



# Vertical variation of a black soil's properties in response to freeze-thaw cycles and its links to shift of microbial community structure

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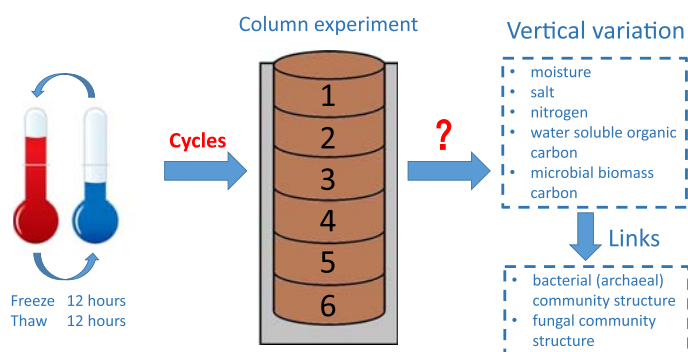
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## HIGHLIGHTS

- Electrical conductivity (EC) and moisture vertically varied after freeze-thaw cycles.
- Ammonium and nitrate nitrogen in top layers of soil column increased.
- Greater increment of water soluble organic carbon in middle layers was observed.
- More decrease of microbial biomass carbon in middle layers was noticed.
- EC, pH, water soluble organic carbon and moisture affected microbial community.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Soil freeze-thaw cycles (FTCs) change soil physical, chemical, and biological properties, however information regarding their vertical variations in response to FTCs is limited. In this work, black soil (silty loam) packed soil columns were exposed to 8 FTCs, and soil properties were determined for each of vertical layer of soil columns. The results revealed that after FTCs treatment, moisture and electrical conductivity (EC) salinity tended to increase in upper soil layers. Increments of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) in top layers (0–10 cm) were greater than those in other layers, and increments of water soluble organic carbon (WSOC) and decrease of microbial biomass carbon (MBC) in middle layers (10–20 cm) were greater than those in both ends. Overall, microbial community structure was mainly influenced by soil physical properties (moisture and EC) and chemical properties (pH and WSOC). For bacterial (archaeal) and fungal communities, soil physical properties, chemical properties and their interaction explained 79.73% and 82.66% of total variation, respectively. Our results provided insights into the vertical variation of soil properties caused by FTCs, and such variation had a major impact on the change of structure and composition of soil bacterial and fungal communities.

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## 1. Introduction

Freeze-thaw cycles (FTCs) have effects on soil physical and chemical processes (Zhang et al., 2016), biogeochemical cycles, and microbial communities' structure and composition (Schimel et al., 2007; Sharma et al., 2006). It is well known that FTCs may change the turnover of soil nutrient (Fuss et al., 2016; Yu et al., 2011), transport of soil moisture

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and water soluble salts (Bing et al., 2015), greenhouse gases release (Liu et al., 2016). Therefore, it is important to investigate the effect of FTCs on soils fertility, health, and global climate change.

Previous studies investigated the effects of FTCs on soil physico-chemical properties in wetland, tundra, and permafrost. FTCs were believed to affect soil aggregate stability and soil particle size (Zhang et al., 2016). More importantly, FTCs might lead to cell lysis and release of nutrients that could be better substrates for the survivors from FTCs. The sterilization effect of FTCs was a function of their amplitude, frequencies, and freezing and thawing rates. As a consequence of the insulating effect of soil, a temperature gradient might form across the soil columns. Within the same temperature range, the degree to which the soil structure was being destroyed and soil microbe was being lysed might vary between upper soil layers and lower soil layers (Lipson et al., 2000). In this case, vertical variation of soil physical, chemical, and microbial properties across a soil column was expected. A recent study showed that increments of ammonium nitrogen ( $\text{NH}_4^+$ -N) levels decreased in selected soils with the increase of soil depth after FTCs, while the increments of  $\text{NO}_3^-$ -N concentrations caused by FTCs was largely dependent on soil types (Yu et al., 2011). However, it is not known on vertical variation of other soil properties after FTCs.

Soil microbial community structure changes in response to FTCs (Feng et al., 2007). After FTCs, microbial habitat characteristics were vertically different due to redistribution of soil moisture, salts, pH, redox potential, and oxygen (Hu et al., 2015). Through the integration of soil properties data and microbial community structure data, we would have a more comprehensive understanding of freeze-thaw phenomenon. However, very few studies have successfully linked the changes in microbial community structure and composition to vertical variation of soil parameters after FTCs.

Black soil located in northeast China plays an important role in ensuring food security of China. On early spring in this region, spring ploughing just comes before FTCs, which may influence soil fertility. Therefore, it is necessary to monitor the change of microbial community composition and diversity in response to FTCs. Such monitoring would be helpful to improve land management, since biodiversity is a representative indicator to evaluate overall quality and health of soil (Gardi et al., 2009). The objectives of this work were to 1) study the vertical variation of a black soil (silty loam) physical, chemical, and microbial properties caused by FTCs, and 2) quantitatively correlate soil properties with bacterial (archaeal) and fungal community structure after FTCs.

## 2. Materials and methods

### 2.1. Site description

A black soil (silty loam) used in this study was collected in the fall of 2016 at 43.822955°E, 125.27803°N (altitude, 269.6 m), a place located at Siping area, Jilin Province, China. The sampling site was a part of Songnen plain. The parent material was quaternary sedimentary rock, vegetation was poplar, and land-use was shelter forest for farmland. The soil sample was a composite of 3–5 individual soil cores taken at 5-m intervals, and triplicate samples were taken (Ma et al., 2012). Freeze-thaw phenomenon was common in this region. According to the ranges of average lowest and extreme lowest temperatures ( $-7.6$  to  $-27.4$  °C), as well as average highest and extreme highest temperatures ( $+1.7$  to  $+20.7$  °C) during the freeze-thaw seasons in 1971–2000, we chose  $-15$  °C and  $+10$  °C as freezing and thawing temperatures, respectively, in lab-simulated column experiments. Top 30 cm of the soil was collected and transported to the laboratory under ice. Vegetation, roots and stones were removed, and the soil was sieved (2 mm), mixed, bagged, and stored at 4 °C in the dark until use. A portion of soil was air dried for soil physicochemical characterization.

### 2.2. Experimental design

Soil column (Fig. S1) was double-walled, 30 cm in height, and 10 cm in inner-diameter. The space between outer and inner wall as well as the bottom of the column was filled with polyurethane as main thermal insulation material (Timmis, 2010), which can guarantee that heat interchange only appears on the soil surface, thus better mimic the natural environmental condition. Soil moisture was added to reach 50% water holding capacity (WHC), and then packed into the column to make the bulk density equal to the field value ( $1.35 \text{ g/cm}^3$ ). Six replicate soil columns were prepared, 3 were left at 10 °C fridge as unfrozen control group, the other 3 were put into a high and low temperature experimental chamber (Yiheng, Shanghai, China) as FTCs treatment group. The soil columns were initially frozen at  $-15$  °C for 24 h, and then were subject to 8 FTCs, each of them consisted of 12 h freezing at  $-15$  °C and 12 h thawing at  $+10$  °C. Our preliminary test showed that the soil in the bottom of column could freeze or thaw completely within 12 h.

### 2.3. Analytical methods

After the experiments were completed, the soil columns were horizontally cut into 6 5 cm-height layers using a sterile saw blade. From top to bottom, the corresponding layers were labeled as C1 to C6 for unfrozen control group, and T1 to T6 for FTCs treatment group, respectively. Soil sample was taken from each of the layer for soil characterization and DNA extraction. Soil moisture (%) was determined by weight loss after drying the samples under 105 °C. Soil texture (clay, silt, and sand content in %) and volume mean diameter (VMD,  $\mu\text{m}$ ) were determined with a laser particle size analyzer (Bettersize 2000, Dandong, China). Soil pH and electrical conductivity salinity (EC,  $\mu\text{S/cm}$ ) were determined with pH meter and conductivity meter (soil-to-water ratio, 1:2.5), respectively.  $\text{NH}_4^+$ -N (mg/kg),  $\text{NO}_3^-$ -N (mg/kg), total nitrogen (TN, mg/kg), and total dissolved phosphorus (TDP, mg/kg) were determined by UV-Vis spectrophotometer (MapData, Shanghai, China). Soil water soluble organic carbon (WSOC, mg/kg) was extracted by 0.5 M  $\text{K}_2\text{SO}_4$  solution and determined by dichromate oxidation (Mebius, 1960). Soil microbial biomass carbon (MBC, mg/kg) was determined by the chloroform fumigation-extraction method (Vance et al., 1987).

### 2.4. Soil DNA extraction, sequencing, and sequencing data processing

Our preliminary experiments showed that FTCs could significantly lower the microbial biomass, thus combined soil samples were used for DNA extraction, soil samples of top 2 layers, middle 2 layers, and bottom 2 layers from each group of soil columns were combined, thus a total of 6 DNA samples were obtained. The DNA samples were labeled as C12, C34, and C56 for control group, T12, T34, and T56 for treatment group. Soil DNA was extracted by Tiangen soil DNA extraction kit (Tiangen, Beijing, China) following the manufacturer's protocol. Quality of DNA was assayed on 1% agarose gel and quantity of DNA was determined by a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

A total of 30–50 ng DNA was used to generate amplicons using a MetaVx™ Library Preparation kit (GENEWIZ, Inc., South Plainfield, NJ, USA). For bacterial 16S rDNA amplicon library construction, a protocol developed previously was used (You et al., 2016). For fungal 18S rDNA amplicon library construction, hypervariable regions V3, V4, V7 and V8 of fungi were selected. The 3' part of V3 and full V4 regions were amplified using forward primer containing sequence "GGCAAGTCTGGTGC C" and reverse primer containing sequence "ACGGTATCTRATCRTC". The entire V7 and V8 regions were amplified using forward primer containing sequence "CGWTAACGAACGAG" and reverse primer containing sequence "AICCATCAATCGG". Next generation sequencing was conducted on an Illumina MiSeq platform (Illumina, San Diego, USA) at GENEWIZ, Inc. (Suzhou, China).

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