Hungarian University of Agriculture and Life Sciences

Doctoral School of Biological Sciences

Ph.D. Thesis

N_2O fluxes from nitrification and denitrification processes in agricultural soils

By

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Gödöllő, Hungary

2021

Title: N_2O fluxes from nitrification and denitrification processes in agricultural soils

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1. BACKGROUND AND OBJECTIVES

Globally, agricultural soils constitute an important source of greenhouse gases (GHGs). Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are important climate-relevant trace gases. Nitrous oxide acts to deplete stratospheric ozone and also act as a GHG: it has a global warming potential being 306 times greater than that of CO_2 in the atmosphere for around 100 years on average (World Meteorological Organization and Global Atmosphere Watch, 2019). The atmospheric N_2O concentration in 2018 was 331.1 ppb (World Meteorological Organization and Global Atmosphere Watch, 2019). According to the estimates, more than 60% coming from fertilized agricultural soils (Reay *et al.*, 2012). Therefore, the emission of N_2O from agricultural soils represents a very important aspect of the global N cycle and the energy balance of the surface (Paustian *et al.*, 2016). In order to design effective strategies for N_2O mitigation, it is necessary to understand the different biotic and abiotic factors that control N_2O emissions (Han, Walter and Drinkwater, 2017).

N_2O is produced through the processes of nitrification, denitrification, dissimilatory nitrate reduction to ammonium and chemo-denitrification, and others. However, due to the different processes of production and consumption in the soil, soil N_2O flux can be bi-directional (Flechard *et al.*, 2005).

In spite of the complex and multiple ways of N_2O formation, nitrification and heterotrophic denitrification are assumed to be the key predominant sources of the N_2O emissions from soil ecosystems. Nitrification, as an aerobic process, controlled by ammonium and oxygen concentrations, and by certain bacteria such as *Nitrosomonas*, *Nitrosolobus*, genus (Singh and Tyagi, 2009) have been established as the principal N_2O source in soils with low water availability. On the other hand, denitrification - by which NO_3^- is reduced to gaseous compounds such as NO, N_2O, and N_2 (Tao *et al.*, 2018) - is the main process responsible for N_2O emission under anaerobic conditions and is performed by denitrifying bacteria through a series of steps catalyzed by intracellular enzymes. Apart from contributing to N_2O emissions, denitrification is the only known biological sink of N_2O by the reduction of N_2O to N_2 (Putz *et al.*, 2018).

The microbe-mediated processes of nitrification and denitrification are coupled and affected by the combination of different abiotic and biotic factors and the physical and biochemical soil properties (Smith, 2017) as organic carbon and

nitrogen content, microbial community, vegetation type, soil acidity and soil temperature, soil water content and more specifically WFPS. All of those factors may be affected by the type, intensity and timing of different management practices such as tillage, fertilization (Signor and Cerri, 2013).

N_2O emission from agricultural soils has been considered to be the most uncertain emission category due to the lack of knowledge about emission-generating processes and their natural variability including large spatial and temporal variability. Moreover, the limitations of the methodologies commonly used to quantify GHG emissions also increase uncertainty in the results. Static and dynamic chamber methods are widely used, but the high degree of spatiotemporal heterogeneity in emissions - generally characterized as "hot spots and hot moments" (Butterbach-Bahl *et al.*, 2013) – should also be taken into consideration.

These findings all suggest that more detailed knowledge needs to be gained in long term studies carried out under various environmental conditions for a better understanding of the underlying causes of spatiotemporal variability and also for reducing uncertainties of greenhouse gas emission measurements.

1.1. Objectives

The combination of long-term field experiment (2 years) conducted in a conventional management system and laboratory experiments performed under different emission drivers has also been rarely carried out in Hungary.

As croplands are the most common form of agricultural land-use in Hungary, covering more than 50% of the country's territory, the aim of the present study was to describe the temporal variability of cropland N_2O emission and to determine the effects of different environmental factors and management practices on soil N_2O emissions.

We have focused on the key variables controlling N_2O emissions i.e. temperature, soil WFPS, N fertilizer application, plant growth, and carbon source.

2. MATERIALS AND METHODS

2.1. Field N_2O measurement

2.1.1 Study site

A two-year-long field experiment (November 2017 - November 2019) was conducted in Kartal (47.658°N, 19.532°E, 153 m a.s.l.) in the middle part of Hungary, and which had a continental (pannonian) climate. The soil is a chernozem brown forest soil. The study site has a running eddy-covariance (EC) station for CO_2/H_2O gas exchange and meteorological measurements.
Management data during the study period are shown in Table 1.

Table 1**.** Dates of agronomical activities and fertilizer inputs in kg N ha^{-1}; CAN 27%N (calcium ammonium nitrate, NPK 15-15-15 (nitrogen, phosphorus and potassium), Nikrol 30% (N30), MAS 27%, (lime, ammonium nitrate) in the study period. (CAN 27%, NPK 15-15-15 and MAS 27% were used in the field as granular, Nikrol 30% was used as a liquid form).

Cropping season	Crops	Sowing date	Harvesting date	Fertilizer application date	Fertilizer type and N%	N input (kg Nha^{-1})
2017-2018	Winter wheat	03/10/2017	14/07/2018	01/10/2017	CAN 27%	100
				15/03/2018	Nikrol30%	140
2018-2019	Rapeseed	10/09/2018	no harvest	29/08/2018	NPK 15-15-15	200
2019	Sorghum	03/05/2019	30/09/2019	03/05/2019	MAS 27%	200
2019-2020	Winter wheat	14/10/2019	21/07/2020	10/04/2019	MAS 27%	100

2.1.2 Field sampling of soil N_2O emissions

N_2O emissions were measured from November 2017 to November 2019 generally bi-weekly with the exceptions when the soil was frozen or covered by snow. The sampling campaign was done using static (closed) chambers. Sampling time was between 10.00 and 12.00 h. Ten polyvinyl chloride (PVC) collars were inserted into the soil (2.7 cm depth) at 1 m apart along a 10 m transect. The collars were left permanently there to avoid the sudden emission peaks after its installation, the collars removed only at harvesting and tillage, after they were immediately returned to the initial location. During the measurements, the collars were covered by lids only for the duration of the sampling. The area of the chambers formed was 81.71 cm^2 and the volume was 523 cm^3. Air samples from the chambers were taken at 0, 10, 20, and 40 min after closure with a Hamilton

syringe. A total of 10 ml of air samples were injected into evacuated vials of 10 ml. After sampling the samples in the hermetically closed vials were transported immediately to the laboratory for the analysis.

2.1.3 N_2O detection of the field samples

Nitrous oxide concentrations were determined with an HP 5890 II gas chromatograph.

Soil N_2O emissions were calculated as follows (Horváth *et al.*, 2010):

Equation 1. N_2O flux calculation :

$$F = \frac{\Delta N2O \times 2 \times AN \times Vch \times f}{Vm \times Ach \times t},$$

where F is the emission [µg $Nm^{-2}h^{-1}$], $\Delta N2O$ is the slope of N_2O mixing ratio in the chamber during sampling (1/60 h) [ppb], *AN* is the atomic weight of N, *Vch* is the volume of the chamber [m^3], *f* is the factor taking into account the residual pressure in the evacuated vials (1.233), *Vm* is the molar volume [L] (Vm = 24 L at t=20 °C laboratory temperature during measurements), *Ach* is the surface of soil covered by the chamber [m^2], *t* is the sampling time [1/60 h].

2.1.4 Ancillary measurements

Net ecosystem exchange of CO_2 (NEE) was measured by the eddy covariance (EC) station representing the activity of the vegetation. Leaf area index (LAI), VIgreen, soil water content (SWC), soil temperature (T_s), and soil bulk density (BD) were measured close to each collar simultaneously at the air sampling.

2.1.5 Microbial investigations.

Soil sampling from the same used field was also performed for microbiological investigations. 5 soil samples were chosen based on the N_2O emissions for the analysis of metabolic functions of soil samples microbial communities by Biolog Eco microplates, and also for the enumeration of microbial populations using plate count methods for bacteria population, actinomyces, ammonificans, and fungi. Also, Denitrifying bacteria were enumerated by the Most Probable Number (MNP) technique.

2.2. Lab measurements

Successive laboratory experiments were done under different treatments includes SWC, N fertilization, presence and absence of plant, and carbon source amendment.

2.2.1 Soil sampling

Using soil from the same field (Kartal), four laboratory experiments were done in order to measure the N_2O emission. Where soil was collected from the top 15 cm layer from the field site and transported into the lab. After that, the soil was air-dried before establishing the experiments and passed through a 2-mm mesh while visible roots and organic residues were removed and then mixed thoroughly before use. PVC tubes (10.2 cm in diameter and 20 cm height) were used as pots filled up to 15 cm with about 1.6 kg soil to achieve a bulk density of 1.30 g cm^{-3}. The top 5 cm layers of the tubes were used as static chambers during the N_2O emission measurements. SWC of soil was measured on a weight basis. Then the soil were preincubated at the selected SWC for 4 days. N fertilizer was applied on the surface of the soil at the beginning of the measurements and the pots were kept under favorable conditions (12 hours of light, 20°C air temperature).

2.2.2 Lab N_2O emission experiments design

The first three lab experiments were divided into bare and planted soils, treated with ammonium nitrate fertilizer: the 1st experiment was planted with wheat, it had two series, the 1st one treated with 0, 50 and 100 kg N ha^{-1}, under 20% SWC, contrary to the 2nd series that treated with 0, 75 and 150 kg N ha^{-1}, under 25% SWC. The second experiment containing repeated series of combinations of bare and planted soil (with maize), treated with 0, 75, and 150 kg N ha^{-1}, two SWC levels <30 (15, 20 and 25%) and >30 (35 and 40%). For the third experiment which was divided into bare and planted soil (with maize) two soil water content levels were chosen (20% SWC and 40% SWC) under 0, 75, and 150 kg N ha^{-1} ammonium nitrate fertilizer addition.

During the 1st experiment, N_2O flux measurement were done as weekly during 4 and 5 weeks, for the first and the second series, respectively. Later on, we decided to increase the frequency of the measurements in the other experiments. In this 3rd experiment the N_2O flux measurements which were performed for a period of 445 h in which a D(+) glucose monohydrate ($C_6H_{12}O_6.H_2O$) (250 mg glucose kg^{-1} soil) addition was done after 241 and 439 h from fertilization.

Concerning the 4th experiment, it was done from our principal study site (cropland soil), and a second soil that was a forest one that sampled in the Botanical Garden of the Hungarian University of Agriculture and Life Sciences, and as the third type we used sterilized sand. Each one received the same fertilizer type: ($NaNO_3$), except for forest soil another experiment was done using (NH_4NO_3) fertilizer. This experiment was done from bare soil under three N rates 0, 75, and 150 kg N ha^{-1} and under 80 % water filled pore space. Concerning sterilized sand since it does not contain any microbes, a 1 ml of microbial solution was added to the pots together with a portion of carbon source that's is D(+) glucose monohydrate (250 mg glucose kg^{-1} soil) before adding the fertilizer and starting the measurement.

After measuring the N_2O emission for several days, glucose addition, microbial solution, and other fertilizer portions were also added in several portions and during different times based on the N_2O emission tendency. N_2O measurement in this experiment was done at 869.5 h, 909 h and 965 h in the case of cropland soil, sand, and forest soil, respectively.

2.2.3 Lab N_2O concentration measurement and flux calculation

For the 1st lab experiment a gas sampling was done manually and N_2O concentration using an HP 5890 II gas chromatograph, electron capture detector technique. While for the rest of the lab N_2O emission experiments, the top part of the pots served as closed chambers connected to an N_2O gas analyzer Thermo Scientific 46i were used for the N_2O concentration measurements, each measurement lasted 20 minutes.

Soil N_2O emissions were calculated using the measured concentration change by equation 1.

2.3. Data Elaboration and Statistical Analysis

Data processing and statistical analysis were performed in R (R Core team, 2018). Gaussian error propagation was used to calculate propagated uncertainties of the field averages and the uncertainties of the cumulative sums of lab N_2O emission measurements (2nd lab experiment).

The cumulative emissions were calculated using the following formula:

Equation 2. Cumulative N_2O emissions

$$T = \sum_{i=1}^{n}[(X_i + X_{i+1})/2 \times (t_{i+1} - t_i) \times 24\ /1000]$$

where T (mg N m^{-2}) is the cumulative N_2O emissions, X (µg N m^{-2} h^{-1}) is the average daily N_2O emission rate, i is the i^{th} measurement, and ($t_{i+1} - t_i$) is the number of days between two adjacent measurements.

3. RESULTS AND DISCUSSION

3.1. Field experiment

3.1.1 Seasonal variations of the N_2O emissions

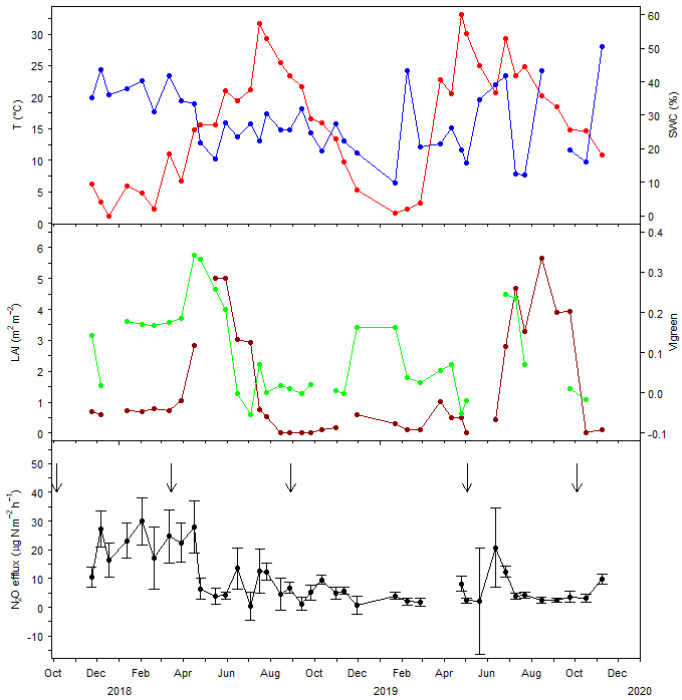

Figure 1. Temporal variations of soil temperature (Ts, °C, red dots) at a depth of 5 cm, soil moisture (SWC, %, blue dots) in the 0-7.5 cm soil layer (upper panel), VIgreen index (VIgreen, green dots), Leaf area index (LAI, $m^2.m^{-2}$, brown dots) (middle panel) and nitrous oxide (N_2O) emission (lower panel) over the study period (November 2017- November 2019). Error bars represent standard deviation. Arrows show fertilizer application.

The seasonal variations of the N_2O emissions are presented in Figure 1 (lower panel). During the study period the average N_2O emissions displayed high temporal variation with an average emission of 11.32 ± 9.35 µg N m^{-2} h^{-1} and 5.55 ± 5.24 µg N m^{-2} h^{-1}, for the years 2018 and 2019, respectively.

The highest N_2O emission peak (29.24 ± 8.11 µg N m^{-2} h^{-1}) was recorded during the freezing-thawing period at the beginning of February 2018 which is similar to a study reported by Kurganova and de Gerenyu (2010) reporting that

the freeze-thaw processes abruptly increased the emission of N_2O from the soils with high water contents. This emission could be caused by anoxic conditions, created by the higher soil water content (40.3%) and by the triggered plant residue decomposition which both stimulated denitrification, and N_2O production.

N fertilizer application on the 15th of March 2018 resulted in the second highest N_2O emission peak (27.95 ± 9.07 μg N m^{-2} h^{-1}) on 16th of April 2018 that coincided with a SWC of 33.53 % and a T_s of 14.87°C. This emission peak was detected 4 weeks after the fertilization with 140 kg N ha^{-1} Nikrol and during the physiological peak of winter wheat crop and it was associated with the highest value of VIgreen (0.342). The value of the third highest emission was approximately the same as the second peak (27.23 ± 6.31 μg N m^{-2} h^{-1}) and was measured at 43.55 % SWC and 3.37°C on 6th of December 2017, 8 weeks after N application with 100 kg N ha^{-1} CAN 27% and winter wheat sowing (beginning of the heading physiological stage) in October 2017.

We assumed that the observed high soil moisture conditions were often favorable for denitrification during these N_2O peaks emissions. According to Hayashi *et al.,* (2015) the rate of N_2O emissions increased with soil temperature up to 15–20°C and a negligible soil emission was found at a temperature below 5 °C. In contrast to this study, we found higher emissions even at lower temperatures, which corresponded to the results published by Dobbie and Smith (2003). Our results suggested that high N_2O emissions even at lower temperatures could be caused by a decrease in N uptake by plants which could favor microbial activity (Groffmann *et al.,* 1993). Our results suggest that N fertilization significantly enhanced N_2O emissions even after two months following the applications of N fertilizers. Besides, plant phenology also affects the magnitude of plant effects on N_2O production which was observed in our results.

The lowest N_2O emissions (0.27 ± 4.92 μg N m^{-2} h^{-1}) was observed on the third of July, 14 weeks after fertilization at 27.39 % SWC and 21.13°C, and was associated with the low value of VIgreen (–0.05). The lower emission was probably due the to lack of N in the soil, which is in line with a lot of studies (Shurpali *et al.,* 2016). Low N_2O emission (0.73 ± 3.21 μg N m^{-2} h^{-1}) was also observed on 30th of November 2018, which could be explained by its association with a low SWC of 19% after a long dry period and a T_s of 5.3°C which were not favorable for the N_2O emission.

On the other hand, several studies (Khalil, Mary and Renault, 2004) reported that daily N_2O emissions from the soil could be very low even after

fertilization, as it was observed on 13th September 2018 (1.26 ± 2.23 µg N m^{-2} h^{-1}) two weeks after N application (Figure 1) despite the fact that the SWC and T_s were favorable (31.85% and 21.6°C, respectively) for the N_2O production. Our data corresponded with the results published by Ball, McTaggart and Watson (2002). After this low N_2O emission, an increment in the emission was observed again and it was associated with 22.3% SWC. So our founded results proposed that the absence of the N_2O emission after two weeks from fertilization and its appearance again after several days could be caused by the presence of the easily decomposable carbon together with microbial diversity present in the field and their abundance and activity, which in turn can correlate with plant factors. Added to the environmental factors (precipitation) that affect soil properties like; soil water content.

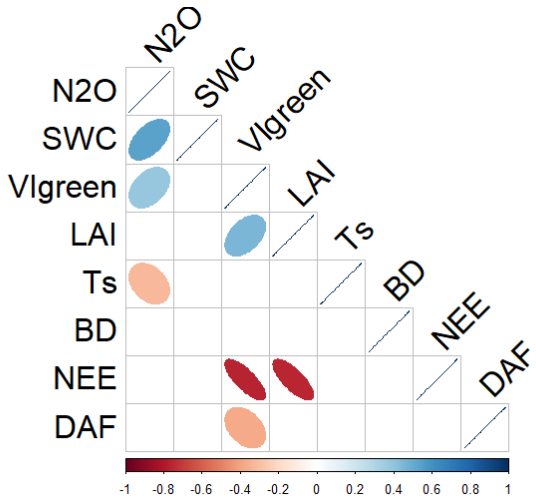

Figure 2. Correlation plot between nitrous oxide efflux and different driving variables, SWC (soil water content), VIgreen (VIgreen index), LAI (leaf area index), T_s (soil temperature), BD (bulk density of the soil), NEE (net ecosystem exchange of CO_2), DAF (day after fertilization). Only statistically significant ($p<0.05$) correlations are presented.

On the basis of the correlation plot and the correlation coefficients between nitrous oxide emission and different driving variables (Figure 2), we demonstrated that SWC and VIgreen had a significant positive (R= 0.53, R= 0.38, respectively) with p-level <0.05, while soil temperature (Ts) had a negative correlation with the N_2O emission (R= –0.32). Apparently there is no consensus about whether plants promote or suppress N_2O emissions; plants take up a large amount of N from the soil for growth (Ciampitti and Vyn, 2012), which leads to a reduction in the available N in the soil and thus reduce soil N_2O emissions

(Wang *et al.*, 2019). Others provided evidence that the presence of plants generally stimulates N_2O emissions which correspond to our data (Hayashi *et al.*, 2015) because the correlation with VIgreen suggests that there is possible effect of plant presence on soil N_2O emission.

Concerning SWC, the positive correlation with the N_2O emission was also reported in many papers (Bouwman, 1998; Ruser and Schulz, 2015). On the other hand, the negative correlation of T_s with N_2O emissions observed in our study conflicted with a report proving that the N_2O emissions from the soils were positively correlated with soil temperature (Sosulski *et al.*, 2014) as the denitrification rate and soil microbial activity are positively related to temperature (Sulzman *et al.*, 2005). We should note that it is difficult to find a clear relationship between T_s and N_2O emission rates because in the field the highest T_s was always related to lower SWC.

More variance can be explained by a multiple linear regression including SWC and VIgreen as independent variables (r^2= 0.5052, p <0.0001).

Equation 3: The multiple linear regression with the fitted parameters.

$$N2O = -8.6039 + 0.6005 * SWC + 24.8447 * VIgreen$$

Our results clearly demonstrate that besides SWC plant activities also have to be taken into account as key drivers influencing N_2O emissions from fields.

3.1.2 Field microbial investigations.

For the field microbiological investigations, it was shown that the five soil microbial communities were capable of metabolizing organic substrates. It was also shown that their capacity of utilization of six-type carbon sources were different, the carbohydrates were the carbon source with the highest degree of metabolic utilization and amines/amides had the lowest degree of metabolic utilization.

In addition, there was a tendency that the numbers of total bacteria, fungi, and ammonificans were higher on the same sample among the 5 sampling dates, contrary to the denitrificans communities that responded differently, and the higher N_2O emissions were not always accompanied with higher denitrifiers population and higher metabolic activity, and the reverse was also observed, that cleary demonstrated that besides microbial communities others factors were influencing the N_2O emission and also affecting microbial communities, additionally, the emitted N_2O was produced by other microbial population rather than bacteria denitrificans, even under higher SWC levels.

3.2. Laboratory incubation experiments

3.2.1 First experiment

Effect of fertilizer addition, soil water content, and plant presence on the N_2O emissions

This experiment had two repetitions (series), during the first series which was done under 20% SWC, and treated with 0, 50, and 100 kg N ha^{-1} ammonium nitrate fertilizer in bare and planted soils (Figure 3). In bare soil, N_2O averages during the first week of the measurement was higher in the N0 than in soil treated with N50 and N100. In planted soil there was a clear difference between the treatments according to the fertilizer addition. After 2 weeks of incubation, there was a difference just between N0 and N50, both in bare and planted soil. After three weeks significant differences between all the treatments were detected in bare soil. In planted soil significant difference was found just between N0 and N50. Then, after 4 weeks clear significant differences were recorded in bare and planted in all the treatments, where bare soil was a bit higher than planted soil. Later on, after 5 weeks, the difference was recorded just between N0 and N50, both in bare and planted soil, and here there is no big difference between bare and planted.

Concerning the second series of the experiment (Figure 3), that treated with ammonium nitrate 0, 75, and 150 kg N ha^{-1}, under 25 % SWC. After one week, in bare soil a difference in the N_2O emission was recorded only between N0 and N75. Nevertheless, in planted soil, the difference was recorded between the three N rates. The same tendency was observed after two weeks. After three weeks significant differences were observed in planted soil between all fertilizer rates: N75 was 1.7 folder times higher than N0, N150 was 1.4 folder times higher than N75. In bare soil, the differences were observed only between N0 and N150, and between N75 and N150. Finally, after four weeks significant increases in the N_2O emission were observed both in bare and planted soils with increasing N fertilizer rate, where the emission was higher in the presence of plant compared with bare soil.

Comparing the N_2O emission from the two different series, it was clearly shown that under N0 both in bare and planted soil, soil under 25% SWC (2^{nd} series) emitted more N_2O than soil under 20% SWC (1^{st} series), with 4.6, 19.6, 6.6, and 1.1 times, during the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} week, respectively. Also with plant presence N0 soil under 25% SWC emitted 3.3, 17, 3.2, and 3.9 times than N0 soil under 20% SWC, aslo during the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} week, respectively.

So from the two series of the 1st experiment, it was clearly shown that the N_2O emission was significantly affected by the soil water content level, plant presence, and fertilizer rate. The N_2O emitted from soil of 20, 25% SWC could be mainly from the nitrification process.

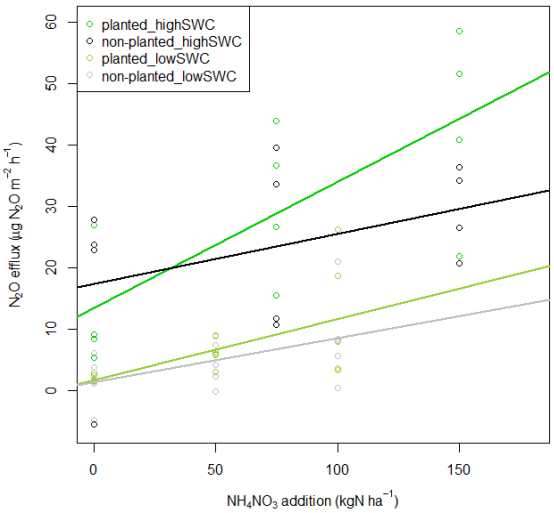

Figure 3. N_2O emission averages in the planted and bare soil under different NH_4NO_3 fertilizer rates (0, 50 and 100 kg N ha^{-1}), under SWC equal to 20% for the first serie and a second serie under NH_4NO_3 fertilizer rates (0, 75 and 150 kg N ha^{-1}), under SWC = 25%, during 5 an and 4 weeks lab experiment, respectively.

Based on the regression presented in the Figure 17 fertilizer application had a positive effect on the N_2O emission, r^2= 0.36, r^2= 0.26, for bare, and planted soil, respectively, under lower SWC with p-level<0.05. Also a significant effect of fertilizer application was recorded in planted soil under higher SWC, r^2= 055, with p-level<0.05. Contrary, the regression between N_2O emission and fertilizer application was not significant on bare soil under higher SWC (r^2= 0.16).

3.2.2 Second experiment

Drivers of N_2O emissions

We found significant correlations between N_2O emissions and SWC (R=0.45), as well as N_2O emissions and fertilizer amount (R=0.25). Increase in denitrification rates and/or N_2O emission rates has been frequently found

following N-fertilizer application. Fu *et al.*, (2012) also demonstrated a correlation between SWC and the N_2O emissions.

Similarly to our field study, we used multiple linear regression between N_2O emissions, SWC, and nitrogen fertilizer treatment to explain more variance. The parameters with their significance level are shown in Table 2.

Table 2. Results of the multiple regression for soil N_2O emissions: r^2 values and regression coefficients with statistical significance levels (intercept, SWC and nitrogen fertilizer addition, ***: $p<0.001$ in all cases).

	r^2	a	SWC	Nitrogen fertilizer
Bare soil	0.26	–38.02 ***	2.08 ***	0.13 ***
Planted soil	0.29	–43.37 ***	2.34 ***	0.16 ***

Effect of N-fertilizer application and plant presence on cumulative N_2O emissions

The cumulative N_2O emissions are illustrated in Figure 4 (left panel), showing temporal variations during the 22 days long period after fertilization. Application of ammonium nitrate fertilizer in doses of 0, 75, 150 kg N ha^{-1} and at SWC >30% significantly increased the cumulative N_2O emissions from bare soil, about 22 days after fertilizer application (DAF>20) with values of 5.77 ± 0.18, 10.66 ± 0.51 and 16.1 ± 0.88 mg N m^{-2}, respectively. The same pattern was found in planted soil. The cumulative N_2O emissions in N0, N75, N150 treatments were 5.93 ± 0.32, 10.44 ± 0.50 and 18.12 ± 1.20 mg N m^{-2}. The values from bare soil of N150 and of N75 were three and two-fold higher compared to N0, respectively. Even in planted soil the highest N_2O emission was observed in soil treated by 150 kg N ha^{-1} ammonium nitrate fertilizer and its value was three and around two times higher compared to the N0 and N75, respectively.

Numerous studies reported that nitrogen content or fertilizer addition was the most important driver determining soil N_2O emission (Myrgiotis *et al.*, 2019) and a lot of studies are in agreement with our results as N fertilizer was identified as having a clear positive effect on the N_2O emissions. Moreover, nitrous oxide emission rates in the soil are not only affected by the nitrogen application rates but also by the rates at which plants and soil microorganisms utilise nitrogen. As a result, under the same N fertilizer conditions N_2O emissions from fields under maize could be less than those from fields without plant cover as reported by Wang *et al.*, (2019). Conversely, in our study and under the same N fertilizer conditions, the cumulative N_2O emissions from planted maize soil were

approximately the same as from bare soil, except the soil treated with 150 kg N ha^{-1} N fertilizer, where the N_2O emission of planted soil was significantly higher than that of bare soil. This could be supported by a recent study which reported that maize growth reduced soil N_2O emission but N application can exert an antagonistic effect (Wang *et al.,* 2019).

Effect of soil water content on cumulative N_2O emissions.

The cumulative N_2O emissions increased significantly with increasing SWC which is shown in Figure 4 (right panel). The cumulative N_2O emission in bare soil observed in SWC>30% treatment (three weeks after fertilizer application) was three-fold higher than at SWC <30%. The same tendency was recorded in maize planted soil where the highest cumulative N_2O emission was measured at higher soil water content (SWC>30%) and it was three times higher than at SWC less than 30%. Comparing the cumulative N_2O emissions from bare and planted soils at the different soil water content levels (SWC<30%, SWC>30%) the cumulative emission in planted soil at the higher soil water content (>30%) was 2 mg N m^{-2} higher than in bare soils and 0.64 mg N m^{-2} higher than at SWC<30%.

In agreement with the model of Davidson (1991), denitrification could be the dominant mechanism in our soil. The main difference between our results and the model was that we measured high N_2O emission at SWC>30% (70-80% WFPS), which is supposed to favor denitrification. Hence from the values of the cumulative N_2O emissions at the different SWC levels, we also confirm that the application of fertilizer in soils of lower water content (<30%) and higher water content (>30%) would increase N_2O production from the nitrification and denitrification processes, respectively.

Concerning the comparison of cumulative N_2O emission between bare and planted soils under the lower SWC, the emission was approximately the same. Even at the higher SWC level only a small difference was recorded between bare and cultivated soil. This corresponded to a report (Sperling 2015), in which N_2O emissions were found to be similar between the bare and planted treatments, especially at 40-60% WFPS, while above 60% WFPS, emissions increased in cores from the planted type and decreased in cores from the bare type.

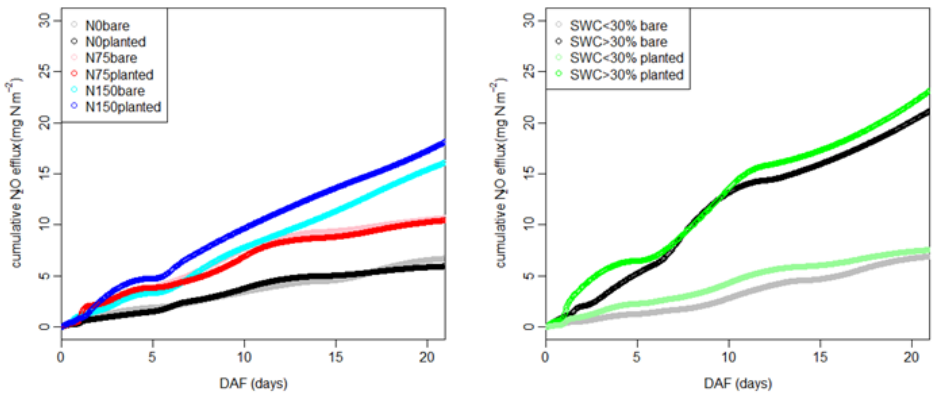

Figure 4. Cumulative N_2O emission in the planted and bare soil during 22 days lab experiment, under different N fertilizer rates (0, 75 and 150 kg N ha^{-1}) (left panel), and under two soil water content levels (SWC < 30%, SWC >30%: the average SWC were: SWC<30 bare 20.2%, SWC>30 bare 36%, SWC<30 planted 20.5%, and SWC>30 planted 35.4%) (right panel) as a function of days after fertilizer application (DAF).

3.2.3 Third experiment

In this experiment we did somethings similar to the 2nd experiment, but complemented with glucose addition.

Effect of N fertilizer rate and plant presence on the N_2O emission.

The results of this experiment showed substantial differences and variations in N_2O emission (Figure 5). Comparing the N_2O emission between bare and planted soil, at the different N fertilizer rate (Figure 5, left panel). After 2 h from fertilization, planted soil had a lower emission than bare soil, except in soil treated with 75 kg N h^{-1}. The fertilizer effect was observed just between N75 and N150 in bare soil, in contrary to the planted soil where emission increased with increasing the N fertilizer rate. During the 12 h after fertilizer application, we observed the same pattern which was observed after 2 h from fertilization, and the emissions were 6.7 and 2 times higher in planted soil than bare soil under N75, N150, respectively. After 24 h, the tendency has changed. The same variation which was observed after 12 h from fertilization was recorded after 72 h and after 144 h. After 157 h, the effect of fertilizer was shown under all the rates just in planted soil. After 228, no effect of both fertilizer rate and plant presence was observed.

Then, after 251 h from the application of N fertilizer and after 10 h from 1st portion of glucose addition, a very higher N_2O amount was emitted again in all the treatments, in bare the fertilizer effect were just between N0 and N150, N75

and N150, but in planted soil, the emissions were lower than in bare soil, but it increased with increases of N rate. 24 h later, in bare soil the emission from the N75, and N150 were increased. In planted soil, just small increases were recorded under N0 and N75. Then from 59 h to 183 h from 1st portion glucose amendment, the N_2O emissions under all the treatments were decreased more and more. Higher increases in the N_2O gas emissions were observed again in bare soil after 6 h from the 2nd portion of glucose, and 445 h from fertilizer application.

So based on the results it was shown that in general the emission was higher in planted soil compared with bare soil. However, in some measurement days bare soil had higher emission than planted soil that can be caused by plant uptake which needs more N for growth. But plant effect can be suppressed under a very high N fertilizer rate (Wang *et al.*, 2019) which is in agreement with our results. The highest emission in N0 soil was reported also in some studies e.g. Oktarita *et al.* (2017). For the effect of fertilizer, a significant relationship as it usual in most studies was found between N_2O emission and fertilizer rate in several measurement days, in other cases fertilizer rate had no clear effect, that was also recorded in a recent study done by Dencső (2021).

Concerning the effect of glucose addition on the N_2O emissions, it was illustrated that N_2O was really affected by carbon source addition, especially in bare soil, where in case of presence of enough glucose the emission was higher in N75 after 34 h than N150, even it was lower after 10 h from glucose addition, and this maybe because bacteria population need more time to use the glucose for N_2O production, that's why the N_2O emission in soil treated with N150, maybe will need also some time to be higher than N75 N_2O emission, also the diversity of microbial population and the presence of hot spots could cause this variation.

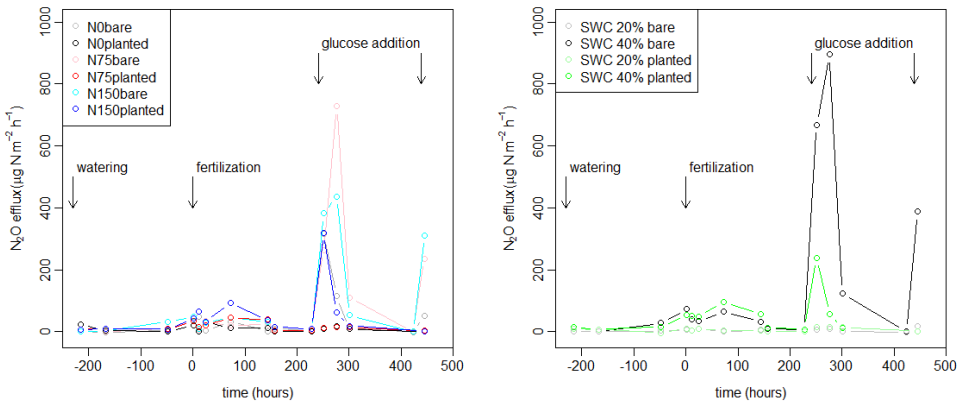

Figure 5. N_2O emission averages in planted and bare soil, under different N fertilizer rates (0, 75 and 150 kg N ha^{-1}), and under two soil water content levels (SWC= 20%, SWC= 40%), during 445 days lab experiment, and amended with glucose.

Effect of soil water content and glucose addition on the N_2O emission

Separating the results of the N_2O emission based on the soil water content (Figure 5, right panel), it was shown that the average N_2O before fertilization was higher at SWC 40%, both in bare and planted soils, then after 2 h from fertilizer application. N_2O emission in bare soil was higher by 11.6 times at 40% SWC than at the lower SWC (20%), and still the N_2O emission at 40% SWC in planted soil was higher than 20% SWC by more than 5 times.

Later, after 72, 144, 157, until 228 h from N fertilizer application, the N_2O emissions in all the treatments both in bare and planted soils were decreased except in bare soil 20% SWC there was no clear decrease. Those low N_2O emission rates increased in all of the treatments after 10 h from glucose amendment, even without addition of N fertilizer (N0), with larger increment at 40% SWC. Those emissions were 7, 4, 32, and 118 times higher than before glucose addition, in case of planted soil 20% SWC, bare soil 20% SWC, planted soil 40% SWC, and bare soil 40% SWC, respectively. Then, after 301 h from N addition and 59 h from 1st glucose addition N_2O emissions decreased again. After the 2nd glucose portion addition, N_2O emissions increased again in bare soil, both under 20 and 40% SWC to highest values 20 and 440 times more under 20% SWC and under 40% SWC, compared to which were recorded before this addition.

So, the results showed that the SWC level had a positive effect on the N_2O emission. Under 20% SWC where there was a variation whether N_2O emission from bare or planted soil was the highest, but in general, in most cases planted

soil had the highest emission. Besides, soil under 40% SWC seemed to be to most affected by the glucose amendment, with the dominance of bare soil emissions.

Concerning the effect of glucose addition on the N_2O emissions, the dependency of the N_2O emission on the carbon source was clearly observed, that is necessary in the denitrification process especially and heterotrophic nitrification. Several studies found that denitrification (N_2O production) was promoted after glucose addition since it is more easily dissolved (Chen, Mothapo and Shi, 2015). The highest N_2O emission after glucose emission was observed in bare compared to the planted soil, because the reason could be that there was no enough N in planted pots.

3.2.4 Fourth experiment

During this experiment only bare soils were used, where the aim was to compare the effect of glucose addition on N_2O emission in different soils and SWC was at 40% level.

N_2O emissions from three different soil types (forest, cropland and sand)

N_2O emission from forest soil

N_2O emission under sodium nitrate fertilizer

N_2O emission averages from forest soil treated at 40% SWC, and sodium nitrate fertilizer was shown in (Figure 6, upper panel), the emission showed a variation depending on the additional treatments. N_2O emission was measured before 24 h from fertilization, it seemed that even without fertilization, forest soil emitted a considerable amount of N_2O. Then after 4 h from $NaNO_3$ fertilizer application, the N_2O emissions for N0, N75, and N150 were increased. Then, after 27.5 h, N150 was increased again. The N_2O emissions were decreased during, 48, 70, 96, 116, 148, 196, and 239 h continuously. Then the N_2O was measured after 16 h from receiving pots the 1st portion of glucose (267 h after fertilizer application), where they emitted a higher amount of nitrous oxide. These emissions were higher 9, 16, and 10 times for N0, N75, and N150, compared to the values before the glucose addition. Then N_2O emissions decreased again.

A second portion of glucose was added to the pots after 362.h from fertilization, and 113.5 h from adding the 1st glucose portion, and other N_2O peaks were detected after 4 h from this 2nd glucose portion addition. Thereafter, they decreased again. Then, a 1ml microbial solution was added (after 535.5 h from fertilization), but still, no significant increases were detected. Later on, a third

portion of glucose was amended and after 2 h from its addition and 607 h from fertilizer addition, peaks of N_2O emissions were recorded, even in the N0. Then the N_2O emissions were deceased. Later, a portion of glucose for the fourth time was added to the pots after 750 h from adding fertilizer, when increases in the emitted N_2O were recorded 19.5 h after the addition. From 769.5 h until 849 h from fertilizer addition the emissions were deceased gradually.

After that, a second sodium nitrate addition was done (after 869.5 h from 1st fertilizer addition), where higher emissions were detected after 2 h . These higher emissions were decreased again after 19 h. For that, the fifth portion of glucose was added (after 72.5 h from the 2nd fertilizer addition), and N_2O emissions were measured after 4.5 h from this amendment, where higher emissions compared to which were detected after just fertilizer addition were recorded. It increased again after 27.5 h from this glucose amendment to another peak for N150, contrary to N0, N75 that decreased.

So, N_2O emission seemed to be responded very fast after glucose amendment, where in most cases, N_2O emission peaks were recorded during the first 28 h. Also, it was shown that the effect of glucose addition was very short since most of the peaks disappeared rapidly and the emission decreased gradually. Concerning the different glucose portions, all of them caused a higher emission, but the emission course was a bit different depending on the other drivers limiting the N_2O emission.

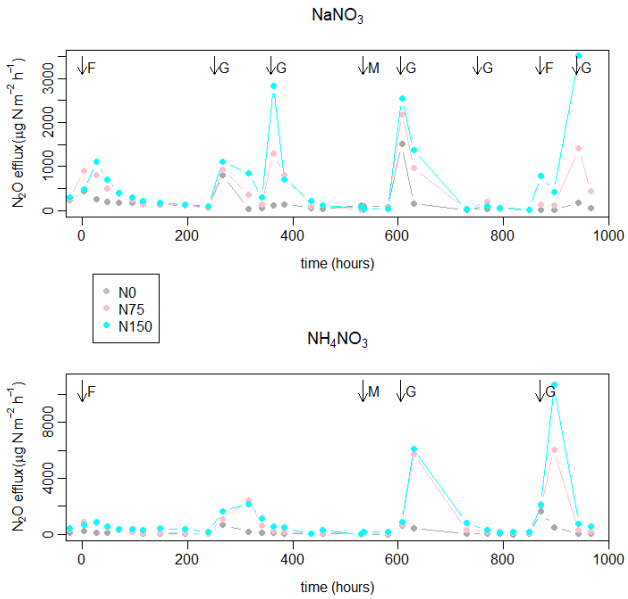

Figure 6. N_2O emission averages from forest soil (bare soil), during 965 h long study period, under 40% SWC, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}) (upper panel), and ammonium nitrate fertilizer (lower panel), and amended with glucose (G) and microbial solution (M).

N_2O emission under ammonium nitrate fertilizer

N_2O emission was measured in a series of pots fertilized by ammonium nitrate (Figure 6, lower panel). The emission showed a different variation compared with soil treated with sodium nitrate, where only fewer additional amendments were used, since we recorded a considerable emission during the first 316 h after fertilizer application. The positive fertilizer effect was observed starting from 4 h from its addition but without a big difference between N75 and N150. The N effect started to decrease after 48 h from its addition and after a long time (267 h from fertilizer addition), it appeared again, without any N addition, which could be caused by the fertilizer type since ammonium was a needed substrate for the nitrification process and our conditions are anaerobic, so maybe it was just needed time to be transformed under such conditions. Also, the microbial solution seemed to have a positive effect on the N_2O emission after 23 h from its addition. Besides, N_2O emissions after 2nd glucose addition were 1.1 and 1.7 times higher than after the 1st portion, for N0 and N150, respectively.

Comparing the emissions under the two different N fertilizer types, it was observed that both fertilizers had a positive effect, also all the glucose additions had a significant positive effect, but the temporal variation of the emission were different. Between 70-239 h from fertilization, the emissions decreased in both N fertilizer type, but it seemed that soil treated with ammonium nitrate emitted on average more N_2O than in soil treated with sodium nitrate, except in soil treated with N150. Soil fertilized by $NaNO_3$ needed glucose addition at 267 h after fertilization, contrary to the other one which emitted higher N_2O amount without any additions. NH_4NO_3 caused the higher emission, so it could be suggested that only denitrification occured in soil with $NaNO_3$ addition, while in soil with NH_4NO_3 nitrification could take place also.

N_2O emission from cropland soil

The N_2O measurement from cropland soil (Figure 7) was started before 72 h from fertilization. Where N_2O was emitted at a significant values, but it deceased with time. Then after 4 h and 27.5 h from fertilizer addition, no very higher emissions were detected. Then after 48, 70 h from fertilization, it decreased a bit. Then after 120 h from fertilizer addition, glucose portion was added, and

N_2O emissions were measured after 4 h from this amendment, where a pulses in the N_2O emissions were recorded, with significant effect of N fertilizer rate. Then after 24 h from it addition (148 h from N fertilizer addition), it increased again to another higher peak, that were higher 357, 116 times higher than before adding glucose portion (94.5 h from N application), for N75, N150, respectively, and 18, 9.8 times higher than the emitted amounts after 4 h, for N75, N150, respectively.

An addition of 1 ml of microbial solution was done after 157 h from fertilizer application, but the decreases continued, except soil treated with N150 that decreased later. Then the second portion of glucose addition with another 1 ml of microbial solution were added (207 h from fertilizer addition), and N_2O measurement was done after 15 h, where no increment in the N_2O emission was recorded. This decrease in the emission continued until 725.5 h from fertilizer addition and 519 h from both 2nd glucose portion and 2nd microbial solution additions, even there was another portion of sodium nitrate fertilizer that was added to the pots on 683.5 h from 1st fertilizer addition. Then after 114 h from this addition (after 779.5 from 1st fertilizer addition), a third portion of glucose was done, and an emitted peacks were observed for N0, N75, and N150 after 18 h from this amendment, thoses values were very higher than the amount emitted before the second N fertilizer portion. Then, it was start to decrease after 42 h.

From the obtained results, it was shown that the maximum emitted N_2O with just sodium nitrate addition, was after 27.5 h from its addition for N150, while for N75, it was after 4 h from its addition. Contrary, in the other experiment that was done in similar conditions (3rd experiment), but with ammonium nitrate fertilizer, the highest emission were recorded after 2 h and 72 h from the N addition. In the case of glucose incorporation, the maximum N_2O emitted from soil treated with 150 kg N ha^{-1} was after 47 h from 1st portion of glucose addition and 14 h from 1st microbial addition, and under N75 the maximum emission was after 24 h from the addition. The maximum emissions obtained with glucose addition were 69 and 66 times higher than with just fertilizer addition, in the case of N75 and N150, respectively, which proved the positive effect of easily decomposable carbon on the N_2O emission. Also, its importance was clearly showed again when a 2nd fertilizer addition was done, but there was no recorded increment, maybe because the 2nd glucose portion which was added before more than 500 h, was used by microbes for their growth, and during the 2nd N addition there was no enough carbon to use it, that's why a 3rd glucose amendment caused a great N_2O emission.

In general, it was clearly shown that during the first hours of measurement after N fertilizer addition, soil treated with ammonium nitrate (3rd experiment) emitted more N_2O compared with soil treated with sodium nitrate fertilizer. For example, when the frequency of the experiment was similar to which was done in this experiment, the emitted N_2O after around 72 h was 37 and 7.6 times higher in soil treated with ammonium nitrate compared with soil treated with sodium nitrate fertilizer (in N75, and N150). Then after 1st glucose addition, soil treated with ammonium nitrate fertilizer emitted more N_2O than soil treated with sodium nitrate fertilizer, this difference may be caused by the difference in the measurement time, and due to the difference in nitrification and denitrification processes. However, after the 2nd glucose addition, the contrary was observed, that could be caused by the addition of microbial solution. Therefore, it can be concluded that both N addition and easily decomposable carbon were key factors influencing and enhancing the N_2O emission when no other factors are limiting.

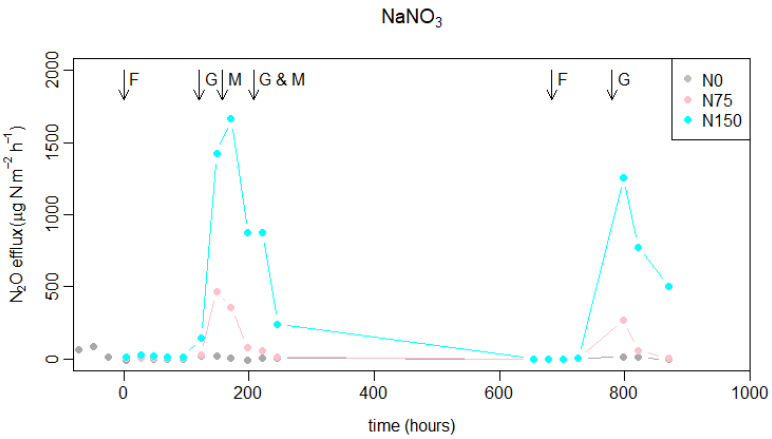

Figure 7. N_2O emission averages from cropland soil (bare soil), during 869.5 h long study period, under 40% soil water content, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}), and amended with glucose (G), microbial solution (M).

N_2O emission from sand

Based on the results illustrated in Figure 8, it was shown that N fertilizer addition positively influenced the N_2O emission, where the presence of N supplies represented a key factor controlling the N_2O production. This effect was observed before N addition to the sand. 48 h after fertilizer addition (accompanied with around 100 h from 1st glucose addition and 1 ml microbial solution) represented the ideal timing for the highest N_2O emissions under 150 kg N ha^{-1} sodium nitrate.

While in soil treated with 75 kg N ha^{-1}, the maximum value was after 43 h from the 2nd glucose addition with the 2 ml of microbial solution. N_2O emission decreased between 70 – 190.5 h from fertilization, even with microbial addition, then it was increased after the addition of the 3rd portion of glucose. All of these findings shed light on the stimulatory role of glucose addition or the easily decomposable carbon which represent a key factor, also the role of microbial addition on the N_2O production and emission.

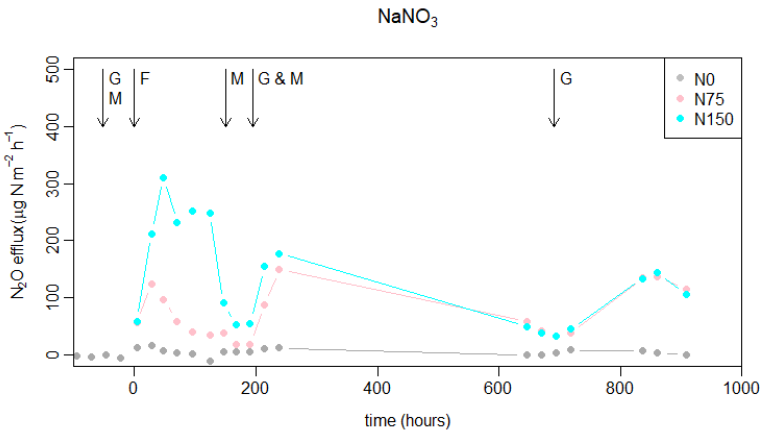

Figure 8. N_2O emission averages from sand (bare soil), during 909 h long study period, under 40% soil water content, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}), and amended with glucose (G), microbial solution (M).

Comparison between N_2O emissions in the three soil types

Based on the observed results from the three different soil types, it could be clearly concluded that N fertilizer addition and the easily decomposable carbon together had a significant effect on the emissions. Their presence enhanced the N_2O emission when no other drivers were limiting, and these results were in accordance with other studies reporting that the N-fertilizers affect the amount of NH_4^+ or NO_3^- available in the soil, which in turn affect N_2O production process (Signor and Cerri, 2013). In a study done by Wang *et al.* (2005), it was reported that supplies of available organic C appeared to be a critical factor controlling denitrification and/or heterotrophic nitrification processes and N_2O emission. Also, several studies found that denitrification (N_2O production) was promoted after glucose addition since it is more easily dissolved (Chen, Mothapo and Shi, 2015).

For that more understanding about the different effects of different C sources on the bacterial community over longer time scales is needed, that may

help in understanding the complex interaction between N_2O and the different drivers as well as its production and reduction.

4. NEW SCIENTIFIC RESULTS

Long-term (2 years) field data of N_2O emission from cropland soil under conventional management system during different crops in Hungary with parallel laboratory experiment on the same soil under different emission drivers has been rarely carried out in Hungary. Hence our study is of primary importance in order to obtain consistent values contributing to the national GHG estimates.

The highlights of the most important results from the present study can be summarized as:

1- Based on lab experiments, the emission increased with increasing N rates in the case where all the other controlling drivers are in favorable conditions. Doubled amount of N fertilizer caused two to three-fold higher increase in N_2O emission, both in bare and planted soil. Fertilizer effect can remain even after a long time from its application (several weeks), while in the field experiment no significant correlation was found between fertilization timing and N_2O emission.

2- Additionally, fertilizer type seemed to have a clear effect on the N_2O emission rates and plays an important role in determining its variation. In laboratory experiment soil treated with ammonium nitrate emitted more N_2O than soil treated with sodium nitrate fertilizer.

3- We described the influence of soil water content level on nitrous oxide emission in a Hungarian agricultural soil. For lab experiments, increasing SWC content resulted in an increase in the N_2O emission in all of the combinations with other drivers, SWC of 36% (on average) caused a three-fold higher increase in N_2O emission compared to the soil under SWC of 21% (on average). And increasing SWC by 5% caused at least one-fold higher increase in N_2O emission. Besides, increasing the SWC level from 20 to 40% caused an increase in the N_2O emission with more than 11 and 5 times in bare and planted soil, respectively. Also, a positive relationship between N_2O emission and SWC was recorded in the field study ($R = 0.53$).

4- We concluded that plant presence generally stimulated N_2O emissions, but this effect depended on the other influencing drivers, especially on the N fertilizer rates, where the enhanced effect appears with increasing N rates. The plant effect was shown both under field and lab conditions. In the field study, VIgreen had a significant positive ($R = 0.38$)

correlation with the emission and planted soil emitted higher amount of N_2O than bare soil in the lab experiments.

5- Carbon source was found as a key factor influencing the N_2O emission, where its presence as an easily degradable form stimulated the emission. Carbon sources played a stimulatory role, especially under anaerobic conditions and in the absence of plants. In cropland soil case (bare soil), glucose addition caused higher emission with more than 65 times compared to N_2O emitted with just N fertilizer addition. While its presence with lower quantities caused a lower emission, and its presence as a not easily decomposable form will cause a late N_2O emission.

6- We found that microbial communities and their activity were affected by the different management practices. Our results clearly showed that the highest N_2O emission was not always correlated with higher denitrificans population, and higher metabolic activity. Other microbial communities, rather than bacteria denitrifiers could play an important role in the N_2O formation process, together with the different other influencing derives.

5. CONCLUSIONS

From the two-year-long N_2O field soil emission and the laboratory experiments, the main results revealed the complexity of N_2O emissions and showed that different factors played major roles throughout the different phases of the study period.

In the field study, the magnitude of emissions varied widely and characterized with a mixed effect of soil water content and crop growth since we found a positive relationship between N_2O emission and both SWC and VIgreen. In contrast, a negative correlation between N_2O emission and soil temperature was found due to the usually dry conditions under high temperatures. For the field microbiological investigations, it was shown that the five soil microbial communities were capable of metabolizing organic substrates, and their capacity of utilization of six-type carbon sources were different. In addition, there was a tendency that the numbers of total bacteria, fungi, and ammonificans were higher on the same sample among the 5 sampling dates, contrary to the denitrifying bacterial communities that responded differently, and the higher N_2O emissions were not always accompanied with higher denitrifiers population and higher metabolic activity, and the reverse was also observed, that cleary demonstrated that besides microbial communities others factors were influencing the N_2O emission and also affecting microbial communities, additionally, the emitted N_2O was produced by other microbial population rather than denitrifiers, even under higher SWC levels.

Besides the field results, a strong positive correlation was found between the amount of N fertilizer and N_2O emission in laboratory experiments. Similarly to the field results, soil water content was a major factor modifying N_2O emission rates, while the effect of plant presence was moderate depending on the other influencing drivers. In addition, carbon source seemed to be another key factor influencing N_2O emission, especially where no other drivers limited the production and the emission of N_2O, (e.g. in bare soil under 40% SWC). Additionally, fertilizer type seemed to have a clear effect on the N_2O emission rates and plays an important role in determining its variation.

This study illustrates and sheds light on the complex effect of agricultural management and the climatic conditions determining N_2O emissions. These relationships could provide valuable additions for modeling studies and GHG inventories as well as for developing management strategies to reduce N_2O emissions from agricultural soils.

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7. BIBLIOGRAPHY (LIST OF WORKS CONSULTED)

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1- Temporal variability and drivers of nitrous oxide emissions from Central Hungarian croplands: field and pot experiments.
Meryem Bouteldja, Insaf Malek, Katalin Posta, Györgyi Kampfl, Szilvia Fóti, Krisztina Pintér, Zoltán Nagy, János Balogh. Eurasian Soil Science, accepted paper

2- Responses of soil respiration to different biotic and abiotic drivers in a temperate cropland.
Insaf Malek, Meryem Bouteldja, Katalin Posta, Szilvia Fóti, Krisztina Pintér, Zoltán Nagy, János Balogh. Eurasian Soil Science. doi. 10.1134/S1064229321070097

Published conference papers:

1- N_2O flux from planted and not planted cropland soils.
Meryem Bouteldja, Insaf Malek, Katalin Posta, Györgyi Kampfl, János Balogh.
18th Alps-Adria Scientific Workshop. p. 30,31
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2- Temporal variability of N_2O emission in agricultural field.
Meryem Bouteldja, Insaf Malek, Katalin Posta, Györgyi Kampfl, János Balogh.
19^{th} Alps-Adria Scientific Workshop. P.63. DOI: 10.34116/NTI.2020.AA

3- CO_2 efflux from agricultural soils in hungary.
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4- The effect of biotic and abiotic drivers on soil respiration in Kartal site.
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