

Hierarchy of factors driving N₂O emissions in non-tilled soils under different crops

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Summary

Nitrous oxide (N₂O) is emitted to the atmosphere as a by-product of nitrification and denitrification by soil microbial processes. Differences in climate, soil and management regulate these processes, causing N₂O emissions to vary in space and time. This study aimed to identify and rank the soil properties that control N₂O emissions in non-tilled soils under different crops. Over a period of 2 years, gas samples were taken from closed chambers and soil properties were determined once per season. N₂O emission rates were highly variable (from –15 to 314 µg N₂O-N m⁻² hour⁻¹). A regression tree analysis allowed us to classify soil N₂O emissions into three groups, separated by topsoil temperature (primary factor) and water-filled pore space (WFPS, secondary factor). N₂O emissions were small (mean 4.22 µg N₂O-N m⁻² hour⁻¹) with topsoil temperature less than 14°C (Group 1), large (mean 61.87 µg N₂O-N m⁻² hour⁻¹) with topsoil temperature between 14 and 23°C and WFPS more than 58.5% (Group 2) and moderate (mean 21.4 µg N₂O-N m⁻² hour⁻¹) with topsoil temperature more than 23°C and WFPS less than 58.5% (Group 3). These emission groups allow for more efficient sampling of N₂O emissions in the field: in winter, when topsoil temperatures are less than 14°C and N₂O emissions are expected to be small or even negligible, sampling frequency can be reduced; in autumn and spring, when topsoil temperatures are more than 14°C and WFPS is more than 60–70%, sampling frequency should be increased.

Introduction

Nitrous oxide (N₂O) is the main greenhouse gas (GHG) generated by cropping systems and is the main focus of efforts aimed at mitigating GHG emissions from agricultural soils (IPCC, 2007; Snyder *et al.*, 2009). Soil N₂O emissions are variable in space and time, giving rise to ‘hot spots’ and ‘hot moments’ that are difficult to predict (McClain *et al.*, 2003). This large variability results from the complex set of environmental variables, such as soil and microbial community heterogeneity, which control the nitrification and denitrification processes responsible for N₂O emissions (Firestone & Davidson, 1989). Often, the cause of the large N₂O emission rates in ‘hot spots’ and ‘hot moments’ can be linked to only one variable.

There is considerable controversy about the main variable driving N₂O emission rates and about the way a given variable can promote or limit N₂O emissions in different situations. For

example, Shelton *et al.* (2000) found a linear relationship between N₂O emissions and soil water content between field capacity (60% water-filled pore space, WFPS) and water saturation (100% WFPS). On the other hand, Schindlbacher & Zechmeister-Boltenstern (2004) observed maximum emissions between 80 and 95% of WFPS, with decreasing N₂O emission rates at more than 95% WFPS. Dobbie & Smith (2001) and Schindlbacher & Zechmeister-Boltenstern (2004) observed a positive relationship between N₂O emissions and topsoil temperature when the WFPS percentage remained large, while Almaraz *et al.* (2009) found a negative relationship between the two variables in a field trial in which N₂O emissions were related to rainfall. Under field conditions, agricultural traffic and zero tillage may increase soil bulk density and give way to anaerobic zones in surface horizons (Sasal *et al.*, 2006). This may give rise to N₂O emissions caused by denitrification processes (Beare *et al.*, 2009).

Nitrous oxide emission rates depend on the sum of variables required by soil microbial populations to carry out nitrification and denitrification processes. These variables can be divided into components such as substrate availability (NO₃⁻, NH₄⁺,

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NO_2^- and labile carbon, C) and factors (O_2 availability, soil moisture and temperature) whose actions are often hierarchical. If one or more of these variables is affected, N_2O emissions are likely to diminish. The ecological stoichiometry controlling emissions is the balance of multiple chemical substances, energy and materials in ecological interactions and processes. This conceptual framework has been successfully applied to topics ranging from population dynamics to biogeochemical cycling. This approach provides a tool for analysing how the balance of the multiple factors required by soil organisms affects N_2O production (Sterner & Elser, 2002; Hessen *et al.*, 2004). Our study aimed to identify and rank the soil variables driving N_2O emission rates across seasons in non-tilled soils under different crops. It was hypothesized that the conceptual framework of ecological stoichiometry would be a useful approach to understanding the variation of N_2O emissions under field conditions.

Materials and methods

A non-manipulative field trial was conducted between April 2009 and February 2011 to determine N_2O emission rates and their main driving factors, in an agricultural field in the Province of Buenos Aires, Argentina ($34^\circ 57' 29''S$, $60^\circ 13' 11''W$). The soil was a loamy Typic Argiudoll (clay 190 g kg^{-1} ; silt 400 g kg^{-1}) from the O'Higgins series (INTA, 2012) with 35.2 g kg^{-1} organic matter and pH (1:2.5 soil:water suspension) of 5.7 in the A horizon. The field was under continuous no-till farming with a three-year crop sequence composed of wheat/double crop soyabean–maize/full season soyabean. In this sequence $85\text{--}95\text{ kg N ha}^{-1}$ as urea was added at the time of wheat sowing and when maize was at the V_{3-5} phenological stage.

Measurements were performed following a systematic stratified design. In the 30-ha experimental area (Figure 1), six field plots were seasonally sampled (approximately every three months) over two years. Measurements were performed in two temporally-shifted three-year crop sequences: (i) Sequence 1, starting with full season soyabean residues; and (ii) Sequence 2, starting with double cropped soyabean residues (Table 1). These cropping sequences allowed for simultaneous measurement of the response variables of interest in the various crops of the typical cropping sequence of the region. In this way, we expected to capture the possible variability in N_2O emissions across seasons. In order to capture the variability caused by the passage of farm machinery typical of a non-tilled topsoil, six samples were taken, within each cropping sequence, at two positions in the plot: (i) border (large traffic intensity); and (ii) away from the border (small traffic intensity). The three chambers in the same position within the plot were 10 m apart; those in different positions were 50 m apart (Figure 1). Each field chamber was considered as an experimental unit.

Gas samples were taken from within static, closed and non-vented chambers (surface = 0.13 m^2 , height = 0.125 m), inserted into the soil to a depth of 0.05 m . Each chamber had a metal base and an aluminum-coated plastic top. As the field trial was carried out on a production farm, we had to remove the chambers

after sampling and re-insert them 24 hours before the subsequent sampling. After each insertion, 15 mm tap water was added to each chamber in order to ensure an adequate seal between the soil and the chamber base before gas sampling. This addition of water sometimes resulted in a small increase in WFPS values at each sampling date.

Sampling was carried out in the morning, as described by Cosentino *et al.* (2012). Gas samples were taken from the chamber headspace at 0, 20 and 40-minute intervals after closing the chambers. Gases were extracted using a vacuum pump, and injected into previously evacuated 25-cm^3 vials sealed with rubber stoppers fixed to the vial with an aluminum flange. We followed this procedure on each sampling date and for each of the six chambers within each plot.

Within seven days of sampling, N_2O was measured in the laboratory with a GC 6890 Agilent Technologies Network gas chromatograph, fitted with a ^{63}Ni electron capture detector (Agilent Network GC System, ÁECD, Santa Clara, CA, USA) and a $30\text{ m} \times 530\text{ }\mu\text{m} \times 25\text{ }\mu\text{m}$ Molsieve HP-Plot column. The oven, injector and detector temperatures were 150, 100 and 300°C , respectively. The carrier gas was N_2 and the injection volume was 0.5 cm^3 .

The N_2O fluxes (f) were calculated as:

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{V_m}, \quad (1)$$

where $\Delta C/\Delta t$ is the change in N_2O concentration in the chamber during the incubation time Δt , V is the volume of the chamber (16.7 dm^3), A is the soil area (0.13 m^2) covered by the chamber, m is the molecular mass of N_2O and V_m is molar volume of N_2O . Gas fluxes were calculated as the increase in concentration during the incubation period. A linear function was fitted to the N_2O emission/incubation time relationship. When the coefficient of determination (R^2) of the fitted linear function was greater than 0.7 the slope of the function was taken to be the rate of N_2O flux over the 0–40 minutes interval. When R^2 was smaller than 0.7 and a linear function could not be fitted, N_2O flux over the interval was considered to be null. In this study, the minimum detectable limits (distinguishable from zero) were either more than $0.3\text{ }\mu\text{g N}_2\text{O-N m}^{-2}\text{ hour}^{-1}$ or less than $-0.3\text{ }\mu\text{g N}_2\text{O-N m}^{-2}\text{ hour}^{-1}$. All measured N_2O emission values were included in the analysis.

At the same time as the flux measurements, topsoil temperature was measured at 0.10 m depth beside each chamber. After gas sampling, soil samples (0– 0.2 m in depth) from inside the chamber perimeter were taken. Nitrate-N was extracted from wet soil samples with a solution of CuSO_4 (Jackson, 1958) and nitrate concentration was determined by colorimetry (Keeney & Nelson, 1982) after reduction of nitrate to nitrite (Markus *et al.*, 1985). Topsoil structural types or classes in each site were described according to Soil Survey Staff (1999). Soil bulk density (BD; 100 cm^3 cylinders; 0.05 m diameter) and gravimetric water content (GWC) were determined on samples taken within the perimeter of each field chamber. Both BD and GWC values

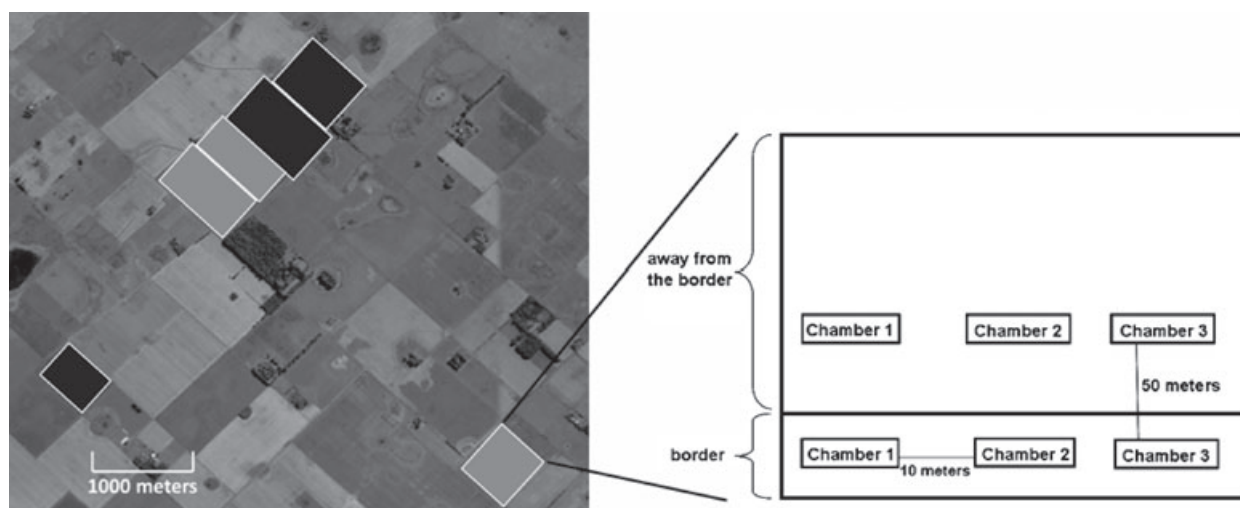


Figure 1 Experimental plot locations within fields (left) and chamber locations within each plot (right). Grey squares correspond to sequence 1, black squares correspond to sequence 2.

Table 1 Chronogram of sampling, sowing, N fertilizer and harvest of the two crop sequences over 2 years

Sequence 1	Soyabean res.			Wheat			W res.			Double crop. soyabean				Soyabean residue				Maize												
	2009												2010												2011					
Date of	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar							
Sampling		X		X			X				X			X				X		X		X								
Sowing				X					X										X											
N fertilization				X																X										
Harvesting							X					X																		
Sequence 2	Soyabean res.						Maize			Maize residue				Full season Soyabean																
	2009												2010												2011					
Date of	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar							
Sampling		X		X			X				X			X				X		X		X								
Sowing							X												X											
N fertilazation							X																							
Hervesting											X																			

w. res. = wheat residue; crop. = cropping.

were used to calculate porosity (P), assuming a particle density (Dp) of 2.65 Mg m^{-3} , and volumetric water content (VWC) using Equations (2) and (3):

$$P = 1 - (BD/Dp), \quad (2)$$

$$VWC = GWC \times BD. \quad (3)$$

The percentage of WFPS was calculated by subtracting VWC from P.

A decision tree analysis, based on a procedure originally proposed by Morgan & Sonquist (1963) and later used by others (cited by Lemon *et al.*, 2003), was used to separate a single group of values into more homogeneous subgroups. This analysis involves a series of decisions, given that a sample is considered as

a single group. The parent group is transformed into two new subgroups to minimize the sum of squares; each subgroup becomes more homogenous in the response variable (N_2O emission rate). In such a way, each subgroup turns into a new parent group. These divisions may be repeated as many times as necessary.

Linear regression analysis was used to fit functions to the relationship between subgroup N_2O emission rates and soil NO_3^- -N concentration. Each point in the regression scatter was the result of the individual measurement of each chamber. The Infostat package was used for decision tree and linear regression analysis (Infostat, 2002).

Results

Away from the border locations, topsoils had mainly granular and subangular blocky aggregates, while those in border locations had

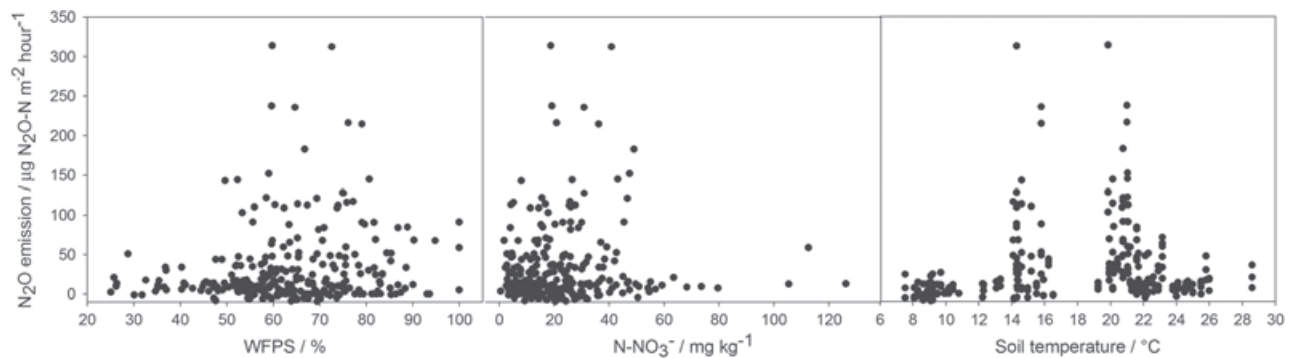


Figure 2 Distribution pattern of N₂O emission rates as a function of water-filled pore space (WFPS), soil NO₃⁻-N concentration and topsoil temperature (left to right).

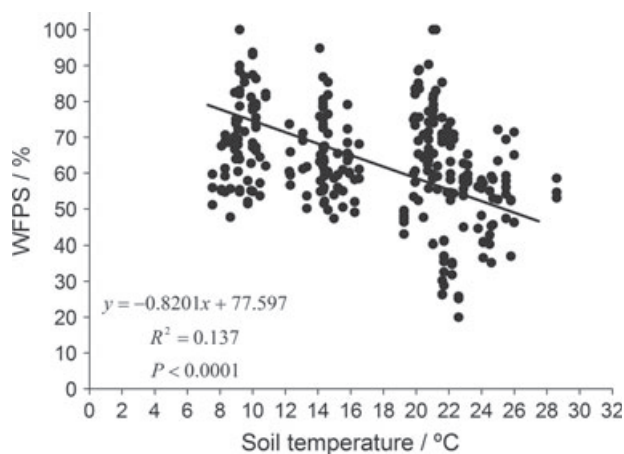


Figure 3 Relationship between water-filled pore space (WFPS) and topsoil temperature.

mainly planar, massive and subangular blocky aggregates. Despite these different structural types, similar topsoil bulk densities (from 1.2 to 1.4 Mg m⁻³) were observed in both locations in the plots: N₂O emission rates were also similar away from the border and in border locations.

During the study period, N₂O emission rates ranged between -15 and 314 µg N₂O-N m⁻² hour⁻¹, with large variability among replicates. When plotted against all measured soil properties, no relationship between emission rates and WFPS or soil NO₃⁻-N concentration was observed, but N₂O emission rates showed a clearer response pattern across the topsoil temperature range (Figure 2). Nitrous oxide emission rates were very variable in the 14–23°C topsoil temperature range, whereas they were smaller and less variable at topsoil temperatures less than 14°C and more than 23°C. A negative relationship between soil temperature and WFPS was observed with $R^2 = 0.137$ and $P < 0.0001$ (Figure 3).

The regression tree analysis showed three groups of N₂O emission rates which differed significantly ($P < 0.001$): Group 1, small N₂O emission rates, 4.22 ± 4.11 µg N₂O-N m⁻² hour⁻¹; Group 2, large N₂O emission rates, 61.87 ± 4.07 µg N₂O-N m⁻² hour⁻¹; and Group 3, moderate N₂O emission rates,

21.4 ± 5.01 µg N₂O-N m⁻² hour⁻¹ (Figure 4). These emission groups coincide with the distribution pattern of topsoil temperature (Figure 2).

The small N₂O emission rates (Group 1) occurred during winter, when topsoil temperatures were always less than 14°C, as was found in both crop sequences in June and August 2009 and 2010 (Figure 5). In this case the rate of N₂O emissions showed no relationship with any of the measured variables. The large N₂O emission rates (Group 2) were associated with topsoil temperatures of more than 14°C and WFPS of more than 58.5% (Figure 4), observed in November 2009 (crop sequence 2, Figure 5) and March and October 2010 (crop sequences 1 and 2, Figure 5). The moderate N₂O emission rates (Group 3) occurred at topsoil temperatures of more than 23°C and WFPS less than 58.5% (Figure 4). They were observed in November 2009 (crop sequence 1, Figure 5), December 2010 and February 2011 (crop sequences 1 and 2, Figure 5).

The large and moderate N₂O emission rates (Groups 2 and 3) were positively related to soil NO₃⁻-N concentration. However, the slope of fitted straight lines describing these relationships was different for each emission group and crop (Figure 6). Good relationships were found for maize and wheat, and in fallow periods with soyabean residues (Figure 6). No clear relationship was found for periods under soyabean crops, regardless of the N₂O emission group and temperature range considered.

Discussion

N₂O emission values were divided into three groups, each of which was associated with one or more of the study variables. The first limiting variable was topsoil temperature, which separated the small emission group (Group 1) from the remainder (Figures 4, 5). In this emission group, topsoil temperature (less than 14°C) had a direct effect, probably because of reduced microbial activity at these temperatures, which influences N₂O emissions (Keeney *et al.*, 1979; Trumbore *et al.*, 1996; Farquharson & Baldock, 2008; Maljanen *et al.*, 2009).

The results of this study are consistent with those observed by others (Trumbore *et al.*, 1996) who found a decrease in microbial

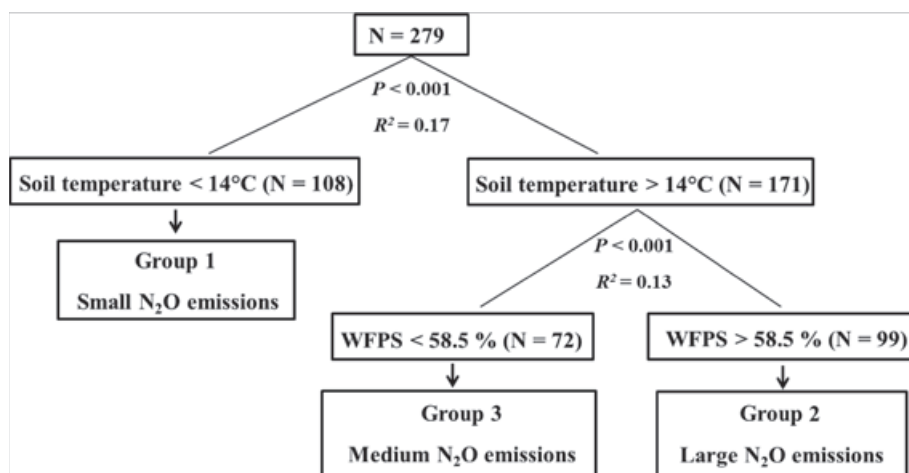


Figure 4 Results of the regression tree analysis, showing the main variables affecting N₂O emission rates.

activity with decreasing topsoil temperature. However, Maljanen *et al.* (2009) found that even in sub-zero temperatures (-6.8°C) N₂O emissions were observed in soils that undergo freezing processes regularly, and where an adaptation of the microbial community to low temperatures could be expected. This is not the case in a temperate region such as the Argentine Pampa, which suggests that, at our study site, microbial communities are probably not adapted to produce N₂O at low temperatures because the soils are never frozen.

Nitrous oxide emissions were large and very variable with topsoil temperatures between 14 and 23°C and WFPS more than 58.3% (Group 2, Figures 4, 5). These variations were positively related to soil NO₃⁻-N concentration, as shown by the fitted relationships for maize and soyabean residues (Figure 6). Events characterized by both large temperature and large WFPS could favour relatively large rates of N₂O production, provided sufficient NO₃⁻ was present in the soil (Castaldi, 2000). In fact, measured NO₃⁻-N concentrations were always more than 5 mg kg^{-1} in the study site, suggesting that soil nitrate never limited N₂O emissions totally, as has been shown by Dobbie *et al.* (1999).

According to Dalal *et al.* (2003) denitrification rate increases with increasing NO₃⁻ content, when the soil is wet and temperature and carbon availability are not limiting. This occurs because the presence of NO₃⁻ inhibits N₂O to N₂ reduction, resulting in a relatively large N₂O:N₂ ratio at similar humidity and oxygen content. Dalal *et al.* (2010) found a positive correlation between N₂O emissions and soil NO₃⁻ content, when topsoil temperature varied between 10 and 30°C and WFPS between 30 and 80%. In contrast, results from a field trial in Denmark with no added fertilizer and WFPS of 50–70% (Ambus, 2005) showed a negative relationship between the rate of N₂O emission and soil NO₃⁻-N concentration. This different result could be due to the smaller WFPS in the Danish field trial, which is expected to promote nitrification instead of denitrification processes.

N₂O emissions were moderate when topsoil temperature was more than 14°C and WFPS less than 58.3% (Group 3, Figures 4, 5). These moderate N₂O emissions were smaller than those observed in experiments performed under controlled conditions,

which showed an increase in N₂O emission rates at temperatures as large as 70°C (Keeney *et al.*, 1979; Schindlbacher & Zechmeister-Boltenstern, 2004). These large N₂O emission values are possible when increasing temperatures lead to an increase in the size of the soil anaerobic zones (Li *et al.*, 2000), as greater respiration rates cause greater O₂ concentration gradients, thus resulting in a greater soil volume devoid of oxygen (Smith *et al.*, 2003). This would lead to an increase in denitrification. Added to this, larger soil temperature causes an increase in microbial activity (Farquharson & Baldock, 2008) and increases gas solubility, causing a greater loss of N₂O to the atmosphere before being reduced to N₂ (Dalal *et al.*, 2010).

Our field results showed that WFPS decreased with topsoil temperature (Figure 3). Soil drying at greater temperatures avoided the development of anaerobic zones, such as those found in experiments where soil water content is controlled (Dobbie & Smith, 2001; Schindlbacher & Zechmeister-Boltenstern, 2004). When a soil dries to less than 60% WFPS, the relative importance of denitrification as a source of N₂O emissions decreases while the relative contribution of nitrification increases (Linn & Doran, 1984). These by-product N₂O emissions from nitrification are usually less than those of denitrification (Castaldi, 2000; Smith *et al.*, 2003), which provides an explanation of why N₂O emission rates in Group 3 were only moderate. In this case, the influence of topsoil temperature was indirect and mediated by soil water content.

The relationship between N₂O emissions and soil NO₃⁻-N concentration differed from Group 2 to Group 3 and between crops within each Group (Figure 6). In Group 2, linear relationships were fitted when soil was cropped to maize and covered by soyabean residues, while no relationship was found in soyabean-cropped soil (Figure 6a–c). In Group 3, soil NO₃⁻-N concentration also influenced N₂O emission rates under maize and wheat and again no relationship was found under soyabean (Figure 6d–f). Nitrous oxide emissions under maize and wheat in Group 3 occurred at smaller rates than those in Group 2, which can be ascribed to the smaller N₂O emissions when nitrification instead of denitrification prevails (Castaldi, 2000; Smith *et al.*, 2003).

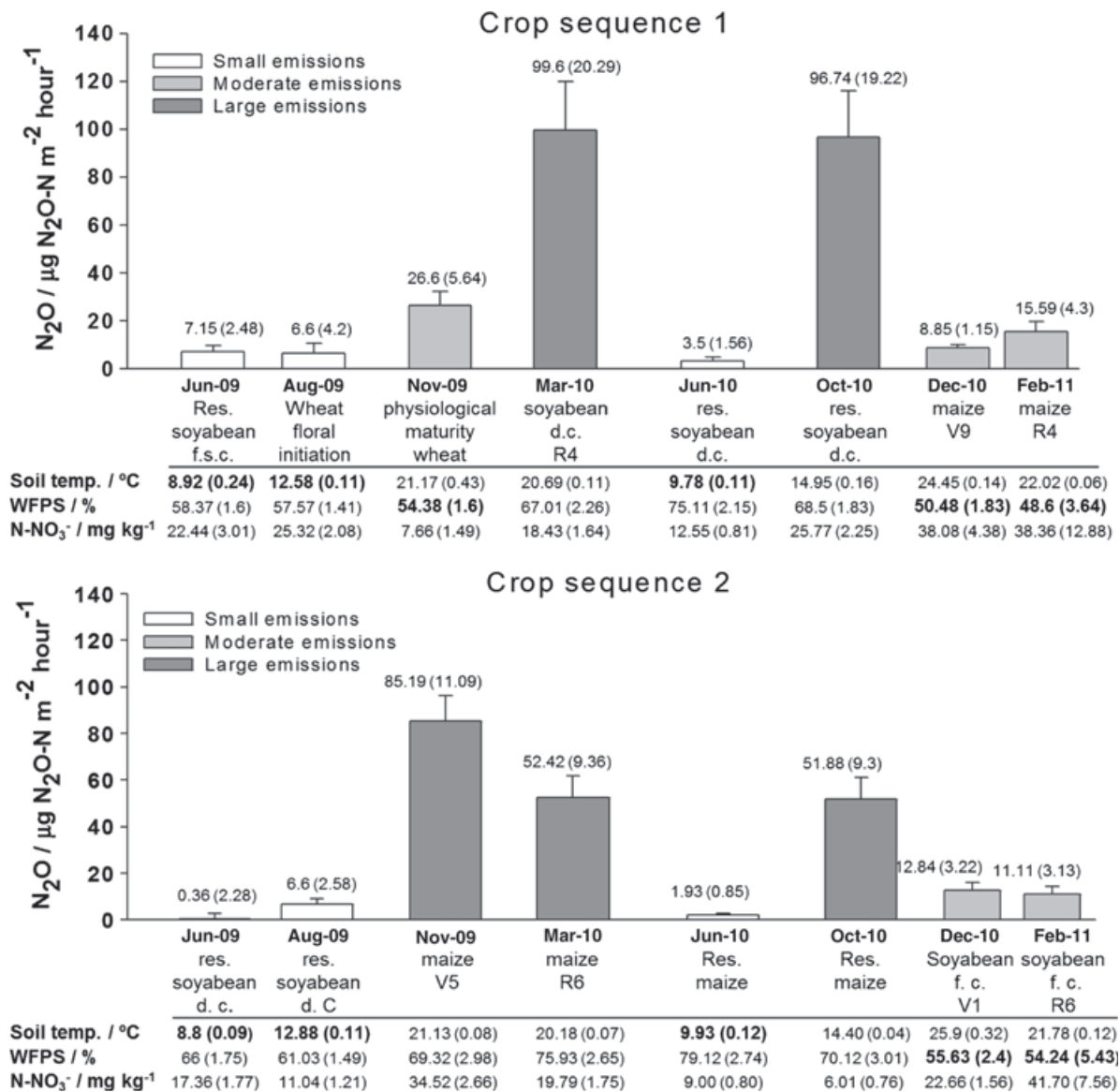


Figure 5 N₂O emissions during the study period for each crop sequence (bars). Capped vertical lines above each bar are one standard error. Values above bars are means and (SE). Below each graph, values for means and (SE) are shown for soil temperature, water-filled pore space (WFPS) and soil NO₃⁻-N concentration.

It is interesting to note that soyabean crops did not show a linear relationship between N₂O⁻ and NO₃⁻-N, regardless of the emission group. It is likely that some unmeasured variable could explain N₂O emissions under soyabean crops, in particular the large variability of N₂O emissions under soyabean in Group 2. Ghosh *et al.* (2002) and Rochette & Janzen (2005) found large N₂O emissions in legume crops, which could be related to other factors such as release of root N exudates. Soil organic C is another possible biophysical factor regulating N₂O emissions. It can influence N₂O emissions in two ways, as a source of energy for denitrifiers and by increasing biological oxygen demand and creating anaerobic zones in the soil ('hot spots'). In fact, additions of degradable organic C may lead to localized depletion

of oxygen at microsites and enhanced N₂O production (Helgason *et al.*, 2005). The results of N₂O emissions under soyabean crops need further clarification. It is likely that the inclusion of other biophysical variables would help explain the origin of large N₂O emissions in soyabean crops better.

Conclusions

The most important factor driving soil N₂O emission rates was topsoil temperature, followed by water-filled pore space and soil NO₃⁻ concentration. From this study of non-tilled soils, a hierarchical arrangement of factors was used to explain N₂O emission rates, which was determined by which particular

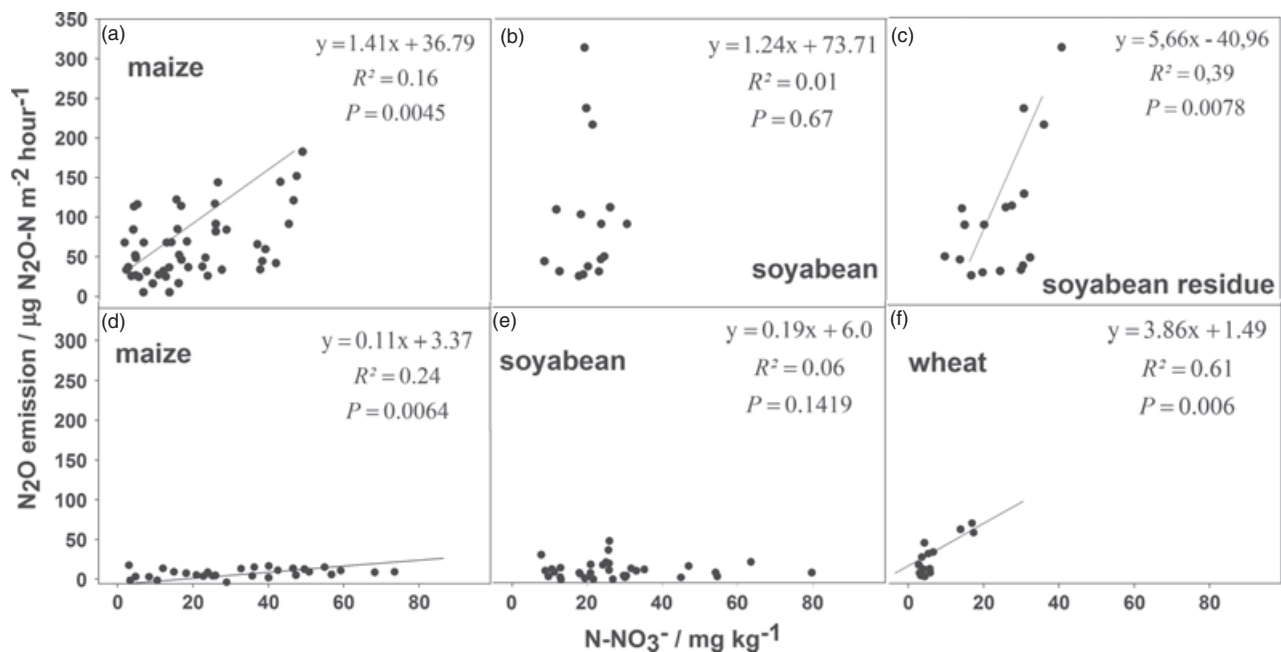


Figure 6 Relationship between N_2O emission rate and soil NO_3^- -N concentration for different crops and residues. (a–c) Large emissions with topsoil temperatures between 14 and 23°C (Group 3). (d–f) Moderate emissions with soil temperatures more than 23°C and WFPS less than 58.5% (Group 2).

variable limited N_2O production. This followed the hypothesized conceptual framework of ecological stoichiometry, which provides a useful tool to understand how the balance of these variables affects N_2O production by soil microbes (Hessen *et al.*, 2004).

The results of this study show the variation and driving factors of N_2O emission rates in an agricultural field under zero tillage and temperate climate, arranged in a hierarchical order dominated by topsoil temperature. These results could be extrapolated to other areas supporting similar soil, climate and management conditions. Nitrous oxide emission groups obtained through regression tree analysis could be helpful when deciding when a soil should be sampled for N_2O emissions, saving time and effort during fieldwork. For example, N_2O emissions are likely to be small or even negligible when topsoil temperatures are less than 14°C, a common occurrence during winter in temperate regions. In this case, checking topsoil temperature would reduce sampling effort. On the other hand, it is important to make measurements when WFPS is more than 60–70% and topsoil temperature more than 14°C, as often occurs during Autumn and Spring. In this case, more frequent N_2O measurements would be recommended to capture all possible environmental variables.

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