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REVIEW

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Relationships of soil physical and microbial properties with nitrous oxide emission affected by freeze-thaw event

Abstract Freeze-thaw event often occurs in regions at mid-high latitude and high altitude. This event can affect soil physical and biological properties, such as soil water status, aggregate stability, and microbial biomass and community structure. Under its effects, the bio-indicators of soil microbes including the kinds and quantities of some specific amino sugars may vary, and the process and intensity of soil nitrogen transformation may change, which can result in an increase in nitrous oxide (N_2O) production and emission, making the soil as the major source of N_2O emission. This paper summarizes the research progress on the aspects mentioned above, and suggests further research directions on the theoretical problems of soil N_2O production and emission under the effects of freeze-thaw event.

Keywords nitrous oxide, freeze-thaw event, soil property

1 Introduction

Nitrous oxide (N_2O) is a greenhouse gas with important impacts on our environment. Its 100-year global warming potential is about 300 and 23 times as strong as that of CO_2 and CH_4, respectively. Because of N_2O reaction with stratospheric ozone, ozone concentration decreases. This may result in UV-radiation increase. N_2O from soils is the main source of greenhouse gases in agriculture which contributes about 60% of total anthropogenic emissions of N_2O (Intergovernmental Panel on Climate Change, 2001). N_2O is produced through the processes of nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and chemo-denitrification (Granli and Bøckman, 1994).

Freeze-thaw event often occurs in regions at mid-high latitude and high altitude, decreasing the stability of aggregate (Oztas and Fayetorbay, 2003), changing the structure and function of microbial communities (Sharma et al., 2006), and releasing more available nutrients to improve microbial activities. All these impacts can increase N_2O emissions. Soils in the freezing-thawing stressed regions become the major emission sources (Intergovernmental Panel on Climate Change, 2001; Röver et al., 1998; Sameshima-Saito et al., 2004). N_2O emission during freezing–thawing period in the field studies is approximately 65% of the total annual emission (Wagner-Riddle et al., 1997). At present, most researchers focus their studies on the N_2O emission in growing seasons; emissions in fallow season are often neglected, however. In the methodology to assess the annual direct biogenic emissions of greenhouse gases (GHG) released from European agriculture, Freibauer (2003) has suggested that the N_2O emission factors for mineral soils that are exposed to severe frost should be larger than those for soils in warmer regions. In China, seasonal gelisol (more than 50-cm frozen layer) accounts for 46.3% of the country's total area, showing a very strong freeze-thaw event (Zhao et al., 1993). More and more attention is now paid to the emission of greenhouse gases because of global warming. The Chinese government issued the document of the National Project Reply to Climate Change in China on June 4, 2007, and initiated a special Sci-Tech Campaign to Cope with Climate Changes on the same day. China will develop key technologies to control greenhouse gases emission and to minimize global climate change. Currently, few findings on the soil N_2O emissions during freezing-thawing period have been reported in China. The present review summarizes the changes of soil physical and biological properties, e.g., water status, aggregate stability, and microbial biomass and community structure. These changes can influence N_2O production

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and emission. Further research fields concerning the theoretical problems of soil N_2O production and emission under effects of freeze-thaw event are suggested. There is a very big significance in reducing soil N_2O emission, enhancing efficacy of nitrogen fertilizer, and developing sustainable agriculture between high crop yield and environmental harmony (Tilman et al., 2002).

2 Relationship of soil physical properties with N_2O emission affected by freeze-thaw event

Freeze-thaw stress can influence soil water flow and heat transport (Hansson et al., 2004), soil solute movement (Radke and Berry, 1998) and soil water infiltration (Zheng et al., 2001). Freezing and thawing can significantly reduce the bulk density of plough pan and increased its porosity and hydraulic conductivity (Deng et al., 1998). Water moves upward to the freezing zone, carrying some solutes along (Radke and Berry, 1998). Soil moisture can be obviously increased due to freezing because of water migration caused by water potential of frozen soil fringe. However, the increased amount depends on the moisture distribution in the soil profile before freezing (Gong et al., 1997). During the steady freezing stage, cumulative infiltration and final infiltration rates may decrease as soil frost depth increases. During the thawing stage, cumulative infiltration and final infiltration rates may increase with the increase of thawing depth (Zheng et al., 2001). Freezing-thawing alternation can change the phase of water and spatiotemporal temperature in soils (Bond-Lamberty et al., 2005). Freezing-thawing stress may change soil partial environment resulting in soil water status difference. During the thawing period, water may infiltrate soil particles to cause different soil water characteristics. The ability of N_2O production and further reduction to N_2 is very different in soil microcosm (Wang et al., 2004). Denitrification is the main process of N_2O production in freezing-thawing soils (Ludwig et al., 2004; Sharma et al., 2006). N_2O is produced by microorganisms during continuous soil freezing in an unfrozen water film on the soil matrix. This thin liquid water film is covered by a layer of frozen water. The frozen water in the form of an ice layer represents a diffusion barrier which can reduce oxygen supply to the microorganisms and partly prevent the release of the N_2O (Teepe et al., 2001). The anaerobic micro-environment is helpful for N_2O production through denitrification. Thawing event may account for a large proportion of N_2O emission. Burton and Beauchamp (1994) suggested that N_2O produced in the unfrozen subsoil was unable to diffuse through the frozen soil surface. The subsurface region beneath the ice layer allowed N_2O accumulation. Thawing of the frozen layer resulted in the release of N_2O from the subsurface region (Burton and Beauchamp, 1994). However, this explanation is in doubt, because any trapped in this way would probably be further reduced to N_2 instead of remaining as N_2O, provided the temperature was too low for denitrifiers to act. Peak N_2O emission could be explained by the production in surface soil and the physical release of trapped N_2O in subsurface soil (Röver et al., 1998; Teepe et al., 2001). The period of seasonal snow-cover significantly affects the soil nitrogen cycle. Though the time of snow-cover had little difference, N_2O could be emitted significantly (Brooks et al., 1998).

The freezing-thawing cycle can also affect soil aggregate stability. Lehrsch et al. (1991) indicated that the effect of freezing-thawing stress on soil aggregate stability depend on the frozen status. They emphasized the non-uniformity of structural changes induced by frost: by freezing a soil sample, some parts of the sample always became wetter and the other got drier. In the wetter part, frost may disrupt aggregates because expansion of ice crystals in pores may break the particle-to-particle bonds. In contrast, drying is believed to cause a shrinkage of soil mass and precipitation of bonding agents at particle-to-particle contacts. These contrasting processes may act in different parts of the soil sample, resulting in the decreased and increased aggregate stabilities respectively. Therefore, the accurate aggregate stability measurements should be based only on the average of these opposing changes. Lehrsch (1998) suggested that freezing–thawing cycle could increase the soil aggregate stability near the surface layer. Most studies indicate that freezing–thawing cycle can decrease aggregate stability and destroy aggregate (Oztas and Fayetorbay, 2003; van Bochove et al., 2000). Freezing may destroy the aggregate stability at higher degree in macroaggregates (>0.25 mm) than in microaggregates (van Bochove et al., 2000). The destruction degree of macroaggregates stability is more remarkable when moisture content is higher at freezing (Oztas and Fayetorbay, 2003; van Bochove et al., 2000). Macroaggregates can accumulate and protect lots of active organic carbon generated recently (Puget et al., 1995). With the destruction of macroaggregates, so much of active organic carbon is released to improve denitrification and more N_2O is produced. Freezing may increase the rates of C mineralization and denitrification activity by 95% and N_2O production by 220% after thawing. The increase in microaggregates is higher (57%) than that in macroaggregates (van Bochove et al., 2000). A study reported that soil could produce an up-to-1000-fold increase in N_2O emission rates during thawing (Priemé and Christensen, 2001). Wang et al. (2005) reported that bioavailability of organic carbon significantly affected the microbial production of N_2O, N_2O production through denitrification was still strong at lower soil water content if enough active organic carbon was supplied to soil microorganisms.

3 Relationship of soil microbial properties with N_2O emission affected by freeze-thaw event

Soil microbial properties are important for the cycling of carbon and nitrogen in soils during the freezing-thawing period. Some microbes may die under frozen stress. Dead microbes may release lots of carbon and nitrogen nutrients (Klemdtsson et al., 1988). Microbes surviving the freezing-thawing cycles will have a high potential for sequestering soil carbon and nitrogen nutrients and enhance the soil carbon and nitrogen mineralization, and N_2O emission may also increase during thawing (Oztas and Fayetorbay, 2003; Sharma et al., 2006).

The structure and function of soil microbial communities can be affected by freezing temperature, freezing time, freeze-thaw frequency and soil water content prior to freezing. Moderate freeze-thaw fluctuations may have minimal influence on microbial biomass pools; nevertheless, severe freeze-thaw fluctuations may have strong contrasting effects on the amount, form, and time of nitrogen and organic carbon supply in soil solution (Grogan et al., 2004). Freezing can increase the rates of carbon and nitrogen cycling in forest soils, but the effects may vary with different freeze intensity. Forest soils in severe freeze treatment ($-13^{\circ}C$) have more significant effect of stimulating soil respiration, N_2O flux, and mineralization, (Neilsen et al., 2001) than those in mild ($-3^{\circ}C$) freeze treatment after being cultured at laboratory temperature. Duration of freezing can influence the N_2O emission during the thawing period. The longer the duration of freezing persists, the greater the N_2O loss during thawing. Surviving microbes rapidly use the nutrients released from dead microbes to produce large amounts of N_2O (Papen and Butterbach-Bahl, 1999). N_2O emission and soil microbial biomass carbon can be increased with the increase of freezing-thawing frequency (Larsen et al., 2002; Priemé and Christensen, 2001). High water content prior to freezing can produce more anaerobic microcosms in which denitrifiers are in favor of producing more N_2O during the thawing period. Röver et al. (1998) measured the maximum emissions of N_2O at 80% WFPS after freezing in an agricultural soil. van Bochove et al. (2000) reported that emission of N_2O from a clay soil was significantly larger at a volumetric water content of 39% than at 28%. Teepe et al. (2004) studied the effects of four water contents prior to freezing on N_2O emission during thawing period. The microbial communities and processes in freezing-thawing fallow season may differ from growing season (Brooks et al., 1997). Freezing-thawing stress may change the structure and function of microbial communities (Sharma et al., 2006). During the winter fallow season, the soil microbial community is dominated by fungi (Schadt et al., 2003), while bacteria appear to dominate during the growing season (Lipson et al., 2002).

Because of the complex composition, quantitative variety, and determination instability of the microbial community, uncertainty is presented by microorganisms themselves as bio-indicators of soil ecological process. Some stable substrates, e.g. amino sugars originated from microorganisms, are used to indicate the changes of the structure and function of microbial communities. Amino sugars are used to indicate the relative contribution of fungi and bacteria to soil ecological impacts (Wang et al., 2003). Different amino sugars originate from different microbial communities in soils (Parsons, 1981; Zhang and Amelung, 1996). In general, four amino sugars exist widely in soils. Glucosamine and galactosamine mainly originate from fungi and bacteria, respectively (Parsons, 1981; Sowden and Ivarson, 1974). Mannosamine may be originated from some bacteria, and muramic acid only originates from bacteria (Kenne and Lindburg, 1983). Zhang and Amelung (1996) first established gas chromatographic simultaneous determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Therefore, amino sugars can be used to indicate the structure and function of microbial communities. The composition and content of amino sugars in soils before and after a freezing-thawing event may indicate the structure and function of microbial communities, combining MPN and PCR-DGGE determination technology to confirm them (Sharma et al., 2006).

4 N_2O production process and its determination affect by freeze-thaw event

Biological nitrification and denitrification are the main processes of N_2O production, while abiotic process contributes negligibly (Granli and Bøckman, 1994; Klemdtsson et al., 1988). N_2O production process is similar in soils under freezing-thawing cycles (Ludwig et al., 2004; Müller et al., 2003; Müller et al., 2002).

Nitrification is either autotrophic or heterotrophic in aerobic soils and autotrophic nitrification is recognized to be more important for N_2O production in most soils (Wood, 1990; Tortoso and Hutchinson, 1990). Autotrophic nitrifiers obtain energy from the oxidation of NH_4^+. NH_4^+ or NH_3 stepwise oxidation to NO_3^- via NO_2^- is catalyzed by ammonia monooxygenase, hydroxylamine oxidoreductase and nitrite oxidoreductase. N_2O, as an intermediate, is formed during NH_3 oxidation (Wrage et al., 2001). C_2H_2 inhibits NH_3 oxidation at concentrations between 0.1 and 10 Pa (Berg et al., 1982). Therefore, autotrophic nitrification can be inhibited. Much more attention is paid to autotrophic nitrification than heterotrophic nitrification. Heterotrophic nitrification has not been studied extensively, and cannot be inhibited by the low concentration of C_2H_2 (Richardson et al., 1998). Heterotrophic nitrifiers use organic substances as

both a carbon and an energy source (Robertson and Kuenen, 1990). Heterotrophic nitrification is considered to be more common among fungi than bacteria, and fungi play important roles in heterotrophic nitrification in soils with a low pH (Odu and Adeoye, 1970). For instance, heterotrophic nitrification is presented in grassland soils as affected by freezing-thawing stress (Müller et al., 2002).

Denitrification is stepwise reduction of NO_3^- to N_2. The reactions are carried out by denitrifiers. Heterotrophic denitrifiers are facultative anaerobes that are able to use NO_3^- in place of oxygen as an electron acceptor in respiration to cope with low-oxygen or anaerobic conditions. Enzymes catalyzing the reactions are nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase. The former three enzymes are involved in N_2O production, but the last enzyme is related to N_2O consumption. N_2O is reduced to N_2 by nitrous oxide reductase catalysis. 10 kPa C_2H_2 is enough to inhibit the reduction of N_2O, which may lead to the accumulation of N_2O as the only end product of denitrification (Klemdtsson et al., 1988; Wrage et al., 2001). Soil water status can affect denitrifying enzyme activity (Wang and Cai, 2005).

Several methods, such as nitrification inhibitor (e.g. dicyandiamide, DCD), ^{15}N isotope and acetylene inhibition, are used to study the processes of N_2O production. Each method has its advantages and disadvantages (Granli and Bøckman, 1994). There are lots of studies on N_2O flux from soils as affected by freezing-thawing stress in northern America and northern Europe (Table 1), but few studies are focused on the processes of N_2O production in soils affected by freezing-thawing stress. Priemé and Christensen (2001) reported that the contribution of nitrification and denitrification to N_2O production from farmed organic soils as affected by freezing-thawing stress was quantitatively researched using acetylene inhibitory method. Denitrification was responsible for the majority of N_2O emission from German ploughed soil, Swedish ploughed and grassland soils. Also, the shortcomings of acetylene inhibitory method were put forward (Priemé and Christensen, 2001). Müller et al. (2002; 2003) reported that denitrification was the major process of N_2O production from German grassland soils as affected by freezing-thawing stress using both acetylene inhibitory method and ^{15}N-labelled method. Müller et al. (2002) analyzed N_2O production process in freezing, thawing and post-thawing stages. N_2O reductase activity could increase with the increase of soil temperature (Müller et al., 2003). At present, a few studies report the investigation of N_2O production process in freezing-thawing events using ^{15}N in experiments (Ludwig et al., 2004; Müller et al., 2002). The underlying processes that occur during freezing-thawing are poorly understood. Immediately after the beginning of the thawing, denitrification may have 83% contribution to N_2O production (Ludwig et al., 2004). Some accurate, convenient and rapid technologies are established to determine $^{15}N_2O$ production processes. These methods include isotope ration mass spectrometry (Stevens et al., 1993), gas chromatography with a thermal conductivity detector (Sameshima-Saito et al., 2004) and ^{15}N–^{18}O dual-isotope labeling method (Wrage et al., 2005). Quantification of N_2O production is helpful to regulate N_2O production and emission.

5 Conclusions

N_2O is an intermediate product of nitrogen cycling. Under stable environment, nitrogen species are relatively stable, and N_2O emission decreases step by step. The changes of environmental factors may lead to nitrogen species changes so as to enhance the N_2O production and the emission. The amount of N_2O emission depends not only on the environmental factors but also on the intensity and frequency of environmental factors. Currently, most studies on N_2O emission are investigated under stable environmental conditions, not after the changes of the environmental situation. Freezing-thawing stress can change water flow, heat conductivity, soil structure and microbial community. These changes can influence the activity of nitrifiers and denitrifiers. The strength of nitrification, denitrification and enzyme activity changes may lead to an increase in N_2O emission. On one hand, freezing-thawing stress may cause water phase changes and destruction of aggregate may result in the release of

Table 1 Field determination of nitrous oxide emission amount in freeze-thaw stressed soils

location	vegetation	freeze-thaw period	N_2O amount (N) /kg·hm^{-2}	freeze-thaw flux per annual flux/%	reference
Guelph, Canada	barley, soybean	Mar. 1994–Apr. 1994	1.50–4.30	65.0	Wagner-Riddle et al. (1997)
New Hampshire, USA	beech, maple, birch	Dec. 1997–Mar. 1998	0.67	8.68	Groffman et al. (2006)
		Dec. 1998–Mar. 1999	0.88	24.6	
		Dec. 1999–Mar. 2000	0.63	17.7	
Jokioinen, Finland	barley	Oct. 1999–Apr. 2000	3.30	53.2	Regina et al. (2004)
		Oct. 2000–Apr. 2001	8.00	53.7	
		Oct. 2001–Apr. 2002	18.9	78.6	
Rovaniemi, Finland	barley	Oct. 2000–Apr. 2001	5.80	80.5	Regina et al. (2004)
		Oct. 2001–Apr. 2002	14.5	77.1	

trapped N_2O and nutrients. On the other hand, lots of active nutrients from dead microbes can be used by surviving microbes to carry on nitrification and denitrification for N_2O production. These changes are found to affect the N_2O production and emission.

Freezing-thawing stress can significantly change the soil physical and microbial properties which affect N_2O production and emission. Further researches should be concentrated on the following aspects: (1) How does freezing-thawing stress affect soil aggregate structure, soil organic carbon activity and available nitrogen? The relationship of aggregates and nutrients to N_2O emission should be considered. The intensity of N_2O emission from different soil aggregates in size should be taken into account. (2) How to use modern molecular bio-techniques to study the relationship of the microbial community to the N_2O production process and its impact factors? The relationship of nitrifying enzyme, denitrifying enzyme and nitrous oxide reductase activity to N_2O production and emission should be paid attention to. What mode of substrate do microorganisms utilize after freezing? Do microorganisms use the same substrate in freezing season as in growing season or others? and (3) How to use microbial bio-indicators (e.g. amino sugars) to assess bacteria and fungi activity, microbial living condition?

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References

Berg P, Klemedtsson L, Rosswall T (1982). Inhibitory effects of low partial pressures of acetylene on nitrification. Soil Biology and Biochemistry, 14(3): 301–303

Bond-Lamberty B, Wang C K, Gower S T (2005). Spatiotemporal measurement and modeling of stand-level boreal forest soil temperatures. Agricultural and Forest Meteorology, 131(1): 27–40

Brooks P D, Schmidt S K, Williams M W (1997). Winter production of CO_2 and N_2O from alpine tundra: environmental controls and relationship to inter-system C and N fluxes. Oecologia, 110(3): 403–413

Brooks P D, Williams M W, Schmidt S K (1998). Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry, 43(1): 1–15

Burton D L, Beauchamp E G (1994). Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. Soil Science Society of America Journal, 58(1): 115–122

Deng X M, Wang J, Zhu W S, Chen D S, Liu L P (1998). Effect of freezing-thawing event on physical properties of plow pan soil. Chinese Science Bulletin, 43(23): 2538–2541 (in Chinese)

Freibauer A (2003). Regionalised inventory of biogenic greenhouse gas emissions from European agriculture. European Journal of Agronomy, 19(2): 135–160

Gong J D, Qi X S, Xie Z K, Wang Y J (1997). Effect of seasonal freezing on soil moisture and its significance for agriculture. Journal of Glaciology and Geocryology, 19(4): 328–333 (in Chinese)

Granli T, Bøckman O C (1994). Nitrous oxide from agriculture. Norwegian Journal of Agricultural Science, 12(Suppl): 1–128

Groffman P M, Hardy J P, Driscoll C T, Fahey T J (2006). Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. Global Change Biology, 12(9): 1748–1760

Grogan P, Michelsen A, Ambus P, Jonasson S (2004). Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biology and Biochemistry, 36(4): 641–654

Hansson K, Šimůnek J, Mizoguchi M, Lundin L C, van Genuchten M T (2004). Water flow and heat transport in frozen soil: Numerical solution and freeze-thaw applications. Vadose Zone Journal, 3(2): 693–704

Intergovernmental Panel on Climate Change (IPCC) (2001). Climate Change 2001: The Scientific Basis. Cambridge: Cambridge University Press, 7–76

Kenne L K, Lindburg B (1983). Bacterial polysaccharides. In: Aspinall G O ed. The Polysaccharides. New York: Academic Press, 287–365

Klemdtsson K, Svensson B H, Rosswall T (1988). A method of selective inhibition to distinguish between nitrification and denitrification as sources of nitrous oxide in soil. Biology and Fertility of Soils, 6(2): 112–119

Larsen K S, Jonasson S, Michelsen A (2002). Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. Applied Soil Ecology, 21(3): 187–195

Lehrsch G A (1998). Freeze-thaw cycles increase near-surface aggregate stability. Soil Science, 163(1): 63–70

Lehrsch G A, Sojka R E, Carter D L, Jolley P M (1991). Freezing effect on aggregate stability affected by texture, mineralogy and organic matter. Soil Science Society of America Journal, 55(5): 1401–1406

Lipson D A, Schadt C W, Schmidt S K (2002). Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microbial Ecology, 43(3): 307–314

Ludwig B, Wolf I, Teepe R (2004). Contribution of nitrification and denitrification to the emission of N_2O in a freeze-thaw event in an agricultural soil. Journal of Plant Nutrition and Soil Science, 167(6): 678–684

Müller C, Kammann C, Ottow J C G, Jäger H J (2003). Nitrous oxide emission from frozen grassland soil and during thawing periods. Journal of Plant Nutrition and Soil Science, 166(1): 46–53

Müller C, Martin M, Stevens R J, Laughlin R J, Kammann C, Ottow J C G, Jäger H J (2002). Processes leading to N_2O emissions in grassland soil during freezing and thawing. Soil Biology and Biochemistry, 34(9): 1325–1331

Neilsen C B, Groffman P M, Hamburg S P, Driscoll C T, Fahey T J, Hardy J P (2001). Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. Soil Science Society of America Journal, 65(6): 1723–1730

Odu C T I, Adeoye K B (1970). Heterotrophic nitrification in soils – a preliminary investigation. Soil Biology and Biochemistry, 2(1): 41–45

Oztas T, Fayetorbay F (2003). Effect of freezing and thawing processes on soil aggregate ability. Catena, 52(1): 1–8

Papen H, Butterbach-Bahl K (1999). 3-year continuous record of N-trace gas fluxes from untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany: I. N_2O emissions. Journal of Geophysical Research-Atmosphere, 104(D15): 18487–18503

Parsons J W (1981). Chemistry and distribution of amino sugars in soils and soil organisms. In: Paul E A, Ladd J N, eds. Soil Biochemistry, Vol.5. New York: Marcel Dekker, 197–227

Priemé A, Christensen S (2001). Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane farmed organic soils. Soil Biology and Biochemistry, 33(15): 2083–2091

Puget P, Chenu C, Balesdent J (1995). Total and young organic matter distributions in aggregates of silty cultivated soils. European Journal of Soil Science, 46(3): 449–459

Radke J K, Berry E C (1998). Soil water and solute movement and bulk density changes in repacked soil columns as a result of freezing and thawing under field conditions. Soil Science, 163(8): 611–624

Regina K, Syväsalo E, Hannukkala A, Esala M (2004). Fluxes of N_2O from farmed peat soils in Finland. European Journal of Soil Science, 55(4): 591–599

Richardson D J, Wehrfritz J M, Keech A, Crossman L C, Roldan M D, Sears H J, Butler C S, Reilly A, Moir J W B, Berks B C, Ferguson S J, Thomson A J, Spiro S (1998). The diversity of redox proteins involved in bacterial heterotrophic nitrification and aerobic denitrification. Biochemical Society Transactions, 26(3): 401–408

Robertson L A, Kuenen J G (1990). Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaerab pantotropha* and other bacteria. Antonie van Leeuwenhoek, 57(2): 139–152

Röver M, Heinemeyer O, Kaiser E A (1998). Microbial induced nitrous oxide emissions from an arable soil during winter. Soil Biology and Biochemistry, 30(14): 1859–1865

Sameshima-Saito R, Chiba K, Minamisawa K (2004). New method of denitrification analysis of *Bradyrhizobium* field isolates by gas chromatographic determination of ^{15}N-labeled N_2. Applied and Environmental Microbiology, 70(5): 2886–2891

Schadt C W, Martin A P, Lipson D A, Schmidt S K (2003). Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science, 301(5638): 1359–1361

Sharma S, Szele Z, Schilling R, Munch J C, Schloter M (2006). Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Applied and Environmental Microbiology, 72(3): 2148–2154

Sowden F J, Ivarson K C (1974). Effects of temperature on changes in the nitrogenous constituents of mixed forest litters during decomposition after inoculation with various microbial cultures. Canadian Journal of Soil Science, 54(3): 387–394

Stevens R J, Laughlin R J, Atkins G J, Prosser S J (1993). Automated determination of nitrogen-15 labeled dinitrogen and nitrous oxide by mass spectrometry. Soil Science Society of America Journal, 57(4): 981–988

Teepe R, Brumme R, Beese F (2001). Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biology and Biochemistry, 33(9): 1269–1275

Teepe R, Vor A, Beese F, Ludwig B (2004). Emissions of N_2O from soils during cycles of freezing and thawing and the effects of soil water, texture and duration of freezing. European Journal of Soil Science, 55(2): 357–365

Tilman D, Cassman K G, Matson P A, Naylor R, Polasky S (2002). Agricultural sustainability and intensive production practices. Nature, 418(6898): 671–677

Tortoso A C, Hutchinson G L (1990). Contribution of autotrophic and heterotrophic nitrifiers to soil NO and N_2O emissions. Applied and Environmental Microbiology, 56(2): 1799–1805

van Bochove E, Prévost D, Pelletier F (2000). Effects of freeze-thaw and soil structure on nitrous oxide produced in a clay soil. Soil Science Society of America Journal, 64(5): 1638–1643

Wagner-Riddle C, Thurtell G W, Kidd G K, Beauchamp E G, Sweetman R (1997). Estimates of nitrous oxide emissions from agricultural fields over 28 months. Canadian Journal of Soil Science, 77(2): 135–144

Wang J, Zhang X D, Xie H T, Zhu P, Jiang G M (2003). New quantificational indexes in modern study of soil organic matter. Chinese Journal of Applied Ecology, 14(10): 1809–1812 (in Chinese)

Wang L F Cai Z C, Yang L F, Meng L (2005). Effect of disturbance and glucose addition on nitrous oxide and carbon dioxide emissions from a paddy soil. Soil and Tillage Research, 82(2): 185–194

Wang L F, Cai Z C (2005). Dynamics of denitrifying enzyme activity in red soils as affected by water treatment. In: Zhu Z L, Minami K, Xing G X, eds. 3rd International Nitrogen Conference Contributed Papers. New York: Science Press USA Inc, 174–177

Wang L F, Cai Z C, Yan H (2004). Nitrous oxide emission and reduction in a laboratory-incubated paddy soil response to water pre-treatment. Journal of Environmental of Sciences, 16(3): 253–257

Wood P M (1990). Autotrophic and heterotrophic mechanisms for ammonia oxidation. Soil Use and Management, 6(1): 78–79

Wrage N, van Groenigen J W, Oenema O, Baggs E M (2005). A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. Rapid Communications in Mass Spectrometry, 19(22): 3298–3306

Wrage N, Velthof G L, van Beusichem M L, Oenema O (2001). Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry, 33(12/13): 1723–1732

Zhang X, Amelung W (1996). Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry, 28(9): 1201–1206

Zhao Q G, Wang H Q, Gu G A (1993). Gelosols of China. Acta Pedologica Sinica, 30(4): 341–354 (in Chinese)

Zheng X Q, van Liew M W, Flerchinger G N (2001). Experimental study of infiltration into a bean stubble field during seasonal freeze-thaw period. Soil Science, 166(1): 3–10