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N_2O emission from a temperate forest soil during the freeze-thaw period: A mesocosm study

Bo Peng [a,b], Jianfei Sun [a], Jun Liu [a], Weiwei Dai [a], Lifei Sun [a,b], Guangting Pei [a,b], Decai Gao [a,b], Chao Wang [a], Ping Jiang [a], Edith Bai [a,c,*]

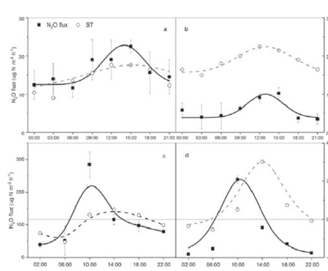
[a] CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110016, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] School of Geographical Sciences, Northeast Normal University, Changchun, 130024, China

HIGHLIGHTS

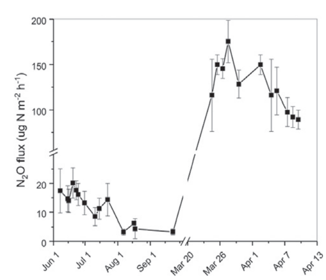
- Soil N_2O emission was high during the freeze-thaw (FT) period in the temperate forest.
- Soil enzyme activities were maintained at high level at the freezing stages during the FT period.
- Physical isolation of previously produced N_2O at the freezing stage was an important mechanism for N_2O emission.
- The biological mechanism could sustain after several numbers of the FT cycles.

GRAPHICAL ABSTRACT

Diurnal dynamic N_2O flux in growing season (a ,b) and FT period (c,d).



N_2O flux during the sampling period.



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ABSTRACT

Nitrous oxide (N_2O) is an important greenhouse gas and is involved in the destruction of ozone layer. However, the underlying mechanisms of the high soil N_2O emission during the freeze-thaw (FT) period are still unclear. Here, we conducted a mesocosm study with high frequency *in situ* measurements to explore the responses of soil microbes to the FT cycles and their influences on soil N_2O emission. We found the high N_2O emission rate during the FT period was mainly due to the release of substrates, the maintenance of high enzyme activities at the freezing stage, and the fast recovery of microbial biomass nitrogen (MBN) and high microbial activities at the thawing stage. Physical isolation of previously produced N_2O was an important mechanism for the higher N_2O flux at the thawing stage. With increasing numbers of the FT cycles, MBN at the thawing stage remained stable and potential dehydrogenase activities at the thawing stage also remained stable after the first eight FT cycles and only declined during the last two cycles, suggesting the sustainability of the biological mechanisms. Our study suggests that although MBN declined, microbial enzymes could maintain high activities at a few degrees Celsius below zero in this temperate forest soil and produce high N_2O fluxes even at the freezing stage, which were trapped under the ice layer and released at the thawing stage, resulting in high soil N_2O emission during the FT period.

1. Introduction

Nitrous oxide is an important greenhouse gas and is involved in the destruction of ozone layer (Abalos et al., 2018; Billings, 2008; Montzka

* Corresponding author at: Institute of Applied Ecology, Chinese Academy of Sciences, No. 72 Wenhua Road, Shenyang, Liaoning 110016, China.
E-mail address: baie@iae.ac.cn (E. Bai).

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et al., 2011; Ravishankara et al., 2009). Soil sourced N_2O emission accounts for 60% of total N_2O emission (IPCC, 2014), and up to 70% of its annual flux might be from the freeze-thaw (FT) period in temperate regions (Kreyling et al., 2008; Yao et al., 2010; Zhang et al., 2003). The underlying mechanisms of N_2O emission during the FT period still need to be explored for better understanding of its high emission rate during the period and better prediction of its effect on global climate change.

N_2O flux during the FT period was found to be mainly emitted during the thawing period (Koponen and Martikainen, 2004). First, the physical release of accumulated N_2O during the freezing period has been considered as a reason of the high flux during the thawing period (Bremner et al., 1980). An ice layer can stop the diffusion of N_2O (Nemeth et al., 2014; Teepe et al., 2001), which could cause the accumulation of N_2O that is later emitted when the ice layer thaws. However, this physical blocking effect alone can only cause higher fluxes during the thawing period than during the freezing period, but cannot cause higher mean fluxes during the FT period than during the growing season (Rover et al., 1998). Most researchers believe N_2O emissions during the FT period were related to biological processes (Muller et al., 2002; Song et al., 2006; Zhu et al., 2009), such as nitrification and denitrification processes (Abbasi and Adams, 2000; Muller et al., 2002). Increased availability of substrates for N_2O production (*e.g.* ammonium, nitrate, dissolved organic N and other liable N) have been found during freezing, which could partly explain the immediacy and magnitude of soil N_2O fluxes during thawing (Deluca et al., 1992; Herrmann and Witter, 2002; Matzner and Borken, 2008; Song et al., 2017). The increased availability of substrates during the freezing period may be attributed to three mechanisms: (i) soil aggregate disruption, (ii) microbial cell lysis or cytoplasmic secretion, and (iii) organic matter mineralization (Congreves et al., 2018). Then during the thawing period, with the increase of soil temperature, microbes could quickly use these substrates and convert them into N_2O *via* nitrification and denitrification processes. In addition, the relatively high soil moisture content during the thawing period is also generally beneficial to N_2O production (Chen et al., 2015; Weitz et al., 2001). Although this biological mechanism could explain the higher N_2O fluxes during the FT period, a few questions still remain about this mechanism. First, whether microbes could respond so quickly to temperature changes remains unclear, especially when they are partly killed or impaired during the freezing period. Second, whether this mechanism is sustainable after a few cycles of freezing and thawing is questionable. For example, soil aggregate disruption might only happen during the first several FT cycles and then no more aggregates could be disrupted. In addition, lab simulations of the freeze-thaw event may not be able to reflect the actual conditions due to the unrealistic temperature changes and the lack of plant presence (Gao et al., 2018). Therefore, an experiment with high frequency *in situ* measurements on soil N_2O fluxes and soil microbial activities and with more numbers of FT cycles is needed to answer these questions.

Changbai Mountain in northeast China has typical temperate forest ecosystems in East Asia, which experiences frequent FT cycles during the spring. To identify the mechanisms of high N_2O emission during the FT cycles, diurnal dynamics of soil N_2O emissions and soil extracellular enzyme activities (*i.e.*, β-glucosidase (BG), cellobiohydrolase (CE) and *N*-acetyl glucosaminide (NAG) and DEH were determined. Due to the reactive nature of soil enzymes, we believe the variations of these enzyme activities would show if microbes were active during the freezing period. Our hypotheses were: 1) Microbial biomass nitrogen (N) would reduce at the freezing stage and could quickly recover at the thawing stage; 2) Microbial enzyme activities related to N production processes could remain high at the freezing stage, resulting in high N_2O production at the freezing stage, which was trapped under the ice layer and later released at the thawing stage; 3) After a few cycles of FT, microbial activities could still remain high and MBN at the thawing stage would not decline, resulting in high N_2O fluxes during the FT period. The objectives of this study were: 1) To understand the roles of physical release and biological processes in N_2O emission during the FT period; and 2) To explore the responses of soil microbes to the FT at a small temporal scale and after a few number of FT cycles.

2. Materials and methods

2.1. Study site

The study was conducted in the Changbai Mountain National Nature Reserve, which was established in 1960 in northeast China (41°42′N, 127°38′E). The study area has a typical temperate climate with long and cold winters and warm summers. Mean annual temperature in the study area is 3.6 °C, with the highest mean monthly temperature in July, and the lowest in January. Mean annual precipitation is 745 mm, mainly falling between May and September. The growing season is from late May to early October (154 days), with the mean temperature at 16.7 °C. Natural Korean pine and broad-leaved mixed forest is distributed from approximately 750–1000 m asl. The soil is classified as a dark brown soil developed from volcanic ash (Albic Luvisol), with mean soil organic carbon at 3.35%, total nitrogen at 0.28% and pH at 5.85 (Table 1).

2.2. Experimental design

Due the high biodiversity in the study area, we used pot experiment to keep the effect of vegetation consistent among treatment. Six separate plots (1 m × 1 m) with buffer zones (1 m at least) were randomly selected in the forest stand in Changbai Forest Ecosystem Research Station. The 0–10 cm soil in each plot was sampled in October 2014 by carefully removing the litter layer and the soil bulk density (0–10 cm mean) and total nitrogen (TN) content were determined. Then, the soils were sieved (2 mm) and homogeneously mixed and then divided into six samples equally. Each sample was evenly put into two pots, with one for gas sampling and the other for soil sampling. A total of 12 pots were established and the bulk density was 0.85 g cm^{-3} in each pot. The pots were cylindrical (diameter ×height: 50 cm × 10 cm) and made of steel for heat conduction. There were three holes on the bottom of each pot to ensure leaching activities. Each pot had one 10-yr old seedling of Korean pine tree and the entire height of the pot was embedded into soils to the depth of 10 cm. The pots were at least 1 m apart from each other and were put under natural conditions for 8 months before the experiment started in the growing season in June 2015.

2.3. Gas sampling and analysis of N_2O

Soil N_2O was measured using the closed chamber method (Hutchinson and Mosier, 1981). A steel-made chamber (diameter × height: 50 × 40 cm) with a septum for gas sampling was placed on each pot every time before sampling gases (n = 6). Water was used to seal the connection between the chamber and base to avoid gas loss. Two fans were equipped in each chamber to increase air circulation. Air and soil temperature (5 cm depth) was recorded *in situ* from June 2015 to April 2016 using a thermometer (TP3001, Boyang China), and the recording frequency was once every 30 mins during the FT period and once after each gas sampling during the growing season. The diurnal dynamics of N_2O production during the growing season (one measurement every 3 h) was determined twice: from 00:00 to 21:00 on June 15, 2015 and from 00:00 to 21:00 on August 17, 2015. The diurnal dynamics of N_2O production during the FT period (one measurement every 4 h) was also determined twice: from 02:00 to 22:00 on March 25, 2016 and from 02:00 to 22:00 on April 10, 2016. These measurements of diurnal variations of N_2O production also helped to find the best time to represent daily average, which was 9:00 to 11:00 during the growing season and 14:00 to 16:00 during the FT period. Therefore, the gas samples were taken 12 times in the growing season between 9:00 and 11:00 and 9 times in the FT period between 14:00