Effects of soil freeze-thaw cycles on microbial biomass and organic matter decomposition, nitrification and denitrification potential of soils

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1. INTRODUCTION

Global warming is one of the biggest environmental issues that we are facing today and causes the melting of permafrost directly and indirectly. Generally speaking, the presence of frozen ground plays an important role in the polar ecology because the main effect of seasonal soil freezing and thawing is to delay the summer warming and the winter cooling of the surface. It also affects the soil hydrology by impeding soil drainage and creating high soil moisture contents in the seasonally thawed upper soil layer (the active layer). A recent simulation study by Poutou et al. (2004) concluded that soil freezing does have a regional impact on the simulation of climate change. As the active layer becomes larger and thicker due to the global warming, it could change soil quality, and stimulate greenhouse gas emission and nutrient discharge (Hatano in this symposium). However, very little is known about how freeze-thaw cycles affect population dynamics of soil microorganisms and their functions, which are significantly associated with ecosystem function such as organic matter decomposition and nutrient supply. Until now, we have been studying about the effects of successive soil freeze-thaw cycles on soil microbial biomass and its functions to estimate ecological significance of possible changes in soil freeze-thaw patterns in temperate regions. Here, we assume that the melting of permafrost increases soils which will be subjected to diurnal soil freeze-thaw cycles and we hope that our results serve some anticipation for this Siberian study in the view point of soil microbial ecology under low temperature and soil freeze-thaw condition.

2. MICROBIAL ECOLOGY IN FROZEN SOIL

2.1 Microbial activity below 0°C

Rivkina et al. (2000) firstly made quantitative measurements of the dynamics of metabolic activity at subzero temperature between +5 and -20°C in the native bacterial population of Siberian permafrost by measuring the incorporation of $^{14}CH_3COONa$ into the lipid fraction. The permafrost samples were aseptically obtained in the Kolyma-Indigirka Lowland in northeast Siberia (69°27'N, 156°59'E) in August 1991 and had -10°C of the native temperature, 23.0% of ice content, 11.5 ~ 12.9 g kg^{-1} of the organic C and -0.8±0.18°C of the freezing point, 1.1×10^8 cells g^{-1} soil and 2×10^5 CFU g^{-1} soil. Sigmoidal incorporation of $^{14}CH_3COONa$ was observed similar to general growth curves at all incubation temperatures and the doubling time increased smoothly as the temperature decrease (1 day at 5, 3 days at 0, 6 days at -1.5, 20 days

Symptom of Environmental Change in Siberian Permafrost Region,
Eds. Hatano R and Guggenberger G, p 177-191, Hokkaido University Press, Sapporo, 2006

at -10, more than 40 days at -15 and about 160 days at -20°C). However, the curves reached different levels of stationary phase, which generally indicates exhaustion of nutrients, depending on the incubation temperature. For microbial community in frozen soils, access to nutrients and the ability to eliminate waste materials are limited by the thickness of the unfrozen films of water. Decreasing temperature leads to thinning of the unfrozen films from about 15 nm at -1.5°C to about 5 nm at -10°C, suggesting that the temperature dependence of the levels of stationary phases is not the absolute exhaustion of nutrients but the inaccessibility of nutrients or accumulation of waste materials due to a diffusion barrier formed at different levels depending on the temperature. Rivkina et al. (2000) quantitatively shows relationships among the levels of stationary phases of the incorporation curve, the amounts of unfrozen water in permafrost and the thicknesses of the unfrozen water layers depending on temperature. These results strongly elucidate importance of soil physical structure to support life of microorganisms in soil, especially in frozen soil, development of unfrozen water film is critical.

2.2 Environmental condition and microbial functions in frozen soil - hydrocarbon degradation

Freezing may not always cease metabolic activity followed by death of microbial community present in permafrost. In other words, belowground conditions during the cold season in permafrost regions are less inhospitable than one might be led to believe from the aboveground conditions (Schimel and Mikan 2005). The cold season does include a long period when soils are frozen solid with temperature as low as -30°C, but at the beginning of the cold season, the active layer is at its maximum thickness, and as the so called ‘zero degree curtain’ is falling, soils are freezing and held at 0°C for several months. It is supposed that part of permafrost melting in summer season will be subjected to diurnal freeze-thaw cycles in both late spring and early fall, however, very little is known about how freeze-thaw cycles affect population dynamics and soil microbial functions, which are significantly related to ecosystem function such as greenhouse gas fluxes, nutrient cycling and purification of contaminants. Demonstration by Eriksson et al. (2001) seems one of the good examples to show stimulation of microbial activity by the soil freeze-thaw cycles.

Eriksson et al. (2001) performed a soil microcosm experiment to study the effect of soil freeze-thaw cycles (-5 to + 7°C) on the biological degradation of weathered diesel fuel in Arctic tundra soil, where the temperature rarely exceeds 10°C and the soil is frozen most of the year. In contrast to continuous incubation at below freezing point (-5°C) where no total petroleum hydrocarbon (TPH) changes and few bacterial metabolic activity (indicated by RNA/DNA ratios) were observed during 48 days of the monitoring period, soil freeze-thaw cycles showed comparatively high bacterial metabolic activity and high TPH degradation to continuous incubation at +7°C. In addition, community composition analysis revealed that incubation under the freeze-thaw condition induced *Rhodococcus* sp., which is likely to be a TPH degrading bacterium isolated from the enrichment culture, to become a predominant community in the soil sample. Hence, the soil freeze-thaw cycles stimulates both TPH degradation and some specific microbial activities. The authors discuss contribution of the partial sterilization effect to explain the eventually stimulatory effect of soil freeze-thaw cycles in TPH bioremediation.

2.3 Frozen soil in permafrost, boreal and temperate regions and microbial functions

On earth, presence of frozen soil is not limited only in the permafrost regions. In boreal and even in temperate regions, temperature fluctuation above and below freezing point causes frequent freezing and thawing of surface soil. Seasonal or diurnal soil freeze-thaw cycles can sensitively affect global warming and cause significant changes in ecosystem function. For example, a snow manipulation experiment in a northern hardwood forest ecosystem at the

Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, United State, revealed that decrease (removal) in the snowpack on the ground induced mild but persistent soil freezing, higher N_2O emission, lower CH_4 uptate, soil NO_3^- level, hydrologic flux of C, N and P (Groffman et al 1999, 2001). These observations are called as "colder soils in a warmer world" and arises the idea that soil freezing might give inherently different patterns of element cycling and loss of nutrients in the ecosystem. Thus, changes in freeze-thaw patterns of ground surface not only in boreal and temperate regions but also permafrost region might have significant effects on ecosystem function associated with microbial metabolic activity in soil by the global warming.

In the next chapter, we will introduce high N_2O emission under the soil freeze-thaw condition which has been observed mainly in temperate regions. We are going to investigate what happen in soil under the freeze-thaw condition.

3. HIGH N_2O EMISSION AND SOIL FREEZE-THAW EVENTS

3.1 Freezing makes uncertain temperature dependency of N_2O emission from soil

N_2O is one of the greenhouse gases with 296 global warming potential (GWP) in 100-year time horizons as well as a potential destroyer of stratospheric ozone layer. More than 50% of N_2O is produced in soil mainly from the soil microbial activity related to the N transformations such as nitrification and denitrification, as a byproduct and an intermediate product, respectively (Bremner and Blackmer 1978). Because N_2O has environmentally risky chemical properties as described above and the relative contribution of these N transformations to total N_2O production of soil varies with environmental factors such as aeration, moisture and temperature (eg. Smith et al. 2003), N_2O dynamics between soil and atmosphere have been a global concern. Generally speaking, the biological processes respond positively to temperature upshift within a range in which the enzymes are stable and retain full activity. Smith (1997) summarized temperature dependency of N_2O emissions from soils under both field and laboratory measurements as Q_{10} (defined as the ratio of a process at T + 10 K to the rate at 10 K) and it shows positive value. Therefore, the microbial metabolic activities associated with N_2O production might be missing in model calculations for an annual budget at temperature near 0°C (Sommerfeld et al. 1993). However, several reports show significance of high N_2O emission during winter even though almost all of the studies dealing with N_2O fluxes were less focused on winter. The potential for high N_2O emission at the time of thawing can be ecologically important to natural ecosystems (Goodroad and Keeney 1984). One of earlier reports by Duxbury et al. (1982) is published in *Nature*, and both field monitoring and laboratory experiment had intensively started since '90 based on detail discussion by Flessa et al. (1995).

Flessa et al. (1995) observed the highest N_2O flux rates (~2700 μg N_2O-N m^{-2} h^{-1}) in the winter under freeze-thaw condition of the soil, during the measurement of annual N_2O emission from July 1992 to August 1993 at 4 different sites in the research farm in southern Germany. Because similar time patterns of N_2O fluxes from arable soils had been reported in several studies, Flessa et al. (1995) divided the phenomenon by two kinds of different scenarios;

(1) N_2O accumulation in the frozen layer, which were produced in the unfrozen subsurface layer, and its release to the atmosphere when the frozen layer thaws

(2) Denitrification stimulated in thawed surface layer during thaw-refreeze and diurnal freeze-thaw conditions

3.2 Case 1: N_2O production in unfrozen subsurface layer under frozen condition

Burton and Beauchamp (1994) monitored soil profile N_2O concentration using a multilevel sampling probe during 2 winter periods. N_2O accumulated at depths of 15 cm from the beginning of February through the middle of March. Accumulated N_2O dissipated following the thawing of the soil in March, indicating that disrupting the continuous frozen layer allowed the release of N_2O trapped below the frozen layer. Calculations of surface flux based on an average concentration gradient for each day indicated 128 μg N_2O-N m^{-2} h^{-1} of average flux for the entire sampling period and peak flux of 660 and 793 μg N_2O-N m^{-2} h^{-1} during thaw events in 1988-1989 and 1989-1990, respectively.

However, since Burton and Beauchamp (1994) did not check N_2O emission from ground surface using the closed chamber method, we only know possible degree of amplification of the N_2O emission caused by thawing of frozen layer, which trapped N_2O produced in subsoil, assuming that concentration gradient of N_2O is caused by reducing reaction in the surface soil layer by denitrifying communities. Moreover, Burton and Beauchamp (1994) can only infer about the process of N_2O production due to the absence of direct measurements of nitrification or denitrification. Nevertheless, the observation by Burton and Beauchamp (1994) strongly indicates significant N_2O production under low temperature conditions (near 0°C) in soil. Koponen et al. (2004) confirmed significant N_2O emissions without soil freezing history in silt and loam soils incubated at 3°C in the laboratory, though the source of N_2O production was not determined. Holtan-Hartwig et al. (2002) reported that low temperature, at or near 0°C, could suppress N_2O reducing activity of denitrifying communities in the soil, therefore, at least, accumulation of N_2O below frozen layer possibly can occur with preventing N_2O reduction to N_2 in the soil profile, whether main source of N_2O is unknown.

3.3 Case 2: Denitrification stimulated in thawed surface layer under thaw-refreeze and diurnal freeze-thaw conditions

The availability of easily degradable C seems to be one of the essential factors for high N_2O emissions during and following freeze-thaw condition. Related to the denitrification enzyme activity, results presented by Christensen and Tiedje (1990), Christensen and Christensen (1991) and van Bochove et al. (2000) clearly showed that soil freeze-thaw cycles caused DOC (dissolved organic carbon) flush. In fact, Herrmann and Witter (2002) conducted laboratory experiment to characterize the source of DOC that becomes available upon soil freeze-thaw cycles and revealed that microbial biomass C contributed 65% to the DOC flush. Röver et al. (1998) and Teepe and Ludwig (2004) clearly showed high N_2O emission following to the peak observed immediately after thawing. Therefore, it could be possible that activity of denitrifying communities is temporarily stimulated by these thaw-refreeze and diurnal freeze-thaw cycles because of rich DOC and depletion of O_2 during the DOC consumption, as long as NO_3^--N is present and N_2O reductase is not induced in soil.

3.4 Different hypothesis: N_2O production in frozen surface layer

Actually, Röver et al. (1998) first reported the source of high N_2O emission observed during winter, soil freeze-thaw condition, as biological processes. They observed high N_2O emission from December 1995 to March 1996 in a fertilized (220 kg N ha^{-1}) and an unfertilized plot on silty loam (pH: 7.1) cropped with winter wheat in winter season in the northern foreland of the Harz mountains in Lower Saxony, Germany, which accounted for as much as 70% of annual N_2O emission. To determine whether the N_2O production can be attributed to biotic or abiotic processes in soil, the incubation experiment was conducted using re-packed soil samples (82.5% of water filled pore space, 1.48 g cm^{-3} of bulk density) collected from the fertilized plot and N_2O emission rates were measured under periodical changes in incubation temperature (-4 to +3°C) in soil samples with or without sterilization by gamma-ray radiation. The sterilized

soil samples showed no N_2O emission (less than 4 μg N_2O-N m^{-2} h^{-1}) whereas the N_2O emission from non-sterilized soil samples showed similar high N_2O emission to that of field observations, indicating that microbial metabolic activities had been the main source for the N_2O emissions that found under soil freeze-thaw conditions in field and laboratory experiments. This is the first demonstration that microbial metabolic activities mainly contribute the high N_2O emission during winter, especially under soil freeze-thaw condition, other than physical release (Burton and Beauchamp 1994) or chemical reaction (Christiason and Cho 1983). Thus, data obtained by Röver et al. (1998) firstly showed that N_2O is biologically produced even in frozen conditions (-4°C).

3.5 Process leading to high N_2O emission from soil under freeze-thaw condition

To identify which N transformation processes occurring during various stages of a freeze-thaw condition are responsible for the high N_2O emission, Müller et al. (2002) separately labeled the ammonium and nitrate pools in an old grassland soil with ^{15}N. Three treatments (addition of $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$, $^{15}NH_4{}^{15}NO_3$) were conducted on the soil (Organic C: 66 g kg^{-1}; pH: 6.2) near Giessen, Germany, which showed distinct N_2O emissions during freeze-thaw events (Kammann et al. 1998). Ten weeks later, they obtained soil samples and subjected the soil samples to a freeze-thaw event (frozen at -20°C for 2 days and then thawed at +20°C). The enrichment of N_2O increased during the thawing period to peak values of 9.3 ($NH_4{}^{15}NO_3$ treatment), 5.6 ($^{15}NH_4NO_3$ treatment) and 17.3 ($^{15}NH_4{}^{15}NO_3$ treatment) atm% ^{15}N which corresponding to the enrichment of the NO_3^- that appeared during the freezing. This indicates that the burst of N_2O is associated with the reduction of NO_3^- which became available during the freeze-thaw cycles.

3.6 Factors affecting high N_2O emission under soil freeze-thaw condition

Independent from such identification of N transformation processes responsible for N_2O production under the soil freeze-thaw condition, Teepe et al. (2001) intensively measured N_2O emission from undisturbed soil samples (16 cm height, 14.5 cm diameter) under controlled freeze-thaw conditions, based on his own field measurement in an agricultural, a fallow and a forest system during the winter of 1995-1995 in Central Germany (Teepe et al. 2000). Teepe et al. (2004) examined the effect of the duration of freezing, initial soil moisture and texture on N_2O emission from soil during cycles of freezing and thawing of soil using undisturbed core (8 cm depth, 14.5 cm diameter) collected from three agricultural sites. When examining the effect of texture, the levels of organic matter content (11-12 g C kg^{-1} and 0.9-1.1 g N kg^{-1}), the inorganic N content (1.7-2.7 mg NO_3^--N kg^{-1} and 1.2-2.1 mg NH_4^+-N kg^{-1}) and the matric potential (-0.5 kPa) were similar but levels of WFPS (%) and pH (KCl) were different among soil samples: sand, 75% and 5.3; silt, 78% and 7.1; and loam, 87% and 7.1, respectively, the effect of the freezing duration was significant (11.5 > 2.4 > 1 days). Even on samples with the same texture (silt), the effect of freezing duration was significant (10 > 9.3, 8.5 > 7 > 2.7 days) and described log-normal distribution-like parabola reaching a peak of 64% of WFPS in each freezing duration. Teepe and Ludwig (2004) examined the effect of tree species and duration of freezing on N_2O emission under alternate freezing and thawing conditions. Undisturbed core samples (14.5 cm diameter, 8+1~5 cm depth depending on the thickness of mineral layer) were collected from a spruce site (Silt-spruce) and three beech sites (Sand-beech, Silt-beech, Loam-beech) in the south of Lower Saxony, Germany, and adjusted matric potential at -0.5 kPa, which was common during soil thawing under field conditions. In the samples collected from the beech forest, the cumulative N_2O emissions were not significantly different between 2.7 and 11.3 days of soil freezing duration, but were significantly different and in the order corresponding to WFPS of the soil sample: Loam, 71.2% and 85.7 mg N_2O-N m^{-2}; Silt, 64.1% and 4.9 mg N_2O-N m^{-2}; and Sand, 44.4% and 0.6 mg N_2O-N m^{-2}. In addition, the cumulative

emission was lower in the samples collected from spruce (0.1 mg N_2O-N m^{-2}) than that from the beech forest in the silty soil samples. However, there were no clear differences in the physicochemical properties of soil samples (pH H_2O, pH KCl, total C and N, CEC, NO_3^- content) to explain the difference in N_2O emission dynamics between the samples collected from different tree species instead of WFPS and NH_4^+ content.

3.7 Summary: Understanding of high N_2O emission during winter related to soil freezing

Higher N_2O emissions observed in the longer duration of freezing period (Teepe et al. 2004) suggested that facultative denitrifying bacteria switch to anaerobic activity in the anaerobic frozen conditions and the number of bacteria changing from aerobic to anaerobic activity increases as the freezing period increases (Röver et al. 1998). This interpretation is supported by the observation that DOC addition without freezing could not reproduce comparably high N_2O emission that observed under controlled freeze-thaw condition in laboratory (Ludwig et al. 2004; Sehy et al. 2004). These results suggested that the unfrozen microsites in soils might have high concentration of DOC and NO_3^- for denitrifying communities as well as O_2 deficiency created by ice surrounding around surface of soil particle which limits gas exchange. Totally, soil freezing causes favorable conditions for denitrification (Teepe et al. 2001; Koponen et al. 2004).

It is certain that both N_2O production and accumulation occur simultaneously in the surface frozen layer, although it is difficult to imagine when comparing with general temperature dependency of microbial metabolic activity as the alternative explanation of high N_2O emission under soil freeze-thaw condition. Thus, contribution of stimulated denitrification under thaw-refreeze and diurnal freeze-thaw conditions and N_2O production in unfrozen subsurface layer to the high N_2O emission observed during winter might be smaller.

3.8 Future approaches to modeling N_2O production and emission from the view point of latest soil microbiology

The change in physiological status of denitrifying communities mentioned above is quite ordinal and, in the future, we can parameterize such a shift of metabolic pattern from environmental parameters surrounding in these microorganisms under the freeze-thaw condition. On the other hand, change in community compositions of microorganisms those involve in N_2O production in soil seems to be interesting and can be a strong tool to characterize the different responses of N_2O emission following the freeze-thaw cycles. Cavigelli and Robertson (2001) find significant diversity in the sensitivity of denitrifying isolates' N_2O reductase to O_2, and Chèneby et al. (2004) demonstrates the diversity of denitrifying bacteria that participate in several steps in the denitrification pathway: reduction of NO_3^- to N_2O or N_2, in soil. However, denitrification by fungi can be ecologically significant, since certain amount of O_2 is necessary for fungal denitrification to occur and the final product of NO_3^- or NO_2^- reduction is not N_2, but N_2O (Zhou et al. 2001). It is reported that, the soil microbial community appears to be dominated by bacteria during the summer season (Lipson et al. 2002), while fungi are dominated during winter in the Colorado alpine (Schadt et al. 2003). Actually, there is little evidence for fungal contribution to N_2O production in soil, even if some fungi genetically have potential for N_2O production via NO_3^- or NO_2^- reduction (Shoun et al. 1992). It's interesting, however, Pietikäinen et al. (2005) found that fungal and bacterial growth rate, examined by ^{14}C-acetate incorporation into ergosterol and thymidine incorporation, respectively, were affected differently by temperature and fungal growth were predominant at low temperature (below 10°C). Therefore, we have come to the conclusion that N_2O production by fungi in frozen layer is one of the current hypotheses that we have been working to explain excessively high N_2O emission under the soil freeze-thaw condition.

4. EFFECTS OF SOIL FREEZE-THAW CYCLES ON SOIL MICROBIAL COMMUNITY

4.1 Background of the study

Soil freezing and thawing affect the ecology not only in the polar region but also in the boreal and temperate regions. Due to the progression of the global warming, the boreal and polar region may get more frequent soil-freezing and -thawing in a year (Groffman et al. 2001; Poutou et al. 2004), whereas temperate regions may get less frequent diurnal soil freeze-thaw cycles during winter to early spring. What is more, soil freeze-thaw event seems to be lost in temperate regions like tropical regions. To understand the potential ecological significance of soil freeze-thaw cycles on soil functions in which microbial activity is highly related, such as organic matter decomposition, nitrification and denitrification, we have started to study the effect of soil freeze-thaw cycles on soil microbial community since 2001 using soil samples collected from temperate and tropical regions. Because one of the earlier reports showed mortality of soil microorganisms by the soil freeze-thaw cycles (Soulides and Allison 1961) and showed that the mortality leads to temporary activation of microbial activity in soil (Skogland et al. 1988; Christensen and Christensen 1991; Deluca et al. 1992), we have compared changes in microbial biomass and activity caused by the freeze-thaw cycles. Our first demonstration using a soil sample collected from a temperate arable field in Tokyo University of Agriculture and Technology (Yanai et al. 2003) showed that soil freeze-thaw cycles (4 times of alternation of -13 to + 4°C) decreased soil microbial biomass (Table 1), determined by the chloroform fumigation and extraction method, to 75% of the unfrozen control (kept at 4°C) and substrate utilizing activity, examined by using Biolog Eco plates®, to 71% of average well color development (AWCD) of the unfrozen control (on day 3 of incubation period; Fig. 1). However, nitrification potential, examined by NO_3^- accumulation in soil after the addition of 200 μg NH_4-N g^{-1} soil of $(NH_4)_2SO_4$ followed by incubation at 60% of maximum water holding capacity at 27°C, did not show any inhibitory effect from the soil freeze-thaw cycles (Fig. 2). Generally speaking, nitrifying communities are sensitive to environmental stresses (Alexander

Table 1. Effect of soil freeze-thaw cycles on population size and microbial biomass.

	Unfrozen	Freeze-thaw
Dilution plate method (CFU g^{-1}soil)		
Culturable bacteria	2.6×10^7	3.0×10^7
Crystal violet-tolerant bacteria	4.0×10^5	5.6×10^5*
Fungi	6.2×10^4	6.5×10^4
Most probable number method (MPN g^{-1}soil)		
Ammonia-oxidizing bacteria	4.1×10^4	1.5×10^4
Nitrite-oxidizing bacteria	2.5×10^4	1.4×10^4
Cellulose decomposing bacteria	7.0×10^4	13×10^4
Chloroform fumigation and extraction method (μg C or N g^{-1}soil)		
Soil microbial biomass C	335	297*
Soil microbial biomass N	56.2	46.2*

* indicates the result of ANOVA ($P < 0.05$). Modified from Yanai et al. (2003).

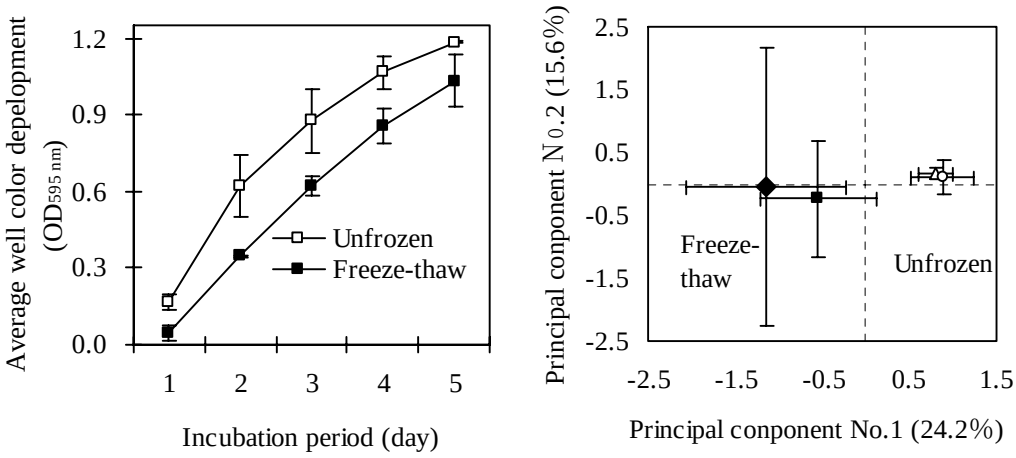

Fig. 1. Effect of soil freeze-thaw cycles on substrate utilization activity of soil (A) and substrate-utilization pattern, based on the readings of days 3 of incubation period (B). Each plot shows mean and SD of duplicate in (A) and triplicate in (B), respectively (modified from Yanai et al. 2003).

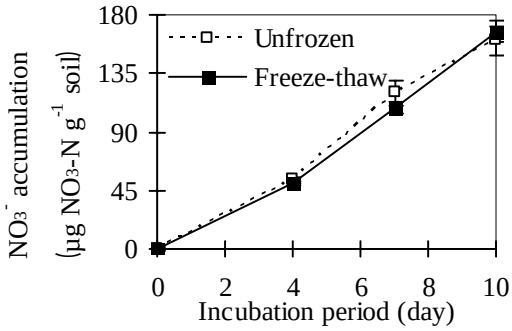

Fig. 2. Effect of soil freeze-thaw cycles on nitrification potential of soil. Soil samples were amended with 200 µg NH_4-N g^{-1}soil of $(NH_4)_2SO_4$, adjusted to 60% of maximum water-holding capacity and incubated at 27°C. Each plot shows mean and standard deviation of triplicate (modified from Yanai et al. 2003).

1977; Killham 1994), so we had expected that the nitrification potential was categorized into one of the soil functions that could be inhibited by the freeze-thaw cycles. Because Neilsen et al. (2001) also observed few inhibitory impacts on nitrification by the freeze-thaw cycles, we conducted extensive studies to analyze the effect of soil freeze-thaw cycles on soil microbial biomass, organic matter decomposition potential and nitrification potential (Yanai et al. 2004a, b). In addition, to explain the high N_2O emission caused by the freeze-thaw cycles, its effect on denitrifying potential has also been conducted.

4.2 Soil microbial biomass

Soil freeze-thaw cycles (Fig. 3) decreased soil microbial biomass N by 2 to 55% (Fig. 4). There were 2 types of soil samples in terms of susceptibility to the freeze-thaw cycles: 3 soil samples in which much higher microbial biomass N remained (88, 97 and 98%) and 5 soil samples in which much lower microbial biomass N remained (55, 57, 63, 65 and 68%) following the freeze-thaw cycles ($P < 0.01$). The remaining rate, called as the microbial survival, was positively correlated with soil pH (KCl) value ($r = 0.890$**, $n = 8$) and pore volume that the diameter size ranged from 9.5 to 6.0 µm- ($r = 0.995$**, $n = 4$) and 6.0 to 3.0 µm-capillary equivalent ($r = 0.970$*, $n = 4$), which was determined by capillary rise relationship (Aluko and Koolen 2000). These results suggested that there might be a protective niche as micro-sites available for large parts of soil microorganisms (Tanaka and Sakamoto

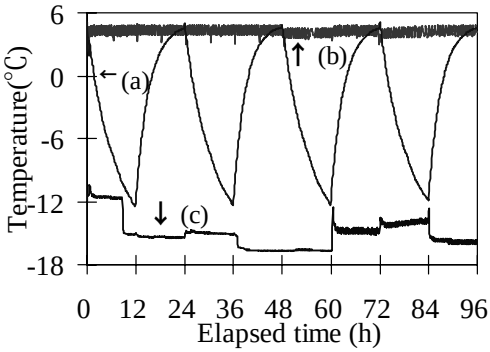

Fig. 3. Temperature treatment for preparation of soil freeze-thaw cycles (a) and the unfrozen control (b) using a freezer (c). Modified from Yanai et al. (2004a).

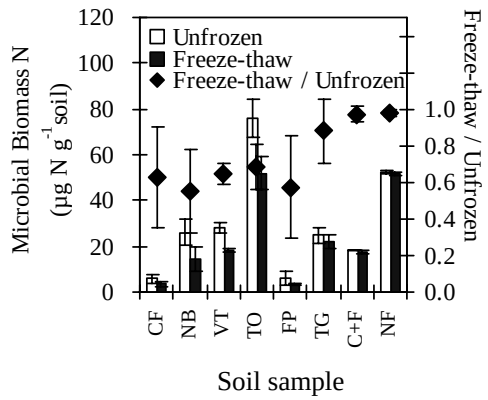

Fig. 4. Effect of soil freeze-thaw cycles on microbial biomass N of soils (modified from Yanai et al. 2004a).

1972) in the presence of unfrozen water and pH level of unfrozen water may control the degree of microbial survival under the soil freeze-thaw condition independent on microbial community structure in each soil.

4.3 Organic matter decomposition potential of soil

Organic matter decomposition potential, one of the most important soil functions to release N and other mineral nutrients from organic matter and examined by rice straw-or chitin-amended respiration at 2% (w/w), showed slightly but opposite responses to the freeze-thaw cycles depending on the substrate examined. The cumulative amount of CO_2 emission (78 days of incubation period) showed that rice straw-mended respiration decreased 6% while chitin-amended respiration increased 7% (Fig. 5). This opposite response seems

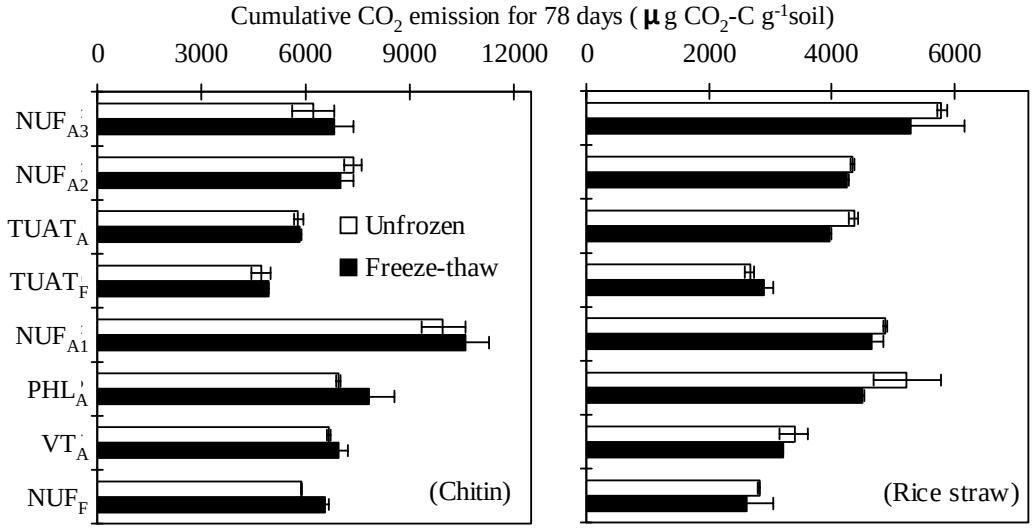

Fig. 5. Effect of soil freeze-thaw cycles on organic matter decomposition potential- chitin amended respiration (left) and rice-straw amended respiration (right). Each substrate was amended at 2% (w/w) to the soil sample. Modified from Yanai et al. (2004a).

possible because our previous result showed decrease in the substrate utilizing activity to 71% of the unfrozen control on an average but utilization of a specific substrate "xylose" increased 2.2 times by the freeze-thaw cycles (Yanai et al. 2003). Although the reason why the increase in utilization of xylose occurred due to the freeze-thaw cycles is still unclear, we discussed the possible contribution of fungal activity in soil in terms of the increase in chitin decomposition potential. In brief, it might be because fungi has higher contribution than bacteria to produce chitinase in soil (Miller et al. 1998; de Boer et al. 1999) and germination of microconidia of *Fusarium oxysporum* in selected 3 soil was significantly enhanced by the freeze-thaw cycles (Fig. 6). Since partial sterilization enhanced germination of fungal spore (de Boer et al. 2003) and soil freeze-thaw cycles significantly decreased soil microbial biomass (Fig. 4), we concluded that soil freeze-thaw cycles caused partial sterilization and it might affect organic matter decomposition potential of soil by disturbing structural composition of soil microbial community (Yanai et al. 2004a).

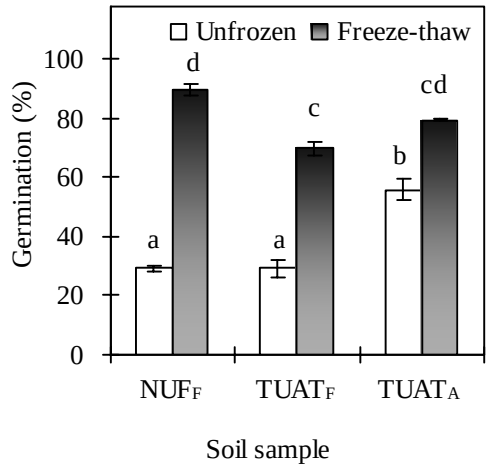

Fig. 6. Germination of fungal spore, tested by microconidia of *Fusarium oxysporum*. Values show mean and SE (n = 2). Values marked with different letters are significantly different, according to the Fisher's LSD ($P < 0.05$). Unpublished data.

4.4 Nitrification potential of soil

Nitrification potential, examined by NO_3^- accumulation in soil after the addition of 200 µg NH_4-N g^{-1} soil of $(NH_4)_2SO_4$ followed by incubation at 60% of maximum water holding capacity at 28°C, was not always inhibited by the freeze-thaw cycles as we observed previously (Fig. 2), and we concluded as follows: (1) as is well known, nitrification potential itself was strongly pH dependent (Fig. 7), that is, the soil samples which had pH (KCl) less than 4.1 showed no NO_3^- accumulation over 70 to 90 days of incubation, and nitrification potential (NO_3^- accumulation rate per g dry soil per incubation day) was higher in the soil samples which had 5.3, 5.5 and 6.5 of

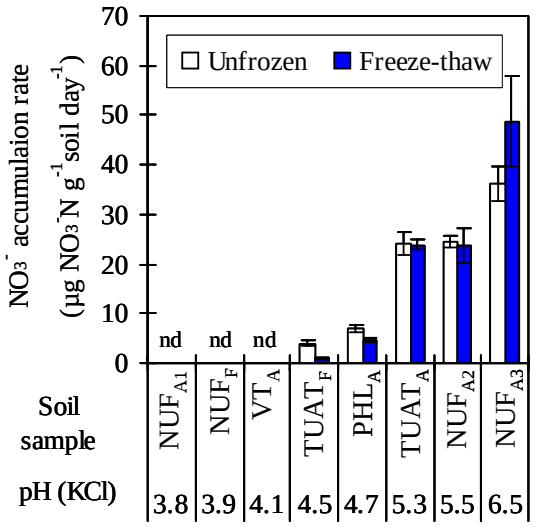

Fig. 7. Effect of soil freeze-thaw cycles on nitrification potential of soils, based on NO_3^- accumulation after the addition of $(NH_4)_2SO_4$ solution to be 200 µg NH_4-N g^{-1}soil and at 60% of maximum water-holding capacity, followed by the incubation at 27**°C**. Values show estimated mean and SE of linear regression (Modified from Yanai et al. 2004b).

pH (KCl) than those 4.5 and 4.7 of pH (KCl) (Fig. 7); (2) the soil freeze-thaw cycles did not inhibit nitrification potential in the 3 soil samples, while clearly inhibited in the 2 soil samples; (3) different responses of the nitrification potential to the soil freeze-thaw cycles might be interpreted as pH decline in the unfrozen water in soil, which covers the soil matrix and microbial cells with a thin water film (Wolfe et al. 2002); and (4) $(NH_4)_2SO_4$ addition, which is the practical method to examine nitrification potential of soil (Schmidt and Belser 1994), had a problematic result in 1 out of 9 soil samples we had examined to monitor NO_3 production in soil, instead, CH_3COONH_4 seemed to be better substrate to monitor NO_3 production in soil (Fig. 8).

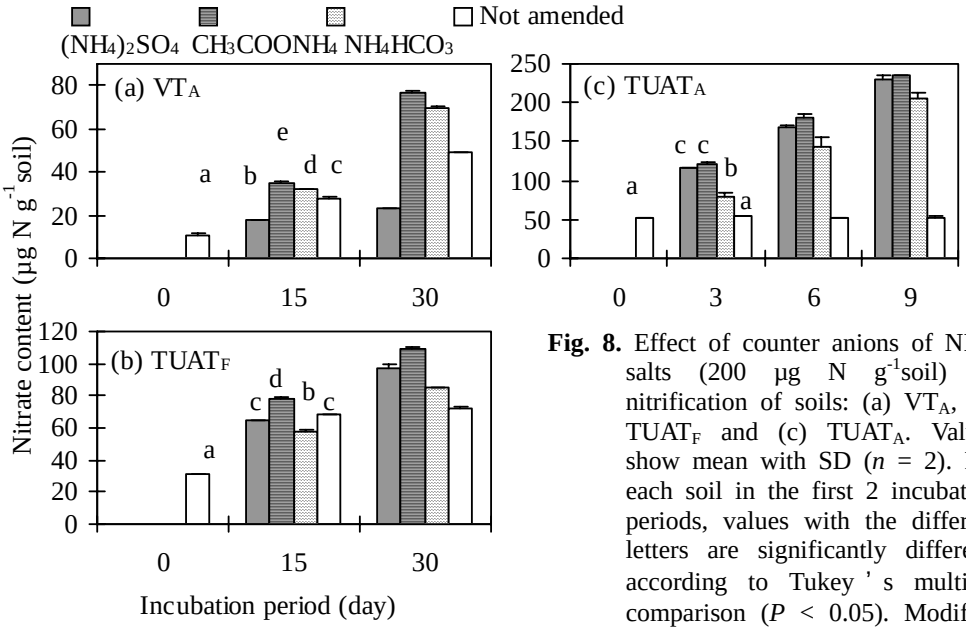

Fig. 8. Effect of counter anions of NH_4^+ salts (200 µg N g^{-1}soil) on nitrification of soils: (a) VT_A, (b) $TUAT_F$ and (c) $TUAT_A$. Values show mean with SD (n = 2). For each soil in the first 2 incubation periods, values with the different letters are significantly different, according to Tukey's multiple comparison ($P < 0.05$). Modified from Yanai et al. (2004b).

4.5 Denitrification potential of soil

Denitrifying potential, examined based on the C_2H_2 inhibition method (Yoshinari et al. 1977), showed a somewhat complicated response to the freeze-thaw cycles (Yanai et al. submitted). Our tentative interpretations are as follows: (1) denitrifying potential could not be characterized by physico-chemical properties of soils unlike nitrification potential (Yanai et al. 2004b); (2) the soil freeze-thaw cycles caused the partial sterilization effect on denitrifying communities themselves; and (3) soil freeze-thaw cycles might cause imbalance of N_2O producing and reducing activity which was characterized as having more severe inhibitory impact on N_2O reducing activity than N_2O producing activity, as demonstrated by Holtan-Hartwig et al. (2002) on low temperature response. We had speculated that imbalanced activity of N_2O production and reduction is a primary trigger to cause high N_2O emission under soil freeze-thaw condition, but further study is needed.

5. DISCUSSION

As we reviewed above, the presence of unfrozen water supports metabolic activity of microorganisms survived in frozen soil, whereas soil freeze-thaw cycles cause apartial sterilization effect-like stimulation of microbial metabolic activity which could result in

changes to ecosystem functions such as organic matter decomposition, nitrification and denitrification. In other word, the mortality of a part of soil microorganisms caused by soil freeze-thaw cycles might release N-rich compounds into the soil (Fig. 4), and subsequent mineralization of these N compounds can be disturbed by the freeze-thaw cycles. Actually, our results demonstrated slightly but significant disturbance in organic matter decomposing potential of soil (Fig. 5). However, the trend was opposite depending on the substrate amended and such disturbance caused by the freeze-thaw cycles is possibly due to the changes in structural composition of soil microbial community, such as activation of fungi (Fig. 6). In addition, the decreased nitrification potential (Fig. 7) and the damages on N_2O reducing activity but not on N_2O producing activity (Yanai et al. submitted) may indicate the stimulation of NH_3 volatilization (Nakahara et al. in this symposium) and N_2O emission from soil, respectively, by the soil freeze-thaw cycles. Therefore, changes in soil freeze-thaw patterns might affect N dynamics in the future, although we do not clearly know current situations on N dynamics in Siberian soil.

Moreover, the indirect effect of soil freeze-thaw cycles on soil microbial community seems also negligible. Poutou et al. (2004) concluded that soil freezing acts to warm the high latitudes in winter and autumn (releasing of heat at the surface) while thawing at spring cools down the surface. As we cited above, it is not the case in Siberia but in the different tundra, the microbial processes that occur in cold soils, under the snow pack, may be complex and surprising, such as during the summer the soil microbial community appears to be dominated by bacteria (Lipson et al. 2002), while the fungi are dominated during winter (Schadt et al. 2003). During such shift of microbial community structure, N-rich compounds might be released that are subsequently mineralized, providing the bulk of annual plant N needs. In addition, such seasonal community structure changes may include changes in organic matter decomposition pattern as shown in Lipson et al. (2002): the utilization of vanillic acid (phenolic compound) was highest in the winter, while the utilization of glycine (amino acid) was lowest in the winter. Changes in soil freeze-thaw patterns could affect mitigation capacity of seasonal climate fluctuation, which can lead to changes in microbial functions, including N transformation processes, in Siberia in the future.

In situ flux monitoring for greenhouse gases and nutrient solutes are critically important works to detect and predict changes in ecosystem functions. We suppose that we need to collaborate more to understand N dynamics in Siberian soil including patterns of soil freeze-thaw cycles. Analysis of the responses to the N transformation processes to soil freeze-thaw cycles can give some ideas about both current and possible future N dynamics in Siberian soil.

6. CONCLUSIONS

Soil freezing plays a significant role in the ecology of the polar region and the soil freeze-thaw pattern may change as an indirect effect of global warming followed by the melting of the permafrost and the active layer getting thicker. Soil freeze-thaw cycles may affect soil microbial community as decreasing microbial biomass followed by release of N-rich compounds into soil or disturb some soil microbial community functions together with community structure. To predict possible consequences how the soil microbial community could be affected by changes in soil freeze-thaw pattern in Siberia, which can affect flux of greenhouse gases and dynamics of nutrient solutes, it may be strongly necessary to characterize and evaluate microbial responses to the soil freeze-thaw cycles, together with the results of field monitoring.

Acknowledgments. The authors are grateful for the invaluable comments of Professor M Okazaki

and Dr. SD Kimura, Tokyo Univ. Agr & Tech. The work was financially supported by the Sasakawa Scientific Research Grant from The Japan Science Society (16-315), the TUA&T 21 Century COE Program (Evolution and Survival of Technology based Civilization), and JSPS Research Fellowships for Young Scientists (17-6518).

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