CRANFIELD UNIVERSITY

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Assessing the balance between greenhouse gases and ammonia emissions from Irish pastures amended with cattle slurry

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Abstract

Agriculture in Ireland is the main source of ammonia (NH_3) and contributes 30% of greenhouse gas emissions (GHG), with the majority of these emissions associated with livestock production. As a result, strategies promoting reductions in NH_3 and GHG emissions are required. The aim of this work was: (i) to assess the impact of various NH_3 abatement techniques on GHG release from a grassland soil; (ii) to investigate the consequences of organic nitrogen (N) applications in terms of carbon (C) sequestration in soils.

The effects of slurry dry-matter content, application technique and timing of application were studied in a fifteen month field-plot experiment where gaseous emissions (CO_2, N_2O, CH_4 and NH_3) post-application were monitored. The natural abundance ^{13}C tracer technique was also used to investigate the short-term dynamic of slurry-derived C and its consequences on soil CO_2 efflux. Finally, ^{15}N labelled slurries, supplemented or not with an additional C substrate, were used in a lysimeter study, under controlled conditions, to characterise the interactions between soil C and N processes post-organic fertilisation.

Trailing-shoe application technique was shown to be and efficient way to lower NH_3 volatilisation from land spread slurry. However, such benefice could be easily offset by an increase in direct N_2O emissions and ecosystem respiration. Conversely, adjusting the timing of slurry spreading to get favourable soil and weather conditions, and to better meet herbage N requirements, had a positive effect on field N balance through a simultaneous reduction of both NH_3 and N_2O emissions.

Emission factors (EF) calculated for slurry-induced N_2O emissions were significantly lower than those calculated for mineral fertiliser and were greatly affected by weather and soil conditions. Such results support the widely spread idea of an inappropriate use of a single default EF value of 1% for both fertiliser types, under the IPCC Tier 1 methodology for national GHG inventories, and calls for the development of region-specific emission factors based on local soil types and climatic conditions.

About 60% of slurry-derived C was shown to remain in the soil, even after 6 months, thus contributing to an increase of SOC pools. However, such incorporation of slurry-derived C may be offset by a positive priming effect of slurry on the degradation of the SOM. Such short-term priming of soil CO_2 efflux may be, under certain conditions, compensated by a subsequent negative PE, thus minimising the impact of such phenomenon on the long-term sequestration of added slurry C. The long-term impact of these priming effects on nutrient and GHG balances remains to be further investigated, as these phenomena may occur on a regular basis in grassland ecosystems.

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Dedication

First, I would like to dedicate this work to my mother who sacrificed so many things for me to succeed in whatever I had chosen. She never criticised my choices, helping me whenever needed, supporting me whenever I was going down… This is for you Mum!

I also cannot forget all my friends, my fellow runners from the Menapians AC: Jimmy, Catherine, Kevin, Mick, Ciaran, Niamh, Helen, Elaine, Barbara, and all those I forget about. Thank you guys for getting me back into running. I would never have thought four years ago that I could become a competitive runner one day. Guys, you kept me alive during such hard time far from my family and I won't forget that… so this is for you as well.

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List of abbreviations

Acetyl CoA: acetyl coenzyme A

AN: ammonium nitrate

ANL: apparent nitrogen loss

ANOVA: analysis of variances

ANR: apparent nitrogen recovery

ATP: adenosine triphosphate

C: carbon

CAN: calcium ammonium nitrate

CH_4: methane

CLRTAP: Convention on Long-Range Transboundary Air Pollution

CO_2: carbon dioxide

$CO_{2\text{-}eq}$: carbon dioxide equivalents

$CO(NH_2)_2$: urea

DM: dry matter

DOC: dissolved organic carbon

ECD: electron capture detector

EF: emission factor

ER: ecosystem respiration

ETS: Emission Traded Sector

EU: European Union

FAD /$FADH_2$: flavin adenine dinucleotide

FID: flame ionisation detector

GC: gas chromatography

GHG: greenhouse gas

GPP: growth primary production

GWP: global warming potential

H_2SO_4: sulphuric acid

HCl: hydrochloric acid

HNO_2: nitrous acid

HNO_3: nitric acid

IPCC: International Panel on Climate Change

IRMS: isotope-ratio mass spectrometer

KCl: potassium chloride

LUC: land use change

MAP: mean annual precipitation

MAT: mean annual temperature

MIT: mineralisation-immobilisation turnover

N: nitrogen

N_2: dinitrogen

N_2O: nitrous oxide

NAD^+ / NADH: nicotinamide adenine dinucleotide

NBP: net biome productivity

NEC: National Emission Ceilings

NECB: net ecosystem carbon balance

NEE: net ecosystem exchange

NEP: net ecosystem production

NH_3: ammonia

NH_4^+: ammonium

NH_4NO_3: ammonium nitrate

NO: nitrogen monoxide

NO_2^-: nitrite

NO_3^-: nitrate

NPP: net primary production

O_2: oxygen

OM: organic matter

PE: priming effect

PEP: phosphoenolpyruvate

PPFD: photosynthetic photon flux density

PPR: Pasture, Paddock and Range

RH: relative humidity

SMB: soil microbial biomass

SOC: soil organic carbon

SOM: soil organic matter

SON: soil organic nitrogen

T: temperature

TAN: total ammoniacal nitrogen

TCA: tricarboxylic acid

TCD: thermal conductivity detector

TIC: total inorganic carbon

TOC: total organic carbon

TON: total oxidised nitrogen

UNFCCC: United Nations Framework Convention on Climate Change

VFA: volatile fatty acid

WFPS: water-filled pore space

Chapter 1. Introduction

1.1. Background

Agriculture in Ireland is characterised by its predominant livestock production system based on grass. Typically, animals graze in the field during the growing season and are housed indoors in winter to avoid the grass and the soil to be damaged (Holden et al., 2004). As a consequence, there is an accumulation of animal excreta during winter housing, which are stored as solid or liquid manures (= slurry) and subsequently applied to agricultural lands.

Recycling manure nutrients is necessary for the sustainability of agricultural systems. However, the application of slurry to grasslands may have adverse effects on the environment when considering processes such as greenhouse gases emissions (Soussana et al., 2010), nitrate leaching (Smith et al., 2003a), transport of pathogens (Smith et al., 2001a, 2001b, Crowther et al., 2002, Brennan et al., 2010) or phosphorus movement in soils (Wang et al., 2004).

The present study addresses the consequences of land application of cattle slurry on soil carbon (C) and nitrogen (N) cycles, focusing more precisely on the emissions of ammonia (NH_3) and greenhouse gases (GHG) from grassland soils.

A brief literature review, as well as the objectives of this study is given in this introductory chapter.

1.2. Literature review

1.2.1. Carbon cycle and soil respiration

1.2.1.1. The global carbon cycle: concepts, terminology and estimates

Carbon dioxide (CO_2) is the most abundant GHG. Its current atmospheric concentration was, in 2005, 379 ppm, with an average increase of 1.4 ppm yr-1 over the period 1960-2005 (IPCC, 2007a). Estimates of the global biomass C pool are about 500 Gt C (Gt= 10^9 tonnes) (WBGU, 1998, IPCC, 2007a). Other actively cycling

C pools are: the atmosphere (730 Gt C), oceans (38,000 Gt C) and soils (1500-2000 Gt C).

All these C pools are connected. The atmospheric CO_2 enter terrestrial biomass, via photosynthesis, at a rate of circa. 120 Gt C yr^{-1}. Photosynthesis is commonly defined as "the process by which plants synthesise organic compounds from organic raw materials in the presence of sunlight" (Hall and Rao, 1994). This amount of C, converted from CO_2 to carbohydrate, is known as gross primary production (GPP) (IPCC, 2001, Janzen, 2004).

About half of this C is soon released as CO_2 by autotrophic respiration (respiration by plants), the other half being incorporated into new plant tissues. This difference between photosynthesis and autotrophic respiration is referred to as net primary production (NPP) and is, hence, about 60 Gt C yr^{-1}. This amount is temporarily stored in the plant biomass, but, most eventually, enters soil upon senescence (Janzen, 2004). Finally, virtually all the C fixed in NPP (~ 60 Gt C yr^{-1}) is returned to the atmospheric CO_2 pool through heterotrophic respiration (mainly by soil microorganisms) and plant biomass combustion in natural or human-set fires (IPCC, 2001).

The difference between NPP and heterotrophic respiration, termed as net ecosystem production (NEP), determines how much C is lost or gained by ecosystems in the absence of disturbances that remove C from them. The global NEP is estimated to be about 10 Gt C yr^{-1}, although this is likely to be an overestimate because of the current biased distribution of flux measuring sites (IPCC, 2000, 2001).

In some cases, dissolved, volatile and depositional organic and inorganic fluxes other than GPP and ecosystem respiration (ER) are not negligible. Therefore, the imbalance between GPP and ER does not necessary equal the net C accumulation rate in ecosystems (Chapin III et al., 2006). For this reason, the authors proposed that the term net ecosystem carbon balance (NECB) be applied to the net rate of C accumulation in ecosystems.

Extrapolated to larger spatial scales, NECB is termed "net biome productivity" (NBP) (Chapin III et al., 2006) and is estimated to have averaged -0.2 ± 0.7 Gt C yr^{-1} during the 1980s and -1.4 ± 0.7 Gt C yr^{-1} during 1990s (IPCC, 2001).

1.2.1.2. Mechanisms for aerobic respiration in soils

Soil respiration, which can be measured at the soil surface, is the result of several soil processes affecting both the CO_2 production in soil and the CO_2 transport from the soil to the atmosphere.

Plants and algae, as well as some Bacteria and Archeae organisms, are able to use basic energy sources, such as sunlight (photosynthetic autotrophs) or inorganic chemical reactions (chemosynthetic autotrophs), to synthesise energy-containing organic compounds from inorganic raw materials (Enger et al., 2012). Most soil microorganisms, however, require organic molecules as food , Indeed, those so called "heterotrophs" get their energy from the chemical bonds of organic compounds such as carbohydrates, fat and proteins, which they obtain from the existing soil organic matter (SOM), as well as from decomposing plant tissues (Brady and Weil, 1999; Enger et al., 2012). Plant residues are mainly constituted of complex organic substances such as cellulose, hemicellulose or lignin (Brady and Weil, 1999), which must be decomposed before being utilised by soil microorganisms.

Cellulose is the most abundant organic compound in the biosphere, typically accounting for 30 to 50% of plant dry weight (Lynd et al., 1999) and is, therefore, an important source of energy for soil microorganisms. As reviewed by Lynd et al. (2002), the ability to degrade cellulose aerobically is widespread among fungi and has been observed for several soil bacterial species. Under aerobic conditions, cellulose is hydrolysed by a group of cellulase enzymes which are produced by both bacterial and fungal cellulose degraders. These enzymes are mainly extracellular (Rapp and Beerman, 1991; Schwarz, 2001), although they can be included complex enzyme systems at the cell surface (Bond and Stutzenberg, 1989; Watchinger et al., 1989). Glucose is the main product of the biodegradation of cellulose, although cellobiose and other low molecular weight oligosaccharides may be directly utilised or released in the soil, thus constituting a good growth substrate for other microorganisms (Lynd et al., 2002; de Boer et al., 2005).

Lignin is the most abundant aromatic plant component in the biosphere, accounting for about 20% of plant litter input into soils (Crawford, 1981; Kögel-Kabner, 2000, Gleixner et al., 2001). In higher plants, lignin is usually chemically associated with cellulose and hemicellulose, forming a complex structure – the

lignocellulose – which provides both rigidity to plant structures and resistance to the biodegradation of plant-derived carbohydrates (reviewed by Thevenot et al., 2010). Due to its complex structure, very few bacteria and fungi are able to degrade lignin, and only basidiomycetes known as "white-rot fungi" can fully mineralise the molecule (reviewed by Thevenot et al., 2010). However, once lignin subunits are separated, they can be further degraded and utilised by many other types of soil microorganisms (Brady and Weil, 1999).

In aerobic conditions, products of the hydrolysis of lingo-cellulolytic compounds, as well as other plant residues (sugars, fatty acids, peptides and amino-acids, etc.) may then be used as an energy source for soil microbial respiration. Furthermore, photosynthesis-derived carbohydrates can also be respired by root cells (Lambers et al., 1996).

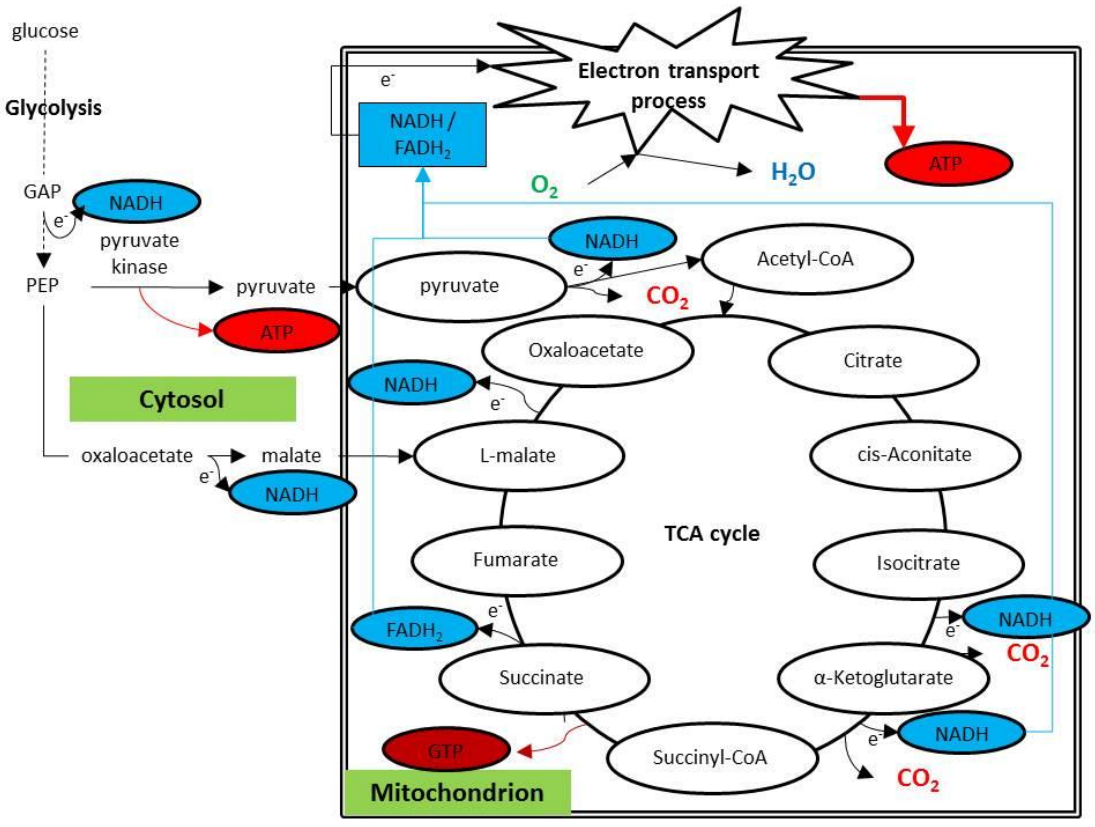


Figure 1: The aerobic cellular respiration in living cells. Glycolysis occurs in the cytosol, while both Krebs cycle and electron transport processes are located inside the mitochondrion (modified from Luo and Zhou (2006) and Enger et al.(2012)).

The most common pathway for the production of CO_2 in soil is the tricarboxylic acid cycle (TCA, also known as "citric acid cycle" or "Krebs cycle", see **Figure 1**). Together with the glycolysis and the mitochondrial electron transport processes, they

constitute the three main pathways for overall aerobic respiration (Fernie et al., 2004, Enger et al., 2012).

Through aerobic respiration pathways, living cells degrade organic compounds such as sugars, fatty acids or proteins to obtain energy through the synthesis of adenosine triphosphate (ATP) (Enger et al., 2012).

Prior to entering the TCA cycle, simple sugars (e.g. glucose) present in the cytosol of eukaryotic cells are converted, via phosphoenolpyruvate (PEP) into pyruvate through the glycolysis pathway (**Figure 1**). Pyruvate molecules are then transported inside the mitochondrion, where they are oxidised to form an acetyl coenzyme A (Acetyl CoA).

Pyruvate is the primary product of glycolysis in animal and microbial cells, whereas plant cells convert PEP mostly to malate (Lambers et al., 1998), which can be transported inside the mitochondrion and directly enter the TCA cycle (**Figure 1**).

In the mitochondrial matrix, acetyl CoA molecules enter the TCA cycle (**Figure 1**), where they undergo a series of oxidations leading to the production of CO_2, as well as to the transfer of hydrogen and electrons from reacting organic compounds to the coenzyme nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), which are converted in NADH and $FADH_2$ molecules (see **Figure 1** and Enger et al., 2012). NADH molecules are also formed in the cytosol, during the glycolytic pathway (**Figure 1**), which may transfer their electrons to mitochondrial NAD^+ and FAD molecules, through protein complex inserted in the mitochondrion internal membrane.

Of the three steps of aerobic cellular respiration, cells generate the greatest amount of ATP from the electron-transport pathway. This is a stepwise sequence, occurring on the mitochondrion internal membrane, during which the energy from NADH and $FADH_2$ molecules, generated in glycolysis and TCA cycle, is used to produce ATP (Figure 1). This pathway involves several protein complex and molecules, most of them being inserted in the mitochondrion internal membrane, and oxygen (O_2) act as the final electron acceptor (Enger et al., 2012). Overall, the chemical reaction for the oxidation of glucose (or other simple sugars) to CO_2 can be described as:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)$$

Fatty acids may also be used as a carbon and energy source for cellular respiration. The process by which those fatty acids are broken down to generate acetyl CoA (among other compounds) is termed as "ß-oxidation". As reviewed by various authors (Kunau et al., 1995; Materson and Wood, 2000; Graham and Eastmond, 2002; Poirier et al., 2006), such enzymatic pathway can occur, inside eukaryote cells, in both mitochondria and peroxisomes. Acetyl CoA generated in the mitochondria by ß-oxidation of fatty acids can then enter the TCA cycle as described above (Enger et al., 2012).

Proteins can also be used as an energy source by soil micro-organisms, such pathway involving their digestion into individual amino acids prior to being respired. Each amino acid then has its amino ($-NH_2$) group removed, during a process called "deamination". The remaining non-nitrogenous part is converted into keto acid before entering the respiratory pathways as acetyl CoA, pyruvate or any other type of molecule found in the TCA cycle (Enger et al., 2012)

The total CO_2 efflux from soil is a combination of: (i) the activity of autotrophic roots and associated rhizosphere organisms, (ii) the bacterial and fungal activity in soil, and (iii) the activity of the macrofauna (Hanson et al., 2000). However, the contribution of macrofauna is usually only a few per cent (Andren and Schnurer, 1985, Konate et al., 2003) and is, therefore, neglected in most studies about soil respiration.

Traditionally, soil CO_2 efflux is partitioned between root and microbial respiration, which are wrongly termed "autotrophic" and "heterotrophic" respiration in many soil studies respectively (Kuzyakov, 2006). Indeed, both terms characterise the strategy of living organisms to acquire C substrate and energy, but are not connected with respiration themselves. Therefore, the authors suggested the terms "microbial respiration" or "respiration by heterotrophs" on one hand, and "respiration by autotrophs" on the other hand.

Kuzyakov (2006) also raised the issue of the rhizomicrobial respiration, which is defined as the "respiration by heterotrophic microorganisms decomposing organic

substances released by living roots" (Kuzyakov and Larionova, 2005) and for which there is a debate about whether it should be included in the microbial soil respiration or in the autotrophic root respiration (Hanson et al., 2000, Bond-Lamberty et al., 2004, Kuzyakov, 2006).

In temperate grasslands, the contribution of root respiration to the soil CO_2 efflux ranges from 17 to 40% (Kucera and Kirkham, 1971, Coleman, 1973, Herman, 1977, Buyanovsky et al., 1987). Root respiration is greatly affected by the supply of assimilates from photosynthetically active plant organs (Xu et al., 2008, Subke et al., 2009).

Most CO_2 evolved by heterotrophic soil organisms is respired by bacteria, fungi and actinomycetes (Kuzyakov, 2006). Their activity tends to be proportional to the amount of decomposable C (Hanson et al., 2000).

Plants are thought to control the activity of heterotrophs, via their control on C supply (rhizodeposits, plant litter, etc.). However, there is strong evidence for a feedback control of plant production by the microbial activity, via their effect on nutrient availability in soils (Raich and Tufekcioglu, 2000).

1.2.1.3. Soil anaerobic processes: fermentation and methanogenesis

When the O_2 is limiting, aerobic respiration is inhibited and anaerobic respiration takes place. This process is a form of respiration using electron acceptors other than O_2.

The anaerobic respiratory processes generally occur during fermentation, which converts glucose (or other sugar compounds) to organic products (Luo and Zhou, 2006). Fermentation, which is inefficient to produce ATP, has multiple pathways, some of which produce CO_2. (e.g. fermentation of glucose to ethanol).

Carbon dioxide may also be produced during the microbial oxidation of methane (CH_4) in the aerobic zone of methanogenic soils (Lidstrom, 1992, Le Mer and Roger, 2001):

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (2)$$

Two forms of oxidation have been identified in soils (Bender and Conrad, 1992, 1993, Hanson and Hanson, 1996):

- The first form, usually termed as "high affinity oxidation", occurs under low (i.e. atmospheric level) CH_4 concentrations and is relatively ubiquitous in soils with low NH_4^+-N inputs (Topp and Hanson, 1991);
- The second form, usually termed as "low affinity oxidation", occurs at relatively high CH_4 concentrations (King et al., 1990, Whalen et al., 1990, Jones and Nedwell, 1993).

Methanotrophs, a term which characterise the bacteria involved in the latest form of CH_4 oxidation, use CH_4 as a carbon and energy source. Their activity is mainly limited by the availability of O_2 in soil (Le Mer and Roger, 2001). Methanotrophy is usually seen as a mechanism which eliminates the atmospheric CH_4 previously released from methanogenic soils.

Methane is the second most important GHG (IPCC, 2007b) and is produced in anoxic conditions by the microbial degradation of organic matter (Chadwick and Pain, 1997, Le Mer and Roger, 2001). Methanogenesis involved a specialised, strictly anaerobic group of anaerobic microorganisms belonging to the domain of *Archaea* (Whitman et al., 1992).

There are two major pathways for the production of CH_4 (Conrad et al., 1989, Schutz et al., 1989):

- Methanogens can use acetate as a substrate, during fermentation in anaerobic conditions, to generate CO_2;

$$CH_3 - COOH \rightarrow CH_4 + CO_2` \quad (3)$$

- Methanogens can also use CO_2 as an electron acceptor to produce methane;

$$CO_2 + H_2O \rightarrow CH_4 \quad (4)$$

In general, production and consumption of CO_2 by anaerobic metabolism is quite low in non-flooded areas. However, applying animal slurries to agricultural soils may locally generate conditions favourable for the production of CH_4 (see paragraph **1.2.3**).

1.2.1.4. Factors controlling soil respiration

Soil respiration processes are influenced by several biotic and abiotic factors, among which C substrate, soil temperature, soil moisture, oxygen concentration, C:N ratio, soil texture and pH are of main importance.

At the ecosystem level, total soil CO_2 efflux is driven by various processes (see **1.2.1.2** and **1.2.1.3**) consuming substrates from various sources. Soil microbial activity, for example, is controlled by the availability and the quality of the organic substrates present in the soil or at the soil surface, while root respiration utilises sugars, proteins, lipid, and other substrates synthesised in plant tissues (Luo and Zhou, 2006).

Soil organic matter is a complex association between various organic components derived from (i) the living biomass, (ii) dead roots and other plant residues, and (iii) the stabilised soil humus (Brady and Weil, 1999). Soil microorganisms consume all kind of substrates, ranging from simple sugars contained in plant residues and roots exudates to hardly decomposable humic acids from SOM (Luo and Zhou, 2006).

Plant residues and root-derived substances contains a great variability of C substrates which were shown, for some of them, to influence soil microbial activity and the subsequent mineralisation of SOM-C, depending on their availability to and decomposability by soil microorganisms: sugars (Dalenberg and Jager, 1981, 1989, Mary et al., 1992, 1993, Wu et al., 1993, Shen and Bartha, 1996, 1997, De Nobili et al., 2001, Falchini et al., 2003, Mondini et al., 2006, Hamer and Marschner, 2005), amino acids (Dalenberg and Jager, 1989, De Nobili et al., 2001, Falchini et al., 2003, Mondini et al., 2006) , root exudates (Mary et al., 1992, 1993, De Nobili et al., 2001),

hemicellulose (Durall et al., 1994), cellulose (Durall et al., 1994, Shen and Bartha, 1997, De Nobili et al., 2001, Fontaine et al., 2004, 2011, Guenet et al., 2010).

The effect of more complex C substrates such as leaf (Wu et al., 1993) and root tissues (Mary et al., 1992, 1993), dung (Bardgett et al., 1998, Amelung et al., 1999, Bol et al., 1999, 2000, 2004, Dungait et al., 2005, 2010) or animal manures (see paragraph **1.2.3.**), which contains a mixture of the various compounds described previously, on soil C mineralisation is also of great importance as they are the main inputs of organic matter in agricultural soils.

Soil CO_2 efflux can be also affected by various abiotic factors, among which soil temperature and soil moisture are of higher importance. When not limited by other factors, root respiration tends to increase with temperature (Atkin et al., 2000). Soil also contains a great diversity of microorganisms with different optimum temperatures for their maximal activity. Therefore, soil respiration usually responds to temperature exponentially within a wide range of values (Mikan et al., 2002, Fierer et al., 2003). However, temperature sensitivity of soil respiration may be affected by soil moisture. Several studies showed, for example, lower temperature sensitivity on well-drained sites than on wetter sites (Davidson et al., 2000, Xu and Qi, 2001, Reichstein et al., 2003).

Soil moisture is another key factor controlling soil respiration. Soil CO_2 efflux tend to be reduced under dry conditions and at high soil moisture content (Bowden et al., 1993, 1998, Liu et al., 2002, Xu et al., 2004). In dry soils, soil microbial activity is mainly limited by the supply in organic substrate while soil respiration is controlled by O_2 diffusion in wet soils (Linn and Doran, 1984).

Soil aeration, N availability, soil texture and pH were also identified as key factor controlling the rate of soil respiration (reviewed by Luo and Zhou, 2006).

1.2.1.5. Carbon sequestration in grassland

Carbon storage in agricultural and forest soils has attracted attention in recent years, due to its potential as a substantial C sink. Under Article 3.3 and 3.4 of the Kyoto Protocol of the United Nations Framework Convention on Climate Change

(UNFCCC), biospheric sinks and sources of carbon can be included by countries as a contribution to reducing GHG emissions (IPCC, 2001, Smith, 2004).

The term "carbon sequestration" can be defined as "the process of removing CO_2 from the atmosphere and storing in C pools of varying lifetime" (Jones and Donnelly, 2004). The main factors determining the amount of C sequestered in soil are: (i) the rate organic matter input, (ii) the decomposability of this organic matter; (iii) the depth in soil at which the organic C is placed, and (iv) the physical protection of this C through either intra-aggregate or organomineral complexes.

Temperate grasslands represent 32% of Earth's natural vegetation (Adams et al., 1990) and about 20% of the land area in Europe (Soussana et al., 2004), being therefore a significant component of the global C cycle. The C sequestered by grasslands is defined as the difference between NPP and C losses as heterotrophic respiration, harvest, fire and other changes in soil C stocks (Jones and Donnelly, 2004). Different options are available to determine the sink or source activity of an ecosystem: (i) to measure the small change in soil C stocks in soil, and (ii) to determine the C balance of the ecosystem. Both approaches are difficult to carry out, but have shown a greater sequestration of C in temperate grasslands than in arable lands. Under existing management conditions, most temperate grasslands worldwide are considered to be C sinks, being able to sequester large amount of C (Jones and Donnelly, 2004).

In Europe, grasslands were shown to be a sink for CO_2 (Vleeshouwers and Verhagen, 2002; Soussana et al., 2007), although the uncertainty around the estimate may be larger than the sink itself (Janssens et al., 2003). Furthermore, it is still uncertain how long the sink activity can continue and what the upper limit of C storage is in soils (Jones and Donnelly, 2004).

As reported by Smith et al. (2005), such C sequestration in grassland soils may be altered under the anticipated climate change and increased atmospheric CO_2 concentration. Indeed, increasing temperatures may speed decomposition processes, wherever soil moisture is not limiting, leading to a potential decrease in soil organic carbon (SOC) stocks for European grasslands. However, following the same authors, this effect may be reduced by an enhanced NPP and projected improvements in

management techniques. Carbon sequestration by grassland soils is largely affected by management practices and land use changes. For example, Guo and Gifford (2002) have shown an increase in soil C up to 30% after conversion from crop to pasture. Conversely, the transition from grassland to arable land led to a decrease in soil C of about 60%. Similarly, Freibauer et al. (2004) estimated that, in Europe (as far as Ural mountains), the conversion of arable land to grassland would lead to an increase in soil C sequestration potential of 9 to 12 Mt C $year^{-1}$, with a further increase of 2,2 to 2,7 Mt C $year^{-1}$ from the conversion of short duration pastures to perennial grasslands. However, such mitigation measures should be restricted only to the surplus of arable land (assuming that the food demand remains the same in the future).

Concerning the impact of grassland management on C sequestration, Soussana et al. (2004) showed that moderately enhanced N fertilisation increases the organic matter input to the soil proportionally more than it increases the process of C mineralisation, whereas intensive fertilisation stimulate mineralisation and, therefore, enhance C losses. Furthermore, it has been shown that the addition of organic manures to grassland plots is more likely to increase C storage in soils, compared to inorganic fertilisers, but also increases rates of soil respiration, leading to a possible net CO_2 loss of 4.9 g CO_2 m^{-2} h^{-1} on cattle slurry treatment (Jones et al., 2006).

Finally, the agricultural sector is expected to help countries to meet the targets set up under the terms of Kyoto Agreement by reducing their own GHG emissions and increasing C sequestration. However, it is important to take into account that any management reducing CO_2 emissions may, at the same time, have the opposite effect on other GHGs (Desjardins et al., 2005).

1.2.2. Nitrogen cycle and gaseous N losses from soils

1.2.2.1. The global N cycle

The nitrogen cycle describes the different forms of N in the atmosphere and the biosphere, as well as the processes involved in N form changes. On the Earth, continental biosphere is a significant N pool, soils and biomass stocking 60 Mt $N.year^{-1}$ (Galloway et al., 2004). This pool is supplied by the symbiotic fixation of atmospheric N, by leguminous, and by synthetic nitrogen fertilizers application. On

the other hand, N losses from the continents to the atmosphere occur mainly as molecular N (N_2) and nitrous oxide (N_2O) emissions, with values of 115 and 11 Mt.year^{-1}, respectively, in 1990 (Galloway et al., 2004).

Recently, N_2O abundance in the atmosphere increased because of human activities. This long-lived GHG has a global warming potential (for a 100-year time horizon and by mass unit) 298 times higher than CO_2 and represents 7.9% of total greenhouse gases emissions (IPCC, 2007a, 2007b). Furthermore, N_2O indirectly takes part to stratospheric ozone depletion through its conversion to nitrogen monoxide (NO) (Cicerone, 1987).

On a global scale, NH_3 can result from natural processes, food production and energy production. Estimated to be 20.6 Tg N year^{-1} in 1860, total NH_3 emissions increased dramatically during the 20th century, following the development of the Haber-Bosch process to synthesise NH_3-based fertilisers for food production and an increase in energy consumption, to reach 58.3 Tg N year^{-1} in 1993 (Galloway et al., 2004).

In the atmosphere, NH_3 reacts with acidic aerosols such as sulphuric acid (H_2SO_4), nitric acid (HNO_3), nitrous acid (HNO_2) or hydrochloric acid (HCl) to form NH_4^+ salts (Finlayson-Pitts & Pitts, 1999). Therefore, emitted NH_3 returns to the biosphere in the form of either dry deposition of NH_3 and particulate NH_4^+ salts, or by wet deposition of hydrated NH_4^+ molecules (Asman et al., 1998).

Ammonia volatilisation, particularly from farming activities, constitutes a loss of valuable N for crops. Furthermore, deposition of ammonia may cause undesirable changes in natural ecosystems, Indeed, significant NH_3 deposition over a long period of time may contribute to a N saturation of the ecosystem (Tamm, 1991), potentially increasing N losses by leaching of NO_3^- (Grennflet & Hultberg, 1986) or denitrification (Magalhaes et al., 1984; Tietema & Verstraten, 1991), Ammonium input to the system may also contribute to the acidification of soils, causing nutrient imbalances and the release of toxic ions such as Al_3^+ (Van Breemen et al., 1984; Reuss et al., 1987; Nilsson et al., 1988; Van Dijk et al., 1989; Tomlinson, 1991). Acidification and N saturation of soils, following NH_3 deposition, were shown to contribute to a decrease of species diversity in both heathlands (Van Breemen & Van

Dijk, 1988) and calcareous grasslands (Bobbink, 1991; Bobbink & Willems, 1987), as well as to a shift towards species with higher nutrient-use efficiency (Bobbink, 1991; Bobbink & Willems, 1987, Van der Eerden et al., 1990). Finally, by affecting their structure and growth, N deposition significantly contributed to a general decline of forests in Europe (reviewed by Krupa, 2003).

Therefore, emissions of NH_3 from many European countries will need to be reduced in compliance with the Convention on Long-Range Transboundary Pollution and the National Emission Ceilings Directive of the European Union (EEA, 2000).

The soil N cycle can be described in terms of eight major processes (Schulten and Schnitzer, 1997, Jarvis, 1997, Murphy et al., 2000): fixation, mineralisation, nitrification, immobilisation, assimilation, volatilisation, denitrification and leaching (**Figure 2**).

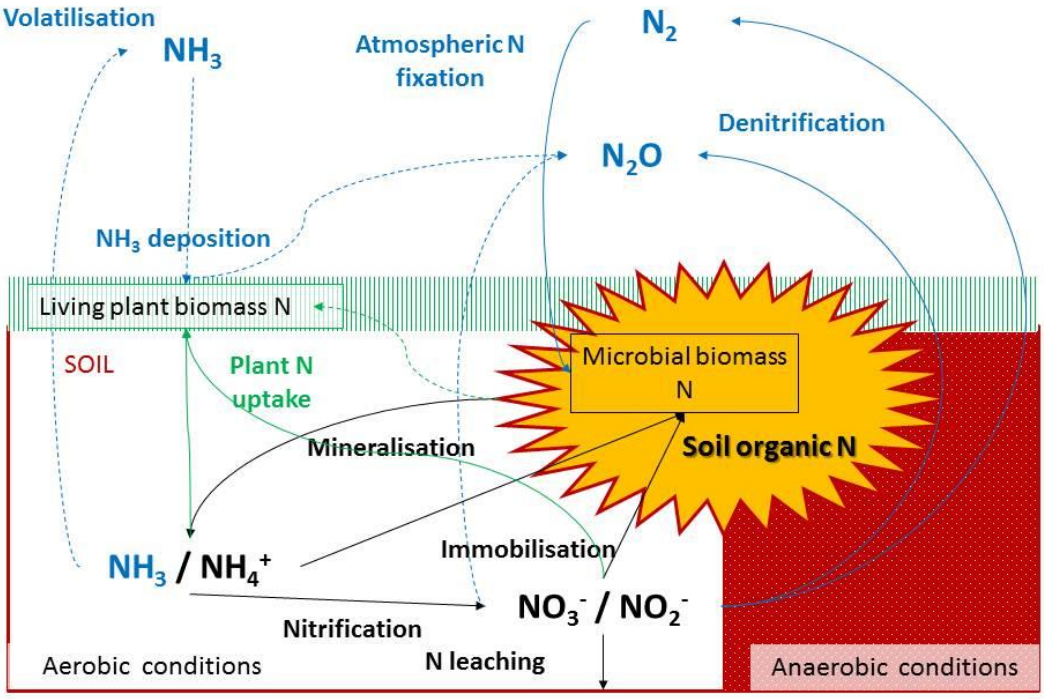


Figure 2: The soil N cycle and its major processes (based on Jarvis, 1997; Schulten & Schnitzer, 1997; Murphy *et al.*, 2000). Input N from the atmosphere (blue arrow), internal fluxes (black), N losses to the atmosphere (red) and N assimilation by plants (green) are presented.

1.2.2.2. N fixation

N fixation is a biological process that involves the reduction of atmospheric N_2 to NH_4^+ (or NH_3) (Beevers, 1976). It is the main process by which atmospheric N is added to terrestrial ecosystems (Galloway et al., 2004).

A few living organisms are able to utilize N_2. The best known are free-living bacteria such as *Azotobacter* spp. and *Clostridium* spp., blue-green algae, and bacteria from the *Rhizobium* genus, associated with legumes in a symbiotic relationship (Troeh and Thompson, 2005). These organisms usually have a nitrogenase enzyme system which converts N_2 to NH_4^+ (Beevers, 1976). This N which is added to the soil system can then undergo various transformations.

1.2.2.3. Mineralisation / Immobilisation of N, plant N uptake and nitrate leaching.

Ammonification (or mineralisation) is the enzymatic decomposition of organic N which is converted to NH_4^+ (Schulten and Schnitzer, 1997, Murphy et al., 2003, Troeh and Thompson, 2005). Low molecular organic N substrates, such as amino acids, amino sugars, purines and pyrimidines, are converted to NH_4^+, which can be subsequently either absorbed by soil microorganisms or utilised by nitrifying bacteria. It may also just accumulate in soils (Murphy et al., 2003).

Mineral N usually represents only a small part of total N in soils, with more than 95% of total soil N originating from the soil organic matter (Whitehead, 2000). The soluble organic N (SON) pool, from which the inorganic N pool is mainly derived, is therefore of major importance (Murphy et al., 2000). The size of such N pool may be as large as , or even larger than, the mineral N pool in soils (Smith, 1987, Jensen et al., 1997, McNeill et al., 1998, Bhogal et al., 2000).

Biological immobilisation is defined as the assimilation of soil by the soil microbial biomass (SMB) (Murphy et al., 2003). Soil microorganisms assimilate mainly NH_4^+ (Jansson and Persson, 1982, Recous et al., 1988, Shen et al., 1989), although NO_3^- can be assimilated when the soil is depleted in NH_4^+ (Azam et al., 1986, Recous et al., 1988).

The mineralisation immobilisation turnover (MIT) theory, which considers that organic N is converted to NH_4^+ before microbial uptake, is widely seen as the main pathway regulating soil-derived N availability (Jansson and Persson, 1982, Drury et al., 1991, Hadas et al., 1992). Alternatively, soil microorganisms can also assimilate compounds from the SON pool directly, as a C substrate, subsequently releasing the excess N (Barraclough, 1997).

Both immobilisation and mineralisation take place simultaneously in the soil, but there is a net mineralisation when microbes are predominantly C-limited, whereas a net immobilisation occurs when soil microbes are predominantly N-limited. The balance between both processes is mainly driven by the relative demand by microbes for N and C (Bardgett, 2005), which depends on the C:N ratio of the substrate compared to that of the decomposer organisms (Schulten and Schnitzer, 1997, Whitehead, 2000).

The release of N through the mineralisation of SOM and plant residues was also shown to be one of the main sources of plant N uptake (Scarsbrook, 1965; Paul and Voroney, 1980). Plants mainly take up inorganic N, with a preference for NO_3^- when it is not limiting in soils (Streeter and Bartha, 1988). However, SON may be a major N source for plants whenever mineralisation rates are limited (Kielland, 1994, Atkin, 1996, Michelsen et al., 1996).

Contrary to NH_4^+ ions that are retained in soils by absorption to negatively charged soil colloids (eg. clay minerals), NO_3^- ions are highly mobile in soil solution (Bardgett, 2005). Hence, when not used by plants or microbes, and not retained by soils (eg. limited anion exchange capacity), they can be readily lost from soils to drainage waters through the process of NO_3^- leaching. This process can be a problem in heavily fertilised agricultural soil where N losses by leaching often lead to pollution of groundwater, lakes and streams. The main factors affecting leaching losses are (Troeh and Thompson, 2005) (i) the rate of nitrification (and fertilisation), (ii) the amount of rain, (iii) the permeability and water-holding capacity of the soil, and (iv) the nature of the crop growing on the soil.

1.2.2.4. Nitrification, denitrification and N_2O production

Nitrification consists in the oxidation of NH_4^+ (or NH_3) to NO_3^-, via NO_2^-, and is performed both by autotrophic and heterotrophic nitrifiers in soils (Granli and Bøckman, 1994, Wrage et al., 2001).

Autotrophic nitrifiers use nitrification as an energy source to fix CO_2 (Granli and Bøckman, 1994). Autotrophic nitrification is an aerobic mechanism which involves two groups of microorganism (Bock et al., 1986):

- NH_4^+ is first oxidised to nitrite (NO_2^-) by NH_3 oxidisers, mainly *Nitrosomonas* spp.;
- NO_2^- is then oxidised to NO_3^- by NO_2^- oxidisers, mainly *Nitrobacter* spp.

N_2O is a by-product which is formed during the oxidation of NH_4^+ to NO_2^-, through the chemical decomposition of the intermediate hydroxylamine (NH_2OH), as well as from the decomposition of NO_2^- itself (Wrage et al., 2001).

As stated above, heterotrophic nitrifiers are also known to play a role in the N cycle. Contrary to autotrophic nitrifiers, that use nitrification as an energy source for fixing CO_2, heterotrophic nitrifiers use organic matter as both a C and energy source (Castignetti, 1990, Robertson and Kuenen, 1990). They can obtain part of their energy from oxidation of NH_4^+ or organic nitrogen compounds (Granli and Bøckman, 1994) Heterotrophic nitrifiers are more common among fungi than among bacteria (Odu and Adeoye, 1970).

However, some bacteria also display nitrifying abilities (Papen et al., 1989). In contrast to conventional denitrifying microorganisms, these heterotrophic nitrifying bacteria are often able to denitrify under aerobic conditions (Robertson et al., 1989). N_2O is then product as an intermediate in the reduction of NO_2- to N_2 (Anderson et al., 1993, Richardson et al., 1998).

Although heterotrophic nitrifiers produce more N_2O than autotrophic nitrifiers under aerobic conditions (Papen et al., 1989, Anderson et al., 1993), they are generally considered as a minor source of N_2O. However, under certain

circumstances, such as a low pH, an elevated O_2 concentration and high organic C content, heterotrophic nitrifiers may produce a significant amount of N_2O

Nitrous oxide may also be lost from soil by the process of denitrification, which is a microbial reduction of NO_3^- to molecular nitrogen (N_2) (Granli and Bøckman, 1994, Wrage et al., 2001). Nitric oxide (NO) and N_2O are two gaseous intermediates that can be accumulated and eventually released, depending on the conditions (Granli and Bøckman, 1994). Denitrification is of particular concern, since it is a major source of N_2O, particularly on grassland soils.

Denitrification is a process carried out by a wide range of microorganisms when there is a high NO_3^- and C supply, as well as hypoxic or anoxic conditions (Bardgett, 2005). Denitrifiers are widely distributed across the bacterial taxa (Firestone, 1982) but fungal denitrification exists and Laughlin and Stevens (2002) have shown evidence for fungal dominance in grassland soils. Most of the denitrifiers can use NO_3^- as their primary electron acceptor to produce energy from organic compounds, under conditions of low availability for O_2: they are heterotrophic denitrifiers and facultative anaerobes. However, some microorganisms can use this NO_3^- for oxidation of inorganic compounds (eg. S_2^-, Fe_2^+), hence carrying out an autotrophic denitrification (Granli and Bøckman, 1994).

Nitrification and denitrification are not two separated processes. Considering a coupling between both pathways, Wrage et al. (2001) suggest a maximal production for N_2O where conditions are sub-optimal for both nitrifiers and denitrifiers.

Other mechanisms for N_2O production are mentioned in the literature. Nitrifier denitrification is a pathway of nitrification, carried out only by autotrophic nitrifiers, in which NH_3 is oxidized to NO_2^-, followed by the reduction of NO_2^- to NO, N_2O and N_2 (Wrage et al., 2001). Another pathway is the chemodenitrification, a non-biological process in which intermediates from the oxidation of NH_4^+ to NO_2^-, or NO_2^- itself, are chemically decomposed with organic (eg. amines) or inorganic (eg. Fe_2^+ or Cu_2^+) compounds (Spott et al., 2011). This process is also closely linked to nitrification.

1.2.2.5. Factors regulating N_2O emissions from soils

N_2O is a gas produced by some microbial processes, but the magnitude of fluxes between soil and atmosphere depends on several abiotic factors such as soil aeration, soil water content, pH and temperature (Granli and Bøckman, 1994).

Soil water-filled pore space (WFPS), which is a good integrator of both soil aeration and moisture content, is one of the key driver for N_2O production in agricultural soils (Smith et al., 2003b). Generally, denitrification rate increases as WFPS rises (Granli and Bøckman, 1994). Nitrification rate also increase with WFPS until O_2 content becomes a limiting factor. As a consequence, N_2O emissions tend to increase with WFPS, when the latter is in its lower range, until the soil reaches values in the range of 70 to 90% WFPS. Above 90% WFPS however, these emissions decrease because of the reduction of N_2O to N_2 after complete denitrification (Smith et al., 1998, Rudaz et al., 1999).

Soil temperature provides another major control on N_2O emissions. Indeed, when nitrification and denitrification are not limited by WFPS or soil mineral N content, N_2O release from soils tend to increase with an increase in soil temperature (Skiba et al., 1998; Conen et al., 2000; Dobbie & Smith, 2003; Smith et al., 2003; Flechard et al., 2007). Furthermore, when there is no other parameter overriding the temperature effect, these emissions tend to follow diurnal cycles observed for temperatures (Smith et al., 1998). However, Rudaz et al. (1999) could not demonstrate such relationship between the production of N_2O from soil and its temperature, supposedly because such effect was offset by the variability of soil water statuses within each of their temperature classes.

Some other factors can have an impact on N_2O fluxes from grassland soils, particularly NO_3^- and NH_4^+ concentration (Clayton et al., 1997, Jones et al., 2007), vegetation (Niklaus et al., 2006) and SOM content (Granli and Bøckman, 1994).

All these factors regulating N_2O fluxes from the soil have a key impact on their temporal and spatial pattern. Climatic phenomena like freezing-thawing cycles (Priemé and Christensen, 2001) or rainfall (Dobbie and Smith, 2003) are important drivers for the seasonality of N_2O emissions. In the same way, these emissions vary

from one year to the other because of the variability of temperatures and precipitation (Jones et al., 2007).

Human activities have also an impact on the seasonality of N_2O fluxes, due to regular N input as fertiliser for example (Jones et al., 2005, Jones et al., 2007). The relationship between N_2O emissions and the amount of N fertilizer applied has given rise to the concept of emission factor (EF), where EF is the amount of N_2O-N emitted expressed as a fraction or a percentage of the N applied (Dobbie and Smith, 2003). The International Panel for Climatic Change (IPCC) propose a value of 1% (uncertainty range: 0.3 – 3%) as EF for N additions from mineral fertilisers, organic amendments and crop residues, and N mineralised from mineral soil as a result of loss of soil carbon (IPCC, 2006).

1.2.2.6. Ammonia volatilisation

Ammonia from deposited animal excreta, as well as from applied manure and urea fertilisers, is liable to volatilise from the soil surface to the surrounding air. Animals such as cows and pigs excrete N primarily as urea (about 70% of the total N excretion), in urine, but also as undigested proteins in their faeces (Groot Koerkamp et al., 1998).

Ammonia is mainly the product of the degradation of these N compounds. Therefore the hydrolysis of urea ($CO(NH_2)_2$), as such or present in urine, is of key importance in the formation of the NH_3 emitted from grassland soils and can be described as follows (Sommer &Husted, 1995):

$$CO(NH_2)_2 + 2H_2O \rightarrow {CO_3}^{2-} + 2{NH_4}^{+} \quad (5)$$

This reaction is catalysed by an enzyme called urease. Its rate follows the law of Michaelis-Menten and is positively influenced by the urease activity, pH and temperature (Groot Koerkamp et al., 1998).

Before being emitted in the air, NH_3 is involved in an equilibrium, in the liquid (l), with its ionic form ${NH_4}^+$ as follows (Sommer et al., 1991; Groot Koerkamp et al., 1998):

$$NH_4^+(l) \leftrightarrow NH_3(l) + H^+ \quad (6)$$

Such equilibrium is characterised by the constant K_{NI} for the dissociation of NH_4^+ (K_{NI}= $[NH_3]x[H^+]/[NH_4^+]$), and is also dependent on pH and temperature (Sommer et al., 1991; Groot Koerkamp et al., 1998). The total ammoniacal N (TAN) content of the ammoniacal solution is defined as the sum of the concentrations of both dissolved NH_3 and NH_4^+ present in the solution.

The subsequent release of NH_3 in the atmosphere is then a two-stepped process, where dissolved NH_3 is first transferred to the air immediately in contact with the ammoniacal solution, before diffusing towards the atmosphere. Those two steps follow Henry's law for dilute systems (Sommer et al., 1991; Groot Koerkamp et al., 1998).

Volatilisation from field-applied liquid manures, for example, is driven by the concentration gradient between the partial pressure of NH_3 in the air, at the slurry / soil surface, and that in the atmosphere, at a height where it is no longer affected by the emission from the slurry-amended soil surface (Sommer et al., 2003). Such process was shown to be dependent on the characteristics of the applied material, as well as on some environmental and management factors.

The amount of NH_3 present at the slurry surface is mainly controlled by its TAN content as well as by its pH and temperature (Sommer & Hutchings, 2001). The last two parameters are key drivers of the equilibrium between both dissolved NH_3 and ionic NH_4^+ present in slurry, as mentioned earlier. In solution, the proportion of TAN present as NH_3 increases as pH increases, whereas increasing temperatures lead to a decrease in the solubility of NH_3 and increases its rate of diffusion (Freney et al., 1981). Therefore, NH_3 volatilisation rates tend to be positively correlated to both the initial pH of slurry and the air temperature.

The transfer of NH_3 from the soil to the free atmosphere occurs then by both diffusion and convection. It is primarily a function of atmospheric stability, which is mainly affected by wind speed and solar radiation (Sommer et al., 2003). Indeed, NH_3 volatilisation was shown to increase with increasing wind speed (Thompson et al., 1990b; Sommer et al., 1991; Sommer et al., 1997). High solar radiation also increases considerably NH_3 volatilisation, due to its positive effect on atmospheric instability

and, primarily, on the resulting increase in soil temperature, which leads to a higher NH_3 partial pressure at the slurry surface (Sommer & Hutchings, 2001; Sommer et al., 2003).

The rate of infiltration of TAN into the soil is also of great importance when dealing with NH_3 losses from surface-applied slurry. Indeed, it reduces the pool of TAN at the soil surface, decreasing therefore the volatilisation of NH_3 (Thompson et al., 1990a; Sommer & Olesen, 1991; Sommer & Jacobsen, 1999; Sommer & Hutching, 2001). Slurry dry-matter (DM) content is one of the main parameter affecting the movement of slurry TAN in the soil and is inversely related to the rate of infiltration of TAN into the soil (Sommer et al., 2006). Therefore, NH_3 volatilisation tends to increase with increasing slurry DM content (Sommer & Olesen, 1991; Sommer et al., 2006).

Rainfall soon after surface-application of slurry may also increase the rate of infiltration of TAN into the soil, lowering any potential NH_3 losses (reviewed by Sommer & Hutchings, 2001). On the other hand, slurry infiltration rate tend to be negatively correlated to the soil moisture content. Sommer et al. (1991), for example, observed lower NH_3 volatilisation rates from dry soils. On the other hand, Donovan and Logan (1983) showed that NH_3 losses could increase when infiltration was limited by a high soil water content.

1.2.3. Slurry application on grassland soils: gaseous C and N losses

Numerous studies have shown an increase of soil CO_2 efflux rates following the application of liquid manures to agricultural soils (Saviozzi et al., 1997, Flessa and Beese, 2000, Rochette et al., 2000a, 2004, Chantigny et al., 2001, Bol et al., 2003b, Jones et al., 2005, Jones et al., 2006, Fangueiro et al., 2007).

The rate of C mineralisation is often maximal a few hours only after slurry spreading (Flessa and Beese, 2000, Rochette et al., 2000a, Chantigny et al., 2001, Kuzyakov and Bol, 2004b) when readily available slurry-derived C substrates, such as volatile fatty acids, are being metabolised (Kirchmann and Lundvall, 1993). It may also increase soil microbial biomass (Saviozzi et al., 1997, Rochette et al., 2000,

2004, Bol et al., 2003a), even though this effect is not always significant (Chantigny et al., 2001, Bol et al., 2003a).

Land application of animal slurries has also been shown to enhance N_2O release from agricultural soils (Clayton et al., 1997, Ellis et al., 1998, Rochette et al., 2000b, Chadwick et al., 2000, Clemens and Huschka, 2001, Tilsner et al., 2003, Wulf et al., 2002b, Rodhe et al., 2006, Jones et al., 2007). Indeed, animal slurries provide both high amount of NH_4^+-N (Morvan et al., 1996) and readily available C (Rochette et al., 2000a) which may enhance N_2O release from soil by stimulating both nitrification and denitrification (Bergstrom et al., 1994, Granli and Bøckman, 1994, Dendooven et al., 1998b, Clemens and Huschka, 2001).

Application of liquid manure to agricultural soils may also increase CH_4 emissions immediately after spreading (Chadwick and Pain, 1997, Flessa and Beese, 2000, Wulf et al., 2002b). Indeed, applied slurry may locally create conditions favourable for the production of CH_4 through the immediate supply of utilisable C and by increasing soil moisture (Chadwick and Pain, 1997). Highest CH_4 emission rates a few hours after application may partly result from the release of dissolved CH_4 which would have been produced during slurry storage operations (Sommer et al., 1996, Chadwick et al., 2000)

Ammonia emissions from soils are usually low and the process of volatilization happens mainly from the high N input occurring after the deposition of excreta or the application of N fertilizer (Whitehead, 2000, Troeh and Thompson, 2005). Liquid manures are a major source of NH_3, which is emitted either from manure stores (e.g. Sommer et al., 1993, Amon et al., 2006) or during, and after, land application (e.g. Weslien et al., 1998, Wulf et al., 2002a). Volatilisation losses of NH_3 from slurry-amended soil are mainly driven by the NH_3 partial pressure gradient between the slurry / soil surface and the atmosphere and can be described as a two-step process (Sommer et al., 2003):

- From the time of application until the moment the infiltration of slurry into the soil is complete, NH_3 emissions depend only on the characteristics of the slurry and the atmospheric transport processes (which are affected by both wind speed and temperature profiles);

- Then, until NH_3 emissions become negligible, these emissions depend on the slurry-soil interactions

1.2.3.1. Ammonia abatement techniques and their consequences on greenhouse gas emissions

Altering manure characteristics through dilution, separation or any other treatment has been seen in the literature as one possible way to reduce NH_3 volatilisation from land-applied manures. For example, NH_3 fluxes have been shown to increase with and increasing DM content in liquid manures (Sommer and Olesen, 1991).

A high DM content contributes to the sealing of soil pores, reducing infiltration of slurry liquid phase in the soil and potentially increasing NH_3 volatilisation (Donovan and Logan, 1983, Dosch and Gutser, 1996). Therefore, Sommer and Olesen (1991) showed that, within a 4-12% range of DM content, dilution of slurry could reduce NH_3 losses. Mkhabela et al. (2009) showed similar results on NH_3 losses when diluting swine slurry and investigated the effect of such dilution on N_2O losses post-application. They found no significant effect of such slurry treatment and could not, therefore, support the widespread idea of a trade-off between NH_3 and N_2O. In a more extreme comparison, Gregorich et al. (2005) found that N_2O after liquid manure application could be increased up to three fold compared to solid manure, with a much larger N fraction lost as N_2O.

The use of low NH_3 application techniques has extensively been covered, focusing mainly on the comparison between conventional broadcast applications and newly developed shallow and deep injection methods in terms of both NH_3 and N_2O emissions (Dosch and Gutser, 1996, Dendooven et al., 1998a, Ellis et al., 1998, Malgeryd, 1998, Weslien et al., 1998, Flessa and Beese, 2000, Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a, Wulf et al., 2002b, Perala et al., 2006, Sistani et al., 2010, Velthof and Mosquera, 2011).

Such methods were shown to reduce NH_3 volatilisation losses by up to 90% (Dendooven et al., 1998a), but could lead to a significant increase in N_2O release from soils in the weeks following slurry application (Dosch and Gutser, 1996, Ellis et al.,

1998, Wulf et al., 2002b, Perala et al., 2006, Velthof and Mosquera, 2011). However, the observation of such trade-off between NH_3 and N_2O across the literature was inconsistent, some studies showing no effect of application technique on the latest emissions (Dendooven et al., 1998a, Weslien et al., 1998). Sistani et al (2010) showed lower N_2O emission following the injection of swine effluent, when comparing with surface application during the first year of their experiment, but found higher emissions from the same plots in the second year.

So far, only few studies have investigated the impact of switching from surface application to direct injection of slurry on CO_2 (Dosch and Gutser, 1996, Dendooven et al., 1998a, Flessa and Beese, 2000, Sistani et al., 2010) and CH_4 (Flessa and Beese, 2000, Wulf et al., 2002b, Rodhe et al., 2006, Perala et al., 2006, Sistani et al., 2010). Soil CO_2 efflux was not (Dendooven et al., 1998a, Sistani et al., 2010) or only slightly increased (Dosch and Gutser, 1996, Flessa and Beese, 2000) when injecting slurry, whereas CH_4 emissions were shown to increase significantly on various occasions (Flessa and Beese, 2000, Wulf et al., 2002b, Sistani et al., 2010).

The trailing-shoe application technique has also been well described as an efficient NH_3 abatement technique when applying slurry onto grassland soils (Malgeryd, 1998, Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a). For many grassland areas, it is considered to be the most effective way to lower NH_3 losses as high stone content of soils and undulating topography make injection unsuitable.

Only few studies have compared N_2O emissions from low trajectory slurry applications, such as the trailing-shoe technique, with the traditional broadcast application. Wulf et al. (2002b) tested four different application techniques: splash plate, trail hose, trail shoe and slurry injection into the soil. There was a clear effect of slurry application technique on N_2O emissions when comparing injection technique to the three other ones. Injecting slurry increases N_2O emissions two- to three-fold. However, the difference between splash-plate and trailing-shoe, in terms of N_2O emissions, was not significant. Furthermore, after summing direct and indirect N_2O, as well as methane, all expressed in CO_2 equivalent, the authors concluded that trailing-shoe technique was the most efficient way to reduce non-CO_2 GHG emissions on grassland.

Wulf et al. (2002b) also measured CH_4 emissions following the application of slurry treatments, but found no significant differences between splash plate and trailing shoe application.

1.2.3.2. Impact of the timing of slurry application on gaseous C and N losses

The timing of manure application also affects NH_3 emissions which increase as a function of temperature and wind speed (Sommer et al., 2003). It is also likely to affect N_2O emissions since N_2O production is mainly driven by N availability, temperature and soil aeration. Most studies investigating the impact of timing of slurry application on NH_3 and N_2O emissions post-application focused on the comparison between autumn (typically after harvest for croplands) and spring application, as reviewed by VanderZaag et al. (2011).

However, slurry in Ireland is mainly applied in summer (Hyde and Carton, 2005) when N volatilisation losses can be high due to warm temperature. Application of slurry in spring can be seen as optimal as N is then applied in a period when uptake by herbage is high and NH_3 losses are relatively low. Although wetter soils in such period may limit the number of days when slurry can be applied on the field using the conventional broadcast method (with a tank and a splash-plate device), Lalor and Schulte (2008) showed in a modelling study that, in Ireland, the number of available spreading days is substantially higher where trailing-shoe is used for slurry application. In terms N_2O emissions, Chadwick et al. (2000) observed, after applying pig and dairy slurries onto perennial ryegrass, higher N_2O losses in spring than in summer, which would support the idea of a trade-off between NH_3 and N_2O emissions. Studies comparing summer and spring applications onto grassland are however still scarce.

However, applying slurry in colder and wetter conditions in spring may also significantly increase CH_4 production from grassland soils (Chadwick et al., 2000)

1.2.4. Ammonia and greenhouse gas emissions in Ireland: impact of the agricultural sector

Ireland is unique among the European Union (EU) countries for the proportion of its GHG emissions which originate from agriculture, representing 30.4% of national and 42% of the non-Emissions Traded Sector (non-ETS) emissions (Duffy et al. 2012). This proportion is high compared to the EU average of 9%, and reflects the relative importance of agriculture, which is predominantly based on export of ruminant livestock products, to the Irish economy (Breen et al., 2010). Amongst the developed economies, only New Zealand has a higher proportion of national GHG emissions associated with agriculture (NZ-MfE, 2012).

The latest reported (2010) emissions from the Irish agricultural sector amount to 17.9 Mt carbon-dioxide equivalents ($CO_{2\text{-eq}}$). These emissions are dominated by CH_4 and N_2O (Duffy et al. 2012). This emissions profile arises because of the dominance of cattle and sheep livestock production in Irish agricultural output. Methane emissions sourced from livestock enteric fermentation is the primary source of greenhouse gases, accounting for almost 50% of total emissions. The two other major sources are CH_4 emissions from manure management (12.5%) and N_2O emissions arising as a result of chemical/organic fertiliser application and animal deposition (36.5%).

Methane emissions have decreased since 1998 and been driven primarily by decreases in the total number of beef cattle and sheep. This decrease is primarily due to enteric CH_4 decrease due to reductions in sheep numbers. Hence CH_4 from manure management has remained relatively static. The total agricultural N_2O emissions have also decreased by 11% relative to 1990 and over 20% relative to 1998 peak emissions (Duffy et al. 2012). Decreased N_2O emissions arising from animal deposition, termed pasture, paddock and range (PPR) emissions, have followed a similar trend to methane emissions, with the principal reductions arising from sheep (38%) and non-dairy cattle (11%). Similarly, reductions in the application of mineral fertiliser resulted in a 28.9% decrease in associated emissions between 1998 and 2008, associated with a 30% increase in fertiliser costs since 2000 (Lalor et al., 2010). Whilst inputs of mineral N have decreased, the use of organic fertiliser (and

associated emissions) has remained constant despite decreases in the total livestock numbers.

The agriculture sector also accounts for 98.5% of NH_3 emissions in Ireland with energy related sources responsible for the remainder (EPA, 2012). Similar to methane, the NH_3 emissions trend is largely determined by the cattle population and shows a steady increase up to almost 120.7 kt in 1998. There was some decline in the populations of cattle and sheep after 1998, as well as a decrease in fertiliser use, which contributes to a downturn in NH_3 emissions in the period 2000 to 2010. The current ammonia emissions in 2010 are 106.2 kt.

1.2.5. Legislation in regards of greenhouse and transboundary gas emissions

Ireland is a Party to the Convention on Long-Range Transboundary Air Pollution (CLRTAP), under which certain transboundary air pollutants (including NH_3) are controlled (UNECE, 1999). As a member of the European Union, Ireland is also subject to the National Emissions Ceilings (NEC) Directive (Anon 2001), which implements the Gothenburg targets for member states. In Ireland the Department of the Environment is the lead agency responsible for transboundary air pollutants.

Ireland's national emission ceiling for NH_3 under the NEC Directive is 116 kilotonnes (kt) to be achieved by 2010 (EPA, 2012). This is equivalent to an 8% reduction in emissions from the 1990 a baseline of 126 kt. In addition, Ireland was limited to increasing GHG emissions by 13.5% relative to 1990 for the period 2008-12 under the terms of the Kyoto Protocol. Ireland is currently compliant with both sets of legislation, with ammonia emissions being some 10kT under target, whilst greenhouse gas emissions are just on target.

However, future transboundary/greenhouse gas legislation and national agricultural production targets appear to be paradoxical. Food Harvest 2020 envisages ambitious expansion, both in terms of the volume and value of output, for the agriculture sector as a whole and particularly for the dairy sector (DAFM 2011). Whilst Food Harvest targets envisage a 50% increase in dairy production, this expansion will occur within an EU policy context whereby GHG emissions must be

reduced. Under the EU 2020 Climate and Energy Package and its associated Effort-Sharing Decision, Ireland has been set a 20% reduction target for its non-Emission Traded Sectors (EU 2010). As agriculture comprises over 40% of this category of emissions, there will be sustained pressure into the future to reduce emissions. In addition, future revised National transboundary targets are likely to be more stringent, although these will not be re-negotiated until 2013.

1.3. Objectives and hypothesis

Whilst much work has been done on abatement strategies for either NH_3 or GHG, there is less information on the trade-offs across the full suite of gaseous emissions for these strategies. Furthermore, little is known about the interactions between N and C cycle when applying organic fertilisers, particularly concerning the possible trade-offs between N emissions and C sequestration.

The input of organic C is likely to enhance C storage in the soil, but it may also stimulate soil microbial activity, subsequently increasing soil respiration. On the other hand, lowering volatilisation N losses may increase the amount of N into the soil, leading to potentially higher N_2O emissions.

The aims of this project were therefore:

- to assess the impact of NH_3 abatement techniques, namely the alteration of slurry DM content, the spreading method and the timing of application on N_2O, CO_2 and CH_4 release from a grassland soil;
- to investigate the consequences organic N application in terms of C sequestration in soils.

The project consists in three different experiments (**Chapter 3** to **5**), which were all based on one unique experimental field site. Therefore, a brief description of the experimental site, as well as a general view of the experimental design, will be presented in the next chapter.

Chapter 2. Field site and general view of the experimental set-up

This PhD project comprised three different experiments, which will be detailed in subsequent chapters, focusing on the impact of cattle slurry application on soil C and N dynamics and subsequent GHG losses, in a permanent grassland system:

- a fifteen month field-plot experiment, aiming at investigating the impact of slurry DM content, application technique and timing of application on the overall GHG balance from cattle slurry applied onto grassland soils (**Chapter 3**);
- a short-term (30 days) field plot experiment using ^{13}C natural abundance tracer technique to investigate the contribution of applied C to the evolved CO_2, its effect on the mineralisation of SOM and whether the application technique affects the subsequent dynamics of applied C in the soil (**Chapter 4**);
- a six month lysimeter experiment, in controlled conditions, focusing on the fate of applied C and N in the system [soil – plant – atmosphere] and the interaction between both C and N cycles (**Chapter 5**).

All these experiments were set up from the same grassland site. Therefore, the aim of this chapter is to present the site location and characteristics, as well as a general view of the experimental design.

2.1. Site location and characteristics

Both field experiments were undertaken on a grassland site in Johnstown Castle, Wexford, Ireland (52°18′N; 6°30′W). The field is under an established (>10years) and uniform ryegrass (*Lolium perenne*) sward.

2.1.1. Climate

The mean annual rainfall and temperature, averaged over 25 years (Rosslare weather station, Met Eireann, 1978 – 2003), are 1044 mm and 10ºC. Monthly rainfall reaches a minimum in July (45 mm) and a maximum in October (111 mm) (**Figure 3**).

Temperatures are the lowest in February (min: 4.1ºC / max: 8.6 ºC) and reach their maximum in August (min: 12.5 ºC / max: 18.3 ºC) (**Figure 3**).

May is, in average, the sunniest month of the year (214h of sun), whereas only 53h of sun are recorded in December.

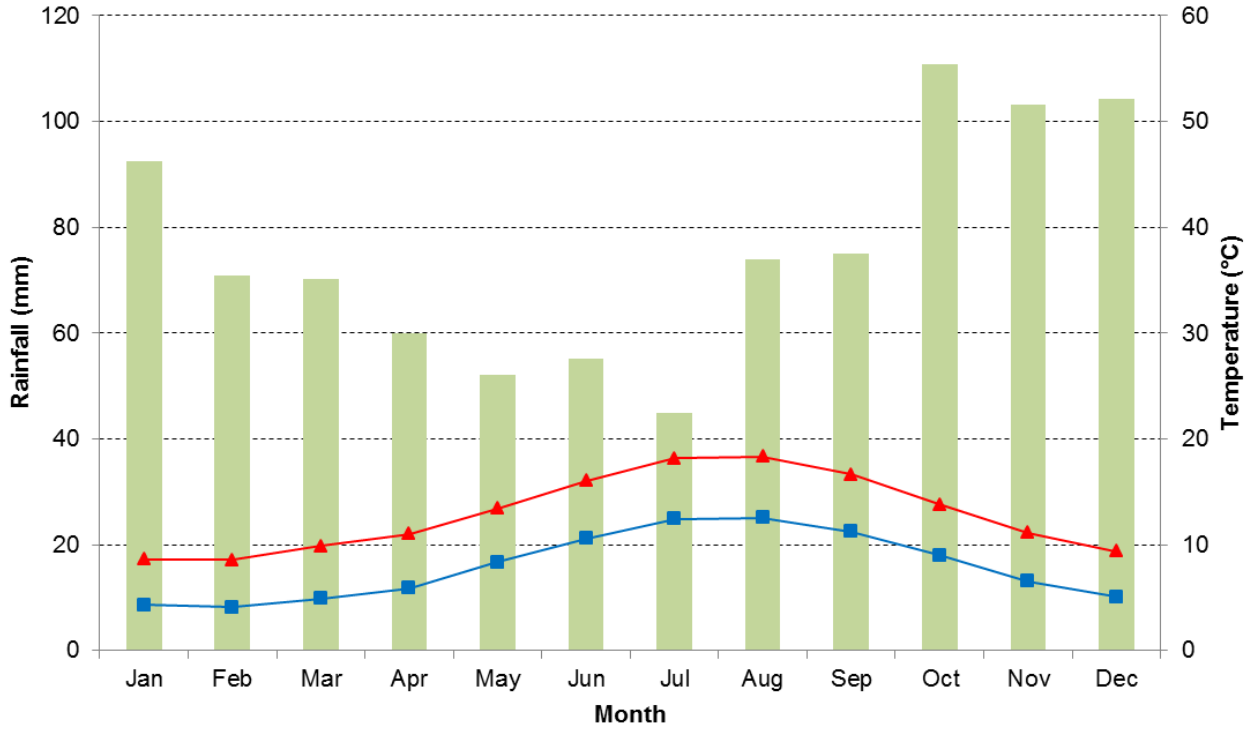
2.1.2. Soil measurements and analysis before the start of the experiments

In March 2009, before applying any treatment on the plots, soil samples were collected on the field, at two different depths (0 – 10 and 10 – 20 cm), around the plots (n = 10) (**Figure 4**).

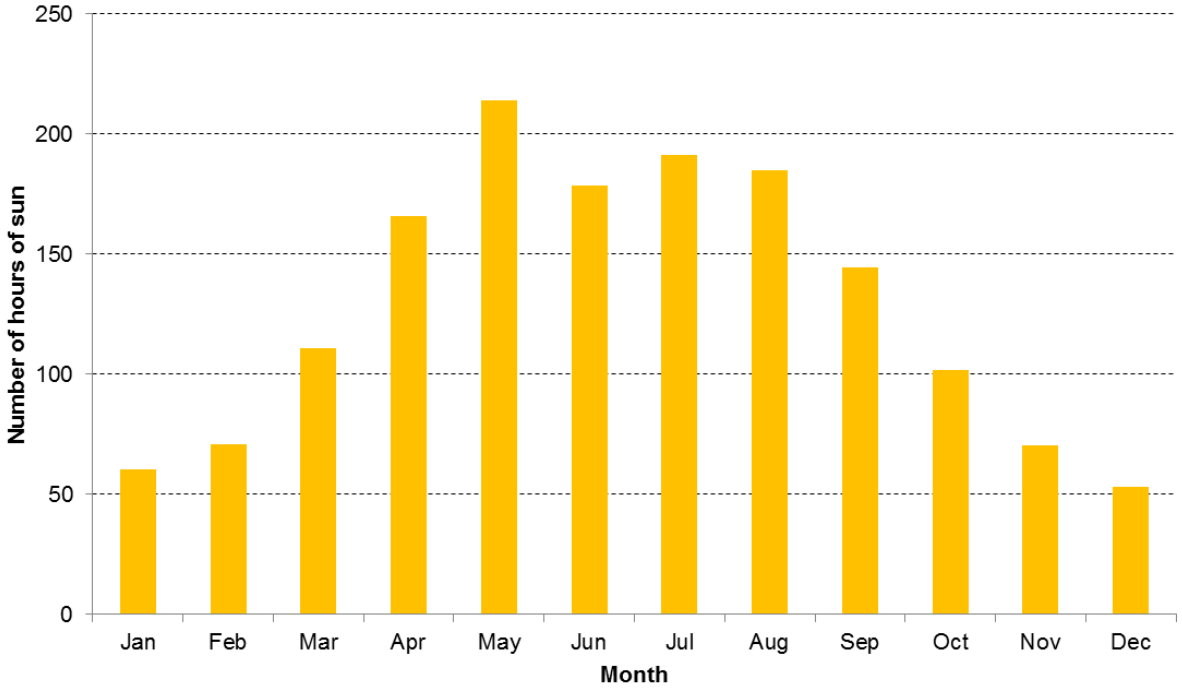
Total C and N content of soil were analysed using a LECO TruSpec CN analyser (LECO Corporation, St. Joseph, MI, USA). Before being run on the analyser, samples were frozen at -80ºC for 16h, freeze-dried for three days and finally ground.

Ammonium nitrogen (NH_4^+-N) and total oxidised nitrogen (TON) fractions were determined by extracting 5 g of fresh soil with 50 ml of a solution of 2M potassium chloride (KCl) (Maynard and Kalra, 1993). Samples were then shaken for 30 min with an automated shaker (New Brunswick Scientific Model G-10 Gyrotory shaker). The extract was then filtered through Whatman N^o. 2 filter paper (Whatmann International Ltd., Maidstone, UK) and the filtrate was stored at 4 ºC prior to colorimetric analysis with an Aquaquem 600 Discrete Analyser (Thermo Scientific, Vantaa, Finland).

The gravimetric soil moisture content of the soil was measured by drying 10 g of field-moist soil overnight at 105ºC.



a)



b)

Figure 3: Monthly weather data, recorded at the Rosslare weather station (Ireland) and averaged for 25 years (1978-2003). The first diagram (a) presents monthly rainfall (columns), daily minimum (blue) and maximum (red) temperatures. The second diagram (b) provides an average value for the number of hours of sun each month.

To get an average value of the dry bulk density for the field site, intact soil cores were collected at the same sampling points as previously stated, using a soil sample ring kit (Eijkelkamp, Em Giesbeek, Netherlands). Ten samples of known volume were taken around the plots (**Figure 4**). The soil surface was prepared with a trowel or knife to remove surface vegetation and the top 20 mm of soil and roots. A stainless steel sample ring (volume = 251.33 cm^3) was pushed into the ground. After digging around them, the cylinder was carefully removed from the ground and the soil trimmed with a sharp knife until the surface was flush with the ends of the cylinder. Plastic caps were placed on both ends of the cylinders. The soil surface was then dug and levelled to a depth of 10cm where another set of ten intact cores was similarly collected. Samples were then brought to the laboratory for analysis.

In the laboratory, samples were weighed up moist, dried overnight at 105ºC and then weighed again for gravimetric soil moisture content determination. For each sample, dry soil was then sieved through a 2 mm sieve and the stones left on the mesh were weight up. Sample bulk density was calculated as the mass of oven-dry soil divided by the volume of the original sample and corrected by mass of stone in the sample.

2.1.3. Soil characteristics

The soil is a well-drained coarse loamy over fine loamy soil, classified as a Brown Earth. Particle size distribution, pH and total C/N content are given in **Table 1** for both 0-10 and 10-20 cm soil layers.

Soil dry bulk density was 947 ± 50 kg m^{-3} for the 0-10 cm layer. Hence, total C and N content for the top soil layer represented, in average, 28.6 ± 0.0 and 2.9 ± 0.1 tons ha^{-1} respectively, while it contained only 10.1 ± 1.1 kg inorganic N ha^{-1}.

Table 1: Particle size distribution, pH, C and N content of the first 20 cm of soil in the experimental site (data collected in March 2009 and expressed as mean values ± standard errors).

Layer	% Clay	% Silt	% Sand	pH	% C	% N	C:N
0-10 cm	13.9 ± 1.2	29.0 ± 1.6	57.1 ± 4.8	5.5 ± 0.1	3.02 ± 0.18	0.30 ± 0.02	9.92 ± 0.32
10-20 cm	14.7 ± 1.5	29.1 ± 1.9	56.2 ± 5.1	5.4 ± 0.1	2.08 ± 0.35	0.23 ± 0.04	9.21 ± 0.17

2.2. General experimental design

Prior to the 15 month experiment, a 180 m^2 area was divided into 40 plots (2 m x 1.5 m, 0.5 m gap between each) (**Figure 4**).

2.2.1. Main field experiment (April 2009-June 2010)

In 2009, 21 of these plots were set out in a randomised block design (n=3) (**Figure 4** and **Table 2**). Twelve plots were spread with cattle slurry, either grass-based or maize-based and with varying DM content (**Table 2**). Slurry was applied to mimic the application pattern of the splash plate technique (**Figure 5**).

In addition another three plots were spread with low DM maize-based slurry, but using a typical trailing shoe pattern of application (**Figure 5**).

The experiment also included control plots (no N applied) and plots fertilised with calcium ammonium nitrate (CAN).

The experimental design is presented in the **Figure 4**. A more detailed description of the experimental set up for this experiment is given in the **Chapter 3**.

Table 2: Slurry treatments used during the experiment

Treatment	Fertiliser type	Application technique	DM content
SP_G_H	Grass-based cattle slurry	Splash plate	High
SP_G_L	Grass-based cattle slurry	Splash plate	Low
SP_M_H	Maize-based cattle slurry	Splash plate	High
SP_M_L	Maize-based cattle slurry	Splash plate	Low
TS_M_L	Maize-based cattle slurry	Trailing shoe	Low

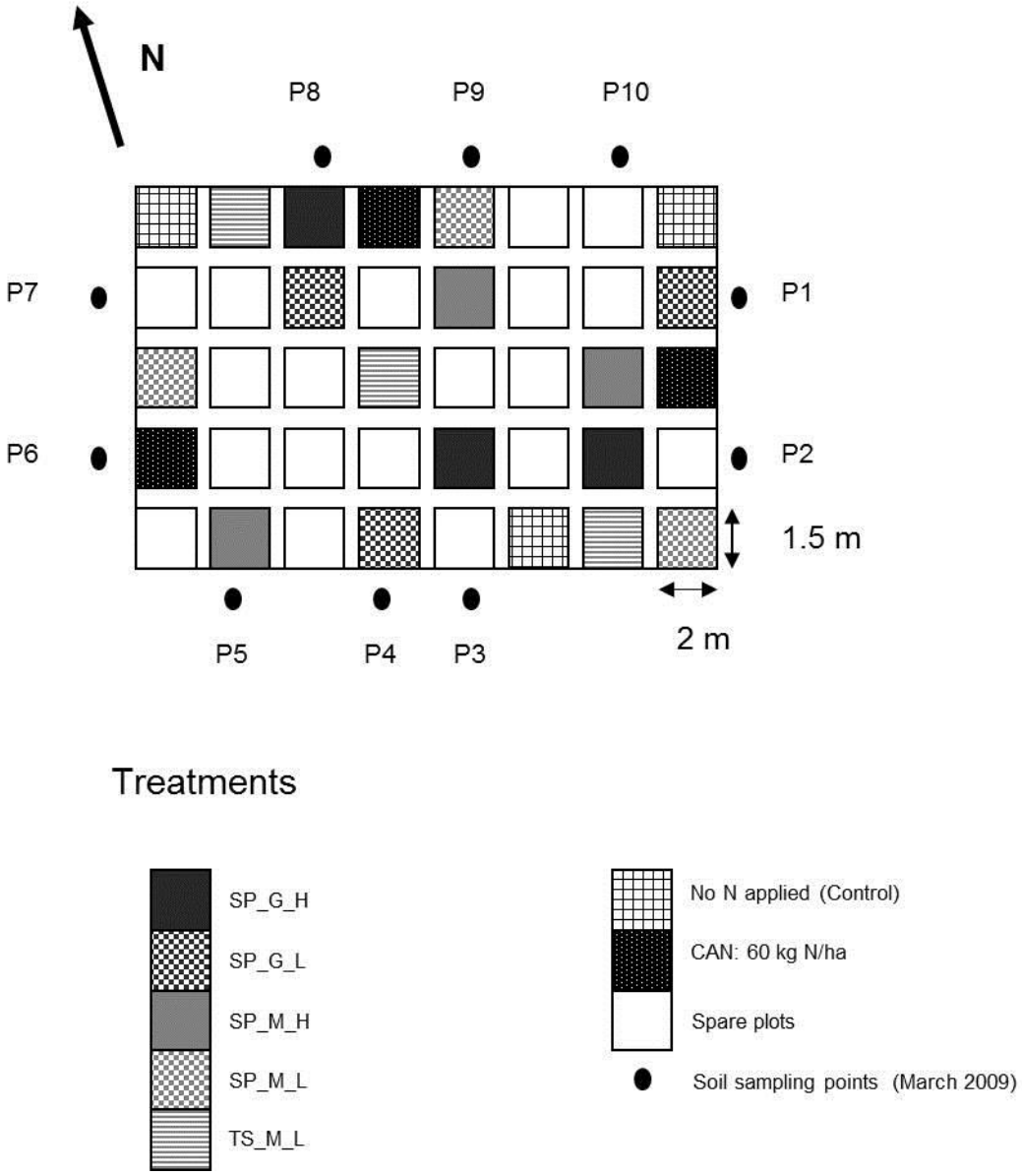


Figure 4: Experimental design for the four slurry application in 2009. Soil characteristics before experiment were determined after sampling points P1 to P10.

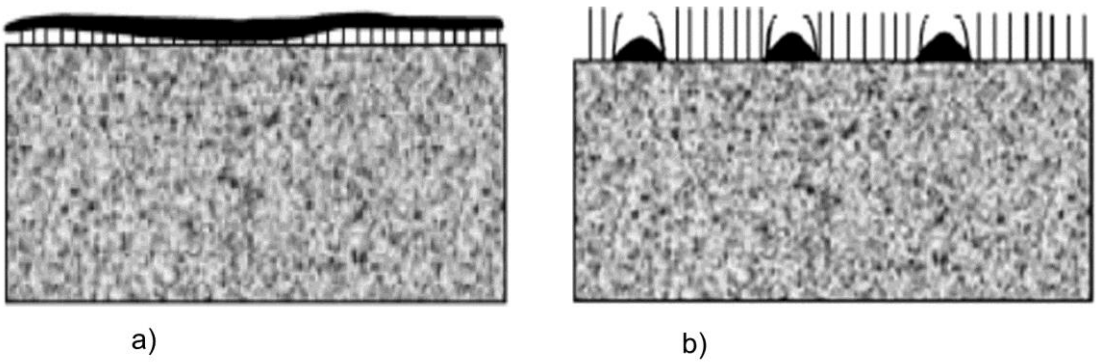


Figure 5: Slurry distribution pattern at the soil surface when a) splash-plate and b) trailing-shoe applied slurry (Misselbrook et al., 2002).

2.2.2. Isotopic partitioning of soil CO_2 efflux post slurry application (August 2009)

The plots used in this experiment (1.5 x 2m) were a sub-sample of those described for the main field experiment. Indeed evolution rates and ^{13}C isotopic composition of CO_2 emitted from plots applied with low DM maize-based slurries (treatments SP_M_L and TS_M_L) were compared with those recorded for the SP_G_L treated plots in order to determine the contribution of slurry-derived CO_2-C to the total CO_2 efflux.

A more detailed description of the experiment is given in the **Chapter 4**.

2.2.3. Dynamic of applied slurry C and N after application to incubated intact soil cores (October 2010 – April 2011).

The experiment was undertaken in Johnstown Castle (Ireland) between October 2010 and April 2011. Small-sized lysimeters (diameter: 15.2 cm), originating from the field described above**,** were applied with various cattle slurry treatments and incubated in controlled conditions for the length of the experiment. Differences between grass- and maize-based slurries and the use of ^{15}N stable isotope tracer technique were used to follow the fate of slurry-derived C and N post-application, either in gas and plant samples, collected during the experiment, or in the soil after destruction of the cores at the end of the experiment.

A more detailed description of the experiment is given in the **Chapter 5**.

Chapter 3. NH_3 abatement techniques and GHG emissions

3.1. Introduction

Ammonia emissions from applied manure contribute about 20-30% of the total N emission from livestock farms in industrial countries (ECETOC, 1994, Misselbrook et al., 2000, Hutchings et al., 2001). Hyde et al. (2003) estimated that spreading of cattle slurry on grassland is the source of approximately 25% of NH_3 emissions in Ireland. Therefore, there is a need to develop NH_3 abatement strategies to reduce volatilisation losses when applying cattle on the field. This can be achieved by modifying slurry application technique, optimising the timing of application or modifying slurry characteristics (Brink et al., 2001). However, such reduction of NH_3 losses from land spreading manures might increase the pool of available N in agricultural soils. This in turn may affect the production of N_2O and/or leached N losses from these soils (Brink et al., 2001, Saggar et al., 2004). In addition, re-deposition of volatilised N can result in indirect emissions of N_2O, which is a potent GHG with a global warming power 298 folds higher than carbon dioxide (CO_2) (IPCC, 2007b).

Greenhouse gas emissions from agriculture, accounted for 31.1% of total GHG emissions in 2010, and are primarily comprised of CH_4 from enteric fermentation in animals, and N_2O from management of animal manures and agricultural soils (Duffy et al., 2012). Nitrous oxide represented 12.7% of national GHG emissions in 2010 and 40.6% of GHG emissions from agriculture, while N_2O sourced from manure application to land, comprises 12.5% of total N_2O emissions (Duffy et al., 2012).

As stated above, a negative interaction between NH_3 and N_2O emissions can occur when applying manure onto agricultural soils (Dosch and Gutser, 1996, Ellis et al., 1998, Chadwick et al., 2000, Wulf et al., 2002b, Gregorich et al., 2005, Perala et al., 2006, Velthof and Mosquera, 2011). As part of the NH_3 lost from the field will be deposited and reemitted somewhere else as N_2O, referring to such emissions as "indirect N_2O emissions" (Mosier et al., 1998), the possible trade-off between both

gaseous N losses is a key element to be investigated when assessing the GHG balance from fertilised grassland soils.

Manure organic matter (OM) is a key driver of N transformations and losses. It can be used by soil micro-organisms, such as heterotrophic nitrifiers and denitrifiers, both as a structural element and, mainly, as an energy source (Granli and Bøckman, 1994, Troeh and Thompson, 2005). Furthermore, it has been shown that the addition of organic manures to grassland plots is more likely to increase C storage in soils, compared to inorganic fertilisers, but also increases rates of soil respiration, leading to a possible net CO_2 loss from amended soils (Jones et al., 2006). Therefore, it is clear that the fate of slurry OM and N are closely linked and that GHG mitigation strategies need to consider both C and N dynamics concurrently (Petersen and Sommer, 2011).

Altering manure characteristics through dilution, separation or any other treatment has been seen in the literature as one possible way to reduce NH_3 volatilisation from land-applied manures. For example, NH_3 fluxes have been shown to increase with and increasing DM content in liquid manures (Sommer and Olesen, 1991). A high DM content contributes to the sealing of soil pores, reducing infiltration of slurry liquid phase in the soil and potentially increasing NH_3 volatilisation (Donovan and Logan, 1983, Dosch and Gutser, 1996). Therefore, Sommer and Olesen (1991) showed that, within a 4-12% range of DM content, dilution of slurry could reduce NH_3 losses. Mkhabela et al. (2009) showed similar results on NH_3 losses when diluting swine slurry and investigated the effect of such dilution on N_2O losses post-application. They found no significant effect of such slurry treatment and could not, therefore, support the widely spread idea of a trade-off between NH_3 and N_2O. In a more extreme comparison, Gregorich et al. (2005) found that N_2O after liquid manure application could be increased up to three fold compared to solid manure, with a much larger N fraction lost as N_2O.

The use of low NH_3 abatements technique has extensively been covered, focusing mainly on the comparison between conventional broadcast applications and newly developed shallow and deep injection methods in terms of both NH_3 and N_2O emissions (Dosch and Gutser, 1996, Dendooven et al., 1998a, Ellis et al., 1998, Malgeryd, 1998, Weslien et al., 1998, Flessa and Beese, 2000, Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a, Wulf et al., 2002b, Perala et al., 2006,

Sistani et al., 2010, Velthof and Mosquera, 2011). Such methods were shown to reduce NH_3 volatilisation losses up by up to 90% (Dendooven et al., 1998a), but could lead to a significant increase in N_2O release from soils in the weeks following slurry application (Dosch and Gutser, 1996, Ellis et al., 1998, Wulf et al., 2002b, Perala et al., 2006, Velthof and Mosquera, 2011). However, the observation of such trade-off between NH_3 and N_2O across the literature was inconsistent, some studies showing no effect of application technique on the latest emissions (Dendooven et al., 1998a, Weslien et al., 1998). Sistani et al (2010) showed lower N_2O emission following the injection of swine effluent, when comparing with surface application during the first year of their experiment, but found higher emissions from the same plots in the second year.

The trailing-shoe application technique has also been well described as an efficient NH_3 abatement technique when applying slurry onto grassland soils (Malgeryd, 1998, Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a). For many grassland areas, it is considered to be the most effective way to lower NH_3 losses as high stone content of soils and undulating topography make injection unsuitable. Only a few studies have compared N_2O emissions from low trajectory slurry applications, such as the trailing-shoe technique, with the traditional broadcast application. Wulf et al. (2002b) tested four different application techniques: splash plate, trail hose, trail shoe and slurry injection into the soil. There was a clear effect of slurry application technique on N_2O emissions when comparing injection technique to the other three, broadcasting clearly increasing N_2O emissions two- to three-fold. However, the difference between splash-plate and trailing-shoe, in terms of N_2O emissions, was not significant. Furthermore, after summing direct and indirect N_2O, as well as methane, all expressed in CO_2 equivalent, the authors concluded that trailing-shoe technique was the most efficient way to reduce non-CO_2 GHG emissions on grassland, but they did not address the fact that such technique could also have a potential adverse effect on ecosystem respiration and C sequestration from soils.

The timing of manure application also affects NH_3 emissions which increase as a function of temperature and wind speed (Sommer et al., 2003). It is also likely to affect N_2O emissions since N_2O production is mainly driven by N availability, temperature and soil aeration. Most studies investigating the impact of timing of

slurry application on NH_3 and N_2O emissions post-application focused on the comparison between autumn (typically after harvest for croplands) and spring application, as reviewed by VanderZaag et al. (2011). However, slurry in Ireland is mainly applied in summer (Hyde and Carton, 2005) when N volatilisation losses can be high due to warm temperatures. Application of slurry in spring can be seen as optimal as N is then applied in a period when uptake by herbage is high and NH_3 losses are relatively low. Although wetter soils in such period may limit the number of days when slurry can be applied on the field using the conventional broadcast method (with a tank and a splash-plate device), Lalor and Schulte (2008) showed in a modelling study that, in Ireland, the number of available spreading days in the first five months of the year (130 days) is substantially higher where trailing-shoe is used for slurry application, ranging from 6.5 to 8 days (median value) for moderate to well drained soils (only 0.5-3 days for splash-plate application). In terms N_2O emissions, Chadwick et al. (2000) observed, after applying pig and dairy slurries onto perennial ryegrass, higher N_2O losses in spring than in summer, which would support the idea of a trade-off between NH_3 and N_2O emissions. Studies comparing summer and spring applications onto grassland are however still scarce.

This experiment was designed to investigate the impact of slurry DM content, application technique and timing of application on the overall GHG balance from cattle slurry applied onto grassland soils. Several hypotheses were drawn prior to the start of the experiment:

- Any reduction in NH_3 losses, following an alteration of slurry DM content, a switch from splash-plate to trailing shoe or from summer to spring / autumn application will increase soil N pools, leading to an increase in measured N_2O emissions (as a result of the trade-off mentioned previously between NH_3 and N_2O fluxes).

- The supply of extra N to the soil (following the implementation of one of the NH_3 abatement strategies tested in this experiment), coupled with an input of organic C, will enhance soil microbial activity, increasing soil (and ecosystem) respiration.

3.2. Materials and methods

3.2.1. Site characteristics and experimental design

The experiment was undertaken between April 2009 and August 2010, on a grassland site in Johnstown Castle, Wexford, Ireland (52°18′N; 6°30′W).

A description of the field site and its characteristics, as well as a presentation of the experimental design and treatments, is given in **Chapter 2**.

3.2.2. Slurry preparation and application in the field

The different slurry treatments used in this experiment (see **Chapter 2**) were prepared directly on the field by mixing thoroughly given proportions of faeces (1/3 to 1/5 of the prepared volume of slurry) and urine in a plastic bucket. The resulting slurry was poured into a watering can to be subsequently applied on the corresponding plots.

Slurry applications were carried out on four separate dates (24 April, 20 July, 17 August and 20 September 2009) to provide a range of contrasting weather conditions. Spreading occurred at approximately midday on each occasion, at a rate of 30 t ha^{-1}. This represented an N supply rate from 26.1 up to 35.7 kg $N.ha^{-1}$ application $^{-1}$ for low DM content slurries, and from 60.3 up to 67.2 kg $N.ha^{-1}$ application $^{-1}$ for high DM content slurries. The C application rate ranged from 380 to 646 kg C ha^{-1} $application^{-1}$ and from 836 to 1022 kg C ha^{-1} $application^{-1}$ for low and high DM content slurries respectively (**Table 3**).

The experiment also included control plots (no N applied) and plots fertilised with CAN at a rate of 60 kg N ha^{-1}. The fertiliser was applied by hand using a granulated form.

Table 3: Characteristics of slurry treatments applied in a) April, b) July, c) August and d) September 2009.

Treatment	DM content (% total weight)	N content (g kg^{-1} fresh slurry)	C:N ratio	N application rate (kg ha^{-1})	C application rat (kg ha^{-1})
SP_G_H	7.40	2.17	12.6	65.1	836
SP_G_L	3.81	1.13	14.7	33.9	496
SP_M_H	7.32	2.75	10.5	82.5	863
SP_M_L	3.98	1.09	16.0	32.7	525
TS_M_L	4.45	1.20	16.3	36	588

(a)

Treatment	DM content (% total weight)	N content (g kg^{-1} fresh slurry)	C:N ratio	N application rate (kg ha^{-1})	C application rat (kg ha^{-1})
SP_G_H	7.42	2.10	15.6	63.0	985
SP_G_L	3.79	1.15	14.0	34.5	482
SP_M_H	7.60	2.08	16.4	62.4	1022
SP_M_L	4.26	1.19	16.1	35.7	575
TS_M_L	4.79	1.31	16.4	39.3	646

(b)

Treatment	DM content (% total weight)	N content (g kg^{-1} fresh slurry)	C:N ratio	N application rate (kg ha^{-1})	C application rat (kg ha^{-1})
SP_G_H	5.28	2.24	13.2	67.2	887
SP_G_L	2.06	0.87	15.9	26.1	416
SP_M_H	6.01	2.18	13.8	65.4	904
SP_M_L	3.29	1.12	14.1	33.6	475
TS_M_L	3.47	1.22	15.3	36.6	559

(c)

Treatment	DM content (% total weight)	N content (kg^{-1} fresh slurry)	C:N ratio	N application rate (kg ha^{-1})	C application rat (kg ha^{-1})
SP_G_H	6.50	2.01	14.6	60.3	882
SP_G_L	2.88	1.13	15.3	33.9	518
SP_M_H	6.34	2.17	15.1	65.4	985
SP_M_L	2.51	1.13	13.8	33.9	468
TS_M_L	2.48	0.89	14.2	26.7	380

(d)

3.2.3. Slurry analyses

For each application, the different slurries were directly sampled on the field, after mixing urine and faeces and prior to application. They were then stored in the cold room until subsequent analysis in the lab.

Slurry DM content was calculated by difference in weight between fresh slurry and slurry oven dried at 105ºC for 24h.

Total C and N content of slurry were analysed using a LECO TruSpec CN analyser (LECO Corporation, St. Joseph, MI, USA). Before being run on the analyser, slurry samples were frozen at -80ºC for 16h, freeze-dried for a week and finally ground. On the TruSpec CN analyser, during what is called combustion phase, the sample is oxidised at high temperature (950ºC, then 850ºC), and moisture and particulates are removed. Once collected, combustion gases are mixed with oxygen before passing through a CO_2 infrared detector. Sample C content is determined from the quantity of CO_2 detected. Gases are then transferred to a helium carrier flow and swept through hot copper to remove oxygen and change NO_x to N_2. After removing CO_2 and water, a thermal conductivity cell is used to determine the N content.

Total ammoniacal N in slurry (data not shown) was also determined by extracting 25 g of fresh slurry with 500 ml of 0.1M hydrochloric acid. Samples were then shaken for 1 hour on a tumble motion RS12 shaker (C. Gerhhardt UK Ltd., Brackley, UK). The extract was then filtered through Whatman No. 2 filter paper (Whatmann International Ltd., Maidstone, UK) and the filtrate was stored at 4ºC prior to colorimetric analysis with an Aquaquem 600 Discrete Analyser (Thermo Scientific, Vantaa, Finland). Unfortunately, results from such analysis were abnormally high in comparison with the total N content of the corresponding slurry, possibly due to a contamination of analysed samples. Due to a lack of remaining sample, analysis of TAN content could not be repeated. However, TAN usually constitutes about 50% of the total amount of N present in cattle slurry (Webb, 2001)

3.2.4. Methods for gaseous sampling and analysis of gas samples

3.2.4.1. Ammonia measurements

Ammonia measurements were made using open dynamic chambers constructed from poly-vinyl chloride (25cm x 30cm), with ammonia concentrations measured at the inlet and outlets to the chambers. Each chamber had a centrifugal air blower (95 CFM ActiveAir blower, Hydrofarm Ltd. USA) with airflow regulated at 15 L min^{-1} through each chamber. The blower was connected to the chamber by means of 10cm diameter air conditioning ducting (Ferns Engineering, Wexford). Prior to entering each chamber, NH_3 contained in the ambient air was sampled using a Leuning shuttle with placed in series with the chamber. Each sampler consisted of a stainless steel sheet coated with oxalic acid (3%) crystals coiled inside a cylindrical plastic body (Leuning et al., 1985). Another set of filters were placed prior to the outlet (**Figure 6**). Fluxes were subsequently calculated as the differential between the inlet and outlet NH_3 concentration, accounting for the mass flow of air across the chamber per time unit. Ammonia scrubbers were replaced after 1, 2, and 6 hours. Chambers were subsequently removed and replaced after a further 6 hours for a 12 hour period and for 24 hours at hour 72 and hour 144.

Interpolation between calculated emission rates was subsequently used to calculate cumulative emissions.

3.2.4.2. Soil carbon dioxide measurements

Soil CO_2 respiration measurements were made using an EGM-4 infra-red gas analyser (PP Systems, Hitchin, Herts, UK) coupled to a closed chamber. In this closed chamber system, the air is sucked from the chamber to the analyser, and recycled back to the chamber after analysis. The flux is then calculated from the increase of the concentration of CO_2 inside the chamber over time.

The chambers, 11.3 cm in diameter and 16.3 cm high, were inserted to a depth of 2 cm into the soil, allowing a 1434 cm^3 volume of air within the chamber. Each chamber was randomly positioned on its corresponding plot. Two rubber septa were

inserted at the top of the chamber and an air circulation was created between the chamber and the analyser using a flexible tubing system.

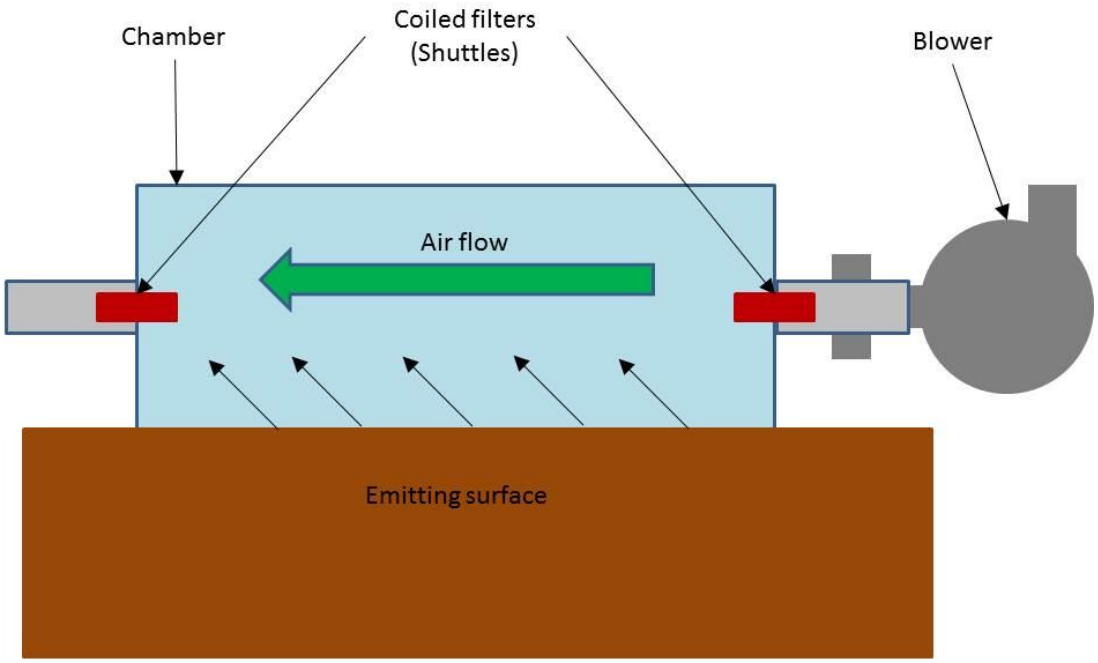


Figure 6: Schematic of dynamic ammonia chambers.

Measurements were taken for a period of three minutes on each plot. During the measurements period, the chamber was maintained onto the soil and air was continuously pumped, at a rate of 500 ml min^{-1}, from the chamber to the analyser where the CO_2 concentration was analysed. The EGM-4 is a non-dispersive infrared gas analyser. When air passes through the sample cell, part of the CO_2 is absorbed at a wavelength of 426 nm and a reading of the CO_2 concentration (in ppm) is then given by the analyser. For the purpose of this experiment, CO_2 readings were saved onto the instrument's memory every 30 s.

CO_2 fluxes (in g CO_2-C m^{-2} s^{-1}) were then calculated from the six measurements made over the sampling period, using the following equation:

$$F_{CO_2} = \frac{PM_{CO_2}}{RTM_C}\frac{V}{A}\frac{\Delta C_{CO_2}}{\Delta t}\times 10^{-6} \quad (7)$$

where P is the air pressure inside the chamber (calculated by the analyser, in atm), M_{CO2}, is the molar mass for CO_2 (= 44.01 g mol^{-1}), M_C is the standard atomic weight for C (= 12.01 g mol^{-1}), R is the gas constant (8.205746 x 10^{-5} m^3 atm K^{-1} mol^{-1}), T is the air temperature at the grass level (taken from Johnstown Castle weather station, in K), V is the volume of the chamber (in m^3), A is the area covered by the chamber (in m^2) and $\frac{\Delta C_{CO_2}}{\Delta t}$ is the slope given by the linear regression of the concentration of CO_2 inside the chamber over the measuring period (in ppm s^{-1}).

Fluxes were then either analysed as such or converted in kg CO_2-eq ha^{-1} day^{-1} for subsequent statistical analyses.

Measurements were taken usually between 10am and 1pm, daily for the first 10 days, and then weekly. Gaseous fluxes were monitored weekly (or fortnightly) until October 2009, and then monthly from November 2009 to March 2010. At the start of the new growing season, fluxes were measured fortnightly until August 2010.

3.2.4.3. Nitrous oxide and methane measurements

N_2O and CH_4 fluxes were measured using closed static chambers (Smith et al. 1995). Accumulation methods consists in allowing the targeted gas to build up inside a sealed chamber, placed onto the soil, during a certain measuring period. Air from the chamber is sampled various times during the measuring period with a syringe, before being analysed on a gas analyser (Smith et al., 1995, Pumpanen et al., 2009). The flux is then calculated from the increase of the concentration of the targeted gas inside the chamber over time.

The chambers, 11.3 cm in diameter and 16.3 cm high, were inserted to a depth of 2 cm into the soil, allowing a 1434 cm^3 volume of air within the chamber. Rubber septa, previously removed to avoid over pressurisation inside the chamber, were then quickly put back in place at the top of the chamber. Each chamber was randomly positioned on its corresponding plot.

Ambient air samples were collected at time zero and measurements were taken after 40 minutes. Samples were taken using a 20-ml gas-tight polypropylene syringe (BD Plastipak, Becton Dickinson, Spain) which was opened fully and fitted with a hypodermic needle. To collect a sample from a chamber, the needle was inserted through the septum and the syringe was flushed to ensure adequate mixing of the air within the chamber prior to the withdrawal of a sample from the chamber. A headspace sample was then immediately injected into and flushed through a pre-evacuated 7-ml gas-tight vial (Supelco, Bellefonte, Pennsylvania, USA), which was then stored for subsequent analysis.

Measurements were taken usually between 2pm and 5pm, daily for the first 10 days, and then weekly. Gaseous fluxes were monitored weekly (or fortnightly) until October 2009, and then monthly from November 2009 to March 2010. At the start of the new growing season, fluxes were measured fortnightly until August 2010.

The N_2O and CH_4 concentration in each sample was analysed using a gas chromatograph (GC) (Varian CP 3800 GC, Varian, USA). From each vial a 0.7 ml sub-sample was automatically injected into the chromatograph using a Combi-Pal automatic sampler (CTC Analysis, Switzerland) under computer control. The sample is then carried through a 3.75 m packed column (Poraplot Q packed column, JVA Analytics, Dublin, Ireland) by the carrier gas Argon at a flow rate of 35 ml min^{-1}.

The sample was split in two before running through the detectors. The N_2O concentration was analysed using an Electron Capture Detector (E.C.D.) at 300ºC while a Flame Ionisation Detector (F.I.D.) was used to detect CH_4. The gas chromatograph was also fitted with a Thermal Conductivity Detector (T.C.D.), prior to the F.I.D., for the non-destructive analysis of CO_2. However, another method was used to measure the CO_2 on the field (see 3.2.4.2).

N_2O (in g N_2O-N m^{-2} h^{-1}) and CH_4 fluxes (in g CH_4-C m^{-2} h^{-1}) were then calculated using the following equations:

$$F_{N_2O} = \frac{PM_{N_2O}}{RTM_N} \frac{V}{A} \frac{\Delta C_{N_2O}}{\Delta t} \times 10^{-9} \quad (8)$$

$$F_{CH_4} = \frac{PM_{CH_4}}{RTM_C}\frac{V}{A}\frac{\Delta C_{CH_4}}{\Delta t}\times 10^{-9} \quad (9)$$

where P is the atmospheric pressure (taken from Johnstown Castle weather station, in Pa), M_{N2O} / M_{CH4} is the molar mass for N_2O (= 44.013 g mol^{-1}) or CH_4 (= 16.04 g mol^{-1}), M_N / M_C is the standard atomic weight for N (= 14.07 g mol^{-1}) or C (= 12.01 g mol^{-1}), R is the gas constant (8.314472 m^3 Pa K^{-1} mol^{-1}), T is the air temperature at the grass level (taken from Johnstown Castle weather station, in K), V is the volume of the chamber (in m^3), A is the area covered by the chamber (in m^2) and $\frac{\Delta C_{N_2O}}{\Delta t}$ (or $\frac{\Delta C_{CH_4}}{\Delta t}$) is the variation of gas concentration over the measuring period (ppb h^{-1}).

Fluxes were then converted either in kg N_2O-N ha^{-1} day^{-1} (kg CH_4-C ha^{-1} day^{-1}) or in kg CO_2-eq ha^{-1} day^{-1} for subsequent statistical analyses. The global warming potential (GWP) of each gas for a 100 year time horizon (IPCC, 2007b) was used for the latest conversion, with the following values: GWP_{CH4}= 25 and GWP_{N2O}=296.

Emission factors for N_2O were calculated for each treatment using the following equation:

$$EF = \frac{F_{N_2O(Treatment)} - F_{N_2O(Control)}}{N_{applied} - F_{NH_3(treatment)}} \quad (10)$$

where F_{N2O} and F_{NH3} were cumulative fluxes of N_2O and NH_3, for the entire experimental period, integrated from measurements on control or treated plots; and $N_{applied}$ the total amount of N applied on corresponding treated plots.

3.2.5. Monitoring of soil and weather parameters during experiment

On most of the soil CO_2 measurements or gas sampling days, soil temperature and soil volumetric water content were measured after inserting a sensor (WET-1, Delta-T Devices, Cambridge, UK), connected to a data logger (HH2 Moisture Meter,

Delta-T Devices, Cambridge, UK), into the first 10 cm of soil. Measurements were taken for each plot straight after measuring CO_2 efflux or while sampling gas for CH_4 and N_2O determination.

Water filled pore space (WFPS, in %) was then calculated as follow (Fanzluebbers, 1999) :

$$WFPS = \frac{\theta . D_P}{1 - \frac{D_B}{D_P}} \quad (11)$$

Where θ is the soil volumetric content (in %), D_B is the soil dry bulk density (see 2.4, in g cm^{-3}) and D_P is the soil particle density (in g cm^{-3}). For the purpose of this study, the average value for mineral soils (2.65 g cm-^{3}) (Skopp, 2001) was used in WFPS calculation.

Throughout the period of the study, hourly rainfall, soil temperature, wind speed and solar radiation were automatically registered by the meteorological station in Johnstown Castle.

3.2.6. Methods used for statistical analyses

Data collected on the field were analysed using the statistical package STATISTICA version 10 (Statsoft, Tulsa, Oklahoma).

Cumulative fluxes for the entire experiment were analysed using either a one-way Analysis of Variance (ANOVA) to investigate the differences between inorganic fertiliser (CAN) and cattle slurry spread plots in terms of gaseous emissions from the field plots, or a factorial ANOVA to study the effect of slurry type, application technique or DM content on NH_3 and GHG emissions from soils. A nested design had to be used in order to take into account the imbalance of the experimental design. The impact of timing of application was investigated using a Repeated Measures ANOVA with four levels of repetition corresponding to the four application dates. In the three types of analysis, differences between individual treatments were assessed using Least Significant Difference (Fisher LSD test, α=0.05).

The structure of the data may have called for the use of the following non-parametric tests:

- Mann-Whitney test, to compare CAN and slurry spread plots in terms of indirect N_2O emissions per unit of N applied;
- Friedmann test, to analyse the effect of timing of application whenever the use of a Repeated Measures ANOVA was not possible.

3.3. Results and Discussion

3.3.1. Closed chamber measurements of trace gas effluxes from the soil

In order to measure CO_2, CH_4 and N_2O emissions, chambers were placed at the soil surface, something which often modifies the gas flux to be measured by changing the vertical profile of the gas concentration, the energy balance and the turbulent regime (Rochette, 2011).

In this experiment, chamber walls were inserted about two centimetres into the soil, to limit lateral gas exchange (Healy et al., 1996). However, subsequent soil disturbances may affect the measured rate of gas exchanges between the soil and the atmosphere (Matthias et al., 1980; Norman et al., 1997). As a consequence, Rochette et al. (2011) suggest the use of collars that are inserted into the ground prior to the chamber deployment. However, collars may also affect soil conditions, by preventing run-off and shading the soil, and gas exchanges, by formation of shrinkage cracks at the collar-soil interface (Rochette et al., 2011). In the present experiment, chambers were directly pushed into the ground, with no collar insertion prior to their deployment. Indeed, the presence of collars prior to the application of treatments would not have permitted a homogeneous slurry application pattern within the plots. Furthermore, the small size of the measuring chambers, relative to the size of the plots, led us to consider each measurement as an isolated sampling point within the plot. Thus, varying the location of the chamber within each plot, from one sampling day to the other, allowed us to integrate more spatial variability in trace gas fluxes.

As already mentioned, the insertion of a closed chamber into the soil inherently disturb the natural fluxes of CO_2 or N_2O, The driving factors of such fluxes are usually no longer constant during the gas sampling time, and therefore no linear increase or decrease of the gas concentration inside the chamber, over time, is expected. Nevertheless, the use of a linear regression for calculating CO_2 fluxes has been justified by keeping the closure time short and assuming the concentration change over time to be linear (Kutzbach et al., 2007).

In terms of N_2O emissions, even though there is an increasing body of evidences that, under certain conditions, the use of a linear approach to calculate N_2O flux rates from closed chamber measurements (Healy et al., 1996; Conen & Smith, 2000; Kroon et al., 2008; Rochette & Eriksen-Hamel, 2008; Rochette, 2011), the choice of using a linear regression model, in the present experiment, was justified by the fact that linearity tests conducted both in the field and in controlled conditions (**Chapter 5**) showed that measured N_2O concentrations were (for controls and slurry treatments at least) within the linear range of the N_2O accumulation curve inside the chamber over time. However, the very high N_2O fluxes derived from CAN-fertilised plots may have been underestimated.

3.3.2. Comparison between mineral fertiliser and cattle slurry over the entire experiment

The amount of ammonia volatilised over the entire experiment, lasting from the 24th April 2009 to the 30th June 2010, was shown, after square root transformation, to be significantly higher for slurry spread plots, compared to controls and plots fertilised with CAN (**Table 4**, $P<0.0001$). Volatilised N losses from slurry, post-application were 11 to 16-fold higher compared to inorganic fertiliser application and amounted to 38.3% to 70.8% of the total l N applied.

NH_3 losses from CAN fertilised plots were not significantly different from control plots and represented only 2.9% of the applied N. When corrected by background emissions (estimated from control plots), this gives us an emission factor of less than 2% for CAN, which is similar to values of 1% to 2.5%, reported from the literature by van der Weerden and Jarvis (1997) for non-calcareous soils. Misselbrook

at al. (2000) also found NH_3 losses equivalent to 1.5% of total N applied for ammonium-nitrate (NH_4NO_3), while 59% of TAN was lost as NH_3 when applying cattle slurry.

On the other hand, plots fertilised with slurry displayed, over the entire period, significantly lower N_2O emissions ($P<0.0001$, log-transformed data) than plots fertilised with CAN (**Table 4**). For slurry treatments, N_2O emissions represented on average only 1.4% of the amount of N volatilised by the same plots, when these N_2O losses amounted for up to 119% of the volatilised N for plots where mineral fertiliser was applied. Similarly, Velthof and Mosquera (2011) observed much higher emissions of N_2O from soils fertilised with CAN than from soils spread with cattle slurry. Ball et al. (2004) also found lower emissions from organic N addition compared to inorganic fertiliser. Jones et al. (2007) showed, in the first year of their field experiment, highest N_2O emissions from NH_4NO_3 than from cattle slurry, with emission factors of 1.4 and 0.5% respectively. However, they did not find any significant difference between both treatments in the second year of their experiment.

The calculated N_2O emission factors (see equation 10) of 1.6% and 0.9% found for CAN and cattle slurry respectively are in the same range that those calculated in other studies (Jones et al., 2007, Velthof and Mosquera, 2011) and support the idea of a distinction between organic and mineral fertilisers regarding the calculation of emission factors for national GHG inventories. This is in contradiction with the current IPCC methodology (IPCC, 2006) which assumes a default of 1% of N applied reemitted as N_2O regardless of the N source.

A significant increase of CO_2 from the soil ($P<0.005$) was also shown when amending experimental plots with slurry. The input of readily available C from manures and slurries has been shown to enhance CO_2 release from soils during the first few days after application (Saviozzi et al., 1997, Rochette et al., 2006). Once the slurry-derived soluble C pool is exhausted, soil respiration rates usually go back to background values given by unfertilised plots (Saviozzi et al., 1997). In this experiment, the additional C mineralisation from slurry spread plots, over the entire measurement period, was equal to 60 ± 7% of total C applied.

Table 4: Cumulative gaseous fluxes integrated from field measurements over the entire experiment (428 days) and expressed per amount of total N applied. Fluxes are expressed as mean values ± standard errors.

	N_2O direct* (% N applied)	N_2O indirect (% N applied)	CO_2 (kg CO_2-C kg^{-1} N applied)	CH_4 (kg CH_4-C kh^{-1} N applied)	Total GHG (kg $CO_{2\text{-eq}}$ kg^{-1} N applied)
CAN	1.75 ± 0.78	0.03 ± 0.01	26.09 ± 1.82	0.01 ± 0.00	104 ± 4
Slurry	0.67 ± 0.05	0.49± 0.03	42.85 ± 2.99	0.02 ± 0.00	163 ± 11

* Indirect N_2O-N emissions = 1% of volatilised NH_3-N losses (IPCC, 2006)

To estimate the total release of greenhouse gases from experimental plots, fluxes of N_2O and CH_4 measured on the field were converted in kg CO_2-eq. Indirect N_2O emissions, resulting from the N volatilised from the experimental plots being redeposited in another place, within a two kilometre radius, and reemitted as N_2O, were calculated using a default value of 1% of measured NH_3-N being converted into an indirect N_2O flux to be accounted into the field-scale greenhouse gas budget (IPCC, 2006). These emissions were also converted into kg CO_2-eq and the three gases were added to the amount of CO_2 measured on the field over the experiment.

The total amount of GHG emitted from the the experimental plots was significantly affected by treatments ($P<0.005$), with the highest value being calculated for slurry spread plots. On the other hand, control plots emitted significantly less GHG than any fertilised plot. When expressed per amount of total N applied (**Table 4**), slurry spread plots emitted on average 57% more GHG from the soil than plots fertilised with CAN This was mainly due to the higher cumulative CO_2 emissions from plots treated with slurry, whose impact dwarfed any potential effect of direct and indirect N_2O emissions on the total amount of GHG emitted from treated soils.

However, it is important to mention that the increase in soil respiration when adding readily available and metabolisable C to the system can be partly offset by an increase in photosynthesis (Soussana et al., 2004), and therefore an increase of GPP, from the grass cover which would further reduce the difference between both types of fertiliser in terms of full field-scale GHG budget. Soussana et al. (2004) showed that moderately enhanced N fertilisation increases the organic matter input to the soil proportionally more than it increases the process of C mineralisation, whereas intensive fertilisation stimulate mineralisation and, therefore, enhance C losses. Furthermore, it has been shown that the addition of organic manures to grassland plots

is more likely to increase C storage in soils, compared to inorganic fertilisers, but also increases rates of soil respiration, leading to a possible net CO_2 loss of 4.9 g CO_2 m^{-2} h^{-1} on cattle slurry treatment (Jones et al., 2006).

Cumulative N_2O and NH_3 data measured on the field tends to fully support the idea of a possible trade off between both fertiliser types, the additional NH_4-N, saved from volatilisation when applying CAN, being nitrified and denitrified by soil microbial communities, leading to additional N_2O losses compared to organic fertilisers applied onto grassland soil. This reduced emission factor associated with slurry application possibly arises due to the fact that urea in slurry must be ammonified prior to nitrification and denitrification, allowing more time for plant uptake. Conversely, other studies have found an increase in N_2O for ammonium/urea-based fertilisers compared to nitrate-based fertiliser, but these were performed on lighter soils where most of the apllied nitrate had been lost from the system via leaching (Breitenbeck et al. 1980).

3.3.3. Effect of DM content and application technique

The amount of NH_3 volatilised over the entire experiment was shown, after square root transformation, to be significantly reduced ($P<0.0005$) when lowering slurry DM content or switching from splash-plate to trailing-shoe application technique (**Table 5**). The observed reduction of NH_3 losses when lowering slurry DM content confirms the results from a few slurry dilution experiments (Sommer and Olesen, 1991, Mkhabela et al., 2009). However, when relating these losses to the quantity of N applied (see indirect N_2O emissions in **Figure 7**), splash-plate plots applied with low dry-matter slurry showed significantly higher ($P<0.0005$, square root transformed data) indirect N_2O emissions than plots spread with an higher DM content. This is in contradiction with what was observed previously by Mkabela et al. (2009) who, in a field experiment evaluating the effect of slurry dilution on NH_3 and N_2O emissions from a grassland soil, always found lower NH_3 losses (in % NH_4^+-N applied) for the diluted slurry. Smith et al. (2000) also found a reduction of NH_3 losses (in % NH_4^+-N applied) when applying slurry with splash-plate and band-spread techniques, but not when using trailing-shoe or shallow injection methods.

Concerning the effect of the application technique, trailing-shoe has been well described as an efficient NH_3 abatement technique when applying slurry onto grassland soils (Malgeryd, 1998, Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a). In the present experiment, the use of trailing-shoe application led to a 24% reduction of NH_3 losses, which is less than the 40 to 60% reduction found in some studies (Smith et al., 2000, Misselbrook et al., 2002, Wulf et al., 2002a).

Direct N_2O emissions measured also showed a significant effect "Spreading technique* DM content" ($P<0.005$). Fluxes were reduced in absolute values when lowering slurry DM content (**Table 5**). However, this reduction of direct N_2O emissions was no longer significant when expressing cumulative emissions of N_2O per unit of N applied. This confirmed the findings of Mkhabela et al. (2009) who did not find any significant effect of diluting slurry on N_2O losses from soils. Concerning the effect of the application technique, N_2O fluxes were significantly increased in absolute values (**Table 5**) when switching from splash-plate to trailing-shoe application. However, this was not the case when fluxes were related to the amount of N applied (**Figure 7**). Only few studies have compared N_2O emissions from low trajectory slurry applications, such as the trailing-shoe technique, with the traditional broadcast application. Wulf et al. (2002b) did not observe any significant differences between splash-plate and trailing-shoe application in terms of direct N_2O emissions, but by reducing indirect N_2O emissions without increasing those directly measured on the field, trailing-shoe application technique was, in their study, the most effective way to reduce non-CO_2 GHG emissions (in CO_2-eq) from grassland soils. In the experiment presented here, the calculated total amount of N_2O emitted from slurry spread plots was not affected by the application technique, but the proportion of indirect N_2O in these emissions was reduced from about 45% for conventional broadcast application to 31% when using trailing-shoe (**Table 6**).

Soil CO_2 efflux was also statistically affected by a reduction in slurry DM content ($P<0.01$), but the reduction of CO_2 emissions with a lower slurry DM content was only significant for plots spread with maize-derived slurry. Once expressed per unit of N applied, this effect no longer existed (**Figure 7**). The change of application technique did not have any significant effect. On the other hand, the use of trailing-shoe was shown to significantly increase ($P<0.05$) CH_4 release from amended plots

(**Table 5**), but, in terms of GHG balance, these emissions had a marginal impact on the total amount of GHG emitted from each plot.

Table 5: Cumulative gaseous fluxes for each slurry treatment (in kg CO_2-eq ha^{-1}), integrated from field measurements over the entire experiment (428 days). Fluxes are expressed as mean values ± standard errors. Significant differences between treatments are given by the letters beside each value (Result from a Fisher LSD test, P<0.05)

Treatment*	N_2O indirect	N_2O direct	CO_2 (x 10^3)	CH_4
Control	26 ± 8^{a}	185 ± 41^{e}	21.4 ± 0.6^{h}	97 ± 10^{k}
SP_G_H	514 ± 39^{b}	657 ± 16^{f}	29.1 ± 0.3^{ij}	199 ± 27^{l}
SP_G_L	369 ± 21^{c}	449 ± 70^{g}	26.4 ± 0.9^{i}	97 ± 24^{k}
SP_M_H	494 ± 11^{b}	640 ± 38^{f}	32.3 ± 2.3^{j}	129 ± 38^{kl}
SP_M_L	371 ± 14^{c}	437 ± 65^{g}	23.2 ± 0.6^{hi}	68 ± 24^{k}
TS_M_L	279 ± 11^{d}	575 ± 32^{f}	29.1 ± 0.4^{ij}	202 ± 62^{l}

* Treatment codes are explained in **Chapter 2**.

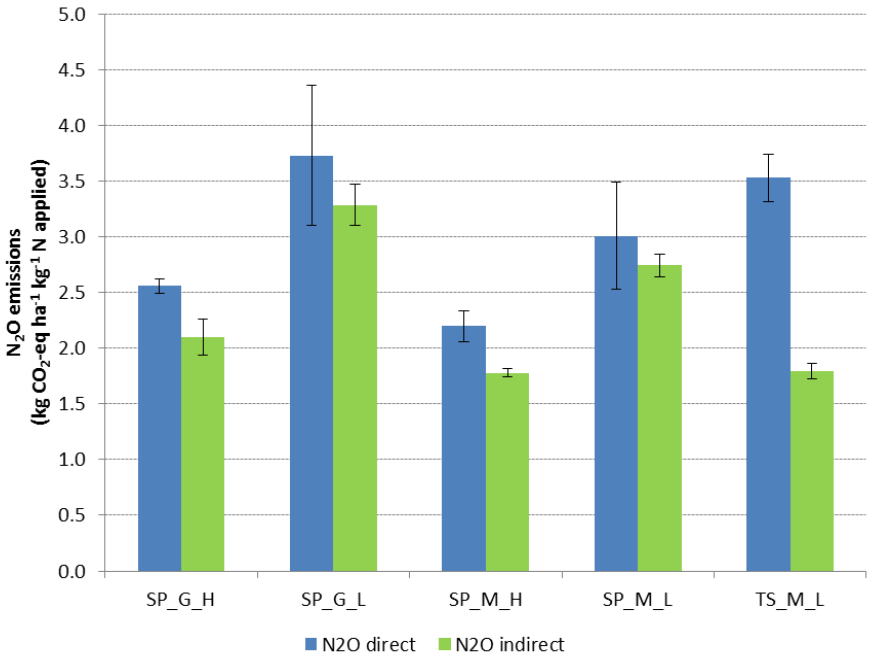
Table 6: Direct, indirect and total N_2O emissions emitted from slurry spread plots over the entire experiment. Fluxes are expressed as mean values ± standard errors.

Treatment*	Direct N_2O (g N_2O-N ha^{-1})	Indirect N_2O (g N_2O-N ha^{-1})	Total N_2O (g N_2O-N ha^{-1})	Proportion of indirect N_2O (%)
SP_G_H	1402 ± 34	1097 ± 84	2499 ± 118	43.9
SP_G_L	958 ± 149	788 ± 44	1746 ± 193	45.1
SP_M_H	1366 ± 80	1055 ± 24	2422 ± 104	43.6
SP_M_L	934 ± 138	793 ± 29	1727 ± 167	45.9
TS_M_L	1311 ± 85	595 ± 23	1907 ± 108	31.2

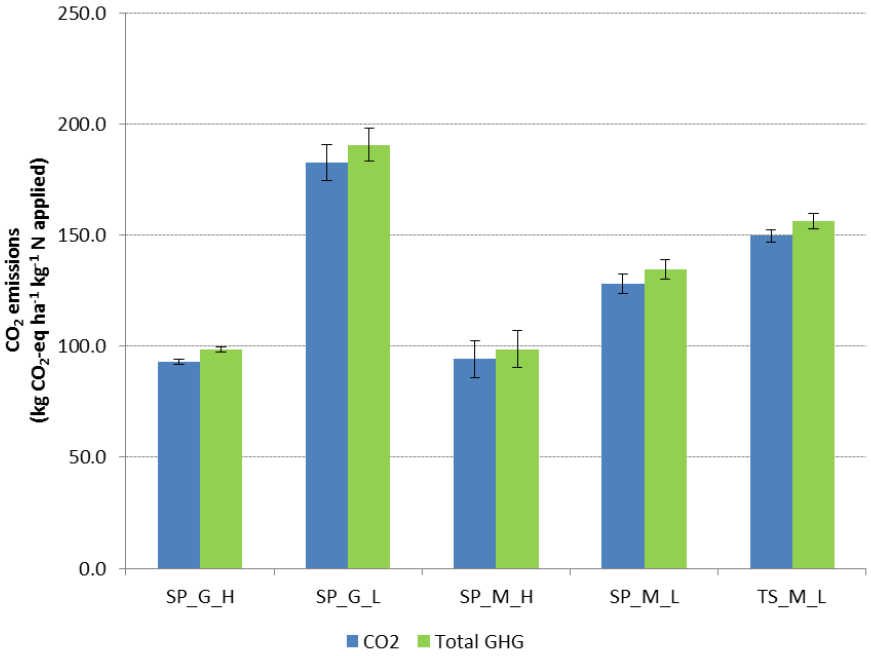
* Treatment codes are explained in **Chapter 2**.

The amount of GHG emitted from slurry spread plots was dominated by CO_2 emissions, which dwarfed any potential impact of other gases, and therefore followed the same patterns as the one observed for soil CO_2 efflux (**Figure 7**). As a consequence, GHG emissions from the soil were significantly affected by a reduction of slurry DM content ($P<0.01$), but this effect no longer existed when those GHG emissions were expressed per unit of N applied.

Cumulative GHG emission from trailing-shoe were, in average, 44% higher than those recorded for the same slurry when it was splash-plate applied, even though this difference was not significant due to a high variability of measured values (**Figure 7**). As mentioned earlier (see **3.3.1**), this increase of ecosystem respiration can easily be offset by an increase in photosynthesis from plants leading to higher GPP and subsequent C storage in plant biomass (Soussana et al., 2004).



(a)



(b)

Figure 7: Mean cumulative (a) direct and indirect N_2O, and (b) CO_2 and total GHG emissions fluxes for each slurry treatment, integrated from field measurements over the entire experiment (428 days) and corrected by controls. For each graph, standard errors are given as error bars (Treatment codes are explained in the Chapter 2).

The slurry application technique significantly affected both direct ($P<0.05$, Box-Cox transformed data with $\lambda=-0.472$) and indirect N_2O emissions ($P<0.0005$ on square root transformed data), the total amount of N_2O originating from experimental plots remaining unchanged over the course of the experiment (**Figure 7**). Once again, this tend to support the idea of a possible trade off between NH_3 and N_2O, even though the increase of direct N_2O when using trailing-shoe was not significant.

So far, only few studies have compared trailing-shoe technique with the conventional broadcasting method (using splash-plate) in terms of N_2O emissions. Contrary to the results exposed here, Wulf et al.(2002b) observed a reduction in total N_2O emissions, when using a trailing-shoe on grassland soils, due to a reduction of NH_3 losses which was not compensated by an increase in directly measured N_2O fluxes.

In terms of strategies to mitigate GHG emissions from grassland soils, it is therefore essential to increase the number of studies assessing the effect of trailing-shoe on such emissions as it is considered to be the most effective alternative to splash-plate appplication in many grassland areas (Smith et al., 2000, Wulf et al., 2002a, Lalor and Schulte, 2008) and tend to offer more flexibility in terms of available spreading days (Lalor and Schulte, 2008).

3.3.4. Effect of timing of application

Cumulative fluxes for the three weeks following each application were compared, after log-transformation of absolute values, using a Repeated measures ANOVA. Results showed a significant effect Timing*Fertiliser type (i.e. control, CAN or slurry) on N_2O ($P<0.0005$), NH_3 ($P<0.005$), CO_2 ($P<0.0005$) and total GHG emissions ($P<0.0001$).

NH_3 volatilisation from slurry-spread plots was considerably higher when applying in July than for any other application (**Figure 8**). Conversely, direct N_2O emissions were the lowest for the same plots whereas these emissions were the highest in August. Overall, total gaseous N losses were the lowest when applying in April, with a 43% reduction compared to July. In terms of farmers' perspectives, this

is of great importance as such reduction of gaseous N losses occurs in a period where the N requirement of the herbage is the largest, optimising N fertilisation and reducing the need for a supplementation with mineral N (Carton & Magette, 1999). Therefore, spring application is not only of environmental interest, but also brings agronomic benefits to the farmer.

NH_3 volatilisation from land spread slurry mainly occurs in the first 24 to 48 hours after application (Sommer and Ersboll, 1994, Misselbrook et al., 2002) and the main environmental driving factors are air / soil temperature, wind speed as well as air humidity and solar radiation (Sommer et al., 2003). Therefore, the high volatilisation rates observed from slurry plots could be linked to the fact that slurry was applied on a warm and dry sunny day (**Table 7**) which favoured high evaporation rates and therefore NH_3 losses. In August, although air temperature was slightly higher than in July, lower solar radiation and higher air moisture reduced N volatilisation losses.

A variation in the pH of applied slurry (not measured in this experiment), from one application date to the other, could have also contributed to variation in the rate of NH_3 volatilisation. Indeed, a higher pH would increase the ratio NH_3:NH_4^+in the slurry (Sommer et al., 2003), thus potentially increasing NH_3 losses. In the present experiment, cow diet was controlled and remained the same throughout the season, thus minimising the variations in the composition of urine and faeces. It was therefore assumed that any slight variation of the pH of applied slurry was unlikely to have offset the effect of environmental factors on NH_3 volatilisation post-spreading.

N_2O losses are driven not only by N inputs, but also by climatic and soil parameters such as soil texture, temperature and WFPS as well as plant N demand (Smith et al., 2003b). Water-filled pore space is directly dependent on both precipitation and the clay content of the soil (which influences the water-holding capacity) and this has been observed to be the primary driver for the seasonality of N_2O emissions (Dobbie and Smith, 2003). In the present study, whenever soil parameters could be measured, fluxes were shown to be mainly and positively correlated with soil WFPS and rainfall data (**Table 8**) which would explain the high emission rates observed after the third application, in August 2009. In addition, grass N response in terms of uptake is reduced in autumn compared to spring with 10.3 kg

dry matter kg^{-1} N in September compared to 15.1 kg dry matter kg^{-1} N in March/April (O'Donovan et al., 2004). Direct emissions in spring and autumn were reduced compared to those measured in July due to lower temperatures and rainfall.

Concerning plots fertilised with CAN, NH_3 volatilisation loses were not significantly different from control plots for any of the application dates with the exception of April (**Figure 8**) and were always considerably lower than for slurry treatments. N_2O release from these plots was significantly higher in July than for the other months.

When subtracting background fluxes to treatment plots and expressing resulting fluxes per unit of N applied, the effect Timing*Fertiliser type could still be observed on direct ($P<0.0001$, log transformed data) and indirect ($p<0.05$, square root transformed data) N_2O fluxes. These effects were similar to those observed for absolute fluxes (**Table 9).** However, timing of application had a significant effect on total N_2O emissions ($P<0.005$, log transformed data) only for slurry treatments. These results clearly showed an existing trade-off between both indirect and direct N_2O, in summer, which led to significant differences between July and August application in terms of measured N_2O and NH_3 fluxes, but not in terms of total N_2O emissions estimated from these measurements. This trade off could not be observed when comparing these two months with April and September, as lower temperatures and low rainfall in spring and autumn (**Table 7**) led to a significant decrease in both N_2O and NH_3 emissions compared to summer applications.

Friedmann tests performed on CO_2, CH_4 and total GHG confirmed ($P<0.0001$) the effect of application timing already observed in **Figure 8**. The timing of fertiliser application had an effect on CH_4 ($P<0.0001$, Friedmann test), but the effect of such emissions of the total GHG budget was usually negligible.

Table 7: Mean weather data collected from Johnstown Castle meteorological station for the three weeks following each application.

a) April 2009

Application date	Mean temperature (ºC)	Cumulative rainfall (mm)	Cumulative solar radiation (MJ m^{-2})
24 April	9.9	12.1	6.18
25 April	7.8	16.5	6.61
1st week	9.1	45.7	77.4
3 weeks	10.0	60.1	296

b) July 2009

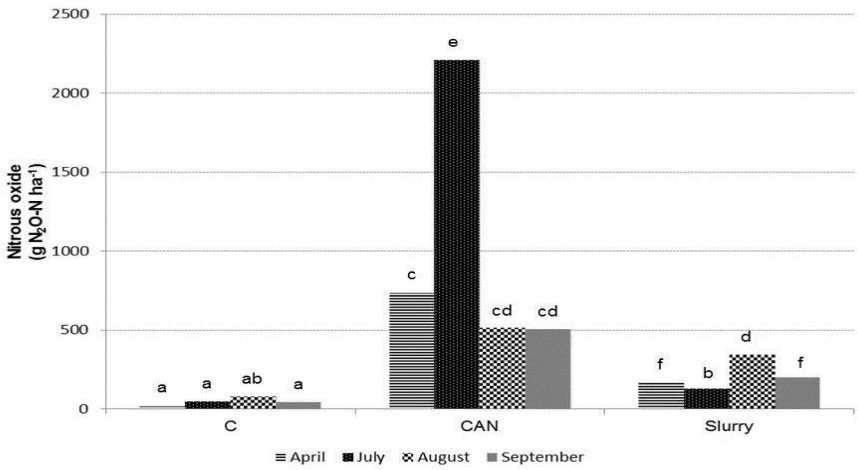
Application date	Mean temperature (ºC)	Cumulative rainfall (mm)	Cumulative solar radiation (MJ m^{-2})
20 July	13.9	0	23.06
21 July	15.4	17.1	16.09
1st week	14.9	42.2	135.5
3 weeks	14.8	108.9	348

c) August 2009

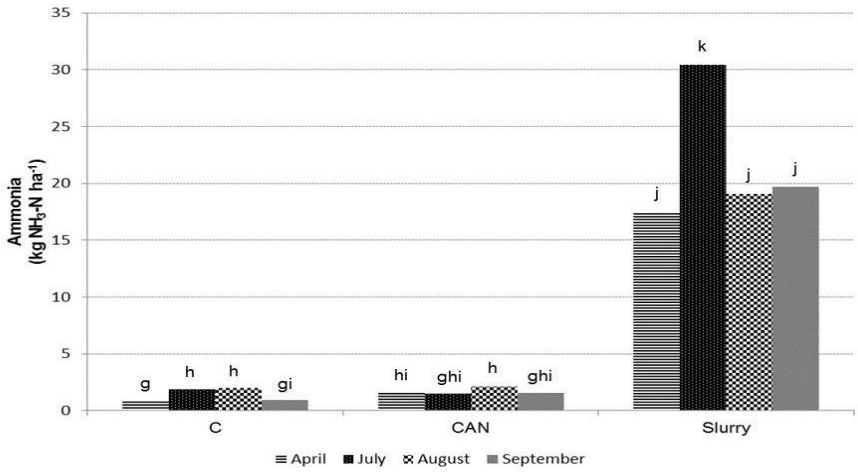
Application date	Mean temperature (ºC)	Cumulative rainfall (mm)	Cumulative solar radiation (MJ m^{-2})
17 August	16.6	0.5	19.6
18 August	16.2	1.4	5.15
1st week	15.1	55.7	83.0
3 weeks	14.1	178	244

d) September 2009

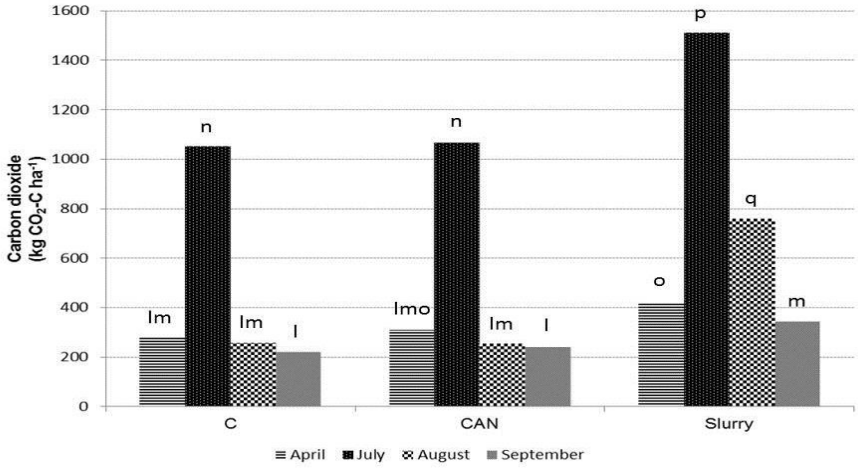
Application date	Mean temperature (ºC)	Cumulative rainfall (mm)	Cumulative solar radiation (MJ m^{-2})
20 September	11.8	0	14.57
21 September	13.1	0	7.25
1st week	13.1	0.7	68.1
3 weeks	12.6	52.3	173



(a)



(b)



(c)

Figure 8: Cumulative fluxes of (a) nitrous oxide, (b) ammonia, and (c) carbon dioxide from experimental plots over the first three weeks following the application of fertiliser. The four application dates were 24 April, 20 July, 17 August, and 20 September 2009.

In terms of total amount of GHG emitted for each three-week period, soil CO_2 efflux dwarfed the other emissions in terms of scale. These CO_2 emissions accounted for about 70% of total GHG emissions from CAN plots, and for up to 88% of those calculated for plots fertilised with slurry. For all treatments, there was a two to four fold increase in July relative to the other spreading dates. Higher summer emissions were to be expected as soil respiration exhibits an exponential response to increases in soil temperature (Lloyd and Taylor, 1994). In addition, the CO_2 losses upon slurry application have to be considered in the context that 1-3 tonnes C ha^{-1} had been applied. As total emissions ranged from 0.2 – 1.6 t C ha-1, there was undoubtedly the potential for additional C sequestration from carbon within the applied slurry.

While some plots did, indeed, appear to retain some of the applied C, some were revealed to release more C than had been applied. A non-parametric Friedmann test was carried out on the amount of CO_2 emitted from slurry treatments (corrected by controls and expressed in % of C applied). There was a significant effect of the application date ($P<0.0001$), with some plots emitting an extra amount of CO_2 (compared to control plots) equal to up to 286% of the applied slurry C. This suggests that an additional amount of soil derived C (in comparison with control plots) was mineralised when cattle slurry was applied at the soil surface. Such phenomenon, known under the generic term of "priming effect", has been widely investigated and is defined, in studies of C turnover, as an extra decomposition of organic C after addition of easily-decomposable organic substances to soil (Dalenberg and Jager, 1989).

Bol et al. (2003b) developed a laboratory method to quantify the priming effect of applying cattle slurry onto grassland soil, and to follow the fate of slurry derived C into the soil. This method, based on the ^{13}C natural abundance tracer technique, uses the natural difference in isotopic signature between C_3 (e.g. rye-grass) and C_4 (e.g. maize) vegetation. By comparing CO_2 effluxes from soils amended with either grass or maize-based slurry and after assuming that CO_2 emission rates from both slurry types are equivalent, it is possible to estimate the proportion of slurry-derived and soil-derived C from the measured total CO_2 efflux. In most statistical analyses carried on in this chapter, there was no significant difference between both types of slurry, which may allow one step further in the investigation of the effect of land spread

cattle slurry on soil C dynamics and the resulting CO_2 balance between the system [soil-grass] and the atmosphere.

Table 8: Non-parametric Spearman rank correlation (P<0.05) calculated between fluxes measured on the field and soil / weather parameters (whenever these were measured). Significant correlations were marked with a star (*).

a) Nitrous oxide

Parameters tested	Spearman rank order correlation
Mean air temperature during sampling period	0.061838*
Cumulative solar radiation during sampling period	-0.055577*
Rainfall (24h before sampling)	0.232639*
Soil temperature	-0.167323*
WFPS	0.294180*

b) Ecosystem respiration

Parameters tested	Spearman rank order correlation
Mean air temperature during sampling period	0.373653*
Cumulative solar radiation during sampling period	0.211320*
Rainfall (24h before sampling)	0.219953*
Soil temperature	0.688280*
WFPS	-0.375021*

Table 9: Direct, indirect and total N_2O emissions measured over the three weeks following each application (in kg CO_2-eq ha^{-1} kg^{-1} N applied). Results from a Fisher LSD test (P<0.05) are given by the letters beside each flux.

a) Calcium Ammonium Nitrate

	Direct N_2O	Indirect N_2O	Total N_2O
April	2.41 (± 0.90) ^{a}	0.12 (± 0.02) ^{f}	2.54 (± 0.88) ^{J}
July	7.26 (± 5.15) ^{b}	0.12 (± 0.04) ^{f}	7.38 (± 5.19) ^{jk}
August	1.69 (± 0.12) ^{a}	0.17 (± 0.05) ^{f}	1.86 (± 0.13) ^{jk}
September	1.66 (± 0.07) ^{ac}	0.12 (± 0.04) ^{f}	1.78 (± 0.09) ^{jk}

b) Slurry

	Direct N_2O	Indirect N_2O	Total N_2O
April	0.73 (± 0.06) ^{d}	1.84 (± 0.16) ^{g}	2.56 (± 0.19) ^{k}
July	0.61 (± 0.10) ^{e}	3.23 (± 0.19) ^{h}	3.84 (± 0.24) ^{l}
August	1.60 (± 0.14) ^{a}	2.20 (± 0.18) ^{i}	3.79 (± 0.22) ^{l}
September	0.96 (± 0.07) ^{cd}	2.29 (± 0.14) ^{i}	3.25 (± 0.17) ^{m}

However, there was no characterisation of the impact of slurry application on CO_2 via photosynthesis. If there had been a greater potential for plant growth (and the remaining growing season is longer) when applying slurry in spring, this might lead to higher C sequestration rates in plant biomass (Soussana et al., 2004, Soussana et al., 2007, Smith et al., 2010) and, therefore, counterbalance the differences in respiratory losses between treatments.

3.4. Conclusion

The results presented in this experiment clearly showed a difference between CAN-fertilised and cattle slurry-spread plots in terms of GHG emitted from the soil. Most particularly, cumulative direct N_2O emissions, and corresponding emission factors, were shown to be significantly higher when applying synthetic fertilisers. Furthermore, those emission factors were seemingly affected by soil and weather conditions, as could be seen from the effect of timing of application on N_2O emissions. It is therefore clear that the use of a single EF value for national GHG inventories, independent from the fertiliser type, the soil conditions and the climate, is unsuitable to an accurate assessment of direct N_2O emissions from the agricultural sector at both national and global scales.

Slurry application technique and the timing of application are of key importance in terms of NH_3 abatement strategies. For many grassland areas, the trailing shoe is considered to be the most effective alternative to conventional splash-plate application, as high stone content of soil and undulating topography make injection unsuitable. However, it was showed in this study that, in terms of GHG field balance, the decrease in indirect N_2O emissions, following a reduction in NH_3 volatilisation losses, could be easily offset by an increase in direct N_2O emissions and ecosystem respiration, potentially leading to a positive global GHG balance. Switching from summer to spring application was by far more efficient to mitigate both NH_3 and GHG as all gaseous emissions from the soil were reduced, due to favourable soil and climatic factors. Any potential trade-off between NH_3 and N_2O emissions was cancelled, leading to an overall positive effect on reactive N losses and offering agronomic benefits to farmers. In addition, applying slurry during spring reduced the risk of 'priming effects' on soil CO_2 efflux, as soil temperature and hence microbial respiration rates, were reduced. In order, to maximise the benefit of applying slurry in

spring, the use of trailing-shoe is also of a great interest as it increases the number of available spreading days (Lalor and Schulte, 2008), offering more flexibility for slurry application management.

The next chapter will focus on the short-term dynamics of slurry-derived C following land application of cattle slurry, with an emphasis on comparing slurry application techniques and identifying possible priming effects.

Chapter 4. Short-term dynamics of slurry-derived C post-application

4.1. Introduction

Over the past two decades, various methods have been suggested to mitigate greenhouse gas emissions to the atmosphere. Among these has been the use of terrestrial C sinks, such as forests and soils, to remove CO_2 from the atmosphere through photosynthesis and store the C in organic forms, including plant biomass and SOM (Houghton, 2001). This process is known as "Carbon sequestration" and is widely seen as an opportunity to reduce GHG emissions from agricultural systems (Ogle et al., 2004, Soussana et al., 2004). Indeed, under Article 3.4 of the Kyoto Protocol of the United Nations Framework Convention on Climate Change, signatory Annex 1 countries may take into account this sequestration as a contribution to reducing greenhouse gas emissions.

The total global soil C pool (including organic and inorganic C) is estimated at 2.3 Gt for the 0-1 m depth interval, representing therefore the main terrestrial C pool (Lal, 2003, Jones and Donnelly, 2004). This is particularly true for grassland ecosystems, where up to 98% of carbon can be stored below-ground (Hungate et al., 1997).

Most studies investigating the potential of soils to sequester C focused on the impact of land-use change (LUC). It is estimated that 14% of global GHG emissions come from LUC (IPCC, 2006). Guo and Gifford (2002) showed in their meta-analysis that the conversion from cropped soils to pastures had the greatest impact on C sequestration. Indeed, grassland soils can sequester relatively large amounts of C, significantly more than arable crops (Conant et al., 2001), but it is still uncertain how long after the conversion such soils will keep storing C and whether there is an upper limit to soil C stocks (Franck, 2002).

Temperate grasslands account for about 20% of the land area in Europe (Soussana et al., 2007) and 45% of the area in Ireland (Gardiner & Radford, 1980). Following estimates from Soussana et al. (2004, 2007), European grasslands may act as a sink for atmospheric CO_2, but uncertainties in such estimations remain very high

(Janssens et al., 2003). Management of temperate grasslands is known to modify SOC storage through practices which affect both C inputs and outputs from soils (Ogle et al., 2004) and should therefore be considered as one possibility to increase soil C stocks (Soussana et al., 2004).

Most long-term pastures are probably near equilibrium with respect to C and will not be significant sinks without some additional inputs such as fertiliser (Bruce et al., 1999). In general, organic manure inputs SOC pool and help to maintain or to increase soil C stocks (Lal, 2003, Soussana et al., 2004, Jones et al., 2006). In a cutting regime, part of the primary production is usually exported as hay or silage, and compensated by farm manure and slurry application (Soussana et al., 2004). However, C import caused by manure or slurry application might not compensate C exports (Soussana et al., 2007).

Sequestration and losses of C from liquid manures incorporated or applied to agricultural soils has already been considered by various studies, either in laboratory incubation experiments (Saviozzi et al., 1997, Dendooven et al., 1998a, Flessa and Beese, 2000, Glaser et al., 2001, Bol et al., 2003a, 2003b, 2004, Kuzyakov and Bol, 2004b, 2005, 2006, Fangueiro et al., 2007) or under field conditions (Martinez and Peu, 2000, Rochette et al., 2000a, 2004, 2006, Chantigny et al., 2001, Jones et al., 2005, 2006, Sauheitl et al., 2005, Angers et al., 2007). Incorporation of slurry into agricultural soils tend to increase soil respiration rates, particularly in the first few days following application (Flessa and Beese, 2000, Rochette et al., 2000a, 2004, 2006, Chantigny et al., 2001, Bol et al., 2003b, Jones et al., 2005, 2006, Angers et al., 2007). It may also increase soil microbial biomass (Saviozzi et al., 1997, Rochette et al., 2000a, 2004, Bol et al., 2003a), even though this effect on SMB-C is not always significant (Chantigny et al., 2001, Bol et al., 2003a). Rochette et al. (2000a) showed that the SMB-C peak usually correspond with a peak of respiratory activity, which indicates a significant microbial contribution to soil CO_2 efflux. Furthermore, Sauheitl et al. (2005) found evidence of a significant microbial utilisation of slurry-derived C in the first few days following manure application. Therefore, incorporation of slurry C in bulk soil (Glaser et al., 2001) is controlled by microbial uptake (Bol et al., 2003a).

In the end, most of these studies suggested multi-stage models for the incorporation and degradation of slurry-derived C in the soil (Rochette et al., 2000a, Chantigny et al., 2001, Glaser et al., 2001, Bol et al., 2003a, 2003b, Fangueiro et al., 2007). However, some of the laboratory studies (Saviozzi et al., 1997, Dendooven et al., 1998a, Flessa and Beese, 2000) and nearly all field studies (Martinez and Peu, 2000, Rochette et al., 2000a, 2004, 2006, Chantigny et al., 2001, Jones et al., 2005, 2006) mentioned previously did not accurately quantify the amount of slurry-derived C sequestered or lost from the soil. Indeed, by only subtracting control fluxes from treatments, they did not take into account the potential priming effects of slurry addition on the mineralisation of native SOM (Bol et al., 2003b).

The term "priming effect" (PE) was first introduced by Bingemann et al. (1953), but the phenomenon itself was discovered by Löhnis (1926) when studying the decomposition of green manure of legume plants in soil. PEs are generally defined as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil" (Kuzyakov et al., 2000). When considering C turnover studies, such phenomenon is usually defined more precisely as the extra-decomposition of SOM following the addition of easily mineralisable C and N to soil (Dalenberg and Jager, 1989). Priming effect on soil CO_2 efflux have been observed following the incorporation of cattle slurry into grassland soils (Bol et al., 2003b, Kuzyakov and Bol, 2006, Fangueiro et al., 2007). Such phenomenon could also be measured on C leaching losses following the deposition of dung patches onto the soil in the field (Bol et al., 1999).

The development and use of C isotope tracer techniques is an invaluable tool for tracing the fate of C and elucidating different components of the C cycle. The natural abundance ^{13}C tracer technique exploits the natural difference in stable isotope ^{13}C content between C_3 and C_4 vegetation (Farquhar et al., 1982). It was developed to investigate the dynamic of 'native' and 'new' soil C following a C_3-C_4 vegetation change (Balesdent et al., 1987, Rochette and Flanagan, 1997, Boutton et al., 1998, Rochette et al., 1999, Dorodnikov et al., 2007). It was also used to trace the fate of newly incorporated C following the addition of C_4-derived substrates such as glucose (Hogberg and Ekblad, 1996, Blagodatskaya et al., 2007) , dung (Amelung et al., 1999, Bol et al., 1999, 2004, Dungait et al., 2005) or slurry (Glaser et al., 2001, Bol et al.,

2003a, 2003b, Kuzyakov and Bol, 2004b, 2005, 2006, Sauheitl et al., 2005, Fangueiro et al., 2007, Angers et al., 2007) to C_3 soils. In most cases, this tracer technique was used to separate two C sources of CO_2 release or dissolved organic carbon (DOC) losses, even though the use of specific experimental designs (Kuzyakov and Bol, 2004b, Kuzyakov and Bol, 2005, Kuzyakov and Bol, 2006) or the coupling with artificial ^{14}C-labelling may allow the separation of three or more C sources.

So far, only few field studies have addressed the fate slurry-derived C after incorporation into agricultural soils. Angers et al. (2007) used the natural abundance ^{13}C tracer technique to study the short-term mineralisation of pig-slurry under field conditions. Sauheitl et al. (2005) coupled such tracer technique with compound-specific analysis to investigate the dynamic of degradation of plant- and microbial derived sugars present in the applied cattle slurry, as well as the dynamic of synthesis of microbial-derived sugars in a grassland soil following slurry application.

The objectives of this study were to a) assess the proportion of field applied slurry C released as CO_2, b) quantify the effects of applied C on the mineralisation of SOM in a permanent grassland system, and c) to investigate whether the application technique affect the subsequent dynamic of applied C in the soil.

4.2. Materials and methods

4.2.1. Experimental site

The experiment was undertaken in August 2009 on a grassland site in Johnstown Castle, Wexford, Ireland (52°18′N; 6°30′W), simultaneously with the third slurry application of the experiment presented in the **Chapter 3**.

The characteristics of the field site are given in **Chapter 2**.

The plots used in this experiment (1.5 x 2m) were a sub-sample of those described in **Chapter 2**. Each slurry treated plot had already been applied in April and July 2009 with the same treatments.

4.2.2. Slurry preparation and application

The experiment was conducted between 17th August and 16th September 2009. Prior to this, beef cattle were fed with either ryegrass (*Lolium perenne*, a C_3 plant with a $\delta^{13}C$ value of ca. ~27‰) or maize (Zea mays, a C_4 plant with a $\delta^{13}C$ value of ca. ~13‰) silages leading to C_3 and C_4 faeces and urine with naturally different ^{13}C contents. Both of them were collected separately and stored at 4ºC for one month before the start of the experiment.

The $\delta^{13}C$ value (expressed in ‰ unit) is used to express the isotopic composition of any sample (S) by relating the $^{12}C/^{13}C$ isotope ratio of the sample (molar abundance ratio R_S) to the stable isotope ratio of the international standard V-PDB (Vienna-Pee Dee Belemnite, $R_{V\text{-}PDB}$) using the following equation:

$$\delta^{13}C_S = \frac{R_S - R_{V-PDB}}{R_{V-PDB}} \times 1000 \qquad (11)$$

The different slurry treatments used in this experiment were prepared by mixing thoroughly 1/5 of faeces with 4/5 of urine. The resulting slurry was poured in a watering can to be subsequently applied on the experimental plots (see **Chapter 3**).

Slurry applications occurred around 10am. Maize-based slurry (C_4, $\delta^{13}C$ = -18.3‰) was applied onto two sets of three plots, either by splash-plate or trailing-shoe technique, at a rate of 30t ha^{-1} (**Table 10**). When applying the C_4 slurry onto a C_3 pasture ($\delta^{13}C$ of soil = -28.2‰), any increase of soil isotopic signal can be attributed to the incorporation of C_4 slurry-C. To account for a possible isotopic fractionation during soil processes, grass-based slurry (C_3, $\delta^{13}C$ = -29.8‰) was also applied onto three plots, in order to level out the effect of microbial fractionation on $\delta^{13}C$ value from slurry (assuming that isotopic discrimination in soil is similar for both slurry types). Finally, three non-fertilised plots were considered as controls.

The difference in $\delta^{13}C$ signatures between C_3 and C_4 slurries was 11.5‰, which is less than the maximum shift of ca. 14‰ observed between maize and ryegrass. This was probably resulting from C_3 concentrate feed which entered the C_4 slurry. Other slurry and soil characteristics, such as DM and total C content, are given in **Table 10**.

Table 10: Soil and slurry characteristics of interest for the isotopic partitioning experiment

	DM content (%)	C content (g C kg^{-1} soil DM or slurry)	$\delta^{13}C$ (‰)
Soil	n.a. (*)	52	-28.2
Maize slurry (SP)	3.29	13.9	-18.3
Maize slurry (TS)	3.47	14.6	-18.3
Grass slurry(SP)	2.06	7.9	-29.8

(*) Soil moisture at the time of slurry application was not measured.

4.2.3. Soil and slurry analysis

At the start of the experiment, soil samples were collected on each plot, passed through a 2 mm sieve and oven-dried 24 hours at 80 ºC. Samples were then acid digested with 1M H_2SO_4, to remove any inorganic C, and ground using a bowl mill). Sub-samples of applied slurries were also oven-dried at 80 ºC and milled.

These samples were then run on a CHN auto-analyser (Carlo Erba NA2000, Milan, Italy) for determination of C and N content. This analyser was coupled to a continuous flow isotope ratio mass spectrometer (IRMS, Finnegan Delta Plus, San Jose, California, USA) for C isotope determination. The $\delta^{13}C_S$ value of each sample was then calculated using the equation 11 (see paragraph 4.2.2.).

4.2.4. C respiration and statistical analysis

Soil CO_2 respiration fluxes were measured after 1, 3 and 6 hours, as well as on days 1, 3, 4, 5, 7, 8, 13, 20 and 30 following slurry application, using a closed static chamber in combination with an infra-red CO_2 analyser (EGM-4, PP Systems, Hitchin, Herts. UK). Fluxes were measured from the linear increase in CO_2 concentration over time (see **Chapter 3**).

The stable isotope ratio $^{12}C/^{13}C$ was measured using the continuous flow IRMS described in **4.2.3**. N_2O was separated from CO_2 by a gas chromatograph with a C13 poropak column. Standards of known $\delta^{13}C$ were periodically injected (after every five samples in order to determine measurement precision). CO_2 concentrations of the samples were determined by integrating the area under of $^{12}CO_2$ and comparing these values with those of a calibration curve generated from injections of standards with known CO_2 concentration. Relationships between $\delta^{13}C$ and CO_2 concentration were generated for each sampling date.

The effect of treatments on CO_2 efflux rates and $\delta^{13}C$ was analysed using the statistical package STATISTICA version 10 (Statsoft, Tulsa, Oklahoma).

Cumulative CO_2 fluxes for the entire experiment were analysed using a one-way ANOVA to investigate the effect of treatments. Differences between individual treatments were assessed using Least Significant Difference (Fisher LSD test, α=0.05).

CO_2 efflux rates at each sampling time were compared using a Repeated Measures ANOVA on square root transformed data, with 13 levels of repetition corresponding to the number of sampling dates. This was followed by post-hoc separation of means using a Fisher LSD test (α=0.05).

In terms of daily $\delta^{13}C$ values for CO_2 respired from the soil, the structure of the data did not allow the use of a Repeated Measures ANOVA to assess the effect of treatment. As a consequence, for each sampling date, the effect of treatments onto these $\delta^{13}C$ values was analysed using a one way ANOVA carried on either raw data or after log-transformation. However, for t = 3 hours after slurry application, the distribution of data did not allow the use of such parametric analysis. Therefore, corresponding $\delta^{13}C$ values were compared using a non-parametric Kruskal-Wallis test, coupled with a multi-comparison of means.

Partitioning of the respired CO_2 into slurry-derived and soil-derived components was estimated using the isotopic mass balance approach described by Bol et al. (2003b). The difference in $\delta^{13}C$ values between the respired CO_2 from the C_3 and C_4 slurry treatments was used to quantify the proportion of slurry- versus soil-derived CO_2-C emitted from the soil.

This calculation was performed as follows for splash-plate applied slurries:

$$sdC_{SP\_M} = \frac{\delta^{13}C_{g(SP_M)} - \delta^{13}C_{g(SP_G)}}{\delta^{13}C_{sl(SP_M)} - \delta^{13}C_{sl(SP_G)}} \times 100 \quad (12)$$

where sdC_{SP_M} was the proportion of slurry-derived C (in %) in the total CO_2 efflux from plots splash-plate applied with maize-based slurry (treatment SP_M),and $\delta^{13}C_g$ and $\delta^{13}C_{sl}$ were the $\delta^{13}C$ values, calculated for C_4 (SP_M) and C_3 (SP_G) slurry treatments, from the emitted CO_2 and the slurry initially applied onto experimental plots respectively.

This simple calculation is only truly valid when daily CO_2 emission rates are identical. This was verified in this study as a Student t-test showed no statistical difference between treatments SP_M and SP_G, whatever the sampling date.

The amount of primed C was then calculated as follows:

$$PE_{SP\_M} = F_{SP\_M} \times \left(1 - sdC_{SP_M}\right) - F_{Control} \quad (13)$$

where any priming effect (PE_{SP_M}, in kg C ha^{-1}) measured onto SP_M treated plots was function of the total CO_2 efflux (F_{SP_M}, in kg C ha^{-1}) from these plots, the proportion of soil-derived C (1-sdC_{SP_M}, in %) and the total efflux measured onto control plots ($F_{Control}$, in kg C ha^{-1}).

In addition, a priming factor (PF_{SP_M}) was calculated by dividing the amount of CO_2 respired from the native soil C in the SP_M treated plot by the amount of CO_2 respired from the control:

$$PF_{SP\_M} = \frac{PE_{SP\_M}}{F_{Control}} \quad (14)$$

Therefore, a value above 1 indicates positive priming; a value equal to 1 indicates the absence of priming and value below 1 indicates a negative priming effect.

Although the application technique was different, there was no significant difference in terms of CO_2 emission rates between the trailing-shoe applied plots (TS_M) and SP_G treated plots, except for t = 1 hour after slurry application

($P<0.001$). Therefore, the equations (12), (13) and (14) were also used in an attempt to calculate, for each sampling date, the proportion of slurry- and soil-derived CO_2-C from TS_M treated plots, as well as the primed C and priming factors.

Cumulative values over the entire experiment were then calculated for the CO_2 efflux from each treatment, as well as for slurry-derived, soil-derived and primed C. When no daily samples were available, it was assumed that the amount of daily respired CO_2 changed linearly between the nearest sampling dates for which samples were available. As there was no statistical difference, regarding cumulative fluxes, between SP_G, SP_M and TS_M treatments (see below), slurry-derived, soil-derived and primed C could be calculated for both splash-plate and trailing-shoe applied plots.

For the parameters calculated using equation (12), (13) and (14), as well as for cumulative values, results were expressed as mean values (± standard error) for each treatment.

Finally, C losses from the initial (soil + slurry) pool were calculated for the 0-2 cm soil depth interval, assuming an average soil bulk density of 947 kg m^{-3}. This gave estimation for the initial soil native C pool of 9845 kg C ha^{-1}, on which the following amounts of slurry-derived C were applied: 417, 439 and 237 kg C ha^{-1} for the SP_M, TS_M and SP_G treated plots respectively.

4.3. Results and Discussion

4.3.1. Analysis of CO_2 efflux rates and $\delta^{13}C$ values measured on the field

The closed chamber method used for the measurement of soil CO2 efflux is discussed in the **Chapter 3** (paragraph **3.3.1**).

The application of cattle slurry onto the soil strongly increased ($P<0.005$) the amount of respired CO_2 from the soil over the 30 days of the experiment (**Figure 9**). On average, CO_2 cumulative losses from slurry amended plots accounted for 8.4 to 10.7% of initial C pool (i.e. the sum of initial soil native C pool and the applied slurry C). However, when subtracting background emissions measured from control plots,

the extra C respired from slurry treated plots represented 215, 143 and 164% of applied slurry C for the SP_G, SP_M and TS_M treatments respectively. Therefore these results showed that high CO_2 losses from slurry amended plots originated not only from the mineralisation of some slurry-derived C but also from an increase of the mineralisation of the native SOM itself.

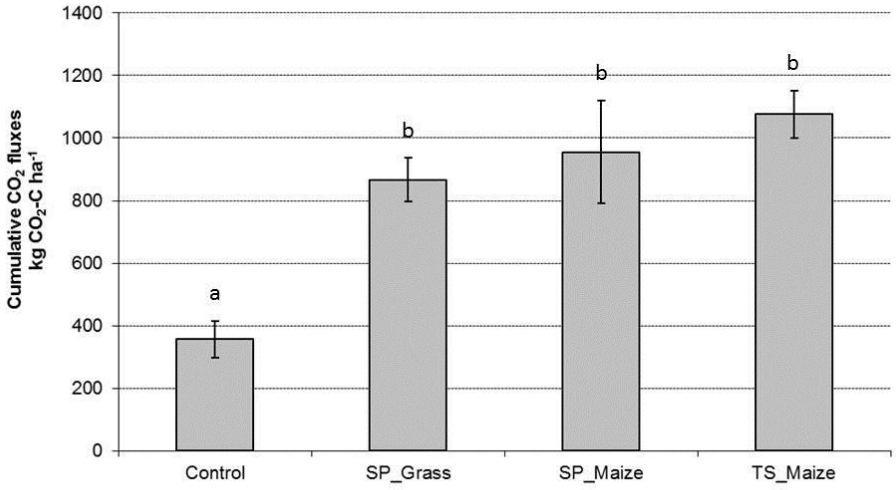


Figure 9: Cumulative CO_2 efflux for each treatment, over the entire experiment (30 days). Results are expressed as mean values, with the standard error being given by the error bars. Homogeneous groups resulting from a Fisher LSD test (α=0.05) are given by small letters.

Cumulative emissions showed no significant differences between slurry treatments over the entire experiment (**Figure 9**), including no effect of the application technique as discussed in the previous chapter (see Chapter 3). In terms of CO_2 efflux rates measured for each sampling date, there was a significant effect "Sampling date * Treatment" ($P<0.005$), with emissions from TS_M treated plots being significantly higher than those SP_G plots after t = 1 hour and t = 1 day, while being lower than SP_M treated plots on day 13 following slurry application (**Figure 10**).

The increase in respired C was significant for the first four days following the application of slurry. It remained significant on day 5 for trailing-shoe applied plots,

but CO_2 emissions were back to background levels for subsequent sampling dates (**Figure 10**). All treatments showed a peak in CO_2 release from soils 6 hours after spreading, with 131, 100 and 75 kg C ha^{-1} day^{-1} emitted by SP_G, SP_M and TS_M treatments respectively, but while plots spread with grass-based slurry steadily decreased then to reach background values on day 5, plots amended with maize-based slurry displayed a second and higher peak on day 3, with 109 and 98 kg C ha^{-1} day^{-1} emitted from SP_M and TS_M treatments respectively (**Figure 10**).

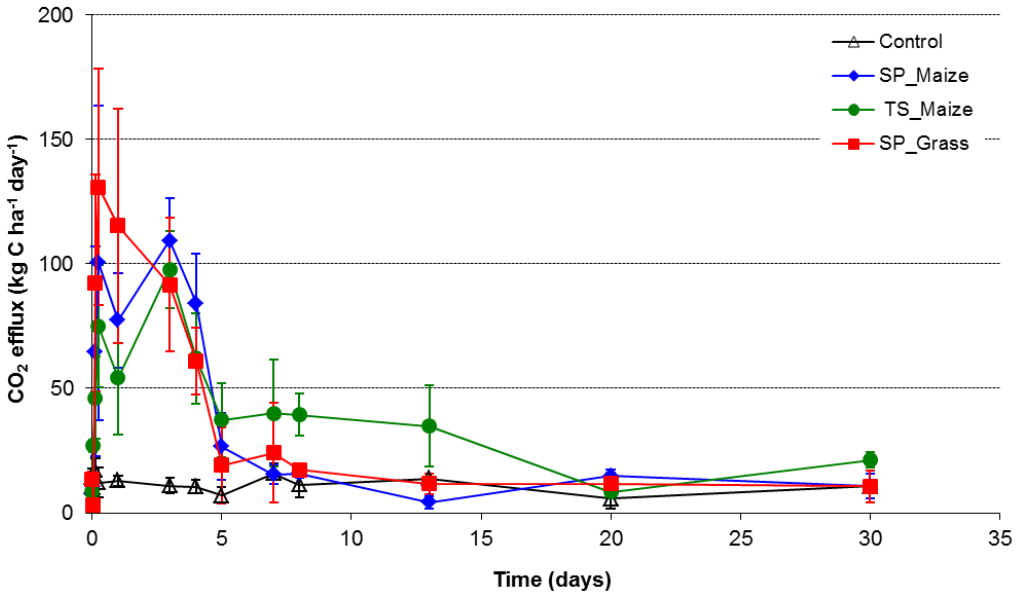


Figure 10: Daily CO_2 efflux rates emitted from field plots during the 30 days of the experiment. Errors bars correspond to standard errors associated with mean value measured at each sampling date.

Similar dynamic for CO_2 efflux from soil were measured onto the same plots after applying cattle slurry in April, July and September 2009 (see **Chapter 3** and **Figure 11**). Such short term patterns of CO_2 fluxes have been observed in the literature after application of slurry to agricultural soils (Flessa and Beese, 2000, Rochette et al., 2000a, Chantigny et al., 2001, Bol et al., 2003b, Fangueiro et al., 2007).

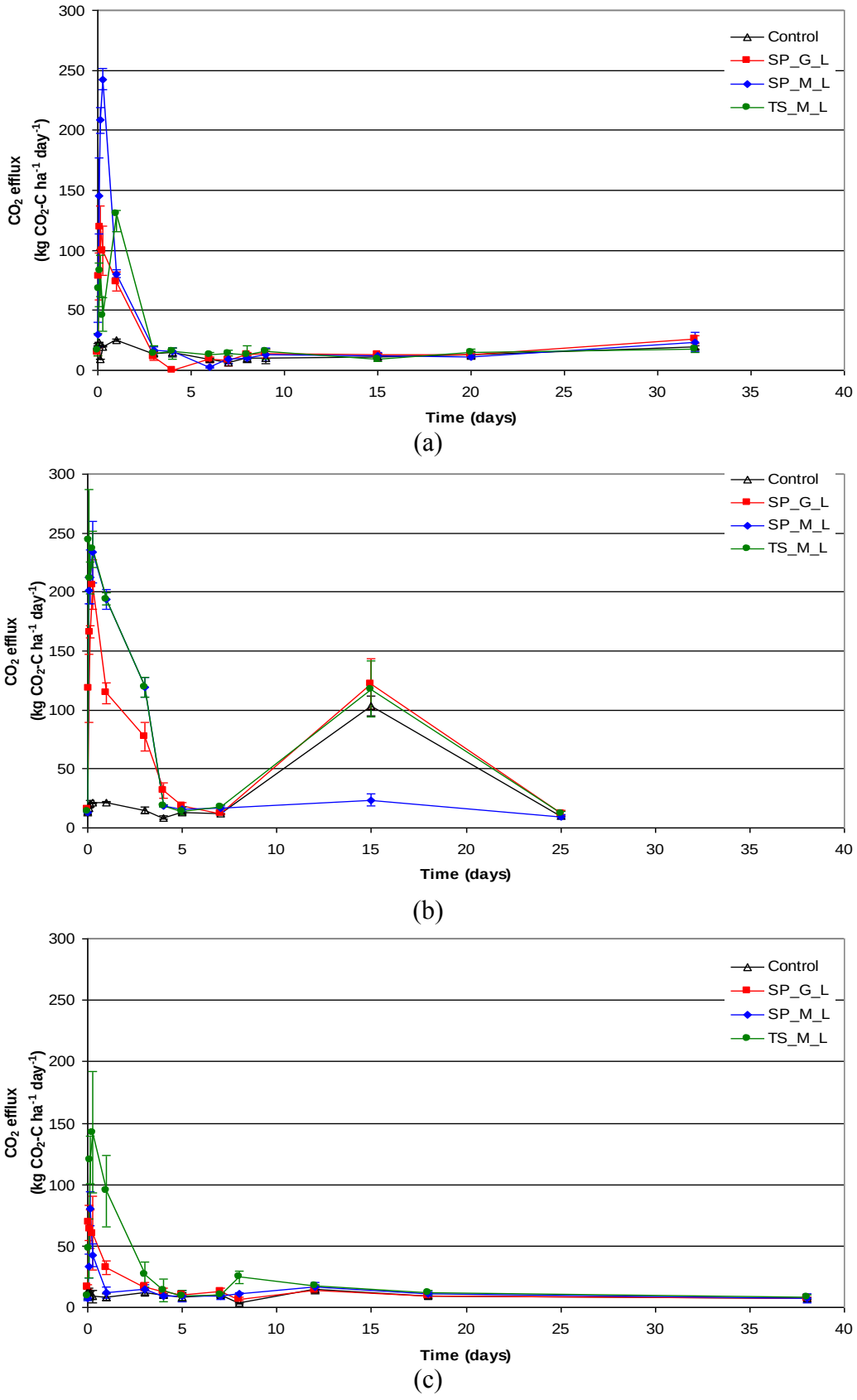


Figure 11: Daily CO_2 efflux rates emitted from field plots following application of cattle slurry in (a) April, (b) July and (c) September 2009 (data from Chapter 3). Errors bars correspond to standard errors associated with mean value measured at each sampling date.

This temporal pattern of CO_2 efflux from soil, following application of cattle slurry, can be split in three periods, as already discussed by Rochette et al. (2000). First, CO_2 fluxes were increased by a factor 6 to 11 only 6 hours after slurry spreading, compared with background values. These short lived bursts of CO_2 were possibly the result of non-biological release of CO_2 (Rochette et al., 2000, Chantigny et al., 2001, Rochette et al., 2004). Carbonate ions are produced during anaerobic slurry storage by hydrolysis of urea and decomposition of organic components in the slurry (Sommer and Husted, 1995, Sommer and Sherlock, 1996) and are likely to be released when the alkaline slurry is applied to an acidic soil.

Following this initial short lived peak of CO_2, a second peak was observed on day 3 for SP_M and TS_M treatments, but not for SP_G treatment, before a gradual decrease of CO_2 emission rates until day 5. Rochette et al. (2000) observed a similar adjustment phase of about 20 days, hence substantially longer than the one observed in this study. They suggested that this period reflected the time during which soil heterotrophs were utilising the easily decomposable C from slurry. It has been shown previously that volatile fatty acids (VFA) are the main source of easily degradable C in anaerobically stored slurries and that they are metabolised within a few days after soil amendment (McGill and Jackson, 1977, Kirchmann and Lundvall, 1993, Sommer and Husted, 1995).

Finally, Rochette et al. (2000) observed, after this adjustment phase, a period of relatively constant CO_2 emission rates from slurry treated plots with a small but significant difference between manured and control plots. They attributed such pattern to the decomposition of more recalcitrant organic substrates. In the present study, such period was observed only for the TS_M treated plots whereas SP_G and SP_M plots displayed a return of CO_2 emission rates to background values after only 5 days. One possible explanation could be the fact that traditional broadcast of cattle slurry tend to dilute applied slurry onto a wider area than when using the trailing-shoe technique, minimising the impact of the low decomposition rates observed for the most recalcitrant organic fractions from slurry (Saviozzi et al., 1997) on fluctuating background fluxes.

4.3.2. $\delta^{13}C$ value of the emitted CO_2 and quantification of the source of the CO_2 emissions

The $\delta^{13}C$ values of CO_2 emitted from control and C_3 slurry amended plots were relatively constant (-23.2 ± 0.1‰ and -23.3 ± 0.2‰ respectively) during the experimental period (**Figure 12)**. The average $\delta^{13}C$ of CO_2 in the control plots was 5‰ higher the value measured for the bulk soil where it originated from (-28.2‰). This difference is likely to result from the slower diffusion of heavier $^{13}CO_2$ molecules that that of the $^{12}CO_2$ molecules (Cerling et al., 1991) and is in agreement with what was already observed in the literature (Angers et al., 2007). The $\delta^{13}C$ values of CO_2 evolved from C_4 slurry applied plots (SP_M and TS_M treatments) quickly increased during the first few hours following the application of slurry, reaching a peak value of -20 ± 0.5‰ after 6 hours and -21.7 ± 0.2‰ after 24 hours, respectively. After a small decrease, the same treatments peaked again on day 4, with $\delta^{13}C$ values of -19.7 ± 0.3‰ and -21.4 ± 0.4‰ for SP_M and TS_M treated plots respectively, before slowly decreasing to reach values of -22.9 ± 0.1‰ and -22.8 ± 0.1‰ after on day 30. The $\delta^{13}C$ values from both treatments only significantly differed after 6 hours, 3 and 4 days following slurry application ($P<0.0001$ each time), with the SP_M treatment showing higher values on each occasion.

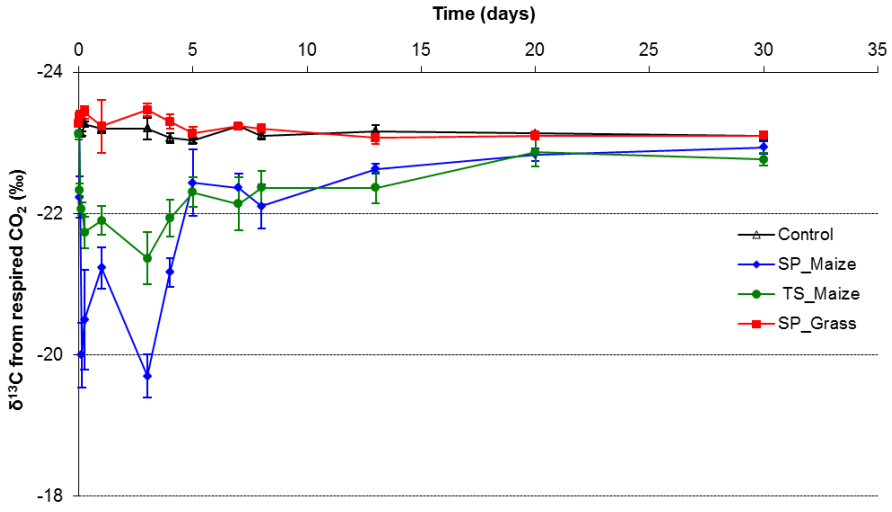


Figure 12: $\delta^{13}C$ values from CO_2 sampled from field plots during the 30 days of the experiment. Errors bars correspond to standard errors associated with mean value measured at each sampling date.

The $\delta^{13}C$ values of CO_2 emitted from C_4 slurry treated plots remained statistically higher than those measured for control and C_3 slurry treated plots for all sampling dates, except for day 5 (P=0.078) and day 20 (P=0.224). The ^{13}C natural abundance tracer technique allowed us then to use these $\delta^{13}C$ values to calculate the contribution of slurry-derived C to the total CO_2 efflux from SP_M and TS_M treated plots (**Figure 13**).

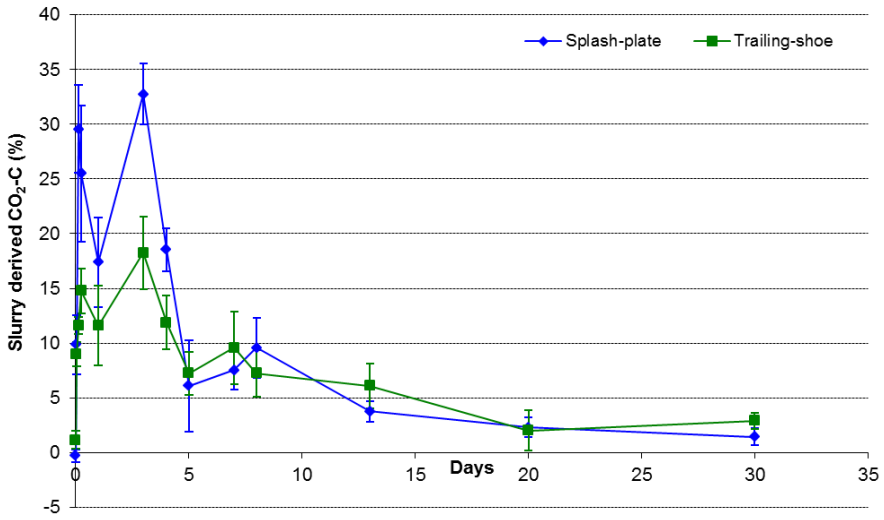


Figure 13: Proportion of slurry-derived CO_2-C estimated from the total CO_2 efflux measured onto field plots applied with maize-based (C_3) cattle slurry. Two application techniques are compared. Errors bars correspond to standard errors associated with mean value estimated for each sampling date.

For both SP_M and TS_M treatments, the contribution of applied slurry C to the total CO_2 efflux from soil increased steeply during the first few hours following slurry application, peaking at 30 and 15% after 3 and 6 hours respectively (**Figure 13**). These peaking values appeared to be synchronised with the first peaks of CO_2 emission rates observed for the same treatments and attributed to the non-biological immediate release of CO_2 dissolved in slurry when applied in the field (see **4.3.1**). In anaerobically stored pig or cattle slurry, carbonates may arise from urea hydrolysis and decomposition of VFAs, which are dominated by acetate (Sommer and Husted, 1995). Anaerobic degradation of acetate results in the production of ^{13}C-depleted CH_4 (Krzycki et al., 1987) and subsequent ^{13}C enriched CO_2 (Boehme et al., 1996). As the pH of slurry usually increase during storage (Portejoie et al., 2003), part of this CO_2 would be solubilised and stored in slurry as carbonates (Sommer and Husted, 1995),

which would explain the enrichment in ^{13}C of the slurry-derived carbonates compared to the overall slurry signature.

However, $\delta^{13}C$ values measured for such rapid release of slurry inorganic C (carbonates) were not as high as those (ca. -9‰) measured by Angers et al. (2007) for CO_2 at the depth of 10 cm below the soil surface, following the incorporation of maize-derived pig slurry (with or without barley straw) into a bare soil. This was probably due to a lower release of non-biological CO_2, as shown by our lower contribution of applied slurry C to such early CO_2 emission peak, compared to the values of ca. 50% observed by Chantigny et al. (2001). Such difference is likely to result from the reported lower total inorganic carbon (TIC) content in cattle slurry than in the pig slurries (Sommer and Husted, 1995). Another explanation would be the fact that, in this study, the CO_2 was sampled at the soil surface rather than down in the soil profile. Therefore, such contribution of slurry-derived carbonates to the total CO_2 efflux was supposedly diluted within the overall CO_2 signal resulting from the various C sources present in soil.

The high $\delta^{13}C$ values of the evolved CO_2 could also be partly attributed to the direct mineralisation of VFAs present in cattle slurry, although it usually takes a few days for such compounds to decompose in soil (Kirchmann and Lundvall, 1993, Chantigny et al., 2004). The analysis of the effect of slurry-derived C on soil biomass activity should give an idea of the delay needed for soil micro-organisms to be activated after applying slurry onto the soil (see **4.3.3**), and therefore confirm or deny the possibility of an early mineralisation of the labile C from slurry.

Another peak in the contribution of applied slurry C to the total CO_2 efflux was observed on day 3 for both treatments, with 33 and 18% respectively (**Figure 13**). Bol et al. (2003b), using similar ^{13}C natural abundance isotope tracer technique in a laboratory experiment, also found an increase in the contribution of slurry C to the total efflux of CO_2 following the incorporation of cattle slurry to two different soils. A few other studies (Kirchmann and Lundvall, 1993, Dendooven et al., 1998, Flessa and Beese, 2000, Rochette et al., 2000, Chantigny et al., 2001, Rochette et al., 2004, Jones et al., 2005, Rochette et al., 2006) report similar contributions of manure-derived C to soil CO_2 efflux during the first few days following their application. However, most of them estimated these contributions by subtracting background fluxes to CO_2 fluxes

measured from amended plots. By doing so, they did not account for a possible positive priming effect of applied manure onto native soil C pools (Kuzyakov et al., 2000, Bol et al., 2003b), therefore overestimating such contribution of manure C to the emitted CO_2. These high values for the contribution of applied slurry C to the total CO_2 efflux were again synchronised with the peak values observed for the same treatments in terms of CO_2 emission, suggesting that the contribution of applied slurry C to the total CO_2 efflux from soil is maximal when the rate of decomposition of the easily degradable C fraction present in this slurry is also at its maximum.

It has been shown previously that the labile C added to soil with manures can increase both microbial biomass and microbial activity (Ritz et al., 1997, Saviozzi et al., 1997, Martin-Olmedo and Rees, 1999, Rochette et al., 2000a). It was also suggested that incorporation of slurry-derived C in the bulk soil is controlled by microbial uptake (Glaser et al., 2001, Bol et al., 2003a). For example, Sauheitl et al. (2004) showed that, during this early phase of slurry decomposition, the soil microbial biomass can use labile soluble C from slurry to synthesise microbial sugars within only a few hours following slurry application.

Following this second peak in terms of the utilisation of slurry C by soil micro-organisms, the contribution of slurry-derived C to the total CO_2 efflux rapidly decreased to reach average values of 6.1 and 7.2% after 5 days (**Figure 5**) for treatments SP_M and TS_M respectively. These contributions keep decreasing then gradually to be less than 3% at the end of the experiment.

All these results can refer back to the two-phase model of decomposition of slurry-derived C suggested by Rochette et al. (2000). This model has already been discussed in several studies and could be developed through the use of the ^{13}C natural abundance isotope tracer technique (Glaser et al., 2001, Bol et al., 2003a, 2003b, Sauheitl et al., 2005, Fangueiro et al., 2007), as well as compound specific analyses (Sauheitl et al., 2005), to investigate the fate of slurry derived C in agricultural soils. Following the initial non biological loss of CO_2, in the first few hours after application, the more liquid phase of slurry, which percolates rapidly into the upper soil, provides simple molecules, such as amino-acids and amino-sugars, to the soil microbial biomass. This readily available and mineralisable C is decomposed exponentially and utilised as a source of energy or for the synthesis of microbial

sugars. Therefore, as mentioned by Sauheitl et al. (2005), it is possible to quantify the microbial turnover of slurry-derived organic matter, in a short term, by compound-specific analysis of microbial sugars and their dynamic of synthesis.

Once the source of labile C is exhausted, soil microbial biomass switches to the decomposition of more recalcitrant C material (Rochette et al., 2000). During this second phase, the input of slurry-derived C to the microbial biomass is dominated by the less mobile, more complex particulate fraction of slurry gradually incorporated to the soil from the solid phase (Glaser et al., 2001, Bol et al, 2003a). However, as suggested by Sauheitl et al. (2005), this apparent two phase incorporation of slurry is caused by a substrate consisting in different particle-size classes which infiltrate at different speeds into soils. The same authors considered that, rather than the classic two-phase model exposed above, where the soil micro-organisms preferentially degrade the labile C fraction from slurry and switch to the more recalcitrant one once their first C source is exhausted, we have a new C input from slurry that is only available for decomposition in two time frames. In such model, there is a preferential incorporation of small particles into soil during the beginning of decay of surface applied organic matter. Larger size fractions first have to become smaller by decay on the soil surface before they can be incorporated into the soil.

Concerning the effect of slurry application technique, the $\delta^{13}C$ values and subsequent contributions of slurry-derived C to total CO_2 efflux were significantly lower for the TS_M treatment than for the SP_M treatment from 3 hours to day 4 after the application of slurry (**Figure 13**). Average cumulative values over this period were for these treatments -21.8±0.3 and -20.5±0.6‰ for $\delta^{13}C$ values and 24.8±25.5 and 13.6±13.9% for the contribution of slurry-derived C to the total CO_2 efflux respectively. Trailing-shoe application of slurry concentrate the amount of applied C and N on a smaller area than when using the conventional splash-plate broadcasting with a similar application rate. Therefore, this technique leads to locally higher amounts of applied slurry. Furthermore, this slurry is in direct contact with the soil and, because of the protection from the grass cover, is less affected by NH_3 and VFAs losses in the atmosphere (Webb et al., 2009). As a consequence, the quantity of slurry-derived labile and easily mineralisable C and N that is effectively incorporated in the soil is higher when using the trailing-shoe application technique.

4.3.3. Priming effect on CO_2-C flux and cumulative losses at the end of the experiment

The comparison of the contribution of the SOM to the total CO_2 efflux, on SP_M and TS_M treated plots, with the background fluxes given by control plots (using the equation (13) given in **4.2.4**) suggested positive and negative PEs on the native SOM mineralisation (**Figure 14)**. Both SP_M and TS_M showed a similar pattern for the daily value of this primed C in the first 5 days following the application of slurry. Both treatments displayed two peak values for the amount of primed C of 62.7±48.3 and 62.6±12.3 kg CO_2-C ha^{-1} day^{-1}, for SP_M treatment, and 51.8±21.8 and 69.0±13.4 kg CO_2-C ha^{-1} day^{-1}, for TS_M treatment, after respectively 6 hours and 3 days following the application of slurry. During the first five days, there was no difference between both treatments (except for t = 1hour, but the value given for TS_M treatment at that date might not have been correct as the assumption of equal CO_2 emission rates between SP_G and TS_M treatments at that date was not verified). During this period, the priming factors, calculated using the equation (14) (see **4.2.4**), were 6.0 ± 6.7 and 6.4 ± 9.8 for the treatments SP_M and TS_M respectively.

However, SP_M treated plots displayed relatively constant high values for this primed C (PE>50 kg CO_2-C ha^{-1} day^{-1}) from 6 hours to day 4, whereas values calculated for the TS_M treatment were more variable over the same period (**Figure 14**). After these initial two peaks, a quick decrease was observed for SP_M plots, with a relative stabilisation then between -9.7 and +8.7kg CO_2-C ha^{-1} day^{-1} for the period going from day 7 to day 30. Concerning the TS_M treated plots, there was a quick decrease of the amount of primed C, between day 4 and day 5, but there was a stabilisation then around 20-25 kg CO_2-C ha^{-1} day^{-1} until day 13, before decreasing to less than 10 kg CO_2-C ha^{-1} day^{-1} on days 20 and 30. Consequently, the amount of primed C from TS_M treatment was significantly higher than for the SP_M treatment between day 5 and day 13 following the application of slurry (**Figure 14**).

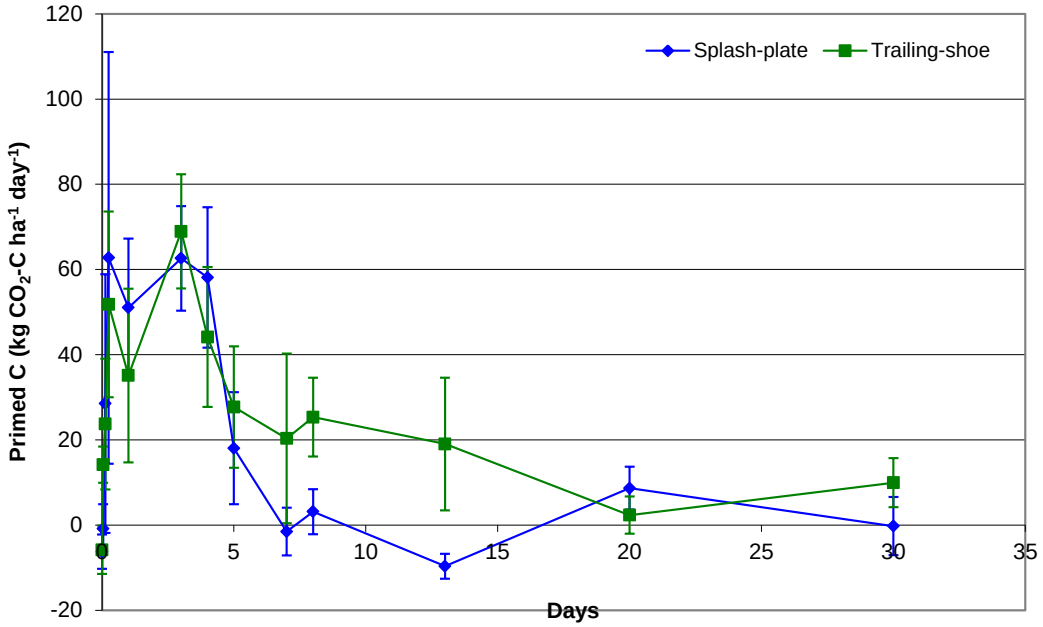
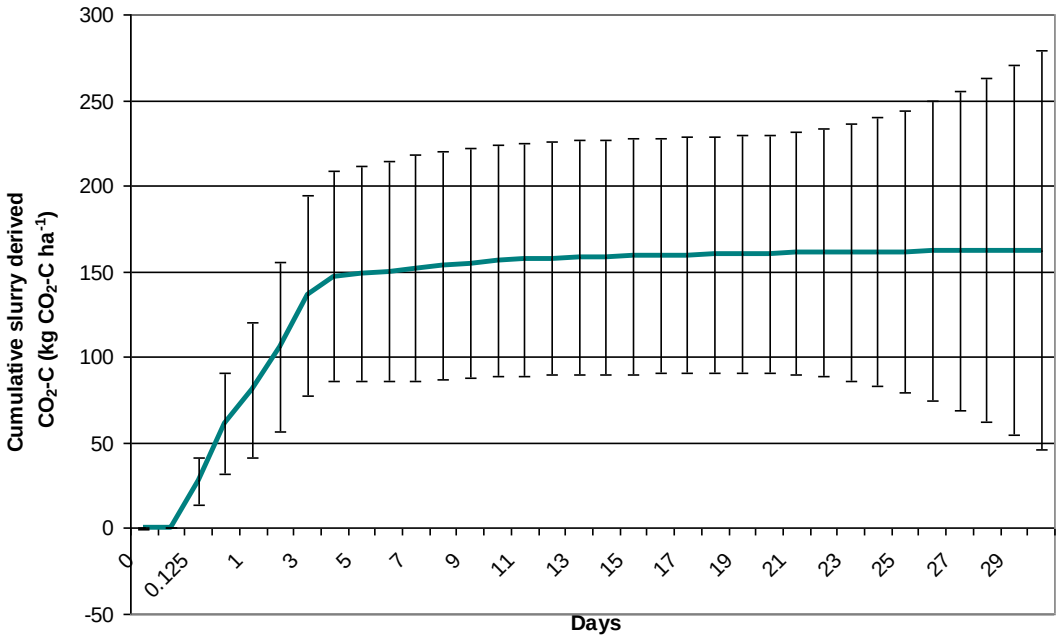


Figure 14: Amount of primed C from plots treated with maize-based slurry calculated by comparing the contribution of SOM-derived C to the total CO_2 efflux from these plots with the CO_2 fluxes measured on control plots. Two application techniques are compared. Error bars correspond to standard errors associated with mean value estimated for each sampling date

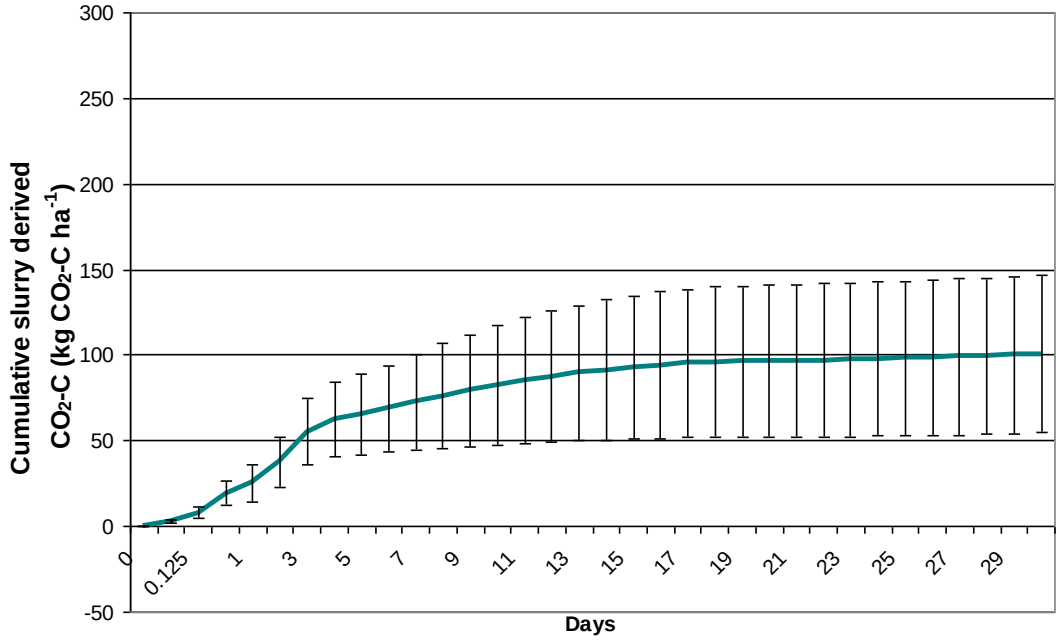
These existing positive PEs during the first five days following application of cattle slurry, for both treatments (**Figure 14)**, were in agreement with what was previously reported in the literature after deposition of dung onto (Bol et al., 1999, 2000) or the incorporation of slurry in (Bol et al., 2003b) grassland soils. Our study showed that the priming of native SOM started only a few hours after applying slurry, reaching a first peak value after only 6 hours, which is in agreement with Bol et al. (2003b) who observed, in an incubation experiment, a strong positive PE, with a priming factor of 37.5±3.4, 5 hours only after incorporating cattle slurry to two different types of grassland soils. This gives a clear indication that it did not take more than a few hours for the soil microbial biomass to respond to the input of organic matter into the soil system. It is therefore likely that part of the early burst in CO_2 emissions from slurry treated plots described earlier, and attributed to the non-biological release of CO_2 dissolved in the liquid phase of slurry, was in fact partly derived from the rapid mineralisation of the most labile and degradable C fraction

present in the slurry. Previous studies (Bol et al., 2003a, 2003b, Sauheitl, 2005, Fangueiro, 2007) showed strong elements to support this idea of a possible, maybe not systematic, nearly immediate activation of the soil microbial biomass following the input of quickly mineralisable slurry-derived C into the soil. Bol et al. (2003a) observed, for example, a contribution of slurry-derived C to the soil microbial biomass C pool as high as 36% only two hours after the incorporation of slurry. Similarly, Sauheitl et al. (2005) observed a rapid synthesis of rhamnose (a microbial sugar) in the first few hours following the input of slurry C into the soil. This is in contradiction with Angers et al. (2007) who assumed that the contribution of any kind of microbial degradation of slurry-derived C was small during the first 10 hours of their experiment, which involved the incorporation of pig slurry into cropped soils, despite the observation of high $\delta^{13}C$ values for the evolved CO_2. The second peak value in terms of primed C could be linked to an important rainfall event (42 mm of rain on days 2 and 3) which could have accelerated the decay of the slurry-derived solid phase on the soil surface and led to a new flush of labile C and N through the upper layers of soils. This new input of easily mineralisable C in the soil would have further enhanced the degradation of native SOM by soil micro-organisms.

As reported by Bol et al. (2003b), priming effect is a short-term phenomenon. Therefore, the amount of primed C became negligible, after day 7, for the SP_M treated plots (**Figure 14**). However, TS_M plots showed a different pattern with the amount of primed C being lower from day 5 onwards than for the previous days, but still significant until day 13. Ohm et al. (2007) showed the extent of PEs is likely to be affected by the amount of substrate added to the soil. Their results displayed higher and / or broader emission peaks of CO_2, in comparison with controls, after adding fructose or alanine to incubated soil. This was related to much higher priming effects caused by the higher addition of substrate. In our study, it was assumed (see **4.3.2**) that trailing-shoe application leads to locally higher amounts of applied slurry. Furthermore, this applied slurry is protected from the atmosphere by the grass canopy. As a consequence, the quantity of slurry-derived labile C and N that is effectively incorporated in the soil is higher when using the trailing-shoe application technique. This would have resulted in a longer lasting PE compared to SP_M treated plots, rather than a higher peak value.



(a)



(b)

Figure 15: Cumulative amount of slurry-derived CO_2-C estimated from the total CO_2 efflux measured onto field plots applied with maize-based (C_4) cattle slurry. Two application techniques are compared: a) splash-plate and b) trailing-shoe application techniques. Errors bars correspond to standard errors associated with mean value estimated for each day.

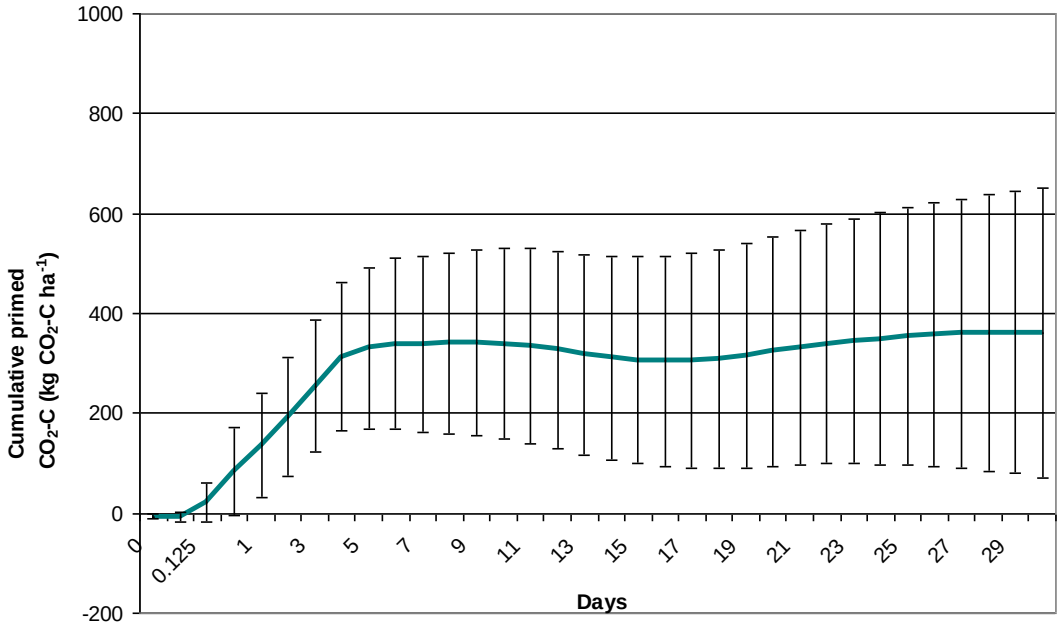
Overall, for the 30 days of the experiment, applied maize-based (C_4) slurries contributed 163 ± 116 kg C ha^{-1} and 101 ± 46 kg C ha^{-1} to the CO_2 emitted from SP_M and TS_M plots, respectively, over the entire measurement period. This contribution was maximal during the first four days following the application of slurry (**Figure 15**). This slurry-derived C accounted respectively for 39 ± 28 and 23 ± 10 % of the amount of applied C. This is within the 17-63% range of values found by Bol et al. (2003b) when calculating the amount of slurry-derived CO_2-C emitted from their [soil-cattle slurry] mixtures over the first 9 days of their incubation experiment.

Cumulative primed C, for the entire experiment, reached 361 ± 289 kg C ha^{-1} and 621 ± 355 kg C ha^{-1} for SP_M and TS_M treatments respectively (**Figure 16**), accounting for 82 ± 69% and 141 ± 81% of the amount of C initially applied on corresponding plots. The calculation of the resulting balance between the incorporation of slurry-derived C and the extra-mineralisation of soil-derived C, with values of -107 ± 194 and -283 ± 358 kg C ha^{-1} for both treatments respectively, showed in this experiment that the priming of soil-derived C after application of cattle slurry onto the soil is likely to have totally offset the incorporation of slurry-derived C in the soil. Previous studies (Dalenberg et al., 1989; Ohm et al., 2007) suggested that positive priming effects do not necessarily decrease C sequestration from soils as the extra amount of native soil C mineralised following the stimulation of soil microbial biomass may be offset by the partial incorporation and stabilisation of the added organic C, something which was not verified here.

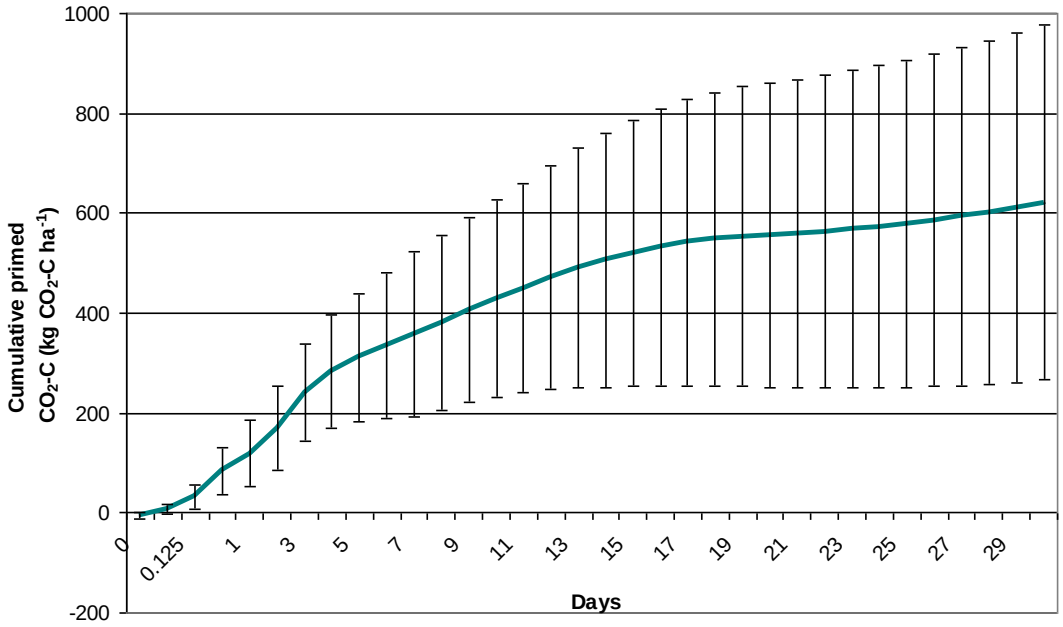
However, soil CO_2 efflux was only measured and partitioned for a period of 30 days. In a longer term, an increase of plant primary production, following the application of slurry (Jones et al., 2006; Shimizu et al., 2009), may contribute to an additional organic C input to the soil through rhizodeposition or the decay of non-harvested plant biomass (Loiseau & Soussana, 1999; Jones & Donnelly, 2004; Franzluebbers, 2005;Rees et al., 2005; Jones et al., 2006). As a consequence, C uptake from grasses should also be taken into account when addressing the impact of cattle slurry amendments on C sequestration from grassland soils. Jones et al. (2006), for example, applied cattle slurry two years in a row in a Scottish pasture which was cut for silage. They measured both grass DM yield and the plant C offtake (through harvest) and found an increase of the remaining plant biomass C (after harvest) of 69

and 96%, in both years respectively, compared to control plots. Those plots displayed a smaller net ecosystem exchange (NEE) value than the one measured for mineral fertiliser during the same period, despite similar plant growth, suggesting higher respiration rates induced by manure application. Nevertheless, the authors reported, over a 6-year period of similar management, an increase of soil C stock (in the top 40 cm soil layer) of about 60% of the total amount of applied slurry C, confirming a C sequestration in grassland soils after application of cattle slurry.

Even though they are short-term phenomena, priming effects may occur on a regular basis, being therefore of a significant influence when calculating long-term nutrient balances (Gerzabek et al., 1997). Several years managing grasslands with synthetic fertilisers or manures generally enhance soil C sequestration (Conant et al., 2001), but, in certain circumstances, C lost by the priming of SOM mineralisation may exceed the additional input of organic C from applied slurry, particularly when applied at a moderate rate (Angers et al., 2010). In their study, Angers et al., (2010) tried to investigate the effect of 20 years of pig slurry application on C storage in a perennial grassland field. They compared two different application rates (50 and 100 m^3 ha^{-1}) and observed a significant decrease of soil C stocks, relative to unfertilised grasslands, at a moderate application rate, whereas soil C stocks were greatly increased at a higher rate. At higher application rate, additional C input from slurry and increased forage productivity likely counterbalanced the PE and maintained soil C levels, but this was not the case when slurry was applied at a moderate rate. On the other hand, a significant proportion of slurry-derived C may remain in the soil for several years after application, contributing (to some extent) to long-term soil C sequestration (Grilo et al., 2011).



(a)



(b)

Figure 16 : Cumulative amount of primed C from plots amended with maize-based (C_4) cattle slurry, estimated from the total CO_2 efflux measured onto such plots. Two application techniques are compared: a) splash-plate and b) trailing-shoe application techniques. Errors bars correspond to standard errors associated with mean value estimated for each day.

Concerning the methodology used for this study and as mentioned in the paragraph **4.2.4**, the accuracy of the calculations, using the equations (12), (13) and (13), could be discussed for TS_M treated plots, as fluxes from SP_G and TS_M treatments were statistically different at t = 1 hour after slurry application, probably leading to significant errors in the estimation of the amounts of slurry-derived and primed C for that time. However, the first 3 hours following the application of cattle slurry accounted for only 5 and 8% of the total amount of slurry-derived and primed C lost from TS_M plots respectively. Therefore, this study gave us a good estimation of the amount of slurry-derived C re-emitted in the atmosphere or incorporated in the soil during the first month following the application of slurry, as well as the amount of extra soil-derived C mineralised during the same time.

4.4. Conclusion

The natural ^{13}C abundance tracer technique was an interesting tool to partition soil CO_2 efflux between the native SOM-C source and the applied slurry-derived C. We observed a nearly immediate activation of the soil microbial biomass, through a peak in the soil CO_2 efflux, leading to a rapid utilisation of slurry-derived C in the first 5 days following the application of slurry. This period of high microbial activity was characterised by a double peak in terms of utilisation of added C (as well as for the primed SOM-C) and followed by a more or less rapid decrease of such activity.

These results suggest, in our example, a three-stage mechanism for the degradation of slurry-derived C in grassland soils:

1. Immediately after applying slurry onto the soil, the soluble C fraction present in the liquid manure either is non-biologically released in the atmosphere as CO_2 or rapidly percolates through the top soil where it can be utilised by the microbial biomass (supposedly r-strategists) within a few hours.

2. During the first few days after manure application, some simple C molecules (id. oligosaccharides, fatty acids, amino-acids) which were associated with the solid slurry-phase can be gradually released and incorporated in the soil, following the decay of surface applied liquid manure. This was suggested by

the second peak of CO_2 and microbial utilisation of slurry C which was observed after 3 days.

3. Finally, once the labile C sources are exhausted, soil microbial biomass and activity rapidly decrease. The remaining micro-organisms (supposedly slow-growing K-strategists) are likely to remain the only active decomposers, degrading the more recalcitrant slurry-derived compound at lower rates but in a longer time frame than previously mentioned r-strategists.

Approximately 60% of applied slurry C remained in the soil at the end of the experiment. However, the application of cattle slurry onto grassland soils rapidly led to a short-term PE which, in our study, nearly totally offset the incorporation of slurry-derived C into the soil, whichever the application technique we used. The long-term impact of these priming effects on nutrient and GHG balances remains to be further investigated, as these phenomena may occur on a regular basis in grassland ecosystems.

The next chapter will focus on the impact of the quality of the applied C substrate on the GHG emissions from a grassland soil and on the fate of applied organic C and mineral N in the [soil – plant – atmosphere] system.

Chapter 5. Quality of the slurry C and its consequences on soil C and N dynamics

5.1. Introduction

Land management is thought to offer opportunities to offset rising atmospheric CO_2 levels and mitigate the potential damaging effects of climate change. As temperate grassland accounts for 32% of global, 20% of European and 90% of national vegetation cover, these ecosystems comprise significant components of both the C and N cycle across all these scales (Adams et al., 1990, Soussana et al., 2004, Abdalla et al., 2010). Several studies have demonstrated that grassland tends to sequester C although the extent of this sink activity is unknown (Janssens et al., 2003, Soussana et al., 2004). Conversely, grasslands are also substantial global reactive N sources, primarily due to the application of organic and mineral N inputs (Flechard et al., 2007). As a result, management may offer both a large N mitigation and C sequestration potential (Soussana et al., 2004, Soussana et al., 2007).

Fertilisation of grasslands can increase sequestration, either via increased net primary productivity, or if organic manures are used, via the direct input of C into the system (Rochette et al., 2004, Sousanna et al, 2004). In addition, in another experiment, increasing cattle slurry application rates also led to higher herbage yields resulting from a 46% increase in N uptake after 38 years (Muller et al., 2011).

Conversely, addition of exogenous C may also lead to enhanced CO_2 respiration, due to 'priming effects' associated with an increased heterotrophic respiration due to the addition of a C source into the system (Bol et al, 2004). These 'priming effects' may also increase N emissions, with a shift occurring toward a predominance of mineralisation from labile organic N in slurry treatments (Muller et al., 2003, Muller et al., 2011). Indeed it has also been shown that the presence of easily available C, which stimulates denitrification activity and O_2 consumption in combination with high mineral N levels, may lead to an increase in N_2O emissions relative to inorganic fertiliser (Velthof et al., 2003).

Therefore, the principle aims of this study were to:

a) monitor the effect of cattle slurry, supplemented with either an easily available or a more recalcitrant C source, on the emissions of GHG from a grassland soil;

b) investigate the consequence of such addition of exogenous C on the dynamics of applied C and N in the [soil – plant – atmosphere] system using combined ^{13}C and ^{15}N tracing

Natural abundance isotope technique (Glaser et al., 2001, Bol et al., 2003a) was used to trace the fate of applied C in the system. Half of the treated lysimeters were also applied with ^{15}N-labelled slurries, so that the dynamic of applied inorganic N could be followed through the analysis of the ^{15}N recovery in soil, plant and N_2O fractions.

5.2. Materials and methods

The experiment was undertaken in Johnstown Castle (Ireland) between October 2010 and April 2011. Small-sized lysimeters (diameter: 15.2 cm), originating from the field described in **Chapter 2,** were applied with various cattle slurry treatments and incubated in controlled conditions for the length of the experiment.

Differences between grass- and maize-based slurries and the use of ^{15}N stable isotope tracer technique were used to follow the fate of slurry-derived C and N post-application, either in gas and plant samples, collected during the experiment, or in the soil after destruction of the cores at the end of the experiment.

5.2.1. Collection and preparation of the lysimeters prior to the experiment

5.2.1.1. Lysimeter design

Lysimeters used in this experiment were designed according to **Figure 17.**

Each lysimeter consisted of a PVC pipe section, 35 cm long and about 15.2 cm diameter (**Figure 18**), closed at the bottom with a PVC collar and a matching stopper.

A 7 mm hole was drilled in the centre of each stopper and a plastic tubing system was fitted through it (**Figure 17**). This drainage system, aiming at collecting water percolating through the soil, was sealed with technical silicone. Inside the collar, the hole was covered by a square piece of metal mesh and 2-3cm gravel layered on top of it. A 5 cm free space was left above the soil surface to allow cattle slurry application onto the grass cover.

As shown on **Figure 17**, a 5 mm space between the inner surface of the pipe and the soil was filled with heated petroleum jelly in order to minimise the risk of preferential water flow down the sides of the lysimeter casing (see **5.2.1.2**.).

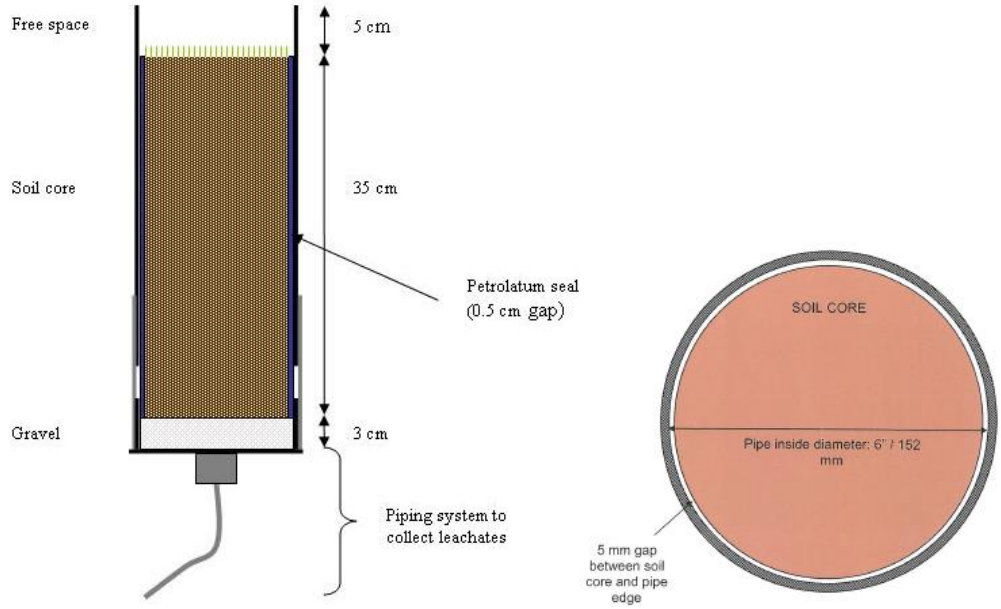


Figure 17: Design and dimensions of the lysimeters

Figure 18: The PVC pipes used to contain intact soil cores

5.2.1.2. Lysimeter collection and preparation

In March 2010, 60 intact soil cores (depth: 35 cm) were collected in the field. These were excavated using a special heavy-duty metal cutting cylinder constructed in order to excavate those soil cores. The cylinder was designed so that it could accommodate a PVC pipe (**Figure 18**) and was sharpened at one end so that it could be pushed into the ground with a digger. The inner diameter of the sharpened edge was such that a 5 mm gap would be created between the inner surface of the pipe and the soil when pushing the metal cylinder into the soil.

Prior to pushing the cutting cylinder into the ground, two parallel trenches were dug, about 1.5 m apart, leaving a continuous block of intact grass-covered soil from which all lysimeters were subsequently extracted (**Figure 19**). A PVC pipe (placed inside the cutting cylinder) was then pushed into the soil until having only 5 cm left over the ground. The pipe, with the soil inside, was then carefully excavated, taken out and trimmed of excess soil.

Meanwhile, the drainage system (collar / stopper + tubing) was prepared by placing a piece of metal mesh over the tubing outlet and adding the gravel layer on the top of it (**Figure 20**). Once the soil core was excavated and as shown on **Figure 17**, this system was tightly fitted to the bottom end of the lysimeter.

a) b) c)

Figure 19: Lysimeter excavation protocol consisting in digging two parallel trenches (a), pushing the cutting cylinder into the ground (b), taking out the cylinder with the PVC pipe and the sampled soil core enclosed inside it (c)

a) b) c)

Figure 20: Preparation of the drainage system to collect leachates, including placing the metal mesh (a, b) and covering it with a layer of gravel (c).

Lysimeters were then carefully disposed on a wooden indoors stand, to avoid damaging the tubing system, where they were sealed with heated petroleum jelly (**Figure 21**). Sealed lysimeters were left to adapt while petrolatum cooled down and solidified, before being transferred to two plant growth rooms, with controlled environmental conditions.

a) b)

Figure 21: Material used to prepare the liquefied petrolatum (a) and freshly sealed lysimeters (b).

5.2.2. Experimental design

5.2.2.1. Initial soil and plant characteristics

Intact soil cores were sampled from the same site as both field experiments (see **Chapter 3** and **4**). Therefore, the soil characteristics are as described in the previous chapters.

Additional soil analysis was performed on archived soil samples which had been previously collected on site at the same time the lysimeters were excavated.

These samples were kept at 4 ºC, in a cold room, until further analysis of C and N content, as well as ^{13}C and ^{15}N isotopic ratios. Corresponding analytical protocols are given in paragraph **5.2.3.3.**

Those additional soil characteristics are presented in **Table 11**.

Table 11: Soil C and N pool size and isotopic ratios at the start of the experiment (n=5, mean value ± standard error).

Soil layer	N content (kg N ha-1)	C:N ratio	$\delta^{13}C$ (‰)	^{15}N isotopic ratio (atom % ^{15}N)
0-10 cm	2219 (± 275)	10.8 (± 0.1)	-29.30 (± 0.11)	0.377 (± 0.004)
10-20 cm	1685 (± 254)	10.4 (± 0.2)	-28.49 (± 0.12)	0.372 (± 0.001)

Six days before applying slurry treatments on each core, the grass was cut on all the lysimeters so that the remaining plants were not taller than 5 cm high. Removed grass samples were pooled together and 4 subsamples were taken for subsequent C and N analyses (see paragraph **5.2.3.3**.). On average, these samples had a 1.33 (± 0.03) % N content and a C:N ratio of 35.5 (± 1.4). They also displayed a $\delta^{13}C$ value of -33.4 (± 0.2) ‰ and a ^{15}N isotopic ratio of 0.394 (± 0.002) atom % ^{15}N.

5.2.2.2. Treatments

The experiment was conducted between the 12th October 2010 and the 31st March 2011. Prior to this, beef cattle were fed with either ryegrass (*Lolium perenne*, a C_3 plant with a $\delta^{13}C$ value of ca. ~-27‰) or maize (Zea mays, a C_4 plant with a $\delta^{13}C$ value of ca. ~-13‰) silages leading to C_3 and C_4 faeces and urine with naturally different ^{13}C contents. Both of them were collected separately and stored at 4ºC for one month before the start of the experiment.

For both maize-based and grass-based slurries, two different batches were prepared in 2L urine containers by thoroughly mixing faeces and urine and

homogenising the resulting liquid manure with a mixer. Dry matter and TAN content of each batch are given in **Table 12**.

One batch of each slurry type was then labelled with ^{15}N by adding a very small amount of highly-enriched ^{15}N labelled ammonium sulphate ($(NH_4)_2SO_4$, 99 atom % ^{15}N) so that the amount of added $^{15}NH_4^+$-N would represent about 5% of the initial TAN content for each slurry type (**Table 12**).

Table 12: Slurry DM and TAN content analysed prior to the ^{15}N labelling operation (mean± se). The amount of ^{15}N labelled ammonium sulphate ($^{15}(NH_4)_2SO_4$, 99% at ^{15}N) subsequently added to each slurry batch is also given in the table.

Slurry type	Batch	DM content (%)	TAN content (g kg^{-1} fresh slurry	$^{15}(NH_4)_2SO_4$ addition (g NH_4^+-^{15}N kg^{-1} slurry)
Grass	1	9.38 (± 0.10)	3.35 (± 0.02)	0.168
Grass	2	9.41 (± 0.18)	3.29 (± 0.05)	-
Maize	1	8.72 (± 0.07)	2.34 (± 0.01)	0.117
Maize	2	8.75 (± 0.10)	2.34 (± 0.03)	-

Both prepared maize-based slurries were then split into three subsamples of equal size. Either cane sugar or pure cotton cellulose (both substances originating from C_4 plants) were added to two of the slurries so that exogenous C amounted for 1% of slurry-derived C. To limit the number of treatments and keep the experiment manageable, grass-based slurry was only split into two C treatments, with- and without- added cane sugar.

Finally, ten different slurry treatments (see **Table 13**) were applied to incubated lysimeters (n = 4) at a rate of 30 t ha^{-1}, representing an N application rate of 94 to 127 kg total N ha^{-1}. This rate of slurry was selected as being typical of the application rate applied annually to grassland in Ireland (Lalor et al., 2011). A 100 ml syringe (Becton Dickinson, Oxford, UK) was used to split grass canopy and dispose the slurry directly onto the soil surface. Characteristics of each amended material are given in **Table 13**.

Four lysimeters were not amended and used as controls.

Table 13: Nitrogen content, C:N ratio and isotopic data for the different treatments

Treatment name	^{15}N labelling	Slurry type	Additional C	Total N (g N kg^{-1} slurry)	^{15}N isotopic ratio (at % ^{15}N)	C:N ratio	$\delta^{13}C$ (‰)
GS	No	Grass	No	2.86	0.375	12.3	-28.09
GSCS	No	Grass	Sugar	3.85	0.385	9.3	-28.05
MS	No	Maize	No	3.23	0.385	10.1	-20.33
MSCS	No	Maize	Sugar	2.92	0.375	11.6	-20.42
MSCel	No	Maize	Cellulose	3.07	0.378	11.0	-20.54
^{15}N_GS	Yes	Grass	No	3.90	0.834	8.8	-27.91
^{15}N_GSCS	Yes	Grass	Sugar	3.35	0.852	10.4	-27.90
^{15}N_MS	Yes	Maize	No	2.70	0.784	12.4	-20.43
^{15}N_MSCS	Yes	Maize	Sugar	2.90	0.750	11.6	-20.33
^{15}N_MSCel	Yes	Maize	Cellulose	2.71	0.777	12.4	-20.34

CS = cane sugar; Cel = pure cotton cellulose

5.2.2.3. Experimental set up and incubation conditions

The experiment was carried out in two growth-rooms, which are walk-in chambers with controlled environmental conditions (TAS, Temperature Applied Sciences Ltd., Goring-by-Sea, UK). The idea behind separating lysimeters in two batches was to isolate those applied with ^{15}N labelled manures from the non-labelled treatments.

The use of such growth-rooms allowed sequential programming of temperature and humidity, as well as the light intensity.

In the purpose of the experiment, lysimeters were pre-incubated for 2 months in the conditions planned for the first 30 days of the experiment (See **Tables 14** and 1**5**). During this time, the protocol for gas sampling (see paragraph **5.2.3.1.)** was tested to ensure the sealing between the chamber and the pipe was such that any risk of gas leak was minimised.

Slurry application occurred on 12th October 2010, marking the start of the experiment (Day 0). On the day before the start of the experiment, the grass was cut to a height of 5cm (as mentioned in **5.2.2.1.**). Two days prior to applying treatments, each lysimeter was watered so that the soil would be saturated with water. The lysimeters were left to freely drain for the next 36 hours. Excess water was collected before the start of the experiment. In terms of incubation condition, a daily routine was then programmed on the growth-room with a 16/8 hour photo-period in order to optimise the daily integrated irradiance as the maximum photosynthetic photon flux density (PPFD) was 500 µmol photon m^{-2} s^{-1} (see **Table 14**):

Table 14: Parameters set up for the growth room daily routine

Phase	Phase length	Temperature	PAR (light intensity)	Humidity
Night	8h	According to averaged monthly weather data	0%	70%
Day	16h	According to averaged monthly weather data	100%	70%

Temperature and precipitation were simulated using a 25 year averaged monthly temperature and rainfall dataset, recorded from the Rosslare meteorological station for the period 1978-2005, were used to simulate a spring application (set on 1st May) (see **Table 15)**. Minimum and maximum temperatures inside the growth room were modified every 30 or 31 days to follow the annual pattern of average monthly temperatures. Each lysimeter was watered every 2 or 3 days, with a 100 ml syringe, so

that the amount of water added over a month period was equal to the average rainfall recorded for the same month.

Table 15: Monthly temperature set up and watering regime according to averaged 30 years monthly data from the nearby Rosslare weather station

Month	Simulated month	Averaged monthly rainfall (mm)	Corresponding watering regime (ml water day^{-1} $core^{-1}$)	Minimum temperature / Temperature "day" (^oC)	Maximum temperature / Temperature "night" (^oC)
1 (31 days)	May	52	25	13.4	8.4
2 (30 days)	June	55	28	16	10.6
3 (31 days)	July	45	22	18.1	12.5
4 (30 days)	August	74	36	18.3	12.5

In each growth room, the lysimeters were set up on the shelves according to the treatment to be applied:

- ^{15}N_GS, ^{15}N_GSCS, ^{15}N_MS, ^{15}N_MSCS and ^{15}N_MSCel in the growth room A.
- Controls, GS, GSCS, MS, MSCS, and MSCel in the growth room B;

Each lysimeter had an individual marked leachate container placed under the shelf with the appropriate tubing put inside (**Figure 22**).

In each growth-room, there were two main sensors – one for temperature, the other for relative humidity (Vaisala Inc., Finland) used in a system of control and auto-correction of conditions of incubation. Furthermore, each growth-room contained two free sensors, disposed on the central shelf about 40 cm above the top of the cores, which were used as an independent control of temperature and relative humidity inside the growth-room.

There was also a Vaisala CO_2 detector which recorded the CO_2 concentration (in ppm) inside each growth-room.

Data from these detectors were automatically saved twice per minute. However they were manually computed and integrated only for each gas sampling date, at the beginning of the experiment, and then on the first day of each month.

a) b)

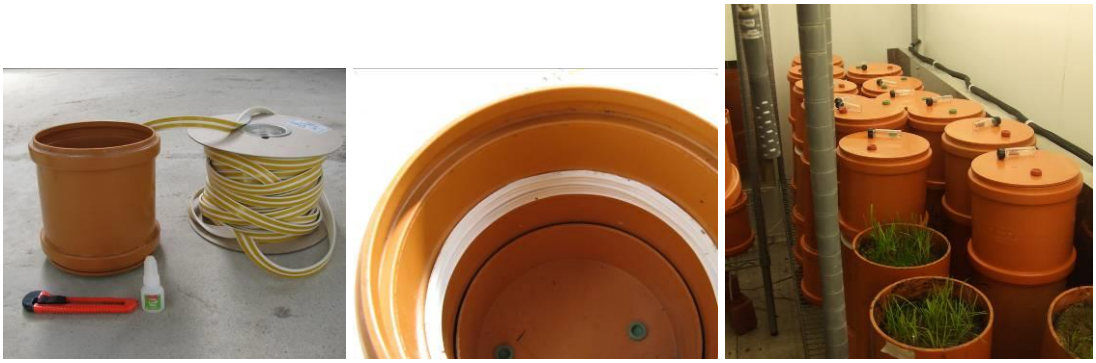
Figure 22 Temperature and humidity sensors in the growth room (yellow circle) and a humidifier (red circle) (a), and the lysimeters set up with the leachate containers.

5.2.3. Sample collection and analysis

5.2.3.1. Gas samples

N_2O, CO_2 and CH_4 fluxes were measured using closed static chambers (Smith et al., 1995). As stated in **Chapter 3**, this method consists in allowing the three gases to build up inside a sealed chamber, placed onto the soil, during a certain measuring period. Air from the chamber was sampled various times during that time with a syringe, before being analysed on a gas chromatograph. The fluxes were then calculated from the increase of the concentration of each gas inside the chamber over time.

Plastic chambers, 7.1 cm in diameter and 20 cm high, were designed for gas samples collection at the top of the lysimeters (**Figure 23c**). The chambers were insulated with neoprene gasket to ensure tight fitting over the lysimeters and, therefore, airtight headspaces at the time of measurement (**Figure 23a** and **b**). Two holes were also drilled in each chamber, through which rubber septa were inserted (**Figure 23b** and **c**).



a) b) c)

Figure 23: Design of chambers used for gas sampling. Each chamber was insulated on the inside with neoprene gasket (a, b) and equipped with two rubber septa (b, c) so that they could be tightly fit at the top of each lysimeter (c) where gas samples would be collected.

At the start of a gas sampling period, chambers were placed onto lysimeters (Becton Dickinson, Oxford, UK), with chamber pressure equilibrated by the use of a syringe needle inserted into one septum as a pressure vent.

Air samples were then collected from the chamber headspace at time 0, 5 and 40 min using a 20 ml syringe and needle mentioned above (1 syringe per treatment).To collect a sample from a chamber, the needle was inserted through the septum and the syringe was flushed to ensure adequate mixing of the air within the chamber prior to the withdrawal of a sample from the chamber. A 20 ml headspace sample was then immediately injected into and flushed through a pre-evacuated 7 ml gas-tight vial (Supelco, Bellefonte, Pennsylvania, USA), which was stored afterwards for subsequent analysis. Over pressurising vials was performed in order to prevent inward leaks to vials. Measurements were taken during the 'daytime' period on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 23 and 36.

The N_2O, CO_2 and CH_4 concentration in each sample was analysed using a GC (Varian CP 3800 GC, Varian, USA). More information about the GC procedure is given in **Chapter 3**.

The N_2O concentration was analysed using an Electron Capture Detector (E.C.D.) at 300ºC. Flame Ionisation Detector (F.I.D.) was used to detect CH_4 while CO_2 was analysed using a Thermal Conductivity Detector (T.C.D.).

N_2O (in g N_2O-N m^{-2} h^{-1}) and CH_4 fluxes (in g CH_4-C m^{-2} h^{-1}) were then calculated using the following equations:

$$F_{N_2O} = \frac{PM_{N_2O}}{RTM_N} \frac{V}{A} \frac{\Delta C_{N_2O}}{\Delta t_{40}} \times 10^{-9} \quad (15)$$

$$F_{CH_4} = \frac{PM_{CH_4}}{RTM_C} \frac{V}{A} \frac{\Delta C_{CH_4}}{\Delta t_{40}} \times 10^{-9} \quad (16)$$

where P is the atmospheric pressure (value set at P = 1.013 kPa), M_{N2O} / M_{CH4} is the molar mass for N_2O (= 44.013 g mol^{-1}) or CH_4 (= 16.04 g mol^{-1}), M_N / M_C is the standard atomic weight for N (= 14.07 g mol^{-1}) or C (= 12.01 g mol^{-1}), R is the gas constant (8.314472 m^3 Pa K^{-1} mol^{-1}), T is the air temperature set inside the growth room (in K), V is the volume of the chamber (in m^3), A is the area covered by the chamber (in m^2) and $\frac{\Delta C_{N_2O}}{\Delta t_{40}}$ (or $\frac{\Delta C_{CH_4}}{\Delta t_{40}}$) is the variation of gas concentration between t = 0 and t = 40 min (ppb h^{-1}).

A similar equation was used to calculate CO_2 fluxes (in g CO_2-C m^{-2} h^{-1}):

$$F_{CO_2} = \frac{PM_{CO_2}}{RTM_C} \frac{V}{A} \frac{\Delta C_{CO_2}}{\Delta t_5} \times 10^{-9} \quad (17)$$

Where M_{CO2} is the molar mass for CO_2 (= 44.01 g mol^{-1}) and $\frac{\Delta C_{CO_2}}{\Delta t_5}$ is the variation of gas concentration between t = 0 and t = 5 min (ppb h^{-1}).

Fluxes were then converted either in kg N_2O-N / CH_4-C / CO_2-C ha^{-1} day^{-1}or in kg CO_2-eq ha^{-1} day^{-1} for subsequent statistical analyses. The GWP of each non-CO_2 gas for a 100 year time horizon (IPCC, 2007b) was used for the latest conversion, with the following values: GWP_{CH4}= 25 and GWP_{N2O}=296.

To calculate cumulative emissions of CO_2, CH_4 and N_2O over the entire sampling period (36 days), missing daily fluxes were linearly extrapolated between each sampling date.

At t = 40 min, triplicate gas samples for isotopic analysis were also collected into evacuated 12 ml glass vials (Labco, UK). Following the guidelines of the external laboratory doing the analysis (see below), a 12 ml sample was injected into the vial so

that the sample would be stored at atmospheric pressure. Due to financial considerations, only gas samples collected on days 0, 1, 2, 3, 5, 7 and 12 were sent away for determination of ^{13}C isotope ratios whereas only samples from days 0, 3, 5, 7, 12, 16 and 23 were analysed for ^{15}N.

Isotopic analyses were carried out at the Stable Isotope Facility (S.I.F.) of the University of California (UC Davis, Davis, CA). Both ^{13}C and ^{15}N isotope ratios were measured, for CO_2 and N_2O respectively, using Thermo Scientific GasBench-PreCon trace gas system interfaced to a Delta V Plus continuous flow IRMS (Thermo Scientific, Bremen, Germany), but both isotopic analyses (^{13}C and ^{15}N) were run on separate samples as these analyses were carried out using two different protocols on two different instruments. This was required as the three main stable isotopes of both N_2O and CO_2 had the same mass charge (m/z): 44, 45 and 46 and so could not be distinguished simultaneously.

In order to analyse for $^{15}N_2O$, gas samples were purged from vials through a double-needle sampler into a helium carrier stream (20 mL/min). The gas sample passed through a CO_2 scrubber (Ascarite) and N_2O was trapped and concentrated in two liquid nitrogen cryo-traps operated in series such that the N_2O is held in the first trap until the non-condensing portion of the sample gas has been replaced by helium carrier, then passed to the second, smaller trap. Finally the second trap is warmed to ambient and the N_2O was carried by helium to the IRMS via a Poroplot Q GC column (25m x 0.53 mm, 25°C, 1.8 mL/min). This column separated N_2O from any remaining CO_2. A reference N_2O peak was used to calculate provisional isotope ratios of the sample N_2O peak.

In order to analyse for $^{13}CO_2$, CO_2 was sampled by a six-port rotary valve (Valco, Houston TX) with a 100µL loop programmed to switch at the maximum CO_2 concentration in the helium carrier gas; for lower concentrations, the entire CO_2 content was frozen in a trapping loop then released to the GC column. The CO_2 was then separated from N_2O and other residual gases by a Poroplot Q GC column (25m x 0.32mm ID, 45°C, 2.5 mL/min). A pure reference gas (CO_2) was used to calculate provisional delta (δ) values of the sample peak. Final $\delta^{13}C$ values were obtained after adjusting the provisional values such that correct $\delta^{13}C$ values for laboratory standards

are obtained. Two laboratory standards are analysed with every 10 samples. The laboratory standards are calibrated directly against NIST 8545.

All results were expressed in the delta notation as per **Chapter 4**, Section **4.2.3.**

5.2.3.2. Leachate samples

As described in the paragraph **5.2.1.1**, each lysimeter was built with a tubing system at the bottom designed to collect residual leachates. Each lysimeter was therefore connected to an individual 500 ml plastic container where draining water could accumulate and be collected.

Leachates were collected before applying treatments and then on days 35, 106 and 124. Volume of the leachate was recorded and sub-sample taken into 50ml plastic test tube (Sarstedt Ltd. ,Wexford, Ireland) for NH_4^+ , NO_2^- and total oxidised nitrogen (TON) analysis on the Aquakem 600 discreet analyser (Thermo Electron OY, Vantaa, Finland), as described by Hoekstra et al. (2010). Samples were also analysed for total N by chemoluminescence, using a Shimadzu TOC-VCPH analyser coupled with a TNM-1 total Nitrogen unit (Shimadzu, Duisbourg, Germany). Nitrate concentrations were not directly measured, but calculated by difference between measured TON and NO_2^- concentrations.

The various concentrations of N species were given in mg N L^{-1}, but such N losses were subsequently converted in kg N ha^{-1}. Whenever a detection limit was known for the instrument (NH_4^+, NO_2^- and TON), concentration below detection limit were considered to be equal to half such detection limit for the calculation of N budgets. However, when no leachates were collected for a lysimeter, corresponding N leaching losses were considered to be zero.

5.2.3.3. Solid (plant, soil) and manure samples

As mentioned in **5.2.2.2**, prepared slurry batches were analysed for DM and TAN content prior to ^{15}N labelling operations. These analyses were carried out as

presented in **Chapter 3**. Amended substances were analysed for total C and N content as presented in the same chapter.

Above ground plant biomass was cut on all the lysimeters on days 22 and 50 so that, each time, the remaining standing biomass was not taller than 5 cm high. Immediately after sampling, plant samples were oven dried at 60 ºC. Due to small sample sizes, samples from both dates were pooled together for each lysimeter. At the end of the experiment, after 170 days, the above ground plant biomass was entirely removed from each lysimeter and analysed. Dried samples were ground in two steps, using successively a commercial crop grinder and a ball mill, in order to generate uniform samples for further analyses. Finally, at the end of the experiment, each lysimeter was destroyed for soil sampling. Soil samples were collected at 0-10 cm and 10-20 cm depths with an auger.

Total C and N contents of soil and plant samples were measured using a LECO TruSpec CN analyser (LECO Corporation, St. Joseph, MI, USA). More information about the analytical method is given in **Chapter 3**. For N (and C) balance calculations, data were converted in kg N (C) ha^{-1}.

Subsamples of dried soil, grass and slurry were also weighed out and encapsulated in tin capsule for subsequent ^{13}C and ^{15}N content analysis. Encapsulated samples were placed in a 96 well micro tray and analysed at UC Davis Stable Isotope Facility. This laboratory runs samples on PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Information on the analysis method can be found on the laboratory's website (http://stableisotopefacility.ucdavis.edu/13cand15n.html).

In one occasion, on day 50, the amount of grass was not enough to carry out both elemental analyses on-site and subsequent subsampling for isotopic analyses to be carried out at UC Davis. As a consequence, on that occasion, the grass was directly weighed up and encapsulated to be sent away for isotopic analysis. However, to avoid the loss of one replication for the corresponding treatment (^{15}N MSCS), two values were arbitrarily attributed to the low yielding lysimeter, for C yield and N uptake respectively, in order to take into account the potential detection limit of the method (i.e. the minimum amount of grass needed to carry out both elemental and isotopic

analyses). Such detection limits were calculated by maximising the C and N content potentially measurable on such small samples, using the maximum values measured on the other lysimeters (49.8 % C and 2.4 % N respectively).

5.2.4. Data processing and statistical analysis

The various C and N fluxes, as well as soil C and N content at the end of the experiment, were analysed using the statistical package STATISTICA version 10 (Statsoft, Tulsa, Oklahoma).

Due to technical difficulties during the destructive soil sampling at the end of the experiment, several treatments were repeated only three times on day 170, in terms of soil and plant C and N measurements. Therefore, all statistical analyses were carried on considering only three repetitions per treatment.

5.2.4.1. Processing of C data and use of the ^{13}C natural abundance tracer technique

As stated in **Chapter 4**, when applying the C_4 slurry onto a C_3 pasture (Initial $\delta^{13}C$ of soil = -29.3 (± 0.1) ‰ in the present experiment), any increase of soil isotopic signal can be attributed to the incorporation of C_4 slurry-C. This is based on the difference between the soil and the applied material in terms of $\delta^{13}C$. To account for a possible isotopic fractionation during soil processes, the temporal pattern of soil $\delta^{13}C$ post-application of C_4 slurry is then compared to a control which consists in the same soil applied with C_3 slurry.

In the present experiment, C_4 slurries (with no additional C or after addition of C_4 sugar / cellulose) were applied onto a C_3 grassland soil. The $\delta^{13}C$ value of those applied materials ranged from -20.54 to -20.33 (see **Table 13**).

Therefore, using a similar isotopic mass balance approach (Bol et al., 2003b) as the one used in Chapter 4, respired CO_2 was partitioned into amendment-derived (cattle slurry only or with additional C source) and soil-derived components. The difference in $\delta^{13}C$ values between the respired CO_2 from the C_3 and C_4 slurry

treatments (with or without additional sugar- / cellulose-derived C) was used to quantify the proportion of amendment- versus soil-derived CO_2-C emitted from the soil. The calculation was performed using the following equation:

$$sdC_{aC4_i} = \frac{\delta^{13}C_{g(aC4_i)} - \delta^{13}C_{g(aC3_i)}}{\delta^{13}C_{sl(aC4_i)} - \delta^{13}C_{sl(aC3_i)}} \times 100 \quad (18)$$

sdC_{aC4i} was the proportion of amendment-derived C (in %) in the total CO_2 efflux measured from each lysimeter on the day *i*. $\delta^{13}C_g$ and $\delta^{13}C_{sl}$ were the $\delta^{13}C$ values (in ‰) from the emitted CO_2 and the material initially applied onto soil cores, calculated for each C_4 slurry treatment and their C_3 counterpart (aC4 and aC3 respectively, see **Table 16**).

Table 16: List of C_4 treatments for which amendment-derived C was calculated. In order to carry on such calculation, each C_4 treatment was associated to a corresponding C_3 treatment.

C4 treatment (aC4)	C3 control (aC3)
Maize-based slurry (MS, ^{15}N MS)	Grass-based slurry (GS, ^{15}N GS)
Maize-based slurry + Cane sugar (MS_CS, ^{15}N MS_CS)	Grass-based slurry + Cane sugar (GS_CS, ^{15}N GS_CS)
Maize-based slurry + Cotton cellulose (MS_Cel, ^{15}N MS_Cel)	Grass-based slurry (GS, ^{15}N GS)

The calculation of sdC_{aC4i} using Equation (18) is only truly valid when daily CO_2 emission rates are identical. This was verified for nearly all sampling days in this study (see paragraph **5.3.3.**) as a Student t-test showed no statistical difference between treatments MS, MSCS, ^{15}N_MS and ^{15}N_MSCS, and their grass-based counterpart (GS, GSCS, ^{15}N_GS and ^{15}N_GSCS respectively).

To reduce the number of treatment, cellulose was not added to grass-based slurry (to create the counterpart of MSCel and ^{15}N_MSCel treatments). However, as there was no statistical difference between daily CO_2 emissions from ^{15}N_MSCel and ^{15}N_GS treatments on one side, and considering that daily CO_2 fluxes from MSCel

and GS treatments were statistically different at only one occasion (day 3, $P < 0.05$), the equation (18) was still used to estimate the amount of both (slurry + cellulose)- and soil-derived C in the total CO_2 efflux.

The amounts of amendment- and soil-derived (Am_C_{C4i} and $Soil\_C_{C4i}$ respectively, in kg C ha^{-1} day^{-1}) were then calculated as follows:

$$Am\_C_{C4_i} = F_{C4_i} \times \frac{sdC_{C4_i}}{100} \quad (19)$$

$$Soil\_C_{C4_i} = F_{C4_i} \times \left(1 - \frac{sdC_{C4_i}}{100}\right) \quad (20)$$

In these equations, F_{C4i} represent the total CO_2 efflux (in kg CO_2-C ha^{-1} day^{-1}) from the corresponding lysimeters on the day i. The amount $Soil\_C_{C4i}$ of soil-derived CO_2-C from amended lysimeters could then be compared to the total CO_2 efflux measured from controls in order to estimate any amount of primed-C (see **Chapter 4** for more information).

Cumulative values over the measuring period were then calculated for the CO_2 efflux from each treatment, as well as for slurry-derived, soil-derived and primed C (whenever those parameters were calculated). When no daily samples were available, it was assumed that the amount of daily respired CO_2 changed linearly between the nearest sampling dates for which samples were available.

For soil and plant samples, within each growth-room and for each sampling date, there was no significant differences between treatments in terms of $\delta^{13}C$ values (see **paragraph 5.3.3.**). Therefore, no attempt was made to estimate amendment-derived C using an isotopic mass balance.

5.2.4.2. ^{15}N enriched tracer technique: methodology and calculations

As mentioned in **paragraph 5.2.2.2.**, grass and maize-based slurry treatments were either not labelled or labelled with ^{15}N by adding a very small amount of highly ^{15}N labelled ammonium sulphate (AS, 99 atom % ^{15}N). The amount of added AS-NH_4^+-^{15}N were 0.168 and 0.117 g NH_4^+-^{15}N kg^{-1} slurry, for labelled grass-based and

maize-based slurries respectively (**Table 12**), representing application rates of 5.02 and 3.50 kg AS-derived NH_4^+-^{15}N ha^{-1}.

The difference in measured isotopic composition between ^{15}N labelled and non-labelled treatments in gas, grass and soil samples was then used to trace the fate of AS-derived ^{15}N, following its application.

First, for a given treatment, the apparent N recovery (ANR) from slurry in herbage was calculated as (Hoekstra et al., 2010):

$$ANR(\%) = \frac{(Nuptake_{slurry} - Nuptake_{control})}{Napplied} \times 100 \quad (21)$$

where N was the total amount of N taken up by grass over the entire experiment or applied in slurry, respectively. Similar equation was used to calculate the apparent N loss from slurry as N_2O (ANL, in %).

Following the ^{15}N isotopic analyses of grass and soil samples, the total amount of ^{15}N (in kg ^{15}N ha^{-1}) was calculated as follows:

$$m_{15} = \frac{14 \times (R/100) \times m}{15 - (R/100)} \quad (22)$$

where m was the total amount of N in the grass or soil sample (converted in kg N ha^{-1}) and R was the molar ratio of the heavy ^{15}N isotope on the total amount of N atoms in the sample (in at % ^{15}N). This equation was derived from the conversion of the molar ratio R into a mass ratio using the value 14/15 as the ratio between the atomic mass of the light ^{14}N isotope and the heavy ^{15}N isotope.

The proportion $ASd^{15}N$ (in %) of this ^{15}N derived from the AS-^{15}N added to labelled slurries was then calculated, by comparison between the R ratios of the labelled materials (L) and their non-labelled counterpart (NL), using the following equation:

$$ASd^{15}N = \frac{E_L - E_{NL}}{E_L} \times 100 \quad (23)$$

Such proportion of AS-derived ^{15}N was calculated for grass and soil samples, to trace the applied labelled ^{15}N. The amount of AS-derived ^{15}N (in kg ^{15}N ha-1) from analysed labelled materials (L) was then calculated from equations (22) and (23) as follows:

$$m_{ASd^{15}N} = (ASd^{15}N / 100) \times m_{15(L)} \quad (24)$$

Finally, for a given labelled treatment, the percentage of AS-derived ^{15}N recovered in herbage samples cut on days 50 and 170 ($^{15}NRH_{50}$ and $^{15}NRH_{170}$ respectively) was calculated as follows:

$$^{15}NRH_i(\%) = \frac{m_{ASd^{15}N(Grass)_i}}{m_{ASd^{15}Nadded}} \times 100 \quad (25)$$

where, *i* was the sampling day for grass, $m_{ASd^{15}N(Grass)_i}$ was the amount of AS-derived ^{15}N in the grass cut on that day (in kg ^{15}N ha^{-1}), and $m_{ASd^{15}Nadded}$ was the amount of AS-NH_4^+-^{15}N initially added.

Similar equation was used to calculate the percentages of AS-derived ^{15}N (^{15}NRS) recovered in soil, at both 0-10 and 10-20 cm depths, at the end of the experiment (170 days).

Following the ^{15}N isotopic analyses of gas samples, the amount of ^{15}N emitted from a given lysimeter as N_2O on each sampling date (in g N_2O-^{15}N ha^{-1} day^{-1}) was calculated as:

$$^{15}N - N_2O_{total} = \frac{\frac{15}{29}a + b}{1 + a + b} \times F_{N_2O} \quad (26)$$

where *a* and *b* were the isotopic mass ratios 45/44 and 46/44, respectively, in the sample, and F_{N_2O} was the daily flux of N_2O (in g N_2O-N ha^{-1} day^{-1}) from the lysimeter surface.

The equation (23) was then used to calculate the proportion of this ^{15}N-N_2O derived from the AS-^{15}N initially added to the slurry.

The amount of AS-derived ^{15}N ($^{15}N\text{-}N_2O_{AS}$, in kg N_2O-^{15}N $ha^{-1}day^{-1}$) from the emitted N_2O could then be calculated using the equation (24) with $^{15}N\text{-}N_2O_{AS}$ and $^{15}N\text{-}N_2O_{total}$ replacing $m_{ASd^{15}N}$ and $m_{15(L)}$ respectively.

To calculate the cumulative amount of AS-derived ^{15}N over the 36 days, $ASd^{15}N$ (as % N_2O) was linearly extrapolated between sampling dates. From day 24 to day 36, these proportions were assumed to be the same as for day 23. The cumulative amount of AS-derived ^{15}N from the emitted N_2O could then be calculated using gap filled daily N_2O fluxes (see paragraph **5.2.3.1**) and summing the subsequently estimated daily amounts of AS-derived ^{15}N.

The equation (25) was then used to calculate the percentage ^{15}NRG of AS-NH_4^+-N initially added which was recovered in the N_2O fraction over the first 37 days (day 0 to 36) of the experiment.

5.2.4.3. Statistical analyses

Data collected during the experiment were analysed using the statistical package STATISTICA version 10 (Statsoft, Tulsa, Oklahoma).

All C and N fluxes, as well as soil C and N content at the end of the experiment, were compared for each sampling date using a factorial ANOVA (n=3) to study the effect of three different factors on such measured values:

- a factor "Growth-room" (G), with two levels (one for each growth-room used in the experiment);
- a factor "Slurry type" (S), with three levels (controls, grass-based or maize-based slurry);
- a factor "C addition" (C), with three levels (no, sugar or cellulose addition)

For soil samples, a factor "Depth" (D) was also introduced (0-10 or 10-20 cm). A nested design was used to take into account the unbalanced experimental design.

Similar analysis was also carried on cumulative fluxes and on all $\delta^{13}C$ values and on ^{15}N isotopic ratios (in at % ^{15}N)

As mentioned in paragraph **5.2.4.1.**, a Student t-test was also carried on between treatments MS, MSCS, MSCel, $^{15}N\_MS$, $^{15}N\_MSCS$ and $^{15}N\_MSCel$, and their grass-based counterpart (GS, GSCS, $^{15}N\_GS$ and $^{15}N\_GSCS$ respectively), in order to valid some ^{13}C isotopic calculations.

Averaged C and N pools or fluxes, as well as all calculated parameters, were expressed as mean values (± standard error) for each treatment.

5.3. Results

5.3.1. Analysis of incubation conditions

Air temperature (T) and relative humidity (RH) were identically set up for both growth-rooms (see paragraph **5.2.2.3.**). However, the analysis of data recorded from free sensors clearly showed that both growth-rooms did not behave similarly during the course of the experiment (**Figures 24** and **25**).

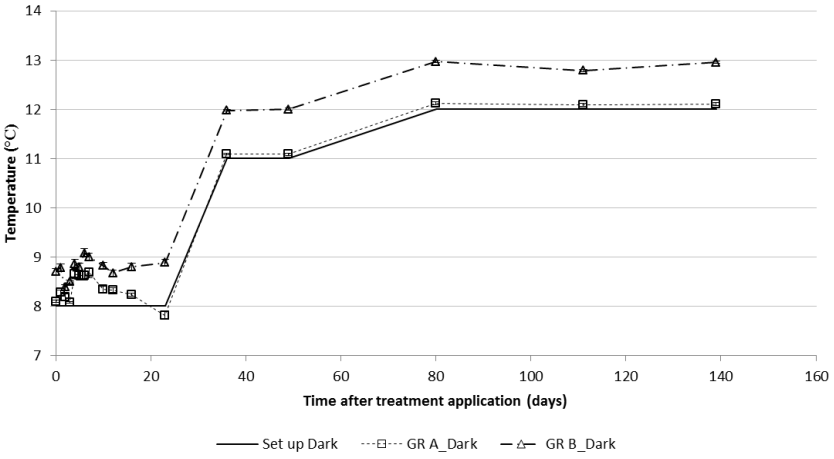
During the daily 8h dark period, when lights were switched off, the growth-room B displayed significantly higher temperatures than the growth-room A (**Figure 24a**) .With the exception of day 23, the difference ΔT_{B-A} between both growth-rooms never exceeded 1 °C.

Due to these temperature differences, there were also differences in terms of relative humidity (**Figure 25a**), both growth-rooms displayed higher RH values than the set 70% on all occasions during night-time (except for day 139 for the growth-room B). This difference exceeded 20% from day 0 to day 10 for the growth-room A and 10% for growth-room B. resulting in a 10 to 15% difference between both growth-rooms for the first 10 days, with values being higher for the growth-room A (ΔRH_{B-A} <0). On the following three sampling dates (days 12, 16 and 23), RH was slightly higher in the growth-room B (ΔRH_{B-A} = 7 to 10%). From day 36 onwards, relative humidity was again the highest in the growth-room A, particularly on day 139 (ΔRH_{B-A}=-29.90 %).

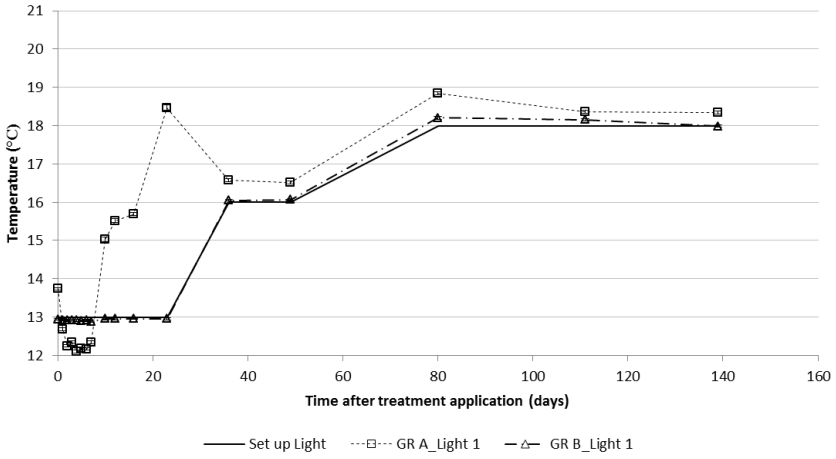
The daily 16h bright period was split in two blocks of 8h for the analysis of T and RH ("Light 1" and "Light 2", **Figures 24b, 24c, 25b** and **25c**) to reflect the structure of the program monitoring the growth-room incubation conditions, where the command lines driving this bright phase was split in two blocks.

During the "Light 1" period, growth-room A displayed significantly higher temperature values than the other growth-room (ΔT_{A-B} = 2 to 6 °C) on days 10, 12, 16 and 23 (**Figure 24b**). Although significant, the difference between both growth-rooms never exceeded 1 °C on the other dates. In the remaining part of the bright phase ("Light 2"), the growth-room B displayed significantly higher values for T than the other growth-room, in the first three days (**Figure 24c**), but the opposite difference ($\Delta T_{B-A}<0$) was observed for the remaining part of the experiment. The growth-room A displayed much higher values for T than the other growth-room (ΔT_{A-B}= 1 to 5 °C) on days 3, 5, 7, 10, 12 and 23 (**Figure 24c**). This difference did not exceed 1 °C subsequently.

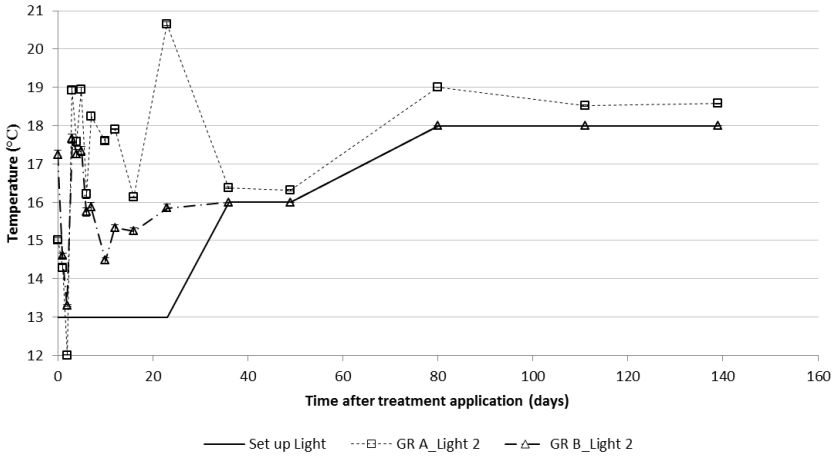
In terms of relative humidity, except on day 0 (ΔRH_{B-A} = 3.2 and 3.9% for "Light 1" and "Light 2" respectively) and day 1 (ΔRH_{B-A} = 8.8% for "Light 2"), the difference ΔRH_{B-A} did not exceed 1% until day 10 (**Figure 25b**). On days 10, 12, 16 and 23, growth-room A displayed much lower values than growth-room B (ΔRH_{B-A} = 10 to 30%). On the following dates, the difference between both growth-rooms was smaller, ranging from 2.7 to 4.4%, except on the last control date (day 139) when the growth-room B displayed much lower RH values than the growth-room A (ΔRH_{B-A} = -33.8 and -42.6% for "Light 1" and "Light 2" respectively). On all dates with the exception of day 139, RH values from the growth-room B closely matched the set value of 70%. Therefore, the difference between both growth-rooms simply resulted from the RH values measured in the growth-room A drifting away from the initially set RH value. On day 139 however, there was a large and abnormal decrease of RH following the switch from simulated July conditions to August averaged temperatures (see paragraph **5.2.2.3.** and **Table 11**).



a)

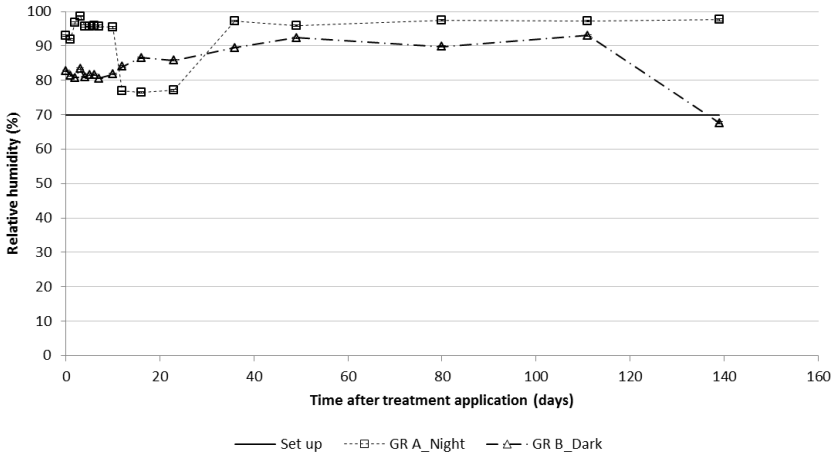


b)

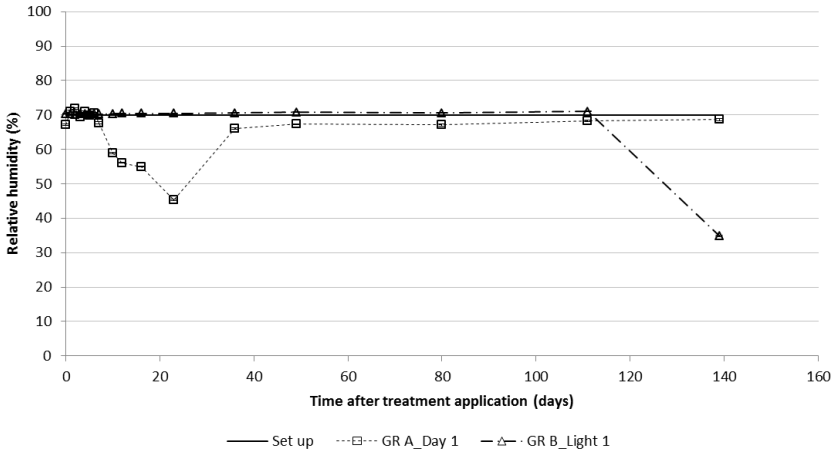


c)

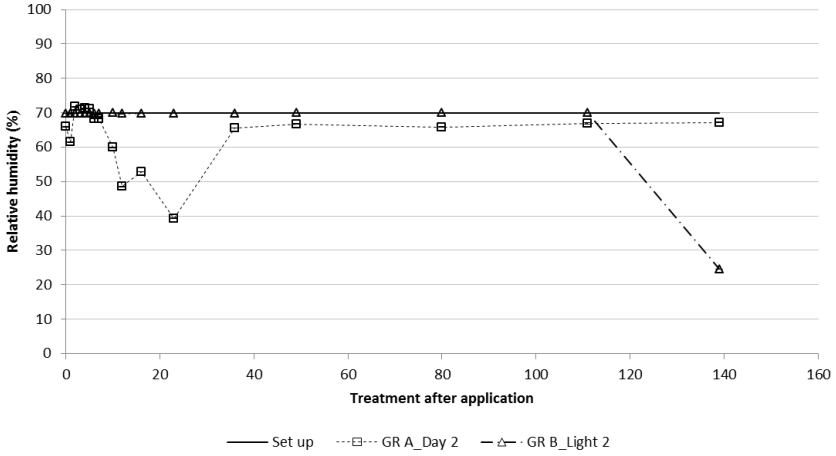
Figure 24: Temperature measured by the free sensor inside each growth-room over the entire experiment. Data were averaged for a) the "night" period, b) the first 8 hours of the bright phase ("Light 1") and c) the last 8 hours of this "bright" phase ("Light 2"). The temperature set up in the monitoring program for the corresponding phase is given by the continuous line.



a)



b)



c)

Figure 25: Relative humidity measured by the free sensor inside each growth-room over the entire experiment. Data were averaged for a) the "night" period, b) the first 8 hours of the bright phase ("Light 1") and c) the last 8 hours of this "bright" phase ("Light 2"). The humidity set up in the monitoring program for the corresponding phase is given by the continuous line.

Concerning ambient CO_2 concentrations (data not shown), the difference between both growth-rooms exceeded 50 ppm only on three occasions, always in the "Light 2" phase, on days 2 ($\Delta CO2_{B-A}$ = -62 ppm), 7 ($\Delta CO2_{B-A}$ = -52 ppm) and 36 ($\Delta CO2_{B-A}$ = -54 ppm). Overall, CO_2 concentrations inside growth-rooms ranged from 464 to 521 ppm, from 448 to 541 ppm and from 452 to 586 ppm, for the "Dark", "Light 1" and "Light 2" phases respectively.

5.3.2. Cumulative greenhouse gas emissions

Carbon dioxide was shown to be the main greenhouse gas emitted by incubated soils over the first 36 days of the experiment, representing in average 98.4% ± 0.3% of the cumulative GHG emissions. These emissions were not significantly affected either by the location of the lysimeter (i.e. growth-room A or B) or by the applied treatment (**Figure 28**).

Cumulative N_2O fluxes over the first 36 days of the experiment (expressed in CO_2 equivalents) accounted for 1.5 ± 0.3% of the direct GHG emissions over the same period. These fluxes were significantly affected by the location of the lysimeters ($P<0.0001$), with average emissions of 342 ± 43 and 96 ± 37 g N_2O-N ha^{-1} for treated lysimeters in growth rooms A and B respectively (**Figure 26**). However, variability within growth-rooms was quite high, with cumulative fluxes ranging, for each growth-room, from 150 to 719 g N_2O-N ha^{-1} and from -98 to 429 g N_2O-N ha^{-1} respectively.

Within each growth-room, there was also a significant effect of the "Slurry type" (effect S*G, $P<0.005$), with a trend toward higher N_2O release from soils applied with grass-based slurries compared to those applied with maize-based slurries. However, this difference was statistically significant only for the coupled treatments ^{15}N_GSCS/^{15}N_MSCS and GS/MS, in both growth-rooms (**Figure 26).** This was clearly related to differences in TAN, rather than total N, content between treatments (**Table 2**). Indeed the effect S*G was no longer significant when cumulative N_2O fluxes were related to the TAN content of the initial slurry (**Table 17**).

There was also an effect of carbon additions to slurry (G * S) effect (see paragraph **5.2.4.3.**, P<0.05), but this effect was only significant with respect to higher N_2O emissions, in growth-room A (^{15}N labelled cores), when grass-based slurry was supplemented with cane sugar (**Figure 26** and **Table 17**).

Over a total number of 33 lysimeters, five of them displayed a cumulative net uptake of N_2O (up to 98 g N_2O-N ha^{-1}, data not shown). As a consequence, cumulative N_2O fluxes were not significantly different from zero for corresponding treatments MS, MSCS and MSCel (**Figure 26** and **Table 17**).

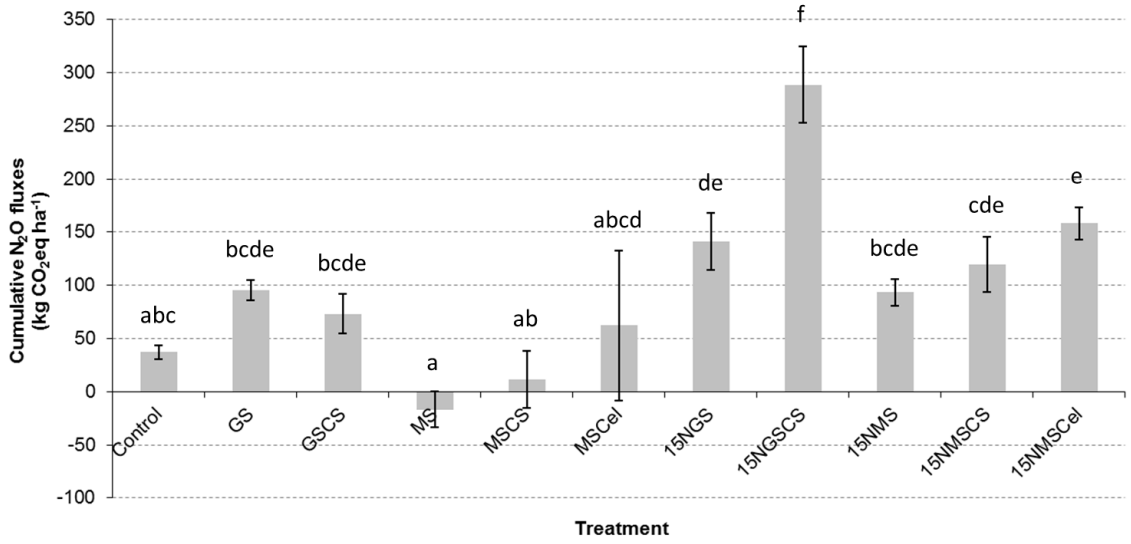
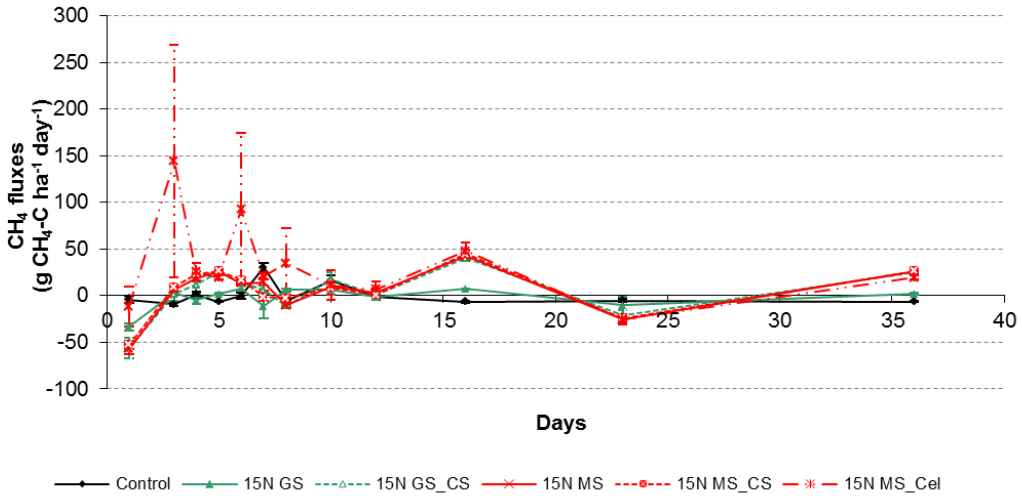


Figure 26: Cumulative N_2O fluxes, over the first 36 days of the experiment, for each treatment. Errors bars represent the standard error of the means. Small letters symbolizes the results of a Fisher LSD test ($p < 0.05$)

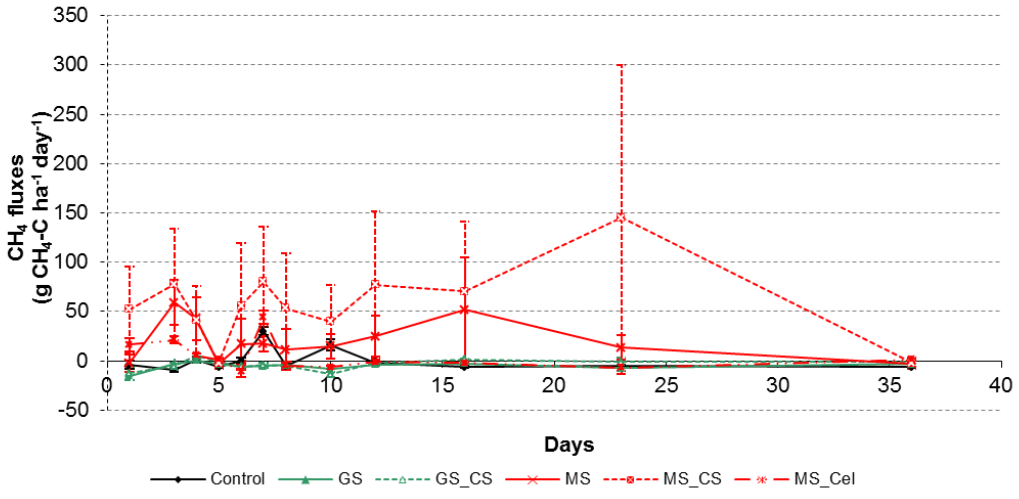
Table 17: Cumulative N_2O fluxes (in % N applied) for each treatment. Results are given as Mean (± se). Small letters symbolizes the results of a Fisher LSD test ($p < 0.05$)

Treatment	N_2O emissions (%N applied)	N_2O emissions (%TAN applied)
GS	0.22 (± 0.02)^{ab}	0.20 (± 0.02)^{abc}
GS_CS	0.12 (± 0.03)^{ac}	0.16 (± 0.04)^{abc}
MS	-0.03 (± 0.03)^{c}	-0.05 (± 0.05)^{a}
MS_CS	0.03 (± 0.06)^{c}	0.04 (± 0.08)^{ab}
MS_Cel	0.13 (± 0.15)^{ac}	0.19 (± 0.21)^{abc}
^{15}N_GS	0.23 (± 0.04)^{b}	0.30 (± 0.06)^{cd}
^{15}N_GS_CS	0.56 (± 0.07)^{d}	0.62 (± 0.08)^{e}
^{15}N_MS	0.22 (± 0.03)^{b}	0.28 (± 0.04)^{bcd}
^{15}N_MS_CS	0.27 (± 0.06)^{b}	0.36 (± 0.08)^{cde}
^{15}N_MS_Cel	0.38 (± 0.04)^{bd}	0.48 (± 0.05)^{de}

Methane emissions were usually low, averaging 342 ± 255 g CH_4-C ha^{-1} over the first 37 days of the experiment (data not shown). However, these CH_4 fluxes were highly variable between lysimeters, with three of them emitting over 1 kg CH_4-C ha^{-1} while another 14 soil cores (including all controls) displayed a net uptake of CH_4 over the measuring period.

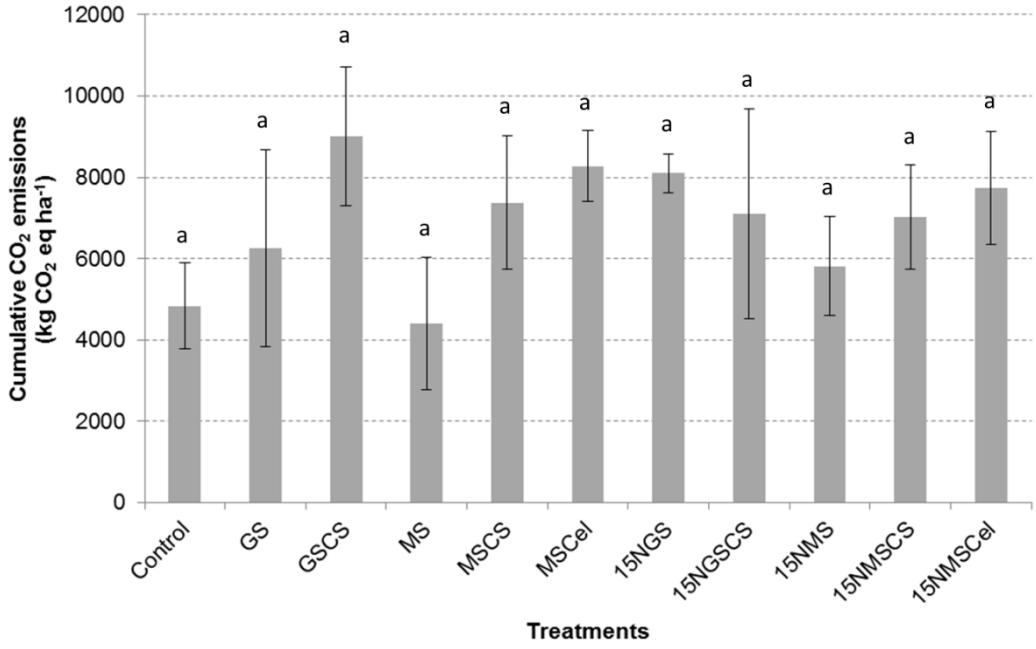


a) ^{15}N labelled cores (Growth-room A) + Control (Growth-room B)

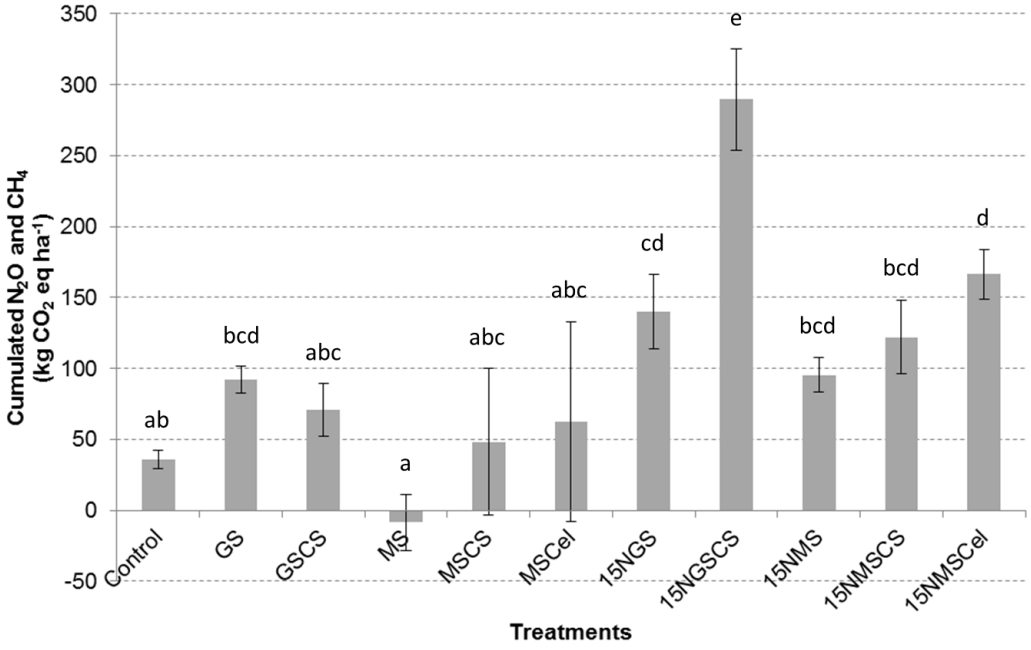


b) Non labelled cores + Control (Growth-room B)

Figure 27: Temporal pattern of daily CH_4 fluxes from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. Daily CH_4 fluxes from controls are shown for comparison in both graphs, even though those controls, incubated in growth-room B, were not replicated in growth-room A.



(a)



(b)

Figure 28: Direct cumulative CO_2 (a) and non-CO_2 (b) greenhouse gases emissions, over the first 36 days of the experiment, from incubated soils. Errors bars represent the standard error of the means. Small letters symbolizes the results of a Fisher LSD test ($p < 0.05$)

Two of these high CH_4 net emitters were in the growth-room B (belonging to treatment MS and MSCS) while the third one was applied with ^{15}N_MSCel slurry in the growth-room A (**Figure 27**). These emissions occurred either as short-lived peaks of up to 393 g CH_4-C ha^{-1}, on days 3, for the lysimeter treated with ^{15}N_MSCel slurry,

or in potentially longer periods of consistently high CH_4emission rate (F_{CH4} > 100 g CH_4-C ha^{-1} day^{-1}) as it was observed for one lysimeter treated with MSCS slurry.

Overall, total direct greenhouse gas emissions were primarily driven by soil respiration (**Figure 28**), and showed no significant effect of any experimental factor.

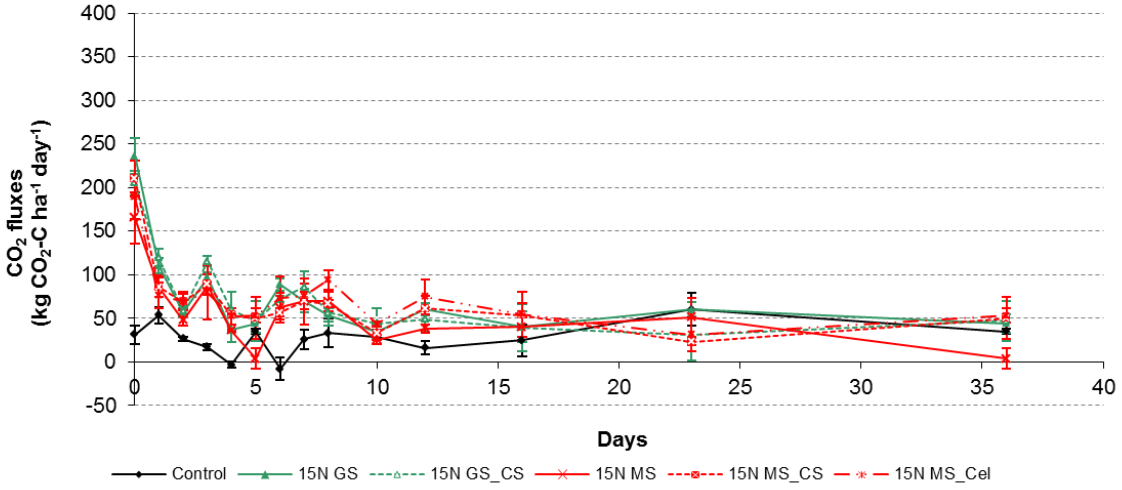
5.3.3. Daily pattern of CO_2 emission and fate of amendment-derived C into the system

5.3.3.1. Dynamic of soil CO_2 efflux from soils

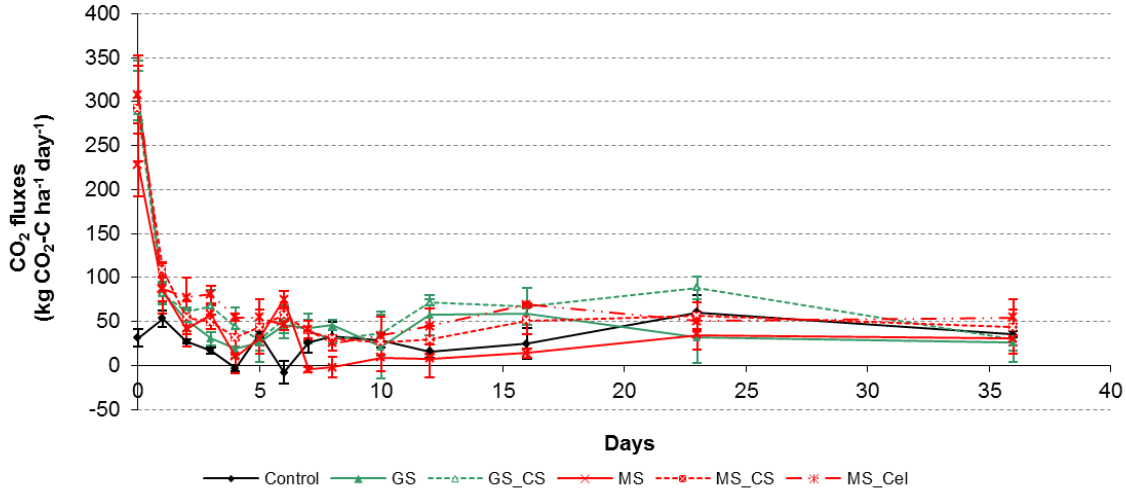
CO_2 fluxes were significantly affected by the location of the lysimeters on days 0 ($P<0.0001$), 1 ($P<0.05$), 3 ($P<0.0001$), 4 ($P<0.01$), 6 ($P<0.0001$), 7 ($P<0.0005$) and 8 ($P<0.0001$). On each occasion, soil respiration rates were significantly higher in the growth-room A than in growth-room B, except on day 0 where soil CO_2 efflux was significantly higher in growth-room B (**Figure 29**). On all these dates but day 7 and day 8, controls displayed significantly lower CO_2 effluxes than all treated lysimeters.

Within each growth-room, there was also a significant effect of the "Slurry type" (effect S*G) on days 1, 8 and 12 ($P<0.05$ each time). In the growth-room A, lysimeters applied with grass-based slurry displayed significantly higher CO_2 effluxes than those applied with maize-based slurry on day 1, but lower emissions on day 8 (**Figure 29a**). In the other growth-room (Growth-room B), lysimeters applied with maize-based manures emitted significantly less CO_2 than those applied with grass-based manures on days 8 and 12 (**Figure 29b**).

On all sampling dates, there was not any effect of the addition of exogenous C (sugar or cellulose).



a) ^{15}N labelled cores (Growth-room A) + Control



b) Non labelled cores (Growth-room B) + Control

Figure 29: Temporal pattern of daily CO_2 fluxes from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. Daily CO_2 fluxes from controls (black line) are shown for comparison in both graphs

5.3.3.2. Slurry-derived CO_2-C and priming effects

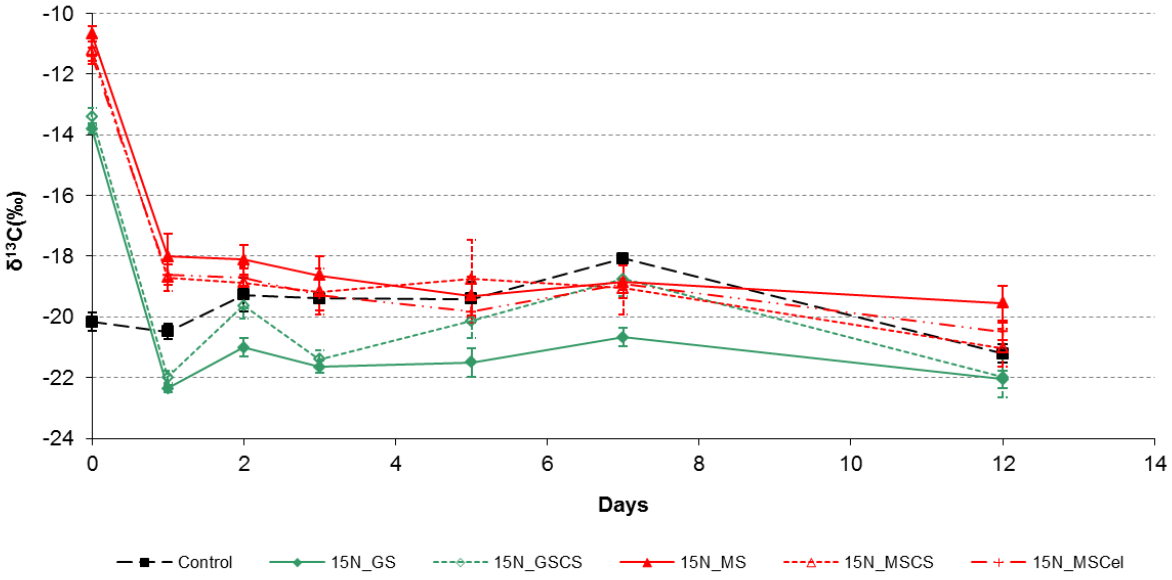
The $\delta^{13}C$ values of sampled CO_2 were significantly ($P < 0.0001$) affected by the location of the lysimeters on two occasions, on day 0 and day 3 (**Figure 30**).

Those initial $\delta^{13}C$ values derived from maize slurry treatments were significantly higher than those from grass-derived slurry (effect S*G, $P < 0.0001$) until day 3. In growth-room A, these values ranged, from -10.2 to -11.9‰ for maize-

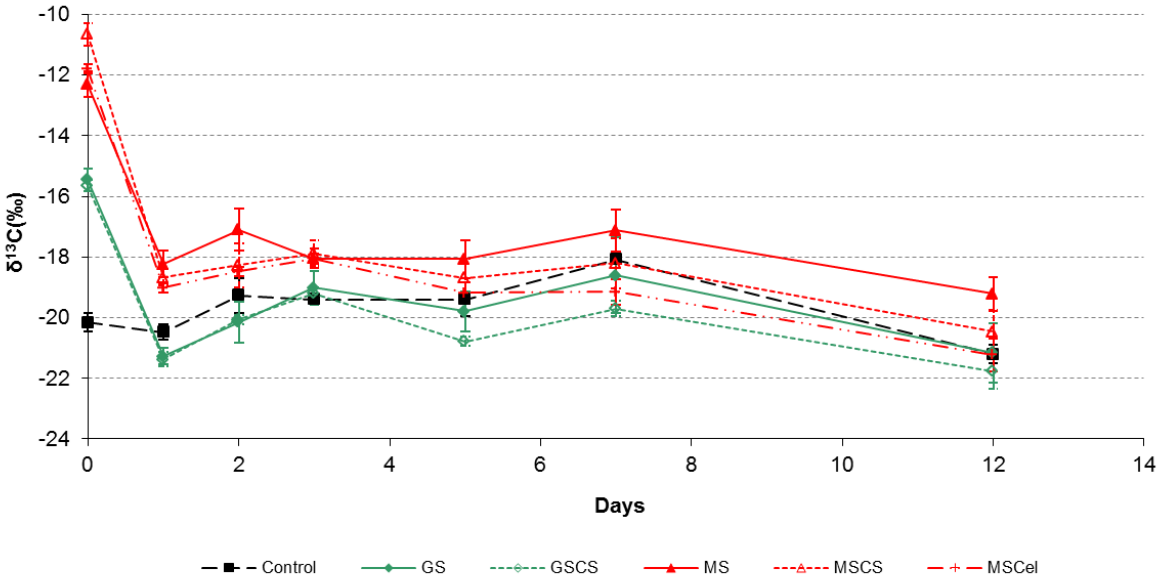
slurry and from -13.8‰ to -14.0‰ for grass-slurry respectively (**Figure 30a**), while they ranged from -10.0 to -13.0‰ and from -14.8 to -16.1‰ for maize and grass slurry in growth-room B (**Figure 30b**). The $\delta^{13}C$ values of CO_2 emitted from controls were then -20.2 ± 0.2‰, which was significantly lower than for any treated lysimeter (**Figure 30**). Surprisingly, the $\delta^{13}C$ of CO_2 respired from the grass slurry treatment, ranging from -14.8 to -16.1‰, was initially enriched in ^{13}C relative to the control. The effect S*G was also significant ($P<0.005$) on day 5 and 12.

On this first gas sampling, the treatment MS_CS, in the growth-room B, displayed $\delta^{13}C$ values significantly higher than those observed for MS and MSCel ($P<0.05$), whereas there was no statistical difference between maize-based slurry treatments in the other growth-room. All treatments displayed initially high values of $\delta^{13}CO_2$-C values on day 0. Variability was lower for ^{15}N labelled lysimeters (growth-room A), with values ranging from -13.8 ± 0.2‰, for ^{15}N_GS treated cores, to -10.7± 0.3‰, for ^{15}N_MS treatment.

On days 1 and 3, the difference between controls and the ^{15}N_GS treatments remained significant. However, after three days, there was not any significant difference between those controls and any lysimeter applied with grass-based manure. Over the twelve days where $\delta^{13}CO_2$-C was monitored, these values remained relatively constant (-19.7 ± 0.7‰) for control lysimeters. Those $\delta^{13}CO_2$-C values remained significantly higher for maize-based treatments than for their grass-based counterparts until day 5 (**Figure 30**).



a) ^{15}N labelled cores (Growth-room A) + Control



b) Non labelled cores + Control (Growth-room B)

Figure 30: Temporal pattern of $\delta^{13}C$ values for CO_2 emitted from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. $\delta^{13}C$ values from controls are indicated by the black line.

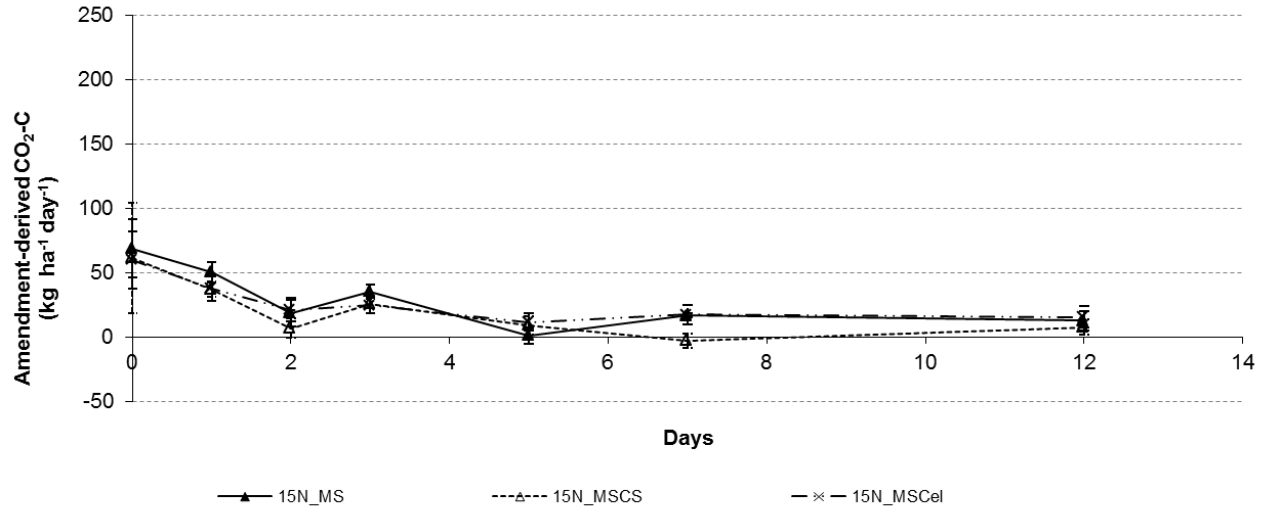
The ^{13}C natural abundance tracer technique allowed these $\delta^{13}C$ values to be used to calculate the contribution of amendment-derived and soil-derived C to the total CO_2 efflux from maize-based (C_4) treatments (**Figure 31**) using the equations (18) (see paragraph **5.2.4.1.**).

Initially (day 0), this contribution was significantly different from one growth-room to the other, due to differences in respiration rates and $\delta^{13}C$ values (**Figure 31**). In the growth-room A (^{15}N labelled treatments), the contribution of amendment-derived C to the total CO_2 efflux ranged, 3 hours after spreading, from 29 to 42%. Treatment-derived CO_2 losses accounted for 69±14, 61±14 and 60±11 kg CO_2-C ha^{-1} for treatments ^{15}N MS, ^{15}N MSCS and ^{15}N MSCel respectively, representing a loss of 5 to 6% of the initial amount of applied C (**Figure 31a)**. This contribution, in proportion, increased on day 1 to reach 43 to 58%, before quickly decreasing.

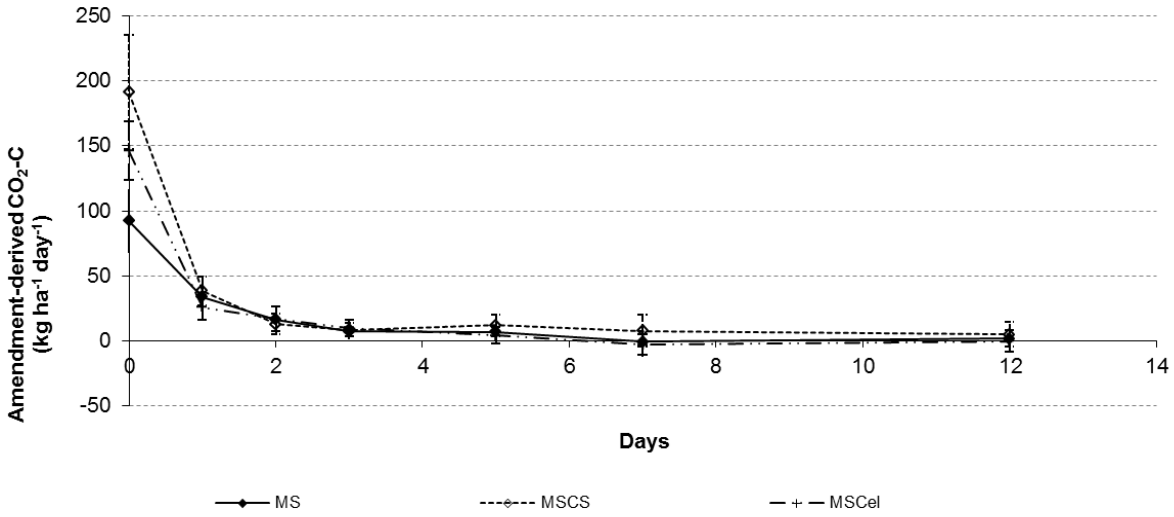
In the growth-room B (non-labelled treatments), contribution of amendment-derived C to the total CO_2 efflux, at day 0, was 40±7 and 48±5% for treatments MS and MS_Cel, but was much higher for the treatment MS_CS (65±5%). These manure-derived C losses were 92±22, 191±43 and 146±23 kg CO_2-C ha^{-1} for treatments MS, MS_CS and MS_Cel respectively, representing 8 to 17% of the amount of applied C (**Figure 31b**). These treatment-derived C losses decreased sharply on day 1, ranging from 25 to 38 kg CO_2-C ha^{-1}, to be less than 20 kg CO_2-C ha^{-1} from day 2 onwards.

Overall, cumulative amendment-derived C losses ranged, after 12 days, from 161 to 311 kg CO_2-C ha^{-1}, representing 15 to 28% of applied C.

The proportion of the total CO_2 efflux which was not derived from manure-derived C was assumed to be derived from SOM pool. It was calculated following the equation (20) (see paragraph **5.2.4.1**). The difference between this estimated SOM-derived CO_2-C and the total CO_2 efflux from controls was used to identify any existing priming effect (PE) following the application of treatments (**Figure 32**).



a) ^{15}N labelled cores (Growth-room A)



b) Non labelled cores

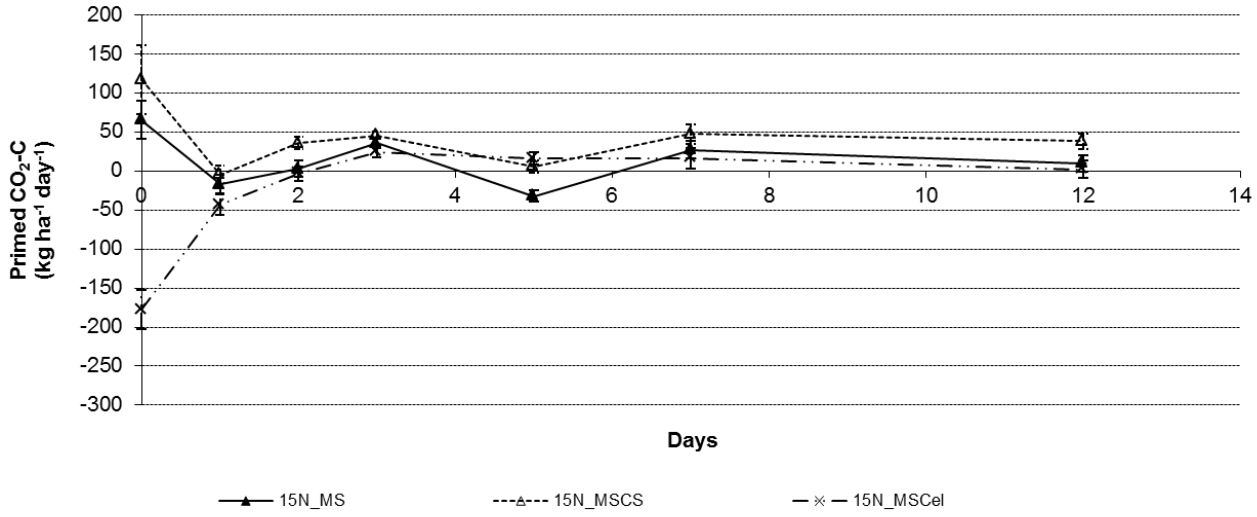
Figure 31: Daily amount of amendment-derived C in the CO_2 emitted from soils applied with ^{15}N labelled (a) or non-labelled (b) liquid manures.

In both growth-rooms, the application of cattle slurry onto soil cores (treatments MS and ^{15}N MS) led to a significant positive PE on day 0, of 105±25 and 65±17 kg CO_2-C ha^{-1} respectively (**Figure 32**). For both treatments, there was no significant PE on days 1 and 2, but a second positive PE of 34±6 and 36±9 kg CO_2-C ha^{-1}, respectively, occurred on day 3. In both growth-rooms, a negative PE was then observed, on day 5, but was consequent only for treatment ^{15}N MS (-11±7 and -32±5 kg CO_2-C ha^{-1} respectively). There was no significant PE in days 7 and 12.

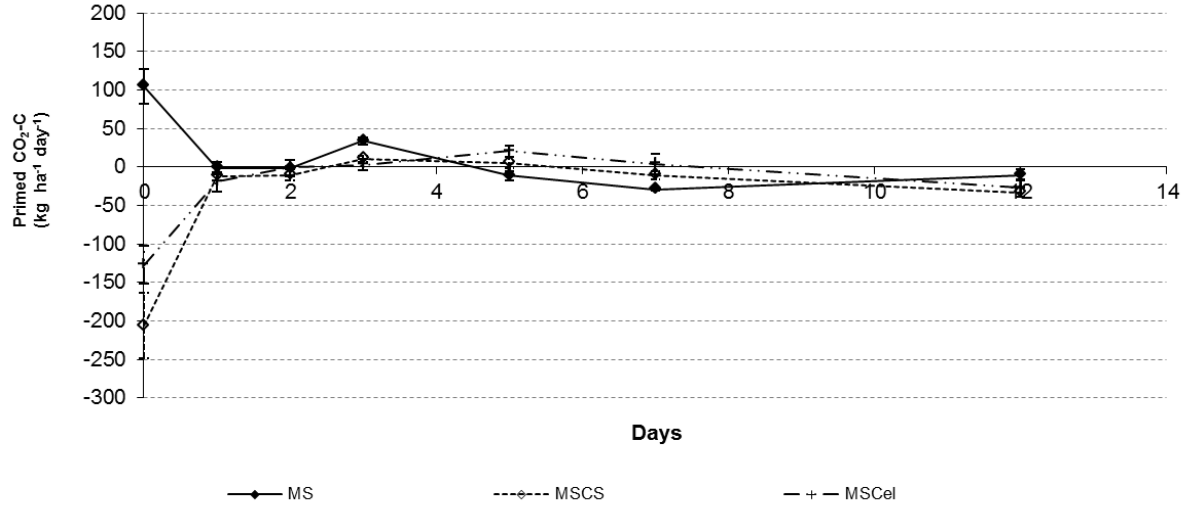
Cumulative primed C on day 4 was 147±61 and 88±53 kg CO_2-C ha^{-1} for both treatments respectively. In both cases, these values decreased to be barely significant against cumulative standard errors after day 5.

The addition of sugar to slurry, in growth-room A (treatment ^{15}N MS_CS), led to a positive PE of 117±18 kg CO_2-C ha^{-1} on day 0 (**Figure 32**). This PE remained positive on days 2, 3, 7 and 12, ranging from 35±7 to 47±15 kg CO_2-C ha^{-1}, but was not significant on days 1 and 5. Over the first 13 days, cumulative primed C was estimated to be 331±133 kg CO_2-C ha^{-1}, being equivalent to 30% of applied C and representing an additional loss of 1.4% of the initial soil C pool (0-10 cm soil layer) compared to controls. On the other hand, in growth-room B (treatment MS_CS), sugar addition to slurry led, on day 0, to a significant negative PE of -206±44 kg CO_2-C ha^{-1}. This PE was barely significant then, except on day 12 where it was estimated to be -33±9 kg CO_2-C ha^{-1}. Over the first 13 days, mineralisation of SOM-derived C was reduced by 234±126 kg C ha^{-1} compared to controls. This reduction represented 1.0% of the initial soil C pool and 21% of the initially applied C.

Addition of cellulose to slurry led, in both growth-rooms, to a significant negative PE on day 0. The corresponding values were -127±25 and -178±15 kg CO_2-C ha^{-1} for treatments MS_Cel and ^{15}N MS_Cel (C_4 slurry + C_4 cellulose treatments) respectively (**Figure 32**). For both treatments, this PE increased in day 1, remaining slightly negative. For the remaining sampling dates, PEs were either not or only marginally significant, being always within the range of -50 to +50 kg CO_2-C ha^{-1}. Globally, cumulative PE was no longer significant from day 7 onwards.



a) ^{15}N labelled cores (Growth-room A)

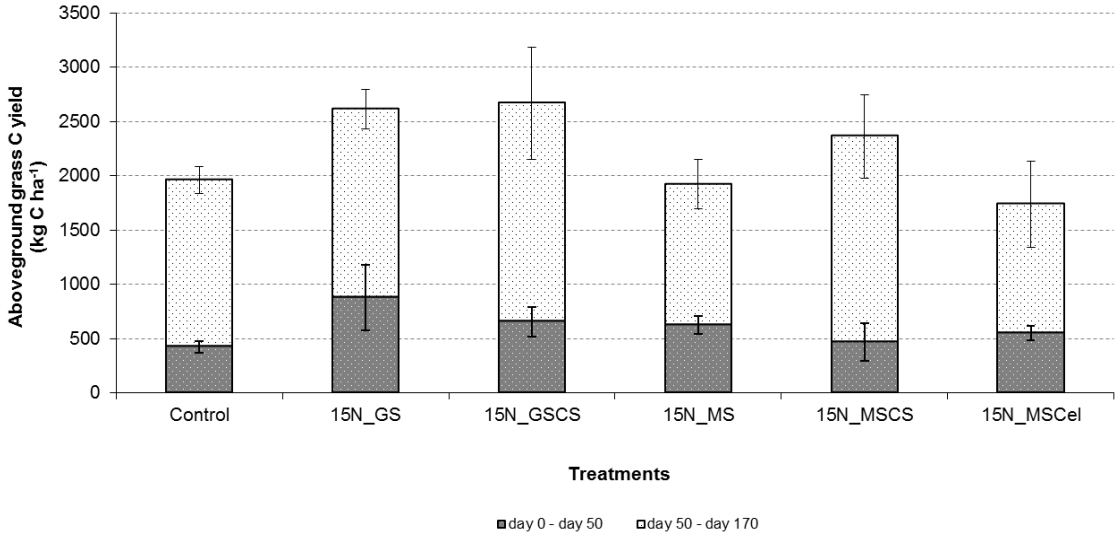


b) Non labelled cores

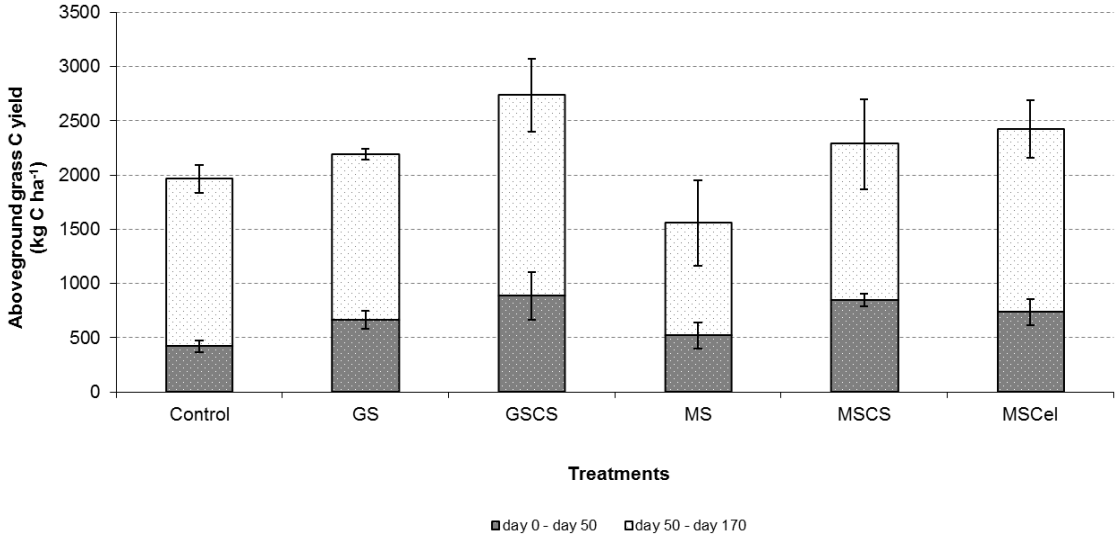
Figure 32: Daily amount of primed C in the CO_2 emitted from soils applied with ^{15}N labelled (a) or non-labelled (b) liquid manures.

5.3.3.3. Fate of applied C in the [Soil – Plant] system

The above-ground C yields after 170 days of incubation are shown, for each treatment, in **Figure 33**. There was not any significant effect of the applied treatment on yields.



a) ^{15}N labelled cores (Growth-room A) + Control (Growth-room B)

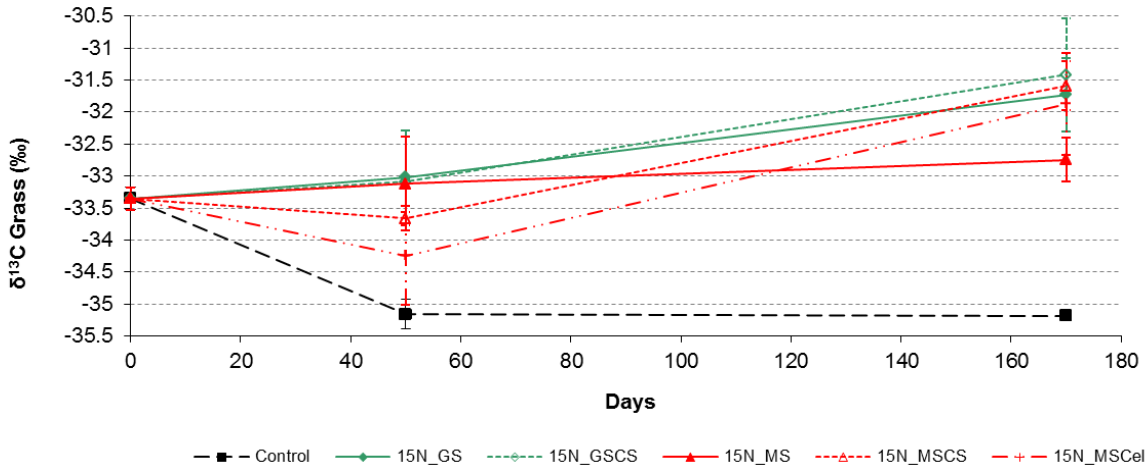


b) Non labelled cores + Control (Growth-room B)

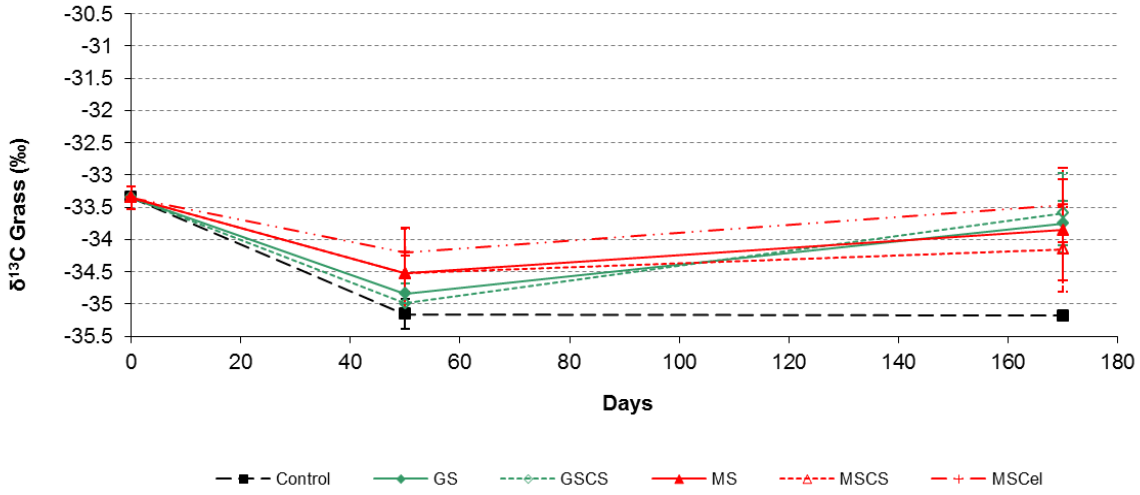
Figure 33: Above ground C yield from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. C yields are given for two measuring periods, as mean values. Error bars represent standard errors.

In terms of $\delta^{13}C$ values for grass bulk organic material, there was a clear effect of the lysimeter's location on day 50 ($P < 0.0005$) and 170 ($P < 0.0001$). However, within each growth-room, there was not any effect of the slurry treatment applied to

each lysimeter (**Figure 34**). The results also showed a clear trend towards an increase of $\delta^{13}C$ values with time in the growth-room A compared to the growth-room B **(Figure 34**).



a) ^{15}N labelled cores (Growth-room A) + Control (Growth-room B)



b) Non labelled cores + Control (Growth-room B)

Figure 34: Temporal pattern of $\delta^{13}C$ values for grass samples from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. $\delta^{13}C$ values from controls are shown by the black line.

In April 2010, the average soil C contents from the field where lysimeters were excavated were 24±1.3 and 31.6±3.1 t C ha^{-1} for the 0-10 and 10-20 cm layers respectively. After 170 days of experiment, soil C stocks from treated lysimeters had increased, being 42±2 and 43±3 t C ha^{-1} for each of the soil layer respectively.

Before the start of the experiment, sampled soil had $\delta^{13}C$ values of -29.3±0.1 and -28.5±0.1‰. Data presented in the **Table 18** shows that these values tended to increase during the experiment. After 170 days, there was a clear effect "Depth" ($P<0.0001$) (**Table 18**), $\delta^{13}C$ values being generally higher in the deeper soil layer. The effect "Depth" x "Growth-room" was also significant ($P<0.005$). Hence, for a given soil depth, $\delta^{13}C$ values were higher in the growth room A (^{15}N labelled cores) than in the growth room B.

There was no isotopic analysis performed on leached organic C, but TOC content of water samples collected at the bottom of the lysimeters was generally low (less than 15 kg C ha^{-1} for all of the soil cores) and only MS treated cores displayed significantly higher losses of dissolved organic C ($P<0.05$, data not shown).

Table 18: Soil C content and $\delta^{13}C$ at the end of the experiment (170 days). Values in the table are expressed as mean ± standard error. Results of two Fisher LSD tests (P<0.05) are given as homogeneous groups by small letters.

Treatment	Soil C content (t C ha^{-1})		$\delta^{13}C$ value (‰)	
	0 – 10 cm layer	10 – 20 cm layer	0 – 10 cm layer	10 – 20 cm layer
Control	35.3±3.8^{abcd}	36.8±3.5^{abcd}	-29.1±0.2^{hi}	-28.3±0.1^{jklm}
GS	40.4±4.6^{abcde}	45.4±4.9^{cdef}	-28.8±0.2^{hijk}	-28.3±0.1^{jklm}
GS_CS	31.6±3.1^{abc}	33.0±7.8^{abcd}	-29.2±0.2^{h}	-28.7±0.2^{hijkl}
MS	34.8±3.7^{abcd}	29.0±4.9^{a}	-29.2±0.1^{hi}	-28.6±0.2^{ijkl}
MS_CS	41.3±3.4^{abcde}	39.8±8.3^{abcde}	-29.2±0.1^{hi}	-28.9±0.2^{hij}
MS_Cel	36.7±2.1^{abcd}	29.7±1.8^{ab}	-28.9±0.1^{hij}	-28.2±0.6^{klm}
^{15}N GS	41.8±2.4^{abcde}	53.7±6.5^{efg}	-28.8±0.1^{hijkl}	-28.2±0.1^{klm}
^{15}N GS_CS	39.8±6.2^{abcde}	53.3±7.5^{efg}	-28.5±0.4^{ijklm}	-28.1±0.1^{lm}
^{15}N MS	57.6±1.1^{fg}	48.1±5.9^{def}	-27.9±0.3^{m}	-28.3±0.1^{klm}
^{15}N MS_CS	44.8±1.8^{bcdef}	35.0±9.9^{abcd}	-28.4±0.3^{jklm}	-28.2±0.1^{klm}
^{15}N MS_Cel	52.7±7.3^{efg}	66.8±3.4^{g}	-28.8±0.2^{hijk}	-28.4±0.2^{jklm}

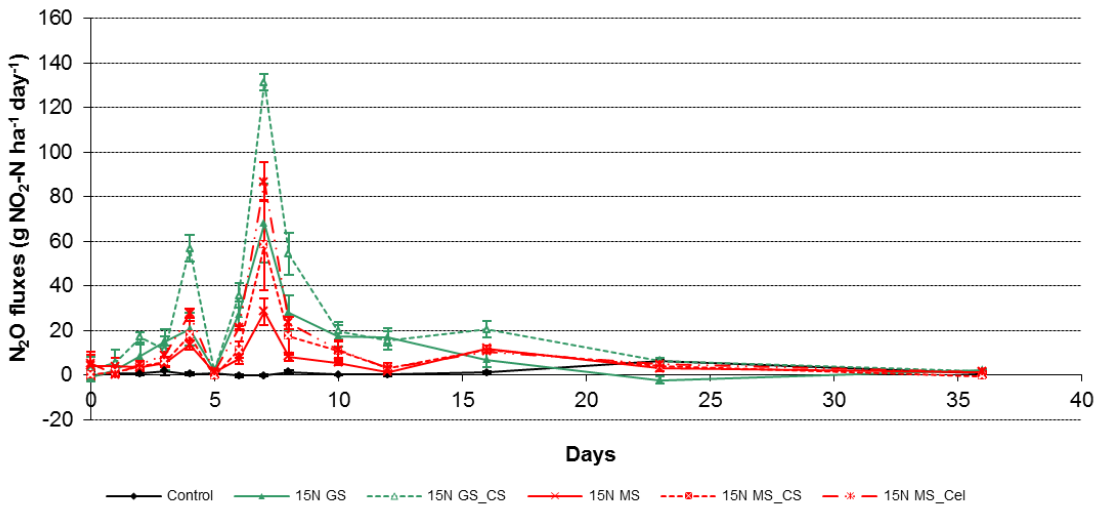
5.3.4. Daily N_2O emissions and recovery of amendment-derived ^{15}N-NH_4^+

5.3.4.1. Temporal pattern of N_2O fluxes

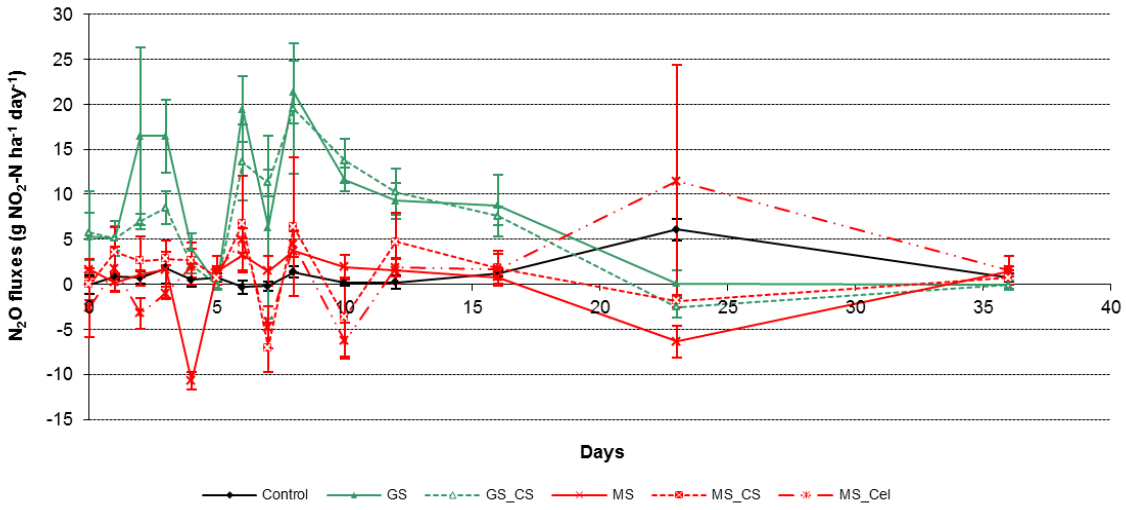
Cumulative N_2O fluxes were significantly influenced by location (see paragraph **5.3.2.**). Such effect could also be observed on most sampling dates, whether the analysis was carried on raw data – on days 6 ($P<0.0001$) and day 12 ($P<0.01$) – or on log-transformed data – on days 2 ($P<0.005$), 3 ($P<0.001$), 4, 7 ($P<0.0001$ both), 8 and 10 ($P<0.0005$ both).In all cases but day 7, there was even a combined effect S*G ($P<0.0001$ on days 3, 6, 10 and 12, $P<0.0005$ on days 2, 4 and 8.

On all these dates, lysimeters applied with maize-based treatments emitted significantly less N_2O (in absolute values) than those applied with grass-based slurries (**Figure 35**). However, such difference between both slurry types no longer existed when expressing N_2O emissions relative to the amount of TAN applied. On all days but day 12, these emissions from maize-based treatments were also lower in the growth-room B than in the other growth-room. The same difference between growth-rooms was found for grass-based treatments on days 4, 6, 8 and 12. In the growth-room B, controls were significantly different from maize-based treatments only on day 4.

Temporally, all ^{15}N labelled treatments displayed two N_2O emissions peaks, on days 4, ranging from 14 ± 2 to 57 ± 6 g N_2O-N ha^{-1} day^{-1}, and on day 7, ranging from 28 ± 6 to 131 ± 4 g N_2O-N ha^{-1} day^{-1} (**Figure 35a**). On both occasions, ^{15}N MS treatment had the lowest emissions while ^{15}N GS_CS treated cores emitted the highest amount of N_2O. Subsequently, those emissions decreased to be less than 20 and 12 g N_2O-N ha^{-1} day^{-1}, for grass-based and maize-based slurries respectively, from day 10 onwards. N_2O emissions from ^{15}N GS treated lysimeters were barely significant, compared to controls, for the last 3 sampling dates whereas those from ^{15}N GS_CS treatment remained above 15 g N_2O-N ha^{-1} day^{-1} until day 16 and were not significant only on day 36. For all soils treated with ^{15}N labelled maize-based slurries, those N_2O fluxes were low and barely significant on days 12, 23 and 36, while they displayed a slight increase to about 10 to 12 g N_2O-N ha^{-1} day^{-1} on day 16 (**Figure 35a**).



a) ^{15}N labelled cores (Growth-room A) + Control (Growth-room B)



b) Non labelled cores + Control (Growth-room B)

Figure 35: Temporal pattern of daily N_2O fluxes from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. Daily N_2O fluxes from controls are shown for comparison.

In the growth-room B, lysimeters applied with grass-based slurry displayed three emission peaks. GS_CS treated cores displayed emission peaks of 7 ± 1 and 9 ± 2 on day 3 and 4 respectively, 14 ± 4 g N_2O-N ha-1 on day 6 and 19 ± 7 g N_2O-N ha-1 on day 8. For GS treated cores, the corresponding peaks were 16 ± 10 and 16 ± 4 g N_2O-N ha^{-1}, on days 3 and 4 respectively, 19 ± 4 g N_2O-N ha^{-1}, on day 6, and 21 ± 3 g N_2O-N ha^{-1} on day 8, (**Figure 35b**). For both treatments, emissions gradually decreased to circa 8 g N_2O-N ha^{-1} day^{-1}on day 16. N_2O fluxes for lysimeters treated

with maize-based slurries were generally low, also displaying only two significant emission peaks, on days 6 and 8, from 3 to 4 g N_2O-N ha^{-1} day^{-1} for MS treatment to 6 to 7 g N_2O-N ha^{-1} day^{-1} for MS_CS treatment.

Significant N_2O uptake episodes could be observed on days 4 and 23, for treatment MS, on day 7 for treatment MS_CS and on days 2, 7 and 10 for treatment MS_Cel respectively (**Figure 35b**). Individually, those episodes of net N_2O uptake were mainly located in the growth-room B, particularly on days 7 and 23, when 53 and 87% of lysimeters displayed negative values for F_{N2O} respectively (**Table 9**). Furthermore, on a total of 29 occurrences for F_{N2O} < -5 g N_2O-N ha^{-1} day^{-1} in the course of the experiment, 26 of them were observed in the growth-room B, mainly on days 7, 10 and 23 (**Table 19**).

N_2O release from non-amended soils (i.e. controls) was low. It was not significantly different from zero on days 0, 4, 6, 7, 10 and 12, while it ranged from 0.7 to 1.8 g N_2O-N ha^{-1} day^{-1} on the other dates, except on day 23 when it reached 6 ± 1 g N_2O-N ha^{-1} (**Figure 35**).

Table 19: Inventory of episodes of net N_2O uptake among lysimeters. The number of occurrences of N_2O uptake measurements and F_{N2O} < -5 g N_2O-N ha^{-1} day^{-1} were given for treated lysimeters in each growth-room, as well as for controls.

Day	Controls (n = 3)		GR_A (n=15)		GR_B (n=15)	
	N_2O uptake	F_{N2O} < -5 g N_2O-N ha^{-1} day^{-1}	N_2O uptake	F_{N2O} < -5 g N_2O-N ha^{-1} day^{-1}	N_2O uptake	F_{N2O} < -5 g N_2O-N ha^{-1} day^{-1}
0	1	0	7	1	4	2
1	0	0	6	0	4	0
2	0	0	0	0	5	1
3	1	0	1	1	4	2
4	1	0	0	0	3	3
5	1	0	2	0	6	0
6	2	0	0	0	0	0
7	2	0	0	0	8	6
8	0	0	0	0	1	1
10	1	0	0	0	4	4
12	2	0	1	0	1	0
16	0	0	0	0	2	0
23	0	0	3	1	13	7
36	1	0	2	0	6	0

5.3.4.2. Isotopic analysis of applied materials

Prior to application, ^{15}N-enriched ammonium sulphate (AS-^{15}N) was added to grass and maize-based slurries at a rate of 5.02 and 3.50 kg additional NH_4^+-^{15}N ha^{-1} respectively (**Table 20**).However, the actual amount of AS-derived ^{15}N recovered in each slurry subsample was calculated from equations (22), (23) and (24) (see paragraph **5.2.4.2.**). From the results of such calculations, this amount of AS-derived ^{15}N in analysed slurry samples was only 8.6 to 10% of the amount of AS-^{15}N initially added (**Table 20**).

Table 20: Comparison between the added amount of ^{15}N enriched AS and the amount of AS-^{15}N recovered in analysed slurry samples for each treatment in the growth-room A

Treatment	Added AS-^{15}N (kg NH_4-^{15}N ha^{-1})	AS-derived ^{15}N calculated from slurry analyses (kg NH_4-^{15}N ha^{-1})	AS-derived ^{15}N calculated from slurry analyses (% added AS-^{15}N)
^{15}N GS	5.02	0.503	10.0
^{15}N GS_CS	5.02	0.439	8.7
^{15}N MS	3.50	0.302	8.6
^{15}N MS_CS	3.50	0.305	8.7
^{15}N MS_Cel	3.50	0.303	8.7

5.3.4.3. Losses of ^{15}N enriched N_2O in the atmosphere

The analysis of the temporal pattern of ^{15}N isotopic ratios for each treatment, in the growth room A, showed a similar trend for all ^{15}N labelled treatments (**Figure 36**).

On day 0, those values were similar to those measured for controls and non-labelled treatments. They ranged from 0.353 ± 0.014 to 0.379 ± 0.006 for treatments ^{15}N GS and ^{15}N MS_Cel respectively.

Isotopic ratios increased to reach peak values on days 5 and 7 (**Figure 36**). On both days, isotopic ratios from emitted N_2O were significantly higher ($P<0.005$) for

grass-based treatments (due primarily to higher initial enrichment) with average values for both days of 1.91% and 2.42% for treatments ^{15}N GS and ^{15}N GS_CS respectively – than for maize-based treatments - with average values of 1.27, 1.61 and 1.69 for treatments ^{15}N MS ^{15}N MS_CS and ^{15}N MS_Cel respectively.

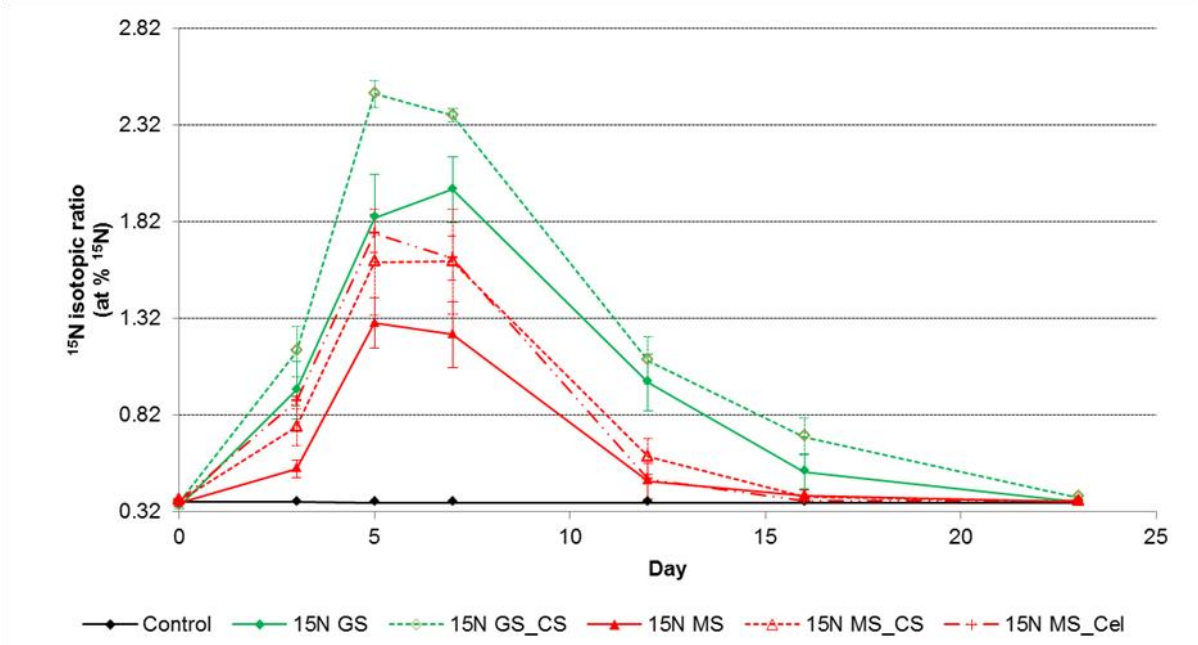


Figure 36: Temporal pattern of ^{15}N isotopic ratio for N_2O emitted from lysimeters applied with ^{15}N labelled liquid manures (Growth-room A). The isotopic ratio from controls is also shown.

During those two days, the contribution of the AS-derived ^{15}N pool to the total amount of N_2O-^{15}N emitted was 83 and 85% for treatment ^{15}N GS and ^{15}N GS_CS respectively, while it ranged from 73 to 82% for maize-based treatments (**Figure 37**).

For all treatments, ^{15}N isotopic ratios subsequently decreased on days 12 and 16 to be barely different from controls on day 23 (**Figure 36**). Hence, the calculated contribution of the AS-derived ^{15}N pool to the total amount of N_2O-^{15}N emitted also decreased to be 65 and 68% on day 12 for ^{15}N GS and ^{15}N GS_CS treated soils respectively. Due to a high variability, this contribution was no longer significant for maize-based treatments. On days 16 and 23, none of these contributions were significant.

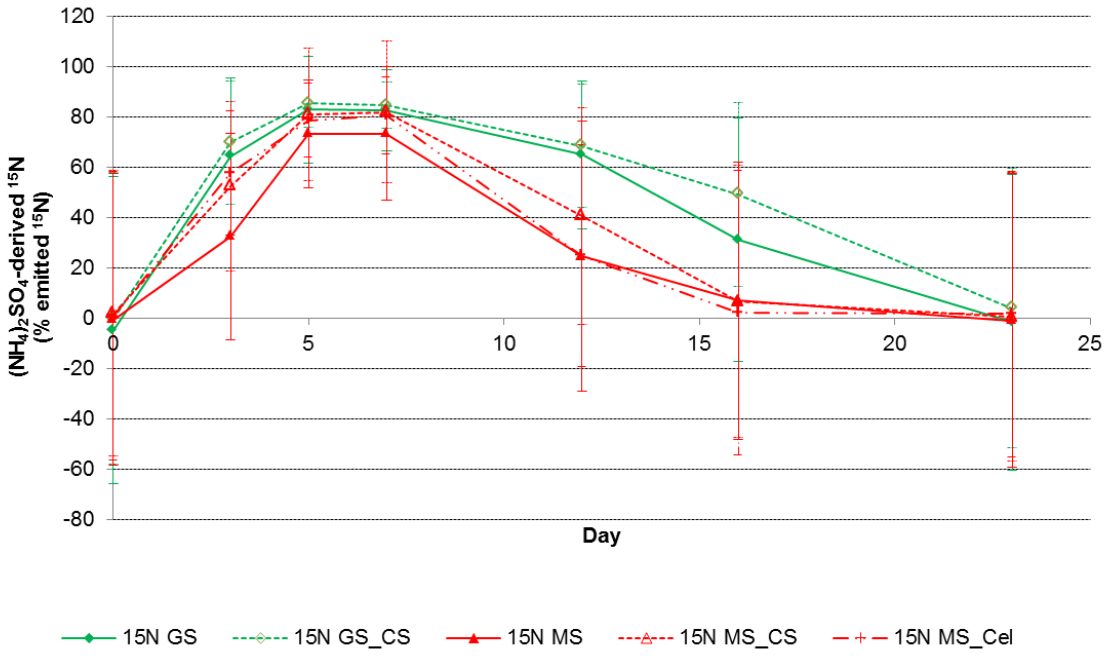


Figure 37: Calculated amount of AS-derived ^{15}N emitted as N_2O (in % of total N_2O-^{15}N) from lysimeters applied with ^{15}N labelled slurries (Growth-room A). Standard errors are given by vertical bars.

After 37 days of measuring N_2O release from incubated soils, ANL values, calculated from the difference between treated lysimeters (both growth-rooms) and controls (growth-room B), ranged from 0.13 to 0.49% in the growth-room A (**Table 21**) and from -0.11 to 0.13% in the other growth-room (data not shown).

The cumulative amount of AS-derived N_2O-^{15}N was generally low but higher for grass-based treatments due to higher initial labelling; with a maximum of 2.35 ± 0.22 g N_2O-^{15}N ha^{-1} for ^{15}N GS_CS treated lysimeters (**Table 21**). All maize-based treatments emitted, in average, less than 1 g N_2O-^{15}N ha^{-1}.

When related to the amount of initially applied AS-derived ^{15}N, the recovery of AS-derived ^{15}N in the gaseous N_2O fraction (^{15}NRG) displayed values ranging from 0.010 to 0.047% (**Table 21**). This recovery tended to be higher for grass-based slurries and following the addition of exogenous C to the initial slurry.

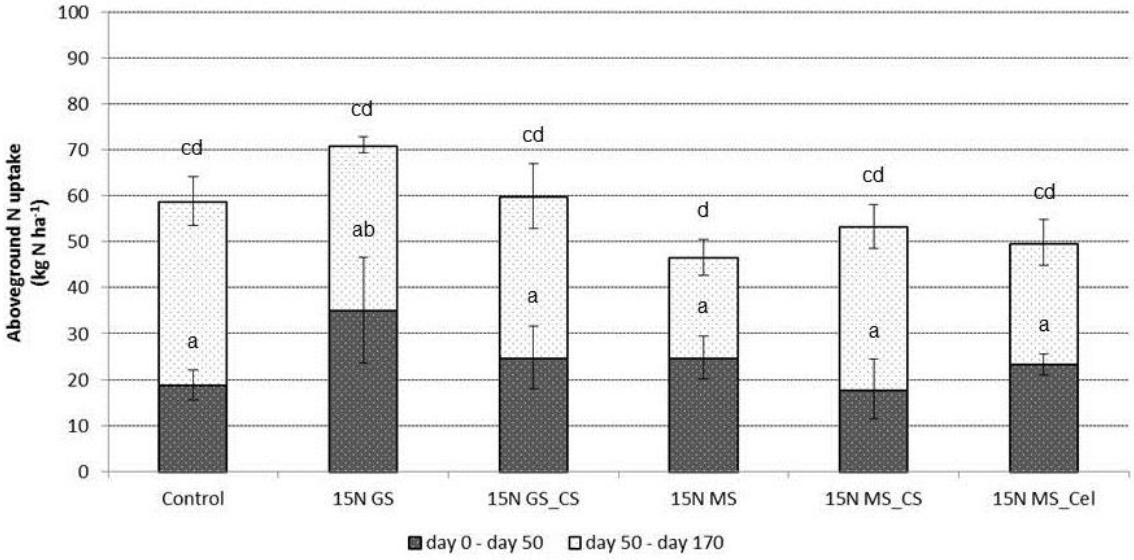
Table 21: Apparent N losses (ANL) and AS-derived ^{15}N recovery (^{15}NRG) in the N_2O emitted over the first 37 days of the experiment. Results are given as mean values ± standard errors.

Treatment	ANL (%)	Emitted AS-derived N_2O-^{15}N (g N_2O-^{15}N ha^{-1})	^{15}NRG (%)
^{15}N GS	0.17 ± 0.05	1.27 ± 0.19	0.025 ± 0.004
^{15}N GS_CS	0.49 ± 0.07	2.35 ± 0.22	0.047 ± 0.004
^{15}N MS	0.13 ± 0.03	0.34 ± 0.13	0.010 ± 0.004
^{15}N MS_CS	0.18 ± 0.06	0.69 ± 0.21	0.020 ± 0.006
^{15}N MS_Cel	0.29 ± 0.04	0.93 ± 0.14	0.009 ± 0.001

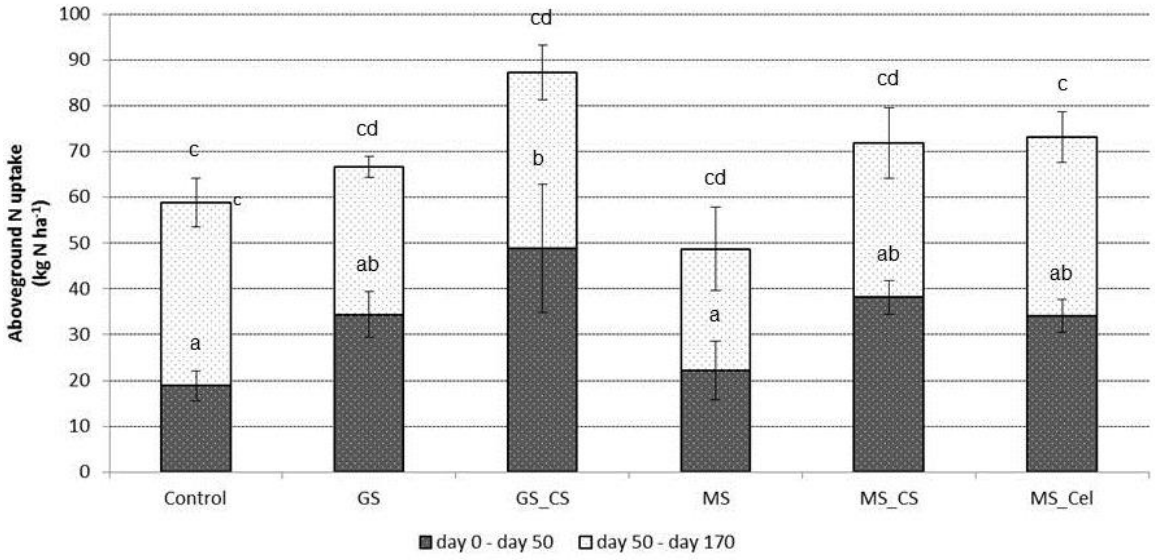
5.3.4.4. Fate of labelled ^{15}N in the [Soil – Plant] system and total ^{15}N recovery

Above ground N uptake over 170 days of incubation are shown, for each treatment, in **Figure 38**. There was a barely significant "Growth room" effect on these N uptake values for the period day 0-50. Overall and for the period day 50-170, this effect was not significant. The applied treatments (C or N) did not significantly affect either N uptake values or the dry matter yield (data not shown).

The average grass N content was 1.88±0.05 % on day 50, being significantly higher than the value of 1.3% measured prior to slurry application. Such grass N content decreased in the remained part of the experiment to reach 0.88±0.03%. The N content of above ground plant tissues (in %) was significantly higher in the growth-room A than in the other growth-room on both day 50 ($P < 0.01$) and day 170 ($P < 0.05$, data not shown).



a) ^{15}N labelled cores (Growth-room A) + Control (Growth-room B)



b) Non labelled cores + Control (Growth-room B)

Figure 38 Above ground N uptake from lysimeters applied with ^{15}N labelled (a) or non-labelled (b) liquid manures. N uptake is given for two measuring periods, as mean values. Error bars represent standard errors. Results of two Fisher LSD tests (P<0.05) are given as homogeneous groups by small letters.

In terms of isotopic ratios, for ^{15}N labelled cores, there was an effect "Slurry type" (P<0.05) on day 50, with higher values for grass-based treatments (**Figure 39**). This effect was absent on day 170.

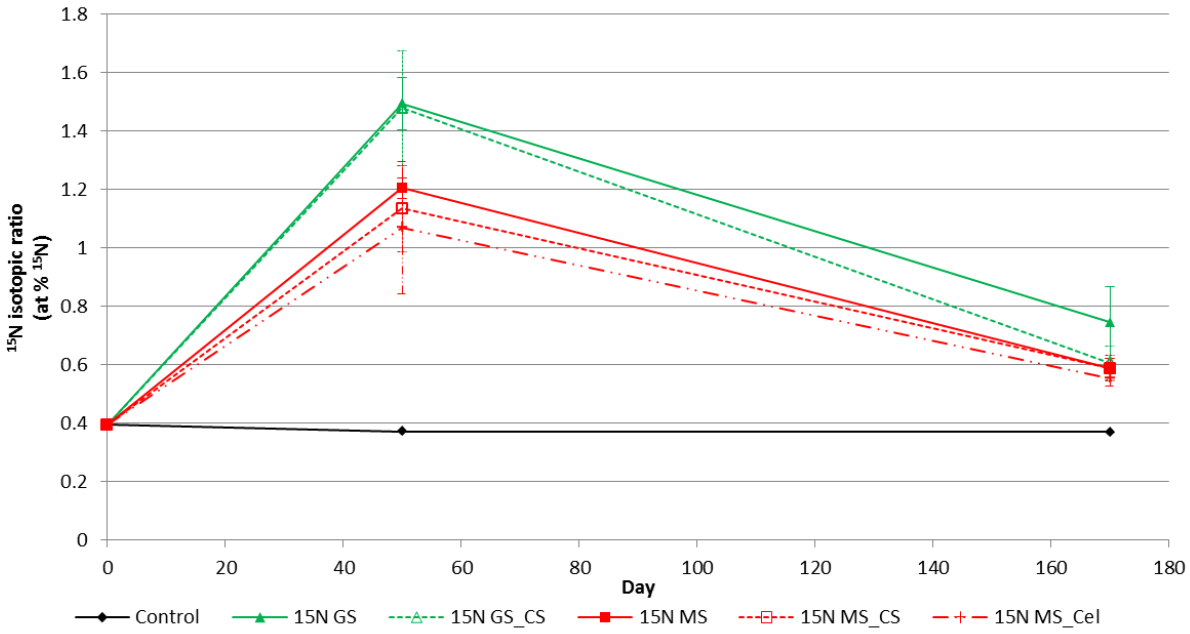


Figure 39: Temporal pattern of ^{15}N isotopic ratio for grass grown from lysimeters applied with ^{15}N labelled liquid manures (Growth-room A). The isotopic ratio from controls is shown for comparison even though those controls, incubated in growth-room B, were not replicated in growth-room A.

Apparent recovery of applied N (ANR), on day 50, ranged from -1.0 to 12.6% in the growth-room A (**Table 22**). In the growth-room B, ANR values ranged from 3.1 to 23.6% (data not shown). In both growth-rooms, those values tended to be higher for grass-based treatments. However, on four occasions in the growth-room A, ANR values were not significantly (or barely) different from 0. For the second growing period (days 50 to 170), ANR values were lower than during the first 50 days. During this period there was a trend for negative ANR, even though, for most treatments, they were not or barely significantly different from 0 (**Table 22**).

When related to the amount of initially applied AS-derived ^{15}N, the total recovery of AS-derived ^{15}N in herbage (^{15}NRH) displayed values ranging from 5.8 to 10.1% (**Table 22**). Again this recovery tended to be higher for grass-based treatments and lower when exogenous C was added to slurry. The proportion of AS-derived ^{15}N recovered in the first 50 days accounted for 69 to 82% of the total ^{15}NRH.

Table 22: Apparent N recovery (ANR) and AS-derived ^{15}N recovery (^{15}NRH) in the herbage samples collected on days 50 and 170 of the experiment. Results are given for each growing period, as well as for the entire experiment, as mean values ± standard errors.

Treatment	ANR (%)			^{15}NRH (%)		
	Day 0 - 50	Day 50 - 170	Total	Day 0 - 50	Day 50 - 170	Total
^{15}N GS	12.6 ± 9.3	-3.1 ± 4.3	9.5 ± 11.3	7.53 ± 2.81	2.60 ± 1.25	10.13 ± 3.08
^{15}N GS_CS	5.4 ± 6.9	-4.3 ± 8.0	1.0 ± 6.7	5.60 ± 2.74	1.44 ± 0.46	7.04 ± 2.77
^{15}N MS	6.7 ± 6.5	-20.4 ± 7.3	-13.7 ± 7.9	5.50 ± 1.21	1.21 ± 0.35	6.70 ± 1.26
^{15}N MS_CS	-1.0 ± 7.6	-4.7 ± 7.4	-5.7 ± 7.7	4.01 ± 2.16	1.81 ± 0.45	5.82 ± 2.21
^{15}N MS_Cel	5.1 ± 4.5	-15.1 ± 8.1	-10.0 ± 9.9	4.84 ± 2.68	1.13 ± 0.27	5.97 ± 2.69

In April 2010, the average soil total N content from the field where lysimeters were excavated were 2.22±0.12 and 1.68±0.11 t N ha^{-1} for the 0-10 and 10-20 cm layers respectively. After 170 days of experiment, soil N stocks from treated lysimeters had increased, being 2.54±0.05 and 2.05±0.06 t N ha^{-1} for each of the soil layer respectively. Soil N content significantly decreased with the sampling depth ($P<0.0001$) (**Table 22**), but there was not any treatment effect.

Table 23: Soil N content and ^{15}N isotopic ratio measured for each treatment at the end of the experiment. Results are expressed as mean values ± standard errors.

Treatment	Soil N content (t N ha^{-1})		^{15}N isotopic ratio (at % ^{15}N)	
	0 - 10 cm	10 - 20 cm	0 - 10 cm	10 - 20 cm
Control	2.53 ± 0.24	2.07 ± 0.11	0.370 ± 0.000	0.370 ± 0.000
GS	2.49 ± 0.22	1.89 ± 0.27	0.387 ± 0.009	0.378 ± 0.004
GS_CS	2.64 ± 0.26	2.22 ± 0.25	0.378 ± 0.006	0.376 ± 0.004
MS	2.58 ± 0.02	2.03 ± 0.07	0.380 ± 0.009	0.374 ± 0.003
MS_CS	2.39 ± 0.27	2.04 ± 0.15	0.393 ± 0.001	0.378 ± 0.001
MS_Cel	2.61 ± 0.13	2.45 ±0.27	0.398 ± 0.005	0.383 ± 0.001
^{15}N GS	2.53 ± 0.07	2.21 ± 0.21	0.442 ± 0.012	0.387 ± 0.008
^{15}N GS_CS	2.51 ± 0.22	1.88 ± 0.13	0.447 ± 0.006	0.394 ± 0.001
^{15}N MS	2.63 ± 0.21	2.04 ± 0.21	0.430 ± 0.010	0.382 ± 0.001
^{15}N MS_CS	2.51 ± 0.10	1.81 ± 1.00	0.433 ± 0.011	0.387 ± 0.006
^{15}N MS_Cel	2.48 ± 0.22	1.96 ± 0.24	0.427 ± 0.005	0.399 ± 0.012

Prior to the start of the experiment, the ^{15}N isotopic ratios of sampled soil were 0.377 ±0.004 and 0.372±0.001 at% ^{15}N for both depths respectively. Data from ^{15}N labelled treatments, presented in the **Table 12**, show that these values increased in the top 10 cm of soil over the course of the experiment, ranging from 0.427 ± 0.005 to

0.447 ± 0.006 at% ^{15}N on day 170. Similar increase of ^{15}N isotopic ratios could be observed in the 10 – 20 cm soil layer, but of a much smaller magnitude (**Table 23**). Neither the slurry type nor the supplementation of slurry with exogenous C affected the ^{15}N isotopic ratios of sampled soil.

Averaged apparent recovery of applied N in soil was -85 ± 348 % in the growth-room A (^{15}N labelled cores) but calculated ANR values were highly variable and never significantly different from 0 (**Table 24**).

When related to the amount of initially applied AS-derived ^{15}N, the total recovery of AS-derived ^{15}N in soil (^{15}NRS) was, in average, 33.2 ± 8.0 % (**Table 24**). For all treatments but ^{15}N MS_Cel, the proportion of AS-derived ^{15}N recovered in the 0 – 10 cm soil layer accounted for 83 to 89% of the total ^{15}NRS. Such proportion was only 68% for the MS_Cel treatment.

Overall, the total recovery of AS-derived ^{15}N in the system, over the course of the experiment, was, on average, 40.4 ± 8.4 %, ranging from 33.7 to 46.2% (data not shown).

With no exogenous C added, both calculated ^{15}N recoveries tended to be higher for maize-based slurry (^{15}N MS) than for their grass-based counterpart (^{15}N GS). However, the addition of exogenous C increased such ^{15}N recovery for grass-based slurry (^{15}N GS_CS), while it had the opposite effect on maize-based treatments (^{15}N MS_CS and ^{15}N MS_Cel).

Table 24: Apparent N recovery (ANR) and AS-derived ^{15}N recovery (^{15}NRS) in the soil samples collected at the end of the experiment. Results are given for two different depths as mean values ± standard errors.

Treatment	ANR (%)			^{15}NRS (%)		
	0 – 10 cm	10 – 20 cm	Total	0 – 10 cm	10 – 20 cm	Total
^{15}N GS	0 ± 241.9	136.9 ± 228.2	136.9 ± 332.5	25.93 ± 7.11	3.51 ± 3.65	29.45 ± 7.69
^{15}N GS_CS	-12.3 ± 250.2	-144.6 ± 130.4	-156.9 ± 282.2	31.92 ± 5.05	6.44 ± 1.62	38.36 ± 5.30
^{15}N MS	88.6 ± 286.4	-25.7 ± 216.1	62.8 ± 358.8	35.08 ± 10.06	4.44 ± 1.97	39.52 ± 10.25
^{15}N MS_CS	-17.7 ± 288.7	-287.3 ± 164.2	-305.0 ± 332.1	26.58 ± 7.89	4.53 ± 2.79	31.11 ± 8.37
^{15}N MS_Cel	-46.2 ± 338.6	-118.8 ± 275.8	-165.1 ± 436.7	18.95 ± 4.92	8.80 ± 6.51	27.76 ± 8.16

5.3.4.5. Analysis of N leaching losses

Cumulative N leaching losses (**Table 26**), estimated after analysis of water samples regularly collected at the bottom of each lysimeter until day 124, were quite low and were significantly affected by the location of each lysimeter ($P<0.0001$).

Table 25: Cumulative leaching losses and apparent N recovery (ANR) of both inorganic and total N, for each treatment, over the entire experiment. Results are expressed as mean values ± standard errors.

Treatment	N losses (% N applied)		ANR (% N applied)	
	Inorganic N	Total N	Inorganic N	Total N
GS	1.22 ± 0.21ab	1.64 ± 0.23de	-0.78 ± 1.35	-0.94 ± 1.76
GS_CS	1.24 ± 0.18a	1.55 ± 0.19def	-0.24 ± 1.12	-0.36 ± 1.42
MS	1.08 ± 0.23abc	1.82 ± 0.17d	-0.77 ± 1.35	-0.52 ± 1.90
MS_CS	1.24 ± 0.29ab	1.77 ± 0.20d	-0.65 ± 1.21	-0.68 ± 1.61
MS_Cel	0.75 ± 0.21abc	0.92 ± 0.15f	-1.17 ± 1.22	-1.55 ± 1.56
15N GS	0.51 ± 0.24c	0.73 ± 0.32f	-1.21 ± 1.14	-1.47 ± 1.48
15N GS_CS	0.57 ± 0.17c	0.96 ± 0.08f	-0.98 ± 0.89	-1.89 ± 1.19
15N MS	0.60 ± 0.30bc	1.06 ± 0.25ef	-1.22 ± 1.03	-1.35 ± 1.36
15N MS_CS	0.51 ± 0.24c	1.19 ± 0.04def	-1.57 ± 1.27	-1.46 ± 1.74
15N MS_Cel	0.71 ± 0.18abc	1.17 ± 0.11def	-1.31 ± 1.20	-1.45 ± 1.60

The amounts of inorganic and total N leached by controls during this period were 1.89 ± 0.16 and 2.43 ± 0.16 kg N ha^{-1} (data not shown). Treated lysimeters displayed significantly lower N leaching losses than controls, leading negative calculated ANR values (**Table 26**). However, within each growth-room, there were no significant differences between treatments, with the exception of MS_Cel treated cores which displayed lower total N losses than MS and MS_CS in the growth-room B.

5.4. Discussion

5.4.1. Fate of applied C in the system [soil-plant-atmosphere]

The closed chamber method used for the measurement of soil CO_2 and N_2O efflux is discussed in the **Chapter 3** (paragraph **3.3.1**). In the present experiment, chambers were fitted at the top of each soil core, rather being inserted into the soil, thus minimising soil disturbance and its effect of measured emissions.

5.4.1.1. Effect of the addition of cattle slurry on total soil CO_2

Numerous studies have shown an increase of soil CO_2 efflux rates following the application of liquid manures to agricultural soils (Saviozzi et al., 1997, Flessa and Beese, 2000, Rochette et al., 2000a, 2004, Chantigny et al., 2001, Bol et al., 2003b, Jones et al., 2005, Jones et al., 2006, Fangueiro et al., 2007).

In the present experiment, there was no overall significant increase of cumulative CO_2 emissions, but on several occasions during the first week of the experiment, controls displayed significantly lower CO_2 effluxes than all treated lysimeters. Globally, CO_2 efflux from soils amended with slurry displayed the same pattern whatever the treatment and incubation conditions. Indeed, rates of CO_2 emissions were highest after a few hours post-slurry application, quickly decreasing then to be back to background values after about a week. Similar patterns were observed in the literature (Flessa and Beese, 2000, Rochette et al., 2000a, Chantigny et al., 2001, Kuzyakov and Bol, 2004b).

This temporal pattern of CO_2 efflux from soil, following application of cattle slurry, could be split in three periods. First, CO_2 fluxes were increased by a factor 5 to 10 only two to three hours after slurry spreading compared with background values. The high CO_2 emissions in the first few hours following slurry application may resulted from the release of the CO_2 dissolved in slurry or formed from carbonate ions present in anaerobically stored liquid manures (Rochette et al., 2000a, 2004, Chantigny et al., 2001). Carbonate ions are produced during anaerobic slurry storage by hydrolysis of urea and decomposition of organic components in the slurry (Sommer and Husted, 1995, Sommer and Sherlock, 1996) and are likely to be released when the alkaline slurry is applied to an acidic soil.

Following this initial short lived peak of CO_2, we observed a second phase of about six days where soil CO_2 efflux rates from treated lysimeters were variable but remained significantly higher than controls on most occasions. Rochette et al. (2000a) observed a similar adjustment phase of about 20 days, hence substantially longer than the one observed in this study. They suggested that this period reflected the time during which soil heterotrophs were utilising the easily decomposable C from slurry. It has been shown previously that VFAs are the main source of easily degradable C in anaerobically stored slurries and that they are metabolised within a few days after soil amendment (McGill and Jackson, 1977, Kirchmann and Lundvall, 1993, Sommer and Husted, 1995).

Finally, Rochette et al. (2000) observed, after this adjustment phase, a period of relatively constant CO_2 emission rates from slurry treated plots with a small but significant difference between manured and control plots. They attributed such pattern to the decomposition of more recalcitrant organic substrates. In the present study, such period could not be observed as CO_2 fluxes decreased to be no longer different from controls from day 7 onwards.

On most sampling dates, there were no differences between grass- and maize-based slurries in regards of the subsequent CO_2 release from the soil following their application. However, soil respiration rates were significantly affected by the slurry type on two occasions in each growth-room. Therefore, even though all treatments had similar C contents, both slurry types were clearly not identical, thus leading to different mineralisation rates in the soil after application.

The ^{13}C natural abundance tracer technique allowed the $\delta^{13}C$ values from CO_2 samples to be used to calculate the contribution of the mineralisation of slurry-derived C to the total CO_2 efflux from lysimeters applied with maize-based slurries.

All treated lysimeters – including those applied with grass-based slurries – displayed much higher $\delta^{13}C$ values from emitted CO_2 than controls on day 0. For example, the difference in $\delta^{13}C$ values between treatment GS and controls was about 1.5 fold the difference treatments MS and GS. These high values for $\delta^{13}CO_2$-C appeared to be synchronised with the high CO_2 emission rates observed on the same date and attributed to the non-biological immediate release of CO_2 dissolved in slurry. In anaerobically stored pig or cattle slurry, carbonates may arise from urea hydrolysis and decomposition of VFAs,

which are dominated by acetate (Sommer and Husted, 1995). Anaerobic degradation of acetate results in the production of ^{13}C-depleted CH_4 (Krzycki et al., 1987) and subsequent ^{13}C enriched CO_2 (Boehme et al., 1996). The pH of slurry usually increases during storage with increasing concentrations of dissolved CO_2, bicarbonate and carbonate (Sommer and Husted, 1995), which would explain the enrichment in ^{13}C of the slurry-derived carbonates compared to the overall slurry signature.

Therefore, the difference of $\delta^{13}C$ values between controls and grass-based treatments may result from the non-biological release of CO_2 dissolved in slurry while the difference between grass- and maize-based, used in the equation (18) to calculate the contribution of slurry-derived C to total CO_2 efflux, may in fact only reflect the decomposition of the slurry-derived organic C fraction. This difference on day 0 could then be partly attributed to the direct mineralisation of VFAs present in cattle slurry, although it usually takes a few days for such compounds to decompose in soil (Kirchmann and Lundvall, 1993, Chantigny et al., 2004).

In the growth-room B, the contribution of slurry-derived C to total CO_2 efflux, as calculated from equation (18) was maximal on the first day of the experiment, with values ranging from 29 to 40 % o in the growth-room A, and from 40 to 65% in the other growth-room. This amount of slurry-derived CO_2-C usually decreased within only a few days. Overall, cumulative amendment-derived C losses, after 12 days, represented 4 to 9% of cumulative CO_2 emissions and ranged from 15 to 28% of applied C.

5.4.1.2. Contribution of treatments MS and ^{15}N_MS to soil CO_2 efflux and priming effect.

The proportion of the total CO_2 efflux which was not derived from manure-derived C was assumed to be derived from SOM pool as suggested by Bol et al. (2003b). The difference between this estimated SOM-derived CO_2-C and the total CO_2 efflux from controls was used to identify any existing priming effect (PE) following the application of treatments.

Positive PEs observed on days 0 and 3 for treatments MS and ^{15}N_MS were in agreement with what was previously reported in the literature after deposition of dung onto (Bol et al., 1999) or the incorporation of slurry in (Bol et al., 2003b) grassland soils. The first priming effect recorded only a few hours after spreading, on day 0, which was in agreement

with Bol et al. (2003b) who observed, in an incubation experiment, a strong positive PE, with a priming factor of 37.5±3.4, five hours only after incorporating cattle slurry to two different types of grassland soils. This gives a clear indication that it did not take more than a few hours for the soil microbial biomass to respond to the input of organic matter into the soil system. Therefore the immediate high CO_2 release from soil resulted partly from the rapid mineralisation of the most labile and degradable C fraction present in the slurry. Previous studies (Bol et al., 2003a, Bol et al., 2003b, Sauheitl et al., 2004, Fangueiro et al., 2007) provide support for this immediate activation of the soil microbial biomass following the input of easily oxidised slurry-derived C into the soil.

The second positive PE observed on day 3 could be linked to the watering of all lysimeters one hour before collecting gas samples. Such event could have accelerated the decay of the slurry-derived solid phase on the soil surface and led to a new flush of labile C and N into the soil. Such input of easily metabolised C in the soil could have further enhanced the degradation of native SOM by soil micro-organisms.

A negative PE was then observed on day 5 which was related to a decrease in SOM decomposition induced by a switch of soil micro-organisms from the decomposition of recalcitrant SOM to more available organic compound derived from slurry. Negative PEs were described in the literature following immediately the addition of various C compounds to soils (Kuzyakov and Bol, 2005, 2006, Smith et al., 2007). These priming effects were related to a preferential microbial utilisation of easily available substrates, such as sugar, compared with SOM. However, in this study, the activation of soil microbial biomass in the first five days of the experiment (positive PE), was followed by a decrease in the native SOM-C decomposition. This delayed inactivation of soil microbial activity may be induced by a shortage of easily respirable C following the initial flush of labile slurry-derived C (Fangueiro et al., 2007).

5.4.1.3. Effect of sugar and cellulose supplementation on the activity of slurry and SOM decomposers

Liquid manures are constituted of various organic compounds of different lability and decomposability (monosaccharides, volatile fatty acids, hemicellulose, cellulose, lignin, etc.). The addition of glucose to incubated soils was shown to induce a peak of CO_2 release from

soils shortly after its addition (Dalenberg and Jager, 1981, 1989, Mary et al., 1992, 1993, Wu et al., 1993, Shen and Bartha, 1996, 1997, De Nobili et al., 2001, Falchini et al., 2003, Mondini et al., 2006, Smith et al., 2007, Blagodatskaya et al., 2007). Similar observations were made for other simple and soluble carbohydrates such as fructose (Hamer and Marschner, 2005) or sucrose (Hogberg and Ekblad, 1996, Nottingham et al., 2009). When supplemented to slurry treated soils, small amount of sugar (1% of applied slurry C) added to soil tend to induce faster, but not necessarily higher, decomposition of slurry-derived organic matter (Kuzyakov and Bol, 2004b, 2005, 2006). However, in the present study, observed peaks of CO_2 release from soils were of similar magnitude for both [slurry alone] and [slurry + sugar] treatments. Furthermore, both were recorded on the first gas sampling date (day 0). Therefore, no difference could be observed between those treatments in terms of dynamic of CO_2 release from soils.

Many of the studies mentioned above used natural ^{13}C stable (Mary et al., 1992, Hogberg and Ekblad, 1996, Nottingham et al., 2009) or enriched ^{14}C radioactive (Dalenberg and Jager, 1981, Dalenberg and Jager, 1989, Wu et al., 1993, Shen and Bartha, 1996, Shen and Bartha, 1997, Falchini et al., 2003, Smith et al., 2007, Blagodatskaya et al., 2007, Hamer and Marschner, 2005) isotope tracers to separate substrate-derived CO_2-C from the mineralisation of native soil C. The amount of primed C could then be calculated from the comparison between the latest and the CO_2 efflux from a given control.

Addition of low molecular weight sugars (i.e. glucose, fructose or sucrose) to soils can affect the amount and/or the activity of soil microbial biomass. However, contradictory results were reported from the literature. Indeed, authors reported positive (Dalenberg and Jager, 1981, 1989, Mary et al., 1993, Wu et al., 1993, Shen and Bartha, 1996, 1997, De Nobili et al., 2001, Falchini et al., 2003, Hamer and Marschner, 2005, Mondini et al., 2006, Blagodatskaya et al., 2007, Nottingham et al., 2009), negative (Shen and Bartha, 1997, Blagodatskaya et al., 2007, Smith et al., 2007) or no priming effect (Wu et al., 1993), depending, among other factors, on the amount of substrate added (Wu et al., 1993, Shen and Bartha, 1997, Blagodatskaya et al., 2007).

To test whether the soil microbial biomass could be triggered into activity by addition of small quantities of various compounds, De Nobili et al. (2001) added a very small quantity (11.3 µg C g^{-1} soil) of glucose, among other substrates, to a soil previously amended or not with cellulose. They found that the addition of glucose to cellulose-amended soils could lead

to a positive priming effect on native soil C which would last longer and be of a greater magnitude than the one observed on unamended soils. On the other hand, Kuzyakov and Bol (2005, 2006), showed that, when added to soils previously amended with cattle slurry, sucrose could induce a short-term (two days) decrease in the mineralisation of SOM while enhancing the decomposition of slurry-derived C.

In the present study, supplementing slurry with sucrose prior to application on lysimeters led to contrasted results. Indeed, the addition of cane sugar to maize slurry induced a clear positive PE in the growth-room A, which was maximal on day 0 and then decreased then but remained significant on most sampling dates until day 12.On the other hand, the treatment MS_CS (in the growth-room B) led to a significant negative PE on both day 0 and 12 (being not significant in between). After 12 days, the same treatments induced an additional loss of 1.4% of native soil C in the growth-room A whereas there was a calculated 1.0% reduction of SOM decomposition in the growth-room.

One possible explanation for such differences in PE values could be an underestimation of control CO_2 fluxes in the growth-room A, when calculating the amount of primed CO_2-C (see paragraph **5.2.4.1**.). Indeed, actual control lysimeters, incubated in the growth-room B, were not replicated in the growth-room A, where the CO_2 evolved from treated lysimeters was shown to be emitted at a lower rate than in the other growth-room, on day 0, but at a higher rate on days 1, 3, 4, 6, 7 and 8. Therefore, the amount of primed CO_2-C was likely to be underestimated on the first day whereas it would have been overestimated in the remaining part of the experiment. Overall, it is quite unlikely that the wrong estimation of control CO_2 fluxes led to such a large difference in the calculated amount of primed C between both growth-rooms.

However, it was shown in this study that whether lysimeters were located in one growth-room or the other could have affected both the amount and $\delta^{13}C$ value of daily evolved CO_2, potentially affecting the calculation of the amount of slurry-derived, SOM-derived and primed C. The effect of incubation conditions on C dynamics will be further discussed in the paragraph **5.4.3.**

Adding pure cellulose to maize-based slurry did not have any effect on soil CO_2 efflux rates, possibly because the decomposition of added cellulose, whose mineralisation rate may have been similar to the one of the bulk slurry (Dendooven et al., 1996), was diluted in the

CO_2 signal from the decomposition of bulk slurry. Cellulose added to soil mixtures was shown to decompose much slower than glucose (Dalenberg and Jager, 1989, Dendooven et al., 1996). However, size and activity of cellulolytic microbial communities may be controlled by an energy supply, such as the addition of soluble and easily decomposable C (Dalenberg and Jager, 1989). For example, the authors showed that the decomposition of wheat straw-derived cellulose started after the decomposition of soluble and easily decomposable substances, which also served as an energy supply for cellulose decomposers.

It has been frequently reported that adding cellulose to soils leads to a positive PE (Shen and Bartha, 1997, Fontaine et al., 2004, 2011). However, such stimulation of native soil C mineralisation may not occur immediately after substrate addition. Indeed, Shen and Bartha (1997) for example observed that a positive PE on SOM mineralisation only two weeks after adding ^{14}C labelled cellulose to an incubated sandy loam. They even recorded significant negative PE values in the first 10 days of their experiment. However, studies reporting a significant reduction of the degradation of SOM following cellulose amendment are still scarce. In a study where they added cellulose to a fallow soil that was kept free from vegetation and manure addition for 80 years, Guenet et al. (2010) showed that such substrate could induce a negative PE on stabilised SOM, particularly when microorganisms decomposing fresh organic matter were simultaneously added to soil. In the present study, both treatments MSCel and ^{15}N_MSCel led to a significant negative PE on SOM decomposition, in the first two days of the experiment. This may have resulted from a switch of soil microorganisms from the decomposition of recalcitrant SOM to the mineralisation of a readily available and more labile C source derived from the [slurry + cellulose] treatment. This phenomenon is often called “preferential substrate utilisation” (Kuzyakov and Bol, 2006). However, the cumulative amount of primed C was generally no longer significant after a week, implying that the early reduction in SOM decomposition was somehow balanced by a slight increase of native soil C mineralisation rates after exhaustion of the more available and utilisable C pools

5.4.2. N_2O emissions and recovery of ^{15}N after application of cattle slurry

5.4.2.1. Dynamic of N_2O release from soil and recovery of applied ^{15}N in the N_2O-N fraction

Land application of animal slurries has been widely shown to enhance N_2O release from agricultural soils (Clayton et al., 1997, Ellis et al., 1998, Rochette et al., 2000b, Chadwick et al., 2000, Clemens and Huschka, 2001, Tilsner et al., 2003, Wulf et al., 2002b, Rodhe et al., 2006, Jones et al., 2007). In the present study, over the 37 days of gas measurements, the amount of cumulative N_2O evolved from each core was highly variable. Therefore, only three treatments (^{15}N_GS, ^{15}N_GSCS and ^{15}N_MSCel) displayed significantly higher N_2O fluxes than controls. However, on all sampling dates but two, between day 2 and day 12, lysimeters treated with grass-based slurry emitted significantly more N_2O than controls. During the same period, maize-based treatments, in the growth-room A, emitted significantly more N_2O than controls, whereas there was usually no differences between those controls and maize-based treatments in the growth-room B. Indeed, animal slurries provide both high amount of NH_4^+-N (Morvan et al., 1996) and readily available C (Rochette et al., 2000a) which may enhance N_2O release from soil by stimulating both nitrification and denitrification (Bergstrom et al., 1994, Granli and Bøckman, 1994, Dendooven et al., 1998b, Clemens and Huschka, 2001).

However, the amount of applied NH_4^+-N remaining in the soil solution and, therefore, available for nitrification may be reduced by both fixation of NH_4^+ onto clay minerals (Nommik & Vahtras, 1982) or by immobilisation of NH_4^+ in the SOM pool (Jansson & Persson, 1982). Fixation of NH_4^+ to clay minerals is generally dependent on several factors such as the amount of NH_4^+ added with slurry or the temperature, water status, pH and texture from the soil (reviewed by Nommik & Vahtras, 1982). The specific sorption of NH_4^+ to the clay interlayers may lead to a high temporary fixation. However, under normal conditions, particularly when a good potassium supply is given, large amount of fixed NH_4^+ are released within a few weeks after fixation (reviewed by Dittert et al., 1998). Differences in NH_4^+ fixation rates, from one soil core to the other, may have contributed to the variability of measured N_2O fluxes.

From day 2 to day 12, lysimeters applied with maize-based treatments emitted significantly less N_2O than those applied with grass-based slurries. This could be related to a

lower NH_4^+-N content of maize-based slurry compared to its grass-based counterpart. Indeed, when expressing cumulative N_2O fluxes as % of TAN applied, the difference between both slurry types was no longer significant. Treatments ^{15}N_GS and ^{15}N_GSCS also emitted more AS-derived N_2O-^{15}N. Considering that ^{15}N-labeled AS was added to all treatments in the same proportions (in relation to their initial TAN content), it is likely that higher losses of AS-derived N_2O-^{15}N from grass-based slurries also resulted from a greater amount of NH_4^+-^{15}N applied with those treatments than with their maize-based counterparts. Indeed, a direct relationship exist between N_2O emissions and N input (Tilsner et al., 2003), which makes the emitted fraction of either total N or TAN applied a better estimate of the treatment effect than the daily or cumulative emissions rate.

Denitrification, which requires high NO_3^- and organic C content under anaerobic conditions (Granli and Bøckman, 1994), is usually the main process contributing to N_2O production in managed temperate grasslands. Application of animal slurries, which usually contains a large amount NH_4^+-N and easily decomposable organic C, can stimulate both nitrifiers (Muller et al., 2003) and denitrifiers (Bergstrom et al., 1994) in soils. However, increased N_2O production following slurry application may have resulted from an increase in the denitrification of nitrified NH_4^+-N rather than from the nitrification process itself (Bergstrom et al., 1994). In the present study, the peak in N_2O emission directly followed the rapid but short-lived increase of soil respiration. This fully support Miller et al. (2009) who found a strong (R^2= 0.77) positive relationship between soil respiration and total denitrification.

Supplementing grass-based slurry with cane sugar induced both a longer lasting stimulation of N_2O-releasing soil processes and higher cumulative N_2O emissions (in % TAN applied) in the growth-room A than in the other growth-room. Although cumulative CO_2 respired from soil was not affected by the supplementation of slurry with sugar, such increase of the N_2O release from the ^{15}N_GSCS treated soil could be related to a significant positive priming effect of the added substrate (slurry + sucrose) on SOM mineralisation. Similarly, Miller et al. (2009) found a strong relationship between respiration and total denitrification, while Tiemann and Billings (2008) showed that glucose addition to soil may increase the activity of denitrifying enzymes. Organic C amendment, which increases microbial activity, may also induce changes in the abundance of denitrifying communities under anoxic soil conditions (Miller et al., 2012). However, in their study, Miller et al. (2009) obtained a

negative relationship between soil respiration and the N_2O molar ratio, suggesting that high C availability may promote a reduction of N_2O to N_2.

On the other hand, any recorded negative priming effect did not significantly affect either N_2O losses from soils or the contribution of the labelled NH_4-^{15}N pool to the emitted N_2O-^{15}N. Indeed, the peak in N_2O emissions originated mainly from the slurry NH_4-N pool (as it was suggested by the high contribution of the labelled NH_4-^{15}N pool to the emitted N_2O-^{15}N). Therefore, the probable switch of nitrifying and denitrifying microorganisms from SOM to slurry-derived organic C compounds, as an energy and C supply for their metabolism, did not affect the nitrification (and subsequent denitrification) of the NH_4^+-N pool. In the case of the positive PE described in the previous paragraph, both nitrifiers and denitrifiers were, to some extent, involved in the enhanced mineralisation of SOM-C, which they used as a C supply for their metabolism. As a consequence, both nitrification and denitrification rates probably increased, leading to a higher consumption of the slurry NH_4^+-N pool (even though the high contribution of the labelled NH_4-^{15}N pool to the emitted N_2O-^{15}N was not modified).

Increased N_2O emissions following a single application of liquid manure on grasslands soils are generally concentrated within a few weeks post fertilisation (Clayton et al., 1997, Ellis et al., 1998, Chadwick et al., 2000), with the occurrence of N_2O peaks emission also often related to rainfall events (Ellis et al., 1998, Dobbie and Smith, 2003). In the present experiment, the losses of N_2O from soils in the first 10 days following slurry application were somehow related to the watering of lysimeters on days 2 and 5.

When describing the dynamic of N_2O exchanges between soils and the atmosphere, it is also important to address the possibility for episodes of net N_2O uptake. Indeed, although agricultural soils are considered mainly as net sources of atmospheric N_2O, several studies have reported evidence that these soils can act, at least temporarily, as sinks for N_2O (Ryden, 1981, Clayton et al., 1997, Glatzel and Stahr, 2001). That was also the case in the present study where negative N_2O fluxes were recorded on various sampling dates, mainly in the growth-room B. Such phenomenon of N_2O uptake from soils was widely reviewed by Chapuis-Lardy et al. (2007). The production and consumption of N_2O occur simultaneously in most soils and net negative fluxes are often confined to N limited ecosystems where N_2O production rates are low and no longer mask growth consumption rates.

Various factors may favour net N_2O uptake from soils, such as a high WFPS and a low N availability. The reduction of N_2O to N_2 is also negatively correlated with the pH and O_2 content of the soil (Chapuis-Lardy et al., 2007). Apparent N_2O uptake can also be recorded when emissions are close to detection limits.

5.4.2.2. Recovery of applied ^{15}N in the [Soil-Plant-Atmosphere] system

Dry matter yield and grass N uptake from slurry treated lysimeters did not differ from controls, neither in the first 50 days nor in the remaining part of the experiment. This contrasts with various studies published so far (Sorensen and Jensen, 1995, Morvan et al., 1997, Hoekstra et al., 2010, 2011) which generally showed an increase of crop dry matter yield and N uptake following the application of liquid manures to soil. However, the herbage N content was sensibly reduced in the remaining part of the experiment.

The ^{15}N stable isotope methodology has been used in various studies to determine the amount of manure-derived N taken up by plants (Sorensen and Jensen, 1995, Morvan et al., 1997, Sorensen and Amato, 2002, Powell et al., 2005, Hoekstra et al., 2010, 2011). In a two years field experiment, Hoekstra et al. (2010) investigated, on a field adjacent to the one where lysimeters were collected for the present study, the impact of the slurry application technique and timing of application on the slurry NH_4-^{15}N recovery in herbage and soil. Following a bandspreading application in spring, they found a recovery, for the applied slurry NH_4-^{15}N, of 31.7 and 27.7% after 6 months and three grass cuts. In the lysimeter experiment presented here, ^{15}NRH was much lower, ranging from 5.8 to 10.1% after 170 days, and 74 to 82% of this AS-derived ^{15}N was recovered by day 50. Treatments $^{15}N\_GS$ and $^{15}N\_GSCS$ displayed higher ^{15}N grass uptake (particularly in the first 50 days), probably related to a greater amount of NH_4^+-^{15}N applied with those treatments than with their maize-based counterparts.

At the end of the growing season, after the third grass cut, (Hoekstra et al., 2010) estimated the recovery of slurry NH_4-^{15}N in soil (top 15 cm) to be 24.1 and 21.1% for both years respectively. In the present experiment, ^{15}NRS (top 20 cm) was slightly higher, ranging from 27.8 to 39.5% after 170 days.

Overall, the total recovery of AS-derived ^{15}N in the N_2O, plant and soil fractions accounted for 33.7 to 46.0% of initially added NH_4-^{15}N. Therefore, the percentage of slurry NH_4-^{15}N not recovered in those three fractions ranged from 54 to 66.3%. This "missing" ^{15}N may have been lost either in the atmosphere or by NO_3^- leaching down the soil column.

N leaching losses during the experiment were shown to be low, ranging from 0.51 to 0.71% of total N applied in the growth-room A (where ^{15}N labelled treatments were applied). In terms of atmospheric N losses, N_2O fluxes were back to background values by the end of the measuring period (37 days). Considering that N_2 fluxes are usually only up to one order of magnitude greater than N_2O efflux rates (Stevens and Laughlin, 1998), contribution of nitrification and denitrification to the "missing" ^{15}N flux was unlikely to be significant. Therefore, most unrecovered slurry NH_4-^{15}N was lost following NH_3 volatilisation at the [slurry-atmosphere] interface, on the soil surface.

The amount of slurry-derived N lost through volatilisation appeared to be sensibly higher than the 44% estimated from field measurements by Hoekstra et al. (2010). Even though soil characteristics were similar for both studies, mean air temperatures measured on the field (9.5°C), in the 24h following spring application of slurry, were quite lower than incubation temperatures recorded in the growth-room A in the first few days of the experiment. Warmer conditions in the present study would explain the possible greater NH_3 volatilisation losses. Indeed, temperature is one of the key drivers of the equilibrium between both dissolved NH_3 and ionic NH_4^+ present in slurry (Sommer et al., 1991; Groot Koerkamp et al., 1998). In solution, increasing temperatures lead to a decrease in the solubility of NH_3 and increases its rate of diffusion (Freney et al., 1981). Therefore, NH_3 volatilisation rates tend to be positively correlated with air temperature. Ammonia loss rate is usually the highest in the first 6 hours following spreading and was shown, during that period, to increase linearly (Moal et al., 1995) or exponentially (Sommer et al., 1991) with an increase in air temperature. After 6 hours, NH_3 losses are usually lower and linearly related to the air temperature (Sommer et al., 1991; Moal et al., 1995).

5.4.3. Impact of incubation conditions on C and N dynamics post-application of slurry

Both growth-rooms used in this study were set up in the exact same way, so that incubating conditions for soil cores were theoretically the same for both sets of lysimeters (^{15}N labelled or not). However, environmental data recorded from sensors positioned in each growth-room clearly showed that incubation conditions in each growth-room were not identical. Indeed, there were probably some issues, in the growth-room A, with the ventilation system allowing air to circulate inside the compartment. With no air circulation, conditions inside the growth-room became more heterogeneous, which altered the feedback control mechanism of air temperature and RH. This issue was fixed before day 36, as the differences between both growth-rooms became minimal from this day onwards. Towards the end of the experiment, the system regulating RH in the growth-room B broke down, hence the difference between both growth-room regarding RH values.

Statistical analyses on various data collected during the experiment, such as CO_2 and N_2O efflux rates, or $\delta^{13}C$ values calculated for grass and soil samples, showed that those measured parameters were clearly affected by the location of the lysimeters.

5.4.3.1. Effect on GHG emissions

When comparing both growth-rooms in terms of soil respiration rates and N_2O emissions, it appears that their temporal pattern is closely related to the pattern of measured air temperature and relative humidity inside each growth-room.

On the day when treatments were applied (as well as on the previous days), the growth-room B was in average 2.2°C warmer than the growth-room A in the second half of the bright period. The difference in temperature, between both growth-rooms, was not significant in the 8h dark phase before applying treatments and measuring gas fluxes, but the relative humidity was about 10% lower in the growth-room B. As a consequence, at the time slurry treatments were applied on each lysimeters, the soil surface was likely to be warmer but drier in the latest. Until day 10, during the dark periods, air relative humidity remained much higher in the growth-room A (up to 15% differences).

Soil CO_2 efflux can be interactively affected by various factors, among which soil temperature and soil moisture are of higher importance. When not limited by other factors, root respiration tends to increase with temperature (Atkin et al., 2000). Soil also contains a great diversity of microorganisms which different optimum temperatures for their maximal activity. Therefore, soil respiration usually responds to temperature exponentially within a wide range of values (Lloyd and Taylor, 1994, Mikan et al., 2002, Fierer et al., 2003). However, temperature sensitivity of soil respiration is also affected by soil moisture. Several studies showed, for example, lower temperature sensitivity on well-drained sites than on wetter sites (Davidson et al., 2000, Xu and Qi, 2001, Reichstein et al., 2003). Indeed, soil moisture is another key factor controlling soil respiration. Soil CO_2 efflux tends to be reduced under dry conditions and at high soil moisture content (Bowden et al., 1993, Bowden et al., 1998, Liu et al., 2002, Xu et al., 2004). In dry soils, soil microbial activity is mainly limited by the supply in organic substrate while soil respiration is controlled by O_2 diffusion in wet soils (Linn and Doran, 1984).

Therefore, higher CO_2 efflux rates measured in the growth-room B, on day 0, were probably due to higher soil temperature. However, the watering effect of applying slurry only lasted a few hours and relatively lower soil moisture may have subsequently limited the activity of soil microorganisms (in comparison with the growth-room A). As a result, from day 1 to day 8, soil respiration rates were sensibly lower in the growth-room B than in the other growth-room.

Daily N_2O efflux rates from treated lysimeters did not differ from controls in the first two days following slurry application. However, once these emissions started to increase on day 2, lysimeters located in the growth-room A emitted more N_2O than those located in the growth-room B. Soil WFPS has been shown to be one of the main soil physical factors controlling N_2O emissions from soils (Smith et al., 1998, Rudaz et al., 1999, Dobbie and Smith, 2003, Smith et al., 2003b). These emissions tend to increase when WFPS increase. However, for really high WFPS (more than 90%), N_2O release from soils may be limited because of the reduction of N_2O to N_2 after complete denitrification (Smith et al., 1998, Rudaz et al., 1999). In the present experiment, all soil cores received same volume of water on each watering event. However, in the growth-room A, humidity was higher particularly, during the first 10 days of the experiment. Therefore, soil moisture in the corresponding soils was probably higher than for the soils incubated in the other growth-room, even when the

lights were on, leading to higher WFPS and N_2O release from these soils. In addition, the lower %RH and higher temperatures recorded in growth room B may have resulted in higher ammonia volatilisation. Indeed, higher volatilisation rates may explain the consistently extremely low emission factors observed in Growth Room B (**Table 7**).

5.4.3.2. Effect on ^{13}C isotopic fractionation from plants and soil microorganisms

Isotopic fractionation expresses the changes in the partitioning of the heavy and light isotopes between a substrate and its product(s), because of physical and chemical processes (Dawson et al., 2002). Biologically mediated isotope fractionation is usually termed as isotope discrimination Δ. It can be calculated using the following equation:

$$\Delta = \frac{\delta^{13}C_{source} - \delta^{13}C_{poduct}}{1 + \delta^{13}C_{product}/1000} \quad (28)$$

The second term in the denominator is usually small. Therefore the equation (28) can be simplified:

$$\Delta \approx \delta^{13}C_{source} - \delta^{13}C_{product} \quad (29)$$

As reviewed by various author (Oleary, 1981, Farquhar et al., 1982, 1989, Lloyd and Farquhar, 1994, Bowling et al., 2008), higher plants have been shown to discriminate against ^{13}C during photosynthesis and respiration. As a consequence, plant tissues tend to be depleted in ^{13}C compared to ambient CO_2. In the C_3 photosynthetic pathway, there are two main fractionating steps:

- the gaseous diffusion of CO_2 through the boundary layer and the stomata of the leaf (a = 4.4‰, O'Leary, 1981)
- the carboxylating activity of the enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) (b ≈ 30‰)

Taking into account these two limiting steps, the discrimination Δ_A associated with the photosynthetic assimilation of C by C_3 plants can be modelled as linear function of the ratio P_i/P_a where P_a and P_i are the partial pressures of CO_2 in the ambient air and in the sub-

stomatal cavity respectively (Farquhar et al., 1982). Following this model, when stomata are sufficiently closed, the discrimination associated with the assimilation of carbon by leaves should be close to 4.4‰. On the other hand, when those stomata are fully open and place no limitation on the diffusion of CO_2, C_3 leaves should discriminate ^{13}C by about 30‰ (Farquhar et al., 1982, Farquhar et al., 1989). Actual photosynthetic discrimination is usually intermediate between both cases.

Any reduction of stomatal conductance induced by a water stress will lead to a reduction in P_i and, subsequently, a reduction in Δ_A (or an increase in $\delta^{13}C$ for the bulk leaf) (Farquhar et al., 1982). Therefore, RH may have a significant impact on the stomatal conductance and, on the subsequent photosynthetic discrimination against ^{13}C. When growing C_3 plants at four different levels of relative humidity, Madhavan et al. (1991) showed that the isotopic fractionation could decrease by up to 3‰, when decreasing RH, which they attributed to a decrease of stomatal conductance at low RH values. In the present experiment, $\delta^{13}C$ of grass samples were found to be up to 1.9‰ higher in the growth-room A than in the growth-room B on day 50, which implied, for the latest, a lower discrimination against $^{13}CO_2$ during photosynthesis. Considering that the growth-room A also displayed significantly lowest RH values in the bright periods, between day 10 and day 36, these results confirm the idea of a reduction of the isotopic fractionation when RH decreases. The difference of $\delta^{13}C$ values between both growth-rooms did not increase after day 50, possibly due to more similar RH values.

Respiratory processes such as photorespiration, day-time or dark respiration may also affect the net discrimination (Δ) against $^{13}CO_2$ (Gillon and Griffiths, 1997, Ghashghaie et al., 2003, Igamberdiev et al., 2004, Lanigan et al., 2008) calculated from the measured ^{13}C isotope content of bulk plant tissues. Day-time respiration is usually low and subsequent isotope fractionation may be neglected (Ghashghaie et al., 2003). On the other hand, both dark respiration and photorespiration may have a significant impact on the Δ value. Indeed, dark-respired CO_2 is usually ^{13}C-enriched compared to leaf material (Duranceau et al., 1999, 2001, Ghashghaie et al., 2001, Klumpp et al., 2005) while photorespiratory processes discriminate against ^{13}C (Gillon and Griffiths, 1997, Tcherkez, 2006, Lanigan et al., 2008). However, the extent to which these processes were affected by incubation temperature and relative humidity, in our experiment, and the consequences on the isotopic content of grass samples remain unknown.

Finally, the input of plant material with different isotopic composition, as well as a different uptake and utilisation of such substrate by soil microorganisms, may have altered the ^{13}C isotopic composition of the topsoil (0 – 10 Cm), leading to significant difference between both growth-rooms regarding their $\delta^{13}C$ values (Ehleringer et al., 2000, Werth and Kuzyakov, 2010). Indeed, as reviewed by these authors, ^{13}C fractionation can occur during both microbial uptake and respiration of C. Similarly to what could be observed for C_3 plant tissues and organs, Werth and Kuzyakov (2010) showed a negative correlation between $\delta^{13}C$ values from SOM or soil microbial biomass and mean annual precipitation (MAP) on various sites. The relationship they found between these $\delta^{13}C$ values and mean annual temperature (MAT) data were reverse to the ones with MAP. These observations were in accordance with findings from the present experiment; however, it remains unclear to which extent incubation temperatures and relative humidity affected ^{13}C isotopic fractionation from incubated soils.

5.5. Conclusion

Similar to the previous chapter (**Chapter 4**), the natural ^{13}C abundance tracer technique allowed the partitioning of soil CO_2 efflux between that derived from native SOM-C and the applied slurry-derived C.

Application of slurry (alone) immediately stimulated the activity of the soil microbial biomass, leading to a quick utilisation of the readily available C compounds from slurry and to net priming on SOM-C. Active soil microorganisms were quite sensitive to nutrient supply and to environmental conditions such as soil water status. These observations fully confirm the three-stage mechanism suggested in the previous chapter (**Chapter 4**) for the degradation of slurry-derived C in grassland soils.

However, once the slurry-derived labile C sources are exhausted, this study shows that the remaining microbial communities may switch from the decomposition of the recalcitrant SOM to more available organic compounds derived from slurry. Therefore, short-term enhanced degradation of SOM may be, under certain conditions, compensated by a subsequent negative PE, thus minimising the impact of priming effects on the sequestration of added slurry-derived C.

The quality of the applied organic C substrate was shown to have a major impact on the activation of soil microorganisms and on the subsequent fate of slurry-derived C. For instance, supplementation of slurry with a C substrate of medium degradability by soil microbes, such as cellulose, induced an immediate and significant decrease in the mineralisation of native SOM-C. This finding clearly supports the hypothesis suggested by Kuzyakov and Bol (2004a, 2005) of a sequential contribution of C substrates to the CO_2 efflux, from the most available and readily decomposable to the most recalcitrant organic C compound.

This work also showed that both nitrifying and denitrifying microbial communities may be involved in the dynamics of slurry-derived C in soil and the subsequent priming on soil native C. However, differences between treatments in the short-term responses to the addition of organic C did not seem to affect the longer term recovery of applied N in the plant and soil fractions.

Finally, the activation of soil microorganisms, in response to the supply of organic C and energy, was shown to be greatly affected by incubation conditions such as the temperature and relative humidity. For example, variations of such parameters could affect both the sign and intensity of measured PEs.

Chapter 6. General discussion and future work

6.1. Mineralisation of slurry-derived C in a grassland soil

Sequestration and losses of C from liquid manures incorporated or applied to agricultural soils have been investigated in various studies, either in laboratory incubation experiments (Saviozzi et al., 1997, Dendooven et al., 1998a, Flessa and Beese, 2000, Glaser et al., 2001, Bol et al., 2003a, 2003b, 2004, Kuzyakov and Bol, 2004b, 2005, 2006, Fangueiro et al., 2007) or under field conditions (Martinez and Peu, 2000, Rochette et al., 2000a, 2004, 2006, Chantigny et al., 2001, Jones et al., 2005, 2006, Sauheitl et al., 2005, Angers et al., 2007). Incorporation of slurry into agricultural soils tend to increase soil respiration rates, particularly in the first few days following application (Flessa and Beese, 2000, Rochette et al., 2000a, 2004, 2006, Chantigny et al., 2001, Bol et al., 2003b, Jones et al., 2005, 2006, Angers et al., 2007) due to the input of labile C compounds. Data presented in this document clearly confirmed such observation. Indeed, surface application of cattle slurry to a grassland soil, on-site or under controlled conditions, clearly stimulated soil CO_2 efflux immediately after spreading.

Slurry application may also locally increase soil microbial biomass (SMB) (Saviozzi et al., 1997, Rochette et al., 2000a, 2004, Bol et al., 2003a), even though this effect on SMB-C is not always significant (Chantigny et al., 2001, Bol et al., 2003a). Rochette et al. (2000a) showed that the SMB-C peak usually corresponds with a peak of respiratory activity, which indicates a significant microbial contribution to soil CO_2 efflux. Furthermore, Sauheitl et al. (2005) found evidence of a significant microbial utilisation of slurry-derived C in the first few days following manure application. Increases in enzymes activities (phosphatase, urease, catalase and dehydrogenase), basal respiration and microbial metabolic quotient have also been observed on the application of slurry (Plaza et al., 2004).

The response of soil microorganisms to the addition of organic C to soil is greatly affected by the quality of the added substrate. Supplementing cattle slurry with C substrates of different degradability and utilisability (i.e. sucrose or cellulose) by soil microbes was shown to affected the contribution of each individual C pool to the total CO_2 efflux (**see Chapter 5**). Liquid manures are complex mixtures of various organic compounds which differ by their availability to and their degradability by the SMB. For instance, Saviozzi et al.

(1997) showed in a laboratory incubation experiment that extracting soluble C compound from pig slurry significantly decreased the amount of rapidly mineralisable C, as well as the size of the SMB pool. Indeed, the amount of soluble C compounds in animal wastes, which may include low molecular compounds such as sugars, amino sugars and VFAs, tend to be highly correlated with the early rates of C mineralisation (Morvan et al., 2006). Another example was given by Saviozzi et al. (1997) and Chantigny et al. (2001) who observed higher soil CO_2 efflux from a combined addition of pig slurry and straw (wheat or barley) to soil than from the separate application of both substrates.

The results from the partitioning of total soil CO_2 efflux between slurry-derived and SOM-derived CO_2-C (see **Chapters 4 and 5**) suggest a three stage mechanism for the decomposition of slurry-derived C in grassland soils:

1. Immediately after applying slurry onto the soil surface, dissolved CO_2 bicarbonates and carbonates are released to the atmosphere. Meanwhile, easily metabolised organic C percolates into the top soil where it can be utilised by the microbial communities (supposedly r-strategists) within a few hours;

2. During the first few days after manure application, simple C molecules (e.g. sugars, amino-acids, fatty acids) are released from solids in the slurry and gradually incorporated into the soil.

3. Once the slurry-derived labile C sources are exhausted, the remaining microbial communities (supposedly slow-growing K-strategists) may switch from the decomposition of the recalcitrant SOM to more available organic compounds derived from slurry, utilising such organic C pool at lower rates but over a longer time frame than has been previously considered.

Other authors also suggested multi-stages models for the decomposition of slurry-derived C in the soil (Rochette et al., 2000a, Chantigny et al., 2001, Glaser et al., 2001, Bol et al., 2003a, Bol et al., 2003b, Kuzyakov and Bol, 2005, Kuzyakov and Bol, 2006, Fangueiro et al., 2007).

Some of the laboratory studies (Saviozzi et al., 1997, Dendooven et al., 1998a, Flessa and Beese, 2000) and nearly all field studies (Martinez and Peu, 2000, Rochette et al., 2000a, Rochette et al., 2004, Rochette et al., 2006, Chantigny et al., 2001, Jones et al., 2005, Jones et

al., 2006) mentioned previously did not accurately quantify the amount of slurry-derived C sequestered or lost from the soil. Indeed, by only subtracting control fluxes from treatments, they did not take into account the potential priming effects of slurry addition on the mineralisation of native SOM (Bol et al., 2003b). In the present document, data from both the field and incubation experiments gave evidence for an alteration of the mineralisation of soil native C following the application of cattle slurry. However, none of the experiments reported in this thesis targeted specifically the SMB-C pool and its activity. Therefore, it was not possible to identify the sources and mechanisms for such priming of SOM mineralisation.

6.2. Priming effects on SOM-C: sources and mechanisms

Priming effects are usually defined as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil" (Kuzyakov et al., 2000). When considering C turnover studies, this phenomenon is usually defined more precisely as the extra-decomposition of SOM following the addition of easily mineralisable C and N to soil (Dalenberg and Jager, 1989). The extra-release of unlabelled CO_2, when investigating the PE of a given C substrate (^{13}C or ^{14}C labelled), can have two origins: (i) an acceleration of microbial C turnover (Dalenberg and Jager, 1981, 1989) and (ii) an increased decomposition of SOM following a stimulation of soil microbial activity (Asmar et al., 1994, Fontaine et al., 2003, 2004, Smith et al., 2007). The former is usually termed as "apparent priming effect" whereas only the latter imply a turnover of SOM-C and is therefore called "real" (Kuzyakov et al., 2000, Blagodatskaya and Kuzyakov, 2008).

Until now, no "clear-cut approach" exists to separate apparent from real PEs (Blagodatskaya and Kuzyakov, 2008). Apparent PEs observed after addition of fresh organic matter may result: (i) from a triggering effect, which is an activation of SMB by trace amounts of substrate with an immediate increase in the respiratory activity (De Nobili et al., 2001, Mondini et al., 2006), or (ii) from a pool substitution with acceleration of microbial turnover (Jenkinson et al., 1985). Real PEs, on the other hand, usually depend on the quality of added substrate and may be related to a low nutrient (usually N) availability. These 'priming effects' most likely occur because the amount of labile C in slurry is adequate to increase microbial activity. However, while there is plentiful N supply within slurry to sustain protein metabolism, labile C sources within the slurry are quickly depleted (Kuzyakov

et al., 2000). This shortage can be supplemented by an enhanced decomposition of SOM, supplying soil microbes with N and leading to an additional release of mineral C (real PE) (Blagodatskaya and Kuzyakov, 2008, Fontaine et al., 2011).

Priming effects on soil CO_2 efflux have been observed following the incorporation of cattle slurry into grassland soils (Bol et al., 2003b, Kuzyakov and Bol, 2006, Fangueiro et al., 2007). Those studies, as well as data presented in this document (see **Chapter 4 and 5**), indicate both short-term (a few hours) and long-term (a few days to a week) responses of soil microorganisms to the application of cattle slurry. This indicates that added substrate may have accelerated both microbial turnover and decomposition of recalcitrant SOM, implying a combination of both real and apparent PE. However, the only way to distinguish the former from the latter would be to relate the calculated amounts of primed CO_2-C with changes in the SMB-C pool. Unfortunately, such analyses of microbial pools could not be done during the course of this project.

Various mechanisms were suggested in the literature (Kuzyakov et al., 2000, De Nobili et al., 2001, Fontaine et al., 2003, 2004, Blagodatskaya et al., 2007) to explain the PEs recorded following the addition of various substrates to soil. However, these mechanisms were elaborated for and tested with single organic compounds (e.g. glucose, amino acids, cellulose…) and were therefore quite difficult to relate to the decomposition of cattle slurry on the soil surface and its associated PEs.

Kuzyakov and Bol (2006) used the results from an experiment where they partitioned the total CO_2 efflux from a grassland soil between three sources (i.e. sucrose, cattle slurry and native soil) to suggest a possible mechanism for the decomposition of slurry-derived C in soil. Assuming that the PE dynamics depend on the utilisability of substrates as well as on the composition of SOM pools, they suggested an alternative mechanism for PEs, which can be summarised as follows: a) following the addition of organic matter to soil, there is a switch from r-strategists onto the utilisation of freshly added substrate; b) if there is enough substrate, there is an increase in the biomass and activity of such active micro-organisms; c) once the most easily utilisable substrate is exhausted, activated micro-organisms decompose other available substrates according to their utilisability; and d) soil microbial activity finally decline and the initial balance between the different SOM pools is restored.

6.3. Consequences of slurry-derived PEs on long-term C sequestration in grassland soils

Under current management conditions, most grasslands in the world are considered to be C sinks for atmospheric CO_2 (Gifford et al., 1992; Bruce et al., 1999; Post & Kwon, 2000; Conant et al., 2001). In Europe, grasslands were shown to be a potential sink for CO_2 (Vleeshouwers and Verhagen, 2002; Janssens et al., 2003; Soussana et al., 2007), although the uncertainty around the estimate may be larger than the sink itself (Janssens et al., 2003). Furthermore, it is still uncertain how long the sink activity can continue and what the upper limit of C storage is in soils (Franck, 2002; Jones and Donnelly, 2004).

Improving grasslands with management practices such as fertilisation, irrigation or seedling legumes are known to increase to SOC storage (Conant et al., 2001; Ogle et al., 2004). Long-term (>50 years) pasture, for example, are probably near equilibrium with respect to C and will not be significant sinks without some additional inputs such as fertilisers (Bruce et al., 1999). In addition to this, Soussana et al. (2004) showed that moderately enhanced N fertilisation increases the organic matter input to the soil proportionally more than it increases the process of C mineralisation, whereas intensive fertilisation stimulate mineralisation and, therefore, enhance C losses.

In typical Irish grasslands, nutrient supplied with cattle slurry is expected to increase SOC by increasing C input from enhanced grass productivity and plant residues returned to soil. Even though slurry C inputs were likely not to be enough, in my experiments, to directly compensate C exports from the harvested grass (Soussana et al, 2007), above-ground C biomass usually represents less than 10% of grassland organic C, the reminder being located in both the root biomass and the SOM pool (Burke et al., 1997; Milchunas et al., 1998; Schuman et al., 1999). Therefore, amending grasslands with slurry may have increased SOC stocks both directly, through the input of organic C, or indirectly, through an increase of litter production or rhizodeposition from the root biomass (Loiseau & Soussana, 1999; Jones et al., 2006; Leifeld et al., 2010).

On the other hand, as shown previously (**chapter 4** and **5)** and as discussed in the **paragraph 6.2**, slurry application onto grassland soil may not only constitute an additional organic C input to the SOC pool, but may also enhance the mineralisation of native SOM (positive PE), something which could (at least partly) offset the sequestration of slurry-

derived C (see **chapter 4**). However, positive priming effects, in the first few days following slurry spreading onto grassland soils, may be, under certain conditions, compensated by a subsequent reduction of SOM mineralisation rates (negative PE, see **chapter 5**), thus minimising the impact of priming effects on C sequestration in grassland soils.

Furthermore, priming effects are short-term phenomena which may be counterbalanced by a longer term increase in CO_2 uptake, through an enhanced NPP (Jones et al., 2006; Shimizu et al., 2009) which contribute to an additional organic C input to soil through rhizodeposition or the decay of non-harvested plant biomass (Loiseau & Soussana, 1999; Jones & Donnelly, 2004; Franzluebbers, 2005; Rees et al., 2005; Jones et al., 2006).Even though they are short-term phenomena, priming effects may occur on a regular basis, being therefore of a significant influence when calculating long-term nutrient balances (Gerzabek et al., 1997). Several years managing grasslands with synthetic fertilisers or manures generally enhance soil C sequestration (Conant et al., 2001), but, in certain circumstances, C lost by the priming of SOM mineralisation may exceed the additional input of organic C from applied slurry, particularly when applied at a moderate rate (Angers et al., 2010). On the other hand, Grilo et al., (2011) showed that a significant proportion of slurry-derived C may remain in the soil for several years after application, contributing (to some extent) to long-term soil C sequestration.

6.4. Interactions between soil C and N processes: soil respiration vs. N_2O release

Nitrous oxide release from grassland soils tends to be closely related to the activity of soil microbes and the subsequent amount of CO_2 evolved, as was suggested by the data presented in the previous chapters. Indeed episodes of high N_2O release, following the application of cattle slurry to the soil, were always related to periods of enhanced soil respiration. However, there was usually a time lag (2-3 days) between the nearly immediate increase of soil microbial activity and the slightly delayed peak of N_2O release. Interestingly, evidence for a positive relationship between soil respiration and N_2O emissions were also observed when switching from conventional (i.e. splash-plate) to a low NH_3 volatilising application technique (i.e. trailing-shoe, see **Chapter 3**). Last, but not least, the partitioning of total CO_2 efflux between slurry-derived and SOM-derived CO_2-C, under controlled

conditions (**see Chapter 5**), clearly showed that N_2O losses followed a rapid activation of the SMB.

Denitrification is usually seen as the main process contributing to N_2O production in managed grasslands. This is a microbial process carried out by a wide range of microorganisms when there is a high NO_3^- and C supply, as well as hypoxic or anoxic conditions (Granli and Bøckman, 1994). The availability of C substrate was shown to be one of the main controls over denitrification as it regulates the soil microbial activity (Firestone, 1982, Tenuta et al., 2000).

Liquid manures are sources of readily decomposable organic C compounds which may trigger denitrification by enhancing respiration (through the creation of anoxic microsites) and by providing energy for denitrifiers (Firestone, 1982, Weier et al., 1993). Tenuta et al. (2000) which measured several indices for the availability of C, such as the water-soluble, extractable and aerobically respired C fractions, showed that these indices were strongly correlated with the denitrification rate. Similarly, Miller et al. (2009) observed a positive relationship between soil respiration and total denitrification. However, the same authors found a negative relationship between soil respiration and the N_2O molar ratio.

Nitrification is the other main process producing N_2O in agricultural soils. Autotrophic nitrifiers use CO_2 as a carbon source and obtain their energy by oxidation of NH_4^+. Heterotrophic nitrifiers, on the other hand, use organic matter as both a carbon and energy source (Granli and Bøckman, 1994). Application of animal slurries, which usually contain large amounts of NH_4^+-N and easily decomposable organic C, was shown to stimulate the oxidation of NH_4^+ to NO_3^- due to a combination of autotrophic and heterotrophic transformations (Muller et al., 2003). However, Bergstrom et al. (1994) suggested that increased N_2O production following slurry application onto grassland soils may result from an increase in the denitrification of nitrified NH_4^+-N rather than from the nitrification process itself.

While the timescale of this study was unable to investigate C and N sequestration into the soil, there is evidence that optimised N application from slurry can also lead to enhanced N sequestration as well as C sequestration. Moderate N fertilisation has been shown to result an increase in C sequestration by stimulating NPP (Soussana et al., 2004, Jones et al., 2006). Manure C inputs may also directly increase SOC stocks (Franzluebbers, 2005, Jones et al.,

2006, Hopkins et al., 2009). However, intensive N management alters the balance between NPP and C mineralisation, with increased in NPP outweighed by proportionately higher rates of mineralisation (Loiseau and Soussana, 1999, Soussana et al., 2004). N uptake is also enhanced as a result, and increased cattle slurry application has been shown to result in substantial N sequestration (Muller et al., 2011). The soil type mediates the this effect, with histosols and mineral soils of a high organic carbon content having a small sink capacity and indeed, management inevitably leads to both C and N loss via mineralisation (Soussana et al., 2004).

6.5. Timing of application: effect of weather and soil conditions

Both in the field and in the growth-room experiments, temperature and overall water status (soil moisture, rainfall, relative humidity, etc.) were key drivers for measured gaseous C and N losses. Nitrous oxide emissions measured in the field were shown to be mainly correlated with rainfall and soil WFPS (**Chapter 3**). Data from the lysimeter experiment (**Chapter 5**) further confirmed the importance of the hydric status of the [soil – plant – atmosphere] in the regulation of N_2O-producing soil processes. Once the watering effect of slurry application had faded, soil respiration also appeared to be limited by the relative humidity inside the growth-room. However, soil CO_2 effluxes measured on the field were highly correlated with the soil temperature. Soil moisture and relative humidity were probably such in the field that they did not induce water-limiting conditions for soil microbial activity.

In the field, weather conditions in spring were both cooler and wetter than on the subsequent summer application dates, resulting in lower N_2O and CO_2 emissions. Timing of manure application also affects NH_3 emissions which increase as a function of temperature and wind speed (Sommer et al., 2003). Most studies investigating the impact of timing of slurry application on NH_3 and N_2O emissions post-application focused on the comparison between autumn (typically after harvest for croplands) and spring application, as reviewed by VanderZaag et al. (2011). However, slurry in Ireland is mainly applied in summer (Hyde and Carton, 2005) when N volatilisation losses can be high due to warm temperature. Spring

application may be considered as an optimal strategy as N is applied in a period when uptake by herbage is high and NH_3 losses are relatively low.

Although wetter soils in such period may limit the number of days when slurry can be applied using the conventional broadcast method, Lalor and Schulte (2008) showed in a modelling study that, in Ireland, the number of available spreading days is substantially higher where trailing-shoe is used for slurry application. Although, switching to trailing-shoe application in order to reduce volatilisation N losses may induce adverse effect on soil respiration rates and N_2O emissions, these effects are likely to be offset by a greater benefit of switching from summer to spring application.

6.6. Dependence of N_2O emission factors on fertiliser type, climate and soil conditions.

N_2O emission factors, calculated from field measurements of soil N_2O emissions for the entire experimental period (**Chapter 3**) and corrected for volatilisation NH_3 losses, were 0.5% to 0.7% for high DM slurry treatments, 0.9% to 1.2% for low DM slurry treatments, and 1.6% for CAN fertilised plots. Cattle slurry-derived EFs (not corrected for NH_3 losses) calculated from the growth-room incubation experiment (**Chapter 5**) were much lower, ranging from -0.1% to 0.5%. Generally, the values reported here were lower than the 1% default EF reported by the IPCC (IPCC, 2006).

Previous studies had reported substantial differences between organic and mineral fertiliser in terms of calculated EFs for N_2O. Some authors reported higher N_2O emissions from applied liquid manures (Flessa & Beese, 2000; Rochette et al., 2000b, 2004; Perälä et al., 2006), whereas grassland soils fertilised with either ammonium nitrate (AN) or CAN were shown to emit much more N_2O than similar plots applied with cattle slurry (Tilsner et al., 2003; Velthof & Mosquera, 2011). Stevens & Laughlin (2002) also found lower EF associated with cattle slurry application. This reduction in EF may be linked to an increased soil pH which, in turn, results in NH_4^+ inhibition of nitrification (de Klein et al., 2003). In another study, Jones et al. (2005, 2007) observed, during a two year measurement period on a managed grassland, significantly lower N_2O emissions from urea treated plots but higher N_2O release from plots applied with poultry manure or sewage sludge pellets, in comparison with AN fertilised soils. The same authors found significantly lower EF values from cattle slurry

applied plots only in the first year of their experiment. Most studies reporting an increase in EF when applying animal manures to agricultural soils (in comparison with synthetic fertilisers) were dealing with poultry or liquid hog manures (Flessa & Beese, 2000; Rochette et al., 2000b, 2004; Jones et al., 2005, 2007), but some papers (Chadwick et al., 2000; Velthof et al., 2003; Jones et al., 2005, 2007; Velthof & Mosquera, 2011) indicate a trend for lower N_2O emissions from cattle slurry than from other types of animal manures. Such difference between both slurry types may have resulted from a lower N and TAN content for cattle slurry (Chadwick et al., 2000) or from a higher fraction of TAN (in relation to total N content), as well as from a higher amount of easily degradable C compounds (e.g. VFAs) and a higher water content in pig slurries (Velthof et al., 2003, Velthof & Mosquera., 2011).

Annual N_2O emission factors from slurry-applied soils were also shown to be affected by the application technique (Weslien et al., 1998; Flessa & Beese, 2000; Perälä et al., 2006, Rodhe et al., 2006; Thomsen et al., 2010; Velthof & Mosquera, 2011). This was confirmed by the results from my field experiment (**Chapter 3**) where, in three application events (April, August and September), soil N_2O emissions were increased in trailing shoe-applied plots, in comparison to their splash plate-applied counterparts, even though the emission factor calculated for trailing shoe-applied plots, for the entire experiments, was only slightly increased.

As shown above, there may be large differences, in terms of EFs, between types of fertiliser and manure, and between various application techniques. As a consequence, there is a need to refine the IPCC methodology for regional and global N_2O inventories, so that GHG mitigation options based on changes in fertiliser type, manure composition and application technique can be reported (Velthof et al., 2003, Lesschen et al., 2011). The research work presented here also highlights the requirement for good constraint of the amount of NH_3 volatilisation, as this is the largest determinant of the EF variability across year for liquid manures.

In the study presented here, there was also a significant variability of N_2O emissions, and subsequent emission factors, across the year. Indeed, EFs were the highest following slurry application in August, whereas there was a trend for lower N_2O emissions after the first application in April. Conversely, Chadwick et al. (2000), who applied pig and cattle slurry onto a grassland soil both in April and July of the same year, found the highest EF values in spring. However, when calculating EF values, they did not subtract volatilisation NH_3 losses

to the amount of N applied, attributing therefore their low EFs in July to the warmer conditions which led to higher volatilisation NH_3 losses. Rochette et al. (2004) also found an effect of the application technique on the fraction of applied pig slurry N reemitted as N_2O, with higher emissions from spring application in comparison to autumn application. Nevertheless, these two studies, as well as the work presented in **Chapter 3**, clearly showed that the effect of timing of application on N_2O emissions was related to the climatic and soil conditions at the time of application (see paragraph **6.5**.).

A few field studies also showed an inter-annual variability of calculated EFs from fertilised grasslands (Clayton et al., 1997; Flechard et al., 2005, 2007; Jones et al., 2005, 2007; Velthof & Mosquera, 2011), which was mainly related to a climatic variability from one year to the other, particularly in terms of rainfall pattern (Clayton et al., 1997; Flechard et al., 2005, 2007, Velthof & Mosquera, 2011), and its impact on soil WFPS (Flechard et al., 2007, Thomsen et al., 2010). Those annual differences in EFs suggests that climatic variability significantly affects the N_2O source strength of grassland ecosystems, from one year to the other, and should therefore be taken into account when estimating N_2O emission for regional inventories (Flechard et al., 2007).

Despite similar climate and management practices, N_2O emissions factors from grassland soils can greatly vary from one soil type to the other (Velthof & Mosquera, 2011), as the amount of N_2O emitted from managed grassland is strongly dependent on soil and climatic conditions. Therefore, there is a need to replace the current Tier 1 approach from IPCC (IPCC, 2006), when dealing with N_2O emissions from fertilised grasslands, by emission factors which are adapted to regional soil and weather characteristics (Dechow & Freibauer, 2010). Cardenas et al. (2010), suggested, for example, the setting up of new region-specific EFs for United Kingdom, based on climatic conditions. Some work was also carried on recently to develop new modelling approaches allowing the estimation of EFs, at a regional or continental scale, as a function of climate and soil conditions (Flechard et al., 2007; Dechow & Freibauer, 2010; Lesschen et al., 2011). Such new region-specific approaches should reduce the uncertainty around calculated EFs, leading to more realistic estimates of soil N_2O emissions (Lesschen et al., 2011).

6.7. Extrapolation of results toward long term predictions of management and climate effects: a modelling perspective.

In this PhD project, we only carried NH_3 and GHG emission measurements on one soil type. However, to some extent, we assessed the impact of climate on these emissions. Nevertheless, as mentioned in the previous paragraph (**6.6**), soil N_2O emissions from agricultural soils are function of both climatic and soil conditions. As a consequence, the interaction between climate and soil type, something we did not look at, is a key drivers of N_2O emission from grassland soils.

In order to extrapolate those emissions both temporally and spatially, there is a need to use process-based models such as, for example, DNDC (Li et al., 1992, Li., 2000), DayCent (Parton et. al., 1998; Del Grosso et al., 2000), FASSET (Bernsten et al., 2003) or PaSim (Riedo et al., 1998; Schmidt et al., 2001, Riedo et al., 2002).

These models provide insights into the drivers of biogeochemical cycles, and can be used to assess long-term effects of grassland management (e.g. Chatskikh et al., 2005; Rafique et al., 2011; Li et al., 2012) and climate change (e.g. Chatskikh et al., 2005; Hsieh et al., 2005; Abdalla et al., 2010; Graux et al., 2012) on C and N cycling, as well as on trace gas emissions. The application of process-based models not only allows the simulation of agricultural GHGs at a range that scales up to national or global level (Giltrap et al., 2010; Kim et al., 2010), but it also allows the exploration of potential mitigation strategies along with a range of climate change scenarios.

Chapter 7. General conclusion

7.1. Conclusion

This PhD was aiming at a) assessing the impact of cattle slurry characteristics, application techniques and timing of application on the NH_3 and GHG emissions from a grassland soil, and b) investigating the consequence of organic N application in terms of C sequestration in cut grasslands.

It was hypothesised that:

- reducing NH_3 volatilisation losses from land spread slurry would increase the soil inorganic N pool, thus leading to potentially higher N_2O emissions;
- the input of organic C at the soil surface would participate to an increase of the SOC pool;
- such input of organic C would also stimulate soil microbial activity, thus increasing soil respiration rates.

Trailing-shoe application technique was shown to be and efficient way to lower NH_3 volatilisation from land spread slurry. However, such benefice could be easily offset by an increase in direct N_2O emissions and ecosystem respiration. Conversely, adjusting the timing of slurry spreading (e.g. switching from summer to spring application) to get favourable soil and weather conditions, and to better meet herbage N requirements, had a positive effect on field N balance through a simultaneous reduction of both NH_3 and N_2O emissions.

Emission factors calculated for slurry-induced N_2O emissions were significantly lower than those calculated for CAN and were greatly affected by weather and soil conditions. Such results support the widely spread idea of an inappropriate use of a single default EF value of 1% for both fertiliser types, under the IPCC Tier 1 methodology for national GHG inventories, and calls for the development of region-specific EFs based on local soil types and climatic conditions.

A significant proportion of applied slurry C is rapidly utilised by soil microorganisms and returned to the atmosphere as CO_2. Nevertheless, about 60% of slurry-derived C may

remain in the soil, even after 6 months, thus contributing to an increase of SOC pools. However, such incorporation of slurry-derived C may be (at least partially) offset by a positive PE of slurry on the degradation of the SOM. Such short-term priming of soil CO_2 efflux may be, under certain conditions, compensated by a subsequent negative PE, thus minimising the impact of such phenomenon on the long-term sequestration of added slurry C. The long-term impact of these priming effects on nutrient and GHG balances remains to be further investigated, as these phenomena may occur on a regular basis in grassland ecosystems.

7.2. Future work

Interactions between soil C and N dynamics, following the application of animal manure are primarily controlled by the size and activity of the SMB pool, as well as by the quality of the added substrate. The work presented here primarily focused on gaseous C and N losses from the system [soil – plant –atmosphere] and considered C and N pools in the bulk soil only. Therefore, there is now a need to open the "black box" and investigate interrelated soil C and N dynamics at a microbial or even at a molecular scale:

Several mechanisms have been suggested to explain the degradation of various organic substrates in soils, but their validity on the field remains to be proven. Furthermore, although they are now highly documented, short-term phenomena such as PEs are still poorly understood due to a lack of information on the sources of primed C and on the microorganisms involved in such processes:

- Some work should be done to evaluate PEs as a function of microbial biomass and community structure. In particular, the role of fungi in mediating both C sequestration and N_2O emissions should be investigated.

- C isotopic methods, based on a combined ^{13}C and ^{14}C labelling, should be used to identify as many C pools / fluxes as possible.

- Recently developed compound-specific analyses should be used to target the different C pools involved in the microbial response to the input of animal manures into the soil.

In addition, future studies should target the organic N fraction of slurry, whose fate in soils remains poorly understood.

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