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Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles

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Abstract

Two Finnish agricultural soils (peat soil and loamy sand) were exposed to four freeze-thaw cycles (FTC), with a temperature change from $-17.3\pm0.4\,^{\circ}C$ to $+4.1\pm0.4\,^{\circ}C$. Control cores from both soils were kept at constant temperature ($+6.6\pm2.0\,^{\circ}C$) without FTCs. Soil N_2O and CO_2 emissions were monitored during soil thawing, and the effects of FTCs on soil microbes were studied. N_2O emissions were extremely low in peat soil, possibly due to low soil water content. Loamy sand had high N_2O emission, with the highest emission after the second FTC. Soil freeze-thaw increased anaerobic respiration in both soil types during the first 3–4 FTCs, and this increase was higher in the peat soil. The microbial community structure and biomass analysed with lipid biomarkers (phospholipid fatty acids, 3- and 2- hydroxy fatty acids) were not affected by freezing-thawing cycles, nor was soil microbial biomass carbon (MIB-C). Molecular analysis of the microbial community structure with temperature gradient gel electrophoresis (TGGE) also showed no changes due the FTCs. These results show that freezing and thawing of boreal soils does not have a strong effect on microbial biomass or community structure.

Keywords: N_2O; CO_2; Freeze-thaw cycle; Biomass; Microbial community structure; Lipid biomarkers; TGGE

1. Introduction

Microbial processes at low temperatures have been suggested to be responsible for up to 70% of annual nitrous oxide (N_2O) emissions from agricultural soils (Röver et al., 1998, Syväsalo et al., 2004). Even though the bulk soil is frozen, the water films on the surfaces of soil particles can remain unfrozen down to $-20\,^{\circ}C$, allowing microbial metabolism, and probably also N_2O production, to take place below 0° (Rivkina et al., 2000). In boreal and temperate regions, soils are exposed to freeze-thaw cycles (FTC) mainly during autumn and spring and also during mild winters. Soil thawing-related N_2O emissions have been reported in several studies (e.g. Christensen and Tiedje, 1990; Röver et al., 1998). N_2O and carbon dioxide (CO_2) emissions have been reported to increase in northern soils during FTC (Schimel and Clein, 1996). These FTC-induced emissions have decreased with repeated FTC (Schimel and Clein, 1996, Priemé and Christensen, 2001, Koponen and Martikainen, 2004). The decrease in gas production with repeated FTC suggests either depletion in microbial nutrient availability or damage to soil microbes.

Soil freezing-thawing events have been suggested to destroy microbial cells, releasing nutrients from the destroyed cells for the surviving microbes, which then are highly active during soil thawing (Christensen and Tiedje, 1990). The extra substrates might also originate from the physical disruption of soil aggregates due to frost action (Christensen and Christensen, 1991, Edwards and Cresser, 1992). Herrmann and Witter (2002) reported that easily

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decomposable material becomes available during FTCs, and microbial biomass C contributes, ca. 65% of the C flush during FTCs. In agricultural soils, the denitrifying population may benefit more from this extra nutrient load than the overall heterotrophic microbial community (Koponen and Martikainen, 2004). Schimel and Clein (1996) suggested that FTC may have an effect on the composition and function of microbial communities.

We studied the effects of multiple soil FTC on soil chemical and microbiological variables in two Finnish agricultural soils. The key nitrogen transforming processes, nitrification and denitrification, as well as the soil microbial biomass and the community structure were studied to obtain a comprehensive picture of the effects of freezing-thawing on soil microbes.

2. Materials and methods

2.1. Study sites

Two different typical Finnish agricultural soil types were studied (Table 1). The soils originated from the experimental fields of the Agrifood Research Finland in Jokioinen (southern Finland, 60°49′N 23°30′E). The mean annual precipitation (measured in the period 1971–2000) of the area is 607 mm, of which approximately 224 mm is snow. The mean annual air temperature is +4.3 °C, with February being the coldest month (mean −6.5 °C) and July the warmest (average +16.1 °C). The topsoil generally freezes in November and thaws in April (Finnish Meteorological Institute, 2002).

Soil samples were taken from depths of 0–25 cm of the uncultivated sectors of the fields. These sectors were kept free from vegetation by regular ploughing. The samplings were carried out on 29 October 2001 (loamy sand) and on 5 November 2001 (peat). The soils were kept at +4 °C for 4 months before the experiments began.

2.2. Experimental set up

Soil material was homogenised by sieving (mesh size 5.6 mm). The soil moisture content expressed as WFPS was 61±4% in peat and 86±5% in loamy sand. The soils were packed into PVC tubes (inner diameter 105 mm, height 300 mm), and soils were compressed manually to equal to the field value of the bulk density (0.34 g cm^{-3} for peat and 1.33 g cm^{-3} for loamy sand). The FTC consisted of freezing the soil cores (six replicates) to −17.3±0.4 °C (5 d) and thawing them at +4.1±0.4 °C (7 d). This FTC was repeated four times. Temperatures applied were selected to mimic the extreme freezing conditions and natural thawing-temperatures in autumn and spring at our study sites. After the fourth cycle the cores were kept at +4.1±0.4 °C for 23 d, in order to study the longer-term effects of multiple FTCs on soil microbiology. Five replicate control cores from both soils were incubated simultaneously without FTC at +6.6±2.0 °C. After each cycle (72 h after the beginning of thawing), 13 d after the fourth FTC (FTC4 + 13 d) and 23 d after the fourth FTC (FTC4 + 23 d), one replicate control and treatment core was destroyed and analysed for soil physical, chemical and microbiological variables.

The temperature of the incubation chambers (LMS Cooled Incubator, model 250) was measured continuously by data loggers (HOBO®).

Table 1
Soil physical-chemical properties

	Peat	Loamy sand
Soil type (FAO)[a]	Terric Histosol	Eutric Cambisol
Total C %[a]	24	2.4
Total N %[a]	1.1	0.16
C/N-ratio[a]	21	15
pH	6.0±0.1	5.4±0.1
WFPS %	61±4	86±5
Bulk density (g cm^{-3})	0.35±0.02	1.33±0.09
Particle density (g cm^{-3})	1.80±0.03	2.56±0.14

[a]From Pihlatie et al. (2004).

2.3. Gas sampling and analysis of CO_2 and N_2O

Measurements of N_2O and CO_2 were done with a closed chamber technique as described by Koponen and Martikainen (2004). Since soil thawing started the measurements were done every 2–4 h during the first 12 h and then 2–3 times a day during the following 2.5 d. The individual cores were covered by chambers, giving each core a headspace of 1.3–1.9 l. The headspaces were flushed continuously with air when incubating the cores in the temperature-controlled chambers to avoid the accumulation of gases in the headspace. The air flush was cut off just before making the gas flux measurements. The concentrations of N_2O and CO_2 were determined with a Hewlett Packard 5890 Series II gas chromatograph equipped with ^{63}Ni electron capture (EC) and thermal conductivity (TC) detectors for N_2O and CO_2, respectively (Nykänen et al., 1995). The flux rates were calculated from the linear increase in the gas concentrations during the measurement period of 35 min, and the cumulative flux was calculated by integrating the fluxes over the entire incubation period of 72 h from the beginning of the soil thawing.

2.4. Soil physical and chemical characterization

Soil particle density was determined using pycnometers (Blake, 1965), and gravimetric moisture content was determined by drying the soil at +105 °C for 24 h. Soil nitrate (NO_3–N) was analysed from the soil:water suspension (1:5 v/v, 175 rpm, 1 h) and ammonium NH_4–N from the soil:KCl suspension (1:5 v/v 2 M KCl, 175 rpm, 1 h). The extractions were filtered (Blauband 589^3 Blue Ribbon filter paper (Schleicher & Schuell MicroScience Gmbh,