, insecticides and fungicides were applied as recommended.

Quantification of N₂O fluxes

The N₂O fluxes were measured from May 2013 until March 2015. Sampling for quantification of N₂O fluxes was performed between 08:00 and 10:00 a.m., following the recommendation of Alves et al. (2012), who found this time best represented the mean daily flux. Fluxes were sampled throughout the winter and summer crop cycle, starting before soil tillage and continuing until the end of the cycle.

The sampling protocol was used daily for at least seven days after planting, and then every two days, and weekly sampling was performed in the periods of least expectation of high fluxes (dry periods). Gas monitoring was done using manual static chambers, positioned longitudinally on the seed row to enclose three wheat rows. Doing this, the effects of seedbed fertilization and side-dressing with urea were captured. During soybean monitoring, chambers were positioned longitudinally to enclose one seed row for the first three weeks, when the chamber base was moved to the side of the plants to avoid plant damage.

The inferior part of the chamber consisted of a rectangular metal frame (0.40 × 0.60 m) with a height of 7.5 cm inserted into the soil to a depth of 5 cm. The upper part of the frame was equipped with a trough (2.0 cm wide × 2.0 cm high) welded on the top of the frame. This trough was filled with water to ensure the seal at the time of coupling the top of the chamber. The top consisted of a polyethylene tray (0.40 × 0.60 m, 0.12 m high), coated with foam and aluminum foil to ensure insulation to reduce temperature changes when exposed to sunlight. The top was fitted with a three-way valve used at the time of sample withdrawal. The incubation time was 30 min, the first sample being taken immediately at the time of chamber closure, another after 15 min, and the last at the end of the incubation period. Polypropylene syringes (60 mL) were used to remove samples from the chamber. Approximately 40 mL of the gas within the chamber was withdrawn, of which 25 to 30 mL were transferred to chromatography flasks sealed with chlorobutyl rubber septa, the remainder being used to expel air from the dead volume of the gas transfer system. Thereafter, syringes were coupled to a vacuum pump to transfer 30 mL of the gas samples to 20-mL chromatographic vials. A volume of ~5 mL was initially discarded to purge the pumping system. The analysis of N₂O concentrations was performed in the Embrapa Agrobiologia gas chromatography laboratory using a Shimadzu GC 2014 gas chromatograph (Shimadzu, Tokyo, Japan) whose configuration and quality control of analysis was reported in Paredes et al. (2015). The N₂O flux (μg m⁻² h⁻¹) was calculated according to the equation described by Jantalia et al. (2008).

The amount of N emitted from each area was obtained by the numerical integration of the N₂O fluxes in time, using the rectangles method. The open Newton-Cotes formula was employed, as follows in equation 1:

$$\int_a^b f(x)dx \cong (b - a)f\left(\frac{a + b}{2}\right) \quad \text{Eq. 1}$$

in which: $f(x)$ is the mean flux obtained from the previous day 'a' and from the day after 'b' the period when field measurements were not taken.

To obtain the emission factor for the fertilizer, the emission of N_2O from the area with nitrogen fertilizer was subtracted from the emission of the unfertilized area and divided by the amount of N applied.

Soil and climate variables

Rainfall and air temperature were monitored throughout the study by a meteorological station present in the same experimental field. On the days of gas sampling, soil samples (0.00-0.10 m) were also taken to determine soil moisture and soil density to determine the water-filled pore space (% WFPS), as described in equation 2:

$$\%WFPS = \left(\frac{Vm}{p} \right) \times 100 \quad \text{Eq. 2}$$

in which: Vm is the volumetric moisture content of the soil ($Vm = Mg \times BD$; Mg is the gravimetric moisture content [$g\ g^{-1}$] and BD , the bulk density [$g\ cm^{-3}$]) and p the porosity of the soil. Total porosity (%) is equal to $[1 - (\text{bulk density}/\text{particle density})] \times 100$, where the particle density was assumed to be $2.96\ g\ cm^{-3}$ as determined by Morais et al. (2013) for soil of the same classification as that taken from the same field station.

For ammonium and nitrate analysis, 20 g of soil from the layer of 0.00-0.10 m were agitated on a rotary shaker for 1 h with 60 mL of $K_2SO_4\ 0.5\ mol\ L^{-1}$. The suspension was filtered and the NO_3^- concentration was determined in the resultant solution by UV spectrometry following the procedure described by Miyazawa et al. (1985), but measuring the absorbance at the wavelengths 220 and 275 nm. The absorbance at 275 nm was multiplied by two and then subtracted from the absorbance at 220 nm to determine the absorbance of NO_3^- , as described by Olsen (2008). The same filtered extract was used to quantify NH_4^+ using the salicylate colorimetric method of Kempers and Zweers (1986). The soluble C was extracted in saline solution ($NaHSO_4\ 0.05\ mol\ L^{-1}$), with a soil-to-extractant ratio of 1:5. Soluble C was quantified in the extract using the colorimetric method of Bartlett and Ross (1988) using $KMnO_4$ as the oxidizing agent. The absorbance was determined at 495 nm.

Statistical analyses

Tests of normality and homogeneity of errors between treatments were performed for subsequent ANOVA, assuming the tillage system as the main plot and N fertilization as a subplot. Differences between treatments were separated by the Fisher's l.s.d. test, at 5 % probability. Pearson correlation analysis was performed to identify the intensity with which the different factors contributed to the soil N_2O emissions.

RESULTS AND DISCUSSION

The area planted with wheat was divided into two for the two sub-treatments with and without N fertilizer. For the soybean, no N fertilizer was added, and the whole area unfortunately was harvested as one for the two years. This problem also happened with wheat in the second year when the whole area was harvested together, so it was impossible to examine the N fertilizer's impact on yield. The yield of wheat was not significantly influenced by the tillage treatment in either year (Table 2). Yields in 2013 were considerably lower than in 2014, which may be attributed to the lack of rainfall after the end of June in the phase of anthesis and early grain development in 2013 (Figure 1). In 2014 rainfall was more evenly distributed. For both years (2013-14 and 2014-15) soybean yields were much higher under NT. This was probably due to the better stand of the crop, which was adversely affected in both years by the lack of rainfall immediately after planting. Under NT surface, mulch and the absence of plowing

Table 2. Wheat and soybean yields obtained from soil tillage and fertilization treatments on a soybean-wheat succession in southern Brazil

Treatment	Wheat 2013	Soybean 2013-14	Wheat 2014	Soybean 2014-15
kg ha ⁻¹				
NT				
+N	2098	2843a	3422	2407a
-N	2603	-	-	-
CT				
+N	2462	1568b	3421	1198b
-N	2660	-	-	-
Average	2456	2206	3422	1803
L.S.D.	-	373.4	-	295.3
P	0.288	<0.001	0.997	<0.001

Means followed by different letters within the column of the same crop are statistically different by the L.S.D. Fisher's test ($p < 0.05$).

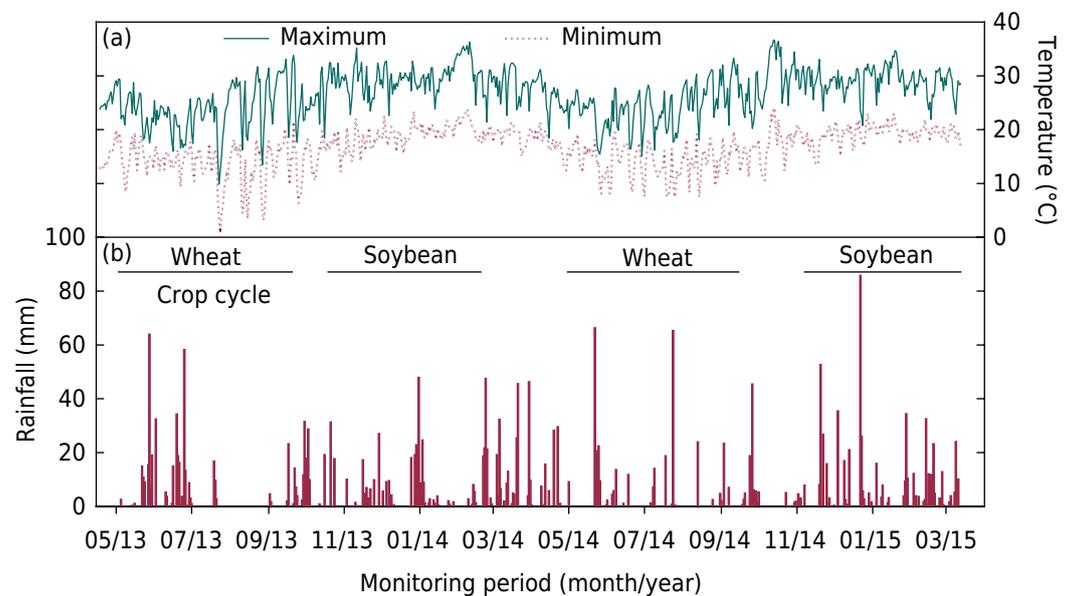


Figure 1. Air temperature and rainfall data for a wheat-soybean succession under different tillage systems and N fertilization in Southern Brazil.

preserved soil moisture and germination was much more successful. In addition, the higher soil nutrient availability under NT contributed somehow to the better yield performance under CT (Table 1).

In the cropping year 2013/2014, in the wheat cycle in CT when no N fertilizer was added, N_2O emissions varied from -12 to $89 \mu g N_2O-N m^{-2} h^{-1}$ (Figure 2b) and in NT, from -12 to $133 \mu g N_2O-N m^{-2} h^{-1}$ (Figure 3b). In the N fertilized plots, the values remained between -12 and $124 \mu g N_2O-N m^{-2} h^{-1}$ for CT, and -13 and $214 \mu g N_2O-N m^{-2} h^{-1}$ for NT. In the summer, when soybean was preceded by wheat not fertilized with N, N_2O emissions in CT ranged from -15 to $181 \mu g N_2O-N m^{-2} h^{-1}$ (Figure 2b) and in NT, from -15 to $164 \mu g N_2O-N m^{-2} h^{-1}$ (Figure 3b). In areas where wheat was fertilized with N before the soybean crop, emissions varied from -6 to $134 \mu g N_2O-N m^{-2} h^{-1}$ in CT, and in NT, from -9 to $108 \mu g N_2O-N m^{-2} h^{-1}$.

In 2013, the N_2O fluxes in the days immediately after the wheat was planted were of low magnitude, on average of $6.5 \mu g N_2O-N m^{-2} h^{-1}$, in both tillage treatments even

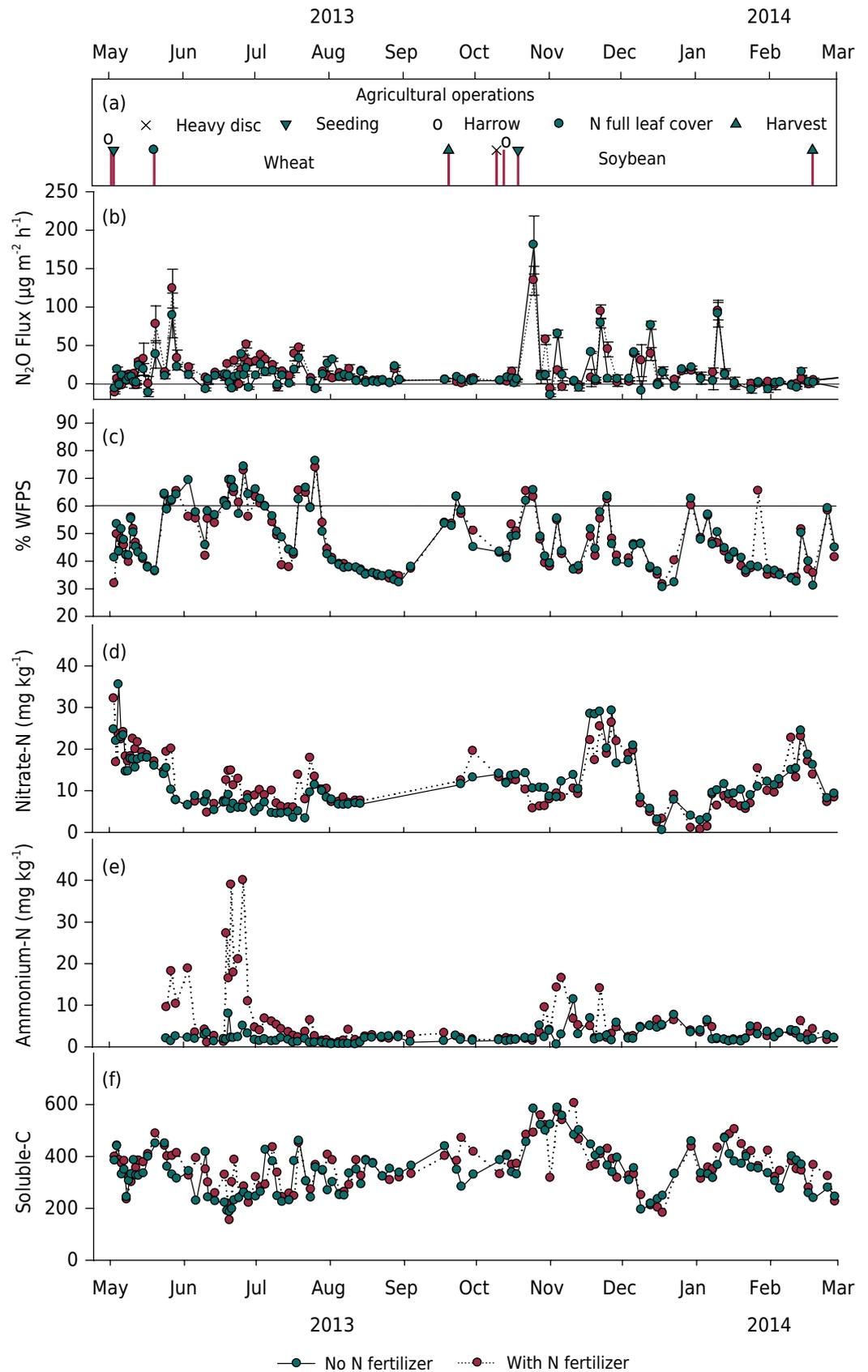


Figure 2. Soil N₂O fluxes (b) and soil water-filled pore space (c), soil nitrate (d), ammonium (e) and soluble carbon (f) in the 0.00-0.10 m soil layer measured during the first cycle of a wheat-soybean crop succession under conventional tillage (CT) with and without N fertilization of wheat.

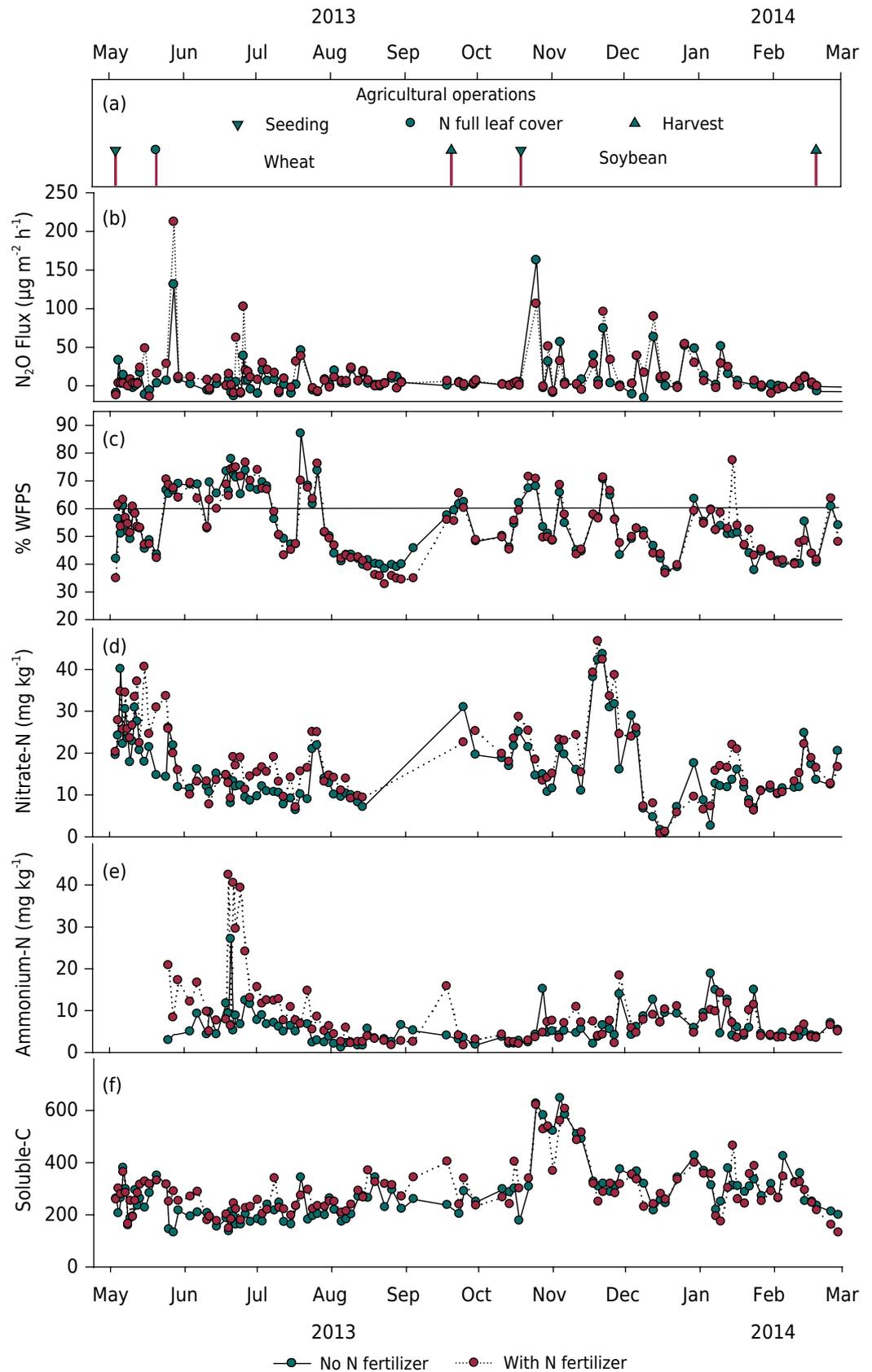


Figure 3. Fluxes of N₂O (b) and water filled pore space (c), nitrate (d), ammonium (e) and soluble carbon (f) in the 0.00-0.10 m soil layer measured during the first cycle of a wheat-soybean crop succession under no-tillage (NT) with and without N fertilization of wheat.

when nitrogen fertilizer was added at planting (Figure 2b). Low N_2O emission at the beginning of the cycle can be attributed to the low rainfall after sowing, only 4.1 mm in 18 days (Figure 1), together with the low rate of fertilizer application (21 kg N ha^{-1}). In addition, the fact that the fertilizer was buried in the furrow can reduce N_2O emissions (van Kessel et al., 2013). The N_2O emission peak occurred on May 27, 2013, seven days after the N fertilizer addition at tillering stage. Even in the treatments without N fertilization, the highest N_2O flux was observed in this period. The occurrence of precipitation after a long period of dry season is likely to explain this behavior, indicating that the N_2O emissions were more limited by the low water content than nitrate availability in the soil (Figures 2d and 3d - Dobbie et al., 1999). Out of the six days before May 27, on four days, daily rainfall ranged between 9 and 15 mm and totaled 50.5 mm. de Morais et al. (2013) also observed the highest peaks of N_2O after soil tillage for elephant grass production after rainfall events.

After N fertilizer application to wheat, rainfall was regular until June 30, totaling a further 304 mm. In the period from May 24 until the end of June, the water filled pore space (%WFPS) was evaluated 22 times and exceeded 60 % on 12 and 19 occasions for the CT and NT treatments, respectively, and on 1 and 5 occasions, exceeded 70 % (Figures 2c and 3c). It is clear that the absence of tillage and the surface mulch present at this time in the NT treatment, preserved soil moisture. Despite the high %WFPS after the initial peak of N_2O emission on May 27, N_2O emissions were low, with peaks not exceeding $40 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$, except in the NT treatment where N fertilizer was added and the emission reached $104 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$. The concentration of nitrate in the soil at this time was low which probably explains these low emissions and there was a small peak of nitrate in the NT treatment, marginally larger where N fertilizer was added (Figures 2d and 3d). After June, rainfall was consistently low, only amounting to 62 mm until the wheat harvest on September 20. As both mineral N concentrations and soil moisture were low throughout this period (Figures 2c, 2d, 2f, 3c, 3d, and 3f) it is not surprising that N_2O emissions remained low.

In the 2014 wheat crop, when no N fertilizer was added, N_2O emissions ranged from -6 to $57 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ under CT, and from -6 to $52 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ in NT (Figures 4b and 5b). With nitrogen fertilization, N_2O emissions under CT remained between -5 and $157 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ and -5 and $86 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ in NT. As in the first year, emissions immediately after sowing were low, averaging $10.7 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$, with the emission peak occurring only after the N fertilizer addition. From planting (April 30) until N fertilization (May 21), there was a total of only 9 mm of rainfall, but in the five days following N fertilization, total rainfall was 120 mm. The WFPS increased from 30 to 40 % to over 70 % in this period (Figures 4c and 5c) and resulted in significant N_2O emissions, especially in the N-fertilized treatments. After this phase of high N_2O fluxes following N fertilizer application, rainfall volume was still high (213 mm until final harvest), and WFPS rose above 60 % on various occasions, but mineral N levels were low (Figures 4d, 4e, 5d, and 5e), which probably explains the decrease in N_2O emissions (below $20 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$) until final harvest.

In the second year (2014-15), when no N fertilizer was applied for the preceding wheat crop, the soybean presented emissions between -6 and $114 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ under CT, and from -4 to $111 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$ under NT. In areas where wheat had been fertilized with nitrogen, under CT soybean emissions varied from -5 to $81 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$, and under NT from -4 to $113 \mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$.

In both years, during the summer soybean crops, peaks of N_2O emission comparable to those registered in the wheat cycles were observed. The most significant peak of N_2O emission in the first year occurred just four days after planting and in the second year six days after planting. In the five days prior to these peaks in N_2O emission, there were 49 and 88 mm of rainfall for the first- and second-year crops,

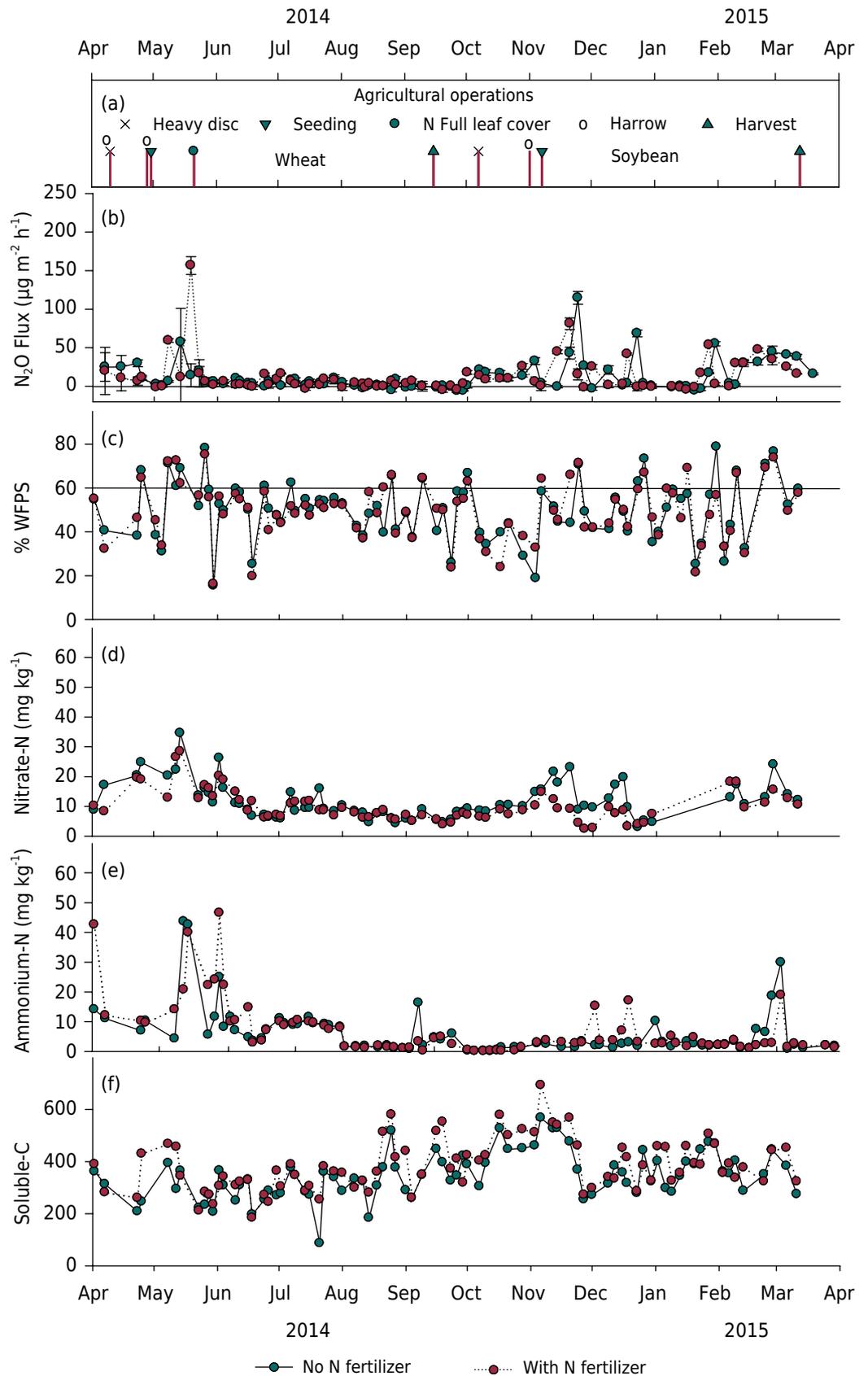


Figure 4. Fluxes of N₂O (b), water-filled pore space (c), nitrate (d), ammonium (e), and soil soluble carbon (f) in the 0.00-0.10 m soil layer measured during the second cycle of a wheat-soybean crop succession under conventional tillage (CT) with and without N fertilization of wheat.

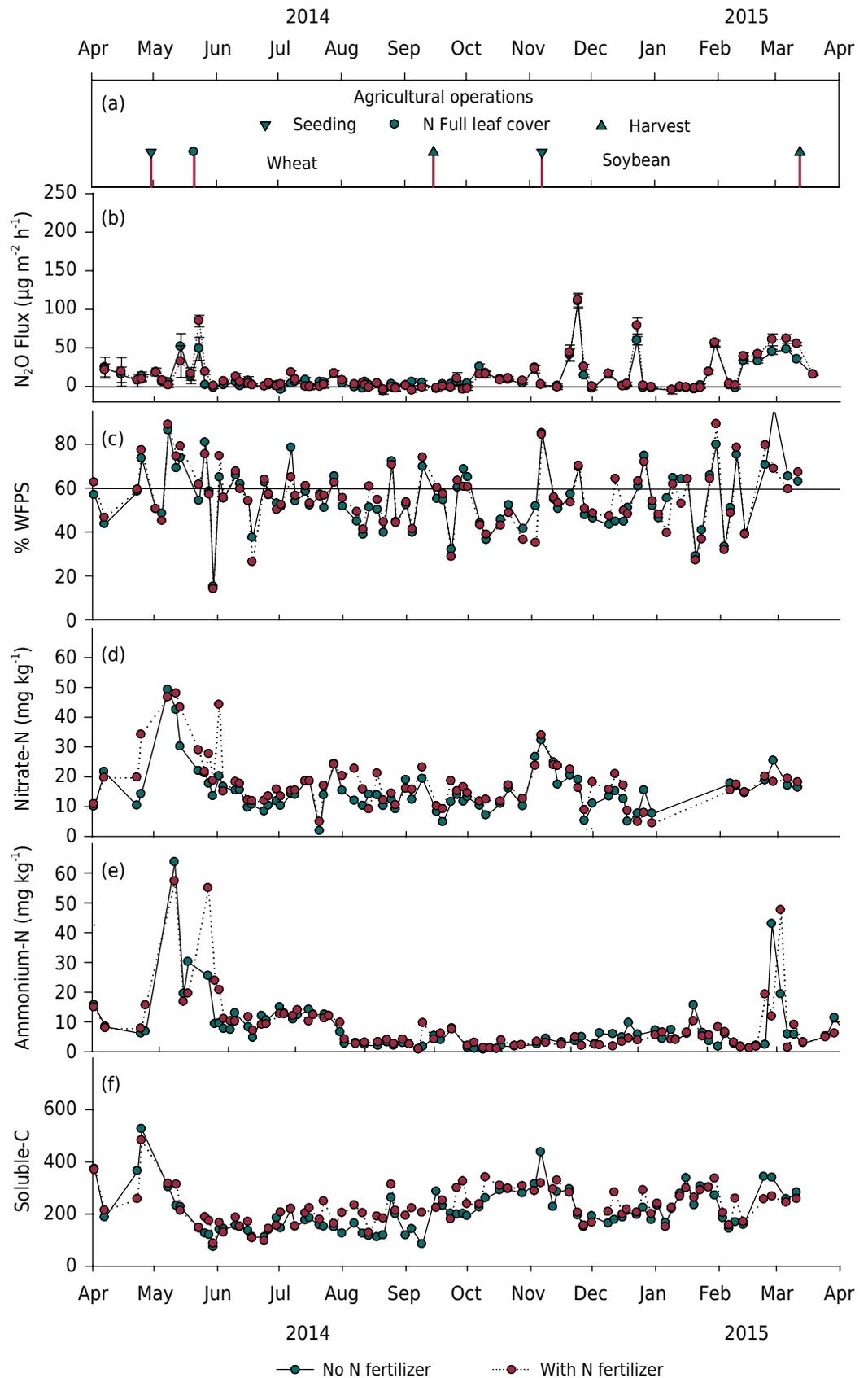


Figure 5. Fluxes of N₂O fluxes (b), water filled pore space (c), nitrate (d), ammonium (e) and soil soluble carbon (f) in the 0.00-0.10 m soil layer measured during the second cycle of a wheat-soybean crop succession under no-tillage (NT) with and without N fertilization of wheat.

respectively, and in both cases, the WFPS of over 60 % coincided with the emission peak (Figures 2c, 3c, 4c, and 5c).

The increase in the %WFPS seemed to be the most important factor for higher emissions throughout the soybean crops. The peaks of N₂O emission occurred during the soybean crop even when nitrogen fertilizer was not applied to the previous wheat crop. Because of its ability to obtain N from BNF, soybean is thought to remove less N-mineral from the soil compared to wheat, allowing a “sparing effect” of the soil mineral N (Chalk et al., 1993). Thus, the nitrate content of the soil was generally higher under the soybean than under wheat especially in the final stages of grain filling when N rich residues were deposited on the soil surface (Figures 2d, 3d, 4d, and 5d) and with the increase in the %WFPS due to rainfall, conditions were created for the production of N₂O.

In the case of soybean, the rainfall was greater in the second year, with the %WFPS remaining high throughout most of the cycle, sometimes close to saturation (Figures 2c, 3c, 4c, and 5c). At these times of very high %WFPS, the emission of N₂O was low. When soil moisture values are very high, close to saturation, the main product of the denitrification process is N₂ from N₂O reduction (Butterbach-Bahl et al., 2013). In fact, on several occasions when %WFPS was high, N₂O emissions were negative. Negative N₂O fluxes were observed on many occasions during the two years of the experiment. Seventeen percent of the samples had negative N₂O fluxes, most less than -10 µg N₂O-N m⁻² h⁻¹. Similar results were reported from the same region by Bayer et al. (2015), from central Brazil by Campanha et al. (2019) as well elsewhere (Syakila et al., 2010). Cowan et al. (2014) suggested that the negative fluxes observed in the literature are lower and associated with the devices’ detection limit. However, when the fluxes were associated with high %WFPS in all four treatments (e.g., June 21 and July 23, 2013; August 21, 2014; and January 16, 2015) it is tempting to suggest that these negative values were due to N₂O reduction. It is reasonable to assume that the occurrence of negative soil flux means the consumption of N₂O by soil microorganisms. However, the mechanisms and factors that induce consumption when %WFPS is not high are still unknown. On a global scale, omitting negative N₂O flux values from the calculations is not a problem as they do not represent a large debt, but this may be different on a national scale (Chapuis-Lardy et al., 2007; Syakila and Kouze, 2011). Although uncertainty persists concerning negative fluxes, in all calculations, negative N₂O fluxes were taken into account.

Availability of C is essential for the heterotrophic microorganisms involved in the denitrification process (Firestone and Davidson, 1989). It is known that the increase in available C promotes increases in N₂O emissions (Ruser et al., 2006). In both years, the soluble C content was higher under CT, and in the soybean cycle. However, it was not possible to establish a clear relationship between soluble C contents and N₂O emissions.

Production of N₂O is regulated by the interaction of the various factors that affect the nitrification and denitrification reactions (Firestone and Davidson, 1989). In each cycle, to evaluate the relative importance of the different variables on the N₂O emissions, a regression analysis was performed (Table 3). For wheat, the parameter with the greatest influence on the N₂O emissions was the N-mineral content, while for soybean it was rainfall, represented by the %WFPS. The differentiated behavior of the variables throughout the year is in line with the growing season of the crops. The highest contribution of %WFPS to soybean emissions is explained by its growth during summer, a time of higher incidence of rainfall. In wheat, besides the fact that lower precipitation occurred during the cycle, N fertilizer was added increasing levels of mineral N in the soil. The coefficients differed between the years, and it was not possible to observe a significant correlation in all cycles. This phenomenon indicates that the variables interacted differently in the two years.

In the first year, nitrogen fertilization significantly increased N₂O emissions during the wheat crop, but in the second year, this increase was not statistically significant ($p < 0.05$; Table 4). Apart from spatial variability, the overall conditions for N₂O formation could

Table 3. Pearson correlation coefficients of the relationships between soil nitrous oxide emissions and the soil properties: concentrations of nitrate, ammonium and soluble carbon and the water-filled pore space (%WFPS) in four treatments over two years of a wheat/soybean sequence at Londrina, Paraná State. Treatments were conventional plow tillage (CT), no-tillage (NT) and with (+N) and without N fertilizer addition (-N) to the wheat crop

Treatment	%WFPS	NO ₃ ⁻	NH ₄ ⁺	C
Wheat 2013				
CT	0.017	-0.121	-0.218	0.161
CT+N	0.150	-0.047	0.172	0.175
NT	0.118	0.095	-0.149	-0.052
NT+N	0.158	0.015	-0.027	0.177
Soybean 2013-2014				
CT	0.394*	0.079	-0.128	0.262
CT+N	0.327*	-0.055	0.107	0.192
NT	0.551*	0.175	-0.063	0.312*
NT+N	0.511*	0.190	-0.013	0.188
Wheat 2014				
CT	0.177	0.767*	0.719*	-0.158
CT+N	0.124	0.355*	0.781*	-0.279
NT	0.251	0.428*	0.788*	0.164
NT+N	0.242	0.432*	0.631*	-0.097
Soybean 2014-2015				
CT	0.322	-0.092	-0.032	-0.165
CT+N	0.356	0.138	0.105	0.457*
NT	0.356	0.319	-0.130	0.228
NT+N	0.324	0.080	0.613*	-0.108

Regression coefficients in bold followed by asterisks (*) indicate significant correlations between the variable and the N₂O flux.

Table 4. Cumulative emissions of N₂O-N for each crop and each cropping year and the net N₂O-N emission owing to N fertilization and the respective emission factor (EF%) in a two-year succession of wheat-soybean managed either under conventional plough tillage (CT) or no-till (NT)

Crop/season	CT + N	CT	Net-CT ¹	EF	NT + N	NT	Net-NT ¹	EF
	kg N ha ⁻¹			%	kg N ha ⁻¹		%	
Wheat 2013	0.57a ²	0.38b	+0.19	0.31±0.11	0.50a ²	0.26b	+0.24	0.39±0.09
Soybean 13/14	0.64	0.64	0.00		0.66	0.64	+0.02	
Wheat - Soybean 13/14	1.22	1.03	+0.19	0.31±0.10	1.17a	0.91b	+0.26	0.42±0.07
Wheat 2014	0.39	0.27	+0.12	0.17±0.12	0.32	0.29	+0.03	0.04±0.16
Soybean 14/15	0.63a	0.77b	-0.14		0.82a	0.70b	+0.12	
Wheat - Soybean 14/15	1.07B ³	1.06	+0.01	0.02±0.18	1.21A	1.04	+0.17	0.37±0.20

⁽¹⁾ Difference in N₂O emission between the area amended with N fertilizer and that without, within the same tillage treatment. ⁽²⁾ Means in the same row followed by different lowercase letters indicate that the difference in N₂O emission between the area amended with N fertilizer and that without, within the same tillage treatment was significant at $p < 0.05$ (Fisher L.S.D. test). ⁽³⁾ Means in the same row followed by different uppercase letters indicate that was a significant difference ($p < 0.05$; Fisher L.S.D. test) in N₂O emission between tillage treatments for the same N fertilizer treatment.

have limited the emission magnitude. The high correlation (Table 3) of N₂O emissions with soil mineral N, especially nitrate suggests nitrification could prevail during the second year of wheat crop (Liang and Robertson, 2021). In addition, soil mineral N levels were high. According to Machado et al. (2021), indigenous soil N is the major contributor to soil N₂O emission even under N fertilization, the former accounting for over 60 % of the total emitted against a contribution of about 30 % as the fertilizer effect. However, when one crop follows another in succession, fertilization of the first crop can influence the N₂O emissions of the next crop, and this was evident in the emissions from the soybean crop in the second year. Theoretically, nitrogen fertilization increases plant growth and contributes to a greater quantity of root and shoot residues deposited in/on the soil. As a result, there may be increased mineralization of organic matter and increase in N₂O emissions from the subsequent crop (Hellebrand et al., 2008).

Total N₂O emissions from CT and NT presented a statistically significant difference in the 2014/2015 two-crop sequence when N fertilizer was added to the wheat crop (Table 4). Neither the total emissions for the 2013/2014 agricultural year, nor for any of the other individual crops was there a statistically significant difference between the soil tillage systems. The adoption of NT may increase N₂O emissions (Liu et al., 2006, 2007; Rochette et al., 2008), due to the increase in microbial activity, higher O₂ consumption, and conditions of higher O₂ restriction all factors which favor denitrification (Linn and Doran, 1984). However, when comparing different soil tillage systems, in addition to all the soil and climatic factors and management factors that influence N₂O emissions, the time of implantation of the system also appears as an important factor. Recently-installed NT systems emit more N₂O than CT systems, but after the first few years, the emissions are similar (Six et al., 2004; Van Kessel et al., 2013).

Higher N₂O emission in newly implanted systems is thought to be related to a decrease in soil porosity and a consequent increase of WFPS in the soil due to increased soil density (Pelster et al., 2011). A possible explanation for the reduction of N₂O emissions over time would be increased macroporosity and decreased soil density in long-term NT systems (Zhang et al., 2007). The results observed in the present study are similar to others (Plaza-Bonilla et al., 2018), indicating that there is evidence that the behavior of emissions of long-term NT systems is similar to that observed in temperate climates.

An important indicator that could be drawn from crop yield and the respective N₂O emission is the N₂O emission intensity, or the quantity of N₂O emitted per mass of grain produced, giving a cleaner production dimension. In the case of soybean, the highest yield under NT compared to CT and the small differences in N₂O emissions resulted in a 44 % reduction in N₂O emission intensity for both years. Under CT, N₂O emission intensities for soybean were 0.41 and 0.58 g kg⁻¹ for the first and second year, respectively, while for NT these numbers reduced respectively to 0.23 and 0.32 g kg⁻¹. For the wheat crop under N fertilization, a business-as-usual practice, the N₂O emission intensities under CT were 0.23 and 0.11 g kg⁻¹ for the first and second year, which was very similar to NT with respectively 0.24 and 0.09 g kg⁻¹. These evidences of more sustainable grain production under NT were also highlighted by Campanha et al. (2019) in a study carried out in the Central region of Brazil, but in this case, with much greater contrast.

No significant differences were observed in emission factors (EF) for the N fertilization between the years and between the systems of soil tillage (Table 4). For CT, the mean %EF estimate for the first wheat-soybean cycle was 0.31 ± 0.10, and for NT, the mean was 0.42 ± 0.07. For the second cycle (2014-2015), EFs were 0.02 ± 0.18 and 0.37 ± 0.20 for CT and NT, respectively. There was a trend of a higher EF for wheat-soybean under NT than CT, but no statistical differences were observed.

All EF estimates suggest that the default value advocated by the IPCC (1 %) would overestimate the emissions in the area, and these results are in agreement with those

of other studies on crops grown in Ferralsols in Brazil (Jantalia et al., 2008; Martins et al., 2015; Campanha et al., 2019). The two-year monitoring study showed that estimates of N₂O losses may vary from year to year, showing the importance of monitoring for more than one year. The studied area is representative of the Brazilian grain production area; the results indicate that for a real estimation of N₂O losses, the development of a specific EF is required.

CONCLUSIONS

Nitrous oxide is a powerful greenhouse gas that also has harmful effects on the ozone layer, and efforts to mitigate its production from agricultural areas are urgently required. This study showed that N₂O emissions will not necessarily increase due to the use of no-tillage (NT) compared to conventional tillage (CT). The estimated N₂O emission factors for the N fertilization of wheat were under half of the default emission factor of IPCC without a significant difference between NT and CT. This was also true when N₂O emissions were considered during the wheat-soybean cropping period. Although not significant for the year-round N₂O emission factor, there was evidence of a residual effect of the N fertilizer applied to wheat on N₂O emission from soybean crop.

While the N₂O emission magnitude is a key point to be tackled, aiming at mitigating climate change, food security is a theme of similar importance. Combining yield data and accumulated N₂O emissions for each crop to calculate the emission intensity gives another dimension to GHG mitigation potential of cropping systems. For soybean production, the highest yield observed under NT brought about a reduction in N₂O emission intensity (kg N₂O kg⁻¹ grain) of 44 %, reinforcing the general concept of NT as a climate-smart practice.

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