# Hierarchy of factors driving N<sub>2</sub>O emissions in non-tilled soils under different crops

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# Summary

Nitrous oxide (N<sub>2</sub>O) 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 N<sub>2</sub>O emissions to vary in space and time. This study aimed to identify and rank the soil properties that control N<sub>2</sub>O 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. N<sub>2</sub>O emission rates were highly variable (from -15 to  $314 \,\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> hour<sup>-1</sup>). A regression tree analysis allowed us to classify soil N<sub>2</sub>O emissions into three groups, separated by topsoil temperature (primary factor) and water-filled pore space (WFPS, secondary factor). N<sub>2</sub>O emissions were small (mean  $4.22 \,\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> hour<sup>-1</sup>) with topsoil temperature less than  $14^{\circ}$ C (Group 1), large (mean  $61.87 \,\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> hour<sup>-1</sup>) with topsoil temperature more than 58.5% (Group 2) and moderate (mean  $21.4 \,\mu$ g N<sub>2</sub>O-N m<sup>-2</sup> hour<sup>-1</sup>) with topsoil temperature more than  $23^{\circ}$ C and WFPS less than 58.5% (Group 3). These emission groups allow for more efficient sampling of N<sub>2</sub>O emissions in the field: in winter, when topsoil temperatures are less than  $14^{\circ}$ C and N<sub>2</sub>O emissions are expected to be small or even negligible, sampling frequency can be reduced; in autumn and spring, when topsoil temperatures are more than  $14^{\circ}$ C and WFPS is more than 60-70%, sampling frequency should be increased.

# Introduction

Nitrous oxide ( $N_2O$ ) 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  $N_2O$  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  $N_2O$ emissions (Firestone & Davidson, 1989). Often, the cause of the large  $N_2O$  emission rates in 'hot spots' and 'hot moments' can be linked to only one variable.

There is considerable controversy about the main variable driving  $N_2O$  emission rates and about the way a given variable can promote or limit  $N_2O$  emissions in different situations. For

Correspondence: M. A. Taboada. E-mail: mtaboada@cnia.inta.gov.ar Received 22 July 2013; revised version accepted 22 July 2013 example, Shelton et al. (2000) found a linear relationship between N<sub>2</sub>O 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 N<sub>2</sub>O 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 N<sub>2</sub>O 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 N<sub>2</sub>O 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 ( $NO_3^-$ ,  $NH_4^+$ ,

doi: 10.1111/ejss.12080

NO2<sup>-</sup> and labile carbon, C) and factors (O2 availability, soil moisture and temperature) whose actions are often hierarchical. If one or more of these variables is affected, N<sub>2</sub>O 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 N2O production (Sterner & Elser, 2002; Hessen et al., 2004). Our study aimed to identify and rank the soil variables driving N2O 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<sub>2</sub>O emissions under field conditions.

# Materials and methods

A non-manipulative field trial was conducted between April 2009 and February 2011 to determine N<sub>2</sub>O 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<sup>-1</sup>; silt 400 g kg<sup>-1</sup>) from the O'Higgins series (INTA, 2012) with 35.2 g kg<sup>-1</sup> 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  $85-95 \text{ kg N ha}^{-1}$  as urea was added at the time of wheat sowing and when maize was at the V<sub>3-5</sub> 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 temporallyshifted 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 N2O 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 nonvented chambers (surface =  $0.13 \text{ m}^2$ , 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  $25 \text{-cm}^3$  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<sub>2</sub>O 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 m  $\times$  530  $\mu$ m  $\times$  25  $\mu$ m Molsieve HP-Plot column. The oven, injector and detector temperatures were 150, 100 and 300°C, respectively. The carrier gas was N<sub>2</sub> and the injection volume was 0.5 cm<sup>3</sup>.

The N<sub>2</sub>O fluxes (f) were calculated as:

$$f = \frac{\Delta C}{\Delta t} \times \frac{\mathrm{V}}{\mathrm{A}} \times \frac{\mathrm{m}}{\mathrm{V}_{\mathrm{m}}},\tag{1}$$

where  $\Delta C/\Delta t$  is the change in N<sub>2</sub>O concentration in the chamber during the incubation time  $\Delta t$ , V is the volume of the chamber (16.7 dm<sup>3</sup>), A is the soil area (0.13 m<sup>2</sup>) covered by the chamber, m is the molecular mass of N<sub>2</sub>O and V<sub>m</sub> is molar volume of N<sub>2</sub>O. Gas fluxes were calculated as the increase in concentration during the incubation period. A linear function was fitted to the N<sub>2</sub>O 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<sub>2</sub>O flux over the 0–40 minutes interval. When  $R^2$  was smaller than 0.7 and a linear function could not be fitted, N<sub>2</sub>O 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 µg N<sub>2</sub>O-N m<sup>-2</sup> hour<sup>-1</sup> or less than  $-0.3 \mu g N_2O$ -N m<sup>-2</sup> hour<sup>-1</sup>. All measured N<sub>2</sub>O 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<sub>4</sub> (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<sup>3</sup> 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

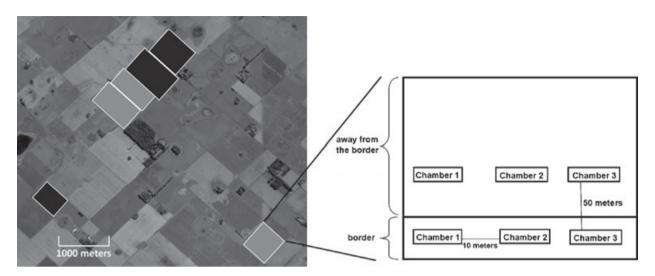


Figure 1 Experimental plot locations within fields (left) and chamber locations within each plot (right). Grey squares correspond to sequence 1, black squares correspond to sequence 2.

Table 1	Chronogram of	sampling,	sowing, N	N fertilizer	and harvest	of the	two crop sequence	s over 2 years
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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	Х	X X		Х	Х	Х	Х	Х	
Sowing		Х		Х		Х			
N fertilization		Х					Х		
Harvesting		Х		Х					
Sequence 2	Soyabean res. Maiz		ze	e 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	Х	X X		Х	Х	Х	Х	Х	
Sowing		Х				Х			
N fertilazation		Х							
Hervesting				Х					

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

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

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

$$VWC = GWC \times BD.$$
(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

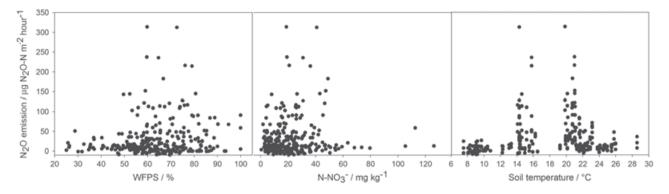


Figure 2 Distribution pattern of  $N_2O$  emission rates as a function of water-filled pore space (WFPS), soil  $NO_3^-$ -N concentration and topsoil temperature (left to right).

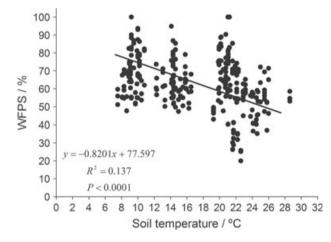


Figure 3 Relationship between water-filled pore space (WFPS) and topsoil temperature.

mainly planar, massive and subangular blocky aggregates. Despite these different structural types, similar topsoil bulk densities (from 1.2 to  $1.4 \,\text{Mg}\,\text{m}^{-3}$ ) were observed in both locations in the plots: N<sub>2</sub>O emission rates were also similar away from the border and in border locations.

During the study period, N<sub>2</sub>O emission rates ranged between -15 and  $314 \mu g N_2 O \cdot N m^{-2} hour^{-1}$ , with large variability among replicates. When plotted against all measured soil properties, no relationship between emission rates and WFPS or soil NO<sub>3</sub><sup>--</sup> N concentration was observed, but N<sub>2</sub>O emission rates showed a clearer response pattern across the topsoil temperature range (Figure 2). Nitrous oxide emission rates were very variable in the  $14-23^{\circ}$ C topsoil temperature range, whereas they were smaller and less variable at topsoil temperatures less than  $14^{\circ}$ C and more than  $23^{\circ}$ 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 N<sub>2</sub>O emission rates which differed significantly (P < 0.001): Group 1, small N<sub>2</sub>O emission rates,  $4.22 \pm 4.11 \,\mu g \, N_2 O \cdot N \, m^{-2} \, hour^{-1}$ ; Group 2, large N<sub>2</sub>O emission rates,  $61.87 \pm 4.07 \,\mu g \, N_2 O \cdot N \, m^{-2} \, hour^{-1}$ ; and Group 3, moderate N<sub>2</sub>O emission rates,

 $21.4\pm5.01\,\mu g~N_2O\text{-N}\,m^{-2}\,hour^{-1}\,$  (Figure 4). These emission groups coincide with the distribution pattern of topsoil temperature (Figure 2).

The small N<sub>2</sub>O 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 N<sub>2</sub>O emissions showed no relationship with any of the measured variables. The large N<sub>2</sub>O 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 N<sub>2</sub>O 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  $N_2O$  emission rates (Groups 2 and 3) were positively related to soil  $NO_3^-$ -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  $N_2O$  emission group and temperature range considered.

# Discussion

 $N_2O$  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  $14^{\circ}C$ ) had a direct effect, probably because of reduced microbial activity at these temperatures, which influences  $N_2O$  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

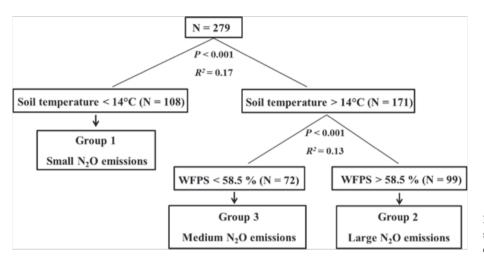


Figure 4 Results of the regression tree analysis, showing the main variables affecting N<sub>2</sub>O emission rates.

activity with decreasing topsoil temperature. However, Maljanen *et al.* (2009) found that even in sub-zero temperatures ( $-6.8^{\circ}$ C) N<sub>2</sub>O 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 N<sub>2</sub>O 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 NO<sub>3</sub><sup>--</sup>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 N<sub>2</sub>O production, provided sufficient NO<sub>3</sub><sup>--</sup>N concentrations were always more than 5 mg kg<sup>-1</sup> in the study site, suggesting that soil nitrate never limited N<sub>2</sub>O emissions totally, as has been shown by Dobbie *et al.* (1999).

According to Dalal *et al.* (2003) denitrification rate increases with increasing  $NO_3^-$  content, when the soil is wet and temperature and carbon availability are not limiting. This occurs because the presence of  $NO_3^-$  inhibits  $N_2O$  to  $N_2$  reduction, resulting in a relatively large  $N_2O:N_2$  ratio at similar humidity and oxygen content. Dalal *et al.* (2010) found a positive correlation between  $N_2O$  emissions and soil  $NO_3^-$  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  $N_2O$  emission and soil  $NO_3^-$ -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.

 $N_2O$  emissions were moderate when topsoil temperature was more than 14°C and WFPS less than 58.3% (Group 3, Figures 4, 5). These moderate  $N_2O$  emissions were smaller than those observed in experiments performed under controlled conditions, which showed an increase in N<sub>2</sub>O emission rates at temperatures as large as 70°C (Keeney *et al.*, 1979; Schindlbacher & Zechmeister-Boltenstern, 2004). These large N<sub>2</sub>O 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 O<sub>2</sub> 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 N<sub>2</sub>O to the atmosphere before being reduced to N<sub>2</sub> (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 N<sub>2</sub>O emissions decreases while the relative contribution of nitrification increases (Linn & Doran, 1984). These by-product N<sub>2</sub>O emissions from nitrification are usually less than those of denitrification (Castaldi, 2000; Smith *et al.*, 2003), which provides an explanation of why N<sub>2</sub>O 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  $N_2O$  emissions and soil  $NO_3^{-}-N$  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  $NO_3^{-}-N$  concentration also influenced  $N_2O$  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  $N_2O$  emissions when nitrification instead of denitrification prevails (Castaldi, 2000; Smith *et al.*, 2003).

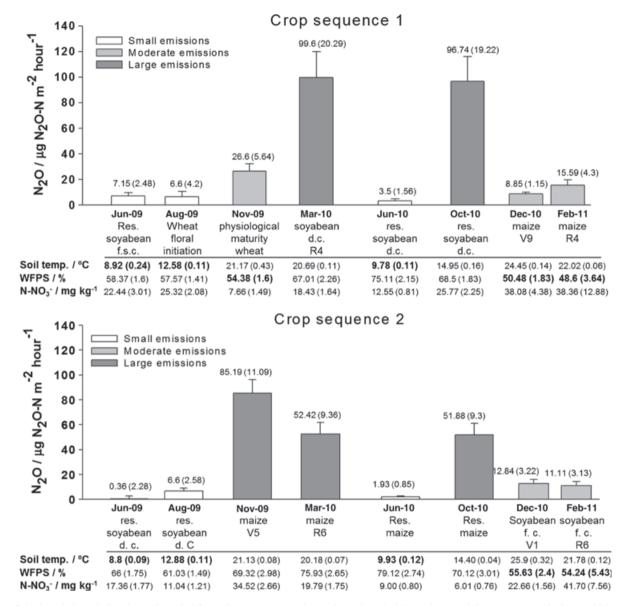


Figure 5  $N_2O$  emissions during the study period for each crop sequence (bars). Capped vertical lines above each bar are one standard error. Values above bars are means and (SE). Below each graph, values for means and (SE) are shown for soil temperature, water-filled pore space (WFPS) and soil  $NO_3^{-}-N$  concentration.

It is interesting to note that soyabean crops did not show a linear relationship between  $N_2O^-$  and  $NO_3^-$ -N, regardless of the emission group. It is likely that some unmeasured variable could explain  $N_2O$  emissions under soyabean crops, in particular the large variability of  $N_2O$  emissions under soyabean in Group 2. Ghosh *et al.* (2002) and Rochette & Janzen (2005) found large  $N_2O$  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  $N_2O$  emissions. It can influence  $N_2O$  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  $N_2O$  production (Helgason *et al.*, 2005). The results of  $N_2O$  emissions under soyabean crops need further clarification. It is likely that the inclusion of other biophysical variables would help explain the origin of large  $N_2O$  emissions in soyabean crops better.

# Conclusions

The most important factor driving soil  $N_2O$  emission rates was topsoil temperature, followed by water-filled pore space and soil  $NO_3^-$  concentration. From this study of non-tilled soils, a hierarchical arrangement of factors was used to explain  $N_2O$  emission rates, which was determined by which particular

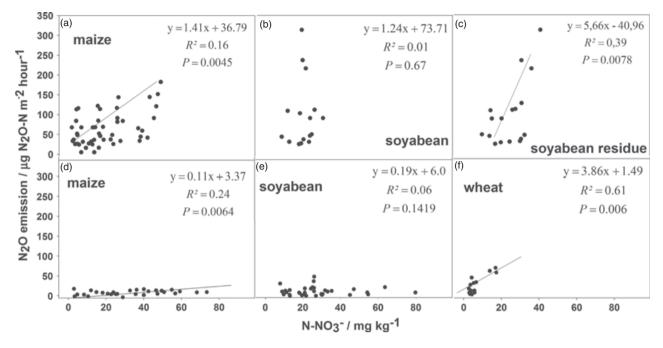


Figure 6 Relationship between  $N_2O$  emission rate and soil  $NO_3^-$ -N concentration for different crops and residues. (a-c) Large emissions with topsoil temperatures between 14 and 23°C (Group 3). (d-f) Moderate emissions with soil temperatures more than 23°C and WFPS less than 58.5% (Group 2).

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 N2O 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 N2O emissions, saving time and effort during fieldwork. For example, N<sub>2</sub>O emissions are likely to be small or even negligible when topsoil temperatures are less than14°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 N2O measurements would be recommended to capture all possible environmental variables.

# Acknowledgements

We are very grateful to Estefania Cartier and her family, on whose farm the study was conducted, Flavio Gutiérrez Boem for his statistical support in the data analysis, and the anonymous reviewers, whose suggestions and criticisms helped us to improve the quality of our manuscript.

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