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**Factors controlling episodic soil CO₂
and N₂O emissions from managed
grassland and corn field in southern
Hokkaido, Japan**

(北海道南部の草地、飼料畑土壌からの突発的 CO₂
および N₂O 排出の制御因子)

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Division of Environmental Resources Doctor Course

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Chapter 1

GENERAL INTRODUCTION

Carbon dioxide (CO₂) is the dominant anthropogenic greenhouse gas (GHGs) in the earth's atmosphere (Keppel-Aleks et al., 2013), accounting for 60 % of global warming. This is a concern because nitrous oxide (N₂O) is a greenhouse gas with a global warming potential of 298 over a 100 years period (IPCC, 2007) and it also is the dominant stratospheric ozone-depleting substance emitted by humans in the twenty-first century (Ravishankara et al., 2009). The contribution of N₂O to the anthropogenic greenhouse effect has been estimated to 6 % (IPCC, 2001). Nitric oxide (NO) is also important components influencing the atmospheric chemistry (IPCC, 2007). It is a precursor of tropospheric ozone, which is a greenhouse gas formed by photochemical reaction, and is a precursor of nitric acid, which is a major component of acid rain (Akiyama et al., 2000). Increase in the GHGs concentration in the atmosphere is an important global issue, which has discernible impact on global climate (IPCC, 2007). Atmospheric N₂O and CO₂ concentrations have been increased to 324.2 ppb and 391 ppm in 2011 at a rate of $2.0 \pm 0.1 \text{ ppm yr}^{-1}$ and $0.73 \pm 0.03 \text{ ppb yr}^{-1}$, respectively (IPCC, 2013).

Globally, agricultural soils are a major source of anthropogenic N₂O and NO emissions (IPCC, 2007; Sánchez-García et al., 2014). Agricultural soils accounted for 60 % of the global anthropogenic N₂O flux and released approximately 1.6 Tg NO-N yr⁻¹ globally (IPCC, 2007). Increases in soil CO₂ emissions also have the potential to exacerbate increasing atmospheric CO₂ levels and to provide a positive feedback to global warming (Raich and Tufekcioglu, 2000). Soil is believed to contribute about 20 % of the total CO₂ emissions (Sauerbeck, 2001).

Rates and reactions of biogeochemical processes vary in space and time to produce both hot spots and hot moments of elemental cycling. Biogeochemical hot moments are defined as short periods of time that exhibit disproportionately high reaction rates

relative to longer intervening time periods (McClain et al., 2003). Although our knowledge of the sources and sinks of N₂O and CO₂ in different environments is increasing, prediction of the GHGs exchange in time and space remains challenging (McClain et al., 2003; Bouwman et al., 1995) because of hot spots and hot moments frequently account for larger proportion of the GHG budgets, especially for N₂O (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999). This arises from the heterogeneity of the systems from which these gases are emitted, and the complex interactions which occur between the chemical, physical and biological variables which control their generation (Duxbury and McConnaughey, 1986; Clayton et al., 1994). As for the hot-moment flux, it included not only the peak emission of GHGs but also the low-frequency negative observations. The mechanism and environmental drivers responsible for the uptake of GHGs are also important. In order to divide these two types of CO₂ and N₂O fluxes at hot moment, the episodic peak flux of GHGs was named as “hot moment” and the uptake observations as “cold moment” in this study. Previous studies mostly concentrated on the first type of fluxes at hot moment due to the large contribution to GHGs emission.

The extremely great contribution of N₂O hot moment has been reported by several literatures, 40 ~ 51 % in manure-fertilized fields in Harford, New York (Molodovskaya et al., 2012), 73 % mainly after thawing at the Höglwald forest in Bavaria (Papen and Butterbach-Bahl, 1999), 55 ~ 80 % mainly after heavy rain in a paddy field in Tsukuba, Japan (Akiyama et al., 2013) and 51.1 % of the observed cumulative N₂O flux from intensely-grazed grassland in Ireland was contributed by three major emission events covering a timeframe of only 6.6 % of the measurement period (Scanlon and Kiely, 2003). This large variability of N₂O might be caused by the complex set of environmental variables such as soil temperature and soil moisture, as well as nutrient availability, which control the nitrification and denitrification processes responsible for N₂O emissions (Farquharson and Baldock, 2008). Biological CO₂ variability shows wide swings at a range of timescales, and complicates the identification of CO₂ abnormalities (Risk et al., 2013). The high spatial and temporal variations of soil respiration in natural systems have been

reported by Epron et al. (2004) and Zheng et al. (2010). And the variability of CO₂ was associated with various factors such as soil moisture, soil temperature (Yan et al., 2014; Epron et al., 2004), soil organic matter (SOM) content and substrate quality (Bilgili et al., 2013). Therefore, knowledge of episodic soil CO₂ and N₂O fluxes is essential to reliably quantify these gases fluxes and to develop future mitigation strategies (Imer et al., 2013).

Temporal heterogeneity, represented as hot moment, is accentuated in agricultural relative to non-managed landscapes. Land management causes physical and biogeochemical disturbance of extreme magnitude and in pulsed events (Groffman et al., 2009). Soil properties including organic carbon (C) and mineral N contents, bulk density, aggregate size and moisture condition would be changed by agriculture practice, such as fertilization and tillage, and these soil characteristics strongly affected the flux of GHGs. The decreased bulk density by tillage, enhancing primarily soil gas transport processes, could induce large flushes of GHGs already in the soil immediately and these increases were always short-lived (Reicosky et al., 1997, 2005; Ellert and Janzen, 1999). The better aeration of soil after tillage also gave N₂O a better chance of escaping before being denitrified to N₂ (Elmi et al., 2003; Ball et al., 1999), and stimulated the consumption of organic matter by aerobic microorganisms (Doran and Smith, 1987). The breakdown of soil aggregate would be promoted by tillage, which increased contact among microbial biomass, organic C and N, and soil moisture, thereby stimulating CO₂ production and providing substrates for nitrifying and denitrifying microorganisms (Six et al., 2004; Velthof et al., 2010; Pinto et al., 2004). And the number of anaerobic micro-sites increased due to O₂ consumption and created conditions favorable for N₂O production (Doran et al., 1997).

Episodic fluxes of N₂O in hot moments have been reported after events causing a convergence of reactants, e.g., drying-rewetting (Groffman et al., 2009; Ruser et al., 2006; Dobbie and Smith, 2001), rainfall (Liengaard et al., 2012; Dobbie and Smith, 2003) and freezing-thawing events (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999). Rapid transient pulses of CO₂ have also been observed after precipitation (Savage et al., 2014). In addition to soil and climatic factors, the

agricultural activities also control the emissions of CO₂ and N₂O from agricultural soils (Ussiri et al., 2009; Jarecki and Lal, 2006). N₂O fluxes at hot moment were also dominated by agricultural management practices, such as fertilizer and manure additions (Imer et al., 2013; Dobbie and Smith, 2003; Dobbie et al., 1999), harvest (Imer et al., 2013; Dobbie and Smith, 2003) and tillage (Groffman et al., 2009; Xu et al., 2000), and CO₂ hot moment was stimulated by tillage (Rochette and Angers, 1999). Peng et al. (2011) stated that management impacts on soil flux magnitudes of CO₂ are rather small compared to environmental drivers.

Therefore understanding the temporal variability of CO₂ and N₂O fluxes is key importance to reliably estimate these gases fluxes and to develop mitigation strategies. However there were few previous studies focused on the identification and analysis of gas hot moments or environmental factors triggering gas hot moments (Molodovskaya et al., 2012). The lack of information on hot moment quantification is attributed to the fact that there is no agreed-on operational definition of a hot moment, and the magnitude and time scale of N₂O flux events are highly variable even among similar crop, climate, or soil types (Molodovskaya et al., 2012). In the present study, CO₂ and N₂O hot moments in managed grassland and cornfield was evaluated by box plot method, and laboratory incubation of soil core was conducted out for a period of 10 days to measure CO₂, NO and N₂O production in different aggregate sizes, gravimetric water contents, bulk densities and nutrient managements. The objectives of this study were to:

- 1) improve prediction and understanding of episodic soil N₂O and CO₂ fluxes at hot moments by box plot analysis, including investigate the reasons inducing potential hot moments and the contribution of hot moments at annual timescales.

- 2) study the effects of bulk density, soil moisture content, aggregate size and nutrient management on CO₂, N₂O, NO emissions from agriculture soil, and then to identify key factors affecting the episodic CO₂, N₂O emission.

This dissertation is divided into 7 chapters. Chapter 1 is the general introduction to the background and objectives of this study. Chapter 2 provides a review of the

relevant literature on CO₂, NO and N₂O productions in soils. Chapter 3 describes the study sites, soil used and the methodologies used in this study. Chapter 4 shows the results of evaluation of CO₂ and N₂O hot moments in managed grassland and cornfield. Chapter 5 reports on the discussions of the key factors affecting CO₂, NO and N₂O productions from the results of incubation study by using aggregated soil with different moisture contents, nutrient managements and bulk densities along with the soil chemical properties. A general discussion on the results and the connection of each chapter of this study is presented in Chapter 6. Finally, the entire study is summarized in Chapter 7.

Chapter 2

LITERATURE REVIEW

2.1 Greenhouse gas emission from soil

Carbon dioxide (CO₂) is the dominant anthropogenic greenhouse gas (GHGs) in the earth's atmosphere (Keppel-Aleks et al., 2013) that traps a portion of the Sun's radiant energy and thereby warms the earth (Mitchell, 1989), accounting for 60 % of global warming. Nitrous oxide (N₂O) is another important climate relevant trace gas that contributes to global warming (Smith, 1990; Yung et al., 1976) by acting as a shield from damaging ultraviolet-B radiation (Crutzen, 1970). The contribution of N₂O to the anthropogenic greenhouse effect has been estimated to 6 % (IPCC, 2001). Despite N₂O concentration in atmosphere was low, it is also very important since it has 298 times the global warming potential of CO₂ (IPCC, 2007; Ravishankara et al., 2009) and 23 times longer lifetime of CO₂ (about 150 years) in atmospheric (IAEA, 1992; IPCC, 2007). Additionally, N₂O is the dominant stratospheric ozone-depleting substance emitted by humans in the twenty-first century (Ravishankara et al., 2009), reacting with oxygen radicals in the stratosphere to form nitrogen monoxide (Graedel and Crutzen, 1993). Nitric oxide (NO) is also important components influencing the atmospheric chemistry (IPCC, 2007). It is a precursor of tropospheric ozone, which is a greenhouse gas formed by photochemical reaction, and is a precursor of nitric acid, which is a major component of acid rain (Akiyama et al., 2000). Despite great uncertainties regarding the magnitude of biogenic production of NO in soil, it represents a significant source of global atmospheric NO_x (Davidson and Kinglerlee, 1997).

Increase in the GHGs concentration in the atmosphere is an important global issue, which has discernible impact on global climate (IPCC, 2007). Atmospheric N₂O and CO₂ concentrations have been increased to 324.2 ppb and 391 ppm in 2011 at a rate of $2.0 \pm 0.1 \text{ ppm yr}^{-1}$ and $0.73 \pm 0.03 \text{ ppb yr}^{-1}$, respectively (IPCC, 2013). Globally, agricultural soils are a major source of anthropogenic N₂O and NO emissions (IPCC,

2007; S á nchez-Garc ía et al., 2014), and there is little doubt that the use of nitrogen (N) fertilizer and manure is driving the increase in atmospheric N₂O (Davidson, 2009). Agricultural soils accounted for 60 % of the global anthropogenic N₂O flux (IPCC, 2007) and released approximately 1.6 Tg NO-N yr⁻¹ globally (IPCC, 2007). Increases in soil CO₂ emissions also have the potential to exacerbate increasing atmospheric CO₂ levels and to provide a positive feedback to global warming (Raich and Tufekcioglu, 2000). Soil is estimated to contribute about 20 % of the total CO₂ emissions (Sauerbeck, 2001).

2.1.1 Soil CO₂ emission

CO₂ is produced in soil as a result of decomposition of SOM by micro-organisms and root respiration (Ball et al., 1999). Soil respiration activity is the result of the activity of all the microbial population in the soil. Therefore it depends on the composition, size and metabolic activity of this population and these are, in turn, influenced by factors such as temperature, moisture, oxygen availability, SOM decomposability and mineral nutrients (Rodrigo et al., 1997). Crop residue decomposition depends on additional factors such as the quality and location of the residues (Garnier et al., 2003).

Temperature is an important parameter known to determine soil respiration. Normally the respiratory maximum temperature in soils is not reached, and an increase in O₂ uptake or CO₂ production rate is observed with temperature rise. Oorts et al. (2007a) found that higher temperatures and higher soil moisture will both lead to larger SOM and crop residue decomposition under temperate climates.

Several reports mentioned that optimal soil moisture conditions for heterotrophic activity in this soil could be provided within a wide range of 40 % ~ 90 % WFPS (Ruser et al., 2006; Frank et al., 2002; Drewitt et al., 2002). Weier et al. (1993) reported that the restriction imposed on aerobic microbial respiration by high WFPS might be compensated by the available C to fermenters. The effect of soil moisture on CO₂ production was a delicate balance between having sufficient water for

substrate transport and microbial requirements, and adequate O₂ for respiration (Skopp et al., 1990). Soil moisture controls not only the diffusion of O₂, but also substrate availability (Stark and Firestone, 1995) and microbial activity. Water availability will limit the soil respiration when WFPS is lower since water is essential for microbial survival and activity (Sey et al., 2008), while CO₂ production was reduced by restricted O₂ accessibility at higher WFPS (Ruser et al., 2006). Skopp et al. (1990) noted that soil respiration is optimal near 60 % WFPS for many soils, but could be lower if soil microbial growth was limited due to insufficient substrates. The relationship between WFPS and soil respiration in varied textured soils was proposed to be described by quadratic models and the optimal WFPS value for soil respiration rates was concluded to be from 40 to 70 % by Doran et al. (1990). Linn and Doran (1984) found that O₂ consumption, CO₂ production and nitrification by aerobic microorganisms reached a plateau between 40 % ~ 60 % WFPS and declined as soils became progressively more anaerobic.

Soil porosity also influenced soil CO₂ fluxes, which indicated the soil pore network plays a major role in transportation of CO₂ through soil to the soil surface. And furthermore, the increased soil porosity favoured the aeration of soil making more O₂ available to microbes to act on SOM and crop residues. This is in agreement with Al-Kaisi and Yin (2005) who attributed the non-significant relationship between soil CO₂ and different forms of C to the government by soil pore characteristics and the sufficient soil organic C substrate (Mangalassery et al., 2013).

Soil bulk density and aggregate size greatly influenced soil moisture desorption, the depth of soil to which O₂ could diffuse, and the thickness of water films at equal air porosities (Grable and Siemer, 1968), and then affected CO₂ fluxes from soil. Several reports showed organic C and potentially mineralized organic matter increased with the increase in aggregate sizes (Jastrow et al., 1996; Semenov et al., 2010; Wright and Hons, 2004). This is because macro-aggregates not only contain micro-aggregates, which themselves contain organic matter, but also contain inter-microaggregate particulate organic matter (POM) as well (Denef et al., 2004). The rates of microbial CO₂ production are strongly related to the size of the soil organic C and microbial

biomass pools (Lundquist et al., 1999). Seto and Yanagiya (1983) also declared that water extractable organic C (WEOC) pool was usually associated with CO₂ production. Puget et al. (1995) suggested that the larger concentrations of SOM associated with macro-aggregates was due to either its protection within these large aggregates or that only those large aggregates enriched with organic matter could survive slaking. SOM is the largest global terrestrial C pool (Kern and Johnson, 1993) and changes within this reservoir have direct implications on atmospheric CO₂ (Janzen et al., 1998).

2.1.2 Soil NO and N₂O emissions

2.1.2.1 Processes of soil NO and N₂O emissions

N₂O and NO produced in soils results mainly from two contrasting microbiological processes of denitrification and nitrification as by-products (Yamulki and Jarvis, 2002; Bremner, 1997; Conrad, 1996; Tortoso and Hutchinson, 1990; Wrage et al., 2001; Marusenko et al., 2013). On the global level, > 65 % of atmospheric N₂O comes from soil as a result of nitrification and denitrification (Bouwman, 1990a).

Denitrification is an anaerobic microbial process which sequentially converts NO₃⁻ to N₂ via NO₂⁻, NO and N₂O utilizing organic C as the electron donor; a form of respiration in the absence of O₂ (Zumft, 1997; Firestone and Davidson, 1989; Ye et al., 1994), which maintains the sustainability of an agricultural system (Jha et al., 2011) because it removes reactive N from terrestrial and aquatic ecosystems (Davidson and Seitzinger, 2006; Groffman et al., 2006; Seitzinger et al., 2006). If incomplete, it contributes to global warming by emitting NO and N₂O as intermediate products (Jha et al., 2011). According to a model of Davidson (1991), N₂O is primarily derived from denitrification at soil moisture contents greater than 60 % WFPS due to a decreased O₂ supply. Denitrification is the predominant biological process responsible for global increases in atmospheric N₂O (Baggs, 2008) mainly due to the large inputs of N fertilizers to arable land to meet or increase food productivity (Morley et al., 2014).

N_2O production in soils at moisture contents below field capacity is generally attributed to nitrification (Tortoso and Hutchinson, 1990; Davidson, 1992; Hutchinson et al., 1993). Nitrification is reported to make a substantial contribution to the N_2O emissions under aerobic conditions (Williams et al., 1998). The first stage of nitrification is the oxidation of ammonia (NH_3) with ammonia monooxygenase to NO_2^- via hydroxylamine (NH_2OH), and N_2O and NO can be produced as a by-product by several pathways (Firestone and Davidson, 1989; Wrage et al., 2005). This process takes place in aerobic microsites of soils (Wrage et al., 2005) by chemoautotrophs using NH_3 as an energy source (Hollocher et al., 1981). Ammonia oxidation, the first step in the N-cycle, is important for N_2O emissions as it is the rate-limiting step, supplying substrate for denitrification (Di et al., 2010). The second step of nitrification is the conversion of NO_2^- to NO_3^- . Different groups of bacteria are involved at the two steps, namely NH_3 -oxidizers and NO_2^- -oxidizers, respectively (Bock et al., 1986). Tortoso and Hutchinson (1990) concluded that chemoautotrophic NH_4^+ oxidizers, rather than chemoautotrophic and heterotrophic NO_2^- oxidizers, are the predominant source of NO and N_2O production during nitrification in soil. Hence, gas production by mixotrophic growth of NH_4^+ oxidizers (Stuven et al., 1992) cannot be discounted as a source of NO and N_2O . Hutchinson et al. (1993) found that chemoautotrophic NH_4^+ oxidizers were the predominant source of gaseous N oxides at water contents $\leq 10\%$ (ca. -10 kPa) in a sandy loam.

A substantial part of the NO_3^- produced by nitrification in an oxic zone can diffuse towards an anaerobic zone where it can be denitrified into N_2 (Nielsen et al., 1996; Arah, 1997). The development of aerobic and anaerobic microsites within close proximity, indeed in the same soil aggregate, permits nitrification and denitrification to occur simultaneously (Azam et al., 2002).

In addition to these two main processes, other soil microbial processes were also involved in the production of N_2O (Baggs, 2011; Spott et al., 2011), including the dissimilatory nitrate reduction to ammonium (DNRA), nitrifier denitrification and codenitrification. Nitrifier denitrification could contribute significantly to N_2O production in soil under limiting O_2 concentrations (Venterea, 2007; Wrage et al.,

2005) and it is a pathway that ammonia oxidisers are thought to turn to under short-term O_2 limitation whereby NO_2^- is reduced to N_2 via N_2O (Poth and Focht, 1985). This reduction is thought to be similar to denitrification, whereby heterotrophic denitrifiers use NO_3^- or NO_2^- as an electron acceptor under low O_2 conditions (Wrage et al., 2005). Codenitrification has also been identified as a relevant N_2O formation pathway in soils (Spott et al., 2011). No evidence has been reported that would serve to unambiguously define the cause for the observed variations (increase or decline) in soil N_2O fluxes since the extremely complex set of reactions leading to N_2O formation and consumption in soils (Sánchez-García et al., 2014).

Denitrification is the only process that can account for both production and consumption of NO under anaerobic soil conditions (Remde and Conrad, 1991a; Baumgärtner and Conrad, 1992). Both microbial processes of nitrification and denitrification could produce NO as by-products (Yamulki and Jarvis, 2002; Remde and Conrad, 1991b). However, the turnover of NO in soil is very dynamic due to the consumption of NO through both the oxidation of NO to NO_3^- by aerobic heterotrophic bacteria (Baumgärtner et al., 1996; Koschorreck et al., 1996; Rudolph et al., 1996; Dunfield and Knowles, 1999; Gölde and Conrad, 2000) and the reduction of NO to N_2O by denitrifiers in anaerobic microniches (Remde and Conrad, 1991a; Schäfer and Conrad, 1993; McKenney et al., 1982). And the relative consumption by denitrification seems to be higher (Skiba et al., 1993) by the reason of the NO consumption through the unspecific oxidation to NO_2^- during denitrification (Rudolph et al., 1996). The affinity of denitrifiers to NO is very high and they can consume it as a substrate even in well-aerated soil (Conrad, 1996). Furthermore, denitrifying bacteria tend to utilise oxidised forms of N such as NO_2^- , NO_3^- and NO in preference to N_2O (Yamulki and Jarvis, 2002). Therefore, nitrification is considered as the main source of NO flux (Anderson and Levine, 1986; Adak et al., 2002).

2.1.2.2 Factors controlling soil N_2O and NO emissions

The magnitude of N_2O and NO emitted varied with complex interactions between

soil properties, climatic factors and agricultural practices (Granli and Bøckman, 1994). Key controlling factors include soil mineral N (Ball et al., 1997; Castaldi and Smith, 1998; Seneviratne and Van Holm, 1998; Yao et al., 2010); oxygen availability and soil moisture content (Carran et al., 1995; Teira-Esmatges et al., 1998; MacKenzie et al., 1998; Yao et al., 2010); presence of soluble organic matter and easily mineralisable C which promotes microbial activity (Ineson et al., 1998; Kaiser et al., 1998; Burford and Bremner, 1975); soil pH and soil temperature (Mahmood et al., 1998); physical soil processes and properties (Yao et al., 2010; Diba et al., 2011).

N₂O emission is expected to be higher than NO emission when WFPS is higher, (Davidson, 1991). Davidson (1991) proposed an optimum moisture content for maximum NO flux between 30 % ~ 70 % WFPS. Within the range of optimum soil moisture content, changes in % WFPS are not expected to change NO flux significantly. Below WFPS of 30 %, NO flux is expected to change significantly with changes in % WFPS (Sullivan et al., 1996). The optimum conditions for N₂O emissions via denitrification have been assumed to exist at WFPS of 70 ~ 90 % (Bateman and Baggs, 2005; Dobbie et al., 1999), whereas N₂O emissions at lower WFPS have often been attributed to nitrification (Dobbie et al., 1999; Davidson, 1991; Venterea et al., 2010). Ruser et al. (2006) reported that N₂O emission rates were generally small at WFPS < 60 % and increased significantly at WFPS > 70 % with the highest N₂O fluxes occurring at the highest soil moisture level. Increasing N₂O emission rates with increasing soil moisture content have been reported from many laboratory and field studies and attributed to an increased denitrification activity induced by a reduced O₂ diffusion into the soil (Mosier et al., 1986; Clayton et al., 1994; Flessa and Beese, 1995; Corre et al., 1996; MacKenzie et al., 1997; Dobbie and Smith, 2001; Ruser et al., 2001). With increasing WFPS from approximately 70 to 90 %, there was a greater than 50-fold increase in denitrification (Scholefield et al., 1997). Wolf and Russow (2000) and Russow et al. (2000) found that N₂O emission from soils originated mainly from nitrification at soil moistures below 60 % WFPS. N₂O emission at 90 % WFPS was influenced by the availability of organic C (Ruser et al., 2006).

O₂ as a controlling factor in regulating the magnitude and pathway of N₂O and NO production is very important (Firestone and Davidson, 1989; Tiedje, 1988; Wrage et al., 2001). Concentration of O₂ is the main factor controlling denitrification through the activity and synthesis of denitrifying enzymes in soil (Tiedje, 1988). Denitrification has been considered for long as a strictly anaerobic process, but many soil denitrifying micro-organisms are able to produce N₂O over a wide range of O₂ concentration, even in well-aerated soils, because presumably in anaerobic microsites where the biological O₂ demand exceeds supply (Khalil et al., 2004). However, nitrification is a strictly aerobic process since the NH₄⁺ oxidation enzyme of nitrifying organisms requires O₂ for activation (Wood, 1986). Bollmann and Conrad (1998) showed that N₂O emitted by nitrification in soils was important at partial pressures higher than 0.1–0.5 kPa O₂. Goreau et al. (1980), using sediment slurries, found that production of N₂O by nitrification reached a maximum at 0.2 kPa O₂ pressure. O₂ availability greatly influences the nitrification rates, which were reduced by a factor of 6–9 when O₂ pressure decreased from 20.4 to 0.35 kPa (Khalil et al., 2004). Khalil et al. (2004) found that the amount of N₂O production by nitrification increased rapidly from 0.16 to 1.48 % (the amount of N₂O-N emitted per unit of NH₄-N oxidised) when O₂ pressure fell from 20.4 to 0.76 kPa. Bollmann and Conrad (1998) found that the maximum soils N₂O emission by nitrification occurred at 0.5 kPa O₂. Anaerobic conditions may lead to the incomplete degradation of organic matter and the accumulation of small organic compounds, including acetate, fatty acids (Tsusuki and Ponnamperna, 1987; Dassonville et al., 2004; Dassonville and Renault, 2002) that may be consumed later in aerobic conditions and decrease temporarily the pH of the soil solution (Dassonville et al., 2004). Such changes in easily mineralisable C compounds and pH might affect potential respiration and denitrification activities (Khalil et al., 2005). Oxygen concentrations are rarely measured, and soil moisture content has generally been accepted as a measurable proxy of O₂ availability (Linn and Doran, 1984).

Trace gas fluxes from soil to atmosphere are regulated by the production, consumption, and transport of the gas within the soil, by the thickness of the laminar

boundary at the soil-air interface, and by the atmospheric concentration of the gas (Sanhueza et al., 1994). The turnover of NO in soil is very dynamic due to more consumptive fates (Davidson, 1991), and the transport could have a major impact on its emission (Yamulki and Jarvis, 2002; Galbally and Johansson, 1989; Remde et al., 1993; Rudolph et al., 1996; Rudolph and Conrad, 1996). Gas diffusion condition was not only related to the exchange rate of trace gas emission within soil to the surface but also to maintain optimum soil aeration. The limited gas diffusion could decrease the “short lifetime” NO flux from soil by prolonging the residence time within soil resulting in higher consumption rate, and by restricting the production rate due to inadequate supply of O₂ for nitrifying bacteria.

Bulk density and aggregate size greatly influenced soil moisture desorption, the depth of soil to which O₂ could diffuse, and the thickness of water films at equal air porosities (Grable and Siemer, 1968). Greenwood (1975) that anaerobic conditions would form in the centre of soil aggregates when the aggregate radius exceeded 9 mm. However, Sexstone et al. (1985) did measure anaerobic conditions in a 4 mm aggregate. Similarly, Khalil et al. (2004) measured denitrification in 3mm aggregates. Effect of aggregate on N₂O and NO emissions was not only associated with the aerobic volume but also the nutrient supply. Aggregates with diameters of 20 to 25 mm often contained anaerobic centers but exhibited only low rates of denitrification due to a limiting availability of electron donors (Højberg et al., 1994). Sexstone et al. (1985) also found that denitrification rates did not correspond to aggregate size or anaerobic volume and surmised that other factors, such as C supply, probably determined the observed denitrification rates. The difference in aggregate size was also linked to the pool size of SOM, which was very important for N₂O and NO emission from soil.

Most biological reactions are influenced by temperature. The decrease in NO flux with increase in soil temperature is also associated with soil moisture stress (Sullivan et al., 1996). A decrease in NO flux at high (> 33 °C) soil temperature was observed by Aneja et al. (1995). The response of NO flux to an increase in soil temperature has been observed by several other researchers (Williams et al., 1988; Shepherd et al.,

1991; Slemr and Seiler, 1991; Valente and Thorton, 1993; Kim et al., 1994). The effect of soil temperature on emission of NO from agriculturally managed soil was modeled as an exponential function as suggested by several other investigators (Williams et al., 1988; Shepherd et al., 1991; Slemr and Seiler, 1991; Valente and Thorton, 1993). Optimum soil temperature for nitrification of NH_4^+ to NO_3^- is 25 to 35 °C, although some nitrification occurs over a wide temperature range. Denitrification increases rapidly in the 2 to 5 °C range. Denitrification will proceed at slightly higher rates when temperature is increased to 25 to 60 °C, but is inhibited by temperatures > 60 °C. The increase in denitrification at elevated soil temperatures suggests that thermophilic microorganisms play a major role in denitrification. Thus, denitrification losses coinciding with spring thawing are related to accelerated denitrification rates when soils are quickly warmed from 2 to 12 °C or higher.

Matson et al. (1990) and Le Roux et al. (1995) pointed out that the rates of N transformations for given environmental conditions were positively correlated with SOC contents. Li et al. (2005) also concluded that higher N trace gas emissions were promoted by higher SOC contents due to higher soil microbial activity based on a literature review. Availability of C not only supports the activity of denitrifiers per se but also has the indirect effect of causing microsite anaerobiosis due to increased respiratory demand for O_2 (Azam et al., 2002). It is well-known fact that microorganisms require a readily decomposable substrate before reduction of NO_3^- can occur (Burford and Bremner, 1975). An increase in microbial activity and concurrent high microbial consumption of O_2 , particularly in the presence of NH_4^+ , is certain to cause microsite anaerobiosis and encourage denitrification (Williams et al., 1998). These phenomena are affected positively by the availability of organic C and the latter is often correlated positively with the rate of denitrification (Burford and Bremner, 1975; Simek and Hopkins, 1999). In a study of the kinetics of the denitrification enzymes in Rowden soil, Dendooven et al. (1994) showed that each milligram of $\text{CO}_2\text{-C}$ produced was associated with the formation of 1.17 and 0.73 mg $\text{N}_2\text{O-N}$, with or without added glucose, respectively (Scholefield et al., 1997).

Nitrification takes place over a wide range in pH (4.5 to 10), although the optimum

pH is 8.5. Denitrifying bacteria are sensitive to low pH, thus, microbial denitrification is negligible at $< \text{pH } 5.0$ but increase with pH. At $\text{pH } < 6.0$ to 6.5 , N_2O represents more than half the N loss. Formation of NO occurs at $\text{pH } < 5.5$. Increasing pH was associated generally with both decreasing denitrification and N_2O -to- N_2 ratio (Scholefield et al., 1997).

It has been widely reported that, with increasing concentration of soil NO_3^- , denitrification changes from being dependent on NO_3^- , with either first order or Michaelis-Menten kinetics, to being independent of NO_3^- , that is, following zero order kinetics (Scholefield et al., 1997). Ellert and Janzen (2008) also demonstrated that N_2O emissions were constrained by inorganic N as N content was low, while controlled by other factors and no longer closely linked to inorganic N as N levels increased. Low NO_3^- concentrations can exert denitrification rate control, high NO_3^- can result in the inhibition of N_2O and NO reductase activities (Firestone et al., 1979; Lalis-Grundmann et al., 1988; Nommik, 1956; Blackmer and Bremner, 1978), which increases N_2O and NO productions. And the other mechanism for the increase in N_2O emission at very high NO_3^- concentration was C substrate limitation of denitrification, which easily occurred at very high NO_3^- and led to incomplete denitrification (Wang et al., 2013; Senbayram et al., 2012). The relationship between soil extractable N values with NO flux had also been described by several models (Williams et al., 1988; Bawkin et al., 1990; Davidson, 1991; Shepherd et al., 1991; Slemr and Seiler, 1991; Skiba et al., 1992; Serca et al., 1994). There was a general increase in NO flux with extractable soil N, as shown in the study of Slemr and Seiler (1991).

The rate of N_2O emission not only depends on the overall denitrification rate but is also strongly affected by parameters that influence the proportion of N_2O relative to the total gaseous N-losses during denitrification. An increase in $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio could result from a decrease in soil pH (Knowles, 1982; Scholefield et al., 1997) and temperature (Nommik, 1956), an increase in NO_3^- (Blackmer and Bremner, 1979; Scholefield et al., 1997) due to the inhibition of the conversion of N_2O to N_2 , an decrease in C substrate concentration (Weier et al., 1993; Wang et al., 2013), or a

decrease in water content (Parkin, 1987; Scholefield et al., 1997; Del Grosso et al., 2000; Parton et al., 1996; Davidson, 1992). The addition or presence of an easily decomposable substrate in soil when soil moisture contents are high will increase anoxic conditions and again increase the conversion rate of N_2O to N_2 (Weier et al., 1993). Blackmer and Bremner (1977) also suggested that the addition of glucose-C promotes the growth of soil microorganisms and increases uptake of N_2O in soils. Due to the many environmental factors altered by field management that influence denitrification and the production ratio, it is not recommended to estimate denitrification from N_2O field measurements by using an average $\text{N}_2/\text{N}_2\text{O}$ ratio (Weier et al., 1993).

2.2 Episodic CO_2 and N_2O fluxes at hot moment from soil

2.2.1 CO_2 and N_2O hot moments and its contributions

Rates and reactions of biogeochemical processes vary in space and time to produce both hot spots and hot moments of elemental cycling. Biogeochemical hot moments are defined as short periods of time that exhibit disproportionately high reaction rates relative to longer intervening time periods and it occur when episodic hydrological flow paths reactivate and/or mobilize accumulated reactants (McClain et al., 2003). Because of hot spots and hot moments frequently accounting for larger proportion of the GHGs budgets, especially for N_2O (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999), the prediction of the GHGs exchange in time and space remains challenging (McClain et al., 2003; Bouwman et al., 1995). This arises from the heterogeneity of the systems from which these gases are emitted, and the complex interactions which occur between the chemical, physical and biological variables which control their generation (Duxbury and McConnaughey, 1986; Clayton et al., 1994). Yamulki and Jarvis (2002) also stressed that many studies have examined the contribution of soil to the budgets of greenhouse gases, but the uncertainties are large because of the extreme variability of emissions in both time and space. According to the definition of hot moment, GHGs fluxes at hot moment actually included not only

the peak emission of GHGs but also the low-frequency negative observations. The mechanism and environmental drivers responsible for the uptake of GHGs are also important and interesting. In order to divide these two types of hot-moment CO₂ and N₂O fluxes, the episodic peak fluxes were named as “hot moment” and the uptake observations as “cold moment” in this study. Previous studies mostly concentrated on the first type of fluxes at hot moment due to the large contribution to GHGs emission.

The extremely great proportion occupied by N₂O hot moment has been reported by several literatures, 40 ~ 51 % in manure-fertilized fields in Harford, New York (Molodovskaya et al., 2012), 73 % mainly after thawing at the Höglwald forest in Bavaria (Papen and Butterbach-Bahl, 1999), 55 ~ 80 % mainly after heavy rain in a paddy field in Tsukuba, Japan (Akiyama et al., 2013). Scanlon and Kiely (2003) found that 51.1 % of the observed cumulative N₂O flux from intensely-grazed grassland in Ireland was contributed by three major emission events covering a timeframe of only 6.6 % of the measurement period. These results showed that the bulk of the annual flux was dominated by relatively few flux peaks. This large variability of N₂O might be caused by the complexity of interactions between various factors such as soil temperature and soil moisture, as well as nutrient availability, which control the nitrification and denitrification processes responsible for N₂O emissions (Farquharson and Baldock, 2008; Choudhary et al., 2002). However, Garcia-Montiel et al. (2003) found that N₂O hot moment after rainfall accounted for only 1.8 % in forest and pasture systems in Rondonia, Brazil due to the low N availability. Biological CO₂ variability shows wide swings at a range of timescales, and complicates the identification of CO₂ abnormalities (Risk et al., 2013). The high spatial and temporal variations of soil respiration in natural systems have been reported by Epron et al. (2004) and Zheng et al. (2010). And the variability of CO₂ was associated with various factors such as soil moisture, soil temperature (Yan et al., 2014; Epron et al., 2004), SOM content and substrate quality (Bilgili et al., 2013).

2.2.2 Factors inducing CO₂ and N₂O hot moments

In addition to soil and climatic factors, the agricultural activities also control the emissions of CO₂ and N₂O from agricultural soils (Ussiri et al., 2009; Jarecki and Lal, 2006). It is widely accepted that the main anthropogenic source of N₂O is agriculture (Delwiche, 1981) which contributes 70-80 % of the anthropogenic N₂O emissions (Isermann, 1994; Dalal et al., 2003). Episodic fluxes of N₂O in hot moments have been reported after events causing a convergence of reactants, e.g., drying-rewetting (Groffman et al., 2009; Ruser et al., 2006; Dobbie and Smith, 2001), rainfall (Liengaard et al., 2012; Dobbie and Smith, 2003) and freezing-thawing events (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999). Temporal heterogeneity, represented as hot moment, is accentuated in agricultural relative to non-managed landscapes. Land management causes physical and biogeochemical disturbance of extreme magnitude and in pulsed events (Groffman et al., 2009). Agricultural management practices, such as fertilizer and manure additions (Imer et al., 2013; Dobbie and Smith, 2003; Dobbie et al., 1999), harvest (Imer et al., 2013; Dobbie and Smith, 2003) and tillage (Groffman et al., 2009; Xu et al., 2000) also dominate N₂O fluxes besides environmental drivers. Although rapid transient pulses of CO₂ have also been observed after precipitation (Savage et al., 2014) and tillage (Rochette and Angers, 1999), management impacts on soil flux magnitudes of CO₂ are rather small compared to environmental drivers (Peng et al., 2011).

The simultaneous occurrence of nitrification and denitrification in soil associated with N fertilisation has been reported by several studies (Zanner and Bloom, 1995; Nielsen et al., 1996; Abbasi and Adams, 1998). Ammonium should be nitrified and hydrolysed to NO₃⁻ when it was associated with producing N₂O (Xu et al., 2000). Therefore, fertilizer application in the form of ammonium triggered N₂O hot moment due to providing plentiful NH₄⁺ for nitrification, and subsequent NO₃⁻ for denitrification as N source and a good promoter for all the denitrification enzymes (Zumft, 1997). The formation of NH₄⁺ by mineralization mainly restricted nitrification under normal field conditions (Dalal et al., 2003). Placement of NH₄⁺ or

urea-N produced more N_2O than did NO_3^- under aerobic conditions due to the increased substrate for nitrifying organisms was reported in the study of Wang and Rees (1996). Bremner and Blackmer (1981) observed N_2O emissions in ‘well-aerated soils’ and the emissions were not correlated with NO_3^- but were significantly correlated with NH_4^+ concentrations. While the magnitude of N_2O flux responded to fertilizer application was varied with mineral N content in soil, which was attributed to that N_2O emissions were constrained by inorganic N as N content was low, while controlled by other factors and no longer closely linked to inorganic N as N levels increased (Ellert and Janzen, 2008). Magdoff (1982) reported a similar much stronger response to N fertilizer in hay plot in contrast with corn plot since the lower soil NO_3^- -N levels.

Drying-wetting event affected N_2O production in the ways of enhancing the activity of microbial biomass and the N availability (Mummey et al., 1994) by the release of easily-decomposable organic matter, and the mineralization (Groffman and Tiedje, 1988), and succeeding denitrification was also initiated by the reduced soil O_2 levels by intensive aerobic respiration and more substrates for the denitrifying bacteria (Groffman and Tiedje, 1988). Koga et al. (2004) also found high N_2O fluxes were largely affected by the amount and timing of rainfall. Priemé and Christensen (2001) found that denitrification was only responsible for 23-53 % of N_2O emission following wetting. While at high initial WFPS, the stimulation of N_2O hot moment by rainfall might be primarily resulted from denitrification due to the rapid decreases in soil O_2 concentration induced by relatively high soil moisture condition. The episodic high N_2O emissions after rainfall event were not only attributed to the high overall rate of denitrification but also the fractions of nitrogenous products of denitrification. The conversion of aerobic condition to anaerobic condition activated the NO_3^- and NO_2^- reductases quicker than the N_2O reductase, leading to higher $\text{N}_2\text{O}/\text{N}_2$ ratio of the denitrifier (Knowles, 1982; Otte et al., 1996). Morley et al. (2008) demonstrated that the N_2O reductase became inactive after the re-exposure of anaerobic soil to O_2 , but other denitrification enzymes still kept active. Dendooven and Anderson (1994) also found that NO_3^- reductase was more persistent than N_2O reductase when anaerobic

condition was rapidly induced. And the magnitude of N₂O peak emission after re-wetting was strongly influenced by the length of drought period and decreased with successive rainfall events (Groffman et al., 2009; Denmead et al., 2000). Scholes et al. (1997) demonstrated that N₂O peak emissions on savanna soil after the first wetting were significantly higher than those on the previously wetted treatment. In addition to high WFPS, soil mineral N was required for high episodic N₂O emissions (Akiyama et al., 2013). Therefore rainfall tended to enhance the amount of N₂O high fluxes followed fertilization since it promotes the chemical and physical breakdown of the fertilizer and manure, vertical transport of NO₃⁻ to subsurface horizon, and the wetting of the soils (Smith et al., 1998; Akiyama et al., 2013). And Ball et al. (2004) also reported extremely high N₂O flux peaks following heavy rain from an imperfectly drained gleysol grassland after fertilizer addition. An episode of very high N₂O emission in response to rainfall soon after fertilization as NH₄-N was also captured from continuous cornfield in Ohio, USA by Jacinthe and Dick (1997).

Soil temperature influences N₂O emissions through its effect on the activity of microorganisms and enzymes (Farquharson and Baldock, 2008). The dominant sources of CO₂ production are microbial decomposition of SOM and root respiration (Savage et al., 2014), which also increased with soil temperature and moisture (Ellert and Janzen, 2008). Kirschbaum (1995) indicated that soil temperature strongly affected respiratory activity contributing to CO₂ production during decomposition of organic material, which was also reported by Savage et al. (2014). Drury et al. (2008) found that CO₂ emission peaks in corn and soybean fields in Ontario, Canada corresponded to soil temperature peaks. Savage et al. (2009) reported a transient pulse of CO₂ following rainfall event. CO₂ hot moments followed rainfall were mainly attributed to the enhanced the activity of microbial biomass and the N availability (Mummey et al., 1994), which led to the reduced soil O₂ levels (Groffman and Tiedje, 1988). When soil moisture content was high, microbial activity always kept a high level and the stimulation of mineralization by rainfall event was limited due to the rapid creation of anaerobic zones following rainfall event. Hence the stimulation of rainfall on CO₂ hot moment depended on the soil moisture content.

Hot moments of N_2O and CO_2 induced by harvest and manure application were primarily attributed to the fact that the residues from harvested materials and the addition of manure remarkably enhances the organic C content and promotes SOC decomposition and N mineralization, which increases soil NO_3^- and NH_4^+ concentrations (Xu et al., 2000). Harvest is a further pulse disturbance event, instantaneously removing plant N demand, leaving a de-vegetated landscape vulnerable to N loss during fall rains (Dobbie et al., 1999). Hence the enhancement of harvest on N_2O production was also related to the low N uptake by plants. Residues resulting from harvest and manure application also could lead to the concomitant increase in denitrification potential, which was related to provide a source of energy for denitrifiers, and stimulate the O_2 consumption by microbial respiration to establish anaerobic microenvironments in soil (Farquharson and Baldock, 2008). And the latter one was more important since SOC was not easily to limit denitrification as electron donor caused by the low competition for C under anaerobic condition (Groffman et al., 1988). The ways in which SOC affected N_2O flux can be influenced by other factors such as soil moisture, temperature and N dynamics (Farquharson and Baldock, 2008).

The immediately flush of CO_2 induced by tillage was attributed mainly to enhanced transport of CO_2 already in soil (Ellert and Janzen, 1999). Meanwhile, tillage also introduced large amounts of O_2 into the soil, stimulating the consumption of organic matter by aerobic microorganisms (Doran and Smith, 1987), which induced CO_2 hot moment. Furthermore, cultivation alters the architecture of surface soil by breaking open plant residues and soil aggregates, re-arranging pore space and redistributing soil moisture. These changes could increase contact among heterotrophic organisms, mineralizable C and soil moisture, thereby stimulating CO_2 production (Tester, 1988). Tillage also increased the rates of N_2O production both from nitrification by promoting soil aeration (Linn and Doran, 1984) and from denitrification by O_2 consumption. Nitrification was likely to be the primary process contributing to the larger emission after tillage when WFPS was low. A higher proportion of N_2O production by denitrification stimulated by tillage was observed with the increase in WFPS since the relatively high WFPS range measured in ploughed treatments was

optimum to promote both nitrification and denitrification (Estavillo et al., 2002). When soil structure was disrupted by tillage, a greater potential of mineralization and a sudden increase of mineral N was enhanced by SOC accumulation (Estavillo et al., 2002). Soil organic matter is a source of nutrient elements for plant growth, yielding N, P, and S upon decomposition (Yang et al., 2007).

2.3 The effect of tillage on emissions of CO₂, NO and N₂O

Tillage is widely used in agriculture management and it strongly affects the flux of GHGs (Elder and Lal, 2008). The drastic changes in physical characteristics of soils by tillage affecting trace gas fluxes were summarized by Sanhueza et al. (1994) as: (1) loosening induces an increase in soil porosity and subsequent an enhancement in gas transport within the soil; (2) an increase in the surface area interacting with the atmosphere; (3) a mobilization of nutrients and a burial of surface litter; and (4) aeration produces an increase in microbial metabolism (oxidation) and also a possible switch from anaerobic to aerobic processes.

2.3.1 The effect of tillage on CO₂ emission

Soils, as the largest surface terrestrial C pool, contain approximately 1500 Pg of C (Post et al., 1990; Eswaran et al., 1993). Terrestrial ecosystems have contributed as much as half of the increases in CO₂ emissions from human activity in the past two centuries (Post et al., 1990). Of the past anthropogenic CO₂ additions to the atmosphere, about 50 Pg has been contributed by cultivated soils (Paustian et al., 1997), through the mineralization of SOC. Six et al. (1998, 1999) suggested that increased macro-aggregate turnover under conventional tillage is a primary mechanism causing decreases of soil C. Many studies have reported that CO₂ emission with respiration was often stimulated by tillage (Roberts and Chan, 1990).

Immediately after tillage, large flushes of CO₂ from soil were observed by many studies and these increases were always short-lived (Reicosky et al., 1997, 2005; Ellert and Janzen, 1999). Short-term effects occurred within hours after tillage and the

tillage-induced flush of CO₂ was ascribed mainly to the changes in soil physical properties enhancing primarily soil gas transport processes, particularly the initial flux of CO₂ already in the soil (Reicosky et al., 1995), and to a lesser extent with changes of SOM turnover rates, which require some time for enhanced soil microbial activity (Reicosky et al., 1997; Ellert and Janzen, 1999). These CO₂ fluxes reflect both the concentration gradient as maintained by heterotrophic production of soil CO₂, and the transfer coefficient as determined by soil physical properties (Ellert and Janzen, 1999). CO₂ flux immediately after tillage is due to physical processes, which was supported by the fact that soil respiration rate never increased after tillage (Jackson et al., 2003). However, the relative contributions of the increased microbial activity and physical changes of soil structure enabling rapid exchange of the soil atmosphere had not been firmly quantified (Otten et al., 2000; Jackson et al., 2003; Reicosky et al., 2005), but immediate-term changes in CO₂ flux rates may serve as an indicator for longer-term changes in soil C stocks (Reicosky et al., 1997). CO₂ has a relatively long lifetime within soil and that emission to the atmosphere depends mainly on its production rather than the transportation (Yamulki and Jarvis, 2002; Sanhueza et al., 1994). The medium-term effects of tillage was observed within weeks and months after soil tillage and involve changes in availability of organic matter for microbial turnover and adaptation of the soil microbial fauna (Doran et al., 1998) which require some time for enhanced soil microbial activity (Reicosky et al., 1997; Ellert and Janzen, 1999).

Tillage introduced large amounts of O₂ into the soil, stimulating the consumption of organic matter by aerobic microorganisms (Doran and Smith, 1987). In addition to enhancing physical release or exchange of O₂ and CO₂, tillage also increased the surface area available for microbial attack by breaking apart large pieces of plant residue (Blevins et al., 1984). The incorporating plant residues with soil after tillage also could affect the soil microclimate by creating a moist, aerated environment favorable for microbial activity (Blevins et al., 1984) and flourish microorganisms as the fresh food source which was placed in contact with moisture and O₂ (Reicosky et al., 1995; Mikha and Rice, 2004). This will increase residue decomposition (Beare et

al., 1992; Cambardella and Elliott, 1993) and soil C concentration (Coppens et al., 2006). Therefore, the higher emissions of CO₂ induced by tillage partly may originate from the plant residues incorporation (Oorts et al., 2007b).

SOM is physically protected from decomposition when it is located within aggregates or in pores small enough to limit microbial accessibility and preclude microbial attack (Sollins et al., 1996). Jastrow et al. (1996) also stated that a major factor controlling SOM dynamics is believed to be the differing degrees of protection from decomposition afforded by the spatially hierarchical organization of soil aggregate structure and old C associated with microaggregates may be both biochemically recalcitrant and physically protected. Tillage would disrupt soil structure and expose protected SOC to increases C availability for the microbial biomass (Rasmussen and Collins, 1991). The breakdown of aggregate structure could be promoted not only by the compressive and shearing forces during tillage (Tisdall and Oades, 1982; Schjønning and Rasmussen, 2000; Elliott, 1986; Angers et al., 1992) but also by soil inversion and mixing, exposing new soil to wet-dry and freeze-thaw cycles at the surface (Rovira and Greacen, 1957; Beare et al., 1994) to reduce aggregate stability (Mikha and Rice, 2004), and also by decreasing soil bulk density, thus increasing pore space and altering pore size distribution (Schjønning and Rasmussen, 2000). The enhanced effect of drying and rewetting by tillage increases macroaggregates susceptibility to disruption. Six et al. (2000a) found tillage increases the decomposition rate and turnover of macroaggregate. Beare et al. (1994) observed that macroaggregates from tillage were much less stable, reflecting their greater susceptibility to dispersion. Similarly, Elliott (1986) and Six et al. (2000b) observed aggregates of cultivated soil were more susceptible to slaking. Tillage has been found to induce a loss of C-rich macroaggregates and a gain of C-depleted microaggregates (Six et al., 2000b). A large portion of physically protected SOM within soil aggregates was released as CO₂ emission (Rasmussen and Collins, 1991). Therefore SOM content would influence the effect of tillage on CO₂ flux. The less readily decomposable SOM could result in lower partial pressure of CO₂ in the soil atmosphere so that a tillage event does not produce such a large burst of CO₂ by

degassing, as well as less substrate for microbial decomposition in the subsequent period after tillage has occurred (Jackson et al., 2003). Ellert and Janzen (1999) also reported that the short-term influence of tillage on atmospheric CO₂ transferring from soil C was small under semi-arid conditions in southern Alberta, Canada.

Rochette and Angers (1999) reported the effects of tillage on CO₂ flux was varied with date of plowing due to the differences in soil microclimatic conditions following plowing events. They found that cumulative CO₂ fluxes following spring plowing were lower than on a non-tilled control, during which soil temperatures in the plow layer were < 10 °C, while summer plowing increased CO₂ emissions by as much as 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for a period of 65 d. Tilled soil tended to show slightly warmer temperatures and lower moisture content compared with no-till soils (Jackson et al., 2003). Dao (1998) largely attributed the higher amounts of cumulative CO₂ flux over a growing season as well as higher microbial biomass, activity, and respiration from tilled soils to the higher soil temperature in the tilled soils.

The stress imposed on the microbial activity and substrate transport by the stimulated evaporation of soil moisture followed tillage would be compensated by other factors. The optimal moisture conditions for heterotrophic activity in this soil could be provided within a wide range (Ruser et al., 2006), which was also reported in the studies of CO₂ emissions from grassland (Frank et al., 2002) and forest soils (Drewitt et al., 2002).

2.3.2 The effect of tillage on NO and N₂O emission

Due to the high consumption rate, the transport of NO flux could have a major impact on its emission (Yamulki and Jarvis, 2002) and it was mainly produced at the top surface layer of the soil (Sanhueza et al., 1994). Therefore the improved soil aeration and a possible stimulation of organic matter mineralization followed tillage stimulated the nitrification activity (Yamulki and Jarvis, 2002; Lee et al., 2009), resulting in high NO emission from soil. Sanhueza et al. (1994) stated that the increase of NO in the plowed soil was resulted mainly from the changes in the

physical characteristics of the soil after plowing and especially to the larger surface area that is in contact with the atmosphere. A better aeration of the soil after tillage also gave the N_2O a better chance of escaping before being denitrified to N_2 (Elmi et al., 2003; Ball et al., 1999).

Tillage led to physical disruption of soil structure protecting SOM (Necpřovet al., 2013) and organically bound N, decreased surface bulk density, increased soil gaseous exchange and aeration and the accessibility of crop residues for soil microbes, and consequently increased microbial decomposition of organic C and N (Karlen et al., 1992; Yamulki and Jarvis, 2002) which released inorganic N and provides substrates for nitrifying and denitrifying microorganisms (Six et al., 2004; Velthof et al., 2010; Pinto et al., 2004). The disruption also enhanced moisture evaporation, increased maximum soil temperature and thus accelerated the mineralization process (Angers et al., 2010). Studies in irrigated corn fields in the U.S. (Liu et al., 2005; Lee et al., 2009), and in grassland or arable soils in the UK (Yamulki and Jarvis, 2002; Skiba et al., 2002) also showed that tillage promotes NO emissions likely due to a stimulation of nitrification rate, which is considered as the main source of NO flux under aerobic soil conditions (Anderson and Levine, 1986). And the enhanced water evaporation by tillage (Yamulki and Jarvis, 2002) decreased the residence time of NO in the soil matrix and the likeliness that NO is further consumed by denitrification (Conrad and Bollmann, 1998; Russow et al., 2009), which was also partly responsible for the NO flux induced by tillage. It is well known that NO diffusion rates are typically more than 105 times slower through water than through the equivalent thickness of air (Galbally and Johansson, 1989; Yamulki et al., 1995). Then O_2 consumption, promoted by decomposition of SOM and microbial activity after tillage, tended to exceed the diffusive capacity of soil could happen even in drier conditions, which increased the number of anaerobic micro-sites and created conditions favorable for N_2O production (Doran et al., 1997; Lemke et al., 1998). Therefore tillage could promote both nitrification and denitrification deriving N_2O production, a higher proportion of N_2O lost by denitrification was observed as WFPS increased (Estavillo et al., 2002).

The consumption of O_2 stimulated by tillage sometimes could be compensated by the greater porosity and higher gaseous exchange rate followed tillage, resulting in sufficient aerobic micro-sites and higher nitrification rate, especially at low soil organic C content and soil moisture content, otherwise the diffusion of O_2 to microsites was limited and denitrification was induced. The varied effects of tillage on N_2O flux at low soil moisture content had been reported by several studies. Ussiri et al. (2009) stated that in drier soils, where production of N_2O through denitrification was limited, the increased decomposition of organic matter stimulated by tillage may result into more nitrification. The contrary result was reported by Doran et al. (1997), who indicated the stimulated microbial activity as a result of mixing of decomposing crop residues as O_2 uptake by microorganisms exceeded the diffusive capacity of the soil even in drier conditions, at WFPS values below 45 %. Grant et al. (2004) found lower N_2O emission from no tillage compared to conventional tillage in a situation when denitrification was limited by low soil moisture content and SOM decomposition was slowed down.

The magnitude of N_2O flux after tillage also depended on SOC and mineral N surplus. Organic C not only created the anaerobic condition for denitrification by decomposition but also provided C substrate for denitrification (Myrold and Tiedje, 1985a). When the diffusion of O_2 to micro-sites within soil was limited, initial NO_3^- concentration tended to be the key controlling factor of the N_2O emission induced by tillage. Nitrification was inhibited due to the decreased O_2 availability and the succeeding denitrification rate will be restricted if the initial NO_3^- content in soil was insufficient. And the depleting of NO_3^- stimulated the reduction of N_2O to N_2 (Højberg et al., 1994) resulting in lower N_2O emission. This was similar to a model study result of Stolk et al. (2011), who found that the effects of changing environmental conditions on reduction of N_2O strongly depended on NO_3^- content of soil. More anaerobic conditions hardly affected on the production-to-reduction ratio of N_2O if NO_3^- is abundant, but will decrease this ratio if NO_3^- is limiting. The former increased N_2O emissions, whereas in the second case the emissions will decrease (Stolk et al., 2011). In field, tillage could lead to considerable losses of the

mineralized N from the soil system via a number of pathways including N leaching and/or N₂O emissions, particularly when high soil inorganic N content coincide with high moisture conditions (Nepštová et al., 2013).

Chapter 3

MATERIALS AND METHODS

In the managed grasslands and corn fields in Shizunai and Shin-hidaka (Fig. 3.1), long term monitoring of soil CO₂, N₂O and NO emissions to investigate manure application effect on C and N₂O budgets has been conducted since 2004. And the laboratory incubation of soil core was conducted out for a period of 10 days to measure CO₂, NO and N₂O production in different aggregate sizes, gravimetric water contents, bulk densities and nutrient managements. This chapter describes the precise information of the materials and methods.

3.1 Study Sites

Shizunai site (SZN) located in the Experimental Livestock Farm of Hokkaido University, Southern Hokkaido, Japan (42°26'N, 142°29'E). As shown in Fig.3.1, Shin-hidaka site (SHD) is very close to Shizunai site, which located in Niikapu Station, National Livestock Breeding Center, Shin-Hidaka city, Southern Hokkaido, Japan (42°24'N, 142°28'E). The climate of our study sites is humid continental climate with cold winters and cool summers, but without apparent wet or dry seasons. The mean annual precipitation and air temperature are 1365 mm and 7.9 °C, respectively with the mean monthly temperature ranging from 23.6 °C in August to -8.1 °C in January (Sasayama AMeDAS station). The site is covered with snow from the end of December to the beginning of March.

The soil in Shizunai is derived from Tarumae (b) volcanic ash and is classified as Thaptic Melanudands (Soil Survey Staff 2006; Mollic Andosol (IUSS Working Group WRB 2006)). The C and N contents in the Ap horizon were 3.7 % and 0.33 %, respectively, and the C/N ratio was 11.0 (Shimizu et al., 2009). The soil matric potential ranged from 1 to 100 kPa. The dominant grass species in the managed grassland were reed canary grass (*Phalaris arundinacea* L.) and foxtail grass (*Alopecurus pratensis* L.). Soil texture of Shin-hidakais also classified as Mollic

Andosol (IUSS Working Group WRB, 2006) with pH value of 5.5. The thickness of the Ap-horizon was 26 cm with a dry bulk density of 0.63 mg N m^{-3} and TC and TN contents of the Ap-horizon were 8.69 % and 0.78 %, respectively, and the C/N ratio was 11.14. The grassland was established more than 100 years ago, and renovated in 2004 from pasture. Dominant grass species in this site were orchard grass (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* Huds.).



Fig.3.1 Location map of the study sites in Shizunai and Shin-hidaka

3.2 CO₂, NO and N₂O fluxes at hot moments

3.2.1 Experimental setup

In Shizunai site, grassland had been established in the study field (100 m × 210 m) for more than 30 years before 2004. The average of chemical fertilizer application rates from 1984 to 2004 was $133 \pm 36 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (Shimizu et al., 2010) and the grass was harvested twice per year (June and August). The experimental managed grassland started on November 26, 2004. The grassland was converted to cornfield on November 08, 2009, and corn was cultivated in the field until November 05, 2012. In both of grassland and cornfield, three plots with different nutrient managements were established and the position of each plot in cornfield corresponded to those in the

previous grassland. A half of the study field was applied with both manure and chemical fertilizer as MF plot (100 m × 100 m), the other half with only chemical fertilizer as F plot (100 m × 100 m). MF plot was 10 m away from F plot. In the major F plot, a part of field was avoided from fertilization as control plot (CT plot). In each major plot, 4 experimental subplots (5 m × 4 m for grassland; 10 m × 10 m for cornfield) were set up for gas flux measurements.

In Shin-Hidaka site, permanent grassland was established for grazing from 1973 ~ 2004. Managed grassland was renovated in 2004 from pasture, and gas sampling started from May 04, 2007. Three experimental plots including CT (40 m × 40 m), F (130 m × 170 m) and MF (130 m × 170 m) plots were also set up. MF plot was 10 m away from F plot. Similar to SZN site, 4 experimental subplots in each major plot were set up for gas flux measurements (10 m × 10 m). The grassland was converted to cornfield on November 05, 2012, and the study in cornfield finished in October 12, 2014.

Agriculture managements conducted in these two sites were similar except for one more time harvesting and fertilizer application in the grassland of SHD. During the study period, harvest was conducted in June and August in the grassland of SZN, in June, August and October in the grassland of SHD, and September in cornfield; fertilizer was applied at the beginning of plant growing period (May, June or July in the grassland of SZN, May, July and August in the grassland of SHD, May in cornfield); manure was spread in May in grassland and October or November in cornfield; plough was only conducted in November in cornfield; seeding, harrowing, and rolling was conducted in May in cornfield. Annual average fertilizer and manure application rates were shown in Table 3.1. Chemical fertilizer is comprised of ammonium sulfate, ammonium phosphate, potassium sulfate and potassium magnesium sulfate, and beef cattle manure with bedding litter (bark) was applied as manure (Shimizu et al., 2010). The C/N ratio, pH and water content of manure were in the range of 12.0 ~ 34.6, 6.39 ~ 9.25 and 41 ~ 74 %, respectively.

Table 3.1 Average annual fertilizer and manure application rates of SZN and SHD

Site	Fertilizer	Nutrient	Grassland			Cornfield		
			CT	F	MF	CT	F	MF
SZN	Manure (kg ha ⁻¹)	Fresh weight	-	-	42294 ± 4506	-	-	42810 ± 5268
		C	-	-	6980 ± 1019	-	-	7497 ± 1243
		N	-	-	350 ± 125	-	-	408 ± 168
		P	-	-	350 ± 202	-	-	496 ± 87
		K	-	-	376 ± 207	-	-	436 ± 126
	Fertilizer (kg ha ⁻¹)	C	29 ± 33	11 ± 24	0	-	41 ± 8	41 ± 8
		N	0	59 ± 32	32 ± 47	-	100 ± 7	100 ± 7
		P	11 ± 5	18 ± 13	1 ± 3	-	139 ± 10	139 ± 10
		K	61 ± 25	89 ± 49	22 ± 37	-	77 ± 5	77 ± 5
SHD	Manure (kg ha ⁻¹)	Fresh weight	-	-	10000 ± 0	-	-	39500 ± 14849
		C	-	-	1997 ± 407	-	-	4767 ± 959
		N	-	-	168 ± 65	-	-	259 ± 120
		P	-	-	138 ± 73	-	-	152 ± 73
		K	-	-	288 ± 167	-	-	301 ± 170
	Fertilizer (kg ha ⁻¹)	C	16 ± 27	17 ± 28	16 ± 26	58 ± 82	122 ± 12	113 ± 3
		N	0	29 ± 16	17 ± 10	0	130 ± 24	107 ± 6
		P	53 ± 36	52 ± 32	34 ± 27	20 ± 29	222 ± 194	157 ± 221
		K	33 ± 30	31 ± 34	0	0	0	0

SZN, Shizunai site; SHD, Shin-Hidaka site; CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; Values for SZN site are mean ± stand deviation for 5 years in grassland and 3 years in cornfield respectively; Values for SHD site are mean ± stand deviation for 5.5 years in grassland and 2 years in cornfield respectively.

3.2.2 Gas sampling, measurement and calculation

Generally, gas fluxes were measured at 2-28 day intervals during the crop growing season and 10-30 day intervals during the non-growing season (Shimizu et al., 2010). The crop growing season was defined as the period of time with 7-day moving average of daily air temperature above 5 °C and the non-growing season as the rest (Shimizu et al., 2010). The time interval of sampling after each management was 5-20 day for plough in cornfield, 3-20 day for harvest in cornfield, 2-15 day for seeding, harrowing and rolling in cornfield, 2-25 day for harvest in grassland, 2-15 day for manure application in cornfield. Measurement frequency was increased to twice per week after fertilizer application in both grassland and cornfield, and manure application in grassland.

Gas fluxes from soil were measured by closed chamber method manually in all the plots. Fluxes were measured between 8:00 and 11:00 hours on each measuring day to minimize the influence of diurnal temperature variation. The size of stainless steel chamber (diameter × height) was 40 cm × 30 cm in F and MF plots of grassland, and

20 cm × 25 cm in CT plot of grassland and in all plots of cornfield. 12 hours before gas flux measurements, the chambers were placed into the soil to a depth of around 3 cm and the green parts of the plants inside the chamber were cut and removed. Before closing chamber, a 250 ml gas sample from the headspace of each chamber was extracted into a Tedlar bag for CO₂ and NO analysis and a 20 mL gas sample was injected into an evacuated vial (10 mL) for N₂O analysis as time 0 min. After 6 min under a closed-chamber condition, a 250 ml of headspace gas sample was extracted from each chamber into Tedlar bag for CO₂ analysis according to Nakano et al. (2004). And after 20 or 30 min, another 250 mL gas sample was extracted into a Tedlar bag for NO analysis and 20 mL was injected into a vial for N₂O analysis.

N₂O concentration of gas samples stored in vials was determined within 1 month using an electron capture detector (ECD) gas chromatograph with the detection limits of 0.03 ppmv (model GC-14B; Shimadzu, Kyoto, Japan). CO₂ concentration was analyzed within 8 h using an infrared CO₂ gas analyzer with detection limits of ±1.0 ppmv (ZFP9GC11, Fuji Electric System, Tokyo, Japan). NO concentration was analyzed within 16 h by Chemiluminescence N Oxide Analyzer (Model 265P, Kimoto Electric, Osaka, Japan). Gas flux was estimated from the change in gas concentration in the chamber against closure time:

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T} \times \alpha$$

Where F is the gas flux ($\mu\text{g N m}^{-2} \text{ h}^{-1}$, $\text{mg C m}^{-2} \text{ h}^{-1}$); ρ is the density of each GHG at the standard conditions ($\text{CO}_2 = 1.977 \times 10^6 \text{ mg m}^{-3}$, $\text{N}_2\text{O} = 1.978 \times 10^6 \text{ mg m}^{-3}$, and $\text{NO} = 1.340 \times 10^6 \text{ mg m}^{-3}$); 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 rate of change in gas concentration in the chamber during the sampling time; T is the air temperature inside the chamber ($^{\circ}\text{C}$) and estimated as the average of temperatures at the beginning and the end of gas sampling; and α ratio of molar mass of C or N of the molecular weight of each respective gas ($\text{CO}_2 = 12/44$, $\text{N}_2\text{O} = 28/44$, and $\text{NO} = 14/30$). Based on this equation a positive flux value indicates that gas emission from soil surface into the atmosphere occurred, while negative flux indicates the gas sink from the atmosphere

into the soil. The cumulative gas fluxes were calculated as follows:

$$\text{Cumulative gas flux} = \sum_{i=1}^n (Ri \times 24 \times Di)$$

where Ri is the mean gas flux ($\text{mg m}^{-2} \text{ h}^{-1}$) of the two successive sampling dates, Di is the number of days in the sampling interval and n is the number of sampling times. The cumulative periods of grassland and cornfield in SZN were calculated from November 26, 2004 to November 08, 2009 and from November 09, 2009 to November 05, 2012, respectively, and from May 04, 2007 to November 05, 2012 in grassland of SHD and from November 15, 2012 to October 12, 2014 in the cornfield of SHD.

3.2.3 Meteorological and soil parameters

The SZN and SHD sites were close to each other, and the daily precipitation of these two sites was obtained from the Sasayama AMeDAS (Automated Meteorological Data Acquisition System) station, 5 km away from the experimental field, by Japan Meteorological Agency. Air temperature and soil temperature at 5 cm depth were measured by a thermistor thermometer (CT220; CUSTOM, Tokyo, Japan) during gas sampling period. Soil moisture content at a depth of 0–6 cm was measured by the frequency domain reflectometry (FDR) method (DIK-311A; Daiki, Saitama, Japan) in the grassland and gravimetrically in the cornfield. Total porosity, in a 100 mL soil core, was measured using a three-phase meter (DIK-1110; Daiki Rika, Saitama, Japan). Water-filled pore space (WFPS, %) was calculated from the FDR device reading ($\text{m}^3 \text{ m}^{-3}$) and the total porosity (Linn and Doran, 1984).

Soil samples at a depth of 0–5 cm were collected from all plots with 3 replications and were sieved through a 2 mm sieve within 48 hours, and any stones and roots were removed. Fresh soil samples were immediately extracted with deionized water (1:5) for measuring the concentration of WEOC, NO_2^- -N, NO_3^- -N and soil pH, and with 2 mol L^{-1} KCl (1:10) for NH_4^+ -N analysis. All the extracts were filtered through 0.2- μm membrane filters and stored at 4 °C until analysis. WEOC content, NO_2^- -N and

NO₃⁻-N concentration and soil pH were analyzed using a total organic C (TOC) analyzer (Model TOC-5000A; Shimadzu, Japan), ion chromatography (QIC Analyzer; Dionex Japan, Japan) and a combined electrode pH meter (F-8 pH meter; Horiba, Japan), respectively. The concentration of NH₄⁺-N was determined by indophenol-blue method (UV mini 1240; Shimadzu, Japan).

3.2.4 Episodic emission analysis

Box plot analysis (SPSS 16.0), widely used for outlier analysis (Dowdy et al., 2011), was chosen to pick up episodic N₂O and CO₂ events as potential hot and cold moments of N₂O and CO₂ emissions. Even when the distribution of flux data is not normality, this method can be used because it depends on the median and not the mean of the data (Walfish, 2006). Box plot method fixed the upper fence (UF) and lower fence (LF) at 75th percentile, and 1.5 and 3 times distance from the inter-quartile range separately. Molodovskaya et al. (2012) found that the episodic event contribution and covered timeframe in the 1.5 times outlier category were the least variable between years based on the daily N₂O fluxes, which also agreed with the findings of Scanlon and Kiely (2003). And Molodovskaya et al. (2012) also concluded that the multiday peaks in this category showed the best agreement with the definition of a hot moment, since it was the lowest threshold category in which most of peak events responded to the environmental changes and formed the most distinct seasonal patterns that were observed across multiple years. Therefore here we choose 1.5 times UF and LF, the mild level, as the standards of hot and cold moments respectively, shown as: $UF = Q3 + 1.5 \times (Q3 - Q1)$; $LF = Q1 - 1.5 \times (Q3 - Q1)$, where $Q1$ and $Q3$ is the lower quartile (25 percentile) and the upper quartile (75 percentile) respectively. When the magnitude of CO₂ and N₂O fluxes is higher than UF, they are considered as hot-moment fluxes and as cold-moment fluxes when they are lower than LF. However there was no N₂O or CO₂ flux below than LF as the episodic flux in cold moments in our study. The events with potential to induce hot moments discussed in this study included rainfall event, manure or fertilizer

applications, tillage (the practices of harrowing, rolling or plough), harvest and temperature > 20 °C.

3.2.5 Determination of denitrification and nitrification N₂O

The N₂O–N/NO–N ratio was calculated to determine the mechanism of N₂O production. Bouwman (1990b) concluded that the N₂O–N/NO–N ratio was less than 1 during nitrification, 1–100 during both nitrification and denitrification and more than 100 during denitrification based on the results reported by Anderson and Levine (1986) and Lipschultz et al. (1981).

3.2.6 Statistical analyses

The differences of the frequency of hot moment induced by each event were analyzed with Fisher's Exact Test (R i386 3.0.3 for Windows). The normality of N₂O and CO₂ fluxes were analyzed by Shapiro-Wilk test. Kendall's tau rank correlation coefficient (Kendall's R) and simple linear regression analyses were calculated between gas productions and soil chemical properties.

3.3. The mechanism of key factors inducing episodic CO₂ and N₂O fluxes

3.3.1 Soil sample preparation

Soil samples at 0–20 cm depth of F and MF plots in managed grassland in Shin-Hidaka site were collected on 30 May 2013 and brought to the laboratory and immediately stored in refrigeration at 4 °C. The fresh soil samples were then spread on trays and air dried at room temperature. Air-dried soil samples were softly crushed manually and sieved into two classes of 0–2 mm and 2–4.5 mm aggregates. Aggregate preparation was conducted at room temperature (20 °C). Roots and stones were removed from soil samples by hand.

3.3.2 Incubation design

For determining the effects of variations in soil aggregate size, soil moisture

content, nutrient management and bulk density on N₂O, NO and CO₂ production, incubation treatments were arranged by a randomized complete design according to Table 3.2. Gravimetric water content (GWC) of soil sample was set as 0.35 g g⁻¹ and 0.45 g g⁻¹ respectively. GWC of 0.35 g g⁻¹ is around 60 % of max water holding capacity in field which is the matric potential of around -10 kpa in our site, and it was larger than the minimum GWC observed in study plots which means it can happen in field. GWC of 0.45 g g⁻¹ is about the 80 % of the maximum water holding capacity which is the matric potential of around -3 kpa which near the field water capacity. Distilled water was added by sprayer to achieve the desired moisture conditions.

Table 3.2 Incubation Setup

Aggregate size (mm)	Gravimetric water content (g g ⁻¹)	Nutrient management	Bulk density (g cm ⁻³)
0~2	0.35	F	0.45
2~4.5	0.45	MF	0.66

Adjustment of moisture contents were conducted at room temperature (20 °C). Immediately following the water additions, soil was packed into plastic rings (diameter 4 cm, height 4 cm, volume 50.24 cm³) with different dry bulk densities (BD) (0.45 g cm⁻³, 0.66 g cm⁻³) manually to achieve a range of water filled pore space (WFPS) from 29 % to 83 % (Table 3.2). The pre-determined level of BD in our study was relatively lower than other studies due to the soil type. Andosols, characterized by low BD (Classification Committee of Cultivated Soils, 1996), is formed by weathering of volcanic ash in well-drained conditions and approximately 50 % of upland fields in Japan are covered by this type soil (Akiyama et al., 2000).

Nylon net was covered at the bottom of plastic ring for keeping the soil and the bottom of the core was fitted with a part to exchange the gas inside of the soil cores, and then put all rings into 1.5 L Mason jars and sealed air tightly (Fig.3.2). Each treatment has 3 replications and there were 48 soil cores in total. Ambient wet air (the compressed air used for flushing the jar was connected to a flask with stopper and distilled water to stabilize soil moisture content during incubation) was passed

through a vinyl tube connected to the jar at a rate of 0.375 L min^{-1} for 20 min to replace the gas in the jar completely, and at the same time ambient air was also injected into a vinyl tube connected to bottom of soil cores by syringe 3 times to flush soil sample in the core from the bottom completely (Fig.3.3). After each gas sampling, the soil atmosphere inside of the jar was replaced by ambient wet air in the same way again. A jar without a soil sample was also prepared and labeled as a blank. The same compressed ambient air was flushing through the blank jar by the same way initially and after each gas sampling, which assured the concentrations of target gases were the same in all jars initially. Incubation was conducted by housing all the jars in a temperature controlled incubator for 10 days. Three replications were conducted in the experiment.



Fig.3.2 Incubation plastic ring and jar

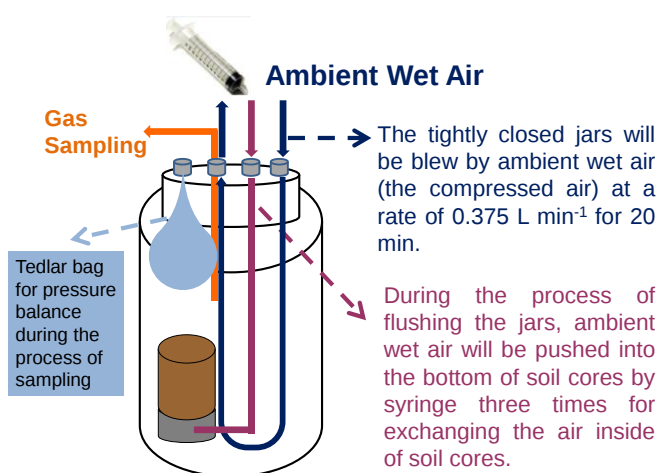


Fig.3.3 Incubation procedure

Gas sampling was carried out 7 times during incubation, at 1, 2, 3, 4, 6, 8, 10 days, respectively. A Tedlar bag inside of the jar was used for pressure balance during sampling. About 400 ml of an air sample was extracted from the headspace of the blank jar into a Tedlar bag by syringe for NO and CO₂ analysis, and a sub-sample of 20 ml was taken from the Tedlar bag and injected into an evacuated 10 ml vial by syringe for N₂O analysis. These air samples were regarded as time 0 min. The Mason jars were left at 15 °C, and then air samples were again taken in the same manner. Incubation condition of 15 °C was chosen since tillage usually happened in May and November. The air temperature of November is too low for incubation; and the mean temperature and max daily temperature of May is 14 and 16 °C, respectively. After each gas sampling, replaced the headspace air of the jar again by purging the jars immediately.

3.3.3 Soil properties analysis

Additional soil cores were packed for analyzing initial physical properties of each treatment with 3 replications. Actual volume (m³) of each soil core was measured by digital actual volumenometer (DIK-1150, Daiki Rika Kogyo, Saitama, Japan), and then water-filled pore space (WFPS, %), porosity (m³ m⁻³), air ratio (m³ m⁻³), liquid ratio (m³ m⁻³), solid ratio (m³ m⁻³) and Tortuosity of pores (mm⁻¹) were calculated. Relative gas diffusion coefficient (D/D₀) was also measured and calculated by using the method developed by Osozawa and Kubota (1987) under steady-state conditions using O₂ as the diffusing gas. First, we measured the gas diffusion coefficient (D) of the soil core samples and then calculated D/D₀ [D₀ (O₂) = 0.178 cm² S⁻¹ under the standard condition, 273 K, 760 mmHg]. D was calculated as: $D = \frac{\text{Air Ratio}}{\alpha^2 \times (t_2 - t_1)} \times \ln\left(\frac{C_{t1} - C_{t0}}{C_{t2} - C_{t0}}\right)$, where C_{t0}, C_{t1} and C_{t2} are the concentrations of O₂ at 0, t₁ and t₂, respectively; α is estimated by solve process ($\text{Air Ratio} = \alpha \times \tan \frac{\alpha}{L_{\text{soil core}}} \times L_{\text{container}}$), where L_{soil core} is the height of soil core and L_{container} is the height of the diffusion container.

The other physical items were calculated as: Air ratio ($\text{m}^3 \text{ m}^{-3}$) = (Core volume—Actual volume)/ Core volume; liquid ratio ($\text{m}^3 \text{ m}^{-3}$) = (Gravimetric water content $\times$ Soil weight)/ Core volume; Solid ratio ($\text{m}^3 \text{ m}^{-3}$) = 1—Air ratio—liquid ratio; Porosity = Air ratio + liquid ratio; WFPS = liquid ratio/Porosity; Tortuosity of pores = $\sqrt{\frac{\text{Air filled porosity}}{D/D_0}}$ (Schjønning et al., 2002).

Just after water adjustment and after incubation, soil chemical properties were measured with 3 replications. Fresh soil samples were immediately extracted with deionized water (1:5) about two hours for measuring the concentration of WEOC, NO_2^- -N, NO_3^- -N, PO_4^{3-} -P, SO_4^{2-} -S, Cl^- , soil EC and soil pH, and with 2 mol L^{-1} KCl (1:10) about two hours for NH_4^+ -N analysis. All the extracts were filtered through 0.2-um membrane filters and stored at 4 °C until analysis. The WEOC content was measured by using a total organic C (TOC) analyzer (Model TOC-5000A; Shimadzu, Japan). The concentration of NO_2^- -N, NO_3^- -N, PO_4^{3-} -P, SO_4^{2-} -S and Cl^- were analyzed by ion chromatography (QIC Analyzer; Dionex Japan, Japan). Soil EC and pH were tested by a combined electrode pH meter (F-8 pH meter; Horiba, Japan) and conductivity meter (CM-30V; TOA electrochemical measuring instruments, Tokyo, Japan), respectively. NH_4^+ -N content was determined by indophenol-blue method (UV mini 1240; Shimadzu, Japan). Net ammonification and net nitrification (mg N kg^{-1}) are estimated as the difference of NH_4^+ or $\text{NO}_3^- + \text{NO}_2^-$ before and after incubation respectively. Net mineralization is calculated as the sum of net ammonification and net nitrification.

Without the limiting of all the factors influencing the denitrifying rate, except enzyme quantity, the potential N_2O production rate was assumed to be proportional to enzyme content (Raymond et al., 1992), named as denitrifying enzyme activity (DEA). DEA was determined after incubation immediately by acetylene block technique, which inhibits the final conversion of N_2O to N_2 gas (Tiedje, 1982). Soil samples were incubated under $\text{N}_2 + 10\% \text{ C}_2\text{H}_2$ atmosphere at 25 °C with solution treated with chloramphenicol (1 g L^{-1}), NO_3^- -N (50 mg $\text{KNO}_3\text{-N L}^{-1}$) and glucose (300 mg glucose-C L^{-1}). Chloramphenicol was used to prevent de novo synthesis of

denitrification enzymes (Genthner et al., 2013), and NO_3^- and glucose was added as electron donor and acceptor for denitrification. A 6 g of wet soil sample and 6 ml treated solution were placed into a 115 ml glass flask. The flasks were vacuumed and flushed with N_2 to ensure anaerobic conditions for 3 times, and then flushed with $\text{N}_2+10\%$ C_2H_2 gas for 2 times. The headspace gas was sampled by a syringe and DEA was calculated as N_2O concentration in headspace between 2 and 4 h.

Soil microbial biomass C (MBC) and N (MBN) were measured by chloroform fumigation-extraction method before incubation. MBC and MBN were calculated by the following formula:

$$\text{MBC or MBN} = \frac{\Delta C}{k}$$

where ΔC is the difference in concentration of organic C or N extracted by 0.5 mol L^{-1} K_2SO_4 (1:10 soil: solution ratio) between fumigated and non-fumigated soil samples (mg kg^{-1}), and k is a factor (0.43, Martens, 1995).

3.3.4 Gas sampling, measurement and calculation

N_2O concentration of gas samples kept in vials was determined by a gas chromatography with electron capture detector (model GC-14B, Shimadzu, Kyoto, Japan). CO_2 concentration was analyzed with infra-red CO_2 gas analyzer (ZFP9GC11, Fuji Electric System, Tokyo, Japan). NO concentration was analyzed with Chemiluminescence N Oxide Analyzer (Model 265P, Kimoto Electric, Osaka, Japan). Gas flux was estimated using the following equation:

$$F = \rho \times \frac{V}{W} \times \frac{\Delta c}{\Delta t} \times \frac{273}{T}$$

where F is the gas flux ($\mu\text{g N m}^{-2} \text{ h}^{-1}$, $\text{mg C m}^{-2} \text{ h}^{-1}$); ρ is the density of each GHG at the standard conditions ($\text{CO}_2 = 1.96 \times 10^6 \text{ mg m}^{-3}$, $\text{N}_2\text{O} = 1.97 \times 10^6 \text{ mg m}^{-3}$, and $\text{NO} = 1.34 \times 10^6 \text{ mg m}^{-3}$); V (m^3) is the volume of the incubation jar; W (g) is the oven soil weight; Δc ($\text{m}^3 \text{ m}^{-3}$) is the gas concentration change in the jar; Δt is the incubation period (h) and T ($^\circ\text{C}$) is the absolute temperature. A positive flux denotes emission from the soil and a negative flux indicates uptake from the headspace. The cumulative gas flux during incubation ($\text{mg kg}^{-1} 10 \text{ d}^{-1}$) was calculated as follows

equation:

$$\text{Cumulative gas flux} = \sum_{i=1}^n (R_i \times D_i)$$

where R_i is the mean gas flux rate ($\text{mg kg}^{-1} \text{ day}^{-1}$) of the two successive sampling intervals, D_i is the number of days in the sampling interval (day) and n is the number of sampling times.

3.3.5 Ratio of $\text{N}_2\text{O-N/NO-N}$

The $\text{N}_2\text{O-N/NO-N}$ ratio was calculated to determine the mechanism of N_2O production. Bouwman (1990b) concluded that the $\text{N}_2\text{O-N/NO-N}$ ratio was less than 1 during nitrification, 1–100 during both nitrification and denitrification and more than 100 during denitrification based on the results reported by Anderson and Levine (1986) and Lipschultz et al. (1981).

3.3.6 Statistical analyses

The calculations of mean values and standard deviation were performed by Excel. The effects of nutrient management, soil moisture, aggregate size and bulk density on the cumulative gases production and soil properties were proceeding with analysis of one-way ANOVA by SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). The significant level of the changes in soil chemical properties during incubation, and the statistical differences of cumulative gas production and soil properties due to different treatments were also evaluated with one-way ANOVA. Pearson correlation coefficient between cumulative N_2O production and soil properties was carried out using SPSS 16.0.

Chapter 4

Evaluation of N₂O and CO₂ hot moments in managed grassland and cornfield, southern Hokkaido, Japan

4.1 Introduction

Rates and reactions of biogeochemical processes vary in space and time to produce both hot spots and hot moments of elemental cycling. Biogeochemical hot moments are defined as short periods of time that exhibit disproportionately high reaction rates relative to longer intervening time periods (McClain et al., 2003). Although our knowledge of the sources and sinks of N₂O and CO₂ in different environments is increasing, prediction of the GHGs exchange in time and space remains challenging (McClain et al., 2003; Bouwman et al., 1995) because of hot spots and hot moments frequently account for larger proportion of the GHG budgets, especially for N₂O (Stehfest and Müller, 2004; Lamers et al., 2007; Groffman et al., 2009; Papen and Butterbach-Bahl, 1999). Episodic fluxes of N₂O at hot moments have been reported after events causing a convergence of reactants, e.g., drying-rewetting (Groffman et al., 2009; Ruser et al., 2006; Dobbie and Smith, 2001), rainfall (Liengaard et al., 2012; Dobbie and Smith, 2003) and freezing-thawing events (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999). Rapid transient pulses of CO₂ have also been observed after precipitation (Savage et al., 2014). In addition to soil and climatic factors, the agricultural activities also control the emissions of CO₂ and N₂O from agricultural soils (Ussiri et al., 2009; Jarecki and Lal, 2006). N₂O fluxes at hot moment were also dominated by agricultural management practices, such as fertilizer and manure additions (Imer et al., 2013; Dobbie and Smith, 2003; Dobbie et al., 1999), harvest (Imer et al., 2013; Dobbie and Smith, 2003) and tillage (Groffman et al., 2009; Xu et al., 2000), and CO₂ hot moment was stimulated by tillage (Rochette and Angers, 1999). Therefore, knowledge of episodic soil CO₂ and N₂O fluxes is essential to reliably quantify these gases fluxes and to develop future mitigation strategies (Imer et al., 2013). However there were few previous studies focused on the identification

and analysis of gas hot moments or environmental factors triggering gas hot moments (Molodovskaya et al., 2012). The lack of information on hot moment quantification is attributed to the fact that there is no agreed-on operational definition of a hot moment, and the magnitude and time scale of N₂O flux events are highly variable even among similar crop, climate, or soil types (Molodovskaya et al., 2012). In this chapter, episodic soil N₂O and CO₂ fluxes at hot moments was evaluated by box plot analysis to improve prediction and understanding of the temporal variability of long-term N₂O and CO₂ fluxes from managed grassland and cornfield in southern Hokkaido, Japan.

4.2 Material and methods

As described in Chapter 3, long term monitor of CO₂ and N₂O fluxes by manual closed chamber was conducted in managed grassland (2004 to 2009 for SZN; 2007 to 2012 for SHD) and cornfield (2009 to 2012 for SZN; 2012 to 2014 for SHD) with 4 replications. In both of grassland and cornfield, three plots with different nutrient managements were established, including F plot (applied with fertilizer), MF plot (applied with manure plus fertilizer), and CT plot (no-fertilizer). The hot moments of N₂O and CO₂ flux were analyzed by box plot method, using the upper fence at 1.5 times distance from the inter-quartile range as a threshold. Soil NO₃⁻-N and NH₄⁺-N, soil temperature and WFPS was also measured.

4.3 Results

4.3.1 Environmental variables

In SZN, daily air temperature ranged from -9.4 to 27.1 °C from 2005 to 2009, in grassland and -12 to 25.9 °C from 2010 to 2012, in cornfield (Fig. 4.1 a). In SHD, the range of daily air temperature was -8.8 to 32.3 °C from 2007 to 2012, in grassland and -16.1 to 31.2 °C from 2013 to 2014, in cornfield (Fig. 4.2 a). The locations of these two sites were close to each other and in the same climatic region. Annual pattern of change in the air temperature of these two sites was same, less than zero from the beginning of December to the middle of March, then increased, reaching the

maximum in August and then decreased steadily to December. Annual mean air temperature ranged from 8.0 to 8.9 °C for 2005 to 2014. Highest monthly precipitation usually occurred from August to October and snowfall was happened from December to May (Fig. 4.1 b; Fig. 4.2 b). Maximum daily precipitation of 90.5 mm d⁻¹ happened on July 23, 2008 in SZN, and 97.5 mm d⁻¹ for SHD on September 16, 2013. Annual precipitation was in the range of 961 to 1590 mm for 2005 to 2014.

Soil temperature had a similar pattern to the air temperature (Fig. 4.1 c; Fig. 4.2 c). WFPS value of SZN ranged from 43 to 90 % in grassland and from 40 to 99 % in cornfield (Fig. 4.1 d). For SHD site, WFPS ranged from 50 to 94 % in grassland and 37 to 99 % in cornfield (Fig. 4.2 d). WFPS of SZN was always high in 2005 with the average of 78 %, and it was lowest in 2011, 68 %.

In SZN, NO₃⁻ ranged from 0.05 to 51.66 mg N kg⁻¹ in grassland and 0.03 to 102.95 mg N kg⁻¹ in cornfield (Fig. 4.1 e). In SHD, soil NO₃⁻ concentration was from 0.007 to 88.50 mg N kg⁻¹ in grassland and 1.17 to 284.09 mg N kg⁻¹ in cornfield (Fig. 4.2 e). NH₄⁺ ranged from 0.14 to 245.13 mg N kg⁻¹ in grassland and 0.27 to 692.63 mg N kg⁻¹ in cornfield of SZN (Fig. 4.1 f), and from 0.134 to 218.09 mg N kg⁻¹ in grassland and 2.48 to 252.96 mg N kg⁻¹ in cornfield of SHD (Fig. 4.2 f). NH₄⁺-N increased after fertilization and following that the small peak of NO₃⁻-N was found. In 2010 in the cornfield of SZN, no NH₄⁺-N peak was found and NO₃⁻-N concentration was generally higher than other years, which implied that the higher nitrification rate occurred in the first year after the land use change from grassland to cornfield. However this kind of phenomenon was not observed in SHD site.

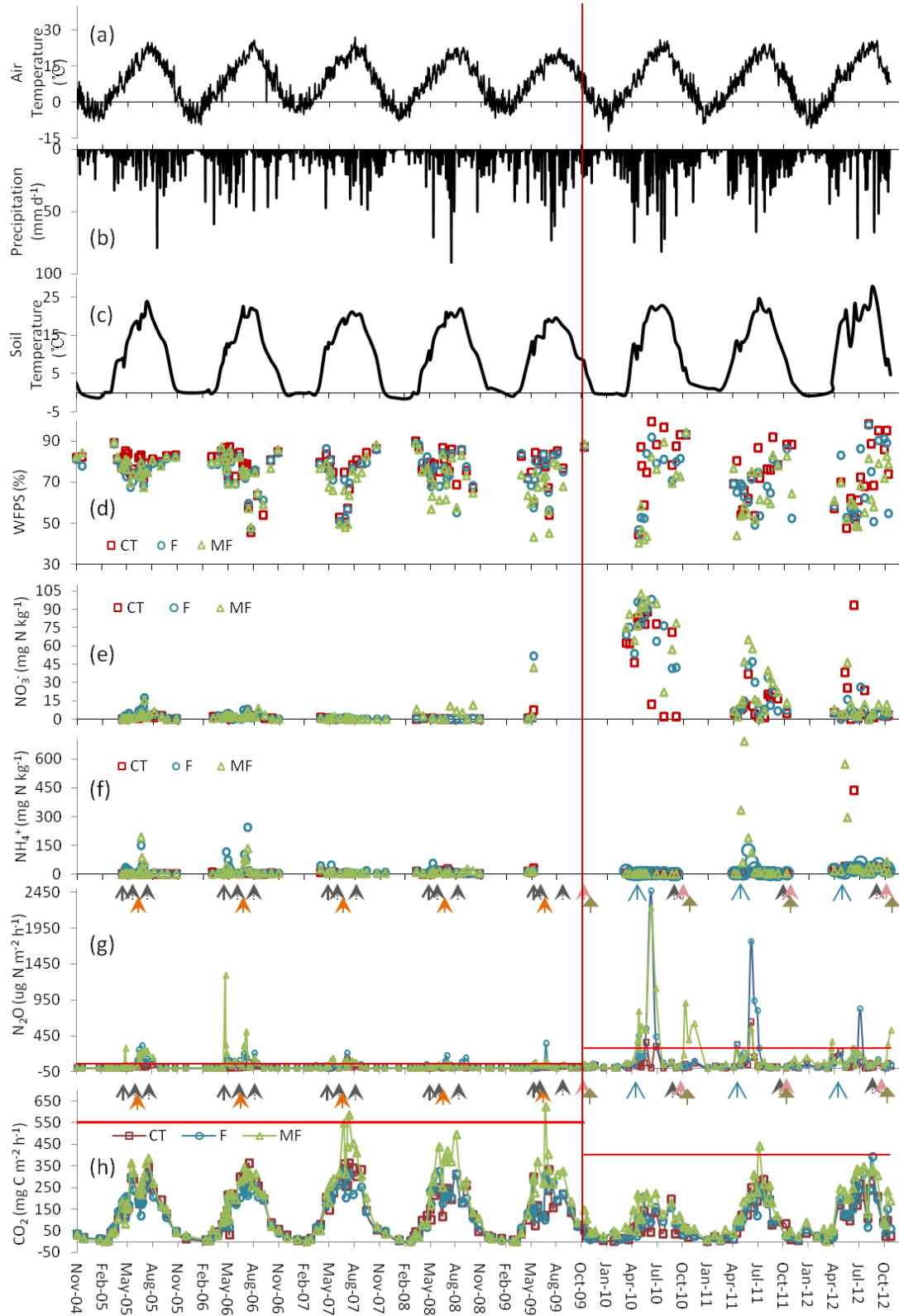


Fig.4.1 Seasonal changes of N_2O and CO_2 fluxes, meteorological and soil chemical properties in SZN

(the left side of vertical red line is grassland ; the right side of vertical red line is cornfield; solid lines in (g) (h) are the UFs of N_2O and CO_2 fluxes in hot moment respectively; Arrows in (g) (h) shows the practices of different managements: $\uparrow$ Fertilizer and manure applications; $\uparrow$ Harvest; $\uparrow$ Fertilizer application; $\uparrow$ Manure application; $\uparrow$ Plough; $\uparrow$ Harrowing, rolling, seeding and fertilizer application).

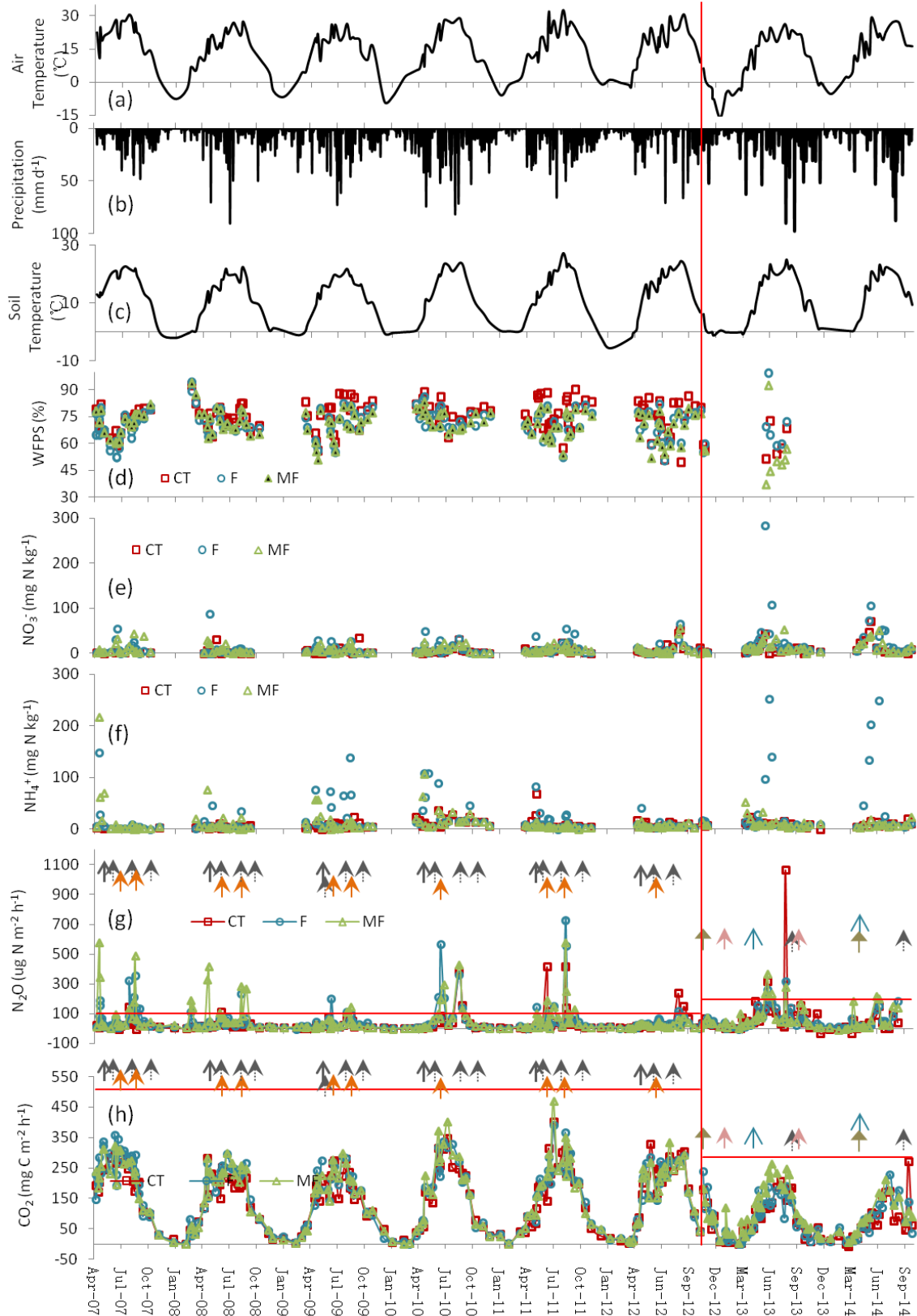


Fig.4.2 Seasonal changes of N_2O and CO_2 fluxes, meteorological and soil chemical properties in SHD

(the left side of vertical red line is grassland ; the right side of vertical red line is cornfield; solid lines in (g) (h) are the UFs of N_2O and CO_2 fluxes in hot moment respectively; Arrows in (g) (h) shows the practices of different managements: $\uparrow$ Fertilizer and manure applications; $\downarrow$ Harvest; $\uparrow$ Fertilizer application; $\uparrow$ Manure application; $\uparrow$ Plough; $\uparrow$ Harrowing, rolling, seeding and fertilizer application).

4.3.2 Gas fluxes

N₂O fluxes in grassland and cornfield of SZN are shown in Fig. 4.1 g, respectively. In both grassland and cornfield, N₂O fluxes were significantly higher in F and MF plots than in CT plots ($p < 0.01$). As can be seen from Fig. 4.2 g, N₂O flux from CT plots also tended to be lower than F and MF plots both in grassland and cornfield of SHD, whereas the differences of the magnitude of N₂O flux between plots was much smaller than SZN. There was a tendency in N₂O fluxes in MF plots were higher than F plot in grassland and cornfield except for the CT plot of cornfield in SHD. N₂O fluxes usually tended to increase after fertilization, harvest, and rainfall in summer. For SZN site, the maximum peak N₂O flux of 1291 $\mu\text{g N m}^{-2} \text{h}^{-1}$ was observed in MF plot of grassland, while that of 2461 $\mu\text{g N m}^{-2} \text{h}^{-1}$ was measured in F plot of cornfield. The highest N₂O flux of cornfield in SHD was measured in CT plot, up to 1065 $\mu\text{g N m}^{-2} \text{h}^{-1}$, and the largest N₂O flux of 729.63 $\mu\text{g N m}^{-2} \text{h}^{-1}$ was observed in F plot of grassland. There were some negative N₂O fluxes (N₂O uptake by soil). For SZN site, proportions of positive N₂O fluxes were 87.0 % for CT plot, 95.1 % for F plot, 92.7 % for MF plot in grassland, and 93.4 % for CT plot, 98.7 % for F plot and 100 % for MF plot in cornfield. In the cornfield of SHD site, 96.8 % of N₂O observations in CT plot were positive, and 96.6 % for F plot and 94.9 % for MF plot. The percentages of positive N₂O fluxes in grassland of SHD were 96.1 % for CT plot, 98.0 % for F plot and 98.7 % for MF plot. Except for the CT plot in cornfield of SHD, which showed higher value of mean N₂O flux than F plot, MF plots always had the largest mean N₂O fluxes and followed by F plot in both grassland and cornfield. The mean N₂O fluxes were significantly larger in cornfield than in grassland for both sites, but the coefficient of variation (CV) of N₂O fluxes in grassland was higher than that in cornfield (Table 4.1). Normality test showed that N₂O fluxes in all plots were skewed to the left and not normally distributed. The mean N₂O flux of grassland in SZN was similar to that of SHD, while that of cornfield in SZN was 2-fold higher compared with SHD.

Table 4.1 Descriptive statistics of N₂O and CO₂ fluxes from each plot

Site	Plot	N	25 %	50 %	75 %	Max	Mean	SD	CV (%)	Normality Test
SZN Grassland N ₂ O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$)	CT	123	1.47	5.92	12.52	114.46	9.14	13.7	149.8	p<0.001
	F	122	3.39	10.18	45.48	337.86	42.07	67.1	159.4	p<0.001
	MF	123	2.75	13.80	61.03	1290.66	58.99	139.7	236.7	p<0.001
	Whole Site	368	2.47	8.25	24.84	1290.66	36.72	92.0	250.5	p<0.001
SZN Cornfield N ₂ O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$)	CT	76	7.33	21.37	61.11	638.48	53.40	97.0	181.6	p<0.001
	F	76	14.19	37.97	132.79	2461.37	163.88	375.8	229.3	p<0.001
	MF	76	30.08	73.06	234.82	2223.51	198.23	323.3	163.1	p<0.001
	Whole Site	228	14.24	40.07	120.95	2461.37	138.50	296.9	214.3	p<0.001
SZN Grassland CO ₂ fluxes ($\text{mg C m}^{-2} \text{h}^{-1}$)	CT	123	48.90	150.54	241.66	363.52	154.22	105.9	68.7	p<0.001
	F	122	59.92	168.32	219.32	329.58	146.19	96.3	65.9	p<0.001
	MF	123	50.56	202.41	304.02	622.82	197.30	146.0	74.0	p<0.001
	Whole Site	368	53.31	169.23	253.26	622.82	165.95	119.89	72.2	p<0.001
SZN Cornfield CO ₂ fluxes ($\text{mg C m}^{-2} \text{h}^{-1}$)	CT	75	21.35	58.72	127.60	309.72	87.52	81.5	93.1	p<0.001
	F	75	23.76	74.87	148.81	390.81	94.81	84.7	89.3	p<0.001
	MF	75	54.80	120.27	212.31	442.10	142.34	102.6	72.1	p<0.001
	Whole Site	225	30.96	81.44	179.21	442.10	108.28	92.9	85.8	p<0.001
SHD Grassland N ₂ O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$)	CT	153	7.02	14.48	30.42	419.03	32.4	62.93	194.23	P<0.001
	F	153	10.07	21.08	53.71	729.63	51.47	95.63	185.80	P<0.001
	MF	153	9.26	22.62	64.68	579.23	61.97	104.44	168.53	P<0.001
	Whole Site	459	8.08	19.19	44.73	729.63	48.62	90.11	185.34	P<0.001
SHD Cornfield N ₂ O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$)	CT	62	3.56	37.81	94.86	1065.71	68.74	142.64	207.52	P<0.001
	F	59	14.21	38.05	81.00	318.50	61.26	66.58	108.67	P<0.001
	MF	59	20.71	40.73	94.42	364.98	70.09	80.51	114.87	P<0.001
	Whole Site	180	13.16	39.61	86.08	1065.71	66.73	102.40	153.45	P<0.001
SHD Grassland CO ₂ fluxes ($\text{mg C m}^{-2} \text{h}^{-1}$)	CT	153	70.73	185.1	241.02	401.51	166.34	96.46	57.99	P<0.001
	F	153	78.07	199.2	250.95	392.29	173.96	99.91	57.43	P<0.001
	MF	153	70.45	204.45	248.55	469.7	175.57	102.23	58.23	P<0.001
	Whole Site	459	72.82	194.98	247.06	469.7	171.96	99.42	57.82	P<0.001
SHD Cornfield CO ₂ fluxes ($\text{mg C m}^{-2} \text{h}^{-1}$)	CT	62	24.36	63.12	122.05	272.21	80.69	63.24	78.37	P<0.05
	F	61	23.30	74.07	133.52	239.30	82.55	63.70	77.16	P<0.05
	MF	59	45.05	86.55	163.67	264.13	106.72	70.77	66.31	P<0.05
	Whole Site	182	35.44	76.86	135.12	272.21	89.75	66.61	74.22	P<0.001

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; SD, Standard Deviation; CV, Coefficient of variation; Normality Test was analyzed by Shapiro-Wilk test.

The varied trend of CO₂ fluxes in SZN and SHD was similar. In both grassland and cornfield, CO₂ fluxes were significantly higher in MF plot than in CT and F plots (p<0.01) and no significant difference between F and CT plots (Fig. 4.1 h; Fig. 4.2 h). The seasonal variation in CO₂ fluxes followed a similar trend to air and soil temperature. CO₂ fluxes increased from May to August, and then decreased to

December. In winter season positive CO₂ fluxes were also found in these two study sites. For SZN, the maximum peak CO₂ flux of 623 mg C m⁻² h⁻¹ was found in MF plot of grassland, while that of 442 mg C m⁻² h⁻¹ was found in MF plot of cornfield. The highest CO₂ flux in the grassland of SHD was 470 mg C m⁻² h⁻¹, captures in MF plot, and that of cornfield was up to 272 mg C m⁻² h⁻¹ measured in CT plot. For both sites, mean CO₂ fluxes were significantly larger in grassland than in cornfield, however, the CV of CO₂ fluxes in cornfield was slightly higher than that of grassland (Table 4.1). Those were opposite tendency to that of N₂O fluxes. Normality test showed that the distribution of CO₂ fluxes did not show normality, similar to N₂O fluxes (Table 4.1).

4.3.3 Threshold and frequencies of N₂O and CO₂ fluxes at hot moments

The upper fence (UF) values of gas hot moments, calculated by box plot method, of N₂O and CO₂ fluxes in each plot of SZN and SHD are presented in Table 4.2. The varying patterns of UFs were similar with the mean gas fluxes (Table 4.1). The UFs of N₂O fluxes were higher in cornfield than in grassland, and MF plot tended to show the highest UFs of N₂O fluxes except for the CT plot of cornfield in SHD with the highest UF, followed by F plot in both cornfield and grassland. On the other hand, UFs of CO₂ fluxes were higher in grassland than in cornfield, however, the highest and lowest UFs of CO₂ fluxes were also found in MF and CT plots, respectively, in both grassland and cornfield. The UFs of gas fluxes of each site in grassland and cornfield were used as the baseline to pick up N₂O and CO₂ fluxes in hot moment, and the number and frequencies of gas hot moments are shown in Table 4.3.

Table 4.2 The UF values of N₂O and CO₂ hot moments in all plots

Site	Gas	Land use	CT	F	MF	Whole Site
SZN	N ₂ O (μg N m ⁻² h ⁻¹)	Grassland	29.1	108.6	148.5	58.4
		Cornfield	141.8	310.7	541.9	281.0
	CO ₂ (mg C m ⁻² h ⁻¹)	Grassland	530.8	458.4	684.2	553.2
		Cornfield	287.0	336.4	448.6	401.6
SHD	N ₂ O (μg N m ⁻² h ⁻¹)	Grassland	65.5	119.2	147.8	99.7
		Cornfield	231.8	181.2	205.0	195.5

CO ₂ (mg C m ⁻² h ⁻¹)	Grassland	496.5	510.3	515.7	508.4
	Cornfield	268.6	298.8	341.6	284.6

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; UF, Upper Fence; There is no CO₂ or N₂O fluxes below than lower fence (LF, data was not shown).

Table 4.3 Number and frequencies of N₂O and CO₂ hot moments

Site	Gas	Land use	Period	CT		F		MF		Whole Site	
				N	Frequency (%)	N	Frequency (%)	N	Frequency (%)	N	Frequency (%)
SZN	N ₂ O	Grassland	5 years	2	1.6	27	22.1	32	26.0	61	16.6
			1 st yr (04.11.26-05.11.13)	0	0.0	9	31.0	9	31.0	18	20.7
			2 nd yr (05.12.19-06.11.13)	1	3.9	11	42.3	11	42.3	23	29.5
			3 rd yr (06.12.14-07.11.29)	1	4.6	2	9.5	6	27.3	9	13.9
			4 th yr (08.01.17-08.10.28)	0	0.0	4	17.4	1	4.4	5	7.3
			5 th yr (08.12.01-09.11.08)	0	0.0	1	4.4	5	21.7	6	8.7
		Cornfield	3 years	4	5.3	9	11.8	15	19.7	28	12.3
			1 st yr (09.11.09-10.11.16)	2	7.4	4	14.8	10	37.0	16	19.8
			2 nd yr (10.12.05-11.11.28)	2	8.7	4	17.4	2	8.7	8	11.6
			3 rd yr (11.12.12-12.11.05)	0	0.0	1	3.9	3	11.5	4	5.1
	CO ₂	Grassland	5 years	0	0.0	0	0.0	2	1.6	2	0.5
		Cornfield	3 years	0	0.0	0	0.0	1	1.3	1	0.4
SHD	N ₂ O	Grassland	5.5 years	10	6.5	16	10.5	23	15.0	49	10.7
			1 st yr (07.05.04-08.05.08)	1	3.6	3	10.7	8	28.6	12	14.3
			2 nd yr (08.05.11-09.05.02)	1	4.0	1	4.0	4	16.0	6	8.0
			3 rd yr (09.05.06-10.05.04)	0	0.0	2	7.1	2	7.1	4	4.8
			4 th yr (10.05.16-11.05.07)	2	9.1	5	22.7	4	18.2	11	16.7
			5 th yr (11.05.11-12.05.09)	4	12.1	4	12.1	5	15.2	13	13.1
			6 th yr (12.05.17-12.11.05)	2	11.8	1	5.9	0	0.0	3	5.9
		Cornfield	2 years	2	3.2	2	3.4	7	11.9	11	6.1
			1 st yr (12.11.15-13.11.07)	2	5.3	2	5.3	5	13.2	9	7.9
			2 nd yr (13.12.03-14.10.12)	0	0.0	0	0.0	2	9.5	2	3.0
	CO ₂	Grassland	5 years	0	0.0	0	0.0	0	0.0	0	0.0
		Cornfield	2 years	0	0.0	0	0.0	0	0.0	0	0.0

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; N, Number; Frequency = (Number of hot moment) / (Total number of data) × 100.

For both SZN and SHD sites, N₂O hot moment frequency was significantly larger in MF plots than in F plots compared with CT plots ($p < 0.001$) (Table 4.3). N₂O hot moment frequency was largest in MF plot, followed by F plot and CT plot in both grassland and cornfield. In SZN, the frequency of N₂O hot moments over the whole study period in F and MF plots ranged from 11.8 % in cornfield to 26.0 % in grassland, and it was less than 5.3 % in CT plots. In SHD, the frequency of N₂O hot moments in MF plots of grassland and cornfield was 15.0 % and 11.9 % respectively,

which was in contrast with that in CT plot was 6.5 % and 3.2 %. In both sites, N₂O hot moments in F and MF plots of grassland occurred more frequently than the corresponding plot in cornfield, which was same as in the CT plot in SHD but on the contrary in the CT plot in SZN. Regardless of the land-use type, the frequency of N₂O hot moments over the whole study period in SZN was higher than that of SHD.

In SZN, largest N₂O hot moment frequency in grassland was 29.5 % in second year (December 19, 2005 to November 13, 2006), followed by 20.7 % in first year (November 26, 2004 to November 13, 2005) and 13.9 % in third year (December 14, 2006 to November 29, 2007), then decreased to 7.3 % and 8.7 % in fourth year (January 17, 2008 to October 28, 2008) and in fifth year (December 01, 2008 to November 08, 2009), respectively (Table 4.3). In cornfield, after conversion from the grassland, N₂O hot moment frequency increased to 19.6 % in the first year (November 09, 2009 to November 16, 2010), then decreased to 11.6 % in second year (December 05, 2010 to November 28, 2011), then 5.6 % in third year (December 12, 2011 to November 05, 2012) (Table 4.3). In SHD, the N₂O hot moment frequency did not changed regularly, which was different from SZN site. The frequency of N₂O hot moments in grassland kept a relatively high level in the first (14.3 %), fourth (16.7 %) and fifth year (13.1 %), and the lowest frequency was found in the third year, down to 4.8 % (Table 4.3). The sharply increase in the N₂O hot moment frequency after the land-use change from grassland to cornfield was not observed in SHD, which happened in SZN.

On the other hand, few CO₂ hot moments, 0.5 % and 0.4 % respectively, were found in grassland and cornfield of SZN (Table 4.3). CO₂ hot moment in SZN was not found in CT and F plots. CO₂ hot moment frequency in MF plots was 1.6 % and 1.3 % in grassland and cornfield, respectively. In SHD, no CO₂ hot moment was recognized.

4.3.4 Events inducing N₂O and CO₂ hot moments

N₂O and CO₂ hot moments in SZN and SHD were often found in July and August in both grassland and cornfield (Fig. 4.1 g h; Fig. 4.2 g h), and the average daily

temperature of July and August was 20.1 °C during the study period. This indicated that the occurrence of temperature > 20 °C might induce N₂O and CO₂ hot moments.

Rainfall is an important factor for variability of N₂O fluxes, and 34 % and 39 % of N₂O hot moments in SZN responded to rainfall (>0 mm d⁻¹) within a day in grassland and cornfield, respectively (Fig. 4.1 b g; Fig. 4.2 b g). Within 3 days after rainfall greater than 15 mm d⁻¹, 39 % of N₂O hot moments in grassland and 57 % in cornfield were observed in SZN, and 59 % for grassland and 55 % for cornfield in SHD, respectively. In all plots of cornfield of SZN, N₂O hot moments were captured at 2 days after the rainfall of 28 mm d⁻¹ on June 18, 2010 and 3 days after the rainfall of 43.5 mm d⁻¹ on June 27, 2011, respectively. In F plot of cornfield in SZN, N₂O hot moment of 931 µg N m⁻² h⁻¹ was found on July 08, 2011 just after the rainfall of 19 mm d⁻¹. In MF plot of cornfield in SZN, N₂O hot moment of 898 µg N m⁻² h⁻¹ was found on November 04, 2010 just one day after rainfall of 15.5 mm d⁻¹ and 7 days after manure application (Fig. 4.1 g). In CT plot of grassland in SHD, the N₂O peak emission of 151 µg N m⁻² h⁻¹ was measured at 3 days after the rainfall of 28 mm d⁻¹ on September 13, 2012. N₂O hot moments in both F and MF plots of grassland in SHD responded to the rainfall of 21 mm d⁻¹ within 2 days (July 05, 2010) and the rainfall of 29.5 mm d⁻¹ within 3 days (July 06, 2011). In all plots of cornfield in SHD, N₂O hot moments were captured at 2 days after the rainfall of 17 mm d⁻¹ on June 20, 2013 and 1 day after the rainfall of 90 mm d⁻¹ on August 19, 2013. But no N₂O hot moment was observed in all plots of SZN after the rainfall of 14 mm d⁻¹ on June 14, 2011. Similarly, N₂O hot moments did not occurred at 3 days after the rainfall of 14 mm d⁻¹ on September 23, 2007 and at 1 day after the rainfall of 14 mm d⁻¹ on December 05, 2010 in all plots of grassland in SHD. A CO₂ hot moment in grassland of SZN was also observed at 17 days after harvest and 2 days after a rainfall of 44.5 mm d⁻¹. These indicate that within 3 days of rainfall greater than 15 mm d⁻¹ N₂O and CO₂ hot moments were induced.

In grassland of SZN, N₂O hot moment was found 9 days after harvest in F plot without rainfall (0 mm d⁻¹) from 3 days ago (Fig. 4.1 g). There was no influence of rainfall (0 mm d⁻¹) from 2 days ago, N₂O hot moment was also measured in the CT

plot of grassland in SHD at 8 days after harvest on August 20, 2007 and 15 days after harvest on June 17, 2011 (Fig. 4.2 g). The highest N_2O hot moment of $114 \mu\text{g N m}^{-2} \text{h}^{-1}$ in CT plot of grassland in SZN was observed 30 days after harvest without rainfall (0 mm d^{-1}) (Fig. 4.1 g). These indicate that harvest was an event inducing N_2O hot moments.

In MF plot of cornfield in SZN, N_2O hot moment of $336 \mu\text{g N m}^{-2} \text{h}^{-1}$ was observed 1 day after harrowing, rolling and fertilizer application without rainfall for 4 days before the N_2O hot moment. And another N_2O hot moment of $524 \mu\text{g N m}^{-2} \text{h}^{-1}$ was found at 24 days after harrowing and rolling with very little rainfall of 0.5 mm d^{-1} . These indicate that tillage induced N_2O hot moments.

In grassland of SZN, N_2O hot moments in CT plot occurred in July and August, but in F and MF plots mainly occurred between May to September when chemical fertilizer and manure were applied in grassland (May, June and July) (Fig. 4.1 g). In SHD, most of N_2O hot moments in grassland were also measured from May to September and from June to August in cornfield. In grassland of SHD, N_2O hot moment of $143 \mu\text{g N m}^{-2} \text{h}^{-1}$ was captured at 5 days after fertilizer application in F plot and peak N_2O fluxes were also observed both in F and MF plots at 3 days after fertilization on May 15, 2007 without rainfall (0 mm d^{-1}) from 2 days ago. While on July 18, 2007 and May 24, 2008, no N_2O hot moment was found in F or MF plots at 14 days and 18 days after fertilizer addition, respectively (Fig. 4.2 g). In SZN, N_2O hot moments of 207 and $110 \mu\text{g N m}^{-2} \text{h}^{-1}$ were recorded at 13 days after fertilizer application in F and MF plots of grassland on the same day, and there was no rainfall for 4 days before the N_2O hot moments, but N_2O hot moments were not found in MF plot of grassland at 19, 22 and 23 days after fertilizer application (on July 24, 2007, August 01, 2006 and June 01, 2006, respectively) (Fig. 4.1 g). These indicate that fertilizer and manure applications are events inducing N_2O hot moment within 2 weeks.

4.3.5 Events inducing N₂O and CO₂ fluxes in hot moment significantly

Based on the finding about the events inducing hot moments mentioned in last section (chapter 4.3.4), different thresholds of each event were chosen to analyze the effect of each event on increasing the frequency of hot moments, and more clear results of significant events would be concluded when the threshold of each event was determined as: for rainfall event, 3 days within the occurrence of precipitation of more than 15 mm d⁻¹; for nutrient management event, within 15 days from the day of manure or fertilizer applications; for tillage event, within 30 days from the day of the practices of harrowing, rolling or plough; for harvest event, within 30 days from the harvest day; and occurrence of temperature > 20 °C. The numbers of gas fluxes in hot and non-hot moments under each event were counted and the effect of each event on the frequency of hot moment was analyzed with Fisher's Exact Test. The overall influence of each event, including the single effect and combined effects with other events, on the frequency of N₂O and CO₂ hot moments are shown in Table 4.4 (detail information of combined events was not shown).

Table 4.4 Number and frequency of N₂O and CO₂ fluxes in hot moment and non-hot moment with the effects of events

Events	Number of hot moments					Number of non-hot moments		Frequency of hot moment (%)		P value
	Single effect	Combined effect with other events	Overall effect	No effect	Total	Overall effect	No effect	Overall effect	No effect	
N ₂ O—Grassland SZN										
T>20°C	4	13	17	44	61	27	280	38.6	13.6	< 0.001
Rainfall	3	21	24	37	61	66	241	26.7	13.3	< 0.05
Harvest	4	30	34	27	61	64	243	34.7	10.0	< 0.001
Fertilizer	1	28	29	32	61	19	288	60.4	10.0	< 0.001
Manure	4	6	10	51	61	8	299	55.6	14.6	< 0.001
N ₂ O—Cornfield SZN										
T>20°C	1	2	3	25	28	30	170	9.1	12.8	0.775
Rainfall	7	9	16	12	28	53	147	23.2	7.6	< 0.05
Harvest	0	0	0	28	28	18	182	0.0	13.3	0.139
Fertilizer	0	4	4	24	28	14	186	22.2	11.4	0.250
Manure	0	2	2	26	28	2	198	50.0	11.6	0.075
Tillage	1	7	8	20	28	49	151	14.0	11.7	0.645
CO ₂ —Grassland SZN										
T>20°C	1	0	1	1	2	43	323	2.3	0.3	0.225
Rainfall	0	1	1	1	2	89	277	1.1	0.4	0.430
Harvest	0	1	1	1	2	97	269	1.0	0.4	0.462
CO ₂ —Cornfield SZN										

T>20℃	1	0	1	0	1	32	192	3.0	0.0	0.147
N ₂ O—Grassland SHD										
T>20℃	0	26	26	23	49	52	358	33.3	6.0	<0.001
Rainfall	3	26	29	20	49	127	283	18.6	6.6	<0.001
Harvest	4	29	33	16	49	117	293	22.0	5.2	<0.001
Fertilizer	2	18	20	29	49	77	333	20.6	8.0	<0.01
Manure	0	4	4	45	49	12	398	25.0	10.2	0.08
N ₂ O—Cornfield SHD										
T>20℃	0	3	3	8	11	18	151	14.3	5.0	0.122
Rainfall	3	3	6	5	11	30	139	16.7	3.5	<0.01
Harvest	0	0	0	11	11	15	154	0.0	6.7	0.603
Fertilizer	0	0	0	11	11	8	161	0.0	6.4	1.0
Manure	0	0	0	11	11	4	165	0.0	6.3	1.0
Tillage	0	0	0	11	11	45	124	0.0	8.1	0.07

T>20 ℃, air temperature on sampling day is higher than 20 ℃; Rainfall, 3 days within the occurrence of precipitation of more than 15 mm d⁻¹; Harvest, within 30 days from harvest day; Fertilizer, within 15 days from the day of fertilizer application; Manure, within 15 days from the day of manure application; Tillage, within 30 days from the day of the practices of harrowing, rolling or plough; P value, the significant level of the comparison between the frequency of hot moments related to event (overall effect) and non-related to the event (overall effect) by Fisher's Exact Test; Frequency = (Number of hot moment) / (Total number of data) × 100.

N₂O in Grassland: Except the effect of manure application event in SHD was not significant (p=0.08), the events of temperature > 20 ℃ (p<0.001 for SZN and SHD), rainfall (p<0.05 for SZN; p<0.001 for SHD), harvest (p<0.001 for SZN and SHD), fertilizer application (p<0.001 for SZN; p<0.01 for SHD), and manure application (p<0.001 for SZN) in grassland all increased the frequency of N₂O hot moments significantly. Harvest event had the largest contribution to the number of N₂O hot moments, with 34 out of 61 in SZN and 33 out of 49 in SHD. The frequency of N₂O hot moment related to each event can be estimated by the percentage of the number of N₂O hot moments affected by the event compared with the total number of N₂O fluxes related to the event. In SZN, although the number of N₂O hot moments related to fertilizer application event was not the largest, equal to 29, the frequency of N₂O hot moments due to fertilizer addition event was the highest (60.4 %), followed by the events of manure application (55.6 %), temperature > 20 ℃ (38.6 %), harvest (34.7 %) and rainfall (26.7 %) (Table 4.4). In SHD, the frequency of N₂O hot moment induced by the event of temperature > 20 ℃ was largest (33.3 %) and followed by harvest (22.0 %), fertilizer application (20.6 %) and rainfall (18.6 %) (Table 4.4).

In SZN, even without the contribution of the events of rainfall, manure addition or temperature $> 20\text{ }^{\circ}\text{C}$, fertilizer application event still increased the frequency of N_2O hot moment significantly ($p<0.05$). And fertilizer application event also played a key role in the combined events affected by it. The effect of the combined event of harvest and fertilizer was always significant ($p<0.001$) even when temperature was lower than $20\text{ }^{\circ}\text{C}$ or there was no rainfall event. Under the event of fertilization combined with rainfall, the frequency of N_2O hot moment was 87.5 %, but without rainfall event, the frequency decreased to 46.9 % (data was not shown).

In contrast to SHD, the effect of fertilizer addition was strongly affected by the rainfall event and the single effect of fertilization was not significant. Harvest event significantly increased the frequency of N_2O hot moment in SHD even without the influence of fertilization event. The frequency of N_2O hot moment associated with fertilizer application combined with rainfall event was up to 36.4 % and 56.3 % with temperature $> 20\text{ }^{\circ}\text{C}$, while down to 12.5 % and 13.6 % without the influence of rainfall event and temperature $> 20\text{ }^{\circ}\text{C}$ respectively.

The frequency of N_2O hot moments related to manure application event was up to 55.6 % in SZN, but its contribution to the total number of hot moments was smallest since manure was only applied in MF plot. The single effect of rainfall event was significant in SZN ($p<0.05$) even though there were only three N_2O hot moments related to it. Episodic N_2O hot moment flux after single rainfall event reached to $140\text{ }\mu\text{g N m}^{-2}\text{ h}^{-1}$ in MF plot (May 29, 2007), but up to $231\text{ }\mu\text{g N m}^{-2}\text{ h}^{-1}$ if rainfall event happened when temperature was $> 20\text{ }^{\circ}\text{C}$ (July 29, 2005) (Fig. 4.1 g). Similarly, the frequency of N_2O hot moment induced by rainfall event increased to 55.6 % when temperature was higher than $20\text{ }^{\circ}\text{C}$ in SHD (Fig. 4.2 g). The idea that temperature $> 20\text{ }^{\circ}\text{C}$ ($p<0.001$) significantly facilitated the frequency of N_2O hot moments in grassland was also supported by the fact that the frequency of N_2O hot moments was small in 2009 in SZN and lowest in the 3rd year in SHD (Table 4.3), when the air temperature during most sampling days was lower than $20\text{ }^{\circ}\text{C}$.

N_2O in cornfield: However, only rainfall event induced episodic N_2O flux in hot moment significantly ($p<0.05$ for SZN; $p<0.01$ for SHD) in the cornfields of both

sites, which was also supported by the fact that the inter-annual patterns of N₂O hot moments frequency were generally similar with the number of rainfall events. In SZN, the highest N₂O hot moment frequency and the largest number of rainfall event were all measured in the 1st year after the conversion from grassland to cornfield and lowest in the 3rd year (Table 4.3). And in SHD, the number of rainfall event in the 1st year (12 months) and 2nd year (10 months) of the study period was 9 and 3, with the N₂O hot moment frequency of 7.9 % and 3.0 %, respectively (Table 4.3). N₂O hot moments driven by rainfall event ranged from 352 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ (CT plot) to 800 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ (F plot) in SZN (Fig. 4.1 g). The highest N₂O peaks in F (2460 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) and MF (2220 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) plots of SZN all happened on July 05, 2010 after rainfall event under high temperature ($> 20\text{ }^{\circ}\text{C}$) (Fig. 4.1 g). The largest N₂O fluxes in CT and F plots of SHD were captured within 2 days after a heavy rainfall of 90 mm d⁻¹ when temperature $> 20\text{ }^{\circ}\text{C}$ on the same day (August 19, 2013) (Fig. 4.2 g).

CO₂: CO₂ hot moment was only measured occasionally in SZN (Fig. 4.1 h). The frequency of CO₂ hot moments in grassland was influenced by the events of temperature $> 20\text{ }^{\circ}\text{C}$, rainfall and harvest, and in the cornfield by only temperature $> 20\text{ }^{\circ}\text{C}$ event, while all the effects were not significant.

4.3.6 Ratio of N₂O-N/NO-N

N₂O-N/NO-N ratio was higher during hot moment than non-hot moment (Table 4.5). N₂O-N/NO-N ratio in hot moment was always larger than 1, and even in the non-hot moment. N₂O-N/NO-N ratio less than 1 accounted for less than 6.6 % of N₂O fluxes at non-hot moment in SZN and 5.8 % in SHD.

In SZN, N₂O-N/NO-N ratio was higher in cornfield than in grassland. During hot moment in the cornfield, N₂O-N/NO-N ratio more than 100 accounted for 67 to 100 %, which implied that denitrification was the main pathway of N₂O emissions in hot moment in cornfield, and in grassland, it ranged from 22 to 50 %. In SHD, the N₂O-N/NO-N ratio of N₂O hot moment in cornfield was slightly higher than that in grassland, but the contribution of denitrification to N₂O emission at hot moment was

less than that of SZN due to the less proportion of N_2O -N/ NO -N ratio > 100. During non-hot moment in SZN and SHD, N_2O -N/ NO -N ratio more than 100 accounted for 4 to 9 % in grassland and 12 to 25 % in cornfield. More than 75 % of N_2O fluxes in non-hot moment were produced by both nitrification and denitrification processes, and the contribution of denitrification to non-hot moment N_2O flux was also higher in cornfield than in grassland.

Table 4.5 Percentage of the N_2O -N/ NO -N ratio in hot moment and non-hot moment within each threshold (< 1, 1 ~ 100, > 100) (%)

	Land use	Plot	Hot moment			Non-hot moment		
			< 1	1 ~ 100	> 100	< 1	1 ~ 100	> 100
SZN	Grassland	CT	0.0	50.0	50.0	4.1	91.8	4.1
		F	0.0	70.4	29.6	1.1	94.7	4.2
		MF	0.0	78.1	21.9	6.6	84.6	8.8
		Whole Site	0.0	73.8	26.2	3.9	90.6	5.5
	Cornfield	CT	0.0	0.0	100.0	0.0	86.1	13.9
		F	0.0	33.3	66.7	1.5	77.6	20.9
		MF	0.0	26.7	73.3	1.7	80.3	18.0
		Whole Site	0.0	25.0	75.0	1.0	81.5	17.5
SHD	Grassland	CT	0.0	60.0	40.0	0.7	94.4	4.9
		F	0.0	75.0	25.0	0.7	94.9	4.4
		MF	0.0	65.2	34.8	2.3	90.8	6.9
		Whole Site	0.0	67.3	32.7	1.2	93.4	5.4
	Cornfield	CT	0.0	50.0	50.0	0.0	75.0	25.0
		F	0.0	50.0	50.0	3.5	82.5	14.0
		MF	0.0	71.4	28.6	5.8	82.7	11.5
		Whole Site	0.0	63.6	36.4	3.0	79.9	17.2

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot.

4.3.7 Contribution of N_2O and CO_2 hot moments to annual emission

Table 4.6 showed that linear regressions between N_2O flux and CO_2 flux in non-hot moment and soil temperature, soil moisture content. In SZN, N_2O fluxes at non-hot moment in each plot were significantly correlated with soil temperature at 5 cm in grassland of SZN, and with WFPS in cornfield. While in SHD, there was significant positive relationship between N_2O fluxes at non-hot moment with soil temperature and WFPS in each plot of grassland, and with soil temperature in cornfield.

Table 4.6 Linear regressions between N₂O and CO₂ fluxes in non-hot moment and soil temperature, soil moisture content

Site	Gas	Land use	Plot	Linear regression	P value	R ²
SZN	N ₂ O	Grassland	CT	N ₂ O-N = 0.730 × T soil 5cm − 0.478	< 0.001	0.29
			F	N ₂ O-N = 1.238 × T soil 5cm + 0.847	< 0.001	0.24
			MF	N ₂ O-N = 0.985 × T soil 5cm + 2.064	0.001	0.23
		Cornfield	CT	N ₂ O-N = 134.506 − 121.109 × WFPS	< 0.05	0.16
			F	N ₂ O-N = 245.458 − 241.551 × WFPS	< 0.05	0.22
			MF	N ₂ O-N = 334.624 − 370.434 × WFPS	0.001	0.30
	CO ₂	Grassland	CT	CO ₂ -C = 12.401 × T soil 5cm + 16.047	< 0.001	0.81
			F	CO ₂ -C = 11.213 × T soil 5cm + 26.092	< 0.001	0.75
			MF	CO ₂ -C = 16.268 × T soil 5cm + 18.469	< 0.001	0.79
		Cornfield	CT	CO ₂ -C = 7.918 × T soil 5cm − 2.124	< 0.001	0.65
			F	CO ₂ -C = 8.530 × T soil 5cm + 0.523	< 0.001	0.68
			MF	CO ₂ -C = 10.041 × T soil 5cm + 29.253	< 0.001	0.70
SHD	N ₂ O	Grassland	CT	N ₂ O-N=1.873×T soil 5cm+89.758×WFPS−73.545	< 0.001	0.41
			F	N ₂ O-N=2.479×T soil 5cm+116.679×WFPS−87.682	< 0.001	0.38
			MF	N ₂ O-N=2.570×T soil 5cm+120.367×WFPS−92.699	< 0.001	0.33
		Cornfield	CT	N ₂ O-N=4.871×T soil 1cm+15.457	< 0.05	0.13
			F	N ₂ O-N=4.459×T soil 5cm+18.322	< 0.001	0.33
			MF	N ₂ O-N=4.107×T soil 5cm+32.780	0.001	0.20
	CO ₂	Grassland	CT	CO ₂ -C = 10.784 × T soil 5cm + 26.044	< 0.001	0.84
			F	CO ₂ -C = 11.188 × T soil 5cm + 29.055	< 0.001	0.84
			MF	CO ₂ -C = 11.462 × T soil 5cm + 25.876	< 0.001	0.83
		Cornfield	CT	CO ₂ -C = 3.827 × T soil 5cm − 37.496	< 0.001	0.29
			F	CO ₂ -C = 4.546 × T soil 5cm + 39.152	< 0.001	0.42
			MF	CO ₂ -C = 6.427 × T soil 5cm + 47.051	< 0.001	0.61

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; T soil 5 cm, soil temperature at the depth of 5 cm; WFPS, Water filled pore space.

Annual total N₂O emission in SZN ranged from 0.64 to 2.71 kg N ha^{−1} yr^{−1} in grassland and 4.45 to 15.83 kg N ha^{−1} yr^{−1} in cornfield. The range of annual N₂O emission in SHD was from 2.50 to 3.61 kg N ha^{−1} yr^{−1} in grassland and from 4.77 to 5.83 kg N ha^{−1} yr^{−1} in cornfield. Based on the regression equations shown in Table 4.6, cumulative N₂O background emission at hot moment was estimated. Cumulative N₂O production in hot moment was estimated by subtracting the cumulative N₂O background emission at hot moment and cumulative N₂O non-hot moment emission from the annual total N₂O emission. Contribution of N₂O production in hot moment to annual total N₂O emission in SZN ranged from 53.2 % (CT plot of cornfield) to 68.6 %

(MF plot of grassland), except in the CT plot of grassland where it contributed only 8.4 % (Table 4.7). In SHD, 32.8 % (F plot) ~ 42.7 % (MF plot) of cumulative N₂O emission was emitted at hot moment in grassland and 5.9 % (F plot) ~ 19.4 % (MF plot) in cornfield.

Non-hot moment CO₂ fluxes in both sites were always significantly positively related to soil temperature at 5 cm in both grassland and cornfield (Table 4.6). And annual CO₂ production in SZN ranged from 10337 to 13735 kg C ha⁻¹ yr⁻¹ in grassland and 6914 to 10964 kg C ha⁻¹ yr⁻¹ in cornfield (Table 4.7). In SHD, cumulative CO₂ emission was from 12445 to 13073 kg C ha⁻¹ yr⁻¹ in grassland and only from 7182 to 8355 in cornfield, which was much lower than SZN (Table 4.7). Because of the low frequency of CO₂ hot moments in SZN, the contribution of CO₂ fluxes in hot moment to annual emission was below 1.7 % (Table 4.7).

Table 4.7 Cumulative N₂O and CO₂ emissions and the contributions of N₂O and CO₂ hot moments to annual emissions

Site	Land use	Plot	Hot moment emission	Non-hot moment emission + Background emission of hot moment	Total emission
SZN					
			Cumulative N ₂ O (kg N ha ⁻¹ yr ⁻¹)		
	Grassland	CT	0.05 ± 0.08 (8.4 %)	0.59 ± 0.09 (91.6 %)	0.64 ± 0.13
		F	1.36 ± 0.81 (60.9 %)	0.87 ± 0.07 (39.1 %)	2.23 ± 0.76
		MF	1.86 ± 1.73 (68.6 %)	0.85 ± 0.18 (31.4 %)	2.71 ± 1.70
	Cornfield	CT	2.37 ± 0.65 (53.2 %)	2.08 ± 0.36 (46.8 %)	4.45 ± 0.50
		F	8.69 ± 5.88 (66.0 %)	4.47 ± 0.58 (34.0 %)	13.16 ± 5.32
		MF	10.02 ± 8.54 (63.3 %)	5.80 ± 1.55 (36.7 %)	15.83 ± 7.71
			Cumulative CO ₂ (kg C ha ⁻¹ yr ⁻¹)		
	Grassland	CT	--	--	10914 ± 1126
		F	--	--	10337 ± 647
		MF	224 ± 308 (1.6 %)	13511 ± 1732 (98.4 %)	13735 ± 1938
	Cornfield	CT	--	--	6914 ± 2229
		F	--	--	7425 ± 2136
		MF	184 ± 318 (1.7 %)	10780 ± 2443 (98.3 %)	10964 ± 2318
SHD					
			Cumulative N ₂ O (kg N ha ⁻¹ yr ⁻¹)		
	Grassland	CT	0.94 ± 0.89 (37.6 %)	1.56 ± 0.43 (62.4 %)	2.50 ± 1.28
		F	1.08 ± 1.24 (32.8 %)	2.21 ± 0.51 (67.2 %)	3.29 ± 1.33
		MF	1.54 ± 1.19 (42.7 %)	2.06 ± 0.48 (57.1 %)	3.61 ± 1.53
	Cornfield	CT	0.98 ± 1.38 (16.8 %)	4.85 ± 1.06 (83.2 %)	5.83 ± 2.44
		F	0.28 ± 0.40 (5.9 %)	4.49 ± 1.08 (94.1 %)	4.77 ± 1.48
		MF	1.06 ± 0.47 (19.4 %)	4.39 ± 1.34 (80.6 %)	5.45 ± 1.82
			Cumulative CO ₂ (kg C ha ⁻¹ yr ⁻¹)		

Grassland	CT	--	--	12445 ± 3819
	F	--	--	13073 ± 3992
	MF	--	--	12928 ± 3267
Cornfield	CT	--	--	7182 ± 285
	F	--	--	7573 ± 445
	MF	--	--	8355 ± 1885

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; Values within brackets shows the contributions of N₂O and CO₂ hot moments, and the sum of background emission and non-hot moments to annual emissions; Values are mean ± stand deviation for 5 years in grassland and 3 years in cornfield of SZN and for 5 years in grassland and 2 years in cornfield of SHD.

4.4 Discussion

4.4.1 General mechanism of N₂O emission in hot moments

Compared with grassland, relatively larger pool of both NO₃⁻ and NH₄⁺ were always found in the cornfield of both SZN and SHD sites, even in CT plots (Fig. 4.1 e f; Fig. 4.2 e f), which implied that the higher N mineralization in cornfield in addition to the higher rate of fertilizer placement (Table 3.1). The mean WFPS in cornfield was 70 ± 15 % in SZN (Fig. 4.1 d) and 69 ± 12 % in SHD (Fig. 4.2 d), which implied that soils were probably aerobic when WFPS < 70 %. Davidson (1991) also concluded that N₂O was mainly produced by nitrification at WFPS < 70 % typically, whereas denitrification becomes the main process at higher WFPS. The high nitrification rate in cornfield confirmed the effectiveness of this statement. However the contributions of denitrification to N₂O emission both in hot moment and non-hot moment in cornfield were greater than in grassland (Table 4.5), especially in SZN site. The percentage of N₂O hot moments in SZN emitted by denitrification was 75 % on average in cornfield while 26.2 % in grassland. In SHD, the contribution of denitrification to N₂O hot moment in F and MF plots of cornfield was 1.25 and 2 times higher than that in grassland, respectively. At non-hot moment, 17.5 % and 17.2 % of N₂O fluxes in SZN and SHD could be explained by denitrification in cornfield respectively, but less than 8.8 % and 6.9 % in grassland (Table 4.5). The increase volume of anaerobic zones due to rapid reduction of oxygen ability by N mineralization could lead to the high potential of denitrification in cornfield. And much greater NO₃⁻ surplus in cornfield not only provided more sufficient N supply for

denitrification and also led to higher $\text{N}_2\text{O}/\text{N}_2$ ratio in denitrification, resulting in the higher contribution of denitrification to N_2O emission. Inhibition of high NO_3^- on N_2O reductase had been reported by Blackmer and Bremner (1978). And C substrate limitation of denitrification easily occurred in the soil with very high NO_3^- , leading to an increase in N_2O as the main product of incomplete denitrification (Wang et al., 2013; Senbayram et al., 2012). Consequently, anaerobic condition tended to be the key controlling factor of denitrification, the main pathway of N_2O hot moment, rather than N supply in cornfield. Akiyama et al. (2013) stated similar results that the controlling factor of episodic N_2O emissions, mainly produced by denitrification, was WFPS rather than NO_3^- content.

The mean WFPS in grassland ($75 \pm 9\%$ for SZN, $73 \pm 8\%$ for SHD) was higher than cornfield, especially for MF plots (Figs. 4.1 d and 4.2 d), which suggested that grassland tended to have high potential of both nitrification and denitrification due to the moderate moisture condition. The $\text{N}_2\text{O}\text{-N}/\text{NO}\text{-N}$ ratio during hot moments in both SZN and SHD were always higher than 1 in grassland (Table 4.5), implying N_2O hot moments were likely to be primarily produced by denitrification, which corresponded with the fact that there were positive correlations between N_2O fluxes in hot moments with WFPS in these two sites, although the relationships were not significant (Table 4.8). Katayanagi et al. (2008), Toma and Hatano (2007) also concluded similar results that the high N_2O fluxes were caused by high denitrification activity, supported by the increase of N_2O flux with an increase in $\text{N}_2\text{O}\text{-N}/\text{NO}\text{-N}$ ratio. Significant positive relationships between NH_4^+ content with N_2O emission in hot moment were also found in grassland of SZN and SHD (Table 4.8), which suggested that nitrification also contributed parts of episodic N_2O hot moments. Different from cornfield, N was often limited in grassland ecosystems (Flechard et al., 2007; Magdoff, 1982). The substantial grassland root exudates and residues with high C/N ratio was likely to promote more rapid immobilization of mineral N, the accumulation of organic matter and possibly asymbiotic N_2 -fixation (Huntjens and Albers, 1978) than cultivated soil, which resulted in the relatively low level of mineralized N in grassland soils (Robertson et al., 1993; Huntjens and Albers, 1978). Less contribution of

denitrification in grassland than that in cropped fields was also found by Bouwman (1996) and due to quick and high N uptake and a longer growing period in grassland compared to annual crops. Therefore, N limitation might be the controlling factor of N₂O production in hot moment in grassland compared with WFPS.

Table 4.8 Kendall's tau rank correlation coefficient of relationship between N₂O flux and environmental factors

Site	Land use	P-value	T soil (°C)	WFPS (%)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)
SZN	Grassland	All	0.480**	-0.237**	0.241**	0.207**
		Hot moment	0.051	0.155	0.102	0.233*
		Non-hot moment	0.466**	-0.225**	0.031	0.135*
	Cornfield	All	0.168**	-0.196**	0.129*	0.078
		Hot moment	0.265*	0.143	0.211	0.084
		Non-hot moment	0.140**	-0.368**	0.082	0.139*
SHD	Grassland	All	0.451**	0.045	0.225**	0.065
		Hot moment	0.161	0.079	0.083	0.208*
		Non-hot moment	0.432**	0.009	0.148**	0.005
	Cornfield	All	0.316**	-0.142	0.190**	0.068
		Hot moment	0.127	0.357	-0.500	-0.500
		Non-hot moment	0.266**	-0.125	0.185**	0.092

T soil, soil temperature at the depth of 5 cm; WFPS, Water filled pore space; **, p < 0.01; *, p < 0.05; Kendall's tau rank correlation coefficient of relationship between CO₂ flux and environmental factors cannot be analyzed because the number of CO₂ hot moment is limited (data was not shown).

4.4.2 Events inducing episodic N₂O flux peaks

Molodovskaya et al. (2012) firstly used box plot method to identify the N₂O fluxes in hot moment from manure-fertilized corn and alfalfa field in Harford, New York, and the UFs of N₂O-N ranged from 7.1 to 10.7 mg m⁻² d⁻¹ (296 to 446 µg m⁻² h⁻¹). UF values in F and MF plots of cornfield were 311 and 542 µg N m⁻² h⁻¹ in SZN (Table 4.2), which is similar to the UF values of Molodvskaya et al. (2012). The UFs of N₂O hot moment in F and MF plots of SHD were slightly lower. UF values of N₂O in cornfield were around 5 and 2 times larger than those in grassland in SZN and SHD respectively (Table 4.2). This might be as a result of the higher N input (Table 3.1) and higher mineral N concentration in cornfield (Fig. 4.1 e; Fig. 4.2 e). Those were able to increase N₂O production in field (Katayanagi et al., 2008; Koga et al, 2004)

and this finding was similar to the results of Bouwman (1996). Lower UF of N_2O in grassland was also related to an increased demand for N_2O as a terminal electron acceptor due to the low NO_3^- content (Burton et al., 1997).

In both SZN and SHD sites, the effect of fertilizer application event on increasing the frequency of N_2O hot moment was significant in grassland but not significant in cornfield (Table 4.4), which can be mainly attributed to the large gap in NO_3^- and NH_4^+ contents under different land-use types. Ellert and Janzen (2008) demonstrated that N_2O emissions was constrained by inorganic N as N content was low, while controlled by other factors and no longer closely linked to inorganic N as N levels increased. N_2O emission from the grassland was more sensitive to fertilizer application due to the N limit. Magdoff (1982) also reported a similar much stronger response to N fertilizer in hay plot in contrast with corn plot since the lower soil $\text{NO}_3\text{-N}$ levels. Ammonium should be nitrified and hydrolysis to NO_3^- when it was associated with producing N_2O (Xu et al., 2000). Therefore, fertilizer application in grassland significantly triggered N_2O hot moment due to providing plentiful NH_4^+ for nitrification, and subsequent NO_3^- for denitrification as N source and a good promoter for all the denitrification enzymes (Zumft, 1997). The formation of NH_4^+ by mineralization mainly restricted nitrification under normal field conditions (Dalal et al, 2003), which was certified by the significant effect of fertilizer addition event in our grassland. The combination of harvest and fertilization events increased the frequency of N_2O hot moment in grassland of both SZN and SHD significantly. N_2O hot moments in both F and MF plots of grassland occurred more frequently than in cornfield of these two sites, which might be as a result of the higher number of fertilizer application in grassland and the effects of combined events.

Rainfall event increased the frequency of N_2O hot moment significantly both in grassland and cornfield of SZN and SHD (Table 4.4), which agrees with denitrification processes were very important for N_2O hot moments as can be seen from the result of $\text{N}_2\text{O-N/NO-N}$ ratio which was more than 1 in all plots (Table 4.5). Koga et al. (2004) also found high N_2O fluxes were largely affected by the amount and timing of rainfall from arable lands in Tokachi, Hokkaido, Japan. Drying-wetting

event affected N₂O production in the ways of enhancing the activity of microbial biomass and the N availability (Mummey et al., 1994) by the release of easily-decomposable organic matter, and the mineralization (Groffman and Tiedje, 1988), and succeeding denitrification was also initiated by the reduced soil O₂ levels by intensive aerobic respiration and more substrates for the denitrifying bacteria (Groffman and Tiedje, 1988). Priemé and Christensen (2001) also concluded that denitrification was only responsible for 23-53 % of N₂O emission following wetting. However, the stimulation of N₂O hot moment by rainfall event in our two studied sites might be primarily resulted from denitrification due to the rapid decreases in soil oxygen based on relatively high soil moisture condition. Firestone and Davidson (1989) also found that the prompt increase in N₂O emission by several orders of magnitude directly after rewetting was more typical for denitrification than for nitrification and the increased C availability and O₂ consumption induced by rewetting favored N₂O production by denitrification. The episodic high N₂O emissions after rainfall event were not only attributed to the high overall rate of denitrification but also the fractions of nitrogenous products of denitrification. The conversion of aerobic condition to anaerobic condition activated the NO₃⁻ and NO₂⁻ reductases quicker than the N₂O reductase, leading to higher N₂O/N₂ ratio of the denitrifier (Knowles, 1982; Otte et al., 1996). Morley et al. (2008) also demonstrated that the N₂O reductase became inactive after the re-exposure of anaerobic soil to O₂, but other denitrification enzymes still kept active. Dendooven and Anderson (1994) also found that NO₃⁻ reductase was more persistent than N₂O reductase when anaerobic condition was rapidly induced. Therefore, the rapid decrease in oxygen availability of soil due to rainfall was likely to promote the accumulation abundant N₂O emissions as the intermediates of denitrifying.

The frequency of N₂O hot moments induced by fertilizer application event was 60.4 % in SZN and 20.6 % in SHD (Table 4.4), and it increased up to 87.5 % and 36.4 % with the impact of rainfall event respectively, which means it is better to apply fertilizer in grassland avoiding the influence of rainfall event. This resulted from that the fact rainfall event promotes the chemical and physical breakdown of the fertilizer

and manure, vertical transport of NO_3^- to subsurface horizon, and the wetting of the soils (Smith et al., 1998). Ruser et al. (2006) also shown that different soil moisture contents considerably influenced the N_2O emission factor of fertilizer and the emission factor strongly increased at a WFPS > 70 %. This phenomenon was also stressed by Dobbie et al. (1999), who found a strong positive correlation between the amount of rainfall during the first 4 weeks after N application and the cumulative N_2O emission in field measurements. Ball et al. (2004) reported extremely high N_2O flux peaks (up to $490 \text{ mg N m}^{-2} \text{ d}^{-1}$ ($20417 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$)) following heavy rain from an imperfectly drained gleysol grassland over the first 4 days after fertilizer addition. An episode of very high N_2O emission in response to rainfall soon after fertilization as NH_3 was also captured from continuous cornfield in Ohio, USA by Jacinthe and Dick (1997). In grassland, the combined event of fertilizer application with rainfall was more important for N_2O hot moments in SHD might be resulted from the difference in the appearance of rainfall event. The number of rainfall event in SHD was 8.4 per year while only 6 per year in SZN. The influence of soil moisture content on N_2O emission factor of fertilizer may be a key factor for the great inter-annual variability of N_2O emission factors found in field studies even if soil management and fertilization was nearly unchanged (Kaiser et al., 1998; Kaiser and Ruser, 2000).

A study in the lucerne-ryegrass pasture at Wagga Wagga, in southeast Australia showed that N_2O flux peak after rain was as high as 100 to $200 \text{ mg N m}^{-2} \text{ d}^{-1}$ (4167 to $8333 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$) (Denmead et al., 2000) which was much higher than the maximum N_2O flux peak value in our study site ($2461 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$). Annual precipitation in that site was 538 mm which was less than half of our study site (1365 mm). Higher N_2O peak value found by Denmead et al. (2000), compared to this study, was ascribed to the drought effect before precipitation. The magnitude of N_2O peak emission after re-wetting was strongly influenced by the length of drought period and decreased with successive rainfall events (Groffman et al., 2009). Scholes et al. (1997) demonstrated that N_2O peak emissions on savanna soil after the first wetting were significantly higher than those on the previously wetted treatment.

N_2O flux peak due to rainfall in CT plot ($352 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$) was lower than that in F

plot $800 \mu\text{g N m}^{-2} \text{ h}^{-1}$) in cornfield of SZN (Fig. 4.1 g), indicating that soil mineral N, in addition to high WFPS, was required for high episodic N_2O emissions, as shown also by Akiyama et al. (2013). In SZN, N_2O flux peaks with rainfall were $4.88 \sim 6.59$ times higher in cornfield and $2.14 \sim 2.38$ times higher in grassland than the respective mean N_2O fluxes. Higher NO_3^- concentration in cornfield than in grassland (Fig. 4.1 e; Fig. 4.2 e), could explain the difference in N_2O fluxes induced by rainfall in the two land uses. Akiyama et al. (2013) also stated larger N_2O emissions after rainfall were possibly emitted by denitrification of the leached NO_3^- in the subsurface soil. Garcia-Montiel et al. (2003) attributed the small N_2O emission after rainfall in pastures to the low N availability that characterizes pasture soils. Kessavalou et al. (1998) reported up to five-fold increase in N_2O emissions after wetting. However in the cornfield of SHD, the magnitude of N_2O hot moment induced by rainfall event in CT plot was greater than that of F and MF plots on the same day. Peak N_2O flux of $1066 \mu\text{g N m}^{-2} \text{ h}^{-1}$, the maximum N_2O flux in SHD during study period, was captured after rainfall event in CT plot while N_2O flux of $319 \mu\text{g N m}^{-2} \text{ h}^{-1}$ in F plot and $280 \mu\text{g N m}^{-2} \text{ h}^{-1}$ in MF plot was measured at the same time. This might be attributed to the low WFPS in F ($67 \pm 14 \%$) and MF plot ($53 \pm 15 \%$) in contrast with CT plot ($72 \pm 8 \%$).

In the grassland of both SZN and SHD, the combined event of harvest and fertilizer application, and the combined event of fertilizer and manure application caused largest N_2O flux peak in F and MF plots, respectively (Fig. 4.1 g; Fig. 4.2 g). Manure application in cornfield conducted in October or November stimulated N_2O flux in MF plot of SZN (Fig. 4.1 g), which resulted in N_2O hot moments during low temperature occasionally. This could be attributed to the fact that the residues from harvested materials and the addition of manure remarkably enhances the organic C content and promotes soil N mineralization, which increases soil NO_3^- and NH_4^+ concentrations and N_2O production (Xu et al., 2000). The enhancement of harvest event on N_2O production was also related to the low N uptake by plants. Residues resulting from harvest and manure application also could lead to the concomitant increase in denitrification potential, which was related to provide a source of energy

for denitrifiers, and stimulate the O₂ consumption by microbial respiration to establish anaerobic microenvironments in soil (Farquharson and Baldock, 2008). And the latter one was more important since SOC was not easily to limit denitrification as electron donor caused by the low competition for C under anaerobic condition (Groffman et al., 1988). The non-significant influence of manure addition on N₂O hot moment in cornfield of SZN and SHD might be driven by the low temperature. The ways in which SOC affected N₂O flux can be influenced by other factors such as soil moisture, temperature and N dynamics (Farquharson and Baldock, 2008).

Tillage increased the rates of N₂O production from nitrification by promoting soil aeration (Linn and Doran, 1984) and also from denitrification by O₂ consumption. Nitrification was likely the primary process contributing to the larger emission after tillage when WFPS was low. A higher proportion of N₂O production by denitrification stimulated by tillage was observed with the increase in WFPS since high WFPS range was optimum to promote both nitrification and denitrification (Estavillo et al., 2002), which was probably similar to SZN site. Several N₂O hot moments were measured after tillage event although tillage did not significantly increase the frequency of N₂O hot moments in SZN (Table 4.4). The number of N₂O hot moments associated with tillage event was 7 in MF plot and 1 in F plot in cornfield, and the difference might result from organic C levels. The slightly lower content of SOC in SHD compared with SZN also partly resulted in the fact that no N₂O hot moment followed tillage was measured in the cornfield of SHD. When soil structure was disrupted by tillage, a greater potential of mineralization and a sudden increase of mineral N was enhanced by SOC accumulation (Estavillo et al., 2002). Effect of tillage might be masked by the strong effect of rainfall in cornfield. Additionally, tillage was usually conducted during the low temperature season in the early spring and late autumn. Soil temperature influences N₂O emissions through its effect on the activity of microorganisms and enzymes (Farquharson and Baldock, 2008). This was also one of the reasons for the weak influence of tillage on N₂O flux peak.

After the transformation of land-use type from grassland to cornfield in SZN, N₂O hot moment frequency increased and then decreased with time, which was different

from SHD. Annual average N fertilizer application rate in grassland of SZN was higher than that in SHD (Table 3.1) while the NO_3^- concentration in grassland of SZN was lower than that of SHD, even in CT plot (Fig. 4.1 e; Fig. 4.2 e). This implied that more nitrification happened in SHD, which probably resulted from the slightly lower WFPS in grassland of SHD (Fig. 4.1 d; Fig. 4.2 d) and the different N uptake rates by plant associated with the different grass species in these two sites. Therefore the lower initial NO_3^- concentration in SZN might lead to the response of N_2O hot moment to fertilizer application event at the beginning of the establishment of cornfield. On the other hand, the effect of land use change on increasing of N_2O hot moment in SHD might be limited compared with the effects of other events. Both in SZN and SHD, only rainfall event significantly induced N_2O hot moment in cornfield. The number of rainfall event was 11, 7 and 5 in the 1st, 2nd and 3rd year of cornfield in SZN, while only 9 and 3 in the 1st and 2nd year in SHD, respectively. This also partly contributed to the lower N_2O hot moment frequency after land use change in SHD.

4.4.3 The contribution of N_2O hot moment to annual emissions

N_2O hot moments usually contributed to more than half of the total annual emissions in SZN, ranging from 53.2 % (CT plot of cornfield) to 68.6 % (MF plot of grassland), although it was only 8.4 % in CT plot of grassland (Table 4.7). In SHD, the contribution of N_2O hot moment was less than SZN, ranged from 16.8 % (CT plot of cornfield) ~ 42.7 % (MF plot of grassland) except for 5.9 % in F plot of cornfield. This agreed with the lower frequency of N_2O hot moment in SHD compared with SZN (Table 4.3). In cornfield, the frequency of N_2O hot moment in SZN was approximately 2 fold higher than that in SHD, which conformed with the less occurrence of rainfall event in SHD (6 yr^{-1}) compared with SZN (7.7 yr^{-1}). In grassland, the frequency of N_2O hot moments induced by fertilizer application event was 60.4 % in SZN and 20.6 % in SHD, and for manure application event was 55.6 % in SZN and 25 % in SHD (Table 4.4). The frequencies of N_2O hot moment stimulated by other events except fertilizer and manure application events were similar between

these two sites (Table 4.4), which suggested that the different influences of these two events resulted in the fact that the average N₂O hot moment frequency in SZN (16.6 %) was slightly higher than SHD (10.7 %) (Table 4.3). Compared with SHD, the lower NO₃⁻ concentration in SZN (Fig. 4.1 e; Fig. 4.2 e) might lead to more sensitive response of N₂O hot moment to fertilizer and manure application events. The addition of manure remarkably enhances the organic C content and promotes soil N mineralization, which increases soil NO₃⁻ and NH₄⁺ concentrations and N₂O production (Xu et al., 2000).

Large contribution of N₂O hot moment has been reported previously, 40 ~ 51 % in manure-fertilized fields in Harford, New York (Molodovskaya et al., 2012), 51.1 % in intensely-grazed grassland in Ireland (Scanlon and Kiely, 2003), 73 % mainly after thawing at the Höglwald forest in Bavaria (Papen and Butterbach-Bahl, 1999), 55 ~ 80 % mainly after heavy rain in a paddy field in Tsukuba, Japan (Akiyama et al., 2013). These results showed that the bulk of the annual flux was dominated by relatively few flux peaks. However, Garcia-Montiel et al. (2003) found that N₂O hot moment after rainfall accounted for only 1.8 % in forest and pasture systems in Rondonia, Brazil due to the low N availability.

4.4.4 Less episodic nature of CO₂ flux

Number of CO₂ hot moments was only three in total in SZN and even no CO₂ hot moment was found in SHD (Table 4.3). Our result showed that patterns of CO₂ flux followed soil temperature trends (Fig. 4.1 c h; Fig. 4.2 c h) and CO₂ hot moment was only found in MF plot both in grassland and cornfield of SZN, which revealed that the relative lower SOC in F and CT plot and SHD site might restrict the extreme larger emission of CO₂. The dominant sources of CO₂ production are microbial decomposition of SOM and root respiration (Savage et al., 2014), which increased with soil temperature and moisture (Ellert and Janzen, 2008). Kirschbaum (1995) also indicated that soil temperature strongly affected respiratory activity contributing to CO₂ production during decomposition of organic material, which was also reported by

Savage et al. (2014). Compared with soil moisture condition, soil temperature controlling the CO₂ emission was the main reason for the less episodic nature of CO₂ in our study. Temperature more than 20 °C was the only event influencing CO₂ hot moment both in grassland and cornfield of SZN (Table 4.4). The regression of CO₂ in non-hot moment with soil temperature (Table 4.6), which also indicated that temperature was the main controlling factor of CO₂ fluxes. Drury et al. (2008) found that CO₂ emission peaks in corn and soybean fields in Ontario, Canada corresponded to soil temperature peaks. Savage et al. (2009) reported a transient pulse of CO₂ following rainfall event. The reason for the negligible effect of rainfall event on CO₂ hot moment in our sites would be the high WFPS. Microbial activity always kept a high level and the stimulation of mineralization by rainfall event was limited due to the rapid creation of anaerobic zones following rainfall event, which was supported by N₂O hot moments following rainfall event in both grassland and cornfield (Table 4.4). The small episodic response of CO₂ flux to precipitation was too low to be considered as CO₂ hot moment. Ball et al. (1999) also found CO₂ emission decreased sharply under no-tillage after heavy rainfall and the concurrent increase in N₂O emission in these post-rainfall periods. The sharp decrease in CO₂ emission was attributed to the less macro-porosity under no-tillage and the restriction of the soil macro-porosity by the heavy rainfall, reducing soil air-filled pore space and respiration and increasing anaerobism. This phenomenon was also reported by Linn and Doran (1984), who reported that the sustained high production of CO₂ in the tilled treatments in the wet period may have resulted from greater aerobic respiration as a result of the lower WFPS than under no-tillage.

In both SZN and SHD, a slightly higher CV of CO₂ flux in cornfield than grassland was found (Table 4.1). This is due to the change in shadowing effect by plant cover with corn growth. Hu et al. (2001) also showed that cornfield with higher Q_{10} value was more sensitive to the temperature than grassland.

A significant positive correlation between N₂O fluxes with CO₂ fluxes from bare plots with no fertilizer application has been reported in previous studies (Toma et al., 2010; Mu et al., 2009), however CO₂ fluxes was also contributed by root respiration

and N₂O fluxes was strongly influenced by fertilizer application in our studies. Therefore, CO₂ hot moments did not correspond to N₂O hot moments in this study. Peng et al. (2011) also stated that the impacts of management on the magnitudes of soil CO₂ fluxes were rather small compared to environmental drivers, which was similar to our results. In contrast, N₂O fluxes greatly responded to managements.

Our results of the evaluation of N₂O and CO₂ hot moments in managed grassland and cornfield suggested that mitigating episodic N₂O emissions would greatly reduce annual N₂O production, and appropriate time of fertilization, avoiding rainfall and harvest event in grassland, could be one option. However, fertilizer was usually spread soon after harvest to promote grass production, this kind of combined influence of fertilization and harvest on N₂O hot moments was unavoidable in practice. Because of the simultaneous response of N₂O flux peak to events, the time interval of our N₂O flux measurement might not have caught all the peaks. Therefore, the magnitude of N₂O peak events and total N₂O emissions might be underestimated. Intensified manual sampling is advised, not only during the hot moment events but also after the hot moment events, because peak fluxes may be over-represented if measurements tend to be concentrated around periods of hot moments (Flechard et al., 2007). The pulse N₂O emissions during freeze-thaw cycles were not included in this study, which also might underestimate the contribution of N₂O flux in hot moment (Gregorich et al., 2008).

4.5 Conclusion

CO₂ flux, strongly influenced by soil temperatures, was much less episodic than N₂O flux, and fertilizer and manure application increased the percentage accounted by N₂O hot moments to total number of observation. Threshold of N₂O peak event was higher in cornfield than in grassland due to higher fertilization and higher mineralization rate in cornfield. Temperature more than 20 °C, rainfall, harvest, fertilizer and manure applications (only in SZN) were the events significantly promoting N₂O hot moments in grassland, while only rainfall event significantly

promoted N₂O hot moment in cornfield. More sensitive responding of N₂O hot moment to fertilizers addition event in grassland than in cornfield can be attributed to much lower NO₃⁻ contents in grassland where N tended to be limited. Greater magnitude of N₂O hot moment in cornfield after rainfall event was caused by more sufficient N supply for denitrification. Except the cornfield in SHD, N₂O emission in hot moments accounted for more than one-third of the annual total emissions in fertilizer and manure applications. Mitigating episodic N₂O emissions would greatly reduce annual N₂O emissions. Fertilization avoiding rainfall in grassland could be one option. This paper provides a better understanding of N₂O and CO₂ hot moments, and will further improve our ability to forecast, model and mitigate gas emissions.

Chapter 5

Incubation study: The mechanism of key factors inducing episodic CO₂ and N₂O fluxes

5.1 Introduction

Large contribution of episodic gas fluxes to annual gas production has been reported by several studies (Molodovskaya et al., 2012; Scanlon and Kiely, 2003; Papen and Butterbach-Bahl, 1999; Akiyama et al., 2013), which arises from the heterogeneity of the systems from which these gases are emitted, and the complex interactions which occur between the chemical, physical and biological variables which control their generation (Duxbury and McConnaughey, 1986; Clayton et al., 1994). Temporal heterogeneity is accentuated in agricultural relative to non-managed landscapes. Land management causes physical and biogeochemical disturbance of extreme magnitude and in pulsed events (Groffman et al., 2009). Soil properties including organic C and mineral N contents, bulk density, aggregate size and moisture condition could be changed by environmental events and agriculture management, and these soil characteristics dramatically affected the flux of GHGs. The large variability of N₂O was caused by the complex set of environmental factors such as soil temperature and soil moisture, as well as nutrient availability, which control the nitrification and denitrification processes responsible for N₂O emissions (Farquharson and Baldock, 2008). The variability of CO₂ was associated with various factors such as soil moisture, soil temperature (Yan et al., 2014; Epron et al., 2004), SOM content and substrate quality (Bilgili et al., 2013). Episodic N₂O fluxes at hot moment have been reported after events including drying-rewetting (Groffman et al., 2009; Ruser et al., 2006; Dobbie and Smith, 2001), rainfall (Liengaard et al., 2012; Dobbie and Smith, 2003), freezing-thawing (Groffman et al., 2009; Papen and Butterbach-Bahl, 1999), fertilizer and manure additions (Imer et al., 2013; Dobbie and Smith, 2003;

Dobbie et al., 1999), harvest (Imer et al., 2013; Dobbie and Smith, 2003) and tillage (Groffman et al., 2009; Xu et al., 2000). Rapid transient pulses of CO₂ have also been observed after precipitation (Savage et al., 2014) and tillage (Rochette and Angers, 1999). Therefore the effects of bulk density, soil moisture content, aggregate size and nutrient management on CO₂, N₂O and NO emissions from agriculture soil were studied in this chapter and then to clarify the key factors inducing CO₂ and N₂O hot moments from incubation study.

5.2 Materials and methods

Soil samples at 0-20 cm depth of F and MF plots in SHD site were collected. Air-dried soil samples were sieved into 0~2 mm and 2~4.5 mm aggregates. After the adjustment of gravimetric water content (GWC, 0.35 and 0.45 g g⁻¹), soil was manually packed into plastic rings with different bulk densities (0.45 g cm⁻³, 0.66 g cm⁻³) to achieve a range of water filled pore space (WFPS) from 29 % to 83 %. And then put all rings into 1.5 L Mason jars and sealed air tightly. Each treatment has 3 replications. Ambient wet air was passed through a vinyl tube connected to the jar to replace the gas in the jar completely. After each gas sampling, the soil atmosphere inside of the jar was replaced again. Incubation was conducted at 15 °C for 10 days. Gas sampling for N₂O, CO₂ and NO analysis was carried out 7 times during incubation. Additional soil cores were packed for analyzing initial physical properties of each treatment with 3 replications. Just after water adjustment and after incubation, soil chemical properties were measured with 3 replications. Net ammonification and net nitrification (mg N kg⁻¹) are estimated as the difference of NH₄⁺ or NO₃⁻+NO₂⁻ before and after incubation respectively. Net N mineralization is calculated as the sum of net ammonification and net nitrification. Denitrifying enzyme activity (DEA) was determined after incubation by acetylene block technique. Soil microbial biomass C (MBC) and N (MBN) were measured by chloroform fumigation-extraction method before incubation. Detail information about the incubation processe, gas sampling, gas measurement and calculation were described in Chapter 3.

5.3 Results

5.3.1 Emission dynamics of gas fluxes during incubation

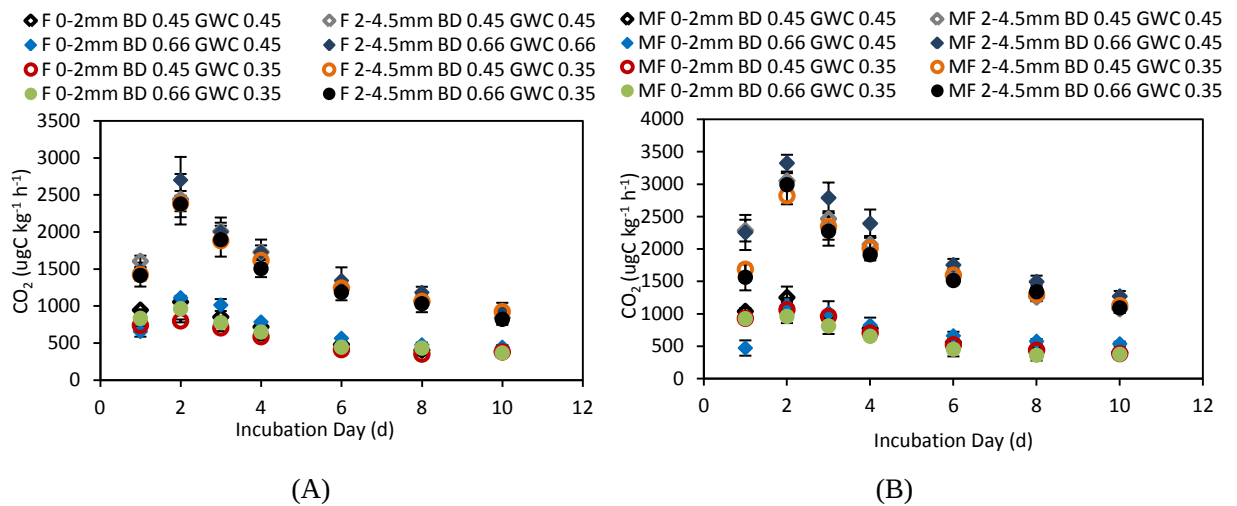


Fig.5.1 Emission dynamic of CO₂ flux during incubation

Fig. 5.1 shows the evolution of CO₂ fluxes over time during incubation. CO₂ fluxes ranged from 822 to 2702 $\mu\text{g C kg}^{-1} \text{h}^{-1}$ in 2-4.5mm of F soil, from 368 to 1113 $\mu\text{g C kg}^{-1} \text{h}^{-1}$ in 0-2 mm of F soil, from 1076 to 3322 $\mu\text{g C kg}^{-1} \text{h}^{-1}$ in 2-4.5 mm of MF soil with and from 362 to 1251 $\mu\text{g C kg}^{-1} \text{h}^{-1}$ in 0-2 mm of MF soil. Regardless of GWC and BD, CO₂ fluxes in 2-4.5 mm treatment were always higher than that in 0-2 mm of both F and MF soils during the whole incubation period. CO₂ fluxes in 0-2 mm were slightly higher in MF soil than F soil, and much higher in 2-4.5 mm. CO₂ fluxes of all treatments peaked at the 2nd day and then decreased until the end of incubation, and the soil respiration after 10 days was still relatively high. CO₂ fluxes were mainly controlled by aggregate size and nutrient management rather than BD and GWC.

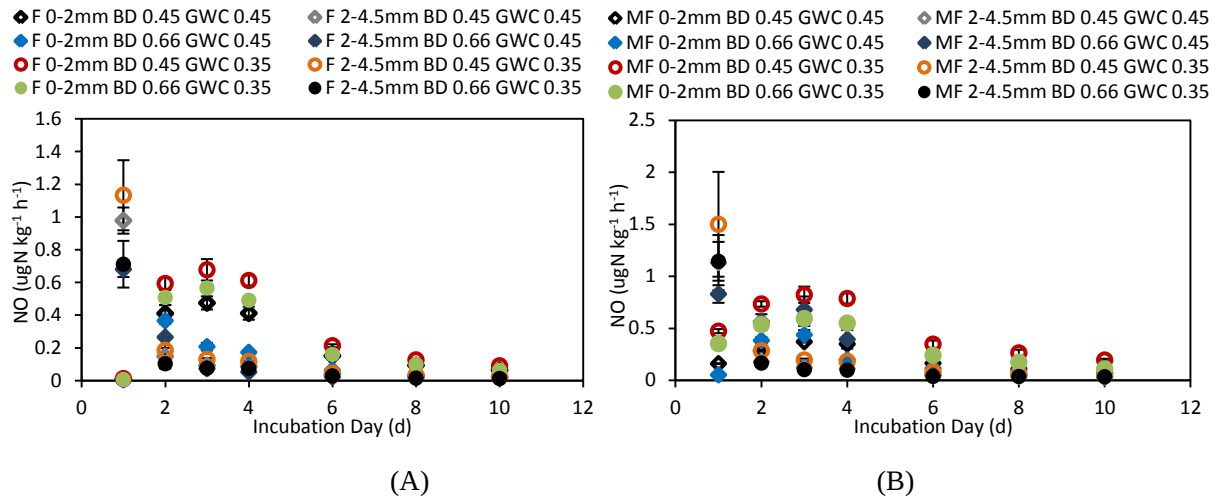


Fig.5.2 Emission dynamic of NO flux during incubation

Fig. 5.2 displays the varied dynamics of NO fluxes during incubation. For 0-2 mm treatment of both F and MF soils, NO fluxes achieved a maximum at the third day with a later decrease, except the F soil with high GWC with high BD treatment peaked at the second day. NO fluxes ranged from 0.005 to $0.678 \mu\text{g N kg}^{-1} \text{h}^{-1}$ in 0-2 mm of F soil and from 0.053 to $0.823 \mu\text{g N kg}^{-1} \text{h}^{-1}$ in 0-2 mm of MF soil. But the peak NO fluxes in 2-4.5 mm treatments were soon recorded at the 1st day and decreased sharply at the second day towards the end. And the NO fluxes at the first day were higher in 2-4.5 mm than in 0-2 mm. NO fluxes in 2-4.5 mm were from 0.013 to $1.133 \mu\text{g N kg}^{-1} \text{h}^{-1}$ for F soil and from 0.029 to $1.500 \mu\text{g N kg}^{-1} \text{h}^{-1}$ for MF soil. NO fluxes generally followed the trend: low GWC treatments > high GWC treatments, MF soil > F soil, 2-4.5mm treatments > 0-2 mm treatments.

For F soil, NO fluxes in low BD treatments were generally higher than those in high BD, which was in contrast with MF soil. The effect of BD on NO flux in MF soil was also influenced by GWC, and there was no obvious difference in the NO fluxes between high BD and low BD treatments when GWC was high, while low BD was slightly lower than high BD when GWC was low.

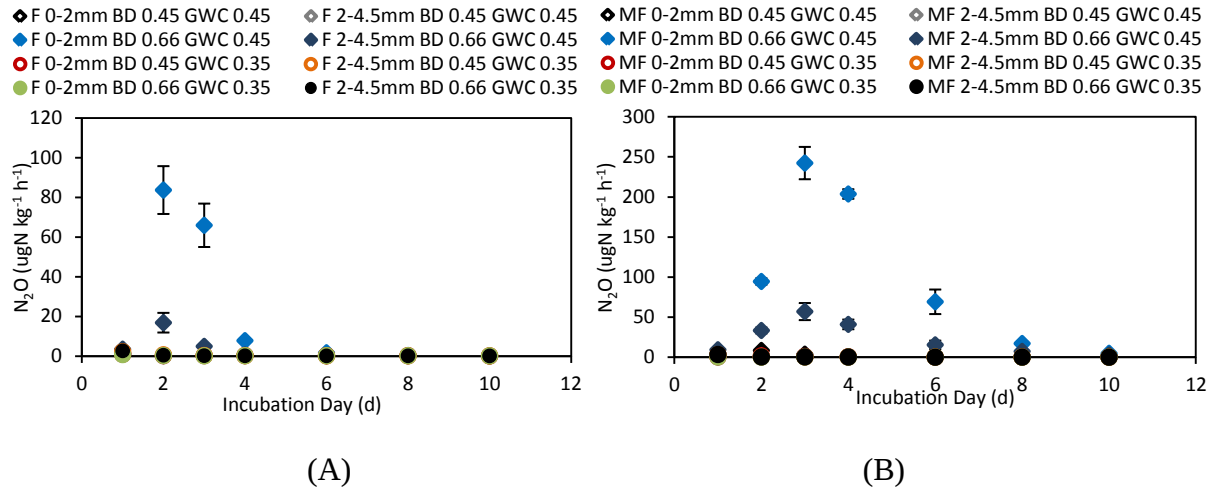


Fig.5.3 Emission dynamic of N_2O flux during incubation (all treatments)

Fig.5.3 shows the change in dynamics of N_2O fluxes with respect to time of incubation. For both F and MF soils, N_2O fluxes in high BD with high GWC were extremely higher than that in other treatments. N_2O flux in high BD with high GWC of F soil was in the range of 0.082 to 83.65 $\mu\text{g N kg}^{-1} \text{h}^{-1}$ in 0-2 mm and 0.089 to 16.775 $\mu\text{g N kg}^{-1} \text{h}^{-1}$ in 2-4.5 mm, while that of MF soil ranged from 0.435 to 242.303 $\mu\text{g N kg}^{-1} \text{h}^{-1}$ in 0-2 mm and 2.678 to 56.979 $\mu\text{g N kg}^{-1} \text{h}^{-1}$ in 2-4.5 mm. The magnitude and the high emission period of the N_2O flux in high BD with high GWC was much greater and longer in 0-2 mm than in 2-4.5 mm. Peaks of the N_2O flux of MF soil were observed after 3 days followed by a decrease to negligible amounts after 8 days of incubation in 0-2 mm and 6 days in 2-4.5mm. And the largest N_2O flux was observed at the 2nd day in F soil which is one day earlier than in MF soil. The treatment effects could not be differentiated clearly from the 4th day in 0-2 mm and from the 3rd day in 2-4.5 mm. At the end of incubation, the flux in high GWC with high BD of MF soil was still relatively higher than in other treatments.

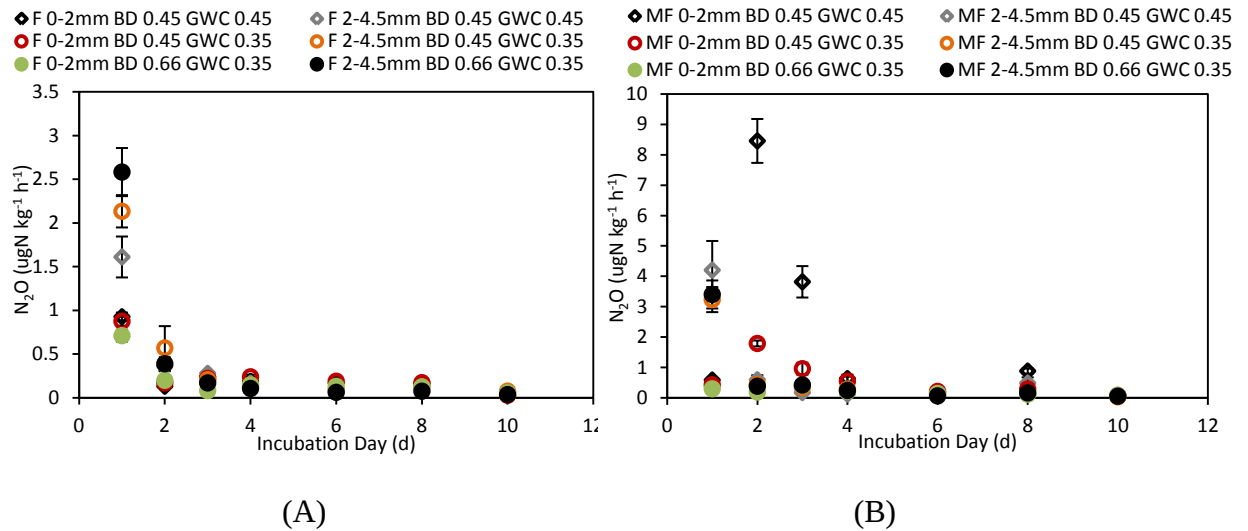


Fig.5.4 Emission dynamic of N₂O flux during incubation of other treatments expect for the high GWC high BD treatments

Fig. 5.4 presents the results of N₂O fluxes in other treatments except for those in the high BD with high GWC treatments. N₂O fluxes of F soil, below 2.582 ug N kg⁻¹ h⁻¹, were highest at 1st day and then decreased, and N₂O emission in 2-4.5 mm treatments was higher than in 0-2 mm. The variation trend of N₂O flux in 2-4.5 mm of MF soil during the incubation was similar to those of F soil, and peaked at the first measurement that was the 1st day and then quickly declined over 1-2 days. And N₂O flux of MF soil varied between 0.044 and 4.208 ug N kg⁻¹ h⁻¹ in 2-4.5 mm treatments and between 0.077 and 8.456 ug N kg⁻¹ h⁻¹ in 0-2 mm treatments. However the highest N₂O fluxes of MF soil were found at the 2nd day in 0-2 mm aggregate and then gradually decreased until the end of incubation. Regardless of GWC, BD, aggregate size, MF soil always tended to emit more N₂O flux than F soil.

5.3.2 Cumulative gases productions

Table 5.1 Cumulative gases emission

Nutrient Management	BD (g cm ⁻³)	Aggregate (mm)	GWC (g g ⁻¹)	CO ₂ (mg C kg ⁻¹ 10d ⁻¹)	NO (ug N kg ⁻¹ 10d ⁻¹)	N ₂ O (ug N kg ⁻¹ 10d ⁻¹)
F	0.45	2-4.5	0.45	332.32 ± 27.01 a	34.75 ± 1.08 eghijk	71.96 ± 4.69 a
F	0.66	2-4.5	0.45	350.30 ± 24.67 ab	27.96 ± 2.78 egijk	613.27 ± 153.56 a
F	0.45	2-4.5	0.35	325.38 ± 3.19 a	42.27 ± 4.70 ck	85.91 ± 4.77 a
F	0.66	2-4.5	0.35	313.76 ± 20.97 a	25.77 ± 5.18 di	85.75 ± 8.94 a

MF	0.45	2-4.5	0.45	418.51 ± 11.27 c	41.98 ± 7.46 cj	158.42 ± 17.10 a
MF	0.66	2-4.5	0.45	468.22 ± 17.80 d	65.03 ± 8.95 af	4518.96 ± 764.78 b
MF	0.45	2-4.5	0.35	400.36 ± 23.04 c	60.84 ± 11.37 af	118.93 ± 14.68 a
MF	0.66	2-4.5	0.35	393.76 ± 13.71 bc	41.54 ± 3.89 ci	121.81 ± 8.30 a
F	0.45	0-2	0.45	142.05 ± 0.43 e	44.45 ± 3.37 ch	50.98 ± 2.07 a
F	0.66	0-2	0.45	152.73 ± 4.95 e	23.79 ± 1.78 dg	3817.28 ± 258.84 c
F	0.45	0-2	0.35	119.88 ± 6.73 e	64.21 ± 2.08 af	53.06 ± 0.97 a
F	0.66	0-2	0.35	134.33 ± 9.34 e	51.82 ± 2.27 cf	41.17 ± 1.74 a
MF	0.45	0-2	0.45	158.40 ± 11.89 e	43.06 ± 1.79 ce	380.54 ± 9.76 a
MF	0.66	0-2	0.45	162.22 ± 14.90 e	38.46 ± 1.86 cde	16727.28 ± 307.53 d
MF	0.45	0-2	0.35	146.94 ± 14.23 e	102.79 ± 6.61 b	116.87 ± 11.18 a
MF	0.66	0-2	0.35	132.85 ± 17.12 e	71.80 ± 6.52 a	43.48 ± 1.77 a
Nutrient Management				P=0.152	P=0.001	P=0.07
Water Content				P=0.452	P < 0.01	P < 0.01
Aggregate Size				P<0.001	P < 0.05	P=0.108
Bulk Density				P=0.824	P=0.06	P < 0.01
Treatment effect				P < 0.001	P < 0.001	P < 0.001

BD, bulk density; GWC, gravimetric water content; F, Fertilizer plot; MF, Manure and Fertilizer plot; Values are mean ± stand deviation.

Table 5.1 suggested the effect of all treatments on cumulative gas emission was significant ($p < 0.001$ for CO_2 , $p < 0.001$ for NO and $p < 0.001$ for N_2O). However, the varied trends of gas production were different among the treatments.

Although there was no significant effect of GWC or nutrient management, the treatments with high GWC tended to emit greater CO_2 than low GWC, and the CO_2 emission of MF soil was higher than F soil. There was no detectable difference in cumulative CO_2 emission between high and low BD treatments. With regard to the aggregate effect, larger aggregate treatments discharged higher cumulative CO_2 emission flux than small aggregate treatments ($p < 0.001$). The cumulative CO_2 flux emission in 2-4.5 mm treatments varied between 313.76 and 468.22 $\text{mg C kg}^{-1} 10\text{d}^{-1}$ while 119.88 and 162.22 $\text{mg C kg}^{-1} 10\text{d}^{-1}$ in 0-2 mm treatment.

Cumulative NO emission was significantly influenced by nutrient management ($p = 0.001$), GCW ($P < 0.01$) and aggregate size ($P < 0.05$). Cumulative NO emission of MF soil, ranging from 38.46 to 102.79 $\text{ug N kg}^{-1} 10\text{d}^{-1}$, was always higher than that of F soil, from 23.79 to 64.21 $\text{ug N kg}^{-1} 10\text{d}^{-1}$. In high BD with high GWC treatment, 2-4.5 mm aggregate produced more NO than 0-2 mm aggregate, especially for MF soil. While other treatments with 2-4.5 mm aggregate emitted less NO than that with

0-2 mm aggregate. In 2-4.5mm aggregate, cumulative NO flux in high BD treatment was slightly higher in high GWC than in low GWC, which was in contrast to other treatments, low GWC was more benefit for NO production. Although the effect of BD on cumulative NO flux was not significant ($p=0.06$), the treatment with low BD tended to emit high cumulative NO flux except for the 2-4.5 mm with high GWC treatment of MF soil.

As for cumulative N₂O flux, the effects of GWC ($P<0.01$) and BD ($P<0.01$) were significant. In low GWC, N₂O production was not influenced by BD in 2-4.5 mm treatments, and low BD emitted slightly higher N₂O flux than high BD in 0-2 mm treatments. In high GWC high BD significantly promoted the N₂O flux. High GWC always stimulated more N₂O production except for low BD of F soil. The N₂O flux of MF soil was greater than that of F soil in all treatments ($P=0.07$). In the high GWC of MF soil and high BD with high GWC of F soil, N₂O flux was higher in 0-2 mm than 2-4.5 mm, but in other treatments was contrary. The N₂O production in high BD with high GWC of both F and MF soils (613.27 to 16727.28 ug N kg⁻¹ 10d⁻¹) was several-fold higher than that in other treatments (41.17 to 380.54 ug N kg⁻¹ 10d⁻¹).

5.3.3 Soil properties

5.3.3.1 Soil physical properties

The soil physical properties of each treatment were shown in Table 5.2. Significant differences between treatments were observed in all physical characteristics ($p<0.001$). Gas diffusion coefficient (D/D_0), air ratio, tortuosity and WFPS were significantly controlled by GWC ($p<0.001$) and BD ($p<0.001$), while the porosity was correlated with aggregate size ($p<0.001$) and BD ($p<0.001$). Although the effect of aggregate size was not significant ($p=0.345$), the WFPS in 0-2 mm was slightly larger than that of 2-4.5 mm at the same level of GWC and BD treatments. The value of WFPS was in the range of 76 ~ 83 % and 29 ~ 33 % in high BD with high GWC and low BD with GWC, respectively and 44 ~ 59 % in other treatments. High BD with high GWC resulted in higher WFPS. The D/D_0 in high BD with high GWC was from 0.002 to

0.006. The D/D_0 in low BD with high GWC was slightly higher ($0.170 \sim 0.223$) than that in high BD with low GWC ($0.097 \sim 0.120$), which implied that the effect of GWC on D/D_0 was more pronounced rather than that of BD. The D/D_0 in low BD with low GWC treatments was largest, fluctuating from 0.286 to 0.308. The varied pattern of air ratio was similar to D/D_0 , low BD and low GWC promoting higher D/D_0 and air ratio, which was opposite to water ratio. Porosity was high in the low BD with low GWC treatment and the low BD with high GWC treatment and low in the other two treatments, which was contrary to the solid ratio. Tortuosity was highest in high BD with high GWC ($9.241 \sim 6.666 \text{ mm}^{-1}$), followed by high BD with low GWC ($2.059 \sim 2.185 \text{ mm}^{-1}$) and other two treatments ($1.468 \sim 1.831 \text{ mm}^{-1}$). High BD with high GWC led to very higher tortuosity value.

Table 5.2 Physical Properties

Aggregate (mm)	GWC (g g ⁻¹)	Nutrient Management	BD (g cm ⁻³)	Solid Ratio (m ³ m ⁻³)	Water Ratio (m ³ m ⁻³)	Air Ratio (m ³ m ⁻³)	Porosity (m ³ m ⁻³)	WFPS (%)	D/D ₀ (----)	T (mm ⁻¹)
0-2	0.45	F	0.45	0.31 ±0.0037 ae	0.37 ±0.0003 a	0.32 ±0.0034 a	0.69 ±0.0037 ae	53 ±0.24 a	0.223 ±0.048 a	1.468 ±0.174 a
0-2	0.45	F	0.66	0.34 ±0.0078 b	0.55 ±0.0017 b	0.11 ±0.0073 b	0.66 ±0.0078 b	83 ±0.92 b	0.003 ±0.002 b	8.961 ±3.907 b
0-2	0.35	F	0.45	0.17 ±0.0049 c	0.24 ±0.0004 c	0.59 ±0.0047 c	0.83 ±0.0049 c	29 ±0.15 c	0.308 ±0.004 c	1.516 ±0.010 a
0-2	0.35	F	0.66	0.27 ±0.0028 df	0.36 ±0.0002 d	0.36 ±0.0029 d	0.73 ±0.0028 df	50 ±0.21 d	0.120 ±0.022 d	2.059 ±0.174 a
0-2	0.45	MF	0.45	0.29 ±0.0077 ad	0.37 ±0.0004 a	0.34 ±0.0078 ad	0.71 ±0.0077 ad	52 ±0.58 a	0.175 ±0.012 eg	1.661 ±0.048 a
0-2	0.45	MF	0.66	0.32 ±0.0063 beh	0.55 ±0.0016 b	0.13 ±0.0079 be	0.68 ±0.0063 gbe	80 ±0.98 e	0.002 ±0.001 b	9.241 ±1.227 b
0-2	0.35	MF	0.45	0.26 ±0.0586 f	0.24 ±0.0012 c	0.50 ±0.0597 f	0.74 ±0.0586 f	33 ±2.64 f	0.263 ±0.015 f	1.599 ±0.024 a
0-2	0.35	MF	0.66	0.38 ±0.0171 g	0.36 ±0.0002 d	0.26 ±0.0173 g	0.62 ±0.0171 h	59 ±1.67 g	0.097 ±0.026 d	2.098 ±0.230 a
2-4.5	0.45	F	0.45	0.17 ±0.0023 c	0.37 ±0.0003 a	0.46 ±0.0026 h	0.83 ±0.0023 c	44 ±0.16 h	0.201 ±0.007 ae	1.665 ±0.030 a
2-4.5	0.45	F	0.66	0.28 ±0.0021 df	0.55 ±0.0011 b	0.17 ±0.0022 e	0.72 ±0.0021 df	76 ±0.24 i	0.006 ±0.003 b	6.666 ±2.112 b
2-4.5	0.35	F	0.45	0.16 ±0.0008 c	0.24 ±0.0007 c	0.60 ±0.0014 c	0.84 ±0.0008 c	29 ±0.11 c	0.295 ±0.011 c	1.552 ±0.031 a
2-4.5	0.35	F	0.66	0.27 ±0.0003 df	0.36 ±0.0019 d	0.36 ±0.0019 d	0.73 ±0.0003 df	50 ±0.26 d	0.113 ±0.012 d	2.116 ±0.112 a
2-4.5	0.45	MF	0.45	0.16 ±0.0026 c	0.37 ±0.0028 a	0.47 ±0.0032 h	0.84 ±0.0026 c	44 ±0.32 h	0.170 ±0.025 g	1.831 ±0.149 a
2-4.5	0.45	MF	0.66	0.30 ±0.0037 adf	0.55 ±0.0006 b	0.16 ±0.0043 e	0.70 ±0.0037 adg	78 ±0.49 j	0.003 ±0.001 b	8.236 ±0.873 b
2-4.5	0.35	MF	0.45	0.16 ±0.0032 c	0.24 ±0.0006 c	0.60 ±0.0038 c	0.84 ±0.0032 c	29 ±0.18 c	0.286 ±0.006 cf	1.576 ±0.019 a
2-4.5	0.35	MF	0.66	0.28 ±0.0044 df	0.36 ±0.0014 e	0.36 ±0.0032 d	0.72 ±0.0044 df	50 ±0.16 d	0.106 ±0.002 d	2.185 ±0.019 a
Nutrient Management				P = 0.322	P = 0.978	P = 0.677	P = 0.322	P = 0.839	P = 0.509	P = 0.735
Water Content				P = 0.216	P < 0.001	P < 0.001	P = 0.216	P < 0.001	P = 0.001	P < 0.001
Aggregate Size				P < 0.001	P = 0.988	P = 0.125	P < 0.001	P = 0.345	P = 0.964	P = 0.699
Bulk Density				P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Treatment effect				P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

BD, bulk density; GWC, gravimetric water content; F, Fertilizer plot; MF, Manure and Fertilizer plot; WFPS, water filled pore space; D/D₀, relatively gas diffusion coefficient; T, tortuosity of pores; Values are mean ± stand deviation.

5.3.3.2 Soil chemical properties

Table 5.3 and table 5.4 presents the soil chemical properties of each treatment before (just after the water adjustment) and after incubation.

Before incubation, NH_4^+ concentration was $43.39 \sim 44.33 \text{ mg N kg}^{-1}$ in 2-4.5 mm and $16.01 \sim 16.77 \text{ mg N kg}^{-1}$ in 0-2 mm of F soil, and $60.31 \sim 60.67 \text{ mg N kg}^{-1}$ in 2-4.5 mm and $18.23 \sim 18.82 \text{ mg N kg}^{-1}$ in 0-2 mm of MF soil. Much more NH_4^+ content in larger aggregate was measured than smaller aggregate ($p < 0.001$). NH_4^+ concentration was higher in MF soil than in F soil, although the difference was not significant ($p = 0.086$). Although NO_2^- concentration in all treatments was low, it was significantly higher in 2-4.5 mm than in 0-2 mm ($p < 0.001$). NO_3^- concentration was significantly higher in MF soil ($31.63 \sim 37.71 \text{ mg N kg}^{-1}$) than in F soil ($10.10 \sim 16.83 \text{ mg N kg}^{-1}$) ($p < 0.001$), but there was no clear effect of aggregate sizes ($p = 0.118$), slightly higher NO_3^- surplus was found in 2-4.5 mm of F soil while 0-2 mm of MF soil. The MBN was mainly influenced by aggregate size ($p < 0.001$) and nutrient management ($p < 0.001$). The MBN was higher in 2-4.5 mm of MF soil than in 0-2 mm of F soil, it is similar to the effect of other chemical properties except for NO_3^- . The MBC and WEOC were also primarily influenced by aggregate size ($p < 0.001$) rather than nutrient management ($p = 0.061$ for MBC, $p = 0.164$ for WEOC). The MBC and WEOC in 2-4.5 mm aggregate were 1.8 $\sim$ 2.4 times and 2.2 $\sim$ 3.4 times higher than those in 0-2 mm, respectively. MF soil tended to have more MBC than F soil. The mean value of MBC: MBN ratio of 2-4.5 mm in F soil, 2-4.5 mm in MF soil, 0-2 mm in F soil and 0-2 mm in MF soil was 8.48, 6.58, 5.17 and 4.53, respectively. The larger aggregates tended to have a higher ratio of MBC: MBN. There was a significant difference of soil pH between in F soil ($5.51 \sim 5.65$) and in MF soil ($5.64 \sim 5.77$) ($p < 0.001$). Soil pH in 0-2 mm tended to slightly higher than that in 2-4.5 mm ($p < 0.001$). Cl^- content of F and MF soil was $15.01 \sim 17.18 \text{ mg kg}^{-1}$ and $38.05 \sim 41.53 \text{ mg kg}^{-1}$, respectively and significantly different ($p < 0.001$). Nutrient management ($p < 0.001$) and aggregate size ($p < 0.001$) were the main controlling factors of EC and SO_4^{2-} . SO_4^{2-} content in 2-4.5 mm of MF soil was higher than those in 0-2 mm of F

soil.

Except for SO_4^{2-} and Cl^- , soil chemical properties showed significant change between the beginning and the end of incubation. NH_4^+ concentration of all treatments significantly increased by 1.1 ~ 2.6 folds ($p < 0.001$) after incubation, especially for the larger aggregate size. NO_2^- pool was depleted at the end of incubation. NO_3^- content increased by 1.78 ~ 4.68 mg N kg^{-1} in 0-2 mm and 20.52 ~ 47.61 mg N kg^{-1} in 2-4.5 mm after incubation, except for 2-4.5 mm with high BD and high GWC of MF soil, NO_3^- concentration decreased after incubation. WEOC concentration was significantly decreased in all treatments at the end of incubation ($p < 0.05$) and the WEOC consumption was higher in 2-4.5 mm (46.93 ~ 98.60 mg C kg^{-1}) than in 0-2 mm (19.50 ~ 44.22 mg C kg^{-1}). The value of pH was slightly decreased in 0-2 mm, while increased in 2-4.5 mm. EC obviously rose in 0-2 mm treatments, while it slightly changed in 2-4.5 mm.

All the chemical properties of soil samples at the beginning and the end of incubation showed significant differences between the treatments ($p < 0.001$) which indicates the remarkable combined effect of these four factors.

Table 5.3 Soil chemical properties (1)

Management	BD (g cm ⁻³)	Aggregate (mm)	GWC (g g ⁻¹)	MBN (mg N kg ⁻¹)		NH ₄ ⁺ (mg N kg ⁻¹)		NO ₂ ⁻ (mg N kg ⁻¹)		NO ₃ ⁻ (mg N kg ⁻¹)		MBC (mg C kg ⁻¹)		WEOC (mg C kg ⁻¹)	
				before	after	before	after	before	after	before	after	before	after	before	after
F	0.45	2-4.5	0.45	65.67	43.39	106.97 ±3.35	1.14	0.0 ±0.0	16.44	19.40 ±1.05	527.01	212.86	156.77 ±5.51		
F	0.66	2-4.5	0.45	±1.70	±0.92	111.89 ±12.58	±0.26	0.0 ±0.0	±1.59	18.91 ±0.67	±271.27	±14.55	152.30 ±19.25		
F	0.45	2-4.5	0.35	60.56	44.33	95.80 ±3.19	1.55	0.0 ±0.0	16.83	20.42 ±0.62	539.95	223.69	156.98 ±3.84		
F	0.66	2-4.5	0.35	±5.07	±5.05	96.52 ±0.78	±0.16	0.0 ±0.0	±3.09	19.75 ±2.42	±13.37	±25.80	132.54 ±4.28		
MF	0.45	2-4.5	0.45	111.99	60.31	132.62 ±4.14	2.62	0.0 ±0.0	31.64	36.32 ±3.47	723.05	275.18	199.93 ±15.33		
MF	0.66	2-4.5	0.45	±7.27	±1.11	143.56 ±2.25	±0.19	0.0 ±0.0	±3.29	28.01 ±2.12	±2.89	±5.27	228.25 ±16.65		
MF	0.45	2-4.5	0.35	111.47	60.67	130.82 ±3.37	2.29	0.0 ±0.0	31.63	34.77 ±1.62	747.33	289.32	206.89 ±13.87		
MF	0.66	2-4.5	0.35	±15.01	±1.77	136.05 ±4.80	±0.38	0.0 ±0.0	±2.61	33.41 ±1.59	±52.35	±3.16	190.73 ±6.91		
F	0.45	0-2	0.45	64.69	16.77	18.05 ±1.12	0.48	0.0 ±0.0	10.59	39.20 ±0.47	292.62	94.99	62.50 ±4.80		
F	0.66	0-2	0.45	±5.11	±0.50	22.04 ±1.28	±0.09	0.0 ±0.0	±0.45	31.11 ±1.30	±53.04	±3.53	75.50 ±9.18		
F	0.45	0-2	0.35	50.57	16.01	21.01 ±1.57	0.59	0.0 ±0.0	10.10	33.65 ±1.96	293.76	99.16	62.80 ±7.12		
F	0.66	0-2	0.35	±0.38	±2.73	19.24 ±1.71	±0.08	0.0 ±0.0	±0.24	38.30 ±0.65	±70.06	±0.72	54.94 ±5.28		
MF	0.45	0-2	0.45	73.88	18.23	19.78 ±1.79	0.63	0.0 ±0.0	37.46	77.23 ±2.80	341.84	94.22	61.54 ±3.23		
MF	0.66	0-2	0.45	±7.64	±1.59	29.16 ±2.86	±0.04	0.0 ±0.0	±2.78	44.36 ±9.99	±131.54	±1.86	74.06 ±8.25		
MF	0.45	0-2	0.35	70.37	18.82	22.35 ±0.70	0.59	0.0 ±0.0	37.71	82.77 ±2.34	311.35	84.09	48.13 ±0.50		
MF	0.66	0-2	0.35	±11.58	±1.47	22.66 ±1.24	±0.04	0.0 ±0.0	±1.30	85.32 ±2.85	±25.96	±3.51	49.95 ±2.32		
Nutrient Management				P<0.001	0.086	0.22	P<0.01	--	P<0.001	0.769	0.061	0.164	0.167		
Water Content				0.380	0.986	0.74	0.870	--	0.23	0.645	0.969	0.661	0.471		
Aggregate Size				P<0.001	P<0.001	P<0.001	P<0.001	--	0.118	P<0.001	P<0.001	P<0.001	P<0.001		
Bulk Density				1	1	0.778	1	--	1	0.227	1	1	0.986		
Treatment effect				P<0.001	P<0.001	P<0.001	P<0.001	--	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		

BD, bulk density; GWC, gravimetric water content; F, Fertilizer plot; MF, Manure and Fertilizer plot; MBN, microbial biomass N; MBC, microbial biomass C; WEOC, water extractable organic C; Values are mean ± stand deviation.

Table 5.4 Soil chemical properties (2)

Management	BD (g cm ⁻³)	Aggregate (mm)	GWC (g g ⁻¹)	pH		EC (mS m ⁻¹)		Cl ⁻ (mg kg ⁻¹)		SO ₄ ²⁻ (mg S kg ⁻¹)		MBC: MBN
				before	after	before	after	before	after	before	after	before
F	0.45	2-4.5	0.45	5.51 ±0.06	5.79 ±0.20	4.88 ±0.25	4.67 ±0.34	15.01 ±0.68	20.63 ±4.41	9.61 ±0.94	10.90 ±1.40	8.03
F	0.66	2-4.5	0.45		5.80 ±0.14		4.78 ±0.32		17.08 ±1.95		11.00 ±1.42	
F	0.45	2-4.5	0.35	5.51 ±0.05	5.73 ±0.13	5.55 ±0.15	5.62 ±0.35	15.97 ±1.42	16.37 ±2.80	9.20 ±0.85	10.76 ±0.80	8.92
F	0.66	2-4.5	0.35		5.87 ±0.15		5.32 ±0.35		15.81 ±0.94		10.12 ±0.66	
MF	0.45	2-4.5	0.45	5.71 ±0.08	6.01 ±0.13	7.88 ±0.12	7.94 ±0.36	38.27 ±0.74	44.30 ±0.95	10.64 ±1.25	13.33 ±0.45	6.46
MF	0.66	2-4.5	0.45		6.05 ±0.15		7.91 ±0.32		41.69 ±4.79		14.14 ±0.41	
MF	0.45	2-4.5	0.35	5.64 ±0.07	6.01 ±0.15	9.68 ±0.48	9.29 ±0.72	38.05 ±3.05	44.75 ±7.13	10.70 ±0.72	13.12 ±1.53	6.70
MF	0.66	2-4.5	0.35		5.94 ±0.14		9.06 ±0.61		42.60 ±3.95		12.47 ±0.88	
F	0.45	0-2	0.45	5.65 ±0.03	5.45 ±0.01	2.46 ±0.11	4.78 ±0.07	17.18 ±0.87	18.68 ±0.95	6.67 ±0.37	6.45 ±0.70	4.52
F	0.66	0-2	0.45		5.50 ±0.02		4.30 ±0.27		16.31 ±0.86		7.48 ±0.70	
F	0.45	0-2	0.35	5.62 ±0.01	5.47 ±0.02	2.77 ±0.06	5.00 ±0.13	16.20 ±1.45	17.49 ±1.60	6.28 ±0.35	5.94 ±0.45	5.81
F	0.66	0-2	0.35		5.45 ±0.01		5.43 ±0.05		12.21 ±1.26		5.89 ±0.21	
MF	0.45	0-2	0.45	5.77 ±0.01	5.59 ±0.02	6.33 ±0.21	9.14 ±0.22	41.53 ±6.74	28.26 ±1.39	7.31 ±0.44	7.59 ±0.85	4.63
MF	0.66	0-2	0.45		5.81 ±0.12		6.71 ±1.04		31.60 ±1.50		8.34 ±0.43	
MF	0.45	0-2	0.35	5.77 ±0.02	5.58 ±0.01	7.00 ±0.18	11.00 ±0.05	40.63 ±2.20	35.25 ±1.64	6.97 ±0.74	7.20 ±0.15	4.42
MF	0.66	0-2	0.35		5.57 ±0.02		11.21 ±0.15		36.29 ±1.05		7.12 ±0.07	
Nutrient Management				P<0.001	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.05	--
Water Content				0.188	0.457	P<0.01	P<0.05	0.719	0.936	0.979	0.318	--
Aggregate Size				P<0.001	P<0.001	P<0.001	0.585	0.405	0.085	P<0.001	P<0.001	--
Bulk Density				1	0.538	1	0.617	1	0.662	1	0.848	--
Treatment effect				P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	--

BD, bulk density; GWC, gravimetric water content; F, Fertilizer plot; MF, Manure and Fertilizer plot; MBN, microbial biomass N; MBC, microbial biomass C; Values are mean ± stand deviation.

5.3.3.3 The N₂O-N/NO-N ratio and DEA

Table 5.5 provides an overview of the N₂O-N/NO-N ratio of cumulative gas production during incubation and the DEA after incubation for the various treatments. GWC ($p < 0.01$) and BD ($p < 0.01$) dominated the N₂O-N/NO-N ratio significantly. The treatments with high GWC high BD showed the extremely higher value of N₂O-N/NO-N ratio ($p < 0.001$), 21.77 in 2-4.5 mm of F soil, 69.59 in 2-4.5 mm of MF soil and > 100 in 0-2 mm of F and MF soils. The N₂O-N/NO-N ratio in other treatments ranged from 1.97 to 3.80 in 2-4.5 mm and 0.60 to 7.63 in 0-2 mm.

Denitrifying enzyme activity (DEA) was used as an indirect measure of enzyme concentration. The varied trend of DEA did not correspond to N₂O-N/NO-N ratio, and all the factors affected DEA value significantly. The DEA in 2-4.5 mm was significantly lower than that in 0-2 mm ($p < 0.001$). High BD treatments showed greater DEA than low BD treatments ($p < 0.01$). The differences between all treatments were also notable ($p < 0.001$). Extremely high DEA was measured in 0-2 mm with high BD and high GWC of MF and F soils. In contrast, 2-4.5 mm with high GWC of F soil showed lowest value of DEA.

Table 5.5 The N₂O-N/NO-N ratio and DEA

Management	Aggregate (mm)	BD (g cm ⁻³)	GWC (g g ⁻¹)	N ₂ O-N/NO-N	DEA (ug N h ⁻¹)
F	2-4.5	0.45	0.45	2.07 ±0.20	74.45 ±3.40 a
F	2-4.5	0.66	0.45	21.77 ±3.54	66.95 ±4.12 a
F	2-4.5	0.45	0.35	2.05 ±0.25	106.55 ±32.79 ab
F	2-4.5	0.66	0.35	3.44 ±0.94	147.94 ±9.96 ac
MF	2-4.5	0.45	0.45	3.80 ±0.25	112.26 ±22.88 ad
MF	2-4.5	0.66	0.45	69.59 ±7.30	187.49 ±57.76 bcdef
MF	2-4.5	0.45	0.35	1.97 ±0.14	137.72 ±9.07 aeg
MF	2-4.5	0.66	0.35	2.94 ±0.17	222.58 ±88.55 cgh
F	0-2	0.45	0.45	1.15 ±0.09	243.79 ±2.20 ch
F	0-2	0.66	0.45	124.04 ±53.88	343.92 ±17.20 i
F	0-2	0.45	0.35	0.74 ±0.08	238.23 ±31.80 cj
F	0-2	0.66	0.35	0.86 ±0.13	246.28 ±33.23 fhjk
MF	0-2	0.45	0.45	7.63 ±2.51	228.99 ±5.72 cgj
MF	0-2	0.66	0.45	444.20 ±31.71	385.34 ±26.30 i
MF	0-2	0.45	0.35	1.14 ±0.53	172.09 ±12.81 bcdgk
MF	0-2	0.66	0.35	0.60 ±0.06	314.31 ±1.83 hij
Nutrient Management				0.143	0.188
Water Content				P<0.01	0.798
Aggregate Size				0.063	P<0.001

Bulk Density	P<0.01	P<0.01
Treatment effect	P<0.001	P<0.001

BD, bulk density; GWC, gravimetric water content; F, Fertilizer plot; MF, Manure and Fertilizer plot; DEA, denitrifying enzyme activity; Values are mean $\pm$ stand deviation.

5.4 Discussion

5.4.1 CO₂ emission and its controlling factors

The peak time of CO₂ flux was not influenced by any factors studied here, and the maximum CO₂ evolution of all treatments was recorded at 2nd day which suggested the activity of aerobic microorganism peaked one day after the water adjustment. The added water could activate the microbial processes inside the soil.

The dominant source of CO₂ production from bare soil is microbial decomposition of SOM (Savage et al., 2014). The significantly higher cumulative CO₂ production in 2-4.5 mm than in 0-2 mm, indicating the more intensive of microorganism activity in larger aggregate, would be attributed to the high concentration of MBC and WEOC of soil sample (Table 5.3, p<0.001). In the studies of Elliott (1986), more labile and readily mineralized SOM was associated with macro-aggregates than micro-aggregates and was the primary source of nutrients lost during cultivation. Several reports showed organic C and potentially mineralized organic matter increased with the increase in aggregate sizes (Puget et al., 1995; Jastrow et al., 1996; Semenov et al., 2010; Wright and Hons, 2004; Diba et al., 2011). This is because macro-aggregates not only contain micro-aggregates, which themselves contain organic matter, but also contain inter-microaggregate particulate organic matter (POM) as well (Denef et al., 2004). Hence, macro-aggregates are generally associated with larger concentrations of SOC, mineralizable nutrients and microbial biomass compared with micro-aggregates (Gupta and Germida, 1988). This was in contrast to Seech and Beauchamp (1988), they declared that CO₂ emission during aerobic incubation was greatest in micro-aggregates (<0.25 mm) and decreased with increasing aggregate size due to the deficiency in C substrate in large macro-aggregates. As can be deduced from Fig.5.5, CO₂ production generally increased with the initial (WEOC+MBC) content in soil, but this trend was not

obvious within 0-2 mm treatments, which suggested that the stimulated effect of (WEOC+MBC) content on CO₂ emission was more apparent at the higher level of organic C. The rates of microbial CO₂ production are strongly related to the size of SOC and microbial biomass pools (Lundquist et al., 1999). Seto and Yanagiya (1983) also declared that WEOC pool was usually associated with CO₂ production. Much higher WEOC consumption was also observed in 2-4.5 mm treatment (Table 5.6, $p < 0.001$). However, at the same level of aggregate size, CO₂ emission did not respond to the increase in WEOC consumption positively (Fig.5.5), which implied the contribution of other kinds of organic C to CO₂ loss from soil might increase with time. Comparing WEOC consumption and CO₂ production, all samples produced higher CO₂ than WEOC consumption (Fig.5.5). This result suggests that WEOC was consumed only by decomposition and assimilation did not happen. Tillage event could increase C availability to the microbial biomass by disrupting soil structure and exposing protected organic C (Rasmussen and Collins, 1991) and by breaking apart large pieces of plant residue (Blevins et al., 1984). Compared with the larger aggregate soil, the much lower contents of WEOC and MBC in smaller aggregate size suggested the great potential of organic C decomposition during the degradation of soil aggregate promoted by tillage. However, there was no CO₂ hot moment was measured after tillage event in the cornfield of our study sites, which implied that other factors limited the effect of tillage on CO₂ flux, such as the low soil temperature during tillage event. Rochette and Angers (1999) also reported the effects of tillage on CO₂ flux was varied with date of plowing due to the differences in soil microclimatic conditions following plowing events. They found that cumulative CO₂ fluxes following spring plowing were lower than on a non-tilled control, during which soil temperatures in the plow layer were $< 10\text{ }^{\circ}\text{C}$, while summer plowing increased CO₂ emissions by as much as $2\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ for a period of 65 d.

MF soil emitted higher CO₂ compared with F soil due to the slightly higher level of MBC and WEOC in MF soil (Table 5.3). However the effect of manure application on promoting CO₂ production during incubation was not significant ($p=0.152$), which corresponded to the fact that manure application did not stimulate episodic high CO₂

fluxes at hot moment both in grassland and cornfield of our study sites. Manure was spread in May in grassland and October or November in cornfield. Low temperature during manure application in cornfield might restrain the effect of manure application event. Additionally, the released WEOC and MBC from manure might be time-consuming and limited, which also restrained the effect of manure application on initiating CO₂ hot moment in grassland.

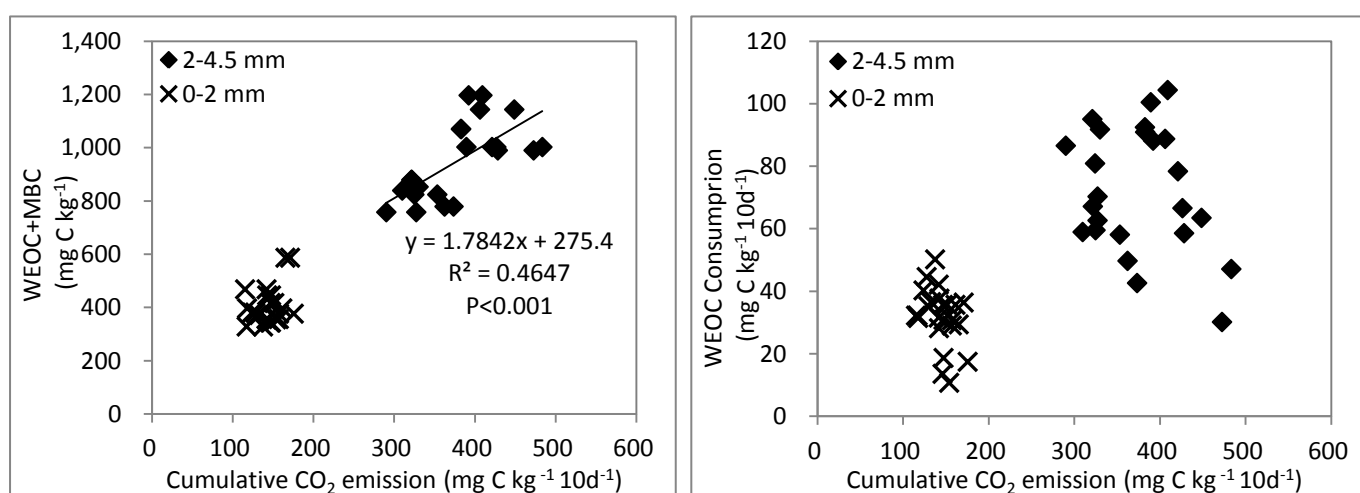


Fig.5.5 The relationship between cumulative CO₂ production with initial (WEOC+MBC) level and WEOC consumption

Emission of CO₂ in high GWC was slightly greater than that in low GWC, which was resulted from the higher activity of microorganism in high GWC treatments. The insignificant effect of GWC, from 0.35 ~ 0.45 g g⁻¹, on CO₂ emission indicated that optimal moisture conditions for heterotrophic activity in this soil could be provided within a wide range (Ruser et al., 2006). This result was also published in the studies of CO₂ emissions from grassland (Frank et al., 2002) and forest soils (Drewitt et al., 2002). This observation also agreed with the fact that rainfall event did not significantly induce CO₂ hot moment. Drying-wetting event could enhance the activity of microbial biomass and the N availability (Mummey et al., 1994) by the release of easily-decomposable organic matter, and the mineralization (Groffman and Tiedje, 1988). While in our study, microbial activity was not restrained by low soil water content (GWC, 0.35 g g⁻¹; WFPS, 29 %) and always kept a high level. On the other hand, anaerobic zones could be rapidly created by rainfall due to the high WFPS

value in field, which was also not benefit for promoting CO₂ emission.

The relationship between WFPS and soil respiration in varied textured soils was proposed to be described by quadratic models and the optimal WFPS value for soil respiration rates was concluded to be from 40 to 70 % by Doran et al. (1990). Linn and Doran (1984) reported that O₂ consumption, CO₂ production and nitrification by aerobic microorganisms reached a plateau between 40 % ~ 60 % WFPS and declined as soils became progressively more anaerobic. Water availability limited substrate transport and soil respiration when WFPS was lower, while CO₂ production was reduced by restricted O₂ availability at higher WFPS (Ruser et al., 2006; Sey et al., 2008). However, such kind of relation between soil respiration and WFPS was not found in our results and no significant effects of BD, air ratio or D/D₀ on CO₂ production were measured (Table 5.6). This suggested that the organic C concentration was the key factor controlling CO₂ producing process rather than the O₂ available to microbes to act on organic matter and crop residues or the gas diffusion condition of soil. This partly explained the negligible effect of tillage event on episodic CO₂ fluxes at hot moment in our field study. Many literatures have reported that CO₂ emissions with respiration were often stimulated by tillage (Roberts and Chan, 1990). Episodic CO₂ flux followed tillage was not only attributed to the enhanced aerobic area within soil but also the promoted water evaporation by tillage (Yamulki and Jarvis, 2002). Linn and Doran (1984) reported that the sustained high production of CO₂ in the tilled treatments in the wet period may have resulted from greater aerobic respiration as a result of the lower WFPS than under no-tillage. While CO₂ production of our incubation was WFPS-independent, which also implied that the stimulation of tillage on consumption of organic matter by aerobic microorganisms (Doran and Smith, 1987) through introducing large amounts of O₂ into soil and enhancing soil water evaporation was not obvious. Even in the high BD with high GWC treatment with approximately 80 % WFPS, the restriction of O₂ availability imposed on aerobic microbial respiration by high WFPS might be compensated by the available C (Weier et al., 1993). Corresponded to the exceeding high cumulative N₂O emission at WFPS of around 80 %, CO₂ might be partly from

the N₂O-producing bacteria (Sey et al., 2008).

CO₂ emission in 2-4.5 mm treatments tended to increase with the net N mineralization ($p < 0.001$) (Fig. 5.6), which suggested the contribution of organic C decomposition to the significant larger increment of NH₄⁺ in 2-4.5 mm treatments could not be ignored. Soil organic matter was a major source of inorganic nutrient for plant growth, yielding N, P and S, and microbial energy upon decomposition (Yang et al., 2007; Lal, 2004). There was no other clear relationship between mineral N content, MBN or net nitrification with CO₂ production was observed during incubation (Fig5.6; Fig.5.7), which suggested that the dominating mechanism controlling C and N dynamics in the soil was probably different.

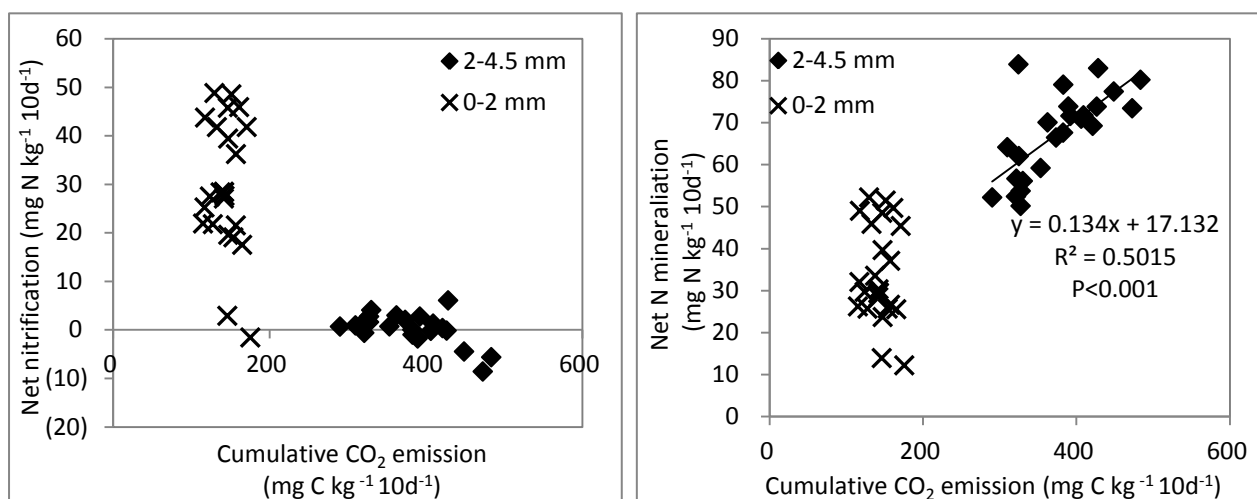


Fig.5.6 The relationship between cumulative CO₂ with net nitrification and mineralization

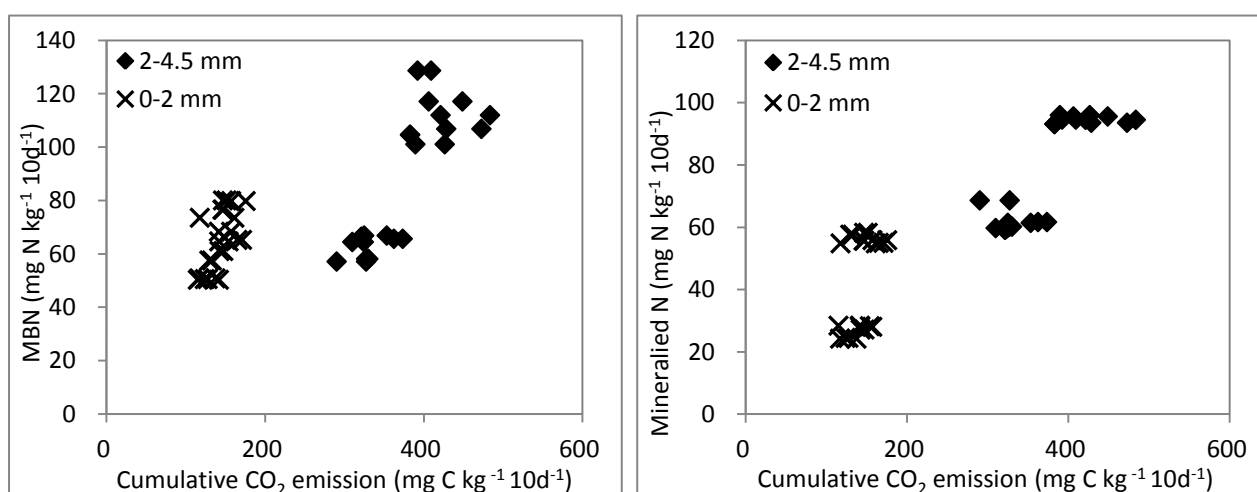


Fig.5.7 The relationship between cumulative CO₂ with MBN and mineralized N

Table 5.6 Pearson Correlation between cumulative CO₂ production and soil properties

Chemical properties	Cumulative CO ₂	Chemical properties	Cumulative CO ₂	Physical properties	Cumulative CO ₂
Before-WEOC	.972**	Before-(NO ₃ ⁻ +NO ₂ ⁻)	.229	Air Ratio	.116
△WEOC	-.705**	Net nitrification	-.822**	Water Ratio	.110
Before-MBC	.116	Net mineralization	.873**	Solid Ratio	-.432**
Before-NH ₄ ⁺	.976**	Net ammonification	.977**	WFPS	-.028
Before-MBN	-.875**	DEA	-.632**	Porosity	.432**
Before-mineral N	.860**			D/D ₀	-.102
				T	.060

**, Correlation is significant at the 0.01 level (2-tailed); mineral N = NH₄⁺+NO₃⁻+NO₂⁻; Net ammonification, the difference of NH₄⁺ before and after incubation; Net nitrification, the difference of NO₃⁻+NO₂⁻ before and after incubation; Net mineralization, the sum of net ammonification and net nitrification; WEOC, water extractable organic C; MBN, microbial biomass N; MBC, microbial biomass C; DEA, denitrifying enzyme activity; WFPS, water filled pore space; D/D₀, relatively gas diffusion coefficient; T, tortuosity of pores.

There was a positive relationship between cumulative CO₂ emission with total porosity (Table 5.6), indicating the soil pore network had a profound influence on driving CO₂ produced by microbial respiration to the soil surface (Mangalassery et al., 2013).

The insignificant effects of BD, GWC or nutrient management on CO₂ flux during incubation also conformed to the less episodic nature of CO₂ fluxes in the field study, which was primarily controlled by temperature.

5.4.2 NO emission and its controlling factors

NO flux rate of 2-4.5 mm treatments got peak very soon, at the 1st day, and the peak time of NO flux of 0-2 mm soil was two days later than 2-4.5 mm soil which indicated the establishment of the optimal environment for NO producing was quicker in big aggregate size. Both microbial processes of nitrification and denitrification could produce NO as by-products (Yamulki and Jarvis, 2002; Remde and Conrad, 1991b). However, the turnover of NO in soil is very dynamic due to the consumption of NO through both the oxidation of NO to NO₃⁻ by aerobic heterotrophic bacteria (Baumgärtner et al., 1996; Koschorreck et al., 1996; Rudolph et al., 1996; Dunfield

and Knowles, 1999; G  dde and Conrad, 2000) and the reduction of NO to N₂O by denitrifiers in anaerobic microniches (Remde and Conrad, 1991a; Sch  fer and Conrad, 1993; McKenney et al., 1982). And the relative consumption by denitrification tended to be higher (Skiba et al., 1993; Rudolph et al., 1996). Therefore, nitrification is considered as the main source of NO flux (Anderson and Levine, 1986). The effect of aggregate size on cumulative NO production was significant ($p<0.05$). NO production of high GWC high BD treatments was promoted by larger aggregate size, which was in contrast to other treatments. At the same soil moisture condition, much higher net nitrification rate in 0-2 mm aggregate size ($p<0.001$) resulted in the higher NO emission. But for the high GWC high BD treatment with extremely low D/D_0 , higher NO production was found in larger aggregate size treatments with relatively higher value of D/D_0 , which implied that the effect of D/D_0 on NO emission might be more important than the difference of the net nitrification rate between the varied aggregate sizes. Therefore, when gas diffusion condition of soil was not limited, NO emission was mainly controlled by the production process whereas NO emission primarily depended on the transport of gas. The significant positively relationship between cumulative NO emission with D/D_0 (Table 5.7, $p<0.01$) agreed with the results of Yamulki and Jarvis (2002) that NO was characterized by “short-lived” and the transport could have a major impact on its emission. At high D/D_0 , both the exchange of NO between soil and atmosphere, and the diffusion of O₂ to microsites might be inadequate, resulting in increase of denitrification (Alvarez et al., 1998).

Matson et al. (1990) and Le Roux et al. (1995) pointed out that the rates of N transformations for given environmental conditions were positively correlated with SOC contents. Li et al. (2005) also concluded that higher N trace gas emissions were promoted by higher SOC contents due to higher soil microbial activity based on a literature review. However such kind of relationship between NO production with WEOC or MBC was not found in our incubation. Therefore the significant higher NO emission from MF soil than F soil ($p<0.001$) should be attributed to the higher initial NO₃⁻ level in MF soil ($p<0.001$) rather than the higher organic C content. And cumulative NO emission was significantly positively related to NO₃⁻ ($p<0.01$) in our

study. The relationship between soil extractable N values with NO flux had been described by several models (Williams et al., 1988; Bawkin et al., 1990; Davidson, 1991; Shepherd et al., 1991; Slemr and Seiler, 1991; Skiba et al., 1992; Serca et al., 1994). There was a general increase in NO flux with extractable soil N, as shown in the study of Slemr and Seiler (1991).

Lower GWC treatments ($p < 0.01$) and lower BD treatments ($p = 0.06$) resulted in higher NO evolution which was primarily attributed to greater O₂ availability. This corresponded with the significantly positively correlation between cumulative NO with air ratio (Table 5.7, $p < 0.05$) and negatively with water ratio (Table 5.7, $p < 0.01$). The increase in soil moisture content not only led to the decreased NO production by nitrification but also the increased N₂O production and NO consumption by denitrification due to the establishment of anaerobiosis in soils (Linn and Doran, 1984; Davidson, 1991; Sanhueza et al., 1994; Dobbie et al., 1999; Skiba et al., 2002; Russow et al., 2009). It is also well known that NO diffusion rates are typically more than 105 times slower through water than through the equivalent thickness of air (Galbally and Johansson, 1989; Yamulki et al., 1995). Thereof, low water content also decreased the residence time of NO in the soil matrix and the likeliness that NO is further consumed by denitrification (Conrad and Bollmann, 1998; Russow et al., 2009), which was also partly responsible for the higher NO production promoted by lower GWC ($p < 0.01$).

Sullivan et al. (1996) stated that increasing soil moisture content had no effect on NO emissions above a value of 30 % WFPS and increased NO production below 30 % WFPS, and lower soil moistures led to decreased NO flux. The WFPS in our study ranged from 29 % ~ 83 % and NO production was negatively related to WFPS (Table 5.7, $p < 0.01$) which implied that the increase in WFPS above 30 % tended to restrict NO emission from our soil. This was confirmed by the result that cumulative NO emission of treatments with low GWC low BD, around 30 % WFPS, was relatively higher than the same soil with the same aggregate size. Cardenas et al. (1993) put the range of optimum soil moisture content for NO flux at 9 ~ 18 % for sandy loam soils (BD 1.59 g cm⁻³). 50 % WFPS was proposed to be the optimum value for NO flux by

a model of NO production from soils (Davidson, 1991) and no significant change of NO flux is to be expected between 30 and 70 % WFPS.

Table 5.7 Pearson Correlation between cumulative NO production and soil properties

Chemical properties	Cumulative NO	Chemical properties	Cumulative NO	Physical properties	Cumulative NO
Before-WEOC	-.229	Before-(NO ₃ ⁻ +NO ₂ ⁻)	.422**	Air Ratio	.327*
△WEOC	.208	Net nitrification	.506**	Water Ratio	-.473**
Before-MBC	-.179	Before-MBN	.414**	Solid Ratio	-.014
Before-NH ₄ ⁺	-.166	Before-PH	.495**	WFPS	-.398**
Net ammonification	-.251	DEA	.064	Porosity	.014
Before-mineral N	.068	N ₂ O/NO Ratio	-.203	D/D ₀	.398**
Net mineralization	.033			T	-.276

**, Correlation is significant at the 0.01 level (2-tailed); mineral N = NH₄⁺+NO₃⁻+NO₂⁻; Net ammonification, the difference of NH₄⁺ before and after incubation; Net nitrification, the difference of NO₃⁻+NO₂⁻ before and after incubation; Net mineralization, the sum of net ammonification and net nitrification; WEOC, water extractable organic C; MBN, microbial biomass N; MBC, microbial biomass C; DEA, denitrifying enzyme activity; WFPS, water filled pore space; D/D₀, relatively gas diffusion coefficient; T, tortuosity of pores.

5.4.3 N₂O emission and its controlling factors

Cumulative N₂O production of MF soil was always higher than F soil (p=0.07) due to the higher initial NO₃⁻ concentration, significantly positively correlating to N₂O emission (p<0.05, Table 5.8). High NO₃⁻ content not only led to higher overall denitrification rate but also the higher N₂O/(N₂O+N₂) ratio of denitrification, inducing higher N₂O emission from MF soil. The inhibition of higher NO₃⁻ content on the reduction of N₂O to N₂ (Simarmata et al., 1993) was caused by the preferential acceptance of electrons from NO₃⁻ compared with N₂O (Firestone and Tiedje, 1979). This revealed that the stimulation of manure application event on N₂O hot moment in grassland of SZN (p<0.001) and SHD (p=0.08) was likely to be attributed to the increased NO₃⁻ by manure application. The poor influence of manure application on provoking CO₂ hot moment in field and on promoting CO₂ production during incubation also implied that the enhancement of microbial activity and oxygen consumption by this event was limited. Hence the higher NO₃⁻ content in the grassland of SHD compared with SZN (Table 5.9) might be the reason for the

relatively weaker effect of manure application event on increasing the frequency of N₂O hot moment in the grassland of SHD (Table 4.4).

Table 5.8 Pearson Correlation between cumulative N₂O production and soil properties

Chemical properties	Cumulative N ₂ O	Chemical properties	Cumulative N ₂ O	Physical properties	Cumulative N ₂ O
Before-WEOC	-.201	Net mineralization	-.397**	Air Ratio	-.545**
△WEOC	.407**	Before-(NO ₃ ⁻ +NO ₂ ⁻)	.275	Water Ratio	.590**
Before-MBC	.221	Net nitrification	-.193	Solid Ratio	.327*
Before-NH ₄ ⁺	-.190	Before-MBN	.247	WFPS	.594**
Net ammonification	-.140	Before-PH	.383**	Porosity	-.327*
Before- NO ₃ ⁻	.301*	DEA	.556**	D/D ₀	-.523**
△NO ₃ ⁻	.044	N ₂ O/NO Ratio	.986**	T	.703**
Before-mineral N	-.019				

**, Correlation is significant at the 0.01 level (2-tailed); mineral N = NH₄⁺+NO₃⁻+NO₂⁻; Net ammonification, the difference of NH₄⁺ before and after incubation; Net nitrification, the difference of NO₃⁻+NO₂⁻ before and after incubation; Net mineralization, the sum of net ammonification and net nitrification; WEOC, water extractable organic C; MBN, microbial biomass N; MBC, microbial biomass C; DEA, denitrifying enzyme activity; WFPS, water filled pore space; D/D₀, relatively gas diffusion coefficient; T, tortuosity of pores.

Table 5.9 Soil properties in SZN and SHD

Site	Land use	Plot	NO ₃ ⁻ -N (mg N kg ⁻¹)	NH ₄ ⁺ -N (mg N kg ⁻¹)	WEOC (mg C kg ⁻¹)	WFPS (%)
SZN	Grassland	CT	1.24 ± 1.68	7.21 ± 7.40	70.05 ± 25.61	77 ± 9
		F	2.85 ± 6.44	21.60 ± 37.09	52.73 ± 25.29	75 ± 9
		MF	2.93 ± 5.49	15.89 ± 29.99	99.80 ± 71.45	73 ± 10
		All	2.61 ± 5.49	16.66 ± 30.95	75.31 ± 54.16	75 ± 9
	Cornfield	CT	24.99 ± 30.54	21.25 ± 59.58	50.56 ± 37.10	73 ± 15
		F	27.55 ± 31.91	17.53 ± 21.65	54.62 ± 44.32	70 ± 15
		MF	31.84 ± 32.81	45.71 ± 119.76	69.97 ± 57.94	67 ± 13
		All	28.43 ± 31.80	29.65 ± 83.21	59.33 ± 48.72	70 ± 15
SHD	Grassland	CT	6.43 ± 8.74	8.46 ± 8.69	47.84 ± 22.90	75 ± 8
		F	11.19 ± 14.17	16.64 ± 26.83	48.75 ± 29.57	71 ± 8
		MF	9.78 ± 9.96	12.97 ± 24.53	70.37 ± 43.08	71 ± 8
		All	9.24 ± 11.42	12.89 ± 22.19	55.96 ± 34.80	73 ± 8
	Cornfield	CT	10.26 ± 13.69	8.26 ± 4.80	51.83 ± 58.33	72 ± 8
		F	28.77 ± 47.12	34.31 ± 63.76	61.50 ± 74.38	67 ± 14
		MF	15.29 ± 13.55	11.10 ± 9.53	69.54 ± 97.33	53 ± 15
		All	17.64 ± 29.54	17.39 ± 37.90	60.36 ± 76.95	69 ± 12

CT, Control plot; F, Fertilizer plot; MF, Manure and Fertilizer plot; WEOC, water extractable organic C; WFPS, water filled pore space; Values are mean (during whole study period)± stand deviation.

In low GWC of 0.35 g g^{-1} , effect of BD on N_2O production was negligible while high BD promoted exceedingly higher N_2O emission in high GWC of 0.45 g g^{-1} . Similarly, the acceleration of high GWC on N_2O was more obvious when BD level was high. This fact illustrated the combined effect of GWC and BD on the enhancement of N_2O production was more noteworthy. The treatment with high GWC high BD produced greatly higher cumulative N_2O emission than the other treatments. WFPS value of this treatment was approximately 80 % which provide more comfortable environment for denitrification. This was consistent with the several studies, which also reported that the greatest N_2O production was measured at 80 % WFPS (Linn and Doran, 1984; Myrold and Tiedje, 1985a). Ruser et al. (2006) announced that N_2O emission rates were generally small at $\text{WFPS} < 60 \%$ and increased significantly at $\text{WFPS} > 70 \%$ with the highest N_2O fluxes occurring at the highest soil moisture level. Denitrification was considered as the main pathway of N_2O emission in this treatment due to the higher $\text{N}_2\text{O-N/NO-N}$ ratio, especially for the 0-2 mm group. Davidson (1991) concluded that N_2O emission was mainly produced by nitrification at $\text{WFPS} < 70 \%$ typically, whereas denitrification becomes the primary process at higher WFPS. The optimum WFPS for N_2O emissions via denitrification was also assumed to be $70 \sim 90 \%$ (Bateman and Baggs, 2005; Dobbie et al., 1999), whereas N_2O emission was attributed to nitrification at lower WFPS (Dobbie et al., 1999; Davidson, 1991; Venterea et al., 2010). Gas diffusion condition of this treatment was very limited with D/D_0 of $0.002 \sim 0.006$, while N_2O production in this treatment was extremely high, which demonstrated that the transport of N_2O was not the key factor controlling N_2O emission in contrast with the produce process.

Ruser et al. (2006) reported that that N_2O emission rates were generally small at $\text{WFPS} < 60 \%$ and increased significantly at $\text{WFPS} > 70 \%$ with the highest N_2O fluxes occurring at the highest soil moisture level. This was similar to the result of our incubation that N_2O production was generally small at WFPS ranged from 29 % to 59 % and extremely high at WFPS of about 80 %. The mean value of WFPS in field was higher than 53 %, which means that there was a high potential of N_2O hot

moment induced by rainfall event due to the rapid establishment of anaerobic area after rainfall event in field. This was in accordance with the fact that rainfall event significantly increased the frequency of N₂O hot moment in both SZN and SHD.

The big gap of N₂O production between the treatments with the same GWC but different WFPS proposed that predicting N₂O flux from soil using only GWC was not valid. This observation agrees with the results of Davidson (1991), WFPS was a more suitable expression of soil moisture content due to reducing the confounding factors of soils (particle density, pore space, and bulk density) (Sullivan et al., 1996). The calculation of WFPS standardizes the amount of space water occupies in soil by accounting for each soil's differing bulk density (Sullivan et al., 1996).

The theoretical aerobic volume in 2-4.5 mm treatments was greater than in 0-2 mm based on the higher porosity ($p < 0.001$), higher air ratio ($p = 0.125$) and lower WFPS ($p = 0.345$) in larger aggregate at the same condition. It was predicted by Greenwood (1975) that anaerobic conditions would form in the centre of soil aggregates with the radius exceeding 9 mm. Højberg et al. (1994) found that aggregates with diameters of 20 to 25 mm often contained anaerobic centers but exhibited only low rates of denitrification due to a limiting availability of electron donors. The smallest aggregate exhibiting an anaerobic center had a radius of 4 mm was stated by Sexstone et al. (1985). Robinson et al. (2014) reported less gas diffusion and therefore lower O₂ in the small aggregates of 1-2 mm due to the lower permeability when all soil aggregates were packed to the same bulk density (1.0 g cm⁻³). However, significant higher growth of NO₃⁻ during incubation was found in all 0-2 mm groups, which implied the quicker net nitrification rate in 0-2 mm aggregate treatments. This result might be attributed to the much higher activity of SOC decomposition in larger aggregate sizes resulting in insufficient O₂ availability for nitrification, and the following limited NO₃⁻ supply suppressed the denitrification. Nitrification is limited when the NH₄-N concentration falls below 0.5 mg N kg⁻¹ (Garrido et al., 2002), while NO₃-N concentrations less than 20 mg N kg⁻¹ may limit denitrification (Myrold and Tiedje, 1985a,b). This kind of NO₃⁻ restriction on denitrification might occur in 2-4.5 mm, especially for F soil with 18.91 ~ 20.42 mg N kg⁻¹ NO₃⁻ after incubation. Seech and

Beauchamp (1988) reported similar results that the considerable differences in the available substrate C related to different aggregates size could attributed partly to the large spatial variability of denitrification occurring over relatively short distances in field soil. Sexstone et al. (1985) also stated that steep O_2 gradients was usually found over very small distances from the aggregate surface when aggregates were incubated under air. Macro-aggregates may simultaneously provide a microhabitat for heterotrophic microorganisms and facultative or obligate anaerobes like denitrifiers, depending on substrate availability and O_2 diffusion to microbial cells (Sey et al., 2008). Therefore, both the microbial activities of nitrification and denitrification were relatively lower in 2-4.5 mm than 0-2 mm, which was consistent with the lower DEA value (Table 5.5, $p < 0.001$) and net nitrification rate ($p < 0.001$) in 2-4.5 mm. The DEA provides an assessment of the concentration of functional denitrifying enzymes and reflects the environmental history of the soil (Tiedje, 1982), the lower DEA value in 2-4.5 mm could be explained by the less microbial biomass and lower availability of these enzymes.

While this kind of differences in the overall rates of nitrification and denitrification did not result in the significant varied cumulative N_2O production between 0-2 mm and 2-4.5 mm with low GWC of 0.35 g g^{-1} . The reason might be the different mechanisms controlling N_2O production from different aggregate sizes soil with low GWC. According to the $N_2O\text{-N}/NO\text{-N}$ ratio (Table 5.5), N_2O emission in 0-2 mm at GWC of 0.35 g g^{-1} was mainly produced by nitrification ($N_2O\text{-N}/NO\text{-N} < 1$), whereas the contribution of denitrification in 2-4.5 mm was greater due to the $N_2O\text{-N}/NO\text{-N}$ ratio > 1 . This observation implied that the N_2O/NO_3^- ratio of nitrification tended to be smaller than that of denitrification. In contrast, in high BD with high GWC treatment, N_2O emission in 0-2 mm was 6.2-fold for F soil and 3.7-fold higher for MF soil than that in 2-4.5 mm. This was attributed to the higher denitrification rate caused by greater NO_3^- supply by net nitrification and higher contribution of denitrification to N_2O emission in smaller aggregate, which was supported by the higher DEA and higher $N_2O\text{-N}/NO\text{-N}$ ratio of 0-2 mm (> 100) than that of 2-4.5 mm. This concurs with the findings of Uchida et al. (2008), who documented that the higher N_2O emissions

occurred from the smaller aggregates due to varying gas diffusivities and higher rates of denitrification when treated with bovine urine, and larger aggregates only producing N_2O after significant compaction. Beside the more N surplus by intensive nitrification, the increased surface area of 0-2 mm aggregate to microbial attack might also partly caused greater denitrification (Drury et al., 2004). The original NO_3^- level in soil was regulated by the nutrient management ($p < 0.001$) while by aggregate size ($p < 0.001$) after incubation which also pointed to the prominent effect of aggregate size on the transformation of NO_3^- surplus relating positively to the N_2O production ($p < 0.01$). The diffusion of NO_3^- was limited when soil aggregate radius was larger than 2 mm under anaerobic condition by a model for NO_3^- reduction and diffusion in aggregated soils (Myrold and Tiedje, 1985b), which also might restrain denitrification in the larger aggregate treatments.

The high WEOC content of 2-4.5 mm treatments tended to create more anaerobic ozone during incubation, and denitrification rate and N_2O production were limited by insufficient NO_3^- supply at WFPS of about 80 %, which suggested high potential of the stimulation of N_2O hot moment by fertilizer application in grassland, where had lower NO_3^- concentration and higher WFPS value (mean WFPS was 75 % in SZN and 73 % in SHD). And the frequency of N_2O hot moment related to fertilizer addition event in grassland of SZN (up to 60.4 %) was around three times higher than that in SHD (20.6 %) (Table 4.4). This was partly explained by the higher WEOC content and more anaerobic microsites for denitrification in the grassland of SZN (Table 5.9). While at WFPS of 29 % ~ 59 %, N_2O production during incubation was mainly promoted by high WEOC content in 2-4.5 mm treatments due to more contribution of denitrification, which implied that anaerobic environment was the key controlling factor of high N_2O production.

There was a significant positive relationship between N_2O production and $\text{N}_2\text{O-N/NO-N}$ ratio of 1 to 100 (Fig.5.8), which implied that denitrification was responsible for N_2O production of these treatments. Katayanagi et al. (2008), Toma and Hatano (2007) and Toma et al. (2010) also reported similar results that the high N_2O fluxes could resulted from high denitrification activity as N_2O flux increased

with an increase in $\text{N}_2\text{O}/\text{NO}$ ratio. Consequently, denitrification was generally the main pathway of N_2O production in our incubation, even sometimes the soil condition was conducive to nitrification, which was supported by the significantly positive relationship between N_2O emission and WFPS ($p < 0.01$), water ratio ($p < 0.01$) and DEA ($p < 0.01$) (Table 5.8). The fact that high N_2O production during incubation was mainly produced by denitrification was also supported the result that N_2O hot moment was dominantly attributed to denitrification in field study. Wolf and Russow (2000) concluded that even under conditions promoting rapid nitrification rates, the NO_3^- produced may be lost via denitrification. Rapid nitrification may promote the creation of anaerobic microsites stimulating denitrifying activity (Azam et al., 2002) and the NO_3^- produced could reach anaerobic zones by diffusion, thereby denitrification was provoked if easily oxidizable C not limited (Azam et al., 2002). High DEA value was even found in the treatments with low WFPS, which also means that the presence of large quantities of denitrifying enzymes in aerobic soils and microsite anaerobiosis often occurred during the build-up of these enzymes (Azam et al., 2002).

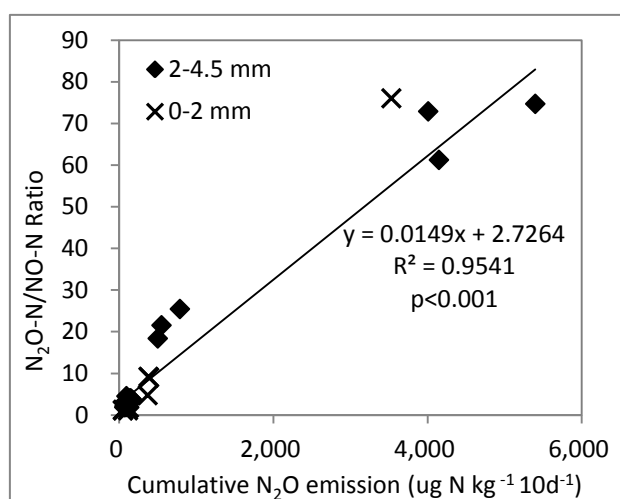


Fig.5.8 The relationship between cumulative N_2O emission with $\text{N}_2\text{O-N}/\text{NO-N}$ ratio (1~ 100)

Tillage increased the rates of N_2O production from nitrification by promoting soil aeration (Linn and Doran, 1984) and also from the denitrification resulted from quick O_2 consumption by the enhanced decomposition of soil organic C and plant residue (Estavillo et al., 2002; Necplovet al., 2013; Yamulki and Jarvis, 2002; Doran et al.,

1997). Denitrification started as soon as the trapped O_2 was utilized (Ottow and Fabig, 1985). And a higher proportion of N_2O production by denitrification stimulated by tillage was observed with the increase in WFPS since high WFPS range was optimum to promote both nitrification and denitrification (Estavillo et al., 2002). In our study, denitrification induced by the rapid O_2 consumption was probably more important for N_2O hot moment after tillage event. Jackson et al. (2003) also found the anaerobic micro-sites created by tillage in soil increased denitrification rate, which was accompanied by an increase in anaerobic eubacteria. N_2O production was mainly promoted by high WEOC content due to greater contribution of denitrification in 2-4.5 mm treatments at low WFPS (29 % ~ 59 %), which suggested that SOM content tended to be the first key factor of N_2O flux after tillage due to the promoted anaerobic volume by quicker SOM decomposition. Estavillo et al. (2002) also reported that when soil structure was disrupted by tillage, a greater potential of mineralization and a sudden increase of mineral N was enhanced by soil organic C accumulation. After the establishment of sufficient anaerobic ozone within soil after tillage, NO_3^- concentration would be the controlling factor of N_2O emission from denitrification, which was revealed by the great difference in N_2O production between 0-2 mm and 2-4.5 mm treatments at WFPS of about 80 % during incubation.

Therefore, more N_2O hot moments associated with tillage event in MF plot (7) compared with F plot (only 1) in the cornfield of SZN was mainly attributed to the higher levels of WEOC and NH_4^+ , resulting in more anaerobic microsites for denitrification in MF plot (Table 5.9). And higher NO_3^- concentration was also measured in MF plot, which further promoted the N_2O production by denitrification after tillage event. It is also worth noting that although not significantly, the frequency of N_2O hot moments in the cornfield of SZN was increased by tillage event from 11.7 % to 14.0 %, whereas tillage event tended to decrease the frequency of N_2O hot moment in SHD ($p=0.07$; Table 4.4). Similarly, this was attributed to the higher NH_4^+ and NO_3^- concentrations and WFPS value in the cornfield of SZN, providing more appropriate environment for N_2O production.

The magnitude of N_2O production at low WFPS (29 % ~ 59 %) was generally small

during incubation, which also implied that episodic N_2O flux after tillage event might not be so large due to the enhanced aeration in soil. This agreed with the fact that the effect of tillage event on inducing N_2O hot moment in cornfield was not significant.

In SZN, the N_2O fluxes at hot moment and non-hot moment in cornfield were always higher than that in grassland. More than 10 times lower NO_3^- concentration was measured in grassland compared with cornfield (Table 5.9), which implied that the inadequate mineral N was the limited factor for N_2O emission from grassland. Even in CT plot, much higher NO_3^- concentration was also found in cornfield, which suggested that besides the higher N input (Table 3.1), higher nitrification rate in cornfield also resulted in the higher level of NO_3^- . The lower WEOC and WFPS in cornfield led to higher nitrification rate (Table 5.9), which corresponded with the incubation study that higher WEOC in 2-4.5 mm led to more anaerobic condition, and then limited NO_3^- produced by nitrification and N_2O production at high WFPS (> 76 %). Similarly, in SHD, lower WFPS, higher NO_3^- content and higher mean N_2O flux were also found in all plots of cornfield compared with grassland. While the magnitude of the largest episodic N_2O fluxes in F and MF plots in cornfield (induced by rainfall event) was lower than that of grassland (induced by fertilizer application event). The difference in NO_3^- between grassland and cornfield in SHD was not as much as that in SZN, while much lower WFPS, especially for MF plot, was found in cornfield (Table 5.9). Therefore, higher denitrification rate was stimulated by the increased NO_3^- by fertilizer addition in grassland rather than the increased WFPS by rainfall in cornfield.

The magnitude of N_2O flux peaks with rainfall were 4.88 ~ 6.59 times higher in cornfield and 2.14 ~ 2.38 times higher in grassland than the respective mean N_2O fluxes in SZN, which was supported by the observation during incubation that the increased in BD at GWC of 0.45 g g^{-1} (WFPS from 44 ~ 53 % to 76 ~ 83 %) resulted in 8.5 and 28.5 folds growth in N_2O production in 2-4.5 mm F and MF soil, respectively, while 74.9 and 44.0 folds increase in 0-2 mm soil, and much higher N_2O production of high GWC high BD treatment was measured in MF soil with higher initial NO_3^- supply compared with F soil. This indicated that soil mineral N, in

addition to high WFPS, was required for high episodic N_2O emissions, as shown also by Akiyama et al. (2013).

Scholefield et al. (1997) declared that the increasing pH was generally associated with both decreasing denitrification and N_2O -to- N_2 ratio. However the higher pH ($p < 0.001$) and DEA was measured simultaneously in 0-2 mm soil which implied that pH was not the key factor of denitrification activity in out incubation.

Even in the high BD with high GWC treatment, both NH_4^+ and NO_3^- contents increased after incubation. Wang and Cai (2008) reported a similar result where NO_3^- -N contents increased after incubation even at a soil moisture content of 100 % of field water capacity. The growth of NH_4^+ content in this treatment was relatively higher than other treatments (Table 5.3), which implied that the reduction of NO_3^- to NH_4^+ by dissimilation might happen in our study. Stanford et al. (1975) reported NH_4^+ production from added NO_3^- under anaerobic, acidic conditions by NO_3^- dissimilatory reduction pathway, and in enhanced amounts with added glucose-C. However there was no accurate data to estimate the contribution of this process to N_2O production. Therefore, the relative lower increment NO_3^- of this treatment was attributed to both denitrification and dissimilation.

The extremely high peak fluxes of N_2O were measured only in the treatments of high BD with high GWC during incubation (Fig.5.3). The peak time of MF soil was one day later than F soil might be induced by the lower NO_3^- level of F soil, leading to quicker conversion of N_2O to N_2 and the lower D/D_0 of MF soil. It took little time that the consumption rate of N_2O became to be greater than the producing rate N_2O in F soil and the exchange of N_2O from soil was relatively reduced in MF soil. The peak fluxes of N_2O in this treatment was of shorter duration in 2-4.5 mm soil than that in 0-2 mm soil, which due to the NO_3^- limitation in larger aggregates. The depleting NO_3^- stimulated the reduction of N_2O to N_2 (Højberg et al., 1994).

5.4.4 The interaction of different gases

Nitrogen mineralization and the following transformation, i.e. nitrification and

denitrification whereby N_2O is produced as an immediate product, are intimately linked to the organic C decomposition (Wrage et al., 2001). Although there was no clear relationship between cumulative productions of N_2O and CO_2 (Fig.5.9), the indirect effect of SOC decomposition on N_2O emission through controlling O_2 availability in our study was very important and the main reason for the differences of N_2O emission between smaller and larger aggregate soil. And this also suggested that the differences in N_2O production between treatments cannot be readily attributed to the influence of BD, GWC or nutrient management on the microbial growth rate and respiratory consumption of O_2 .

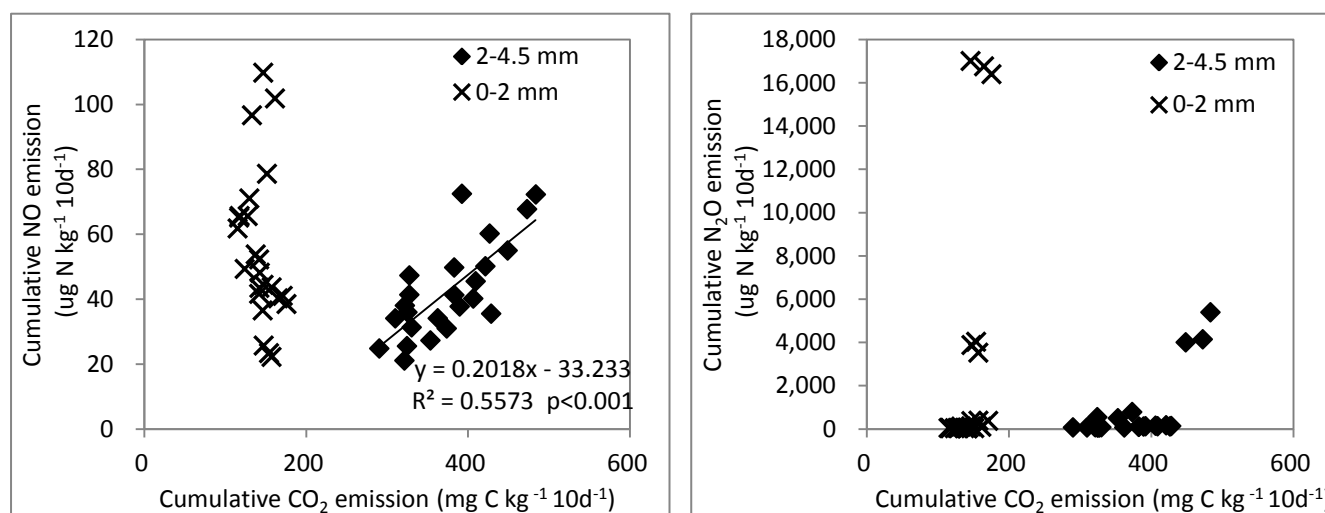


Fig.5.9 The relationship between cumulative CO₂ with NO and N₂O

Cumulative production of NO showed significant correlation with the CO₂ emission in 2-4.5 mm aggregates (Fig.5.9, $p < 0.001$), which indicated that SOM decomposition was closely related to the NO production at the high concentration of (MBC+WEOC). While there was no effect of soil C decomposition on NO emission of 0-2 mm was observed. Schutter and Dick (2002) attributed a lack of the relationship between CO₂ production, N mineralization and aggregate size to the temporal variation in substrate availability and microbial biomass within aggregates.

In our study, CO₂ production did not vary significantly with moisture content, but N₂O production significantly varied with moisture content and higher moisture content resulted in high N₂O production, which indicated that the microorganism

producing N_2O was more sensitive to the changes in soil moisture content. Linn and Doran (1984) observed a linear relationship between CO_2 and N_2O productions at 60 % WFPS. They documented that microbial activities was considered to be limited at water content below 60 % WFPS and aerobic microbial activities was decreased when water content above 60 % WFPS.

Soil moisture content has generally been accepted as a measurable proxy of O_2 availability (Linn and Doran, 1984). Actually, soil moisture controls not only the diffusion of O_2 , but also substrate availability (Stark and Firestone, 1995) and microbial activity. Except EC, all chemical properties studied here were not affected by GWC significantly (Table 5.3; Table 5.4) before and after the incubation. And the CO_2 emission was only significantly controlled by aggregate size instead of GWC. These results pointed to the fact that the main influence of GWC on N_2O emission was associated with O_2 level rather than the substrate availability and microbial activity in our study.

Since fungi have a considerably greater C/N ratio than bacteria (Kushwaha et al., 2001), fungi was probably dominant in 2-4.5 mm aggregates and bacteria dominate in 0-2 mm aggregate due to higher MBC: MBN ratio in larger aggregate, which was similar as other reports (Tisdall and Oades, 1982; Gupta and Germida, 1988). The different kinds of microorganism within soil aggregate might also affect the CO_2 , NO and N_2O emission from soil, which need more study in the future.

5.5 Conclusion

Manure application improved, but not significantly, CO_2 production due to the increased levels of SOC. Greater CO_2 production in larger aggregate due to higher MBC and WEOC contents suggested high potential of SOM decomposition during the degradation of soil aggregate promoted by tillage, which might be restrained by low soil temperature in field. The enhanced aeration by tillage was not likely to induce CO_2 hot moment due to the non-significant effect of BD on CO_2 production. Optimal moisture conditions for soil respiration activity could be provided within a

wide range from 29 ~ 83 % WFPS, partly explaining the weak effect of rainfall event on CO₂ hot moment in field. The insignificant effects of BD, GWC or nutrient management on CO₂ flux contributed to the less episodic nature of CO₂ fluxes in field. NO emission was mainly controlled by net nitrification rate when soil physical condition tended to reduce residence time, whereas NO production primarily depended on the transportation at the limited gas diffusion condition. Low soil moisture and low bulk density resulted in higher NO evolution because of the greater O₂ availability and gas exchanges. The enhancement of N₂O production by manure application due to higher initial NO₃⁻ concentration was not significant. Denitrification was the main pathway of N₂O production during incubation even soil condition sometimes was conducive to nitrification. The extremely higher N₂O production improved by high GWC and high BD was owed to the higher denitrification rate. N₂O production at low WFPS was generally small implied that the prompt increase in N₂O emission by rainfall event was more typical for denitrification than for nitrification, and the stimulation of tillage event on N₂O hot moment might be limited. Larger aggregate soil emitted more N₂O at low WFPS (29 % ~ 59 %) due to more contribution of denitrification, resulting from enhanced anaerobic environment by high WEOC content. N₂O production was much higher in smaller aggregate soil at WFPS of 80 % caused by greater NO₃⁻ supply produced by nitrification. Therefore in addition to high WFPS, soil mineral N was required for high episodic N₂O emissions. Besides higher N input rate, higher nitrification rate also resulted in more NO₃⁻ surplus in cornfield due to the larger aeration in soil. Lower NO₃⁻ concentration and higher WFPS led to high potential of N₂O hot moment induced by fertilizer application in grassland. The effect of tillage on N₂O hot moment depended on the stimulation of sufficient anaerobic ozone by soil organic C decomposition and nitrification, WFPS and NO₃⁻ concentration.

Chapter 6

GENERAL DISCUSSION

6.1 Achievements of this study

Knowledge of episodic soil CO₂ and N₂O fluxes is essential to reliably quantify these gases fluxes and to develop future mitigation strategies (Imer et al., 2013). However there were few previous studies focused on the identification and analysis of gas hot moments or environmental factors triggering gas hot moments (Molodovskaya et al., 2012). The lack of information on hot moment quantification is attributed to the fact that there is no agreed-on operational definition of a hot moment, and the magnitude and time scale of N₂O flux events are highly variable even among similar crop, climate, or soil types (Molodovskaya et al., 2012). Molodovskaya et al. (2012) suggested the numerical threshold used to pick up episodic gas fluxes at hot moment should to be linked to the flux measurement range and firstly used box plot method to distinguish potential hot moments from the more generic concept of disturbance. Box plot analysis can be used for outlier analysis even when the flux data are not normally distributed because it depends on the median and not the mean of the data (Walfish, 2006). There are also other methods of detecting outliers, including generalized ESD test and Tietjen-Moore test. Generalized ESD test is an excellent tool to investigate multiple outliers for data sets that have more than ten observations from an approximately normally distributed sample (Walfish, 2006), and it is restricted to two-sided tests and requires only an upper bound on the suspected number of outliers so it is preferred when the exact number of outliers is not known (NIST, 2012). Tietjen-Moore test requires that the suspected number of outliers must be specified exactly. If the number of outliers is not specified correctly, this can distort the conclusions of the tests. Therefore the box plot method tended to be the most suitable analysis method to investigate potential hot moment in our study since the distribution of both CO₂ and N₂O fluxes were non-normal in our study and the number of outlier was unknown. Furthermore the hot-moment flux included not only the peak emission

of GHGs but also the low-frequency negative observations according to the definition of hot moment. The mechanism and environmental drivers responsible for the uptake of GHGs are also important. While previous studies mostly concentrated on the first type of fluxes at hot moment due to the large contribution to GHGs emission. In this study, the episodic peak flux of GHGs was named as “hot moment” and the uptake observations as “cold moment” in order to divide these two types of CO₂ and N₂O fluxes at hot moment. The box plot method provided statistical thresholds to define both of these two types of flux at hot moment although there was no GHGs flux at cold moment was picked up in our sites.

In the present study, temporal variability of long-term N₂O and CO₂ fluxes from managed grassland and cornfield in southern Hokkaido, Japan was assessed, and the frequency of gas fluxes at hot moment and contribution of hot moments at annual timescales was evaluated by box plot method. Through further research about the potential importance of hot moment phenomena, we can obtain a better mechanistic understanding of the factors that create hot moments, which is necessary to move toward quantitative models and to predict their occurrence (McClain et al., 2003). And mechanism inducing episodic gas fluxes at hot moment was discussed based on the incubation study of CO₂ and N₂O productions affected by different aggregate sizes, gravimetric water contents, bulk densities and nutrient managements. Although the cumulative gas emissions from the incubation experiment is not possible to extrapolate directly to field conditions, due to the controlled and static laboratory conditions used, these findings about the controlling factors of CO₂ and N₂O production by incubation still could provide better understanding about the episodic CO₂ and N₂O fluxes induced by events.

Therefore hot moment analysis could be a promising tool for estimating and predicting the most productive N₂O events, especially when direct correlations between individual parameter time series are difficult to determine (Molodovskaya et al., 2012).

6.2 Shortages and perspective

Because of the simultaneous responses of CO₂ and N₂O flux peaks to events, the time interval of gas fluxes measurement in our field study might not have caught all the peaks. Especially the changes in soil physical properties by agriculture practice and environmental events could induce immediately large flushes of GHGs already in the soil and these increases were always short-lived (Reicosky et al., 1997, 2005; Ellert and Janzen, 1999). Therefore, the magnitude of N₂O peak events and total N₂O emissions might be underestimated, and the transient gas flux peaks immediately after events were missed. More continuous high-resolution gas fluxes and environmental data are needed for both quantification and refining criteria of hot moment estimates. And the intensified sampling is advised, not only during the hot moment events but also after the hot moment events, because peak fluxes may be over-represented if measurements tend to be concentrated around periods of hot moments (Flechard et al., 2007). The more frequent gas sampling could provide more chances of studying the combined effect of events on inducing episodic gas fluxes in field. In our study site, there was freeze-thaw event in field, while the pulse N₂O emissions during freeze-thaw cycles were not included in this study, which also might underestimate the contribution of N₂O flux in hot moment (Gregorich et al., 2008). Due to the lack of the influence of plants, and the controlled and static laboratory conditions used, the relevance of the findings about the controlling factors of CO₂ and N₂O production by incubation to microbial processes controlling episodic CO₂ and N₂O fluxes at hot moment in the field remains to be confirmed in the future. The big difference in the ratio of MBC: MBN between small and big aggregate size soil suggesting the different kinds of microorganism within soil aggregate might also affect the CO₂, NO and N₂O emission from soil, which need more study in the future.

Chapter 7

SUMMARY

Introduction

Atmospheric nitrous oxide (N₂O) and carbon dioxide (CO₂) concentrations have been increased to 324.2 ppb and 391 ppm in 2011 at a rate of 0.73 ± 0.03 ppb yr⁻¹ and 2.0 ± 0.1 ppm yr⁻¹, respectively. Soil is a major source of anthropogenic GHGs emissions. N₂O and CO₂ emissions from soil were highly variable in time caused by the complex set of environmental variables such as soil temperature and moisture, soil organic matter (SOM) content, O₂ status as well as nutrient availability. Gas fluxes at hot moment frequently accounts for larger proportion of the GHG budgets. Temporal heterogeneity is accentuated in agricultural relative to non-managed landscapes. Land management causes physical and biogeochemical disturbance of extreme magnitude and in pulsed events. Episodic CO₂ and N₂O fluxes at hot moment have been reported after events including drying-rewetting, rainfall, freezing-thawing, fertilizer and manure additions, harvest and tillage. Therefore understanding the temporal variability of CO₂ and N₂O fluxes is key importance to reliably estimate these gases fluxes and to develop mitigation strategies. In this study, episodic N₂O and CO₂ fluxes at hot moments in managed grassland and cornfield was evaluated by box plot method, and the effects of nutrient management and soil properties associated with tillage including bulk density, soil moisture content, aggregate size on CO₂, N₂O and NO emissions were studied by laboratory incubation, and then to identify key factors affecting the episodic CO₂ and N₂O emission.

Material and methods

Hot moment analysis: Field study was conducted in Shizunai (SZN) and Shin-Hidaka (SHD), southern Hokkaido, Japan. Soil N₂O and CO₂ fluxes from managed grassland (2004 to 2009 for SZN; 2007 to 2012 for SHD) and cornfield (2009 to 2012 for SZN; 2012 to 2014 for SHD) were monitored with 4 replications by closed chamber method at three plots applied with fertilizer (F), manure plus fertilizer (MF), and no-fertilizer (CT), respectively. The hot moments of N₂O and CO₂ flux

were analyzed by box plot method, using the upper fence at 1.5 times distance from the inter-quartile range as a threshold. Soil NO_3^- -N and NH_4^+ -N, soil temperature and water-filled pore space (WFPS) was also measured.

Incubation study: Soil at 0-20 cm depth of F and MF plots of SHD was collected, and was sieved into 0~2 and 2~4.5 mm aggregates. After the adjustment of gravimetric water content (GWC, 0.35 and 0.45 g g^{-1}), soil was packed with different dry bulk densities (BD, 0.45 and 0.66 g cm^{-3}) manually. Put all soil cores into Mason jars and sealed air tightly. Incubation was conducted at 15 °C for 10 days. Ambient wet air was used to flush the jars before incubation and after each gas sampling. Gas sampling was carried out 7 times. Additional soil cores were packed for analyzing physical properties. After water adjustment and incubation, soil chemical properties were measured. Denitrifying enzyme activity (DEA) was determined after incubation under $\text{N}_2 + 10\% \text{ C}_2\text{H}_2$ atmosphere. Soil microbial biomass C (MBC) and N (MBN) were measured before incubation. All measurements were conducted with 3 replications.

Evaluation of N_2O and CO_2 hot moments in managed grassland and cornfield

The characteristics of N_2O and CO_2 hot moments were generally similar in both sites but varied between grassland and cornfield. Peak N_2O flux in SZN and SHD was up to 1291 and 730 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ in grassland, respectively, and 2461 and 1066 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ in the cornfield. Maximum CO_2 fluxes of 623 and 470 $\text{mg C m}^{-2} \text{ h}^{-1}$ were observed measured in grassland of SZN and SHD, respectively, and 442 and 272 $\text{mg C m}^{-2} \text{ h}^{-1}$ in cornfield. Threshold of N_2O hot moment was higher in cornfield than in grassland due to higher fertilization and higher NO_3^- concentration. The number of N_2O hot moments accounted for 17 % and 11 % in grassland of SZN and SHD to total number of observations, and 12 % and 6 % in cornfield, respectively. The frequency of N_2O hot moment was always largest in MF plot, followed by F and CT plots. But CO_2 hot moment in SZN was only 0.5 % in grassland and 0.4 % in cornfield, even no CO_2 hot moment was found in SHD. In grassland, temperature > 20 °C, rainfall, harvest, fertilizer application and manure application (only in SZN) contributed to N_2O hot moment significantly. While in cornfield, only rainfall event significantly promoted

N₂O hot moment. More sensitive responding of N₂O hot moment to fertilizers addition event in grassland than in cornfield can be attributed to much lower soil NO₃⁻ contents in grassland where N tended to be limited. Greater magnitude of N₂O hot moment in cornfield after rainfall event was caused by more sufficient N supply for denitrification. Except the contribution of N₂O hot moment in the cornfield of SHD ranged from 5.9 % ~ 19.4 %, N₂O emission in hot moments accounted for more than one-third of the annual total emissions in F and MF plots.

The mechanism of key factors inducing episodic CO₂ and N₂O fluxes

Manure application improved, but not significantly, CO₂ production due to the increased levels of SOC. Greater CO₂ production in larger aggregate due to higher MBC and WEOC contents suggested high potential of SOM decomposition during the degradation of soil aggregate promoted by tillage, which might be restrained by low soil temperature in field. The enhanced aeration by tillage was not likely to induce CO₂ hot moment due to the poor relationship between BD, air ratio or D/D₀ with CO₂ production. Optimal moisture conditions for soil respiration activity could be provided within a wide range from 29 ~ 83 % WFPS, partly explaining the weak effect of rainfall event on CO₂ hot moment in field. The insignificant effects of BD, GWC or nutrient management on CO₂ flux contributed to the less episodic nature of CO₂ fluxes in field. The enhancement of N₂O production by manure application due to higher initial NO₃⁻ concentration was not significant. Denitrification was the main pathway of N₂O production during incubation even soil condition sometimes was conducive to nitrification which suggested the importance of denitrification for N₂O hot moment in field. The extremely higher N₂O production improved by high GWC and high BD with WFPS of around 80 % was owed to the higher denitrification rate. N₂O production at low WFPS was generally small implied that the prompt increase in N₂O emission by rainfall event in field was more typical for denitrification than for nitrification. Larger aggregate soil emitted more N₂O at low WFPS (29 % ~ 59 %) due to more contribution of denitrification, resulting from enhanced anaerobic environment by high WEOC content. N₂O production was much higher in smaller aggregate soil at WFPS of 80 % caused by greater NO₃⁻ supply produced by

nitrification. Therefore in addition to high WFPS, soil mineral N was required for high episodic N₂O emissions. Besides higher N input rate, higher nitrification rate also resulted in more NO₃⁻ surplus in cornfield due to the larger aeration in field. Lower NO₃⁻ concentration and higher WFPS led to high potential of N₂O hot moment induced by fertilizer application in grassland. Higher NO₃⁻ concentration and the enhanced anaerobic condition by greater soil organic C decomposition and nitrification could improve the stimulation effect of tillage on N₂O hot moment.

Conclusion

CO₂ flux, strongly controlled by soil temperatures, was much less episodic than N₂O flux, and the insignificant effects of soil bulk density, soil moisture or nutrient management on CO₂ flux contributed to the less episodic nature of CO₂ flux in field. Temperature more than 20 °C, rainfall, harvest, fertilizer and manure applications were the events significantly promoting N₂O hot moments in grassland, while only rainfall event significantly induced N₂O hot moment in cornfield. Great anaerobic condition created by high WFPS or quick SOM decomposition in addition to sufficient mineral N contents were required for episodic peak N₂O emissions. Mitigating episodic N₂O emissions would greatly reduce annual N₂O emissions. Fertilization avoiding rainfall in grassland could be one option.

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