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N_2O EXCHANGES BY THREE AGRICULTURAL PLOTS IN SOUTHERN BELGIUM:
DYNAMICS AND RESPONSE TO METEOROLOGICAL DRIVERS AND AGRICULTURAL PRACTICES

Margaux LOGNOUL

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Promoteurs: Bernard HEINESCH & Bernard BODSON
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Abstract

In Belgium, managed soils are responsible for more than 60 % of N_2O emissions from agriculture. This thesis aimed at gaining insight on the influence of farming practices and meteorological conditions on N_2O emissions and flux dynamics in crops and grassland in Belgium. We worked on three research focus points: (1) the suitability of eddy covariance to measure N_2O fluxes, (2) the influence of farming practices on N_2O exchanges and (3) the weight of N_2O in the greenhouse gas budget of managed soils.

Three measurement campaigns were setup in Southern Belgium. In 2015, two sets of automated dynamic closed chambers were used to monitor N_2O exchanges in a maize crop with the aim of studying the long-term effect of contrasting tillage practices (conventional tillage vs. reduced tillage) in experimental plots. In 2016, at the Lonzée ICOS Station, N_2O emissions were measured by eddy covariance in a sugar beet crop from fertilization to harvest. Finally, in 2018, at the Dorinne ICOS Station, we set up a paired-flux tower measurement campaign to assess the impact of pasture restoration vs. business-as-usual on a control parcel.

After 8 years of contrasting tillage practices, N_2O and CO_2 emissions were respectively 10 times and twice larger in the parcel under reduced tillage than in the conventionally tilled parcel. Reduced tillage practices, by limiting the mixing of crop residues to the topsoil layer, favor microbial development and increase N and C availability for microorganisms.

In the sugar beet crop, we observed a large N_2O emission peak following fertilization. While this event was expected, a surprising result was the inhibition of this burst after the seed-bed preparation, which constituted a first-time observation. We hypothesized that the microbiome was disturbed by this shallow soil disturbance. This hypothesis would benefit from further investigations. We estimated that N_2O emissions accounted for at least 20 % of the net greenhouse gas budget of the crop, highlighting the importance of including N_2O when assessing budgets from fertilized soils.

Finally, the paired-flux tower experiment in a pasture revealed that, during the first year, restoration practices can trigger high N_2O emissions, although not as great as fertilizer and cattle excreta-induced N_2O peaks in non-restored parcels, and with different flux dynamics.

All in all, these results confirmed that N_2O emissions by managed lands are influenced by farming practices, particularly those implying soil disturbance, on the long and short-term. More research is needed to fully understand how N_2O production mechanisms are affected by tillage and soil preparation practices.

Key words: Nitrous oxide, Crop, Pasture, Tillage, Fertilization, Closed chambers, Eddy covariance.

Résumé

En Belgique, les sols sont responsables de plus de 60 % des émissions de N_2O du secteur agricole. Cette thèse avait pour objectif d'étudier *in situ* l'influence des pratiques agricoles et de la météo sur les émissions de N_2O et leur dynamique dans des cultures et pâtures en Belgique. Trois questions de recherche ont été abordées : (1) l'adéquation de l'eddy covariance à la mesure des flux de N_2O, (2) l'influence des pratiques agricoles sur les échanges de N_2O et (3) l'importance du N_2O dans le bilan gaz à effet de serre des sols cultivés.

Trois campagnes de mesures ont eu lieu en Wallonie (Belgique). En 2015, deux sets de chambres fermées automatiques ont permis de suivre les échanges de N_2O dans des parcelles expérimentales de maïs pour étudier l'effet à long terme de pratiques contrastées de travail du sol (conventionnel vs. réduit). En 2016, à la Station ICOS de Lonzée, une mesure par eddy covariance du N_2O échangé par une culture de betterave, de la fertilisation jusqu'à la récolte, a été effectuée. Enfin, en 2018, à la Station ICOS de Dorinne, un système de tours d'eddy covariance jumelées a permis d'évaluer l'impact de la restauration d'une prairie en comparaison avec une prairie pâturée normalement.

Après 8 ans de pratiques contrastées de travail du sol, les émissions de N_2O et CO_2 étaient respectivement 10 fois et 2 fois plus importantes dans la parcelle en travail réduit que dans la parcelle en labour conventionnel. En travail du sol réduit, l'incorporation des résidus de culture est limitée en profondeur, ce qui favorise le développement microbien et une plus grande disponibilité en N et C.

Dans la culture de betterave, comme attendu, un important pic d'émission de N_2O a suivi la fertilisation. Cependant, ce pic a été inhibé suite à la préparation du lit de semis, ce qui est une observation inédite. L'hypothèse d'un microbiome perturbé par ce travail peu profond du sol a été proposée et mériterait plus d'investigations. Concernant le bilan gaz à effet de serre de la culture, le N_2O a été estimé comme pesant pour minimum 20 % des émissions totales, ce qui confirme l'importance d'une mesure directe de ces émissions pour obtenir un bilan de GES réaliste des sols fertilisés.

Enfin, le suivi d'une restauration de pâture avec des tours d'eddy covariance jumelées a montré que, après un an et malgré des dynamiques d'émission différentes, cette pratique ne produisait pas d'émissions de N_2O plus élevées que celles liées à la fertilisation et aux excrétas bovins dans la parcelle non restaurée.

Dans l'ensemble, ces résultats ont confirmé que les émissions de N_2O des cultures et pâtures sont influencées par les pratiques agricoles, en particulier celles impliquant une perturbation superficielle du sol, à long comme à court terme. De plus amples recherches seront nécessaires pour comprendre comment les mécanismes de production de N_2O sont affectés par le travail du sol.

Mots-clés : Protoxyde d'azote, Culture, Pâture, Travail du sol, Fertilisation, Chambres fermées, Eddy covariance.

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First and foremost, my deep appreciation goes to the F.R.S.-FNRS and Gembloux Agro-Bio Tech for funding and hosting my research project.

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Spilling the tea

From primary school to university, I’ve heard plenty of diminishing comments, such as “you passed because the teacher likes you” or “you owe your grades to your smile” (sure, smiling at blank sheets is my secret weapon to ace exams). So, when I joined the world of research a few years back, I thought I would finally be preserved from sexism and discriminatory behaviors. It’s always been a male dominated community, but I thought I’d only meet educated people who would never judge me based on my gender. I thought I’d be treated like any other scientist. Well, I thought wrong.

The first time I presented a poster in an international conference, a guy came to my stand. Great, let’s discuss! Well, not exactly. Let *him* discuss. For the entire poster session (and the coffee break that followed), I had to suffer his presence and hear all the reasons why my results were wrong. His proof was a single paper from 1990 (I wasn’t even born).

The next year, at the same gigantic event, I encountered a group of male scientists wandering around in the poster alley. They stopped at my stand, and proceeded to discuss my results… without even acknowledging my presence. No “hi”, no “excuse me, is this your poster?”, nothing. I guess I’d make a great statue.

The first (and last) time I did an oral presentation in a big conference, some guy monopolized the time dedicated to questions. And what a surprise: he had none! He just wanted me and the whole audience to know that he had published an awesome paper and thus probably knew better than me about my own experiments.

Another memorable experience happened after a colleague’s presentation that contained several figures of mine. After the talks, as everyone was walking out of the auditorium, I was cornered by a male scientist who proceeded to criticize my graphs, telling me that I would never publish my work and that I had a poor understanding of my own dataset. Of course, that man had not raised a hand after the presentation to express his grievances. I tried to respond, only to be cut off and called “defensive”. I left the meeting after that encounter.

I wish that was it. But I also need to mention the countless times I was interrupted by male peers while talking. The intrusive questions from male peers about my pregnancy. The unsolicited comments from male peers about my looks. The “it’s all in your head” from male peers when talking about sexism happening at conferences. Most of the time, I didn’t even know these men.

It’s neither easy nor enjoyable to navigate through a thesis in this context. I should be proud about completing a PhD. Instead, I’m left with a bittersweet feeling because these experiences made me doubt about my legitimacy.

If you are reading this and are looking for resources about sexism in research: a great way to start is to visit the website “didthisreallyhappen.net”! They share comics about sexist behaviors happening in the science world. Some drawings will probably resonate with you. You can also read their last publication: “Drawing everyday sexism in academia: observations and analysis of a community-based initiative” (Bocher et al., 2020). I’ll end this rant with a comic of my own. I had to spill the tea.


BABY AND PHD DON'T GO TOGETHER
YOU WILL NEVER PUBLISH YOUR DATA!
Why are you being so defensive?
You should smile more
You could write during your maternity leave
I DON'T HAVE ANY QUESTION BUT...
YOU'RE OVER REACTING
YOU'RE WRONG
MALO

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List of acronyms

ANOVA	Analysis of variance
BA	Block average
BE	Belgium
DNA	Deoxyribonucleic acid
CEC	Cation exchange capacity
EC	Eddy covariance
EEF	Enhanced efficiency fertilizers
EF	Emission factor
GHG	Greenhouse gas
GWP	Global warming potential
ICOS	Integrated Carbon Observation System
IPCC	Intergovernmental Panel on Climate Change
LOD	Limit of detection
LSU	Livestock unit
NBP	Net biome production
NEE	Net ecosystem exchange
NGB	Net greenhouse gas budget
PCR	Polymerase chain reaction
RM	Running mean
RMSE	Root-mean-square error
RNA	Ribonucleic acid
SCF	Spectral correction factor
SD	Standard deviation
SE	Standard error
SOC	Soil organic carbon
SWC	Soil water content
UV	Ultra-violet
WFPS	Water-filled pore space

I

INTRODUCTION

I. Introduction

1. GLOBAL CONTEXT

Nitrous oxide (N_2O) is known as the second most impactful non-CO_2 greenhouse gas (GHG) after methane (CH_4), and its global warming potential (GWP) is 265 times that of CO_2 over a period of 100 years (Smith, 2017). It is also a major contributor to stratospheric ozone depletion (Portmann et al., 2012). Its current atmospheric concentration reached 328 parts per billion (ppb), which represents a 20 % increase since the pre-industrial era (Smith, 2017). With annual global emissions estimated at 17.9 Tg N_2O-N y^{-1} (Ciais et al., 2013), it is responsible for 6 % of anthropogenic radiative forcing (Myhre et al., 2013).

While the majority of N_2O is produced in natural ecosystems (atmosphere, oceans, unmanaged soils), about 40 % originate from anthropogenic sources (**Figure** I. 1). The most critical human activity is agriculture (**Figure** I. 2) and researches indicate that the atmospheric concentration keeps rising at an increasing rate due to the extension of land used for agriculture and the intensification of use of nitrogen fertilizers (Ussiri and Lal, 2013).

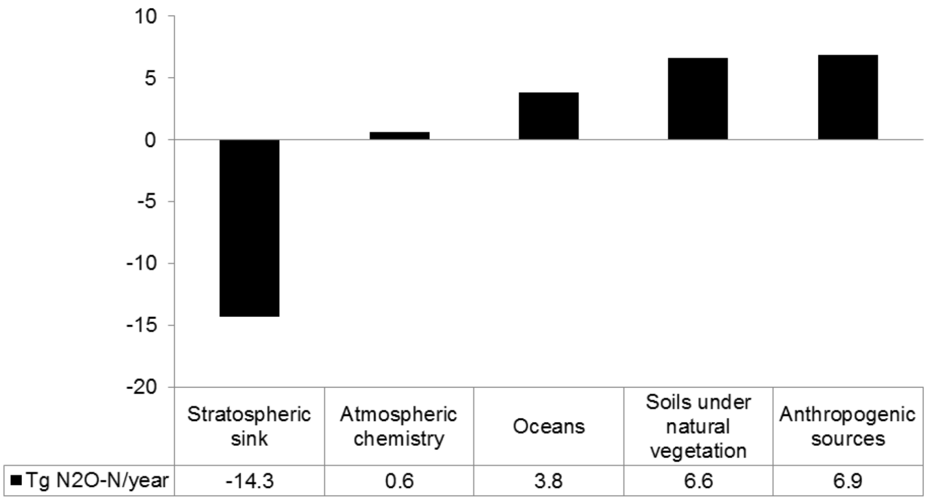

Figure I. 1 - N_2O sources and sinks (based on Ciais et al., 2013)

Several abiotic and biotic mechanisms are implied in N_2O emissions by cultivated soils, with nitrification and denitrification being the most determining processes (Hénault et al., 2012). Studies have shown that the water-filled pore space (WFPS) and thus oxygenation conditions were decisive drivers of N_2O production (Rabot et al., 2016). Soil nitrogen and carbon content have also been identified as key variables driving N_2O exchanges (Senbayram et al., 2012). Farming practices, such as fertilization or tillage, can have an impact on these variables and therefore influence N_2O emissions by managed soils (Plaza-Bonilla et al., 2014; Wang and Dalal, 2015).

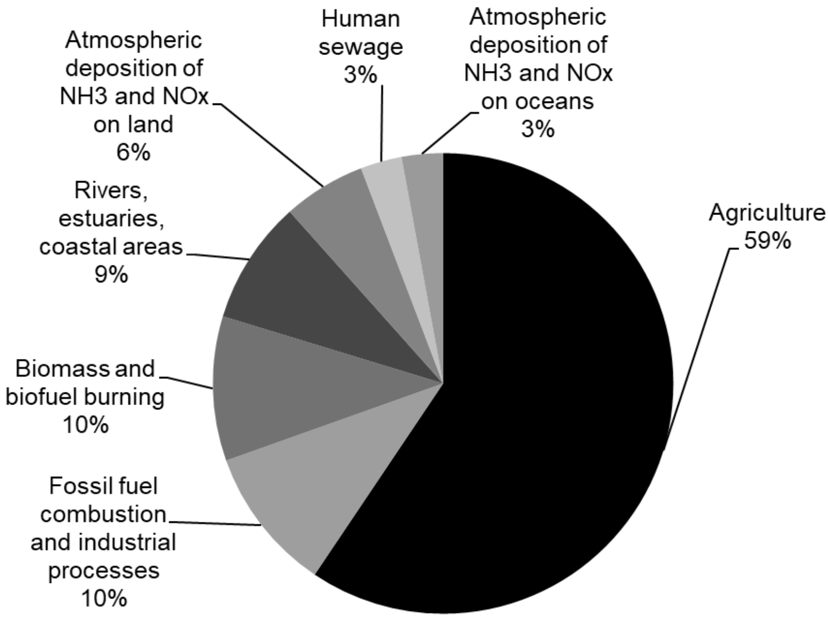

Figure I. 2 - Anthropogenic sources of N_2O (based on Ciais et al., 2013)

Although the main drivers have been identified, there remain knowledge gaps and uncertainties concerning (i) the magnitude and dynamics of their impacts, (ii) the understanding of microbial and physical processes and (iii) interactions between the factors involved (Butterbach-Bahl et al., 2013; Nicolini et al., 2013), making it complicated to implement adequate mitigation strategies. By establishing N_2O emission factors for managed soils (De Klein et al., 2006), the Intergovernmental Panel on Climate Change (IPCC) helped to build inventories at a national or continental scale, but these are sometimes uncertain and do not allow explaining processes at a smaller spatial and temporal scale.

2. N_2O-PRODUCING MECHANISMS IN SOILS

2.1. N_2O IN THE SOIL NITROGEN CYCLE

N_2O is mainly produced in soils and results from several transformations within the nitrogen cycle (**Figure** I. 3). These various reactions can occur simultaneously in different microsites (Baggs, 2008) and their understanding is essential for the study of N_2O emissions.

The production of N_2O by soils can be due, on the one hand, to abiotic mechanisms: the surface decomposition of ammonium nitrate (NH_4NO_3), the chemical decomposition of hydroxylamine (NH_2OH) and chemical denitrification (also called chemodenitrification).

On the other hand, N_2O is also produced via biological mechanisms (completed by bacteria and soil fungi): ammonification (mineralization of nitrate into nitrite, then ammonium), nitrification (oxidation of ammonium to nitrite then nitrate), denitrification (alternative respiration by nitrate reduction) and "nitrifier denitrification" (alternative respiration performed by some nitrifying bacteria). Microbial production of N_2O in soils is the dominant source, which has increased with the use of nitrogen fertilizers (Davidson, 2009).

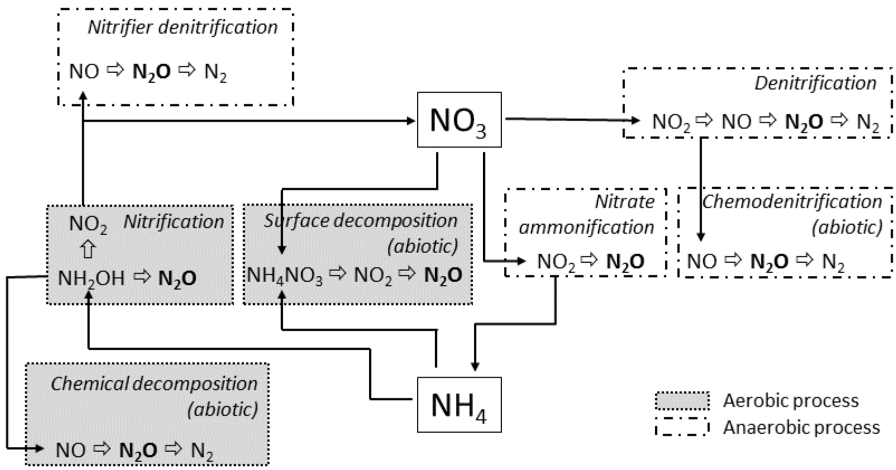

Figure I. 3 - N_2O production in soils (from Lognoul, 2015)

Nitrification and denitrification are the two main processes contributing to emissions from soils (Braker and Conrad, 2011; Hénault et al., 2012), accounting for almost 90 % of the annual global N_2O budget (Syakila and Kroeze, 2011). They are detailed below.

2.2. NITRIFICATION

Nitrous oxide is a by-product of nitrification, which is carried out by autotrophic[1] (mostly) and heterotrophic microorganisms. They are chemolithotrophs, that is, they obtain energy by oxidizing a nitrogen molecule of inorganic origin such as ammonium, which is the source of electrons[2]. The electron acceptor is O_2 in the case of nitrification, meaning that it is an aerobic mechanism.

[1] Whose source of carbon is inorganic.

[2] It seems however that some heterotrophic nitrifiers do not couple nitrification to the generation of energy (Prosser, 2005).

Autotrophic nitrification takes place in two steps involving two groups of bacteria of the Nitrobacteraceae family: those oxidizing ammonium and those oxidizing nitrite.

Heterotrophic nitrification is also carried out in two stages (the first by bacteria, and the second by fungi). Some nitrifying bacteria can also achieve denitrification ("nitrifier denitrification", see **Figure** I. 3) when free oxygen is less common. However, NO production is faster than the corresponding N_2O production, so this process is less important than nitrification by the bacteria mentioned (Smith et al, 2003).

Finally, searchers recently discovered that the nitrite oxidizer *Nitrospira,* a chemolithoautotrophic bacteria, was also capable to oxidize ammonia (Daims et al, 2015). Indeed, the genus of “comammox” (complete ammonia oxidizer) codes for both processes. *Nitrospira* was identified in a variety of environments, including agricultural soils. However, this bacterium is thought to produce little N_2O compared to ammonia-oxidizing bacteria (Kits et al., 2019).

2.3. DENITRIFICATION

N_2O is an intermediate product of denitrification. This process can be achieved by fungi and a wide variety of heterotrophic bacteria (Robertson and Groffman, 2007) which, in the event of a decrease in the availability of oxygen, use nitrate as the acceptor of electron (Smith et al., 2003). Each one of the four stages of the reaction is controlled by a specific enzyme, and each is inhibited by the presence of O_2. Denitrification occurs under anaerobic conditions[3] (Ferguson, 1994). Although nitrous oxide is only an intermediate product of this series reduction, its production rate may be greater than its consumption rate, creating an imbalance that can lead to N_2O emissions. There are several reasons for this.

Since enzymatic induction occurs in sequence, like the denitrification steps (**Figure** I. 4), there is a gap between the production of an intermediate product through one enzyme and its consumption through another (Robertson and Groffman, 2007; Richardson et al., 2009). At each step, there may be an intermediate product exchange with the environment; this is particularly the case of N_2O which can escape into the gaseous phase of the soil matrix. In addition, the accumulation of an intermediate product may be due to an imbalance in the enzymatic activities, generating unequal reaction kinetics between the different stages and favoring the net production of nitrous oxide (Pan et al., 2013). Such imbalance may result from the availability of other electron acceptors, such as nitrate, that are preferred over N_2O, which will not be reduced to N_2 (Chapuis-Lardy et al., 2007). Finally, it should be noted that nearly a quarter of the identified denitrifying fungi are incapable of producing the enzyme "nitrous oxide reductase" (N_2O-reductase) which allows the reduction of N_2O to N_2 (Shoun et al., 1992), and that denitrifying bacteria do not always perform this fourth step because the energy generated is low (Richardson et al., 2009).

[3] Conditions tend to be anaerobic when the rate of O_2 respiration by soil micro-organisms and plant roots exceeds the rate of O_2 diffusion from the atmosphere. Such conditions are also encountered when the gaseous phase of the soil matrix is replaced by water.

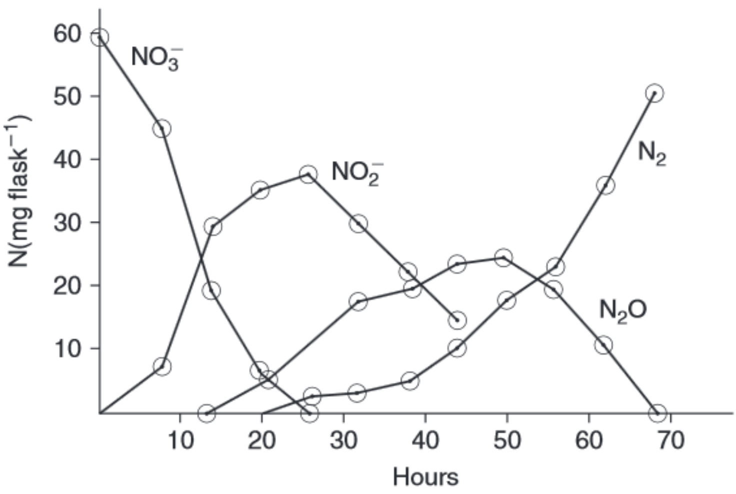

Figure I. 4 - Production sequence of denitrification (from Robertson and Groffman, 2007)

Denitrification is therefore considered the main source of N_2O in soils by some authors (Dobbie et al., 1999, Robertson and Groffman, 2007), despite the fact that nitrification may be the dominant process under certain conditions (Bateman and Baggs, 2005).

That being said, denitrification can also act as a sink when environmental conditions favor the reduction of N_2O (e.g. when nitrate availability is low but labile carbon content is high, see Section 3 of this Chapter). Denitrification is recognized as the only significant biological sink (Chapuis-Lardy et al., 2007, Vieten et al, 2007).

3. DRIVING VARIABLES OF N_2O EXCHANGES

As explained above, the production and consumption of nitrous oxide in soils result mainly from microbial processes, themselves being controlled by the various factors that influence the activity of microorganisms.

3.1. PEDO-CLIMATIC CONDITIONS

3.1.1. Water-filled pore space

The water-filled pore space (WFPS) is defined as follows (**Equation** I. 1):

Equation I. 1 $$WFPS\ (\%) = 100 * \theta_V / P$$

Where θ_v is the volumetric water content ($m^3\ m^{-3}$) and P is the total porosity ($m^3\ m^{-3}$). This variable directly characterizes soil pore oxygenation and gas exchange and is used in many studies on nitrous oxide emissions from soils. Indeed, WFPS is considered an adequate indicator of microbial activity by some authors because it provides information on water and oxygen availability (Linn and Doran, 1984; Robertson and Groffman, 2007). The microbial processes of interest are known to be strongly dependent on the soil water content (**Figure** I. 5).

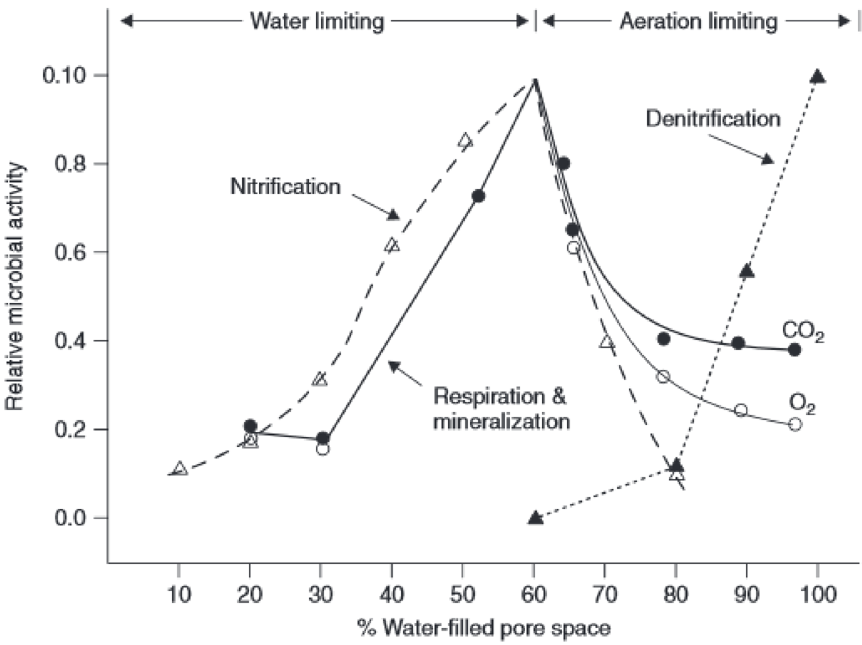

Figure I. 5 - Schematic representation of the intensity of microbial activity depending on the WFPS (from Linn and Doran, 1984)

Below 20 % WFPS, microbial activity is inhibited due to limited water availability (Smith et al., 2003). Between 20 % and 60 % WFPS, nitrification is predominant and increases linearly with WFPS, as well as microbial respiration and mineralization. The optimal nitrification rate is thought to occur at a WFPS of 60% (Linn and Doran, 1984). Such an observation has also been demonstrated in other laboratory experiments on soil samples, measuring a maximal rate of aerobic respiration of nitrifying populations at this value (Bateman and Baggs, 2005). Indeed, under these conditions, both water and free oxygen are available for microorganisms. Above 60 %, free oxygen availability decreases and areas of anaerobic conditions in the soil become larger, favoring denitrification.

As a result, the relative contribution of the two main N_2O-production mechanisms (nitrification and denitrification) changes according to the WFPS (Bateman and Baggs, 2005; **Figure** I. 6). According to Smith et al. (1998) and Dobbie et al. (1999), N_2O emissions are expected to increase as WFPS rises, with exponential evolution due to denitrification above 60-65 %.

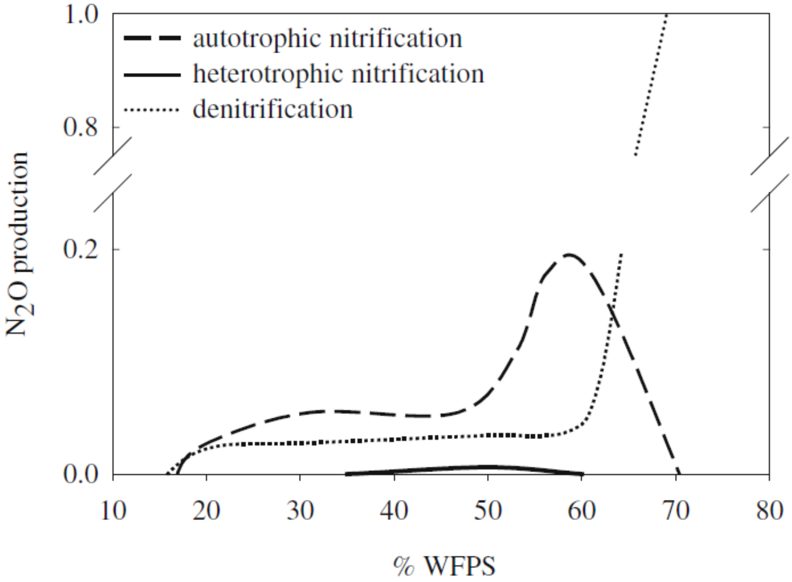

Figure I. 6 – Modelled contribution of autotrophic and heterotrophic nitrification and denitrification (from Bateman and Baggs, 2005)

However, this is not always observed: Laville et al. (2011), after laboratory experiments, found that beyond a WFPS of 70 %, emissions decreased. Castellano et al. (2010) observed, during free drainage of soil samples, a Gaussian distribution of fluxes as a function of WFPS. *In situ* measurements have shown the same phenomenon (Smith et al., 2003; Flechard et al., 2007, Figure 5): no N_2O emission was detected beyond a WFPS of 85-90%. This is explained by the fact that the N_2O produced cannot diffuse from the soil, which is almost saturated with water, and the gas can therefore be completely reduced to N_2 (Chapuis-Lardy et al., 2007).

Measurements in managed grassland (Dobbie et al., 1999, Flechard et al., 2007; van der Weerden et al., 2012) or in cultivated soils (Skiba and Ball, 2002; Adviento-Borbe et al. 2007; Laville et al., 2011; Saggar et al., 2013) corroborated these observations. However, these studies also found that the emissions observed in the WFPS range described above also depended on other variables (e.g. the availability of N substrate, provided by fertilizers). This is discussed in the following paragraphs.

3.1.2. Soil temperature

The soil temperature controls the activity of micro-organisms, a higher temperature favoring N_2O production (Smith et al., 2003). This causes two things: on the one hand, in aerobic and anaerobic zones, the respective development of nitrifying and denitrifying bacteria is stimulated; on the other hand, because of the increasing activity of bacteria, the availability of free oxygen decreases, increasing the anaerobic zones, which favors all the more denitrification, and therefore the production of N_2O (Flechard et al., 2007).

In an attempt to model the response of N_2O emission from fertilized soil to temperature and WFPS, Flechard et al., (2007) analyzed post-fertilization measurements from ten European grassland sites. In a range of non-limiting water content, the emission factor increased exponentially with soil temperature (**Figure** I. 7), as shown by Smith et al. (2003).

The same study investigated the relationship between flux from non-fertilized plots and soil temperature, and showed similar behavior, with a clear trend for negative N_2O fluxes at temperatures below 10 °C (within non-limiting WFPS). It can be hypothesized that, since denitrification seems less sensitive to drops of temperature than nitrification (Parton et al., 2001; de Bruijn et al., 2009), it is the predominant mechanism below 10 ° C and the low availability of nitrate (no fertilization) results in a reduction of N_2O in N_2.

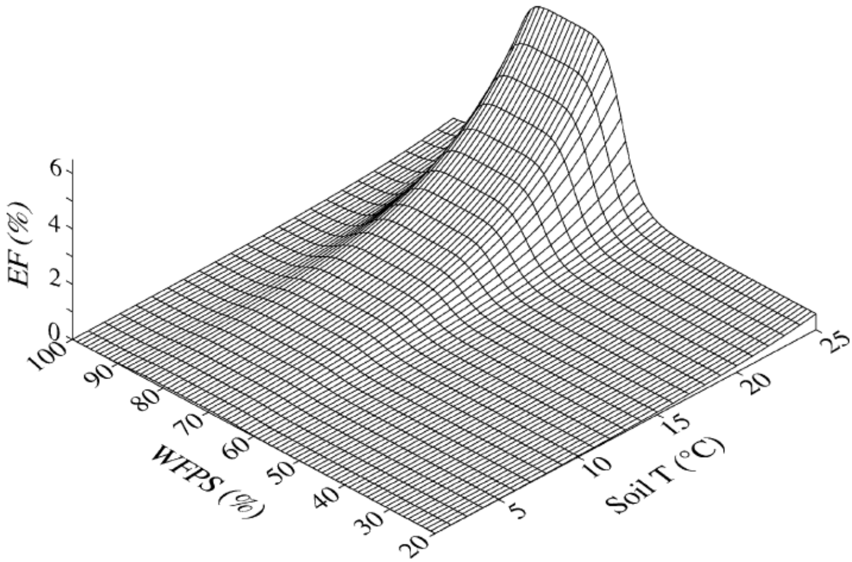

Figure I. 7 - Emission factor (EF) of fertilized soils as a function of soil temperature and WFPS (from Flechard et al., 2007)

In cultivated soils, winter GHG emissions and particularly nitrous oxide can represent up to 50 % of the annual balance depending on climatic conditions (Flessa et al., 1995; Kaiser et al., 1998), despite a reduced microbial activity due to low temperatures (Laville et al., 2011). However, winter weather phenomena such as freeze-thaw cycles are not often investigated *in situ* because they can only be observed in certain climates. Studies carried out on soil samples subjected to natural climatic conditions (Wagner-Riddle et al., 2008) and in the field in cultivated soils (Furon et al., 2008) have shown that the origin of N_2O releases during the thaw was a peak of denitrification in the soil surface layers rather than the release of N_2O accumulated during the freeze.

3.1.3. Soil texture and structure

The texture of a soil is defined by the dimensions of particles and their relative proportions. Soils are divided into textural groups according to their percentage of sand, silt and clay. The structure of a soil is determined by the arrangement of particles in aggregates. The size and shape of aggregates depend on the presence of microorganisms and roots, as well as on climatic and mechanical constraints on the soil. Both of these characteristics have an impact on nitrous oxide fluxes: by determining pore size and connectivity, they directly influence water behavior and oxygenation conditions within the soil.

In fine-textured soils, anaerobic conditions appear at lower water contents than in coarser ones, and last longer (Gödde and Conrad, 2000; Stehfest and Bouwman, 2006). This may lead to higher N_2O emissions, as shown by several studies based on *in situ* measurements in hayfield and cropland (Bouwman et al., 2002; Skiba and Ball, 2002; Chatskikh et al., 2005).

Regarding soil structure, van der Weerden et al. (2012) found that large macroporosity led to faster soil drainage in pastures and thus to a reduced duration of anaerobic conditions after rainfall, resulting in lower emissions. Skiba and Ball (2002) showed a link between low total porosity and high N_2O emissions.

In Belgium, annual N_2O emission measurements in cultivated soils suggested that the influence of soil texture is smaller compared to that of farming practices, such as fertilization or compaction caused by traffic (Goossens et al. 2001; Mosquera et al., 2007). However, soil texture should not be underestimated when assessing N_2O budgets: it partially defines soil cation exchange capacity (CEC), and Henault et al. (2019) found that pH, CEC and clay content were the best predictors of the capacity of a soil to reduce N_2O.

3.1.4. Soil pH

Scientific literature reports increased production of nitrous oxide in soils with acidic pH. The autotrophic nitrifying microorganisms remain active at low pH, and it is assumed that heterotrophic nitrifiers also contribute to nitrification under such conditions (De Boer and Kowalchuk, 2001). This mechanism is therefore minimally affected by soil pH.

Denitrification appears to be the most pH-sensitive process. Although there is no direct link between soil pH and denitrifying microorganism activity (Simek et al., 2002), soil acidity influences enzyme induction: the enzyme N_2O-reductase is inhibited by acid pH and the N_2O/N_2 production ratio is negatively correlated with soil pH (Chapuis-Lardy, 2007; Liu et al., 2010). Most of these observations come from laboratory studies because it is difficult to compare soils of different pH *in situ* without other variables, such as soil texture or organic matter, varying as well. One study focusing on this matter measured the impact of a pH change in grassland: a negative correlation of the ratio $N_2O/(N_2O + N_2)$ with the pH of the soil was observed (Cuhel et al., 2010).

In a recent study *in situ* in France, Henault et al. (2019) tested the impact of liming acidic fertilized soils. Their results showed that increasing the soil pH towards neutrality reactivated N_2O reduction.

3.1.5. Soil nitrogen content

Soil mineral nitrogen includes ammonium (NH_4^+) and nitrate (NO_3^-) and comes mainly from the mineralization of organic compounds and fertilizer inputs. Ammonium and nitrate are the basic substrates of nitrification and denitrification mechanisms, since they serve as electron donor and acceptor respectively (**Figure** I. 3). It is therefore logical to find a correlation between their availability in the soil and the production of nitrous oxide (Conrad et al., 1996). Even though N_2O fluxes can be correlated to several variables, several *in situ* studies have shown that the availability of mineral N in soils was largely responsible for N_2O emissions (Adviento-Borbe et al., 2007; Laville et al., 2011; Wang and Dalal, 2015).

The supply of nitrate or ammonium brought to agricultural lands can take different forms, which influences the emission mechanisms and N_2O exchanges observed. This question is discussed in Section 3.2 (p. 39). The nitrogen availability for microorganisms is also linked to the plant uptake. Finally, soil N content can also be influenced by dry deposition of reduced and oxidized nitrogen from the atmosphere. However, the impact of this phenomenon on N_2O emissions is negligible in managed lands (Hicks et al., 2014).

3.1.6. Soil carbon content

Labile carbon is the carbon fraction with the shortest degradation time (a few weeks to a few years). In cultivated soils, it mainly comes from degraded crop residues or organic fertilizers. It is the most important carbon source for heterotrophic micro-organisms, as it is the case with denitrifiers and some nitrifiers. Labile carbon is also involved in denitrification as a source of electrons for the energy-generating redox chain[4].

A high content of labile carbon thus favors mechanisms of heterotrophic nitrification and denitrification. Indeed, under such conditions and if mineral nitrogen substrates (ammonium and nitrate) are available, the activity of microorganisms is stimulated and the oxygen consumption by the nitrifying bacteria generates on the one hand a production of N_2O and nitrate, and on the other hand an increase in anaerobic zones, which favors denitrification (Del Grosso et al., 2000; Bouwman et al., 2002; Senbayram et al., 2012; Van Zwieten et al., 2013).

However, if a high content of labile carbon is combined with low levels of inorganic nitrogen, a consumption of N_2O and thus low or negative fluxes can be observed. On the one hand, nitrification is impacted by the non-availability of ammonium; on the other hand, denitrification uses N_2O as an electron acceptor and reduces it to N_2 (Mathieu et al., 2006, Chapuis-Lardy et al., 2007).

[4] Denitrifying bacteria are hetero-(chemo)-organotrophic microorganisms, meaning that their sources of carbon and electrons are organic molecules. The labile carbon fulfills both roles.

The role of soil content in labile carbon is therefore closely dependent on that of mineral nitrogen, which will determine the influence on N_2O fluxes of the different types of fertilizer.

3.2. FARMING PRACTICES

Farming practices can influence nitrous oxide emissions either by acting on nitrogen and carbon availability or by modifying soil characteristics, which will affect nitrification and denitrification.

3.2.1. Fertilizer application rate

Nitrogen is an essential element in the development and growth of plants, which is taken up in the form of nitrate, or ammonium under anaerobic conditions or under acidic pH (Maathuis, 2009). It is then accumulated as amino acids and proteins. To meet the needs of crops, farmers apply fertilizer. The rate of application is defined as the amount of nitrogen fertilizer (mineral or organic) applied to a crop per unit area and time, without considering a possible splitting of the total input over the cropping season.

In their review of 139 studies, Bouwman et al. (2002) showed a general trend towards a significant increase in cumulative N_2O emissions over the growing season with the application rate of nitrogen fertilizer, as fertilization increases the availability of mineral nitrogen. Similar observations have also been reported by other journals and studies (Stehfest and Bouwman 2006; Snyder et al., 2009; Laville et al., 2010; Hénault et al., 2012; Plaza-Bonilla et al. 2014). The fertilization rate is therefore a determining factor to explain N_2O emissions. Grant et al. (2006) added a nuance: the relationship between N_2O fluxes and nitrogen inputs during the growing season also depends on previous fertilizer inputs and nitrogen residues in the soil. Indeed, they will influence the assimilation capacity in the cultivated plot.

Finally, the dynamics over time of N availability should be taken into account when studying N_2O emissions. Indeed, crops have different N assimilation curves and are fertilized following different schedules (split input, before or after seeding…).

3.2.2. Type of fertilizer

The type of nitrogen fertilizer used to increase the yield of a crop can influence N_2O emissions from the crop for three reasons:

- Depending on the form of nitrogen contained in the fertilizer (ammonium, nitrate or organic nitrogen), it will be more or less rapidly available and used by the bacteria, and will favor nitrification or denitrification;
- The presence of rapidly degradable organic carbon in the fertilizer will favor the micro-organic activity related to the production of N_2O;
- The fertilizer could, when spread, modify soil characteristics such as moisture content or pH, which could influence emissions by the crop plot (e.g. the application of liquid manure can increase soil humidity).

Velthof et al. (2003) studied the effect on N_2O emissions of nine types of mineral and organic fertilizers on samples from cultivated soils. The highest emission peaks corresponded to fertilizers with high inorganic or easily mineralizable organic N content, and high easily mineralizable C content, and to fertilizers in liquid form, which allowed a better distribution of C and N in the soil matrix. This study also found that emissions from other fertilizers, although less intense during the first days, lasted longer after application. This phenomenon was attributed to the succession of organic matter mineralization, nitrification (production of nitrate by ammonium oxidation) and denitrification (reduction of nitrate) (Chadwick et al., 2000). Similar observations have been drawn from the experiment of Van Zwieten et al. (2013). Regarding mineral fertilizers, no difference in N_2O emissions is expected between solid (granular) and liquid forms, as both release their nitrogen content very quickly into the soil.

However, the type of fertilizer does not seem to influence emissions on the long term. Indeed, when looking at the N_2O budget of a crop, it appears that other variables take precedence in the control of emissions, such as the rate of application of fertilizer, the type of crop in place, climatic events as well as soil pH (Stehfest and Bouwman, 2006). This observation is corroborated by the *in situ* study of Plaza-Bonilla et al (2014): although the emission dynamics were different just after application, no significant difference in the flux balance after several years was observed between a mineral and an organic fertilizer.

3.2.3. Type of crop/agricultural land

Different types of crops, subject to similar soil and climatic conditions, could result in different nitrous oxide budgets, depending on how plants are involved in the nitrogen cycle, whether by assimilating N or by being mineralized in the soil after harvest. However, there are few long-term studies comparing crops with each other and calculating annual balances (Snyder et al., 2009).

After comparing N_2O budgets from several studies, Stehfest and Bouwman (2006) determined that legume crops emitted more N_2O than cereal crops, which in turn emit more than other types of crops. For leguminous plants, the important balances observed could come from the remaining roots and nodules or from the incorporation of residues after harvest, thus constituting a substantial supply of organic nitrogen for the next crop (Dick et al., 2006; Mosier et al., 2006). Other studies of interest have, on the contrary, observed very low positive fluxes, or even negative fluxes (Glatzel and Stahr, 2001), and thus seem to have failed to take into account the period of decomposition of residues following harvest. It should be noted that while the incorporation of legume residues generates significant emissions, it can reduce the mineral fertilizer input for the next crop and thus possibly lead to a reduction in the overall balance of a crop rotation (Schwenke et al., 2015).

Wang and Dalal (2015) suggest that it is also important to consider bare soil periods when establishing budgets, as significant emissions can be observed during winter periods without cultivation.

Finally, it is also interesting to look at nitrate-fixing intermediate crops. These intercrops are most often set up to fight against nitrate lixiviation and soil erosion caused by precipitation. Their impact on greenhouse gas emissions is often poorly considered (Bodson and Vandenberghe, 2013). A nitrate-fixing intercrop, by reducing the soil nitrate content, can lead to a reduction in N_2O emissions during its growth (Mitchell et al., 2013). However, it can have an entirely opposite effect when it decomposes on the soil surface or after incorporation into the soil (Sanz-Cobena et al., 2014). Peyrard (2016) obtained more moderate results regarding legumes: while they tended to increase N_2O emissions when used as fertilizer instead of mineral N, they did not significantly influence emissions during their growing period.

3.2.4. Tillage

Tillage and other types of soil preparation can have an impact on its structure and porosity, thus modifying the aeration conditions of pores and the way water is retained. This can have significant consequences for the production of N_2O by soil microorganisms. Several long-term studies (several years) have examined the impact of different tillage methods, without being able to identify a general trend (Snyder et al., 2009; van Kessel et al., 2013). Indeed, some searchers speak of larger cumulative emissions for plowed crops (van Kessel et al., 2013; Plaza-Bonilla et al., 2014; Wang and Dalal, 2015), while others have observed the opposite (Skiba et Ball, 2002).

To find some answers, it would be interesting to determine if tillage generates direct effects, that is to say in the short term (in the days or weeks that follow), in particular via the incorporation of crop residues that will then be mineralized and increase the availability of C and N (Laville et al., 2011). To make such observations, a high-definition temporal measurement system is necessary. It would also be relevant to study the long-term influence of plowing on N_2O-producing communities of micro-organisms: soil tillage and crop residue management practices that promote the development of micro-organisms, such as reduced tillage, can show higher long-term emission rates (Jahangir et al., 2011).

It should also be noted that regular tillage can also cause a reduction of the poral space and alter the aeration conditions at low or medium depth: we can expect that the compaction caused by farm machinery traffic has an impact on N_2O emissions by favoring denitrification (Mosquera et al., 2007).

4. N_2O FLUX CHARACTERISTICS

4.1. *TEMPORAL VARIABILITY*

N_2O exchanges by managed soils are characterized by continuous and small fluxes (called background fluxes) and by short and intense sporadic peaks (Groffman et al., 2009). These two very different dynamics obey the variables detailed in Section 3 (page 33).

Emission peaks can contribute to more than 50 % of fluxes while occurring during very short periods of time and are usually triggered by a large and abrupt change in key variables in N_2O production (Butterbach-Bahl et al., 2013). These bursts can last for one or more days (Clayton et al., 1997) and may be internally fluctuating (Smith and Dobbie, 2001).

On the other hand, background N_2O exchanges are low fluxes that occur continuously throughout the year depending on the activity of the N_2O-producing microorganisms. They can be positive or negative and differ from one site to another depending on pedo-climatic characteristics and crop management (van Groenigen et al., 2004). A few studies have shown intraday fluctuations in background fluxes, linking them to temperature (Laville et al., 2011) or to photosynthetic activity (Shurpali et al., 2016).

4.2. SPATIAL VARIABILITY

Several studies have shown significant spatial variability in N_2O fluxes across agricultural fields (Glatzel and Stahr 2001; Mathieu et al., 2006; Flechard et al., 2007; Groffman et al. 2009; Hénault et al., 2012) at the scale of the square meter. Locations with high emission rates are called "hot spots" and can contribute significantly to ecosystem emissions (van den Heuvel et al., 2009).

This variability is most likely due to the heterogeneity of soil conditions, caused either by an uneven distribution of organic matter and a slightly irregular topography influencing soil moisture (Mathieu et al., 2006; van den Heuvel et al., 2009) or by an unequal horizontal distribution of the abundance of N_2O-producing microorganisms (Jahangir et al., 2011). At the end of a measurement campaign on winter wheat using dynamic closed chambers, F. Broux (unpublished results) measured the soil nitrate content at the chambers locations and found that it was strongly correlated to the cumulated N_2O emission in each chamber during the last month of the experiment.

5. N_2O EMISSIONS IN BELGIUM

In Belgium, almost 50 % of land surface is dedicated to crops and permanent grasslands (StatBel, 2018). According to the National Inventory Report (NIR, 2019), greenhouse gases from agriculture accounted for 8.5 % of total emissions in the country in 2014, and N_2O emissions from soils represent a third of agricultural emissions.

Belgian estimates allocate N_2O emissions from agriculture to two source categories:

- Manure management, including the storage and handling of animal fertilizers;
- Agricultural soils, including the application of organic and mineral N fertilizer, and on-site production of urine and dung by grazing animals.

Indirect N_2O emissions from both categories are taken into account, respectively as those originating from the deposition of volatile nitrogen and those from leaching and run-off in soils.

N_2O flux estimates are based on the IPCC 2006 Guidelines (Tier 1 and 2) that provide emission factors (EF) for the mentioned categories (De Klein et al., 2006). Said EF are mostly based on large inventories. Belgian numbers for years 1990, 2014 and 2015 are illustrated in **Figure** I. 8. Direct emissions from manure management and agricultural soils are the biggest sources of N_2O. However, they have decreased by 24 % between 1990 and 2015 because of a smaller and optimized use of N fertilizer and a reduction of livestock. The decrease of direct emissions has induced a similar trend for indirect emissions (-32 %).

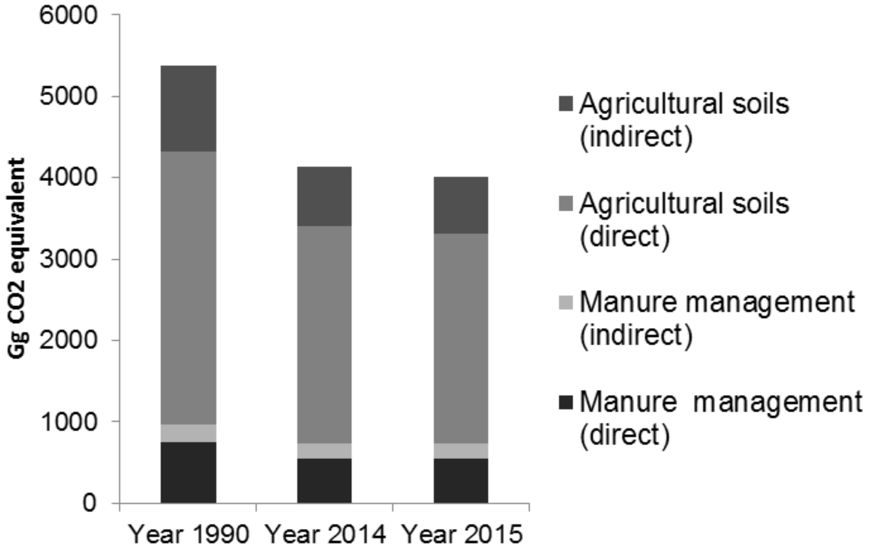

Figure I. 8- N_2O emissions from agriculture in Belgium (based on NIR, 2019)

6. FLUX MEASUREMENT TECHNIQUES

The most common tools for measuring N_2O fluxes are soil chambers. Although simple in concept and operation, the chambers have a limited coverage over space and time. As a consequence, these systems show limitations when it comes to capturing the temporal variability of fluxes and can be biased due to spatial variability. As a result, uncertainties on annual estimations can reach up to 50 % (Flechard et al., 2007). In addition, chamber-based measurements are intrusive and tend to modify the environmental conditions during measurements. Finally, the need to dismantle the system during farming operations often leads to loss of data at critical periods.

Micrometeorological measurements have become feasible following the development of fast and reliable analyzer technology. Eddy covariance flux measurements allow non-intrusive quantification of N_2O exchanges over time. They integrate fluxes over large areas (i. e. field scale) and are continuous, thus providing data even during and right after farming operations. Consequently, they capture well the temporal N_2O exchange variability while minimizing the risk for spatial bias. This method is probably the most suitable for the experimental determination of controlling factors and for the assessment of emission factors. Moreover, the measured fluxes can be used to verify the performance of process-based models (Pattey et al., 2007).

The eddy covariance method is not very widespread for N_2O fluxes yet given the analyzer prices. It is expected to become more and more common in the next few years. However, the eddy covariance technique requires high expertise and continuous watch. Until today, no Walloon team possessed skills in eddy covariance N_2O measurements.

At the start of this thesis in 2015, long-term measurements of N_2O exchanges at the ecosystem level and integrated over a long period were uncommon and experiments were mostly focused on forest and grassland ecosystems. To our knowledge, only two studies had performed such measurements on agricultural parcels (Molodovskaya et al, 2012; Huang et al., 2014). Moreover, no protocol on measurement and data treatment for non-CO_2 gases had been published at this moment (Nemitz et al., 2018).

7. THESIS OBJECTIVES AND COLLABORATIONS

The research project presented in this document falls within the scope of climate change and greenhouse gas emissions. By focusing on N_2O exchanges by agricultural soils, this work aims at contributing to a better understanding of how farming practices applied in Belgium may influence N_2O emissions. The following research questions were investigated:

- **Can we measure N_2O fluxes with eddy covariance:**
  - Is this technique suitable to monitor N_2O exchanges over managed lands?
  - Do the existing procedures (data collection, data treatment, etc.) need to be adapted for this specific trace gas?

- **What was the impact on N_2O emission dynamics and/or budget of farming practices in three specific experiments:**
  - A long-term “conventional vs. reduced tillage” comparison in a maize crop?
  - A conventionally-run sugar beet crop?
  - The first year of a pasture restoration?

- **What is the weight of N_2O in the greenhouse gas budget of a sugar beet crop:**
  - How much N_2O is emitted by a sugar beet crop in comparison to other GHG?

The following paragraphs list the detailed objectives of the next chapters as well as collaborations.

- ***Chapter II: Material and methods***

This chapter aims at describing the two measurements techniques that were used for this thesis, i.e. automated closed chambers and eddy covariance. Both experimental set-ups are detailed, as well as data acquisition and treatment procedures. Finally, the experimental sites are presented.

- ***Chapter III: Impact of tillage on greenhouse gas emissions by an agricultural crop***

This chapter is based on the following publication: *LOGNOUL, M., THEODORAKOPOULOS, N., HIEL, M.-P., REGAERT, D., BROUX, F., HEINESCH, B., BODSON, B., VANDENBOL, M., AUBINET, M., 2017. Impact of tillage on greenhouse gas emissions by an agricultural crop and dynamics of N_2O fluxes: Insights from automated closed chamber measurements. Soil and Tillage Research 167, 80–89.*

The study was part of the project "AgriGES" led within Gembloux Agro-Bio Tech (University of Liège). It focused on the impact of two common tillage practices in Belgium (conventional tillage *vs.* reduced tillage) on GHG emissions in a maize crop using automated closed chambers. It was initiated by Donat Regaert who set up the experiment, collected flux data and built a dedicated data processing software. I was in charge of controlling quality and filtering data, analyzing fluxes and writing the cited publication. We received the technical help of Alain Debacq regarding the flux measurement system and the scientific support of Dr. Nicolas Theodorakopoulos for microbial analyses.

- ***Chapter IV: N_2O short-term response to topsoil disturbance and temperature in a crop***

This chapter is based on the following publication: *LOGNOUL, M., DEBACQ, A., DE LIGNE, A., DUMONT, B., MANISE, T., BODSON, B., HEINESCH, B., AUBINET, M., 2019. N_2O flux short-term response to temperature and topsoil disturbance in a fertilized crop: An eddy covariance campaign. Agricultural and Forest Meteorology 271, 193–206.*

The study focused on four objectives:

- Setting up a continuous GHG monitoring of a representative Belgian production crop for an entire cropping season (from fertilization to harvest);
- Establishing data processing procedures for EC N_2O data, as at the time of publication, no reference document was available;
- Estimating the weight of N_2O in the GHG budget of the crop;
- Investigating flux dynamics and its relation to farming practices and meteorology.

Alain Debacq was responsible for the technical follow-up of the experiment, including the maintenance of the instruments and the collection of flux data. Anne De Ligne and Tanguy Manise were respectively in charge of processing CO_2 fluxes and following up farming practices at the study site. I took care of data processing and filtering, flux analyses and the writing of the cited scientific paper.

- ***Chapter V: How do emission dynamics of N_2O respond to pasture restoration?***

This chapter is based on unpublished results from an EC measurement campaign led in a grazed pasture from March 2018 to February 2019. Thanks to the collaboration with The Research Centre of Excellence Plants and Ecosystems (Antwerp University, Belgium), we were able to set up a paired-flux tower experiment to investigate the impact of pasture restoration on N_2O flux dynamics.

Instruments and conditioning boxes were installed by Alain Debacq, who took care of maintenance and data collection during the whole experiment along with Bernard Douxfils. Louis Gourlez de la Motte provided meteorological data. Bi-monthly soil samples were performed by myself and Mélissa Lonneux. I performed data processing and treatment as well as analyses.

II

MATERIAL AND METHODS

II. Material and methods

1. INTRODUCTION

Reliable *in situ* measurement methods are needed in order to improve the understanding of N_2O production mechanisms in soils. Several techniques are available, ranging from simple closed chambers to micro-meteorological methods, of various degrees of complexity. The most commonly used techniques for measuring N_2O exchanges between the atmosphere and ecosystems are closed chambers and eddy covariance, which are presented in this chapter. An overview of the experimental sites used in this thesis is given at the end of this Chapter.

2. AUTOMATED CLOSED CHAMBERS

2.1. *GENERAL PRINCIPLE*

Determining N_2O fluxes with closed chambers consists in hermetically isolating a volume of air above the studied surface (usually from 0.05 to 0.5 m²) and measuring the evolution of N_2O concentration over time. To do so, an enclosure with a removable lid is slightly inserted into the ground. When the lid closes, the N_2O concentration in the enclosed air varies depending on the exchanges between the soil and the air above the studied surface. This variation is measured by analyzing the concentration over time.

In static closed chambers, air samples are periodically taken by means of a syringe, and then analyzed in a laboratory. In dynamic chambers (**Figure** II. 1), the air circulates in a hermetic circuit to an analyzer which measures the N_2O concentration at regular intervals and during the entire enclosure time.

2.2. *EXPERIMENTAL SET-UP*

Closed chambers systems are either manual or automated. Manual systems require an operator to close the chamber lid and, in the case of static chambers, to perform sampling with a syringe during the measurement period. Automated systems make it possible to overcome human presence and to make more frequent measurements (up to several per day).

In this research project, we used a homemade system of automated dynamic closed chambers (**Figure** II. 2). Chambers were made out of PVC pipes connected to a gas analyzer (**Figure** II. 3). The air was pumped through the inlet, which was equipped with a filter to prevent tube plugging by dust or damages to downstream instruments. Each chamber had a motorized lid closing for half an hour, while the gas analyzer performed N_2O concentration measurements every 30 seconds. A 2-mm layer of polyurethane closed-cell foam covered the inside of the chamber lids to ensure the sealing when closed. A data logger (Micrologger ® CR3000, Campbell Scientific, Logan, UT, US) was used to control the closing cycle of the chambers (by regulating the lid motors and opening the solenoid valves between each chamber and the gas analyzers), and to collect GHG concentration data. When placing the measuring sets on the experimental parcels, each chamber was slightly inserted into the ground (a few centimeters), avoiding tractor wheel tracks and parcel borders.

For more details on the equipment, you can refer to page 76 in Chapter III.

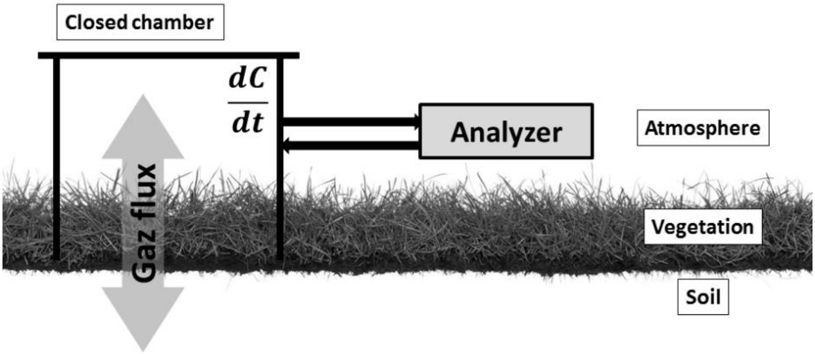

Figure II. 1 - Schematic representation of a closed chamber set-up to measure the evolution of gas concentration (*dC/dt*) in an enclosed space

Figure II. 2 – Homemade set of automated closed chambers in a maize field. The bottom left chamber is performing a measurement sequence (lid closed)

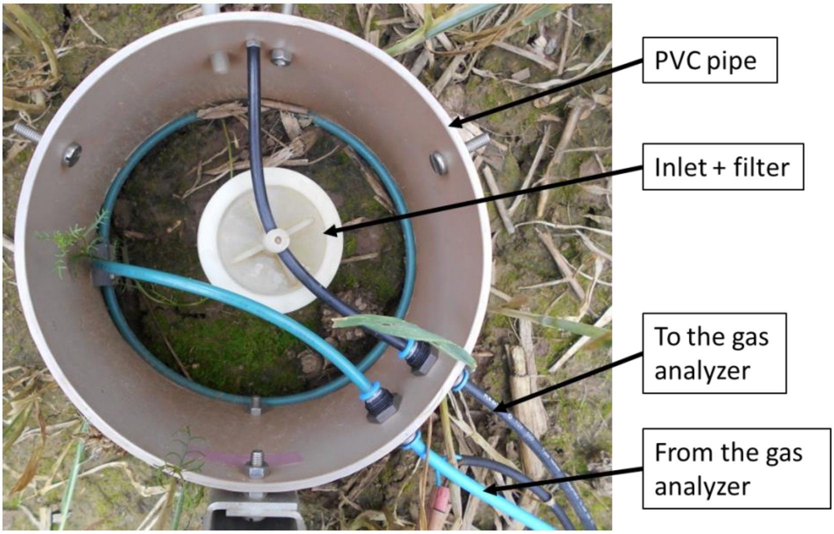

Figure II. 3 - Inside of an automated dynamic closed chamber

2.3. *DATA TREATMENT*

Flux calculation is performed by fitting a regression on the concentration time series. The purpose is to derive the slope at time = 0 (i.e. when the lid closes) to evaluate the flux as if no chamber was installed on the studied surface. Indeed, the gas exchange can be influenced by the evolution of concentration in the chamber during the enclosure time (Kutzbach et al., 2007).

Two models are frequently suggested in the literature: the linear model (**Equation** II. 1) and the exponential model **Equation** II. 2). The latter is well suited if gas concentration reaches a plateau during the chamber closing time. In this case, the flux is related to the slope at the origin (E2).

Equation II. 1 $$C(t) = L_1 + L_2 * t$$

Equation II. 2 $$C(t) = E_1 + \frac{E_2}{E_3}(1 - exp(-E_3 * t))$$

Figure II. 4 shows an example of a concentration time series with both regression models tested on measurements. The choice of fit is usually based on the best R^2 with a control on the other parameters of the chosen model.

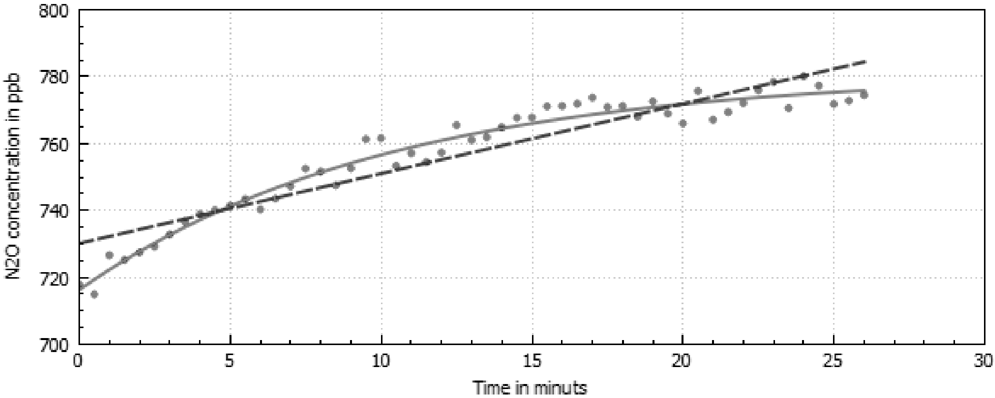

Figure II. 4 – Example of a concentration time series and regressions (dots: measurements, dashed line: linear fit, plain line: exponential fit)

Measurements can be affected by several events unrelated to N_2O production mechanisms in soils, such as technical problems (failure of the lid engine, plugged filter, pump failure, etc.) or field hazards (insects in the inlet, flooded chamber, vegetation preventing the lid from closing). These impaired data should be removed from the dataset prior to flux calculation to avoid artefacts. In this thesis, we based the filtering process on (1) visual observations during frequent visits to the experimental field, (2) sample pressure and N_2O absolute concentration measured by the gas analyzer and (3) the goodness of fit parameters (R^2 and RMSE).

The detailed procedures for flux calculation and data quality control are explained in page 77.

3. EDDY COVARIANCE

3.1. *GENERAL PRINCIPLE*

Eddy covariance is a micro-meteorological technique based on measuring the transport of molecules by air turbulence. To calculate GHG exchange, the eddy covariance technique requires simultaneous measurements at high frequency (usually 10 Hz) of the vertical wind and the trace gas concentration. Under some assumptions, the flux can be equaled to the covariance of the vertical component of the wind speed and the concentration of the trace gas (**Equation** II. 3):

Equation II. 3 $$F_{wc} = \frac{1}{n_s}\sum_{k=1}^{n_s} w'_k c'_k = \overline{w'c'}$$

In this equation, F_{wc} is the flux over the period considered (on which is determined the average to calculate the fluctuations), n_s is the amount of measurements during this period (e.g. 18000 if measuring at 10 Hz during 30 min), *w'* is the fluctuation of vertical wind speed and *c'* the fluctuation of concentration of the trace gas. More details on the assumptions behind this equation and how it was obtained are given in Aubinet et al. (2012).

This technique has two specificities differentiating it from closed chambers: GHG measurements are integrated over large areas (the measurement footprint covers a surface of the order of one hectare) and they are carried out with an important temporal resolution (usually semi-hourly).

3.2. EXPERIMENTAL SET-UP

3.2.1. Eddy covariance mast

To implement the eddy covariance technique, wind speed and N_2O concentration of eddies are to be measured simultaneously over the studied area at a high frequency rate. The experimental set-up consists of a mast rising above the vegetation, on which a sonic anemometer and the gas analyzing system are attached.

Figure II. 5 illustrates the eddy covariance installation at the Lonzée ICOS station (Belgium). The inlet of the sampling tube connected to the gas analyzer is located as close as possible to the anemometer on top of the tower.

3.2.2. Closed-path N_2O analyzer

In this section, we present the technical specifications related to our closed-path N_2O analyzer.

a) Sampling line

A schematic representation of the closed-path sampling line is given in **Figure** II. 6. Air is sampled through a rain cap protecting the inlet from raindrop and insects. It then goes through a tube made out of inert material to limit signal attenuation (Nordbo et al., 2014) before reaching the analyzer sampling cell. The tube is protected by insulating foam traversed by a heating resistor to prevent condensation. Several filters and a water trap are placed along the sampling line to prevent dust and liquid water to reach the gas analyzer. The air flow is controlled by a manual valve before entering the gas analyzer. Both the analyzer and the vacuum pump are placed in thermo-regulated containers.

b) Laser spectrometer

In order to measure the concentration of N_2O, a very precise instrument is necessary because its absorption lines are very close to those of other gases such as water vapor (McManus et al., 2010). Such technology has only been available for a few years, but has shown to be reliable and can detect very low exchanges (Rannik et al., 2015). In our experiments, we used a quantum cascade laser (QCL) spectrometer (Aerodyne Research Inc., Billerica, MA, USA).

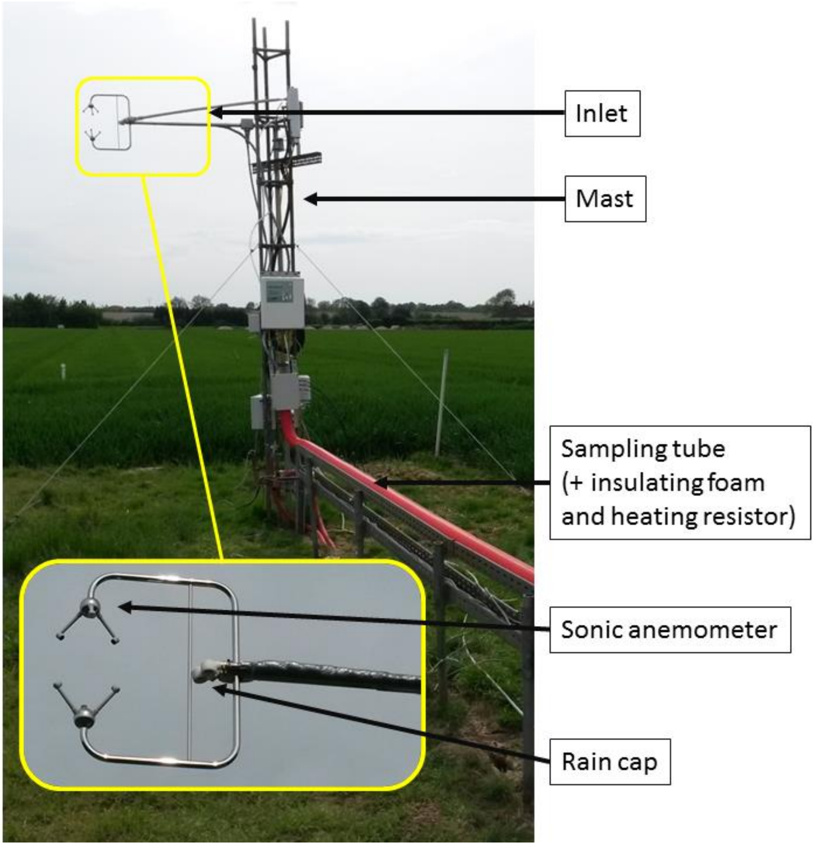

Figure II. 5 - Eddy covariance set-up in the field (Lonzée ICOS Station, BE)

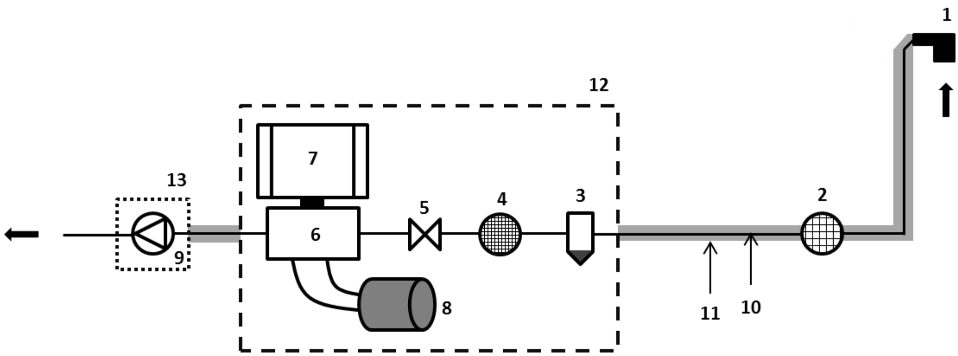

Figure II. 6 – Gas sampling line (1: inlet with raincap, 2: filter, 3: water trap, 4: filter, 5: manual valve, 6: spectrometer, 7: computer and controlling software, 8: internal thermo-regulation system, 9: vacuum pump, 10: sampling tube, 11: insulating foam with heating resistor, 12-13: thermo-regulated containers). Arrows indicate the air flow direction.

A continuous infrared light signal is produced by an emitter made out of semi-conductor material. This emitter can work in a very narrow wavelength range, which is needed for N_2O. The laser signal is reflected by a series of mirrors towards the analyzer sampling cell and then transmitted to a detector. The detected and emitted signals for a determined wavelength are then compared to calculate the trace gas concentration in the sampling cell.

c) Thermo-regulation

This instrument, as compared to older ones, can work at room temperature, preventing the need for liquid nitrogen (Nelson et al., 2004). An internal thermo-regulation system (water and ethanol) is used to maintain the laser temperature around 25°C (see number 8 in **Figure** II. 6). The gas analyzer is placed into an insulated container. Although the device naturally produces heat during normal operation, the container is equipped with a heating system to maintain a stable temperature during winter months. An air-cooling device is also used to temper high temperature variations that can occur during the summer.

d) Sampling cell pressure

The spectrometer operates with a vacuum pump (TriScroll 600 Dry Scroll Pump, Agilent Technologies, Santa Clara, CA, USA) able to maintain a very low pressure in the sampling cell. This is needed to minimize collisional broadening in the spectrometer, although it does not eliminate it (Mappe Fogain, 2013). In our experiments, the cell pressure was set at 26.7 hPa with a nominal flow rate of 6 standard l/min.

e) Controlling software

The QCL spectrometer is operated by a software called TDLWintel (Aerodyne Research Inc., Billerica, MA, USA). This program controls the emitter and its wavelength range, ensures data acquisition at high frequency and calculates trace gas concentrations (N_2O, H_2O and CH_4) and dry mixing ratios.

We set the nominal acquisition frequency at 10 Hz. However, it can slightly vary (< 5 %). Therefore, the software performs a resampling to provide data at exactly 10 Hz, which is needed to calculate the covariance with the wind velocity signal (**Equation** II. 3).

When calculating N_2O and CH_4 mixing ratios, the program also accounts for gas dilution and pressure broadening caused by the presence of water vapor. However, users should be aware that a residual cross-talk between H_2O and N_2O could persist and affect very low fluxes (Nemitz et al., 2018).

f) Comparison with other systems

Following the approach of Bachy et al. (2016), we compared the H_2O fluxes measured with the Aerodyne QCL with those obtained using an infrared gas analyzer (LI-7200, LI-COR, Lincoln, NE, USA). The half-hourly fluxes correlated well ($R^2 = 0.86$ for a slope of 0.99), reinforcing the idea that no systematic bias originated from our instrument.

3.3. *DATA TREATMENT*

3.3.1. Pre-processing

The pre-processing procedures presented in this section were performed using EddyPro® (LI-COR Environmental, LI-COR, Lincoln, NE, USA), a software dedicated to the calculation of eddy covariance fluxes. Technical details regarding the following paragraphs can be found in the product manual.

a) Statistical tests on time series

Before calculating fluxes, assessing the quality of eddy covariance data is necessary. Quality control is performed on 10 Hz time series from the gas analyzer and the sonic anemometer.

A series of statistical tests is proposed in Vickers and Mahrt (1997); they are summarized in **Table** II. 1. Data that do not satisfy these quality tests should be removed from the dataset used to calculate GHG fluxes.

Note that the test evaluating the skewness and kurtosis of time series was discarded: while a visual examination of data did not detect instrument malfunction, this test tended to flag them regardless. It was not possible to adapt the test parameters to suit our dataset and it was therefore deemed irrelevant.

Table II. 1 – Statistical tests performed on EC timeseries

Issue tested	**Test purpose**
Spikes	Spikes due to electronic issues during data transmission or recording can appear in the data. Spikes are counted and replaced by linear interpolation.
Signal resolution	The signal resolution is evaluated to ensure that concentration and wind speed variations are well recorded.
Drop-outs and discontinuities	This test detects if data in the time series have a value statistically different from the rest during a consequent amount of time (i.e. different from one-time spikes).
Absolute limits	Data are flagged if they exceed plausible limits (note that this test is performed after the removal of spikes).
Skewness and kurtosis	This test evaluates the shape of data distribution which can be an indicator of instrument malfunction.

b) Evaluation of micrometeorological conditions

Theoretical conditions used to build **Equation** II. 3 include the steady state of time series and the development of good turbulent conditions.

The steady state test (Foken and Wichura, 1996) requires that the covariance between vertical wind velocity and gas concentration over a whole averaging period (usually 30 minutes) be less than 30 % different from the covariance calculated over shorter periods of time (5 minutes).

The test on turbulent conditions is based on the comparison between modelled and observed turbulence parameters. Detailed equations for the calculation of said parameters can be found in Foken et al. (1991).

Mauder and Foken (2006) combine both these quality criteria to offer a single flag ranging from 0 to 2 (0: best quality data, 2: bad quality data). They suggest to discard flag-2 data and to use flag-1 data only for general analyses such as averaging fluxes over long periods.

c) *Rotation of anemometer coordinates*

When placing a sonic anemometer in field conditions, it is difficult to align its axis perfectly with the terrain horizontal line. Its coordinates are thus corrected so that the average vertical wind velocity is equaled to zero.

The most commonly used methods are the double rotation and the planar-fit corrections. In this work, the planar fit method (Wilczak et al., 2001) was used to rotate the anemometer coordinates, as recommended by ICOS (Sabbatini et al., 2016). Note that the results were very similar to those obtained with the double rotation (see Chapter IV, page 102).

d) *Detrending method*

Wind speed and concentration fluctuations of **Equation** II. 3 are determined based on the mean of the variable calculated over an averaging period. Most searchers choose a period of 30 minutes when the contribution of low frequency turbulence is negligible. Indeed, with an averaging period of 30 min (i.e. 1800 s), eddies characterized by a frequency lower than 1/1800 Hz are note taken into account.

The two mostly used methods to calculate the mean of the variable are the following:

- Block average (BA): the mean is calculated over the whole period (30 min);
- Running mean (RM): for each sample in the half-hour time series, the deviation from the mean is calculated within a smaller averaging window (usually a centered rectangular window).

The method used can influence the resulting flux and must therefore be chosen with caution. We compared these two methods using H_2O fluxes from the Lonzée ICOS station (**Figure** II. 7). Three averaging windows were tested for the RM method (150 s, 300 s and 500 s).

The smaller the averaging window, the more fluxes calculated with the RM method tended to be smaller than those based on the BA method. Indeed, when detrending using a window shorter than 300 s (i.e. 5 min), the influence of larger eddies (i.e. with a signal at lower frequencies) in the time series was not taken into account. Therefore, we chose to implement the BA detrending method in the following chapters to avoid underestimating fluxes.

e) *Time-lag determination*

There exists a time-lag between the time series of wind speed and gas concentration. This is due to the instrumental set-up, especially when the air is pump through a tube to the sampling cell of the gas analyzer (i.e. if the analyzer is a closed-path instrument). This lag is usually estimated by maximizing the covariance function between the gas concentration and wind speed time series in a narrow plausible time window. After estimating the time-lag, data need to be resynchronized in order to calculate fluxes.

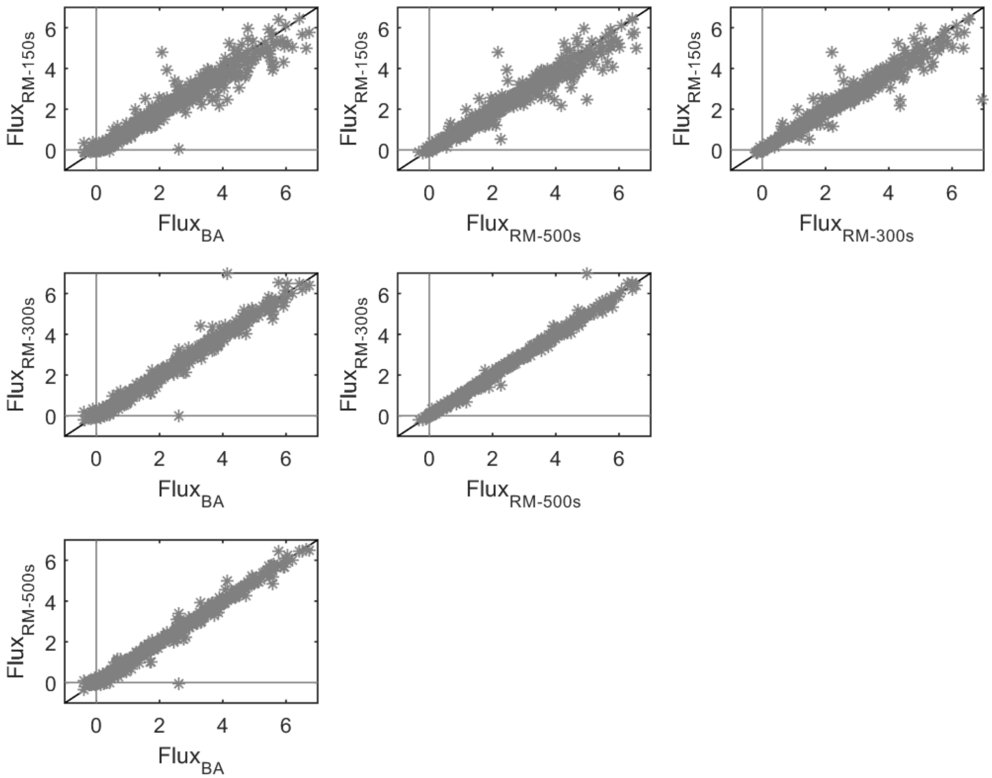

Figure II. 7 – Comparison of H_2O fluxes (unit: mmol m^{-2} s^{-1}) obtained in the Lonzée ICOS Station with different detrending methods (RM: running mean, BA: block average)

3.3.2. Post-processing

After calculating fluxes, post-processing data is necessary. The two most important procedures are briefly presented in this section. You can refer to Aubinet et al. (2012) for a complete overview of post-processing treatments.

a) Spectral corrections

The time response of the gas analyzer and the instrumental set-up (e.g. a closed-path sampling line) can act as low-pass filters and thus lead to high-frequency attenuation of the eddy covariance signal.

This high-frequency loss can be evaluated with empirical equations or with an experimental approach. We used the latter in this thesis, as proposed by the software EddyPro: the signal losses due to the sensor separation (i.e. the distance between the anemometer and the inlet of the gas analyzer) were assessed as suggested by Horst and Lenschow (2009) while those due to the sampling tube were evaluated with power spectra (Fratini et al., 2012). These assessments provided two spectral correction factors that were multiplied to obtain a single factor to correct fluxes.

b) U* filtering

It has long been recognized that during periods of low turbulence conditions (e.g. at night), the flux that is measured appears lower due to the incapacity of the instruments to capture gas exchanges. The friction velocity (u*) can be used as a criterion to discriminate low and well mixed periods (Papale et al., 2006) in order to discard artificially low fluxes.

A method described in Aubinet et al. (2012) involves the visualization of temperature-normalized fluxes against u* to detect a u* threshold. While this approach is commonly used with CO_2 fluxes, we were not able to implement it with N_2O data: we did not manage to untie the relationship between temperature, u* and N_2O fluxes to normalize them. We thus decided to use CO_2 fluxes to retrieve a u* threshold that was then used to filter N_2O exchanges in both our eddy covariance experiments. More details on this procedure can be found in Chapter IV, Section 2.4.2 (page 104).

4. METHODOLOGICAL CHOICE

Closed chambers and eddy covariance are two very different techniques and are thus meant to be used in different contexts.

Automated closed chambers can be homemade and the associated N_2O analyzer is way less expensive than the high-frequency spectrometer required for eddy covariance. The set-up can be easily moved from one site to another and can be implemented on small surfaces. It is therefore ideal to work on experimental parcels of a dozen squared meters to compare the effect of farming practices. This system can also be used *in labo* in incubators. However, results can be greatly influenced by spatial variability (Glatzel and Stahr, 2001) as they do not cover a large surface. Thus, enough chambers should be used in comparative studies to reveal potential differences between treatments. Although the adequate number will of course depend on the spatial variability of each site, we can share an example based on Chapter III: we were able to observe a significant difference between tillage treatments by simultaneously replicating measurements 8 times on each treatment.

The eddy covariance technique is more expensive to implement and demands specific expertise for data collection, control, correction and filtering. EC towers are usually set-up for long-term measurements. Given the large footprint and the high temporal resolution, EC is particularly suitable for long monitoring campaigns of agroecosystems in local farms. The major advantage is that instruments can be left in place, whatever the farming practice performed (e.g. ploughing), while chambers need to be removed and put back afterwards.

In conclusion, closed chambers should be dedicated to experimentations on small parcels while eddy covariance should be favored for close-up monitoring and budgeting gas exchanges over long periods (Lognoul et al., 2019). Chambers could possibly come as a support for eddy covariance, in particular to help gap-filling N_2O time series. Indeed, when EC is not able to capture fluxes (e.g. during low mixed conditions, see Section 3.3.2 above), closed chambers will provide observations. Such duo could help build mechanistic gap-filling solutions for N_2O.

The comparison between the two techniques is summarized in **Table** II. 2.

Table II. 2 – Comparison of automated closed chambers and eddy covariance specificities for measuring N_2O fluxes

	Automated closed chambers	**Eddy covariance**
Can be homemade	Yes	No
Is available as a "plug-and-play" system	Yes	No
Fast-response N_2O analyzer required	No	Yes
Can be used in labo	Yes	No
Specific expertise required for data treatment	No	Yes
High temporal resolution achievable (several measurements/day)	Yes	Yes
High spatial integration (scale of an ecosystem)	No	Yes
Can stay in place during farming operation	No	Yes

5. EXPERIMENTAL SITES

This section gives a rapid overview of the experimental sites in which we led our studies. For thorough descriptions, please refer to the dedicated chapters. All sites were located in Southern Belgium (**Figure** II.8).

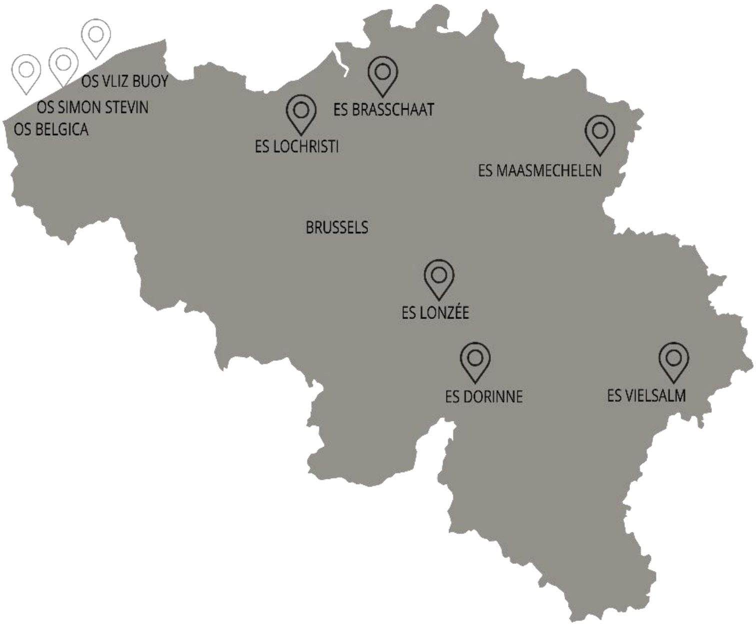

Figure II. 8 – Location of Belgian ICOS stations (OS: ocean station, ES: ecosystem station). ES Lonzée and ES Dorinne are the two EC sites used in this thesis. The "Soil-residues" plots of Gembloux are located in the same municipality as the ES Lonzée. (Map adapted from www.icos-belgium.be)

5.1. "SOIL-RESIDUES" PLOTS OF GEMBLOUX AGRO-BIO TECH (BE)

5.1.1. Site description

This site is located in Gembloux (central Belgium) in the loess region (**Figure** II. 9 and **Figure** II. 10, page 64). Experimentations have been conducted by searchers of Gembloux Agro-Bio Tech (Liège University, BE) since 2008. Several farming practices are tested on the long term, such as tillage (reduced vs. conventional) and the management of crop residues (exported or returned to soil). The experimental parcels are placed in a Latin square design.

N_2O fluxes were measured at this site with dynamic closed chambers in 2015. The activity of microorganisms producing N_2O was also investigated (Theodorakopoulos et al., 2017). The experiment is described in Chapter III (page 73).

Figure II. 9 – Experimental site « Soil – residues », Gembloux Agro-Bio Tech (Liège University). Satellite view imported from WALONMAP (2015)

Figure II. 10 - Parcels disposed in a Latin square design in the site « Soil – residues », Gembloux Agro-Bio Tech (Liège University)

5.1.2. Complementary information

To complement the information provided in Chapter III, we describe in this section (1) the method applied to measure the soil porosity, needed to calculate the WFPS and (2) the calibration procedure of the humidity probes.

1) Soil porosity

Soil samples were carried out in 2014 using metal rings to extract intact soil cores. Four samples were retrieved from each parcel. By dividing the weight of soil cores after drying by the volume of the ring, we were able to assess the average bulk density (*D'*) in each experimental parcel. The soil porosity (*P*) was calculated as follows (**Equation** II. 4) using a particular density (*D*) of 2.65 g cm^{-3}.

Equation II. 4 $$P = \left(1 - \frac{D'}{D}\right) * 100$$

The bulk densities of the parcel under reduced tillage and the parcel under conventional tillage were respectively 1.43 and 1.46 g cm^{-3}, resulting in the respective porosities of 46.2 and 44.8 %.

2) Humidity probes

The humidity probe Delta-T ML2x can provide a 1 %-accuracy according to the manufacturer. When placed horizontally, such probe has to be buried under at least 50 mm of soil. A generalized calibration was performed prior to the experiment following the manufacturer's instructions. However, we recommend that future experimenters perform the soil-specific calibration (using an undisturbed soil sample) to gain on accuracy.

5.2. LONZÉE ICOS STATION (BE)

The study site of Lonzée (Gembloux, BE) is located in the loess region (**Figure** II. 11, page 66). It is labelled as a "Level-2 ICOS station" since 2018. It is a 12-ha field belonging to a local farmer and has been cultivated for 80 years. The soil is characterized by a pH(H_2O) of 7.9 (measured in 2017).

An eddy covariance mast has been set up in the very center of the parcel and equipped with CO_2, H_2O, CH_4 and N_2O analyzers, with a weather station and with several probes measuring pedo-climatic conditions.

In 2016, we followed a sugar beet crop from seeding to harvest and evaluated the influence of conventional farming practices on N_2O emissions, as explained in Chapter IV (page 97). In **Table** II. 3, the published studies investigating GHG at this site are presented.

Figure II. 11 – Eddy covariance instruments in a sugar beet crop at the Lonzée ICOS station, Belgium

Table II. 3 – Site-specific publications on GHG at the Lonzée ICOS station

Authors (year)	Title of the publication
Moureaux et al. (2006)	Annual net ecosystem carbon exchange by a sugar beet crop
Hoyaux et al. (2008)	Extrapolating gross primary productivity from leaf to canopy scale in a winter wheat crop
Moureaux et al. (2008)	Carbon balance assessment of a Belgian winter wheat crop (Triticum aestivum L.)
Suleau et al. (2009)	Wind velocity perturbation of soil respiration measurements using closed dynamic chambers
Aubinet et al., 2009	Carbon sequestration by a crop over a 4-year sugar beet/ winter wheat/seed potato/winter wheat rotation cycle
De Ligne et al. (2010)	New transfer functions for correcting turbulent water vapor fluxes
Dufranne et al. (2011)	Comparison of carbon fluxes, growth and productivity of a winter wheat crop in three contrasting growing seasons
Suleau et al. (2011)	Respiration of three Belgian crops: Partitioning of total ecosystem respiration in its heterotrophic, above- and below-ground autotrophic components
Bachy et al. (2013)	Long-term measurements of volatile organic compounds exchanges above a maize field at Lonzée (Belgium)
Buysse et al. (2017)	Carbon budget measurement over 12 years at a crop production site in the silty-loam region in Belgium
Lognoul et al. (2019)	N_2O flux short-term response to temperature and topsoil disturbance in a fertilized crop: An eddy covariance campaign

5.3. *DORINNE ICOS STATION (BE)*

The study site of Dorinne (BE) is located in the Condroz region, in Southern Belgium (**Figure** II. 12, page 68). It is part of a local cattle farm that has been in place for several generations of farmers. The current herd is made of Belgian Blue Beef, which is a breed reared for meat.

In 2018, the farmer decided to entirely restore one of his pasture. This implies the application of a total herbicide, followed several weeks later by harrowing and reseeding. For a year, we monitored N_2O fluxes in this parcel and in an adjacent non-restored plot that was used as control. In **Table** II. 4, the published studies investigating GHG at this site are presented.

Table II. 4 - Published studies on GHG at the Dorinne ICOS station

Authors (year)	Title of the publication
Jérôme et al. (2014)	Impact of grazing on carbon dioxide exchanges in an intensively managed Belgian grassland
Gourlez de la Motte et al. (2016)	Carbon balance of an intensively grazed permanent grassland in southern Belgium
Mamadou et al. (2016)	Sensitivity of the annual net ecosystem exchange to the cospectral model used for high frequency loss corrections at a grazed grassland site
Dumortier et al. (2017)	Methane balance of an intensively grazed pasture and estimation of the enteric methane emissions from cattle
Gourlez de la Motte et al. (2018)	Rotational and continuous grazing does not affect the total net ecosystem exchange of a pasture grazed by cattle but modifies CO_2 exchange dynamics
Gourlez de la Motte et al. (2019)	Herd position habits can bias net CO_2 ecosystem exchange estimates in free range grazed pastures
Dumortier et al., (2019)	Point source emission estimation using eddy covariance: Validation using an artificial source experiment

Figure II. 12 – Eddy covariance instruments in a grazed pasture at the Dorinne ICOS station, Belgium

III

IMPACT OF TILLAGE ON GREENHOUSE GAS EMISSIONS BY AN AGRICULTURAL CROP

III. Impact of tillage on greenhouse gas emissions by an agricultural crop

This chapter has been published as:

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Abstract

Our experiment aimed at studying the impact of long-term tillage treatments – reduced tillage (RT) and conventional tillage (CT), on CO_2 and N_2O emissions by soil and at describing the dynamics of N_2O fluxes. Gas measurements were performed from June to October 2015 in a Belgian maize crop, with homemade automated closed chambers, allowing continuous measurement at a high temporal resolution. After 7 years of treatment, CO_2 and N_2O average emissions were significantly larger in the RT parcel than in the CT parcel. This observation was attributed to the effect of tillage on the distribution of crop residues within the soil profile, leading to higher soil organic C and total N contents and a greater microbial biomass in the upper layer in RT. A single N_2O emission peak triggered by a sudden increase of water-filled pore space (WFPS) was observed in the beginning of the measuring campaign. The absence of large emission afterwards was most likely due to a decreasing availability of N as crop grew. N_2O background fluxes showed to be significantly correlated to CO_2 fluxes but not to WFPS, while the influence of soil temperature remained unclear. Our results question the suitability of reduced tillage as a “climate-smart” practice and suggest that more experiments be conducted on conservation practices and their potent negative effect on environment.

Keywords

Nitrous oxide; Carbon dioxide; Tillage management; Automatic chambers; Maize; Cropland.

1. INTRODUCTION

Nitrous oxide (N_2O) is a greenhouse gas (GHG) considered as the third largest contributor to global warming (Ciais et al., 2013). It is also a precursor of molecules causing stratospheric ozone depletion (Portmann et al., 2012). Since 1750, its atmospheric concentration has increased by 20 % (Meure et al., 2006) and reached 327 nmol mol^{-1} in 2014 (NOAA, 2015). Agricultural soils constitute the major anthropogenic sources of N_2O, and the rise of its atmospheric concentration is primarily due to an increase in land conversion for agriculture and the intensified use of nitrogen fertilizers (Ussiri and Lal, 2013).

Nitrification and denitrification are considered to be the most important pathways of N_2O production in agricultural soils (Braker and Conrad, 2011). Nitrification is the oxidation of ammonium to nitrite and nitrate, and is performed by auto- and heterotrophic bacteria under aerobic conditions (Butterbach-Bahl et al., 2013). N_2O is a byproduct of nitrification. Denitrification is the reduction of nitrate to nitrite, NO, N_2O and finally N_2. It is performed by heterotrophic bacteria and fungi under anaerobic conditions (Robertson and Groffman, 2007). N_2O emissions by soils show high spatial variability (Molodovskaya et al., 2012) and are characterized by low continuous background fluxes and intense sporadic emission peaks due to the intensification of microbial activity (Butterbach-Bahl et al., 2013).

N_2O production is driven by oxygenation conditions in soil pores, which can be approximated by the commonly used water-filled pore space (WFPS in m^3 m^{-3}, Robertson and Groffman, 2007). Soil nitrogen and organic carbon contents are also important drivers as they are substrate of nitrification and denitrification, and carbon source of heterotrophic microorganisms respectively (Wang and Dalal, 2015). Soil pH, temperature, texture and pore structure also play a substantial role in N_2O emissions, by influencing microbial activity and the behavior of water in the soil matrix (Rees et al., 2013; Stehfest and Bouwman, 2006).

Farming practices can have an impact on drivers of N_2O production and therefore influence emissions by soils. While the impact of fertilization rate and type on N_2O emissions have been extensively reviewed (Hénault et al., 2012; Plaza-Bonilla et al., 2014; Velthof et al., 2003), tillage effects are not so well understood. In Europe, reduced tillage practices (as an alternative to conventional tillage) have been increasingly implemented with the purpose of diminishing production costs and soil compaction (Holland, 2004). Reduced tillage has shown to reduce surface erosion by improving soil pore structure and stability (Oades, 1984) and to increase water retention (Copec et al., 2015; Lampurlanès et al., 2001) and C sequestration in the uppermost soil layer (Alvarez, 2005).

However, the impact of tillage management on GHG emissions by soils is uncertain and one should note that practices labelled as “reduced” are not always uniquely defined. Increased C sequestration induced by conservation practices is often paired with reduced CO_2 emissions from soils (Abdalla et al., 2013), nonetheless other authors observed higher respiration rates in soils under reduced tillage (D’Haene at al., 2009; Kainiemi et al., 2015). Concerning N_2O, past studies have reported contradictory flux behavior as affected by tillage practices: several authors reported higher N_2O fluxes under reduced tillage than conventional tillage (Abdalla et al., 2013; Ball et al., 2008; D’Haene et al., 2008; Goossens et al., 2001), while others observed enhanced N_2O emissions under conventional tillage (Koga, 2013; Mutegi et al., 2010; Plaza-Bonilla et al., 2014; Wang et Dalal, 2015) or no difference between the two treatments (Chatskikh et al., 2008; Negassa et al., 2015). Abdalla et al. (2013) highlighted in their review that although there was a trend for larger N_2O emissions under reduced tillage, the impact of tillage practices also depended on soil texture and climate. History of tillage practices should also be taken into account (Six et al., 2004). Up to now, no consensus on the impact of tillage practices on GHG fluxes has emerged.

Our experiment aimed to study two practices (conventional tillage – CT; and reduced tillage – RT), and bring answers to the following questions: (1) what is the impact of tillage on CO_2 and N_2O emissions by a crop after 7 years of contrasting tillage practices, (2) can we link these emissions to soil physicochemical properties and (3) how can we characterize the dynamics of N_2O fluxes and connect them to climatic drivers?

Our study was conducted in an experimental corn maize field in the Belgian loess belt. CO_2 and N_2O fluxes were measured using a fully automated system of dynamic closed chambers. Soil moisture and temperature were monitored continuously and soil samples were taken in order to measure pH, total nitrogen and soil organic carbon, along with microbial abundance during the measurement campaign.

2. MATERIAL AND METHODS

2.1. *EXPERIMENTAL SITE*

The study was conducted in Gembloux, Belgium (50°33’53.94’’ N, 4°42’32.97’’ E). The local climate is oceanic temperate characterized by humid summers and mild, rainy winters. The soil type in this location is classified as Cutanic Luvisol (World Reference Base) with a silt loam texture (18-22 % clay, 70-80 % silt, and 5-10 % sand) and a C:N ratio between 10 and 12.

The experimental field has been subjected to farming practices experiments since 2008, comparing two tillage treatments (conventional and reduced tillage) in a Latin square design; one parcel was selected in each tillage modality to conduct our experiment. Both parcels were subjected to residues restitution (harvestable straw was returned to the field, as well as stubbles and chaffs).

Crop history at the field is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011 and 2012), *Vicia faba* (2013), *Triticum aestivum* (2014) and *Zea mays* (2015). In March 2015, all parcels were treated with glyphosate. On April 20, 2015, 122 kg N ha^{-1} fertilizer were applied prior to seedbed preparation (7 to 10 cm-depth) and maize sowing on April 22, 2015. Each plot was then treated with weed killer on May 28, 2015. Maize harvest took place after October 15, 2015.

2.2. *TILLAGE TREATMENTS*

After winter wheat harvest in summer 2014, residues were buried in each parcel by stubble breaking at a depth of 7 to 10 cm. On January 6, 2015, winter ploughing at 25-cm depth was performed using a moldboard plow on conventionally tilled parcels only. Seedbed preparation and sowing (performed for both tillage treatments on April 22, 2015) consisted of three steps: a first passage with a tine stubble cultivator, followed by a passage of the tractor mounted with a dual cultivator in front and at the back a rotary harrow, wedge ring roller and finally a third passage with a row drill. None of these agricultural tools disturbed the soil below 10 cm.

2.3. CO_2 AND N_2O FLUXES

2.3.1. Automated dynamic closed chambers

Measurements were performed with dynamic closed chambers (Norman et al., 1992). The system is based on the following principle: the chamber placed on the ground encloses a small volume of air in which GHG concentrations are measured repeatedly over a short period of time and the flux (F [mol m^{-2} s^{-1}]) is given by the evolution of concentration (C [mol m^{-3}]) inside the chamber (Longdoz et al., 2000). In these conditions, the flux may be calculated as follow (**Equation** III. 1):

Equation III. 1 $$F = \frac{V}{S}\frac{dC}{dt}$$

where V is the volume of the chamber and pneumatic circuit [m^3] and S is the surface covered by the chamber [m^2]. We conducted CO_2 and N_2O flux measurements using an innovative system of automated dynamic closed chambers. 16 PVC collars (height: 145 mm, diameter: 192 mm) were each equipped with a motorized lid and with a dynamic vent to prevent pressure gradient related artificial fluxes (Suleau et al., 2009). An enclosed air circuit ran from each chamber to gas analyzers measuring CO_2 and N_2O concentrations (respectively OEM Gascard® NG, Edimburgh, UK, and Thermo Scientific™ 46i, Waltham, USA[5], both calibrated prior to the measurement campaign and connected in series). The 16 chambers were divided into two sets of 8. Each set operates according to a 4.5-hour closing cycle described as follow. Every half-hour, a new chamber begins its measurement sequence: while all the others are left open, the chamber closes for 27 minutes during which CO_2 and N_2O concentrations are measured every 30 seconds (i.e. 54 measurements per closure period). Then the chamber opens again for 3 minutes to purge the system. At the end of a cycle, all 8 chambers open for 30 minutes to allow control of the analyzer stability. On each parcel, 8 chambers were disposed in between maize rows and slightly inserted into the ground to prevent leaking. The two automated sets operated continuously from June 16 to October 15, 2015.

[5] Additional information on the N_2O analyzer: Lower detectable limit = 0.02 ppm; zero noise = 0.01 ppm RMS (30 s averaging time).

2.3.2. Flux calculation

Equation III. 1 is implemented by fitting a regression on the concentration time series. Different models may be used; in this case we chose either an exponential regression (**Equation** III. 2):

Equation III. 2 $$C(t) = E_1 + \frac{E_2}{E_3}(1 - \exp(-E_3 * t))$$

From which the initial slope of **Equation** III. 1 is deduced (**Equation** III. 3):

Equation III. 3 $$\left(\frac{dC}{dt}\right)_{t=0} = E_2$$

Or a linear model (**Equation** III. 4):

Equation III. 4 $$C(t) = L_1 + L_2 * t$$

From which the average slope, L_2, is derived. Linear regression is frequently used for chambers measurements. However, using the linear model implies a risk of flux underestimation: indeed, the gas flux itself can be significantly influenced by the evolution of concentration in the chamber, in which case a non-linear regression should be used (Kroon et al., 2008; Kutzbach et al., 2007).

2.3.3. Data treatment

Prior to flux calculation, quality criteria were applied to every half hour in order to remove data affected by technical problems such as unclosed chamber lid or air circulation failure.

CO_2 fluxes were calculated by fitting an exponential model to concentration data given their non-linear behavior (Kutzbach et al., 2007). Fluxes with a regression coefficient of determination R^2 below 0.75 were discarded in order to eliminate data concerned by technical problems but undetected by the previous quality criteria (this threshold did not systematically remove low fluxes).

N_2O fluxes were calculated using either a linear or an exponential regression. The choice of fit was based on the greatest adjusted R^2 and when the exponential model was chosen, further filtering was performed based on the E_3 fitting parameter as expressed in **Equation** III. 2 (Matthias et al., 1978). For this experiment, the acceptable range for E_3 was determined to be 0 to 0.143 s^{-1}. When the fit RMSE was greater than 12 ng N m^{-2} s^{-1}, N_2O fluxes estimates were removed from the dataset unless the fit was characterized by a R^2 greater than 0.75, thus avoiding systematical removal of high fluxes. Although both these values were chosen arbitrarily, they did not affect average N_2O emissions (less than 5 % variation). Finally, CO_2 and N_2O half-hourly concentration data series presenting an erratic behavior or several minute-long gaps were manually removed from the dataset. In order to avoid averaging bias within a cycle, missing fluxes for one individual chamber were gap-filled by linear interpolation between preceding and following cycle. 4.5-hour flux averages were calculated by averaging the 8 individual chamber fluxes over a cycle.

High wind velocities can affect closed chamber measurements by creating a depression between the chamber and ambient air despite the vent, leading to flux overestimation (Suleau et al., 2009). In order to estimate wind velocity induced perturbations, we tested the response of 4.5-hour averaged CO_2 flux measurements to mean horizontal wind velocity, which was measured on a nearby site by a sonic anemometer (HS-50, Gill Instruments, Lymington, UK). This test showed no significant impact of wind velocity on chamber flux measurements for either tillage treatment, suggesting that such perturbation was not critical in our experiment, presumably because of a windscreen effect from maize plants.

Cumulated emissions were calculated by integrating 4.5-h averaged data over the measurement period.

2.4. *CLIMATE AND SOIL VARIABLES*

In each parcel, two soil humidity probes (Delta-T ML2x for RT and Campbell CS-616 for CT) and two temperature probes (PT1000 sensors) were placed in between chambers at 5-cm depth. The water-filled pore space (WFPS) was obtained by dividing the volumetric water content by soil porosity.

Soil chemical properties were also characterized: soil samples were made by mixing together three subsamples taken at 0-10 cm depth nearby the chambers. This procedure was repeated three times in each parcel at each sampling date (June 17, July 6, August 17, September 4 and October 15, 2015). All samples were analyzed for pH (KCl 0.1 N), total organic carbon content (Walkley-Black method – Neslon and Summers, 1982) and total nitrogen content (Kjeldhal method – Bremner and Mulvaney, 1982).

Plant material was collected in each parcel several times during crop growth (July 6, July 23, September 17 and October 14) on 4.5 m² plots and placed in a stove at 60°C until dried, in order to assess maize aerial dry weight.

2.5. *MICROBIAL BIOMASS*

2.5.1. DNA extraction

Bacterial, archaeal and fungal abundance was approximated by respectively quantifying bacterial and archaeal 16S ribosomal ribonucleic acid (rRNA) gene and fungal 18S rRNA gene per g dry soil^{-1} in the soil sampled (sampling procedure described in 2.4). Soil deoxyribonucleic acid (DNA) was recovered following the direct extraction method of Zhou et al. (1996), with several modifications (described in Biver and Vandenbol, 2013). DNA was extracted by a combination of lysozyme, proteinase K, sodium dodecyl sulfate, and incubation at 65°C. The crude lysate was then purified by chloroform–isoamyl alcohol extraction followed by a single precipitation step with polyethylene glycol/NaCl 5%. To normalize DNA extraction yield, 10 ng of internal standard (plasmid pHT01, MoBiTec GmbH, Germany) was added to each soil extraction prior to DNA processing. The remaining plasmid quantity at the end of the extraction was then quantified by quantitative polymerase chain reaction (qPCR). Total DNA extracted was quantified by spectrophotometry

(Thermo Scientific NanoDrop 2000 UV-vis spectrophotometer). Soil humidity was taken into account to express the quantity of DNA recovered per gram of dry soil.

2.5.2. *Quantitative PCR*

Quantitative PCR was performed on a StepOnePlusTM Real-Time PCR system (Life Technologies). Each reaction was carried out in 20 µl reaction mix containing ORA™ qPCR Green ROX H Mix, 2X (HighQu, Germany), each primer at 1 µM, and 1 µl of DNA sample. To obtain a representative sequence and build the standard calibration curve, a single amplicon was isolated and used as template. Briefly, after a PCR amplification step, PCR products were cloned into a plasmid (TOPO TA cloning vector, Invitrogen, U.S.) and used to transform DH5 alpha E. coli. Then, a single colony was selected and the isolated plasmid containing the amplicon was purified and linearized with an appropriate restriction enzyme. The amplicon concentration was determined by spectrophotometry (Thermo scientific NanoDrop 2000 UV-vis Spectrophotometer).

For each gene, the experiment was conducted in a single run on the same plate containing all the samples and the standards. All samples were analyzed in duplicate. The primers employed and the PCR amplification efficiencies are summarized in **Supplementary Table** III. 1 (page 90). The PCR protocol quantification was as follows: 10 min at 95 °C, and then 40 cycles consisting of 15 s at 95 °C, 1 min at 60 °C. A melting curve analysis was performed at the end of each run to ensure that the amplification had yielded a single amplicon.

2.6. *DATA ANALYSIS*

For each soil sampling date, a one-way analysis of variance (ANOVA) was performed to compare the impact of tillage treatments on soil chemical properties and microbial abundance. CO_2 and N_2O half-hour flux data were averaged over the entire 4-month period for each chamber, and mean fluxes were then compared by performing a one-way ANOVA to evaluate the impact of tillage treatment. N_2O fluxes were separated into two categories, peak and background, based on visual evaluation of emission behavior. The peak start was determined by a sudden increase of N_2O emissions and its end by a return to background level.

Background fluxes were analyzed by fitting a linear model to N_2O data, with soil temperature (T_{soil}), water-filled pore space (WFPS) and CO_2 fluxes as independent variables. For each regression test (N_2O vs. T_{soil}, N_2O vs. WFPS and N_2O vs. CO_2), bootstrapping was performed to evaluate the influence of individual points on the goodness of fit. The regression R^2 was calculated for 1000 bootstrap samples of 100 points. The frequency distribution of R^2 was then calculated. Multiple linear regressions were also tested by adding progressively other independent variables to the simple linear model. All statistical analyses were made using Matlab Statistics Toolbox (MathWorks, Natrick, MA, USA).

3. RESULTS

3.1. *SOIL-RELATED VARIABLES*

Evolutions of WFPS and T_{soil} from June 16 to October 15 are presented in **Figure** III. 1a-b. T_{soil} ranged from 6.2 to 30.2 °C, slightly decreasing throughout the summer. WFPS varied from 0.45 to 0.97 m^3 m^{-3} in the parcel under conventional tillage, and from 0.47 to 0.86 m^3 m^{-3} in reduced tillage. High spatial variability of this variable in the surface layer was noted, as illustrated by the standard deviation on the graph. WFPS rises were caused by rainfall, the first increase being observed on June 22 at 5:00 am and followed by free drainage 9 h later.

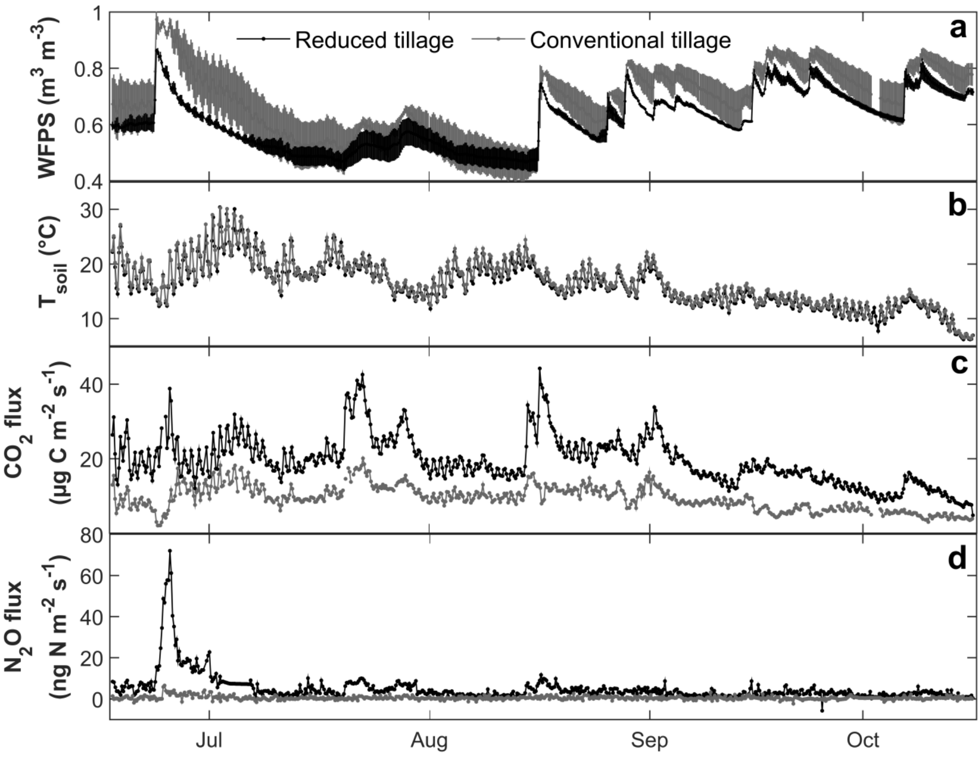

Figure III. 1 - Evolution of water-filled pore space and standard deviation (a), soil temperature (b), carbon dioxide fluxes (c) and nitrous oxide fluxes (d) under reduced tillage (black) and conventional tillage (grey). Data are given from June 16 to October 15, 2015

Soil pH, SOC and total N contents are presented in **Figure** III. 2a-b-c. Respectively for CT and RT and over the entire measurement period, mean soil pH were 6.18 and 5.67, mean SOC were 1.59 and 1.97 kg C m^{-2}, and mean total nitrogen content 0.18 and 0.21 kg N m^{-2}. For each sampling date, the three variables differed significantly between treatments (higher pH and lower SOC and total N contents characterizing the parcel under CT), except for total N on the first sampling date (June 17).

Maize dry weight (DW) recorded during the measurement period is illustrated in **Figure** III. 2d. Plant dry weight was always higher in the parcel under CT.

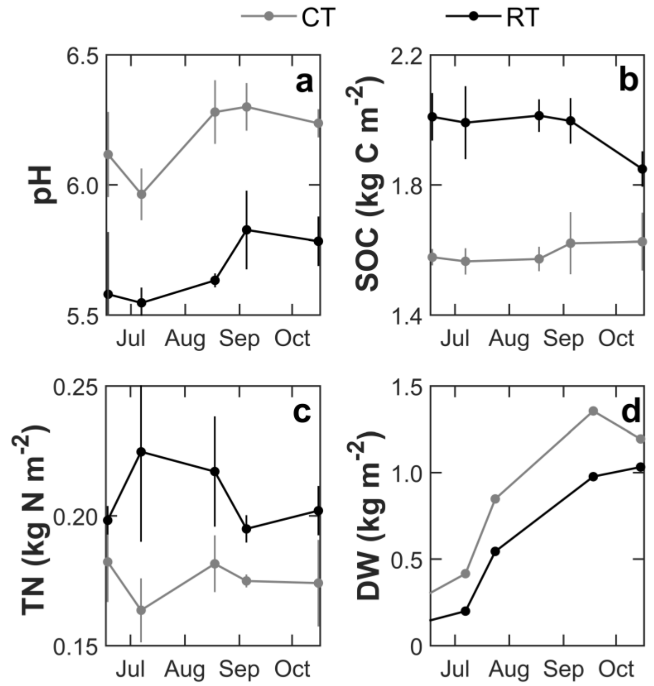

Figure III. 2 - Evolution of soil pH (a), soil organic carbon (b), total nitrogen content (c) in the upper soil layer (0-10 cm) and maize dry weight (d) under reduced tillage (black) and conventional tillage (grey). *Error bars represent standard errors*

3.2. *MICROBIAL BIOMASS*

16S rRNA and 18S rRNA genes target numbers were used as proxies for bacterial, archaeal and fungal biomass. The gene quantification approach revealed a greater abundance of microorganisms in the top 10 cm of soil in the RT parcel than in CT throughout the experimental period (+5 % of archaea, +9 % of fungi and almost +50% of bacteria based on *gene copies* g^{-1} dry soil). In mid-June, both archaeal 16S rRNA and fungal 18S rRNA gene concentrations were significantly higher in RT than in CT, but differences between treatments were no longer significant afterwards. Bacterial biomass was significantly higher in RT than in CT in mid-June, mid-July and mid-October. These results are illustrated in **Figure** III. 3.

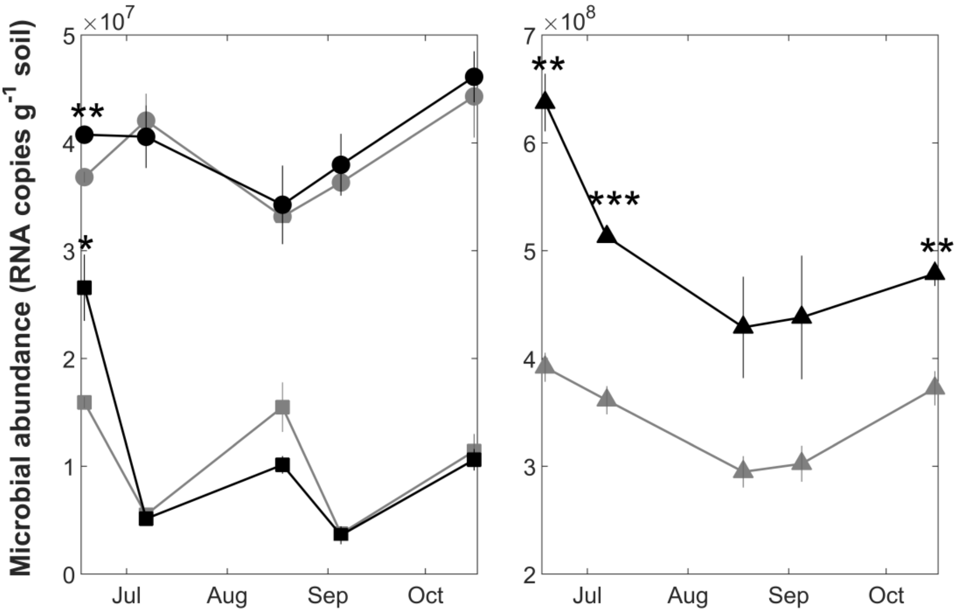

Figure III. 3 - Fungal (squares) 18S, archaeal (circles) and bacterial (triangles) 16S gene abundance in the top soil (0-10 cm) under reduced tillage (black) and conventional tillage (grey). *Error bars represent standard errors and stars indicate a significant difference between treatments (***: p-value < 0.001, **: p-value < 0.01, *: p-value < 0.05)*

3.3. CO_2 AND N_2O FLUXES

Around 5800 half hour flux data were collected by the end of the experiment. Prior to N_2O flux calculation, the monitoring test (technical problems detection) removed 267 half hours for CT and 141 for RT. After flux calculation and filtering, around 70% of N_2O fluxes remained for both treatments. Concerning CO_2, filtering removed 267 half hours for CT and 108 for RT.

RT CO_2 fluxes measured by the chambers were on average significantly higher than CT CO_2 fluxes, respectively 19.5 ± 0.9 µg C m^{-2} s^{-1} and 9.6 ± 1.8 µg C m^{-2} s^{-1} (**Figure** III. 4). Concerning this gas, important variations were observed, as well as a daily pattern (**Figure** III. 1c). Mean N_2O fluxes were also significantly higher in RT (5.00 ± 0.66 ng N m^{-2} s^{-1}) than in CT treatment (0.537 ± 0.072 ng N m^{-2} s^{-1}, **Figure** III. 4), resulting in cumulated N_2O emissions almost 10 times greater in RT (533 g N ha^{-1}) than in CT (57 g N ha^{-1}).

A N_2O emission peak was observed in both treatments, starting on June 23 (27 h after rainfall for CT and 9 h for RT) and ending approximately around July 5 for CT and July 13 for RT (**Figure** III. 1d). The rest of time was characterized by less important background fluxes. No daily dynamics was observed in N_2O fluxes.

A regression analysis was performed on N_2O background fluxes and the other variables measured at the same temporal resolution (**Figure** III. 5). Adding independent variables (i.e. multiple linear regressions) did not significantly improve the model (p-value > 0.05). Therefore, simple linear fits were retained. In conventional tillage, only daily CO_2 fluxes were significantly correlated to N_2O data (p-value < 0.001), explaining 13 % of variability (**Figure** III. 5c). In reduced tillage, soil temperature explained about 20 % of daily fluxes variability (p-value < 0.001, **Figure** III. 5d), while CO_2 fluxes explained 65 % of variability (p-value < 0.001, **Figure** III. 5f). Bootstrapping the data showed that for all the significant regressions, the calculated R^2 were distributed quite narrowly around the R^2 value obtained with the original data (**Figure** III. 6), suggesting a good robustness of the regression analysis.

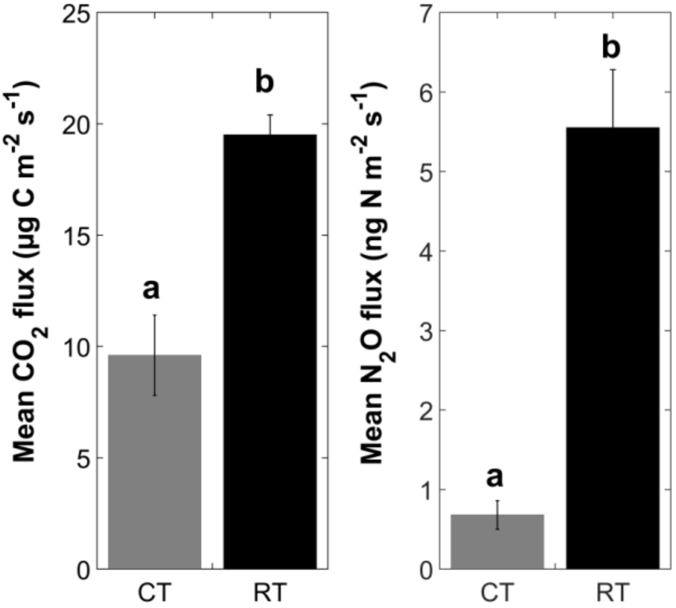

Figure III. 4 - Mean CO_2 (left) and N_2O (right) fluxes measured by the chambers in the parcels under conventional tillage (grey) and reduced tillage (black). *Different letters indicate a significant difference between groups. The standard error given with each mean (see error bar) illustrates a spatial variability between the 8 chambers of one set*

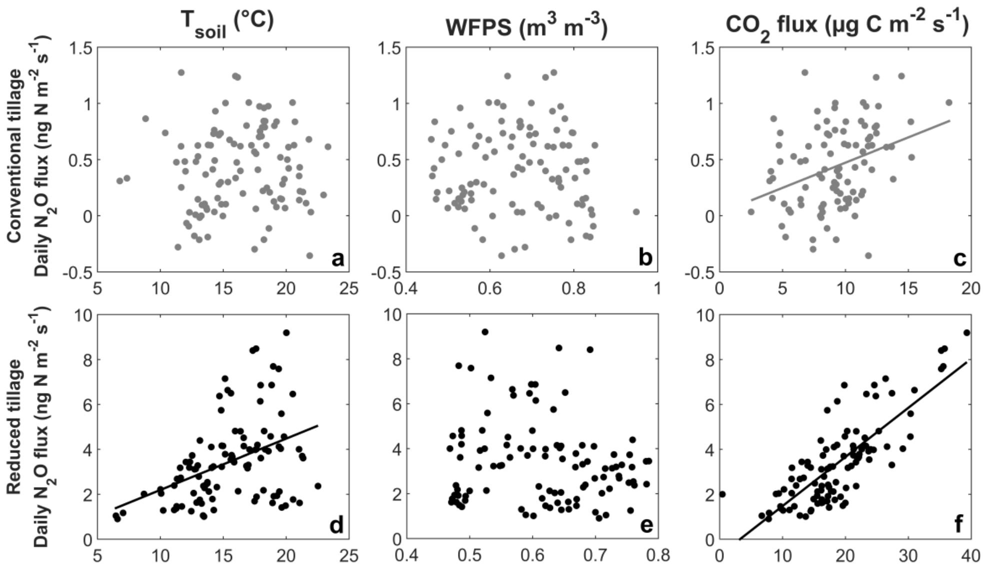

Figure III. 5 - Regression analysis of N_2O background fluxes tested against T_{soil} (a and d), WFPS (b and e) and CO_2 fluxes (c and f). Top: conventional tillage, bottom: reduce tillage. *Regression parameters and determination coefficients are given in* **Table** III.1

Table III.1- Regression analysis of N_2O background fluxes. *SE: standard error*

	Independent variable	Intercept (SE)	Slope (SE)	R^2	p-value
Conventional tillage					
	CO_2	**0.024** (0.119)	**0.045** (0.012)	0.13	0.000
Reduced tillage					
	T_{soil}	**-0.087** (0.766)	**0.23** (0.05)	0.18	0.000
	CO_2	**-0.71** (0.33)	**0.22** (0.02)	0.63	0.000

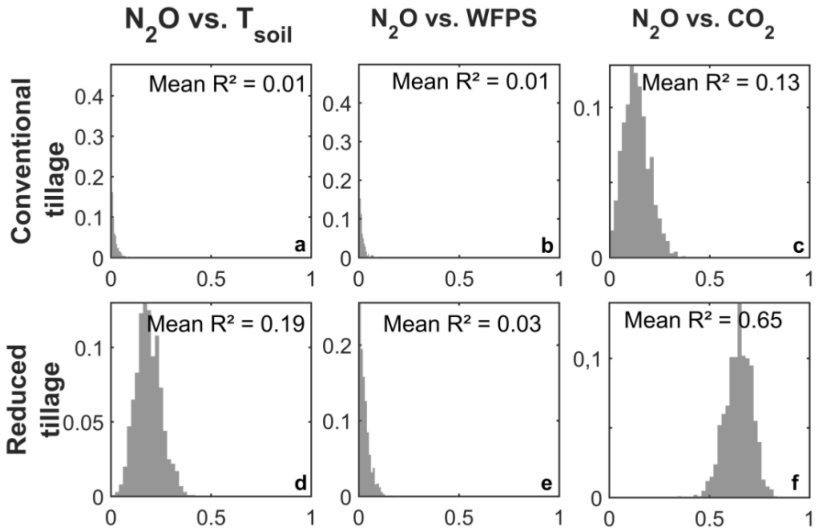

Figure III. 6 - Bootstrap analysis of correlations: frequency distribution (y axis) of R^2 (x axis) for 1000 samplings

4. DISCUSSION

4.1. *IMPACT OF TILLAGE ON CO_2 AND N_2O EMISSIONS*

4.1.1. CO_2 emissions

Our results showed a significant impact of tillage on soil respiration, with RT CO_2 fluxes on average twice as large as CT CO_2 fluxes (**Figure** III. 4). This can be linked to the observation of greater substrate quantity in the upper soil layer in RT (Conrad et al., 1996), illustrated by significantly higher SOC and total N content in the first 10 cm. This is consistent with the findings of Harrison-Kirk et al. (2013) who showed that CO_2 production was significantly affected by soil organic content and D'Haene at al. (2009) who observed higher C mineralization rates in the 0-5 cm layer of RT soil samples. The higher respiration rate in the parcel under RT is also most likely connected to the greater bacterial biomass found in the concerned parcel (**Figure** III. 3), as also mentioned by other authors (Jahangir et al., 2011; Kandeler et al.,1999; Vian et al., 2009).

Root respiration in crops is thought to account for most of soil respiration (Buysse and Aubinet, 2010), but it is unlikely that the greater soil respiration under RT is due to greater plant root systems under this tillage treatment: biomass measurements showed smaller aerial dry weight for the parcel under RT (**Figure** III. 2d), which can also be expected for roots if we consider that maize root/shoot ratio is not influenced by tillage (Anderson, 1988).

Our results are however in contrast with other experiments: several authors observed either no difference between treatments or higher soil respiration in soils under CT

(Abdalla et al., 2013; Chatskikh et al., 2008). The absence of difference might be inherent to an early stage of experimentation; e.g. in years 2009 to 2011 of our experiment, the tillage treatments showed no significant influence on heterotrophic respiration (Dufranne, personal communication). Higher respiration, for its part, was attributed to a higher mineralization rate caused by ploughing, leaving SOC less protected in micro-aggregates (Abdalla et al., 2013; Drury et al., 2006). However, this explanation could be challenged: Kainiemi et al. (2015) concluded that CT decreased CO_2 emissions compared to RT by producing large aggregates and clogs resulting in lower respiration, and by burying crop residues in deeper layers, which could slow down decomposition.

4.1.2. N_2O emissions

Our observations showed mean N_2O fluxes to be significantly higher in RT than in CT. The differences in WFPS observed between treatments could possibly explain the latter results. However, it is difficult to assess as WFPS differences between treatments remained rather small (less than 10 % on average) and this variable showed high spatial variability within each parcel.

Greater total N and SOC observed in the uppermost soil layer in RT were more likely responsible for such results. Indeed, the production of N_2O is correlated to the availability of N substrate (Conrad et al., 1996). Combined with a greater SOC, the activity of microorganisms is stimulated and the oxygen consumption by the nitrifying bacteria generates N_2O and nitrate, and an increase of anaerobic zones, promoting denitrification (Bouwman et al., 2002; Del Grosso et al., 2000; Senbayram et al., 2012; Van Zwieten et al., 2013). This being said, further experiments should investigate in detail the nitrogen fractions to have information on ammonium and nitrate availability rather than on total N, as the latter also includes organic and cellular N in microorganisms. Experiments conducted the following year showed that NH_4^+ from fertilization was rapidly oxidized and was under the limit of detection in both parcels 4 weeks after fertilizer application (Theodorakopoulos, personal communication). Therefore, we assume that the N_2O emissions presented in our results (7 weeks after fertilization) mostly originated from the mineralization of crop residues, which were assessed by SOC measurements.

The greater bacterial biomass in the upper soil layer under RT, in addition to our observation of a greater soil respiration, could also explain the difference in N_2O emissions between the two tillage treatments.

Tillage also had significant although very little effect on soil pH. This variable is less likely to have majorly influenced N_2O fluxes as a more acid pH can have divergent effects (Kemmitt et al., 2006, Liu et al., 2010) and the difference between treatments were small.

A difference in soil structure could exist too, as tillage affects soil organic content and the distribution of pore size at such depth. This could have influenced N_2O production and emission by impacting oxygenation conditions and water retention (Ball, 2013; Mosquera et al., 2007). As the soil structure was not investigated by measurements in the present experiment, this assumption cannot be verified.

Lastly, we were not able to measure fluxes between fertilization (April) and mid-June and thus missed potent peaks directly related to fertilizer application that might have accentuated or tempered our results. However, N_2O emissions in RT were continuously superior to emissions in the other treatment, as for CO_2 fluxes, suggesting that our results are representative of the effect of tillage practices on greenhouse gas emissions.

4.2. *VARIABILITY OF N_2O FLUXES*

4.2.1. N_2O emission peak

A single N_2O emission peak was observed in both CT and RT in June, which happened shortly after a rain event that caused the WFPS to increase sharply (**Figure** III. 1). The soil moisture sudden elevation acted as a trigger. Indeed, it has been widely reported that a rise of soil moisture can cause N_2O emission bursts to a certain extent (Dobbie et al., 1999; Robertson and Groffman, 2007; Smith et al., 2003).

In our case, the emission started to exceed background level 9 h and 27 h after the WFPS increase respectively for RT and CT and maximum N_2O emissions were obtained after 27 h and 31.5 h. We assumed that several hours are needed for microbial activity to be stimulated and to produce N_2O, which is emitted almost instantly. This hypothesis is supported by other studies observing similar results: in an experiment on incubated soil cores of texture similar to our experimental parcels, Mikha et al. (2005) observed a regain of microbial activity 8 h after soil rewetting; using a similar experimental set-up, Baere et al. (2009) observed a rise of N_2O production 24 hours after the soil moisture increase. The assumption that the observed delay was due to gas confinement in water-clogged pores, with N_2O being able to escape to the soil surface only when soil began to drain, as assumed by Rabot et al. (2014 and 2015), was not retained in this case for two reasons: first, the soil remained close to saturation for only a couple of hours and secondly, during the WFPS peak, significant CO_2 emissions persisted, suggesting that gas diffusion was still effective in the soil during this time.

The question remains of knowing which mechanisms were responsible for the emission peaks. During the peak event, soil moisture reached high WFPS values, generally thought to favor denitrification over nitrification (Bateman and Baggs, 2005). However, nitrification cannot be discarded; in a parallel experiment conducted on the parcel under RT, Theodorakopoulos et al. (2017) observed that this mechanism was a substantial contributor to N_2O emissions at high WFPS. This is in agreement with Baere et al. (2009) who highlighted that nitrification could be an important N_2O producing mechanism in uncompacted soils. Furthermore, in reduced tillage, we observed a stronger response of N_2O fluxes to the WFPS increase than in conventional tillage, along with higher SOC and greater soil respiration. These results could support the nitrification hypothesis as this mechanism is thought to be dependent of the availability of SOC (Harrison-Kirk et al., 2013) and of its mineralization after soil rewetting (Baere et al., 2009).

While a N_2O emission peak was observed in June, no peak appeared during the second half of the experiment, despite high WFPS. This absence of peak is most likely related to a lower availability of mineral N substrate for soil microorganisms, caused by high N uptake by plant due to need for growth. Indeed, the highest growing rate in our parcels was recorded in the month of July, with a tripling plant dry weight (**Figure** III. 2d). More information about the evolution of mineral N fractions (i.e. NH_4 and NO_3) could help verifying this hypothesis in future experiments. One could also hypothesize that the soil moisture threshold necessary to trigger emission peaks was not reached. Nonetheless this assumption appears unlikely, as WFPS reached around 90% of the maximum WFPS observed in June in both parcels, and literature reported emission bursts for much lower WFPS values (Flechard et al., 2007). One other possible explanation for the absence of peak could be microbial biomass diminution following hot and dry weather (Mikha et al., 2005), as WFPS was at its lowest in the first weeks of August. However, this hypothesis was discarded too as neither the biomass of microorganisms in the topsoil nor CO_2 fluxes showed a decreasing trend.

4.2.2. N_2O background fluxes

A positive correlation between daily N_2O background fluxes and T_{soil} was observed in RT but not in CT (**Figure** III. 5). This suggests that the stimulating effect of temperature on N_2O production by soil microorganisms (Smith et al., 2003) might be neutralized by a limited availability of N and SOC in the surface layer, or taken over by the influence of WFPS (Flechard et al., 2007), although the latter seems unlikely as WFPS showed no significant correlation to N_2O background fluxes in both treatments. Furthermore, no daily cycle in N_2O fluxes following the evolution of T_{soil} was observed. Such a pattern might only appear under drier conditions (Laville et al., 2011).

The absence of correlation between N_2O background fluxes and WFPS highlights the role of soil moisture in triggering large emissions when N and C substrate is available, rather than as an all-time proportional driver. Indeed, although several authors reported N_2O flux observations in function of the soil moisture content (expressed as WFPS) and showed a Gaussian evolution, with a limitation of fluxes above a WFPS of 70 to 85 % (Castellano et al., 2010; Flechard et al., 2007; Smith et al., 2003), such line of attack might be reductive as it assimilates WFPS to a tuning button.

Finally, N_2O background fluxes were found to be significantly related to CO_2 fluxes in both tillage treatments (**Figure** III. 5). The positive correlation appears logical because the biotic mechanisms involved in CO_2 and N_2O production share substrates and are driven by common variables (Xu et al., 2008). However, this assumption is not thorough; during the N_2O emission peak in June, low CO_2 emissions were recorded in the parcel under CT.

5. Conclusion

Our experiment aimed at studying the impact of reduced and conventional tillage on greenhouse gas exchanges by a cultivated soil. CO_2 and N_2O fluxes were measured using automated dynamic closed chambers during four months.

The results showed that after 7 years of contrasted tillage practices, reduced tillage significantly enhanced greenhouse gas emitted by the soil, with mean CO_2 fluxes 2 times larger and mean N_2O fluxes 10 times larger than emissions measured in conventional tillage. This observation was attributed to higher SOC and total N, and a larger bacterial biomass in the uppermost soil layer, caused by limited burying and mixing of crop residues under reduced tillage. The question remains of knowing if such difference between reduced and conventional tillage would be observed in conditions of crop residues retrieval. Our results, added to the variety of findings on tillage management, question the suitability of reduced tillage as a “climate-smart” practice and suggest that more experiments be conducted on conservation practices and their potent negative effect on environment.

A several day-long N_2O emission peak was observed in both tillage treatments hours after an increase of WFPS, suggesting that microorganisms producing N_2O were stimulated rapidly. Denitrification could be thought to prevail as high WFPS (> 90 %) were recorded, however nitrification could also play a substantial role as reported by other studies. N_2O background fluxes did not correlate with WFPS, suggesting that this variable mostly acts as a trigger for large emissions rather than as a tuning button. Further experiments analyzing microbial activity and dynamics of NH_4 and NO_3 in the surface soil layer should help improving the understanding of underlying mechanisms. It should be noted that the N_2O peak happened two months after fertilization and was thus not directly related to fertilizer application; this highlights the risk of missing potentially large emissions when conducting measurement campaigns only based on farming practice events.

6. Acknowledgment

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7. SUPPLEMENTARY DATA

Supplementary Table III. 1- Primers used in this study for quantitative gene analysis

Gene	Primer	Sequence	Reference	Efficiency (%)	Expected size
bacterial 16S rRNA	Bac786R Bac518F	**CTA CCA GGG TAT CTA ATC** **CCA GCA GCC GCG GTA AT**	Baker *et al.*, 2003; Muyzer *et al.*, 1993	81,74	208
fungal 18S rRNA	FR1 FF390	**AIC CAT TCA ATC GGT AIT** **CGA TAA CGA ACG AGA CCT**	Vainio and Hantula (2000)	76,4	389
archaeal 16S rRNA	727R 519F	**GCT TTC RTC CCT CAC CGT** **CAG CMG CCG CGG TAA**	Park *et al.*, 2010	91,53	268

8. ADDITIONAL INFORMATION (UNPUBLISHED SECTION)

8.1. *CAMPAIGN REPRESENTATIVITY*

Although the maize crop was fertilized and sowed in April, the measurement campaign only started two months later (mid-June) due to technical constraints. The homemade chambers ran until harvest (mid-October). Therefore, the weeks following fertilization are not included in the average emissions presented in this Chapter (page 82). The latter should thus not be interpreted as representative of the whole crop season. However, if measurements had been performed since the beginning, differences between treatments might still have been observed overall. Each parcel was fertilized with the same amount of N fertilizer and two reasonable scenarios can be hypothesized: (1) either the microbial abundance was already significantly higher in the parcel under reduced tillage in April, leading to observing a difference in N_2O emissions between treatments, (2) or the microbial abundances were similar in both treatments in April and May, leading to similar N_2O emissions from the soil following fertilization which, added up to the rest of the campaign, would have dampen the difference. Nevertheless, when setting up dynamic closed chambers campaigns in the future, users should ensure that the whole crop season is covered when possible.

Another limitation of this particular campaign is that it covers only one crop. The absence of repetition could hide a yearly effect that would have led to different conclusions due to meteorological conditions or the crop in place. Therefore, generalizing about the effect of tillage on N_2O emissions should be done with caution. More will be known when the data from the following crops will be analyzed. For example, the year following the publication of the paper, data over winter wheat at the same experimental site were released. Similar differences of soil characteristics and N_2O fluxes between tillage treatments were observed. Those yet unpublished results are discussed in Section 2.2.1 of Chapter VI.

8.2. *INSTRUMENTAL LIMITATIONS*

The WFPS in both parcels was derived from soil humidity measurements. The latter were performed using probes that were calibrated following manufacturer-based equations. This can lead to overestimation of soil moisture compared to a specific calibration in soil cores (N. Parvin, personal communication), and we indeed obtained very high WFPS in our experiment. A specific calibration should thus be advised for future campaigns for more precise WFPS data.

The lack of precision of the humidity probes questions the higher WFPS observed in the CT parcel than in the RT parcel during most of the campaign. However, this observation becomes less surprising when looking at the water retention curves of both parcels (**Figure** VI. 1).

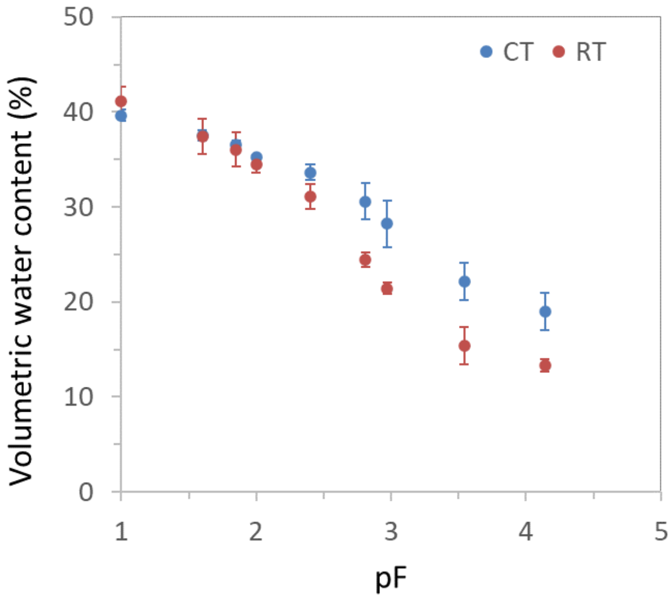

Figure VI. 1 – Water retention curve of the parcel under conventional tillage (CT, blue) and the parcel under reduced tillage (RT, red) measured in 2014.The vertical bars indicate the standard deviation from the mean (4 samples). *Data: courtesy of N. Parvin*

The curves were obtained in 2014 by following the Richards pressure plate method (DIN ISO 11274, 2012) using four undisturbed soil samples from each parcel. In this graph, pF is equal to *log(-ψ)*, with *ψ* being the soil water potential expressed in cm. The greater the pF, the bigger the difference between treatments, with the volumetric content in the CT parcel being higher. This suggests that the WFPS observed would be similar in both parcels when the soil is close to saturation, but higher in the CT parcel when drying.

8.3. *CHAMBERS POSITIONING*

Because of technical constraints, the 16 homemade chambers were separated into two groups and placed in two parcels only. Although this set-up allowed to have 8 repetitions in each parcel, we did not have any repetition of parcels within each tillage treatments. Thus, the difference observed between treatments could be the result of a variability inherent to the two chosen parcels. However, our results regarding soil chemical properties are consistent with those of Hiel et al. (2018), who observed a progressive differentiation of crop residues stratification at the experimental site depending on the tillage treatment, with significant differences appearing in 2012. The observations on the individual plots chosen in our experiment did not stand out from the rest of the parcels under similar treatment (M.-P. Hiel, personal communication).

Chambers were placed two-by-two in between adjacent maize rows. This seemed to be the best strategy to deal with the technically limited spatial expansion of the system (only two gas analyzers) while avoiding the risk of placing all chambers in a single row and facing a preferential run-off path during heavy weather. In future experiments in these parcels, additional gas analyzing stations and soil chambers should be acquired if searchers want to place them on more than one plot per treatment while preserving the high temporal resolution (several measurements per day and per parcel).

IV

N_2O SHORT-TERM RESPONSE TO TOPSOIL DISTURBANCE AND TEMPERATURE IN A CROP

IV. N_2O short-term response to topsoil disturbance and temperature in a crop

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Abstract

Using the eddy covariance technique, half-hourly N_2O fluxes were measured over a sugar beet crop (ICOS Station, Lonzée, BE) from fertilization to harvest. Several parameters of the data quality control tests were adapted to suit the characteristics of N_2O. No u* filtering threshold could be seen for N_2O fluxes; therefore, it was determined based on CO_2 data. The uncertainty of N_2O fluxes was assessed for several aspects of data treatment (total random uncertainty, spectral correction, u* filtering, gap-filling), which were combined to determine the uncertainty of the N_2O budget.

Between fertilization and harvest, the crop emitted 1.83 (± 0.21) kg N_2O-N ha^{-1} corresponding to 1.2 % of N supplies. Flux variability was characterized by three episodes of high emissions across the experiment, interspersed with lower background fluxes. These peak events were driven by soil moisture and temperature, dependent on the time-scale. Soil water content at 5 cm was identified as the single trigger for N_2O emission peaks given sufficient N availability, while intraday oscillations were positively correlated to the variations in surface temperature rather than deeper soil temperatures.

For the first time, an inhibiting and short-term effect of topsoil disturbance (seed-bed preparation) on N_2O fluxes was recorded, which interrupted the peak that followed fertilization, and delayed the start of the next high emission episode. This observation, along with the synchronicity found between surface temperature and diel oscillations of N_2O fluxes, supports the hypothesis of a N_2O-producing microbial community located in the topmost soil layer. Given that a third of the overall N_2O emissions during the measurement campaign occurred between fertilization and seed-bed preparation, further investigation into the timing of farming operations as mitigation strategies is needed. The contribution of N_2O emissions to the net greenhouse gas balance (which comprises CO_2 and N_2O fluxes) was estimated at between 20 and 66 %. Our results stress the importance of including nitrous oxide when measuring gas exchanges in fertilized crops, and to do so at high temporal resolution for improved estimates.

Keywords
Nitrous oxide; Eddy covariance; Sugar beet; Crops; Fertilization; Soil disturbance; Temperature.

1. INTRODUCTION

Nitrous oxide (N_2O) is a long-lived greenhouse gas (GHG) considered to be the third largest contributor to global warming (Ciais et al., 2013) and a major factor in stratospheric ozone depletion (Portmann et al., 2012). The agricultural sector contributes 72.2% of European Union N_2O emissions (Mandl et al., 2018). N_2O atmospheric concentration reached 330 nmol mol^{-1} in 2017 (NOAA, 2018) and the estimated growth of 0.80 nmol mol^{-1} per year is mainly explained by an intensified use of nitrogen fertilizers (Ussiri and Lal, 2013). In Belgium, 33.7% of agricultural GHG emissions originate from N_2O produced by soils (IRCEL, 2017). Although national estimates show a 24% decrease for the period between 1990 and 2014, N_2O emissions rates were predicted to increase by 3 kg N_2O-N ha^{-1} every year between 1990 and 2050 (Roelandt et al., 2007).

In agricultural lands, N_2O mostly originates from microbial nitrifying and denitrifying processes occurring in soil (Bracker and Conrad, 2011). The basic substrates for nitrification and denitrification are ammonium and nitrate (respectively acting as electron donor and acceptor), and N availability in soils correlates with N_2O production (Conrad et al., 1996). Labile C also plays a role in these processes as a source of C for heterotrophic nitrifiers and denitrifiers (Robertson and Groffman, 2007). N_2O biotic production is driven by pedo-climatic conditions, dependent on weather and farming practices (Ussiri and Lal, 2013). N_2O fluxes are usually characterized by high temporal variability with emission bursts (known as “hot moments”) interspersed with low background fluxes (Butterbach-Bahl et al., 2013; Molodovskaya et al. 2012). Spatial variability was also observed (with “hot spots”) in agricultural ecosystems (Hénault et al., 2012), due to heterogeneous soil physicochemical properties (Mathieu et al., 2006) and uneven distributions of microbial abundance (Jahangir et al., 2011).

Given sufficient N availability, N_2O emission bursts can be triggered under high soil water content following precipitation (Castellano et al., 2010; Plaza-Bonilla et al., 2014), irrigation (Trost et al., 2013) or freeze-thaw events (Ludwig et al., 2004). Such emission peaks are usually observed after fertilization, although peaks attributed to organic matter mineralization several months after N input are also documented (Lognoul et al., 2017).

Soil temperature plays a role in N_2O emissions by influencing microbial activity (Flechard et al., 2007). Moreover, increasing temperatures, by stimulating soil respiration, can result in greater anaerobic zones that are favorable to denitrification (Smith et al., 2003). Short time-scale variations are not well understood: a positive diel correlation between N_2O emissions and soil surface temperature was observed by Laville et al. (2011) over a very short period of time (9 days), while negative correlations were also recorded (Shurpali et al., 2016). Alves et al. (2012) reported a positive relationship with temperature deeper in the soil profile.

Regarding farm management and soil disturbance, mainly tillage practices have been studied in relation to N_2O emissions, especially their long-term effects (D'Haene et al., 2008; Drury et al., 2006; Vian et al., 2009). When compared with practices involving ploughing, reduced tillage tends to enhance N_2O emissions in the long term (Abdalla et al., 2013) by concentrating crop residues in the uppermost soil layer and favoring microbial development (Lognoul et al., 2017). However, the short-term effect (daily to monthly scales) of soil disturbance is rarely investigated, mostly because of a lack of sufficient temporal resolution due to limiting measurement techniques. In particular, to our knowledge the short-term effect on N_2O fluxes of soil disturbance by seedbed preparation in croplands has not yet been studied.

Up to now, most *in situ* studies on crops were performed using manual closed chambers at low temporal resolution. However, this technique prevents researchers from adequately capturing the sudden variations in N_2O emissions (Kroon et al., 2008) and can be source of error when measurements are scaled up spatially (Lammirato et al., 2018). Automated chambers that allow an improved temporal resolution have also been used (Laville et al., 2011, Theodorakopoulos et al., 2016), but the uncertainty related to spatial variability remains. The eddy covariance (EC) technique represents a suitable alternative because it can provide continuous flux estimates at high resolution (half-hourly scale) integrated over a large surface area (Aubinet et al., 2012), thus obviating spatial heterogeneity. However, the EC data treatment typically used for CO_2 and H_2O needs to be adjusted for N_2O. This includes reviewing parameters of quality tests and determining the time-lag, which can be challenging when the signal-to-noise ratio is low (Langford et al., 2015). It is only recently that a reference document concerning non-CO_2 gases was published by the European network ICOS (Nemitz et al., 2018).

On the whole, the main drivers of N_2O exchange have been identified. However, it remains unclear how the driving variables affect fluxes in terms of amplitude and timescale, and how they are influenced by crop management. These knowledge gaps and low-precision budget estimates make it difficult to implement mitigation guidelines for farmers. Therefore we set up an eddy covariance measurement campaign on a managed cropland with the aim to (1) determine the appropriate EC data treatment for our N_2O dataset, (2) assess the short-term response of N_2O fluxes to farming practices together with meteorological variables *in situ*, and (3) estimate the N_2O budget of a production crop, and its uncertainty, from fertilization to harvest.

2. MATERIALS AND METHODS

2.1. STUDY SITE

The study site is a 12-ha crop field located in the Lonzée Terrestrial Observatory (Level-2 ICOS Station) in central Belgium. The site has been cultivated for at least 80 years. It is owned and managed by a local farmer, and is subject to a 4-year rotation: winter wheat (*Triticum aestivum* L.) / sugar beet (*Beta vulgaris* L.) / winter wheat (*Triticum aestivum* L.) / seed potato (*Solanum tuberosum* L.). The climate is oceanic temperate (mean annual temperature: 10.2°C, annual precipitation: 743 mm). The soil is characterized by a silt-loam texture (FAO classification: Luvisol) with 20 % clay, 7.5 % sand, and 72.5 % silt. A detailed description of the site can be found in Moureaux et al. (2006).

The present study was conducted from March to October 2016, focusing on a sugar beet crop which represents the second most important root crop in Belgium and in Europe (StatBel, 2018). To our knowledge, it is the first EC dataset collected for N_2O on this type of crop. The sequence of farming operations is reported in **Table** IV. 1.

Table IV. 1 - Calendar of farming operations during the experiment

Date	Farming operations	Specifications
07-Dec-2015	Winter ploughing	Over mustard using a moldboard plow (30-cm depth)
22-Mar-2016	Liquid nitrogen application	NH_4NO_3 (136.5 kg N ha^{-1})
9-Apr-2016	Weeding	Glyphosate (972 g ha^{-1})
12-Apr-2016	Seed-bed preparation	Stubble cultivator followed by a rotary harrow (7 to 10-cm depth)
13-Apr-2016	Sugar beet sowing	Row drilling of *Beta Vulgaris* L. cv Lisanna KWS
3-9-19-May-2016 23-Jun-2016	Weeding	Phenmedipham, ethofumesate, metamitron, triflusulfuron-methyl, dimethenamid-P
11-Jun-2016	Urea application	4 kg N ha^{-1}
1-14-Aug-2016	Fungicide application	Epoxyconazole, fenpropomorph, pyraclostrobin
27-Oct-2016	Harvest	Yield of 83 ton ha^{-1} (16 % sugar)
29-Oct-2016	Ploughing and soil preparation Winter wheat sowing	/

The application of N fertilizer was performed 22 days before seeding. It is usually recommended to wait at least 10 days to avoid excessive ammonium soil concentrations that can be toxic for germinating seedlings (Laudinat et al., 2015). Seeding was itself preceded by a seed-bed preparation the day before, to level the soil surface and break up aggregates to favor plant emergence.

The sugar beet crop in this study was preceded by winter wheat (October 2014 to August 2015). A mustard cover crop was established between the two main crops (August to December 2015).

2.2. MEASUREMENT SYSTEM

2.2.1. Meteorological and ancillary measurements

Meteorological conditions were monitored by a weather station located in the center of the field. The station was equipped with a radiometer measuring global and net radiation (CNR4, Kipp and Zonen, Delft, NL) and photo-receptor cells measuring photosynthetic photon flux density (PAR Quantum sensor SKP 215, Skye Instruments Limited, Llandrindod Wells, UK). A weighing rain gauge (TRw S415, MPS system s.r.o., Bratislava, SK) recorded precipitation. Air temperature and humidity were measured by a thermistor and an electrical capacitive hygrometer (RHT2nl, Delta-T Devices Ltd, Cambridge, UK). An infrared remote temperature sensor (IR 120, Campbell Scientific, Logan, UT, US) monitored the surface temperature. This temperature was considered to be the canopy temperature once the leaf area index (LAI) exceeded 0.5 m^2_{leaf} m^{-2}_{soil}, corresponding to a canopy height of 15 cm.

In addition, pedoclimatic conditions were monitored at 5, 15, and 25 cm depth for three locations around the station to derive averages for each depth. The ground instrumentation included electrical resistance thermometers for soil temperature (PT 107, Campbell Scientific, Logan, UT, US) and time domain reflectometers for soil moisture (EnviroSCAN-Probe, Sentek Sensor Technologies, Stepney, SA, Australia).

The LAI was determined by regular destructive sampling in six sub-plots from the beginning of June to October. Green leaves were cleaned and air-dried, then scanned to determine their surface area, which was divided by the sampling area.

2.2.2. Flux measurements

N_2O and CO_2 fluxes were measured continuously at the field scale using the eddy covariance technique (Aubinet et al., 2012). The eddy covariance tower and related instruments were located in the center of the field. The CO_2 concentration was measured at 10 Hz by an infrared gas analyzer (LI-7200, LI-COR, Lincoln, NE, US). Flux calculations and data treatments for CO_2 are described in Buysse et al. (2017).

The N_2O concentration was measured at 10 Hz using a quantum cascade laser (QCL) spectrometer controlled by the TDLWintel data acquisition program (Aerodyne Research Inc., Billerica, MA, USA). H_2O concentration was also measured by the instrument. Air sampling was ensured by a vacuum pump downstream (TriScroll 600 Dry Scroll Pump, Agilent Technologies, Santa Clara, CA, USA) and through an 8.2 m long and 4.5 mm wide sampling tube upstream of the QCL. The tube was equipped with an inlet rain cap (Intake Tube Rain Cap V3, LI-COR, Lincoln, NE, USA) and protected by insulating foam traversed by a heating resistor. The flow and cell pressure were controlled by a needle valve at the inlet of the sampling cell. The cell pressure was set at 26.7 hPa with a nominal flow rate of 6 standard l/min. This low pressure helps to limit collisional broadening in the spectrometer but does not eliminate it (Mappe Fogain, 2013). Therefore, the data acquisition program computes the dry mixing ratio of N_2O using a built-in correction, accounting for gas dilution and pressure broadening caused by the presence of water vapor. However, a residual cross-talk between H_2O and N_2O could persist and affect very low fluxes (Nemitz et al., 2018). Verification was done on small N_2O fluxes (< 0.3 nmol m^{-2} s^{-1}), which were grouped by class of temperature (class range: 2.5°C) to obviate a potential co-dependency of N_2O and H_2O on this variable, and the correlation between the two gases was tested. No significant relationship was found for any of the temperature classes; the correction performed by TDLWintel was thus considered sufficient for our experiment.

The temperature of the sampling cell was maintained at 25 °C using a 20 % ethanol-fluid recirculating chiller (OASIS THREE chiller, Solid State Cooling, Wappingers Falls, NY, USA). Wind tridimensional velocities were measured at 20 Hz by a sonic anemometer (Solent Research HS-50, Gill Instruments, Lymington, UK). The inlet of the sampling tube and the anemometer were both mounted on the tower at 2.8-m height above ground, with a 19.7 cm separation (eastward separation: 18.9 cm; southward separation: 6.8 cm; no vertical separation).

2.3. N_2O FLUX COMPUTATION

The computation of half-hourly fluxes was performed using the eddy covariance processing software EddyPro® (LI-COR Environmental, LI-COR, Lincoln, NE, USA).

Means were calculated using block averaging over 30 min. As recommended by ICOS (Sabbatini et al., 2016), the planar fit method (Wilczak et al., 2001) was used to rotate the anemometer coordinates. When compared to the double rotation, the two methods correlated very well (R^2 = 0.99) with a slope of 1.01.

The time-lag between the data streams of the N_2O mixing ratio and vertical wind velocity was determined by searching for the maximum of the covariance function in a narrow time window, for which limits ([0.9 – 3.0] s) were assessed by a visual examination of the frequency distribution of time-lags in a larger window (20 s) for the entire data set (see **Supplementary Figure** IV. 1, page 123). 62 % of time-lags fell into the narrow time window. It should be noted that even during periods of low fluxes (< 0.5 nmol m^{-2} s^{-1}), valuable time-lags that fell into the narrow frame window were found. If no covariance maximum could be found within such frame, a default value was applied, corresponding to the mode of the frequency distribution (1.9 s).

The season-averaged cospectrum of N_2O was compared to the cospectrum of temperature which was considered to be ideal (Foken et al., 2012). A loss of high frequency information was clearly identified (**Figure** IV. 1). A suiting correction that accounted for low-pass filtering was thus applied to N_2O fluxes. We performed the two-part method suggested by EddyPro®. This combines by multiplication two spectral correction factors, compensating respectively for losses due to sensor separation (Horst and Lenschow, 2009) and for those due to the sampling tube (Fratini et al., 2012).

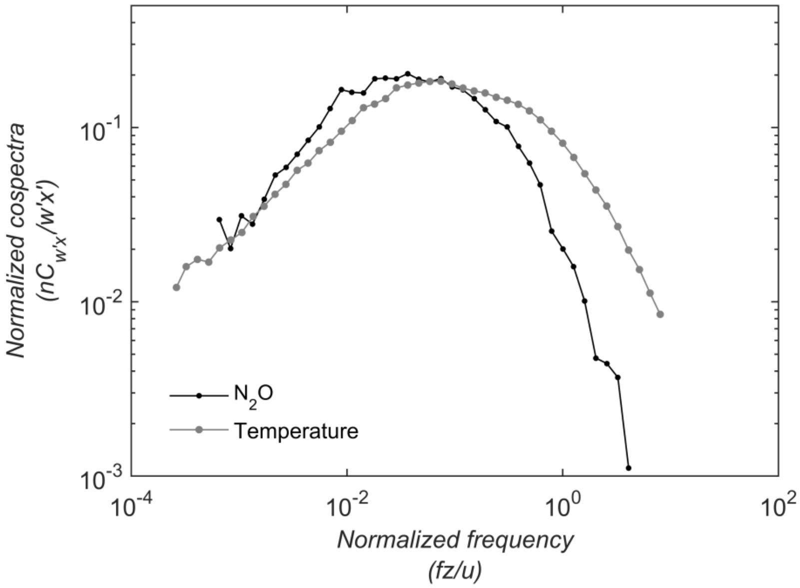

Figure IV. 1 - Normalized cospectral density of temperature and N_2O (f: frequency, z: height, u: wind velocity)

The correction by Fratini et al. (2012) is based on the comparison of temperature and N_2O power spectra to derive the experimental transfer function. A Lorentzian curve is then fitted to that function between 0.005 and 0.5 Hz, for which we found a cut-off frequency of 0.11 Hz. For both stable and unstable conditions, the method selects good quality power spectra ($n_{unstable}$ = 86, n_{stable} = 77) to calculate a linear relationship between spectral correction factors (SCF) and half-hourly averaged wind speeds. The linear model is then used to correct the rest of the dataset by retrieving SCF based on the wind speed of individual half-hours. The quality criteria for selecting good power spectra are given in **Table** IV. 2. The confidence bounds of the regression were used to assess the uncertainty related to the spectral correction (see Section 2.6).

Finally, storage fluxes were assumed to be negligible at the experimental site. The storage term is usually small in short ecosystems like croplands and it is frequently measured by the one-point method rather than by profile integration (Moureaux et al., 2012). De Ligne (2018) validated this assumption for our experimental site after conducting a two-week experiment on winter wheat. They compared a three-level profile (0.1, 0.8 and 2.4 m) of CO_2 concentration measurements with the one-point method (2.4 m) and concluded that profile integration was not needed, given the low weight of storage fluxes. This was verified for N_2O fluxes on sugar beet: 95 % of storage fluxes calculated with the one-point method amounted to less than 1 % of total fluxes (EC flux + storage flux) and the storage term integrated over the crop season accounted for less than 0.5 % of the N_2O budget.

Table IV. 2 - Quality criteria for selecting power spectra and performing spectral correction

	Friction velocity ($m\ s^{-1}$)	**Sensible heat flux** ($W\ m^{-2}$)	**Latent heat flux** ($W\ m^{-2}$)	**N_2O flux** ($nmol\ m^{-2}\ s^{-1}$)
Minimum, unstable	0.2	20	20	1
Minimum, stable	0.05	5	3	0.5
Maximum (stable and unstable)	5	1000	1000	200

2.4. Data quality control

2.4.1. Maintenance and statistical tests

Half-hour fluxes were discarded during periods of instrument maintenance (e.g. change of filter). Whenever the QCL shut down and was restarted (mainly after power cuts), the laser tuning rate took several hours to readjust. Therefore, data following a restart (up to 12 h) were also discarded.

The quality of time series was controlled using statistical tests as detailed in Vickers and Mahrt (1997). Settings for CO_2 served as the main basis for N_2O settings and were adapted to best suit the control of N_2O time series quality (see **Supplementary Table** IV. 1, page 124). The tuning of said parameters was mainly guided by visual examination of time series and with the intent to avoid systematic flagging of behaviors linked to physical events captured by the QCL.

Adequate conditions of stationarity and turbulence were assessed following the tests by Foken and Wichura (1996) with quality classes (Mauder and Foken, 2006); data categorized as level 2 were discarded.

All tests and criteria combined, a total of 29 % of N_2O fluxes were discarded. Out of the 8082 half-hours left, 7 were identified as outliers and removed; these fluxes exceeded the weekly median by two to three orders of magnitude.

2.4.2. Influence of friction velocity

Eddy covariance flux data can show sensitivity to friction velocity (u*) which can come from an artifact rather than from actual mechanisms responsible for producing or absorbing the gas of interest (Aubinet et al., 2012). In the case of N_2O, known producing or absorbing mechanisms are not known to be directly influenced by u* and we would expect decreasing fluxes with decreasing u* to be artifact-related.

Assessing the u* threshold under which fluxes are underestimated is usually done by the visualization of temperature-normalized fluxes against u* and the identification of the limit of a plateau at low u* (Aubinet et al., 2012). However, we did not follow this approach with our N_2O measurements: after selecting a range of data less likely to be influenced by fertilizer (4 months after N application) or by precipitation (low soil water content), it was not possible to retrieve a robust relationship between temperature and N_2O fluxes to normalize the latter. Therefore, the u* threshold was instead determined based on CO_2 fluxes measured at the experimental site. First, **Equation** IV. 1 was fitted to CO_2 night fluxes (R_{meas}) using the air temperature (T_{air}) as predictor (with R_{10}: ecosystem base respiration at 10 °C, and Q_{10}: temperature sensitivity parameter). Night fluxes were then normalized (R_{norm}) by means of **Equation** IV. 2:

Equation IV. 1 $$R_{fit,10} = R_{10} * Q_{10}^{(T_{air}-10)/10}$$

Equation IV. 2 $$R_{norm} = \frac{R_{meas}}{R_{fit,10}}$$

After plotting R_{norm} by classes of friction velocity, it was visually assessed that the u* threshold fell between 0.05 and 0.1 m s^{-1} (**Figure** IV. 2). This range is very similar to that which Moureaux et al. (2006) found at the same experimental site for a previous sugar beet crop in 2004. The uncertainty related to u* filtering is discussed in Section 2.6.

After applying the u* filter (0.1 m s^{-1}), 65 % of the dataset remained for the analyses (i.e. an average of 31 flux measurements per day).

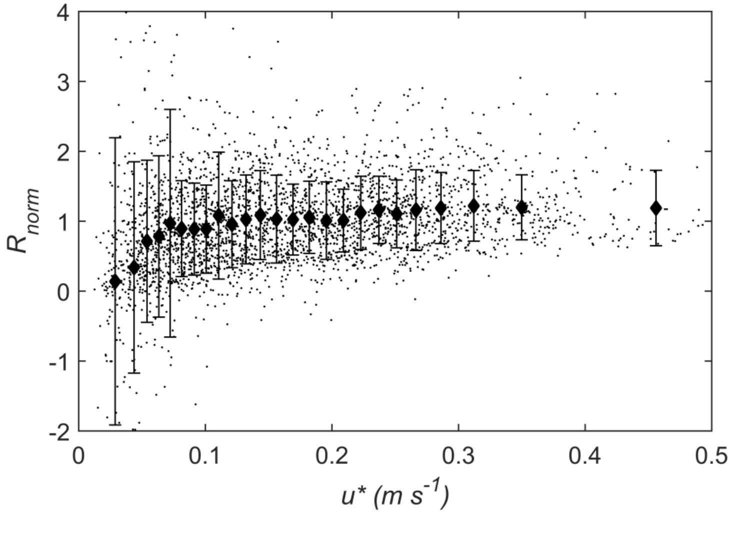

Figure IV. 2 - Normalized CO_2 night fluxes (R_{norm}) by equal-sized classes of friction velocity (u*)

2.5. DATA TREATMENT AND ANALYSES

2.5.1. Gap-filling

Data gaps were filled at the daily-scale according to the following approach (Mishurov and Kiely, 2011):

Case 1 - If less than 18 half-hours were missing for a day, the daily mean was calculated based on the 30-min fluxes available for that day;

Case 2 - For days missing more than 18 half-hours, daily means were assessed with a moving average using a 7-day rectangular window.

The threshold of 18 missing half-hours was chosen based on 1000 random samplings of [48–N] half-hours out of a complete day. The average daily mean and the associated confidence interval were computed for N varying from 0 to 47. For N under 18, we considered that the gain of confidence in the daily mean weighted by the number of available half-hours did not substantially improve. Therefore, N = 18 represents a suitable trade-off between representativity and data availability. Days missing less than 18 half-hours represented 88 % of the dataset.

Two gaps longer than a day occurred in the dataset (July 20 to 25 and November 1 to 4). Missing days were replaced by the mean of three days before and three days after the gap (*Case 3*). We assumed that this method did not introduce substantial bias into the budget as both gaps occurred during periods of low and constant fluxes. Enlarging the periods to take into account up to a week of data to fill such gaps did not change its value by more than 5 %.

The uncertainty related to gap-filling is discussed in Section 2.6.

2.5.2. N_2O budget

The N_2O budget (or Net Ecosystem Exchange of N_2O, NEE_{N2O}) of the sugar beet crop was calculated based on the gap-filled dataset as the sum of daily fluxes from fertilization to harvest. The crop emission factor was calculated as described in the Tier 1 equation of the IPCC guidelines (De Klein et al., 2006), by dividing the amount of N emitted as N_2O by the amount of N supplied, including fertilizer and crop residues (mineralized nitrogen from land-use change and management was considered negligible). N-amount from crop residues was determined as the yield of buried dry biomass from the previous crop (mustard leaves, stems and roots, and sparse wheat regrowth) multiplied by the estimated content of potentially mobilized N in the previous year[6] (Destain et al., 2010).

NEE_{N2O} was converted to CO_2-equivalent ($NEE_{N2O,\ CO2\text{-}eq}$) by multiplying it by 298, which represents the global warming potential of N_2O over 100 years (Myhre et al., 2013).

2.5.3. Management and meteorological controls of N_2O flux

Daily N_2O fluxes were averaged by class of SWC-5 (< 25 %, 25 – 30 %, 30 – 35 %, > 35 %) and compared by means of a one-way ANOVA. A one-way ANOVA test

[6] The amount of N brought to the crop by residues was estimated at 20 kg N ha^{-1}.

was also performed on fluxes averaged over 7 days preceding and 7 days following seed-bed preparation and sowing.

To investigate half-hourly N_2O flux dynamics, fluxes were compared to several temperature dynamics (air, surface, and soil) as temperature is known for its daily cyclic variation. Two contrasting periods were examined: the first emission peak from March 25 to April 12, and a period of background fluxes (daily average < 0.5 nmol m^{-2} s^{-1}) with dry soil from mid-August to mid-September. For each period of interest, half-hourly data (x_i) were standardized with regard to the daily mean ($\bar{x}_d$) and daily standard deviation (s_d) following **Equation** IV. 3. Then standardized variables ($x_{stand,i}$) were averaged by time of day, resulting in 48 values per period. Such analyses were performed on non-gap-filled data.

Equation IV. 3 $$x_{stand,i} = \frac{x_i - \bar{x}_d}{s_d}$$

To further analyze the episodes of emission peaks, a linear regression model was fit to daily averaged N_2O fluxes to evaluate a potential correlation with daily averaged CO_2 fluxes (Lognoul et al., 2017).

All statistical analyses were made using Matlab Statistics Toolbox: Matlab Version 2015a (MathWorks, Natrick, MA, USA).

2.6. *EVALUATION OF UNCERTAINTIES*

The total random uncertainty of individual 30-minute fluxes (ε_{30min}) was assessed as proposed by Langford et al. (2015) by calculating the root mean squared deviation from zero in a region of the covariance function far away from the true time-lag (150 – 200 s). The total random uncertainty was integrated over a day and over the crop season (ε_{season}) following the rules of error propagation (Taylor, 1982), assuming that errors were independent from one period to another (**Equation** IV. 4 and **Equation** IV. 5).

Equation IV. 4 $$\varepsilon_{day} = \sqrt{\sum_{i=1}^{48} (\varepsilon_{30min,i})^2}$$

Equation IV. 5 $$\varepsilon_{season} = \sqrt{\sum_{j=1}^{219} (\varepsilon_{day,j})^2}$$

When multiplied by 1.96, the individual random error provides an estimate of the measurement precision at the 95 % confidence intervals, which can be used as individual N_2O flux limits of detection, or LOD (Langford et al., 2015).

The budget over the crop season is also subject to other uncertainties. In the case of our dataset, three potential sources were identified: (1) spectral correction, (2) u* filtering, and (3) gap-filling.

The uncertainty related to the spectral correction (δ_{SC}) was approximated via the 99 %-confidence interval of the regression between SCF and wind speed used to correct half-hourly fluxes.

The uncertainty inherent to u* filtering (δ_{UF}) was approximated by the variation of the non-gap-filled budget over the whole campaign after filtering N_2O fluxes within the plausible range for the u* threshold (from 0.05 to 0.1 m s^{-1}).

The uncertainty related to gap-filling (δ_{GF}) was calculated depending on the number of missing half-hours in a day (SD: standard deviation, 1.96: normal score used to estimate the 95 % confidence intervals).

- If less than 18 half-hours were missing, the uncertainty of the daily flux was calculated as 1.96*SD of the daily mean;
- If more than 18 half-hours were missing, the uncertainty of the daily flux was calculated as 1.96*SD of the moving average;

During the two longer gaps, the uncertainty of the daily mean was calculated as 1.96*SD of the 3 days preceding and 3 days following the gap.

We assumed that between days, errors were random and independent, and we combined the three gap-filling cases to obtain the overall gap-filling uncertainty.

Finally, the four sources of uncertainty (total random uncertainty, spectral correction, u* filtering, and gap-filling) were combined assuming their non-correlation between one another at an annual scale (**Equation** IV. 6 – Taylor, 1982).

Equation IV. 6 $$\delta_{total} = \sqrt{(\varepsilon_{season})^2 + (\delta_{SC})^2 + (\delta_{UF})^2 + (\delta_{GF})^2}$$

3. RESULTS

3.1. ANCILLARY MEASUREMENTS

The evolution of pedo-climatic variables is presented in **Figure** IV. 3. The daily soil temperature varied between 5 and 22 °C during the crop season, with warmest temperatures reached in the months of June, July, and August (**Figure** IV. 3a).

Cumulative rainfall between March and October 2016 was 454 mm, keeping the global soil water content (SWC) between 20 and 44 %. Before fertilization (March 22), the soil water content in all three soil layers (5, 15, and 25 cm) was around 35 % (**Figure** IV. 3b). Precipitation started 3 days after fertilization, immediately followed by an increase in SWC (reaching about 40 %). The rainy weather lasted until the end of April with a weekly average of 14 mm. The soil water content at 5 cm (SWC-5) remained above 32 %. The first weeks of May were much drier with SWC-5 decreasing to 25 %. Besides a rainy month of June, fewer precipitation events were observed during the summer, followed by a gradual drying of the soil (SWC-5 at its lowest of the cropping season) until mid-October when rainfall resumed. When compared to data collected by the Royal Meteorological Institute of Belgium between the years 1981 and 2010, precipitation at the experimental site in 2016 fell within the Belgian average range (Figure 4), except a rainy month of June (+66 %) and a drier period between August and November (–57 %). Note that the month of May was not particularly drier than average but precipitation events were distributed towards the end of the month (**Figure** IV. 3b).

The leaf area index of sugar beet is shown in **Figure** IV. 3c. After a slight increase in the first two months after sowing, the LAI increased to 3.93 m^2_{leaf} m^{-2}_{soil} to then decrease by 30 % until the harvest date. The observed development curve is typical for sugar beet (Theurer, 1979).

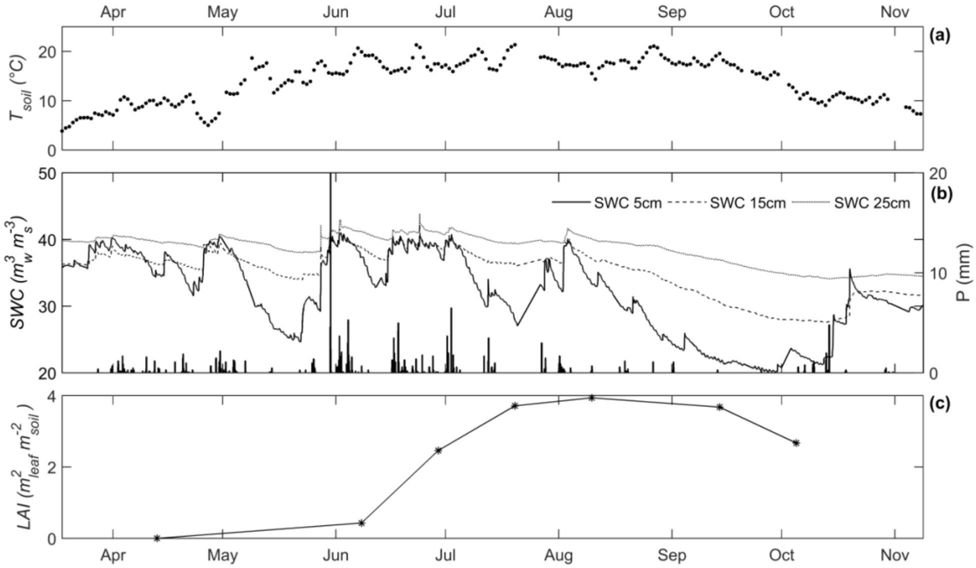

Figure IV. 3 - Evolution of (a) daily averaged soil temperature, (b) 30 min soil water content at 5, 15, and 25 cm, and 30 min precipitation, and (c) leaf area index

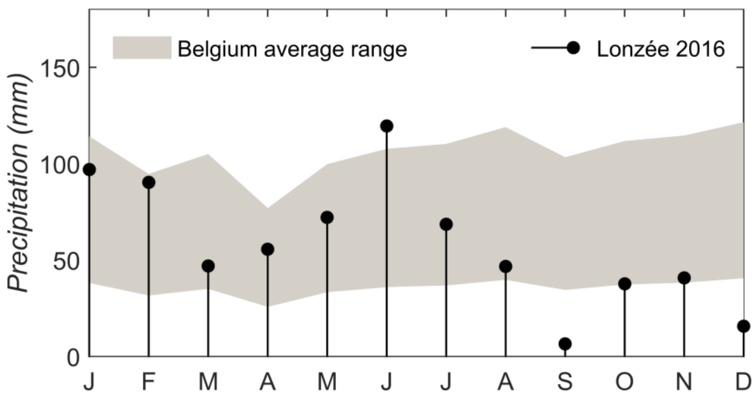

Figure IV. 4 - Belgian average precipitation range between 1981 and 2010 (upper and lower limits: average ± 1*standard deviation) and monthly precipitation at Lonzée

3.2. N_2O FLUXES

3.2.1. Cumulated N_2O fluxes

The evolution of CO_2 fluxes, N_2O fluxes, and cumulated N_2O emissions are presented in **Figure** IV. 5. Fluxes throughout the crop season fluctuated between –0.81 nmol m² s^{-1} and 5.56 nmol m² s^{-1}, with an average of 0.34 nmol m² s^{-1}.

The total amount of N_2O emitted by the sugar beet crop between fertilization and harvest was 6520 µmol N_2O m^{-2}, corresponding to 1.83 kg N_2O-N ha^{-1}. With regard to the N inputs (as fertilizer and crop residues), the sugar beet crop is characterized by an emission factor of 1.2 %. We compared this result to various studies including non-cereal crops, rotations, and managed grasslands (**Table** IV. 3). Our sugar beet crop had a higher EF_1 than other sites fertilized with similar amounts of inorganic N and was one of the few presenting an EF_1 higher than 1 % (**Figure** IV. 6).

The NEE_{N2O} converted to CO_2-eq was 230 kg CO_2-C m^{-2}. CH_4 was assumed to be negligible in our experiment: the experimental field is located on soil with good drainage, therefore excluding situations of continuous waterlogging that are favorable to methane production (Smith et al., 2003).

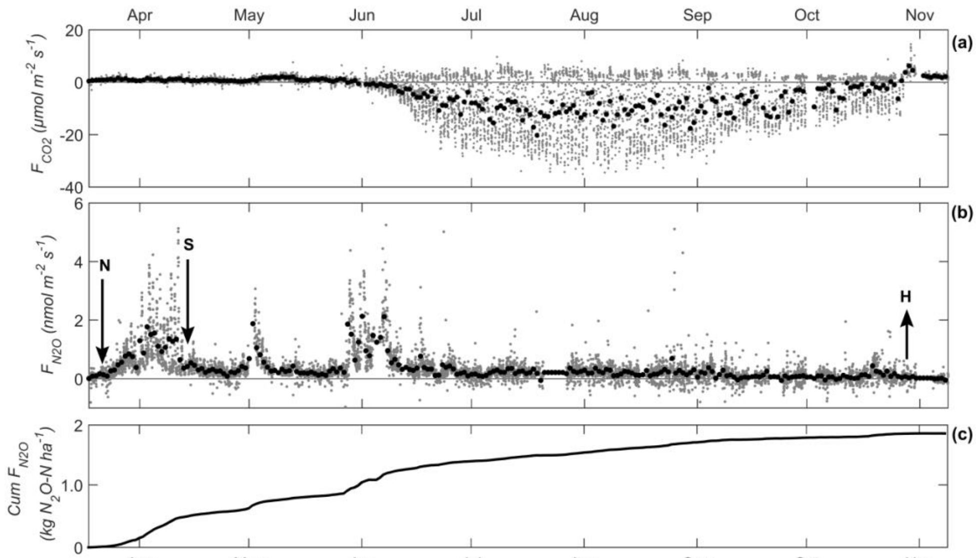

Figure IV. 5 - Evolution of (a) CO_2 fluxes (grey: half-hourly flux, black: daily averaged flux), (b) N_2O fluxes (grey: half-hourly flux, black: daily averaged flux), and (c) cumulated N_2O fluxes. *The arrows indicate the farming practices (N: fertilization on March 22, S: soil preparation and sowing on April 12–13, H: harvest on October 27)*

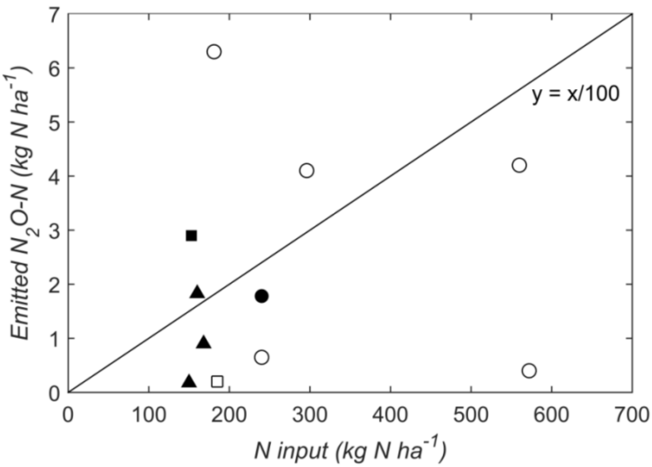

Figure IV. 6 - Comparison of emission factors from studies featuring different types of fertilizer (circles: organic fertilizer, triangle: inorganic fertilizer, square: both) and types of vegetation (filled: crop and grassland conversion to crop, empty: managed grassland). The diagonal line represents an emission factor (EF) of 1 %. Exact values and references for each study are given in **Table** IV. 3

Table IV. 3 - Emitted N_2O (kg N ha^{-1}), N inputs (kg N ha^{-1}) and associate emission factor (%) from studies including crops, grassland conversion to crop and mowed grassland

Study	**Measurement method (temporal resolution)**	**Type of crop (measurement period)**	**Type of fertilizer**	**Emitted N_2O**	**N inputs**	**EF**
Present study [a]	EC (30 min)	Sugar beet (219 d)	Inorganic fertilizer	1.83	160	1.2 (±0.1)
Merbold et al., 2014 [a, b]	EC (30 min)	Restored grassland (365 d)	Manure and inorganic fertilizer	29.10	198	14.71
Fuchs et al., 2018 [c]	EC (10 min)	Mowed grassland – year 1 (365 d) Mowed grassland – year 2 (365 d)	Cattle and pig liquid slurry	4.1 6.3	296 181	1.39 3.48
Koga, 2013 [c]	SCh (10 days)	Sugar beet (156 d)	Inorganic fertilizer	0.18	150	0.12
Seidel et al., 2017	SCh (2 – 7 days)	Perennial grassland – min (300 d) Perennial grassland – max (300 d)	Various types of cattle slurry	0.40 4.20	*572* *560*	0.07 0.75
Pugesgaard et al., 2017	SCh (2 – 4 weeks)	Rotation mean – barley, faba bean, potato, wheat (347 d)	Inorganic fertilizer	0.90	168	0.54
Reinsch et al., 2018 [c]	SCh (1 week) SCh (1 week)	Permanent grassland (365 d) Grassland conversion to maize (365 d)	Cattle slurry Cattle slurry	0.65 1.78	240 240	0.27 0.74
Flechard et al., 2005 [c]	ACh (2 hours)	Managed grassland (365 d)	Cattle slurry and inorganic fertilizer	0.2	185	0.6
Laville et al., 2011 [a]	ACh (1.5 hour)	Maize (365 d)	Cattle slurry and inorganic fertilizer	2.90	*153*	1.90

EC: eddy covariance. SCh: manual static closed chambers. ACh: automated closed chambers. Italics: value deduced from the two other columns when not provided.

[a] Nitrogen from land use change and management was not included or deemed negligible.

[b] Not included in the associated graph (**Figure** IV. 6).

[c] The factor was determined by comparing a treated plot and a control plot.

3.2.2. Farming and meteorological controls of N_2O fluxes

N_2O exchanges showed high temporal variability: three episodes of intense emission peaks were observed (from March 25 to April 12, from April 30 to May 4, and from May 28 to June 17) and, in between, N_2O fluxes stayed at a background level which hovered around 0.20 nmol m² s^{-1}. Over the whole dataset, daily emissions were significantly higher for SWC-5 above 35 % (**Figure** IV. 7) although rainfall did not systematically trigger emission bursts.

The first emission peak started after fertilization, almost simultaneous with heavy rainfall causing the SWC-5 cm to increase to 40 %. This episode lasted until the day of seed bed preparation and sugar beet sowing in the parcel. Immediately after, the emission intensity significantly went down ($p < 0.001$), being reduced by 70 % to return to background levels (**Figure** IV. 8). Almost a third of the N_2O emitted during the whole crop season was exchanged during this peak episode (**Figure** IV. 5c). During this first peak, daily N_2O fluxes were significantly correlated to CO_2 fluxes (**Figure** IV. 9). No similar significant relation was found for further peak episodes in the dataset or for background fluxes.

A second peak started between April 30 – 17:00 and May 2 – 10:00 (about two days of missing data), at least five days after precipitation and the sudden rise of SWC-5. The burst lasted for 4 to 5 days. By this time, the SWC-5 had decreased to 34 %.

The third N_2O peak episode began on May 28 – 10:00 (more than two months after fertilization), about 15 hours after a rainstorm (more than 25 mm of water fell in an hour). Precipitation events went on for a week and were interrupted for a few days (SWC-5 decreased below 35 %). It then started to rain again almost every day until the beginning of July. The period that followed was characterized by background N_2O fluxes that lasted until harvest without any noticeable emission burst.

The characteristics of the three peak episodes are summarized in **Table** IV. 4.

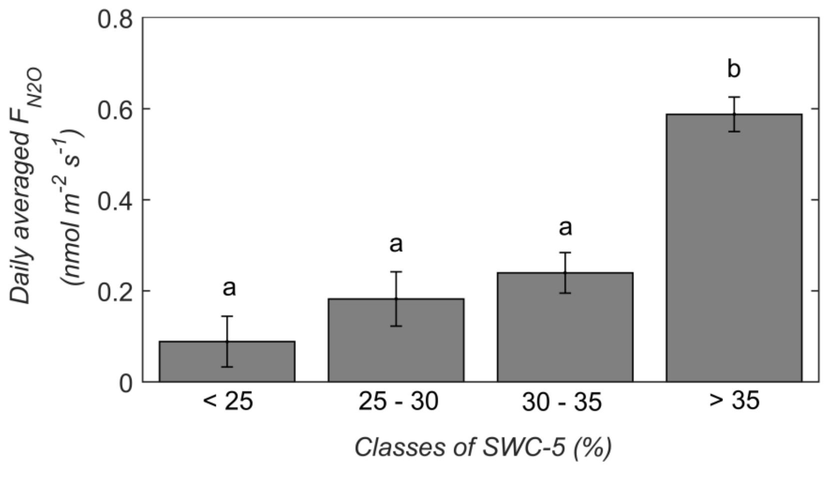

Figure IV. 7 - Daily averaged N_2O flux by classes of soil water content at 5 cm (SWC-5). *Different letters over bars indicate a significant difference ($p < 0.001$)*

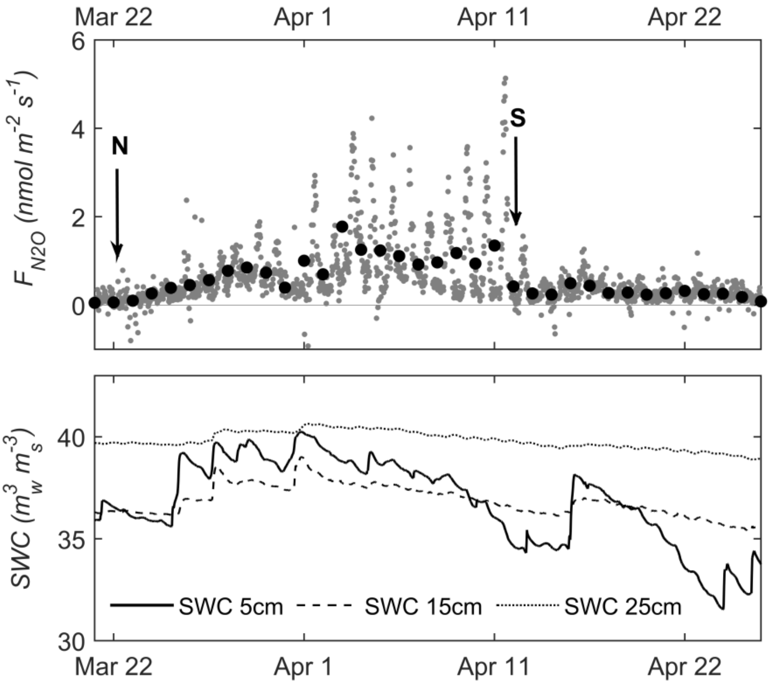

Figure IV. 8 - Focus on the soil preparation and sowing event. *Top: N_2O fluxes (grey: 30 min flux, black: daily averaged flux). Bottom: 30 min SWC at 5 (plain line), 15 (dashed line), and 25 cm (dotted line)*

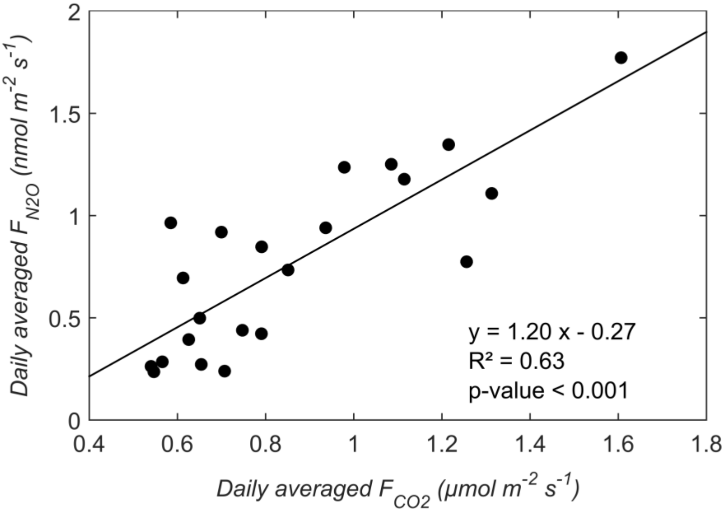

Figure IV. 9 - Correlation between daily averaged CO_2 flux and N_2O flux during the first episode of peak emissions

Table IV. 4 - Summarized specificities of episodes of N_2O burst during the crop season

	First episode	**Second episode**	**Third episode**
Specificities			
Start date	March 25	April 30	May 28
Duration	18 d	5 d	21 d
Time after fertilization	3 d	39 d	67 d
Time after last rainfall	1 h	5 d	15 h
Average SWC_5	38.3 %	38.4 %	37.7 %
Average T_{air}	15.0 °C	15.8 °C	21.4 °C
Average daytime flux	1.26 nmol m^{-2} s^{-1}	1.10 nmol m^{-2} s^{-1}	0.90 nmol m^{-2} s^{-1}
Average nighttime flux	0.59 nmol m^{-2} s^{-1}	0.87 nmol m^{-2} s^{-1}	0.71 nmol m^{-2} s^{-1}
Part in total emissions over the measurement campaign	28 %	7 %	24 %
Correlation with CO_2	***	NS	NS

3.2.3. Daily variations in N_2O fluxes

Daily oscillations in N_2O fluxes can be seen, especially between March and July, with daytime fluxes up to 20 times larger than nighttime fluxes. This observation was examined in more detail during two specific periods: one with high emission (first peak from March 25 to April 12) and one with low emission (background flux period from mid-August to mid-September).

During the first N_2O emission peak (March-April), the N_2O fluxes showed a clear daily oscillation with a maximum reached in early afternoon and minimum values during the night (**Figure** IV. 10a). This oscillation was better in phase with the surface temperature ($T_{surface}$) than with T_{air} – although with a slight delay between 09:00 and 12:00 – and the two normalized variables were well correlated ($R^2 = 0.92$, p-value < 0.001). It was not in phase with T_{soil} at 5 cm, with an offset of 2 to 3 h.

The N_2O diel cycle was less smooth and its amplitude of variation was smaller during the background period than during peaks (**Figure** IV. 10b). However, normalized night fluxes were still significantly lower than day fluxes (p-value < 0.01).

The daily cycle seemed in phase with the air temperature and was better correlated with this variable ($R^2 = 0.33$, p-value < 0.001) than with T_{soil} at 5 cm and with $T_{surface}$ (note that, by this time of the year, the surface temperature was considered to be the sugar beet canopy temperature).

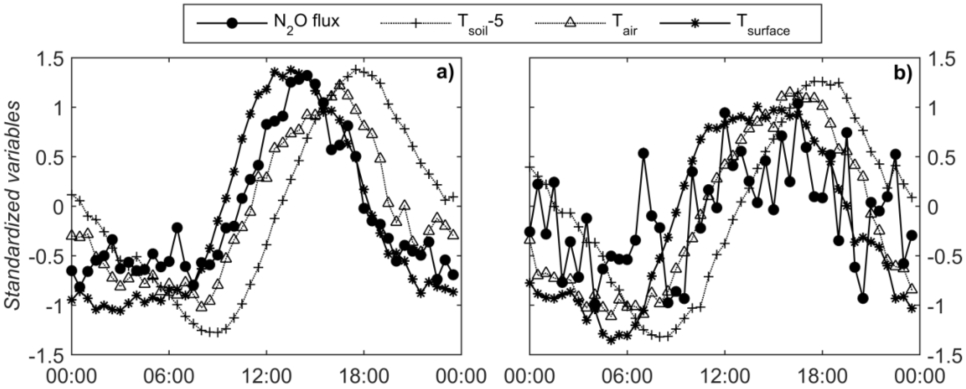

Figure IV. 10 - Daily averaged variation of standardized N_2O fluxes (circles), surface temperature (stars), air temperature (triangles) and soil temperature at 5 cm depth (crosses) during (a) the first N_2O burst episode (March 25 to April 12) and during (b) the background emission period in the summer (August 15 to September 20)

3.3. *EVALUATION OF UNCERTAINTIES*

The absolute total random error (ε_{season}) over the crop season was 5 g N_2O-N ha^{-1}. This corresponds to a relative error of 0.3 %, whereas the relative error at the 30 min scale ranged from 2 to 850 %, with an average of 30 % (20 % during unstable conditions and 60 % during stable conditions). 23 % of N_2O fluxes lay under their respective LOD. However, these were not excluded from our dataset as doing so might induce a bias in flux averages over longer periods (Langford et al., 2015). The absolute and total random errors on individual 30 min fluxes, as well as LOD are illustrated in **Figure** IV. 11. Negative fluxes were observed during the whole measurement campaign. Such fluxes could be seen as biophysically implausible in our experimental site (Chapuis-Lardy et al., 2007) and most of them were inferior to their LOD. However, they were also not discarded to avoid a systematic bias.

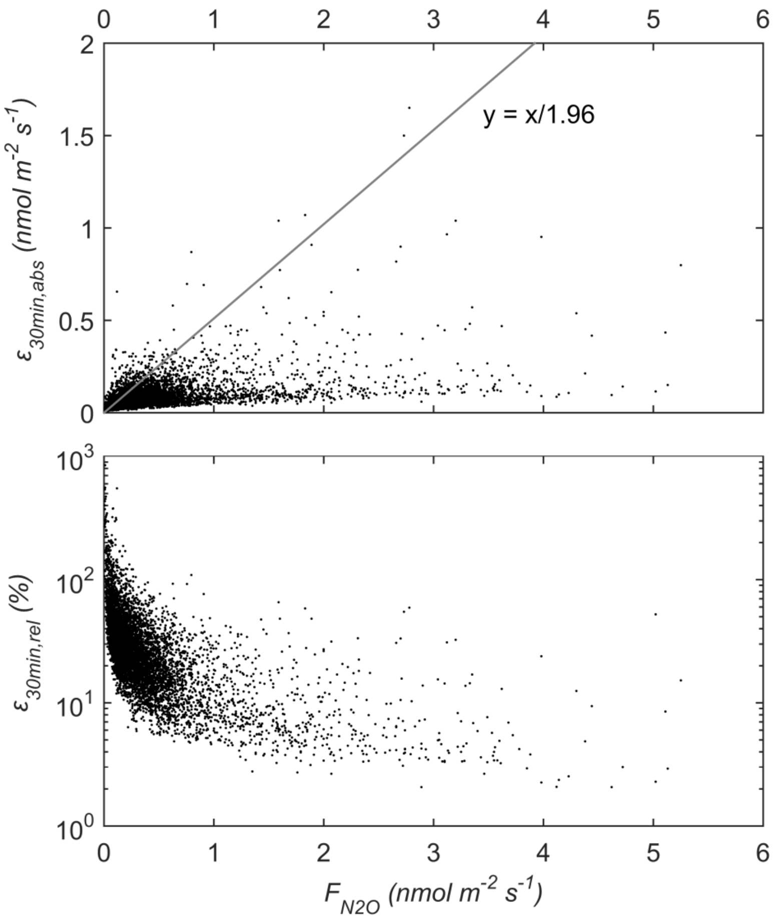

Figure IV. 11 - Total random uncertainty of absolute 30 min N_2O fluxes (top: absolute uncertainty, bottom: relative uncertainty*). Observations lying above the line fall under their individual limit of detection ($LOD_{30min}=1.96*\varepsilon_{30min}$)*

The uncertainty from spectral correction of the N_2O budget over the whole campaign was 52 g N_2O-N ha^{-1} (relative uncertainty: 2.8 %). On average, the relative uncertainties for unstable and stable conditions were respectively 1.4 % and 3.9 %.

Regarding u* filtering, an absolute uncertainty of 149 g N_2O-N ha^{-1} (relative uncertainty: 8.2 %) for the N_2O budget was found for the u* threshold range (from 0.05 to 0.1 m s^{-1}).

Regarding gap-filling, the three gap cases (Cases 1, 2, and 3) combined resulted in an overall uncertainty of 148 g N_2O-N ha^{-1} (8.1 %), after considering this, between days errors were random and independent. We noted that the absolute uncertainty of daily means during long gaps in background fluxes (0.20 nmol m^{-2} s^{-1}) was 6 times smaller than the uncertainty for a one-day gap on May 1 during the second emission peak (1.29 nmol m^{-2} s^{-1}). The relative uncertainty during the background emissions was smaller as well (96 % and 190 % for background and peak respectively).

The total uncertainty of the N_2O budget reached 217 g N_2O-N ha^{-1} (12 %). The absolute and relative uncertainties are summarized in **Table** IV. 5.

Table IV. 5 - Summarized uncertainties of the N_2O budget over the whole crop season

Source of uncertainty	Absolute uncertainty (g N_2O-N ha^{-1})	Relative uncertainty (%)
Total random error (ε_{season})	5	0.3
Spectral correction (δ_{SC})	52	2.8
u* filtering (δ_{UF})	149	8.2
Gap-filling (δ_{GF})	148	8.1
Total uncertainty (δ_{tot})	217	12

4. DISCUSSION

4.1. *DYNAMICS OF N_2O FLUXES*

4.1.1. Short-term effects of farming practice

Three episodes of high N_2O emissions were observed over the crop season. They were characterized by different durations and flux variations (**Table** IV. 4), illustrating the great variability of N_2O emissions (Molodovskaya et al., 2012).

The first peak episode was triggered within hours by heavy precipitation following N application. Such a rapid increase of water content and N availability in the soil is well-known to favor N_2O production by micro-organisms (Butterbach-Bahl et al., 2013; Plaza-Bonilla et al., 2014), and during the whole measurement period, N_2O emissions were on average higher for SWC-5 greater than 35 % (**Figure** IV. 7). It was, however, impossible to identify the production mechanism responsible for the high fluxes. Although high soil water contents are usually associated with denitrification (Linn and Doran, 1984; Dobbie et al., 1999; Bateman and Baggs, 2005), a recent study, conducted under soil and climate conditions similar to our study, found that N_2O emissions were positively correlated to nitrification gene transcripts despite an elevated water-filled pore space (Theodorakopoulos et al., 2017). Meanwhile, when looking at soil temperatures during that peak episode, nitrification could be thought to prevail: while nitrifiers are still active above 5 °C, denitrification rates are usually low below 15 °C (Bouwman, 1990).

Seed-bed preparation and sowing were performed 18 days after the first peak started; consequently reducing N_2O fluxes for two weeks. Although the SWC had decreased, it returned to previous levels within that period without N_2O fluxes rising again (**Figure** IV. 8). The soil temperature remained stable (around 10°C) for 12 days after sowing. These two observations suggest that soil disturbance was the sole cause of this inhibition. To our knowledge, such immediate impact of soil disturbance has not yet been observed.

The influence of tillage in the long term has already been studied without an emerging consensus. On the one hand, some have measured enhanced emissions under reduced or no tillage in comparison with conventional tillage, attributing their observations to poorer soil aeration promoting denitrification (Mutegi et al., 2010) or to crop residues left near the soil surface (Lognoul et al., 2017). On the other hand, others have attributed greater N_2O emissions to ploughed fields: such practice would promote microbial N_2O production in the long term by incorporating manure and residues into soil aggregates (Koga, 2013). The short-term effect of soil disturbance is rarely investigated, mostly because of a lack of sufficient temporal resolution or because tillage practice and fertilization are performed at the same time (Žurovek et al., 2017). Soil perturbation has been shown to enhance N_2O emission in the medium term (Žurovek et al., 2017). Ebrahimi and Or (2016) modelled N_2O-related activities in soil to find that anaerobic activity was favored in large aggregates. It could be inferred that soil disturbance inhibits denitrification (and therefore emissions), by breaking up bulks and leading to aerobic conditions. However, we cannot confirm that the peak resulted solely from denitrification and this hypothesis alone cannot explain why the second peak episode started 5 days after the last rainfall, while the third one followed the last rainfall by only 15 h.

The delay between rainfall and the second peak episode suggests that the microbial community took some time to build back up after being disturbed by seed-bed preparation. The soil temperature decreased during that associated rain event but stayed high enough for nitrifiers to be active, and remained mostly similar to the temperatures observed in the beginning of the first high emission episode. We thus hypothesized that the microbiome producing N_2O was active at the very surface of the soil before either being shut down by disturbed surface conditions or being relocated deeper. More research is needed to verify this hypothesis since the reactivity to perturbations of N_2O producers in aggregates and the related regulating factors have not yet been studied (Wang et al., 2019).

A third of the total N_2O produced by the crop was emitted between fertilization and seed-bed preparation, and the inhibiting effect observed immediately after sowing was attributed to soil disturbance. Further research on mitigation strategies should thus investigate the impact of the timing between such farming operations.

We excluded the predominance of abiotically produced N_2O at the soil surface as during the first peak episode daily N_2O fluxes were significantly correlated to CO_2 fluxes (**Figure** IV. 9), which represent soil respiration at this stage of the crop season. This correlation hints at a significant role of microbial activity following fertilization. The emergence of sugar beet photosynthetic organs reversing CO_2 net exchange explains the absence of correlation during the other two episodes of high flux.

The last peak episode occurred two months after fertilization. Comparable late bursts have been observed under similar soil and climate conditions, which were attributed to crop residue mineralization (Lognoul et al, 2017). Although mustard residues were incorporated prior to the sugar beet crop, it cannot be excluded that this peak originated from remaining N fertilizer rather than solely from mineralization. Sugar beet usually absorbs less than 15 % of its nitrogen requirements in April and May (Legrand and Vanstallen, 2000), which corresponds to seed germination and emergence (Didier, 2013). N content can thus remain high until three months after fertilization under such conditions of crop type, climate, and soil texture (Lenz, 2007).

4.1.2. Daily variation of N_2O fluxes

Day fluxes were higher than night fluxes throughout the whole experiment and a diel pattern was clearly identified during peak episodes (**Figure** IV. 10a). These observations align with the known control of temperature on N_2O-related microbial activity (Smith et al., 2003). Moreover, intense microbial activity during the day can further decrease O_2 availability in soil pores, acting as a positive feedback on anaerobic production of N_2O by denitrifiers when nitrate substrates are available (Flechard et al., 2007). This would mean that denitrification could be stimulated in conditions of hot and dry weather, with anaerobic zones extending without being necessarily due to precipitation. Denitrification could thus have played a significant role in the higher day emissions observed during the background flux period (**Figure** IV. 10b).

The diel cycle observed during peak episodes was in phase with the surface temperature rather than with deeper temperatures. This supports the assumption issued earlier of a N_2O-producing microbial community located in the topmost soil layer. Similar observations were made by Laville et al. (2011) for a fertilized crop over a period of 9 days, while others found a correlation with deeper temperatures (Alves et al., 2012; Livesley et al., 2008). Keane et al. (2017) observed a similar correlation but their interpretation of this as an effect of photosynthetically active radiation mediating exuded photosynthetate-C in the root zone, favoring microbial mechanisms producing N_2O (Van Zwieten et al., 2013) doesn't hold in the present case as sugar beet had still not emerged. The hypothesis of a prevailing effect of temperature on microorganisms is thus retained for the first months of our experiment.

The daily pattern observed during the second and third peak episodes was less obvious (**Table** IV. 4). On the one hand, we observed larger night emissions than during the first peak episode, which could originate from a higher range of temperatures in May and June promoting nightly microbial activity. On the other hand, day fluxes were smaller than in April: day emission potential could be dampened by reduced N availability over time. Although direct measurements of soil N content were not available for the present study, N availability should be taken into account along with soil temperatures when explaining N_2O flux variability, as these are poor predictors if considered separately (Lai and Denton, 2017).

Our results highlight that when studying N_2O flux dynamics, several time-scales can be considered. Increases in soil water content near the surface (5 cm depth), in combination with active microorganisms and sufficient N availability, can trigger large emission episodes during the cropping season (immediately or with delay), while the surface temperature drives daily variations in N_2O emissions.

4.2. CUMULATED N_2O EMISSIONS

A total of 1.83 (± 0.21) kg N_2O-N ha^{-1} was lost through N_2O emissions between the fertilization of the sugar beet crop (March 2016) and its harvest (October 2016). This corresponds to an emission factor (EF_1) of 1.2 %, which is slightly larger than the annual estimate of 1 % given by the IPCC guidelines (De Klein et al., 2006) used in Belgium. We believe that our EF_1 could have been even higher if the whole year had been monitored: emissions during bare soil periods, although presumed to be low, could have been added to the N_2O budget. In addition, more N_2O emissions could have been expected if the month of May had not been as dry for the most part. Higher soil water content would have promoted higher N_2O emissions, as at that time N availability in the soil was not yet limiting. The EF_1 of our crop was higher than other sites fertilized with similar amounts of inorganic N, and was one of the few presenting an EF_1 higher than 1 % (**Figure** IV. 6). Given the variety of results that can be obtained with different types of fertilizers and crops, a refinement of emission factor estimates is needed to take farming specificities into account when assessing N_2O emissions. However, our comparison is to be taken with caution, as studies monitoring N_2O fluxes at a sub-daily temporal resolution (using eddy covariance or automatic chambers) tended to give significantly higher EF that the others. It is not excluded that lower EF_1 values originate from (1) a lack of observations late after fertilization (Lognoul et al., 2017), or (2) an insufficient temporal resolution leading to missing short peaks similar to the second emission episode observed in this experiment. More measurements at higher temporal resolution are needed to refine the comparison.

Buysse et al. (2017) estimated the Net Biome Production (NBP) of the experimental site over 12 years (i.e. three 4-year crop rotations). When including harvest and C importation (manure, slimes), they found that the site was a net source of C with an annual average loss of 820 kg C ha^{-1}. Sleutel et al. (2003) calculated the annual NBP of Belgian cropland in the silt-loam region (based on C stock inventories over 10 years) and found a similar value (NBP = 900 kg C ha^{-1}). A much lower annual estimate was found by Goidts and van Wesemael (2007) for cropland in Southern Belgium from 1955 to 2005 (120 kg C ha^{-1}). After adding the $NEE_{N2O,\ CO2\text{-}eq}$ to these NBP estimates, we found a net greenhouse gas balance (NGB) varying from 350 to 1130 kg C ha^{-1}. Thus, N_2O emissions would account for 20 % to 66 % of the NGB, highlighting the importance of including N_2O when assessing the GHG budget from a fertilized crop.

4.3. EVALUATION OF UNCERTAINTIES

Uncertainties in eddy covariance fluxes are not often investigated (Kroon et al., 2010) although such information is important for the purposes of inter-site comparison and accurate estimates of GHG exchanges. In this study, uncertainties were addressed separately depending on the source, to then be combined in the N_2O budget as uncorrelated uncertainties.

The relative total random error of individual eddy fluxes was rather important (up to 850 %) which is similar to the findings of Kroon et al. (2007). This error increased with decreasing flux amplitude (**Figure** IV. 11), due to larger signal-to-noise ratios in small fluxes (Langford et al., 2015). This error was reduced to less than one percent after integrating N_2O fluxes over several months. Total random error of the budget was thus negligible compared to the other sources of uncertainty.

The uncertainty related to spectral correction was smaller than 3 %, most likely because of the good amount of high-quality fluxes used to build the spectral correction regressions (SCF as a function of wind speed). This source of uncertainty was small in comparison to those others linked to data treatment (respectively three and four times larger for u* filtering and gap-filling, **Table** IV. 5). In future experiments, a reduction of the uncertainty might be achieved by refining data treatment procedures, e.g. upgrading the gap-filling technique to a mechanical approach, or narrowing the u* threshold range by using more data.

Regarding gap-filling, our results also highlight the importance of performing continuous measurements during high emissions, as the uncertainty associated with missing data is larger during peaks than during background flux periods. This is due to greater flux variability during high emission bursts, leading to larger confidence intervals on the gap-filling value.

While the total uncertainty of the N_2O budget was estimated at 12 % (217 g N_2O-N ha^{-1}), it represented less than 3 % when considering the NGB at the site. Thus, using eddy covariance to measure N_2O fluxes appears to be a reliable method when it comes to NGB assessment.

5. CONCLUSION

Our study aimed to assess the short-term response of N_2O fluxes to weather and farming practices in a sugar beet crop. Measurements were performed from fertilization to harvest using the eddy covariance technique, which enabled the recording of a continuous stream of data at a high temporal resolution.

N_2O emissions were characterized by a diel pattern that correlated well with variations of surface temperature. This suggests that microorganisms responsible for N_2O production were active at the very surface of the soil. The activity of those microorganisms was significantly inhibited by seed-bed preparation. This is the first time that a short-term effect of soil disturbance on N_2O flux has been observed in a crop. An important part of the total N_2O-N loss over the crop season occurred between fertilization and seed-bed preparation. The influence of these farming practices should thus be investigated further to implement mitigation strategies. For example, (i) how does the timing between fertilization and seed-bed preparation affect the total N_2O emissions, and (ii) do N_2O emissions display an inhibited behavior following other tillage practices, such as stubble breaking or ploughing?

To better understand the underlying mechanisms behind the immediate effect of soil disturbance on N_2O emissions, more *in situ* studies are needed measuring N_2O fluxes at high temporal resolution in crops. We also recommend that researchers assess bulk density and mineral N content (ammonium and nitrate) before and after each farming practice to have a better view of the evolution of soil characteristics and how these are affected by soil disturbance. Finally, investigating the real-time activity of nitrifiers and denitrifiers will help to understand the dynamics of N_2O production in soil aggregates.

6. ACKNOWLEDGMENTS

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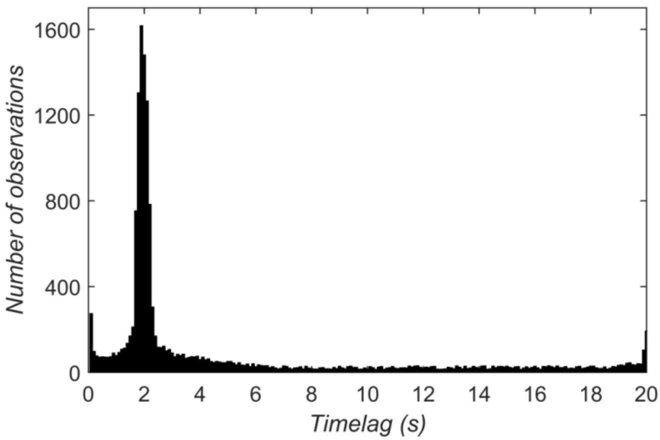
7. SUPPLEMENTARY DATA



Supplementary Figure IV. 1 - Frequency distribution of N_2O time-lags in the large time window (20 s). *The mode of the distribution is 1.9 s*

Supplementary Table IV. 1 - Tests and corresponding parameters as set in EddyPro® for the control of N_2O time series

Test	**Parameters**
Spike count/removal	Plausibility range: 5.0 σ Maximum number of consecutive outliers: 3 Accepted spikes: 10%
Amplitude resolution	Range of variation: 7.0 σ Number of bins: 100 Accepted empty bins: 70%
Drop-outs	Default parameters set by the software were maintained, as they seemed suitable to detect actual drop-outs that were not flagged also by the "Absolute limits" test
Absolute limits	Minimum: 0.030 µmol mol^{-1} Maximum: 3.000 µmol mol^{-1} Outranged values were filtered
Skewness and kurtosis	This test was not retained because it was not considered relevant for our dataset.

V

HOW DO EMISSION DYNAMICS OF N_2O RESPOND TO PASTURE RESTORATION?

V. How do emission dynamics of N_2O respond to pasture restoration?

This chapter is based on unpublished results from an eddy covariance campaign on a grazed pasture located in Dorinne (BELGIUM).

Authors: LOGNOUL M., BODSON B., DEBACQ A., DUMORTIER P., GOURLEZ DE LA MOTTE L., LONNEUX M., HEINESCH B. and AUBINET M.

Abstract

A paired eddy covariance flux tower experiment was set up in Southern Belgium to investigate the response of N_2O emissions to pasture restoration. Fluxes, meteorological conditions and soil C and N contents were monitored during one year, from March 2018 to February 2019. A parcel subjected to restoration (RE) was treated with a total herbicide, then harrowed and finally sowed with a new mix of grasses. The adjacent parcel served as control (CO); it was fertilized and grazed.

The RE parcel emitted significantly less N_2O than the CO parcel (- 36 %). Most likely the restoration process, although bringing substantial organic matter to the soil, did not provide as much instantly accessible substrate to the N_2O producing microorganisms as did the farming operations in the CO parcel (fertilization + grazing). Moreover, the exceptionally dry weather might have dampened the N_2O flushes. The total herbicide (glyphosate) was also thought to have decreased microbial activity in the RE parcel. More research is needed to explore the long-term effect (> 2 years) of pasture restoration on N_2O emissions.

Harrowing, by mixing the dead plants with the topsoil layer, was identified as the single trigger for N_2O emission bursts in the RE parcel during spring. Modifying this practice could thus be a potential lever for mitigation.

Keywords

Nitrous oxide; eddy covariance; grazed pasture; pasture restoration; harrowing.

1. INTRODUCTION

Nitrous oxide (N_2O) is a greenhouse gas with a global warming potential of 265, meaning that, at equivalent mass, its impact on climate change is 265 times larger than this of CO_2 (Smith, 2017). Its atmospheric concentration reached 329.9 ppb in 2017, resulting from a 20 % increase since the pre-industrial era (WMO, 2019). Agriculture is currently the greatest anthropogenic source of N_2O, with 67 % of emissions attributed to this human activity (Myrhe et al., 2013). N_2O is produced by soil microorganisms as a result of nitrification and denitrification, depending on the type of N substrate available and on pedo-climatic conditions (Braker and Conrad, 2011) which are influenced by parental soil material, climate and farming practices. In Belgium, almost half of the land surface is dedicated to agriculture, with 35.2 % of arable lands being permanent grasslands (StatBel, 2018). Those agro-ecosystems are substantial sources of N_2O (Saggar et al., 2004), with emission factors that can exceed those of crops (Oenema et al., 1997; Hortnagl et al., 2018).

A pasture can be considered “worn-out” when a depletion of the vegetation or a decreased nutritional quality for cattle is observed. This can be caused by over grazing, destruction by wild boars or repeated poor climatic conditions. A common[7] practice to restore worn-out pastures consists of the mechanical or chemical destruction of grasses, followed by optional tillage and finally the sowing of a new grass mix. This practice is strictly controlled by the third Nitrate Directive in the Walloon Region (PGDA, 2014): the destruction of grasslands in autumn is forbidden to prevent nitrate lixiviation, and restored pastures cannot be fertilized with organic amendments for two years after destruction (one year in case of mineral fertilizer). Despite those precautions, pasture restoration can lead to N loss through N_2O emissions. Vegetal residues are usually characterized by a low C:N ratio and, when returned to the soil, can substantially increase its organic N content (Davies et al., 2001), resulting in a flush of N mineralization (Velthof et al., 2010). Both these studies reported increased N_2O emissions following restoration. Velthof et al. (2010) also observed that ploughing reduced N_2O losses. Other authors also found increased N_2O emissions following grassland restoration; however, N_2O fluxes were mostly explained by pedoclimatic conditions (WFPS and soil temperature) or by fertilization (Merbold et al., 2014) rather than farming operation directly linked to restoration.

This paper aims at studying the response of N_2O emissions to pasture restoration during the first year following this event, with a focus on the immediate effect (within days) of farming operations on fluxes. N_2O exchanges were monitored in two adjacent grazed pastures conventionally managed by a local farmer. One of the parcels was restored in the spring, while the other parcel served as control. While comparative studies are usually implemented with soil chambers, we used the eddy covariance technique (Aubinet et al., 2012): this paired flux tower experiment allowed N_2O measurements at a high temporal resolution (30 min) and the instruments were left in place when farming operations were performed.

2. Material and methods

2.1. *Study site*

The study was conducted in the Terrestrial Observatory of Dorinne (level-2 ICOS station, coordinates: 50° 18′ 44″ N; 4° 58′ 07″ E) located in the hilly region of Condroz

[7] While no statistics on the frequency of total restoration in Belgium are available, such practice is not systematically performed when the quality of the pasture performance is decreasing. Some farmers restore their parcels every 7 to 8 years, while others favor over-seeding first (D. Knoden, Fourrages Mieux NPO, personal communication).

in Southern Belgium. The area is subjected to temperate suboceanic climate with an average air temperature of 9.4 °C and annual precipitation of 905 mm, which is slightly colder and wetter than the country average according to the Royal Meteorological Institute of Belgium (IRM, 2018). The soil is characterized by a loamy texture with a calcareous or clay substrate and a pH of 5.6. The bulk density was not measured, but it is estimated at 1.3 g cm^{-3} for pastures in this area according to ministerial guidance regarding the control of nitrate lixiviation hazard. The research site is a permanent grassland grazed by Belgian blue cattle within a farm managed by a local farmer. The vegetation includes 66 % grasses (mostly *Lolium perenne* L.), 16 % legumes (mostly *Trifolium repens* L.) and 18 % other species. It is annually fertilized with organic and mineral fertilizers (average fertilization rate: 120 kg N ha^{-1} y^{-1}) and grazed with an average annual stocking rate of 2.3 livestock unit (LU) ha^{-1} and a grazing season of 160 days. None of the parcels in this farm had been restored for the past 50 years.

An extended description of the study site can be found in Gourlez de la Motte et al. (2016).

2.2. PASTURE RESTORATION

A paired flux tower experiment was set up to study the influence of pasture restoration on N_2O fluxes from March 2018 to February 2019 (**Figure** V. 1).

Figure V. 1 – Dorinne ICOS station, satellite view imported from WALONMAP (2018).Stars indicate the locations of the eddy covariance towers (blue: RE parcel – length: 460 m, red: CO parcel – length: 360 m). *This image was taken shortly after the restored parcel was harrowed*

A 3.3-ha parcel was restored in spring 2018 (restoration treatment – RE) by (1) killing the vegetation in place with a broad-spectrum systemic herbicide (glyphosate), (2) harrowing to mix plant residues to soil aggregates and (3) reseeding afterwards.

An adjacent 4.2-ha parcel, managed with business-as-usual operations, served as control (control treatment – CO). Granular mineral fertilizer was applied in March, followed by lime to counter the acidifying effect of fertilizing and cattle excreta. The control parcel was fertilized again in May.

The detailed schedule of the pasture restoration is given in **Table** V. 1, along with the farming practices performed in the CO parcel. The CO parcel was grazed from April to November 2018, while the RT was grazed from June to November 2018. Note that the average grazing density in the CO parcel for 2018 was almost twice as high as the usual grazing habits in this parcel.

Table V. 1 - Farming operations implemented on the restored and the control parcels

Restoration treatment (RE)	
Date	**Farming operations**
March 23, 2018	Herbicide (Glyphosate, 4.5 l ha^{-1})
April 13 & 16, 2018	Harrowing
April 22, 2018	Reseeding (60 % ray-grass – 20 % meadow fescue – 10 % Timothy seed – 10 % white clover, 36 kg seed ha^{-1})
June 26, 2018	Mowing
Control treatment (CO)	
Date	**Farming operations**
March 6, 2018	Fertilizer (slow release granules, ammonitrate, 40 kg N ha^{-1})
March 27, 2018	Lime
May 22, 2018	Fertilizer (slow release granules, ammonitrate, 40 kg N ha^{-1})

2.3. *N_2O FLUXES*

2.3.1. Eddy covariance set-ups

The exchanges of N_2O between the parcels and the atmosphere were measured using the eddy covariance technique. Each parcel was equipped with the eddy covariance instruments described in the following paragraphs. Both set ups were built identically, using the same materials and analyzers.

The wind vertical velocity and direction were measured at 10 Hz with a sonic anemometer (CSAT3, Campbell Scientific Ltd, UK). The N_2O concentration was measured at 10 Hz with a quantum cascade laser (QCL) spectrometer (Aerodyne Research Inc., Billerica, MA, USA). This spectrometer also measured H_2O concentration to provide dry mixing ratios. The air was pumped through an 8.2 m long and 4.5 mm wide insulated polyethylene tube with a vacuum pump (TriScroll 600 Dry Scroll Pump, Agilent Technologies, Santa Clara, CA, USA). The tube inlet was protected by a rain cap (Intake Tube Rain Cap V3, LI-COR, Lincoln, NE, USA).

In the CO parcel, the eddy covariance mast was 2.6-m high and the anemometer and the inlet of the QCL spectrometer were separated by 34 cm (horizontal separation: 25 cm; vertical separation: 23 cm). In the RE parcel, the mast was 1.97-m high and the vertical sensor separation was 23 cm (no horizontal separation).

2.3.2. N_2O flux computation

The computation of N_2O fluxes was performed using the software EddyPro® (LI-COR Environmental, LI-COR, Lincoln, NE, USA) following the directions given in Lognoul et al. (2019), which are in accordance with ICOS guidelines (Nemitz et al., 2018).

The detection of the time-lag between N_2O concentration and vertical wind velocity was done by covariance maximization in a [1–4] s window with a default value of 2 s for N_2O, and in a [1–6.5] s window with a default value of 2.5 s for H_2O. The default time-lag was only applied to 16 % and 14 % of data, and the average time-lags were 2.1 ± 0.6 s (standard deviation) and 2.2 ± 0.8 s for CO and RE treatments respectively. The anemometer coordinates were rotated following the planar fit method (Wilczak et al., 2001). Timeseries detrending was performed at the half-hourly scale by block averaging.

The quality of raw data was controlled as preconized by Vickers and Mahrt (1997) with the following tests: spike count and removal, amplitude resolution, drop-outs, absolute limits, discontinuities and steadiness of horizontal wind. The conditions for good turbulence and stationarity were also controlled (Foken and Wichura, 1996). Using the results of these tests, the software ranked half-hours in a scale from 0 (good quality) to 2 (bad quality). Level 2 data were discarded prior to our analyses.

Spectral corrections were performed to compensate for low-pass filtering effects due to the sampling tube (Fratini et al., 2012) and the sensor separation (Horst and Lenschow, 2009). The average spectral correction factors applied to N_2O fluxes were 1.41 ± 0.33 (mean ± standard deviation) and 1.46 ± 1.20 for CO and RE datasets respectively.

Data were further filtered to obviate the artificial influence that friction velocity can have on eddy covariance fluxes. We used a friction velocity threshold of 0.13 m s^{-1} as determined by Gourlez de la Motte et al. (2016) and based on CO_2 fluxes, and applied it to N_2O data.

Finally, we used a footprint model (Kljun et al., 2015) to evaluate the contribution of the parcels to the measured fluxes. Data with a parcel contribution lower than 50% were discarded. Note that 82 % of data flagged for insufficient contribution were also flagged according to the u* threshold criterion.

2.3.3. Comparison between treatments

In order to compare N_2O fluxes from both treatments, a one-way analysis of variance (ANOVA) was performed. Prior to testing the difference between means, the response variable (N_2O flux) was log-transformed in order to meet the criteria of normal distribution required to do the ANOVA. Three sets of data were tested: data collected during the whole experiment, data collected during periods of high N_2O emissions (DOY < 180 i.e. June 29) and data collected during periods of background fluxes (DOY > 180). The significance level (alpha parameter) was set to 0.05.

2.4. *ANCILLARY MEASUREMENTS*

2.4.1. Meteorological measurements

Meteorological measurements were taken in the CO parcel. Precipitation (P) was recorded with a tipping bucket rain gauge (Model 52202/52203, R.M. Young Company, Michigan, USA). The soil water content was measured at 5 cm depth (SWC5) with a dielectric sensor (ThetaProbe, DELTA-T Devices Ltd Cambridge, UK). The soil temperature was measured at 2 cm depth (TS2) with platinum resistors (Pt1000, JUMO GmbH & Co. KG, Fulda, Germany).

2.4.2. Soil chemical analyses

Soil sampling was carried out biweekly. In each parcel, 3 samples (each made out of 5 sub-samples mixed together) were taken in the eddy covariance footprint in a depth from 0 to 10 cm. Sampling over dried cow dung or in compacted zones (i.e. preferential path of cows) was avoided. Before analyses, aerial vegetation was removed from samples and soil was sieved through an 8-mm mesh. Samples were stored at 4 °C in hermetic bags and analyzed less than 24 h after sampling.

The soil pH was measured after dilution in a solution of potassium chloride 0.1 N. The total organic carbon (SOC) content was quantified by the Dumas method (Dumas, 1826). Nitrate and ammonium contents were determined in field-moist samples by flow injection analysis derived from the ISO standard 14256 2:2005.

2.4.3. Grazing intensity

Grazing intensity was evaluated using livestock units (LU). One unit is the grazing equivalent of one adult cow. A heifer was counted as 0.6 LU and a calf as 0.4 LU. The cattle count was performed at least twice a month.

3. RESULTS

3.1. *N_2O FLUXES*

N_2O fluxes were measured in both parcels from March 2018 to February 2019 (**Figure** V. 2). After quality control and filtering using footprint and friction velocity, 46 % and 33 % of data remained for CO and RE respectively. **Figure** V. 2 also shows the grazing intensity (filled area).

In the CO parcel, an average N_2O emission (± standard deviation) of 1.56 ± 2.40 nmol m^{-2} s^{-1} was measured. In the RE parcel, the average flux was 36 % lower (0.99 ± 2.89 nmol m^{-2} s^{-1}). During periods of high N_2O emissions (e.g. in May and June), both parcels emitted N_2O within a range of similar magnitude, reaching past

40 nmol m^{-2} s^{-1}. However, the one-way ANOVA revealed that N_2O emissions were on average significantly higher in the CO parcel (p-value < 0.01), regardless of the period considered (whole data set, high emission period and background emission period). Sample means and associated standard errors are presented in **Table** V. 2.

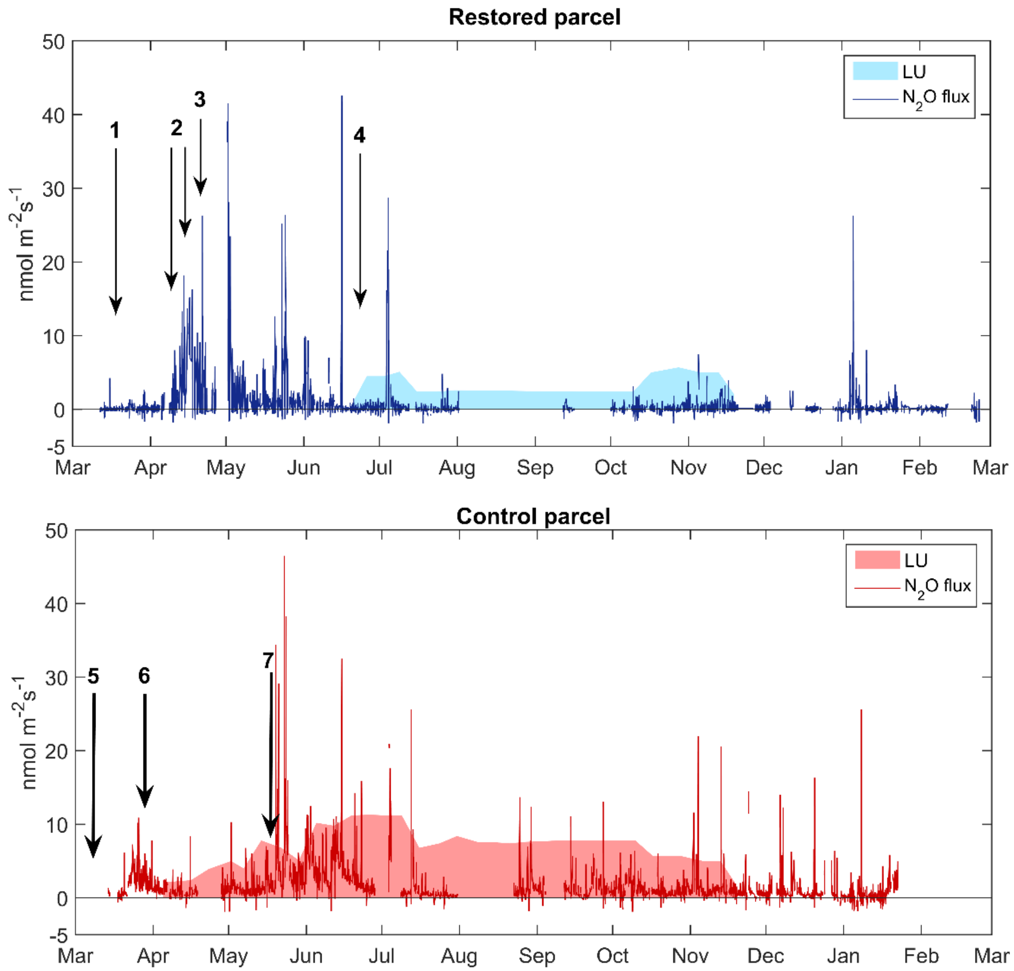


Figure V. 2 - N_2O 30-min flux (black line) and livestock units per hectare (grey area) in the RE parcel (top) and the CO parcel (bottom). Numbers indicate farming operations (1: herbicide, 2: harrowing, 3: reseeding, 4: mowing, 5: fertilizer, 6: lime and 7: fertilizer)

In the RE parcel, several episodes of high N_2O emission were observed (**Figure** V. 2, top). The first one occurred a few hours after harrowing for the first time (mid-April) and went on for 13 days. In May, high emissions, although more erratic, were also observed. In the CO parcel (**Figure** V. 2, bottom), high emissions were mostly observed following fertilizer applications in the end of March and in the end of May.

Table V. 2 - Mean N_2O flux (F_{N2O}) in the CO and RE parcels during the whole experiment and during periods of high and low emissions

Period considered	CO F_{N2O} [nmol m^{-2} s^{-1}] (mean ± standard error)	RE F_{N2O} [nmol m^{-2} s^{-1}] (mean ± standard error)
Whole experiment	**1.55** ± 0.04	**0.99** ± 0.05
High emissions (DOY < 180)	**2.50** ± 0.05	**1.80** ± 0.07
Low emissions (DOY > 180)	**0.95** ± 0.02	**0.37** ± 0.03

3.2. *ANCILLARY MEASUREMENTS*

3.2.1. Meteorological conditions

Meteorological conditions during the experiment are presented in **Figure** V. 3. It rained 595 mm during the 12 month-experience (from March 2018 to February 2019), which is 34 % lower than the average annual precipitation in the area. The year 2018 was the hottest and the fourth driest summer in Belgium since 1981 (IRM, 2018).

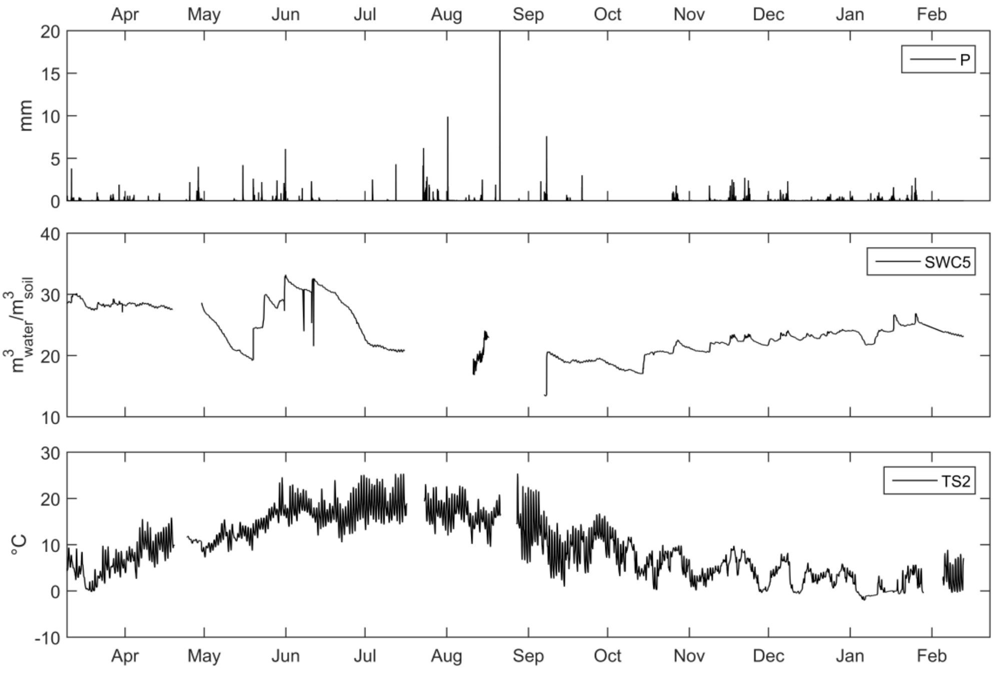


Figure V. 3 - Precipitation (P), soil water content at 5 cm depth (SWC5) and soil temperature at 2 cm depth (TS2) in the CO parcel (30 min resolution)

The soil water content was measured at 5 cm depth (SWC5). In the CO parcel, it varied from 13.4 and 33.2 m^3_{water} m^{-3}_{soil}. The highest and lowest SWC were respectively observed in the spring and during the summer. Note that many data are missing for the summer, thus the true minimum value during the experiment might be even lower. Soil water content data in the RE parcel were not usable due to numerous instrument failures.

The soil temperature at 2 cm depth (TS2) was measured in the control parcel. It ranged from - 2.1 to 25.5 °C. A few episodes of frozen soil were observed in December 2018 and in January 2019.

3.2.2. Soil chemical properties

The results of soil analyses are presented in **Figure** V. 4. During the measurement campaign, the soil organic carbon content (SOC) averaged at 42.2 ± 3.1 g C kg^{-1}_{soil} in the CO parcel and at 34.3 ± 2.2 g C kg^{-1}_{soil} in the RE parcel. When averaged over the whole experiment, SOC was higher in the CO parcel (+ 23%).

Soil pH in the two parcels were very close (5.7 and 5.6 in the CO and RE parcel respectively). A non-lasting increase occurred in the CO parcel; this is commonly observed after urea application.

Nitrate content ranged from 2 to 195 kg $N\text{-}NO_3$ ha^{-1} in the CO parcel and from 5 to 263 kg $N\text{-}NO_3$ ha^{-1} in the RE parcel, while ammonium content ranged from 5 to 46 kg $N\text{-}NH_4$ ha^{-1} in the CO parcel and from 6 to 31 kg $N\text{-}NH_4$ ha^{-1} in the RE parcel. To further understand N_2O production dynamics, the evolution of mineral N compounds during specific N_2O efflux events were estimated. Production rates (or consumption if negative) were calculated as the average slope during a given period. In the CO parcel, the ammonium content was stable while nitrate production was high (8.2 kg $N\text{-}NO_3$ ha^{-1} d^{-1}) in the beginning (May) of an episode of high N_2O emissions (**Figure** V. 5). At the end of this episode (June), ammonium was produced at a rate of 1.4 kg $N\text{-}NH_3$ ha^{-1} d^{-1} while nitrate was consumed at a rate of – 5.3 kg $N\text{-}NO_3$ ha^{-1} d^{-1}.

In the RE parcel, ammonium and nitrate evolutions were also quite different before and after harrowing. Before harrowing, ammonium was produced at a rate of 0.75 kg $N\text{-}NH_3$ ha^{-1} d^{-1} and nitrate content was stable while after harrowing ammonium showed a consumption rate of – 11.28 kg $N\text{-}NH_3$ ha^{-1} d^{-1} and nitrate showed a production rate of 9.76 kg $N\text{-}NO_3$ ha^{-1} d^{-1} (**Figure** V. 6).

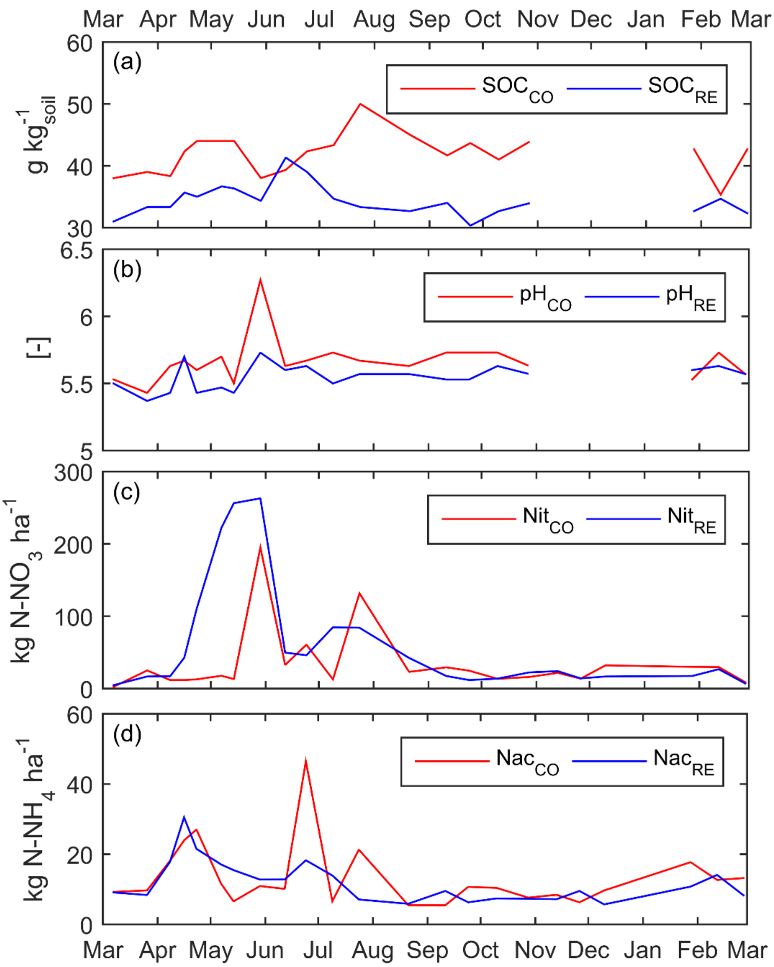


Figure V. 4 - Soil organic carbon (SOC), pH, nitrate (Nit) and ammonium (Nac, i.e. ammoniacal nitrogen) contents in the CO parcel (red) and the RE parcel (red)

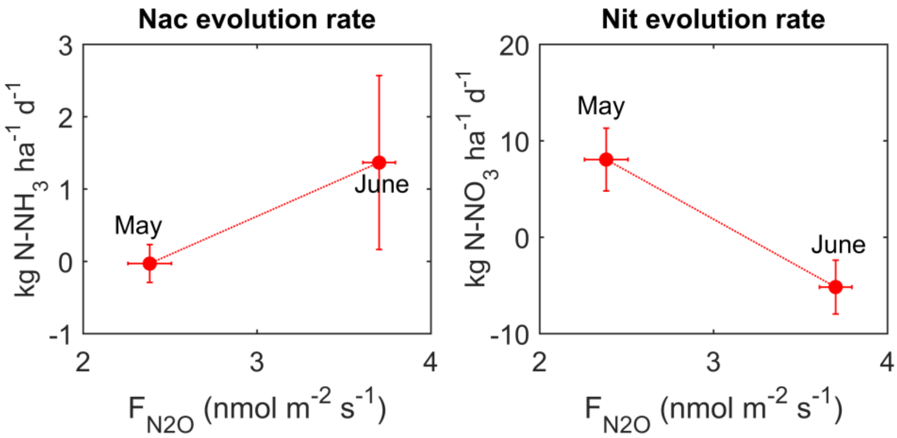


Figure V. 5 - Evolution of ammonium (Nac, i.e. ammoniacal nitrogen) and nitrate (Nit) soil content in the CO parcel in the beginning (from May 8 to May 30) and at the end (from May 30 to June 25) of an episode of high N_2O emissions (F_{N2O}). *Mind that the graphs have different vertical scales*

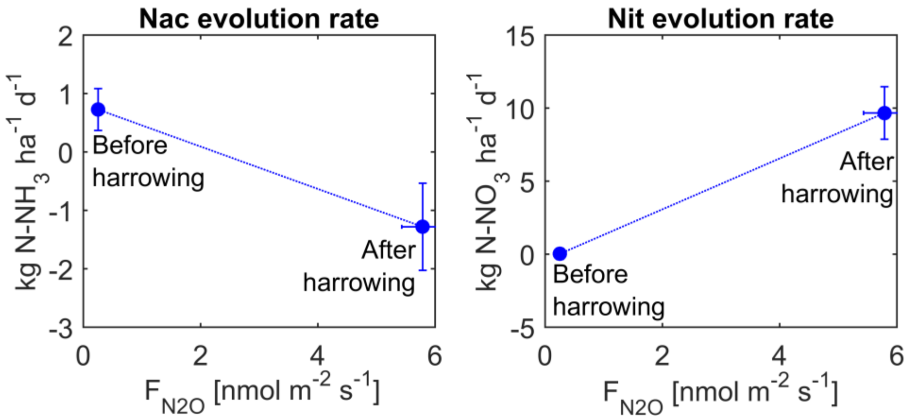


Figure V. 6 - Evolution of ammonium (Nac, i.e. ammoniacal nitrogen) and nitrate (Nit) soil content in the RE parcel before harrowing (from March 8 to April 4) and after harrowing (from April 17 to April 24) and N_2O fluxes (F_{N2O}). *Mind that the graphs have different vertical scales*

4. DISCUSSION

4.1. *INFLUENCE OF RESTORATION ON AVERAGE N_2O EMISSIONS IN THE FIRST YEAR*

Our results show that the parcel subjected to the control treatment (CO) emitted on average 50% more N_2O than the restored parcel (RE) during the year following the restoration (**Table** V. 2). The significant difference between treatments was also observed when taking the period characterized by emission peaks (DOY < 180) and the period of background fluxes (DOY > 180) separately.

A higher availability of C and N in the control parcel could explain those results. Indeed, C substrate can enhance ammonium-driven N_2O production (i.e. nitrification) (Bergstrom et al., 1994) and we measured higher SOC content in the CO parcel. Regarding the N supply, we can hypothesize that it was greater for N_2O-producing microorganisms in the CO parcel than in the RE parcel. In the control treatment, fertilizer application was performed twice, the cattle brought dung and excreta from March to November and the grazing intensity (i.e. livestock units) was almost twice as high. Restoration did not seem to rise to the level of the CO parcel (de Klein et al., 2010), though it provided organic matter following herbicide application that could have compensated fertilization (Ammann et al., 2020) and that sparked off N_2O emission bursts after harrowing. Indeed, while organic compounds undergo decomposition when returned to the soil, the mineralization process often restitutes less than 45% of green manure-N in the first year (Destain et al., 2010). Note that the yearly average content of nitrate and ammonium measured in the topsoil layer were not significantly different between treatments, but this might come from the sampling method, as we avoided cow dungs. To further investigate the comparison of SOC, nitrate and ammonium supply between treatments, analyses of C and N contents in the pasture biomass prior to restoration and during decomposition should be performed. Unfortunately, no biomass measurements were made prior to the herbicide application in our experiment, nor did we analyze the floral composition.

Although N_2O emissions were on average higher in the CO parcel, the amplitude of N_2O fluxes in the RE parcel right after harrowing was in times similar to those in the control treatment following fertilization. Longer measurement campaigns could reveal that restoration has the potential to trigger as much N_2O emission as the conventional treatment on the long term: De Toffoli et al. (2013) showed that the destruction of permanent grassland leads to substantial enrichment of soil mineral nitrogen for several years. Such observation was not made in our experiment, probably because our analyses only focused on the topsoil layer (10-cm depth). Given that applying organic fertilizer in a grazed parcel is legally forbidden in the two years following restoration (one year in case of mineral fertilizer), it would thus be interesting to investigate if the degradation of the residues incorporated reaches the N_2O-triggering level of fertilization in a non-restored plot.

The smaller average N_2O emission in the RE parcel could also originate from the glyphosate application: herbicide is thought to impair the enzymatic activity linked to

N_2O in crops (Jiang et al., 2015). The effect on microbial population could have lasted beyond DOY = 180: while nitrogen contents in both parcels are similar, average N_2O emissions are still higher in the control treatment. In a laboratory experiment, Kyaw and Toyota (2007) observed that glyphosate almost completely suppressed N_2O production, while soil respiration remained unaffected. More research is needed to understand the impact of glyphosate application on microbial activity (Zhang et al., 2018), especially in the context of pasture restoration.

4.2. *RESPONSE OF N_2O FLUX DYNAMICS TO FARMING OPERATIONS*

The restoration process in our experiment implied the incorporation of vegetation residues to the soil. Harrowing was performed twice mid-April, i.e. three weeks after herbicide application. Immediately after harrowing, N_2O emission bursts were observed (**Figure** V. 2), simultaneously with an increase of nitrate, while no rise in SWC or TS were recorded (**Figure** V. 3). Thus, soil disturbance, rather than meteorological factors, is thought to have triggered N_2O production. Similar N_2O flux behavior was observed by Baggs et al. (2000) in ploughed grassland: emission peaks lasting up to two weeks after soil disturbance reflected on the increased N and C substrate for nitrification and denitrification. Peterson et al. (2019) also observed a direct response of N_2O efflux following the incorporation of residues in a crop. In addition, they observed that the effect was greater with soil humidity. This suggests that a stronger response after harrowing could have been expected if 2018 had not be such a dry and hot year. More precipitation might have led to higher N_2O emissions overall in the RE parcel. As highlighted by Ammann et al. (2020) in a paired plot experiment, inter-annually varying climatic conditions can affect N_2O emissions from restored parcels with similar strength than the restoration operations themselves.

Since the practice of incorporating dead plant residues to soil has shown to spark off N_2O emission peaks, it could be an interesting lever for GHG mitigation in grassland (Vellinga et al., 2004). Delaying harrowing to a period with dryer soil conditions could be an option, as long as the chosen seed mix can be sowed later in the season. Harrowing sooner (lower temperature and thus reduced microbial activity) might also dampen N_2O emission. However, the latter is not permitted before February according to Belgian laws because of the nitrate lixiviation hazard. Direct seeding could also be considered, as it would imply suppressing harrowing. However, the absence of tillage after chemical destruction has been reported as enhancing N_2O emissions (Velthof et al., 2010). More research is therefore needed to consider the harrowing schedule as a mitigation option.

4.3. *IDENTIFYING THE MECHANISMS PRODUCING N_2O*

In order to investigate the mechanisms responsible for N_2O emissions, the production (or consumption) rates of N compounds before and after specific flux events were calculated.

In the CO parcel, the N_2O peaks in May and June were studied (**Figure** V. 5). In May, nitrate soil content was increasing at a rate close to 10 kg ha^{-1} d^{-1}. In June, N_2O emissions were much larger and nitrate content was decreasing, as indicated by the negative evolution rate. Since low rainfall and a decrease in soil moisture were observed, it is unlikely that the nitrate decrease was due to lixiviation. Therefore, we attribute nitrate production and consumption to nitrification and denitrification respectively. Note that plant uptake can also be responsible for nitrate decrease; however, the rate was considered constant during spring and summer. Our results thus indicate that both mechanisms were behind N_2O emissions (Theodorakopoulos et al., 2017) and most likely successively. This should be kept in mind when implementing mitigation technologies implying biological inhibitors (Misselbrook, 2014).

In the RE parcel, the focus was put on the high emission episode triggered by harrowing (**Figure** V. 6). Before soil disturbance, the nitrate soil content remained constant while ammonium was slightly increasing, evoking the mineralization process resulting from the glyphosate application. After harrowing, N_2O fluxes to the atmosphere raised while ammonium consumption and nitrate production were observed, reflecting the activity of nitrifiers.

Nitrification seems to have occurred simultaneously in both experimental parcels in the second half of spring (i.e. April-May). This is likely a consequence of a mineralization peak, which occurs when soil warms up after winter while SWC is high enough before summer (Grosclaude, 1999). The enhanced microbial activity produces ammonium that fuels nitrifying processes in the soil. This phenomenon was not interrupted by the restoration process.

5. CONCLUSION

This study focused on how restoring a worn-out pasture can affect N_2O emissions. We measured N_2O exchanges with paired eddy covariance towers in a restored parcel and in an adjacent control parcel (subjected to business-as-usual farming practices) during one year.

The restoration led to significantly smaller average N_2O emissions than the control treatment. Although the application of a total herbicide led to N mineralization followed by N_2O emission peaks, it was likely not enough to match the mineral and cow excreta fertilization in the control parcel and to trigger similarly high emissions during the first year. Furthermore, on the one hand the N input from livestock was likely greater than usual in the CO parcel due to the higher grazing density in 2018, and on the other hand the exceptionally dry conditions most likely dampened the response to harrowing and the average emissions that year in the RE parcel. Glyphosate application might also have been unfavorable to microbial activity. Despite those observations, N transformation in soil did not seem affected by restoration: nitrification occurred in both parcels in spring, as a likely consequence of ammonification.

In the restored parcel, harrowing was identified as a practice to act on in order to mitigate N_2O emissions. Indeed, it appeared to be the single trigger of emission bursts after glyphosate application, and the N_2O peaks reached at times the same magnitude than fertilization-induced emissions in the control parcel. However, more research is needed to assess the impact of anticipating or delaying residues incorporation with meteorological conditions in mind.

Future studies should consider measuring beyond the first year to explore the N_2O emission behavior and soil N contents in restored pastures, in relation with the new floral composition and grazing. In addition, mitigation strategies concerning pasture restoration should take into account N_2O emissions, but also the nitrate lixiviation hazard, the dynamics of mineralization in spring, and most importantly the feasibility of mitigation practices in regards to periods of plant growth.

6. ACKNOWLEDGMENTS

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VI

DISCUSSION AND PERSPECTIVES

VI. Discussion and perspectives

The overall objective of this thesis was to investigate how farming practices affect N_2O emissions in crops and grasslands in Belgium. For this purpose, my research team mobilized three different study sites and set up three experiments:

- The comparison of long-term conventional vs. reduced tillage practices on experimental plots in a maize crop, using two sets of 8 automatic closed chambers;
- The monitoring (from seeding to harvest) of a sugar beet crop managed by a local farmer, using eddy covariance;
- The evaluation of the immediate impact of restoration on a grazed grassland managed by a local farmer, using paired eddy covariance towers.

The first research question of my thesis focused on **the suitability of eddy covariance to measure N_2O fluxes**. While EC has been used for several decades to measure CO_2 fluxes, the measurements of N_2O fluxes using this technique are more recent and guidelines concerning methodology are rare. In Section 1 of this Chapter (page 148), we gather observations from all three experiments to highlight the advantages of eddy covariance when it comes to capture the full variability of N_2O fluxes.

The second research question aimed at investigating **the influence of farming practices on N_2O fluxes** (Section 2, page 150). While the monitoring of the sugar beet crop and of the "control" grassland parcel allowed us to assess the importance of direct fertilization, the "restored" grassland plot and the comparative study of tillage practices highlighted the influence of crop residues. All three Chapters also provided insights on the impact of tillage and soil disturbance on N_2O emissions on the long and short terms.

Finally, the third issue investigated in this work was **the weight of N_2O in greenhouse gas budget of cultivated lands**, which we were able to assess using GHG measurements over the sugar beet crop (Section 3, page 154).

In the following pages, we attempt to answer the research questions and share a few perspectives (Section 4, page 156).

This Chapter ends with a Section dedicated to recommendations for future studies in light of our three experiments (page 158).

1. MEASURING N_2O FLUXES WITH EDDY COVARIANCE

1.1. CAPTURING THE FLUX VARIABILITY

N_2O fluxes from managed soils present an important temporal variability. Indeed, they are characterized by low background fluxes interspersed with emission bursts, also called “hot moments”. While occurring over very short periods of time (a few days to a few weeks), those bursts can contribute to more than 50 % of the net N_2O emissions (Butterbach-Bahl et al., 2013).

In this thesis, several aspects of N_2O flux variability were highlighted. We have confirmed the occurrence and importance of hot moments. Although majorly triggered by fertilization in wet soil conditions, they can also be observed several months after N input (Lognoul et al., 2017). Because these “late” peaks are driven by soil organic matter mineralization, they are much more difficult to predict. Other farming practices such as the incorporation of residues can also lead to significant N_2O emissions, as it was observed in a restored pasture (Chapter V). Our results also highlighted a diel variability of N_2O fluxes (Lognoul et al., 2019): emissions were significantly higher during the day than the night, and the flux dynamics were closely related to the diel evolution of soil surface temperature. This latter finding is unpreceded, given that earlier studies associated N_2O flux dynamics with deeper soil temperatures.

To fully capture such variability, one must choose an adequate method. Up to now, most of the studies investigating N_2O emissions by crops and grasslands implied the use of closed chambers to measure fluxes. While these constitute an affordable and easily set up solution *in situ*, the risk to miss hot moments is great (Kroon et al., 2008). Indeed, biased results can emerge if searchers mostly focus on fertilization events and do not question the time of measurements during the day. The eddy covariance technique appears to be the most suitable method to fully capture the N_2O flux variability. Emissions are indeed continuously monitored at a high temporal resolution (30 min in most studies) and integrated over large areas, reducing the risk to miss hot spots. Unlike chambers, the EC mast can stay in place during farming operations such as soil preparation. Eddy covariance is thus very well suited to investigate N_2O flux dynamics *in situ* at a short time scale.

1.2. ASSESSING UNCERTAINTIES

In Chapter IV, several sources of uncertainty of N_2O data were investigated.

The relative random error of individual 30-min fluxes ranged from 2 to 850 %, which is similar to other findings (Kroon et al., 2007). However, when integrated over the whole crop season, it became negligible (0.3 %). The uncertainty related to spectral corrections was also small (3% of N_2O budget of the sugar beet crop). On the contrary, the error due to gap-filling was larger and we noted that the uncertainty associated with missing data was greater during peaks than during periods of background flux. This stresses the importance of continuous measurements during high emissions.

All in all, we estimated the total uncertainty of the N_2O budget at 12 %. When considering the net greenhouse gas budget of the site, the total uncertainty was less than 3 %. Therefore, eddy covariance is an adequate measurement method to assess GHG budgets over agricultural ecosystems.

1.3. DATA TREATMENT

Computing N_2O eddy fluxes may be done using software packages originally implemented for CO_2 fluxes. However, they require adaptation of data treatments. While most of the common tests can be utilized to control the quality of N_2O data, test parameters should be adapted to avoid over-flagging (e.g. when doing spike removal). Indeed, N_2O time series might not behave as CO_2 time series do and a case-to-case assessment should be done. When it comes to data filtering (for example with a u* threshold) or to gap-filling, methods built for CO_2 dataset might also be used and modified to best suit the specific features of N_2O fluxes.

All this being said, we wish to draw the searcher's attention to the matters of time-lag and spectral corrections. These two corrections imply the need for high quality fluxes, either to assess a narrow time window to look for the true time-lag and to define a default time-lag value (**Supplementary Figure** IV. 1, page 123), or to build the relationship between spectral correction factors and wind velocity (**Table** IV. 2, page 104) to correct the entire dataset. The quality of N_2O data is often related to the amplitude of the fluxes; small fluxes are characterized by low signal-to-noise ratios (Langford et al., 2015). Because of this, correcting fluxes measured over low-emitting sites could be challenging and the need to resort to artificial N_2O sources might arise (Nemitz et al., 2018).

1.4. EDDY COVARIANCE AS A ROUTINE MEASUREMENT IN EXPERIMENTAL FIELDS

In Chapters II and IV, the complexity of eddy covariance measurements was highlighted. The understanding of physical processes on the one hand and of the numerous data treatment procedures on the other hand is essential. When using EC data processing software, such as EddyPro®, the user should always avoid using the "basic mode" and deeply look into the "advanced mode". Indeed, many steps (such as filtering and spectral corrections to only name a few) and many parameters have to be taken into consideration: the risk to bias results by blindly operating software is high.

Thus, operating eddy covariance measurements and especially treating EC fluxes demands expertise. When this specific expertise is detained by a PhD student, it questions the ambition to exploit EC data as a routine operation on the long-term, given the difficulties in obtaining doctoral grants, the precarity of students' contracts and the high staff turnover it entails.

2. THE INFLUENCE OF FARMING PRACTICES

2.1. NITROGEN INPUTS

According to a synthesis report of the United Nations (UNEP, 2013), N_2O emissions attributed to fertilization and crop residues returned to soils are significantly larger than those from other sources related to food production.

In the preceding Chapters, we came across these two forms on N inputs with the fertilization of the sugar beet crop and of the "control" grassland parcel, and with the incorporation of wheat straw residues in the experimental plots and of the grassland vegetation in the restored parcel.

2.1.1. Directly assimilable nitrogen

The application of nitrogen directly assimilable by plants generates a rapid increase of easily accessible nitrogen supply for microorganisms. Depending on the provided form of nitrogen (NH_4^+, NO_3^- or a combination of both), nitrifiers or denitrifiers will produce N_2O molecules, given that soil conditions are favorable to microbial activity (sufficient humidity and adequate temperature). Direct fertilization is a well-known trigger of N_2O emission bursts. In Chapters IV (sugar beet crop) and V (grazed grassland), the application of N fertilizer resulted, as expected, in high emission peaks. In both situations, the large emissions lasted for a few weeks before returning to background level. Regarding the studied crop, the N_2O peaks constituted more than a third of the net N_2O budget of the crop.

While our observations confirm the critical impact of fertilization with directly assimilable N on N_2O emissions from managed lands, it is not possible to discuss the influence of fertilization rate on N_2O emissions with our results. Indeed, both study sites mobilized in Chapters IV and V (Lonzée and Dorinne ICOS stations) are run by local farmers in the purpose of production and we, as researchers, had no say in the technical itineraries. While this can be seen as a drawback because some aspects of practices cannot be studied (here, the rate of fertilization), it is a major advantage to study practices that are really applied by producers in the country.

Although directly assimilable N input has been identified as a critical driver of N_2O emissions, it remains challenging to established mitigation strategies using this practice as a lever. N application is a cornerstone of crop itineraries. Farmers must already deal with the increasing needs for high yields while limiting the ecological impact of their work (Venterea et al., 2011). In Belgium, efforts are being made by producers to minimize nitrate lixiviation to ground waters, as dictated by the Nitrate Directive. This is mostly done by strategizing direct N input according to the desired yield and to the crop needs, and by integrating nitrate-fixing intercrops into rotations. Rather than act on the amount and rate of N application to mitigate N_2O emissions, the efficiency of fertilizers would be a solution worth exploring. This matter is discussed below in Section 4.2.

2.1.2. Crop residues management

Fertilization with directly assimilable N is not the sole source of nitrogen for soil microorganisms in managed lands. The soil organic matter represents a substantial reservoir of N and C, which can be mineralized more or less rapidly after the plant death. Crop residues contribute to this pool.

In Chapter III, we observed a significant N_2O emission burst occurring in June, i.e. more than two months after the direct fertilization of the maize crop. This emission peak was attributed to the mineralization of the residues from the preceding crop (wheat straw, which was incorporated during the previous summer). At this time of the year, mineralizing processes are enhanced: the temperature increases and stimulates the microbial activity while the soil moisture is still sufficient for microorganisms (Grosclaude, 1999).

In Chapter V, we observed that the incorporation of grass residues (previously treated with a total herbicide) also triggered large N_2O emissions. However, the peaks occurred almost immediately after harrowing, not months later. The amplitude of this event was similar to the emission peak originating from the mineral fertilization in the adjacent "control" parcel.

This difference in the timing of N_2O release can be explained by the carbon-to-nitrogen ratio of plant residues (C:N ratio, i.e. the ratio between the mass of carbon and the mass of nitrogen in a substrate). Wheat straw is characterized by a C:N ratio > 50 (Cheshire et al., 1999), while the C:N ratio of grassland aboveground biomass is < 20 (Heyburn et al., 2017).

According to Juarez (2013), the kinetics of soil organic matter decomposition mostly depends on environmental factors, including the substrate composition. In order to degrade organic matter and benefit from its carbon content, bacteria and fungi also have to consume nitrogen. When the C:N ratio of a substrate is high (> 20), microorganisms take up C and N from the substrate itself, but also the nitrogen from the soil to compensate the low N content of the substrate. This results in partial N immobilization, which can cause "nitrogen hunger" for plants (**Figure** VI. 2, grey line). Such substrates are characterized by longer decomposition time (Taylor et al., 1989). In contrast, when the C:N ratio of the substrate is low, excess nitrogen is freed during the decomposition of microorganisms (**Figure** VI. 2, black line). In addition, such residues degrade more rapidly.

In conclusion, our results regarding the incorporation of wheat straw and grassland residues highlight two pieces of information:

- Incorporating crop residues can generate substantial N_2O emissions, as shown in the comparative study of grassland restoration;
- The timing of N_2O release depends on the C:N ratio of the residues.

Our observations are corroborated by those of Frimpong and Baggs (2010), who found a negative correlation between N_2O emissions and the C:N ratio of residues.

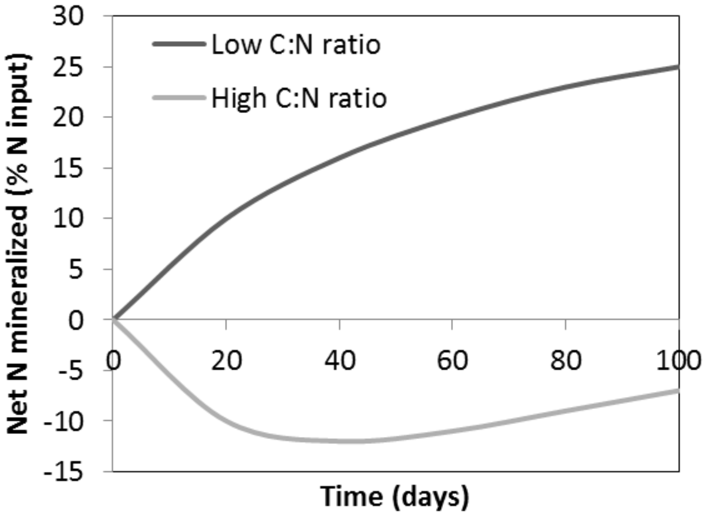

Figure VI. 2 – Illustration of the mineralization kinetics of organic matter depending on the C:N ratio

2.2. TILLAGE AND SOIL DISTURBANCE

The word "tillage" covers various practices mainly aiming at improving the soil structure and incorporating plants residues. They can be performed before seeding, after harvest or during periods of bare soil.

2.2.1. Impact of long-term tillage practices

In this thesis, we first took interest in N_2O emissions from parcels undergoing long-term contrasting tillage treatments (Chapter III). In an 8-years old Latin square experimental plot, some parcels were subjected to conventional tillage practices (winter ploughing + shallow incorporation of crop residues) while others were subjected to reduced tillage (shallow incorporation of crop residues).

We observed that GHG emissions (N_2O and CO_2) were significantly higher from the maize plot subjected to reduced tillage than from the plot under conventional tillage. By limiting the mixing of crop residues to the topsoil layer, where microbial life is concentrated, reduced tillage practices was thought to enhance N_2O emissions. This stratification of crop residues leading to higher N and organic C content near the soil surface was observed in all the RT parcels of the experimental site between 2012 and 2015 (Hiel et al., 2018). Following the maize crop, winter wheat was planted (2016) and a similar trend in N_2O fluxes was observed (F. Broux, unpublished results): the average emissions from the RT treatment were almost twice higher than those from the CT treatment. This observation was made during the peak episodes but also during background flux periods. However, the behavior of CO_2 emissions was the opposite: soil respiration was twice higher in the CT parcel than in the RT parcel. This could be explained by the fact that the emergence of winter wheat in the RT parcels was hampered by the maize residues remaining in the topsoil layer, leading to a smaller plant biomass, and therefore a lower root respiration.

The slightly lower pH observed in the topsoil layer of the RT parcel might also have played a role in the higher N_2O emissions. Indeed, while nitrification is relatively unaffected by soil acidity, the reduction of N_2O by denitrifiers is correlated to soil pH, leading to greater net N_2O production (Liu et al., 2010).

Lower soil disturbance, obtained via reduced tillage itineraries, is thought to promote C sequestration (Haddaway et al., 2017), thus contributing to the reduction of the atmospheric carbon pool. However, an increase of organic carbon stocks in soils may generate adverse effects: those practices can affect N_2O emissions and thus be counterproductive in regards to the GHG budget. On this matter, authors do not agree. In their review, Mangalassery et al. (2015) reported that, although a trend for enhanced N_2O emissions is reported for reduced tillage, recent studies observed a reduced net warming potential after the adoption of zero-tillage strategies. By contrast, Lugato et al. (2018) found that after 30 years, the CO_2 mitigation achieved by C sequestration by soils was overridden by increased N_2O emissions, questioning the "4p1000 initiative" launched at the COP21 in 2015.

The question of the long-term effect of tillage practices on N_2O emissions remains open. It should be investigated simultaneously with that of other greenhouse gas to better understand the link between tillage and net warming potential of agricultural soils.

2.2.2. Immediate response to soil disturbance

The instant effect of soil disturbance on N_2O emissions is rarely studied, mainly because instrumental setups, such as soil chambers, have to be removed from the parcel before the tractor passage. Eddy covariance allows overcoming this drawback by capturing fluxes continuously. In this thesis, we benefitted from two eddy covariance campaigns during which short-term effects of soil disturbance were observed.

In Chapter V, a pasture was restored with the application of a total herbicide, later followed by two harrow passes to break down and incorporate grass residues. Within hours, large N_2O emissions were recorded, reaching magnitudes similar to those of fertilizer-related N_2O peaks in the adjacent control parcel. This efflux increase was attributed to the mineralization of grass residues, which were no longer protected from soil microorganisms. Although the increased microbial activity and CO_2 efflux after residues incorporation are well documented (Reicosky, 1997; Gaillard et al., 1999; Gaillard et al., 2003), *in situ* observations of associated N_2O emissions are somewhat more seldom (Baggs et al., 2000; Peterson et al., 2019). In this case, the effect of harrowing on N_2O emissions was most likely due to the incorporation of organic matter to be degraded by microorganisms, rather than to the disturbance of soil aggregates.

In Chapter IV, a parcel was amended with mineral N fertilizer, which triggered a major N_2O burst. Three weeks later, the farmer proceeded to a seedbed preparation (i.e. a shallow soil disturbance to even the soil surface and break down big aggregates, and sowed his field with sugar beet, leading to a finer and packed structure near the surface. The N_2O peak was immediately shut down and emissions returned to background level. This observation was unpreceded in literature and raised the question of the mechanisms responsible for the dampening of the N_2O peak. Two hypotheses emerged from our results: (1) the reduction of the size of soil aggregates, and thus of anaerobic zones, decreased the activity of denitrifiers (Ebrahimi and Or, 2016), and (2) the microbiome was relocated deeper, under soil conditions less favorable to microbial activity. However, we did not have the means to verify these suggestions, and more research on the link between soil aggregates and microbial N_2O producers is needed (Wang et al., 2019).

Both these studies, in a pasture and in a sugar beet crop, suggest that the effect of soil disturbance on N_2O emission is closely linked to other farming practices that have to do with N inputs (N fertilizer or crop residues). A few perspectives to better understand the short-term influence of tillage practices are suggested in Section 4 of this Chapter (page 156).

Note that, although we benefitted from several parcels submitted to winter ploughing on a bare soil, the short-term effect on N_2O emissions of this tillage practice was not investigated in this thesis. However, we can hypothesize that it would not likely generate large N_2O efflux, because of the absence of residues and the low temperatures. Therefore, it does not appear to us as a significant focal point for further studies.

3. THE WEIGHT OF N2O IN THE NET GHG BUDGET OF CULTIVATED LANDS

In order to assess the Net GHG Budget (NGB) of parcels in cropland and grassland, it is essential to set up measurement campaigns of relevant trace gases. In rain fed crops, CO_2 and N_2O are identified as main contributors to the NGB, while CH_4 must also be taken into account when studying pastures.

In **Figure** VI. 3, we present the NGB of two of our study sites. Keep in mind that the purpose of these graphs is merely to provide estimates, as the assessments are based on both measurements and calculated estimates, sometimes based on different years.

In **Figure** VI. 3A, we present the NGB of a sugar beet crop at the Lonzée ICOS Station. The net N_2O exchange (expressed in CO_2-equivalent: 230 kg CO_2-C ha^{-1}) was measured with EC in 2016, while the CO_2 budget (820 kg CO_2-C ha^{-1}) was taken from the estimates of Buysse et al. (2017) who studied the net biome production at this site for 12 years. The site was a global source for both GHG and N_2O amounted to almost a third of the NGB (28 %).

In **Figure** VI. 3B, the NGB of a grazed pasture of the Dorinne ICOS Station is given. The estimate of the annual net N_2O exchange (expressed in CO_2-equivalent: 1575 kg CO_2-C ha^{-1}) was based on the extrapolation of the average 30-min N_2O flux in the control parcel (two fertilizations + grazing from April to November) in 2018.

We divided the year into two periods depending on flux amplitude: (i) a *peak period* from mid-March to mid-July (4 months, i.e. 365*4/12 days), and (ii) a *background period* during the rest of the year (8 months, i.e 365*8/12 days). Gaps in each period were filled with the mean of N_2O fluxes available within that period. Note that while this estimate could obviously be more precise by refining the gap-filling procedure, it is 3 times larger than the assessment of Gourlez de la Motte (2019). Using the equation of Tier 1 in 2006 IPCC guidelines (De Klein et al., 2006) and data from 2010 to 2014, they found an annual emission rate of 498 kg CO_2-C ha^{-1} with 68 % originating from cattle excreta. Based on our 2018 measurements, it was not possible to discriminate emissions from excreta and those from fertilization. However, we can note that the *peak period* occurred consequently to the fertilization operations in the parcel. The part of N_2O emissions originating from N fertilizer could thus be bigger than what was estimated by Gourlez de la Motte (2019). More long-term measurement campaigns are needed to better assess the N_2O budget of the Dorinne ICOS Station, along with the part of cattle excreta and yearly fertilization in the origins of emissions.

A - Lonzée ICOS Station (sugar beet)   *B - Dorinne ICOS Station (pasture)*

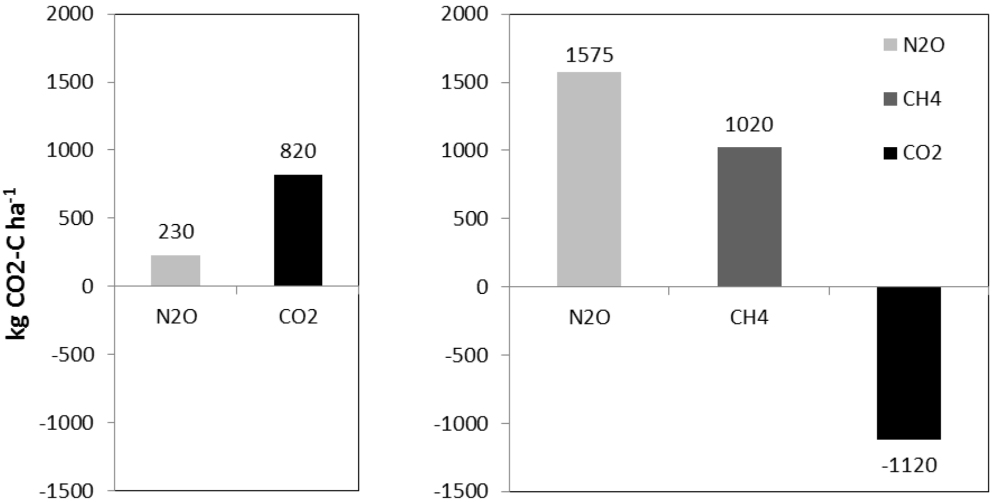

Figure VI. 3 - **A**: NGB of a sugar beet crop (Lonzée ICOS Station) (N_2O: 230 kg CO_2-C ha^{-1} measured by EC; CO_2: 820 kg CO_2-C ha^{-1} estimated by Buysse et al., 2017). **B**: NGB of a grazed pasture (Dorinne ICOS Station) (N_2O: 1575 kg CO_2-C ha^{-1}, measured by EC; CH_4: 1020 kg CO_2-C ha^{-1} from Dumortier et al. (2017); CO_2: -1120 kg CO_2-C ha^{-1} from Gourlez de la Motte (2019))

The annual net CH_4 exchange (expressed in CO_2-equivalent: 1020 kg CO_2-C ha^{-1}) was estimated by Dumortier et al. (2017) based on EC measurement coupled with a GPS campaign to locate the cows in the footprint and identify grazing behavior. Finally, the annual CO_2 exchange of the pasture, or net biome production (-1120 kg CO_2-C ha^{-1}) was assessed by Gourlez de la Motte (2019).

When considering both ours and Gourlez de la Motte's estimates of annual N_2O emissions, the pasture remains a GHG source because the carbon uptake by the parcel is no match to compensate the other GHG source (offset between 40 % and 70 %). N_2O therefore appears critical in the pasture budget. Nitrogen management in pastures, especially mineral fertilization, would constitute a subject of interest for further studies to find ways to lower the weight of N_2O in the NGB.

4. OTHER PERSPECTIVES

4.1. *SOIL DISTURBANCE AND NITROGEN INPUTS*

As discussed above, soil disturbance can have very contrasting results on N_2O efflux, depending on the circumstances. The short-term effects of tillage practices should thus be investigated further, particularly in relation to the management of N fertilizer and crop residues.

4.1.1. Soil disturbance and crop residues

Amongst practices performed in Belgium, the cultivation of nitrate-fixing intermediate crops during autumn and winter is common, and in some region even mandatory. They serve as green manure for the following crop and are usually selected with a low C:N ratio to prevent nitrogen hunger after incorporation. Chapter V highlighted that the incorporation of crop residues with low C:N ratio can have a critical impact on N_2O emissions, and other authors have confirmed a significant tillage/residues interaction (Baggs et al., 2003).

An aspect worth investigating would be the influence of the method of destruction (frost, herbicide or mechanical destruction), in addition to evaluating the impact of shredding the crop prior to incorporation, as Iqbal et al. (2014) suggested that, during the first year, the speed of degradation processes is inversely correlated to the size of plant particles. The comparison of incorporation vs. mulching would also be needed. Indeed, while mulching as a soil conservation practice is increasingly used to prevent erosion and maintain soil humidity, Peyrard (2016) highlighted that it was a risky practice in regard to N_2O emissions. In autumn 2014, an experiment was conducted at the experimental farm of Gembloux Agro-Bio Tech (D. Eylenbosch, unpublished results): four cover crops were mechanically killed and residues were placed in perforated bags on the soil surface. Four months later, the dry biomass in the bags had dropped by 90 %, while analyses of bag contents revealed carbon losses up to 560 kg C ha^{-1} and nitrogen losses up to 55 kg N ha^{-1}. This hints at great volatilization losses; CO_2 and N_2O measurements would shed light on this hypothesis. Studying the impact of plastic mulching on N_2O fluxes in Belgium would also be interesting, as this practice is performed in several areas of the country for vegetable and maize crops.

Finally, coupling research on low nitrate leaching potential (which is controlled in farms by the Belgian administration every year) and N_2O emissions would be of interest and could perhaps lead to the implementation of bonuses for agro-environmental services in the future.

4.1.2. Soil disturbance and directly assimilable N

The inhibition of fertilizer-related N_2O emissions following soil disturbance (Chapter IV) was to our knowledge a first-time observation that would require more investigation.

First of all, the link between soil disturbance and efflux inhibition should be confirmed. Additional measurement campaigns should be performed over crops that are fertilized before sowing, or which are subjected to mechanical weeding after fertilization. Those should be done using instruments that allow a fine temporal resolution and that do not require to be removed from the site during farming operations.

Then, it would be interesting to test how the delay between fertilization and soil disturbance affect the inhibition of N_2O emissions, and to assess how much of a lever for mitigation it represents. While modifications in the calendar of farming operations are limited due to crop specifications (e.g. sowing date) and meteorological conditions, fertilizing closer to the seedbed preparation might attenuate the impact of fertilizer-induced N_2O bursts on the greenhouse gas budget. Indeed, it is not uncommon for farmers to add N or NPK fertilizer weeks before planting spring crops, thus dissociating fertilization and soil preparation by a long period of time (B. Bodson, personal communication).

Finally, investigations on the mechanisms behind the inhibition are required to understand how soil disturbance affects soil conditions and microbial communities responsible for N_2O production in this very situation. Future studies should provide a detailed description of soil disturbance (faming tools employed, depth of disturbance, weather conditions, etc.). In addition, the use of microtomographic imagery (to characterize soil structure and poral connections) combined to microbial RNA analyses (to evaluate nitrification and denitrification rates) could help shed light on how soil disturbance affects N_2O-producing and emitting mechanisms.

4.2. EFFICIENCY OF FERTILIZERS

Although N application has been identified as a critical driver of N_2O emissions, it remains challenging to established mitigation strategies using this practice as a lever. Farmers must already deal with the increasing needs for high yields while limiting the ecological impact of their work (Venterea et al., 2011). In Belgium, efforts are already being made by producers to minimize nitrate lixiviation to ground waters, as dictated by the Nitrate Directive. This is mostly done by strategizing direct N input according to the desired yield and to the crop needs, and by integrating nitrate-fixing intercrops into rotations. It thus seems that modifying the rate of fertilization would not be a relevant mitigation strategy in the Belgian context and that research should focus on nitrogen use efficiency (Uchida and von Rein, 2018).

In order to act on fertilization practices to mitigate N_2O emissions, interest could be taken in the efficiency of fertilizers. Enhanced efficiency fertilizers (EEF) have been developed to limit the loss of nutrients to the environment through leaching or gaseous losses, either because they contain nitrification inhibitors or because they offer more control over the release of nitrogen to the soil (Halvorson et al., 2014). Comparison of N_2O emissions induced by various EEF could be performed on experimental parcels with the aim to issue a degree of risk to the different products available on the market. Economic and ecological impacts should also be taken into account (Ushida and von Rein, 2018).

The use of organic fertilizers (manure or slurry) allows the recycling of farmyard products. In cropping systems depending mostly on this practice rather than on the input of inorganic fertilizer, Hansen et al. (2019) found in their review that the correlation between yearly total N input and N_2O budget was weak. On the contrary, they highlighted that the risk for N_2O emissions was linked to the amount of readily available N and C in manure. Characterizing the latter in the most commonly used organic fertilizers in Belgium in parallel with assessing the induced N_2O emissions (total emissions and flux dynamics) would help understanding the importance of this kind of N input in N_2O emissions. The use of organic fertilizers such as composted manure should also be looked into, as reduction of N_2O emissions up to 30 % have been recorded (Lim et al., 2018).

5. RECOMMENDATIONS FOR FUTURE STUDIES

5.1. N_2O FLUX MEASUREMENTS

5.1.1. Automated dynamic closed chambers

When doing comparative studies using chambers, searchers should perform intercalibration with the gas analyzers to discard instrumental bias. The instrumental drift (zero and span) should also be quantified. When setting up the measurement system in the field, chambers should be placed with care to avoid soil compaction. The strategy of positioning in different experimental plots will depend on the number of available chambers and should be detailed (e.g. how many chambers on each plot, how far from each other, were there plants in the chambers…).

In our experiment, the measuring cycle lasted 4.5 h (8 chambers closing for 30 min each + 30 min to purge the system and control drifts), thus allowing between 5 and 6 averaged fluxes per set (i.e. per parcel) per day. The closing duration was slightly reduced to bring the total cycle to 4 h for the three campaigns that followed. In the future, it would be interesting to investigate how much the closing duration can be cut in order to increase temporal resolution while maintaining a low contribution of the instrumental noise over the total signal (total = natural signal + instrumental noise). One way to evaluate the signal-to-noise ratio is to calculate the auto-covariance function, as explained in Lognoul (2015). Note that this analysis should be experiment-specific, given that all sites and lab conditions will not offer the same natural signal.

5.1.2. Eddy covariance

Regarding eddy covariance, it is crucial that users understand the precise background of N_2O data processing before proceeding to flux analyses. Data treatment should be detailed in the papers.

Authors should also investigate the impact of their methodological choices on budgets. Using the dataset from Lonzée (Chapter IV), we compared the N_2O budgets obtained after correcting raw fluxes with spectral correction factors using (a) the method implemented in EddyPro® using spectra (based on Fratini et al., 2012 and Horst and Lenschow, 2009) and (b) the experimental approach using cospectra (described in Aubinet et al., 1999). Although no impact on flux dynamics was observed, a 6 % difference in cumulated corrected fluxes between methods was found. It originated from greater spectral correction factors obtained with EddyPro®. The biggest differences between methods were observed for stable conditions at low wind speed.

Finally, gap-filling strategies for EC datasets could be investigated. For example, the N_2O dataset from the sugar beet crop (Chapter IV) could be used to evaluate if modelling can serve a gap-filling purpose. By faking gaps of various lengths and at different moments of the crop season in this dataset, searchers will be able to assess the ability of a model to predict N_2O fluxes and the associated error in conditions of high or low emissions and for a variety of gap sizes. Another path worth exploring is the use of chambers as a back-up system to keep measuring N_2O during failures of the EC system. Indeed, during the year following the sugar beet crop, many technical issues were encountered: several weeks of EC data were missing, making it impossible to confidently gap-fill the dataset and assess the influence of fractioned fertilization in the winter wheat crop.

5.2. ANCILLARY MEASUREMENTS

In addition to monitoring N_2O exchanged by the ecosystem, searchers should do the following measurements (**Table** VI.1). Some are already routinely performed in most experimental sites, while other could be adopted without demanding too much manpower or financial resources.

Soil sampling should be implemented as a biweekly to monthly routine (depending on the period of the year and the farming operations) to evaluate soil characteristics such as nitrate and ammonium soil contents in the topsoil layer. The cost of such analyses remains reasonable and these observations would allow a better understanding of the biomechanisms producing N_2O. Because of the lack of such data in the campaign of 2016 in Lonzée (Chapter IV), our hypotheses regarding the role played by nitrification and denitrification in emission peaks could not be verified.

The crop development and the biomass quality could be assessed to better evaluate the N uptake and its restitution to the soil at the different stages of a crop season or crop rotation.

Table VI. 1 - Recommended measurements and frequencies on experimental sites

Soil chemical properties	
Total organic carbon content Nitrate content Ammonium content pH*	At least twice a month
Soil physical conditions	
Bulk density	Yearly + before and after each tillage operation
Temperature (surface, topsoil layer) Moisture (topsoil layer) Precipitation	Continuously
Other	
Crop development indicator (LAI, biomass…)	At least once a month between emergence and harvest
Biomass quality (floral composition, C:N ratio…)	Before destruction and/or incorporation (intermediate crop, crop residues, pasture…)

**pH its analysis is often included in basic soil analyses packages.*

Finally, when studying the immediate or long-term response of N_2O emissions to soil disturbance, searchers should assess the soil physical properties as a routine every year for long-term studies, but also before and after tillage operations. This could include measuring the soil bulk density and water retention curve, or even evaluating the soil poral network using X-ray tomography as a one-time experiment to better understand gas and water transfers through the soil matrix and to the surface (Smet et al., 2018) and how they influence N_2O production and emission. The activity of N_2O-producing microorganisms by analyzing microbial RNA could also be assessed. However, those measurements are more expensive and require a specific expertise.

5.3. *METADATA*

When comparing results from different studies, it is essential to have enough information in hands. However, sufficient metadata or data are sometimes missing to complete and discuss inter-site comparisons. In this Section, we share a non-exhaustive list of information (within the scope of this thesis) that searchers should include in their papers for a better use of their data by the community and for a better understanding of N_2O flux behavior.

When it comes to site description, authors should mention the following information:

- Precise localization and average climate conditions. This will allow comparing with the weather conditions during the experiment (e.g. in **Figure** IV. 4);
- Soil characteristics: texture, bulk density, pH and any additional information that could help interpreting the behavior of N_2O emissions and comparing different sites.

The dataset of Lonzée allowed us to observe the N_2O immediate response to soil disturbance and rain events all along the crop season. Given the good coverage, we were able to assess an emission factor (1.2 %) and compare it to other studies. This value was higher than that of other crops fertilized with a similar type and amount of N (**Figure** IV. 6). While the different methods used to monitor N_2O exchanges were suspected to have influenced the compared EF, soil pH also plays a role in regional disparities. In their meta-analysis, Wang et al. (2017) found evidence of a negative correlation between site pH and N_2O EF. That would imply that the soil pH of other sites would be higher than the pH at the Lonzée ICOS Station, which is rather high due to the frequent liming (pH (H_2O) = 7.9). Unfortunately, pH is not always mentioned in papers metadata.

Regarding farm management, the following details should be shared:

- History of soil management (tillage mode, grazing intensity, etc.);
- Recent history of crop management (at least the preceding crop and intercrop);
- Equipment used for tillage operation during the experiment (tools and their purpose, depth of soil disturbance, etc.);
- N-related crop information, such as their theoretical N uptake curve (along with biomass measurements when available) to better assess the competition between plants and microorganisms.

VII

CONCLUSION

VII. Conclusion

The present thesis investigated the influence of farming practices on N_2O emissions from agricultural lands in Southern Belgium. Three measurements campaigns were set up, using automated dynamic closed chambers and eddy covariance to focus on the following issues: (1) the suitability of eddy covariance to measure N_2O fluxes, (2) the influence of common farming practices on N_2O flux dynamics and N_2O budget and (3) the weight of N_2O in the GHG budget of managed lands.

Here are the key findings of this work:

- In managed lands, where high N_2O flushes are expected, eddy covariance constitutes an adequate measurement system to monitor N_2O exchanges, as data treatment procedures can be adapted and we estimated that total error on a crop-season GHG budget was low.
- In an experiment on a maize crop, significantly higher N_2O and CO_2 emissions were observed in a parcel under long-term reduced tillage in comparison with conventional tillage. This difference was explained by the different stratification of crop residues in the soil profile between the two tillage treatments.
- The restoration of a pasture including the use of a total herbicide combined to harrowing has the potential to immediately trigger emissions as high as fertilizer-induced N_2O burst. The low C:N ratio of residues was identified as critical for the short delay between harrowing and N_2O flushes.
- For the first time, an inhibiting effect on fertilizer-induced N_2O emissions was observed after soil disturbance in a sugar beet crop. The diel evolution of N_2O fluxes during the crop season was synchronized with that of soil surface temperature. Both these findings indicate that N_2O producing mechanisms were located in the topsoil layer.
- N_2O can play a major role in the net GHG budget of crops (between 20 and 66 %), highlighting the need for systematic implementation of N_2O measurements when assessing the GHG budget of managed lands.

Several perspectives to this thesis were identified, such as the investigation of the inhibiting effect of soil disturbance on fertilizer-induced emissions, and the different ways to use crop residues (e.g. destruction and incorporation methods).

Finally, we would like to stress that further research should at all time consider the current pressure to produce on farmers and the legal requirements they have to comply with in the context of N inputs in cultivated lands.

VIII

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VIII. References

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