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N_2O fluxes from nitrification and denitrification processes in agricultural soils

By

Meryem Bouteldja

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Name of Doctoral School: Doctoral School of Biological Sciences

Head: Prof. Dr. Zoltán Nagy

Department of Plant Physiology and Plant Ecology

Institute of Agronomy

Hungarian University of Agriculture and Life Sciences

Supervisor: Dr. János Balogh,

Department of Plant Physiology and Plant Ecology

Institute of Agronomy

Hungarian University of Agriculture and Life Sciences

....

Approval of Head of Doctoral School Approval of Supervisor

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ABBEVIATIONS

AWCD	AVERAGE WELL COLOR DEVELOPMENT
AMO	AMMONIA MONOOXYGENASE
BMP_S	BEST MANAGEMENT PRACTICES
$CaCO_3$	CALCIUM CARBONATE
$CaCl_2$	CALCIUM CHLORIDE
CFC_S	CHLOROFLUOROCARBONS
CH_4	METHANE
CO_2	CARBON DIOXIDE
DNDC	DENITRIFICATION-DECOMPOSITION
EDC	EASILY DEGRADABLE CARBON
GC	GAS CHROMATOGRAPHY
GHGs	GREENHOUSE GASES
HNO_3	NITRIC ACID
HNO	NITROXYL
IPCC	INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE
LAI	LEAF AREA INDEX
LULUCF	LAND USE, LAND-USE CHANGE AND FORESTRY
MnO_4^-	PERMANGANATE
Mt CO_2-EQ	MILLION TONNES OF CARBON DIOXIDE EQUIVALENT
N0	0 kg NITROGEN PER HECTARE
N_2O	NITROUS OXIDE
N_2H_4	HYDRAZINE

N50	50 kg NITROGEN PER HECTARE
N75	75 kg NITROGEN PER HECTARE
N100	100 kg NITROGEN PER HECTARE
N150	150 kg NITROGEN PER HECTARE
$NaNO_3$	SODIUM NITRATE
NH_3	AMMONIA
NH_4^+	AMMONIUM
NH_4NO_3	AMMONIUM NITRATE
NH_2OH	HYDROXYLAMINE
$^{15}NH_4\ NO_3$	SINGLE-LABELLED AMMONIUM NITRATE
NO	NITRIC OXIDE
NO_2^-	NITRITE
NO_3^-	NITRATE
NOB	NITRITE-OXIDIZING BACTERIA
$KMnO_4$	POTASSIUM PERMANGANATE
ppb_V	PARTS PER BILLION BY VOLUME
SOC	SOIL ORGANIC CARBON
SOM	SOIL ORGANIC MATTER
SWC	SOIL WATER CONTENT
Tg	TERAGRAM
T_s	SOIL TEMPERATURE
WFPS	WATER-FILLED PORE SPACE
WRB	WORLD REFERENCE BASE FOR SOIL RESOURCES

1. INTRODUCTION

1.1. Foreword

Globally, agricultural soils constitute an important source of greenhouse gases (GHGs), therefore it is of crucial importance to develop a better understanding of the source and sink activities of agricultural systems (Oertel *et al.*, 2016). Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are important climate-relevant trace gases (Oertel *et al.*, 2016). Nitrous oxide acts to deplete stratospheric ozone and also acts as a GHG: it has a global warming potential being 306 times greater than that of CO_2 persisting in the atmosphere for around 100 years on average CO_2 (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) which is about 20% higher than its pre-industrial value (IPCC, 2014). According to the estimates, 87.2% of N_2O emissions has originated mainly from animal waste management and agricultural soils (Cerri *et al.*, 2009) with 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, 2017a).

Based on global population growth rates, fertilizer use is likely to be amplified and business-as-usual scenarios even project an 18% increase in N_2O emissions by 2030 (Reay *et al.*, 2012). That means that the global N demand is estimated to increase by $\sim$1.8 Tg year^{-1} (FAO, 2017). As a result, there is an urgent need for comprehensive research to evaluate the potential reductions in N_2O emissions that may be achieved through appropriate management practices for increasing cropland nitrogen use efficiency and reducing N_2O emissions (Ussiri and Lal, 2012).

N_2O is produced through the processes of nitrification, denitrification, dissimilatory nitrate reduction to ammonium and chemo-denitrification (Stevens and Laughlin, 1998), and others. However, due to the different processes of production and consumption in the soil, soil N_2O fluxes can be bi-directional (Flechard *et al.*, 2005).

The reviews of the biological pathways for N_2O production show that all microorganisms involved in the catabolic branch of the N-cycle could contribute to N_2O production (Schreiber *et al.*, 2012). In spite of the complex and multiple ways of N_2O formation, nitrification (including nitrifier denitrification and ammonia oxidation) and heterotrophic denitrification are assumed to be the key predominant sources of the N_2O emissions from soil ecosystems (Zhu, Burger, Doane, *et al.*, 2013). Nitrification, as an aerobic process, controlled by ammonium and oxygen

concentrations, and by certain bacteria such as *Nitrosomonas*, *Nitrosolobus*, *Nitrosovibrio* genus (Singh and Tyagi, 2009), nitrification 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 (Ananyeva *et al.*, 2015) and is performed by denitrifying bacteria through a series of steps catalyzed by intracellular enzymes including nitrate reductase, nitrite reductase and nitric oxide reductase (Saggar *et al.*, 2013). The optimal conditions for denitrification include soil with a high proportion of water-filled pore space (WFPS), with sufficient NO_3^- and available carbon (C) sources (Shelton, Sadeghi and McCarty, 2000). 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 catalyzed by nitrous oxide reductase (Putz *et al.*, 2018) and induced by anoxic environment, low NO_3^- availability and low soil temperature (Flechard *et al.*, 2005).

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 (C) and nitrogen (N) content (Hayakawa *et al.*, 2009), microbial community (Graf *et al.*, 2016), vegetation type (Pilegaard *et al.*, 2006), soil acidity and soil temperature (Vor *et al.*, 2003), soil water content and more specifically WFPS which represents a key indicator of oxygen availability in soils and has an important effect on N_2O emissions influencing both nitrification and denitrification processes (Butterbach-Bahl *et al.*, 2013).

All of those factors regulating gas production processes and emissions may be affected by the type, intensity and timing of different management practices such as tillage (Chirinda *et al.*, 2010), fertilization (Allen *et al.*, 2010), and irrigation (Franco-Luesma *et al.*, 2020).

Numerous studies reported that nitrogen fertilizer rates positively influenced N_2O emissions which could be described by linear or exponential relationships (Hoben *et al.*, 2011; Kim, Hernandez-Ramirez and Giltrap, 2013) but growing crops could also have an effect on emission rates.

These findings show that there is considerable variability regarding the effects of different biotic and abiotic factors controlling the N_2O emission from agricultural soils resulting in higher uncertainty of soil N_2O emission estimations. Therefore, 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 (Monni, Perälä and Regina, 2007) including large spatial (Jungkunst *et al.*, 2008) and temporal (Konda *et al.*, 2010) 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 (Loreau and de Mazancourt, 2013).

1.2. Objectives

Accurate quantification of nitrous oxide and greenhouse gas (GHG) emissions is of primary importance to climate scientists. Although spatial heterogeneity and temporal dynamics of emission patterns have been widely studied being the potential cause of the uncertainty in N_2O emission estimates (Fóti *et al.*, 2018; Tian *et al.*, 2019), we still have knowledge gaps in the GHGs quantification. Current national estimates of GHG emissions are still highly uncertain (Butterbach-Bahl *et al.*, 2013) due to the lack of integrable measured datasets and the variability of the measured emission rates. Better quantification of the N_2O emission based on intensive measurements (long study period) could help to understand the agricultural N_2O emissions, especially in East-Central Europe due to the lack of studies available on croplands.

As croplands are the most common agricultural land-use in Hungary, covering more than 50% of the country's territory, the aim of the present study is 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 combined long term field experiment (2 years) conducted in a conventional management system and laboratory experiments performed under different emission drivers. We focused on the key variables controlling N_2O emissions i.e. temperature, soil WFPS, N fertilizer application, plant growth, and carbon source.

A hypothesis was formulated that N_2O emission from cropland soil might be controlled by soil moisture, N fertilizer, temperature, carbon sources, and plant presence. Field N_2O emission measurements in a conventional management system were combined with pot experiments on N_2O emission under specific conditions to determine how the N_2O emission was influenced by these drivers.

2. LITERATURE REVIEW

2.1. Greenhouse gases and climate change

Among the GHGs there are some which do not occur naturally in the atmosphere. Those are artificial compounds including hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF_6). But, the most important GHGs occur naturally in the atmosphere and are responsible for the natural greenhouse effect making life possible on Earth (Le Treut, 2007). Their presence accounts for less than 1% of the total volume of dry air in the atmosphere, and are known as trace gases, but they are the most important forcing factors of climate change (Ussiri and Lal, 2012; Moron, 2014), characterized by their permeability to short wave radiation from the Sun, but in contrast, they are impermeable to longwave radiation from the earth (Ussiri and Lal, 2012). Because GHGs absorb infrared radiation, therefore, such change in their atmospheric concentration alters the energy balance of the climate system, where an increase in atmospheric GHGs concentrations produces a net increase in absorption of energy of the Earth, leading to the warming of Earth's surface (Ussiri and Lal, 2012). This is why they play important role in Earths'energy budget by absorbing and re-emitting infrared radiation emitted by Earth's surface, preventing it from escaping to space in order to stabilize the heating of Earth's atmosphere and surface, thus, global warming (Kweku *et al.*, 2018).

Among these natural gases causing the greenhouse effect, water vapor, carbon dioxide, methane, and nitrous oxide, which all perform as effective global insulators (Met Office Hadley Centre, 2011). Although water vapor is the main GHG in the atmosphere, it is not very affected by human activities (Forster *et al.*, 2007), while CO_2, CH_4 and N_2O are greatly influenced by them (Signor and Cerri, 2013). Thus, these three gases are considered the most important ones related to the greenhouse effect (Signor and Cerri, 2013). Since the 1980s, a scientific consensus has proved that the natural greenhouse effect had intensified due to human activities, which set in motion a global warming trend by increasing the concentration of GHGs in the atmosphere (Houghton, 2001; EPA, 2007; Solomon *et al.*, 2007). During the past few decades, atmospheric concentrations of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) have been increasing at rates of 0.8, 0.5, and 0.3% year^{-1}, respectively (Wang *et al.*, 2013) which have been implicated with global climate change (Solomon *et al.*, 2007). For example, CO_2 in the atmosphere has increased from about 280 ppm in the pre-industrial era (1750) (Moron, 2014; Blasing, 2016) to the current 407.8 ± 0.1 ppm (World Meteorological Organization and Global Atmosphere Watch, 2019). Similarly, concentrations of CH_4 and N_2O have increased from 722 and 270 ppb in the pre-industrial era (Snyder *et al.*, 2009; Blasing, 2016) to current levels of 1869 ± 2 and 331.1

± 0.1 ppb, respectively (World Meteorological Organization and Global Atmosphere Watch, 2019). In spite of the lower atmospheric concentration of CH_4 and N_2O compared to the CO_2, they each contribute to the atmospheric anthropogenic greenhouse effect in relation to their concentrations in the atmosphere, about 15%, and 6% for methane and N_2O, respectively due to the global warming potential 23 times (CH_4) and 306 times (N_2O) that of CO_2 on a 100-year timescale (Ussiri and Lal, 2012; World Meteorological Organization and Global Atmosphere Watch, 2019).

The atmospheric concentration of CO_2 has increased mostly due to fossil fuel use of power generation and transportation, deforestation, and accelerated processes of organic matter decomposition (Cheng and Johnson, 1998; Yoro and Daramola, 2020). While for the CH_4, its concentration was increased mainly due to agriculture (rice and livestock farming), coal mining, oil and gas production and distribution; biomass combustion; and municipal landfills (Flores-Jiménez *et al.*, 2019; Turner, Frankenberg and Kort, 2019), N_2O concentration has increased mainly as a result of agricultural soil management and N fertilizer use, also livestock waste management, mobile, and stationary fossil fuel, combustion, and industrial processes contribute to the N_2O emission, besides soils and oceans also emit N_2O naturally (Syakila and Kroeze, 2011; Uchida and von Rein, 2018). Therefore, an alteration in the chemical composition of the global atmosphere was caused by anthropogenic activities (Crutzen and Lelieveld, 2001). And it is predicted that changes in the concentration of trace gases will have a dramatic influence on the habitability of the earth, like; food insecurity, and destruction of the stratospheric ozone layer. According to the models, the Earth's surface is likely to warm by 3-5°C for the next century with the current trends (Le Treut, 2007). Such warming would have adverse impacts on ecosystems because ecosystems will not be able to adjust to such rapid temperature changes (Ussiri and Lal, 2012).

Among all sources, soils are major sources of atmospheric GHGs (Deng *et al.*, 2020), with the main share (37%; especially of N_2O and CH_4) of agricultural emissions (Tubiello *et al.*, 2015). Agriculture and associated land-use change remain a source when considering all three major biogenic GHGs (Paustian *et al.*, 2016). Where 25% of the contribution of total global anthropogenic GHG emissions was from land-use: 10-14% directly from agricultural production, especially via livestock management and GHG emissions from soils, and another 12-17% from land cover change, including deforestation (Smith *et al.*, 2014; Tubiello *et al.*, 2015).

Recently, agriculture greenhouse gas (GHG) emissions have received much attention (Wysocka-Czubaszek *et al.*, 2018) because of the worldwide GHG reduction policy and predicted growing food demand in following decades, caused by an increase in population and which

probably reach 9.8 billion in 2050 (World Population Prospects The 2017 Revision, 2017). Based on the Annual Greenhouse Gas Inventory of the European Union from 1990 to 2018 and the Inventory Report 2020, the total GHG emissions (excluding LULUCF) in 2018 reach 4234 Mt CO_2 equivalent, where total emissions from agriculture were 436 Mt CO_2-eq with contributions of CH_4, N_2O, and CO_2 of 55%, 42.6% and 2.4% of total agricultural emissions, with 240 Mt CO_2-eq, 186 Mt CO_2-eq, and 10.6 Mt CO_2-eq, respectively (EEA, 2020).

Belowground gas fluxes of CO_2, methane (CH_4), and N_2O are the result of a variety of (micro)biotic processes (Kuzyakov and Blagodatskaya, 2015): CO_2 is produced by soil respiration including root, faunal, and microbial respiration; (Rastogi, Singh and Pathak, 2002; Vargas *et al.*, 2020), CH_4 through methanogenesis (Dutaur and Verchot, 2007), while N_2O is produced by a combination of microbial transformation processes, mostly denitrification, as well as nitrification and nitrifier-denitrification (Opdyke, Ostrom and Ostrom, 2009; Wrage-Mönnig *et al.*, 2018). Their emissions from soils are the result of complex production, consumption, and transport processes, and are affected by a wide range of environmental and management factors (Wang *et al.*, 2013), also their production and consumption in soils are related to microbiological processes where microorganisms and their controlling factors are very important (Chen, Tam and Ye, 2010). Hence, the microbial activities are controlled by environmental conditions, including temperature, rainfall, and soil biological, chemical, and physical characteristics (Wang *et al.*, 2013). As a result, emissions of GHGs from soils have been related to climate, management activities (e.g. soil cultivation, irrigation, fertilizer application), and various soil characteristics, e.g. soil organic carbon and nitrogen contents, dissolved organic C and N contents, mineral N contents, soil bulk density, salinity and redox potential (Huang, Yu and Gambrell, 2009; Ogle *et al.*, 2014). However, the relationships between GHGs emissions and the different driving factors are often confused due to the spatio-temporal variations in emissions, in part because of complex interactions between GHGs productions-consumptions-transports in the soil profile (Panikov, Mastepanov and Christensen, 2007). This makes soil GHG emissions a key topic in global change issues, climate research, agriculture, and management (Oertel *et al.*, 2016).

Therefore, GHG emissions from soils need to be better quantified for global budgets (Oertel *et al.*, 2016), in the hope of reducing GHG emissions to the atmosphere because there is an urgent need to mitigate the adverse impacts of climate change. Specifically, the close relationship between soil-derived greenhouse gas (GHG) emissions and soil processes such as biogeochemical cycling of C and N, that can either increase or decrease the initial climate forcing (Crowther *et al.*, 2015; Van Nes *et al.*, 2015).

2.2. The role of nitrous oxide in climate change

Nitrous oxide (N_2O) is a colorless gas of slightly sweet odor and taste under ambient conditions. It was discovered by Joseph Priestly in 1772 (Gillman, 2019), while its first presence in the atmosphere has been known since 1939 (Adel, 1939). However, its importance to the global environment was only realized in the early 1970s when atmospheric scientists hypothesized that N_2O released into the atmosphere through denitrification of nitrates in soil and waters triggers reactions in the stratosphere that may lead to the destruction of the ozone layer, which in turn protects the earth from biologically harmful ultraviolet (UV) radiations from the Sun (Crutzen, 1970, 1972, 1974; Ussiri and Lal, 2012). Later it was classified as an important greenhouse gas (GHG) that could modify the radiation energy balance of the earth-atmosphere system based on the investigations of its radiative properties (Wang *et al.*, 1976; Ramanathan *et al.*, 1985).

Nitrous oxide is present in the atmosphere at a considerably low concentration (1200-fold lower than CO_2). In spite of its very small concentration in the atmosphere, its contribution to global warming makes it an important long-lived greenhouse gas (121 years), also it have a high global warming potential (GWP), 306 times higher than CO_2 on a 100-year timescale (World Meteorological Organization and Global Atmosphere Watch, 2019), with an estimated contribution to the global warming of 6% (Butterbach-Bahl *et al.*, 2013; Ciais *et al.*, 2014; Nie *et al.*, 2016; IPCC, 2014). For this reason, its emission has a long-term influence on climate, since, it becomes well mixed throughout the atmosphere much faster than it is removed (Solomon *et al.*, 2007). In addition to its potential global warming as mentioned Ussiri and Lal (2012), this trace gas also plays important role in the stratosphere chemistry which was stimulated the interests in atmospheric chemistry of N_2O, when the photochemical degradation of N_2O in the stratosphere leads to ozone-depleting nitric oxide (NO), nitrogen dioxide (NO_2), and to other important free radical reservoir species (e.g., HNO_3) (Crutzen and Schmailzl, 1983; Montzka *et al.*, 2011). In the current atmosphere, because of the large historic emissions and long lifetimes of the CFCs, it leads to much more ozone depletion than does N_2O , but it is expected to decrease more in the future because the CFCs is now declining with the implementation of the 1989 Montreal Protocol (Hartmann *et al.*, 2013; Rigby *et al.*, 2013), whereas the N_2O is increasing. Owing to the decline in chlorofluorocarbons (CFCs) emission, it is probable that N_2O will become the dominant ozone-depleting substance in Earth's atmosphere in the twenty-first century (Ravishankara, Daniel and Portmann, 2009). These characteristics, in combination with its increasing concentration in the atmosphere, make the N_2O an important factor in the global climate system and atmospheric chemistry and as consequence, it has attracted much attention in the last decades (Ravishankara, Daniel and Portmann, 2009; Nadeem *et al.*, 2012).

The pre-industrial source of N_2O is estimated at 11 (8-13) Tg N_2O-N $year^{-1}$ (Ehhalt *et al.*, 2001; Ruddiman, 2010), Pre-agricultural N_2O emission from soils was 6-7 Tg N_2O-N $year^{-1}$ (Bouwman *et al.*, 1993), 3-4 Tg N_2O-N $year^{-1}$ from deep oceans (Nevison, Weiss and Erickson III, 1995) and other aquatic and atmospheric deposition sources contributed <1.0 Tg N_2O-N $year^{-1}$ (Seitzinger and Kroeze, 1998). Hence, roughly one-third of the pre-industrial N_2O sources are attributed to the oceans and about two thirds to soil (Smithson, 2001).

Earlier studies showed an increase in the N_2O concentration since the beginning of the industrial era (MacFarling Meure *et al.*, 2006), which was also recorded by the ice-core measurements indicating a relative stability of the N_2O mixing ratio at about 270 ppbv (270 nmol mol^{-1}), over thousands of years until the beginning of the industrial era (Prather, Holmes and Hsu, 2012). The mixing ratio exceeded 280 ppbv for the first time in 1905; it reached 300 ppbv by the mid-1970s; and it has continued to increase steadily since, reaching a global average of 322 ppbv in 2010 and 328 ppbv in 2016 (Blasing, 2016), (Figure 1 showed the changes in atmospheric N_2O concentration based on the data of the Advanced Global Atmospheric Gases Experiment (AGAGE), which represent different concentrations than which were reported by Blasing (2016), but the same trend was observed, differences were too small), to reach a mixing ratio of 331.1 ± 0.1 ppb in 2018 (World Meteorological Organization and Global Atmosphere Watch, 2019), which is by 1.2 ppb higher as compared with 2017 and by 123% higher as compared with the pre-industrial period (270 ppb) (Kudeyarov, 2020). The rise of the concentration is accelerating, the fastest increases in the atmospheric N_2O concentration were seen in the recent 10 years with an average of 0.95 ppb/year (World Meteorological Organization and Global Atmosphere Watch, 2019).

There is a consensus in the science that human activities have increased the concentration of GHGs in the atmosphere causing the intensification of the natural greenhouse effect and set in motion a global warming trend (Smithson, 2001; Solomon *et al.*, 2007). The generally accepted explanation for the increase in the atmospheric mixing ratio of N_2O since the nineteenth century is the increase in the emission from sources related to human activity (Smith, 2017). For now, the anthropogenic N_2O emissions compared with their estimated level in 1900 are greater by a factor of eight (Smith, 2017). This increase is due mainly to the increasing use of nitrogen fertilizers applied to agricultural soils caused by the agriculture expansion (Hartmann *et al.*, 2013), especially since the invention of the Haber-Bosch process in the early 20^{th} century (Gruber and Galloway, 2008). Therefore, global N_2O emissions reach about 17.7 Tg of N per year (Denman *et al.*, 2007), and microbial processes in soils and aquatic ecosystems are responsible for ~89% of its annual

contribution, where more than 90% of N_2O content in the atmosphere are from the biological sources of the earth's surface (Nie *et al.*, 2016).

Beside the emission processes, the photolytic reactions in the stratosphere are the only known sinks for the atmospheric N_2O is estimated to remove approximately 13.5 (12.4-14.6) Tg N yr^{-1} (Tian *et al.*, 2020).

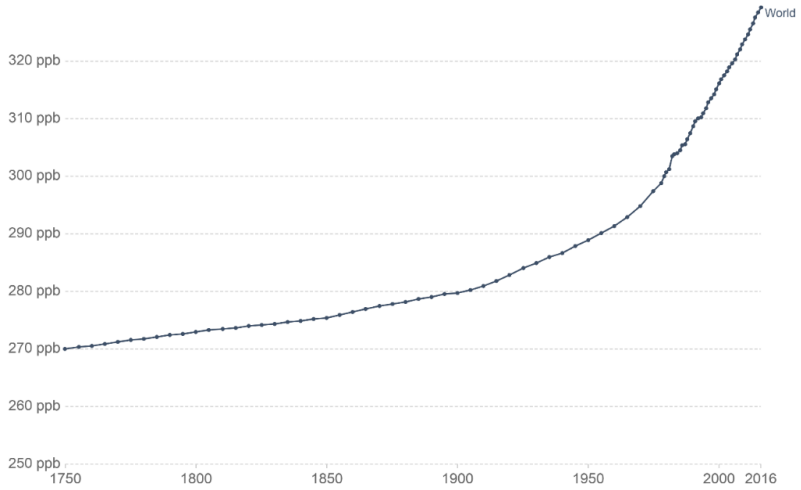

Figure 1. Trend in the global annual averaged atmospheric mixing ratio of nitrous oxide (N_2O) in parts per billion (ppb), between 1750 and 2016.

European Environment Agency (EEA). Original data is derived from the Advanced Global Atmospheric Gases Experiment (AGAGE), available at: https://www.eea.europa.eu/data-and-maps/data/external/agage-measurements.

2.3. Nitrogen Cycle

To a great extent, the N cycle of the Earth can be described as a network of oxidation-reduction reactions mediated by plants, animals, fungi, bacteria, and archaea, which are essential to maintaining the balance between reduced and oxidized forms of N in the ecosystems (Coskun *et al.*, 2017) (Figure 2).

On the other hand, anthropogenic disturbance of the biogeochemical cycles is perhaps today's greatest environmental challenge, where N-cycling is one of the most profoundly affected (Bakken and Dörsch, 2007). Human activities are the biggest contributor of nitrogen and have a significant impact on the nitrogen cycle nowadays (Ghaly and Ramakrishnan, 2015), through the industrial production of reduced-N fertilizer using the Haber-Bosch process, the fixation of N_2 by cultivated legumes, and the combustion of fuels, which now result in more fixed nitrogen per year than all natural processes combined (Fowler *et al.*, 2013). In particular the use of synthetic nitrogen (N) fertilizer, have doubled global annual reactive N inputs in the past 50-100 years, causing

deleterious effects on the environment through increased N leaching and nitrous oxide and ammonia emissions (Qiao *et al.*, 2015).

Atmospheric dinitrogen gas which represents the largest pool of N in the biosphere enters the living world naturally via biological N_2 fixation by diazotrophic prokaryotes as well as geochemically, e.g., via lightning, but it is not directly available to most organisms (Vitousek *et al.*, 2013).

Organic N depolymerization (N mineralization in the soil) which conducts the production of inorganic NH_3/NH_4^+ is carried out both under aerobic and anaerobic conditions (Schimel and Schaeffer, 2012). Contrary, the oxidation and reduction of inorganic N are relatively tight processes: nitrification which is responsible for NH_4^+ oxidation by soil microbes producing hydroxylamine, nitrite, and nitrate and a reverse process, and denitrification, involving the reduction of NO_3^- to NO_2^-, nitric oxide, nitrous oxide, and finally back to N_2, are largely restricted to aerobic and anaerobic environments, respectively (Coskun *et al.*, 2017; Zhu, Castellano and Yang, 2018). Added to that, another important pathway of N loss can occur in dry and hot conditions in soils with high pH and where NH_4^+ has accumulated in the surface being responsible for the NH_3 release (Tian *et al.*, 2018). Other reactions that participate in terrestrial N cycling include dissimilatory nitrate reduction to ammonia (DNRA), and anammox which is the formation of N_2, through the direct oxidation of NH_4^+ and nitrite (NO_2^-) under anoxic conditions. In agricultural soils still little information is documented about anammox microbial community structure (Zhou *et al.*, 2017).

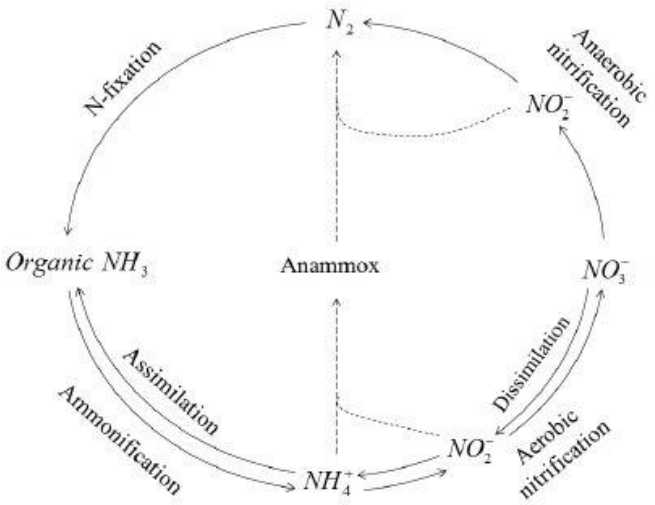

Figure 2. Simplified N cycle showing the major processes that can take place in the soil (Trimmer, Nicholls and Deflandre, 2003).

2.4. Nitrous oxide sources

Globally, N_2O share about 6% of total GHG emissions (Olivier, Schure and Peters, 2017), it can be produced from natural sources such as uncultivated soils, oceans, wetlands, and other aquatic systems, when soils and oceans represent the largest sources, or anthropogenic sources such as agriculture, combustion of fossil fuel, adipic acid and nitric acid production, and biomass burning (US Environmental Protection Agency, 2010; Syakila and Kroeze, 2011; Harter *et al.*, 2014; Dencső *et al.*, 2021). On the other hand, some sources can be related to both natural and anthropogenic processes, such as riparian zones, rivers, estuaries, and continental shelves, which may be polluted by agricultural runoff and drainage, and forest and grassland fires which can be human-initiated (e.g. land clearing) or by lightning ignition (Ussiri and Lal, 2012).

Recently, the global N_2O emissions based on bottom-up and top-down estimates (Tian *et al.*, 2020): were 17.0 (minimum-maximum estimates: 12.2-23.5) Tg of nitrogen per year and 16.9 (15.9-17.7) Tg of nitrogen per year, respectively, between 2007 and 2016. While global human-induced emissions, which are dominated by nitrogen additions to croplands, increased by 30% over the past four decades to 7.3 (4.2-11.4) Tg of nitrogen per year (Tian *et al.*, 2020).

2.4.1 Natural N_2O sources

2.4.1.1 Soils under natural vegetation

Estimates of the global total emission from soils under natural vegetation vary from 3.3 to 9.9 Tg N yr^{-1} (Xu-Ri *et al.*, 2019). This amount is similar to the sum of all anthropogenic sources, including agriculture (Ciais *et al.*, 2014). However, some other global budgets of N_2O emissions from natural sources based on both bottom-up modeling approaches have been established (Tian *et al.*, 2020), where they estimate the natural soil flux at 5.6 (4.9-6.5) Tg N yr^{-1} in the decade between 2007 and 2016. Some microbiological, chemical, physical, and environmental parameters that determine N_2O emissions create complex interactions that make extrapolating global emissions budgets difficult and uncertain, but the publication of the IPCC fourth assessment report has helped to add some improvements in N_2O budgets due to the increased number of new measurements from natural soils but still increased the number of field measurement is needed for better comprehensive estimates because there is still a lack in the in many vegetation types (Ussiri and Lal, 2012).

2.4.1.2 Aquatic nitrous oxide sources

The emission from aquatic ecosystems involve; marine and freshwater sources which including oceans, estuaries, rivers, and lakes, N_2O emissions from aquatic ecosystems were enhanced by the increased N availability which caused an unintended environmental consequence

(Ussiri and Lal, 2012). For example, for the ocean representing an important source of N_2O (Thomson *et al.*, 2012), a flux of 3.4 (2.5-4.3) Tg N yr^{-1} was estimated by Tian *et al.* (2020) during their bottom-up estimates in the decade between 2007 and 2016. Previously, Duce *et al.* (2008) concluded that the deep oceans are also a source of anthropogenic N_2O, Where N_2O formation in the deep ocean can be enhanced by atmospheric deposition of nitrogen compounds, in particular nitrogen oxides, and this nitrogen deposition is partly from fossil fuel combustion (Syakila and Kroeze, 2011). Concerning the dominant N_2O formation pathway; water column nitrification during subsurface oxidation of organic matter is widely accepted as the main source for the majority of the open oceanic N_2O emissions (Ussiri and Lal, 2012). Also Freing, Wallace and Bange (2012) estimated that oceanic N_2O production is dominated by nitrification with a contribution of only approximately 7 percent from denitrification, indicating that previously used approaches may have overestimated the contribution from denitrification.

However, few studies provided information on the N_2O yield in other aquatic ecosystems due to the measurement difficulties of N_2O emission in aquatic environments (Ussiri and Lal, 2012).

2.4.1.3 Wetlands

Wetlands are minor contributors to global N_2O emissions (Bouwman *et al.*, 1993). Major factors that control production and emission of N_2O emission in wetlands include organic inputs and water level, which determine the balance between aerobic and anaerobic soil environments (Ussiri and Lal, 2012). Lu and Xu (2014) recorded that both temperature rise and exogenous organic matter inputs increased N_2O emission rates and cumulative amount from wetland soil. Added to that Chapuis-Lardy *et al.* (2007) suggested that low availability of NO_3^- and conditions in soils that slow diffusion, such as the water-saturation of wetlands may promote N_2O consumption. Audet *et al.* (2014) recorded low and deposition rates in relatively preserved natural wetlands. Therefore, more studies are needed on the wetlands acting as the source or sink for N_2O emission, because the comprehensive understanding of the processes is still limited.

2.4.2 Anthropogenic sources

Many different sources can be accounted as anthropogenic sources, but microbial nitrification and denitrification from agricultural soils, fossil fuel combustion, and biomass burning are the most important anthropogenic sources of N_2O (Ussiri and Lal, 2012). Besides, industrial processes of adipic acid and nitric acid production produces N_2O as a byproduct and represent a major contributor to the emission. Adipic acid produced is used in the production of nylon. Recently, Tian *et al.* (2020) reported that the anthropogenic sources contributed, on average 43% to the total N_2O emission (mean: 7.3; min-max: 4.2-11.4 Tg N yr^{-1}), of which direct and

indirect emissions from nitrogen additions in agriculture and other sectors contributed around 52% and around 18%, respectively. The remaining anthropogenic emissions (about 27%) were originated from other direct anthropogenic sources including fossil fuel and industry (around 13%) (Tian *et al.*, 2020).

2.4.2.1 Agriculture

Agricultural soils represent an important source of nitrous oxide (Guenet *et al.*, 2021), mainly generated directly from inorganic and organic forms of N added to soils as fertilizers, manures, and composts, some of the inorganic N added to soils as fertilizers undergo microbial nitrification and denitrification processes in soils, releasing N_2O to the atmosphere, some additional N_2O may arise through biological N fixation, manures in animal housing and storage, urine and feces deposited onto soils during animal grazing (Rochette *et al.*, 2008; Ussiri and Lal, 2012). Where most of the emitted N_2O emissions from agricultural soils are the result of nitrification and denitrification of mineral N following the application of synthetic fertilizers and organic amendments (Charles *et al.*, 2017). Therefore, bottom-up models have used N fertilizer input as the sole predictor to estimate agricultural N_2O inventories, using an emission factor (Shcherbak, Millar and Robertson, 2014).

Globally, agriculture including direct and indirect N_2O emissions accounted for about 75% of total N_2O emissions, where manure in pastures, rangelands and paddocks and synthetic fertilizers represents the main sources of N_2O emissions in 2016 for about (22% and 18%, respectively) (Olivier, Schure and Peters, 2017). While in Hungary in 2018, 87 per cent of total N_2O emissions were generated in agriculture (Kis-Kovács *et al.*, 2020) (Figure 3).

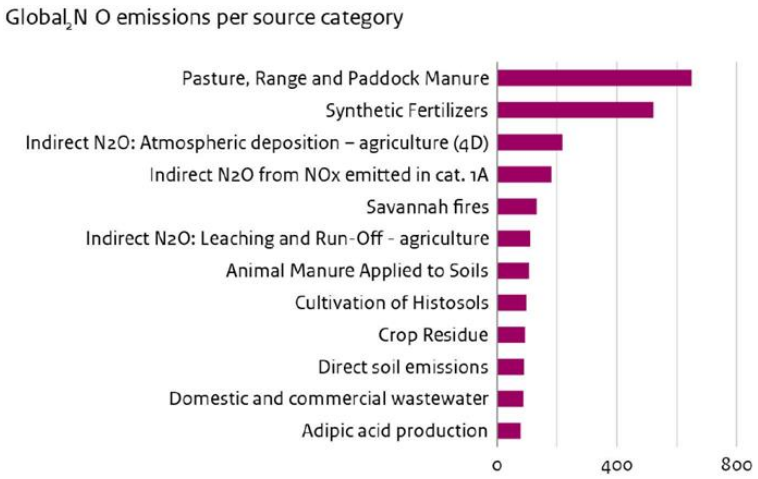

Figure 3. Top 12 sources of global nitrous oxide emission (megatonnes CO_2 equivalents) (1A: Public Electricity Generation, fossil fuel combustion (other), international air transport and international marine transport (bunkers)) (Olivier, Schure and Peters, 2017).

N_2O emission from agricultural soils is a very important subject not only because of its direct effects but due to its indirect effect, agricultural nitrogen (N) leaching and runoff in water bodies which contribute significantly to the global atmospheric N_2O budget, and which is also the largest source of uncertainty in the bottom-up inventory (Turner *et al.*, 2015; Tian, Cai and Akiyama, 2019). And since a considerable amount of nitrogen can be leached from agricultural fields to aquatic systems (Syakila and Kroeze, 2011), Kroeze and Seitzinger (1998) suggested that N_2O emissions from rivers, estuaries, and continental shelves may increase from 1.9 Tg N_2O-N in 1990 to 4.9 Tg N_2O-N in 2050 mainly due to an increase in fertilizer use to feed a growing world population, which is also expected to increase from 105.6 Tg N in 2009 to >135 Tg N in 2030 (FAO, 2011). Generally, the amounts of the emitted N_2O increase exponentially with increasing nitrogen inputs, for every 1000 kg of applied nitrogen fertilizers, it is estimated that around 10-50 kg of nitrogen will be lost as N_2O from soil (Shcherbak, Millar and Robertson, 2014), natural increases in N_2O emission are also expected, with a doubling of anthropogenic N_2O emissions by 2050 as it was reported by Davidson and Kanter (2014), also it is expected that agricultural soils will contribute up to 59% of total N_2O emissions in 2030 (Hu, Chen and He, 2015).

On the basis of bottom-up approaches, anthropogenic N_2O emissions increased from 5.6 (3.6-8.7) Tg N yr^{-1} in the 1980s to 7.3 (4.2-11.4) Tg N yr^{-1} in 2007-2016, at a rate of 0.6 ± 0.2 Tg N yr^{-1} per decade ($P < 0.05$). Up to 87% of this increase resulted from direct emission from agriculture (71%) and indirect emission from anthropogenic nitrogen additions into soils (16%) (Tian *et al.*, 2020).

That's why a comprehensive assessment of soil N_2O emissions is of paramount importance, especially the emissions from agricultural soils, because it's a major aspect of the global N cycle and it represents a key contribution of modern agriculture which, in turn, poses a serious threat to agriculture itself. Moreover it is also important to understand climate-ecosystem interactions and the effect of climate change (Paustian *et al.*, 2016; Tian *et al.*, 2019). Understanding the roles of the different drivers controlling the emissions is crucial for adopting the most appropriate agricultural management in order to meet the growing food demand together with high requirements of environmental protection.

2.4.2.2 Other anthropogenic sources

Other anthropogenic emissions of N_2O are associated with biomass burning, fossil fuel combustion involving different byproducts, such as nitric oxide (NO) and hydrogen cyanide (HCN), and industrial processes of synthesis of adipic acid and nitric acid (HNO_3) producing N_2O

as a byproduct of adipic and nitric acids, also N_2O sources include sewage and wastewater treatment which produces N_2O by nitrification and denitrification of N present in the form of urea, NH_4^+ and NO_3^- (Wargadalam *et al.*, 2000; Ussiri and Lal, 2012; UNEP, 2013). The IPCC assigns 2 Tg N year^{-1} to industrial, energy generation, and biomass burning processes but still, the level of uncertainty is large enough and those 2 Tg N year^{-1} are presented within a range of 0.7-3.7 Tg N year^{-1} (Colorado, McDonell and Samuelsen, 2017).

Recently Tian *et al.* (2020) reported that the contribution from fossil fuel combustion and industrial emissions decreased rapidly between 1980 and 2000, largely due to the installation of emissions-abatement equipment in industrial facilities that produce nitric and adipic acid. Added to those, the tropical land conversion constitutes a source because accelerated decomposition and mineralization of litter, root material, and SOM in the first few years after forest clearing may cause a pulse of N_2O emissions, except in older clearing (more than 10 year old) (Ussiri and Lal, 2012).

2.5. N_2O formation pathways and uptake

Soils can act both as a source and a sink of N_2O (Syakila and Kroeze, 2011). However, on the global scale, the source activity largely dominates the sink one (Hénault *et al.*, 2012). Various microbial metabolic pathways and abiotic processes for the formation of N_2O exist (Weller *et al.*, 2019). The multiple pathways of N_2O production and consumption include nitrification including ammonia (hydroxylamine) oxidation, heterotrophic denitrification, nitrifier denitrification, dissimilatory nitrate reduction to ammonium (DNRA, or nitrate ammonification), anaerobic ammonium oxidation (anammox) and chemodenitrification, with each process modulated by specialized groups of microbial assemblages (Figure 4).

2.5.1 Overview of the major biological pathways for N_2O emissions

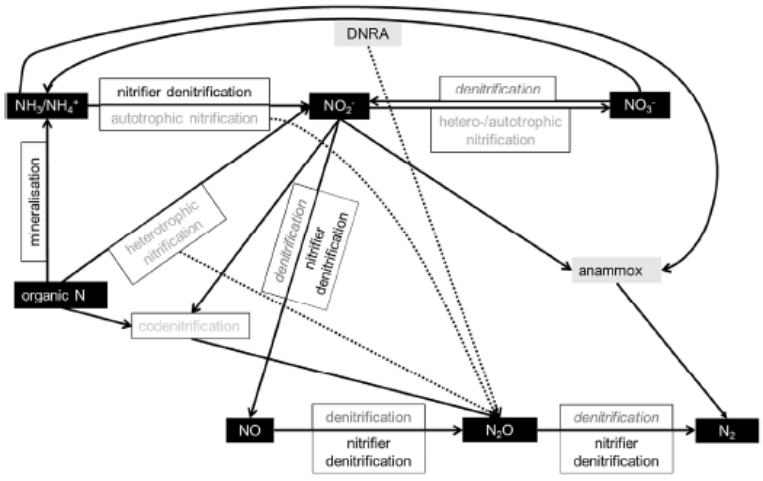

Figure 4. Soil processes and pathways Pathways responsible for N_2O production. Black boxes show N pools, other boxes identify microbial pathways; here, different text style and colour represent different pathways. Note that the pools are not uniform. For example the NO_2^- pool consists of at least three individual pools (NO_2^- from denitrification in anoxic microsites, NO_2^- from nitrification in microoxic/oxic microsites, NO_2^- from heterotrophic nitrification in oxic microsites associated with the presence of organic N and a specific microbial community). Arrows with solid lines show pathways, dotted lines show the production of possible byproducts. DNRA: dissimilatory nitrate reduction to ammonia, also known as respiratory ammonification (Wrage-Mönnig *et al.*, 2018).

2.5.1.1 Nitrification

Nitrification is the aerobic oxidation, can be either complete or shared between different microorganisms, in which ammonia is oxidized to nitrate via nitrite with each step performed by a specialized group of prokaryotes generally belong to *Nitrosomonas* and to *Nitrobacter*, The majority of bacteria involved are autotrophs and use CO_2 as a source of carbon (Hu, Chen and He, 2015; Beeckman, Motte and Beeckman, 2018; Velthof, 2018).

The first step is ammonia oxidation ($NH_3 \rightarrow NH_2OH/HNO \rightarrow NO_2^-$), performed by the amoA gene encoding the ammonia monooxygenase (AMO) enzyme, is known to be catalyzed by microorganisms termed ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) (Purkhold *et al.*, 2000; Brochier-Armanet *et al.*, 2008), as well as newly discovered taxa called comammox belonging to the *Nitrospira* lineage II (Daims *et al.*, 2015; Van Kessel *et al.*, 2015), contributing to a growing appreciation that nitrifiers are more diverse than originally thought (Duan *et al.*, 2019). This process is followed by oxidation of NO_2^- to NO_3^- catalyzed by nitrite-oxidizing bacteria (NOB); which can convert nitrite to nitrate (Duan *et al.*, 2019). Ammonia oxidation is critical for the production of nitrification-originated N_2O (Hu, Chen and He, 2015), and ammonia oxidizers are considered major contributors to atmospheric N_2O (Wang *et al.*, 2015).

N_2O formation pathway for AOB includes NO_2^- reduction via nitrite reductase (NIR) and nitric oxide reductase (NOR) (Kozlowski, Price and Stein, 2014) and the incomplete oxidation of the intermediate product of nitrification (Caranto, Vilbert and Lancaster, 2016; Velthof, 2018), Hydroxylamine might be oxidized to NO by the hydroxylamine oxidoreductase, possibly through the chemical decomposition (Ritchie and Nicholas, 1972), followed by reduction to N_2O catalyzed by the nitric oxide (Hu, Chen and He, 2015). But the latter process is not completely characterized and is still a subject of debate (Schreiber *et al.*, 2012). Also, N_2O production in AOA mechanism(s) are not fully resolved (Kozlowski et al., 2016), but recent studies on agricultural soil showed that AOA has lower N_2O yields than AOB (Hink, Nicol and Prosser, 2017; Hink *et al.*, 2018).

2.5.1.2 Heterotrophic denitrification

Heterotrophic denitrification is a major microbial respiratory process that serves to the reduction of nitrate (NO_3^-) and NO_2^- to nitric oxide (NO), and nitrous oxide (N_2O), and finally dinitrogen (N_2) under anaerobic conditions (Philippot, Hallin and Schloter, 2007; Hallin *et al.*, 2018). However, heterotrophic denitrification in the presence of O_2 has been also reported in physiological studies of pure denitrifier strains isolated from soils and sediments (Patureau *et al.*, 2000), and could even occur in anaerobic microsites of aerated arid or semiarid soils caused by intensive respiration (Abed *et al.*, 2013).

The process is carried out predominantly by heterotrophic microorganisms being facultative anaerobes that are able to use NO_3^- instead of oxygen as an electron acceptor in respiration (Velthof, 2018). However, any other N oxides (NO_2^-, NO, or N_2O) can also serve as a substrate (Coyne, 2008). Denitrification capacity is distributed among microbial groups in Archaea, Proteobacteria, and eukaryotic fungi (Zumft, 1997).

The process is facilitated by four enzymes systems: nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS) (Zumft, 1997). The first step ($NO_3^- \rightarrow NO_2^-$) is catalyzed by the narG or napA, this step could be carried out by a large proportion of soil microorganisms; the second step ($NO_2^- \rightarrow NO$) is catalyzed by the nirK or nirS genes; the third step leading to N_2O formation ($NO \rightarrow N_2O$) is mediated by the cnorB or qnorB genes encoding the nitric oxide reductase, while as the last step, the reduction of N_2O to N_2 which catalyzed by the nosZ gene encodes for NOS, is the only known microbial process which could reduce N_2O to N_2 in the biosphere which would represent only 0.1% to 5% of the soil bacteria (Philippot and Germon, 2005; Philippot, Hallin and Schloter, 2007; Jones *et al.*, 2013; Tao *et al.*, 2018). The genes encoding NIR and NOS (i.e., nirK/nirS and nosZ, respectively) are frequently used as functional markers to analyze the denitrifier communities (Cui *et al.*, 2016; Azziz *et al.*, 2017; Yang, Zhang and Ju, 2017). Previously, Harter *et al.* (2014) observed that emissions of N_2O were inversely related to nosZ gene expression.

Nearly one-third of nirS or nirK-containing denitrifiers, such as *Agrobacterium tumefaciens* and some strains within the genus Thauera, lack the nosZ gene (Philippot *et al.*, 2011; Bakken *et al.*, 2012), and therefore nitrous oxide reductase ability is absent. As such, N_2O may be formed, but a complete reduction to N_2 cannot occur (Tao *et al.*, 2018).

Fungi could also play vital role as key producers of N_2O via heterotrophic denitrification in a wide variety of soils (Thamdrup, 2012; Matsuoka *et al.*, 2017). The fungal denitrification system comprises a copper-containing nitrite reductase together with cytochrome P450 nitric oxide

reductase to reduce nitrite to N_2O (Shoun *et al.*, 2012). The primary product of fungal denitrification is N_2O because fungi generally lack the nosZ gene to further reduce N_2O to N_2 (Philippot *et al.*, 2011), but their in situ contribution to N_2O has yet to be directly measured (Hu, Chen and He, 2015). Although numerous studies have been carried out, there is still contradictory information related to linkages between N_2O emission and the abundance, diversity, and structure of the wider denitrifier community (Tao *et al.*, 2018). These facts suggest that soil N_2O emissions are highly variable both spatially and temporally, which makes measuring and predicting soil N_2O particularly difficult (Cowan *et al.*, 2014).

2.5.2 Other important sources of soil N_2O production

Apart from the above-mentioned nitrification and heterotrophic denitrification pathways, other microbial sources are also reported to occasionally contribute to N_2O production in soil ecosystems.

2.5.2.1 Nitrifier denitrification

Another N_2O formation route namely nitrifier denitrification, recorded also as nitrification related pathway (Hu, Chen and He, 2015), in this process, NH_3 is oxidized to NO_2^-, followed by reduction of NO_2^- to NO by nitrite reductases and further reduction to N_2O by NO reductases, with the whole process carried out solely by AOB (Hu, Chen and He, 2015). This process may account for up to 100% of nitrous oxide emissions derived from ammonium (NH_4^+) in soils and could be more significant than classical denitrification under some conditions (Wrage-Mönnig *et al.*, 2018). High ammonium concentrations, low organic carbon contents, low O_2 levels, and low pH are conditions under this process seems to be an important source of N_2O, but still not much is known about this mechanism of N_2O production (Wrage *et al.*, 2001; Velthof, 2018).

2.5.2.2 Dissimilatory Nitrate Reduction to Ammonium (DNRA)

Dissimilatory NO_3^- reduction to ammonia (DNRA), also termed nitrate ammonification, is a microbially-mediated pathway of N cycle that transforms NO_3^- first to NO_2^-, and then to NH_4^+, carried out by fermentative organisms (Ussiri and Lal, 2012; Friedl *et al.*, 2018), while the contribution of the produced N_2O by this process to the total N_2O budget is likely marginal since the amounts are small (Stremińska *et al.*, 2012). Concerning DNRA conditions are similar to those for denitrification. However, DNRA is favored by a high ratio of available C, and low oxygen (O_2), and under NO_3^- limiting conditions (Tiedje, 1988), it is also suggested that this process may not be strictly confined to highly reducing and high C:N conditions as traditionally understood (Schmidt, Richardson and Baggs, 2011). Some DNRA-performing bacteria, such as the most investigated *Wolinella succinogenes* and *Anaeromyxobacter dehalogenans*, possess a gene

encoding the nitrous oxide reductase (Simon *et al.*, 2004; Sanford *et al.*, 2012), and could constitute an important net sink for N_2O (Hu, Chen and He, 2015).

2.5.2.3 The anaerobic ammonium oxidation

Anammox pathway [$(NO_2^- \rightarrow NO) + NH_4^+ \rightarrow N_2H_4 \rightarrow N_2$] involves the reductive combination of NO from nitrite reduction with ammonium as an electron donor to form hydrazine (N_2H_4), which is subsequently oxidized to N_2 (Hu, Chen and He, 2015), and the entire process is mediated by slow-growing anammox bacteria affiliated within the Planctomycetales order of the Planctomycetes phylum (Kartal *et al.*, 2011, 2013). The intermediate NO could serve as an important substrate for N_2O formation by the nitric oxide reductases in AOA, AOB, NOB, denitrifiers or DNRA bacteria (Figure. 4), but cannot be directly reduced by anammox bacteria (Strous *et al.*, 2006). Although a subordinate role of this process compared to denitrification in agricultural soil was recently shown by Zhu *et al.* (2018).

2.5.2.4 Chemodenitrification

Chemical denitrification is the process by which NO_2^- and NH_2OH are chemically reduced to N_2O (Heil, Vereecken and Brüggemann, 2016). Among the several involved reactions in this process, small amounts of N_2O may be produced through the chemical decomposition of nitrite (or chemodenitrification) (Bremner, 1997). The process could be driven by the presence of Fe (II) that is produced by heterotrophic Fe (III)-reducing microorganisms (Melton *et al.*, 2014), as well as by the availability of nitrite, that is produced during the reduction of nitrate by heterotrophic denitrifying bacteria (Torres Porras *et al.*, 2016). The extent of N_2O production via chemodenitrification versus denitrification is still poorly understood (Otte *et al.*, 2019), but the latter could be more significant, and more work is needed (Matocha, Dhakal and Pyzola, 2012).

2.5.3 Nitrous oxide emission from plants

Since it is unclear if all major sources of N_2O have been identified, the global nitrous oxide budget still has major uncertainties (Keppler and Lenhart, 2017). As an example, plant contribution to the N_2O emission was a controversial subject, whether via its indirect role as conduits of nitrous oxide produced by soil microorganisms (Pihlatie *et al.*, 2005) or directly via its production in their leaves (Dean and Harper, 1986). Therefore, to make clear its global budget it is necessary to recognize all sources of N_2O and implicit mechanisms (Timilsina *et al.*, 2020)**.**

Several studies have concluded the important role of plants in the N_2O emission: Yang and Cai (2005) reported that in a soybean pots experiment the cumulative N_2O emission during the growing season was 5.9 times greater than that from the identical but unplanted pots, but the difference in N_2O fluxes between the two treatments was not significant until the grain-filling

stage. Also, Chen *et al.*(2002) found that the amount of N_2O emitted directly from soybean, maize plants accounted for 6 to 11% and 8.5 to 16% of the total soil-plant N_2O emissions, respectively. Similarly Zou *et al.* (2005) recorded a wheat contribution with 10% at wheat tillering to 62% at the heading stage, but the source of this emitted gas was the point of debate. Where some studies proposed that the N_2O can be transferred from roots in the transpiration stream of upland plants to leaves and then emitted to the atmosphere, as N_2O is a soluble gas, as an example, Chang *et al.* (1998) recorded that barley (*Hordeum vulgare*) and canola (*Brassica napus*) plants can serve as a conduit for dissolved N_2O from the root zone to the atmosphere. Besides, in a study by Pihlatie *et al.* (2005) using a ^{15}N-enrichment approach, it was demonstrated that all of the ^{15}N-N_2O emitted from Fagus sylvatica leaves was derived from soil-applied $^{15}NH_4NO_3$. On the other hand, in a laboratory experiment (Smart and Bloom, 2001) plants were found as potential sources of N_2O from crop fields. They mentioned that ^{15}N isotopic signatures of N_2O emitted from leaves supported that N_2O can be formed enzymatically inside wheat leaves by plant NO_3^- assimilation and it was not N_2O produced by microorganisms on root surfaces and emitted in the transpiration stream. They estimated that this production could account for 5-6% of the total amount of N_2O thought to be emitted by agricultural plant-soil systems alone. They also found in their investigation that leaves did not emit N_2O when plants exposed to NH_4^+ despite the high rate of N_2O production in the rhizosphere.

Furthermore, the hypothesis that plants just serve as conductor of N_2O produced by soil microorganisms is not supported by a recent study by Lenhart *et al.* (2019), where stable isotope measurements ($\delta^{15}N$, $\delta^{18}O$, and $\delta^{15}N^{sp}$) of N_2O emitted by plants clearly show that the dual isotopocule fingerprint of plant-derived N_2O differs from that of currently known microbial or chemical processes. All of those studies indicated that N_2O emitted by plants might not be produced by soil microorganisms as it was mentioned by Timilsina *et al.* (2020). Despite the reports that plants are N_2O producers it is still a hitherto unknown mechanism. For this reason, very recently, and based on the experimental evidence from various studies, Timilsina *et al.* (2020) proposed a pathway that is active only when cells experience hypoxia or anoxia, and that plant N_2O production can be in the mitochondria from nitric oxide (NO). While, NO_3^- in the cytosol is metabolized to produce nitrite (NO_2^-) during hypoxia and anoxia, which is reduced to form NO via the reductive pathway in the mitochondria. Under low oxygen conditions, the latter is further reduced to N_2O by the reduced form of cytochrome c oxidase.

Although studies have proved the agricultural contribution to the total N_2O emissions from soil-plant systems, the underlying mechanisms are still unknown, and as the emissions estimates are based on the soil enclosures, thus, there is a likelihood of underestimating the whole soil-plant

N_2O emissions (Pihlatie *et al.*, 2005; Ussiri and Lal, 2012). A multidisciplinary approach, including studies of processes in soils and plants, canopy and ground flux measurements, stable isotope techniques, together with modeling is needed (Lenhart *et al.*, 2019) for more understanding of the N_2O produced by plants, thus decrease the uncertainty of global nitrous oxide budget.

2.5.4 N_2O uptake

Despite the reports on the production and emission of N_2O, soils can sink N_2O from the atmosphere (Signor and Cerri, 2013). Globally, the consumption of nitrous oxide in soils is not likely to exceed 0.3 Tg N yr^{-1}, indicating that the projected sink is not more than 2% of the currently estimated sources of N_2O in the atmosphere (Schlesinger, 2013). Therefore, the current budget for N_2O is unbalanced, showing an excess of sources over sinks (Schlesinger and Bernhardt, 2013) and in contrast to the other major greenhouse gases CO_2 and CH_4, the underlying controls of soil N_2O sink capacity have rarely been studied despite N_2O consumption in soil being frequently reported (Chapuis-lardy *et al.*, 2007). Formerly, many researchers were believed that the negative fluxes (i.e., uptake) were inaccurate or not significantly different from zero (Schlesinger, 2013). Some publications reported uptake in early field studies (Freney, Denmead and Simpson, 1978; Ryden, 1981). Since then, both significant and frequent net negative N_2O fluxes have been reported, but without any consideration in the discussion other than an occasional remark on the lack of information on the extent to which soils act as a sink for N_2O (Longoria-Ramirez *et al.*, 2003; Xu *et al.*, 2004).

Others have also reported net negative fluxes of N_2O into the soils in the field, indicating N_2O consumption by the microbial community (Chapuis-Lardy *et al.*, 2007). Nevertheless, N_2O uptake in fertilized fields has been observed (Maggiotto et al. 2000; Glatzel and Stahr 2001), despite the fact that agricultural soils are not likely to be as sinks for N_2O (Syakila and Kroeze, 2011).

Until recently, the only known sink for N_2O in the biosphere is its enzymatic reduction to dinitrogen (N_2) by N_2O reductase encoded by the nosZ gene is found among microorganisms capable of complete denitrification (Chapuis-Lardy *et al.*, 2007; Richardson *et al.*, 2009; Spiro, 2012; Jones *et al.*, 2014). Significant proportion of denitrifying microorganisms produce N_2O as a terminal product due to the lack of this gene encoding the catalytic subunit of the N_2O reductase (Jones *et al.*, 2008). On the other hand, several microorganisms with an N_2O reductase that can use exogenous N_2O as the sole electron acceptor do not possess the preceding steps in the denitrification pathway (Sanford *et al.*, 2012). This is why studies revealed that the abundance and diversity of these potential N_2O consumers with their environmental role, also denitrifiers having

nosZ role in net N_2O emissions have been underestimated and remains undefined (Jones *et al.*, 2013, 2014).

However, a new lineage of the N_2O-reductase (nosZ clade II) has been identified, and it is abundant and widespread in soils, (Sanford *et al.*, 2012; Jones *et al.*, 2013; Orellana *et al.*, 2014). A recent survey of microbial genomes done by Graf, Jones and Hallin (2014) has shown that about 51% of the organisms belonging to nosZ clade II unable to denitrify because of the lack of nitrite reductase. Also, Domeignoz-Horta *et al.* (2016) provided unambiguous evidence in their results that the overlooked non-denitrifying NosZ II-type bacteria can contribute to N_2O consumption in soil. But, the importance of nosZ clade II for net N_2O emissions in the rhizosphere is still not known (Graf *et al.*, 2016).

Factors influencing the consumption of N_2O by soils are still unclear (Signor and Cerri, 2013), where net N_2O consumption has been measured under various conditions from the tropics to temperate areas, in natural and agricultural systems (Chapuis-Lardy *et al.*, 2007). It was reported that the consumption of N_2O by soils is controlled by environmental factors including pH, water content, soil temperature, and availability of labile organic C and N, often, not always, associated to low availability of N and O_2 in soils, i.e., favorable conditions to reduce N_2O to N_2 (Signor and Cerri, 2013; Assémien *et al.*, 2019). Therefore, any modifications of soil conditions due to land management practices may affect N_2O uptake (Guenet *et al.*, 2020) which makes it difficult to identify a set of conditions generally suitable for N_2O uptake (Chapuis-Lardy *et al.*, 2007).

As the IPCC Guidelines do not include surface uptake of N_2O, Syakila, Kroeze and Slomp (2010) argued that N_2O uptake needs to be investigated whether or not the surface sink of N_2O is negligible, both at the global and national scales and considered it as an omission. However, fundamental questions about the capacity of soil microbial communities to act not only as sources but also as sinks for N_2O remains unanswered together with the factors regulating N_2O consumption which are not yet well understood and which merit further study (Chapuis-Lardy *et al.*, 2007; Domeignoz-Horta *et al.*, 2016) which could help account for the current imbalance in estimated global budgets of N_2O. That's why a systematic investigation into N_2O consumption is necessary in both field and laboratory studies before definite conclusions in order to be able to consider it in budgets and models and to close the global N_2O budget in order to close the global N_2O budget (Chapuis-Lardy *et al.*, 2007).

2.6. Nitrous oxide flux measurements

The measurements of the greenhouse gas fluxes have been ranged within different scales from a few grams of soil to several hectares of land area, which has participated in the current

understanding of biosphere-atmosphere exchange of GHGs (Denmead, 2008), and in order to assess their contribution and the potential mitigation options, quantitative information on gaseous fluxes also are needed.

The design of N_2O monitoring and observation protocols pose considerable challenges, because the emissions notoriously exhibit a high degree of spatial and temporal variability (Ussiri and Lal, 2012; Butterbach-Bahl *et al.*, 2013) due to the dependence of microbial N_2O production and consumption processes on environmental controls such as redox potential, substrate availability, temperature, and land management on soil (Butterbach-Bahl *et al.*, 2013).

To determine the rate of soil surface-atmosphere exchange of N_2O different methods and approaches exist: simple and widely used chamber methods, sub-surface methods, mass balance, micrometeorological methods with various degrees of complexity (eddy covariance, eddy accumulation, relaxed eddy accumulation, flux gradient methods), laboratory experiments, airborne measurements, and some empirical models like the emission factor (EF) method developed by Intergovernmental Panel on Climate Change (IPCC) and boundary line approach. As well as process-based modeling which represents important tools that provide emission estimates.

This section describes some commonly used techniques for measuring the N_2O flux, emphasizing the principles behind, strengths, and weaknesses associated with each technique. Although the emphasis is placed on chamber technique, which receives more attention because most of the global understanding of GHG fluxes and their control by physical, chemical, and microbial processes has largely arisen from this method (Ussiri and Lal, 2012), and more specifically on closed chamber technique, because it was used in our work.

2.6.1 Flux chamber systems

Flux chamber-based analysis is the most common field measurement technique used and represents the smallest scale, which has been conducted for almost a century, and widely used in soil emission studies of trace gas fluxes, the approach is also suitable for understanding the processes that regulate N_2O fluxes from the soil, and contributed most to the current understanding of the magnitude and spatiotemporal variability of N_2O fluxes and soil and environmental variables regulating it (Ussiri and Lal, 2012; Šimek, Hynšt and Šimek, 2014; Pavelka *et al.*, 2018). Chamber design depends on the purpose of the measurements, but in general there are opaque cylinders or boxes inserted into the soil to form an airtight enclosure (Oertel *et al.*, 2016).Chamber systems need to be easily and rapidly moved in order to measure multiple predetermined spots

(Oertel *et al.*, 2015). Besides, chambers should be installed on a collar (of steel or inexpensive polyvinyl chloride) to avoid gas leakage from the chamber to the atmosphere (Oertel *et al.*, 2016).

To minimize the influence of the collar on the soil structure and plant roots, the collar should, pushed to a depth of a few centimeters as mentioned by Heinemeyer *et al.* (2011) and collars need to be installed at least 24 h before the first measurement to prevent their influence on flux measurement since they affect the concentration in the soil profile (Bahn *et al.*, 2009). However, a proper design and measurement time schedule should be done to minimize the effects of chamber design on fertilizer addition/spreading and rain inside the measured area as mentioned (Pavelka *et al.*, 2018).

In addition, chamber systems should be equipped with auxiliary sensors (for air temperature, pressure and relative humidity should be installed inside and outside the chamber) to record the necessary drivers influencing soil emission (Oertel *et al.*, 2016). Besides, gas concentration profiles can be evaluated if gas production in different soil depths is of interest (Chirinda *et al.*, 2014). The frequency of chamber measurements is usually made weekly and rarely more frequently than once daily (Ussiri and Lal, 2012). Concerning the chamber technique, it is based on the increase (or decrease in case of sink) in gas concentration within the enclosed headspace, the change of mixing ratio can be analyzed with various gas sensors, e.g., gas chromatography for the N_2O (Hedley, Saggar and Tate, 2006; Oertel *et al.*, 2016).

Chamber systems are classified as open or closed chambers based on whether or not they are open to the atmosphere, respectively, with closed chambers being subdivided into closed static and closed dynamic ones (Rochette *et al.*, 1997; Kutzbach *et al.*, 2007). Closed dynamic chambers may also be referred to as non-stade state flow-through chambers (Oertel *et al.*, 2016).

2.6.1.1 Closed chambers

Closed chambers are designed to be sealed, to cover a known area of soil and that allows the gas exchange between the soil below the chamber and the chamber headspace (Pihlatie *et al.*, 2013). They can subdivide into static or dynamic ones, which differ in how ambient conditions inside the chamber are restored (Oertel *et al.*, 2016).

In static chambers, the monitored soil surface area ranges from very small surface, to ~0.5 m^2 (Clayton, Arah and Smith, 1994), depending on the dimensions of the gas chambers. While this kind of method represents a most commonly used tool for measuring N_2O fluxes from soil (Pihlatie *et al.*, 2013) and because N_2O chamber measurements are commonly used to assess N_2O mitigation strategies or to calculate national greenhouse gas inventories via country-specific emission factors

(EFs) determination, it is important that statistical analysis of the data robustly estimated since it is challenged by the heterogeneous nature of N_2O fluxes (de Klein *et al.*, 2020). Such chambers can be operated manually or automated, for example, using the manual one, gas sampling from the headspace with the gas syringe and gaseous concentration measurement in the laboratory using a GC and electron capture detector (ECD) or is the usual practice. While the manuals are able to cover spatial variability, the automatic systems do not have to be assisted. However, latters involve higher material costs, but they can be used for continuous monitoring (Oertel *et al.*, 2016).

Dynamic chamber systems represent a more complex method, generally automated and consists of a dynamic chamber and gas analyzer, where the air is circulated between the headspace and a gas analyzer in a closed-loop, in order to have a linear increase (Ussiri and Lal, 2012). Chambers design allows an automatic opening and closing of the lid (Almand-Hunter *et al.*, 2015). N_2O emission can be analyzed as well with closed dynamic chambers (Cowan *et al.*, 2015), but compared to static chambers was rarely used (Oertel *et al.*, 2016).

Since as it is reported Oertel *et al.* (2016), the accumulation time for the gas measurements need to be adapted to the emission rates of the different gases, N_2O measurements accumulation time lies between 30 and 90 min (Hayakawa *et al.*, 2009) due to the low emission rates. Cavity ring-down spectroscopy (CRDS) exists for monitoring systems, where N_2O is analyzed from one sample, similar to gas chromatography with higher precision and without additional equipment such as gas generators or gas cylinders, thus providing better portability (Fleck *et al.*, 2013). Nonetheless, high acquisition costs are involved (Oertel *et al.*, 2016), and recently a high-sensitivity nitrous oxide (N_2O) sensor based on mid-infrared continuous-wave cavity ring-down spectroscopy techniques were developed for environmental trace-gas measurements (Tang, Li and Wang, 2019).

Advantages of closed chamber method

The system characterized by its simplicity and easy applicability. When, static chambers are the most commonly used method for measuring nitrous oxide (N_2O) fluxes from agricultural soils (de Klein *et al.*, 2020), a static manual closed chamber, does not require any power in the field. Besides, operating with a simple principle, inexpensive, and can be used under a wide range of conditions, while samples can be collected with a syringe, stored in vials, and transported for later analysis in the laboratory, which makes it very easy to adopt (Conen and Smith, 1998; Rochette and Eriksen-Hamel, 2008). Also, to provide continuous records of gas fluxes from the same location, the automated chambers can also be designed (Ussiri and Lal, 2012).

Disadvantages of closed chamber method

Despite their advantages, the chambers can cover only a small area of soil surface. Therefore, to provide a representative estimate of the GHG fluxes a large number of chambers are required (Ussiri and Lal, 2012). The closed static chamber method significantly underestimates seasonal annual N_2O emissions since it may not able to capture the intensive emission pulses due to the low measurement frequency (Scott, Crichton and Ball, 1999). While it excludes fluctuations of ambient pressure caused by wind turbulence, in consequence, there will be no gaseous mixing of soil air with the atmosphere (Hutchinson and Mosier, 1981). Other weaknesses associated with the gas chambers technique particularly for the closed chambers includes: increases in gas concentration in the chamber headspace, which may affect the gas fluxes, where the accumulation of N_2O in the chamber inhibits the emission. Also, a flux change from linear to nonlinear was caused during one gas sampling which is taken at a certain time interval (Ussiri and Lal, 2012).

2.6.1.2 Open chambers

Another type of chamber system is the open dynamic chamber or flow-through flux chambers. This technique generates a continuous gas flow (Kutsch, Bahn and Heinemeyer, 2009), where gas concentrations are analyzed at the air inlet and outlet of the chamber, and gas flux is calculated by the difference of the concentrations at both ends, consequently, since the flux is analyzed continuously, there are no accumulation times needed (Oertel *et al.*, 2016). So evidently it can be clear that open dynamic chambers are technically more sophisticated and more expensive as compared to closed systems (Oertel *et al.*, 2016). Added to that, the major disadvantage of this method is limited by the small magnitude of concentration increase when fluxes are small (Ussiri and Lal, 2012). That's why closed dynamic chambers are still the most common systems (Pumpanen *et al.*, 2004).

2.6.1.3 Data evaluation

Soil flux for all gases can be calculated by linear and non-linear (exponential) regression, using the slope of the concentration change inside the chamber headspace (Christiansen *et al.*, 2011). But as mentioned by Oertel *et al.* (2016) during calculating flux N_2O sometimes values calculated with a non-linear model delivered lower values than values calculated with a linear model.

2.6.2 Mass Balance Approaches

The mass balance technique has been used widely in the past few decades also for N_2O (Denmead *et al.*, 2000). The technique is suitable for small-defined source areas, from tens to a few thousand square meters in extent, and can be applied on a closed or an open system (Ussiri

and Lal, 2012). Mass balance methods equate the rate of production of a gas in a control volume with the difference between the rate at which the gas is carried out and in the control volume by the wind (Denmead, 2008), where the missions are calculated from the difference in the rates at which the gas is carried into control volume above the source area and out by the wind (Ussiri and Lal, 2012).

The major strength of mass balance methods is that it fills the gap between the gas chamber method and micrometeorological approaches, it was appropriate for measuring gas fluxes from small well-defined source areas, very suitable for both homogenous and heterogeneous source distributions in the case of closed systems (Ussiri and Lal, 2012). Also appropriate for determining N_2O from fertilizer applications (Prasertsak *et al.*, 2001).

2.6.3 Micrometeorological methods

Frequently known as top-down approach, characterized by its high temporal resolution the methods use the flux gradient technique (Waldo *et al.*, 2019). In these techniques, temperature, wind, and gas concentrations at two or more points above the soil or vegetation surface are measured by gas sensors placed on towers (Denmead, 2008). The approach has been utilized to measure gas fluxes over a large area (Dalal *et al.*, 2003), without changing the physical condition of the observed surface, i.e. non-intrusive (Li *et al.*, 2008) which can reduce the spatial variability problems related static chamber techniques (Lapitan, Wanninkhof and Mosier, 1999). The difficulties in measuring GHG concentration for micrometeorological techniques flux quantification generally arises from slow time response instruments, and the need for detection of small concentration differences or fluctuations against a large background concentration (Wagner-Riddle, Thurtell and Edwards, 2005), also their use has been limited hitherto because capital costs are high, and a requirement for specialized personnel to operate it (Sapkota *et al.*, 2016; Smith, 2017).

Three common methods that fall under this category include (1) eddy covariance (EC), (2) eddy accumulation (EA), and (3) flux gradient methods.

2.6.3.1 Eddy Covariance Technique

The EC technique is the most direct method for measuring a flux over a surface (Pattey *et al.*, 2007). The EC technique is becoming popular for ecosystem assessment of gaseous fluxes due to its characteristics as a scale-appropriate method allowing the assessment of whole ecosystem gaseous exchange, also it produces a direct measurement of net gaseous exchange across the canopy-atmosphere interface. In addition to that, it is able to measure ecosystem gaseous exchange across a spectrum of timescales ranging from hours to years (Baldocchi *et al.*, 2001). This

technique is best applied when three conditions are met: (i) flat terrain, (ii) steady environmental conditions, and (iii) extending upwind for an extended distance of the underlying vegetation, while systematic errors in interpretation of EC measurements can cause by a violation of these conditions (Baldocchi, 2003). While the first EC flux measurements of N_2O was not made until the 1990s in contrast to the EC flux measurements of CO_2. Now, because the latest generation of analyzers is better, it is now possible to measure N_2O fluxes near their background level, which can still add up to a considerable fraction of the annual budget (Nemitz *et al.*, 2018).

Advantages and Disadvantages of Eddy Covariance technique

Eddy covariance is the direct preferred micrometeorological approach method, it uses vertical turbulences to analyze the turbulent heat and gas exchange between the soil surface and atmosphere (Launiainen *et al.*, 2005), it is independent of atmospheric stability and does not require some of the simplifying assumptions made in other micrometeorological technique (Denmead, 2008). Despite its capability to measure continuously and incorporate areas of up to several square kilometers (Myklebust, Hipps and Ryel, 2008), there are some practical problems in EC measurement for N_2O fluxes: dealing with the effects of simultaneous fluxes of heat and water vapor either by measuring them and apply the corrections to the apparent values of gaseous flux, accounting for lags between measuring vertical wind speeds and gas concentrations, and accounting for possible damping of gas fluctuating by sampling down tubes (Ussiri and Lal, 2012). Even, the EC method is ideally suited for capturing the high emission events with good spatial representativeness and temporal coverage, but it may well be challenged, for example, for N_2O flux measurements, sometimes inadequate sensitivity to detect small fluxes can be recorded in the tunable diode lasers and quantum cascade lasers used for flux measurements by eddy covariance technique (Kroon *et al.*, 2007; Kroon, Vesala and Grace, 2010; Nemitz *et al.*, 2018).

2.6.3.2 Eddy Accumulation

In the EA air associated with updrafts and downdrafts is collected into two separate containers at a rate proportional to the vertical wind speed (Brut *et al.*, 2004; Pattey *et al.*, 2006). Eddy accumulation techniques utilize a fast response solenoid valve, allowing air to be sampled, thereby eliminates the need for a fast response gas analyzer which required for the EC approach, so it is particularly appropriate for trace gases (Denmead, 2008; Ussiri and Lal, 2012).

2.6.3.3 Flux Gradient Methods

In this technique, the vertical flux is determined as was mentioned by Rapson and Dacres (2014) by measuring gas concentrations at two or more different heights and recording the horizontal wind speed rather than both horizontal and vertical wind speeds. Three approaches

commonly used for determining gas fluxes are: the aerodynamic method (Prueger and Kustas, 2005) which was used in a study for N_2O emission measurements from a vegetable farm following manure application using an open-path Fourier transform infrared (OP-FTIR) concentration sensor with retro reflectors (Bai *et al.*, 2019), the tracer technique (Denmead, 2008), and the energy balance (Bowen ratio) method (Denmead, 2008).

2.6.4 Laboratory experiments

Laboratory approaches are a helpful method in order to assess the influence of single parameters (e.g., soil temperature or nutrient availability) on soil emissions (Oertel *et al.*, 2016). Single parameters can be changed, while others are kept constant (Schaufler *et al.*, 2010). Also small field chamber systems can be used in the laboratory, while some research groups use chambers specially designed for laboratory use (Schaufler *et al.*, 2010; Yao *et al.*, 2010).

2.6.5 Airborne measurements

The nature of an airborne study is to deliver data over a short time period only and to provide a spatial survey of the prevalence and spatial distribution of such high-emission locations along with any other distributed sources in an area that is difficult to access (Desjardins *et al.*, 2010; Wilkerson *et al.*, 2019), the measurement uses direct sampling approaches to collect gases from transects. For instance, air samples on an ascending and descending flight path of an airplane were collected by D'Amelio *et al.* (2009). These samples were stored in flasks and analyzed in the laboratory by gas chromatography for the N_2O as an example.

2.7. Modeling soil GHG emissions

2.7.1 Empirical Models

2.7.1.1 IPCC Emission Factor Method

The default methodology of IPCC was used by most countries in order to calculate anthropogenic emissions from agricultural soils, including those from fertilizers animal waste, N fixed, and crop residues (Lokupitiya and Paustian, 2006). The direct N_2O emissions from agricultural soils are calculated by multiplying total soil N input from various sources such as synthetic fertilizer N, and excretal N from grazing animals by an appropriate emission factors (EFs) (Ussiri and Lal, 2012) recommended by the Intergovernmental Panel on Climate Change (IPCC) for national N_2O inventories (Kudeyarov, 2020). However, the IPCC approach is limited by the uncertainty in emission factors and in indirect emissions, limited data on the type and amount of N excreted by grazing animals and by spatial and temporal variability of N_2O emissions. Therefore, the IPCC approach represent only a first approximation of actual emissions, simple and

generalized (Saggar *et al.*, 2008), which make it not useful for assessing mitigation options (Kudeyarov, 2020).

2.7.1.2 Boundary Line Approach

Boundary line analysis (BLA) is a technique used for defining bivariate relationships for processes that are limited by multiple factors (Farquharson and Baldock, 2008), while in the absence of other, the dependence of N_2O emissions on a specified variable can be established using this approach boundary line approach (Ussiri and Lal, 2012).

2.7.2 Process-Based Modeling

The process-oriented modeling is the most promising tool for accounting for the large spacio-temporal variability of GHG fluxes (Butterbach-Bahl *et al.*, 2004). They can also very useful in the understanding of the complex interactions of biogeochemical processes involved in trace gas production with reducing the uncertainty associated with national and global GHG estimations (Barnsley, 2007). Models generally simulate the GHG exchange at a given site based on the underlying processes, i.e. the dominant physico-chemical, plant, and microbial processes involved in ecosystem C and N cycling and associated GHG exchange (Li *et al.*, 2000), also the exploration of potential mitigation strategies (Giltrap, Li and Saggar, 2010). In the case of nitrous oxide, the underlying assumption in process-oriented modeling is that the N_2O emission is controlled by comparable factors across the climatic zones and land uses e.g. moisture, microbial C and N turnover, temperature, substrate responses, and that by capturing the major biogeochemical processes within an ecosystem it is possible to predict the temporal variability of N_2O fluxes (Ussiri and Lal, 2012). For example, two process-based models, DAYCENT and DNDC, were used in a study done by Smith *et al.* (2008) to estimate N_2O emissions, soil nitrate- and ammonium-N levels, as well as soil temperature and water content, using the same model (DNDC) an estimate of the soil-atmosphere exchange of different gaseous N forms (N_2, NO, N_2O, NH_3) was done in Hungary by Machon *et al.* (2010), also recently a simulation of nitrous oxide emissions at field scale using the SPACSYS model was done by Wu *et al.* (2015) , added to those, the potential of using process-based model ensembles to predict jointly productivity and N_2O emissions at field scale is discussed by Ehrhardt *et al.* (2018).

2.8. Variability of nitrous oxide flux

Despite the recent progress in quantifying the diverse N_2O sources over the last three decades, effort, in order to quantify emissions of N_2O from agricultural fields across the world have been made particularly difficult since it represents a challenge due to a large number of interacting drivers that result in a high degree of temporal and spatial heterogeneity that make N_2O

emissions difficult to characterize at the field scale causing uncertainties in the global N_2O budget (Ciais *et al.*, 2014; Harty *et al.*, 2016; Smith, 2017; Waldo *et al.*, 2019). The variability was generally characterized by "hot spots and hot moments" (Butterbach-Bahl *et al.*, 2013) which is a consequence of heterogeneity of the soil's physical, chemical, and biological conditions, which control the key biogeochemical processes that generate N_2O (Smith, 2017). Furthermore, these factors interact with each other (Ussiri and Lal, 2012), and it is evident that the relationships between these factors and N_2O fluxes are difficult to predict and highly non-linear. As microbial N_2O production and consumption processes were dependent on environmental controls such as substrate availability, redox potential, and temperature, N_2O fluxes from soils are notoriously variable across various temporal and spatial scales (Butterbach-Bahl *et al.*, 2013).

A very high spatial variability of N_2O emissions at different scales, from the microscale one to the regional one has been shown by specific studies (Parkin, 1987; Groffman and Tiedje, 1989; Fóti *et al.*, 2018), with coefficients of variations ranging between 50% and 200% (Mathieu *et al.*, 2006; Konda *et al.*, 2008). Also in a study done by Van den Heuvel *et al.* (2009) that compared N_2O fluxes at scales ranging from 0.00013 to 0.31 m^2, found that "spatial variation was highest at the smallest scale". Furthermore, spatial variability can be linked to the mineral nitrogen availability or to topographic or micro topographic effects at distances beyond a few meters (Hénault *et al.*, 2012), and it occurs not only within fields or paddocks where N is applied or manure deposited but also in areas beyond the field where soluble forms of N are transported through drainage or runoff (Smith, 2017). Recently, McDaniel *et al.* (2017) recorded that the N_2O fluxes showed distinct spatial patterns, and were uniquely related to soil properties. Besides, the variability at the plot scale is often due to the presence of some very high fluxes on "hot spots", which account for a significant part of the whole flux (Hénault *et al.*, 2012). The presence of hot spots was also reported in cultivated fields, which emit at rates several orders of magnitude above the background N_2O fluxes (Ball *et al.*, 1997). While the occurrence of hot spots and hot moments of N_2O emission could be reduced through the N_2O emission mitigation (Wagner-Riddle *et al.*, 2020). Added to this, specific measurement techniques could help for improving the capture of spatial variability (Hénault *et al.*, 2012), where a study done by Fóti *et al.* (2018) using geostatistical tools concluded that topographic differences even the minor ones, had a primary importance in N_2O spatial patterns dynamics in an investigated grasslands and the N_2O was found to follow the patterns of depressions and crests to varying extent. For that, and as an example, the development of fast analyzers based on infrared spectrometry with quantum cascade laser (Guimbaud *et al.*, 2011), will allow to reinvestigating the spatial variability, which also provides very high sensitivity for gas analysis and which will be helpful for studying low fluxes and N_2O uptake by soils (Hénault *et al.*, 2012).

On the other hand, high temporal variability of soil N_2O fluxes is also observed due to climatic and agronomic events, at different scales (hours, days, seasons, years) (Laville *et al.*, 2011). N_2O emissions pulses over a few hours to days as triggered by freezing-thawing, soil rewetting, or fertilization can dominate annual fluxes at a given site (Ussiri and Lal, 2012). For example, in a Michigan (United States of America) wintertime cropland experiment (Ruan and Robertson, 2017) it was recorded that episodic fluxes after freeze-thaw events lasted only hours but accounted for the majority of wintertime N_2O fluxes, which were especially significant under reduced snow cover conditions. As well, Scheer *et al.* (2016) observed high fluxes after a >100 mm rainfall event which resulted in up to 79% of the annual emissions occurring over just 7 days. Also, nitrous oxide emissions commonly show diurnal fluctuations, caused mainly by changes in temperature, consequently, if unsuitable times of day are chosen for gas sampling from chambers, this can potentially lead to a bias in emission measurements (Smith, 2017). For that reason, Alves *et al.* (2012) suggested that the fluxes measured at 09.00-10.00 and 21.00-22.00 most closely matched the daily mean flux. Besides, the sampling frequency may play an important role in the uncertainty of current global N_2O estimates from agricultural soils (Barton *et al.*, 2015; Wang *et al.*, 2020), because in frequent sampling is considered one of the major disadvantages of using manual sampling methods and has the potential to overlook both day-to-day variability and diurnal variability (Reeves *et al.*, 2016).

While protocols have been adopted by researchers operating manual chamber systems that are designed in order minimize the missing important emission events , sampling frequencies can be adjusted to record the outcome of fertilizer applications and also irrigations where they occur (Smith, 2017) (for instance, rain events may be unpredictable). In one recently reported example of this event-related approach which was reported by Bell *et al.* (2016) daily gas samples were taken on ten occasions over the first 2 weeks after fertilizer application, after that sampling frequency was reduced to 2 days per week for the following 3 weeks, later on, for the next 5 months (or until the next fertilizer application) a fortnightly sampling strategy was implemented and then reduced to monthly sampling for the remaining 6 months. Recommendations for experimental design and deployment of chambers to reduce the uncertainty associated with the spatial, temporal, and experimental variability in N_2O fluxes were provided by Charteris *et al.* (2020). Also Waldo *et al.* (2019) recommend the use of chambers to investigate spatiotemporal controls as a complementary method to micrometeorological monitoring, especially in systems with high variability.

The overarching goal of reducing the large uncertainty in the global N_2O budget still remains a formidable task, despite the all currently available global data on N_2O emissions from

various source sectors were used (Shurpali *et al.*, 2016). In the end, the spatiotemporal variability of N_2O emission, which makes it difficult to quantify the N_2O fluxes, may turn into an opportunity for mitigation if we are able to understand it (Hénault *et al.*, 2012).

2.9. Factors influencing agricultural soil N_2O fluxes

Fluxes of N_2O from agricultural ecosystems are the result of complex interactions of various parameters, including, soil physical, biological, chemical properties, and climate, also, the land management practices (Millar *et al.*, 2010). Because N_2O is primarily produced by the microbial nitrification and denitrification processes in agricultural soils (Bateman and Baggs, 2005; Pan *et al.*, 2018). We are focusing on the major factors affecting the emission via the two processes.

2.9.1 Environmental factors and soil characteristics affecting soil N_2O fluxes

In order to better predict and mitigate N_2O emissions, it is essential to identify the key environmental factors which govern the dominant microbial N_2O sources. Among the various factors that influence microorganisms growth and regulate N_2O emissions from soils the most important are the following: soil moisture and aeration, soil temperature, soil pH, carbon available and nitrogen, and other soil characteristics like soil texture and micronutrient content (Mosquera and Dolfing, 2007; Signor and Cerri, 2013; Deng *et al.*, 2015; Hu, Chen and He, 2015).

2.9.1.1 Soil moisture and aeration

Soil moisture is a key driver of N_2O emissions, it can explain 74% of its variation as it regulates the oxygen availability, which in turn affects nitrification through its roles both as a substrate for AMO and as the terminal electron acceptor from cytochrome C oxidase (Schindlbacher, Zechmeister-Boltenstern and Butterbach-Bahl, 2004; Ussiri and Lal, 2012; Butterbach-Bahl *et al.*, 2013). Also, it influences denitrification through its impact on O_2 diffusion. In addition, soil water content not only determines the availability of O_2 but also influences the metabolic activity of microbial cells together with diffusion and transport of nutrients within the soil matrix (Hu *et al.*, 2015), which could cause a confounded relationship between WFPS and rates of N_2O emissions (Hu, Chen and He, 2015). Moreover, N_2O emitted via denitrification depends on the structure and wetness of the soil where it has a higher chance of being emitted to the atmosphere rather than being reduced to N_2 if can easily diffuse from the site of production to an oxygenated pore space (Ussiri and Lal, 2012).

Moisture status is controlled by different factors which are rainfall, plants-through evapotranspiration, and soil texture-which influence water holding capacity (English *et al.*, 2005; Li *et al.*, 2016; Säurich *et al.*, 2019). While the increase in WFPS due to wetting-up events like irrigation, rainfall, and snowmelt not only facilitates soil nitrification and denitrification (Hu *et al.*,

2015) but also promotes N_2O production (Hofstra and Bouwman, 2005), where several studies reported a significant relationship between soil N_2O emission and WFPS (Deng *et al.*, 2015; Rutkowska *et al.*, 2017), with an increase in the emission after atmospheric precipitation (Snowdon *et al.*, 2013). But, in a similar study, Guo *et al.* (2014) reported that the N_2O reductase can increase in activity after prolonged periods of high soil water content, leading to the conclusion that N_2O emissions were driven by both moisture content and the duration of wetness (Sperling, 2015).

In general, N_2O emissions are favored when the soil is sufficiently wet to restrict O_2 availability (Nishio *et al.*, 1988; Butterbach-Bahl *et al.*, 2013; Hayashi *et al.*, 2015), but under super saturation conditions, most part of the N_2O is reduced to N_2 (Davidson *et al.*, 2000). It was suggested that soils with 30% < WFPS < 60-70%, > 80-90% WFPS were the optimum conditions for N_2O production via nitrification-related pathways and heterotrophic denitrification, respectively (Braker and Conrad, 2011; Huang *et al.*, 2014). While under aerobic conditions denitrification rate is typically 0.3-3% of the anaerobic rate (Ussiri and Lal, 2012). Otherwise, many different results were recorded in several studies which were shown in Table 1.

Nevertheless, it is important to highlight that being the main process is not the same thing as having the higher emission rates (Signor and Cerri, 2013). Recently, Balaine *et al.* (2016) showed that because relative gas diffusivity accounted for the interaction of soil bulk density and matric potential it was able to explain the variation in N_2O fluxes better than WFPS, where under field conditions the recognition of the relation between WFPS and N_2O emission has been important in the development of a better understanding of the dynamics of N_2O emissions (Smith, 2017).

2.9.1.2 Soil temperature

Soil temperature represents an important driver controlling N_2O flux (Davidson and Swank, 1986; Signor and Cerri, 2013). Hence, N_2O emissions are not only directly affected by temperature effects on enzymatic processes involved in N_2O production (Butterbach-Bahl *et al.*, 2013). Furthermore, an increase in soil temperature stimulates soil respiration (microbial activity) leading to a decrease in the oxygen content in the soil air, that's mean increasing anaerobic sites in which denitrification can take place, followed by an increase in N_2O emission (Signor and Cerri, 2013; Kudeyarov, 2020).

The N_2O emission from soil grows up to 37 °C, and then the N_2O production decreases, while the Q10 for N_2O varies in the range of 1.7-9.3 (Kudeyarov, 2020). While this effect is not straightforward. For instance, N_2O consumption during denitrification process could be also stimulated by temperature increase (Ussiri and Lal, 2012). Also, temperature thresholds can be

very different in different climatic regions as was illustrated by Cosentino, Figueiro Aureggui and Taboada (2013).

However, it has been observed that the N_2O emission increases exponentially with an increase in temperature (Cantarel *et al.*, 2011; Liu *et al.*, 2011b). Also, recently Bosco *et al.* (2019) reported that daily fluxes of N_2O were correlated positively with soil temperature, but this correlation corresponded with N fertilization. The authors mentioned that the latter was probably caused by the high microbial activity associated with the organic matter mineralization in the warm season, which in parallel with other studies reported that soil temperature may be a driver for N_2O production when substrates are abundant, and the soil water content is optimal for microbial processes (Liu *et al.*, 2011b). This positive effect may be overlain by soil water stress as an example (Fowler *et al.*, 2009), or other factors. Contrary, several research studies have reported a non-linear relationship between temperature and the rate of N_2O emissions (and the rate of total denitrification) (Abdalla *et al.*, 2009; Blagodatskaya *et al.*, 2014). Additionally, studies recorded hot moments of N_2O emission during freeze-thaw events and reported that these events may be responsible for up to 50% of the total annual N_2O emissions, which illustrates the importance of temperature at the boundary of soil freeze-thawing (Groffman *et al.*, 2009; Weller *et al.*, 2019). The N_2O emission during the freeze-thaw event would be explained by a proposed mechanism that low temperatures decrease the rate of N uptake by the plant after top dressing in autumn (Groffmann et al. 1993) and also by O_2 depletion plus the easy decomposable organic carbon and nitrogen to the soil delivered during frost time (Butterbach-Bahl *et al.*, 2013; Weller *et al.*, 2019). All these variations on the effect confirm that the response of N_2O emissions to changes in soil temperature can be complex (Smith, 2017), therefore it's not easy to predict a clear correlation. Table 1 showed some other studies that reported temperature effects on N_2O formation pathways.

2.9.1.3 Soil pH

Changing soil pH is widely considered to influence nitrous oxide production (Dai *et al.*, 2017), represents a major factor influencing N_2O emission pathways (Teutscherova *et al.*, 2017) (some references are in Table 1). Denitrification rates tend to decrease at low soil pH values (Šimek, Jíšová and Hopkins, 2002), contrary, a global meta-analysis of field experiments has revealed that the amounts of N_2O substantially increased in soils with lower pH values (Shcherbak, Millar and Robertson, 2014). This finding was explained by the fact that N_2O-reductase is generally not functional at low pH for the reduction of N_2O to N_2 (Bakken *et al.*, 2012; Shaaban *et al.*, 2018). While in soils with pH of 4.0, N_2O is the main product of denitrification, an increase in one unit of pH may decrease 0.2 units in the molar fraction of the N_2O emitted (Knowles, 1982; Stevens and Laughlin, 1998). Apart from the biological process, chemodenitrification, was also

reported to be favored in acidic soils (pH < 5) with high nitrogen fertilizer inputs (Braker and Conrad, 2011). Although earlier studies from agricultural lands have revealed the sensitivity of N_2O emissions to soil pH (Bakken *et al.*, 2012; Samad *et al.*, 2016), contradictory viewpoints have also been reported both for increases (Qu *et al.*, 2014) or decreases (Shaaban *et al.*, 2018) in soil N_2O emissions in response to pH manipulation.

2.9.1.4 Soil nitrogen availability

As N_2O primarily produced by nitrification and denitrification, and since they are strongly influenced by N content, Therefore, NH_4^+ availability is the factor that most frequently limits the overall rate of nitrification. While, a decrease in the NO_3^- concentration below 20 mg/kg dry soil induced a decrease in the N_2O emission to its complete absence (Senbayram *et al.*, 2012), and when NO_3^- in the soil is high emissions of N_2O will also be greater (Ruser *et al.*, 2006). Different sources of nitrogen exist, and any of them also stimulate the N_2O flux, such as N fertilizers (see separately under management effects), animal manures, crop residues, biological nitrogen fixation (Bateman and Baggs, 2005; Ghaly and Ramakrishnan, 2015; Pan *et al.*, 2018), Besides, litterfall, plays an important role in energy and nutrient transfer, and also in maintaining soil fertility (Lavelle *et al.*, 1993), where the incorporation cover in soil surface with constant leaf litterfall and extensive root systems in the rubber agroforestry systems increased organic carbon and nitrogen in the soil and improving their accumulation rates (Tongkaemkaew *et al.*, 2018).

In addition, N cycling is also affected by the N inputs through deposition, where atmospheric nitrogen deposition has become a large source of nitrogen for terrestrial and aquatic ecosystems worldwide (Galloway *et al.*, 2008). Their excess leads to high N availability and causes N saturation (Aber *et al.*, 1998), and goes beyond the availability of plants and microbes and is lost through leaching (Rustad *et al.*, 2001; Beier *et al.*, 2008) or gaseous emissions (Aber *et al.*, 1998). Different chemical forms of nitrogen in which will eventually deposit (ammonia or ammonium, nitrogen oxides, nitrate), in different physical forms: gases and aerosols (Bleeker, 2018).

The total deposition of nitrogen mainly consists of wet and dry deposition, wet deposition, predominantly rain and snow, carries nitrate and ammonium, and dry deposition involves complex interactions between airborne nitrogen compounds and plant, water, soil, rock, or building surfaces (Kingston, Bowersox and Zorrila, 2000). For the nitrogen deposition to (semi-) natural vegetation in source areas (e.g. agriculture), ammonia dominates the overall deposition (Bleeker, 2018). Other nitrogen compounds (nitric oxide, nitric acid, etc.), may be subject to the deposition pathway.

2.9.1.5 Soil available carbon

Soil organic matter is the main carbon source that is provides C and energy source for soil heterotrophic denitrifying organisms, generally act as electron donors in the denitrification,

although some heterotrophic nitrification can also require a source of SOC (Cameron, Di and Moir, 2013; Quin *et al.*, 2015; Zhang *et al.*, 2020). Additionally, it activated soil respiration, microbial growth, increases the O_2 consumption, which is conducive to the formation of the anaerobic environment, thus indirectly enhanced the soil denitrification process (Signor and Cerri, 2013; Nie *et al.*, 2016). Usually, SOC comes from crop residue and other organic sources, like microbial biomass. In a study conducted by Wang *et al.* (2005), supplies of available organic C appear 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 simple and readily available organic substance compared to the original soil organic carbon (Nishio *et al.*, 1988; Azam *et al.*, 2002; Chen, Mothapo and Shi, 2015; Giles, Daniell and Baggs, 2017).

2.9.1.6 Other soil properties affecting N_2O fluxes

Several other properties have important effects, for example, due to inherent differences in hydraulic conductivity of different soil types resulting in differences of N_2O emission under different soil moisture conditions (Harty *et al.*, 2016). Soil texture can influence soil moisture where soils with a high proportion of large pores promote the emission of gases produced under aerobic conditions because it retaining less water (Weerden *et al.*, 2010), contrary, the formation of N_2O under anaerobic conditions was favored in soils with dominant fine pores (Gu *et al.*, 2013). Recently, Kudeyarov (2020) reported that N_2O emission increases when the soil texture becomes heavier.

In addition, a variety of metal cofactors are important, such as Molybdenum (Mo), Iron (Fe), Copper (Cu), and Zink (Zn) which are required for denitrification enzymes, for example Cu has a critical role and absolutely required for nitrous oxide reductase (Signor and Cerri, 2013). Yet, similarly, Shaaban *et al.* (2019) reported a higher N_2O emission in a Cu addition treatment as compared with the control and an increase in the emission with increasing Cu concentration in soil. Also, it has been recognized that the structure and activity of soil microbial communities, nitrification, denitrification, soil respiration, and N-mineralization have been affected by heavy metals (Holtan-Hartwig *et al.*, 2002).

2.9.2 Management factors affecting N_2O formation

Agricultural management practices such as nitrogen fertilization (mineral or organic), soil tillage, and crop residues are of great importance in N_2O emissions (Signor and Cerri, 2013).

2.9.2.1 Nitrogen fertilization

Once the N_2O emissions by nitrification and denitrification depend on the N content in the soil, N fertilizer also enhances N_2O emissions in circumstances where other factors are not limiting, while the effect of fertilizers can be a directly via the amount of NH_4^+ or NO_3^- available in the soil (Signor and Cerri, 2013) needed for nitrification and denitrification, respectively, as well as indirectly by plant biomass production enhancement, and then more crop residues, what could increase N_2O emissions for a long term (Hellebrand, Scholz and Kern, 2008).

Lot of studies aimed to describe the mathematical relationship between accumulated N_2O emission and amount of N applied, therefore several approach exist: like a simple linear relationship (Chen, Huang and Zou, 2008), Dencső (2021) reported no linear response of N_2O to the different fertilizer rates in no-till agricultural soil, whereas exponential relationship related to N-fertilization rates in maize was also presented (Ma *et al.*, 2010). Van Groenigen *et al.* (2010) obtained stable N_2O emissions to an application rate of 187 kg N/ha and an N rate above 200 kg N/ha induced significant increases in N_2O emissions.

On the other hand, added to the different environmental factors, management practices, and microorganisms abundance and activity, there are other factors influencing fertilizer effect. Among them; fertilizer type, application rate, application technique, application timing (Eichner, 1990) are important. For instance, ammoniacal fertilizers increase N_2O emissions slower than nitric fertilizers, since nitric sources can be denitrified immediately, contrary ammonia sources still have to be nitrified before the denitrification (Signor and Cerri, 2013). Recently, Tao *et al.* (2018) concluded that the organic fertilizers increased denitrifying enzyme activity, increased denitrifying-bacteria gene copy numbers, but reduced N_2O emissions, where nirS- and nos Z-type denitrifiers were more sensitive than nirK-type denitrifiers to the organic fertilizers. Other studies concluded with opposite findings where peaks of N_2O flux were higher after organic N fertilization events than after mineral N fertilization. Bosco *et al.* (2019) explained it by the increment of the soil microbial community due to the N and C availability, thus, led to high O_2 consumption that may create anaerobic conditions suited for the denitrification process from which N_2O is originated. Contrary, other studies reported lower N_2O emissions with organic fertilizers than mineral fertilizers (Aguilera *et al.*, 2013). However, the effect of fertilizers on soil GHG emissions strictly depends on climate and soil specific conditions as well as on the type of the organic fertilizer itself. Indeed, Pelster *et al.* (2012) reported that N_2O emissions responded similarly to organic and mineral N sources in soil with high C content, whereas only manure application increases soil N_2O flux in soils with low C content. Added to fertilizer type, application method also seemed to be important as many authors concluded that deep placement of N fertilizer could

be an effective means to reduce N_2O emissions in no-tillage systems (Van Kessel *et al.*, 2013; Millar, Doll and Robertson, 2014). Therefore, the timing of fertilizer application is so important because the emissions rate in the soil not only affected by soil nitrogen content and application rates but also their utilization by plants and soil microorganisms (the effectiveness of the nitrogen) (Nie *et al.*, 2016). Other findings related to fertilizer application are shown in Table 1. However, based on the literature the exact effect of fertilizers on soil N_2O emissions strictly depends on climate and soil specific conditions as well as on the type of the fertilizer itself. Also, the microbial population present in the soil should be taken into consideration.

2.9.2.2 Soil tillage

Tillage systems (tillage intensity or its absence) may affect N_2O emissions, resulting changes in soil biological and physical conditions like: soil aeration, soil moisture, microbial activity, and the rate of residue decomposition (Signor and Cerri, 2013). Studies on the effects of no-tillage (NT) and tillage on N_2O emissions have shown various results. Some have reported higher N_2O emissions from NT than from conventional tillage (CT), contrary others have shown lower emissions from NT than CT, and still, others have reported no difference among tillage practices (Table 1). While different factors that aggravate N_2O emissions from NT soils compared to CT, among them, soil compaction, the maintenance of greater water content in no-till soils, as a result, the promotion of denitrification (Linn and Doran, 1984; Baggs, Chebii and Ndufa, 2006; Bayer *et al.*, 2015). Moreover, it must be recognized that the largest impact of reducing tillage is a redistribution of SOC towards the soil surface, that's why there has been considerable discussion on whether it leads to N_2O increases or not, because anoxic conditions can increase its reduction to N_2 (Mei *et al.*, 2018; Buchen *et al.*, 2019; Ogle *et al.*, 2019). While plowing increases aeration, thereby increase the biological activity, it also increases the accessibility of crop residues for soil microbes (Khan, 1996; Signor and Cerri, 2013), which may induce pulses of N_2O emissions, Otherwise, in the CT system, O_2 concentration increment in soil may consequently decrease the N_2O emission (Signor and Cerri, 2013).

2.9.2.3 Crop residues

N_2O emissions can be higher or lower depending on the biochemical composition of crop residue added to the soil (Gomes *et al.*, 2009) since their incorporation affect both N mineralization and immobilization, which in turn influences nitrification and denitrification processes via the N availability. N_2O emission negatively correlates with the C/N value, where at $C/N \geq 30$ the N_2O emission being lower, contrary a higher N_2O emission may occur at lower C/N ratio (equal to 11, a typical ratio in the arable soils) because the dominance of mineralization over the immobilization seemed to be in soils with a smallest C/N ratio (lower than 30/1), that promoted available N which

can be absorbed by plants or used in microbial processes, contrary, a higher C/N ratio would decrease denitrification (N_2O emissions) (Signor and Cerri, 2013; Kudeyarov, 2020). Some studies related to crop residue incorporation are indicated in Table 1. Details about the correlation between the soil's organic carbon content and N_2O emission after application of organic and mineral nitrogen fertilizers were showed in a study done by Gu *et al.* (2017). In addition, Cosentino, Minervini and Taboada (2017) indicated that the N_2O emission was affected by the residue position and not by its origin (soybean or corn). The highest emission values were shown during surface treatments, but the effect is not yet fully understood and may well be highly site-specific (Guenet *et al.*, 2020).

Table 1. Research studies reporting the effect of different factors influencing nitrous oxide emissions.

Factor	Relevant findings	Refference
Soil moistue	Higher emissions by nitrification, with a maximum at 20% WFPS.	(Ludwig *et al.*, 2001)
Soil moistue	Optimum soil moisture for N_2O through nitrification at 30-60% water-filled pore space, whereas 60-80% WFPS represents the optimum condition for N_2O production under denitrification.	(Davidson, 1991)
Soil moistue	N_2O production is optimal around 60% WFPS and lowest when WFPS is below 30%.	(Gao *et al.*, 2014)
Soil moistue	N_2O emissions are greatest in anoxic conditions with a WFPS of 70-80% or more.	(Butterbach-Bahl *et al.*, 2013)
Soil moistue	The highest N_2O fluxes were found at between 73 and 95% WFPS, primarily originated from denitrification.	(Säurich *et al.*, 2019)
Soil moistue	Under tropical climatic conditions, the emission peak occurred at around 60% WFPS, but emissions can remain still high at even 80% WFPS	(Van Lent, Hergoualc'h and Verchot, 2015)
Soil moistue	N_2O occurred within a narrow range of soil matric potential of −1.9 to −4.5 kPa, corresponding to a wide range of 63-98% WFPS.	(Castellano *et al.*, 2010)
Soil temperature	An increase in nitrification-derived N_2O production and associated AOAamoA (ammonia monooxygenase) gene abundance with increasing soil temperature (from 25 to 35 °C) under aerobic conditions.	(Liu *et al.*, 2017)
Soil temperature	Denitrification-derived N_2O production increased with temperature and only the nirS type denitrifiers community structure was sensitive to temperature change (from 5 to 35 °C).	(Cui *et al.*, 2016)
Soil temperature	N_2O fluxes were approximately zero when the temperature was less than 10 °C or the WFPS was higher than 70% at various depths.	(Nan *et al.*, 2016)
Soil moistue	Denitrification becomes a dominant source of N_2O between 70 and 90% WFPS, while, above 90% WFPS produces undetectable N_2O emissions.	(Ussiri and Lal, 2012)
Soil pH	Nitrifier denitrification being positively related to pH, and heterotrophic denitrification decreased with increasing pH.	(Kool *et al.*, 2010)
Soil pH	A slightly negative correlation between gross nitrification rates and soil pH.	(Booth, Stark and Rastetter, 2005)
Soil pH	Nitrification activity in acidic soils mainly to AOA attribution.	(Huang *et al.*, 2014)
Soil pH	Denitrifier abundance was influenced by soil pH.	(Tao *et al.*, 2018)
Soil pH	In soils with high pH values, the N_2O derived from chemo-denitrification constituted only 0.1-1.3% of total N_2O production.	(Zhu, Burger, Doane, *et al.*, 2013)
Tillage Systems	Non-tillage in humid areas increases N_2O emissions in the early years and then reduces them, in comparison to conventional tillage (NT for more than 10 years old).	(Van Kessel *et al.*, 2013)
Tillage Systems	A reduction on the N_2O emitted under no-tillage or reduced tillage systems when compared to conventional tillage.	(Rutkowska *et al.*, 2017; Plaza-Bonilla *et al.*, 2018)
Tillage Systems	No effect of soil tillage on the changes in the amount of N_2O emission.	(Bayer *et al.*, 2015)
Tillage Systems	Higher N_2O emissions under reduced tillage compared to the conventional plough tillage.	(Mangalassery *et al.*, 2014)
Crop residues	A single addition of wheat straw (C/N = 78.7) slightly decreased the mineral N content in the soil, due to the high C/N ratio, while the application of N-fertilizer in plots with this straw resulted in higher N_2O emissions than in plots without wheat straw.	(Liu *et al.*, 2011a)
Crop residues	Straw incorporation affected the abundance and compositional diversity of AOA amoA, AOB amoA, nirK, and nosZ communities.	(Huang *et al.*, 2019)
Crop residues	In a lime concretion black soil wheat and maize amendments increased N_2O emissions only at 250 kg N ha^{-1}, contrary a decrease was at N200, indicates that crop residue property and rate of N	(Gao *et al.*, 2016)

	fertilizer are important influencing factors of N_2O emission when crop residues combined with N fertilizer are applied.	
Crop residues	Soybean cake amendment dramatically increased soil N_2O emission.	(He *et al.*, 2019)
N fertilizer	Switching from CAN to any urea formulation significantly reduced direct N_2O emissions.	(Harty *et al.*, 2016)
N fertilizer	Fertilizer applications during dry weather result in small emissions of N_2O than the application under moist conditions.	(Schils *et al.*, 2008)
N fertilizer	The greatest emission of N_2O when the application of fertilizer was concurrent to precipitation events.	(Metay *et al.*, 2007)
N fertilizer	N_2O effluxes were positively correlated with NO_3^- content and NH_4^+ content.	(Nan *et al.*, 2016)
N fertilizer	The annual N_2O flux in the cornfield was equal between mineral and mineral combined with organic fertilizers.	(Nugroho *et al.*, 2015)
N fertilizer	A positive correlation between N_2O flux and AOB abundance with N application, with emission even at a lower N rate.	(Meinhardt *et al.*, 2018)
N fertilizer	Different fertilization showed no distinguishable effect on N_2O emission in the laboratory.	(Dencső, 2021)

2.9.2.4 Crop effects

Incorporation of N fixing crops in a rotation may increase N_2O emissions (Kou-giesbrecht and Menge, 2019), while as reported by Ciampitti and Vyn (2012), crop uptakes large amount of N from the soil for growth, reducing the effective N content in the soil, and thus reducing soil N_2O emissions. Recently, Wang *et al.* (2019) found less N_2O emissions from maize field than that of not planted field under the same N fertilizer conditions, but the effect decreased with N fertilizer increment. Otherwise, root respiration reduced rhizospheric O_2 pressure through created an anaerobic environment (Jarecki *et al.*, 2009), which will favor denitrification, Moreover, plants can exert control over N transformations catalyzed by the fungal and prokaryotic populations in and near the rhizosphere by releasing root exudates (Bardgett, Mommer and De Vries, 2014). That factor linked to crop growth is likely to be involved in increasing N_2O emissions but the exact mechanism remains unclear as it was discussed in the N_2O sources section.

2.10. Mitigation strategies of N_2O emissions from agricultural soils

On the one hand N_2O is expected to be the largest ozone-destroying compound (Thomson *et al.*, 2012), on the other hand it is one of the most important agricultural greenhouse gases, therefore, in response to the increasing food demand and deteriorating climate change, effective mitigation strategies of N_2O emission has paramount importance. For reducing N_2O emissions from cropland a best management practices (BMPs) are recommended in order to ensure adequate available N required by crops and prevent N availability exceeding plant N demand (Ussiri and Lal, 2012). The BMPs option include the fertility management or the four R's of fertility (right rate, right time, right location, right formulation) (Coyne and Ren, 2017). Using this strategy there has been some improvement in fertilizer use efficiency (FUE) (Han, Walter and Drinkwater, 2017b). For example, differences in N_2O emissions between different fertilizer N forms were shown in a meta-analysis of fertilizer types (Venterea, Burger and Spokas, 2005), where during ammonium nitrate/calcium ammonium nitrate application the N_2O loss occurs more quickly and

with higher emission factors (%) compared to urea (Clayton *et al.*, 1997; Dobbie and Smith, 2003; Jones *et al.*, 2007). The use of calcium ammonium nitrate, particularly at wet and/or high organic matter sites can resulted in high N_2O emissions (Watson *et al.*, 2009). Also, in general, NH_4^+ fertilizers emit less N_2O than NO_3^-, so prioritizing the use of low N_2O emission fertilizers together with using a nitrate-based fertilizer rather than ammonium if nitrification is supposed to be the main contributor to N_2O fluxes could limit N_2O production (Hénault *et al.*, 2012; Signor and Cerri, 2013), with avoiding the use of urea in soils prone to low O_2 availability and low pH (Zhu, Burger, Doane, *et al.*, 2013). Optimizing fertilizer type by using organic fertilizer application could help in improving soil quality and in N_2O reductions as it was reported by Tao *et al.* (2018) where organic amendments reduced cumulative N_2O emissions by 4.9-9.9%, reduced the N_2O emission factor by 1.3-42% and increased denitrifying enzyme activities by 14.3-56.2%. However, contradictory results on the reduction in soil N_2O emission have been reported (Yao *et al.*, 2015). Also, crop straw and biochar are two farmer-friendly residues that can be used for reducing the application of mineral fertilizers (Borchard *et al.*, 2019; Huang *et al.*, 2019). Besides N type, N placement, timing, and application rates may also help in minimizing the N_2O emissions. N fertilizers can be applied by various placement methods, in a certain depth near the zone of active root uptake, instead of surface application. It may both reduce surface N loss and increase plant N use resulting in reduced N_2O emissions especially when heavy rains are expected (CAST, 2004; Signor and Cerri, 2013). However, it was also shown in a meta-analysis that the application of N-fertilizer at more than 5 cm depth can decrease N_2O emissions, particularly in humid climates (Van Kessel *et al.*, 2013). The same result was found in another study but at the depth of 10 cm (Chapuis-Lardy *et al.*, 2007). Still it is difficult to generalize the benefits of fertilizer N placement for N_2O mitigation strategy since there are contradictory results (Drury *et al.*, 2006; Velthof and Mosquera, 2011). In addition, synchronizing the timing and rate of fertilizer N with plant N demand is an important N management technique in agriculture, which need to be adapted to plant needs. Not all forms of nitrogen can be taken up by plants at the same rate (Oertel *et al.*, 2016) and non plant-available N amounts could lead to increasing N_2O emissions (McSwiney and Robertson, 2005). Splitting N rates could be also important tool to the proper supply of N during the crop cycle, applied in periods in which it is more requested (Signor and Cerri, 2013). A meta-analysis showed that on average, applying fertilizer at higher than the recommended rates increased N_2O emissions by 55% while applying fertilizer at lower than recommended rates decreased N_2O emissions by 33% (Han, Walter and Drinkwater, 2017b). Also, managing the soil chemistry and microbiology may help in the N_2O mitigation. For instance, when conditions are favorable for incomplete denitrification N_2O is produced instead of N_2. Then, using liming to reduce soil acidity and

increase overall denitrification rates, and adjusting micronutrients, especially Cu contents, can reduce N_2O emissions (Signor and Cerri, 2013; Coyne and Ren, 2017).

In addition to the supply of C containing N inputs, other strategies involved in a broader approach to N management known as ‘‘ecologically based nutrient management’’ may help in reducing N losses such as diversified crop rotations, reduced fallow periods, catch crops which may include leguminous species that fix N biological and can absorb substantial amounts of N unused by the preceding crop (Isse et al., 1999; Collins et al., 2007) and thereby minimize N losses by leaching and N_2O emission (Collins *et al.*, 2007; Delgado *et al.*, 2007; Doltra and Olesen, 2013; Han, Walter and Drinkwater, 2017b).

Different results have been reported regarding the mitigation effect of tillage management on N_2O flux, but still there is no clear response for mitigation of N_2O using conservation/reduced tillage or no-tillage (NT) practices compared to conventional tillage (CT) because the effect is controlled by climate, soil properties, and time of application (Ussiri and Lal, 2012). There are discordant findings on the influence of the tillage system: NT can alter soil properties (by lowering soil temperatures) (Six *et al.*, 2002) and lead to decreased N_2O emission (Omonode *et al.*, 2011), while others found a positive effect of no-till on N_2O emissions and explained this with higher microbial activity (Baggs *et al.*, 2003). In addition, other practices may help minimize the potential for N_2O emissions, which an effective irrigation and drainage that can improve water use efficiency, and avoid moisture excesses associated with reductions in air-filled pore space, promoting yield and suppress N_2O emissions by improving aeration (Monteny, Bannink and Chadwick, 2006; Snyder *et al.*, 2009). Recently, using the DNDC model Deng *et al.* (2018) a reduction of 38% on soil N_2O emissions was predicted under sprinkler irrigation compared with flood irrigation, and a similar result was reported by Franco-Luesma *et al.* (2020).

Another improved crop management technique that has been suggested for limiting N_2O emissions from fertilizers is the use of slow- and controlled-release fertilizer forms, or the use of nitrification inhibitors, which slow the microbial processes leading to N_2O formation or can directly reduce N_2O emissions from the fields (Parkin and Hatfield, 2010). Nitrification inhibitor increases fertilizer use efficiency with positive effects on plant growth and inhibits NH_4^+-N oxidation, and in turn soil NO_3^--N content, thus limiting N_2O production (Vitale *et al.*, 2017). A study done by Menéndez *et al.* (2012) showed that at 40% WFPS, the compound 3,4-dimethyl pyrazolephosphate (DMPP) reduced emissions from 17% to 42%, while at 80% WFPS the DMPP efficiency decreased from 45% to 23%. Nevertheless, this effect can be modified by heavy precipitation events (Venterea *et al.*, 2012). The use of nitrification inhibitors may cause a priming

effect with a subsequent increase, which means after a period this nitrogen will be again involved in nitrification and denitrification processes (Kudeyarov, 2020). Added to those, acetylene has shown to be also a strong inhibitor, but it is difficult to apply and maintain adequate concentrations in the soil (Freney *et al.*, 2000).

Although much of the discussions about increased future yield potential has centered around engineered crop plants in order to reduce the dependence on fertilizers and to fix nitrogen by themselves, or by capitalizing C-N interactions in the rhizosphere, this technique can be useful to minimize the environmental impacts of excessive use of N in crop production (Ussiri and Lal, 2012; Signor and Cerri, 2013).

It is difficult to generalize the benefits of the different N_2O mitigations strategies based on the results of these studies, since there is a contradictory point of views for most of the cited studies. That's why it remained a real challenge due to multiple interacting factors that drive nitrification/denitrification and ultimately determine N_2O emission rates. For that, and for better understanding of successful mitigation strategies of agricultural N_2O emissions, more interdisciplinary studies of N_2O fluxes in agroecosystems with accounting for different biotic and abiotic factors are required (Chapin III, Matson and Vitousek, 2011; Han, Walter and Drinkwater, 2017b).

3. MATERIALS AND METHODS

3.1. Field N_2O measurement

3.1.1 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. The climate is continental (pannonian), characterized by an annual rainfall of 620, 552, and 694 mm, and a mean annual temperature of 11.8 °C, 12.9 °C and 12.9°C for the years of 2017, 2018, 2019, respectively. The soil is a chernozem brown forest soil (WRB, 2015: chernozem), sandy loam clay in texture, consisting of 54.9% sand, 28.1% clay, and 17.1% loam, having the following properties.

Regarding chemical characteristics, it is slightly acidic pH(H_2O): 6.3 which can be attributed to the effect of long term fertilizer application (Székely, 2004). While the amount of $CaCO_3$ of samples investigated was 1.7%. Although the amount of humus (3.6%) of the soil is good, the phosphorus and the potassium contents are (AL-P_2O_5: 160 mg/kg, AL-K_2O: 387 mg/kg), and the NH_4^+-N and NO_3^--N are: 4.5 mg/kg, 8.8 mg/kg, respectively.

The study site has a running eddy-covariance (EC) station for CO_2/H_2O gas exchange and meteorological measurements. Manual measurements were done in the vicinity of the EC station (positions within 25 m from the EC station along a 10 m long transect), while the fluxes measured by the EC system originated mainly from the surrounding 5 ha.

Gödöllő Experimental Farm Ltd. has the land management rights of the site and provided management data. The crops included in the rotation were: 2017-2018 winter wheat, 2018 rapeseed, 2019 sorghum, 2019-2020 winter wheat.

The two-year crop rotation was cultivated under a conventional management system with soil tillage, spraying, sowing, harvesting and mineral fertilizer application. Management data during the study period are shown in Table 2.

Table 2. 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 N ha^{-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

3.1.2 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 (for gas sampling times, see supplementary Table 8). The sampling campaign was done using static (closed) chambers (Christensen, Simkins and Tiedje, 1990), and which are cylinders, and easily moved. Sampling time was between 10.00 and 12.00 h, as this was reported to best represent the average daily emission (Smith and Dobbie, 2001; Van Der Weerden, Clough and Styles, 2013). Ten polyvinyl chloride (PVC) collars were inserted into the soil (2.7 cm depth) to minimize the influence of the collar on the soil structure and plant roots as mentioned by Heinemeyer *et al.* (2011) at 1 m apart along a 10 m transect (Figure 5). 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 to analyze within 24 h (Wang *et al.*, 2018).

Figure 5. Field gas sampling during different seasons.

3.1.3 N_2O detection of the field samples

Nitrous oxide concentrations were determined with an HP 5890 II gas chromatograph (Waldbronn, Germany) equipped with a Porapak Q column (2x1.8 m, 80-100 mesh) and an electron capture detector (ECD) operated at 300 °C. The injecting port temperature was 105 °C. The carrier gas was N_2 (purity of 5.5) at a flow rate of 40.6 ml/min. Calibration was performed using 0.32 ppm N_2O in N_2 gas (Figure 6).

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 N m^{-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].

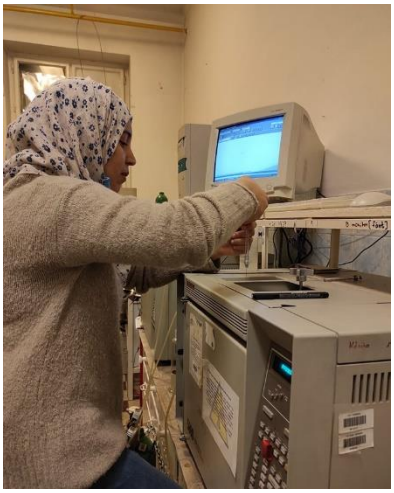

Figure 6. Laboratory N_2O measurement using HP 5890 II gas chromatograph.

3.1.4 Ancillary measurements

Net ecosystem exchange of CO_2 (NEE) was measured by the eddy covariance (EC) station representing the activity of the vegetation. The station consists of a CSAT3 sonic anemometer (Campbell Scientific, USA) and a Li-7500 (Licor Inc, USA), open-path infra-red gas analyzer, both connected to a CR5000 datalogger (Campbell Scientific, USA) via an SDM (synchronous device for measurement) interface. Air temperature and relative humidity (HMP35AC, Vaisala, Finland), precipitation (ARG 100 rain gauge, Campbell, UK), global radiation (dual pyranometer,

Schenk, Austria) incoming and reflected photosynthetically active radiation (SKP215, Campbell, UK), volumetric soil moisture content (CS616, Campbell, UK) and soil temperature (105T, Campbell, UK) were measured half-hourly (Nagy *et al.*, 2007; Pintér, Balogh and Nagy, 2010; Farkas *et al.*, 2011).

Leaf area index (LAI), VIgreen, soil water content (SWC), soil temperature (Ts), and soil bulk density (BD) were measured close to each collar simultaneously at the air sampling.
SWC was measured by time domain reflectometry (ML2, Delta-T Devices Co., Cambridge, UK; Field Scout TDR 300 Soil Moisture Meter, Spectrum Technologies, IL-USA) in the top 0-7.5 cm layer of the soil. Soil temperature was determined at a depth of –5 cm by a digital soil thermometer. Leaf area index was measured by an AccuPar LP-80 ceptometer (Decagon Devices, USA) at each measurement campaign over each plot. VIgreen index was derived from red, green, blue (RGB) values of photographs made by a commercial digital camera (Canon Eos 350D) from the same plots. VIgreen index is the normalized difference of reflected green and red light (Gitelson *et al.*, 2002):

Equation 2. VIgreen index:

$$VIgreen = \frac{Green-Red}{Green+Red},$$

where VIgreen is a dimension less index, *Green* and *Red* are the component values of a digital image. VIgreen was calculated in R (R core Team, 2019).

Bulk density was calculated from the compactness of the topsoil layer measured by a penetrometer (Eijkelkamp, The Netherlands).

3.1.5 Microbial investigations.

In addition to the soil physicochemical properties measurement that were done before starting the the field study, soil sampling was also performed for microbiological investigations, where soil samples were collected from the same used field (Kartal), then were stored in at -20 °C until the measurement times. Samples were chosen based on the N_2O emissions and were correspondingly marked as S1, S2, S3, S4, S4, and S5 for those dates: 06/15/18, 08/27/18, 09/26/18, 04/25/19, and 06/26/19, for the following measurements.

3.1.5.1 Analysis on metabolic functions of soil samples microbial communities by Biolog Eco microplates

The capability of soil samples microbial communities to utilize a variety of carbon sources was assessed by using Biolog Ecoplate. Every plate had 96 wells containing 31 different carbon sources and one blank in three replicate sets. These carbon sources are included in various groups

(Table 3), which are made up of six kinds of carbon sources, including carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides (Gryta, Frąc and Oszust, 2014; Ge *et al.*, 2018). The basic principle is that there is a redox indicator (a tetrazolium salt) in each well, which changed from colorless to purple if added microorganisms utilize the substrate (Cacchio and Del Gallo, 2019). Ecoplate was prepared in the following way (Figure 7):

Preparation of sample solution and plate cultivation

Firstly, 1 g of the collected soil samples were taken and suspended in tubes containing 9 ml of 0.85% stroke-physiological saline solution for each one. The mixture was shaken, and then the suspension was then left to settle again. Then 1 ml from the supernatants were separately diluted to a 10^{-3} gradient. Inoculation was accomplished by pipetting 120 µl of this suspension to each well of the Biolog Ecoplate using a multichannel pipette. Where transferring time of the suspension to the plates should be shortened within 5 minutes, or else the 3 replications in one plate would have difference due to time difference (Xu, Ge and Poudel, 2015). Microplate inoculation should be done under sterile conditions in a laminar-flow hood in order to reduce the interference of microbes from another environment. Then, the microplates were placed in their bags to avoid desiccation and were incubated at a constant temperature (25 °C) continuously for 216 h. However, there are controversial studies concerning the timing which should be used, Cai *et al.* (2010) reported that since fungi will spread after 96 h inoculation, so the time of 72 h or 96 h is the more reasonable, while Jia, Dong and Zhou (2013) supposed that 144 h or 168 h is better because the OD590 nm value is still in fluctuation before that time. So 168 h of incubation results were used in our study for the assessment of microbial functional diversity and statistical analyses.

Finally, and during cultivation, absorbance values of the microplates were read at 590 nm wavelength in each 24 h until 216 h for analyzing the metabolic fingerprints using a Microplate Reader BMR-100 (BORCO Germany). The Biolog Ecoplate method usually measures optical density (OD) at 590 nm because the peak absorbance of the tetrazolium dye occurs at 590 nm (Muńiz *et al.*, 2014). Nevertheless, we used absorbance values at 490 nm as was used in a study done by Feigl *et al.* (2017), because our microplate reader was equipped with 340, 405, 450, 492, and 630 nm filters, but the optimal OD values were provided at 490 nm (Nagy *et al.*, 2013).

Figure 7. Biolog Eco miroplate measurement.

Table 3. The 31 kinds of carbon substrates of Biolog Eco microplate (Ge *et al.*, 2018).

Chemical guild	Plate number	Substrates	Chemical formula
Miscellaneous	B1	Pyruvic acid methyl ester	$C_4H_6O_3$
	G2	Glucose-1-phosphate	$C_6H_{13}O_9P$
	H2	D,L-a-Glycerol phosphate	$C_3H_9O_6P$
Polymers	C1	Tween 40	-
	D1	Tween 80	-
	E1	a-Cyclodextrin	$C_{36}H_{60}O_{30}$
	F1	Glycogen	$(C_6H_{10}O_5)n$
Carbohydrates	G1	D-Cellobiose	$C_12H_{12}O_{11}$
	H1	a-D-Lactose	$C_{12}H_{12}O_{11}$
	A2	Methyl-D-glucoside	$C_7H_{14}O_6$
	B2	D-Xylose	$C_5H_{10}O_5$
	C2	i-Erythritol	$C_4H_{10}O_4$
	D2	D-Mannitol	$C_6H_{14}O_6$
	E2	N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$
Carboxylic acids	F2	D-Glucosaminic acid	$C6H_{13}NO_6$
	A3	D-Galactonic acid latone	$C_6H_{10}O_6$
	B3	D-Galacturonic acid	$C_6H_{10}O_7$
	C3	2-Hydroxy benzoic acid	$C_7H_6O_3$
	D3	4-Hydroxy benzoic acid	$C_7H_6O_3$
	E3	g-Hydroxy butyric acid	$C_4H_8O_3$
	F3	Itaconic acid	$C_5H_6O_4$
	G3	a-Keto butyric acid	$C_4H_6O_3$
	H3	D-Malic acid	$C_4H_6O_5$
Amino acids	A4	L-Arginine	$C_4H_{14}N_4O_2$
	B4	L-Asparagine	$C_4H_8N_2O3$
	C4	L-Phenylalanine	$C_9H_{11}NO_2$
	D4	L-Serine	$C_3H_7NO_3$
	E4	L-Threonine	$C_4H_9NO_3$
	F4	Glycyl-L-glutamic acid	$C_7H_{12}N_2O_5$
Amines/amides	G4	Phenylethylamine	$C_8H_{11}N$
	H4	Putrescine	$C_4H_{12}N_2$

The endpoints calculated from the corrected data were the following: average well color development (AWCDa), Shannon-Wiener diversity index (H′), Shannon evenness index (E), Simpson diversity index (D), and substrate average well color development.

Determination of average well-color development values

Microbial activity in each microplate was expressed as average well color development (AWCD), which measured microorganisms' capability to utilize different carbon sources (Garland and Mills, 1991). Samples with larger variation were thought to have a higher carbon source utilization capability and tend to have higher microbial abundance (Garland, 1997). Average well color development (AWCD) was calculated for all carbon sources with the following equation, according to Ge *et al.* (2018).

Equation 3. Average well color development for all carbon sources

$$AWCD = \sum_{i=1}^{n} (Ci - R)/n$$

where, C_i is the absorbance value of each reaction well at 590 nm, R is the absorbance value of the control well (the blank one (inoculated but without a carbon source), and n is the number of wells. (Ci–R) less than 0.06 of wells are calculated as zero (Classen *et al.*, 2003).

Calculation of metabolic functional diversity indices

Using Biolog Eco microplates calculation method based on functional diversity indices (Zak et al., 1994) the diversity of communities could be investigated. In addition, Strong (2016) extended the concept of evenness to characterize the utilization levels and utilization patterns of microorganisms by carbon source. The following metabolic-ecological indexes were calculated based on the ODs at 168 h, when the community reached the plateau.

(1) Shannon-Wiener diversity index (H') (Keylock, 2005; Spellerberg, 2008)

Equation 4. Shannon-Wiener diversity index (H').

$$H' = -\sum Pi \ln Pi$$

Equation 5. Pi (The ratio of the absorbance of each substrate to the sum of the absorbance for all the substrates).

$$Pi = (Ci - R)/\sum (Ci - R)$$

where Pi is calculated as the ratio of the corrected absorbance value (ODi) in the i^{th} (1 to 31) to the sum of the absorbance value (∑ODi) of all wells in the plate (Ge et al., 2018).

(2) Shannon evenness index (E) (Keylock, 2005), this index focuses on the evenness of ci values across all utilized substrates (Sofo and Ricciuti, 2019).

Equation 6. Shannon evenness index (E)

$$E = H'/\ln S$$

S represents the total number of utilized carbon sources (31 carbon sources), the number of wells that vary in color.

(3) Simpson diversity index (D)

Equation 7. Simpson diversity index (D)

$$D = 1 - \sum Pi^2$$

Principal component analysis

For a more detailed analysis, the AWCD for each group separately of the carbon substrates (six classes of compounds, Table 3) were calculated.

3.1.5.2 Enumeration of microbial populations

In order to estimate the number of colony forming units (CFU) of cultivable microorganisms, plate count methods that rely on bacteria growing a colony on a nutrient medium was used (Figure 8). To ensure that an appropriate number of colonies will be generated several dilutions are cultured. The laboratory procedure involves making serial dilutions of the sample which were prepared by adding 1 g of soil to 9 ml of sterile distilled water. Suspensions were homogenized and shaken. After that serial dilutions were prepared, and 25 µl of dilutions 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were used (Grantina *et al.*, 2011), and cultivating these on different culture media agar in a dish that is sealed and incubated, caseine agar, Frazier agar, Rose Bengal Agar with chloramphenicol, for bacteria population, actinomyces, ammonification, and fungi in a sample, respectively, with repeated replica plating for each dilution. Media were prepared according to the composition and sterilized in an autoclave. The inoculated plates were incubated at temperature of 25 and 30 °C at the duration of 1-3 days for bacteria population, actinomyces, and ammonificans and 5-7 days for fungi (Nakho and Dkhar, 2010). After the incubation period, the suited dilution was chosen (10^{-3} for bacteria population, actinomyces, and ammonificans, for fungi dilution of 10^{-2} was chosen), and the colony forming units were counted and expressed as CFU g^{-1} of soil.

Denitrifying bacteria were enumerated by the Most Probable Number (MNP) technique using both modified media of Alexander and Clark (1965). Each sample was inoculated in 25 tubes for 5 appropriate successive dilutions. All assays were performed in triplicate and all tubes were incubated for 5-8 weeks at 30 °C. Following incubation, the detection of positive samples was based on the counting of the positive tubes which accumulated gas bubble in the inverted Durham tubes together with the color change of the liquid medium. A Most Probable Number (MPN) table was used to determine numbers of denitrifying bacteria on cell/ml.

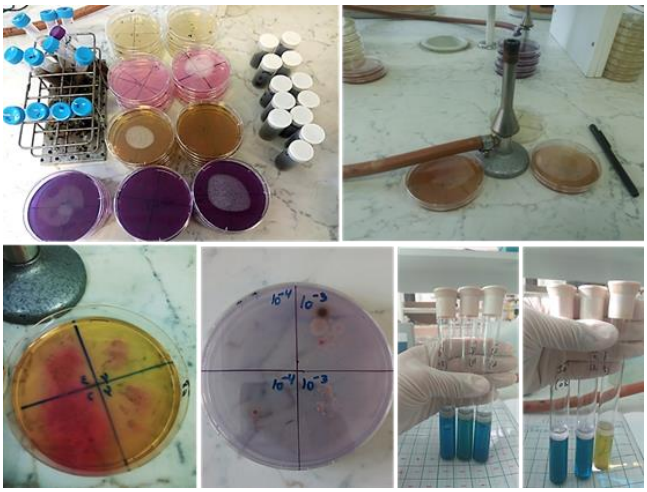

Figure 8. Enumeration of microbial populations.

3.1.5.3 DNA extraction and metagenome analysis

DNA was extracted from soil samples (100 ± 1 mg) using Quick-DNA Fecal/Soil Microbe Microprep Kit (ZYMO Research, CA, USA) following the manufacturer's instructions. The yield and purity of DNA extracts were quantified using an Implen Nanophotometer P300 (Implen GmbH, München, Germany). Purified DNA from five samples per sampling time (MI1: 06/15/18, MI2: 08/27/18, MI3: 09/26/18, MI4: 04/25/19, and MI5: 06/26/19) were pooled and used as a template for sequencing analysis. The abundance of the bacterial and fungal communities of soil samples were estimated using high-throughput sequencing on Illumina MiSeq platform at UD-GenoMed Ltd. (Debrecen, Hungary). The V3-V4 region of 16S rRNA gene (in the case of bacteria) and the ITS1 region (in the case of fungi) were amplified from the microbial DNA extracted from each sample with the following primers: 16S forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', 16S reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3', ITS forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA-3', ITS reverse: 5'-

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGATGC-3'. The next steps were similar in both cases. 12.5 ng DNA and the KAPA HiFi Hot Start Ready Mix (KAPA Biosystems, Wilmington, Massachusetts, US; Roche AG, Switzerland) was used to perform 25 cycles of PCR amplification, with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. Post-amplification quality control was performed by on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). MagSi-NGSPrep Plus (Magtivio B.V., The Netherlands) magnetic beads was used to purify the amplicons away from the free primers and primer dimer species. For the Index PCR the Nextera XT Index Kit was used (Illumina, San Diego, CA, USA) with 502, 503, 504, and 701, 702, 703, 704, 705, 706 index primers. To perform the PCR reaction the KAPA HiFi Hot Start Ready Mix was used with the following parameters; 8 cycles with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. Before the library quantification MagSi-NGSPrep Plus (Magtivio B.V., The Netherlands) magnetic beads was used to clean up the PCR products. For the library validation 1 µl of the diluted final library was run on a Bioanalyzer DNA 100 chip on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Next, each library was normalized, pooled and loaded onto the Illumina MiSeq platform for 2x250 bp paired-end sequencing.

16S rRNA gene and ITS1 paired-end amplicon reads were processed using the Frogs pipeline (Escudié *et al.*, 2018). Briefly, forward and reverse reads were filtered and merged using vsearch (Rognes *et al.*, 2016) with the parameters: min amplicon size: 44; max amplicon size: 550; mismatch rate: 0.15). Merged sequences were clustered using swarm (Mahé *et al.*, 2014). Chimera sequences were removed using remove_chimera.py from the Frogs pipeline. Taxonomic assignment was performed using BLAST (McGinnis and Madden, 2004) against SILVA_SSU_r132_March2018 database (Quast *et al.*, 2013) for ribosomal small-subunit RNA and UNITE Fungi 8.2 database (Abarenkov *et al.*, 2010) for the fungal internal transcribed spacer region.

3.2. Lab measurements.

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

Soil characteristics

Before establishing lab experiments soil samples were collected to measure their characteristics, where SOM (%), the amount of $CaCO_3$ (%), pH(H_2O), pH(KCl), NO_3^- (mg/kg), NH_4^+ (mg/kg), total nitrogen (TN) (mg/kg) and bound (plasticity, K_A) index were investigated, the principal characteristic of the soils samples from eash experiment are presented in Table 4.

Table 4. Soil samples properties of the different lab experiments, a: sample from 1st serie of the 1st experiment, b: sample from the 2nd serie of the 1st experiment, c: sample from cropland soil of the 4th experiment, d: sample from forest soil of the 4th experiment.

Experiment number	SOM (%)	$CaCO_3$ (%)	pH (H_2O)	pH (KCl)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	TN (mg/kg)	KA
1st experiment	7.4 [a]	6.4 [a]	6.4[a]	6.3 [a]	10.5 [a]	5.0 [a]	677.6 [a]	42.3 [a]
	7.7 [b]	6.3 [b]	6.3[b]	6.5 [b]	14.0 [b]	4.5 [b]	1797.3 [b]	42.4 [b]
2nd experiment	7.5	6.4	6.4	6.5	12.0	5.0	2189.3	42.4
3rd experiment	7.2	6.6	6.7	6.5	7.5	6.5	621.6	40.8
4th experiment	7.8 [c]	6.7 [c]	6.7 [c]	6.5 [c]	3.5 [c]	6.5 [c]	1125.6 [c]	41.6 [c]
	6.8 [d]	6.8 [d]	6.8 [d]	5.1 [d]	2.5 [d]	6.0 [d]	894.6 [d]	48.4 [d]

3.2.1 Lab experiments design and N_2O emission measurements

3.2.1.1 First experiment

A first lab experiment was done using soil from the same field (Kartal) under controlled conditions. 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 experiment 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, pots were brought to the selected SWC and were incubated for 4 d in the purpose of avoiding the pulse of respiration associated with wetting dry soils (Kieft and others, 1987). Ammonium nitrate (NH_4NO_3) 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).

This 1st experiment contained two series, each one divided into bare and planted soil (with wheat). The first series of 27 pots was treated with ammonium nitrate fertilizer, 0, 50, and 100 kg N ha^{-1}, under 20% SWC, 3 and 6 repetitions were done for bare and planted soil, respectively. A series of 30 pots was treated with different fertilizer rates, 0, 75, and 150 kg N ha^{-1}, under 25% SWC.

N_2O flux measurements were done weekly during 4 and 5 weeks, for the first and the second series respectively.

After each measurement, an amount of water corresponding to the evaporation losses was added to each pot using distilled water to achieve the target soil water content.

Later on, we decided to increase the frequency of the measurements of the other experiements.

3.2.1.2 Second experiment

A second lab experiment was performed under controlled conditions, containing repeated series of combinations of bare and planted soil (with maize), two SWC levels, and different rates of ammonium nitrate fertilizer (Table 5). These treatments were combined during the experiment resulting 12 combinations with 3 repetitions (36 pots) and the experiment was repeated 3 times (108 pots). This experiment was done using the same soil and the same principle which were used in the previous experiment (1st experiment).

Table 5. Treatments during the 2nd lab experiment. These treatments were combined in each series of the experiment.

Plant presence	SWC V%	N input (kg N ha^{-1})
Planted soil Bare soil	<30 (15, 20 and 25%) >30 (35 and 40%)	0 75 150

3.2.1.3 Third experiment

Cropland soil was used for another experiment which was done using 36 pots, divided into bare and planted soil (with maize), where two soil water content levels were chosen (20% SWC and 40% SWC). 0, 75, and 150 kg N ha^{-1} ammonium nitrate fertilizer was used, with 3 repetitions for each treatments. We used the same experiment steps as it was mentioned in the 1st experiment, except in this experiment maize plant was grown during for around 8 days before their transplanted to the pots (Figure 9). N_2O flux measurements were performed for a period of 445 h in which a D-(+)-glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) (250 mg glucose kg^{-1} soil) addition was done after 241 and 439 h from fertilization.

3.2.1.4 Fourth experiment

N_2O emission from three different soil types

An additional experiment was done, where the N_2O measurements were measured from three soil types: the first soil sampling which was done from our principal study site (cropland soil), while the second was a forest soil that sampled in the Botanical Garden of the Hungarian University of Agriculture and Life Sciences, and as the third type we used sterilized sand.

Soil sampling was done like in the previous experiments, soils were collected from the top 15 cm layer from the sites and transported into the lab. PVC tubes (10.2 cm in diameter and 20 cm height) were used as pots filled up to 15 cm with about 1.69, 1.52, and 2.12 kg for cropland, forest soils and sand, respectively. The top 5 cm layers of the tubes were used as static chambers during the N_2O emission measurements.

The experiment contained a series of 9 pots for each soil type. The soils were preincubated at 80% WFPS for 4 d in the purpose of avoiding the pulse of respiration associated with wetting dry soils (Kieft and others, 1987). The 80% WFPS condition was chosen to ensure anaerobic condition and denitrification occurrence, and soil water-filled pore space was calculated using the gravimetric water content (%), total soil porosity, and soil bulk density (Ding *et al.*, 2007):

Equation 8. Water-filled pore space

$$\text{WFPS (\%)} = \frac{\text{gravimetric water content (\%)}}{\text{total soil porosity}} \times \text{soil bulk density} \times 100$$

where total soil porosity = 1– (soil bulk density/soil particle density).

In order to compare the N_2O emission from the three diffrents soil types, each one received the same fertilizer type: sodium nitrate ($NaNO_3$), except for forest soil another experiment was done using ammonium nitrate (NH_4NO_3) fertilizer to check the effect of fertilizer type on the N_2O emission, at the rate of 0, 75, 150 kg N ha^{-1}. Concerning sterilized sand since it does not contain any microbes, a microbial solution was prepared using 1 g of soil in 9 ml of distilled water, and an amount of 1 ml was added to the pots for creating a microbial environment together with a portion of carbon source that's is D-(+)-glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) (250 mg glucose kg^{-1} soil) before adding the fertilizer and starting the measurement.

After measuring the N_2O emission for several days, a carbon source was added (250 mg glucose kg^{-1} soil) (Giles, Daniell and Baggs, 2017) to all the pots, in order to examine the effect of glucose addition on the N_2O emission from the three soil types. Glucose addition was done in several portions and during different times based on the N_2O emission tendency and the appearance of the N_2O concentration baseline. Also, during the measurement, microbial solution, and other fertilizer portions were added to check which drivers were responsible for the results found. 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.

Figure 9. N_2O laboratory experiment

Easily degradable carbon (EDC)

Before establishing the 4th experiment quantifiable parameters that can be useful for the comparison of the emission between the different soils were measured. The basic physicochemical parameters of the soils are in Table 5. Cropland and forest soil samples were used for measuring the easily degradable carbon (Figure 10) as it was reported by Weil *et al.* (2003) in which diluted potassium permanganate ($KMnO_4$) reacts with the most readily oxidizable (active) forms of soil C, converting Mn(VII) to Mn(II), and proportionally lowering absorbance of 550 nm light. Known also as permanganate oxidisable carbon (POXC) and synonymous with 'active carbon', it was measured as follows.

Air-dried samples were passed through a 2.0 mm sieve to remove large pieces and plant material. After that, a 5 g soil sample was mixed with 2 ml of 0.2 $KMnO_4$ in 1 M $CaCl_2$ (Calcium chloride, pH 7.2), and then using distilled water it was diluted to 20 ml. After 2 min of shaking (about 100 strokes/min), the sample was left for 5-10 min to allow the soil to settle. Tubes were protected from direct light.

Using a clean pipette a 0.5 ml was taken of a clear liquid from the upper 1 cm of the soil-$KMnO_4$ suspension was then added to a tube with distilled water to dilute it to 100 times, and the obtained solution was used for absorbance measurement using spectrophotometric analysis (λ = 550 nm, Hitachi, U-2900). The calibration curve was produced using standards of 0.005, 0.01, and 0.02 M $KMnO_4$, in 0.1 M $CaCl_2$, which were prepared by adding 1.25, 2.50, or 5.00 ml of 0.2 M

$KMnO_4$ stock solution to and diluting to the 50 ml mark with distilled water. Where stock solution was made by 0.2 M $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2). Adjust pH to 7.2 using 0.1 M sodium hydroxide (NaOH).

Calculation. The lower the absorbance reading or the greater the $KMnO_4$ color loss, the greater the amount of oxidizable C in the soil. To estimate the amount of C oxidized, we used the assumption of Blair, Lefroy and Lisle (1995) that 1 mol MnO_4^- is consumed (reduced from Mn^{7+} to Mn^{2+}) in the oxidation of 0.75 mol (9000 mg) of C: EDC was calculated using the following equation (Weil *et al.*, 2003).

Equation 9. Easily degradable carbon

$$\text{Active C}\left(\frac{\text{mg}}{\text{kg}}\right) = [0.02\ \text{mol/l} - (a + b\,\text{absorbance})] \times (9000\ \text{mg C/mol}) \times (0.02\ \text{l solution}/0.005\ \text{kg soil})$$

where 0.02 mol/l is the initial solution concentration, *a* is the intercept and *b* is the slope of the standard curve, 9000 is mg C (0.75 mol) oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{2+}, 0.02 l is the volume of $KMnO_4$ solution reacted, and 0.005 is the kg of soil used.

Some measurements were also performed by the modified version of the above method (Wolińska *et al.*, 2018).

Figure 10. Easily degradable carbon measurement

Lab N_2O concentration measurement and flux calculation

For all 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. Except for the 1st experiment, a gas sampling was done manually using a Hamilton syringe and air samples from the chambers were taken at 0, 10, and 20 min after closure for determining the N_2O concentration using an HP 5890 II gas chromatograph, electron capture detector technique.

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

3.3. 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 10. 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.

For the analysis on metabolic functions of soil samples microbial mommunities by Biolog Eco microplates, the results were expressed as means ± standard deviations. R program was used to create figures, Student's t-test was used to check the significant differences.

4. RESULTS AND DISCUSSION

4.1. Field experiment

4.1.1 Environmental conditions in the study period

The average SWC of the site during the study period varied from 9.9 to 50.5%. Maximum value of soil water content for the year of 2018 was observed in March (41.9%), and for 2019 in November with a value of 50.5 % (Figure 11. upper panel, blue dots).

The lowest SWC in 2018 was 17.12% measured in May during the measurement campaigns, while in 2019, the lowest value of 9.9% was observed in January. During the study period T_s data at 5 cm depth varied between 1.2 and 33.1 °C, with the highest data of 2018 (31.7 °C) obtained in July, and 33.1 °C at the end of April in 2019. The lowest soil temperature data for the years 2018, 2019 were 2.2 and 1.7 °C measured in February and January, respectively (Figure 9. upper panel, red dots).

Air temperature measured by EC station showed a maximum value (34.6 °C) on 12 August 2019 while a minimum of –11.6 °C was recorded on 28 February 2018.

For the years 2018 and 2019, the values of VIgreen varied between –0.06 to 0.34 and –0.06 to 0.26, respectively, with a value lower than 0 meaning no vegetation in the field (fallow periods), while a rapid increase in the values was observed after sowing and germination. The highest VIgreen values were related to the peak green biomass of the crops, which was observed on 16th of April 2018 in wheat (0.34) and on 26th of June 2019 in sorghum (0.24). The values of LAI were equal to 0 m^2 m^{-2} when no vegetation was present in the field and the highest values were observed during the last stages of crop growth, 5.0 and 5.6 m^2 m^{-2} on the 16th of May 2018 and 15th of August 2019, respectively (Figure 11, middle panel).

4.1.2 Seasonal variations of the N_2O emissions

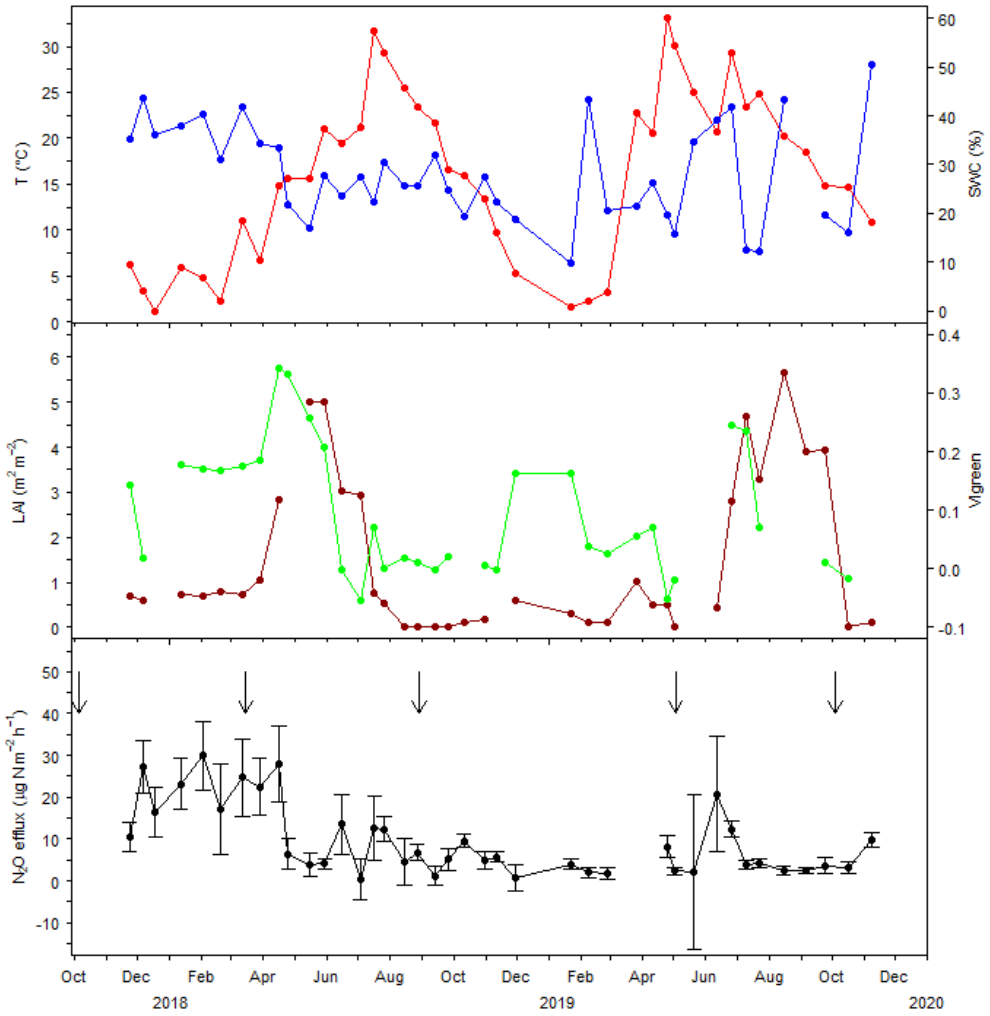

Figure 11. Temporal variations of soil temperature (T_s, °C, red dots) at a depth of 5 cm, soil moisture (SWC, %, blue dots) in the 0-7.5 cm soil layer (upper panel), VIgreen index (VIgreen, green dots), Leaf area index (LAI, m^2 m^{-2}, brown dots) (middle panel) and nitrous oxide (N_2O) emission (lower 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 11 (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 temporal pattern of emissions typically showed distinct emission episodes after fertilizer applications (cf. arrows in Figure 11, lower panel), with the largest emissions often coinciding also with elevated soil water content. The highest emissions during the study were detected during the period of December 2017- April 2018 characterized by higher SWC and crop presence (winter wheat), with another higher emission in 2019 recorded on 12 of June.

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. Peng *et al.* (2019) found in a study that N_2O emission rate was high during the freeze-thaw period and reported that it was mainly due to the release of substrates, the maintenance of high enzyme activities at the freezing stage added to the fast recovery of microbial biomass nitrogen and high microbial activities during this period. Moreover, three of our chambers seemed to function as hot spots on the same sampling day, resulting high variability of emissions.

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 33.5 % and a T_s of 14.9°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 (0.34). 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 43.6% SWC and 3.4 °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. A recent study affirmed the association between higher N_2O emission rates and higher denitrification rates and also reported that the main source of N_2O in the annual crop rotation was the denitrification process (Putz *et al.*, 2018). 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) who reported that high N_2O emission could even be observed at 65% WFPS at a soil temperature of 4.5 °C and NO_3^--N content 5 mg kg^{-1} soil. 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).

Nitrogen content of soil could be the main factor affecting soil N_2O emissions (Nan *et al.*, 2016). Our results suggests that N fertilization significantly enhanced N_2O emissions even after two months following the applications of N fertilizers which was in accordance with a study

reporting that N_2O emissions induced by N-fertilizers are concentrated in some weeks after the fertilizer application (Schils *et al.*, 2008). Several studies pointed out the fact that the presence of plants generally stimulates N_2O emissions. Firstly, roots and heterotrophic organisms could remove oxygen from the rhizosphere increasing O_2 demand, which in turn makes it more prone to denitrification. Secondly, the presence of plants supports denitrification of the rhizospheric organisms by providing electron donors (i.e., easily decomposable OM) once the O_2 is depleted (Hayashi *et al.*, 2015). Besides, plant phenology also affects the magnitude of plant effects on N_2O production which was observed in our results when a higher emission was measured during the physiological maturity stage of winter wheat growth, also during the beginning of the heading stage. Our data correspond to a previous study which indicated that the seasonal contribution of N_2O emissions from plants to ecosystem emissions was significantly higher (62%) at the heading stage than at wheat tillering (10%) (Zou *et al.*, 2005).

The lowest N_2O emissions (0.27 ± 4.92 μg N m^{-2} h^{-1}) was observed on the third of July 2018, 14 weeks after fertilization at 27.4% SWC and 21.1°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 proving that in cases of limited availability of N in the soil or once the effect of applied N subsides, N_2O emissions are reduced and N_2O is emitted at slow rates (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 (8 weeks after 200 kg N ha^{-1} NPK fertilization), this low emission 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 (Conen, Dobbie and Smith, 2000; 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 of September 2018 (1.26 ± 2.23 μg N m^{-2} h^{-1}) two weeks after N application (Figure 11) despite the fact that the SWC and T_s were favorable (31.9% and 21.6 °C, respectively) for the N_2O production. Our data corresponded with the results published by Ball, McTaggart and Watson (2002) who found that N_2O emissions were not always enhanced by the application of N-fertilizers itself.

After this low N_2O emission, an increment in the emission was observed in the next 2 sampling days. On the 26th of September 2018, emission of 5.22 ± 2.59 μg N m^{-2} h^{-1} associated with 24.8% SWC and 16.6 °C was observed. Higher emission was also recorded on 11th of October 2018 (9.63 ± 1.52 μg N m^{-2} h^{-1}), this emission was accompanied by 19.1% and 15.9°C. The fact that we did not observe a high N_2O peak either on 13th of September 2018 could be caused by an

occasionally heavy precipitation after 4 days from the fertilizer application and the lack of measurements during this time (11 days before the 13th of Septembre gas sampling).

Besides, on 13th of September 2018 the SWC level was favorable for denitrification, not nitrification, which makes us propose another hypothesis if we cancel the first suggestion that related to the precipitation and lack of measurements during that time. We can suggest that this low emission could be primarily because during this time there was a lack of the population that mediated the denitrification process. Besides, the low availability of easily decomposable organic C required as an energy source to consume NO_3^- (Wrage *et al.*, 2001) because N_2O production has been reported to be significantly correlated with soil total organic C content (Jahangir *et al.*, 2012). Also, it should be remembered as other several studies (Fierer, Bradford and Jackson, 2007) have shown that the addition of easily degradable organic C was significantly correlated with the abundance of Alpha and Betaproteobacteria. During this sampling time, there were no plants in the field (it was 3 days after rapeseed sowing), so there was a lack of root exudates that can favor the denitrification process as well.

The appearance of the emissions again after a lower one may be due to the microbes diversity and their metabolic activity, with the presence of the bacterial populations responsible for the N_2O production via nitrification process, as we recorded aerobic condition during this time. Also, the higher emissions after a very lower low one could be caused not only by the occurrence of the nitrification process, since denitrification process can also take place in some microsites even under aerobic conditions. In addition and contrary to the previous sampling day (13th of September 2018), plant presence could be a reason that favors denitrification in some microsites. Where a positive interaction between plant and bacterial diversity was apparent in a study done by Zeng *et al.* (2016), consistent with the theory that plant diversity enhances the diversity of soil microbes by increasing the range of food resources available (Van Der Heijden, Bardgett and Van Straalen, 2008). Also, a shift in the community composition between unplanted and planted soils was reported by Philippot *et al.* (2002).

Besides, Enebe and Babalola (2020) reported that maize plants have very significant effects on the selection and enrichment of soil microbes community.

Later, on 31th of October 2018 the emission was decreased a bit 4,92 ± 1,96 μg N m^{-2} h^{-1} with increasing SWC to 27.4 %, and it increased a bit after 12 days to reach on 12th of November 2018, 5.78 ± 1,11 μg N m^{-2} h^{-1} (associated with 22.3% SWC). So it seemed that the N_2O emission during this time increased under aerobic condition and produced via nitrification process,that

supported our proposed causes explaining the N_2O dynamic during this period of field measurement.

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 as reported by Ma *et al.* (2020). Added to the environmental factors (precipitation) that affect soil properties like; soil water content.

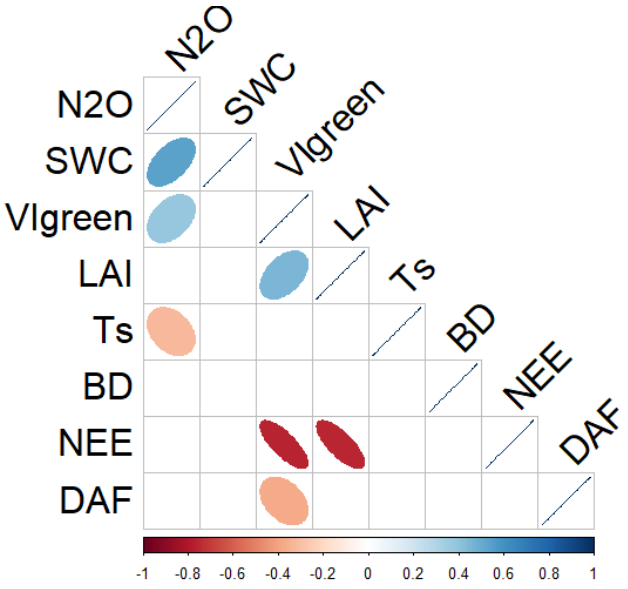

Figure 12. Correlation plot between nitrous oxide efflux and different driving variables, SWC (soil water content), VIgreen (VIgreen index), LAI (leaf area index), T_s (soil temperature), BD (bulk density of the soil), NEE (net ecosystem exchange of CO_2), DAF (day after fertilization). Only statistically 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 12), 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 (T_s) 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.

We aslo used the variable "days after fertilization, DAF" for checking the correlation between fertilization timing and N_2O emission, but we found no significant correlation between them.

More variance can be explained by a multiple linear regression including SWC and VIgreen as independent variables (r^2= 0.5052, p <0.001).

Equation 11: 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.

4.1.3 Field microbial investigations.

4.1.3.1 Analysis on metabolic functions of soil samples microbial communities.

The AWCD of all carbon sources in soil microbial communities.

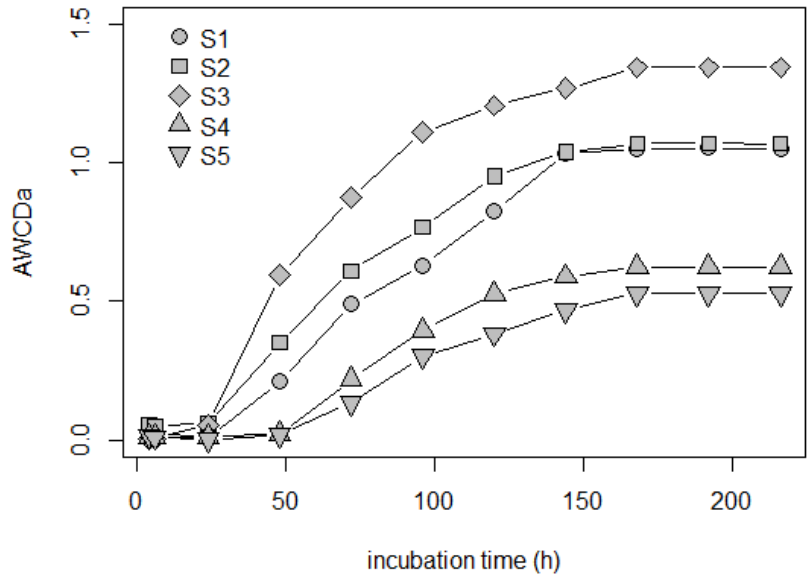

Figure 13. Dynamics of the Average Well Color Development (AWCDa) of five soil samples microbial communities during the incubation time 216 h (9 days), at 28 °C.

Since N_2O emission is mediated by microbial populations we investigated their metabolic activity by using Biolog Ecoplates, where in general it was proportional to the degree of carbon source oxidation of corresponding microbes, which could be characterized by AWCD (Garland and Mills, 1991). Moreover, development phases of the samples were showed from the AWCD graphics, which are lag and exponential phases. The adaptation of the community to substrate degradation may be shown by the lag phase, which may also be an indicator of the low number of microorganisms, in which new enzymes for the organic matter will synthesize by microorganisms (Poyraz and Mutlu, 2017).

The AWCDa (AWCD of all carbon sources) of the five soil microbial communities are shown in Figure 13. The results showed that the AWCDa of the five soil samples exhibited an apparent lag phase on the first day for samples 1, 2, and 3. And around 2 days lag phase for samples 4 and 5. Then significant increases in the average absorbance of all samples in microplates were appeared, which demonstrated that the five soil microbial communities were capable of metabolizing organic substrates in Biolog Eco microplates. The rapid response can be correlated with high population rates. The measurement of the metabolic activity analysis was done during a period of 9 days (216 h), and the slopes of AWCDa curves within this period represented average metabolic rates of the microbial communities (Kong, Wang and Ji, 2013). After 4 days of the incubation period, the increased rate of AWCDa was slower. The average of the AWCDa index for all soil samples (Figure 13) was the highest and reached the peak on day 7 (168 h) of incubation

stating that all cultivable microorganisms enable to steadily use carbon sources during the stable period (Miyake *et al.*, 2016). Where, the highest AWCDa index, after 168 h of incubation, was calculated for sample number three that was sampled on 26th of September 2018, after 27 days from fertilization and 16 days after rapeseed sowing, and it was increased from 0.004 on 4 h to around 1.345 after 7 days, and its metabolic rate was faster than the other soil samples especially S4 and S5. Whereas the lowest (0.529) was in the S5 sampled in 26th of June 2019 after 54 days from fertilizer application, and during the sorghum boot stage, S4 (0.625), and the S1 and S2 were approximately the same (1.054), and (1.070), respectively. In the group with the highest metabolic levels, there were S3, S2, and S1, whereas, the group with a lower metabolism consisted of the S5 and S4 samples, which indicated that the utilization of substrates by S4 and S5 were less efficient than the others.

After 168 h of incubation of the Biolog Eco microplates (Figure 13), it can be noted that there were significant differences in the AWCDa among five soil microbial communities ($p < 0.05$), except between the S2 and S1 no significant differences was recorded, and the order was S3 > S2 > S1> S4> S5, which suggested that soil properties (soil temperature and soil water content) together with soil management practices affected soil microbial communities and their activity.

Metabolism of different biochemical categories of substrates

Ecoplate contained 31 carbon sources in three replicate sets: according to the biochemical properties of carbon sources, the 31 substrates in the Biolog Eco microplates were assigned into six categories, including carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides (Tian-Yuan *et al.*, 2014), the AWCD of those six categories were showed in the Figure 14.

The results indicated that microbial functional diversity changed over time and the utilization of six types of carbon sources by microbes presented an increasing trend with the prolongation of incubation time. For miscellaneous, amines/amides, and polymers there were no significant difference in the utilization among the five microbial communities, however, differed significantly ($p < 0.05$) for carbohydrates, carboxylic acids and, amino acids. For carbohydrates the significant difference was shown between, S1 and S3, S1 and S4, S1 and S5, S2 and S3, S2 and S4, S2 and S5, and S3 and S4, S3 and S5. While for carboxylic acids the difference was recorded between S1 and S4, S1 and S5, S3 and S4, and S3 with S5, for amino acids only beteween S3 and S4 and S5 significant differences were recorded. Where S1 was sampled during the physiological maturity of winter wheat, S2 after around 4 weeks from tillage application, S3 as it was mentionned above was 27 days after fertilizer application and 16 days after rapessed sowing, contrary to S4 which

sampled whene there was no activity, it was just 1 week before sorghum sowing, and finally soil sample number 5 was collected 7 weeks after fertilization and during the sorghum boot stage.

Also, it was shown that the capacity utilization of six-type carbon sources was different. The microbial communities in the five soil samples initially preferred C-substrates from the carbohydrates, but for S2, not just carbohydrate was prefered but miscellaneous and polymers groups were utilized initially. For the other microbial communities of the other soil samples miscellaneous and polymers groups were used from the 48 h of incubation. Later on carboxylic acids amino acid groups were also used in all soil samples. During the exponential phase, an increasing number of substrates (belonging to six five groups) was utilized, except for the amines/amides which was utilized in S1 initially and during the exponential stage it was utilized in all the samples except in S5, that's why it had the lowest AWCD (Figure 14). Thereby illustrating that carbohydrates were the carbon sources with the highest degree of metabolic utilization (S3 has the highest level of metabolic utilization of carbohydrates), refer to the degradation capacity, where a high catabolic capacity may indicate a high number of heterotrophic bacteria (Poyraz and Mutlu, 2017), and the lowest degree of metabolic utilization was amines/amides. Whereas, other studies reported similar results that carbohydrates utilization was the highest whereas the lowest utilization substrates differed from microbial communities (Kong, Wang and Ji, 2013; Tian-Yuan *et al.*, 2014; Ge *et al.*, 2018).

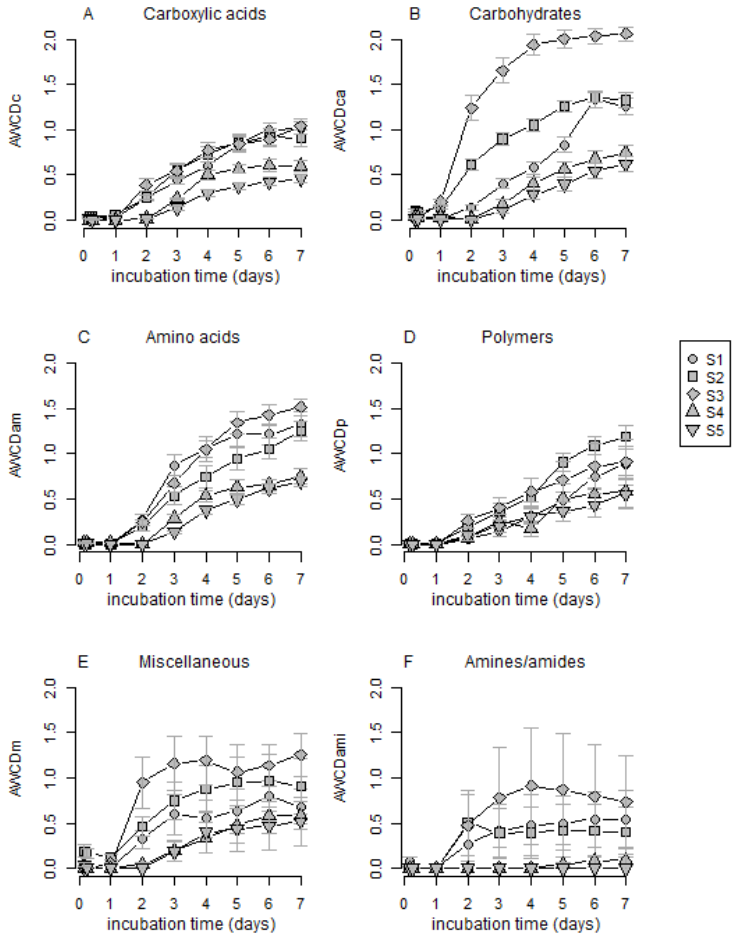

Figure 14. The AWCD of six types of carbon sources in five soil samples microbial communities, including carboxylic acids (A), carbohydrates (B), amino acids (C), polymers (D), miscellaneous (E), and amines/amides (F).

Comparison of metabolic functional diversity indices

Functional diversity indices reflected the metabolic functional diversity of microbial communities (Zhang *et al.*, 2013), the Shannon diversity index (H′) influenced by species richness of communities (Sun *et al.*, 2012), Shannon evenness index (E), and Simpson index (D) of soil microbial communities in the incubation time of 168 h are illustrated in Table 6.

Table 6. Comparison of metabolic functional diversity indices of the rice microbial communities

Sample	Shannon diversity (H′)	Richness (S)	Shannon evenness (E)	Simpson diversity (D)
S1	2.633 ± 0.067	29	0.782 ± 0.002	0.955 ± 0.001
S2	3.198 ± 0.056	28	0.960 ± 0.002	0.955 ± 0.001
S3	3.195 ± 0.057	30	0.939 ± 0.002	0.957 ± 0.001
S4	2.879 ± 0.084	22	0.931 ± 0.004	0.937 ± 0.003
S5	2.902 ± 0.079	21	0.953 ± 0.004	0.939 ± 0.003

As reported by Strong (2016) and based on that, soil microbial communities metabolic functional diversity was larger when a higher diversity index, while the individuals distributed

more equally when the Shannon evenness index (E) was higher (Zhang *et al.*, 2013). The Simpson index (D) is reflected by the most common species (Ge *et al.*, 2018).

We used t-test to detect significant differences among the samples. Table 6 clearly indicated that two indices except Simpson index (D) of the soil microbial communities had significant difference ($p < 0.05$).

Based of the calculated results, Shannon diversity (H′) index ranged from 2.633 to 3.198, in all the samples, The highest H′ index characterized in the microorganisms from sample 2, followed by sample S3, S5, S4 and S1, so it seemed that the soil microbial communities metabolic functional diversity was larger after two weeks from sowing, and lower during the maturity stage of winter wheat plants, so the different management practices had an effect on the soil microbial communities metabolic functional diversity.

Richness index (S) was the highest for microorganisms in soil from S3 (30), whereas it was lowest for S5 (Table 6).

The calculated evenness index (E) was maintained with a level that arranged from 0.782 to 0.960 for all soil microbial communities (Table 6), where a difference between S1 and the other soil samples was detected, contrary to the differences between soils samples S2, S3, S4, and S5 were not very big difference was recorded.

Simpson diversity index (D) was maintained at a similar level (0.937 –0.957) for all soil microbial communities (Table 6) and the differences between soil samples were not significant, which manifested that the most common species of the five soil microbial communities were similar. Furthermore, the different management practices had no impact on the diversity of the species.

To explore the variations in the soil microbial community composition, enumeration of microbial populations was done from the same soil samples.

4.1.3.2 Enumeration of microbial populations

During the six sampling days, the number of soil microbial populations were variable in the field (Figure 15), where a substantial increase of bacterial CFU was detected, the highest value of total bacteria population (5.0 E^{+06} CFU g^{-1} soil) was recorded on 15th of June 2018 (S1), and a second highest value was in the sample of 26th of September 2018 (S3) with a value of 2.6 E^{+06} CFU (g^{-1} soil), and the same value was recorded on 26th of June 2019 (S5), while in the rest of the

sampling days constant values were recorded, 2.9 E^{+05}, 3.0 E^{+05} (CFU) (g^{-1} soil) for the dates of 27th of August 2018 (S2), 25th of April 2019 (S4), respectively.

Similar to bacteria, the first and the second highest values of soil fungi also occurred most frequently in samples collected on 15th of June 2018 and 26th of September 2018 with values of 4500, 3500 CFU (g^{-1} soil), respectively. Constant values were detected on 25th of April 2019, 26th of June 2019, respectively.

On the other hand, denitrificans communities responded differently and were smaller on 15th of June 2018 where a higher bacteria population and fungi were found, and only a value of 360 cell/ml was detected. Contrary, the highest value of 2300 cell/ml was detected on 27th of August 2018, and a lower value of 950 cell/ml was found in the 25 April 2019 soil sample. On 26thof September 2018 and 26th of June 2019 soil samples denitrificans communities were not detected.

Concerning actinomyces, the highest value (2.10 E^{+05} CFU) was recorded on 27th of August 2018, and the lowest was enumerated in the 25th of April 2019 soil sample, with no big difference between the other soil samples, 1.75 E^{+05}, 1.90 E^{+05}, and 1.40 E^{+05} (CFU) (g^{-1} soil) for 15th of June 2018, 26th of September 2018, and 26th of June 2019, respectively.

For the ammonificans, their values during three sampling days were constant, 3.00 E^{+06} CFU (g^{-1} soil) was recorded both on 15th June 2018, 25th April 2019, and 26th June 2019. Whereas, smaller values were detected 2.05 E^{+05} CFU, 1.20 E^{+05} CFU (g^{-1} soil), for 26th September 2018 and 27th August 2018, respectively.

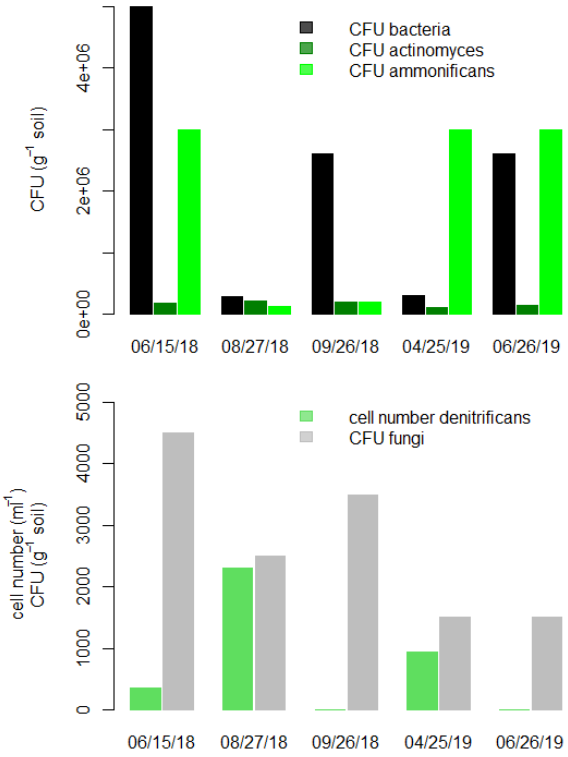

Figure 15. Total number of cultivable microorganisms in the field at six sampling times, total number of bacteria CFU, total number of CFU of actinomyeces, total number of denitrificans cell number (ml^{-1}), total number of cultivable fungi CFU.

Based on the measured soil microbial parameters there was a tendency that on 15th of June in the year of 2018 the numbers of total bacteria, fungi, and ammonificans were the highest among the 5 sampling dates. The lowest number of differing soil microbial parameters was found on the same day suggesting that many biotic and abiotic drivers can determine microorganisms activity and number (Gałązka, Grzęda and Jończyk, 2019).

However, as was reported by Fließbach *et al.* (2007) the main factor limiting their development was the availability of organic matter. While the variance between the different soil microbial populations in our study may be caused by soil properties, and management practices, their communities could be easily disturbed by intensive agricultural practices (Mueller, Belnap and Kuske, 2015; Sun *et al.*, 2015, 2016), which can affect them differently. For example when a highest bacteria population was found in 15th of June 2018 soil sample a lowest denitrificans population was recorded, and when a lowest bacteria population was detected a highest denitrificans population was recorded in 27th of August 2018, which was after around four weeks from tillage application. The founded results could be caused by plowing that can be also affected by soil sampling depth, where a study of tillage plots done by Doran (1980) showed that in the surface soils (0-7 cm) facultative anaerobes, denitrifiers, and aerobic microorganisms, were more

abundant with no tillage than with conventional tillage, while the contrary has been shown in the deeper layer soils (7-30 cm).

In addition, it was known that oxygen is among the key parameters influencing soil microbial activity and soil carbon and nitrogen cycling (Sun *et al.*, 2018). So, as mentioned by Khan (1996) tillage could cause an increase in soil aeration porosity and oxygen diffusion rate, which in turn could increase organic matters degradation (Stępniewski and Stępniewska, 2009), that correlated with soil microbial community (Sun *et al.*, 2018). Many studies have focused on the impact of tillage on soil microbial communities and have found that conservation tillage techniques increase microbial abundance (or biomass), diversity, and enzymes activity (Habig and Swanepoel, 2015; Guo *et al.*, 2016; Zuber and Villamil, 2016), but as reported Keiluweit *et al.* (2017) the contribution of microbial groups with a different preference for oxygen is still unclear.

The remaining straw or organic residues after harvesting can also affect soil microorganisms due to the increases the mineralizable fraction of soil N (Grantina *et al.*, 2011) and as reported Biederbeck, Zentner and Campbell (2005) such increases in the microbial population after green manure incorporation may be short-term or persist for at least one year. In addition, several studies have shown that fertilizer represents important management that promoting crop growth and increases yield (Yu *et al.*, 2019), and it also affects soil microbes (Enebe and Babalola, 2020). In our investigation, the MAS 27%, 200 kg N ha^{-1} fertilizer application on the 3rd of May 2019 was accompanied by a lowest number of fungi, actinomyces, and denitrificans, after around 7 weeks from the N application, also recently, Putri (2017) reported that different fertilizer applications of treatments affected the actinomycetes population. Contrary from the same soil amples a significant number of the the total bacteria was detected. Also, soil bacterial communities are generally more sensitive and smaller than fungal cells and are more easily affected by environmental changes or agricultural practices (Mueller, Belnap and Kuske, 2015; Zhang *et al.*, 2015). In addition, their ability to produce spores, allowing mobility of fungi than bacteria (Sun *et al.*, 2018). In our research, after 4 weeks from 29th of August 2018 fertilizer application, a 2nd highest value of fungi was detected, contrary on the same day the 1st lowest and a 2nd lowest number of denitrificans population and ammonificans were enumerated. These results showed that the effect of fertilizer on different microbial communities can be different, that also reported by studies which have shown that different fertilization treatments have different effects on soil bacterial community diversity and that chemical fertilizers lead to reduced community diversity (Geisseler and Scow, 2014). Similar result was reported recently by Rubiao *et al.* (2020), where fertilizer applications changed the physical and chemical properties of the soil, which in turn affected the soil bacterial community structure (Ling *et al.*, 2016; Wang *et al.*, 2017).

Based on the results of the flux measurements, microbial populations, and the calculation of metabolic functional diversity indices, it was shown that Sample 2 (27th of August 2018) had the highest metabolic functional diversity of microbial communities 3.198 ± 0.056 accompanied with low N_2O flux emission of 6.06 µg N m^{-2} h^{-1}, 25.6% soil water content, and 23.4 °C, soil temperature and 0 leaf area index. This sample had the highest number of denitrifiers 2300 cell/ml, while the number of total bacteria population was not the highest in this sample where 2.90 E^{+05} CFU (g^{-1} soil). It was accompanied with the highest number of actinomyces 2.10 E^{+05} CFU (g^{-1} soil) and the the lowest number of ammonificants (1.20 E^{+05} CFU g^{-1} soil). The lowest metabolic functional diversity (2.633 ± 0,067) was found in S1 (15th June 2018), while it was accompanied with the highest flux among the 5 saming dates (13.51 µg N m^{-2} h^{-1}), 23.6% SWC, 19.4 °C T_s and the highest value for the LAI (3.016). In this sample we enumerated the 3rd highest value of total bacteria population 5.00E^{+06} CFU (g^{-1} soil), the highest number of ammonifiers 3.00 E^{+06} CFU (g^{-1} soil), and just a considerable number of denitrifiers 3.60E^{+02} cell/ml, and the highest value of fungi 4.50 E^{+03} CFU (g^{-1} soil).

Although, when the 2nd highest N_2O emission among the five sampling days was recorded (12.4 µg N m^{-2} h^{-1}), denitrifiers were not detected in the corresponded soil samples, the emission may be caused by the higher soil water content 41.9 C° (on 26th of June 2019) led to anaerobic conditions that can favor fungi denitrification or also some nitrifiers in some aerobic microsites.

Similarly, when denitrifiers were not present in the field, a considerable emission was observed on 26th of September 2018 (accompanied with the 2nd highest number of total bacteria population), that was recorded under aerobic conditions (24.8% SWC), for that, this emission may be caused by nitrification process. Besides, this emission was associated with the 2nd highest number of fungi, that's why denitrification also could take place in some microsites, since fungi could also play a vital role as key producers of N_2O via heterotrophic denitrification in a wide variety of soils (Thamdrup, 2012; Matsuoka *et al.*, 2017). Added to the highest metabolic activity which was observed in this microbial soil sample. The founded results proved our hypothesis suggested during field N_2O emission results, where we supposed that the appearance of N_2O emission after lower one could be primarily due to the microbial diversity present in the field and their metabolic activity, and whether they were favorable for nitrification or denitrification processes. Also, plant presence could cause this microbial diversity difference as was reported above. Besides, SWC have to be taken into account as key driver.

The highest emissions recorded maybe also because the individuals were not distributed equally which characterized by the Shannon evenness index (E), which may refer to the presence

mostly of microbial individuals able to produce the N_2O, added to the other environmental factors influencing the production and emission as it was mentioned above. Where no link between denitrification measures and the abundance of denitrification genes have found in several studies (Miller *et al.*, 2008; Henderson *et al.*, 2010), also, variation in N_2O concentrations does not stringently correlate to variation of denitrifying activity (Schindler *et al.*, 2020).

The reverse was shown when a higher metabolic activity was measured a lower emission was detected on 15th June 2018, maybe due to the lower number of total bacteria together with the absence of denitrifiers, also tillage practices can have an effect on the bacterial disturbance which in turn affect the N_2O production.

So, according to the results we can conclude that not always the lower metabolic activity leads to lower N_2O emission (shown by the results on 27th August 2018). Also, even the denitrifiers which were present with a lower value did not cause a lower emission (shown by the results on 25th April 2019).

Generally, these results proved that other factors added to the microbial diversity and metabolic activity can affect the N_2O production and emission.

That's why soil microbial activities still remain a 'black box' in nitrogen biogeochemical turnover estimation, for that the precise identification of the N_2O microbial mediated processes and a direct linking of the N_2O and microbial metabolic activity can help in the development of microbial ecosystem models (Hu, Chen and He, 2015).

4.1.3.3 Taxonomic and phylogenetic distribution of microbial populations

The soil metagenomes were obtained from five soil samples throughout two-year from an agricultural field which received different managements.

Bacterial relative abundance

From Figure 16, which showed the relative abundances of the bacteria top 10 phyla in the in 5 soil samples, the most abundant phyla were Actinobacteria and Proteobacteria followed by Acidobacteria, and Firmicutes. While the less abundant phyla were Nitrospirae, Bacteroidetes, and Gemmatimonadetes.

Specifically, in the class level (Figure 17), Alphaproteobacteria and Actinobacteria the most abundant classes, which belong to Actinobacteria phyla. Other classes were present withconsiderable percentages, which are Baccili, belong to Firmicutes. Gammaproteobacteria and Deltaproteobacteria belong to Proteobacteria, but with more abundance of Gammaproteobacteria

compared to Deltaproteobacteria.

Among to top 20 species (Figure 18) in the five soil samples, 7 of them were belong to Protobacteria phylum (*Archangium gephyra, Sphingomonas sp., Lysobacter sp, Microvirga sp, Sorangium cellulosum, beta proteobacterium WX53, Aetherobacter rufus*), and five species to Actinobacteria phylum (*Geodermatophilaceae bacterium URHB0062, Mycobacterium sp, Streptomyces sp, Actinoallomurus sp, Luedemannella sp*), where all of them were belong to Actinomycetia class.

Besides, other phyla with several hits were present: Verrucomicrobia, Planctomycetes, and Chloroflexi. Where, Verrucomicrobia that are important members of the rhizosphere, and have been isolated from a variety of plant species, e.g. from *Pinus contorta* (Chow *et al.*, 2002). While, bacteria affiliated within the Planctomycetales order of the Planctomycetes phylum known bacteria involved in anammox pathway (Kartal *et al.*, 2011, 2013). Recently, Ma *et al.* (2020) reported that the relative abundance of Verrucomicrobia and Chloroflexi was negatively correlated with soil nutrients because Verrucomicrobia is generally considered to be oligotrophic (Zhalnina *et al.*, 2015). In our study, the highest abundance of Chloroflexi was detected in sample 1 that sampled during plant presence (physiological maturity of winter wheat), followed by S3 (2 weeks after rapeseed sowing), and S5 (boot stage of sorghum), but the difference between the samples was very small.

At the phylum level, even bacterial distribution was the same in all the five soil samples, a very small difference in the relative abundance was recorded, where the highest percentages of Actinobacteria and Proteobacteria were recorded in S1 and S3 which were sampled during plants presence, but still the difference was very small compared with the rest of the samples. It was reported that plants regulate rhizosphere microbial communities through root exudation in the form of rhizodeposition, temperature, and moisture control, etc. (Denef *et al.*, 2009).

In fact, at the phylum level, many previous studies have shown that N fertilization not only reduces below-ground biodiversity but also shifts bacterial composition, for group such as Proteobacteria, Acidobacteria, and Actinobacteria (Pan *et al.*, 2014; Ling *et al.*, 2017). Contrary, in our results we didn't observe any clear difference in the bacterial composition in the different soil samples, even they were collected during different management practices, as an example, S3 (26^{th} of September 2018) collected after 2 weeks after rapeseed sowing and 4 weeks fertilization, while S5 (26^{th} of June 2019) collected after 7 weeks from fertilization.

Shifts in bacterial composition following N manipulation were previously explained by the copiotrophic hypothesis, where copiotrophic groups that characterized by their fast growth rates are more likely to increase in nutrient-rich conditions, (e.g. Actinobacteria and Firmicutes), contrary to the oligotrophic groups (e.g. Acidobacteria and Chloroflexi) that have a slower growth rate would likely decrease in such conditions (Fierer, Bradford and Jackson, 2007). However, Zeng *et al.* (2015), reported that some copiotrophic organisms (Alphaproteobacteria) did not increase in abundance following N addition. The same was also observed by Fierer *et al.* (2012), and reported that N enrichment had no significant effect in an agricultural field, in contrast in grassland it led to an increase in abundance of the Alphaproteobacteria. This is what was observed in our case, the Alphaproteobacteria which represent the most abundant class among 10 classes didn't vary among the different field management (no very big difference was observed between samples that sampled after short and long time from fertilizer application).

In contrast, Campbell *et al.* (2010) found a decrease in bacterial diversity with N additions. Also, Janssens *et al.* (2010), noted that microbial responses were frequently inconsistent, and the response was affected by both the amount of N added and the duration of the treatment.

These contradictory results suggesting that the effects of N amendments on bacterial diversity levels are variable and likely site-dependent (Fierer *et al.*, 2012). Moreover, other factors may also contribute to soil microbial community changes, where it could be influenced by a wide range of soil characteristics, such as substrate quantity and quality, soil pH, moisture, and oxygen levels, which could vary with soil depth (Eilers *et al.*, 2012) and over seasons (Lauber *et al.*, 2013).

On the other hand, it has been proposed that the ratio between Proteobacteria and Acidobacteria reflects the trophic status of the soil, with lower ratios found in oligotrophic environments (Hartman *et al.*, 2008). A ratio of Alphaproteobacteria to Acidobacteria of 1.44 and 2.25 in bare and vegetated soils, respectively, were observed by Thomson *et al.* (2010). In our case, Proteobacteria and Acidobacteria ratio was ranged from 1.7 to 2.7, but there was no clear difference inthis ratio in the presence and absence of plants.

For Acidobacteria, Cederlund *et al.* (2014) suggested that bacteria belong to this group are to be characterized as oligotrophs, and are thus more likely to dominate in environments of low nutrient availability when examining the relationship between relative abundances of bacterial phyla and net C mineralization. Neverthless, Naether *et al.* (2012) noted that some subgroups of Acidobacteria reacted differently, maybe that's why we didn't observe a difference in the relative abundance of Acidobacteria during the different field management.

Otherwise, Souza *et al.* (2013), reported that Alpha and Betaproteobacteria classes were larger under conventional tillage, whereas the Deltaproteobacteria were more abundant in the No-till system, similarly, in our case, we recorded a higher abondance of Alphaproteobacteria which was the most abundant class, contrary to the Deltaproteobacteria which was classified as 17^{th} among 20 top classes in the 5 soil samples, with the lowest abundance among the five in sample sampled after 3 weeks from tillage (but no big difference compared with other samples). Bacteria belonging to Deltaproteobacteria may have important roles in the availability of some nutrients for both plants and soil microorganisms (Souza *et al.*, 2013).

Besides, the Myxococcales order belonging to the Deltaproteobacteria class was also less abundant in the 5 soil samples where their relative abundance was ranged from 1.6-2.1%. Genera within the Deltaproteobacteria class seemed to be Ncycling generalists, harboring up to six pathways (in addition to ammonia assimilation) (Nelson, Martiny and Martiny, 2016), and it was reported that it is possible that the Myxococcales bacteria were favored by the higher organic matter content with NT (Lueders *et al.*, 2006).

Generally, and as reported Kumar *et al.* (2020), the phylogenetic analysis suggests that most of the denitrifying bacterial communities identified worldwide are affiliated to phyla proteobacteria, actinobacteria, and verrucomicrobia. Further, it is established that the majority of denitrifying bacterial members are affiliated to the class Deltaproteobacteria, Gemmatimonadete, and Bacteroidetes which constitute significant percentages of the N_2O-reducing (i.e. NosZ-containing) bacteria in worldwide soil ecosystems (Hu, Chen and He, 2015).

The Bacteroidetes phylum included plant-growth-promoting and cellulose-decomposing (Verkhovtseva, Kubarev and Mineev, 2007; Soltani *et al.*, 2010) were present in a less abundant level in our case. While, the firmicutes phylum recorded as the fourth most abundant phyla in our soil samples, it was reported that the frequency of denitrification among it is uncertain (Shapleigh, 2013).

At the genus level, it was observed that in the 5 soil samples, the most dominated genus was Bacillus. However, a recent study of denitrification in a large collection of Bacillus strains suggested that denitrification occurred in nearly half (Verbaendert *et al.*, 2011). But in our results, only 2 species belonging to the bacilli genus were recorded (*Paenibacillus sp.and Paenibacillus alginolyticus*). Thus, it appears to be more reasonable to assess the response of bacterial communities at a lower taxonomic level.

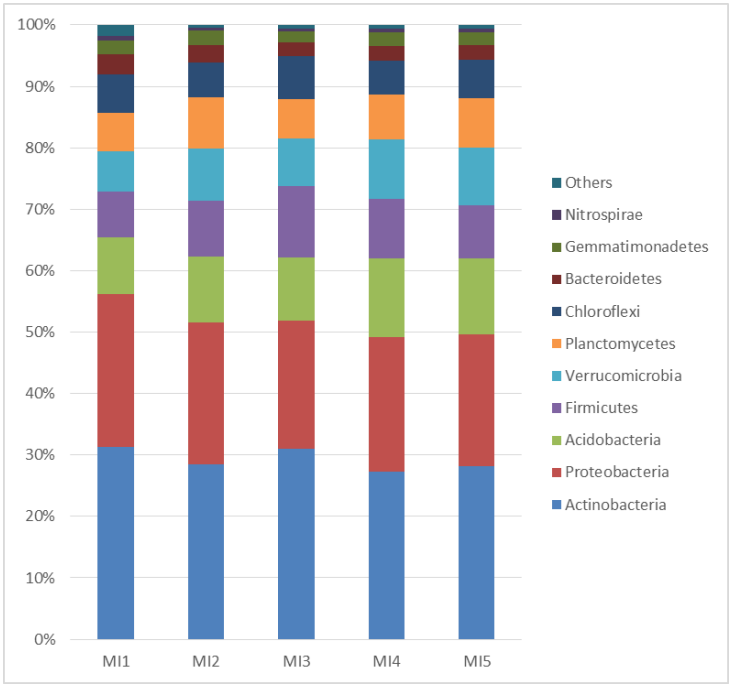

Figure 16. Relative abundance of the top 10 bacterial phyla in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

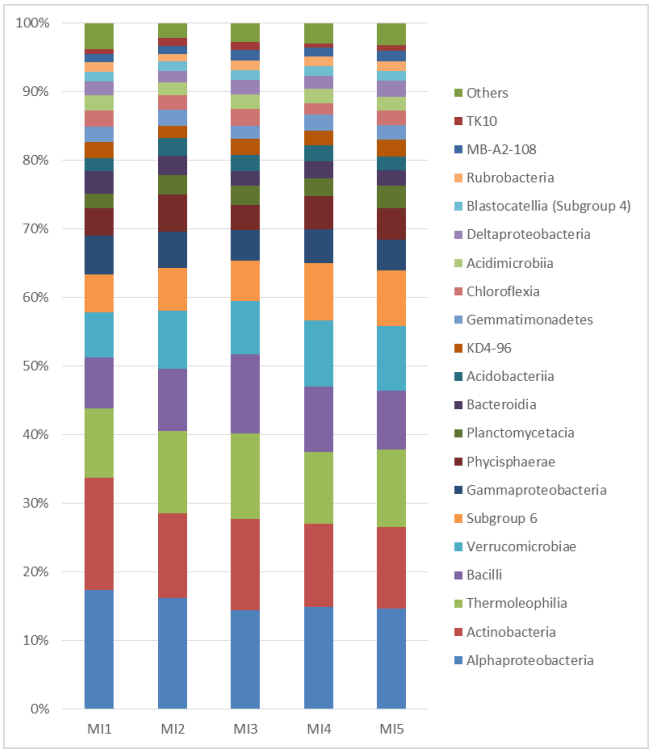

Figure 17. Relative abundance of the bacterial top 20 classes in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

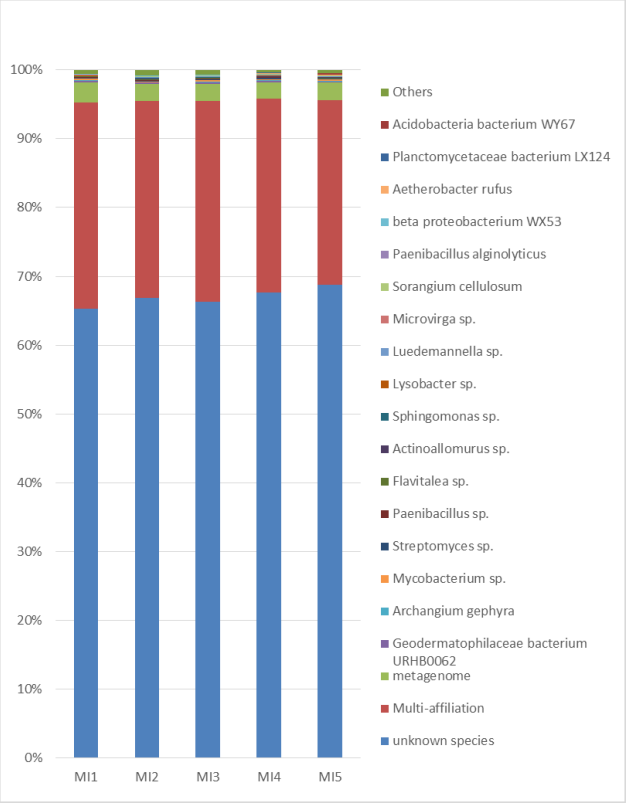

Figure 18. Relative abundance of the bacterial top 20 species in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

Fungal relative abundance

Based on Figure 19 which illustrated the top then fungal phyla in 5 soil samples. The dominant fungal phyla were Ascomycota, Basidiomycota, and Mortierellomycota. Among less the abundant phyla, Zoopagomycota, Olpidiomycota, and Mucoromycota in the five soil sampling dates. It was reported that Ascomycota and its growth rate is correlated with N availability (Fontaine *et al.*, 2011). While, Basidiomycetes are widely recognized as lignin decomposers (Hanson *et al.*, 2008) and thus important for carbon cycling in soil; in the same way, this beneficial function could be adversely affected by high N dose.

While, the three most abundant classes were Sordariomycetes, Dothideomycetes, and Eurotiomycetes (Figure 20), with the most abundant species belongs to Sordariomycetes (*Verticillium_dahliae*) and the second most abundant was belong to Dothideomycetes (*Sclerostagonospora_sp*). These two most abundant species belonged to the Ascomycota phylum.

Among the less abundant species (Figure 21) were, *Schizothecium_sp*, *Trichoderma_atroviride*, *Acremonium_furcatum*, and *Rhizophlyctis_rosea*, where the three first one were belonged to Sordariomycetes class and Ascomycota phylum, while *Rhizophlyctis_rosea* belonged to the

Chytridiomycetes class and Chytridiomycota phylum.

In general, among the 20 top species (Figure 21), 16 of them belonged to the Ascomycota phylum, and 9 of them belongs to Sordariomycetes classes, it was reported that Sordariomycetes of the phylum Ascomycota decrease with soil depth (Ko *et al.*, 2017).

Similarly, to Xu *et al.* (2019), in our study the dominant fungal denitrifying members which belong to Ascomycota including species of *Fusarium*, *Talaromyces*, *Chaetomium*, and *Trichoderma*. Recently, these genera are reported from maize cultivated soils (Xu *et al.*, 2019). In addition to these nirK-gene-bearing denitrifiers are found to have a crucial role in the denitrification process under maize cropping (Dandie *et al.*, 2011). But in our study, we recorded them in the presence and absence of crops but with different relative abondance. For example, *Fusarium_sp* was present with a higher level in S1, S4, and S5. Where S1 was during the physiological maturity of winter wheat, S4 was in the absence of crops, and S5 was 7 weeks after fertilization and sorghum sowing.

The fungal denitrification system comprises cytochrome P450 NO-reductase and copper containing NO_2^--reductase which are primarily responsible for the global perspective of N_2O emissions as fungi lack NosZgene to convert N_2O to N_2 (Hu, Chen and He, 2015).

In fact, some studies reported that fungal diversity was found to decrease significantly with fertilization (Gu *et al.*, 2019). Nevertheless, in our soil samples no differences were observed during the different managements. On the other hand, previous studies reported the adaptation of the dominant microbes to particular soil conditions (Su *et al.*, 2017; Chen *et al.*, 2018).

The metagenomic analysis as an indicator of the potential pathways of the nitrogen cycle showed no big differences in the microbial communities between the different five soil samples (only a small difference in their relative abundances), which reflected that bacterial and fungal communities in our field are the same during this period and not affected by the different management practices.

For that, further research is needed to determine exactly how biotic and abiotic factors influence bacterial community composition, taking into consideration the direct and indirect interactions among plants, soils, and microbes. Also, future studies should focus on the influence of agricultural management practices on rhizosphere soil microbial function to check why the effect has differed from one study to other.

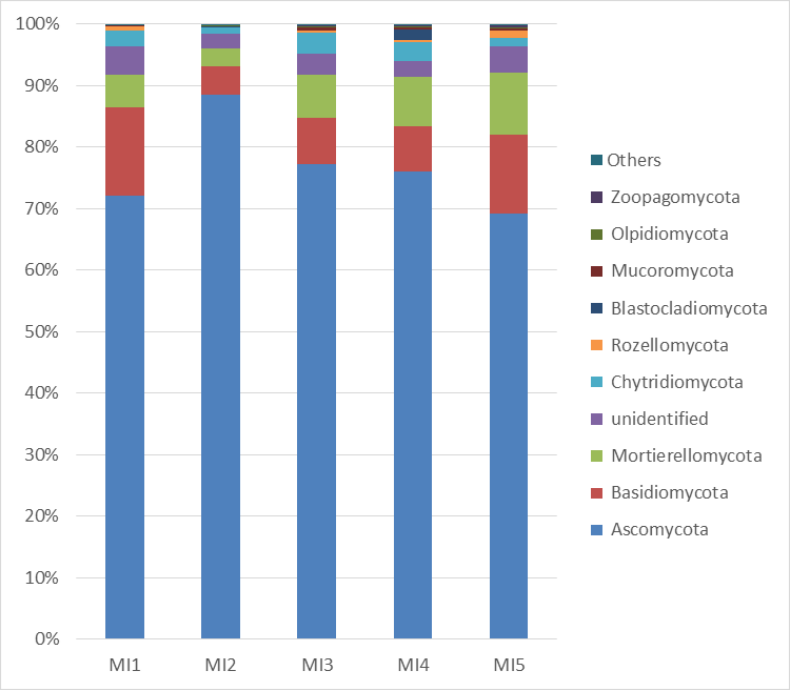

Figure 19. Relative abundance of the top 10 fungal phyla in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

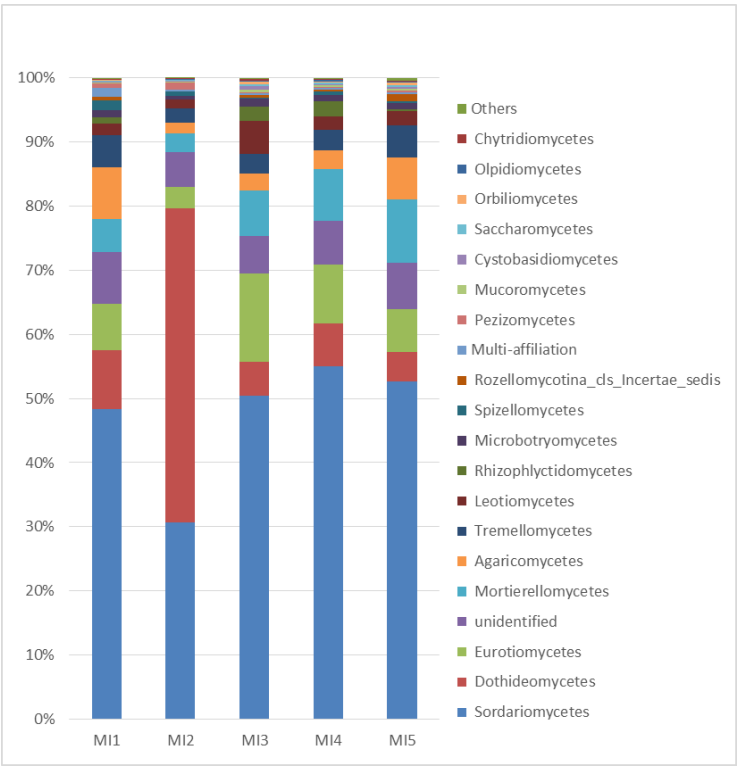

Figure 20. Relative abundance of the fungal top 20 classes in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

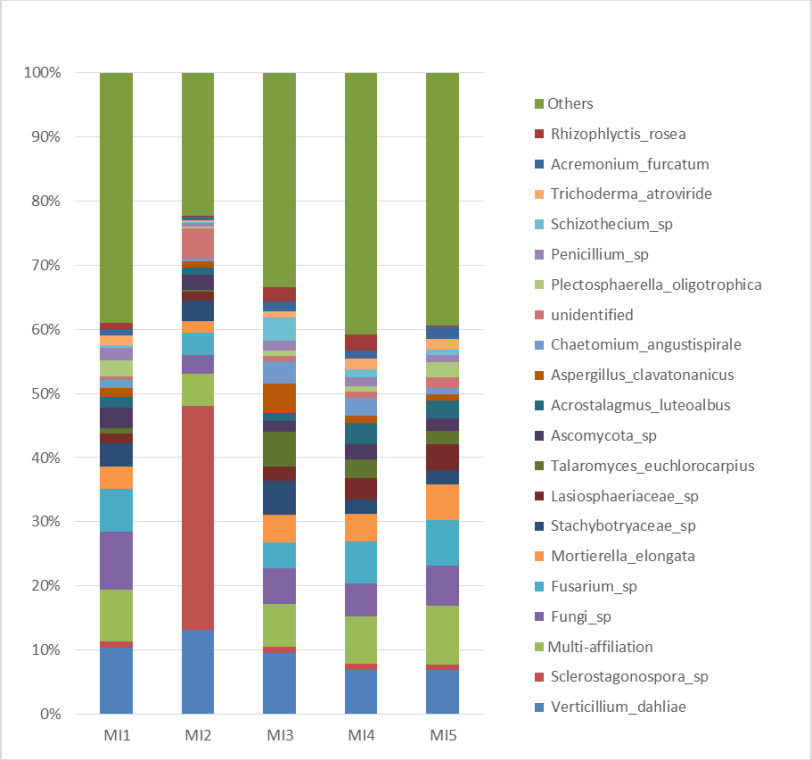

Figure 21. Relative abundance of the fungal top 20 species in 5 soil samples (M1 (S1): 06/15/18, M2 (S2): 08/27/18, M3 (S3): 09/26/18, M4 (S4): 04/25/19, and M5 (S5): 06/26/19).

4.2. Laboratory incubation experiments

4.2.1 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 22), the measured N_2O fluxes ranged from 4.86 ± 25.56 to 26.29 ± 28.45 µg N m^{-2} h^{-1}, when the N_2O averages during the first week of the measurement (6.10 ± 2.88 µg N m^{-2} h^{-1}) was higher in the N0 than in soil treated with N50 (–0.15 ± 4.64 µg N m^{-2} h^{-1}) and N100 (5.70 ± 2.17 µg N m^{-2} h^{-1}). In planted soil there was a clear difference between the treatments according to the fertilizer addition: N50 (5.81 ± 2.21 µg N m^{-2} h^{-1}) was 2 times higher than the N0, N100 was 4.5 times higher than N50. After 2 weeks of incubation, there was a difference just between N0 and N50, in bare soil. In planted soil N50 (3.09 ± 2.52 µg N m^{-2} h^{-1}) was 2 times higher than N0 (1.59 ± 2.29 µg N m^{-2} h^{-1}), while only small difference between N50 and N100 (N100 (bare): 0.49 ± 3.21, N100 (planted): 3.67 ± 1.80 µg N m^{-2} h^{-1}) was recorded, for both cases, bare and planted soil. After three weeks significant differences between all the treatments were detected in bare soil : N_2O flux increased with increasing fertilizer rates with values of 3.75 ± 1.27, 4.25 ± 2.33, 8.41 ± 2.82 µg N m^{-2} h^{-1}, in N0, N50, and N 100, respectively. 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, and also between the bare and planted soil where bare soil was a bit higher than planted soil, in bare soil N50 (7.37 ± 4.0 μg N m^{-2} h^{-1}) was 2.5 times higher than N0, and N150 was around 3 times higher than N50. In planted soil, N100 (18.75 ± 14.40 μg N m^{-2} h^{-1}) was 3 and 13.5 times higher than N0 and N50, respectively. Later on, after 5 weeks, the N_2O emission tendency was differed a bit where 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 because the N_2O emission were approximately the same through all the fertilizer rates, in bare soil the emission were N0: 1.75 ± 9.50, N50: 8.91 ± 7.46, and N100: 8.19 ± 7.26 μg N m^{-2} h^{-1}, while in planted soil, (1.87 ± 4.88, 9.02 ± 11.20, and 7.94 ± 5.46 μg N m^{-2} h^{-1}), respectively.

Concerning the second series of the experiment (Figure 22), 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 between N0 (27.84 ± 29.98 μg N m^{-2} h^{-1}) and N75 (33.61 ± 6.30 μg N m^{-2} h^{-1}), contrary to the N150 which was lower than the previous. Nevertheless, in planted soil, the difference was recorded between the three N rates, where N150 (58.67 ± 29.32 μg N m^{-2} h^{-1}) was 1.6 and 7 times higher than N75 and N0, respectively. The same tendency was observed after two weeks with a difference detected in planted soil, but in bare soil, the difference was just between N0 and N50, the N_2O measured values for N0, N75, and N150 were, (22.98 ± 5.71, 39.60 ± 11.52, 34.23 ± 15.97 μg N m^{-2} h^{-1}) in bare soil, in planted soil were N0: 27.03 ± 11.84, N75: 43.97 ± 2.27, N150: 51.69 ± 62.34 μg N m^{-2} h^{-1}. After three weeks significant differences were observed in planted soil between all fertilizer rates: N75 (15.57 ± 7.79 μg N m^{-2} h^{-1}) 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 (23.74 ± 12.17 μg N m^{-2} h^{-1}) and N150 (36.35 ± 8.76 μg N m^{-2} h^{-1}), and between N75 (11.70 ± 4.74 μg N m^{-2} h^{-1}) 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. In bare soil N150 (26.48 ± 16.84 μg N m^{-2} h^{-1}) was higher 2.5 folder times than N75 which in turn was higher around 3 folder times than N0, while in planted soil N150 (40.90 ± 17.61 μg N m^{-2} h^{-1}) was higher by 1.5, 7.7 times than N75 and N0, respectively.

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 (1st series). Where, for N0 bare soil the 2nd series emitted 4.6, 19.6, 6.6, and 1.1 times than the 2nd series, during the 1st, 2nd, 3rd, and 4th 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. So it seemed that increasing SWC with 5% caused at least one-fold higher increase in N_2O emission.

Besides, during the first series of the experiment plant effect was observed significantly only during the 1st and the 2nd weeks of the measurement, during the 1st weeks planted soil had 4.7, 37, and 0.5 times N_2O emission than bare soil for N0, N50, and N100, respectively. While during the 2nd week, planted soil had 7, 1.3, and 1.4 times higher N_2O in the case of N0, N50, and N100, respectively. Contrary, during the other weeks plants effect was not clear, where bare and planted soils emitted approximately the same amounts. While for the 2nd series, plant presence had an effect during the 4 weeks of the measurement, where it caused emissions that were 1-2 fold higher than bare soil varied with the measurement time and N treatments.

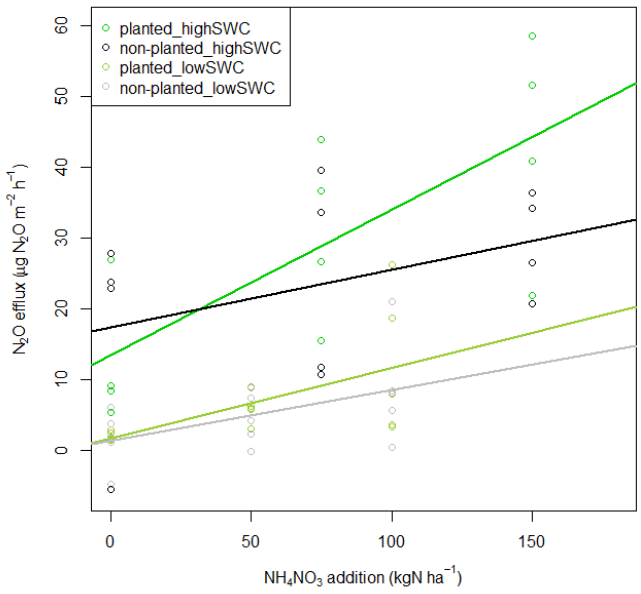

Figure 22. N_2O emission averages in the planted and bare soil under different NH_4NO_3 addition rates rates (0, 50 and 100 kg N ha^{-1}), under SWC equal to 20% for the first series and a second series under N fertilizer rates (0, 75 and 150 kg N ha^{-1}), under SWC = 25%, during 5 an and 4 weeks lab experiment, respectively.

Based on the regression presented in the Figure 22 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= 0.55, 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).

Concerning the effect of soil water content and plant presence separately (Figure 23 and Figure 24), it was shown that SWC and plant had strong effects on the N_2O emission.

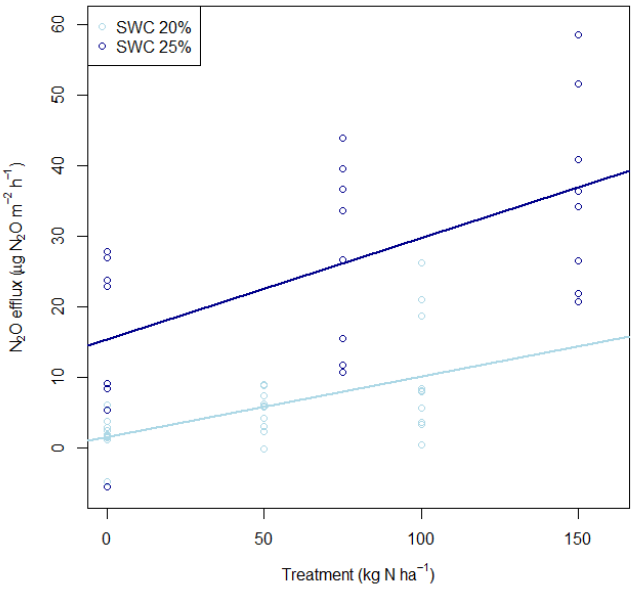

Figure 23. N_2O emission averages under different NH_4NO_3 addition rates (0, 50, 75, 100, and 150 kg N ha^{-1}) and at two different soil water content levels (20 and 25%).

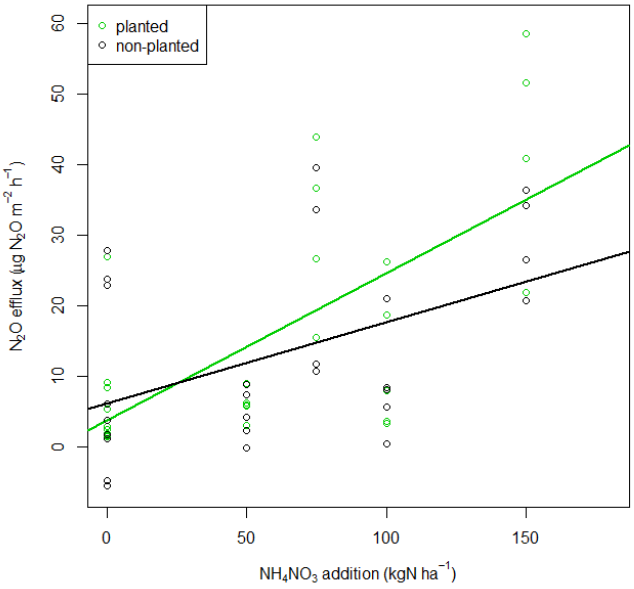

Figure 24. N_2O emission averages from planted and not planted soils under different NH_4NO_3 addition rates (0, 50, 75, 100, 150 kg N ha^{-1}).

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% could be mainly from the nitrification process, as it was mentioned

in several studies (Lan *et al.*, 2014). Also, Davidson (1991) reported that the optimum soil moisture for N_2O through nitrification at 30-60% water-filled pore space, whereas 60-80% WFPS represents the optimum condition for N_2O production under denitrification. Restrict O_2 availability that favor denitrification process, can be induced also by plant presence via root respiration (Jarecki *et al.*, 2009), and subsequently higher N_2O production. Denitrification can occurre even under aerobic conditions in case of the existence of anaerobic microsites created by either microbial growth or the water saturation inside soil aggregates (Renault and Stengel, 1994). In a study done by Klemedtsson, Svensson and Rosswall (1987) it was reported that the denitrification rates in pots planted with barley increased with time along with increased root biomass, and it was 2-22 times compared with the unplanted pots. Added to the other recent studies that proved the contribution of agriculture to the total N_2O emissions from soil-plant systems (Lenhart *et al.*, 2019; Timilsina *et al.*, 2020).

The positive effect of fertilizer rate recorded in this experiment was in accordance with studies proving that N fertilizer enhances N_2O emissions in circumstances where other factors are not limiting, while the effect of fertilizers can be a directly via the amount of NH_4^+ or NO_3^- available in the soil (Signor and Cerri, 2013).

4.2.2 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) with p-level <0.001 (n>500) in both cases. Increase in denitrification rates and/or N_2O emission rates has been frequently found following N-fertilizer application (Kaiser *et al.*, 1998). The level of N-fertilizer application is one of the main factors influencing soil N_2O emission (Zheng, Stewart and Cotrufo, 2012), linear or exponential relation have been reported between N fertilizer and N_2O emissions (Kim, Hernandez-Ramirez and Giltrap, 2013). 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 7.

Table 7. Results of the multiple regression for soil N_2O emissions: r^2 values and regression coefficients with statistical significance levels (a: intercept, SWC and nitrogen fertilizer addition, ***: p<0.001 in all cases (n>100)).

	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 25 (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. However, we should note that the N_2O emissions could also be affected significantly by fertilizer types, for example, N_2O emissions tended to be higher from nitrate-containing fertilizers, particularly in regions, which have high organic matter soils and wet climates (Harty *et al.*, 2016). 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 (Nie *et al.*, 2016). 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 (18.12 ± 1.20 mg N m^{-2}) was significantly higher than that of bare soil (16.1 ± 0.88 mg N m^{-2}). 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). Hence, the effect of maize growth on N_2O emission gradually decreased with an increase in N application (Wang *et al.*, 2019) as N gradually satisfied the need of crop growth, microbial processes of N_2O production obtained more NH_4^+ and NO_3^- (Linquist *et al.*, 2012).

Besides, we must take into consideration that fertilizer applications directly or indirectly induces changes in soil physical and chemical properties, which, in turn, affects the soil bacterial

community structure and the relative abundance of the dominant bacterial groups (Wang *et al.*, 2017; Rubiao *et al.*, 2020). For example, a study of fertilized rice crops showed a larger number of cultivable microorganisms and reported that the application of P and N did not directly affect microbial parameters in the soil, but indirectly by increasing crop yields by means of promoting the accumulation of soil organic matter through increased root turnover (Zhong and Cai, 2007). Moreover, as the application of chemical fertilizers results in low pH of the soils, microbial nitrification and denitrification could also be affected indirectly.

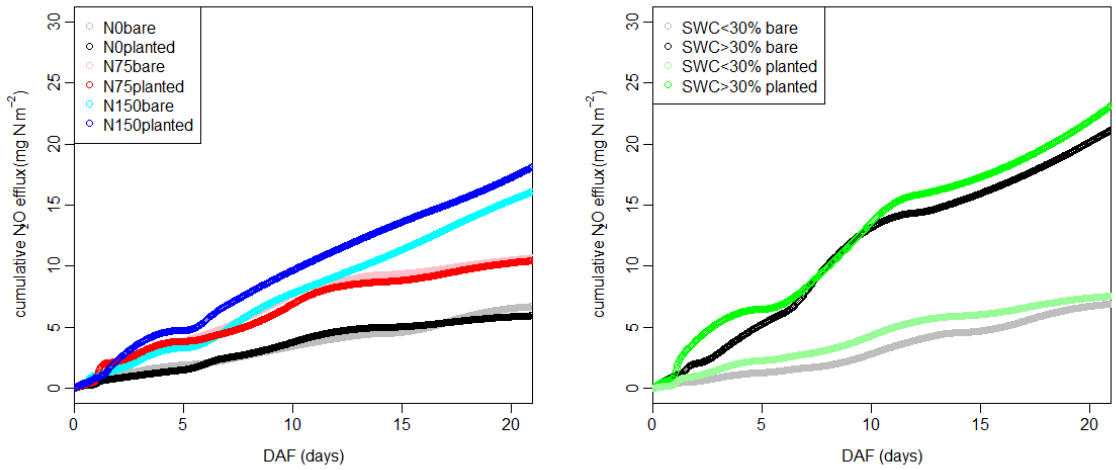

Figure 25. Cumulative N_2O emission in the planted and bare soil during 22 days lab experiment, under different N fertilizer rates (0, 75 and 150 kg N ha^{-1}) (left panel), and under two soil water content levels (SWC < 30%, SWC >30%: the average SWC were: SWC<30 bare 20.2%, SWC>30 bare 36%, SWC<30 planted 20.5%, and SWC>30 planted 35.4%) (right panel) as a function of days after fertilizer application (DAF).

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 25 (right panel). The cumulative N_2O emission in bare soil observed in SWC>30% treatment (21.16 ± 0.84 mg N m^{-2}, three weeks after fertilizer application) was three-fold higher than at SWC <30% (6.89 ± 0.27 mg N m^{-2}). 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%.

There is a consensus in the literature that regardless of the N fertilizer effect soil water content is a key factor affecting the metabolic activity of microorganisms and N_2O emissions (Imer

et al., 2013; Kim *et al.*, 2014). Hayashi *et al.* (2015) reported that moisture levels around 70-80% WFPS caused the greatest emissions and at WFPS level >60% denitrification was reported by Toma *et al.* (2011) as the major source of N_2O emissions, while the dominating source of N_2O switched to nitrification at lower WFPS of 35-60% (Lan *et al.*, 2014). In our study when SWC values exceeded 30% N_2O emission increased dramatically both in bare and planted soils. Besides, 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 of 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.

4.2.3 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 26). Comparing the N_2O emission between bare and planted soil, at the different N fertilizer rate (Figure 26, left panel). Before 48 h from fertilizer application, in general, the emission was a little higher in bare soil than in planted soil. Then, after 2 h from fertilization, still the planted soil had a lower emission than bare soil, except in soil treated with 75 kg N ha^{-1}, the values for bare soil after 2 h were, 36.08 ± 62.45, 34.17 ± 30.40, 49.37 ± 75.64 μg N m^{-2} h^{-1}, for N0, N75, N150, respectively, while for planted soil the values were, 21.59 ± 19.88, 35.12 ± 28.44, and 41.88 ± 56.96 μg N m^{-2} h^{-1}, in N0, N75, and N150, respectively. At this time of measurement, the fertilizer effect was observed just between N75 and N150 in bare soil, in contrary to the planted soil where significant differences were found between all the treatements (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 comparing the emission between planted and bare soil, planted soil N_2O emission was higher than in bare under N75 and N150 only. The emissions were 6.7 and 2 times higher in planted soil than bare soil under N75, N150, respectively. After that after 24 h, the tendency has changed, where the effect of fertilizer was recorded between N0 and N75 also between N0 and N150, in bare soil. While in planted soil, the differebce was observed only between N75 and N150. The same variation which was observed after 12 h from fertilization was recorded after 72 h and after 144 h , where N_2O emissions in planted soil, but with variation on N fertilizer effect. After 157 h, the effect of fertilizer was shown under all the rates just in planted soil, contrary to the bare just N75 was lower than N150. 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 N0 was decreased, contrary under N75, and N150, the emissions 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 fertilizer rates and both in bare and planted soil 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 plant presence caused a variation in the N_2O emission, but 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. As reported about the plant effect that can lead to lower emission compared with no plant presence (Wang *et al.*, 2019), but the effect can be suppressed under a very high N fertilizer rate (Wang *et al.*, 2019) which is in agreement with our found results.

The presence of hotspots maybe caused also more N_2O emission in some pots. The highest emission in N0 soil was reported also in some studies e.g. Oktarita *et al.* (2017) found a higher N_2O emission in N0 compared with 133 kg N ha^{-1} y^{-1}. Moreover, under maize cultivation Van Groenigen *et al.* (2004) reported non linearity of soil N_2O emission regarding different N application.

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, especially after 144 h from

fertilizer application in bare and planted soil, but with more measurement days in planted soil, after 2, 12, 72, 144, and 157 h, in other cases fertilizer rate had no clear effect, that was also recorded in a recent study done by Dencső (2021).

Comparing 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 some time to be higher than N75 N_2O emission, also the diversity of microbial population and hot spots could cause this variation (glucose addition effect will be discussed in next parts also).

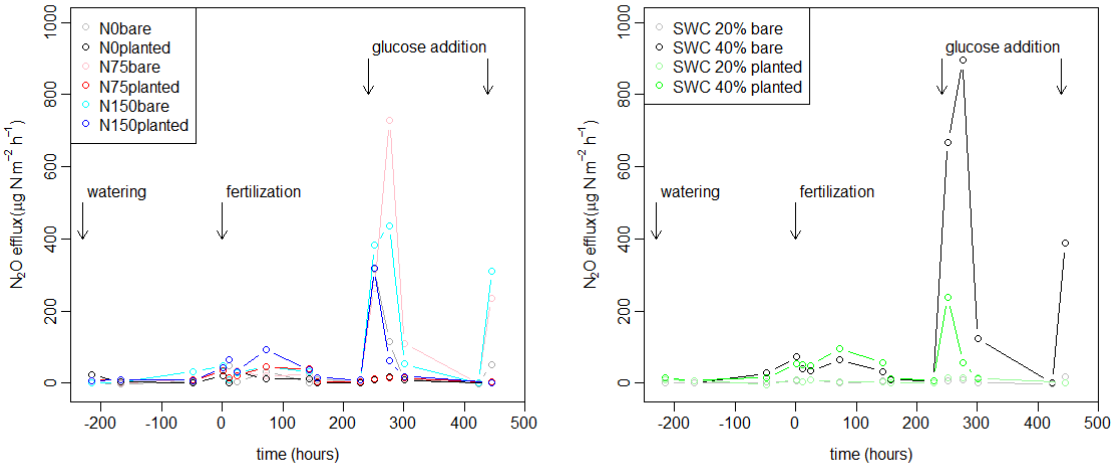

Figure 26. N_2O emission averages in planted and bare soil, under different N fertilizer rates (0, 75 and 150 kg N ha^{-1}), and under two soil water content levels (SWC= 20%, SWC= 40%), during 445 days lab experiment, and amended with glucose.

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 26, 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 6.30 ± 22.91 µg N m^{-2} h^{-1} at the lower SWC (20%), while it was higher by 11.6 times at 40% SWC (73.44 ± 61.86 µg N m^{-2} h^{-1}). In planted soil, the emission was a little higher at 20% SWC, and lower at SWC of 40% compared to bare soil, but still, N_2O emission at 40% SWC (55.56 ± 34.51 µg N m^{-2} h^{-1}) in planted soil was higher than 20% SWC (10.16 ± 20.32 µg N m^{-2} h^{-1}) by more than 5 times. Then the emission decreased a bit in all the treatments and increased again under 20% SWC after 24 h from fertilization, but still the effect of SWC the same, and still planted soil

N_2O emission higher than in bare soil under the same SWC level, with values of, in bare soil 10.0 ± 11.02 μg N m^{-2} h^{-1}, 34.7 ± 41.52 μg N m^{-2} h^{-1}, for SWC 20% and SWC 40%, respectively, for planted soil (at SWC 20%: 8.50 ± 29.52, and at SWC 40%: 48.42 ± 36.21 μg N m^{-2} h^{-1}).

Later, after 72, 144, 157, until 228 h from N fertilizer application, the N_2O emissions in all the treatment both in bare and planted soils decreased under SWC 40%, and SWC 20%, to reach the following values 2.33 ± 1.76, 7.46 ± 7.78, and 5.64 ± 8.18 μg N m^{-2} h^{-1}, for planted soil 20% SWC, planted soil 40% SWC, and bare soil under 40% SWC, except in bare soil 20% SWC there was no clear decrease and it seemed that there was a variation in the tendency. For 72, 144, 157, and 228 h after the ammonium nitrate addition, N_2O emissions from bare at SWC 20% were, 0.29 ± 6.29, 6.45 ± 15.49, 0.86 ± 1.70, and 1.53 ± 2.42 μg N m^{-2} h^{-1}.

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 than at 20% SWC. N_2O emission at 20% SWC were, 5.97 ± 3.60, 14.75 ± 6.0 μg N m^{-2} h^{-1}, for bare and planted soil respectively. While at 40% SWC were, 667.9 ± 580.3, 239.1 ± 643.1 μg N m^{-2} h^{-1}, also for bare and planted soil, respectively. 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 emission in planted soil 40% SWC, planted soil 20% SWC, and bare soil 20%, 40% SWC decreased again to reach the following values, 13.40 ± 18.33, 14.92 ± 10.15, –0.89 ± 20.84, 124.10 ± 125.43 μg N m^{-2} h^{-1}, respectively. After the addition of the 2nd glucose portion (439 h from fertilizer addition), the N_2O emissions increased again in bare soil, both under 20 and 40% SWC to highest values 20 times more under 20% SWC and 440 times under 40% SWC, compared to which were recorded just before the 2nd glucose addition, so the highest emission was always under 40% SWC where it was 20 times higher than under 20% SWC.

So, the results showed that the SWC level had a positive effect on the N_2O emission, in which both in bare and planted soils increased with increasing SWC level. And the emission at 12 h after fertilization under 40% SWC were higher in plant presence plots, compared with the bare soil, except 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.

After the glucose addition, the soil under 40% SWC seemed to be to most affected by this amendment, with the dominance of bare soil emissions.

The effect of soil water content observed in our experiment proved that the N_2O emission increases with increasing SWC level, due to the developing anaerobic conditions which lead in turn to more active denitrification process. It was observed in our case at 40% SWC, where the

emitted N_2O could be of denitrification origin mostly, Säurich *et al.* (2019) recorded the highest N_2O fluxes at WFPS between 73 and 95%, which primarily originated from denitrification, while at 20% SWC mainly from nitrification process.

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 (Ussiri and Lal, 2012; Cameron, Di and Moir, 2013; Quin *et al.*, 2015). Several studies found that denitrification (N_2O production) was promoted after glucose addition since it is more easily dissolved (Nishio *et al.*, 1988; Azam *et al.*, 2002; 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 these pots since plants used the nitrogen for their growth, but still there was a considerable emission from 40% SWS planted soil.

4.2.4 Fourth experiment

During this experiment only bare soils were used and 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 27, upper panel), the emission showed a variation depending on the additional treatments, where during the measurement period, a lot of additions were done (glucose, microbial solution, and N fertilizer), 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 increases again to other higher value, contrary to the rest that decreased, the N_2O emitted amount were 3 and 3.6 times higher than before fertilization. The N_2O emissions were decreased during, 48, 70, 96, 116, 148, 196, and 239 h continuously, especially for N75 and N150. 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, the values were, N0: 803 ± 596 µg N m^{-2} h^{-1}, N75: 937 ± 311 µg N m^{-2} h^{-1}, N150: 1108.2 ± 598.9 µg N m^{-2} h^{-1}. 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 emission 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, the values were, N0: 123 ± 98 μg N m^{-2} h^{-1}, N75: 1286 ± 356 μg N m^{-2} h^{-1}, N150: 2836 ± 1149 μg N m^{-2} h^{-1}. Thereafter, they decreased again, with some fluctuations in the N_2O emission in the soil control (N0). Then, a 1 ml microbial solution was added (after 535.5 h from fertilization), and N_2O was measured after 2 and 23 h from the addition, but still, no significant increases were detected. Later on, a third portion of glucose was amended (after 605 h from fertilization) 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, but not similar to which was observed during the other glucose portions 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 frome 1st fertilizer addition) and measured the emissions after 2 h, where higher emissions were detected. 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 and 77 h from the 2nd fertilizer addition, 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 with a value of 4765 ± 2141 μg N m^{-2} h^{-1}, contrary to N0, N75 that decreased (for N_2O emission values, see supplementary Table 9).

N fertilizer had a significant effect on N_2O emissions in forest soil that was observed in the first hours after fertilization. Also, glucose addition had a positive effect on the N_2O emissions, where the highest values of N_2O emission were recorded always after the glucose additions. 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, and even after 2 h from the glucose amendment. 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 (the presence of enough fertilizer and microbial growth). For example, under the first fertilization, higher N_2O emission values were recorded after the 3rd glucose amendment, compared with the first and the second portions, which may be because the first and 2nd portions were used both for microbial growth and N_2O production, but the third one was amended in time in which was not needed in their growth and used mainly

to produce nitrous oxide. Another factor could be a reason for the difference between the third glucose portion effect and the other, which was the microbes addition that facilitates the uses of glucose and N_2O production very rapidly. The fifth glucose portion also caused a higher N_2O emission, especially in soil treated with N150 may be due to the presence of enough nitrogen after the 2nd fertilizer addition, but in N75 case the emission was lower compared with the 3rd portion addition, maybe because the highest value was emitted before 4 h and there was no measurement during this time.

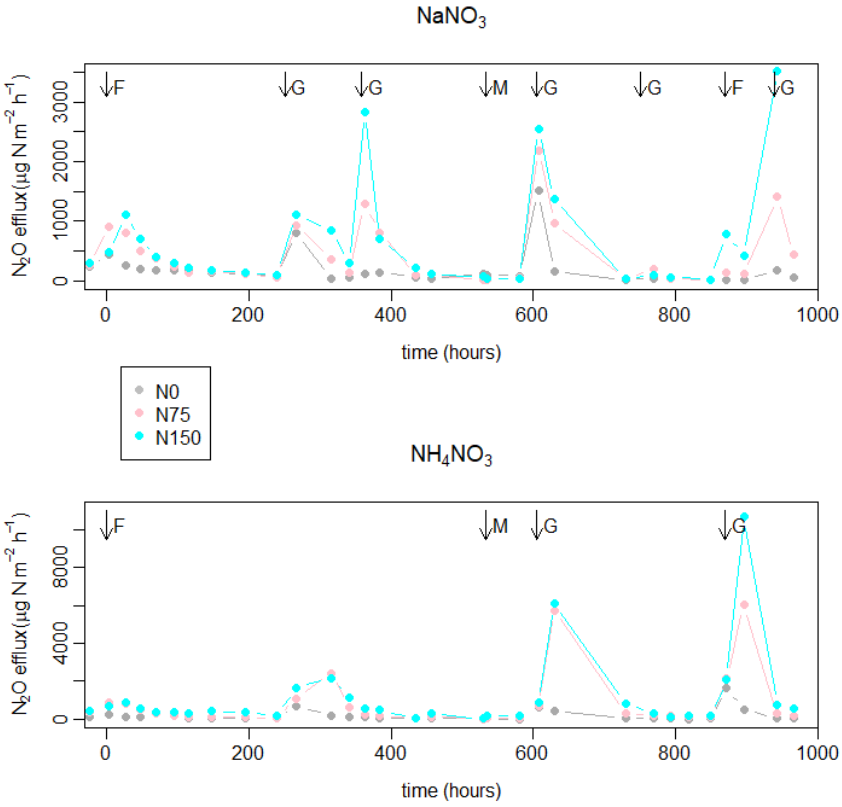

Figure 27. N_2O emission averages from forest soil (bare soil), during 965 h long study period, under 40% SWC, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}) (upper panel), and ammonium nitrate fertilizer (lower panel), and amended with glucose (G) and microbial solution (M).

N_2O emission under ammonium nitrate fertilizer

Similar to the measurement under sodium nitrate fertilizer (Figure 27, lower panel), N_2O emission was measured in a series of pots fertilized by ammonium nitrate (0, 75, and 150 kg ha^{-1}). Measurements started 24 h before fertilization forest soil emitted a large amount of N_2O even without fertilization. After 4 h from N fertilizer addition, N_2O emissions increased but with no significant difference between N75 and N150. Then, it decreased continuously, during 48, 70, and 96 h after fertilization. Then, N75 and N150 were increased a bit after 148 h from fertilization,

with the significant increases in soil treated with N150, Then, it started to decrease again, for N0, N75, N150 after 196 h from fertilizer application.

After 267 h from N addition, 671, 1085, 1658 μg N m^{-2} h^{-1} values were recorded for N0, N75, and N150, respectively. 2.4, 1.3 times higher values for N75, N150, were observed again after 316 h from N addition compared to which were recorded after 267 h. Then it decreased under all the treatments. After these lower emissions, 1 ml of microbial solution was added to each pots (after 533.5 h from fertilizer addition) and then measured after 2 h from its addition, where a significant increase was recorded in soil treated with 150 kg N ha^{-1} ammonium nitrate, and to a bit higher values after 23 h from microbial solution addition which corresponded to 580 h after fertilizer addition. Then, 1st portion of glucose were added (605 h after fertilizer addition), N_2O emissions were measured after 2 h from this addition, which was after 607 h from 1st fertilizer addition, and higher emissions were recorded. Other peaks were detected after around 22 h from glucose addition, which were, 8.6, 7.2 times higher than after 2 h from the addition for N75, and N150, and 147, 133, 32 times higher than before glucose addition. Then, the N_2O emissions were decreased continuously after several measurement days, but still, there is a significant effect of fertilizer rate, N0 < N75 < N150.

Additional glucose portion was added (867.5 h from fertilizer addition), and then we measured the N_2O emissions after 4 h from its addition (871.5 h from fertilizer addition), and higher emissions were recorded. Later on, after 21 h from the addition, N_2O emission peaks were recorded, which were the maximum for soil N75 and N150, and then these values were decreased again continuously (for N_2O emission values, see supplementary Table 10).

The N_2O emission from forest soil, treated with ammonium nitrate showed a different variation compared with soil treated with sodium nitrate, where only fewer additional amendments were used in this soil type, since we recorded a considerable emission during the first 316 h after fertilizer application, which did not need any adjustment. 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 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 1.2, 1.1, and 1.8 times higher than after the 1st portion, for N0, N75, and N150, respectively.

Comparing the emissions under the two different N fertilizer types, it was observed that both fertilizers had a 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, as it was pointed out by several studies (Abbasi and Adams, 2000; Gogina and Gulshin, 2016). Also, after the 1st glucose addition, NH_4NO_3 caused a higher emission than soil under $NaNO_3$, which could be caused by microbes addition, also could be that heterotrophic nitrification was taken place in the production. But in general, under the two different N fertilizer type, N fertilizer addition had a positive effect on the N_2O emission as it was reported by Malchair and Carnol (2009) that nitrogen is frequently the most limiting nutrient in forests, also all the glucose additions had a significant positive effect.

N_2O emission from cropland soil

The N_2O measurement from cropland soil under 40% SWC and treated with 0, 75, 150 kg ha^{-1} sodium nitrate fertilizer, and under different amendments (microbes and glucose additions) was shown in Figure 28. The N_2O measurement was started before 72 h from fertilization, and it was measured for 3 days in each 24 h without any addition. Before fertilization, N_2O was emitted at a significant values, but it deceased with time. Then an addition of fertilizer was done to measure the gas emission after 4 h and 27.5 h from it, where no very higher emissions were detected, but with significant difference between the different N rates. Then after 48, 70 h from fertilization, it decreased a bit, and stabilizes between 70 h and 94.5 h. So during this time, they reach the maximum at 27.5 h from fertilizer application, for N150, and after 4 h for N75. Then after 120 h from fertilizer addition, glucose portion was added, and N_2O emissions were measured after 4 h from this amendment, after this latter addition a pulses in the N_2O emissions were recorded, always 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 (after 94.5 h from N fertilizer 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, and then the N_2O emission was measured after 14 h from this addition and 171 from fertilizer addition, but the decreases continued after 37.5 h from microbes addition, except soil treated with N150 sodium nitrate, the N_2O emission from it was increased a bit after the 14 h from the addition, and then decreased again. 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 from both 2nd glucose and 2nd microbial solution addition (222 h from 1st fertilizer addition), 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, but there was no increment (negligible values) after that.

Then after 114 h from this addition (after 779.5 from 1st fertilizer addition), a third portion of glucose addition was done, and the N_2O emission was measured after 18 h from this amendment, and an emitted peacks were observed for N0, N75, and N150, thoses values were very higher than the amount emitted before the second N fertilizer portion. Then, it was start to decrease from 42 h after the 3rd glucose portion addition (for N_2O emission values, see supplementary Table 11).

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. On the other hand, significant effects of fertilizer addition were clearly also shown during the emission after the first N addition, and also under the different glucose additions there were always a significant difference between the different N addition rates.

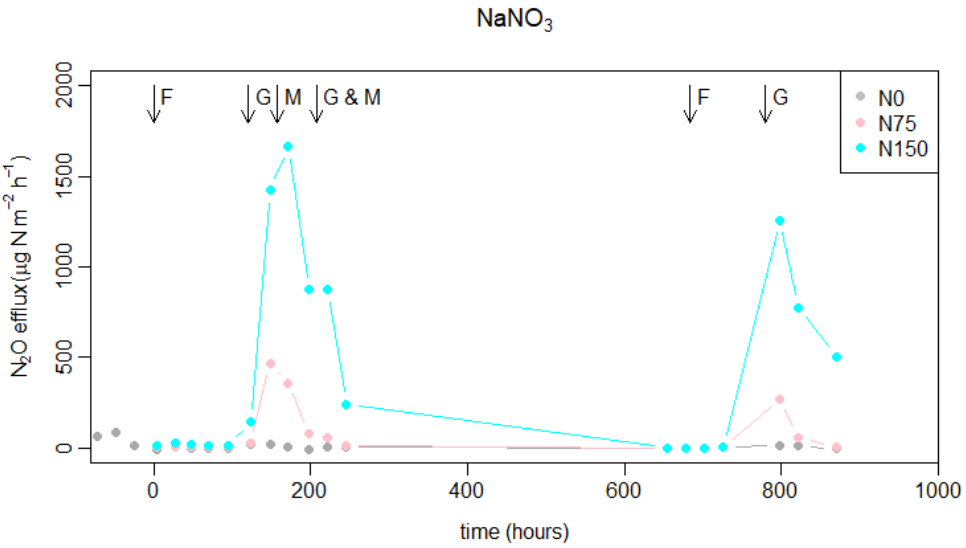

Figure 28. N_2O emission averages from cropland soil (bare soil), during 869.5 h long study period, under 40% soil water content, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}), and amended with glucose (G), microbial solution (M).

In general, and comparing this emission with the other experiments where the same soil type was used, but under ammonium nitrate fertilizer, it was clearly shown that during the first hours of measurement after N fertilizer addition, soil treated with ammonium nitrate emitted more N_2O compared with soil treated with sodium nitrate fertilizer. For example, in the 3rd experiment where 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 cases). 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 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.

N_2O emission from sand

N_2O emission averages from sand treated at 40% SWC, and sodium nitrate fertilizer, microbial and glucose additions were illustrated in Figure 29. N_2O emission was measured from sterilized sand, without any addition, a measurement was done 96, 72 h before adding sodium

nitrate fertilizer, no emissions were detected, the values were close to zero. Then, in order to create favorable conditions, 1st portion of glucose and 1st 1ml of microbial solution were added, and N_2O emissions were measured again after 4 h from this addition, where no emissions were recorded again.

Later on, N_2O emissions were measured after 4 h from fertilizer addition, higher values were detected, these values increased a bit after 27.5 h, to 2.3, 3.7 times higher than after 4 h from N addition, with no significant increase in N0. Thereafter, after 48 h from the N addition, a decreases in the N_2O emission was recorded in soil under no fertilization and treated with 75 kg N ha^{-1}, contrary in N150 an increase in the emission was recorded. A decrease in the N_2O emission, still recorded, from 70-190.5 h from fertilization, but with some fluctuations in soil under N0 and N150. Even there was an addition of another 1 ml of the microbial solution after 150 h from N addition, there was no significant increases. For that, another portion of glucose together with 2 ml of microbial solution were added (after 195 h from fertilizer addition), and the N_2O emissions were measured after 19.5 h from this addition (214.5 h from fertilizer addition), where the emissions were increased to 2, 5, 2.9 times higher than to which were observed before this addition. Then, after 43 h from this addition (238 h from N addition), another higher emission were detected. These higher values were decreased again (after 475 h from addition of the 2nd glucose portion plus the 2 ml of microbial solution).

Then, a 3rd portion of glucose was added (691 h from fertilizer addition), and N_2O emissions were measured after 3 h from that, and 694 h from fertilizer addition, where no significant increases in the N_2O emissions were detected. A similar trend was observed after 27 h from this addition, while a significant increases were detected, for N0, N75, and N150, after 99 h from the addition of the 3rd portion, and they increased more after 123 h from this amendment. Then, it started continously to decrease again (for N_2O emission values, see supplementary Table 12).

Based on the results found, N fertilizer addition clearly influenced the N_2O emission as increased with increasing fertilizer rate, and the presence of N supplies represented a key factor controlling the N_2O production. The N effect was observed before N addition to the sand. Also, in case of the sand, 48 h from fertilizer addition (accompanied with around 100 h from 1st glucose addition portion 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 N75, the maximum value was after 43 h from the 2nd glucose addition with the 2 ml of microbial solution, this difference between the ideal timing, shed light on the role of glucose addition or the easily decomposable carbon on the N_2O production and emission, also the role of microbial addition.

N_2O emission decreased between 70-190.5 h from fertilization, even with microbial addition. It increased after the addition of the 3rd portion of glucose, proving the importance of the easily decomposable carbon as a key factor, but the late increases in the emission after this addition was maybe because the microbes needed time to use it.

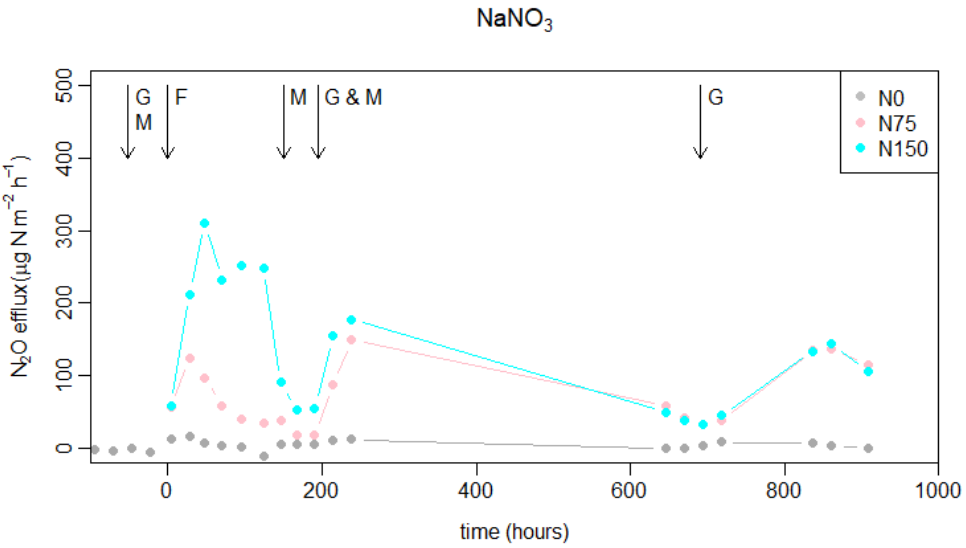

Figure 29. N_2O emission averages from sand (bare soil), during 909 h long study period, under 40% soil water content, treated with different levels of sodium nitrate fertilizer (0, 75, 150 kg N ha^{-1}), and amended with glucose (G), microbial solution (M).

Easily degradable carbon measurement

The easily degradable carbon measurement results from cropland and forest soil samples were illustrated in Figure 30. Using 5 g of soil samples, cropland soil had a lower average value of easily degradable carbon (637.3 mg kg^{-1}), while forest soil sample had a higher value of 706.3 mg kg^{-1}. Even using 2.5 g still the cropland soil had the lower value with 715.9 mg kg^{-1} and forest soil had a higher value of 806.7 mg kg^{-1} (Figure 30). Our results clearly demonstrated that active carbon in forest and cropland soils differed signifiantly, in the forest soil sample EDC was significantly higher than the cropland soil sample, which was shown both using 2.5 and 5 g, where the calculated p-values are: 0.0091, 0.0004, using 2.5 and 5 g, respectively.

The lower value of active carbon in the cropland soil maybe because the soil was affected by soil management practices, as several studies reported that the availability of easily degradable carbon is affected and changed depending on the type of land use (Weil *et al.*, 2003; Wolińska *et al.*, 2014, 2016). Similar result was reported by Wolińska *et al.* (2018) where a reduced ECD was recorded in the agricultural soil.

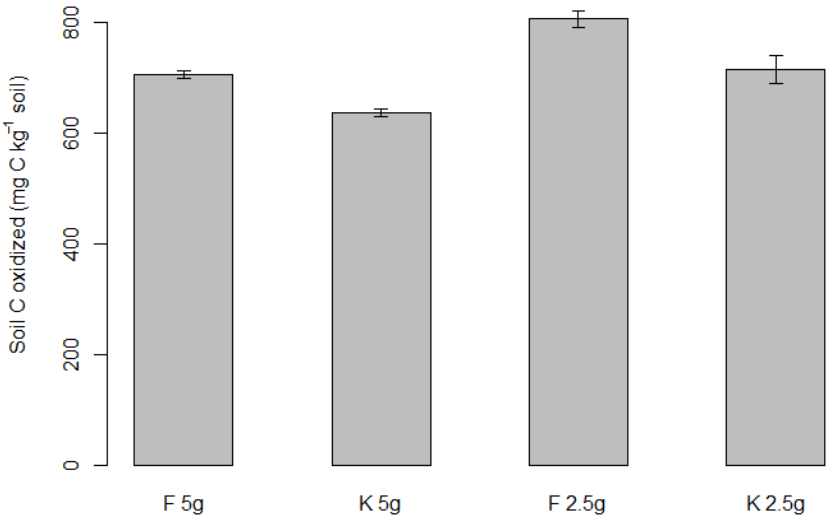

Figure 30. A comparison of active C measured of two different soil samples (cropland and forest soils), using 5 g and 2.5 g.

Comparison between N_2O emissions in the three soil types

Comparing the three soil types, it was shown that forest soil emitted more N_2O than the other soil types. Before fertilization forest emitted more than 18 times higher N_2O compared with the cropland soil, while in the sand no emission was detected due to the unfavorable conditions (no carbon and nitrogen sources, and also no microbes). Even after fertilization, forest soil emitted more N_2O than the cropland soil, also sand emitted more N_2O than cropland soil but not very lower compared to the forest one, but it's not easy to compare sand with the other soil since sand received glucose portion before fertilization. In addition the 1st glucose addition was necessary for forest soil just after 251 h from fertilization, contrary to cropland soil where an addition of glucose was needed after 120 h from fertilization, for the sand a 2nd glucose portion was needed after 195 h from fertilization. After this additions forest soil emitted more N_2O than cropland soil and sand, even there were a higher emission before this addition in forest soil type. The reason of this variation between the two different soils (cropland and forest) was the easily decomposable carbon as it was demonstrated in the previous soil samples analysis (easily degradable carbon measurement), that cropland soil had less active carbon than forest one. The easily decomposable carbon can be affected by management practices as reported by Weil *et al.* (2003) and is closely related to soil productivity and biologically mediated soil properties, also this parameter together with others like N, are critical factors for determination of soil microbiological activities (Anna, Zielenkiewicz and Banach, 2016). For the sand, the lower emission was caused because sand conditions are artificial and the microbial solution added had less denitrifying diversity compared with the original microbial populations. Soil texture could also play an important role since it was reported that during a laboratory experiment N losses from heavily weathered tropical soils were

higher in a clay textured soil variation than from a sandy variation (Sotta, Corre and Veldkamp, 2008).

Based on the observed results from the 3 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 (Nishio *et al.*, 1988; Azam *et al.*, 2002; Chen, Mothapo and Shi, 2015). Weier *et al.* (1993) recorded in their study quite small denitrification rates at high N concentrations in the absence of an available C source but these rates were increased with increasing available C (glucose) because carbon remained as the electron donor for all of the possible reduction steps, so both the production and reduction of N_2O were really controlled by the organic C presence (Weier *et al.*, 1993). Differences in the rates of denitrification, also in the reduction of N_2O to N_2 between different low molecular weight C compound amendments to soil studies were demonstrated in several studies (Morley, Richardson and Baggs, 2014). Additionally, Henry *et al.* (2008) reported that different low molecular weight C additions have been found to lead to varying abundances of narG, encoding for nitrate reductase and nosZ encoding for N_2O reductase. As it was mentioned by Giles, Daniell and Baggs (2017), little is known about the effects of the form of C substrate, or on the interaction between the denitrifying bacterial community and the C substrate. 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.

5. 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.

6. 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. 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 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.

7. SUMMARY

Nitrous oxide is a potent greenhouse gas, with an estimated contribution to the overall greenhouse effect of 6%, and a high global warming potential, 306 times greater than that of CO_2 persisting in the atmosphere for around 100 years on average. From the different natural and anthropogenic N_2O sources, agriculture represents a major source, contributing more than 75% of the global N_2O emissions including direct and indirect emissions, where synthetic fertilizers account for about 18% of N_2O emissions. In Hungary, 87% of the N_2O emission was generated from agriculture. Most of the emitted N_2O from agricultural soils is mainly produced during the microbial mediated nitrification and denitrification processes, with the possibility of the contribution of other microbial metabolic pathways and abiotic processes, including nitrifier denitrification, dissimilatory nitrate reduction to ammonium, anaerobic ammonium oxidation, and chemodenitrification, with each process modulated by specialized groups of microbial assemblages.

N_2O from agricultural ecosystems are the result of complex interactions of various parameters, including soil physical, biological, chemical properties, and climate, as soil available carbon and nitrogen content, microbial community, vegetation type, soil acidity, soil temperature, soil moisture, and other soil characteristics. All of those factors regulating gas production processes and emissions may be affected by the type, intensity, and timing of different management practices such as tillage, fertilization, crop residues, and irrigation. Soil surface-atmosphere exchange of N_2O can be measured using different methods and approaches, where chamber methods are widely used.

As croplands are the most common form of agricultural land-use in Hungary, a two-year-long N_2O field soil emission and laboratory experiments were done to determine the effects of different environmental factors and management practices on soil N_2O emissions focusing on the key variables controlling N_2O emissions i.e. temperature, soil moisture, N fertilizer application, plant growth, and carbon uptake by the plants.

According to the field data we demonstrated that SWC and VIgreen had a significant positive, while soil temperature had a negative correlation with the N_2O emission. It 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. Also, during the field study, a higher N_2O emission was recorded during the freezing-thawing period, and no emission was detected even after fertilization that proved that other drivers were influencing the emission rather than N addition and SWC. Concerning the microbiological investigation, it was shown that five soil

microbial communities were capable of metabolizing organic substrates. It was also shown that the capacity utilization of six-type carbon sources was different, where the carbohydrates were the carbon sources with the highest degree of metabolic utilization and the lowest degree of metabolic utilization was amines/amides. In addition, there was a tendency that othe 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 bacteria denitrificans and higher metabolic activity, and the contrary was also observed.

Besides the field results, different lab experiments were done aiming to study the effect of the different drivers on N_2O emission. In the 1st experiment which contained a 2 series of weekly measurements, positive effects of both N fertilizer and SWC were clearly recorded, increasing SWC by 5% caused at least one-fold higher increase in N_2O emission, while plant presence effect was changed during the weeks, but in general, a positive effect was also observed, in some measurement days it caused emissions that were even 7 times higher than bare soil.

In the next lab experiment, a strong positive correlation was found between the amount of N fertilizer and N_2O emission, as well as N_2O emissions and SWC where significant correlations was observed. Doubled amount of N fertilizer caused two to three-fold higher increase in N_2O emission, Similarly, SWC of 36% (on average) caused three-fold higher increase in N_2O emission compared to soil under SWC of 21% (on average), these effects were observed both in bare and planted soil. While only a minor effect of plant presence was recorded. For the 3rd lab experiment, both plant presence, SWC level, and fertilizer rate had a positive effect on the N_2O emission. Increasing the SWC level from 20 to 40% caused in increase in the N_2O emission with more than 11 and 5 times in bare and planted soil, respectively. And planted soil emitted at least two times higher N_2O than bare soil. Besides, the addition of C source (glucose) had increased the N_2O emissions significantly having bare soil at 40% SWC seemed to be to most affected by this amendment, with emission more than 118 times than before this glucose addition.

Finally, we also compared an N_2O emission of the cropland soil to other soil types (sand and forest soil), where it was clearly concluded that N fertilizer and carbon source represent key factors controlling the N_2O emission, and they had a significant positive effect on the emissions when no other drivers are limiting. In the cropland soil, glucose addition caused higher emission with more than 65 times compared to N_2O emitted with just N fertilizer addition. Fertilizer type also had an effect on the N_2O emission.

Those results illustrate the complex effect of biotic and abiotic factors determining N_2O emissions, which could help to understand the agricultural N_2O emissions. We hope that our results represent a valuable addition to the research on N_2O emission originated from agriculture in East-Central Europe and could be valuable also for developing management strategies to reduce N_2O emissions from agricultural soils.

8. ÖSSZEFOGLALÁS

A dinitrogén-oxid egy rendkívül fontos üvegházhatású gáz, becsült hozzájárulása a felmelgedéshez 6% körüli, üvegházhatás-potenciálja pedig 306-szorosa a szén-dioxidnak (100 éves alapon). A különböző természetes és antropogén forrásai közül a mezőgazdasági eredetű kibocsátás igen jelentős, a teljes dinitrogén-oxid kibocsátásnak a 75%-át adja, míg a műtrágyázás a 18%-át. Magyarországon a kibocsátott N_2O 87%-a származik a mezőgazdasági művelésből. A kibocsátott N^2O nagy része a talajban működő nitrifikációs és denitrifikációs folyamatokból származik, de más anyagcserefolyamatok is hozzájárulhatnak.

A mezőgazdasági talajok N_2O kibocsátása complex interakciók eredménye: a talaj fizikai, biológiai és kémiai tulajdonságiank, illetve a klimatikus tényezők függvénye. Befolyásolja a növényzet minősége, a talajnedvesség, talajhőmérséklet, a talajban rendelkezésre álló N és C források mennyigége és minősége. Minden említett faktort befolyásol továbbá a talajművelés, annak típusa, intenzitása, különös tekintettel a hozzáadott műtrágya mennyiségére. A kibocsátott N_2O mennyiségének mérése többféle módszerrel lehetséges, a leggyakrabban használt módszer – egyszerű kivitelezése miatt és relatív olcsó volta miatt - a kamrás mérési technika.

Magyarország nagy része szántóföldi művelés alá tartozik, ezért vizsgálati helyszínként egy közép-magyarországi szántóföldet választottunk. Két éven keresztül végeztünk terepi N_2O emissziómméréseket és emellett vizsgáltuk a fontosabb ható tényezőket is, így vizsgáltuk a talajhőmérséklet, talajnedvesség, a növényi növekedés és CO_2 felvétel hatását. A terepi mérések adatai alapján szignifikáns pozitív összefüggést találtunk a talajnedvesség (SWC) és N_2O kibocsátás és a VIgreen és az N_2O kibocsátás között, míg a talajhőmérséklettel negatív összefüggést tapasztaltunk, de ez a magasabb hőmérsékletek mellett előforduló alacsonyabb talajnedvességnek volt tulajdonítható. Magas N_2O kibocsátást mértünk a hideg időszakban a talaj fagyása-felengedése mellett, míg alacsony volt a kibocsátás akár közvetlenül műtrágyázás után, alacsony talajnedvesség mellett. A talajmikróbák vizsgálata alapján kimutattuk, hogy a különböző szénforrások közül a szénhidrátok váltották ki a legnagyobb metabolikus aktivitást a vizsgált talajban, míg a legalacsonyabbat az aminok/amidok. Megállapítottuk továbbá, hogy a legmagasabb denitrifikáns aktivitás nem feltétlenül társult magasabb dinitrogén-oxid kibocsátással.

A terepi mérések mellett laboratóriumi kísérleteket is végeztünk a különböző faktorok hatásainak feltárásához. Az első kísérletsorozat során kimutattuk a SWC és a hozzáadott műtrágya mennyiségének N_2O fluxusra gyakorolt pozitív hatását, az SWC 5%-os növekedése is szignifikáns növekedést eredményezett. Emellett a növényi aktivitás pozitív hatását is megállapítottuk. A

második kísérletsorozatban erős összefüggést találtunk a bevitt műtrágya mennyisége és a N_2O kibocsátás között: kétszeres műtrágyamennyiség 2-3-szoros kibocsátásnövekedést eredményezett. A talajnedvesség hatása itt is jelentős volt, a kumulatív kibocsátás háromszor akkora volt átlagosan 36% talajnedvesség mellett, mint 21% mellett. A harmadik kísérletben az eddig vizsgált tényezők mellett vizsgáltuk a hozzáadott szénforrás (glükóz) hatását a dinitrogén-oxid kibocsátásra. A hozzáadott glükóz jelentős mértékben megnövelte az emissziót, különösen magas talajnedvesség mellett.

A vizsgált talajtípus N_2O kibocsátását összehasonlítottuk más talajokkal is (homok és erdőtalaj), ahol a talajokban könnyen hozzáférhető szén mennyisége meghatározónak bizonyult, a szántóföldi talaj esetében a glükóz hozzáadás 65-szörösére növelte az emissziót.

Az eredmények alapján elmondható, hogy a szántóföldi N_2O kibocsátás variabilitása mögött complex hatások állnak, amelyeket részben sikerült feltárnunk a mérések segítségével. Reményeink szerint eredményeink hozzájárulnak a talajok N_2O kibocsátásának megértéséhez és a kapott összefüggések hasznosíthatók annak modellezésében.

9. BIBLIOGRAPHY (LIST OF WORKS CONSULTED)

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11. APPENDICES

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A2. SUPPLEMENTAL TABLES

Table A2.1. Field gas sampling dates during 2 years (November 2017- November 2019).

Samling number	Samling dates
1	23/11/2017
2	06/12/2017
3	18/12/2017
4	11/01/2018
5	02/02/2018
6	19/02/2018
7	12/03/2018
8	28/03/2018
9	16/04/2018
10	25/04/2018
11	16/05/2018
12	30/05/2018
13	15/06/2018
14	03/07/2018
15	17/07/2018
16	26/07/2018
17	15/08/2018
18	27/08/2018
19	13/09/2018
20	26/09/2018
21	11/10/2018
22	31/10/2018
23	12/11/2018
24	30/11/2018
25	22/01/2019
26	08/02/2019
27	26/02/2019
28	25/04/2019
29	02/05/2019
30	21/05/2019
31	12/06/2019
32	26/06/2019
33	10/07/2019
34	23/07/2019

35	15/08/2019
36	06/09/2019
37	24/09/2019
38	16/10/2019
39	08/11/2019

Table A2.2. N_2O emission averages (μg N m^{-2} h^{-1}) 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}), and amended with glucose (G) and microbial solution (M).

Time from fertilization (h)	N0 (0 kg h^{-1})	N75 (75 kg h^{-1})	N150 (150 kg h^{-1})
-24	246 ± 127	264 ± 22.8	307 ± 251
4	450 ± 389	910 ± 677	471 ± 207
27.5	257 ± 237	795 ± 371	1104 ± 492
48	198 ± 177	500 ± 280	706 ± 331
70	167 ± 133	371 ± 24.5	396 ± 266
96	182 ± 137	238 ± 32.1	293 ± 212
116	146 ± 72.4	133 ± 13.6	215 ± 176
148	136 ± 67.3	150 ± 18.2	186 ± 183
196	116 ± 81	124 ± 12.6	126 ± 139
239	85.2 ± 45.6	59.2 ± 23.3	103 ± 87.9
267	803 ± 596	937 ± 311	1108 ± 599
316	40.0 ± 16.4	353 ± 130	848 ± 401
340.5	53.4 ± 25.0	138 ± 43.2	294 ± 263
362.5	123 ± 98.0	1286 ± 356	2836 ± 1149
384.5	144 ± 110	807 ± 39.2	698 ± 485
434.5	60.8 ± 38.7	91.8 ± 21.7	218 ± 240
456.5	43.3 ± 18.5	106 ± 23.9	107 ± 72.2
529.5	111 ± 73.5	8.70 ± 1.96	65.8 ± 50.2
535.5	100 ± 47.6	21.3 ± 7.15	43.8 ± 20.2
580	83.0 ± 46.0	50.5 ± 4.70	25.7 ± 10.9
607	1507 ± 536	2192 ± 1120	2552 ± 1123
629	156 ± 49.3	962 ± 670	1375 ± 140.1
729.5	21.9 ± 9.83	34.0 ± 18.6	31.4 ± 3.10
769.5	28.0 ± 3.51	204 ± 254	97.9 ± 101
793.5	30.2 ± 9.33	35.0 ± 22.0	60.0 ± 61.2
849	10.7 ± 1.38	17.0 ± 7.25	23.3 ± 16.9
871.5	14.9 ± 1.13	133 ± 29.8	784 ± 710

895.5	11.6 ± 13.6	105 ± 13.2	419 ± 185
942.5	176 ± 48.3	1414 ± 1022	3523 ± 492
965	63.6 ± 43.0	441 ± 184	4765 ± 2141

Table A2.3. N_2O emission averages (μg N m^{-2} h^{-1}) from forest soil (bare soil), during 965 h long study period, under 40% SWC, treated with different levels of ammonium nitrate fertilizer (0, 75, 150 kg N ha^{-1}), and amended with glucose (G) and microbial solution (M).

Time from fertilization (h)	N0 (0 kg h^{-1})	N75 (75 kg h^{-1})	N150 (150 kg h^{-1})
-24,0	135	344	408
4,0	229	857	708
27,5	105	833	884
48,0	124	498	559
70,0	284	321	371
96,0	191	192	344
116,0	58.2	95.3	292
148,0	29.2	98.6	410
196,0	13.9	96.1	348
239,0	16.6	33.1	163
267,0	671	1085	1658
316,0	188	2414	2171
340,5	75.4	643	1151
362,5	106	248	579
384,5	67.6	176	473
434,5	14.6	38.7	19.8
457	51.4	104	284
530	5.05	6.15	13.4
536	14.2	22.4	162
581	3.01	43.3	190
607	602	670	844
629	444	5745	6089
730	19.9	305	834
770	12.8	145	274
794	37.6	163	124
818	7.76	104	167
849	12.9	81.9	141
872	1636	2146	2089
896	506	6064	10681
943	31.8	274	731

965	41.4	148	539

Table A2.4. N_2O emission averages (μg N m^{-2} h^{-1}) from the 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).

Time from fertilization (h)	N0 (0 kg h^{-1})	N75 (75 kg h^{-1})	N150 (150 kg h^{-1})
-72	65.6 ± 83.1	/	/
-48	84.1 ± 179	/	/
-24	15.1 ± 18.5	/	/
4	–6.91 ± 10.7	6.76 ± 8.81	9.09 ± 5.85
27.5	1.82 ± 0.46	3.04 ± 1.17	25.3 ± 28.7
48	–0.68 ± 0.16	1.72 ± 1.01	15.8 ± 13.1
70	–3.17 ± 1.27	1.12 ± 0.70	11.9 ± 12.1
94.5	–4.02 ± 1.91	1.31 ± 1.74	12.2 ± 13.8
124	16.7 ± 18.4	25.5 ± 16.2	146 ± 137
148	17.7 ± 1.97	468 ± 432	1421 ± 1911
171	2.2 ± 4.9	359 ± 495	1662 ± 2436
197.5	–11.7 ± 42.5	78.4 ± 104.5	874 ± 1320
222	3.43 ± 1.65	59.0 ± 45.9	872 ± 1108
245.5	7.45 ± 0.86	14.5 ± 5.45	240 ± 289
653.5	–1.21 ± 0.35	–0.76 ± 0.86	–0.79 ± 1.75
677.5	–0.53 ± 0.35	–0.19 ± 0.08	–1.25 ± 1.61
701.5	–0.99 ± 0.84	0.16 ± 0.53	–0.11 ± 2.36
725.5	1.41 ± 0.24	1.25 ± 0.23	2.59 ± 1.51
797.5	13.0 ± 1.88	269 ± 154	1257 ± 615
821.5	8.65 ± 4.04	55.8 ± 73.8	774 ± 277
869.5	–3.36 ± 0.91	1.795 ± 0.73	503 ± 836

Table A2.5. N_2O emission averages (μg N m^{-2} h^{-1}) 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).

Time from fertilization (h)	N0 (0 kg h^{-1})	N75 (75 kg h^{-1})	N150 (150 kg h^{-1})
-96	–3.01 ± 4.82	/	/
-72	–3.72 ± 6.02	/	/
-48	–0.20 ± 2.96	/	/
-24	–5.53 ± 10.2	/	/
4	11.4 ± 1.27	55.7 ± 24.6	58.0 ± 7.81
27.5	15.4 ± 0.95	123 ± 78.5	212 ± 67.3
48	7.45 ± 3.63	96.3 ± 54.2	311 ± 250
70	2.23 ± 0.28	58.7 ± 44.6	232 ± 191
96	0.96 ± 0.94	39.7 ± 33.2	252 ± 267
124	–10.7 ± 3.26	34.1 ± 35.2	248 ± 306
146.5	5.13 ± 5.03	37.5 ± 45.3	90.2 ± 65.3
167.5	5.53 ± 1.33	17.2 ± 15.5	51.8 ± 37.0
190.5	5.72 ± 8.77	17.2 ± 23.5	54.9 ± 44.7
214.5	10.6 ± 1.28	87.6 ± 12.1	154 ± 26.9
238	11.7 ± 1.40	149.9 ± 28.2	178 ± 45.2
646	–1.32 ± 0.40	57.1± 71.4	48.5 ± 5.51
670	–0.79 ± 0.17	41.8 ± 50.0	37.6 ± 26.0
694	3.52 ± 2.06	31.7 ± 34.3	32.2 ± 22.5
718	7.703 ± 1.49	38.6± 41.7	44.7 ± 25.3
837	5.87 ± 1.53	135± 45.2	134 ± 75.6
861	2.91 ± 1.63	137 ± 31.1	144 ± 99.5
909	–0.60 ± 0.20	115 ± 55.2	106 ± 83.4