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**Carbon, Nitrogen and Greenhouse Gas Flux Dynamics
In Cornfield and Managed Grassland: Effects of Land-
Use Change, Manure Management and Liming**

(飼料畑および草地における炭素、窒素、温室効果ガス動態：
土地利用、堆肥施与、酸性矯正の影響)

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1. General Introduction

Increasing food production and ending global food insecurity in a changing global climate is one of the greatest challenges facing agricultural scientists today. This challenge is further complicated by the fact that agricultural production is one of major sources of greenhouse gas (GHG) emissions, which are responsible for driving climate change (Smith et al. 2014). Totally, agricultural GHG sources account for 10–12% of global anthropogenic emissions (Tubiello et al. 2013; Smith et al. 2014) and the rate of emissions has been increasing over the last few decades.

In soils, carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are produced through microbiologically mediated processes that break down organic and inorganic materials. Carbon dioxide fluxes from soil, also called soil respiration (RS), originate from root respiration (RR) and heterotrophic respiration (RH). Heterotrophic respiration is the microbial decomposition of soil organic matter and other carbon (C) sources by bacteria and fungi (Balogh et al. 2016). In upland soils CH₄ is produced under high soil moisture content through anaerobic decomposition of organic matter by methanogenic bacteria (Bayer et al. 2012) and can be consumed through oxidation to CO₂ when sufficient oxygen is present by methanotrophs (Tate 2015). Soil CH₄ emissions depend on the balance between production and oxidation of CH₄ and is influenced by changes in soil moisture content, available C and other biophysical factors (Dunfield et al. 1993; Hütsch et al. 1994; Tate 2015). Denitrification and nitrification are the two most important N₂O producing processes in the soil. Soil N₂O production is influenced by nitrogen (N) and C availability, soil moisture, pH and temperature among other factors (Lampe et al. 2006; Butterbach-Bahl et al. 2013; Li et al. 2013).

Land-use change between grassland and cropland, like cornfield, often results in large changes in soil C and available N and several other soil properties like bulk density, moisture content and microbial activities. Grasslands usually accumulate large amounts of litter and soil organic matter (SOM) that leads to high SOC (~343 Gt C globally) and organic N (Davies et al. 2001; Velthof et al. 2010; Ryals et al. 2014). Converting grassland to cropland will convert the accumulated SOM into available C and N during mineralization (Whitehead et al. 1990; Necpálová et al. 2013) which in turn will increase microbial activity and have significant effect on CO₂ and N₂O emissions (Smith and Conen 2004; Smith et al. 2008; Lang et al. 2015; Sainju 2016). Croplands that undergo annual tillage, such as ploughing, usually tend to have lower SOM content but could have higher decomposition rates. Tillage activities often lead to disruption of soil structure, increased surface area of the contact between SOM and soil microbes, physical release of SOM previously trapped/bound, break down of plant residue and increased aeration which provides aerobic conditions that enhance SOM decomposition, enhanced soil microbial activity, increased soil temperature and exposes SOM to freeze and thaw cycles (Ussiri and Lal 2009; Govaerts et al. 2007; Dalal et al. 2011; Kravchenko et al. 2011; Mbuthia et al. 2015; Govaerts et al. 2006; Katayanagi and Hatano 2012). These changes will have large implications on GHG production and emission in soils. For example while increased aeration can result in higher SOM decomposition and CO₂ fluxes (Bayer et al. 2016), it can lead to increased CH₄ oxidation and consequently decrease soil CH₄ emissions (Omonode et al. 2007; Tate 2015). Converting cropland to perennial crops like grassland has been reported to mitigate GHG emissions by sequestering C in the soil (Guo and Gifford 2002). However, how much C can be sequestered and how much time from conversion of cropland to grassland would be required to achieve significant reductions in GHG emissions is still uncertain. Moreover the effects of land-use change between grassland and cropland can further be complicated by

differences in fertilization management in each system. To fully understand the direction of these changes, long-term studies that monitor changes on the same soil after changes in land-use between grassland and croplands are needed.

Manure and fertilizer management is important for crop production and also for GHG emissions. Adequate fertilization and manure input increase crop production resulting in higher plant biomass and residue input to the soil. This, in addition to the direct C input from manure, can lead to increased SOC (Lal 2004; Jarecki et al. 2005; Rees et al. 2005) and hence could be used as a GHG mitigation practice in agriculture. Manure application has also been reported to have potential to reduce N₂O emissions as the manure N is less available to soil microbes compared to inorganic fertilizer N that can easily be accessed by denitrification and nitrification microbes (Alluvione et al. 2010; Ryals and Silver 2012). However, other studies have found increased N₂O and CO₂ emissions following manure application compared to unfertilized or inorganic fertilized soils (VanderZaag et al. 2011; Owen et al. 2015). These contradictions could be due to differences in manure type, land-use, soil type and properties, local climate or management practices (Skiba et al. 1997; Velthof et al. 2010) and they indicate the need for further research.

As discussed above, a given management practice can have both positive and negative effects on GHG emissions and SOC. It is therefore important to have a full account of both negative and positive effects of a given management option by using a common metric. Global warming potential (GWP) is used to convert CH₄, N₂O and SOC change into a comparable unit by using the radiative forcings of each gas over a given time period relative to CO₂. Global warming potential therefore constitutes the net balance of GHG exchange of a given agro-ecosystem (Robertson and Grace 2004; Mosier et al. 2006) and hence allows us to evaluate the overall effect of a particular management practice.

Some management practices such as inorganic fertilizer application can increase GHG emissions but at the same time they are necessary for increased food production. For instance it has been reported that N_2O and NO emissions will increase by up to 60% by 2030 as a result of increased use of inorganic fertilizers as countries, especially developing countries, seek to produce more food for their increasing populations (FAO and IFA 2001; UNEP 2013; US.EPA 2013). Given the fact that food and feed production is the primary purpose of all agricultural activities, it is essential to evaluate the effect of a given management practice on GHG emissions against its contribution to productivity. Greenhouse gas intensity (GHGI) compares the net greenhouse gas emissions (GWP) to the crop productivity and therefore allows the assessment of the environmental impact and as well as the economic benefit of a given management practice (Robertson and Grace 2004; Mosier et al. 2006). Very few studies have carried out this kind of assessment when reporting GHG emissions from fertilizer and manure applications.

Soil pH has long been known to influence N transformation processes in the soil. In fact soil pH has been called the “master variable” for N cycling because of its effect on several soil chemical, physical and biological properties (Šimek and Cooper 2002). Soil pH has a strong influence on nitrification and denitrification, the two most important N_2O producing processes in the soil (Šimek et al. 2002; Mørkved et al. 2007; Herold et al. 2012). Denitrification is the main source of N_2O fluxes in many agricultural soils (Šimek et al. 2002; Baggs 2008). Denitrification, unlike nitrification, can produce N_2O as well as consume it by reducing it to dinitrogen gas (N_2). Complete denitrification involves four successional steps; nitrate (NO_3^-) reduction to nitrite (NO_2^-), NO_2^- reduction to nitric oxide (NO), NO reduction to N_2O , and N_2O reduction to N_2 . This entire denitrification process is controlled by the transcription of genes for enzymes needed to catalyze each of the four steps. The gene encoding enzymes NO_3^- reductase (*Nar*), NO_2^- reductase (*Nir*) and NO reductase (*Nor*) that

are required for the production of N₂O tend to be expressed much later than N₂O reductase (*NosZ*), gene for the enzyme needed for N₂O reduction to N₂ (Bakken et al. 2012). This suggests that enhancement of the *NosZ* could lead to immediate and increased consumption of the N₂O that is produced as the enzymes needed for N₂O production become expressed. The *NosZ* enzyme has also been reported to have a higher optimum pH than the enzymes (*Nir* and *Nar* and *Nor*) involved in the production of N₂O (Bakken et al. 2012; Pan et al. 2012). This would mean that increasing soil pH would increase N₂O consumption and reduce its emissions. However, whether this can be achieved in soils is still not clear.

The objectives of this study were;

1. To assess the effect of land-use change between grassland and cornfield on soil C, N and GHG dynamics
2. To investigate the effects of inorganic fertilization and manure management on GWP and GHGI in a cornfield and
3. To investigate whether increasing soil pH by liming can mitigate N₂O fluxes in an andosol.

This thesis comprises 8 chapters. The current chapter, general introduction, is followed by an extensive review of C and N cycling, GHG production mechanisms and mitigation options in agro-ecosystems in chapter 2. The third chapter describes the study site, materials and the general methodology used in the study. To answer the first objective of this thesis, the effect of land-use change is discussed in chapters 4 and 5, with chapter 4 focusing on soil C, CO₂ and CH₄ emissions while chapter 5 focuses on soil N, N₂O and NO emissions. Chapter 6 looks at the effect of inorganic fertilization and manure management on GWP and GHGI in cornfield, and chapter 7 looks at an incubation study evaluating the effect of liming on N₂O emissions. Chapter 8 is a general discussion of the achievements of this study and it ends with an outline of further research needs.

2. Literature Review

2.1 Historical increase of greenhouse gas (GHG) emissions and contribution of agriculture sector to global emissions

2.1.1 Global and historical changes in GHG concentrations

There is irrefutable evidence that greenhouse gas (GHG) concentrations in the atmosphere have increased markedly since the industrial revolution in the 19th century (IPCC 2014). Human activities such as burning of fossil fuels, changes in land use such as deforestation and clearing of land for agriculture production are the major drivers of the observed increases in GHGs (IPCC 2007a).

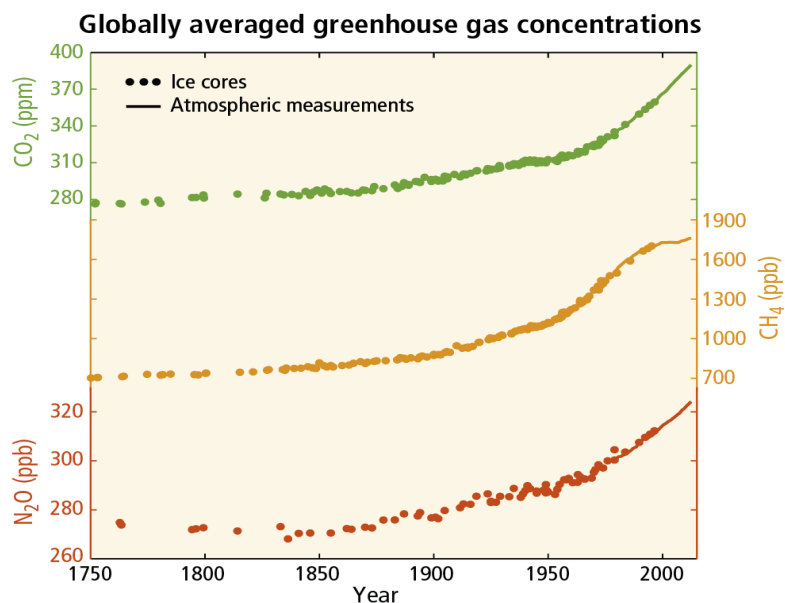


Figure 2.1 Changes in ice core and atmospheric greenhouse gas concentrations since the industrial era (Source: IPCC 2014)

The global average concentrations of carbon dioxide (CO₂) have increased from 280 ppm in the pre-industrial period to as high 400 ppm in 2016 as recorded at the Mauna Loa Observatory in Hawaii (IPCC 2007b; IPCC 2014). Methane (CH₄) and nitrous oxide (N₂O)

concentrations have equally increased at unprecedented rates from pre-industrial levels of around 700 ppb and 270 ppb to over 1700 and 320 ppb respectively (Fig. 2.1)(IPCC 2013).

Global anthropogenic GHG emissions have increased by almost twice as fast from 2000 onwards than from 1970 to 2000 (Fig. 2.2). This unprecedented increase in GHG concentrations and the resulting effect on terrestrial and marine ecosystems has raised the need to reduce GHG emissions to one of most important global agendas of the 21st century.

2.1.2 Contribution of the agricultural sector to global GHG emissions

Agricultural production and agriculture related activities accounted for 13.5% of global annual anthropogenic emissions in 2005 (IPCC 2007a) and was the fourth largest source by sector after energy supply (25%), industry (19.4%) and forestry (17.4%). According to Tubiello et al. (2013), global agriculture GHG emissions increased by 1.6% annually in the period 1961–2010.

When only non-CO₂ GHGs are considered, the agriculture sector is the most important emitter accounting for over 55% of global anthropogenic emissions (US. EPA 2006; Smith et al. 2014). Agricultural soils, through microbial denitrification and nitrification, are responsible for about 56% of global anthropogenic N₂O emissions and the emission rate of soil N₂O increased by 19% from 1990 to 2010 (US. EPA 2013).

Agricultural CH₄ emissions, largely from enteric fermentation, rice cultivation and animal manure, account for over 40% of global emissions (Tubiello et al. 2013; US.EPA 2013). Excluding rice productions systems, agricultural soils tend to be CH₄ sinks rather than sources(US.EPA 2013).

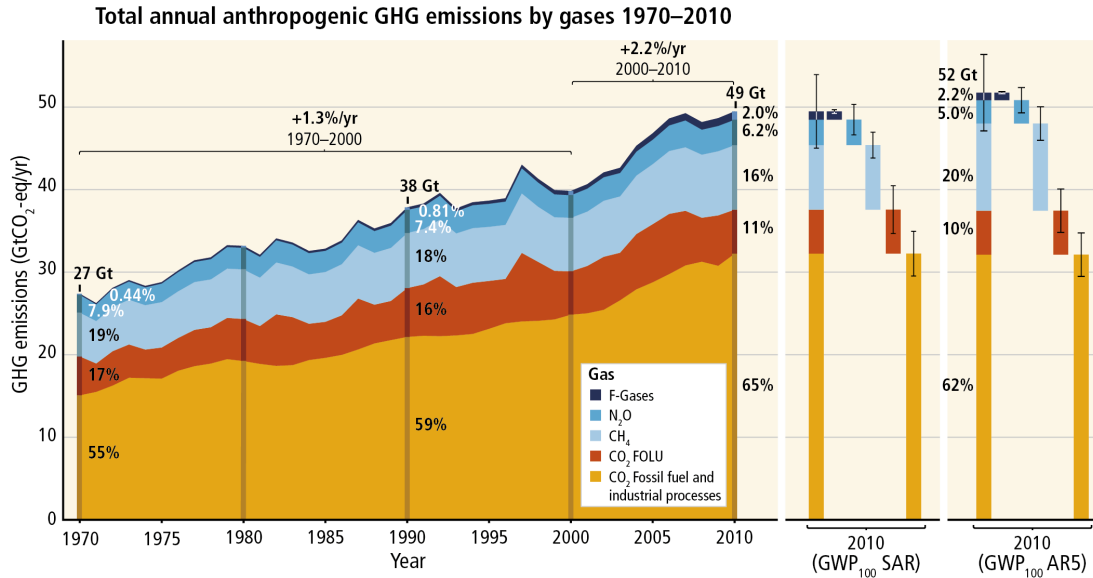


Figure 2.2 Changes in annual anthropogenic greenhouse gas emissions from 1970 to 2010 (source: IPCC 2014)

2.2 Carbon dynamics, carbon dioxide and methane emissions in agricultural soils

The carbon (C) cycle in an agro-ecosystem consists of C input, its transformation from one form to another within the system, and its loss/output from the system. Photosynthesis is the primary source of C sequestration by plants (gross primary production) (Janzen 2004) and it's controlled by climate (solar radiation, rainfall, temperature), soil (type, moisture and nutrient status) and vegetation/plant type. About 50% the C captured by photosynthesis is lost through plant respiration with the remaining half (net primary production) stored in plant tissue. Carbon is added to soils through the fall of plant material (residue, litter and roots) and application of C containing fertilizers such as animal manure (Fig. 2.3). This added C is then transformed by soil microorganisms into soil organic matter (SOM) or soil organic C (SOC). Organic C is then decomposed further by soil microbes and in the process CO₂ and/or CH₄ is produced and released into the atmosphere. Carbon is also lost through

leaching of dissolved organic and inorganic C forms, erosion and through removal by harvested plant materials (Rastogi et al. 2002; Janzen 2004; Lal 2004).

2.2.1 Carbon dioxide production and consumption pathways in soils

There are two main sources of soil CO₂ emissions namely autotrophic and heterotrophic soil (RH) respiration. Collectively these two sources are called soil respiration (RS).

2.2.1.1 Autotrophic soil respiration

Autotrophic soil respiration is CO₂ evolved from plant roots and associated rhizosphere (region of soil directly affected by root secretions) organisms (Hanson et al. 2000) and through mycorrhizal fungi (nodes on roots formed through symbiotic relationship between roots and fungi) (Balogh et al. 2016). However, there is still disagreements on whether root-associated microbial fluxes should be considered part of autotrophic respiration or RH (Chapin et al. 2006), after all such fluxes are not in the strict sense emitted by roots but by microbes that utilize root exudates and other rhizosphere deposits. A review by Kuzyakov and Larionova (2005) differentiated between actual root respiration and rhizosphere microbial respiration components of autotrophic respiration. They defined actual root respiration as the “respiration of roots to obtain energy for maintenance of metabolism and concentration gradient in cells, growth, and active uptake of nutrients” and rhizosphere microbial respiration as “the respiration of heterotrophic microorganisms decomposing organic substances released by living roots”. This study does not differentiate between these two components of root-associated respiration and therefore these two will be combined in the rest of this thesis as root respiration (RR). Root respiration is associated with growth and maintenance of roots and respiration of microbes within the rhizosphere that use products of photosynthesis without a direct effect on storage of soil C (Cahoon et al. 2016). Overall, C

lost through root respiration tends to be lower than the C assimilated through photosynthesis and while it can be a large component of total RS (10–90% according to Ferréa et al. 2012), it is not considered as contributing to global warming. Root respiration generally increases with increased soil C inputs through photosynthesis and increased root biomass (Baggs 2006).

2.2.1.2 Heterotrophic soil respiration

Heterotrophic respiration is the CO₂ produced during microbial decomposition of soil organic matter (SOM) by bacteria and fungi (Balogh et al. 2016). It basically represents the respiration of soil microbes in the soil that utilize pre-formed C as a source of energy. These heterotrophic organisms decompose SOM and other pre-existing soil C forms, and as a result it is this part of RS that generally leads to reduction or loss of soil C.

In soils fungal mycelium absorb nutrients available within the soil or can decompose organic materials such as dead leaves by secreting enzymes that are capable of even hard and resistant plant matter such as lignin and cellulose. By decomposing hard plant materials and requiring much lower N than bacteria, fungi tend to be the most important decomposers in poor soils and for plant residues with high C/N ratio (Chapin et al. 2011). Fungi are also important in plant nutrient uptake by forming symbiotic relationships with plants and in suppressing or causing plant diseases.

Bacteria in soil are mostly saturated around the rhizosphere and they tend to easily decompose fresh plant residue and other organic compounds within or close to the rhizosphere (Chapin et al. 2011).

2.2.1.3 Separating the components of soil respiration

Soil respiration can be partitioned into RH and RR using a number of different approaches that can be grouped into three categories as; component integration, root exclusion and isotopic based approaches (Hanson et al. 2000; Kuzyakov and Larionova 2005). Component integration involves determining the rate of CO₂ emission from each fraction (e.g soil only or root only) and then multiplying the rate with the total mass of each component followed by summation of those results. The root exclusion method involves measurement of CO₂ flux in the presence and absence of roots with the difference between the two results being root respiration. Root exclusion methods include root removal, trenching and gap formation (Hanson et al. 2000). The root exclusion and component integration methods tend to disturb the natural environment, and hence usually alter the rates of both RR and RH. However, they are easy and cheap to use and still give reasonable approximations of the contribution of the different components of RS. Isotopic based approaches make use of C isotopic labelling and tracking the labelled C. The isotopic based approaches are considered to be the most accurate but the high cost associated with such methods limits their use by most researchers.

Separating and partitioning the contribution of RR and RH to total RS is important for predicting the response of an ecosystem to changes in the climate and for understanding feedbacks between soil processes and climate change (Baggs 2006). The contribution of RH or RR depends on a number of factors such land-use type, vegetation species, climatic conditions such as temperature and rainfall, soil properties such as SOM content and texture, and management practices such as fertilization and tillage (Ding et al. 2007a; Schindlbacher et al. 2009; Ferréa et al. 2012; Zhang et al. 2013; Zhou et al. 2014; Luo et al. 2016).

In forest soils RR has been reported to account for a wide range of total RS. For example a review by Chen et al. (2011) found that in mature forests, RR contributed on average 43% of total RS when averaged from a summary of results from 57 sites across the

globe. However, the same study reports that the contribution of RR ranged from 9% to 71%. In a forest in northern Italy, RH was found to be the largest contributor to RS at 68% (Ferréa et al. 2012), while Yevdokimov et al. (2010) found that RR was responsible for 7–56% in a Russian forest and 24–60% in a meadow grassland. In a temperate grassland, Yan et al. (2010) showed that the contribution of RH to total RS was 46–50% while Zhang et al. (2013) found that in wheat and maize fields RH accounted for 36% and 29% of total RS respectively.

2.2.1.4 Factors controlling soil respiration and its components

i. Nitrogen

In a meta-analysis of 295 studies, Zhou et al. (2014) found that N addition increased total RS by 2% across different biomes; but N addition decreased RS by 1.44% in forests while it increased RS by 7.8% and 12.4% in grassland and croplands. The response of RS to N addition was attributed mostly to stimulation of RR by N addition in grassland and cropland but not in forests. On average RR increased by 22.4% due to N addition while RH decreased by 13.7% (Zhou et al. 2014). In grassland and cropland, more photosynthetic products are allocated to roots, which increases the demand for water and nutrients and hence the need for more energy leading to increased RR. Nitrogen addition can decrease RH by inhibiting microbial decomposition through changes in gene expression, shift in microbial communities and decrease in microbial biomass. Another study by Yan et al. (2010) found that N addition did not have a significant effect on total RS but that it increased RR by 19% while it decreased RH by 14%, and that N addition increased RS in a wet year but decreased RS in a dry year. In a maize field, N addition of 0–150 kg N kg⁻¹ was found to increase RS but when N application was higher than 200 Kg N ha⁻¹, RS was suppressed (Song and Zhang 2009). Nitrogen addition could decrease RH and microbial biomass due to N toxicity on soil

microbes such as fungi and by decreasing C availability (Treseder 2008). Other studies have however reported increased MBC due to N fertilization (Luo et al. 2016). The negative effect of N addition on fungi was reported to be due to the progressive inhibition of growth or ligninase activity of fungi or as a result of the formation of larger molecules (condensation) by combining organic C compounds with N-containing compounds (Treseder 2008). High N availability could also lead to increased accumulation of compounds toxic to fungi resulting in their decreased activity. Decreased allocation of C substrates to mycorrhizal fungi which in turn can lead to changes in the structure and composition of soil microbes and further influencing decomposition of SOM is another reported effect of high N availability (Treseder 2008).

ii. Soil temperature

Another important factor controlling RS is soil temperature. A compilation of over 38 studies in different biomes by Chen and Tian (2005) found that Q_{10} values of RS decreased with increasing soil temperature and that soil temperature was a better predictor of RS than air temperature. The response of RS to N addition, described above, is also controlled by annual mean temperature and precipitation (Zhou et al. 2014). Soil temperature is the primary factor affecting seasonal changes in RS and MBC (Wang et al. 2007). According to Song and Zhang (2009), 40–70% of the variation in RS could be explained by soil moisture and temperature. However, soil temperature can have different effects on the different components of RS. Gaumont-Guay et al. (2008) conducted a root-exclusion experiment to check the biophysical controls of RR and RH and found that in cooler seasons (winter, spring and autumn) RH was dominant while RR was the dominant component in summer. Both RH and RR increased exponentially with increasing soil temperature but RR was more sensitive to temperature showing a higher Q_{10} value than that of RH. They also reported that diurnal variations in RR were not only controlled by temperature but also responded to

changes in photosynthetic activity. The rate of supply of photosynthetic products to roots from shoots influences the timing of peak diurnal RR.

iii. Soil moisture

Like all biological processes, CO₂ production through RS and its components need water and therefore are strongly influenced by soil moisture and precipitation. Water addition through irrigation increased RS by 34%, RR by 38% and RH by 28% in a temperate grassland in North China (Yan et al. 2010). Both RH and RR increased with increasing soil moisture until volumetric moisture content reached 15% and 29% respectively and both decreased when moisture was increased further. Increased RR following increase in moisture content is due to increased plant growth and photosynthesis, which increases the supply of C substrates to the roots. Increased RH due to moisture and water addition is due to increased microbial activity. Beyond a certain threshold, increase in moisture content will lead to CO₂ reduction to CH₄ and also it limits the physical movement of CO₂ by limiting diffusivity within the soil profile (Yan et al. 2010; Chapin et al. 2011; Shimizu et al. 2013). 40–70% of variation in RS could be explained by soil moisture and temperature (Song and Zhang 2009). Luo et al. (2016) found that soil moisture accounted for 56–62% of the seasonal variation in RS in a temperate grassland in China. Autotrophic soil respiration was reported to show a stronger negative response to drought conditions than RH in Hungary, central Europe (Balogh et al. 2016). This study also found that the contribution of RR to total RS decreased during drought, as that of RH increased. The non-root associated part of autotrophic respiration (rhizosphere respiration) showed a smaller decrease with drought compared to root-associated autotrophic respiration. Soil water present within the soil colloids is said to be able to supply water to soil microbes within the bulk soil even during drought, thereby maintaining microbial decomposition of organic C, while root metabolism is restricted during drought due to reduced photosynthesis (Balogh et al. 2016).

iv. Management practices

Other field management activities such tillage, harvesting and grazing can lead to changes in C and N supply, moisture content and soil temperature and therefore affect RS. Tillage breaks down soil colloids into smaller particles increasing the surface area of the contact between SOM and soil microbes, leads to the physical release of SOM previously trapped/bound by soil and other cementing agents, breaks down plant residue and increases aeration which provides aerobic conditions that enhance SOM decomposition, enhances soil microbial activity, increases soil temperature and exposes SOM to freeze and thaw cycles (Ussiri and Lal 2009; Govaerts et al. 2007; Dalal et al. 2011; Kravchenko et al. 2011; Mbuthia et al. 2015; Govaerts et al. 2006; Katayanagi and Hatano 2012). An experiment comparing no tillage, shallow tillage to 12cm and deep tillage to 25cm depth in wheat/corn rotations found that ploughing decreased soil moisture by 20% compared to no tillage (Chen and Huang 2009). The same study also found that the influence of ploughing on RS was dependent on whether or not crop residues were incorporated and by the type of crop residues from the preceding season. Comparing no tillage and tillage to 25 cm depth in several sites across east Britain, Mangalassery et al. (2014) found higher bulk density, increased soil shear strength, higher moisture content, higher SOM content and higher MBC in no tillage fields compared to tilled fields. An X-ray scan reviewed not only higher porosity but also soil pores at least twice as large in size and larger pore surface area in tilled soils compared to no tillage (Mangalassery et al. 2014). All these changes have effects not only on the SOM decomposition but also on the physical movement of the resulting CO₂. Land-use change can also result in changes in soil SOM, salinization and acidification; and these will affect microbial activities in soil. For example, in a grassland degraded due to salinization, microbial biomass C (MBC), SOC and root biomass decreased by 55%, 28% and 45%, respectively, compared to the un-degraded site (Wang et al. 2007). Changes in

physical soil properties resulting from land-use change or land management practices can equally influence CO₂ production and emission from soils. Soil pore characteristics (porosity and pore size) can be stronger predictors of CO₂ flux from soil than SOM and MBC (Mangalassery et al. 2014).

2.2.2 Methane production and consumption pathways in soils

Methanogenesis, the process by which CH₄ is produced, occurs under anaerobic conditions as the final step in the anaerobic decomposition of organic C (Fig 2.3). Several bacteria-mediated processes are involved before the final production of CH₄ can occur. Under anaerobic conditions, methanogenic bacteria reduces acetate to CO₂ and finally to CH₄ to produce energy (Le Mer and Roger 2001). Essentially, CH₄ is produced as a by-product of energy metabolism under anaerobic conditions (Meronigal et al. 2005). Methanogenic bacteria can use only a small number of substrates; acetate and di-hydrogen (H₂) are the most important (Segers 1998). Carbon dioxide is typically reduced by H₂ to form CH₄ as the methanogenic bacteria use the C in CO₂ for metabolism. This process yields low energy compared to aerobic decomposition of organic C, and hence when oxygen (O₂) becomes available CH₄ is oxidized to CO₂ by CH₄-oxidizing bacteria or methanotrophs (Meronigal et al. 2005). Methane oxidation occurs in most soils although it is mostly pronounced when CH₄ concentrations are high (above 40 ppm) (Le Mer and Roger 2001). Most of the CH₄ oxidation occurs at or near the sites of CH₄ production within the soil by bacteria (Tate 2015).

Because CH₄ production occurs under anaerobic conditions, in agricultural soils rice paddies are the most important source. In upland soils, such as the site for this study, CH₄ production and emission can occur when soil moisture content is high, typically above 70% water filled pore space (WFPS). Both methanogenesis and CH₄-oxidation can occur

simultaneously in the soil and therefore the balance between these processes will determine whether a soil is a source or sink of CH₄.

2.2.2.1 Factors controlling methane production and oxidation

Factors that control CH₄ production and oxidation include O₂ availability, pH, temperature, availability of organic substrates and nutrients (Segers 1998; Tate 2015). Microbial community abundance and activity of methanogens and methanotrophs is also critical as it determines the balance between CH₄ production and consumption.

i. Soil aeration and moisture content

Methanogenesis is inhibited by O₂ and therefore soil management practices that alter the diffusion of O₂ in the soil are likely to have large influences on CH₄ emissions from soils. A number of studies have reported enhanced CH₄ production or emissions in upland agricultural soils following high rainfall events or when WFPS exceeded 70% (Wu et al. 2010; Wang et al. 2013; Wang et al. 2014). Soil moisture affects CH₄ production by regulating the activity of methanogenes or methanotrophs and it also affects the diffusivity of CH₄ and hence its physical movement within the soil (Wang et al. 2013).

ii. Nitrogen content

Nitrogen fertilization and high N content in soil has been reported to decrease CH₄ oxidation in soil and consequently lead to higher emissions compared to unfertilized soils. In Colorado, USA, N mineralization and nitrification were found to decrease CH₄ uptake/oxidation by grassland soils (Mosier et al. 1991). However, this effect was attributed to mineralization and nitrification processes and not necessarily higher mineral N content. In Germany, high NH₄⁺ and WEOC concentrations led to decreased CH₄ oxidation with a corresponding increase in CH₄ production being observed (Bayer et al. 2012). Up to 41% reduction in CH₄ oxidation in grassland soils was reported in the USA following annual N

fertilization (Mosier et al. 1991). Acton and Baggs (2011) found that CH₄ oxidation was highest in unfertilized soils and that it was 51–76% lower in NH₄NO₃ fertilized treatments. Although CH₄ consumption/oxidation has been reported to decrease with fertilization, especially NH₄ based fertilizers, Flessa (1995) did not find any such effect. The reported increased CH₄ oxidation due to N fertilization or in N rich soils is due to inhibition by N availability. Both NH₃ monooxygenase and methanotroph monooxygenase enzymes have similar substrates. This leads to competition for the enzyme active site between NH₃ and CH₄ and NH₃ is preferred over CH₄ (Flessa 1995; Acton and Baggs 2011). Another possible reason for the decreased CH₄ consumption following N fertilization is the toxicity of NH₂OH or NO₂⁻ produced during nitrification (Acton and Baggs 2011). Despite the many reports of this negative relation between N fertilization and CH₄ oxidation, some studies have found the opposite (Tate 2015). In fact, soil N can inhibit, stimulate or have very little effect on CH₄ oxidation (Bodelier and Laanbroek 2004).

iii. Carbon content

Soil organic matter and labile C forms have a huge influence on CH₄ production and consumption. The content and nature of organic matter influences the intensity of the C reduction processes (Le Mer and Roger 2001). Changes in organic matter can limit the availability of mineralizable C and in turn limit microbial activities such as methanogenesis and CH₄ oxidation (Tate 2015). High water extractable organic carbon (WEOC) can decrease CH₄ oxidation and increase CH₄ emissions (Bayer et al. 2012).

iv. Soil pH

Like most processes dependent on microbes, CH₄ production and oxidation are influenced by soil pH. Methane oxidation can occur over a wide range of soil pH ranging from 2.5–8.0 but the activity of methanotrophs tends to be lower at soil pH below 6 (Tate 2015). A review of several studies by Le Mer and Roger (2001) found that the activity of methanogens is

optimal at neutral to slightly alkaline soil pH while that of methanotrophs is around pH 5.6. Hütsch et al. (1994) reported decreased CH₄ oxidation with decreasing soil pH. Low CH₄ oxidation at low soil pH is due to reduced nitrification and accumulation of NH₄⁺.

v. Soil Temperature

Soil temperature generally increases the activity of both CH₄ production and oxidation. Production of CH₄ showed a marked dependence on temperature with optimum rates at 25–30°C and low rates at low temperature with Q₁₀ values ranged from 5.5 to 16 (Dunfield et al. 1993). In contrast, the same authors found that CH₄ consumption was less dependent on temperature and the optimal range was 20–25°C and significant consumption occurred even at low temperature with Q₁₀ values for CH₄ consumption ranging from 1.4–2.1. Le Mer and Roger (2001) indicated that optimum temperature for CH₄ production is between 30 and 40°C and that low temperatures not only decrease the activity of methanogens but also of other bacteria involved the CH₄ production process; and that methanotrophs on the other hand were still active even at temperatures less than 1°C.

2.2.2.2 Effect of land-use on CH₄ production and oxidation

Changes in land-use and associated management practices alter a lot of soil physical, chemical and biological properties and will no doubt have an effect on CH₄ production and oxidation. In the USA, a woodland had highest rate of CH₄ oxidation, followed by grazed grassland (22% of woodland) and arable land had the lowest (19% of woodland) (Hütsch et al. 1994). These results indicate that the rate of CH₄ oxidation decreased with increasing land/soil disturbance. Land management activities that alter organic matter content will also influence CH₄ production and oxidation in soil. Tillage activities resulted in negative CH₄ fluxes indicating uptake by soils (Omonode et al. 2007). Disturbing natural grasslands by renovation or rotation with annual crops can decrease their CH₄ uptake (Mosier et al. 1991).

Forest soils have higher CH₄ oxidation compared agricultural soils such as wheat/corn rotations despite forest having lower pH and higher NO₃⁻ and NH₄⁺, indicating that land-use influences the inhibition of CH₄ oxidation by N (Hütsch 1998). Tillage, disturbance of soil structure and associated changes, can negatively affect development of methanotrophs because they are slower to recover (Hütsch 1998) and hence leading to reduced oxidation and enhanced emission of CH₄. Tillage of grasslands and conversion to annual crops such as cornfield tends not only to increase aeration but also increase organic matter decomposition, C and N mineralization, alter soil microbial communities and several other soil physical and chemical properties (Davies et al. 2001; Velthof et al. 2010; Necpálová et al. 2013), and therefore have large influence on CH₄ emissions.

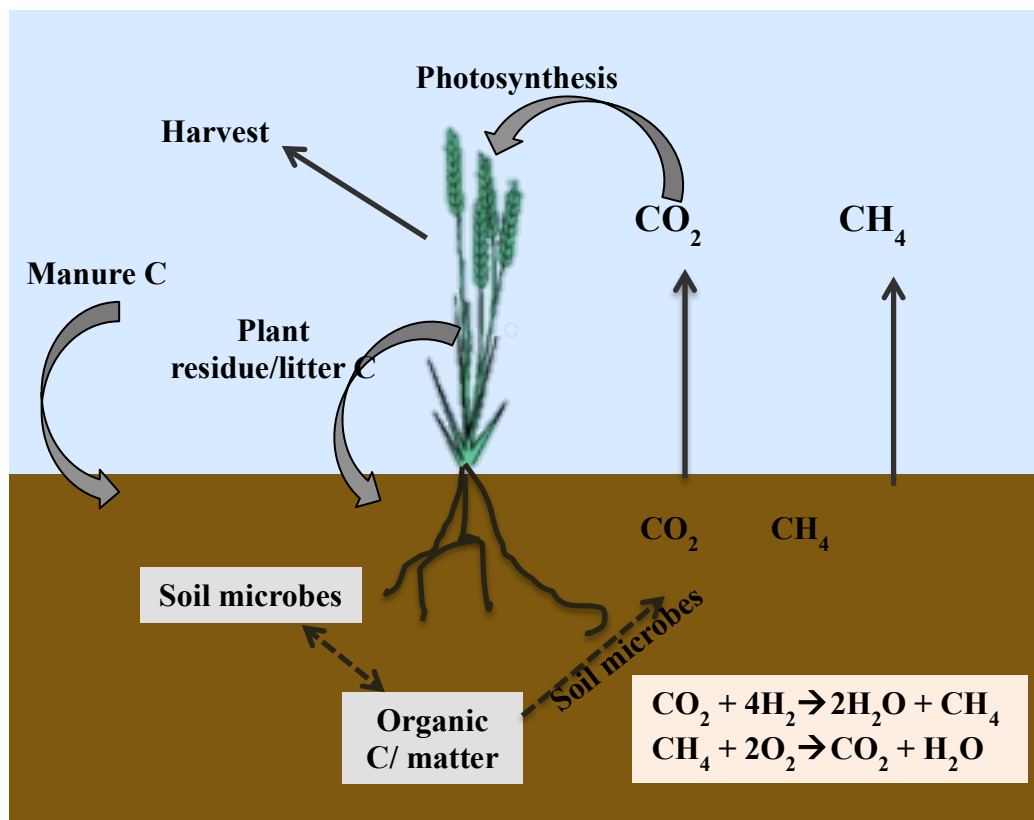


Figure 2.3 Conceptual diagram of carbon cycling in an agro-ecosystem.

While CH₄ production is generally reported to be low in upland soils, periods of high moisture content to could lead to significant emissions. Evaluating CH₄ emissions over long-

term periods is therefore essential to fully understand not only the annual emissions rates but also the biophysical factors that drive the emissions.

2.2.3 Carbon budget concepts and calculation in an agro-ecosystem

The amount of C stored in an ecosystem depends on the balance between C inputs and outputs. Typically this depends on the balance between photosynthesis and respiration (both autotrophic and heterotrophic) plus other episodic C losses (Trumbore 2006)

Net primary production (NPP), which is the net amount of C assimilated by plants through photosynthesis minus the C lost through autotrophic/metabolic plant respiration through above and belowground plant biomass, is the primary source of C input. The C assimilated as NPP remains in the ecosystem and added to the soil as plant residue, which is later transformed to SOM, unless removed through harvest, grazing by animals, RH or by other means such burning, leaching and erosion (Lal 2004). In an agro-ecosystem context NPP is usually measured as the total plant biomass accumulated over a given period (typically one year). This involves measuring (or estimating using various methods as described by Chapin et al. (2011)) both the aboveground and belowground components. Another important source of C input in agro-ecosystems is the application of manure (e.g green manure, compost, animal manure etc). Manure application has been mooted as one of the best ways of increasing SOM and also providing nutrients that lead to increased NPP (Lal 2002).

Carbon is lost primarily through heterotrophic decomposition of plant residues by soil microbes and through removal in the harvested plant material. SOC stock can also be lost through erosion and leaching of water soluble C (Olson et al. 2014). However, C loss resulting from erosion and leaching of both organic and inorganic forms is an important component only over long timescales (centuries and longer), but when considering shorter

timescales (annual to decadal scale), these losses are too small to have a major contribution to net C balance of an ecosystem (Trumbore 2006; Zheng et al. 2008).

When considering SOC changes, direct measurements have been reported to be insensitive to small changes such as seasonal and annual variations but rather work well at sub-decadal and decadal timescales (Mosier et al. 2006; Zheng et al. 2008). Seasonal and annual variations in C flows are however very important and determine the amount of C likely to be sequestered in the soil or in a particular ecosystem. Another problem of using SOC stock to determine the net C gain or loss in the soil is that different methods of calculation can give different results. Assessing the effect of no tillage on SOC sequestration, Olson et al. (2014) found that the comparison method (with conventional tillage as the baseline) showed a $0.45 \text{ Mg C yr}^{-1}$ of SOC sequestration but the pre-treatment method (by comparing the initial and final SOC) showed a reduction in SOC of $0.34 \text{ Mg C yr}^{-1}$ over a 20 year period in the USA. These authors concluded that using the pre-treatment method (by comparing the initial SOC stock to the final SOC at the end of a given period) is a much better approach to determine SOC sequestration than the pairwise comparison method (where one treatment e.g conventional is used as the baseline) under non-steady state (Olson et al. 2014). Having the initial SOC content enables us to determine the absolute changes in SOC stock and hence the SOC sequestration rate. However, initial SOC contents are not always available and using change in SOC cannot show changes in C flows over shorter timescales. To address the problem of timescale and method of calculation, the concept of net ecosystem carbon balance (NECB) has been developed and used by many researchers (Chapin et al. 2006; Zheng et al. 2008; Cui et al. 2014; Zhang et al. 2014b; Yang et al. 2015). Net ecosystem carbon balance is calculated as the difference between C inputs (NPP and manure) and C outputs (RH, CH_4 emission, harvest) (Yang et al. 2015).

Other important parameters/terms used in C calculations and measurement (especially when using micrometeorological methods like eddy covariance) include net ecosystem production (NEP), net ecosystem exchange (NEE), gross primary production (GPP) (Randerson et al. 2002; Chapin et al. 2006; Trumbore 2006; Limin et al. 2015). These are however not discussed in this thesis because our approach used biometric measurement of plant biomass and chamber methods for CO₂ and CH₄ emissions.

2.3 Nitrogen cycling in agricultural soils

Nitrogen is the most limiting element for crop production in most agro-ecosystems in the world. Nitrogen is added to agricultural soils through inorganic N fertilization, manure fertilization and biological N fixation by leguminous crops. Another important source of plant available N is through the decomposition and mineralization of plant litter and SOM by soil microbes. From the soil, N is lost through plant uptake, leaching, emission to the atmosphere in gaseous forms and through erosion. To improve crop productivity to meet the demand for food and feed for the world's growing population, there is an urgent need to develop management practices that can increase SOM, soil N and plant N-use efficiency while minimizing N losses.

The use of organic fertilizers such as manure is an important management practice that increases SOM and provide N for plants. Organic N materials are typically decomposed to inorganic N forms by soil bacteria, fungi and other microbes through a process called mineralization. Soil microbes also need N for their growth and upkeep and will utilize some of the mineralized N or available soil N. The use of available soil N by microbes is called immobilization. The net production of mineral N by the decomposition process (net mineralization) depends on the balance between gross mineralization and gross

immobilization. Net mineralization depends on the quality of the organic material with the C/N ratio being the most important determining factor (Ledgard et al. 1998).

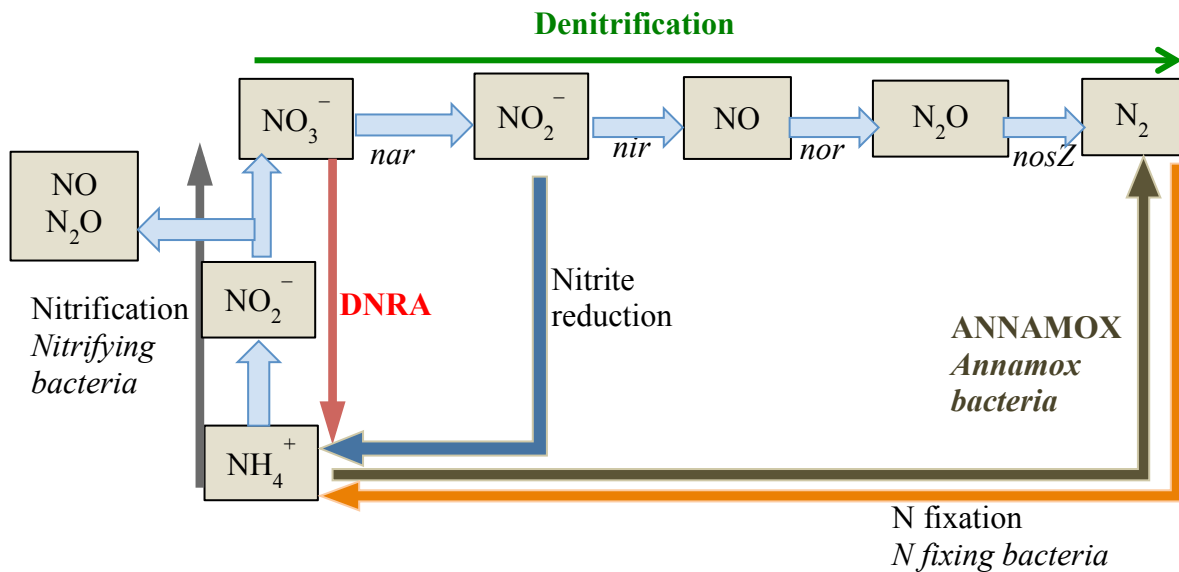


Figure 2.4 Main pathways for transformation of nitrogen between inorganic and gaseous forms. Adapted from Pilegaard (2013).

Figure 2.4 shows the major processes and pathways of inorganic N transformation in the soil. Ammonium (NH_4^+) and nitrate (NO_3^-) are the major forms in which plants take up N and also the most important inorganic forms of the soil N cycle (Chapin et al. 2011). Ammonification, the process by which NH_4^+ is formed from organic N, results from SOM decomposition and also from N fixation by N fixing plants. Once NH_4^+ is formed, it can be taken up by plants, oxidised aerobically to NO_3^- through nitrification, or oxidized anaerobically to N_2 (anaerobic ammonium oxidation; ANAMMOX) (Butterbach-Bahl et al. 2013). Nitrate on the other hand is formed through the nitrification process and can be removed from the soil through plant N uptake, reduction to N_2 through denitrification, reduced to NH_4^+ through dissimilatory NO_3^- reduction to ammonia (DNRA) or lost through leaching (Baggs 2011; Butterbach-Bahl et al. 2013; Lu et al. 2013; Pilegaard 2013). These transformations and losses of inorganic N have huge implications not only on plant production but also on the environment.

While NH_4^+ and NO_3^- transformation, through nitrification and denitrification are important for plant growth, the production of nitric oxide (NO), N_2O and N_2 from these processes is extremely important for the global climate. Nitric oxide is a highly reactive trace gas important in atmospheric chemistry by regulating the photochemical production of ozone in the troposphere and also because it's one of the causes of acid rain (Crutzen 1979; Logan 1983; Davidson et al. 1993; Eickenscheidt and Brumme 2013). Nitrous oxide on the other is a potent GHG with 265 times stronger radiating power than CO_2 (IPCC 2014) and it's the most important substance emitted into the atmosphere causing the depletion of the ozone layer (UNEP 2013). While N_2 does not have any effect on the environment, its emission represents a loss of N from the soil. N_2O and NO are produced as by-products through what is called the “hole in the pipe” model by Firestone and Davidson (1989) and emitted as “leaks” from either nitrification or denitrification processes. The emission (leaks) of NO and N_2O from nitrification and denitrification is controlled by soil moisture, texture, aeration and factors that control the two microbial processes such as N availability and form, temperature, pH and organic C (Firestone and Davidson 1989; Ludwig et al. 2001; Pilegaard 2013).

2.3.1 Nitrification, denitrification and gaseous N production in soils

Nitrification is the aerobic oxidation of NH_4^+ to NO_3^- via nitrite (NO_2^-), with NO and N_2O as by-products, and is carried out by nitrifying bacteria (Fig. 2.4). Nitrification occurs in two distinct steps by two different organisms. The first step, the oxidation of NH_4^+ to NO_2^- , is catalysed by ammonia-oxidizing bacteria (AOB) or archaea (AOA) and the second step, oxidation of NO_2^- to NO_3^- , is catalysed by nitrite-oxidizing bacteria (Costa et al. 2006; Daims et al. 2015). Only a narrow range of bacteria is known to carry out the first step of autotrophic nitrification and only two groups namely *Nitrosospira* and *Nitrosomonas* are known to be NH_4^+ oxidizers (Boer and Kowalchuk 2001). The first step is considered to be

the one limiting the rate of nitrification in soils. It requires the same enzyme, mono-oxygenase, as CH_4 oxidation and hence the reported negative relationship between CH_4 oxidation and nitrification (Hütsch et al. 1994; Boer and Kowalchuk 2001; Le Mer and Roger 2001). Bacteria that can carry out both steps of the nitrification process without relying on a second group of bacteria have been discovered recently (Daims et al. 2015). Nitrifying organisms obtain energy by oxidizing NH_4^+ or NO_2^- and this is usually the only or major source of energy for such organisms (Boer and Kowalchuk 2001). Heterotrophic nitrification, where the oxidation of NH_4^+ and NO_2^- is not related to cellular growth of microbes or energy production, where the substrate is organic N compounds instead of NH_4^+ , by some bacteria and fungi species also exists (Boer and Kowalchuk 2001; Pilegaard 2013; Zhang et al. 2015). Heterotrophic nitrification mostly occurs in acidic soils and soils with high organic matter content (Zhang et al. 2015).

Denitrification is a process, or group of processes, that reduce NO_3^- to N_2 via NO_2^- with NO and N_2O as intermediate products (Firestone and Davidson 1989). It is the only known natural process that can both produce and consume N_2O and NO. Heterotrophic denitrification is the microbial reduction of NO_3^- to N_2 through a four-step sequential process each catalysed by a particular enzyme (Fig 2.4). First NO_3^- is reduced to NO_2^- by NO_3^- reductase enzyme (*nar*), then NO_2^- is reduced to NO by NO_2^- reductase enzyme (*nir*), the next step is the reduction of NO to N_2O by NO reductase (*nor*), and finally N_2O is reduced to N_2 by N_2O reductase (*nosZ*). This process is carried out by denitrifiers which utilize the oxygen in the N-oxide compounds as an electron acceptor to gain energy (Tiedje et al. 1989). When this process occurs to its full completion (complete denitrification), N_2 is the final product. However, incomplete denitrification often leads to large amounts of N_2O and NO being emitted in the process. The rate of denitrification and the relative proportions of the three main gaseous products (NO, N_2O and N_2) depends on several interacting factors

such as climatic factors (e.g. temperature, rainfall), soil properties (e.g. pH, N and C content, aeration and moisture), presence of microbes (type, abundance) and management practices (e.g. tillage, fertilization) (Firestone and Davidson 1989; Tiedje et al. 1989; Saggar et al. 2013). Denitrification can also take place through the chemical reduction of NO_2^- to form NO, N_2O and finally N_2 by metal ions such as iron (Fe^{2+}), copper (Cu^{2+}) and manganese (Mn^{2+}) in a process called chemodenitrification (Cleemput 1998; Butterbach-Bahl et al. 2013). However chemodenitrification only occurs at soil pH below 5 (Pilegaard 2013) and when NO_2^- concentrations are high ($> 250 \text{ mg N g}^{-1} \text{ soil}$) (Cleemput 1998). Another form of denitrification called nitrifier-denitrification or nitrification-coupled-denitrification where NO_2^- produced during nitrification is reduced to N_2 by denitrifiers is reported to play an important role under certain conditions (Bakken et al. 2012).

Another process that produces N_2O and therefore important for N cycling in agricultural soils is dissimilatory nitrate reduction to ammonium (DNRA). This is the reduction of NO_3^- to NH_4^+ by facultative and obligate microorganisms under strictly anaerobic conditions (Butterbach-Bahl et al. 2013). N_2O is produced by the bacterial reduction of NO_2^- produced during the DNRA process.

2.3.2 Factors controlling nitrification and denitrification and associated gaseous N products

2.3.2.1 *Soil microbes*

Both nitrification and denitrification are mostly microbiological processes and therefore dependent on the presence of the necessary microbes. A relatively large range of microbes can carry out denitrification while only a small number of microbes are capable of carrying out the nitrification process (Butterbach-Bahl et al. 2013). The abundance and structure of

the soil microbial community is therefore an important factor in regulating N gases' production from both nitrification and denitrification.

For denitrification to occur the presence of microbes (mostly bacteria), organic C, anaerobic conditions and NO_3^- availability is required. Nitrification process is directly controlled by the availability of NH_4^+ and O_2 (Firestone and Davidson 1989).

2.3.2.2 *Nitrogen and Carbon*

In natural environments, availability of NH_4^+ is dependant on the ammonification process (the mineralization of inorganic N from organic N) while NO_3^- is controlled by the nitrification of the mineralized NH_4^+ (Chapin et al. 2011). Therefore, the availability of organic matter is essential for NH_4^+ production. Factors that control decomposition of SOM, discussed in above sections, will therefore have a large control on both nitrification and denitrification. In agricultural soils, fertilization is an important source of mineral N. The amount and type of fertilizer applied will therefore have a large bearing on nitrification and denitrification rates. Most bacteria and fungi involved in denitrification are capable of carrying out the entire process of reduction from NO_3^- to N_2 , but the relative amounts of each of three gaseous N forms depends on relative abundance of NO_3^- to organic C (Chapin et al. 2011). Higher organic C relative to NO_3^- produces more N_2 while the opposite tends to lead to higher production of N_2O . Organic C also controls the nitrification process. Weier et al. (1993) measured denitrification with different N and available organic C amounts and found that despite high N concentration, denitrification was very low when available C concentration was low. It has been reported that the major form of mineral N in the soil will affect the emissions of N gases. For example, soils with NH_4^+ as the dominant form of mineral N had lower N_2O and NO emissions than those where NO_3^- was dominant, and N_2O was negatively correlated with NH_4^+ but positively correlated with NO_3^- (Davidson et al.

2000). High NO_3^- concentration in the soil relative to NH_4^+ indicates that availability of mineral N in soil exceeds plant N uptake/demand (Davidson et al. 2000) because NO_3^- is preferred by plants over NH_4^+ (Chapin et al. 2011) and therefore is likely to lead to higher N_2O and NO emissions.

2.3.2.3 *Soil moisture*

Soil moisture is arguably the most important environmental factor controlling N_2O production from nitrification and denitrification (Oertel et al. 2016). Nitrifying bacteria require O_2 and at WFPS values below 10% nitrification is limited by low nutrient supply. Rainfall and irrigation increase soil moisture and in turn influence the rates of both nitrification and denitrification. Rainfall events can increase the transport of both N and C substrates needed for nitrification and denitrification and also change aerobic conditions in soils (Saggar et al. 2013) leading to changes in N transformation processes. Increased denitrification when WFPS is above 70% has been reported by many studies (e.g Weier et al. 1993; Saggar et al. 2013) with even higher denitrification rates at WFPS above 85% as oxygen availability becomes even more limited. Emissions of N_2O are at the highest when WFPS is in the range 70–80% depending on soil type (Butterbach-Bahl et al. 2013) because this is the moisture content range when both nitrification and denitrification can occur at relatively high rates. Very high WFPS values however tend to increase the reduction of N_2O to N_2 and hence the reported reduction in N_2O emissions at very high WFPS values (Signor et al. 2013). Soil moisture can also regulate soil temperature, another important factor affecting microbial processes. Soil moisture also influences the physical movement of gases in soils and hence their diffusion and emissions to the atmosphere. For example, at high soil moisture, movement of NO from the soil is inhibited leading to its increased likelihood of

being consumed by denitrification. As a result NO emission tends to be lower under high moisture content (Firestone and Davidson 1989; Pilegaard 2013).

2.3.2.4 *Temperature*

Soil temperature is another key environmental factor driving the rates of nitrification and denitrification in soils. Generally both nitrification and denitrification, and consequently the production N₂O and other gaseous N products, increase with increasing temperature. Temperature does not only affect the microbial activity and production of gaseous N products, but also the rate of diffusion from the soil into the atmosphere (Signor et al. 2013). Denitrification is very low at low soil temperatures with the 2–10°C range as the minimum range for denitrification when C availability is limited (Dorland and Beauchamp 1991). However, high organic C availability can drive denitrification even at very low soil temperatures (Cleemput 1998). Low nitrification at low temperatures and increased nitrification following increased temperatures has been reported in soils and it has been attributed to increased enzyme activity (Hu et al. 2016). Temperature changes can affect the microbial populations in the soil. Hu et al. (2016) found that increasing temperature led to shifting of nitrifiers from bacteria dominated to archaea dominated communities. Although nitrification and denitrification both increase with increasing temperature, very high temperatures can lead to decreased microbial activity. Above 37°C significant there is reduction in N₂O production and emission, denitrification and nitrification rates (Oertel et al. 2016).

2.3.2.5 *Land-use and management practices*

Land-use and land management activities also have an influence on N cycling in soils. Tillage increases soil aeration, reduces moisture due to increased infiltration, exposes SOM

to microbes and increase its decomposition, breaks down plant residue and increases soil temperature (Hütsch 1998; West and Marland 2002; Chatskikh and Olesen 2007; Kravchenko et al. 2011; Abdalla et al. 2013; Campbell et al. 2014; Mbuthia et al. 2015; Congreves et al. 2016; García-marco et al. 2016), and as a result influences not only the production of N gases but also their consumption and diffusion. Increasing N₂O and NO emissions due to tillage activities has been reported by several studies (Ruan and Philip Robertson 2013; Palm et al. 2014; Yonemura et al. 2014). Different vegetation and crop types not only require different amounts of nutrients and fertilization rates, but also have different nutrient uptake rates and affect soil moisture and temperature differently. Therefore the vegetation/crop type will affect nitrification and denitrification. For example, comparing pastures of different ages in South America, Davidson et al. (2000) reported high N₂O and NO emissions in younger pastures than older ones and attributed it to net immobilization due to very low mineralization in older pastures. Annual crops such corn, also require annual tillage compared to perennial crops like grasslands and pastures. Tillage often leads to exposure of the previously physically protected SOM and other substrates, increasing their biochemical availability to soil microbes (Mosier et al. 2006). All these will have a bearing on the cycling of N in the soil.

2.3.3 Soil pH, liming and nitrogen transformation in soils

Soil pH has been termed the “master variable” for N transformation processes in soil (Mørkved et al. 2007; Baggs et al. 2010). Soil pH affects nutrient availability, population and structure of soil microbes and their activity, plant communities and their productivity (Kemmitt et al. 2006). Soil organic matter decomposition is also affected by soil pH through its influence on microbial communities and nutrient availability.

Production and consumption of N_2O and other N gases has been found by many studies to be influenced by soil pH. The reported effect of soil pH on N gas production and consumption is however heterogeneous among studies (Šimek and Cooper 2002; Kemmitt et al. 2006; Baggs et al. 2010; Čuhel et al. 2010; Zaman and Nguyen 2010; Cheng et al. 2013). In tea fields, increased soil pH due to lime-N (CaCN_2) application led to decreased N_2O compared to conventional fertilization (Yamamoto et al. 2014). Liming of an acidic soil led to 4 times higher N_2O than a similar soil with the same final pH and a higher contribution of nitrification (Baggs et al. 2010). Nitrification is usually very low in acidic soils due to the sensitivity of NH_4^+ oxidising bacteria to low pH (Kemmitt et al. 2006). However, significant nitrification can still occur in acid soils, due to presence of pH-neutral microsites and also to acid tolerant NH_4^+ oxidising bacteria (Boer and Kowalchuk 2001). Heterotrophic nitrification is a major pathway of N_2O production in acid soils and soils with large organic matter content (Zhang et al. 2015). Mineralization of N and nitrification has been found to increase with increasing soil pH (Mørkved et al. 2007; Baggs et al. 2010). However, Kemmitt et al. (2006) did not find increased mineralization with increasing soil pH, but found that nitrification was inhibited by low soil pH and they did not observe any effect of pH on denitrification. Baggs et al. (2010) found that when soils were limed to pH 7–8, nitrification was the dominant process producing N_2O . The same study found that at pH below 5, denitrification was the main process for N_2O production. While many studies have reported increased denitrification with increasing pH, with an optimum range around neutral to slightly alkaline pH (Šimek et al. 2002; Čuhel et al. 2010), this relation between soil pH and denitrification may not necessarily be due to soil pH effect but rather to increase in C and N availability with increasing soil pH (Šimek and Cooper 2002). Soil pH also has different effects on the enzyme activities of each of the four steps of the denitrification process. The *NosZ* enzyme becomes very efficient at pH 7 in reducing NO_x compounds all

the way to N₂ with little or no NO and N₂O emissions (Bakken et al. 2012). The *NosZ* enzyme has a higher optimum pH (8.0) than the enzymes; *Nir* (7.5), *Nar* (7.0) and *Nor* (~5), involved in the production of N₂O (Pan et al. 2012). Like N₂O, NO is also produced both by nitrification and denitrification. Generally, NO production is high at pH above 6.5, due to increased nitrification, and high at pH below 5 due to denitrification. Therefore, no proper relationship exists between NO emission and soil pH (Pilegaard 2013).

2.4 Mitigating global warming potential and greenhouse gas intensities in agro-ecosystems

Global warming potential (GWP) is a concept developed by the intergovernmental panel on climate change (IPCC) as a metric to compare the effects of the different GHGs on the climate. It combines the radiative forcing of each GHG in the atmosphere, with CO₂ as the standard, and the residence time of the respective gas in the atmosphere (Zhu et al. 2010). Global warming potential therefore constitutes the net balance of GHG exchange of a given agro-ecosystem (Robertson and Grace 2004; Mosier et al. 2006) and hence allows to evaluate the overall effect of a particular management practice. Because CO₂ is both sequestered through NPP and plant residue incorporation in the soil and lost through RH, a carbon budget approach such as NECB, rather than mere CO₂ emissions, in addition to CH₄ and N₂O emissions, has been used in the calculation of GWP in recent studies (Mosier et al. 2006; Huang et al. 2013; Zhang et al. 2014a; Ali et al. 2015; Yang et al. 2015).

While the reduction of GHGs and GWP from agriculture is of major concern, the primary aim of all agricultural activities is production of food, feed or related materials. An index that evaluates the GWP of a given agricultural management system against the productivity is therefore essential for decision makers. Greenhouse gas intensity (GHGI) is

that index. GHGI is calculated as GWP divided by yield and gives the net GHG emitted for each mass of plant yield (Robertson and Grace 2004; Mosier et al. 2006).

A number of management practices such as tillage management, manure application and residue incorporation have been proposed to have potential to mitigate GHGI from agro-ecosystems. Fertilization strategies that enhance plant nutrient-use efficiency can help to both increase plant productivity and reduce emissions of GHGs.

In upland agro-ecosystems, soil C can be increased through land-use change to permanent pasture or perennial crops (IPCC 2007a; Alberti et al. 2010; Zenone et al. 2011), reducing tillage (West and Marland 2002; Six et al. 2004; Mosier et al. 2006), plant residue management (Follett 2001; Lal 2004) and application of organic manures to soils (Ginting et al. 2003; Shimizu et al. 2009; Alluvione et al. 2010). Practices that increase the input of organic carbons into soils, either as plant litter or root material, reduce soil disturbance, preserve or increase soil quality will ultimately increase soil C. By better matching N supply to crop demand and more closely integrating animal waste and crop residue management with crop production, N₂O emissions could be decreased by about 0.38 Tg N₂O–N (Cole et al. 1997). Improved technologies, such as use of controlled-release fertilizers, nitrification inhibitors, and water management can improve plant N-use efficiency and reduce N₂O emissions (Cole et al. 1997; US.EPA 2013).

However, current experimental evidence indicates that these proposed strategies do not always lead to reduced GWP and GHGI. Several factors are usually at play and more needs to be done to fully understand the interactions between different factors. For example, soil C storage capacity due to reduced tillage depends on rainfall and soil moisture regimes. In humid climates SOC storage can increase over a shorter period (within 5 years) following introduction of no tillage practices. However, in dry climates no/reduced tillage can lead to decreased storage in the short-term (less than 10 years) compared to conventional tillage and

only lead to increased SOC at periods longer than 10 years (Six et al. 2004). This is due to slower incorporation of plant residues due to lower microbial activities in dry climates. Reduced tillage can lead to increased N₂O emissions, especially in the early years, due to increased soil moisture (Six et al. 2004; Mosier et al. 2006) which in turn stimulates denitrification. While manure application can increase SOC, it also increases available N and increase N₂O emissions.

It is clear from the above literature review that the overall effects of tillage, manure application, fertilizer management and liming on GHGI can vary widely with soil types and land-use and hence the need for long-term studies to fully understand the mechanisms involved and how soil and environmental factors interact in driving GHG emissions.

In Hokkaido, and most farming areas in Japan, livestock production relies on a lot of imported feed while at the same time producing a lot of animal manure. It is therefore important to use the manure as a nutrient source to mitigate the overall impact of livestock production practices. Furthermore, the growth in demand of food produced through “clean agriculture” entails that farmers need to use more environment-friendly production systems to stay competitive. The use of manure is one important aspect and its effect on both food production and the environment need to be studied in detail.

3. Materials and Methods

3.1 Study site

The field studies (chapters 4, 5 and 6) were carried out in field W4 at the Hokkaido University's Shizunai experimental livestock farm of the Field Science Center for Northern Biosphere in Shin-Hidaka city, Southern Hokkaido, Japan (Fig. 3.1; 42°26'N, 142°29'E). Soils for the incubation study (chapter 7) were collected from this same site and transported to the laboratory. The site is relatively cool in summer and cold in winter with average annual air temperature and precipitation values of 8.1 °C and 1252 mm respectively. The soil surface is covered with snow from the end of December to the beginning of March. The soil is derived from volcanic ash and is classified as Mollic Andosol (IUSS Working Group WRB 2006). Selected properties of the top Ap horizon soil at the study site are shown in table 3.1.

Table 3.1 Selected soil properties at the study site

pH (H ₂ O)	Sand	Silt	Clay	Total C	Total N	Bulk density
	(%)	(%)	(%)	(%)	(%)	(g/m ³)
5.1	72.7	23.7	3.6	3.8	0.33	0.81

3.2 Field study experimental designs and management

During the study period, land-use was an old grassland (OG) from 2005 to 2009, cornfield (2010-2012) and newly established grassland (NG) (2013-2015). The old grassland had been established more than 30 years prior to the beginning of this study in 2005. The dominant grass species was reed canary grass (*Phalaris arundinacea* L.) and meadow foxtail (*Alopecurus pratensis* L.) in OG, and timothy grass (*Phleum pretense*) in NG.

The average amount of mineral fertilizer applied in OG before commencement of this study was $133 \pm 36 \text{ kg N ha}^{-1} \text{ year}^{-1}$ as NH_4NO_3 . From 1990 to 2004 the grassland was harvested for hay at least twice a year. In September 2009 herbicide was applied and the field ploughed in December.

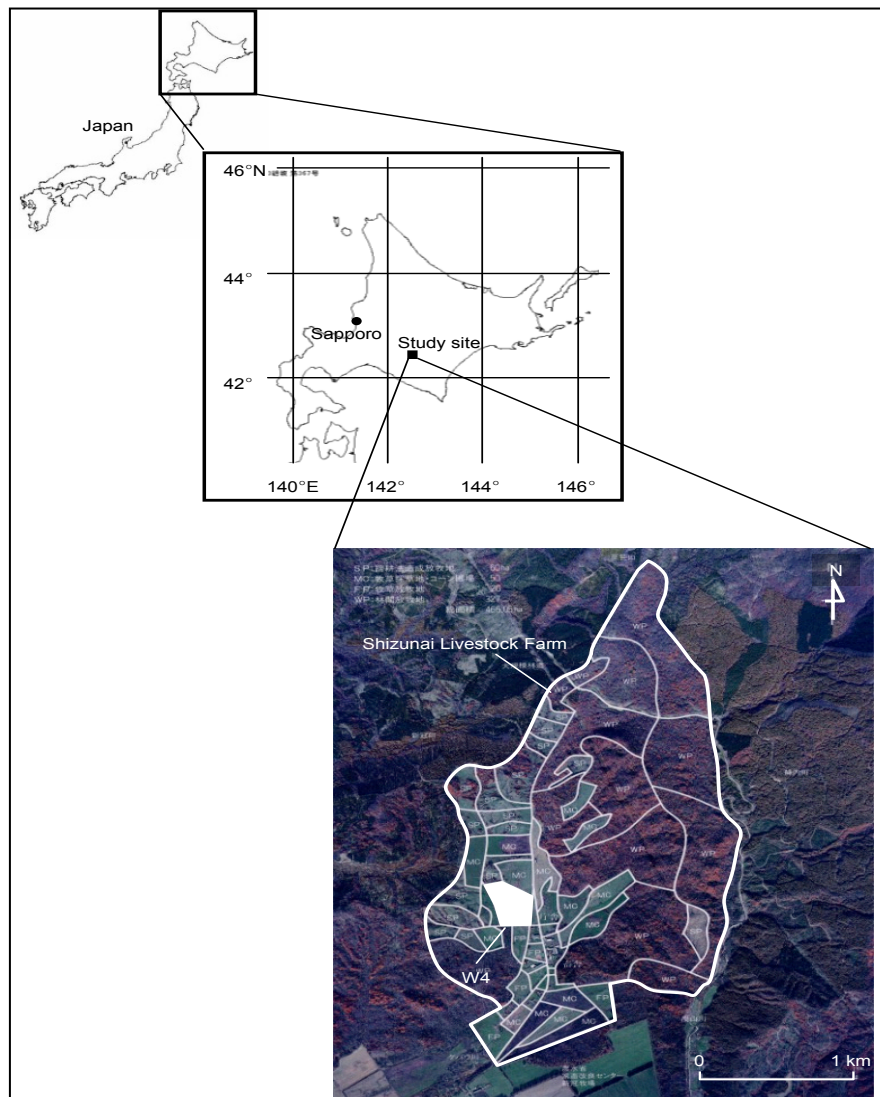


Figure 3.1 Map of study site location.

Field experimental plots were 5x5 m in size and all the treatment plots were replicated four times and arranged in a randomized design. Table 3.2 shows the timing of

fertilizer and manure applications, and other field management practices for field experiments in chapters 4 to 6. In grassland, manure was applied once in spring and inorganic fertilizer was applied twice, in spring and summer. In the cornfield manure was applied in autumn and inorganic fertilizer in spring. The type of inorganic fertilizer was ammonium sulfate and the manure was composited beef cattle manure with bedding litter (bark).

Table 3.2 Timing and kind of field management activities in the field studies from 2005–2015

Land-use	Management activity	Time
Old grassland (<i>Phalaris arundinacea</i> L. and <i>Alopecurus pratensis</i> L.)	Manure application	May
	Fertilizer application	May and June/July
	Harvesting	June and August
Cornfield <i>Zea mays</i> L	Tillage	October/November (ploughing), May (harrowing and planting)
	Manure application	October/November
	Fertilizer application	May
	Harvesting	September/October
New grassland† (<i>Phleum pretense</i> L.)	Tillage	May 2013 (harrowing and planting), September 2013 (herbicide application, ploughing and re-planting)
	Manure application	October 2012, September 2013 and May 2015
	Fertilizer application	May 2013, May and July in 2014 and 2015
	Harvesting	September 2013, June and August 2014 and 2015

† New grassland was established by ploughing and then seeding in Spring of 2013. However, due to poor plant growth after the initial seeding, herbicide was applied in September after which the field was ploughed and reseeded again.

A total of 3 field experiments were conducted. The first one, from 2005 to 2015, investigated the effect of land-use change between grassland and cornfield on C and N cycling. The second experiment, from 2010 to 2015, investigated the effect of different manure and inorganic fertilizer management on GHGI. The third and last field experiment investigated the effect of timing (autumn and spring) of manure application on GHG emissions and plant production.

3.2.1 Field experiment 1 set up and design (Effect of land-use change on C, N and greenhouse gas dynamics)

Change in C, N and GHG dynamics were evaluated as land-use at the study site changed from old grassland (OG; 2005 to 2009) to cornfield (2010-2012) and then back to a newly established grassland (NG; 2013-2015). Date of conversion from OG to cornfield was regarded as the day of herbicide application on 1st October 2009, while date of converting cornfield to NG was 17th October 2012, the day of manure application for next growing season. This will be more precisely described in chapters 4 and 5. Three treatments plots namely; (i) control without N addition (CT plot), (ii) inorganic N fertilizer only (F plot), and (iii) inorganic N fertilizer and composted beef cattle manure (MF plot) were set up from 2005. These treatments were arranged in a complete randomized design and replicated 4 times. The field had been managed as grassland for more than 30 years prior to setting the treatments in 2005 and had received $133 \pm 36 \text{ kg N ha}^{-1} \text{ year}^{-1}$ as inorganic fertilizer from 1984 to 2004. The amount of inorganic fertilizer and manure applied in these plots, from 2005 to 2015, are shown in Table 3.3. The inorganic fertilizer application rates were based on the recommended rates in this region. The manure application rates were optimum rates in this region and based on adequate amounts of potassium application in the fields. Soil organic C and N and GHG fluxes were measured in these plots, as described in sections 3.2.2 to 3.2.7 below, from 2005 to 2015.

Table 3.3 Rates of inorganic fertilizer N, manure N and manure C application from 2005–2015 in F and MF plots (chapter 4 and 5)

Plot	Land-use	Year	Inorganic Fertilizer			Manure				Lime (kg CaCO ₃ ha ⁻¹)
			(kg ha ⁻¹)			(Mg ha ⁻¹)	(kg ha ⁻¹)			
			N	P ₂ O ₅	K ₂ O	C	N	P ₂ O ₅	K ₂ O	
F	OG	2005	164	45	264.5	0	0	0	0	0
		2006	183	67.2	273.1	0	0	0	0	0
		2007	74	23.3	109.4	0	0	0	0	0
		2008	74	20.3	109.4	0	0	0	0	500
		2009	91.4	25.1	135.4	0	0	0	0	500
	Corn	2010	104	144	80.0	0	0	0	0	400
		2011	104	144	80.0	0	0	0	0	400
		2012	96.6	133.7	70.8	0	0	0	0	286
	NG	2013	40	100	60.0	0	0	0	0	300
		2014	150.2	107.9	212.2	0	0	0	0	300
2015		103.8	40	141.3	0	0	0	0	300	
MF	OG	2005	130	6.8	70.4	5.8	236	191.0	266.3	0
		2006	133	6.0	129.5	6.0	310	310.2	254.4	0
		2007	21	0	0	7.7	331	331.4	345.0	0
		2008	0	0	0	8.0	308	259.6	261.6	500
		2009	0	0	0	9.0	491	564.2	698.3	500
	Cornfield	2010	104	144.0	80.0	8.9	599	599.1	572.9	400
		2011	104	144.0	80.0	6.7	216	397.0	325.1	400
		2012	96.6	127.5	70.9	6.7	343	561.3	411.3	286
	NG	2013	40	100.0	60.0	7.3	448.4	715.1	579.9	300
		2014	47	46.9	50.0	0	0	0	0	300
		2015	56.9	22.2	68.8	6.8	165.4	208.5	143.7	300

Note: The unfertilized control plot (CT) did not receive inorganic fertilizer or manure, but received the same amount of lime as the two plots in the table for each year.

3.2.2 Experimental set up and design (Mitigating global warming potential and greenhouse gas intensities through manure management in cornfield)

To evaluate the effect of manure and fertilizer management on global warming potential (GWP) and greenhouse gas intensity (GHGI), two experiments were set up in a cornfield from 2010 to 2013 (chapter 6) in a field that had previously been managed as a grassland.

3.2.2.1 Field experiment 2: Effect of manure and fertilizer application on GWP and GHGI

Five experimental plots were set up and investigated from 2010 to 2012. The treatments plots were; (i) unfertilized control plot (CT), (ii) inorganic fertilizer only plot (F), inorganic fertilizer plus composted cattle manure applied since 2005 plot (MF1), inorganic fertilizer plot plus composted cattle manure applied since 2010 plot (MF2), and composted cattle manure only plot (M). The CT, F and MF1 plots were same as those in the land-use change study (section 3.2.1). The MF2 plot was setup after dividing the large inorganic fertilizer only (F) plot established in 2005 into two, with one half applied with composted manure from 2010 to 2012. The M plot was established in 2011 within the MF1 plot established in 2005 by stopping application of inorganic fertilizer, in this part only, from 2011 onwards. These five plots were replicated 4 times in a complete randomized design with each plot measuring 5x5 m in size. The fertilizer and manure application rates are shown in table 3.4 below.

Table 3.4 Inorganic fertilizer and manure application rates in cornfield (experiment 1, chapter 6)

Plot	Year	Inorganic fertilizer (kg N ha ⁻¹)	Manure		Total N input (kg N ha ⁻¹)
			C input (Mg C ha ⁻¹)	N input (kg N ha ⁻¹)	
F	2010	104	0	0	104
	2011	104	0	0	104
	2012	96.6	0	0	96.6
MF1	2010	104	8.9±0.4	599±31	703
	2011	104	6.7±0.5	216±29	320
	2012	96.6	6.7±0.4	343±11	439.6
MF2	2010	104	8.9±0.4	599±31	703
	2011	104	6.7±0.5	216±29	320
	2012	96.6	6.7±0.4	343±11	439.6
M	2010	0	8.9±0.4	599±31	599
	2011	0	6.7±0.5	216±29	216
	2012	0	6.7±0.4	343±11	343
CT	2010-2	0	0	0	0

3.2.2.2 Field experiment 3: Effect additional spring manure and fertilizer application on soil C and GWP

In experiment 2, plots were set up in the fertilizer only part of the field, described in section 3.2.2.1 above, in 2013. In this experiment, three plots were set up in a complete randomized design with 4 replications to assess the effect of additional manure application in spring season on plant growth and net GHG emissions. The treatments were autumn composted cattle manure application (M1), autumn composted cattle manure application and additional spring inorganic fertilizer application (MF) and autumn composted cattle manure plus additional spring manure application (MM). Autumn manure application was done in November 2012 (40 Mg fresh matter: 450 kg N and 7.3 Mg C per hectare). In May 2013 (spring), additional 29 Mg fresh manure was applied in the MM plot (300 kg N and 5 Mg C per hectare), and inorganic fertilizer (104 kg N, 139 kg P and 77 kg K per hectare) was

applied in the MF plot (Table 3.5). Lime at the rate of 300 kg CaCO₃ was applied in all the plots.

Table 3.5 Rates of inorganic fertilizer N and gross manure C and manure N input in experiment 2 of cornfield (chapter 6).

Plot	Application season	Inorganic fertilizer (kg N ha ⁻¹)	Manure	
			(Mg C ha ⁻¹)	(kg N ha ⁻¹)
M1	Autumn	0	7.3	450
	Spring	0	0	0
MF	Autumn	0	7.3	450
	Spring	104	0	0
MM	Autumn	0	7.3	450
	Spring	0	5.0	300

M1 is autumn composted cattle manure application only plot; MF is autumn composted cattle manure plus spring inorganic fertilizer application plot; MM is autumn composted cattle manure plus additional spring composted cattle manure plot.

3.2.3 Soil and weather measurements

Soil samples were collected at 5 cm depth during each sampling day from April to November (non freezing period) in all treatment plots. Soil samples were sieved (2 mm sieve) and extracted in deionized water or in 2 M KCl solution, and the extracts were stored at 4°C until analysis for dissolved nutrients after being filtered through 0.2 µm membrane filters. From the water extracts; Soil NO₃⁻ concentrations were analyzed by ion chromatography (Dionex QIC Analyzer; Dionex Japan, Osaka, Japan); soil pH was measured by using a combined electrode pH meter (F-8 pH meter; Horiba, Kyoto, Japan); and water extractable organic C (WEOC) was measured using a total organic C (TOC) analyzer (TOC 5000A; Shimadzu, Japan). NH₄⁺-N in the 2 M KCl extract solution was determined using the indophenol- blue method (UV mini 1240; Shimadzu, Kyoto, Japan).

In the grassland (old and new), soil moisture was measured at 0–6 cm depth using the Frequency Domain Reflectometry (FDR) method (DIK- 311A; Daiki, Saitama, Japan).

Calibration curves were made to calculate water-filled pore space (WFPS) from the FDR device reading and percent total porosity. In the cornfield, soil moisture content was measured gravimetrically from soil samples collected at a depth of 0–5 cm.

Daily precipitation and air temperature were obtained from the nearest Automated Meteorological Data Acquisition System (AMEDAS) station of the Japan Meteorological Agency. Thermocouple thermometers (TR-52, T&D, Nagano, Japan) were permanently installed in each plot to measure soil temperature at 5 cm depth at 30-minute intervals. On each sampling day air temperature inside the chamber and soil temperature (5 cm depth) were measured using a hand-held thermometer (CT220; CUSTOM, Tokyo, Japan).

3.2.4 Total soil N and soil C analysis

Samples to determine total organic soil C (SOC) and N (SON) contents were collected every two years from 2006 to 2014 in the inorganic fertilizer only and manure plus inorganic fertilizer (F and MF) plots at 0–15 and 15–30 cm soil depths. These samples were collected at the end of the growing season. The samples were collected every 10 m in a grid covering the entire field resulting in at least 12 points for each plot. Soils were collected from the mineral Ap horizon after removing the root mat. The soils were then air-dried, sieved (2 mm) and roots and other debris removed. Total N and C were analyzed using a C/N analyzer (SUMIGRAPH NC-1000; Sumika Chemical Analysis Service, Ltd., Osaka, Japan). In 2004, prior to the initiation of the treatments, total SON and SOC was measured as 0.33% and 3.7% respectively (Shimizu et al. 2009) and this value was used as the reference for measuring the change in SOC and SON. The amount of SOC stored in the soil layer was calculated based on the equation below:

$$\text{Total C storage (Mg C ha}^{-1}\text{)} = \text{SOC} \times \text{BD} \times \text{H}$$

where SOC is the organic C content (%), BD is the soil bulk density (Mg m^{-3}) and H is the thickness of the soil layer (m). Bulk density was determined gravimetrically from core samples collected at each soil depth.

3.2.5 Gas flux sampling and measurement

All the GHGs (CO_2 , CH_4 , N_2O) and NO fluxes were measured using static closed chambers. The chambers were made of stainless steel painted in white colour and were 20 cm in diameter and 25 cm in height in the cornfield, and 40 cm wide and 30 cm high in OG and NG. The structural outline of the chambers is shown in Figure 3.2. The chambers were placed onto chamber bases (as shown in Fig. 3.2), which were installed permanently during the measurement period to a depth of 4 cm. Chamber bases could not be used in winter due to large amounts of snow, therefore chambers were inserted directly to at least 5 cm depth from the snow-covered surface a day before measurements. We did not remove the snow during winter measurements. After each sampling the chambers were removed from the bases.

Gas samples were taken between 8:00 am and 12:00 pm on each sampling day using a gas tight syringe through a three-way valve fitted onto the chamber cover (Fig. 3.2). The normal sampling frequency was once or twice every fortnight, except in winter when sampling was conducted once or twice every month. A more intensive sampling regime of every two to five days was carried out after fertilization and other events that are known to stimulate gas flux. Gas samples from the headspace of each chamber were collected into pre-vacuumed Tedlar bags for CO_2 and NO analysis or a 20-mL vial bottle for N_2O and CH_4 . Samples for CH_4 , N_2O and NO were taken at 0 and 30 minutes in OG and at 0 and 20 minutes in cornfield after chamber closure. In NG, N_2O samples were taken at 0, 15 and 30 minutes while CH_4 and NO samples were taken at 0 and 30 minutes after chamber closure. For CO_2

analysis samples were taken at 0 and 6 minutes after closing the chambers in all the three land-uses. To check the accuracy of flux calculated using only two headspace concentrations, we compared the slope of the change of N₂O concentration inside the chamber with time using the three headspace concentrations (at 0, 15, and 30 min) and using two headspace concentrations (at 0 and 30 min) for all chambers in the 2013–2015 period (n = 772). The results (Fig. 3.3) showed that the slopes from the three and two headspace concentrations had a 1:1 linear relationship ($y = 1.002x + 0.0003$; $R^2 = 0.9997$). We then compared the slopes of three and two headspace concentrations when N₂O was low (below the median), high (above the median) and the whole data set, and there was no significant difference among the three regression lines ($F = 0.0018$, $p = 0.9981$). This result means flux from the two headspace concentrations could be used for treatment comparisons (Stolk et al. 2009; De Klein and Harvey 2015; De Klein et al. 2003).

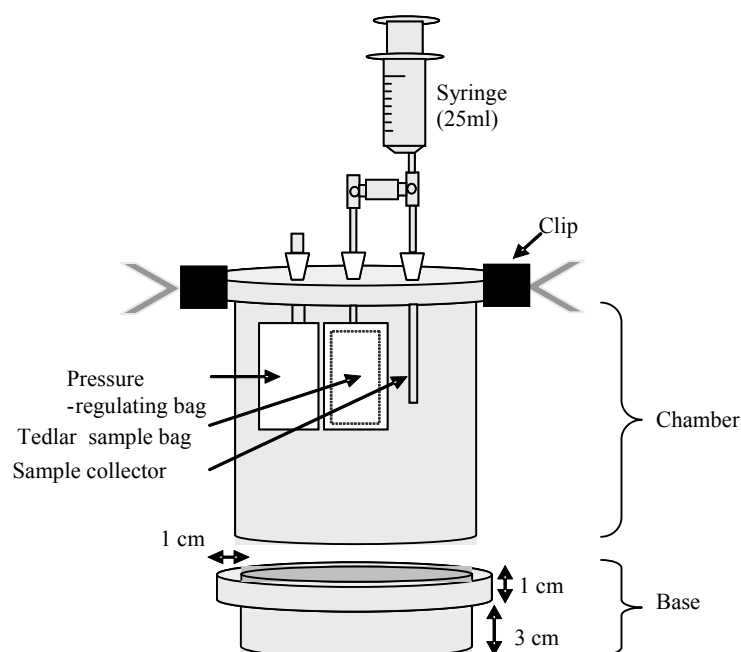


Figure 3.2 Outline of the chamber and base used in the closed chamber method (adapted from Toma and Hatano 2007).

The NO and CO₂ gas concentrations were analyzed in the laboratory within the same day of sampling using a nitrogen oxides (NO_x) analyzer (Model 265P; Kimoto Electric, Osaka, Japan) and an infrared CO₂ analyzer (ZFP9GC11, Fuji Electric, Tokyo, Japan) respectively. CH₄ gas concentrations were determined using a flame ionization detector equipped gas chromatograph (GC-8A; Shimadzu, Kyoto, Japan). N₂O gas concentrations were analyzed within three months using a gas chromatograph fitted with an electron capture detector (Model GC-14B; Shimadzu, Kyoto, Japan).

The gas flux from the soil was calculated using the following linear regression equation (Katayanagi and Hatano 2012).

$$F = \rho \times V/A \times \Delta c/\Delta t \times [273/(273 + T)] \times \alpha$$

where F is the gas flux in $\mu\text{g m}^{-2} \text{ hr}^{-1}$; ρ is the density of each gas at standard conditions (CO₂ = $1.96 \times 10^6 \text{ mg m}^{-3}$, CH₄ = $7.16 \times 10^5 \text{ mg m}^{-3}$, N₂O = $1.97 \times 10^6 \text{ mg m}^{-3}$, and NO = $1.34 \times 10^6 \text{ mg m}^{-3}$); V is the volume of the chamber (m^3), A is the surface area of the chamber (m^2); $\Delta c/\Delta t$ ($10^{-6} \text{ m}^3 \text{ m}^{-3} \text{ h}^{-1}$) is the ratio of change in gas concentration in the chamber during the sampling time; T is the air temperature inside the chamber ($^{\circ}\text{C}$); and α is ratio of molar mass of N of the molecular weight of each respective gas.

Cumulative annual emissions were calculated by linear interpolation between sampling events and numerical integration of underlying area using the trapezoid rule (Ussiri et al. 2009). To calculate the seasonal emissions, winter period was defined as the period from Mid-December, when maximum soil temperature fell below 5°C , to the end of February when maximum temperatures recorded reached 0°C ; the thawing period was defined as the period when minimum daily temperatures reached 0°C , to the time when soils were completely melted (maximum soil temperatures $\sim 5^{\circ}\text{C}$) (Kurganova et al. 2007; Katayanagi and Hatano 2012).

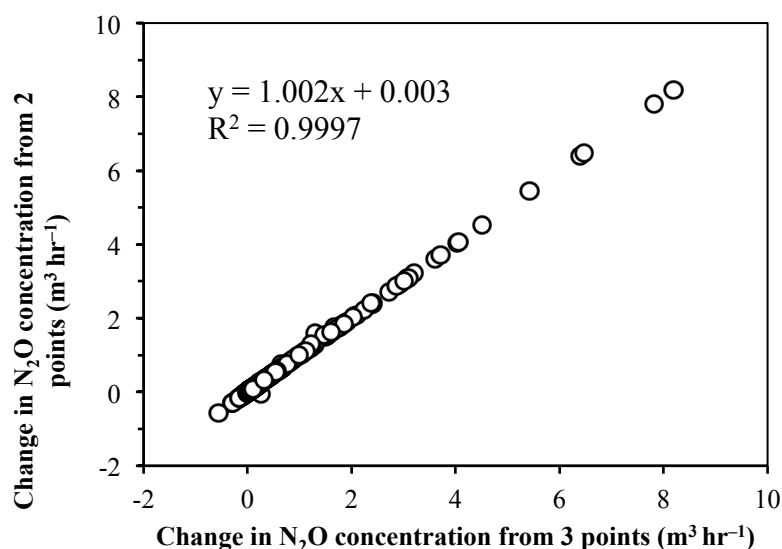


Figure 3.3 Comparison of the slope of the change in N₂O concentration inside the chamber with time using the three headspace concentrations (at 0, 15, and 30 min) and two headspace concentrations (at 0 and 30 min) for all chambers in the 2013–2015 period (n = 772).

3.2.6 Heterotrophic soil respiration and estimation of mineralized N

Heterotrophic respiration (RH) was measured as CO₂ emission from bare soil (plant and root excluded soil) with 4 replications as described by Limin et al. (2015). In the bare plots, the aboveground plants and roots were removed by hand and shovel, and then, a root-proofing permeable sheet (BKS9812, TOYOBO, Osaka, Japan) was vertically inserted to a depth of 30 cm below the ground surface to inhibit regrowth of roots. The surface of the bare plots was covered with a nylon sheet to prevent soil degradation from rainfall, as this would influence the rate of organic matter decomposition. The plants grown on the bare plots were removed by hand regularly to maintain the plot bare. Bare plots were maintained in the same locations within each land-use but moved after each land-use change and after tillage and seeding in cornfield. CO₂ samples in bare plots were collected as described in above.

RH was measured in the CT plot from 2005 to 2009, and in all plots from 2010 to 2015. RH in CT and F plots was regarded as heterotrophic respiration from soil organic

matter decomposition (RHs), while RH from manure amended plots included RHs and heterotrophic respiration from manure decomposition (RHm). Therefore, RHm in MF was estimated by subtracting the RH from F plot, while in M plot by subtracting the RH from CT plot. From 2005 to 2009, RHm was calculated as the difference in total CO₂ emissions in planted plots between MF and F plots (Li et al. 2015; Shimizu et al. 2015).

The total mineralized N was calculated as the sum of soil organic matter N and manure N mineralization. The mineralized N from soil organic matter and manure was estimated by dividing RHs and RHm by the soil and manure C/N ratios, respectively.

3.2.7 Estimation of Q₁₀ values and N₂O emission factors

From the cumulative annual N₂O emissions, we calculated inorganic fertilizer-N N₂O emission factors (EF) using the equation below;

$$EF = N_2O-N (F \text{ plot}) - N_2O-N (CT \text{ plot}) / N \text{ application rate in F plot}$$

The relationship between CO₂ flux and soil temperature was calculated using an exponential function;

$$CO_2 \text{ flux} = \beta_0 \times \exp (\beta_1 \times T)$$

The Q₁₀ values of soil CO₂ flux were then calculated using equation below;

$$Q_{10} = \exp (10 \times \beta_1)$$

where T is the soil temperature at 5 cm depth, β_0 and β_1 are coefficients of the CO₂ flux and temperature function.

3.2.8 Plant N uptake, total N input and soil surplus N

Net primary production (NPP) was measured as the net annual increase in plant biomass (aboveground and belowground biomass).

In grassland the plant biomass was collected four times in a year in April, June, August and October. The aboveground biomass was manually harvested by cutting all the plant biomass within a 0.5 m × 0.5 m quadrat. Two aboveground samples were collected and averaged for each of the four treatment replicates during each sampling event. The belowground biomass was measured by taking a soil block (0.25 x 0.25 x 0.30 m) at each of the 4 replications, from the same points where the aboveground biomass was collected, and then manually separating the roots from the soil.

In cornfield the plant biomass was collected once a year at the end of the growing season just before harvesting. For each of the four treatment replications, corn plants within a 1.5 m x 1 m area were collected by uprooting them (by digging) to 30 cm depth to include all the roots for each plant.

Plant roots were washed in water using a 0.5 mm sieve to completely remove the soil particles and other debris. The plant samples were oven-dried at 70 °C for more than 72 hours and weighed. Each dried sample was analysed for total C and N contents with N/C analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

Surplus soil N was calculated as the difference between total N input (sum of soil and manure mineralized N and chemical fertilizer N) and plant N uptake.

3.3 Incubation study experimental design: Effect of liming on N₂O emissions

From the 3 field experiments, a negative correlation between annual N₂O emissions and soil pH was found (see Chapter 5) and denitrification was determined as the dominant N₂O producing process. Therefore, this incubation experiment aimed at investigating whether increasing pH through liming could mitigate N₂O emissions.

Soil (0–20 cm depth) was collected in 2014 before fertilizer application from the fertilizer only plot in the managed grassland at the Hokkaido University Shizunai experimental

livestock farm (Fig 3.1 and section 3.1). The fertilizer only plot was chosen because it had the lowest soil pH. The soils were air dried and then sieved (2 mm) and kept at low temperature (4°C) until the establishment of the liming treatments.

3.3.1 Experiment 1: Aerobic incubation

Three liming treatments were established as; unlimed soils (L0), low lime rate (L1) at 4 g $\text{CaCO}_3 \text{ kg}^{-1}$ soil, and high lime rate (L2) at 20 g $\text{CaCO}_3 \text{ kg}^{-1}$ soil. After liming, 80 g soil (dry basis) for each lime treatment was repacked into 100 cm^3 cores. The cores were sealed at the bottom to prevent leaching. This amount of soil achieved a bulk density of 0.8 g cm^{-3} ; similar to the bulk density measured in the field. The soils were then pre-incubated for one week at 50% water-filled pore space (WFPS) to allow the soil microbes to re-establish. After the pre-incubation, 200 mg KNO_3 (1 atom% excess ^{15}N) kg^{-1} soil was added in solution form to each of the lime treatments. Unfertilized treatments were also set up for each lime treatment as described above. The final moisture content was then brought to 80% WFPS to create conditions favorable for denitrification but still under aerobic conditions. The fertilizer solution was enough to bring the moisture content to 80% WFPS in the fertilized treatments, while deionized water was added in the unfertilized treatments. Each treatment was replicated four times for gas analysis, and an additional four replications were prepared in a similar manner to measure soil chemical properties. The soils were incubated in 1-liter jars for 16 days from fertilization day in the dark at 15 and 25°C (Only L0 and L2 were incubated at 15°C in experiment 1). The incubation jars were equipped with a sampling port and pressure-regulating valve.

Gas samples were taken twice during the pre-incubation period and on days 1, 2, 4, 7, 10, 13 and 16 after fertilization. Gas samples were taken at 0, 15 and 30 minutes after jar closure on each sampling occasion and the rate of gas production calculated by linear

interpolation between these samples. Gas samples were taken manually using a syringe into a 200 ml Tedlar bag and analyzed within 6 hours after sampling.

^{15}N - N_2O and total $^{15+14}\text{N}_2\text{O}$ were analyzed using an Isotopic N_2O Analyzer (Los Gatos Research Mountain View, CA, USA, model 914-0027). The Isotopic N_2O analyzer is based on off-axis integrated cavity output spectroscopy (OA-ICOS) and it has a precision of 0.2 ppb for N_2O and $\delta^{15}\text{N}$ better than 1 ‰. The analyzer was operated using the batch mode and samples injected manually through the syringe port. The analyzer pumped out air from the measurement cell and then washed the cell with N_2O -free air at least twice before reducing the cell pressure to ~5 Torr in readiness for sample injection. After this, the sample was drawn into the cell and equilibrated with N_2O -free air to a pressure of ~46 Torr inside the cell. Each sample was then measured at a rate of 0.5 Hz for 300 seconds resulting in a total of more than 130 measurements per sample. The average of these measurements was recorded as the value for each individual sample. Detailed operation of the isotopic N_2O analyzer and the calibration of our measurements was done according to Soto et al. (2015).

3.3.2 Experiment 2: Anaerobic incubation

Experiment 2 was conducted using the acetylene (C_2H_2) inhibition technique using unfertilized soils that had been incubated at 25°C in experiment 1. Ten g dry soil was weighed into a 100 ml glass vial and wetted to saturation by adding 10 ml water (for the unfertilized treatments) or 10 ml KNO_3 solution (for the fertilized treatments). Fertilized treatments received an equivalent of 100 mg N kg^{-1} soil as KNO_3 and 600 mg C kg^{-1} soil as glucose. The glucose was meant to provide enough C for microbes in order not to restrict denitrification. The vials were then capped with butyl rubber septa and caps. Immediately after addition of the water or fertilizer solution, the headspace gas inside the vials was

replaced with either pure helium or 10% C₂H₂ gas (90% pure helium). The headspace gas was replaced by vacuuming and refilling with the desired gas (helium or 10% C₂H₂) to atmospheric pressure 3 times to ensure 100% anaerobic and N₂-free conditions. The treatments with or without C₂H₂ were each replicated 4 times for each of the three liming treatments (L0, L1 and L2). All the lime, fertilizer and C₂H₂ treatment combinations were incubated at two temperatures (15 and 25°C). These soils were incubated for 44 hours in the dark.

Gas samples were taken using a 1ml precision syringe at 2, 5, 10, 20 and 44 hours from the start of the incubation. The gas samples were then directly injected into a gas chromatograph fitted with an electron capture detector (Model GC-14B; Shimadzu, Kyoto, Japan) to determine N₂O concentration. By removing only 1% of volume from the vials (1 ml at each time, 4 ml in total by the time of last sampling), we did not alter the pressure inside the vial to significantly affect the gas production rate.

Acetylene inhibits the reduction of N₂O to N₂ gas (Tiedje et al. 1989; Groffman et al. 2006) and therefore N₂O from the samples with C₂H₂ represent the total denitrification (N₂O + N₂). Incubations in experiment 2 were conducted in a 100% anaerobic condition (100% saturation and replacement of the headspace gas with helium) and therefore no N₂O from nitrification was expected. Nitric oxide (NO) is another gaseous product from the denitrification process (Saggar et al. 2013). Under anaerobic conditions, however, NO is rapidly consumed (Davidson et al. 1993; Skiba et al. 1997), and therefore was not considered a major product in this experiment. Therefore, we calculated N₂ gas production as the difference in N₂O concentration between C₂H₂ treatments and the helium only treatments.

3.3.3 Soil sampling and analysis

In experiment 1, soils were destructively sampled on days 1, 4, 10 and 16 after fertilization day. Soils were also sampled twice during the 7-day pre-incubation period after establishment of the liming treatments. In experiment 2, soil analysis was done before and after the 44-hour incubations. In both cases, the soils were incubated in identical conditions to those for gas measurements.

Soil samples were extracted in deionized water or in 2M KCl solution, and the extracts stored at 4°C until analysis for dissolved nutrients after being filtered through 0.2 µm membrane filters. From the water extracts; Soil NO₃⁻ concentrations were analyzed by ion chromatography (Dionex QIC Analyzer; Dionex Japan, Osaka, Japan); soil pH by using a combined electrode pH meter (F-8 pH meter; Horiba, Kyoto, Japan); and water extractable organic C (WEOC) was measured using a total organic C (TOC) analyzer (TOC 5000A; Shimadzu, Japan). NH₄⁺-N in the 2M KCl extract solution was determined using the indophenol-blue method (UV mini 1240; Shimadzu, Kyoto, Japan).

3.4 Data analysis

All statistical analyses were conducted using STATA-13 (Stata corporation, Texas, USA).

In the field experiments (chapters 4 to 6), two-way analysis of variance (ANOVA) was used to evaluate the differences in annual GHG fluxes across years and treatments within each land-use. One-way ANOVA was used to assess the differences in annual GHG emissions and chemical properties among the land-uses for each treatment. Annual N₂O and NO data was natural log transformed [$y = \log(x + 1)$] before analysis of variance. The value of one was added to prevent generation of negative log transformed values. Pearson's correlation test was used to test the relationship between climate and soil variables with

GHG fluxes and cumulative annual emissions. Step-wise single and multiple regression analyses were used to explain the influence of soil and environmental variables on annual GHG emissions.

In the incubation experiment (chapter 7), total N_2O and $^{15}\text{N}_2\text{O}$ concentrations were corrected against the influence of N_2O from ambient lab air already enclosed in the incubation jars by subtracting the N_2O (or $^{15}\text{N}_2\text{O}$) concentration just before closing the jar for incubation from the final concentration inside the incubation jar on each sampling day. One-way ANOVA was used to evaluate the effect of liming and temperature on cumulative N_2O and $^{15}\text{N}_2\text{O}$ emissions in experiment 1. Two-way ANOVA was used to evaluate the effect of lime treatments and incubation temperature on N_2O , N_2 and net denitrification ($\text{N}_2\text{O} + \text{N}_2$) in experiment 2. A three-factor regression analysis was conducted to test the influence of liming, incubation temperature and N addition on the rate of N_2O and N_2 production, and on net denitrification ($\text{N}_2\text{O} + \text{N}_2$) at 5 hours (early stage when soil had not been substantially affected by incubation conditions) and 44 hours (when incubation conditions would have substantially affected the soils and hence the response of denitrifiers) in experiment 2.

4. Effect of Land-use Change on Soil Carbon, Carbon dioxide and Methane Emissions

4.1 Introduction

Increasing atmospheric greenhouse gas (GHG) concentrations is of major global concern due to their effect on global climate change (IPCC 2014). Carbon dioxide (CO₂) and methane (CH₄) are two of the major GHGs emitted from soils with a great effect on the terrestrial carbon (C) cycle (Schlesinger and Andrews 2000; Yan et al. 2010; Bayer et al. 2012; Tate 2015).

Total CO₂ emission from soil (soil respiration; RS) is composed of autotrophic root respiration (RR), from plant roots and associated rhizosphere organisms (Hanson et al. 2000), and heterotrophic respiration (RH), associated with microbial decomposition of organic matter. Soil respiration represents one of the largest flows of C between the terrestrial ecosystem and the atmosphere (Schlesinger and Andrews 2000; Luo et al. 2016). Changes in RS fluxes have large implications not only on soil C storage but also on the potential sequester to C from the atmosphere (Raich and Potter 1995; Peng et al. 2009).

Changes in soil temperature, moisture and nitrogen (N) content can lead to significant changes in RS and soil C (Song and Zhang 2009; Suseela et al. 2012; Zhou et al. 2014). Several studies have reported increased RS with increasing soil temperature (Chen and Tian 2005; Peng et al. 2009; Song and Zhang 2009). However, the magnitude of the influence of temperature on RS is different in different biomes (Chen and Tian 2005) and the components of RS might be stimulated at different rates with increasing soil temperature (Gaumont-Guay et al. 2008). Increasing soil N content and N fertilization have been reported to increase the capacity of soils to sequester C (Schlesinger and Andrews 2000; Lal 2004). However, the reported effect of N fertilization on RS is contradictory among different studies with increasing (Song and Zhang 2009; Luo et al. 2016), decreasing (Bowden et al. 2004) or no response (Brumme and Beese 1992) of RS to N fertilization. These

contradictions could be due to the different response of RS components, RH and RR, to soil N and N fertilization (Yan et al. 2010) or could be due to different responses in different land-uses (Zhou et al. 2014). The effect of soil moisture on RS could also be influenced by the different response of RH and RR (Yan et al. 2010; Balogh et al. 2016). More research is clearly needed to improve our understanding of how RS and its components will respond to a changing climate and N fertilization.

Land-use change between grassland and cropland, like cornfield, often results in large changes in soil C and available N and several other soil properties like bulk density, moisture content and microbial activities are equally modified. Grasslands usually accumulate large amounts of litter and soil organic matter (SOM) that leads to high SOC and organic N (Davies et al. 2001; Velthof et al. 2010; Ryals et al. 2014). Converting grassland to cropland will convert the accumulated SOM into available C and N during mineralization (Whitehead et al. 1990; Necpálová et al. 2013) which in turn will increase microbial activity and have significant effect on CO₂ emissions (Smith and Conen 2004; Smith et al. 2008; Lang et al. 2015; Sainju 2016). Croplands that undergo annual tillage, such as ploughing, usually tend to have lower SOM content but could have higher decomposition rates. Tillage activities often lead to disruption of soil structure, increased surface area of the contact between SOM and soil microbes, physical release of SOM previously trapped/bound, break down of plant residue and increased aeration which provides aerobic conditions that enhance SOM decomposition, enhanced soil microbial activity, increased soil temperature (Govaerts et al. 2006; Govaerts et al. 2007; Ussiri and Lal 2009; Dalal et al. 2011; Kravchenko et al. 2011; Mbuthia et al. 2015). These changes will have large implications on GHG emissions and C storage in soils. For example while increased aeration can result in higher SOM decomposition and CO₂ fluxes (Bayer et al. 2016), it can lead to increased CH₄ oxidation and consequently decrease soil CH₄ emissions (Omonode et al. 2007; Tate 2015).

Converting cropland to perennial crops like grassland has been reported to mitigate CO₂ emissions and to increase sequestration of C in the soil (Guo and Gifford 2002). However, how much C can be sequestered and how much time from conversion of cropland to grassland would be required to achieve significant reductions in GHG emissions is still uncertain. Moreover, the effects of land-use change between grassland and cropland can further be complicated by differences in fertilization management in each system. To fully understand the direction of these changes, long-term studies that monitor changes on the same soil after changes in land-use between grassland and croplands are needed.

Here we present results from a field study that monitored CO₂, soil C and CH₄ continuously for 11 years in a managed grassland that was converted to cornfield and then reverted back to grassland. We discuss the influence of temperature, soil moisture and N fertilization on RS, RH and RR and evaluate the effect of land use change on C dynamics.

4.2 Materials and Methods

This study was conducted from 2005 to 2015 at a field that was an old permanent grassland (OG) that was ploughed and converted to a cornfield (2010–2012) and then converted to a new grassland (NG) (2013–2015) in field W4 at the Shizunai livestock farm of Hokkaido University (Fig. 3.1). Herbicide was applied in September 2009 to kill the grass and the dead grass was incorporated into the soil by ploughing. Date of conversion from OG to cornfield was regarded as the day of herbicide application on 1st October 2009, while date of converting cornfield to NG was 17th October 2012, the day of manure application for next growing season. Changes in soil C, CH₄ and CO₂ emissions were monitored in three treatments; chemical fertilizer plus composted manure (MF), chemical fertilizer only (F) and unfertilized control (CT). A fourth plot with manure only (M) was introduced in 2011 and monitored until 2015. The rates of fertilizer and manure application are shown in Table 3.3. CO₂ fluxes were monitored in planted plots (regarded as soil respiration; RS) and in plant-

excluded bare plots (regarded as heterotrophic soil respiration; RH) at 4 replications for each treatment arranged in a randomized design. Bare plots were maintained in the same locations within each land-use but moved after each land-use change and after tillage and seeding in cornfield. The surface of the bare plots was covered with a nylon sheet to prevent soil degradation from rainfall and sunlight.

Cumulative RH and RS were calculated by linear interpolation of between sampling events and numerical integration of the underlying area using the trapezoidal rule (Ussiri et al. 2009). The difference between cumulative RS and RH was regarded as root respiration (RR). The response of RS and RH to changing soil temperature was calculated as Q_{10} values using the following equations;

$$\text{CO}_2 \text{ flux} = \beta_0 \times \exp(\beta_1 \times T)$$

$$Q_{10} = \exp(10 \times \beta_1)$$

where T is the soil temperature at 5 cm depth, β_0 and β_1 are coefficients of the CO_2 flux and temperature function.

Soil organic C was measured every two years from 2006 to 2014 in F and MF plots at 0–15 and 15–30 cm depth. For each plot and depth, samples were collected using 100-cm³ cores in a grid system covering the entire plot ($n = 16$ –24). Soils were collected from the mineral Ap horizon after removing the root mat. Soils were then air-dried, roots removed, sieved (2 mm) and analysed using a C/N analyzer (SUMIGRAPH NC–1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). A separate core sample was taken to determine soil bulk density, gravimetrically, at each depth. More information on methods is given in chapter 3.

Net primary production (NPP) was measured as annual incremental increase of plant biomass (both above and belowground). Aboveground plant biomass was measured by manually harvesting all plants in a 0.5 m x 0.5 m quadrat 3–times per year in grassland or 1

m 1 m quadrat at the end of growing season in cornfield at 8 replications. The belowground biomass was measured by taking a soil block (0.25 x 0.25 x 0.30 m) at 4 replications in grassland, and by uprooting and digging out all the corn roots to 30 cm depth (in a 1 m x 1 m area) in cornfield. The collected biomass was then oven dried and C content measured using a C/N analyser (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

4.3 Results

4.3.1 Changes in soil C content and stock with land-use change

Soil organic carbon (SOC) content and total SOC stock in F plot did not change much from 2005 to 2009 in OG (Fig. 4.1 and Table 4.1). After conversion of OG to cornfield, both SOC content and SOC stock increased in 2010. However, despite the clear increase in SOC content in the F plot in 2010, there was no statistically significant difference observed among the three land uses. In the MF plot, SOC content increased significantly after conversion of OG to cornfield ($p < 0.01$) at both 0–15 and 15–30 cm depths. A further increase in SOC content in MF plot was observed in 2014 at both measurement depths.

The SOC stock showed a similar trend as SOC content. SOC stock in F plot increased by 5 Mg C ha⁻¹ from 2009 to 2010 in the 0–15 cm depth, despite not being statistically significant, but no increase was observed in the 15–30 cm depth. By the third year of cornfield, SOC stock had increased by about 10 Mg C ha⁻¹ in the F plot and it was significantly higher than that in OG. No significant difference was observed between cornfield and NG. In the MF plot, the increase in SOC stock from 2009 (OG) to 2010 (cornfield) was significant at both measurement depths. A further significant increase in SOC stock was observed in 2012 in the 0–15 cm depth, and a significant reduction was

observed in NG in 2014. In the 15–30 cm depth, SOC stock was not significantly different between cornfield and NG.

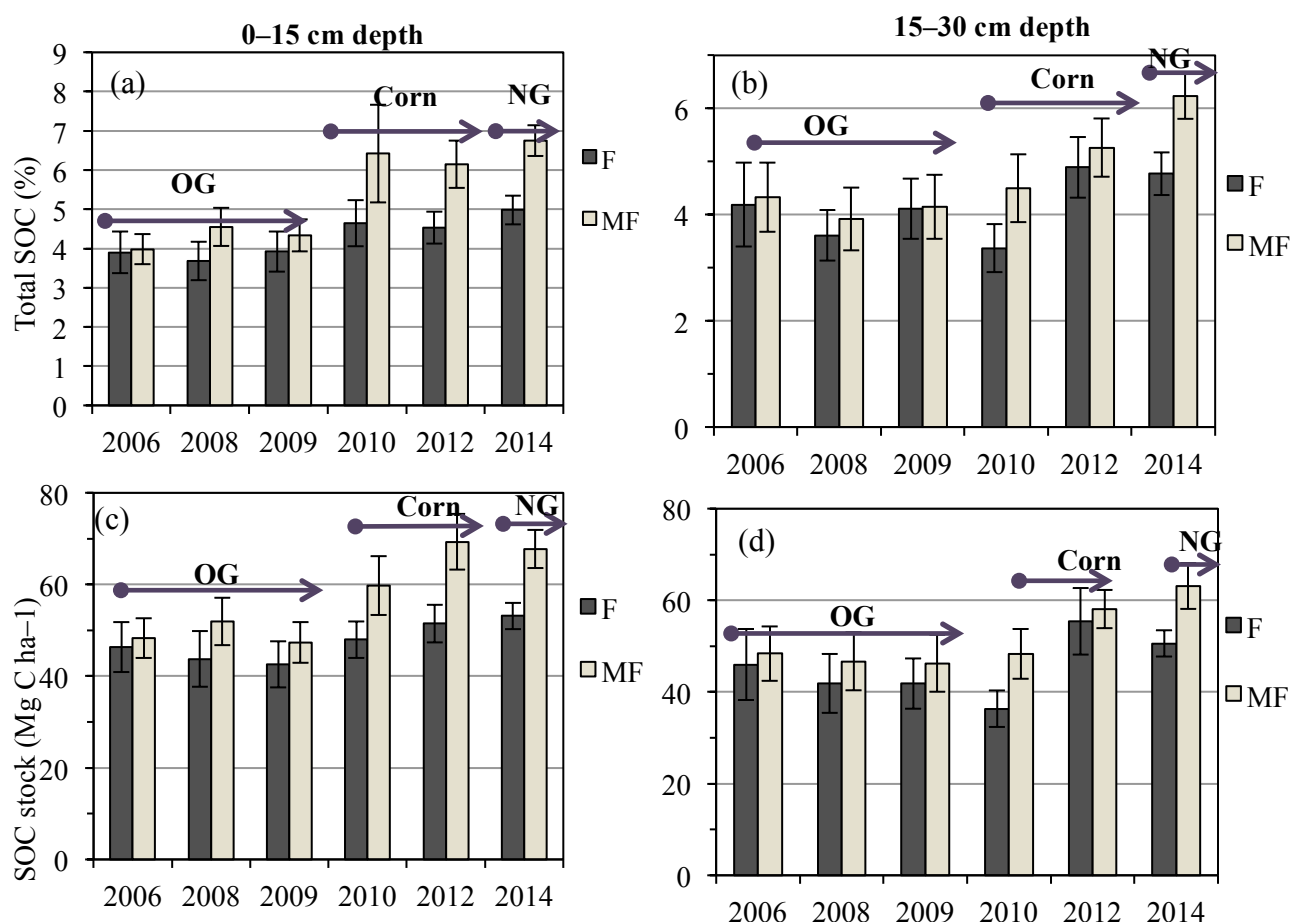


Figure 4.1 Total soil organic carbon (SOC) content (a,b) and SOC stock (c,d) from 2006 to 2014 measured every 2 years at 0–15 and 15–30 cm depths in inorganic fertilizer only plot (F) and inorganic fertilizer plus manure plot (MF). OG is old grassland; NG is new grassland. A higher arrow indicates a significant difference in SOC content or stock among the land-uses ($p < 0.05$). $n=16-24$.

Table 4.1 Bulk density, total soil organic C content (SOC) and SOC stock in F and MF plots.

Year	F plot				MF plot		
	Depth (cm)	Bulk density (g cm ⁻³)	SOC (%)	SOC stock (Mg C ha ⁻¹)	Bulk density (g cm ⁻³)	SOC (%)	SOC stock (Mg C ha ⁻¹)
2006	0–15	0.80±0.09 ^{ce}	3.94±1.07 ^{abcd}	46.31±10.92 ^{abc}	0.81±0.04 ^c	3.98±0.76 ^{cd}	48.27±8.69 ^{bd}
	15–30	0.75±0.07 ^{bcd}	4.19±1.58 ^{abc}	45.99±15.46 ^{abc}	0.76±0.08 ^c	4.33±1.31 ^{ab}	48.38±11.87 ^{ac}
2008	0–15	0.79±0.06 ^c	3.68±0.98 ^{ab}	43.76±12.22 ^{ab}	0.77±0.06 ^c	4.55±0.97 ^{bd}	51.88±10.34 ^{ad}
	15–30	0.77±0.07 ^{de}	3.61±0.96 ^a	41.85±12.84 ^a	0.80±0.06 ^c	3.92±1.19 ^{ac}	46.70±12.64 ^{abc}
2009	0–15	0.73±0.08 ^{abd}	3.92±1.02 ^{abcd}	42.55±10.11 ^{ab}	0.73±0.05 ^{bc}	4.33±0.81 ^{ad}	47.38±8.92 ^c
	15–30	0.68±0.07 ^a	4.11±1.13 ^{abc}	41.85±10.98 ^a	0.75±0.06 ^c	4.15±1.21 ^{abc}	46.27±12.50 ^{ab}
2010	0–15	0.71±0.09 ^{abd}	4.64±1.18 ^{abcd}	47.98±8.00 ^{ab}	0.65±0.10 ^a	6.42±2.49 ^{fg}	59.79±12.87 ^d
	15–30	0.73±0.07 ^a	3.37±0.91 ^{abcd}	36.35±8.05 ^a	0.72±0.07 ^{ab}	4.50±1.28 ^{deg}	48.30±10.81 ^{cd}
2012	0–15	0.76±0.05 ^{abcde}	4.53±0.81 ^{cd}	51.46±8.18 ^c	0.76±0.06 ^c	6.15±1.21 ^{gh}	69.30±12.12 ^f
	15–30	0.76±0.10 ^{abcde}	4.89±1.14 ^{bcd}	55.42±14.58 ^{bc}	0.75±0.08 ^c	5.26±1.10 ^{def}	58.10±8.32 ^{de}
2014	0–15	0.72±0.05 ^{abcd}	4.98±0.73 ^d	53.15±5.77 ^c	0.67±0.05 ^a	6.75±0.79 ^h	67.76±8.32 ^e
	15–30	0.72±0.08 ^{abc}	4.77±0.80 ^{cd}	50.60±5.79 ^{bc}	0.67±0.04 ^{ab}	6.23±0.85 ^{efg}	63.17±10.06 ^{df}

Different letters within the same column indicate significant differences among the years and measurement depths. Data is mean±SD, n = 12–24.

4.3.2 Soil respiration and heterotrophic respiration

Figure 4.2 shows the annual and seasonal variation in RS and RH. The highest RS values for each treatment were recorded in OG and the lowest were recorded in cornfield. In the unfertilized plot (CT) the average RS values were 152, 90 and 128 mg CO₂-C m⁻² hr⁻¹ in OG, cornfield and NG, respectively. A similar trend was observed in the F plot where average RS values were 144, 97 and 132 mg CO₂-C m⁻² hr⁻¹, and in MF plot; 195, 145 and 183 mg CO₂-C m⁻² hr⁻¹ in OG, cornfield and NG, respectively (Fig. 4.2).

The result of two-way ANOVA of effect of land-use and plots showed no significant difference in cumulative annual RS when all data was combined. However, the result of one-way ANOVA of land-use within each plot showed that in F plot, RS was significantly higher

in OG than cornfield ($p < 0.05$), and in manure only plot (M; measured in cornfield and NG only) RS in NG was higher than that in cornfield ($p < 0.05$) (Table 4.2).

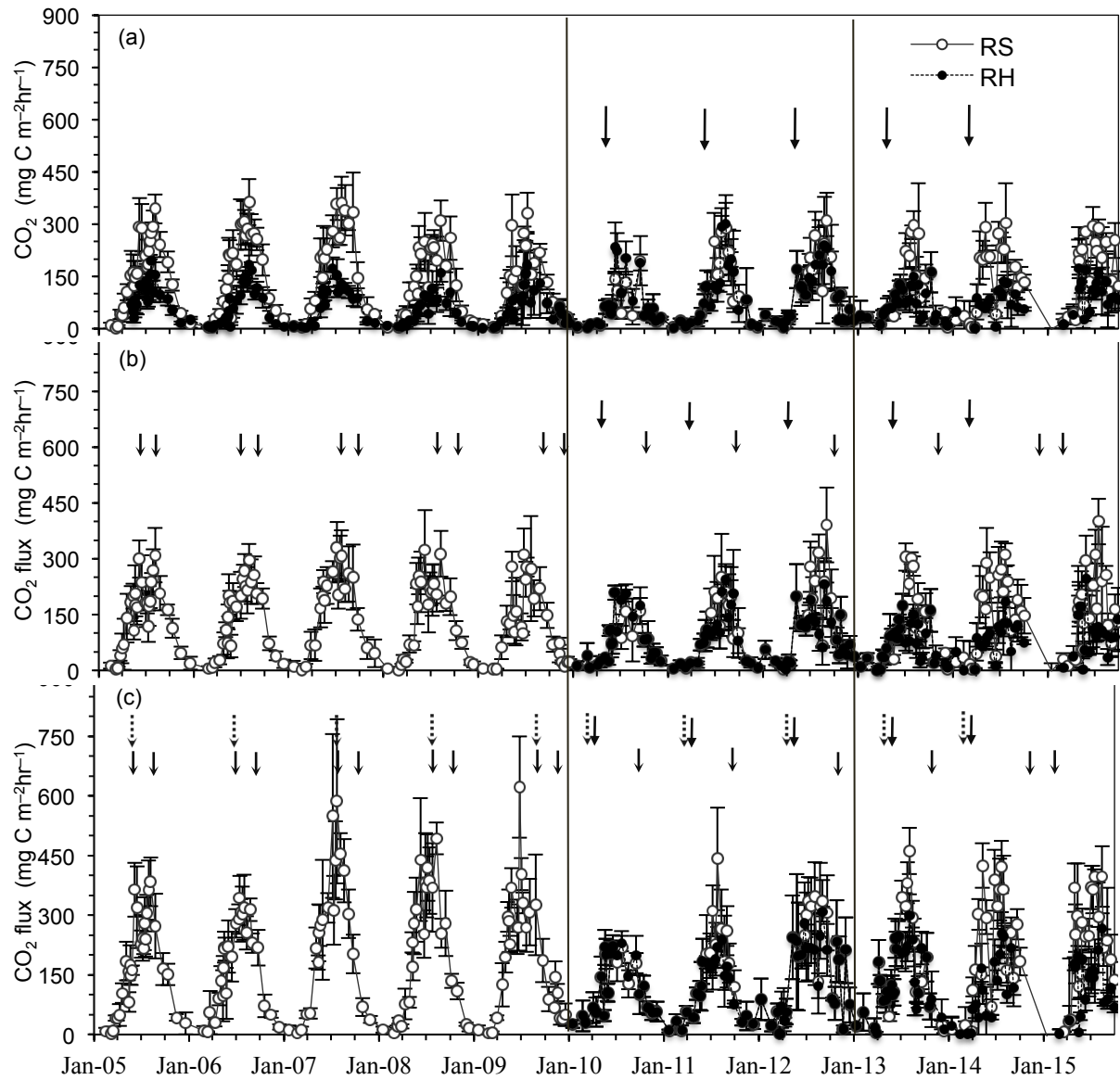


Figure 4.2 Heterotrophic respiration (RH) and total soil respiration (RS) in CT plot (a), F plot (b) and MF plot (c). CT is control plot; F is chemical fertilizer only plot; MF is combined chemical fertilizer and manure plot. Values represent means ($n=4$) $\pm$ SD. Dashed arrows indicate dates of manure application; full arrows with open V shaped tip indicate dates of chemical fertilizer application; full arrows with normal closed tip indicate dates of ploughing. Vertical lines indicate the change from old grassland (2005–2009) to cornfield (2010–2012) and then to new grassland (2013–2015).

Table 4.2 Soil respiration (RS) and heterotrophic respiration (mean $\pm$ standard deviation; SD) from 2005 to 2015

			CT	F	MF	M
OG	2005	RH	4.8±0.8	4.8±0.8	5.3±1.5	–
		RS	10.5±1.0	10.0±1.1	11.4±1.8	–
	2006	RH	4.6±0.7	4.6±0.7	6.4±1.2	–
		RS	11.1±1.3	9.1±1.1	10.9±1.3	–
	2007	RH	4.9±0.5	4.9±0.5	9.2±1.9	–
		RS	12.5±1.5	10.9±1.3	15.2±3.1	–
	2008	RH	4.0±1.1	4.0±1.1	5.1±2.7	–
		RS	10.5±0.9	10.3±1.5	14.6±1.2	–
	2009	RH	4.5±2.5	4.5±2.5	7.8±1.8	–
		RS	8.8±0.9	9.9±1.1	13.1±0.3	–
Average	RH	4.5	4.5	6.7	–	
	RS	10.6	10.0	13.0	–	
Corn	2010	RH	6.8±0.8	6.9±1.1	10.2±0.7	–
		RS	4.0±0.2	4.9±0.7	8.2±0.6	–
	2011	RH	6.5±0.9	6.1±1.1	7.8±1.1	7.8±1.1
		RS	6.8±1.4	6.4±2.3	9.6±1.2	10.1±1.3
	2012	RH	6.8±1.2	4.9±0.3	8.8±0.6	9.7±0.3
		RS	7.3±0.6	8.6±0.7	11.5±1.6	10.3±0.5
	Average	RH	6.7	5.9	8.9	8.7
		RS	6.0	6.6	9.7	10.2
NG	2013	RH	4.4±0.3	4.8±0.3	9.4±0.8	9.2±0.9
		RS	6.7±0.6	7.3±0.8	11.2±1.2	11.7±1.1
	2014	RH	4.0±0.5	4.3±0.4	5.5±0.5	5.9±0.5
		RS	8.5±0.5	9.1±1.1	11.9±1.7	12.0±0.6
	2015	RH	5.0±0.9	4.9±0.9	6.8±1.0	7.0±1.4
		RS	8.9±0.7	8.8±1.8	12.2±1.2	12.7±1.4
	Average	RH	4.5	4.7	7.2	7.3
		RS	8.0	8.5	11.8	12.1
ANOVA		RH		RS		
	MS	F	<i>p</i> value	MS	F	<i>p</i> value
Plot	15.55	14.03	<0.001	149.2	0.60	0.61
Land-use	12.88	11.62	<0.001	350.4	1.42	0.25

Contrary to RS, the highest RH values were recorded in cornfield (Fig 4.2). In the unfertilized plot, in which RH was directly measured in all the three land-uses, RH ranged from 2 to 194, 3 to 300 and 0 to 170 mg CO₂–C m^{–2} hr^{–1} in OG, cornfield and NG

respectively. Cumulative annual RH was highest in cornfield and lowest in OG ($p < 0.01$) (Table 4.2). Surprisingly, in 2010 CO₂ fluxes in the root-excluded plots (RH) were higher than in the non-excluded plots (RS) in all three plots (Fig. 4.2). The annual cumulative RH, in 2010, was also higher than cumulative RS (Table 4.2).

The contribution of RH to RS in CT plot ranged from 38–51% in OG, 93–170% in cornfield and 47–65% in NG. In OG, RH in MF and F plots was not measured directly; hence we estimated RH in MF plot as the sum of RH in CT plot and the difference in RS between MF and F plots, and assumed that RH in F and CT plots was the same. The contribution of RH to RS in MF plot ranged from 34–60% in OG, 76–124% in cornfield and 46–83% in NG. In F plot, the contribution of RH to RS ranged from 38–51% in OG, 56–140% in cornfield and 47–65% in NG. The contribution of RH to RS was significantly higher in cornfield than in OG and NG ($p < 0.05$), and it was higher in NG than in OG but not statistically significant.

4.3.3 Methane fluxes

Methane fluxes were generally low and mostly close to zero or negative in all plots and all land-uses (Fig. 4.3). Disproportionately high fluxes, typically $> 50 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$, were recorded when rainfall greater than 40 mm occurred within 1–6 days prior to gas sampling. The average CH₄ flux when only fluxes less than $50 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ were considered was 1.5 ± 14.2 , -5.8 ± 18.2 and $-10.2 \pm 27.3 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ in OG, cornfield and NG, respectively. On the other hand, when only the fluxes greater than $50 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ were considered, the average fluxes were 186.9 ± 167.2 , 145.5 ± 109.4 and $130.1 \pm 78.2 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ in OG, cornfield and NG, respectively. These high fluxes ($> 50 \mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$) accounted for 7.4%, 8.1% and 6.4% of the total number of flux measurements in OG, cornfield and NG, respectively.

Annual CH₄ emissions ranged from -0.16 to 5.47 kg C ha⁻¹ yr⁻¹ in OG, -0.74 to 5.11 kg C ha⁻¹ yr⁻¹ in cornfield and -0.29 to 2.78 kg C ha⁻¹ yr⁻¹ in NG (Table 4.3). Excluding CH₄ fluxes higher than 50 $\mu\text{g CH}_4\text{-C m}^{-2}\text{ hr}^{-1}$ (observed after rainfall > 40 mm within 1–6 days) which accounted for less than 9% of the total measurements, all plots were CH₄ sinks. Although there were no significant differences in annual CH₄ emissions among the three land-uses, the average fluxes in OG (all plots combined) were slightly higher than those in NG ($p = 0.04$). Annual emissions also tended to be lower in the manure-amended plots except in NG.

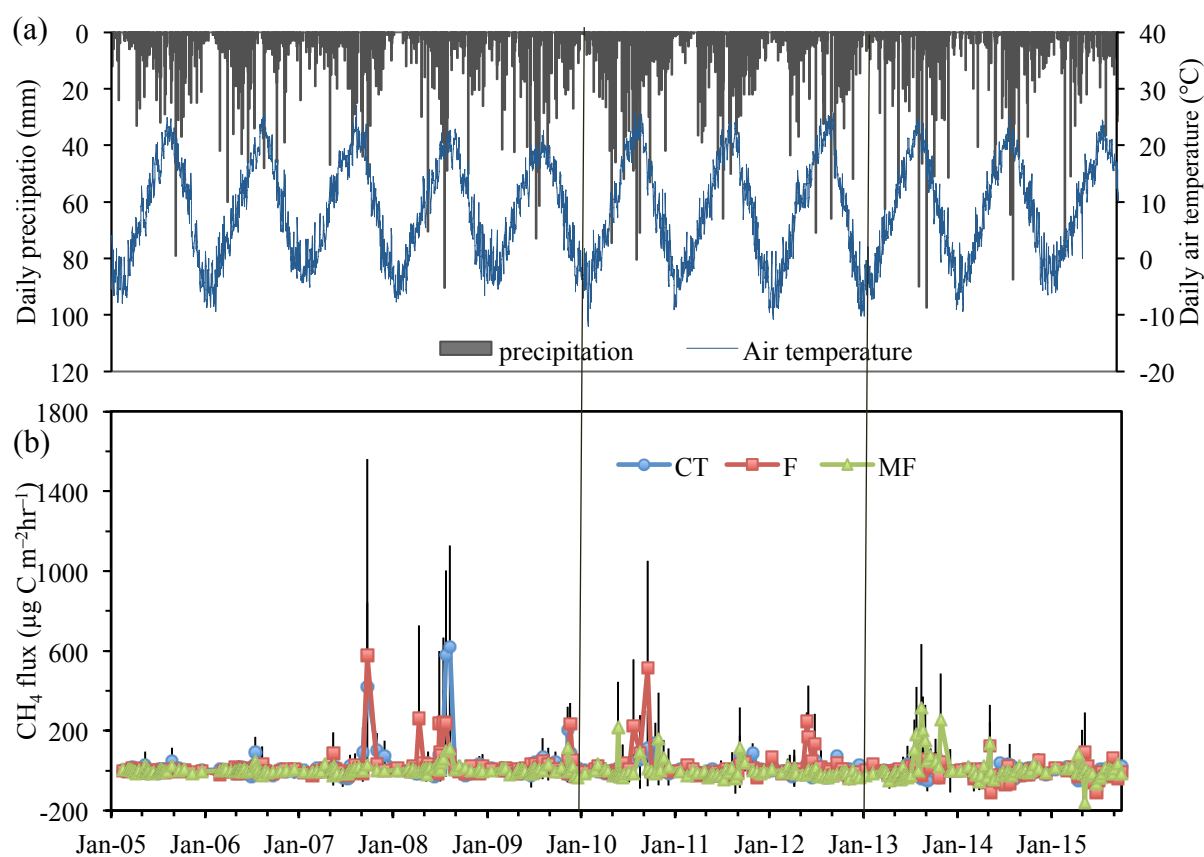


Figure 4.3 Daily precipitation and air temperature (a) and methane (CH₄) flux (b). CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot. CH₄ values represent means ($n=4$) $\pm$ SD. Vertical lines indicate the change from old grassland (2005–2009) to cornfield (2010–2012) and then to new grassland (2013–2015).

Table 4.3 Annual methane emission (mean $\pm$ SD) from 2005 to 2015

Land use	Year	CT	F	MF	M
kg CH ₄ -C ha ⁻¹					
OG	2005	0.28 $\pm$ 0.50	-0.09 $\pm$ 0.34	0.21 $\pm$ 0.30	–
	2006	-0.06 $\pm$ 0.74	-0.12 $\pm$ 0.38	-0.16 $\pm$ 0.36	–
	2007	3.57 $\pm$ 4.53	3.87 $\pm$ 8.36	0.24 $\pm$ 0.63	–
	2008	5.47 $\pm$ 4.10	3.46 $\pm$ 6.11	1.42 $\pm$ 1.27	–
	2009	1.12 $\pm$ 0.50	0.68 $\pm$ 0.31	0.15 $\pm$ 0.83	–
	Average	2.07	1.56	0.37	–
Cornfield	2010	1.51 $\pm$ 1.51	5.11 $\pm$ 3.99	0.51 $\pm$ 1.25	–
	2011	0.31 $\pm$ 0.91	-0.12 $\pm$ 0.59	0.37 $\pm$ 0.22	1.27 $\pm$ 1.82
	2012	-0.16 $\pm$ 0.11	1.88 $\pm$ 2.47	-0.74 $\pm$ 0.22	-0.62 $\pm$ 0.29
	Average	0.53	2.26	0.01	0.35
NG	2013	0.00 $\pm$ 0.20	0.03 $\pm$ 0.32	1.00 $\pm$ 2.04	0.49 $\pm$ 1.08
	2014	2.78 $\pm$ 6.09	2.23 $\pm$ 5.68	1.84 $\pm$ 1.75	1.68 $\pm$ 2.32
	2015	-0.28 $\pm$ 0.30	-0.29 $\pm$ 0.59	-0.01 $\pm$ 0.19	0.02 $\pm$ 0.36
	Average	0.90	0.65	0.94	0.73
ANOVA					
	d.f.	MS	F	p value	
plot	3	2.47	1.00	0.40	
Land use	2	0.85	0.34	0.71	

4.3.4 Soil and Environmental drivers of Carbon dioxide and Methane fluxes

Soil temperature was the most important variable controlling CO₂ fluxes (both RS and RH) with a significant positive exponential relation in all plots and land-uses (Fig. 4.4). Even though the relationship between soil temperature and CO₂ fluxes was significant in all three land-uses, it was stronger in OG and weakest in NG. Q₁₀ values of RS and RH in CT plot of each land-use were estimated from the exponential curves of temperature and CO₂ fluxes in Figure 4.4. Average RS Q₁₀ values were 4.1, 3.2 and 2.0, and RH Q₁₀ values were 3.7, 3.1 and 1.7 in OG, cornfield and NG, respectively. Methane fluxes on the other hand did not always show a significant relationship with soil temperature although the highest fluxes were observed when soil temperature was high (Fig. 4.5).

Both RH and RS CO₂ fluxes decreased with increasing soil moisture, however only in OG there was the correlation significant between WFPS and RH (Fig. 4.6). The negative correlation between WFPS and RS was always significant in all plots in grassland (both OG and NG), but not in cornfield. However, all data combined, the relationship between WFPS and RS was only significant when WFPS was greater than 65% (Fig. 4.7). Methane fluxes showed a weak positive response to increasing soil moisture (Fig. 4.8) and no clear trend of land-use effect on CH₄ response to soil moisture was observed.

Table 4.4 Results of multiple regression on soil respiration (RS), heterotrophic respiration (RH) and CH₄ fluxes using soil temperature and water filled pore space (WFPS) as predictors

Land use	Variable	Regression equation	R ² †
OG	RS	11.56 temperature – 3.38 WFPS + 302.46	0.65***
	RH	5.53 temperature – 1.58 WFPS + 128.02	0.74***
	CH ₄	2.48 temperature + 1.59 WFPS – 134.00	0.05**
Cornfield	RS	10.10 temperature – 2.23 WFPS + 153.64	0.57***
	RH	9.20 temperature – 0.25 WFPS – 7.86	0.63***
	CH ₄	1.67 temperature + 0.85 WFPS – 78.57	0.07**
NG	RS	9.89 temperature – 4.54 WFPS + 379.02	0.43***
	RH	5.82 temperature – 1.32 WFPS + 83.42	0.38**
	CH ₄	0.87 WFPS – 0.49 temperature – 54.28	0.04 ^{ns}

† *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns is non-significant

From the multivariate regression analysis, it was clear that the predicting strength of soil temperature and WFPS on RS and RH was different among the land-uses. Both soil temperature and WFPS had a stronger effect on RS than on RH in all the three land-uses (Table 4.4). However, the amount of influence of environmental variables was different among the land-uses with soil temperature and WFPS explaining 76% and 74% of the

variation in RS and RH fluxes in OG ($p < 0.001$), 59% and 63% in cornfield ($p < 0.001$) and only 34% and 38% in NG ($p < 0.01$) respectively. Individually, soil temperature was the most important explanatory variable on RS and RH. In grassland (both OG and NG) both RS and RH decreased with increasing WFPS while in cornfield RS decreased with increasing WFPS ($p = 0.043$) but RH did not show a significant response to changes in WFPS.

Multiple regression analysis showed that, when all plots within each land-use, soil temperature and WFPS had a significant effect on CH₄ fluxes ($p < 0.01$) in OG and cornfield but explained only 5% and 7% of the variation, respectively (Table 4.4). However only WFPS ($p < 0.05$) had a significant effect on CH₄ fluxes in NG.

The cumulative annual RH showed strong positive correlations with N input ($p < 0.01$) and surplus soil N ($p < 0.05$) in all three land-uses and a strong positive correlation with annual precipitation in OG ($p < 0.05$) (Table 4.5). Weak non-significant positive correlations were also observed between RH and average annual soil NO₃⁻, NH₄⁺ and WEOC concentrations.

Unlike RH, RS did not show significant increase with increasing N input or surplus soil N in all three land-uses and only in OG was a significant positive correlation with annual precipitation ($p < 0.05$) observed. On the other hand, RS (unlike RH) was significantly correlated with WEOC ($p < 0.01$) in all three land-uses and had a significant correlation with average NH₄⁺ concentration ($p < 0.001$) in cornfield. Soil pH had a significant positive correlation with RS in all three land-uses ($p < 0.05$).

Annual CH₄ emissions were negatively correlated with N input, surplus soil N and NO₃⁻ concentration in OG. No other soil or environmental variable showed a strong correlation with CH₄ emissions except for a positive correlation with NO₃⁻ in cornfield (Table 4.5).

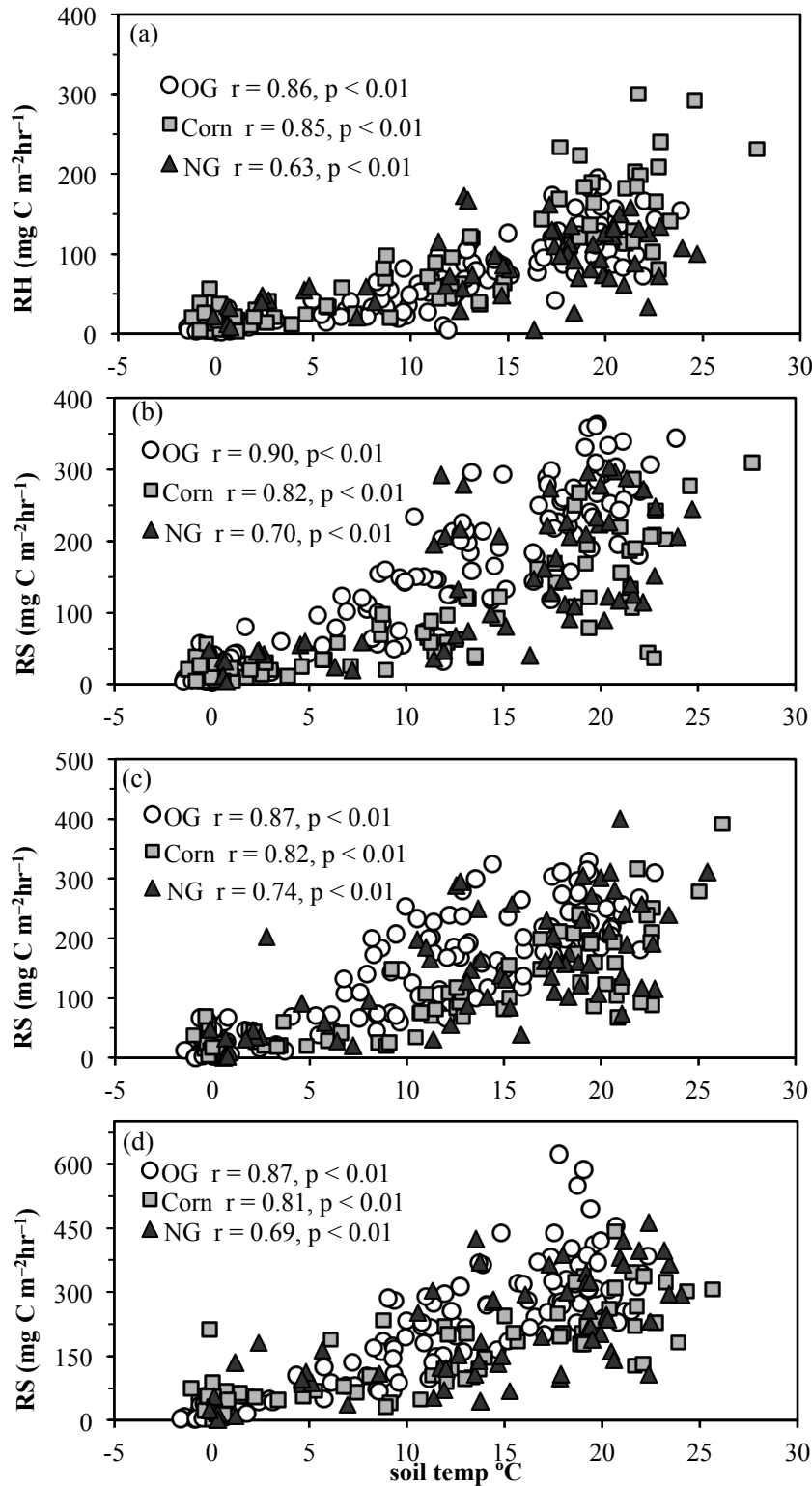


Figure 4.4 Relationship between soil temperature and heterotrophic respiration (RH) in CT plot (a), soil temperature and soil respiration (RS) in CT plot (b), F plot (c) and MF plot (d). OG is old grassland; NG is new grassland. Values represent means ($n=4$). Average RS Q_{10} values were 4.1, 3.2 and 2.0, and RH Q_{10} values were 3.7, 3.1 and 1.7 in OG, cornfield and NG, respectively.

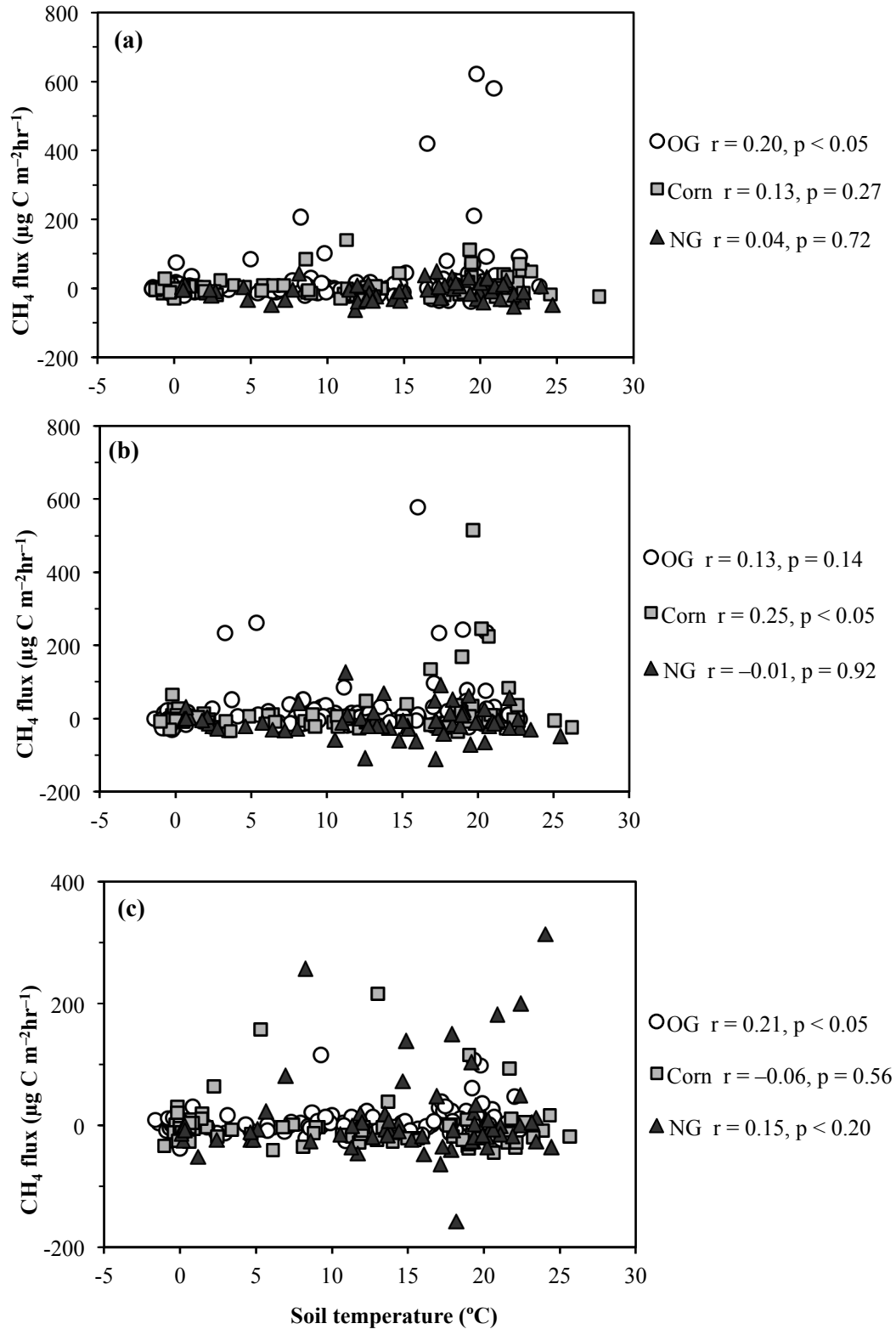


Figure 4.5 Relationship between soil temperature and methane (CH₄) flux in CT plot (a), F plot (b) and MF plot (c). OG is old grassland; NG is new grassland. Values represent means (n=4).

Table 4.5 Linear correlations of annual soil respiration (RS), heterotrophic respiration (RH), root respiration (RR) and CH₄ emissions with soil and climatic variables.

	Variable ^a	RS	RH	RR ^b	CH ₄
Old grassland	Rainfall	0.56*	0.54*	0.19	-0.21
	N input	0.18	0.66**	-0.48	-0.53*
	N surplus	0.31	0.84***	-0.49	-0.59*
	WEOC	0.76**	0.33	0.68*	-0.23
	NO ₃ ⁻	0.04	0.02	0.04	-0.68*
	NH ₄ ⁺	-0.45	-0.08	-0.52	-0.28
	NO ₃ ⁻ -N/NH ₄ ⁺ -N	0.12	-0.05	0.23	-0.48
	pH	0.65*	0.37	0.50	-0.25
Cornfield	Rainfall	0.31	0.41	0.06	-0.26
	N input	0.48	0.74**	-0.46	0.01
	N surplus	-0.21	0.59*	-0.77*	0.32
	WEOC	0.74**	0.28	0.78*	-0.51
	NO ₃ ⁻	-0.51	0.29	-0.85**	0.66**
	NH ₄ ⁺	0.68**	0.45	0.67*	-0.50
	NO ₃ ⁻ -N/NH ₄ ⁺ -N	-0.46	0.50	-0.83**	0.46
	pH	0.70*	-0.13	0.85**	-0.61
New grassland	Rainfall	-0.05	-0.01	0.16	0.09
	N input	0.55	0.86***	-0.11	0.08
	N surplus	0.49	0.93***	-0.34	-0.29
	WEOC	0.60*	0.22	0.42	-0.37
	NO ₃ ⁻	0.00	0.29	-0.29	0.11
	NH ₄ ⁺	-0.25	0.02	-0.20	-0.20
	NO ₃ ⁻ -N/NH ₄ ⁺ -N	0.48	0.77*	-0.23	0.24
	pH	0.76*	0.31	-0.63	0.03

^a WEOC is water extractable organic carbon; WEOC, NO₃⁻, NH₄⁺ and pH are averages of all measurements conducted in each year. ^b RR = RS minus RH. Stars following a number mean statistical significance at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

4.3.5 Net primary production and root biomass

Root biomass ranged from 8.1 to 10.6, 0.1 to 0.6 and 0.2 to 1.9 Mg C ha⁻¹ year⁻¹ in OG, cornfield and NG, respectively (Table 4.6). There were significant differences among the land uses ($p < 0.001$), but no significant differences among the plots. NPP was highest in cornfield and lowest in NG. NPP in the unfertilized plot (CT) was lower than in F and MF plots ($p < 0.001$; Table 4.6).

Table 4.6 Amount of root biomass at end of growing season and annual net primary production (NPP)

Year	Root biomass* (Mg C ha ⁻¹)			NPP (Mg C ha ⁻¹)		
	CT	F	MF	CT	F	MF
2005	–	10.1±3.5	10.0±2.0	3.0±0.3	6.2±0.5	5.3±0.6
2006	–	8.1±2.9	10.6±2.4	3.0±0.5	5.2±0.4	5.1±0.8
2007	–	8.4±2.8	8.9±1.3	3.3±0.9	4.8±0.5	4.2±0.4
2008	–	8.8±1.7	9.1±1.7	2.4±0.2	3.5±0.5	3.4±0.7
2009	–	8.9±3.0	9.7±1.3	2.3±0.2	3.9±0.6	4.0±0.6
2010	0.1±0.0	0.3±0.1	0.3±0.1	2.9±1.1	6.5±2.2	7.0±3.7
2011	0.3±0.1	0.4±0.1	0.5±0.1	3.5±1.3	7.6±1.3	9.9±1.2
2012	0.3±0.2	0.5±0.1	0.6±0.1	2.7±0.4	6.3±0.8	8.4±1.3
2013	0.3±0.2	0.3±0.1	0.2±0.1	0.7±0.1	1.1±0.1	1.3±0.2
2014	1.8±0.3	1.9±0.4	1.3±0.3	3.0±0.3	5.2±0.8	4.9±0.7
2015	0.6±0.2	0.4±0.2	0.5±0.2	3.2±0.5	7.8±0.9	6.1±0.6
ANOVA						
	MS	F	<i>P</i> value	MS	F	<i>P</i> value
Plot	0.30	0.76	0.4805	25.27	10.42	0.0004
Land-use	204.98	522.71	0.0000	16.31	6.72	0.0041

*Only root biomass collected at the end of each growing season reported.

4.4 Discussion

4.4.1 Effect of land use change on Soil C and CO₂ emissions

Ploughing and conversion of grassland to cornfield in 2010 increased organic matter decomposition (RH) regardless of whether manure and inorganic fertilizer were applied or not (Fig. 4.2 and Table 4.2). This significant increase in RH following conversion of grassland to cornfield has been reported previously (Guo and Gifford 2002) and is largely due to increased microbial activity caused by increased aeration, increased surface area of contact between microbes and organic matter and the incorporation of plant residue during tillage (Govaerts et al. 2007; Ussiri and Lal 2009; Kravchenko et al. 2011). However, despite the increase in RH after conversion of grassland to cornfield, total CO₂ emissions (soil respiration) decreased (Table 4.2). This is largely due to higher root biomass and root respiration (RR) in grassland compared to cornfield (Table 4.6). This can be seen from the difference in the contribution of RH to RS in old grassland (34–60%) and cornfield (56–

100%). The finding that RS was higher in grassland than cornfield and that the trend was opposite for RH calls for a clear distinction between the two when reporting changes in CO₂ emissions following land use change.

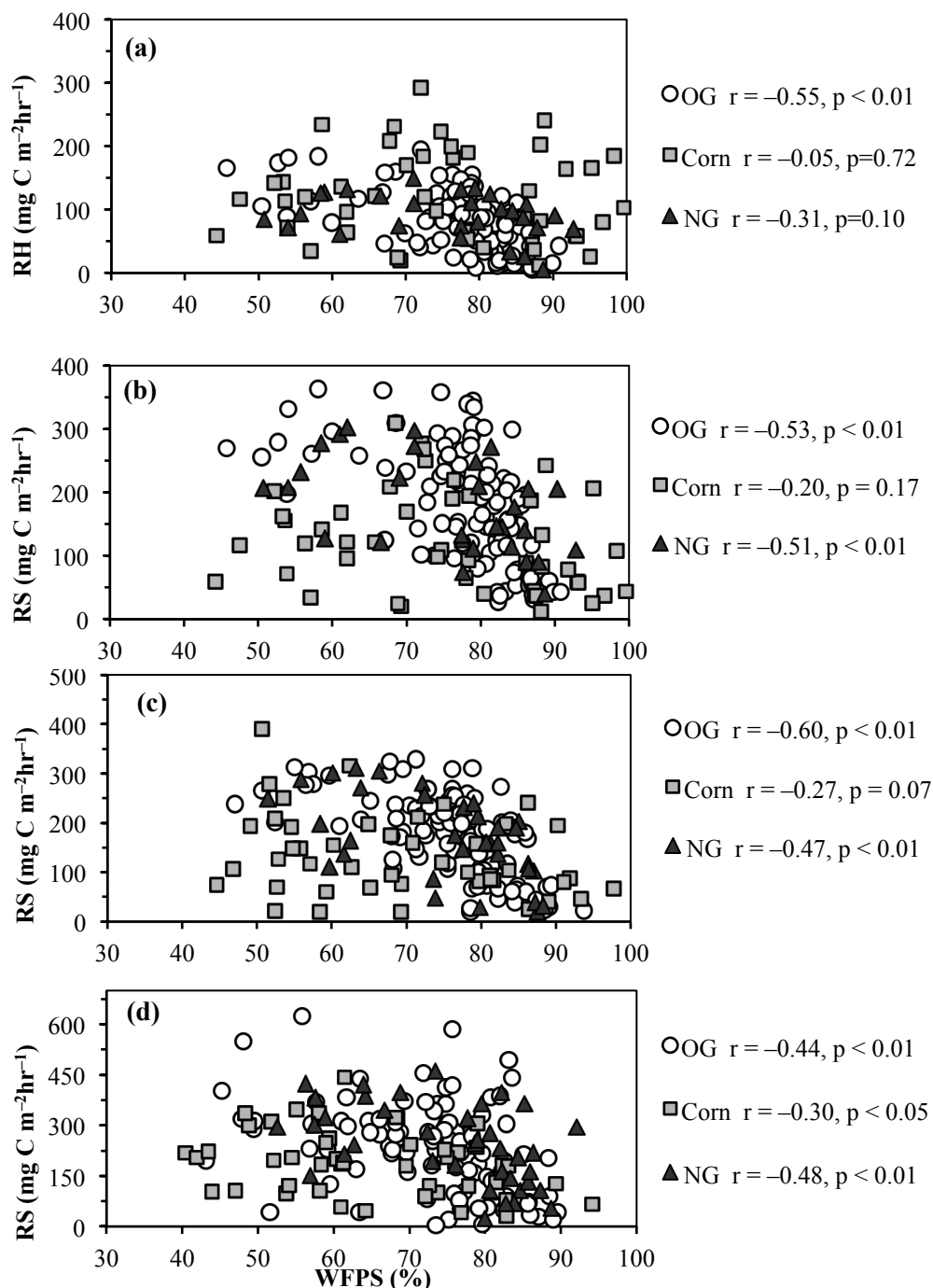


Figure 4.6 Relationship between soil water filled pore space (WFPS) and heterotrophic respiration (RH) in CT plot (a), WFPS and soil respiration (RS) in CT plot (b), F plot (c) and MF plot (d). OG is old grassland; NG is new grassland. Values represent means ($n=4$). Statistical values on the right are for linear correlations.

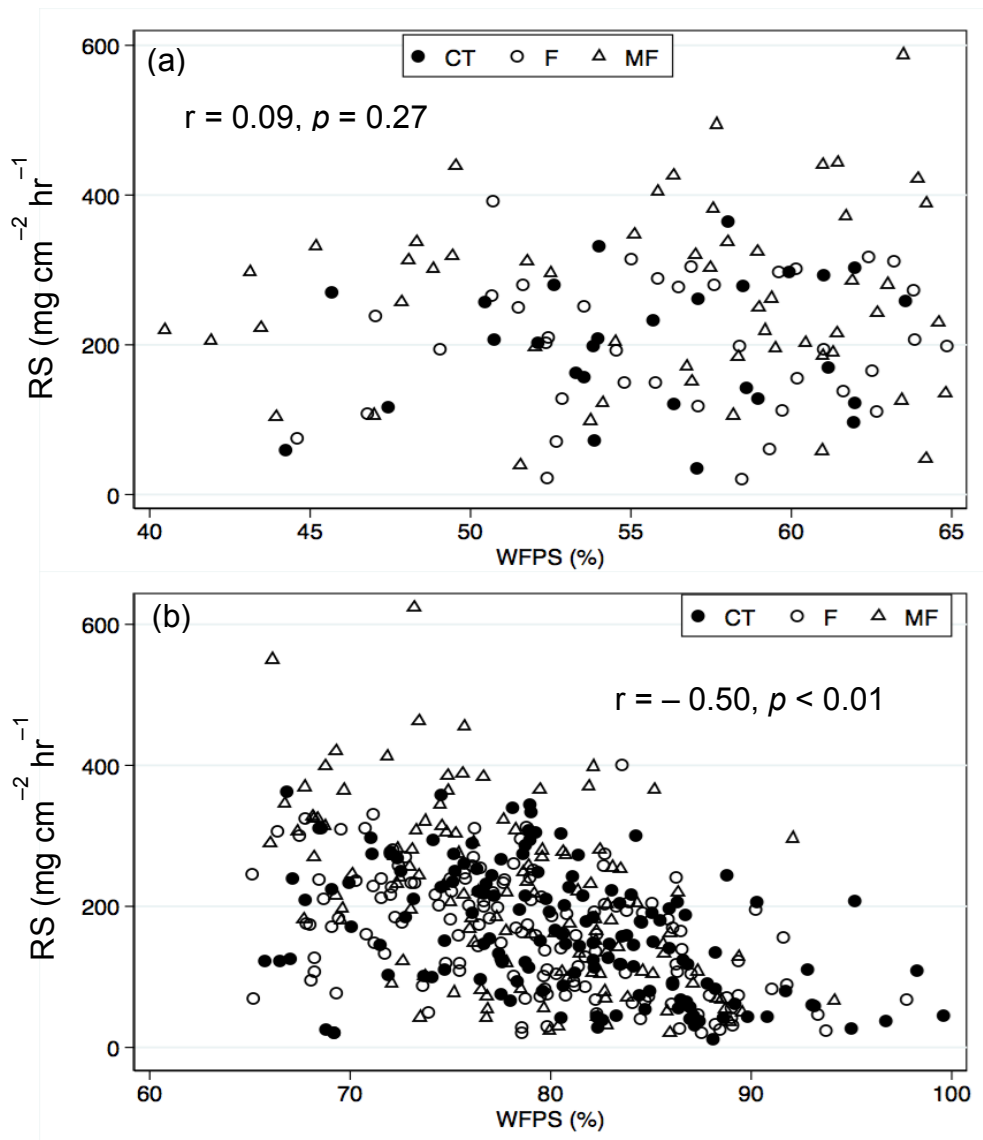


Figure 4.7 Relationship between soil respiration (RS) and soil moisture (WFPS) when WFPS is less than 65% (a) and greater than 65% (b), all data from three land-uses combined. Values represent means ($n=4$).

Surprisingly, in the first year after conversion from grassland to cornfield, in 2010, RH fluxes were higher than RS fluxes in summer (Fig 4.2) and this led to higher annual RH than RS (Table 4.2). This result was contrary to our expectations and different from what is widely known that RH is lower than RS. The possible reason for higher RH than RS in 2010 is that ploughing and incorporation of dead grass from previous year enhanced SOM decomposition due to positive priming effect (Degens and Sparling 1996; Kuzyakov et al.

2000), while the presence of roots in planted plots led to increased competition for the mineralized C between plants and microbes leading to negative priming effect (Cheng 1996; Jingguo and Bakken 1997; Cheng 1999). High N availability relative to C, as was the case in 2010 (see Fig 5.1 in chapter 5), increases competition for available C between roots and microbes (Kuzyakov et al. 2000). However, other studies have reported enhanced SOM decomposition in rooted soils (positive priming effect) compared to root-free soils (Cheng and Coleman 1990; Kuzyakov et al. 2001).

Land-use change from grassland to cornfield increased SOC content and stock at 0–15 and 15–30 cm depth (Fig. 4.1 and Table 4.1). This is contrary to the commonly reported reduction of SOC when grassland is converted to cropland (e.g Post and Kwon 2000; Guo and Gifford 2002; Lal 2002). The main reason for this contradiction between our results and commonly reported findings is that our study evaluated SOC change within 2–3 years after land-use change while most studies focus on periods longer than 5 years or over decades (Post and Kwon 2000). This increase in SOC in the short-term after conversion of grassland to cornfield was likely due to the incorporation of plant residue and manure in the soil following tillage and suggests that conversion or ploughing of managed grassland is important to turn the plant residue/applied manure into actual soil C. Additionally, SOC increased by a greater margin in the top 15 cm depth than the 15–30 cm layer. The depth of ploughing and therefore burial of the plant residue could be important. It is possible that SOC could have decreased had the cornfield been continued for a longer period, however this increase in the short-term shows that occasional short-term rotation of managed grassland with cropland could be an important practice to increase SOC storage in the soil.

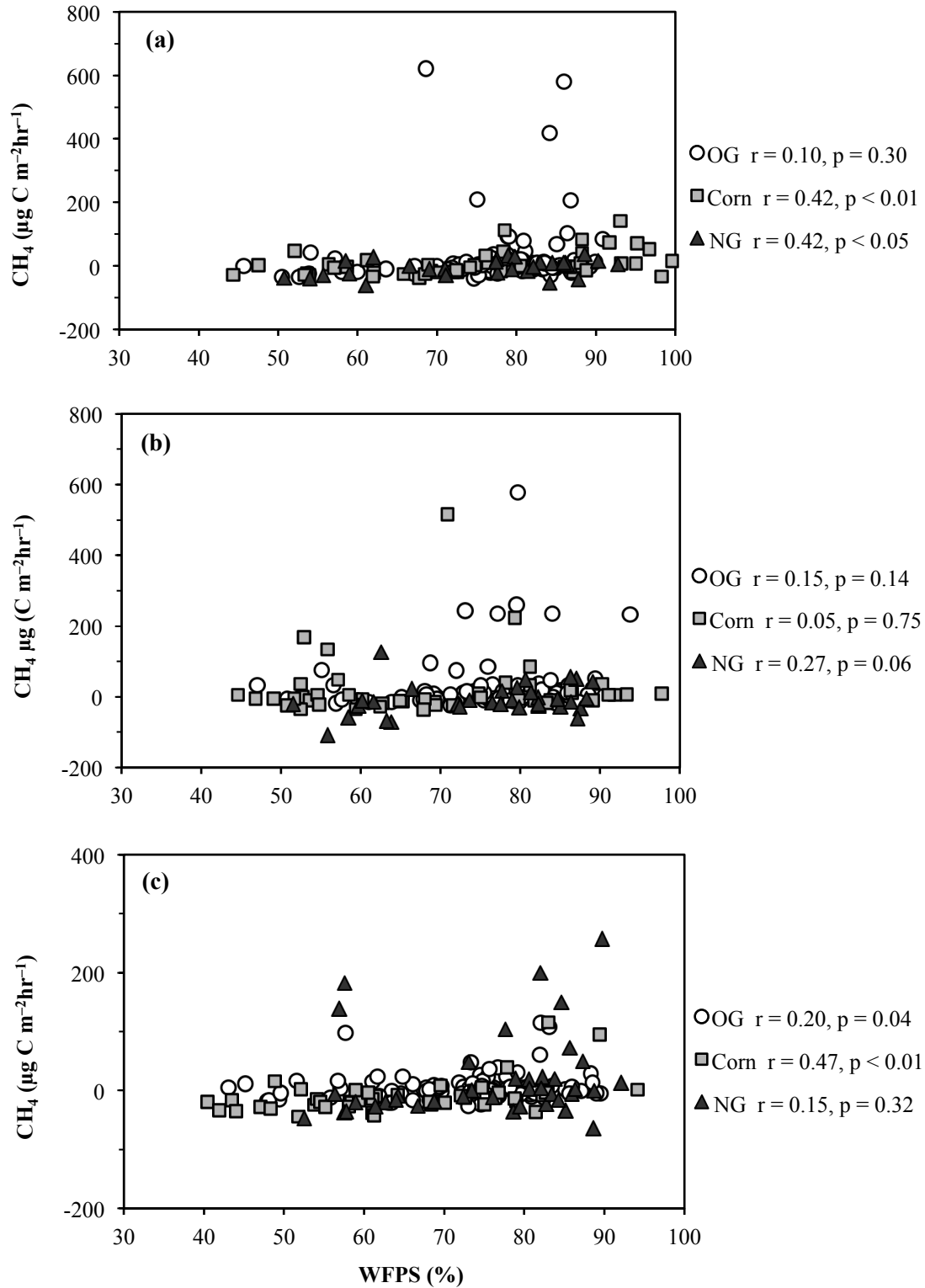


Figure 4.8 Relationship between soil moisture (WFPS) and methane (CH_4) flux in CT plot (a), F plot (b) and MF plot (c). OG is old grassland; Corn is Cornfield; NG is new grassland. Values represent means ($n=4$).

4.4.2 Effect of soil temperature and moisture on RS and RH

It is widely expected that microbial decomposition of SOM and root respiration increase with increasing temperature. Our study, consistent with this expectation and with several other studies (Chen and Tian 2005; Wang et al. 2007; Gaumont-Guay et al. 2008; Song and Zhang 2009; Zhou et al. 2014) found that both seasonal RS and RH fluxes were primarily controlled by soil temperature (Fig. 4.4). The influence of temperature was stronger on RS than RH and both RS and RH showed a stronger response to soil temperature in the undisturbed old grassland than in cornfield and newly established grassland (Table 4.4). Higher sensitivity to soil temperature of RS than RH in this study could be due to additional C from root exudates (Demyan et al. 2016) or from root turnover (Boone et al. 1998; Mäkiranta et al. 2008) which increased respiration. The Q_{10} values of RR have been found to be greater than Q_{10} values of RS and RH (Boone et al. 1998), and this also explains why RS in old grassland, due to higher RR contribution, showed greater response than in the other two land-uses. In this study, average RS Q_{10} values were 4.1, 3.2 and 2.0, and RH Q_{10} values were 3.7, 3.1 and 1.7 in OG, cornfield and NG, respectively. Previous studies have reported RS Q_{10} values of 1.5–3.0 in maize fields and other croplands (Peng et al. 2009; Demyan et al. 2016), 1.5–2.7 in temperate grassland (Chen and Tian 2005; Peng et al. 2009). Much higher RS Q_{10} range of 3.7–14.7 in grassland was reported in a meta-analysis by (Chen and Tian 2005). Higher temperature sensitivity of RR than RH could mean that the predicted continued temperature increase due to climate change will increase CO₂ emissions by greater margins in ecosystems where roots are a larger contributor to RS.

Both RS and RH fluxes were negatively correlated with soil moisture (Fig. 4.6) but the relationship between soil moisture and RH was much weaker than that with RS. Autotrophic respiration is more sensitive to changes in moisture content than RH (Balogh et al. 2016) and hence the stronger response of RS than the response of RH to changes in

moisture. Similar to Boone et al. (1998), our results also show that soil moisture content had a weaker effect on seasonal variation of RS and RH compared to the effect of temperature (Table 4.4). WFPS of 65% seemed to be the threshold below which RH and RS showed slight but non-significant increase with WFPS and beyond which both RS and RH declined significantly with increasing soil moisture in all three-uses in this study (Fig 4.7). This finding is similar to that of Yan et al. (2010) that at low moisture content CO₂ fluxes will increase with moisture but at high moisture content the relationship becomes negative. This is likely due to the reduction of CO₂ to CH₄ as the soil becomes more anaerobic and also to the restricted diffusivity of gases at higher soil moisture (Yan et al. 2010; Chapin et al. 2011; Shimizu et al. 2013). Many studies have reported that RS and RH increases (e.g Yan et al. 2010), decreases (e.g Suseela et al. 2012) or are not influenced (e.g Mäkiranta et al. 2008) by increasing soil moisture. Our findings show that this relationship is not unidirectional but that it could go either way depending on the moisture content range, and therefore the range of soil moisture should be indicated when reporting its relationship with CO₂ fluxes.

4.4.3 Effect of nitrogen input and soil N status on components of RS

Contrary to several reports that N fertilization increases RS due to stimulation of autotrophic root respiration (RR); for example in a meta-analysis in cropland and grassland (Zhou et al. 2014), in an Alfisol planted to maize with 0–250 kg N ha⁻¹ as Urea in China (Song and Zhang 2009) and in a review of several land-use types with different N application rates (Ryan and Law 2005), this study found that RR and RS did not have a significant response to N input. In fact, RR showed a negative response to N input thereby reducing the overall response of RS to N input (Table 4.5). This is contrary to the expectation that root growth and metabolism increase following N addition due to stimulated photosynthesis as the plants invest more energy in the roots to support water and nutrient uptake (Wang et al. 2007; Schindlbacher et al. 2009; Zhou et al. 2014). However, our findings are supported by other

research (Ding et al. 2007a; Alberti et al. 2010). Unlike Ding et al. (2007) who linked the negative effect of N fertilization on RS to suppression of mineralization of native SOC, Table 4.5 shows that it was root respiration that decreased with increased N input and surplus soil N. The negative response of RR to N input and high surplus soil N could be due to high energy requirements for N uptake by roots when N availability is high (Bowden et al. 2004). In this study it is likely that at high N input, C availability became the limiting factor for crop growth and therefore RR. Both RR and RS showed significant positive correlations with available organic C while RH did not (Table 4.5). It has been reported that when C is limited relative to N, increased N addition will significantly increase SOC decomposition but not RR due to inadequate energy or high energy requirement for root growth (Kuzyakov et al. 2000). Zhou et al. (2014) and Bowden et al. (2004) reported that while RS and RR increased with N input, RH was negatively affected by N input. Our findings are quite the opposite. In the current study, N input resulted in a significant increase in RH in both grassland and cornfield (Table 4.5). The increase in RH with increasing N input is consistent with the explanation by Kuzyakov et al. (2000) that N and C addition to soils stimulates microbial activity and SOC decomposition. One of the mechanisms commonly cited to be responsible for some of the reported inhibition of RH following N fertilization is the reduction in soil pH caused by nitrification (Bowden et al. 2004; Foereid et al. 2004). In the current study, liming was applied in all treatment plots leading to annual increases in soil pH, and therefore possibly explaining why we did not observe inhibition of RH by N input.

4.4.4 Factors affecting methane emission

Although the effect of land use on CH₄ emission was not significant, it was clear that on average CH₄ fluxes and cumulative annual emissions were higher in OG than in cornfield and NG (Fig 4.3 and Table 4.3). It has been shown previously that soil disturbance such as tillage can decrease CH₄ emissions (Omonode et al. 2007) and this is likely due to

enhancement of CH₄ oxidation caused by increased aeration. This result is however contrary to that of Mosier et al. (1991) who reported that disturbance of grasslands leads to reduced CH₄ oxidation and Hütsch (1998) that tillage negatively affects the development of methane oxidising bacteria (methanotrophs) leading to increased CH₄ emissions. Soil pH in OG was lower than in cornfield and NG due to annual lime application that started in 2008 in the current study. Low soil pH inhibits the activity of methanotrophs (Hütsch et al. 1994; Tate 2015) due to accumulation of NH₄⁺ which inhibits methanotroph monooxygenase enzyme activity (Flessa 1995; Acton and Baggs 2011). Another reason for higher CH₄ fluxes in OG could have been higher available C relative to available N in OG than in cornfield and NG (Figures 5.1 and 5.7 in chapter 5). High available C can decrease CH₄ oxidation and enhance emissions (Bayer et al. 2012). Correlation of annual CH₄ emissions and available C was negative in all three land-uses despite not being statistically significant (Table 4.5).

Linear regression analysis showed that soil moisture and temperature were the most important factors driving temporal variation of CH₄ fluxes (Table 4.4). However, temperature and soil moisture only explained 5–7% of the variation. Contrary to this study, Wu et al. (2010) found that temperature and moisture explained up to 86% of CH₄ variation in a semi-arid grassland. The annual precipitation in their study was only 335 mm compared to over 1200 mm in this study. In drier areas, occurrence of precipitation (which almost entirely occurred between July and September in the study by Wu et al. 2010) is likely to have large influence than in humid regions. The influence of moisture and temperature on CH₄ fluxes has been reported by several studies (Dunfield et al. 1993; Le Mer and Roger 2001; Wu et al. 2010; Wang et al. 2013). Despite low CH₄ emissions in this study, occurrence of high precipitation 1–6 days prior to gas sampling changed this soil from being a sink to a source of CH₄. This was despite the fact that high fluxes observed after high

precipitation accounted for less than 10% of all the observations. Increase in precipitation could therefore significantly increase CH₄ emissions in what is otherwise a CH₄ sink.

4.5 Conclusions

Grassland to cropland conversion increased heterotrophic soil respiration (RH) due to stimulation of soil organic matter (SOM) decomposition by tillage, but decreased soil respiration (RS) as a result of low root biomass and respiration in cornfield. The conversion from cornfield to grassland increased RS and decreased RH. Despite increasing SOM decomposition, the conversion of grassland to cornfield significantly increased soil organic carbon (SOC). This increase in the first year of conversion indicates that conversion or ploughing of grassland is important to turn plant litter/applied manure into actual soil carbon. These results call for a rethink of the commonly held notion that conversion of grassland to cropland depletes SOC, as the conversion could be beneficial in the short-term. Occasional rotation of managed grassland and cropland could increase SOC storage in the soil.

5. Effect of land use change on soil N and nitrous and nitric oxide dynamics

5.1 Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (IPCC 2007a; UNEP 2013) and is currently the most important substance emitted into the atmosphere causing the depletion of the ozone layer (UNEP 2013), whereas nitric oxide (NO) is a highly reactive trace gas important in atmospheric chemistry as it contributes to acid rain deposition and for its regulation of photochemical production of ozone in the troposphere (Crutzen 1979; Logan 1983; Davidson et al. 1993; Eickenscheidt and Brumme 2013).

The largest source of anthropogenic N_2O emissions is agricultural soils, accounting for about 66% of gross anthropogenic emissions (UNEP 2013). N_2O and NO emissions from soils and agricultural systems are expected to increase further due to increased use of nitrogen (N) fertilizers and manure to meet demand for increased food production (Mosier et al. 1998; US. EPA 2006; Smith et al. 2007; UNEP 2013). Microbial transformation of chemical N is an important source of both N_2O and NO emissions (Vitousek et al. 1997; Medinets et al. 2015). While agricultural soils are not considered to be the major source of NO globally, they are still very important sources especially when fossil fuels are not considered (Bouwman et al. 2002a). Tillage and manure application can increase NO emission by up to 7 times, and as much as 11% of applied fertilizer N can be emitted as NO (Skiba et al. 1997).

It is generally accepted and widely reported that N_2O and NO emission are increased by N fertilization (Jin et al. 2010; VanderZaag et al. 2011; Shimizu et al. 2013; Owen et al. 2015). However, some studies have reported possible reductions in N_2O emissions with improved management of organic materials (Alluvione et al. 2010; Ryals and Silver 2012;

UNEP 2013). Changing fertilizer type to those less susceptible to nitrification, timing of fertilization and use of organic N sources could help mitigate NO emissions (Davidson et al. 1993; Skiba et al. 1997; Smith et al. 1997). There are still many unknowns and high uncertainties in estimating representative annual N₂O and NO emissions from an individual site, as emissions from the same site greatly vary from year to year (Tubiello et al. 2013; Owen et al. 2015).

In grasslands, large amounts of N accumulate in plant biomass resulting in N rich organic matter overtime (Davies et al. 2001; Velthof et al. 2010). When grasslands are ploughed, soil available N increases due to mineralized resulting in increased N losses through leaching (Whitehead et al. 1990; Necpálová et al. 2013) and N gas emissions (Smith and Conen 2004; Smith et al. 2008; UNEP 2013). Compared to grasslands, croplands are ploughed annually, increasing the physical breakdown of soil structure and organic matter and soil aeration, and consequently leading to rapid microbial decomposition of organic matter (Ussiri and Lal 2009; Necpálová et al. 2013). Assessing how N₂O and NO emissions change when land use is alternated between grassland and cropland is important to fully understand the potential for mitigation of the emissions during the transition from one land-use to the other.

Freezing and thawing can stimulate N₂O and NO emissions (Katayanagi and Hatano 2012; Burchill et al. 2014) by releasing carbon (C) and N through microbial lysis and through physical entrapment and release during soil freezing and melting. However, the contribution of winter and thawing periods to annual N₂O and NO emissions, and its annual variation, are not well known.

Long-term field data on N₂O and NO emissions is currently scarce (Tubiello et al. 2013). In this study we report results of continuous monitoring of N₂O emissions for 11

years following manure and chemical fertilizer applications, combined with changing land-use. While many studies have reported the effects of fertilizer, manure and land-use change on N₂O emissions, very few, if any, have measured continuously the changes in the soil properties and N₂O emissions for as long as 11 years with changing land-use, covering a permanent grassland, a cornfield and a newly established grassland. The objectives were: (1) to assess the effect of long-term manure and chemical fertilizer applications on N₂O and NO emissions; (2) to investigate the effect of land-use change (from grassland to cornfield and back) on N₂O and NO emissions; (3) to investigate the factors driving intra and inter-annual variations in N₂O and NO emissions; and (4) to quantify the contribution of winter and thawing periods to annual N₂O and NO emissions.

5.2 Materials and methods

5.2.1 Field experimental designs and plot management

During the study period, land-use was as an old grassland (OG) from 2005 to 2009, cornfield (2010-2012) and newly established grassland (NG) (2013-2015). The old grassland had been established more than 30 years prior to the beginning of this study in 2005. The dominant grass species was reed canary grass (*Phalaris arundinacea* L.) and meadow foxtail (*Alopecurus pratensis* L.) in OG, and timothy grass (*Phleum pratense*) in NG.

The average amount of mineral fertilizer applied in OG before commencement of this study was 133 ± 36 kg N ha⁻¹ year⁻¹. From 1990 to 2004 the grassland was harvested for hay at least twice a year. In September 2009 herbicide was applied and the field was ploughed in December.

Three treatments plots namely; (i) control without N addition (CT plot), (ii) chemical N fertilizer only (F plot), and (iii) Chemical N fertilizer and composted beef cattle manure

(MF plot) were set up in 2005. In 2011, a fourth plot with composted beef cattle manure only (M plot) was added. Each plot was 5 x 5 m in size and all the treatment plots were replicated 4 times and arranged in a complete randomized design.

The timing of fertilizer and manure applications, and other management practices are in Table 3.2 in chapter 3. The type of chemical fertilizer was ammonium sulfate and the manure was composted beef cattle manure with bedding litter (bark). The gross manure N and fertilizer N application rates were as shown in Table 3.3. Lime was applied in all the plots from 2008 to 2015 at an average rate of 400 kg CaCO₃ ha⁻¹year⁻¹.

5.2.2 Soil and weather measurements

Soil samples were collected at 5 cm depth during each sampling day from April to November (non freezing period) in all treatment plots. Air-dried soil samples were sieved (2 mm sieve) and extracted in deionized water for analysis of water extractable organic carbon (WEOC), NO₃⁻, pH or in 2 M potassium chloride (KCl) solution for NH₄⁺-N analysis.

In the OG and NG, soil moisture was measured at 0–6 cm depth using the Frequency Domain Reflectometry (FDR) method (DIK- 311A; Daiki, Saitama, Japan). Calibration curves were made to calculate water-filled pore space (WFPS) from the FDR device reading and percent total porosity (Jin et al. 2010; Linn and Doran 1984). In the cornfield, soil moisture content was measured gravimetrically from soil samples collected at a depth of 0–5 cm.

5.2.3 Measurement of change in soil organic N

Soil organic N was measured every two years from 2006 to 2014 in F and MF plots at 0–15 and 15–30 cm depth. For each plot and depth, samples were collected using 100-cm³ cores in a grid system covering the entire plot (n = 16–24). Soils were collected from the mineral Ap

horizon after removing the root mat. Soils were then air-dried, roots removed, sieved (2 mm) and analysed using a C/N analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). A separate core sample was taken to determine soil bulk density, gravimetrically, at each depth.

5.2.4 Gas flux sampling and measurement

N₂O and NO fluxes were measured using static closed chambers at 4 replications for each treatment. The chambers size was 40 cm in diameter and 30 cm high in grassland and 20cm in diameter and 25 cm in high in cornfield. In cornfield 2 chambers were place in intra-row and the other 2 in inter-row space. Detailed information about gas flux sampling and analysis is described in chapter 3. Briefly, gas samples were taken between 8:00 am and 12:00 pm on each sampling day using a gas tight syringe through a three-way valve fitted onto the chamber cover. The normal sampling frequency was once or twice every fortnight, except in winter when sampling was conducted once or twice every month. A more intensive sampling regime of every two to five days was carried out after fertilization and other events that are known to stimulate gas flux. NO gas concentrations were analyzed in the laboratory within the same day of sampling using a nitrogen oxides (NO_x) analyzer (Model 265P; Kimoto Electric, Osaka, Japan). N₂O gas concentrations were analyzed within three months using a gas chromatograph fitted with an electron capture detector (Model GC-14B; Shimadzu, Kyoto, Japan).

5.2.5 Heterotrophic soil respiration and estimation of mineralized N

Heterotrophic respiration (RH) was measured as carbon dioxide (CO₂) emission from bare soil (plant and root excluded soil) as described in chapter 4 and used to estimate mineralized N. RH was measured in the CT plot from 2005 to 2009, and in all plots from 2010 to 2015.

RH in CT and F plots was regarded as heterotrophic respiration from soil organic matter decomposition (RHs), while RH from manure amended plots included RHs and heterotrophic respiration from manure decomposition (RHm). Therefore, RHm in MF was estimated by subtracting the RH from F plot, while in M plot by subtracting the RH from CT plot. From 2005 to 2009, RHm was calculated as the difference in total CO₂ emissions in planted plots between MF and F plots (Li et al. 2015; Shimizu et al. 2015).

The total mineralized N was calculated as the sum of soil organic matter N and manure N mineralization. The mineralized N from soil organic matter and manure was calculated by dividing RHs and RHm by the soil (10:1) and manure (20:1) C/N ratios, respectively.

5.2.6 Plant N uptake, total N input and surplus soil N

Net primary production (NPP) was estimated as the net increase in plant biomass (aboveground and belowground biomass) annually. Detailed description is provided in chapter 3. The plant samples were oven-dried at 70°C for more than 72 hours and weighed. Each dried sample was analysed for total carbon (C) and N contents with N/C analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Surplus soil N was estimated as the difference between total N input (sum of soil and manure mineralized N and chemical fertilizer N) and plant N uptake.

5.3 Results

5.3.1 Soil and weather variables

Mean annual air temperatures were within long-term normal values for most of the years during this study except for 2007 and 2015, which was recorded at 0.7°C higher than the long-term average value of 8.2°C. 2005 was the coolest as well as the driest year (8.0°C, 999 mm). 2009, 2010, 2011, and 2013 were wetter than average with annual precipitation at least 200 mm higher than the 30-year average of 1252 mm (Table 5.1).

Soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were significantly higher in cornfield than grassland and higher in NG than OG ($p < 0.01$) (Fig. 5.1). NO_3^- significantly increased in 2010 after converting grassland to cornfield and decreased slightly in 2011 and 2012. In the first year after conversion from grassland to cornfield, NO_3^- -N concentration in control plot (without N addition) increased from an average of 1 mg kg⁻¹ to 60 mg kg⁻¹, but decreased to 12 mg kg⁻¹ by the third year of the cornfield (Fig. 5.1) and declined further in the new grassland. NH_4^+ concentration on the other hand did not increase in the first year of cornfield but showed high values in 2012, the third year of cornfield. Soil NO_3^- and NH_4^+ concentrations were higher in chemical fertilizer amended plots (MF and F) compared to CT and M plots in all three land-uses throughout the study period, and were always lowest in the control treatment. Peaks of both soil NO_3^- and NH_4^+ concentrations were observed following chemical fertilizer applications in spring and short-lived peaks in NO_3^- concentrations were sometimes observed after manure application.

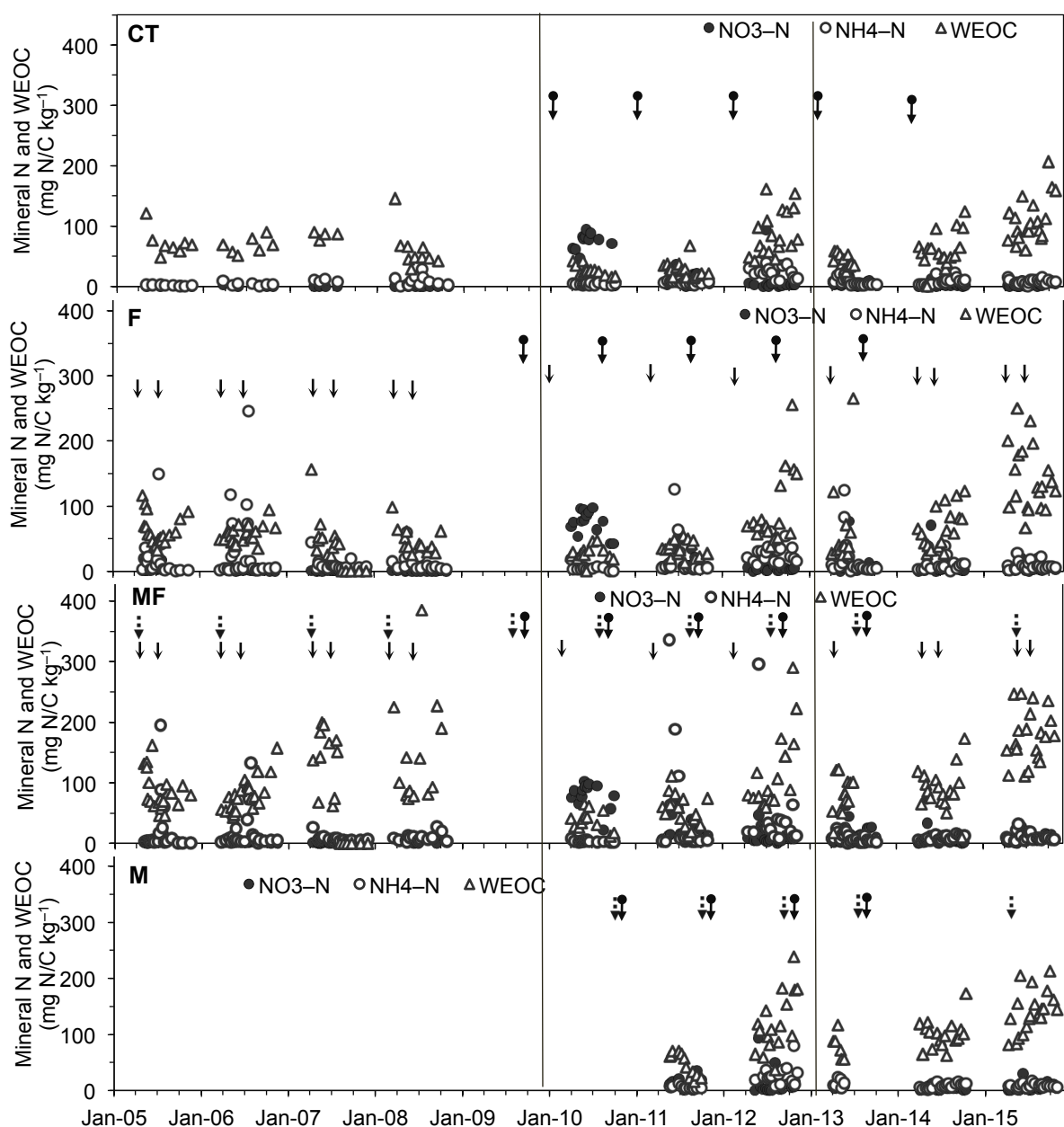


Figure 5.1 Soil nitrate N, ammonium N and water extractable soil organic carbon (WEOC). CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. Dashed arrows indicate dates of manure application; full arrows with open V shaped tip indicate dates of chemical fertilizer application; full arrows with round top and normal closed tip indicate dates of ploughing. Vertical lines indicate the change from old grassland (2005–2009) to cornfield (2010–2012) and then to new grassland (2013–2015).

WEOC was not significantly different among the land-uses but tended to be higher in OG and NG compared to cornfield. WEOC was significantly lower in 2010, the first year of conversion from grassland to cornfield, and increased annually in the 3 years of cornfield. WEOC was higher in the manure-amended plots (MF and M) than in the plots without manure application ($p < 0.01$).

The ratio of WEOC to NO_3^- was highest in OG, followed by NG and lowest in cornfield in all of the plots.

Soil pH was always lower in F plot compared to MF, M and CT plots ($p < 0.001$). Soil pH in MF and M plots (long-term manure application) was higher than that in CT plot (Table 5.1). Soil pH in all plots increased annually from 2008 due to liming.

Table 5.1 Annual precipitation, air temperature and average soil pH in the treatments

Year	Precipitation (mm)	Air temperature (°C)	Average Soil pH		
			CT	F	MF
2005	1007	8.0	5.18	4.63	5.04
2006	964	8.4	5.15	4.54	5.04
2007	804	8.9	5.13	4.53	5.17
2008	785	8.5	4.93	4.43	5.36
2009	1051	8.3	5.24	4.96	5.63
2010	1120	8.6	5.15	4.86	4.99
2011	1006	8.2	5.32	5.18	5.58
2012	1066	8.2	5.71	5.60	5.70
2013	1076	8.2	5.75	5.58	5.99
2014	914	8.2	5.73	5.74	6.29
2015	1015	8.8	6.23	5.93	6.34

5.3.2 Changes in soil N content and stock with land-use change

Soil organic N (SON) content and total SON stock in F plot did not change much from 2005 to 2009 in OG (Fig. 5.2 and Table 5.2). After conversion of OG to cornfield, both SON content and SON stock only increased slightly in 2010. However, a significant increase was observed in the third year of the cornfield ($p < 0.01$). In the MF plot, SON content increased significantly after conversion of OG to cornfield ($p < 0.01$) at both 0–15 and 15–30 cm depths. A further increase in SON content in MF plot was observed in 2014 at both measurement depths.

The SON stock showed a similar trend as SON content. SON stock increased by more than 900 kg N ha⁻¹ from 2009 to 2012 at both 0–15 and 15–30 cm depths in both F and MF plots. No significant difference was observed between cornfield and NG.

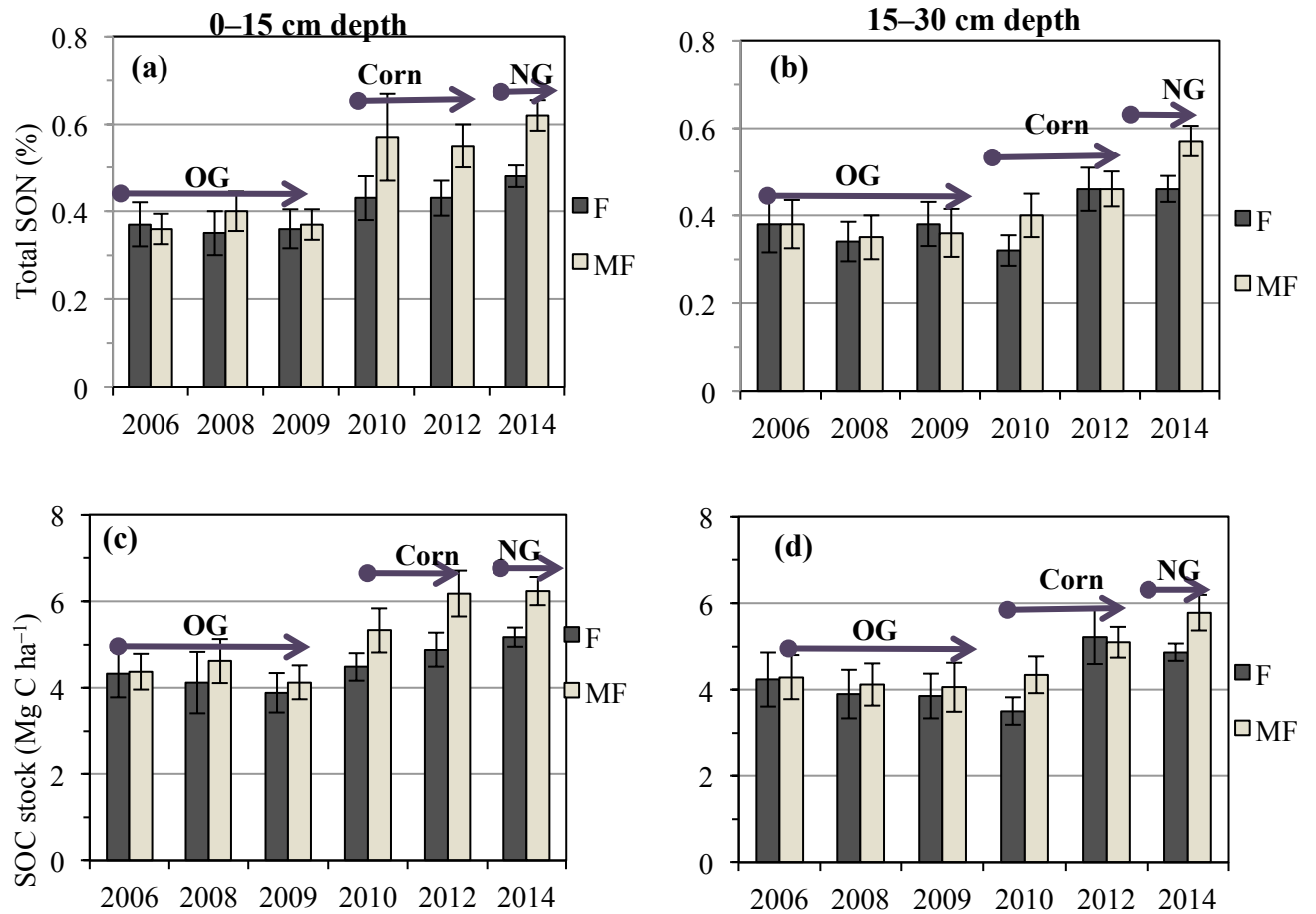


Figure 5.2 Total soil organic nitrogen (SON) content (a,b) and SON stock (c,d) from 2006 to 2014 measured every 2 years at 0–15 and 15–30 cm depths in inorganic fertilizer only plot (F) and inorganic fertilizer plus manure plot (MF). Land-use in 2006–2009 was old grassland (OG), 2010–2012 was cornfield and 2014 was new grassland (NG). n=16–24.

Table 5.2 Soil bulk density, total soil organic N content (SON) and SON stock (mean $\pm$ SD) in F and MF plots.

Year	Depth (cm)	F plot			MF plot		
		Bulk density (g cm ⁻³)	SON (%)	SON stock (Mg N ha ⁻¹)	Bulk density (g cm ⁻³)	SON (%)	SON stock (Mg N ha ⁻¹)
2006	0–15	0.80 $\pm$ 0.09 ^{ce}	0.37 $\pm$ 0.10 ^{abcd}	4.33 $\pm$ 1.09 ^{abc}	0.81 $\pm$ 0.04 ^c	0.36 $\pm$ 0.07 ^{bd}	4.38 $\pm$ 0.83 ^{be}
	15–30	0.75 $\pm$ 0.07 ^{bcde}	0.38 $\pm$ 0.13 ^{abc}	4.24 $\pm$ 1.25 ^{ab}	0.76 $\pm$ 0.08 ^c	0.38 $\pm$ 0.11 ^{ac}	4.29 $\pm$ 1.02 ^{acd}
2008	0–15	0.79 $\pm$ 0.06 ^c	0.35 $\pm$ 0.10 ^{ab}	4.12 $\pm$ 1.42 ^{ab}	0.77 $\pm$ 0.06 ^c	0.40 $\pm$ 0.09 ^{ad}	4.62 $\pm$ 1.01 ^{de}
	15–30	0.77 $\pm$ 0.07 ^{de}	0.34 $\pm$ 0.09 ^a	3.90 $\pm$ 1.12 ^a	0.80 $\pm$ 0.06 ^c	0.35 $\pm$ 0.10 ^{abc}	4.12 $\pm$ 0.97 ^{abc}
2009	0–15	0.73 $\pm$ 0.08 ^{abd}	0.36 $\pm$ 0.09 ^{abcd}	3.89 $\pm$ 0.91 ^{ab}	0.73 $\pm$ 0.05 ^{bc}	0.37 $\pm$ 0.07 ^{cd}	4.13 $\pm$ 0.78 ^{cd}
	15–30	0.68 $\pm$ 0.07 ^a	0.38 $\pm$ 0.10 ^{abc}	3.86 $\pm$ 1.04 ^a	0.75 $\pm$ 0.06 ^c	0.36 $\pm$ 0.11 ^{ab}	4.06 $\pm$ 1.13 ^{ab}
2010	0–15	0.71 $\pm$ 0.09 ^{abd}	0.43 $\pm$ 0.10 ^{abcd}	4.49 $\pm$ 0.64 ^{ab}	0.65 $\pm$ 0.10 ^a	0.57 $\pm$ 0.20 ^{eg}	5.33 $\pm$ 1.02 ^{ef}
	15–30	0.73 $\pm$ 0.07 ^a	0.32 $\pm$ 0.07 ^{abcd}	3.51 $\pm$ 0.63 ^a	0.72 $\pm$ 0.07 ^{ab}	0.40 $\pm$ 0.10 ^{df}	4.35 $\pm$ 0.85 ^{cde}
2012	0–15	0.76 $\pm$ 0.05 ^{abcde}	0.43 $\pm$ 0.08 ^{cde}	4.88 $\pm$ 0.78 ^c	0.76 $\pm$ 0.06 ^c	0.55 $\pm$ 0.10 ^f	6.18 $\pm$ 1.06 ^{gh}
	15–30	0.76 $\pm$ 0.10 ^{abcde}	0.46 $\pm$ 0.10 ^{bcde}	5.22 $\pm$ 1.25 ^{bc}	0.75 $\pm$ 0.08 ^c	0.46 $\pm$ 0.08 ^{de}	5.10 $\pm$ 0.72 ^{ef}
2014	0–15	0.72 $\pm$ 0.05 ^{abcd}	0.48 $\pm$ 0.05 ^c	5.17 $\pm$ 0.45 ^c	0.67 $\pm$ 0.05 ^a	0.62 $\pm$ 0.07 ^g	6.23 $\pm$ 0.65 ^h
	15–30	0.72 $\pm$ 0.08 ^{abc}	0.46 $\pm$ 0.06 ^{de}	4.87 $\pm$ 0.41 ^{bc}	0.67 $\pm$ 0.04 ^{ab}	0.57 $\pm$ 0.07 ^f	5.78 $\pm$ 0.82 ^{fg}

Different letters within the same column indicate significant differences among the years and measurement depths.

5.3.3 Temporal variations of N₂O fluxes

Nitrous oxide fluxes were very episodic and displayed high variations within and across years throughout the study period (Fig 5.3). Intra-annual variations were highly influenced by mean daily temperature and precipitation.

The timing when the highest N₂O fluxes were found differed depending on the land-use and fertilizer application. In OG, the highest fluxes in the MF plot; 275.5, 1290.6, 140.3, and 93.5 $\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$ were found on May 20th, 11th, 29th, and 18th in 2005, 2006, 2007 and 2009 respectively. All of these followed combined chemical fertilizer and manure applications in spring, except in 2008 when the highest flux (71.7 $\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$) was found on 14 July after the second fertilizer application.

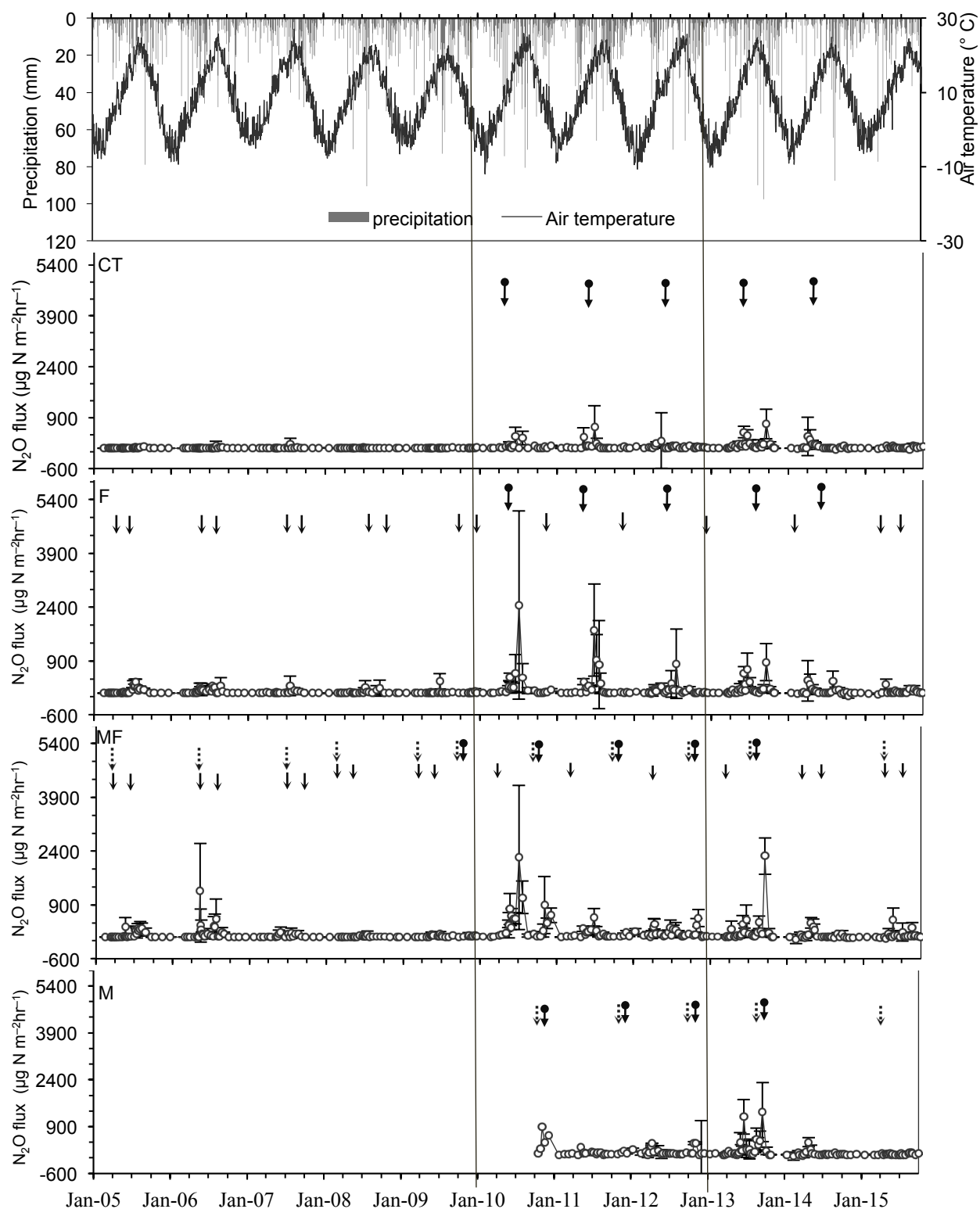


Figure 5.3 Daily precipitation and air temperature (a) and daily N₂O flux. CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. Dashed arrows indicate dates of manure application; full arrows with open V shaped tip indicate dates of chemical fertilizer application; full arrows with round top and normal closed tip indicate dates of ploughing. Vertical lines indicate the change from old grassland (2005–2009) to cornfield (2010–2012) and then to new grassland (2013–2015).

In the F plot the highest fluxes; 313.8, 211.1, 206.8, 175.7 and 333.8 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ were found after the second fertilization on 18, 15, 18, 7 July and 25th June in 2005, 2006, 2007, 2008 and 2009, respectively. This was despite the lower N application rate in the second application compared to the first one in May. In the control plot, the highest fluxes in OG; 50.3, 66.2, 114.5, 22.8 and 30.4 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ in 2005, 2006, 2007, 2008 and 2009, respectively were always found between July and August, and were all preceded by cumulative precipitation of more than 40 mm within 7 days before sampling. In cornfield (2010–2012) the highest fluxes in all the treatment plots were found in either June or July and were preceded by high precipitation. The highest fluxes in cornfield ranged from 223.7 to 638.4 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ in control plot, 822.1 to 2461.4 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ in F plot, and 527.4 to 2223.5 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ in MF plot.

In 2013, the first year of new grassland (but before it was well established), disproportionately high fluxes (713.8, 871.0, 2260.9 and 1359.2 $\mu\text{g N}_2\text{O-N m}^{-2}\text{hr}^{-1}$ in CT, F, MF and M plots, respectively) were found on 18th September two days after very high precipitation (97 mm in 1 day) on 16th September. On June 5 and 20 in 2013, 54 and 40 mm rainfall was recorded and high fluxes were found for samples collected within 5 days. Precipitation higher than 40 mm per day was recorded at least 6 times in 2014 and 2015 but the fluxes were relatively low.

In all of the plots, winter N_2O emissions were very low throughout the study. High fluxes during the thawing period were found in all plots throughout the study period. Nitrous oxide fluxes were highest in the cornfield, followed by NG and lowest in OG (Fig. 5.3 and Table 5.3). Nitrous oxide fluxes in chemical fertilizer-amended plots (F, MF) were higher than those without chemical fertilizer application ($p < 0.01$). The manure-only plot tended to have higher emissions than the control plot.

Table 5.3 Annual N₂O emissions (mean±sd) from 2005-2015 in unfertilized control plots (CT), chemical fertilizer plot (F), manure and chemical fertilizer plot (MF) and manure plot (M).

Land use	Year	CT	F	MF	M
kg N ₂ O-N ha ⁻¹					
OG	2005	0.7±0.4	2.8±0.7	3.6±1.2	–
	2006	0.5±0.3	2.9±0.7	4.9±2.8	–
	2007	0.7±0.5	1.5±0.5	2.2±0.7	–
	2008	0.6±0.1	2.1±1.5	0.9±0.2	–
	2009	0.4±0.1	1.2±0.7	1.4±0.4	–
	Average	0.6	2.1	2.6	–
Cornfield	2010	3.9±1.2	17.4±16.1	22.9±11.3	–
	2011	5.8±2.3	13.6±8.7	14.3±2.2	11.7±2.3
	2012	3.6±0.7	7.1±3.3	7.7±1.2	5.6±1.7
	Average	4.4	12.7	14.9	8.7
NG	2013	5.8±1.2	7.5±2.6	11.1±1.5	13.3±2.3
	2014	2.8±2.5	4.1±2.5	2.9±0.5	2.4±1.4
	2015	1.2±0.2	2.0±0.6	2.3±1.2	1.1±0.4
	Average	3.2	4.5	5.4	5.6
ANOVA					
	d.f.	MS	F	p value	
plot	4	117.31	4.29	0.0065	
Land use	2	216.42	7.91	0.0015	

Inter-annual variations in cumulative N₂O emissions were more pronounced in cornfield and NG than in OG (Table 5.3). Annual N₂O emissions were lower in OG, followed by NG, and highest in cornfield. Averaged over the entire study period for each land-use and compared within each treatment, annual N₂O emissions in cornfield were 6-7 times higher than in the OG ($p < 0.001$) and 1.5-3 times higher than in NG. The emissions in NG were 2-5 times higher than those in OG ($p < 0.001$).

The emissions in the cornfield were highest in the first year after conversion from grassland (2010) and lowest in the third year. After conversion from cornfield to NG, the annual emissions declined slightly in 2013 (first year of conversion), but by the second and third years after conversion, the emissions in NG were significantly lower than in the cornfield. Within each land-use type, there were significant differences in annual N₂O emissions among plots and among the years ($p < 0.01$).

5.3.4 Contribution of winter and thawing periods to annual emissions

In grassland (both OG and NG) contributions of winter N₂O emissions to annual emissions ranged from 0 to 7% in all plots except for 2008 where winter emissions in the CT plot accounted for 25% and 2015 where cumulative winter emissions in F and CT plots were negative (Table 5.4). In cornfield, winter emissions in CT and F plots contributed 2–18%, while in the manure-amended plots, winter emissions contributed as much as 35% to the total annual emissions.

The thawing period tended to make a higher contribution to annual emissions in the unfertilized control treatments (Table 5.5). In 2014, the thawing period accounted for more than 45% of total annual emissions in all plots.

Table 5.4 Winter N₂O emissions (kg N ha⁻¹) and their contribution to total annual emissions in brackets (%)

Year†	CT	F	MF	M
2005	0.02 (2.3)	0.02 (0.7)	0.01 (0.3)	
2006	0.00 (0.0)	0.04 (1.4)	0.02 (0.5)	
2007	0.04 (5.6)	0.07 (4.6)	0.04 (1.9)	
2008	0.12 (24.8)	0.03 (1.6)	0.03 (3.6)	
2009	-0.01 (-2.0)	0.02 (2.0)	0.07 (5.3)	
2010	0.17 (4.3)	0.31 (1.8)	0.36 (1.5)	
2011	0.49 (8.4)	0.69 (5.1)	3.65 (25.4)	3.65 (31)
2012	0.65 (17.9)	0.48 (6.8)	2.00 (26.0)	2.00 (35.8)
2013	0.14 (2.4)	0.32 (4.3)	0.32 (2.9)	0.45 (2.3)
2014	0.11 (5.1)	0.11 (2.6)	0.06 (-1.9)	-0.05 (-2.3)
2015	-0.01 (-1.2)	-0.28 (-13.6)	0.16 (6.9)	0.08 (7.2)

CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. Winter period was defined as the period from Mid-December, when maximum soil temperature fell below 5°C, to the end of February when maximum temperatures recorded reached 0°C. † Winter N₂O emissions were significantly higher in cornfield (2010-2012) than grassland (p<0.01)

Table 5.5 N₂O emissions during the thawing period (kg N ha⁻¹) and their contribution to total annual emissions in brackets (%)

Year*	CT	F	MF	M
2005	0.04 (6)	0.04 (1)	0.03 (1)	
2006	0.01 (2)	0.05 (2)	0.05 (1)	
2007	0.14 (20)	0.18 (11)	0.12 (6)	
2008	0.08 (18)	0.20 (12)	0.08 (10)	
2009	0.03 (7)	0.05 (5)	0.03 (3)	
2010	0.23 (5.8)	0.51 (2.9)	0.87 (3.8)	
2011	2.1 (35)	0.68 (5.0)	0.71 (4.9)	0.71 (6.0)
2012	1.4 (38)	1.03 (14.)	1.43 (18.6)	1.57 (28.1)
2013	0.61 (10.6)	0.73 (9.7)	0.73 (6.6)	0.63 (3.2)
2014	1.32 (61)	1.92 (46.8)	1.75 (60.7)	1.63 (67.8)
2015	0.29 (25.0)	0.83 (40.2)	0.10 (4.5)	0.17 (15.4)

CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. The thawing period was defined as the period when minimum daily temperatures reached 0°C (typically early march), to the time when soils were completely melted (minimum soil temperatures ~5°C) in early May.

*N₂O emissions during thawing were significantly lower in old grassland (2005-2009) than in corn and new grassland ($p < 0.01$).

5.3.5 Temporal variations of NO fluxes

Intra-annual variations of NO fluxes showed a similar trend with N₂O fluxes. However, the NO fluxes were very low throughout the study period with only the MF plot showing higher values (Fig. 5.4). The highest NO fluxes were always found after fertilizer and manure applications.

Annual NO emissions were higher in MF and F plots and lowest in the control plots ($p < 0.05$). Annual NO emissions ranged from 0.01 to 0.18, 0.03 to 0.65, -0.16 to 1.8 and -0.01 to 0.66 kg N ha⁻¹ in CT, F, MF and M plots, respectively. There was no significant difference in annual NO emissions between the grassland and cornfield.

During winter and thawing periods NO fluxes were generally low and varied widely. Winter and thawing period NO fluxes in the CT plot contributed more to annual emissions compared to MF and F plots. The highest contributions of winter and thawing seasons to annual NO emissions were 55% and 32%, respectively, in the CT plot (Tables 5.6 and 5.7).

5.3.6 N₂O-N/NO-N ratio

The ratio of N₂O-N to NO-N (N₂O-N/NO-N) is used as an indicator of the dominant mechanism of N₂O production in the soil. If the ratio is less than 1, nitrification is the main mechanism of N₂O production, if greater than 100, denitrification is the main mechanism (Bouwman 1990). In OG, 2.4%, 79.2% and 18.4% of the N₂O-N/NO-N ratio values were less than 1, between 1-100 and greater than 100, respectively. In cornfield and NG, less than 1% (0.7% and 0.9%, respectively) of the N₂O-N/NO-N values were less than 1. About 69.6% and 64.5 % of the N₂O-N/NO-N values were between 1 and 100, and 29.7% and 34.5% were greater than 100 in cornfield and NG, respectively. Nitrous oxide flux increased with increasing N₂O-N/NO-N values in all plots and land-uses combined.

Table 5.6 Winter NO emissions (g N ha⁻¹) and their contribution to total annual emissions in brackets (%)

Year	CT	F	MF	M
2005	5.8 (10)	10.0 (6)	7.1 (2)	
2006	10.6 (7)	10.0 (4)	8.3 (2)	
2007	12.3 (23)	40.0 (29)	16.0 (5)	
2008	8.0 (55)	6.7 (17)	8.5 (15)	
2009	-4.4 (-33)	20.5 (3)	1.0 (0.5)	
2010	2.4 (6)	0.0 (0)	9.9 (3)	
2011	4.4 (2)	2.0 (0.4)	46.7 (3)	46.7 (7)
2012	8.6 (9)	9.4 (2)	14.3 (2)	14.4 (11)
2013	0.0 (0)	-3.2 (-3)	-3.2 (-2)	7.0 (3)
2014	9.7 (28)	9.7 (14)	-25.8 (-666)	-25.8 (200)
2015	-1.2 (-6)	-2.5 (-2)	-3.6 (2.2)	-1.9 (-8)

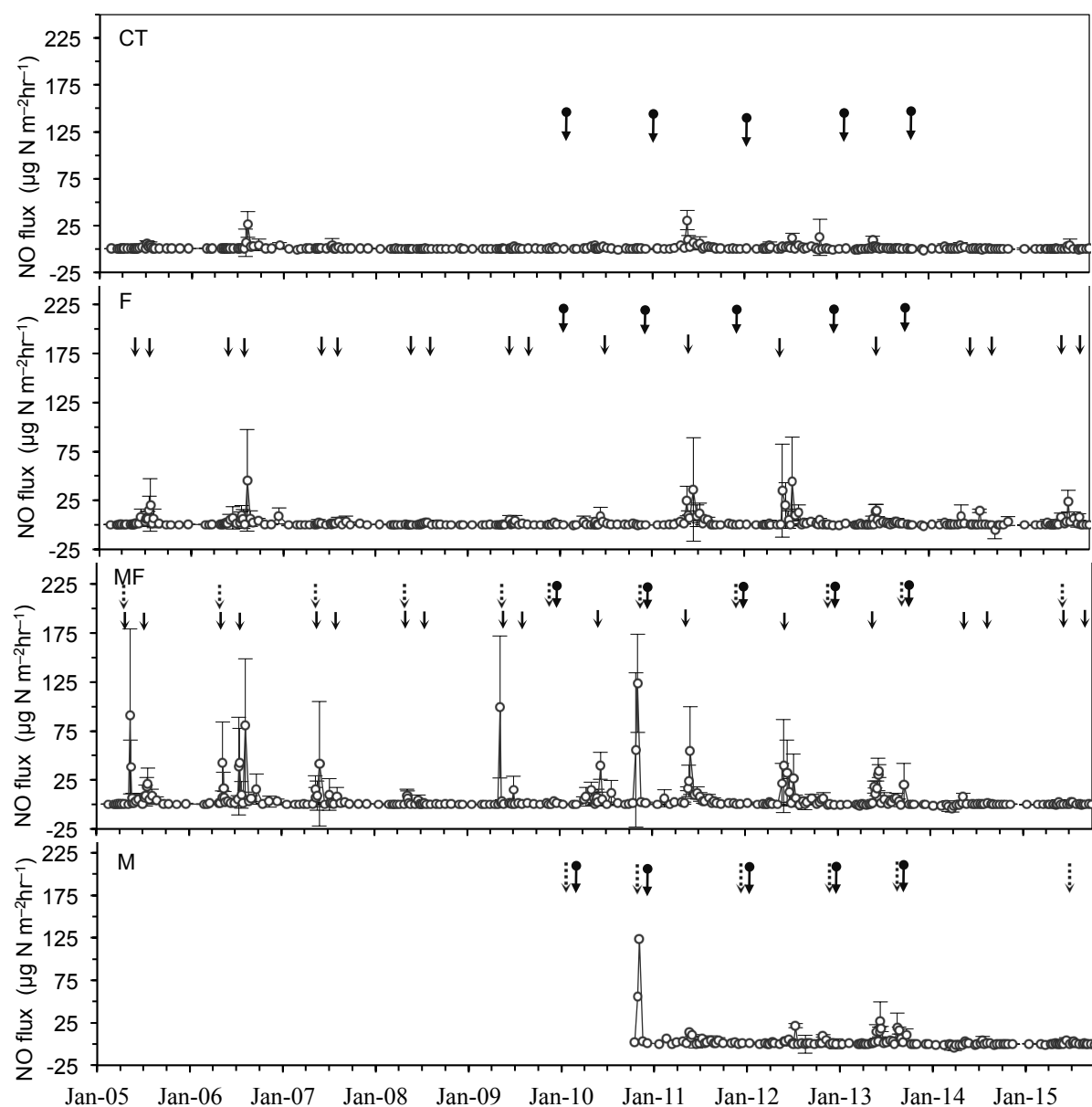


Figure 5.4 Daily NO flux. CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. Dashed arrows indicate dates of manure application; full arrows with open V shaped tip indicate dates of chemical fertilizer application; full arrows with round top and normal closed tip indicate dates of ploughing.

Table 5.7 Thawing period NO emissions (g N ha⁻¹) and their contribution to total annual emissions in brackets (%)

Year	CT	F	MF	M
2005	6.1 (11)	6.3 (4)	7.1 (2)	
2006	9.1 (6)	7.4 (3)	13.7 (3)	
2007	6.0 (8)	1.8 (1)	4.8 (2)	
2008	3.4 (23)	3.5 (9)	14.2 (25)	
2009	-0.9 (-7)	1.0 (0.1)	1.5 (1)	
2010	10.1 (25)	0.0 (0)	86.9 (27)	
2011	60.9 (32)	20.1 (4)	30.3 (2)	30.3 (5)
2012	11.9 (13)	5.0 (1)	121.7 (16)	8.1 (6)
2013	0.8 (1)	-0.9 (-1)	-0.9 (0)	3.2 (1)
2014	10.3 (30.2)	7.9 (11.6)	-17.2 (-442)	-16.1 (130)
2015	0.3 (2)	7.1 (5)	-0.9 (1)	-1.9 (-8)

Table 5.8 Correlation of measured N₂O and NO fluxes with soil variables

	Gas	WFPS	pH	NO ₃ ⁻	NH ₄ ⁺	WEOC	Ts
OG	N ₂ O	-0.0442	-0.0958	0.2875 ^{**}	0.3384 ^{**}	-0.0666	0.2777 ^{**}
	NO	-0.2896 ^{**}	0.0369	-0.0833	-0.0558	0.0472	0.1705 ^{**}
Cornfield	N ₂ O	-0.0163	-0.0813	0.0758	-0.0255	-0.0701	0.1591 ^{**}
	NO	-0.1439	-0.0384	0.0172	0.1792 [*]	0.0307	0.1636 ^{**}
NG	N ₂ O	0.0837	-0.1374	0.2860 ^{**}	-0.0162	-0.0181	0.1328
	NO	-0.1586	0.0384	-0.0164	0.0820	0.0686	0.0050

WFPS=water filled pore space, WEOC=water extractable organic carbon, Ts=soil temperature at 5cm depth. OG is old grassland, NG is new grassland. Double star (**) means $p < 0.01$, and single star (*) means $p < 0.05$.

5.3.7 Factors controlling N₂O and NO emissions

Daily N₂O fluxes were influenced by soil temperature, precipitation, soil pH, moisture content, and N supply (Table 5.8). In OG, the instantaneous N₂O fluxes had significant positive correlations with soil temperature ($p < 0.001$), NO₃⁻ concentration ($p < 0.01$) and NH₄⁺ concentration ($p < 0.001$) and non-significant negative correlations with WFPS, soil pH and WEOC. In the cornfield, correlations were positive with NO₃⁻ (ns) and soil temperature ($p < 0.001$), and negative but non-significant with NH₄⁺, WFPS, pH and

WEOC. In NG, N₂O correlated positively with soil temperature, NH₄⁺ concentration and NO₃⁻ ($p < 0.01$), and negatively with soil pH ($p < 0.05$), WEOC ($p < 0.05$) and WFPS (ns).

Annual N₂O emissions, in all three land-uses, increased with total N input and surplus N in the soil ($p < 0.05$). Annual precipitation had a significant positive linear correlation with annual N₂O emission in cornfield and an exponential relationship in NG (Fig. 5.5). Soil pH showed a negative correlation with annual N₂O emission, but it was significant only in cornfield (Fig. 5.6). However, the ratio of surplus N emitted as N₂O (N₂O-N/surplus N) had a stronger negative correlation with soil pH in all three land-uses (Fig. 5.6).

The ratio of WEOC to soil nitrate (WEOC/ NO₃⁻) was the major driver of changing N₂O emission as the land-use changed (Fig. 5.7). The WEOC/ NO₃⁻ ratio explained 78% of changes in annual N₂O emission as land-use changed in the control plot (Table 5.9).

Nitric oxide fluxes only showed significant correlations with WFPS (negative) in all treatments and with soil NO₃⁻ and NH₄⁺ (positive) in F and MF plots ($p < 0.05$; Table 5.8). N addition was the single most important factor affecting annual NO emissions. Multiple regression analysis of NO fluxes (all data combined) with soil variables (pH, WFPS, WEOC, NH₄⁺, NO₃⁻, temperature) showed that only NH₄⁺ and WFPS were significant predictors ($\text{NO} = 0.026 \text{ NH}_4^+ - 0.364 \text{ WFPS} + 31.923$; $R^2 = 0.05$; $p < 0.001$).

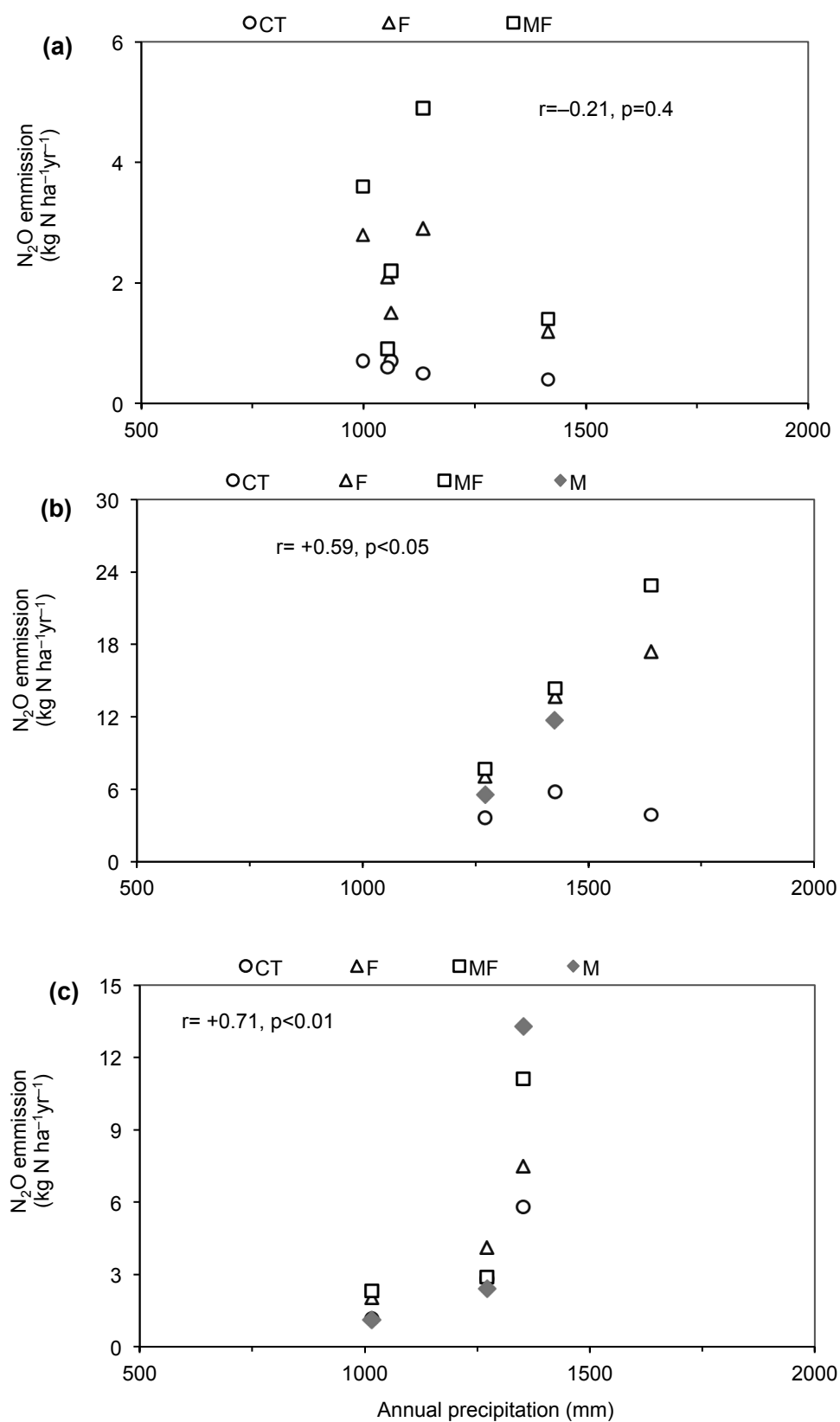


Figure 5.5 Relationship between annual N_2O emission and annual precipitation in old grassland (a), cornfield (b) and new grassland (c). CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot.

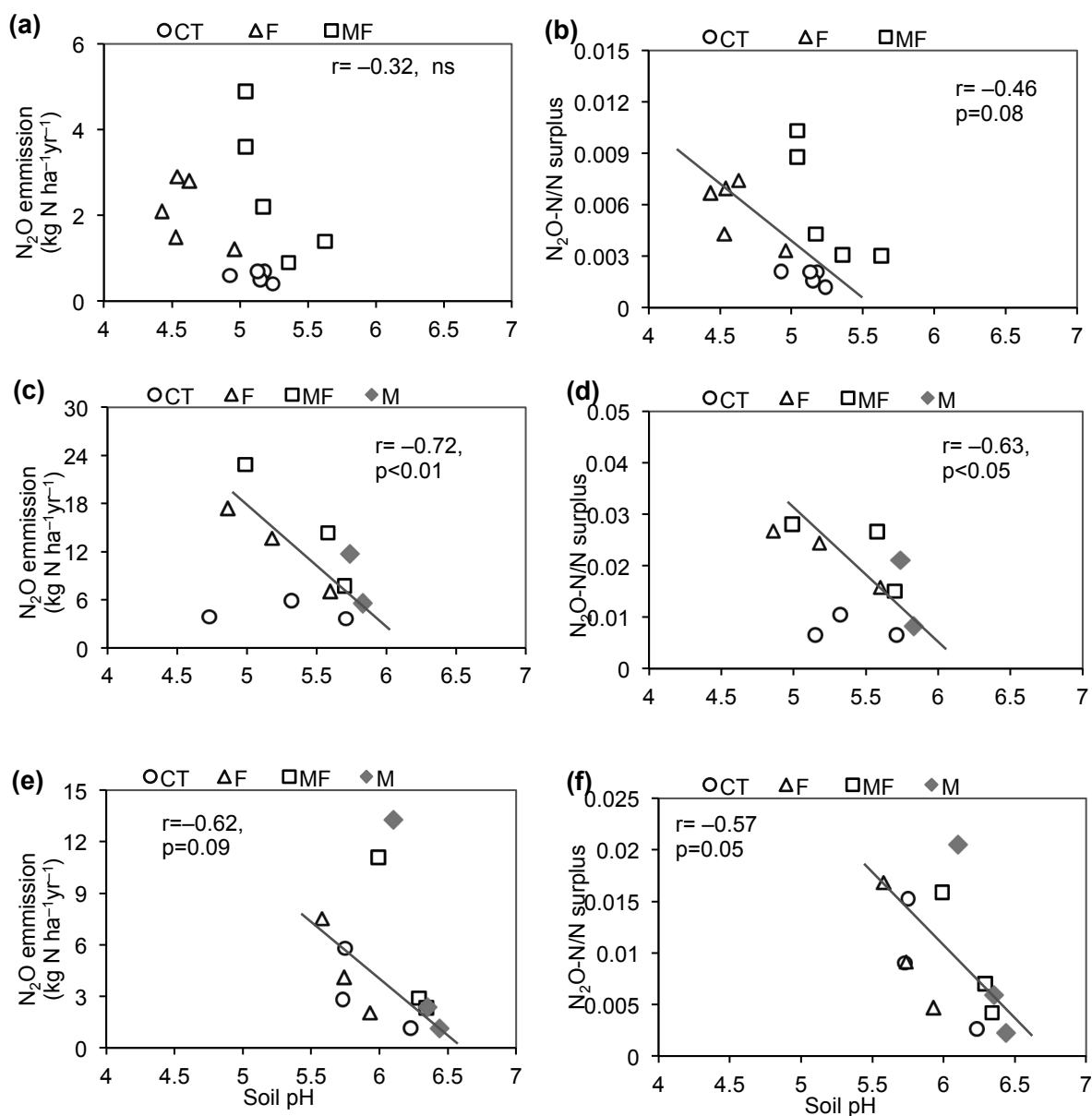


Figure 5.6 Relationship between annual N_2O emission and soil pH and ratio of annual nitrogen emitted as N_2O (N_2O-N) to surplus nitrogen and soil pH in old-grassland (a,b), in cornfield (c,d) and in new-grassland (e,f). CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot.

5.3.8 Heterotrophic soil respiration (RH), mineralized N, plant N uptake and surplus N.

Total RH and total mineralized N were higher in manure-amended plots than F and CT plots, and higher in cornfield than OG and NG ($p<0.05$) (Table 4.2). Plant N uptake in OG and cornfield was not statistically different, but was higher than in NG ($p<0.01$) (Table 5.10).

Surplus soil N in cornfield was higher than in both OG and NG ($p < 0.01$), and higher in NG than OG ($p < 0.05$) (Table 5.10). Chemical fertilization significantly increased plant N uptake ($p < 0.05$)

Table 5.9 Multiple and single linear regression models accounting for change in annual N₂O emission with changing land-use in the unfertilized control plots (CT), chemical fertilizer plot (F) and manure and chemical fertilizer plot (MF).

Treatment†	Variable§	Coefficient	SE	<i>p</i> value	Model R ²
CT	WEOC/NO ₃ ⁻	-0.006	0.002	0.018	0.78
F	WEOC/NO ₃ ⁻	-0.018	0.002	0.001	0.93
	Rainfall	0.011	0.001	0.004	
	pH	-0.705	0.197	0.023	
MF	WEOC/NO ₃ ⁻	-0.024	0.008	0.016	0.55

†Annual N₂O data were transformed using natural log transformation: $\ln(N_2O+1)$

§WEOC/NO₃⁻ is the ratio of the mean annual soil water extractable carbon to soil nitrate, Rainfall is total annual precipitation, soil pH is mean annual values, SE is standard error.

5.4 Discussion

5.4.1 Temporal variation in N₂O and NO emissions

The N₂O fluxes in this study were highly variable and peak emissions occurred either after N addition or after high rainfall. In OG and the last 2 years of NG, all peak emissions occurred after N addition, with less influence of rainfall. In cornfield and the first year of NG rainfall had a larger impact on peak emissions than N addition did. These differences in the response of peak N₂O fluxes among the three land-uses were presumably due to differences in soil mineral N content, aeration and redox conditions. High NO₃⁻ content in cornfield and in the first year of NG provided substrate for denitrifiers, while high precipitation created favourable conditions for denitrification. Occurrence of high rainfall when WEOC/NO₃⁻ ratio was high, in OG and in the last 2 years of NG, would have favoured complete denitrification to N₂ gas and hence lower N₂O fluxes (Burchill et al. 2014; Iqbal et al. 2015).

In OG N₂O peaks following N application were higher and lasted longer after the second fertilization in summer compared to the first application in spring in the F plot. In MF and M plots the peaks were higher in spring when both manure and chemical fertilizer were applied. Manure applications enhance microbial activity, which reduces soil O₂ levels, creating conditions that favour N₂O emissions (Collins et al. 2011; Zhang et al. 2014a), which could explain the observed differences between F and manure plots.

The timing of peak NO fluxes was similar to that of N₂O despite being much smaller in magnitude (Fig. 5.4), which should be expected as both gases are mainly the products of nitrification and denitrification processes and are driven by similar abiotic factors (Davidson et al. 1993; Skiba et al. 1997; Yan et al. 2013; Medinets et al. 2015). Smaller peak NO fluxes relative to N₂O are in agreement with results reported by Yan et al. (2013) of -8 to 169, -10 to 1953 and -3 to 1519 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$ compared to -9 to 111, -11 to 1000 and -11 to 716 $\mu\text{g NO-N m}^{-2} \text{ hr}^{-1}$ at 0, 600 and 200 kg N ha⁻¹ fertilization rate, respectively, in a wheat-maize rotation field study. Wang et al. (2011) and Zhu et al. 2013 also reported lower NO than N₂O fluxes in incubation studies. In this study, the peak N₂O-N fluxes were up to 200 times higher than the peak NO-N fluxes which is significantly higher than those of Wang et al. (2011). However, the higher peak N₂O than NO fluxes found in this study are contrary to results from other studies (Smith et al. 1997; Akiyama et al. 2000; Akiyama and Tsuruta 2002) which reported up to 20 times more NO-N than N₂O-N. This contradiction among different studies could be due to differences in soil moisture and fertilizer types (Smith et al. 1997; Akiyama et al. 2000). When WFPS is greater than 60%, denitrification, which produces more N₂O than NO, is predominant (Davidson et al. 1993; Smith et al. 1997) and diffusion of NO is limited, which allows further consumption of NO by denitrification (Skiba et al. 1997; Smith et al. 1997). The average WFPS value in this study was above 70%. Studies that reported higher NO-N than N₂O-N emission (Smith et al. 1997; Akiyama

et al. 2000; Akiyama and Tsuruta 2002) used urea-based fertilizers singly or in combination with other fertilizer types. Urea-based fertilizers emit more NO (Skiba et al. 1997) and this is related to stimulation of nitrification, which leads to higher NO_x by C from urea (Slemr and Seiler 1991). NH₄⁺ based fertilizer was used in this study. Akiyama et al. (2000) and Akiyama and Tsuruta (2002) used an automated system to measure NO and N₂O fluxes every 4 hours while in our study the gas samples were collected and measure manually once every 1–2 weeks. This difference in methods could also explain the observed differences.

Few studies have reported long-term data of N₂O and NO emissions. There was up to a 10-fold difference in inter-annual N₂O emissions within each land-use and treatment in this study. Differences in annual NO emissions were as high as 6 times. This high variation in annual emissions emphasises the need for long-term studies to reduce uncertainties associated with chamber flux measurements for individual sites.

5.4.2 Influence of N application on N₂O and NO emissions

The N₂O emissions in fertilizer and manure-amended plots were 3-4, 2-5 and 1.4-2 times higher than in the control treatment in OG, cornfield and NG, respectively (Fig. 5.3 and Table 5.3). These results are similar to those of Mosier et al. (1991) who reported an increase of 2-3 times in N₂O emission due to fertilization (20–40 kg N ha⁻¹ as NH₄NO₃) in native grassland and wheat prairies in the USA. Several studies have reported increased N₂O emission with manure and fertilizer applications (Mu et al. 2006; Alluvione et al. 2010; Collins et al. 2011; Ryals and Silver 2012; Zhang et al. 2014a). Manure applications enhance microbial activity, which reduces soil O₂ levels, creating conditions that favour N₂O emissions (Collins et al. 2011; Zhang et al. 2014a).

Our results indicate that soil organic matter mineralization and plant N uptake are important parameters affecting N₂O-N emissions as shown by the significant positive relationship

between N₂O emissions and surplus N and total N input. Therefore, soil organic matter decomposition and plant type should be included when evaluating the emission factors of different soils.

Chemical fertilizer and long-term manure application had a significant influence on soil properties such as pH, mineral N content and organic carbon content (Fig. 5.1). Soil pH was significantly decreased by chemical fertilizer application and increased by long-term manure application. Manure application increased and maintained soil pH probably due to the high pH of the manure (manure pH was around 7). The second reason is that manure increases the buffering capacity of soils due to the presence of carboxyl and phenolic hydroxyl groups in the manure (Whalen et al. 2000). The negative relationship between pH and N₂O emission (Fig. 5.6) suggests that under similar conditions, long-term manure could have benefits of reducing N₂O emissions indirectly by increasing soil pH, while the opposite is true for chemical fertilizer.

Nitric oxide fluxes were stimulated just after fertilization similar to many published reports (Skiba et al. 1997; Akiyama and Tsuruta 2002; Bouwman et al. 2002a; Cui et al. 2012). Although annual NO emissions were higher in inorganic N fertilized plots, regression analysis showed a non-significant increase in annual NO emissions with increasing N input, which disagrees with other studies (Cui et al. 2012; Yan et al. 2013) that have reported a significant linear relation between annual NO emissions and fertilizer N input. One possible explanation for this seemingly non-significant response of annual NO emissions N input is that high moisture content in our site limited the diffusion of NO to the surface (Firestone and Davidson 1989; Skiba et al. 1997; Medinets et al. 2015) which in turn increases the likelihood of NO consumption in the soil by denitrification (Aneja et al. 1996; Akiyama and Tsuruta 2003; Yao et al. 2010; Pilegaard 2013).

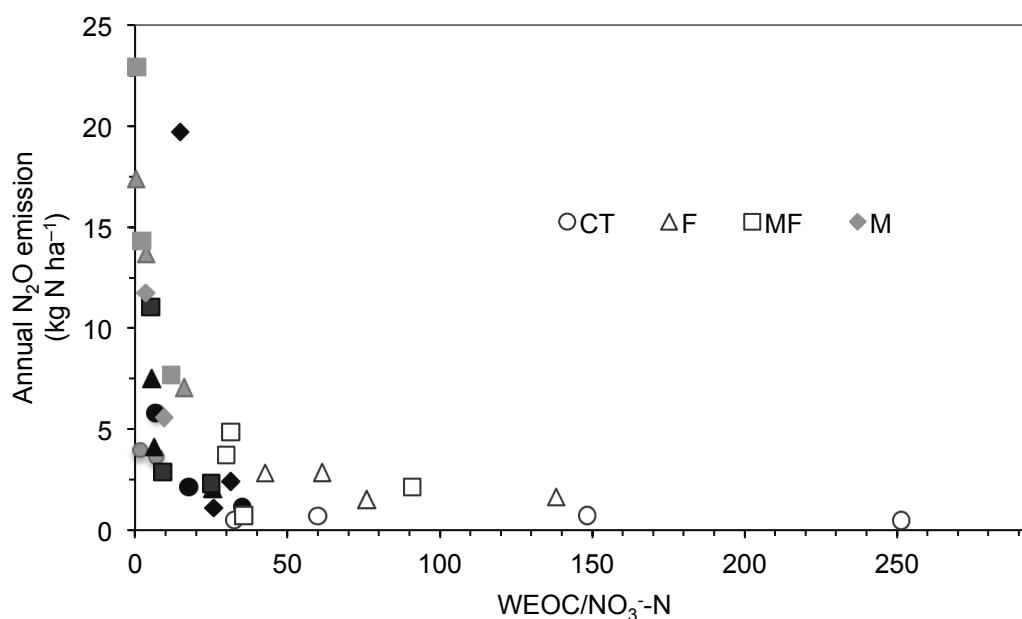


Figure 5.7 Relationship between annual N_2O emission and the ratio of mean water extractable organic carbon to mean soil NO_3^- ($\text{WEOC}/\text{NO}_3^-$). Data in white symbols is in old grassland, grey symbols in cornfield and black symbols in new grassland. CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot.

5.4.3 Soil and environmental factors controlling N_2O emissions

As expected, total N input and surplus N, NO_3^- and NH_4^+ concentrations in the soil were important controlling factors. In cornfield and NG in 2013, the highest N_2O fluxes were recorded following rainfall higher than 40 mm in one day. Other factors such as tillage (Chapin et al. 2011; Li et al. 2015), oxygen availability (Firestone and Davidson 1989; Venterea et al. 2005; Iqbal et al. 2015) and precipitation (Koga et al. 2004) are more important when inorganic N is not limiting in the soil, and hence were very important factors in cornfield. In this study, the higher soil mineral N content (both NO_3^- and NH_4^+) in cornfield and NG even in the control treatment without any N addition, could have been due to enhanced mineralization resulting from tillage (Shimizu et al. 2013). The higher heterotrophic respiration values observed in cornfield and NG compared to OG supports this claim (Table 4.2).

Table 5.10 Plant N uptake and surplus N (kg ha⁻¹ yr⁻¹).

		CT	F	MF	M
2005	Plant N uptake	106.3	231.3	185.3	—
2005	Surplus N	338.2	377.2	409.3	—
2006	Plant N uptake	106.3	194.0	178.3	—
2006	Surplus N	319.7	415.0	474.4	—
2007	Plant N uptake	116.9	179.0	146.9	—
2007	Surplus N	336.8	348.7	512.4	—
2008	Plant N uptake	85.0	130.5	118.9	—
2008	Surplus N	285.4	313.8	293.6	—
2009	Plant N uptake	81.5	145.5	139.9	—
2009	Surplus N	335.2	362.6	459.1	—
2010	Plant N uptake	54.8	106.5	159.0	—
2010	Surplus N	591.9	651.5	817.4	—
2011	Plant N uptake	57.3	117.8	193.6	95.2
2011	Surplus N	555.4	560.6	538.3	558.1
2012	Plant N uptake	86.5	109.9	218.5	113.5
2012	Surplus N	555.7	448.3	507.0	680.0
2013	Plant N uptake	35.2	49.3	75.2	59.4
2013	Surplus N	380.1	446.3	699.1	648.2
2014	Plant N uptake	63.2	108.6	109.7	80.7
2014	Surplus N	313.3	448.3	412.1	403.6
2015	Plant N uptake	43.8	145.3	62.6	66.3
2015	Surplus N	433.3	439.8	546.3	491.7
ANOVA		Plant N uptake		Surplus N	
	d.f.	MS	F	MS	F
Plot	4	9909	8.68**	21202	2.89*
Land-use	2	13043	11.43**	128953	17.6**

CT is control plot; F is chemical fertilizer plot; MF is combined chemical fertilizer and manure plot; M is manure only plot. Surplus N was calculated as difference between total N input (total mineralized N from soil organic matter and manure, and chemical fertilizer N) and the plant N uptake.

**p<0.01, *p<0.05

Effects of soil moisture and rainfall on N₂O production have been reported by many studies (Mosier et al. 1991; Choudhary et al. 2001; Sehy et al. 2003; Alluvione et al. 2010). High N₂O fluxes associated with high soil moisture were likely to have come primarily from denitrification (Sehy et al. 2003; Alluvione et al. 2010; Shimizu et al. 2013). Precipitation enhanced N₂O emission due to stimulation of substrate diffusivity and microbial activity with increased soil moisture content (Kusa et al. 2002; Bateman and Baggs 2005), reduced oxygen diffusivity (Saggar et al. 2013) and the resulting increase in denitrification (Saggar et al. 2013; Li et al. 2015). A negative but non-significant correlation between annual N₂O emissions and precipitation in OG was found. In 2009, when the highest rainfall was recorded in OG, N₂O emissions were very low. This could be due to lower total N input (Table 3.3) and surplus N (Table 5.10) and also lower NO₃⁻ and NH₄⁺ concentrations in OG (Fig. 5.1). Another reason could be that high rainfall in grassland, given the limited drainage in our site and high available carbon relative to NO₃⁻ (Fig. 5.1), might have promoted complete denitrification ((Burchill et al. 2014; Iqbal et al. 2015).

The amount of surplus N emitted as N₂O (N₂O-N/surplus N) had a much stronger negative correlation with soil pH than just N₂O-N and pH in all three land-uses (Fig. 5.6). These results suggest that it's the excess (surplus) N in the soil that is much more influenced by soil conditions and transformed to N₂O. This is supported by a significant positive correlation between N₂O emissions and surplus N. A negative relationship between N₂O and soil pH has been reported by a number of studies (Clough et al. 2004; Pan et al. 2012). Increased activity of N₂O reductase enzyme relative to activities of NO₃⁻ and NO₂⁻ reductase enzymes at high pH may be the main reason for the low N₂O at high pH (Pan et al. 2012). However, this result is contrary to the increased cumulative N₂O production with increasing

pH in grassland and forest soils in Canada reported by Cheng et al. (2013) who unlike our study found that nitrification was the main source of N_2O .

Multiple regression analysis showed that soil moisture and NH_4^+ concentration were the key factors regulating NO fluxes (see section 5.3.6), although NO fluxes showed strong positive correlation with temperature and NO_3^- concentration as single factors (Table 5.8). The negative correlation of NO with WFPS is consistent with the reported impediment of the diffusion of NO at high moisture content and thereby allowing NO consumption (Davidson et al. 1993; Medinets et al. 2015). The fact that NH_4^+ showed a stronger controlling effect on NO than NO_3^- agrees with reports that nitrification was the major source of the NO fluxes (Cui et al. 2012). However, Skiba et al. (1997) reported that denitrification produces more NO than nitrification but net release of NO from denitrification is lower due to impediment of NO diffusivity and NO consumption by denitrifiers.

5.4.4 Importance of winter and thawing periods N_2O and NO emissions.

Winter emissions contributed as much as 35% and 55% in N_2O and NO emissions respectively (Tables 5.4 and 5.6). The contribution of winter N_2O emissions was higher when manure was applied in autumn in cornfield compared to spring in grassland. Winter sampling was done twice or once a month and therefore these values might have been underestimated. However, this study clearly shows that winter emissions contribute a significant amount to annual emissions and this calls for more intensive sampling and inclusion of winter emissions in annual budgets.

The two-months long thawing period (March to early May) contributed as much as 60% to annual emissions in some years (Tables 5.5 and 5.7). In the control plots, thawing period emissions were even more important compared to the other plots. The N_2O emissions increased following soil melting and as soil temperatures became warmer. The high fluxes in

this period could be due to high accumulation of N₂O through denitrification during the freezing period and the physical release as the snow melts (Burchill et al. 2014) and low N₂O reduction rate during thawing (Sehy et al. 2003; Katayanagi and Hatano 2012). Peaks of N₂O emissions in the thawing period may also be due to enhanced mineralization of easily decomposable organic substrates by increased microbial activity (Wu et al. 2010).

5.4.5 Effect of land-use type on N₂O and NO emissions

In this study, the average annual N₂O emissions in grassland (OG and NG) ranged from 0.4 to 4.9 kg N ha⁻¹yr⁻¹, except in 2013 in NG when emissions ranged from 5.8 to 13.3 kg N ha⁻¹yr⁻¹. The high N₂O emissions in NG in 2013 may have been due to ploughing twice, in May and September and reseeding of the grass. Higher precipitation in 2013 just after ploughing and seeding in spring may have further stimulated N₂O emissions. The average annual N₂O emissions in cornfield ranged from 3.6 to 22.9 kg N ha⁻¹ yr⁻¹, and they were significantly higher than values reported by Alluvione et al. (2010) in Italy of 3.9 to 8.7 and 3.9 kg N₂O–N ha⁻¹ yr⁻¹ in a cornfield and those of Choudhary et al. (2001) who found mean values of 2.3 to 3.4 kg N₂O–N ha⁻¹ yr⁻¹ in a silt clay loam soil under cornfield and permanent pastures. Higher N input and precipitation in this study could explain the observed differences in the N₂O emissions.

Higher N₂O emissions in cornfield compared to OG and NG were probably due to higher soil NO₃⁻ concentrations (Fig. 5.1), higher heterotrophic soil respiration and consequently higher mineralized N and higher surplus N. Furthermore, the perennial plants, in grassland, were always in the field and hence capable of taking up available soil N, especially in spring. In the cornfield on the other hand, there was no plant uptake of available N in early spring and autumn, and yet manure was applied in autumn and chemical fertilizer at the time of seeding. This lack of N uptake by plants in some periods, and hence

the lack of synchronisation of plant uptake and soil N availability in some periods, combined with higher precipitation, could have led to overall higher annual emissions in cornfield (FAO and IFA 2001; UNEP 2013; Iqbal et al. 2015). Tillage activities which were conducted every year in the cornfield further influenced heterotrophic soil respiration, N mineralization and hence N₂O emissions. Increasing N₂O emissions due to tillage activities has been reported by several studies (Ruan and Philip Robertson 2013; Palm et al. 2014; Yonemura et al. 2014). The cornfield emissions were not significantly different from NG emissions of 2013 when tillage was conducted.

The average N₂O emissions over the whole study period were higher in NG than OG (Fig. 5.3 and Table 5.3). This could be attributed to higher NO₃⁻ concentration (Fig. 5.1), lower plant N uptake and as a result higher surplus N in NG compared to OG (Table 5.10). This means more applied N in OG was taken up by the plant hence acting as a sink for N (Velthof et al. 2010; Necpálová et al. 2013; Iqbal et al. 2015). In this study, tillage activities and very high precipitation in 2013 in the NG may have played a part in the observed higher emissions. In 2014, the emissions were much lower in NG and by 2015 (3 years after establishment of new conversion) N₂O emissions in NG were not significantly different from those in OG. Our results suggest that within 3 years after conversion from annual cropland to managed grassland, significant reductions in N₂O emissions could be achieved.

Soil NO₃⁻ concentrations in the cornfield and 2013 in NG were significantly higher than in OG, while the WEOC did not differ significantly among the land-use types (Fig. 5.1). The ratio of WEOC to NO₃⁻-N was highest in OG and lowest in cornfield. High abundance of NO₃⁻ relative to labile organic carbon favours N₂O release over N₂ (Firestone and Davidson 1989; Chapin et al. 2011; Iqbal et al. 2015). This is because high NO₃⁻ (electron acceptor) will lead to depletion of the relatively less abundant, electron donor (carbon) (Iqbal et al. 2015) resulting in incomplete denitrification and accumulating higher amounts of N₂O

in the soil. Lower NO_3^- , on the other hand may stimulate the reduction of N_2O to N_2 (Firestone and Davidson 1989; Iqbal et al. 2015). Our results as shown in figure 5.7 are in agreement with this interpretation.

This study shows no significant differences in NO emissions among the three land-uses. This finding is supported by Van Lent et al. (2015). Skiba et al. (1997) reviewed several papers and found conflicting reports of land-use effect on NO emissions. However, other studies have reported lower NO emissions in grassland compared to cornfield and attributed this to greater N-use efficiency due to longer growing seasons in grasslands (Bouwman et al. 2002b).

5.5 Conclusions

Annual N_2O emissions in cornfield were 6–7 times higher than in OG and 1.5–3 times higher than in NG, and NG had 2–5 times higher N_2O emissions than OG. Higher cornfield emissions compared to grassland, and higher emissions in NG compared to OG were due to higher available soil mineral N relative to labile SOC that could have led to incomplete reduction of NO_3^- to N_2 , producing more N_2O in the process. Lack of synchronisation of N availability in the soil and plant N uptake may have further led to the high emissions in the cornfield as well as in the first year of NG. Within the first year of converting grassland to cornfield N_2O emissions increased by more than 500% and remained high 3 years later, while after converting cornfield to new grassland N_2O emissions significantly declined within 3 years. Peaks of N_2O flux following fertilization were heavily influenced by land-use and interacted strongly with rainfall. Nitric oxide emissions were more influenced by nitrogen addition than soil and weather variables.

Winter and thawing period N_2O and NO emissions contributed significantly to annual emissions, highlighting the need for a high frequency of measurements in these

periods. There was up to a 10-fold difference in inter-annual N₂O emissions within each land-use and treatment in this study. Differences in annual NO emissions were as high as 6 times. This high variation in annual emissions emphasises the need for long-term studies to reduce uncertainties associated with chamber flux measurements for individual sites.

6. Mitigating global warming potential and greenhouse gas intensities by applying composted manure in cornfield

6.1 Introduction

A number of studies have been done to evaluate how management practices, such as tillage, manure and fertilizer application, affect GHG emissions (Smith et al. 1997; Rochette et al. 2004; Huth et al. 2010; Cui et al. 2012; Sommer et al. 2015; Barton et al. 2016; Bayer et al. 2016). Adequate fertilization and manure application have been reported to increase soil organic carbon (SOC) through increased biomass production and through direct C input from manure and residue retention in soils (Jarecki et al. 2005; Rees et al. 2005; Huang et al. 2013; Lentz and Lehrs 2014; Ryals et al. 2014; Limin et al. 2015). On the other hand, both manure and fertilizer application can increase N_2O and CH_4 emissions (Clayton et al. 1997; Acton and Baggs 2011; Shimizu et al. 2013) and partly offset the benefits of increased SOC sequestration. Therefore, the overall change in GHG emissions and SOC sequestration should be evaluated when assessing the benefits of any management practice.

To easily compare the effects N_2O , CH_4 and CO_2 emitted in the atmosphere, CO_2 -equivalents (the radiative forcing relative to CO_2 over a 100-year period) are widely used. Net global warming potential (GWP), the sum of CO_2 -equivalents of N_2O , CH_4 and CO_2 , is a good measure of the overall exchange of GHGs in a system (Robertson and Grace 2004). Agricultural ecosystems do not only emit CO_2 but also uptake (remove) CO_2 from the atmosphere through plant photosynthesis. Some of the CO_2 removed through photosynthesis may end up in the soil as SOC. Some researchers have therefore used change in SOC; rather direct CO_2 , when calculating net GWP from agricultural ecosystems. However, direct measurement of SOC content in most cases only detects changes in SOC over 5-10 years time scales (or even longer) and is not sensitive enough to account for small seasonal and annual changes (Zheng et al. 2008). Net ecosystem C balance (NECB) has been proposed to

more accurately account for changes in C accumulation in an ecosystem (Chapin et al. 2006; Zheng et al. 2008). Despite having some limitations such as being influenced by annual climatic variations (Chapin et al. 2006), by measuring the C input, C output and net CO₂ flux from an ecosystem to the atmosphere, NECB is one of the best methods for measuring C changes in cropland over shorter periods such as annually (Yang et al. 2015). Therefore, NECB, in addition to N₂O and CH₄, is used to calculate net GWP in this study.

Since the ultimate goal should be to reduce GHG emissions while being able to increase crop productivity, net GWP must be compared to the crop yield. Greenhouse gas intensity (GHGI), which is calculated by dividing GWP by the crop yield (Mosier et al. 2006), can give a good idea of whether a given management practice can increase productivity without increasing GHG emissions. While many studies have reported the effect of inorganic fertilizer and manure application on CO₂, CH₄ and N₂O emissions, very few have evaluated their effect on GWP and GHGI.

The amount as well as the timing of manure and fertilizer application influences both crop productivity and GHG emissions. The readily availability of inorganic fertilizer N makes it easy to be taken up by plants but also to be easily transformed by microbial processes into N₂O and other gaseous forms. On the other hand, when composted, animal manure is a slow-release fertilizer and it is widely considered to be a good substitute for inorganic fertilizer (Yang et al. 2015). However, low nutrient content of composted manures, compared to inorganic fertilizers, could lead to reduced crop yields. It is therefore important to evaluate the ability of manures to maintain yields if they are to be a real alternative to inorganic fertilizers.

We hypothesized that (1) manure application can increase C sequestration and offset increase in non-CO₂ GHGs; (2) applying manure twice (in autumn and spring) can achieve the same amount of crop productivity compared to conventional inorganic fertilizer (in

spring) without increasing N₂O emissions. To test our hypotheses we initiated a field experiment in 2010 to gain insights into the effects of manure and inorganic fertilizer on GHG emissions, GWP and GHGI in a cornfield. We also explore the major factors driving GHG fluxes in the soil.

6.2 Materials and Methods

Two experiments were set up as described in detail in chapter 3. A brief description is given below

6.2.1 Experiment 1: Effect of manure and inorganic fertilizer

Field measurements were conducted from October 2009 to October 2012 in inorganic fertilizer only (CT), inorganic fertilizer only (F), inorganic fertilizer plus composted cattle manure applied since 2005 (MF1), inorganic fertilizer plus composted cattle manure applied since 2010 (MF2) and manure only (M) plots. Composted cattle manure was applied at the end of October in 2009, 2010 and 2011 and the field ploughed (30 cm depth) within two to three weeks after manure application. The average amount of manure applied in each year was 40 Mg fresh matter representing 390 kg total N and 6.8 Mg total C per hectare. In early May 2010, 2011 and 2012, the field was disc harrowed, after which maize was seeded and inorganic fertilizer (104 kg N as NH₄PO₃, 139 kg phosphorous (P) as P₂O₅ and 77 kg potassium (K) as K₂O per hectare) applied on 18th May in 2010 and 2011 and on 21st May in 2012 in the respective plots. Lime was applied in all the treatment plots at the rate of 300 kg CaCO₃ ha⁻¹.

6.2.2 Experiment 2: Effect of additional spring manure application.

A second experiment was conducted for one year from October 2012 to September 2013. In this experiment, three plots were set up to assess the effect of additional manure application in spring season on plant growth and net GHG emissions. The treatments were autumn composted cattle manure application (M1), autumn composted cattle manure application and additional spring inorganic fertilizer application (MF) and autumn composted cattle manure plus additional spring manure application (MM). Autumn manure application was done in November 2012 (40 Mg fresh matter: 450 kg N and 7.3 Mg C per hectare). In May 2013 (spring), additional 29 Mg fresh manure was applied in the MM plot (300 kg N and 5 Mg C per hectare), and inorganic fertilizer (104 kg N, 139 kg P and 77 kg K per hectare) was applied in the MF plot. Lime at the rate of 300 kg CaCO₃ ha⁻¹ was applied in all the plots.

6.2.3 Total soil N and soil C analysis

In 2010 and 2012, we collected soils in the F, MF1 and MF2 plots at 0–5, 5–15 and 15–30 cm soil depth to measure total soil N and SOC stored each soil layer. These samples were collected at the end of the growing season. The samples were collected every 10 m in a grid covering the entire field resulting in at least 12 points for each plot. Total N and C were analysed using a C/N analyser.

6.2.4 Greenhouse gas flux measurement

The GHGs flux measurements were conducted using the static closed chamber method and samples analysed as described in chapter 3. Cumulative annual emissions were calculated by linear interpolation between each 2 successive sampling events and numerical integration of these areas using the trapezoid rule (Ussiri et al. 2009).

From the cumulative annual N₂O emissions, we calculated N₂O emission factor from inorganic fertilizer N (EF_i) and manure (EF_m) using the equation below:

$$EF_i = (N_2O-N \text{ (F plot)} - N_2O-N \text{ (CT plot)}) / N \text{ application rate in F plot}$$

$$EF_m = (N_2O-N \text{ (M plot)} - N_2O-N \text{ (CT plot)}) / \text{manure N application rate}$$

The relationship between CO₂ flux and soil temperature was calculated using an exponential function. We then calculated the Q₁₀ values of soil CO₂ flux using equation below.

$$CO_2 \text{ flux} = \beta_0 \times \exp(\beta_1 \times T)$$

$$Q_{10} = \exp(10 \times \beta_1)$$

where T is the soil temperature at 5cm depth, β_0 and β_1 are coefficients of the CO₂ flux and temperature function.

6.2.5 Estimation of NECB, GWP and GHGI

Net ecosystem C balance (NECB) was calculated using the following equation (Zheng et al. 2008; Cui et al. 2014; Yang et al. 2015):

$$NECB = C \text{ input} - C \text{ output}$$

$$C \text{ input} = \text{manure C} + NPP$$

$$C \text{ output} = \text{Harvested C} + RH$$

where manure C is the total C in the applied manure, NPP is net primary production (measured as total C in aboveground and belowground dry plant biomass), harvested C is the total C removed through the harvested plant material and RH is heterotrophic soil respiration (measured as the soil CO₂-C from the plant excluded plots). The aboveground biomass was manually harvested by cutting the plant biomass at 8 replications (1.5m x 1m area). The belowground biomass was measured at 8 replications (1.5m x 1m area) by digging out the

corn roots and manually separating the roots from the soil by sieving using a 0.5 mm sieve and then by washing to completely remove the soil particles and other debris. The plant samples were oven-dried at 70°C for more than 72 hours and weighed. Each dried sample was analysed for total C and N contents with a C/N analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

Global warming potential (GWP) was estimated based on the following equation adopted from Zhang et al (Zhang et al. 2014a):

$$\text{GWP} = [E_{\text{CH}_4\text{-C}} \times 16/12 \times 25 + E_{\text{N}_2\text{O-N}} \times 44/28 \times 298] \times 12/44 - \text{NECB}$$

where GWP is the global warming potential (kg CO₂-C equivalents ha⁻¹ yr⁻¹), $E_{\text{CH}_4\text{-C}}$ and $E_{\text{N}_2\text{O-N}}$ are the annual emission rates of CH₄ -C and N₂O -N (kg ha⁻¹ yr⁻¹). The fractions 16/12 and 44/28 are used to convert the mass of CH₄-C to CH₄ and the mass of N₂O-N to N₂O. The constants 25 and 298 are the radiative forcing constants (CO₂ equivalents) of CH₄ and N₂O, respectively, relative to CO₂ over a 100-year time horizon (IPCC 2014). The fraction 12/44 was used to convert CO₂ equivalents to CO₂-C equivalents for easy comparison of NECB contribution to GWP.

By using the above equation, we measured the direct CO₂ equivalents from crop production and we did not consider indirect emissions such as those from fertilizer production and farm operations.

The corn from our study site was used for silage and therefore the GHGI was calculated as GWP divided by biomass yield:

$$\text{GHGI} = \text{GWP} / \text{Yield}$$

6.3 Results

6.3.1 Soil and environmental variables

Monthly average air temperatures ranged from -10.7°C to 30.5°C with the lowest temperatures observed in February, while the maximum temperatures were recorded between July and September. Average air temperatures were less than 0°C from December to March. Soil temperature followed the same trend with air temperature. The soil temperature ranged from -4.0°C to 27°C (Fig 6.1a).

Average monthly precipitation ranged from 5 to 224 mm. The driest months with lowest precipitation were from January to March and the wettest months were from July to September. Annual precipitation was 1120, 1006 and 1066 mm in 2010, 2011 and 2012 respectively.

Soil NO_3^- concentrations increased in May after fertilizer application and were higher in MF2, MF1 and F plots compared to CT and M plots (Fig 6.1b). Short-lived peaks in NO_3^- concentration were sometimes observed after manure application. Nitrate concentrations were higher in 2010 in all plots and lowest in 2012. In the unfertilized control plot, average NO_3^- concentration was 60 mg kg^{-1} in 2010 but decreased to 12 mg kg^{-1} in 2012. The high NO_3^- concentrations 2010 could have resulted from N mineralization, as this was the first year after the conversion from grassland to cornfield.

Ammonium concentrations were highest in 2012 and very low concentrations were observed in 2010. Overall very high variations in NH_4^+ concentration were observed with values ranging from 0 to 900 mg N kg^{-1} . Highest NH_4^+ concentrations were observed in spring just after soil melting and after fertilization and the peaks were more pronounced in MF2 and MF1 plots. The manure amended plots also showed the highest variations in NH_4^+ concentrations.

Water extractable organic C was significantly lower in 2010 and increased annually with highest concentrations found in 2012 (Fig. 6.1d). Water extractable organic C was higher in the manure-amended plots (MF1, MF2 and M) than the plots without manure application ($p < 0.01$).

Soil pH varied among the different treatments and increased from 2010 to 2012 due to liming. Soil pH was generally lower in MF2 and F plots compared to CT, MF1 and M plots. The average annual soil pH ranged from 4.8–5.6, 4.7–5.5 in F and MF2 plots, and 5.2–5.7, 5.0–5.7 and 5.7–5.8 in CT, MF1 and M plots, respectively.

Table 6.1 shows the total C and N contents measured at 0–5, 5–15 and 15–30 cm soil depth in the F, MF1 and MF2 plots. In all the plots, C and N contents were highest in the 0–5 cm soil depth and lowest in the 15–30 cm soil depth except in 2012 in F plot when the topsoil (0–5 cm depth) showed lower C content than the sub soil (15–30 cm depth). Soil C and N contents were higher in the manure-amended plots. MF1 plot which had been receiving manure for a longer period (since 2005) showed higher C and N contents than MF2 that only started receiving manure in 2009/2010 season. From the soil C content, we calculated the total C stock in the top 15 cm depth, and the result clearly shows higher C stock in manure-amended plots, although the differences were statistically significant only in 2012 (Table 6.2).

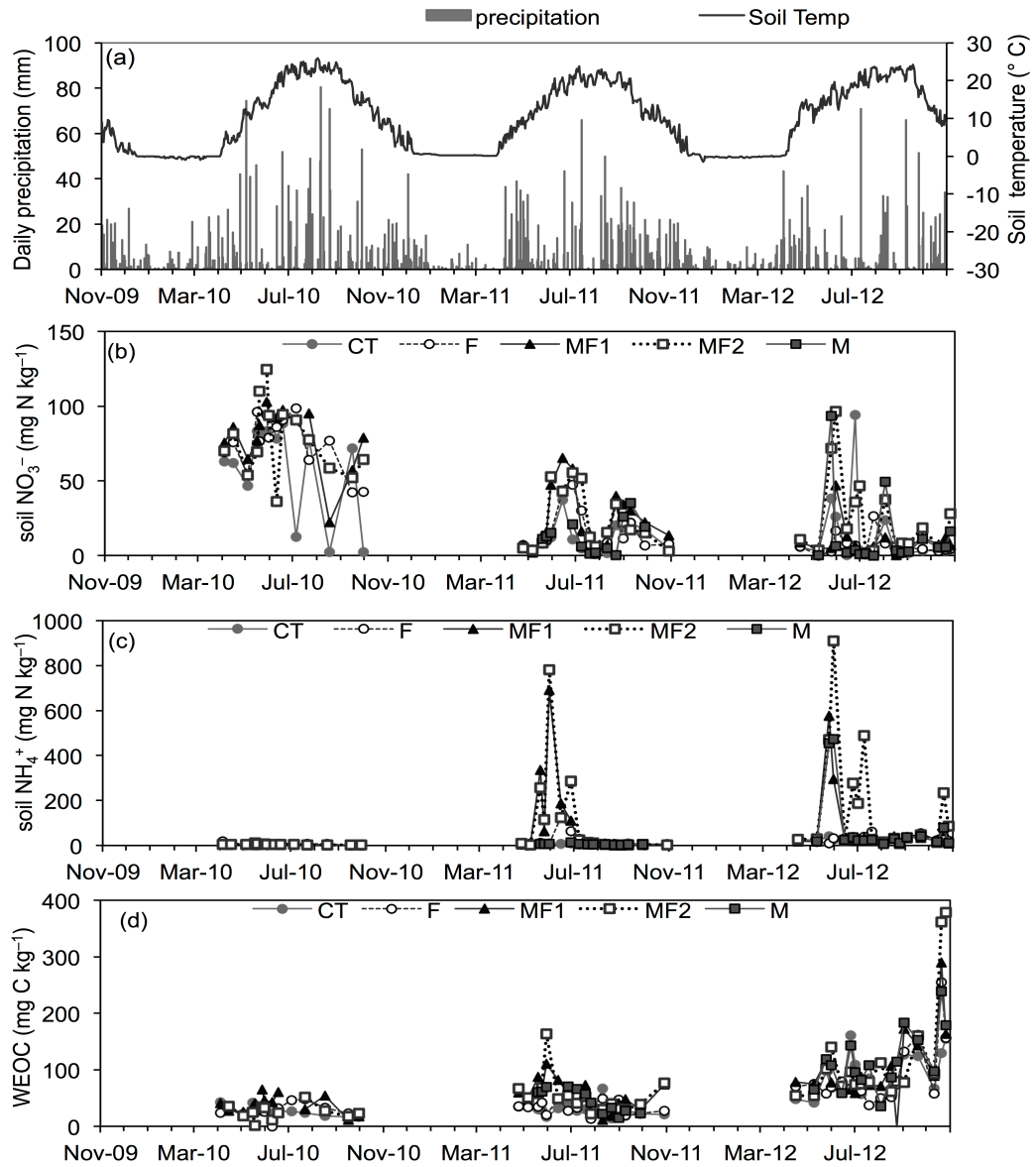


Figure 6.1 Daily average soil temperature and precipitation (a), soil NO_3^- concentration (b), soil NH_4^+ concentration (c) and water extractable soil organic carbon (WEOC) (d) from 2010-2012 in experiment 1. CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot

Table 6.1 Soil C and N content (% per kilogram of soil) in the F, MF1 and MF2 plots (in experiment 1) in each soil depth in 2010 and 2012.

		Organic carbon content (%)			Total nitrogen content (%)		
	Plot	0-5cm	5-15cm	15-30cm	0-5cm	5-15cm	15-30cm
2010	F	4.6±1.4 ^{abc}	4.6±1.0 ^{abc}	3.4±0.9 ^a	0.43±0.12 ^{abcd}	0.42±0.07 ^{abcd}	0.32±0.07 ^a
	MF1	6.7±2.0 ^e	6.5±2.8 ^{de}	4.5±1.3 ^{abc}	0.60±0.16 ^e	0.57±0.23 ^{de}	0.41±0.11 ^{ab}
	MF2	5.2±1.7 ^{abcde}	4.8±2.0 ^{abcd}	4.2±1.3 ^{ab}	0.47±0.14 ^{abcde}	0.45±0.18 ^{abcde}	0.40±0.11 ^{abc}
2012	F	4.4±1.0 ^{abc}	4.6±0.6 ^{abcd}	4.8±1.1 ^{abcde}	0.42±0.09 ^{abcd}	0.44±0.06 ^{abcde}	0.46±0.10 ^{abcde}
	MF1	6.1±1.1 ^{bcde}	6.2±1.4 ^{cde}	5.3±1.1 ^{abcde}	0.53±0.09 ^{bcde}	0.55±0.11 ^{cde}	0.46±0.08 ^{abcde}
	MF2	5.4±1.1 ^{abcde}	5.2±1.1 ^{abcde}	5.0±0.8 ^{abcde}	0.50±0.10 ^{abcde}	0.48±0.10 ^{abcde}	0.46±0.07 ^{abcde}

CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot. Different letters indicate significant differences among plots, depth and years.

Table 6.2 Total soil carbon stock (Mg C ha⁻¹) in the F, MF1 and MF2 plots in 2010 and 2012 (experiment 1)

Year	F	MF1	MF2
2010	84.3±10.3 ^a	110.9±15.9 ^{ab}	96.6±15.9 ^a
2012	107.3±16.6 ^a	127.6±16.7 ^c	119.8±15.7 ^{bc}

6.3.2 Greenhouse Gas Emissions-Experiment 1

6.3.2.1 Nitrous Oxide Emissions

Nitrous oxide fluxes were very episodic and highly varied within and across years (Fig 6.2a).

Intra-annual variations were highly influenced by mean daily temperature and rainfall.

As expected, N₂O emissions were stimulated by fertilization and fluxes were higher in the growing season compared to the non-growing season. However, the highest N₂O fluxes were observed several weeks after fertilization and occurred following rainfall events.

The highest fluxes in 2010 were found on June 18 in CT plot ($352 \mu\text{g N m}^{-2} \text{hr}^{-1}$) and on July 5 in F ($2461 \mu\text{g N m}^{-2} \text{hr}^{-1}$), MF1 ($2223 \mu\text{g N m}^{-2} \text{hr}^{-1}$) and MF2 ($4913 \mu\text{g N m}^{-2} \text{hr}^{-1}$). High rainfall was recorded on June 16 ($>30 \text{ mm}$) and more than 60 mm from July 1st to 4th. In 2011, rainfall of more than 50 mm was recorded between June 23 and 24, and rainfall more than 100 mm was recorded between July 14 and 16. The highest N_2O fluxes in 2011 were found on June 27 in F ($1757 \mu\text{g N m}^{-2} \text{hr}^{-1}$), CT ($638 \mu\text{g N m}^{-2} \text{hr}^{-1}$) and MF1 ($553 \mu\text{g N m}^{-2} \text{hr}^{-1}$) plots, and on July 19 in MF2 plot ($2450 \mu\text{g N m}^{-2} \text{hr}^{-1}$). The highest fluxes in all the five treatments in 2012 were found in either June or July and were all preceded by rainfall of more than 50 mm.

Nitrous oxide fluxes were highest in 2010, followed by 2011 and 2012, respectively and the fluxes were higher in plots with inorganic fertilizer application (F, MF1 and MF2) compared to CT and M plots.

Total annual N_2O emissions averaged 4.4 ± 1.2 , 12.7 ± 5.2 , 14.9 ± 7.6 , 22.4 ± 12.5 and $8.6 \pm 4.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for CT and F, MF1, MF2 and M plots, respectively (Table 6.3). There were significant differences in N_2O emission among plots ($p < 0.001$) and across years. Annual N_2O emissions were highest in MF2 plot, followed by MF1 and F plots, and lowest in M and CT plots, respectively. N_2O emissions in M and CT plots were not significantly different. Based on the annual emissions in Table 2, the average EFs for inorganic fertilizer were 12.9 ± 15.3 , 7.5 ± 6.8 and $3.5 \pm 2.9\%$ in 2010, 2011 and 2012, respectively (Table 6.6). The manure EFs ranged from 0.17 to 0.92%.

6.3.2.2 Carbon Dioxide Emissions (RH)

The pattern of CO_2 fluxes was similar to those of soil temperature with the highest fluxes found in summer and lowest fluxes in winter during soil freezing period (Fig. 6.2b). The fluxes had a significant positive exponential relationship with soil temperature measured at 5

cm (Fig. 6.3). The highest recorded CO₂ fluxes in each plot occurred when soil moisture content (WFPS) was lower than 65%.

CO₂ fluxes tended to be higher in manure plots (MF1, MF2 and M) ranging from 1.2–458 mg C m⁻² hr⁻¹ compare to non-manure plots (F and CT) which ranged from 3.1 – 299 mg C m⁻² hr⁻¹. The average cumulative annual CO₂ emissions from 2010 to 2012 were 6.7±0.2, 5.9±1.0, 8.9±1.2, 9.0±0.7 and 9.0±1.7 Mg C ha⁻¹ yr⁻¹ (mean ±SD) in CT, F, MF1, MF2 and M plots, respectively (Table 6.3). The annual CO₂ emissions in manure-amended plots (MF1, MF2 and M) were significantly higher than in F and CT plots ($p < 0.001$). There was no significant difference among MF1, MF2 and M plots in annual CO₂ emission. Significant differences in annual CO₂ emission among plots ($p < 0.001$) and across years ($p < 0.01$) were observed and the interaction between plots and years was significant ($p < 0.05$).

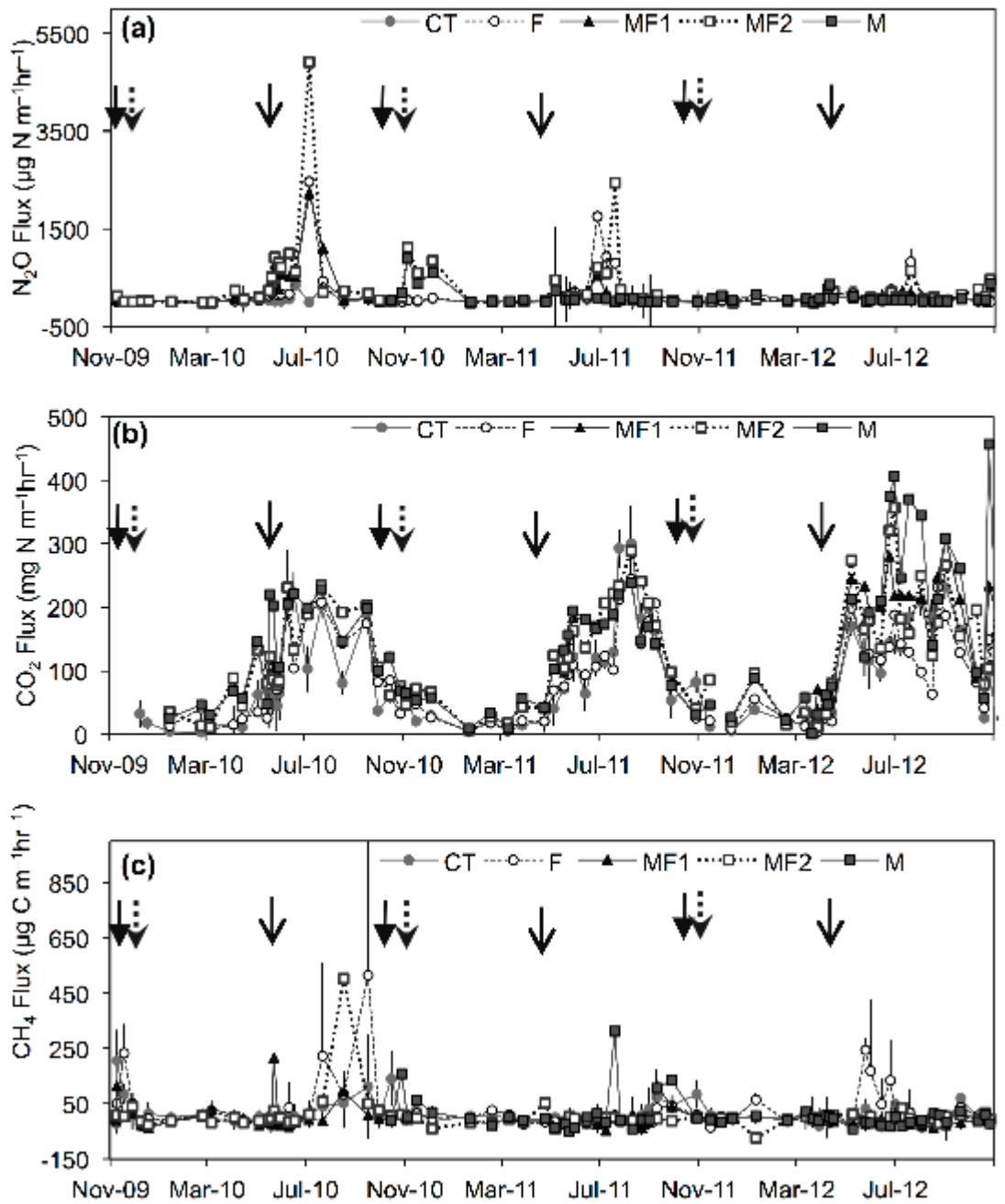


Figure 6.2 Seasonal variation in N₂O flux (a), CO₂ flux (b) and CH₄ flux (c) from 2010-2012 in experiment 1. CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot. Full arrows with closed head indicate dates of manure application, dashed arrows indicate dates of ploughing; full arrows with open V shaped tip indicate dates of inorganic fertilizer application

Table 6.3 Annual manure C input, net primary production (NPP), CO₂ emission, net ecosystem carbon balance (NECB) in Mg C ha⁻¹ yr⁻¹, CH₄ emission (kg C ha⁻¹ yr⁻¹), N₂O emission (kg N ha⁻¹ yr⁻¹) and net global warming potential (GWP) (Mg CO₂-C equivalents ha⁻¹ yr⁻¹) in experiment 1.

Year	Plot	Manure	NPP	CO ₂	NECB	CH ₄	N ₂ O	GWP
			Mg C			kg C	kg N	Mg CO ₂ -C eq
2010	CT	0	2.9±1.1 ^c	6.8±0.8 ^{abc}	-6.5±0.8 ^c	1.5±1.7 ^{ab}	3.9±1.2 ^a	7.1±1.0 ^{cd}
	F	0	6.5±2.2 ^{ad}	6.9±1.1 ^{abc}	-6.2±1.1 ^{bc}	5.1±4.6 ^b	17.4±16.1 ^{abcd}	8.5±2.2 ^d
	MF1	8.9±0.4	7.0±3.7 ^{abd}	10.2±0.7 ^c	-1.7±0.7 ^{ad}	0.5±1.4 ^{ab}	22.9±11.3 ^{bcd}	4.7±1.7 ^{abcd}
	MF2	8.9±0.4	6.1±2.3 ^{ade}	8.9±0.5 ^{bc}	-0.5±0.5 ^a	3.7±3.9 ^{ab}	33.5±2.3 ^d	4.9±0.4 ^{abcd}
2011	CT	0	3.5±1.3 ^{ce}	6.5±0.9 ^{abc}	-5.7±0.9 ^{bc}	0.3±1.0 ^{ab}	5.8±2.3 ^{ab}	6.4±0.9 ^{bcd}
	F	0	7.6±1.3 ^{ab}	6.1±1.1 ^{ad}	-5.1±1.1 ^{bce}	-0.2±0.6 ^a	13.6±8.7 ^{abc}	6.8±1.1 ^{bcd}
	MF1	6.7±0.5	9.9±1.2 ^b	7.8±1.1 ^{abc}	0.3±1.1 ^a	0.4±0.2 ^{ab}	14.3±2.2 ^{abc}	1.5±1.3 ^a
	MF2	6.7±0.5	9.9±1.4 ^b	8.3±1.6 ^{abc}	0.2±1.6 ^a	-0.6±0.7 ^a	25.2±11.9 ^{cd}	3.0±2.8 ^{ab}
	M	6.7±0.5	4.3±1.6 ^{cde}	7.8±1.1 ^{abc}	-0.5±1.1 ^a	1.3±2.1 ^{ab}	11.7±2.3 ^{abc}	2.0±1.4 ^a
2012	CT	0	2.7±0.4 ^c	6.8±1.2 ^{abd}	-6.2±1.2 ^{bc}	-0.2±0.1 ^a	3.6±0.7 ^a	6.6±1.2 ^{bcd}
	F	0	6.3±0.8 ^{ade}	4.9±0.3 ^d	-3.7±0.3 ^{bde}	1.9±2.8 ^{ab}	7.1±3.3 ^{ab}	4.6±0.5 ^{abcd}
	MF1	6.7±0.4	8.4±1.3 ^{ab}	8.8±0.6 ^{abc}	-1.3±0.6 ^{ad}	-0.7±0.2 ^a	7.7±1.2 ^{ab}	2.2±0.7 ^a
	MF2	6.7±0.4	8.7±1.1 ^{ab}	9.8±2.2 ^c	-2.4±2.1 ^{ade}	0.0±0.6 ^{ab}	8.8±4.0 ^{abc}	3.5±2.5 ^{abc}
	M	6.7±0.4	2.9±0.9 ^c	10.3±0.5 ^c	-3.7±0.5 ^{bde}	-0.6±0.3 ^a	5.6±1.7 ^{ab}	4.4±0.8 ^{abc}

Different letters within the same column indicate significant differences among the treatments at $p < 0.05$. CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot

Table 6.4 Average annual CH₄ and N₂O emissions (in carbon dioxide equivalents; CO₂-eq), net global warming potential (GWP) and net greenhouse gas intensity (GHGI) from CH₄ and N₂O in experiment 1.

Year	Plot	CH ₄	N ₂ O	GWP	GHGI
		kg CO ₂ -eq ha ⁻¹ yr ⁻¹		kg CO ₂ -eq kg ⁻¹ yield	
2010	CT	50.6±29.1	1858±299 ^{bcd}	1909±308 ^{bcde}	0.65±0.11 ^{bcdef}
	F	170.4±76.9	8139±3784 ^{bde}	8309±3715 ^{def}	1.27±0.57 ^{cef}
	MF1	17.1±24.1	10743±2646 ^{de}	10760±2628 ^{ef}	1.53±0.37 ^{def}
	MF2	123.3±65.5	15711±554 ^e	15834±557 ^f	2.59±0.09 ^f
2011	CT	10.2±17.5	2731±540 ^{abc}	2741±524 ^{abcd}	0.78±0.15 ^{abcd}
	F	-7.2±11.4	6392±2038 ^{bcd}	6384±2036 ^{bcde}	0.84±0.26 ^{abcde}
	MF1	12.4±4.4	6713±537 ^{bcd}	6725±537 ^{bcd}	0.67±0.05 ^{abcde}
	MF2	-22.4±12.5	11793±2788 ^{de}	11770±2786 ^{ef}	1.19±0.28 ^{bcde}
	M	42.6±35.1	5496±539 ^{abcd}	5538±506 ^{bcde}	1.28±0.11 ^{abcde}
2012	CT	-5.5±10.3	1703±178 ^a	1697±177 ^a	0.62±0.06 ^a
	F	62.8±47.5	3312±769 ^{abc}	3375±810 ^{abc}	0.53±0.12 ^{abd}
	MF1	-24.7±4.3	3594±293 ^{abc}	3569±292 ^{abcd}	0.42±0.03 ^{abc}
	MF2	0.8±10.3	4116±940 ^{bcd}	4116±947 ^{bcde}	0.47±0.11 ^{abcde}
	M	-20.6±5.7	2615±412 ^{ab}	2595±412 ^{ab}	0.89±0.14 ^{abcde}

Note: CH₄ and N₂O emissions were converted to CO₂-equivalents by multiplying annual emissions (shown in Table 6.3) by 25 and 298 respectively; GWP in this table is sum of CO₂-equivalents of CH₄ and N₂O only; GHGI in this table was calculated by dividing GWP from CH₄ and N₂O by total biomass yield. Different letters within the same column indicate significant differences among the treatments.

6.3.2.3 Methane Emissions

Methane fluxes were mostly negative indicating CH₄ consumption in all plots in this study (Fig. 6.2c). However, there was a very wide range of fluxes ranging from -53.6 to 140.7, -36.2 to 515.5, -51.8 to 313.1, -74.0 to 503.0 and -50.2 to 312.8 $\mu\text{g C m}^{-2} \text{ hr}^{-1}$ in CT, F, MF1, MF2 and M plots, respectively. This very wide range was due to very high fluxes ($> 50 \mu\text{g C m}^{-2} \text{ hr}^{-1}$) observed following rainfall of more than 40 mm within 4 days preceding the gas sampling or when WFPS was more than 70% in all the plots. These high CH₄ fluxes accounted for less than 10% of the observed data, but significantly raised the average annual fluxes. Using all data by including the extremely high CH₄ fluxes ($> 50 \mu\text{g C m}^{-2} \text{ hr}^{-1}$), compared to exclusion of these high fluxes, increased the average annual flux ($\mu\text{g C m}^{-2} \text{ hr}^{-1}$) from -1.8 to 7.4 in CT plot, -2.8 to 20.8 in F plot, -7.5 to 1.0 in MF1, -3.2 to 5.1 in MF2 and -6.4 to 6.2 in M plot. Methane fluxes were not significantly different among the plots, but tended to be higher in CT and F plots compared to the manure-amended plots.

Annual CH₄ emissions were not different among all plots but there was a significant difference across the years, and ranged from -0.7 to 5.1 $\text{kg C ha}^{-1} \text{ yr}^{-1}$ (Table 6.3). Methane emissions were significantly higher in 2010 than in 2011 and 2012 ($p < 0.01$). The average total annual CH₄ emissions were 0.5 ± 0.8 , 2.2 ± 2.6 , 0.0 ± 0.6 , 1.0 ± 0.8 and $0.3 \pm 1.3 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ in CT, F, MF1, MF2 and M plots, respectively, over the three-year period from 2010 to 2012. Without the extreme CH₄ fluxes ($> 50 \mu\text{g C m}^{-2} \text{ hr}^{-1}$ observed after high rainfall) all plots were net CH₄ sinks.

6.3.3 Effect of additional spring manure application on Greenhouse Gas Emissions - Experiment 2

The second experiment aimed at comparing the effect of additional manure and inorganic fertilizer application in spring on GHG emissions and crop growth. From Table 6.5, it was

clear that applying manure twice, in autumn and spring, increased the plant productivity (NPP) to the same level as the plot that received inorganic fertilizer, without a net increase in CO₂, CH₄ or N₂O fluxes. This result means farmers in this region could potentially increase productivity by applying manure twice in autumn and spring even without using inorganic fertilizer. Most importantly, the second application of manure in spring led to significant increase in NECB and significant reduction in GWP compared to additional inorganic manure application (Table 6.5).

Table 6.5 Average annual manure C input, net primary production (NPP), CO₂ emission, net ecosystem carbon balance (NECB) in Mg C ha⁻¹yr⁻¹, CH₄ emission (kg C ha⁻¹yr⁻¹), N₂O emission (kg N ha⁻¹yr⁻¹) and global warming potential (GWP) (Mg CO₂-C equivalents ha⁻¹yr⁻¹) in experiment 2.

Plot	Manure	NPP	CO ₂	NECB	N ₂ O	CH ₄	GWP
		Mg C			kg N	kg C	Mg CO ₂ -C eq
M1	7.3±0.3a	3.94±0.46b	8.28±1.70	0.59±1.7b	7.19±1.77	-0.04±0.37	0.33±1.75a
MF	7.3±0.3a	6.99±2.52a	8.71±1.64	0.20±1.6b	10.00±1.83	-0.29±0.24	1.08±1.74a
MM	12.3±0.4b	7.12±1.00a	8.30±1.72	5.22±1.7a	7.41±1.39	0.18±0.23	-4.27±1.81b

Different letters within the same column indicate significant differences among the treatments at $p < 0.05$. M1 is autumn composted cattle manure application only plot; MF is autumn composted cattle manure plus spring inorganic fertilizer plot; MM is autumn composted cattle manure plus additional spring composted cattle manure plot.

6.3.4 Factors controlling GHG emissions.

Nitrous oxide fluxes had statistically significant ($p < 0.01$) positive linear regression relationships with soil temperature and soil NO₃⁻ concentration and a significant negative relationship with soil pH (Table 6.7). Cumulative annual N₂O emissions showed significant positive linear relationships with N input ($r = 0.62$, $p < 0.05$) and precipitation in growing season ($r = 0.56$, $p < 0.05$), and a negative linear relationship with soil pH ($r = -0.72$, $p < 0.05$).

0.01). Average annual N₂O emission in MF1 plot was lower than in MF2 plot despite both plots receiving the same amount of manure and inorganic fertilizer, possibly due to the lower pH in MF2. This seems to suggest that long-term manure application in MF1 buffered against lowering of pH by inorganic fertilizer application.

Methane fluxes were significantly correlated with soil temperature and moisture. Soil moisture seemed to be the most important factor driving CH₄ fluxes and all the disproportionately high fluxes ($>50 \mu\text{g CH}_4\text{-C m}^{-2} \text{hr}^{-1}$) were observed when soil WFPS was more than 70%. Soil chemical properties did not show any significant correlations with the instantaneous CH₄ fluxes (Table 6.7). However, cumulative annual CH₄ emissions had significant linear relationships with soil NO₃⁻ ($r = +0.66, p < 0.01$) and soil pH ($r = -0.61, p < 0.05$).

Soil CO₂ fluxes had positive and significant linear relationships with soil pH and soil temperature and significant negative correlation with soil NO₃⁻ concentration and soil WFPS (Table 6.7). The CO₂ flux increased exponentially with increasing soil temperature (Fig. 6.3) and the resulting Q10 values were 3.2, 2.8, 2.3, 2.5 and 2.3 in the CT, F, MF1, MF2 and M plots, respectively. The annual CO₂ emissions significantly increased with manure application ($p < 0.01$) and this could be largely due to the C input from manure. Multivariate regression analysis showed that soil temperature, WFPS and WEOC concentration explained 55% of the seasonal variation in CO₂ fluxes ($\text{CO}_2 \text{ flux (mg C m}^{-2} \text{hr}^{-1}) = -98.89 + 11.86 \text{ soil temperature} - 2.57 \text{ WFPS (\%)} + 0.32 \text{ WEOC (mg C kg}^{-1})$; $R^2 = 0.55$; $p < 0.001$).

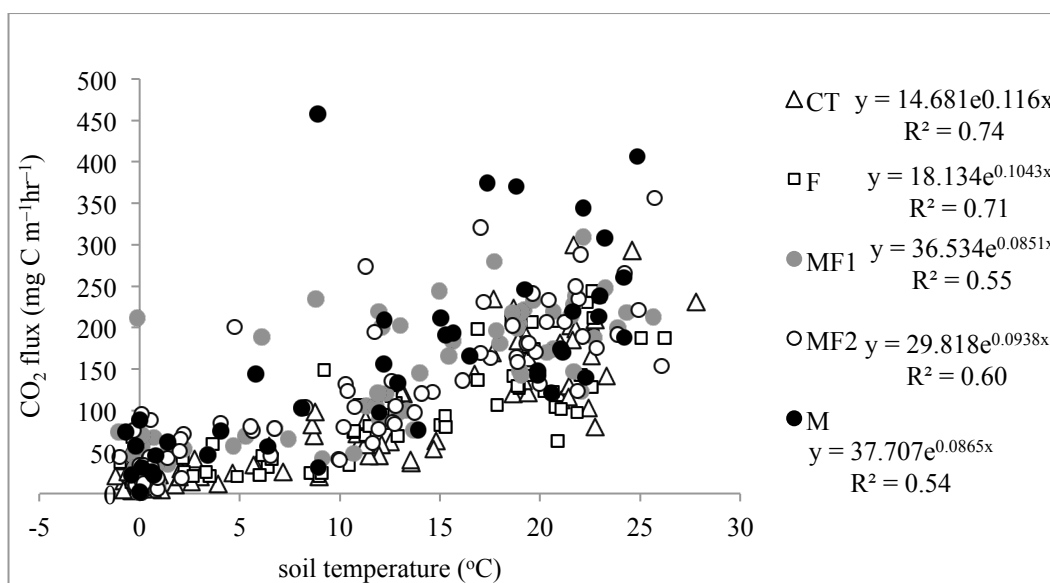


Figure 6.3 Relationship between soil temperature (5 cm) and CO₂ flux in experiment 1. CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot

Table 6.6 Fertilizer and manure N emission factors

Year	Fertilizer EF	Manure EF
2010	12.90±15.35	0.92±1.70
2011	7.52±6.84	0.31±2.00
2012	3.55±2.95	0.17±0.41
Average	7.99	0.47

6.3.5 Effect of fertilizer and manure on NPP, NECB, GWP and GHGI

Inorganic fertilizer significantly increased the net primary production (NPP) in each of the three years in experiment one and also in 2013 in experiment two (Tables 6.3 and Table 6.5). However due to C export from the field through the harvested material, inorganic fertilizer did not increase NECB. Manure application, on the other hand, had a positive but non-significant effect on NPP in experiment 1. However, the second application of manure in spring in experiment 2 significantly increased NPP (Table 6.5), and there was no significant difference between MF and MM plots. Manure input in both experiments 1 and 2 significantly increased NECB.

Global warming potentials, in experiment 1, were lowest in MF1 and MF2 plots and highest in F and CT plots (Table 6.3). Global warming potential ranged from 6.4 to 7.1 Mg CO₂-C eq ha⁻¹ yr⁻¹ in the unfertilized control. Both inorganic and manure fertilization showed potential to reduce the GWPs mainly due to C addition from the manure and also from the increased plant biomass residue left after harvest. In experiment 2, high manure input in MM plot significantly raised the NECB and as a result the GWP was negative. Net ecosystem C balance had the largest contribution to GWP and consequently, manure application seemed to significantly reduce GWP.

Greenhouse gas intensities, presented in Fig. 6.4, were different among the plots. The control plot always had the highest GHGI followed by F plot. Combined manure and inorganic fertilizer plots (MF1 and MF2) always had the lowest GHGIs due to high C input through manure and also high crop residue addition as both had higher NPP and yield. Greenhouse gas intensity in M plot was lower than that in F plot in 2011, but the opposite in 2012.

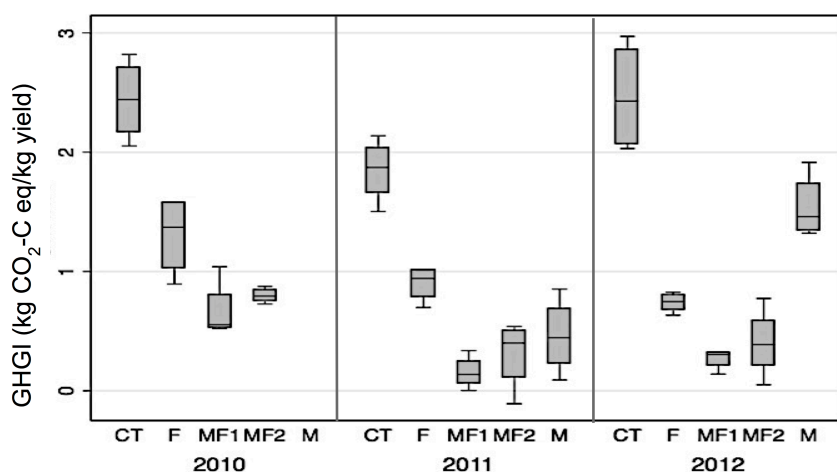


Figure 6.4 Greenhouse gas intensity (GHGI) in 2010-2012 (experiment 1). CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot

Table 6.7 Linear regressions (Pearson's correlation coefficients) for the relationship between greenhouse gas fluxes and environmental variables (experiments 1 and 2 combined)

Variable	N ₂ O flux ($\mu\text{g N m}^{-2} \text{hr}^{-1}$)	CH ₄ flux ($\mu\text{g C m}^{-2} \text{hr}^{-1}$)	CO ₂ flux ($\text{mg C m}^{-2} \text{hr}^{-1}$)
Soil temp	0.174**	0.163**	0.776**
WFPS	0.084	0.205**	-0.341**
Soil pH	-0.281**	-0.060	0.162*
NO ₃ ⁻	0.242**	0.031	-0.265**
NH ₄ ⁺	-0.049	-0.067	0.069
WEOC	0.031	-0.113	0.105

WFPS is water filled pore space, WEOC is water extractable organic carbon. **Significant correlation at $p < 0.01$, * significant correlation at $p < 0.05$

6.4 Discussion

6.4.1 Influence of inorganic fertilizer and manure application on GHG emissions

Application of inorganic and manure fertilizers increased N₂O emissions by 2–5 times compared to the unfertilized control treatment (Table 6.3). Carbon dioxide fluxes only increased following the manure application but not the inorganic fertilizer application. Neither inorganic nor manure fertilization seemed to significantly influence CH₄ fluxes.

Increased N₂O emissions with increased N addition was expected as it is widely reported that the two most important N₂O production pathways in the soils, nitrification and denitrification, are stimulated by high N input (Collins et al. 2011; Sistani et al. 2011; Ni et al. 2012; Saggar et al. 2013; Shcherbak et al. 2014; Li et al. 2015). In this study, the average inorganic fertilizer N₂O emission factor was 7.8% (ranging from 3.5 to 12.9%; Table 6.6), which was significantly higher than the default IPCC value of 1% and also higher than several published values of between 0.1 to 3% (Akiyama et al. 2004; Ding et al. 2007b; Ranucci et al. 2010; Cai et al. 2013; Harty et al. 2016). However, our emission factors were similar to those reported by Flessa (1995) in Germany of 5.1–8.8% in a wheat field and by

MacKenzie et al. (1997) of 0–8% in corn field. High emission factors in this study could be due to the relatively high soil NO_3^- concentrations especially in 2010, leading to high excess N available for microbial transformation. The reason for the high NO_3^- content could have been due to the stimulation of litter and organic matter decomposition and mineralization after the conversion from grassland to cornfield in November 2009. The manure emission factor in this study ranged from 0.17 to 0.92%. These values are similar to those reported by Collins et al. (2010) of 0.05 to 0.1% and Akiyama et al. (2004) of 0.01 to 1.6%. Despite both manure and inorganic fertilizers increasing N_2O emissions, the manure only treatments had significantly lower emissions than the inorganic fertilizer plot. In experiment 2, manure application in MM plot was done twice and at almost twice the amount of that applied in M1 plot but did not lead to increased N_2O emissions (Table 6.5), and almost doubled the NPP. Nitrous oxide emissions and plant productivity in MM and MF plots were similar which suggests that application of manure twice instead of once can enable organic farmers achieve similar productivity levels with conventional farmers using inorganic fertilizers.

More than 5 years of manure application (MF1 and M plots in experiment 1) had an added positive influence of maintaining higher pH values than the inorganic fertilized plot and this could have further benefits in reducing the N_2O emissions given the strong negative relationship between soil pH and N_2O emissions (Fig. 6.5). Manure application increased and maintained soil pH by increasing the buffering capacity of soils due to the presence of carboxyl and phenolic hydroxyl groups in the manure (Whalen et al. 2000) or due the high pH of the manure itself (manure pH was around 7). Presence of cations (like calcium, magnesium etc) and organic N forms in manure could have also increased the soil pH in manure-amended treatments (Whalen et al. 2000).

High CH_4 emission in inorganic fertilizer amended soils due to fertilizer N inhibition of CH_4 oxidation has been reported in many studies (Sistani et al. 2011; Nagano et al. 2012).

We did not find the evidence of higher CH₄ emissions in plots amended with inorganic fertilizer in this study and there was no significant difference in annual CH₄ emissions among the plots. However, the highest CH₄ fluxes were always found in the fertilized plots (Fig. 6.2c) and there was a significant positive correlation between average annual CH₄ flux and average annual soil mineral N concentration ($R^2 = 0.65$, $p < 0.05$). This might suggest inhibition of CH₄ oxidation when soil mineral N is high (MacDonald et al. 1997; Owen et al. 2015; Tate 2015).

Carbon dioxide fluxes and cumulative annual emissions significantly increased due to manure application but not due to inorganic fertilization. Increased availability of C substrates from the manure was likely the major reason for the observed result (Cabrera et al. 1994; Ussiri and Lal 2009; Alluvione et al. 2010; Hu et al. 2013; Lentz and Lehrsch 2014).

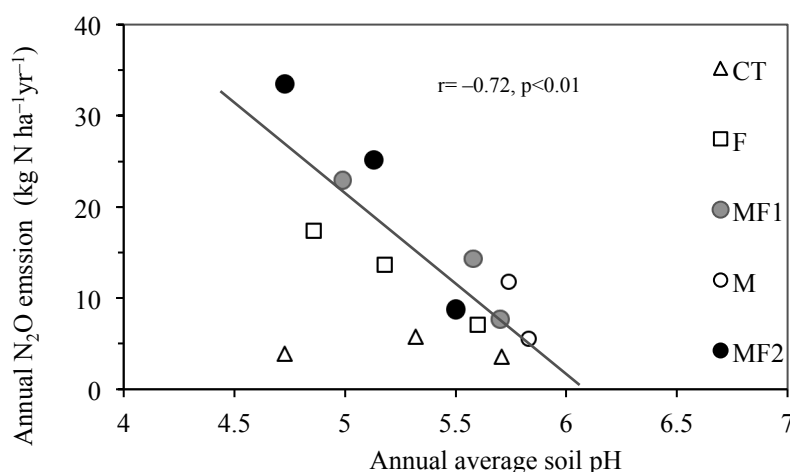


Figure 6.5. Relationship between annual N₂O emission and annual average soil pH (data from experiment 1). CT is unfertilized control plot; F is inorganic fertilizer only plot; MF1 is inorganic fertilizer plus composted cattle manure applied since 2005 plot; MF2 is inorganic fertilizer plus composted cattle manure applied since 2010 plot; M is composted cattle manure only plot. Annual average soil pH was the average of soil pH values measured from all soil samples collected in each year.

6.4.2 Soil and environmental factors controlling GHG emissions

Key soil and climatic variables controlling all the three GHG emissions in this study were soil temperature, soil moisture and rainfall. Soil NO_3^- concentrations and soil pH were important factors for N_2O and CO_2 (Table 6.7)

While soil N_2O fluxes were heavily influenced by N fertilization (both inorganic and manure), the highest fluxes were all found just after rainfall of more than 30 mm within the week preceding the flux measurement date (Figs 6.1 and 6.2). Ryals and Silver (2012) also reported elevated N_2O up to four days from the wetting of soils. Annual N_2O emissions were also very high in 2010, which was the wettest year. Other studies have reported high N_2O emission when rainfall and moisture are high (Mosier et al. 1991; Choudhary et al. 2001; Sehy et al. 2003; Alluvione et al. 2010). The high N_2O fluxes observed after high rainfall were likely due to denitrification (Sehy et al. 2003; Alluvione et al. 2010; Shimizu et al. 2013) as a result of increased soil moisture.

Soil pH was a major factor controlling annual N_2O emissions and this agrees with previous reports (Clough et al. 2004; Pan et al. 2012). The N_2O reductase enzyme is reported to be more active at low soil pH relative to activities of NO_3^- and NO_2^- reductase enzymes (Pan et al. 2012), which could have resulted in the reduced N_2O reduction to N_2 during denitrification and consequently higher fluxes at low soil pH.

The most important factors controlling CH_4 emission in this study were soil moisture and temperature. Very high CH_4 fluxes were observed when WFPS was 70% or higher. Less than 10% of the total CH_4 flux measurements (all recorded when WFPS > 70%) accounted for 50–150% of the cumulative average annual CH_4 emissions. These high CH_4 fluxes significantly reduced CH_4 sink in soil and were solely responsible for the observed positive cumulative annual CH_4 emissions. This indicates that while this upland cornfield was generally a sink for CH_4 under usual moisture levels, increased occurrence of high

rainfall and soil moisture above 70% WFPS could lead to significant CH₄ emissions. Several studies have reported WFPS value of 70–75% as the optimum for high CH₄ production (Wu et al. 2010; Wang et al. 2013; Mazzetto et al. 2014; Wang et al. 2014) and this could be due to both low production of CH₄ and low air permeability at high WFPS levels (Tate 2015).

Carbon dioxide flux is influenced by soil temperature, moisture content and availability of C substrates (Guo and Zhou 2007; Wu et al. 2010; Phillips et al. 2012; Wang et al. 2013; Wang et al. 2014; Xu-bo et al. 2014; Bond-lamberty et al. 2016). The relationship between soil temperature and CO₂ flux (Fig. 6.3) in this study was best described by an exponential curve and this agrees with several published studies (Chen and Tian 2005; Ding et al. 2007a; Peng et al. 2009; Zhou et al. 2014). From the exponential curves, we estimated average Q₁₀ values and these were 3.2, 2.8, 2.3, 2.5 and 2.3 in the CT, F, MF1, MF2 and M plots, respectively. These Q₁₀ values are in agreement with reported values from other studies (Liu et al. 2006; Wu et al. 2010; Phillips et al. 2012; Bond-lamberty et al. 2016). From these values, it seems manure application led to slightly lower Q₁₀ values despite the manure plots having higher WEOC, although we can not make definite conclusions from this study. If this was true, it would be contrary to Liu et al. (2006) who reported higher Q₁₀ values when WEOC was high. The seemingly lower Q₁₀ values in manure-amended plots might be due to the larger influence of manure C on CO₂ emissions.

Soil CO₂ flux negatively correlated to soil WFPS (Table 6.7) with highest fluxes found when WFPS was between 40 and 60%. This WFPS range also showed the lowest CH₄ fluxes. A conclusion can therefore be made that there was high C oxidation when WFPS was low and the opposite was true when WFPS was above 70 % (Ding et al. 2007b; Li-mei et al. 2011).

6.4.3 Effect of fertilizer and manure on NECB and GWP

Carbon sequestration calculations should include all C inputs and losses in an agro-ecosystem (West and Marland 2002; Anthoni et al. 2004; Chapin et al. 2006; Zhang et al. 2014a). The calculated NECB values in this study show that manure application is important for increased C sequestration as a direct C input and it significantly mitigates the C losses from harvested C and CO₂ emission (Table 6.3). While all plots showed negative NECB values in 2010 and 2012, the NECB in manure plots was significantly higher. Results from experiment 2 show that applying manure twice in autumn and spring significantly increased NECB through direct C input but also by increasing the net primary production and hence the C input through residue left in the field. The effect of fertilization on soil C pools is related to the amount of C applied and C produced/retained to the soil (Lal 2004). High NECB in MM plot in experiment 2 was achieved without increased emissions of CO₂, CH₄ or N₂O compared to the plot with combined manure and fertilizer applications (Table 6.5). Higher NECB values, indicating increased C sequestration (Zhang et al. 2014a), in manure amended plots have been reported previously (Anthoni et al. 2004; Limin et al. 2015). Total soil C content and total C stock, measured in 2010 and 2012, were higher in manure-amended plots (Tables 1 and 2) indicating higher C sequestration due to manure application. Compared to the non-manure plot, manure application increased soil C stock in the top 15 cm by $20.7 \pm 11.7 \text{ Mg C ha}^{-1}$ which is similar to 19 Mg C ha^{-1} reported by Owen et al. (2015) in grasslands amended with different manure types. Pathak et al. (2011) also reported higher C accumulation when farmyard manure was applied along with inorganic fertilizer.

Adequate manure application done twice in spring and autumn show a potential to increase not only the plant productivity to the same level of inorganic fertilized plot but also with the added benefit of storing more C in the soil as evidenced from results of experiment 2. Net GWP values (Table 6.3) and GHGIs (Fig. 6.4) show that combined manure and

inorganic fertilization had the highest potential for mitigating net GHG emissions, which we attribute to both increased NPP and increased direct C input into the soil. Composted manure application has been shown in other studies to reduce net GHG emissions (Zhang et al. 2014a; Owen et al. 2015). Without consideration of the C input from manure, GWP values (from N_2O and CH_4) were significantly lower in the unfertilized control plot and highest in the inorganic fertilized treatments, but there were no significant differences in the net GWPs of the manure plus inorganic fertilizer (MF) and inorganic fertilizer only (F) plots (Table 6.4). However, when these GWP values were yield-scaled (GHGI), no significant differences were observed among the plots (Table 6.4). This clearly shows that when evaluating effects of manure and fertilizer management on net GHG emissions, the benefits obtained from increased productivity should be considered. Further research to determine the optimum application rates and ratios of the inorganic fertilizer and composted manure is recommended. Nitrous oxide emissions were more stimulated by inorganic fertilizer than by composted manure, and therefore reduced application of inorganic fertilizer and partial substitution with composted manure could help reduce net GHG emissions, a result which has been found in other studies (Cai et al. 2013; Wang et al. 2013; Yan et al. 2013).

6.5 Conclusions

In this three-year field study we evaluated the effect of inorganic fertilizer and composted cattle manure application on GHGs, GWPs and GHGI in a cornfield. Nitrous oxide emissions increased following both inorganic and manure application although we did not find significant influence of manure and inorganic fertilizer in 2012. Methane fluxes were not influenced by the soil amendments, while CO_2 fluxes increased with manure application. By applying manure twice, in autumn and spring in experiment 2, high plant productivity was achieved without increased GHG emissions compared to one-time manure application

with additional inorganic fertilizer application. Our study found that application of manure led to significant reductions in net GWP and GHGI and this was largely due to direct C input to the soil and also through increased plant productivity.

Measured N₂O fluxes were significantly elevated by rainfall of more than 30 mm within 7 days just before flux measurement. Cumulative N₂O emissions were controlled by soil pH, growing season rainfall and soil mineral N concentration. Less than 10% of CH₄ measurements accounted for 50–100 % of the total annual emissions and these were all observed when WFPS was more than 70 %. Soil temperature influenced the seasonal change in CO₂ emissions, and manure application seemed to reduce the Q₁₀ values of heterotrophic soil respiration.

7. Evaluating the effect of liming on N₂O fluxes from denitrification using the acetylene inhibition and ¹⁵N isotope tracer methods

7.1 Introduction

Denitrification, which is the reduction of nitrate (NO₃⁻) to gaseous nitrogen (N) forms primarily N₂O and dinitrogen (N₂) (Groffman et al. 2006) is the main process, together with nitrification, responsible for N₂O production in soils. However, unlike nitrification, denitrification is not only capable of producing N₂O but can also consume N₂O by reducing it to N₂ (Firestone and Davidson 1989). Denitrification has been reported to be the major N₂O-producing mechanism in the majority of soils (Šimek et al. 2002). Many different factors affect the rate denitrification in soils and these include; available mineral N (particularly NO₃⁻) and carbon (C), temperature, soil moisture, pH and inherent soil microbes (Firestone and Davidson 1989; Šimek et al. 2002; Saggar et al. 2013).

Information on the rate of reduction of N₂O to N₂ is still limited and its determination is hindered by the difficulty of measuring N₂ production under natural conditions. The use of acetylene, which inhibits the reduction of N₂O to N₂ has been widely used to measure denitrification and to estimate N₂O and N₂ separately. Despite the many problems associated with the acetylene inhibition method, such as the inhibition of nitrification and limited inhibition of nitric oxide reductase (Groffman et al. 2006; McMillan et al. 2016), it is still the most widely used method to estimate denitrification largely due to its low cost and easy of usage. The acetylene inhibition method also allows the estimation of the two most important products of denitrification, N₂O and N₂.

The ¹⁵N tracer method is considered as one of the best methods for determining the process involved in N₂O production in soils (Groffman et al. 2006) and its use to determine denitrification has increased over the past few decades due to improvements in instrumentation. One problem with the ¹⁵N tracer method is that it can lead to overestimation

of denitrification when ^{15}N is applied to N limited soils (Saggar et al. 2013). However, this problem does not hold in agricultural soils where N levels are high due to fertilization. Moreover, under controlled laboratory conditions, ^{15}N enrichment by using isotopically labelled N substrates enables us to determine not only how much of the applied ^{15}N is emitted as N_2O from the soil but also deduce the main process by the N_2O was produced (Müller et al. 2014).

The complete heterotrophic denitrification process involves four successional stages from the reduction of NO_3^- to nitrite (NO_2^-), nitric oxide (NO), N_2O and lastly to N_2 . A specific enzyme catalyses each of these stages; nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) (Pan et al. 2012). Some studies have indicated that N_2O reductase is less active at low pH compared to the other three enzymes and hence the tendency to observe high N_2O accumulation in acidic soils as less of it is reduced to N_2 (Šimek and Cooper 2002; Pan et al. 2012; McMillan et al. 2016). This reduced activity of N_2O reductase at low pH suggests that increasing soil pH (e.g., by liming) could be a useful approach to mitigate N_2O emissions from soils. However, the results from different studies have been contradictory (Baggs et al. 2010; Zaman and Nguyen 2010; McMillan et al. 2016). One of the reasons for the observed contradictions among different results is that in soils nitrification and denitrification occur simultaneously and nitrification does not seem to show a clear relationship with pH (Šimek et al. 2002). Although liming as a mitigation technique for N_2O emissions has been previously evaluated, clearly there is still a lack of full understanding of how it affects the total N_2O from denitrification and N_2O reduction to N_2 . Furthermore, very few studies have used acetylene inhibition and ^{15}N - N_2O measurements in a single study to evaluate the effect of liming.

In this study, we investigated how liming affects the N_2O production using ^{15}N - N_2O measurement under aerobic conditions and how it influences N_2O and N_2 production under

anaerobic conditions using the acetylene inhibition method at different temperatures in an Andosol under fertilized and unfertilized conditions.

7.2 Materials and methods

7.2.1 Experiment 1: Aerobic incubation

Three liming treatments were established as; unlimed soils (L0), low lime rate (L1) at 4 g $\text{CaCO}_3 \text{ kg}^{-1}$ soil, and high lime rate (L2) at 20 g $\text{CaCO}_3 \text{ kg}^{-1}$ soil. After liming, 80 g soil (dry basis) for each lime treatment was repacked into 100 cm^3 cores. The soils were then pre-incubated for one week at 50% water-filled pore space (WFPS) to allow the soil microbes to re-establish. After the pre-incubation, 200 mg KNO_3 (1 atom% excess ^{15}N) kg^{-1} soil was added in solution form to each of the lime treatments. Unfertilized treatments were also set up for each lime treatment as described above. The final moisture content was then brought to 80% WFPS to create conditions favorable for denitrification but still under aerobic conditions. The soils were incubated in 1-liter jars for 16 days from fertilization day in the dark at 15 and 25°C (Only L0 and L2 were incubated at 15°C in experiment 1).

Gas samples were taken twice during the pre-incubation period and on days 1, 2, 4, 7, 10, 13 and 16 after fertilization. $^{15}\text{N}\text{-N}_2\text{O}$ and total $^{15+14}\text{N}_2\text{O}$ were analyzed using an Isotopic N_2O Analyzer (Los Gatos Research Mountain View, CA, USA, model 914-0027).

7.2.2 Experiment 2: Anaerobic incubation

Ten g dry soil was weighed into a 100 ml glass vial and wetted to saturation by adding 10 ml water (for the unfertilized treatments) or 10 ml KNO_3 solution (for the fertilized treatments). Fertilized treatments received an equivalent of 100 mg N kg^{-1} soil as KNO_3 and 600 mg C kg^{-1} soil as glucose. The glucose was meant to provide enough carbon for microbes in order

not to restrict denitrification. The vials were then capped with butyl rubber septa and caps. Immediately after addition of the water or fertilizer solution, the headspace gas inside the vials was replaced with either pure helium or 10% C₂H₂ gas (90% pure helium). The treatments with or without C₂H₂ were each replicated 4 times for each of the three liming treatments (L0, L1 and L2). All the lime, fertilizer and C₂H₂ treatment combinations were incubated at two temperatures (15 and 25°C). These soils were incubated for 44 hours in the dark.

Gas samples were taken using a 1ml precision syringe at 2, 5, 10, 20 and 44 hours from the start of the incubation. The gas samples were then directly injected into a gas chromatograph fitted with an electron capture detector (Model GC-14B; Shimadzu, Kyoto, Japan) to determine N₂O concentration. N₂ production was calculated as the difference between C₂H₂ and no C₂H₂ treatments.

7.2.3 Soil sampling and analysis

In experiment 1, soils were destructively sampled on days 1, 4, 10 and 16 after fertilization day. Soils were also sampled twice during the 7-day pre-incubation period after establishment of the liming treatments. In experiment 2, soil analysis was done before and after the 44-hour incubations. In both cases, the soils were incubated in identical conditions to those for gas measurements. Soil NO₃⁻, NH₄⁺, water extractable organic C (WEOC) and pH were analyzed as described in chapter 3.

7.3 Results

7.3.1 Experiment 1: Aerobic incubation

7.3.1.1 *Effect of liming on soil chemical properties*

The addition of 4 and 20 mg $\text{CaCO}_3 \text{ kg}^{-1}$ soil raised the final soil pH from 5.4 ± 0.2 to 6.4 ± 0.1 and to 7.6 ± 0.1 for the L1 and L2 treatments, respectively. The soil pH remained relatively stable during the incubation (Fig. 7.1). In the fertilized soils, soil pH was on average lower by 0.2 units at the end of the incubation compared to the unfertilized soils (Fig. 7.1b).

Lime addition led to higher soil NH_4^+ concentrations in the earlier stage of the incubation (Fig. 7.1). Soil NH_4^+ concentration in the high lime treatment (L2) was approximately double that in the unlimed soil, while NH_4^+ concentration in the low lime treatment (L1) was at least 35% higher than in the unlimed treatment in the first week after lime addition. However, by the 9th day after lime additions, soil NH_4^+ concentrations in the limed treatments significantly decreased ($p < 0.05$), such that no substantial differences among the three lime treatments were observed from the 9th day onwards. Ammonium concentrations decreased gradually in all the treatments as the incubation progressed. There was no noticeable difference in NH_4^+ concentrations between unfertilized and fertilized soils, which was expected since the applied fertilizer was KNO_3 .

Soil NO_3^- concentrations showed an opposite trend to that of NH_4^+ . The NO_3^- concentrations remained relatively unchanged by liming in the first week after lime addition, but increased from the 9th day of the incubation as the NH_4^+ concentrations started to decrease (Fig. 7.1). From the 9th day after lime addition, NO_3^- concentrations in the lime treatments were higher than in the unlimed treatments although the difference was non-significant by the end of the incubation. Less difference in NO_3^- concentrations were observed in the fertilized soils due to a relatively high addition of KNO_3 .

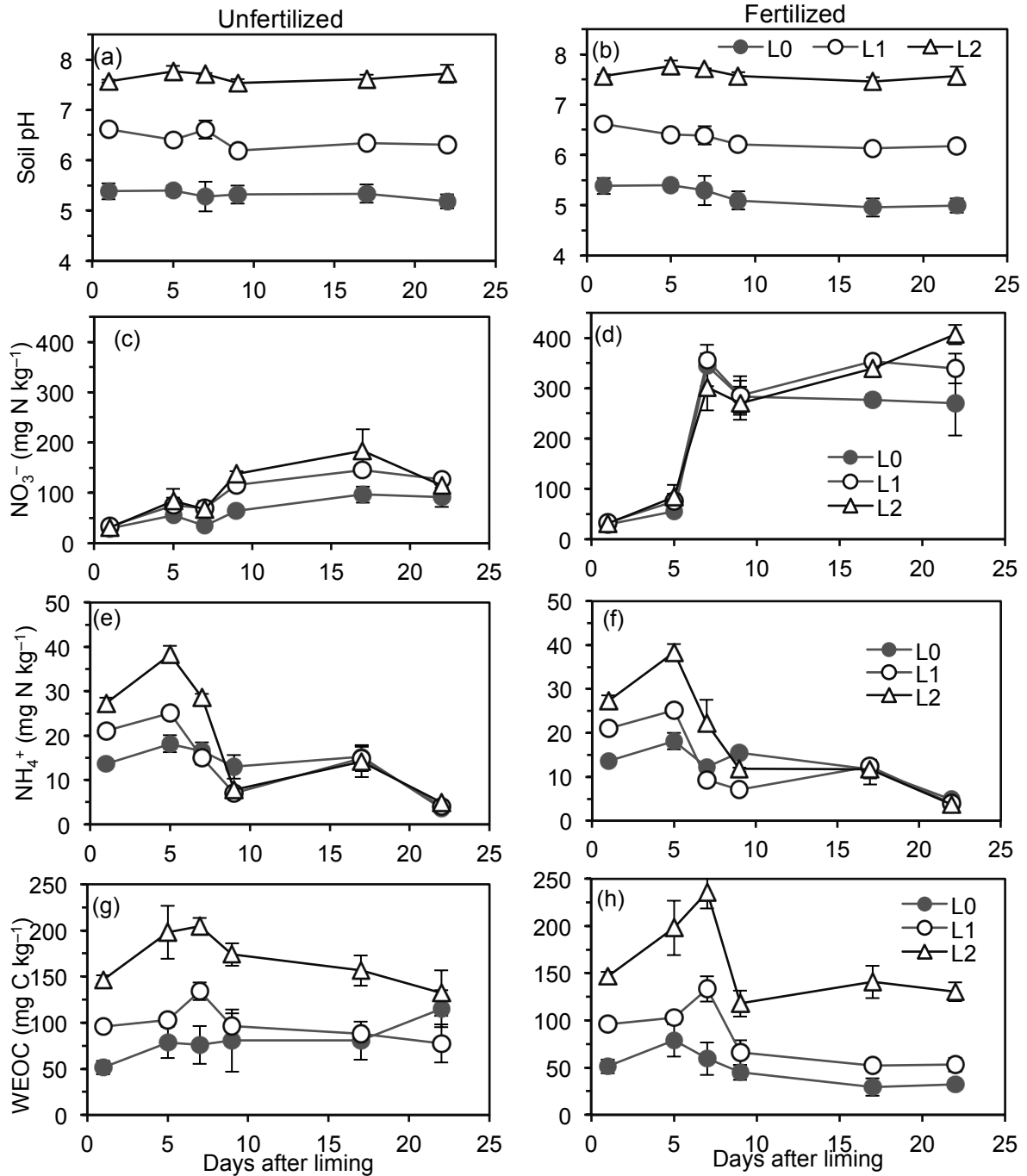


Figure 7.1 Soil pH (a,b), NO_3^- (c,d), NH_4^+ (e,f) and WEOC (g,h) in unfertilized and fertilized soils during the pre-incubation and incubation period in experiment 1. Error bars represent standard deviation ($n=4$). L0 is unlimed soil, L1 and L2 is 4 and 20 mg CaCO_3 kg⁻¹ soil, respectively. Fertilization was done on day 6

7.3.1.2 *Effect of liming on total N₂O emissions*

During the pre-incubation period (day 1 to day 6 after lime additions), when the moisture content was kept at 50% WFPS, N₂O production ranged from -0.2 to 2.2, 1.2 to 3.9 and 4.2 to 14.2 µg N₂O-N kg⁻¹hr⁻¹ in L0, L1 and L2 treatments respectively at both 15 and 25°C incubation (Fig. 7.2). After the addition of KNO₃ in the fertilized soils and deionized water in the unfertilized soils (day 7), the moisture content increased to 80% WFPS, and a very sharp increase in N₂O production was observed even in treatments without KNO₃ addition. Peak N₂O production occurred on the next day after KNO₃ or water addition, and it was higher in the limed treatments when compared to the unlimed soils ($p < 0.05$). Production of N₂O had reduced significantly three days later ($p < 0.01$). From day 8, until the end of the incubation, the high lime treatment (L2) showed the lowest N₂O production rates, at both 15°C and 25°C incubation temperature, not only in the treatments that received KNO₃ but also in the controls where there was no N addition.

Cumulatively, higher amount of N₂O was produced in the L1 treatment (Table 7.1) followed by L0 and lowest in the L2 treatment from the soils incubated at 25°C without N addition ($p < 0.05$). With N addition, L1 had emitted significantly higher amount of N₂O compared to L2 at 25°C incubation ($p < 0.01$), but L0 was not significantly different from L1 and L2. At 15°C, higher amounts of N₂O were produced in L2 treatment than L0 treatment; both in KNO₃ fertilized and unfertilized soils. This result indicates that both N addition and incubation temperature modulated the effect of liming on N₂O production.

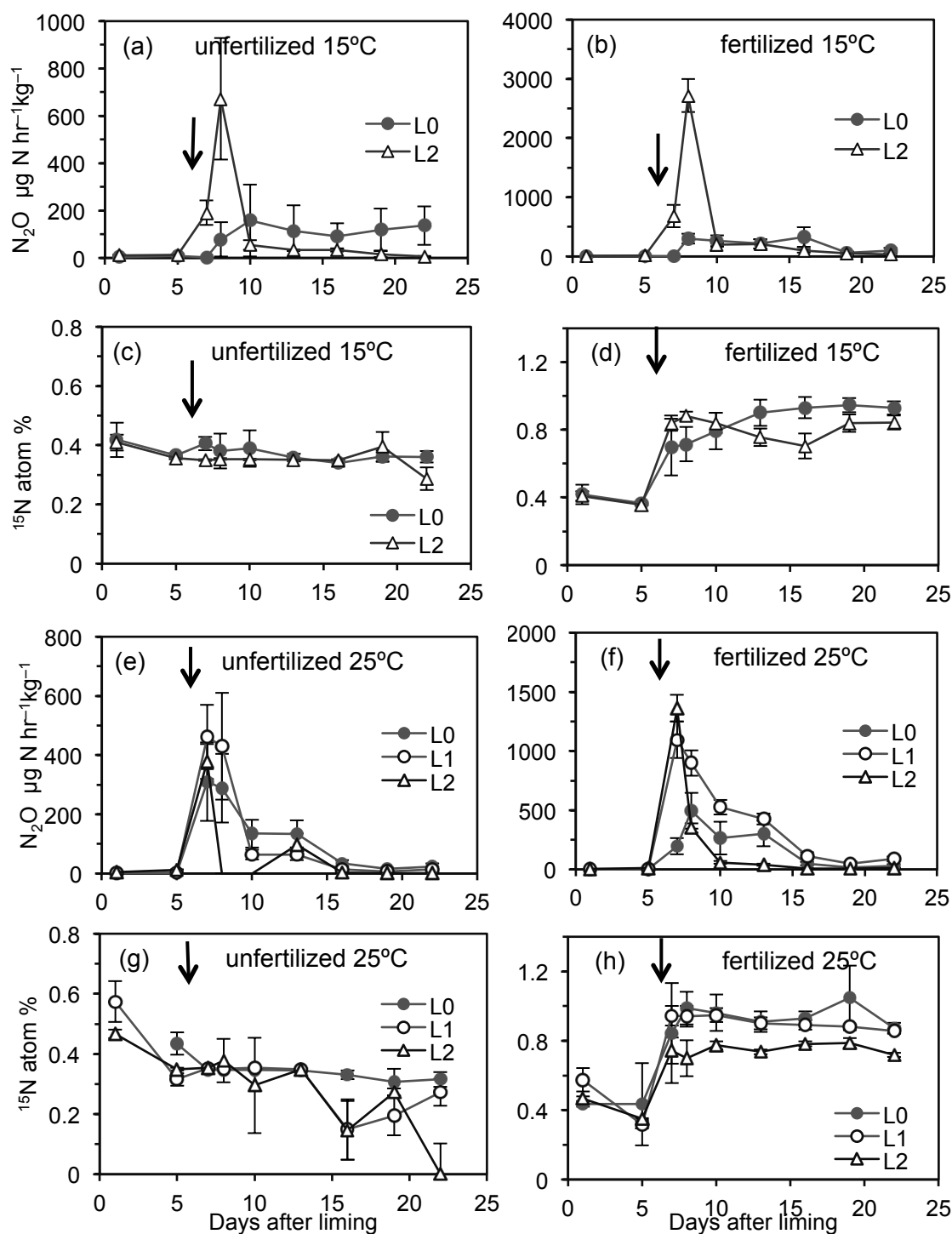


Figure 7.2 Total N_2O production and ^{15}N atom % at 15°C (a-d) and 25°C (e-h) in unfertilized (left) and fertilized (right) soils in experiment 1. L0 is the no lime treatment; L1 and L2 treatments received 4 and 20 mg $\text{CaCO}_3 \text{ kg}^{-1}$ soil, respectively. Fertilizer (KNO_3) in solution form for the fertilized soils and distilled water in the unfertilized soils were added on day 6 (increasing WFPS from 50 to 80%), indicated by the black arrow. Note the difference in scales

7.3.1.3 *Effect of liming on denitrification ($^{15}\text{N}_2\text{O}$ from the fertilizer)*

The time-course of ^{15}N atom% is shown in Fig. 7.2. In unfertilized treatments, ^{15}N atom% remained almost unchanged during the incubation period. In the fertilized soils, the ^{15}N increased from around 0.4 to 0.8–1 atom% after fertilization on day 7. Similar to total $^{14+15}\text{N}_2\text{O}$, the production of $^{15}\text{N}_2\text{O}$ was low in the pre-incubation period and peaked just after increasing the moisture content to 80 % WFPS in both unfertilized controls and fertilized treatment and decreased within 2 days.

The cumulative $^{15}\text{N}_2\text{O}$ was lowest in the L2 treatment only at 25 °C (Table 7.1). The KNO_3 applied contained only 1 atom% excess ^{15}N , and therefore a significant amount of the observed $^{15}\text{N}_2\text{O}$ was from the soil rather than from the applied fertilizer and only 3-12 % of the fertilizer- ^{15}N applied was emitted as $^{15}\text{N}_2\text{O}$. To estimate the amount of $^{15}\text{N}_2\text{O}$ produced from denitrification of the applied ^{15}N we subtracted the $^{15}\text{N}_2\text{O}$ in unfertilized treatments from that in fertilized treatments. At 25 °C, the high lime (L2) and unlimed (L0) treatments emitted less than 4 % of the applied ^{15}N as N_2O , while the low lime (L1) treatment emitted 12 % of the applied ^{15}N as N_2O . At 15 °C, L2 emitted 11 % and L0 emitted 5% of the applied ^{15}N as N_2O (Table 7.1).

Table 7.1 Cumulative $^{15}\text{N}_2\text{O}$ and total $^{14+15}\text{N}_2\text{O}$ production (experiment 1)

Temp	N added	$^{15}\text{N}_2\text{O}$ (mg ^{15}N kg $^{-1}$)			$^{15+14}\text{N}_2\text{O}$ (mg N kg $^{-1}$)		
		L0	L1	L2	L0	L1	L2
15°C	No	0.29±0.46a	–	0.33±0.21ab	41.52±65.76a	–	46.27±29.11ab
	Yes	1.37±1.02bc	–	2.58±1.51c	76.64±25.23bc	–	185.00±21.47c
25°C	No	1.01±1.41 ^{ABC}	0.28±0.17 ^{ABD}	0.11±0.03 ^A	43.37±48.00 ^{AB}	42.08±20.97 ^{BC}	16.33±4.54 ^A
	Yes	1.67±0.54 ^D	2.71±0.10 ^C	0.84±0.06 ^{BCD}	69.41±24.00 ^{CD}	146.44±5.65 ^D	57.56±4.23 ^{BC}

L0 is unlimed soil; L1 is low lime rate at 4 g CaCO_3 kg $^{-1}$; L2 is high lime rate at 20 g CaCO_3 kg $^{-1}$. Different letters (lowercase for 15°C and uppercase for 25°C incubation temperature) indicate significant differences among lime and fertilizer treatments at $p < 0.01$. Analysis of variance was done separately for each incubation temperature.

7.3.2 Experiment 2: Anaerobic incubation

7.3.2.1 Effect of liming on N_2O production

The response of N_2O to liming was quite similar at both low and high-temperature incubations with or without NO_3^- addition (Fig. 7.3). Both the rate and magnitude of N_2O production was greater in the unlimed treatment and lowest in the high lime treatment ($p < 0.01$) throughout the incubation period. The difference in the amounts of N_2O produced between the unlimed and limed soils increased with incubation time. At the early stages (5 hours) of the incubation, in soils without N addition at 25°C, the unlimed treatment had produced 20 times more N_2O than the high lime treatment, but this difference increased to more than 300 times by the end of the incubation. A similar trend was observed at the lower temperature (15°C) despite much lower total N_2O production.

The increase in incubation temperature from 15 to 25°C doubled the rate of N_2O production (both at the early stage (5 hr) and late stage (44 hr) of the incubation) in all the treatments except for the fertilized limed treatments at 44 hours where the N_2O produced was higher at 15°C than 25°C.

The three-factor (lime addition, temperature and nitrogen addition) regression analysis (Table 7.2) showed that in the early stages of the incubation (5 hours) all the three factors had a significant effect on the N₂O production rate. However, at the final stages, incubation temperature did not always significantly affect the N₂O production rate. Figure 7.4 shows the amount of N₂O accumulated at 5 and 44 hours from the start of the incubation. At 5 hours, both at low and high temperature there was no significant difference between L0 and L1 treatments regardless of N addition. The high lime treatment, however, always had the lowest accumulated N₂O regardless of the incubation temperature or whether N was added or not.

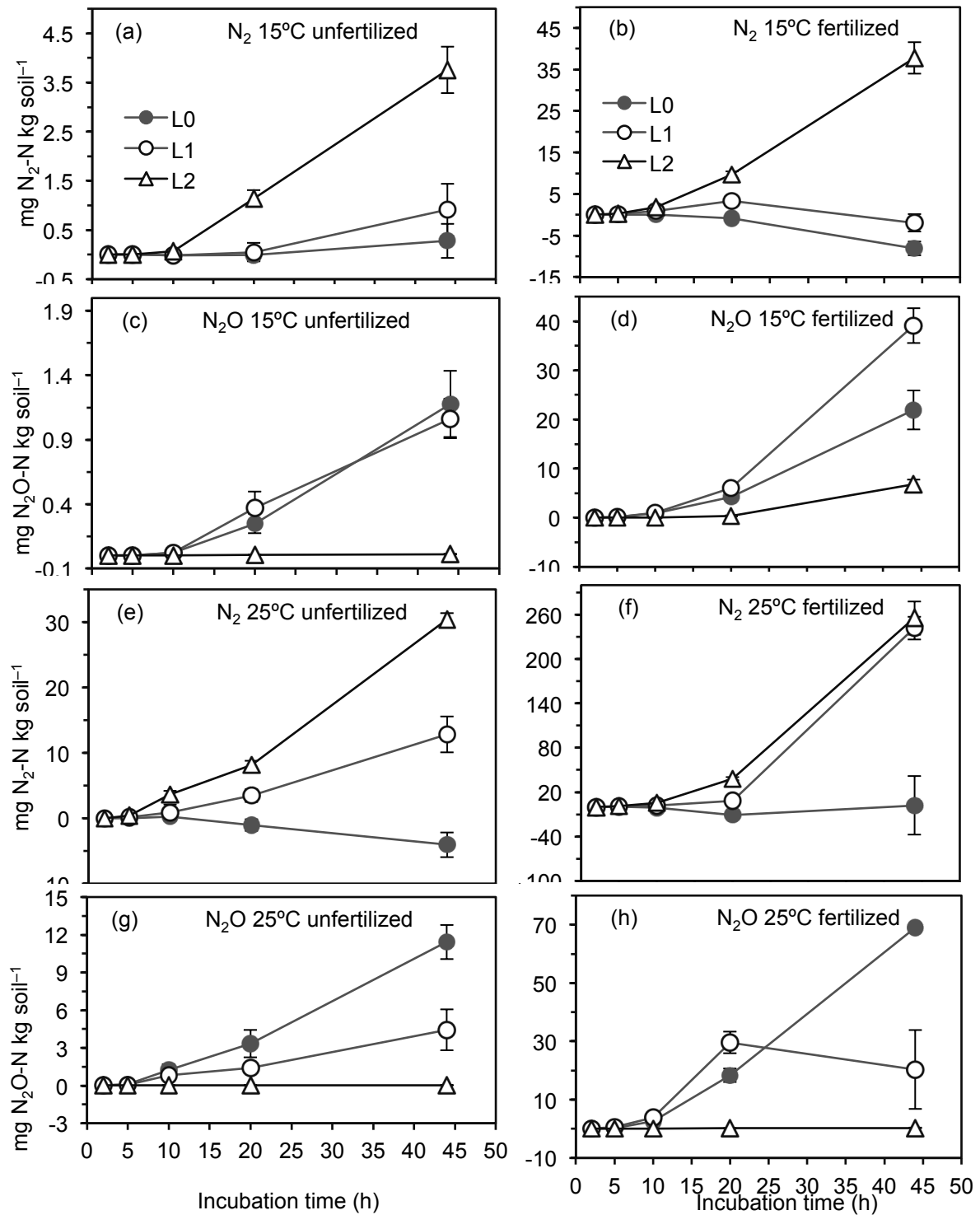


Figure 7.3 Dinitrogen gas (N_2) and nitrous oxide (N_2O) production during the incubation at 15 and 25°C (experiment 2). N_2 gas was calculated as the difference in total N_2O produced between acetylene and no-acetylene treatments. Error bars represent standard deviation (n=4). L0 is unlimited soil, L1 and L2 is 4 and 20 $mg\ CaCO_3\ kg^{-1}$ soil, respectively. Note the difference in scales

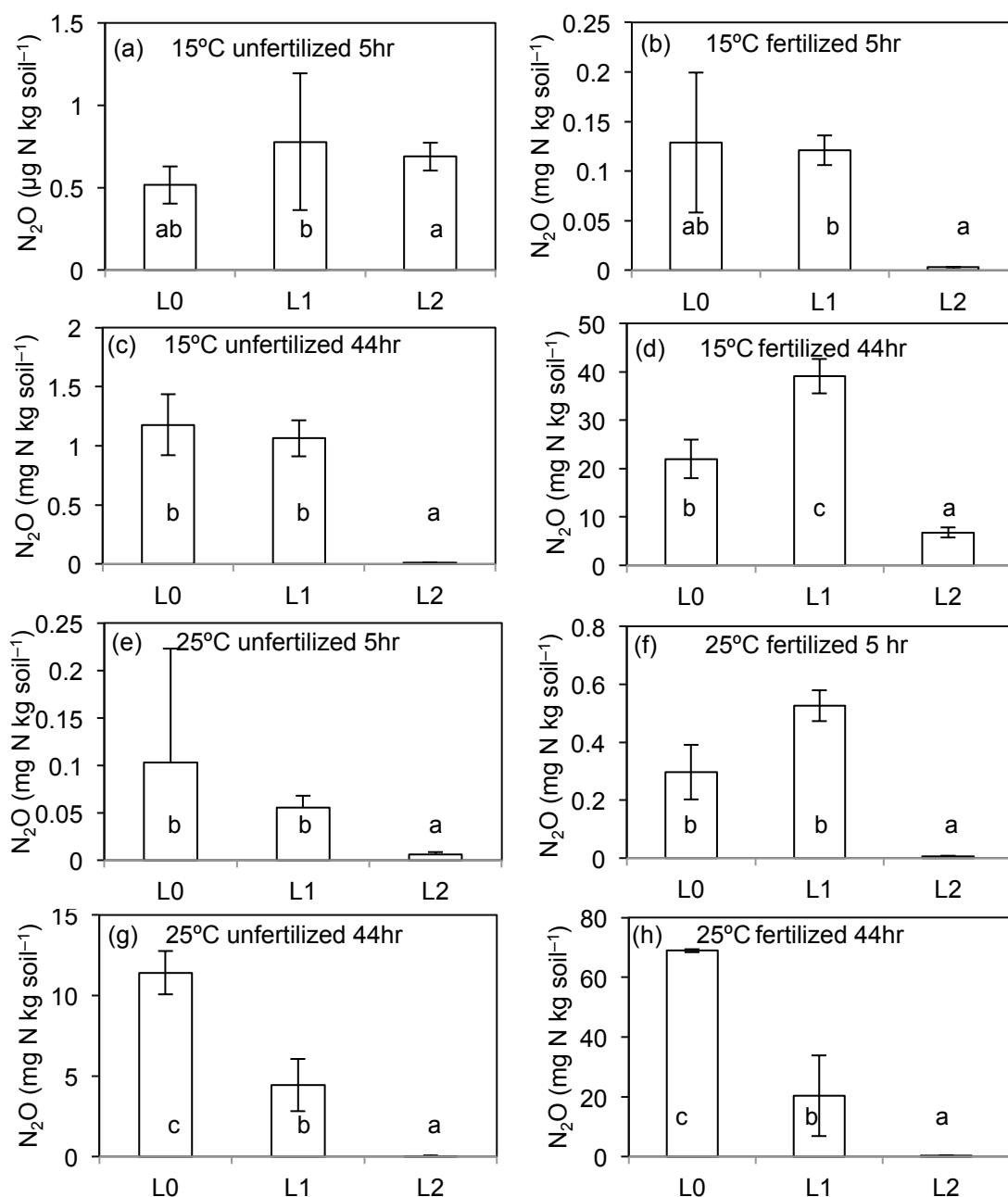


Figure 7.4 Accumulated N_2O at 5 and 44 hours in fertilized and unfertilized soils in experiment 2. L0 is the no lime treatment; L1 and L2 treatments received 4 and 20 mg $CaCO_3 kg^{-1}$ soil, respectively. Note the difference in scales and units. Different letters within each single graph indicate significant differences among the lime treatments at $p < 0.05$

7.3.2.2 Effect of liming on N_2 production

The effect of liming on the N_2 production pattern was the exact opposite to that of N_2O (Fig. 7.3). Increasing liming rate increased N_2 production ($p < 0.01$) at both incubation

temperatures with or without N addition. While the N₂ production increased at an increasing rate throughout the incubation period in the high lime treatment at both incubation temperatures, it decreased over time in the unlimed fertilized soils at 15°C and in the unlimed unfertilized soil at 25°C. In the early stages of the incubation (5 hours), we did not observe significant differences in N₂ production among the treatments at 15°C (Fig. 7.5).

Results of multiple regression analysis of lime rate, N and temperature on N₂ emission (n = 4) showed that lime, N addition and temperature all significantly increased N₂ accumulated both in the early and final stages of the incubation (Table 7.2).

Table 7.2 Multiple regression analysis of the influence of liming rate, incubation temperature and nitrogen addition on N₂O and N₂ production and N₂O/(N₂O+N₂) at 5 and 44 hours from the start of incubation (experiment 2).

N gas	Incubation time (hr)	Regression equation	R ²
N ₂ O	5	−64.01 lime + 12.31 temp + 1.52 N − 154.62	0.48***
	44	−8105 lime + 179.56 N + 9832.51	0.43***
N ₂	5	194.60 lime + 29.08 temp + 2.62 N − 677.70	0.62***
	44	4215 lime + 8434 temp + 805 N − 203482.5	0.54***
N ₂ O + N ₂	5	130.58 lime + 41.40 temp + 4.15 N − 832.32	0.73***
	44	34049 lime + 8499 temp + 985 N − 193650	0.61***
N ₂ O / (N ₂ O + N ₂)	5	−0.44 lime + 1.10	0.19*
	44	−0.61 lime + 1.56	0.64**

Single star (*) is $p < 0.05$; ** is $p < 0.01$; *** is $p < 0.001$; ns is non-significant

7.3.2.3 Effect of liming on net denitrification and denitrification product ratio

Net denitrification (N₂+N₂O emissions) increased with increasing liming rate and with increasing soil pH (Table 7.3). The net denitrification in high lime treatment (L2) was at least 50% higher than in the unlimed treatment at both incubation temperatures and in both unfertilized and fertilized soils. Except for 25°C fertilized soils, L2 treatment had at least 20% higher net denitrification than L1 treatment. Within each lime treatment, the temperature increase from 15 to 25°C more than doubled the net denitrification.

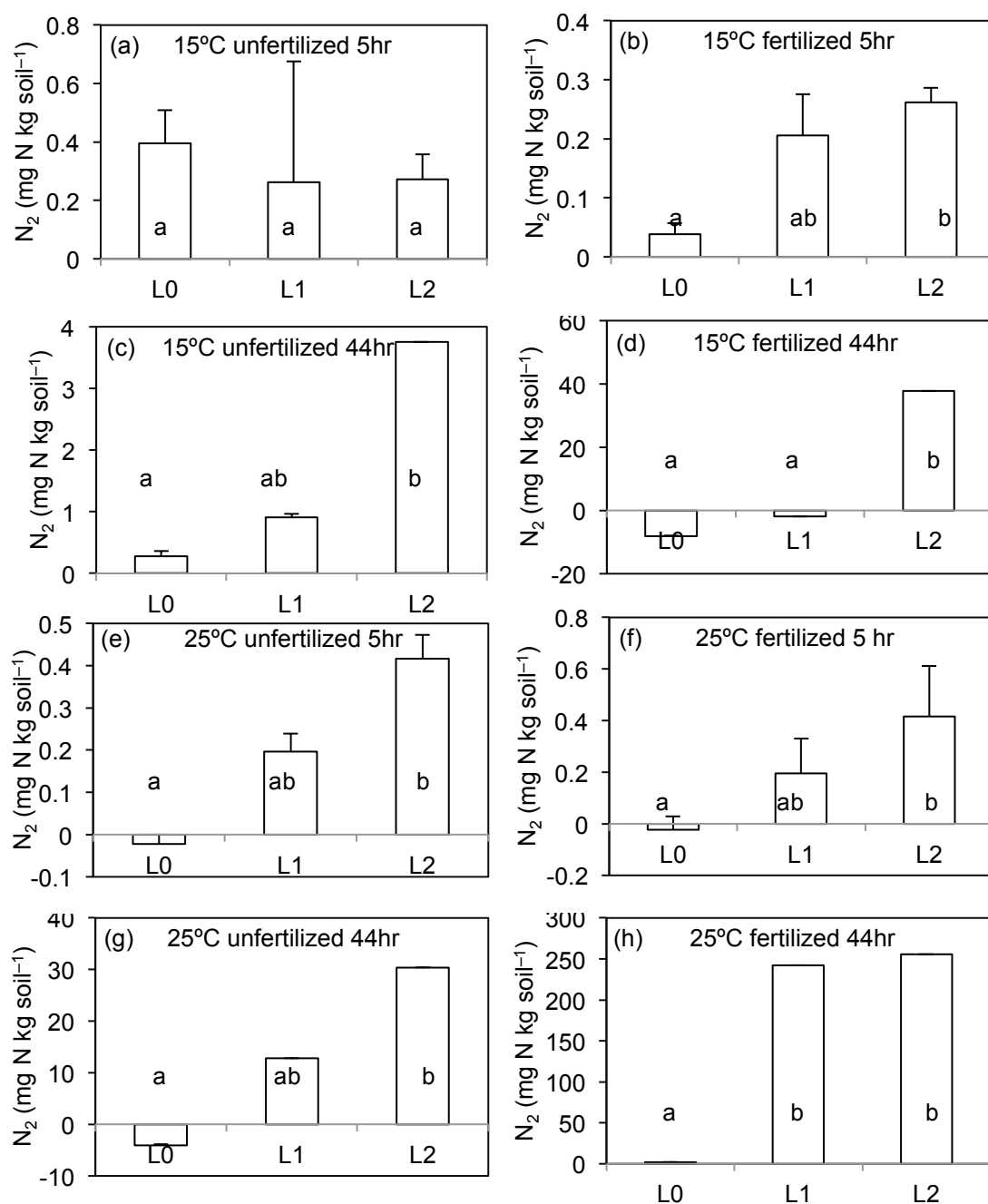


Figure 7.5 Cumulative dinitrogen gas (N_2) production at 5 and 44 hours in fertilized and unfertilized soils in experiment 2. Error bars represent standard deviation ($n=4$). L0 is unlimed soil, L1 and L2 is 4 and 20 $mg\ CaCO_3\ kg^{-1}$ soil, respectively. Note the difference in scales. Different letters within each single graph indicate significant differences among the lime treatments at $p < 0.05$

The denitrification product ratio of $N_2O/(N_2+N_2O)$ was influenced, to a large degree, by liming and, to a lesser degree, by incubation temperature (Table 7.3). In the unlimed (L0) soils, more than 80 % of denitrification was N_2O . In low lime rate treatment (L1), 8–56 % of the denitrification products was N_2O except at 25°C in the fertilized soils. In the high lime

treatment (L2), less than 1% of the denitrification was N₂O and more than 99% was N₂ gas. These results indicate the stimulation of the N₂O-reductase activity by liming. The production ratio of N₂O/(N₂+N₂O) also showed a significant negative correlation with soil pH (Fig. 7.6).

Table 7.3 Cumulative net denitrification (N₂O+N₂) and production ratio (N₂O/(N₂O+N₂))(experiment 2)

Temp	Nitrogen added	Net denitrification (mg N kg ⁻¹)			Product ratio (%)		
		L0	L1	L2	L0	L1	L2
15°C	No	1.45±0.31a	1.97±0.42a	3.76±0.47b	82±10a	56±8b	0.3±0.1c
	Yes	13.83±2.61a	37.12±1.52b	44.56±3.36c	159±4a	105±3b	15±1c
25°C	No	7.36±1.19a	17.26±1.40b	30.46±0.95c	158±18a	26±5b	0.1±0.1c
	Yes	39.51±5.12a	262.27±8.12b	255.77±22.44b	100±50a	8±2b	0.1±0.0c

L0 is unlimed soil; L1 is low lime rate at 4 g CaCO₃ kg⁻¹; L2 is high lime rate at 20 g CaCO₃ kg⁻¹. Different letters within the same row indicate significant differences among lime treatments at p<0.01.

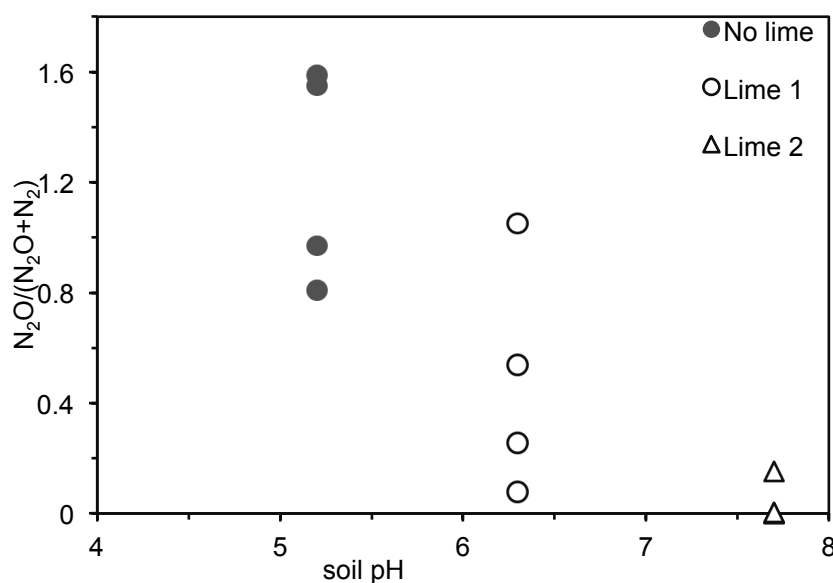


Figure 7.6 Relationship between soil pH and denitrification product ratio (N₂O/(N₂O+ N₂))

7.4 Discussion

The N₂O production during the 7-day pre-incubation period (experiment 1) was higher in limed soils (2.5 and 8.9 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$ in L1 and L2, respectively) compared to unlimed soils (0.9 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$). Several studies have previously found increased N and C mineralization due to liming (Edmeades et al. 1981; Brumme and Beese 1992) and consequently higher N₂O attributed to nitrification (Mørkved et al. 2007; Baggs et al. 2010; Uchida et al. 2013). High peaks of N₂O, in all treatments (experiment 1), on day 7 were due to increase in moisture content and additionally due to fertilization in treatments that received KNO₃. This peak was higher but short-lived in the high liming treatment than in the unlimed soil, and the unlimed soil showed higher fluxes until the end of the experiment (Fig. 7.2). This result indicates that when conditions favoured nitrification (50% WFPS), limed soils emitted more N₂O, but the unlimed soil showed more potential to emit N₂O as WFPS increased to 80%. However, this shift was affected by both temperature and N availability. A significant increase in N mineralization seemed to have been induced by lime application, which led to a 50% increase in soil NH₄⁺ concentration in the limed soils (Figs. 7.1e and f) one day after liming the soils. The increase in NH₄⁺ concentration continued until day 5 and started to drop on day 7, as the NO₃⁻ in limed soils started to show higher values than the unlimed soil (Fig. 7.1c). This suggests that in the first few days liming induced mineralization and thereafter there was consumption of NH₄⁺ and production of NO₃⁻ by the nitrification process (Clough et al. 2003).

Surprisingly, cumulative emissions (both total ¹⁴⁺¹⁵N₂O and ¹⁵N₂O) in the high lime treatment (L2) in experiment 1 were higher at 15°C than at 25°C (Table 7.1). While several studies have found higher N₂O emissions at higher temperatures (Saleh-Lakha et al. 2009; Saggar et al. 2013), it is not impossible to observe higher fluxes at lower temperatures. Dorland and Beauchamp (1991) demonstrated that increasing C availability could lower the

temperature at which optimal denitrification occur. Carbon from lime increased available C in the limed soils (Fig. 7.1) and this could explain why the high lime treatment showed higher cumulative $^{15}\text{N}_2\text{O}$ and total $^{14+15}\text{N}_2\text{O}$ at 15°C than at 25°C. Another possible reason for higher N_2O emission at 15°C than at 25°C in L2 treatment (Table 7.1) could be the reported higher activation energy for the reduction of N_2O to N_2 than the activation energy for production of N_2O (Saggar et al. 2013). At low temperature (15°C), more N_2O might have accumulated than was reduced, while more N_2O could have been reduced to N_2 at higher temperature (25°C).

At 25°C, the soil NO_3^- -N was depleted from the limed fertilized soils by the end of the 44 h anaerobic incubation in experiment 2 (Table 7.4) and emitted as either N_2 or N_2O (Table 7.3). This large depletion of NO_3^- was not observed at 15°C or in the unfertilized treatments. The depletion of NO_3^- -N in the fertilized treatment was most likely stimulated by the addition of glucose as a C source (Clough et al. 2004). The fact that we could not see the same result at 15°C suggests that temperature and low C availability limited denitrification in this soil. Low temperature could have resulted in lower enzyme activity (Saleh-Lakha et al. 2009) which was further limited by low C availability. The increase in denitrification with the addition of N, C and increasing temperature has been reported by other studies (Pfenning and McMahon 1997; Bonnett et al. 2013; Myrstener et al. 2015).

We previously found, in a field study, that N_2O emissions were largely controlled by the ratio of available soil C to NO_3^- -N in this Andosol (Mukumbuta et al. 2017b). A significant interaction between temperature and N or C addition on NO_3^- consumption and N_2O emissions was also found in a Canadian soil (Wertz et al. 2013). Regardless of the amount of NO_3^- consumed in each of the treatments, our study demonstrated that liming has potential to reduce N_2O production substantially under anaerobic conditions. This finding is

in agreement with McMillan et al. (2016), who carried out a similar experiment in two New Zealand soils.

Table 7.4 NO_3^- and water extractable organic carbon (WEOC) at the end of experiment 2

Temp	Nitrogen added	NO_3^- (mg N kg ⁻¹)			WEOC (mg C kg ⁻¹)		
		L0	L1	L2	L0	L1	L2
15°C	No	115.9±26.7	165.2±5.9	168.3±60.3	61.7±22.9	60.9±29.0	148.0±10.8
	Yes	276.6±31.3	325.5±19.9	292.5±9.4	265.2±80.5	299.5±66.3	314.1±42.2
25°C	No	121.6±23.9	187.4±12.5	148.4±10.7	51.9±25.7	71.7±28.2	160.1±32.6
	Yes	188.2±67.0	0.6±1.2	0.1±0.0	186.9±84.2	28.4±10.6	82.6±29.1

A previous study by Li et al. (2014) done using cultured *Pseudomonas* bacteria isolated from the same soil used in this experiment found that addition of a C source (sucrose) together with N increased N_2O fluxes 20-folds compared to addition of N alone at 20°C. Takeda et al. (2012) isolated a number of bacteria species from this same Andosol and found that their optimum pH range was in the acidic range, and that their N_2O emitting activity reduced significantly in the neutral pH range. Nie et al. (2016) identified *Pseudomonas* species as the dominant N_2O emitting bacteria in this Andosol and found that the *Pseudomonas* species that lacked the *nosZ* gene (responsible for N_2O reduction to N_2) had an optimum pH of 5.8–6.3, while those that contained the *nosZ* gene had an optimum pH higher than 6.5. This might explain why a significant reduction in N_2O was always observed in the L2 treatment (pH 7.6) at both 25 and 15°C but not in L1 treatment (pH 6.4). Soil pH in all treatments showed minimal variation, although by the end of the experiment pH of L1

treatment had decreased to 6.1 (Fig. 7.1). It seems therefore that raising the pH of this Andosol to pH above 6.5 would lead to significant reductions in N₂O emissions. In our previous study, part of this field that had a pH above 6 showed lower N₂O emissions than one where the pH was below 5.5 despite both plots receiving the same amount of chemical fertilizer and manure during the study period (Mukumbuta et al. 2017a). Therefore, while several factors would be at play under field conditions, maintaining soil pH above 6.5 would be recommended for this soil. A number of field studies have also reported reduced N₂O fluxes due to liming under field conditions (Brumme and Beese 1992; García-Marco et al. 2016).

The denitrification product ratio (expressed in this study as $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$), a subject that has been studied for decades, is a good indicator of the activity of the N₂O reductase enzyme. In this study, the product ratio was significantly reduced by liming (Table 7.3) and negatively correlated with soil pH (Fig. 7.6). A number of studies (Rochester 2003; Zaman et al. 2007; Bakken et al. 2012) have reported this reduction in the denitrification product ratio with increasing soil pH in different soil types. However, McMillan et al. (2016) found that the product ratio increased with increasing soil pH in a volcanic soil, and attributed it to the high allophane content, which adsorbs copper (an essential co-factor of the N₂O reductase enzyme). Our study was similar to that of McMillan et al. (2016), but contrary to their finding, our results indicate that even in a volcanic soil increasing pH through liming decreases the denitrification product ratio. The higher liming rate in our study compared to theirs could be a reason for the disagreement between their findings and ours. We raised the soil pH from 5.4 (unlimed soils) to 7.6 (highest lime treatment), while the highest lime treatment in McMillan et al. (2016) had a pH of 6.5. In fact, despite not always finding significant differences in N₂O emissions between the unlimed and limed soils, the denitrification product ratio $[\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)]$ in the limed soil was always

significantly lower than that in the unlimed treatments in our study, contrary to McMillan et al. (2016). The reason for this might be that N_2O producing denitrifiers containing the *nosZ* gene in our soil, and therefore capable of reducing N_2O to N_2 , had previously be found to have a higher optimum pH (above 6.5) than those that lacked the *nosZ* gene (Nie et al. 2016). The final pH values in our study were higher than those of McMillan et al. (2016) and could have led to higher rate of N_2O reduction to N_2 (the higher activity of *nosZ* containing denitrifiers), further explaining the different results between our study and theirs.

Liming, however, significantly increased net denitrification (Table 7.3) by increasing the N_2 emission markedly. Liming therefore increases the overall loss of N from soil in gaseous form. Lime treatments showed as much as 6 times more net N ($\text{N}_2 + \text{N}_2\text{O}$) loss from denitrification than the unlimed soil. A review of several studies by Simek and Cooper (2002) also concluded that increasing pH leads to increased combined loss of N gases. Therefore, care must be taken not to solve the problem of N_2O emissions by increasing the net loss or depletion of N from soils through the stimulation of total denitrification. Another issue to consider is that while increasing pH by liming can reduce $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio, it might be offset by the overall increase in total denitrification (Saggar et al. 2013), especially in soils where denitrification might be naturally low.

This study was conducted under controlled laboratory conditions that are obviously very different from field conditions. To fully evaluate and understand the potential of liming to reduce N_2O emissions, field studies are necessary. Our results, similar to other studies (Baggs et al. 2010), suggest that N_2O fluxes respond differently to liming under different moisture levels. Therefore, methods that can distinguish between the nitrification and denitrification N_2O under field conditions should be used. Isotopic analysis of N_2O using laser spectrometry (as in experiment 1 of this study) is one such method.

7.5 Conclusion

This study evaluated how liming affects soil N₂O production via denitrification under aerobic and anaerobic conditions, and how liming affects the denitrification product ratio.

Liming increased N₂O production when soil moisture was low (50% WFPS) but decreased the N₂O production when moisture content increased to 80% WFPS in experiment 1. Under complete anaerobic conditions, liming showed significant potential to decrease N₂O production and decreased the denitrification product ratio (N₂O/(N₂O+N₂)). The N₂O production patterns were influenced by both soil moisture (decreased over time at low WFPS but then showed a sharp increase as WFPS was increased) and liming treatments.

8. General Discussion and Overall Conclusion

8.1 Achievements and contribution of current study

8.1.1 Effect of land-use change between grassland and cornfield on C and N dynamics

Land-use change activities have huge implications on the soil C and N dynamics especially as it relates to GHG emissions and soil C sequestration. Understanding the factors that control changes in C and N during and after land-use change is vital for coming up with appropriate measures that can mitigate any resulting negative effects. Land-use change from perennial grasslands to cropland has been reported to result in significant losses of SOC (Post and Kwon 2000; Guo and Gifford 2002; Lal 2004) and increased CO₂, N₂O and NO emissions resulting from elevated heterotrophic soil respiration and organic matter mineralization (Whitehead et al. 1990; Nécépálová et al. 2013). Changing land-use from cropland to grassland is one of the practices proposed by IPCC's 5th report on climate change to mitigate GHG emissions from agriculture (Smith et al. 2014) and has been echoed by other studies (Lal 2002; Rees et al. 2005). There is therefore need to evaluate how long and how much mitigation can be achieved from this direction of land-use change.

In the current study we evaluated the effect of the land-use change from a permanent grassland to cropland and back on soil C, N and GHG emissions. Indeed similar to several studies mentioned above we found a profound increase in heterotrophic soil respiration (chapter 4) and N₂O emissions (chapter 5) when grassland was ploughed and converted to cropland (cornfield). Most studies (e.g. Guo and Gifford 2002; Rees et al. 2005; Nécépálová et al. 2013), due to their short-term nature, only evaluate a one directional change in land-use. This study, in chapters 4 and 5, however evaluated a two directional change on the same land and we found that converting back to grassland did reduce GHG emissions. Interestingly however, this study also found that while grassland to cropland conversion

increased GHG emissions, it also led to significant increase in SOC (chapter 4) and SON (chapter 5) at 0–15 and 15–30 cm depth. Although both the SOC (Table 4.1 and Fig 4.1) and SON (Fig. 5.2) decreased slightly in the third year of the cropland, the sharp increase in the first year of conversion. This indicates that conversion or ploughing of grassland is important to turn the plant residue/applied manure into actual soil C. Besides the decrease in SOC and SON within the three years of the cropland was lower than the initial increase in the first year. We conclude and recommend that occasional ploughing or conversion of grassland be implemented for a period of at least one or two years. This study (chapters 4 and 5) is also one of very few to indicate that cropland to grassland conversion does reduce GHG emissions but significant gains start to accrue only from the third year (Figures 4.2 and 5.3).

8.1.2 Manure management as a tool to mitigate GWP and GHGI

The use of manure as a means to increase SOC and mitigate GHG emissions has been gaining great interest in the recent past and several studies (Lal 2004). Ginting et al. (2003), Rees et al. (2005), VanderZaag et al. (2011), Ryals et al. (2014) and Barton et al. (2016) have indeed proposed that this is a viable management practice for the aforementioned purpose. However, there are several other studies (Collins et al. 2011; Das and Adhya 2014; Zhang et al. 2014b; Vu et al. 2015) indicating quite the opposite and that while manure does increase soil C, it increases CH₄, N₂O and CO₂ emissions substantially thereby offsetting any gains achieved through the increase in soil C. Most of these studies mentioned above (e.g. Collins et al. 2011; Ryals et al. 2014), however, have either not considered all the GHGs or have not evaluated the resulting increase in GHG against the increase in soil C and/or increased plant/crop productivity. Given the need for increased food production globally and keeping in mind that the primary purpose of agricultural activities is precisely to produce

food and/or feed, any recommendations on whether management practices such as manure application should be adopted must not only look at GHG mitigation potential but must consider the increase/loss in productivity from such practices.

The current study evaluated the effect of manure management and fertilization on net GHG emissions calculated as global warming potential (GWP) and then calculated greenhouse gas intensity (GHGI), calculated as GWP divided by the amount of plant biomass yield, in a cornfield (chapter 6). From our results, manure application increased CO₂ and N₂O emissions (Fig. 6.2). However manure increased net ecosystem carbon balance (Tables 6.3 and 6.5), which is the difference between C inputs (net primary production and manure input) and C losses (harvested C and CO₂ emissions from heterotrophic soil respiration), leading to huge reductions in GWP and GHGI. This is a clear indication of the importance to carry out full accounting of the GHGs as well as productivity of any management practice before giving recommendations on whether to adopt it or not. Findings from chapter 6 also indicate that application of manure twice in a growing season, 40 Mg fresh manure (450 kg N and 7.3 Mg C hectare⁻¹) in autumn and 29 Mg fresh manure (300 kg N and 5 Mg C hectare⁻¹) in spring, can achieve the same level of productivity as manure combined with inorganic fertilizer (40 Mg fresh manure; 450 kg N and 7.3 Mg C hectare⁻¹ in autumn plus inorganic fertilizer in spring; 104 kg N hectare⁻¹) (Table 6.5). This finding means that where manure is available in abundance, complete substitution of manure for inorganic fertilizer would not decrease productivity as is normally reported, but would actually lead to mitigation of net GHG emissions and increased soil C.

8.1.3 Mitigating denitrification N₂O fluxes through liming

Nitrous oxide is arguably the most important GHG emitted from upland agricultural soils. It is 265 times stronger than CO₂ in its effect on global warming potential (IPCC 2014).

Simple technologies and practices that can help mitigate N₂O are urgently needed. The only mechanism of N₂O consumption in soils is its reduction to dinitrogen (N₂) gas, which is controlled by the N₂O reductase enzyme activity during denitrification. The enzymes NO₃⁻ reductase, NO₂⁻ reductase and NO reductase that are required for the production of N₂O have a lower optimum soil pH than N₂O reductase, the enzyme needed for N₂O reduction to N₂ (Bakken et al. 2012; Pan et al. 2012). This would mean that increasing soil pH would increase N₂O consumption and reduce its emissions. However, whether reduction of N₂O emissions following liming can be achieved in soils is still not clear. Some studies (e.g. McMillan et al. 2016) have suggested that liming can not reduce N₂O emissions in volcanic soils due high allophane content, which adsorbs copper (a co-factor of the N₂O reductase enzyme)

This study assessed the effect of liming on denitrification N₂O production under aerobic conditions using the ¹⁵N tracer method. Also, the reduction of N₂O to N₂ was assessed under anaerobic conditions using the acetylene inhibition method. The results indicate that limed treatments (pH 6.4–7.6) showed higher N₂O fluxes at 50% WFPS but lower fluxes when WFPS was increased to 80% (Fig. 7.2). At 25°C cumulative N₂O and ¹⁵N₂O emissions in the high-lime treatment (pH 7.6) were the lowest (emitting at least 30% less ¹⁵N₂O and ¹⁵⁺¹⁴N₂O than the unlimed soil; pH 5.4) (Table 7.1). Under anaerobic conditions, the high-lime treatment showed at least 50% less N₂O than the unlimed treatment with or without N addition and also showed enhanced N₂ production (Figures 7.4 and 7.5). Results in chapter 7 confirmed that increasing soil pH would reduce N₂O emissions when conditions favoured denitrification (Figures 7.2 and 7.3). However, at low soil moisture conditions, the opposite could be true as N₂O fluxes tended to be higher in limed soils when WFPS was kept at 50% (Fig. 7.2). More extensive studies, especially under field conditions are required to further understand the interactions between liming and other soil and

environmental factors such as temperature, moisture and carbon and nitrogen availability on N₂O emissions.

8.2 Perspectives and future research needs

In the current study land-use change from grassland to cropland increased net GHG emissions but it led to increased soil C and N especially in the first year of conversion. Ploughing of grassland is important to turn the accumulated litter into SOC, but our study could not determine the best and practical number of years the cropland should be rotated. Research on how often the grassland/cropland rotation and the number of years for each can help determine the optimal rate turning plant biomass into actual SOC. Additionally, our study found that SOC and SON increased by a greater margin in the top 15 cm depth than the 15–30 cm layer. The depth of ploughing and therefore burial of the plant residue could be important. Deeper ploughing and burial of residue could potentially reduce the decomposition rate of resulting SOM since most microbial activities take place near the surface. Deep ploughing could also potentially lead to more C storage in the deeper soil layers and increase the total C storage. Further research addressing these points is recommended.

Manure application twice in the autumn and spring was found to lead to increased plant productivity and additionally reduced GWP and GHGI. This study was conducted for one year only and a much longer study looking at annual variations and covering different soil and climates is important. The combined application of manure and inorganic fertilizer was the best in terms of increasing productivity and mitigating GHG in the three-year study. However, the best combination ratios of the amounts of manure and inorganic fertilizer were not evaluated in this study. Therefore studies with several different application rates and

ratios of manure to inorganic fertilizer should be considered in future studies for different crops and soils.

Liming showed significant potential to reduce N_2O emissions under anaerobic conditions. Obviously, field conditions are rarely 100% anaerobic and it is not very clear whether how different factors such as temperature, nutrient content and moisture will interact in influencing the response of N_2O emissions to liming. Partitioning and separating field in situ N_2O fluxes from nitrification and denitrification is critical for further understanding the aforementioned interactions. The use ^{15}N isotope techniques (for example incubation and field in situ isotope labelling studies (e.g. Wrage et al. 2004; Baggs 2008) or the use of unique ^{15}N signatures of different processes such site preference (Ryabenko 2013; Snider et al. 2015)) can go a great deal in assisting to understand the influence of liming and other practices on total N_2O emissions while at the same time evaluating nitrification and denitrification separately.

8.3 Conclusion

Overall N_2O emissions and heterotrophic CO_2 increased as land-use changed from grassland with the opposite leading to decreased emissions. Despite leading to increased greenhouse gas emissions, conversion of grassland to cornfield increased soil organic carbon (SOC) and nitrogen contents and stocks. Conversion or ploughing of grassland could be necessary to turn plant residue and manure into SOC. Application of manure can significantly reduce greenhouse gas intensities and global warming potential, while increasing net primary production and net ecosystem carbon balance. Liming reduced N_2O emissions under anaerobic conditions, but could increase emissions when soil moisture is low due to occurrence of both nitrification and denitrification processes under aerobic conditions.

9. Summary

Introduction

Agricultural activities are a major source of the three most important greenhouse gases (GHGs); carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). Management practices that mitigate GHG emissions while enhancing crop productivity are required. Adequate fertilization and manure application increase soil organic carbon (SOC) through increased biomass production and residue retention in soils. On the other hand, both manure and fertilizer application can increase N₂O and CH₄ emissions and offset the benefits of increased SOC sequestration. Therefore, the overall change in GHG emissions and SOC sequestration should be evaluated against crop productivity.

Land-use change can greatly affect carbon (C) and nitrogen (N) dynamics due to differences in vegetation types and management practices among land-uses. Rotations between managed grassland and cornfield is a common practice in Japan. However, the effect of this practice on C and N dynamics is not well documented.

N₂O reduction to dinitrogen (N₂) gas is controlled by N₂O reductase enzyme during denitrification. There are reports that N₂O reductase activity is enhanced at high soil pH (Šimek et al. 2002; Herold et al. 2012), which would suggest that increasing soil pH could mitigate N₂O emissions by increasing its reduction to N₂. However, uncertainty remains on whether liming can reduce N₂O emissions and very few studies have been done in andosol soils.

The objectives of this study were; (i) to investigate the effect of land-use change between grassland and cornfield on C, N and GHG dynamics; (ii) to investigate the effect of manure management (timing and rate) on global warming potentials (GWP) and greenhouse gas intensities (GHGI) in a cornfield; and (iii) to evaluate the possibility of reducing N₂O emissions from denitrification through liming.

Effect of land-use change on C, N and GHG dynamics

A study was conducted from 2005 to 2015 at a field that was an old permanent grassland (OG) that was ploughed and converted to a cornfield (2010–2012) and then converted to a new grassland (NG) (2013–2015) in Shizunai livestock farm, Hokkaido University. Changes in soil C, N and GHGs were monitored in three treatments; chemical fertilizer plus manure, chemical fertilizer only and unfertilized control.

Contrary to what is widely reported, soil organic C and N content and stock increased after conversion of grassland to cornfield. This increase was significant mainly in manure amended plot ($p < 0.01$) at both 0–15 and 15–30 cm depths. A further increase in SOC and N content in the manure plot was observed after land-use change from cornfield to new grassland.

In fertilized plots soil respiration (RS), CO_2 measured in planted plots, was higher in OG (10.0–13.0 $\text{Mg C ha}^{-1} \text{yr}^{-1}$) than in cornfield (6.6–9.7 $\text{Mg C ha}^{-1} \text{yr}^{-1}$) ($p < 0.05$), and then increased again in NG (8.5–11.8 $\text{Mg C ha}^{-1} \text{yr}^{-1}$). RS in the unfertilized plot followed a similar trend as in fertilized plots but was not statistically different among the three land-uses. Contrary to RS, heterotrophic soil respiration (RH) increased due to land-use change from grassland to cornfield by more than 30% and then declined by 20% after converting back to grassland ($p < 0.01$). The contribution of RH to RS was significantly higher in cornfield (60–100%) than in old grassland (38–60%) and new grassland (47–80%) ($p < 0.05$), and it was higher in new than in old grassland but not statistically significant. Lower RH contribution to RS in OG and NG indicates the higher contribution of root respiration in grassland, and shows that organic matter decomposition is the most important source of CO_2 in land-uses that undergo tillage activities.

Annual CH₄ emissions ranged from -0.16 to 5.47 kg C ha⁻¹yr⁻¹ in old grassland, -0.74 to 5.11 kg C ha⁻¹yr⁻¹ in cornfield and -0.29 to 2.78 kg C ha⁻¹yr⁻¹ in new grassland. Excluding CH₄ fluxes higher than 50 µg CH₄-C m⁻²hr⁻¹, which accounted for less than 9% of the total measurements, all plots were CH₄ sinks. Although there were no significant differences in annual CH₄ emissions among the three land-uses, the average fluxes in old grassland (all plots combined) were slightly higher than those in new grassland ($p = 0.04$). Changing land use from grassland to cornfield increased annual N₂O emissions by 6–7 times, from 0.6–2.6 kg N₂O-N ha⁻¹yr⁻¹ in OG to 4.4–14.9 kg N₂O-N ha⁻¹yr⁻¹ in cornfield, while the change from cornfield to grassland decreased N₂O emissions to 3.2–5.4 kg N₂O-N ha⁻¹yr⁻¹ (0.3–0.6 times reduction). N₂O emissions in the newly established grassland were 2–5 times higher than those in the 30-year old grassland. Soil mineral N (NO₃⁻ and NH₄⁺) was higher in cornfield, followed by new grassland and lowest in old grassland, while water extractable organic carbon (WEOC) did not significantly change with changing land use but tended to be higher in old and new grassland than in cornfield. The ratio of WEOC to soil NO₃⁻ was the most important explanatory variable for differences in N₂O emissions as land use changed. High N input, surplus soil N, and precipitation and low soil pH led to increased N₂O emissions.

Mitigating GWP and GHGI in cornfield through manure management in cornfield

To investigate the effect of manure management (timing and rate) on global warming potentials (GWP) and greenhouse gas intensities (GHGI), two experiments were setup in a cornfield. Firstly, in a three-year field study, the effect of inorganic fertilizer and composted cattle manure application on GHGs, GWPs and GHGI was evaluated after setting up unfertilized, inorganic fertilizer only (100 kg N ha⁻¹), manure only (~6.7 Mg C and 300 kg N ha⁻¹) and combined manure and inorganic fertilizer treatments. In the second part, autumn

only, autumn plus additional spring manure or inorganic fertilization (each application rate similar to first part) was evaluated on their effect on GHGI and GWP.

Nitrous oxide emissions increased following both inorganic and manure application. Methane fluxes were not influenced by the soil amendments while CO₂ fluxes increased with manure application. By applying manure twice, in autumn and spring in the second experiment, high plant productivity (7.1 Mg C ha⁻¹ NPP) was achieved without increased GHG emissions (-4.2 CO₂-C equivalents GWP) compared to one-time manure application (3.9 Mg C ha⁻¹ NPP and 0.3 CO₂-C equivalents GWP). Application of manure led to significant reductions in net GWP and GHGI, 4.6–8.5 Mg CO₂-C equivalents GWP and 1.5–2.5 kg CO₂-C equivalents/ kg yield GHGI in non-manure plots compared to 2.2–4.9 Mg CO₂-C equivalents GWP and 0.2–1.3 kg CO₂-C equivalents/ kg yield GHGI in manure plots, and this was largely due to direct C input to the soil and also through increased plant productivity. Measured N₂O fluxes were significantly elevated by rainfall of more than 30 mm within 7 days just before flux measurement. Cumulative N₂O emissions were controlled by soil pH, growing season rainfall and soil mineral N concentration. Less than 10% of CH₄ measurements accounted for 50%–100% of the total annual emissions and these were all observed when WFPS was more than 70%. Soil temperature influenced the seasonal change in CO₂ emissions, and manure application seemed to reduce the Q₁₀ values of CO₂ resulting from RH. This result indicates that when C availability is high, the effect of temperature on RH decreases and therefore increasing global temperature might have greater effect on soils with low C content.

Effect of liming on denitrification N₂O fluxes

Soil pH influences N₂O production processes and liming has been suggested as a tool to mitigate N₂O emissions. The effect of liming on denitrification N₂O production was assessed

under aerobic condition using the ^{15}N tracer, and under anaerobic condition using acetylene inhibition method. A Mollic Andosol with three lime treatments (unlimed soil, 4 and 20 mg $\text{CaCO}_3 \text{ kg}^{-1}$) was incubated at 15 and 25°C for 22 days at 50% and then 80% WFPS (with or without 100 N mg kg^{-1} as 1 atom% excess KNO_3) in experiment 1. For experiment 2, the soil was incubated under completely anaerobic conditions for 44 hours (with or without 100 N mg kg^{-1} as KNO_3) with and without acetylene. The difference in N_2O emission between acetylene and no acetylene treatments was regarded as N_2 emission. In experiment 1, limed treatments showed higher N_2O fluxes at 50% WFPS but lower fluxes when WFPS was increased to 80%. At 25°C cumulative N_2O and $^{15}\text{N}_2\text{O}$ emissions in the high-lime treatment were the lowest (emitting at least 30% less $^{15}\text{N}_2\text{O}$ and $^{15+14}\text{N}_2\text{O}$ than the unlimed soil; $p < 0.05$) with or without fertilization. However, at 15°C without N fertilization, the high lime treatment emitted more N_2O ($0.33 \pm 0.21 \text{ mg } ^{15}\text{N}_2\text{O-N}$ and $46.27 \pm 29.11 \text{ mg } ^{14+15}\text{N}_2\text{O-N}$) than the unlimed treatment ($0.29 \pm 0.46 \text{ mg } ^{15}\text{N}_2\text{O-N}$ and $41.52 \pm 65.76 \text{ mg } ^{14+15}\text{N}_2\text{O-N}$). Under anaerobic conditions, the high-lime treatment showed at least 50% less N_2O emission than the unlimed treatment at both temperatures with or without KNO_3 addition and also showed enhanced N_2 production. These findings indicate that the positive effect of liming on the mitigation of N_2O is influenced by moisture conditions by shifting the balance between nitrification and denitrification .

Conclusions

While grassland to cropland conversion increased GHG emissions, it also led to significant increase in SOC and SON. Although both the SOC and SON decreased in the third year of the cropland, the sharp increase in the first year of conversion indicates that conversion or ploughing of grassland is important to turn plant litter/applied manure into actual soil C. Besides the decrease of SOC and SON within the three years of the cropland was lower than

the initial increase in the first year. These results call for a rethink of the commonly held notion that conversion of grassland to cropland depletes SOC, as the conversion could be beneficial in the short-term of three or less years.

Manure application increased N_2O and CO_2 emissions due to increased available N and C. However, manure application increased plant productivity (NPP) and increased net C balance leading to huge reductions in GHGI and GWP. Autumn plus spring manure application led to higher NPP and C balance without increasing net GHG emissions compared to the autumn only manure application. These findings not only highlight the importance of manure in mitigating GHGI but also the need for a comprehensive accounting of both environmental impacts (such as GHG emissions) and economic benefits (such as increased productivity) when evaluating the influence of management practices such as manure application.

Liming can reduce N_2O emissions from denitrification by enhancing N_2O reduction to N_2 . However, while liming can be recommended as a mitigation strategy, it could potentially enhance the overall loss of N from soils by increasing net denitrification. Furthermore liming might increase N_2O emissions in soils where nitrification is the dominant source of N_2O .

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