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Shifts in soil bacterial and archaeal communities during freeze-thaw cycles in a seasonal frozen marsh, Northeast China

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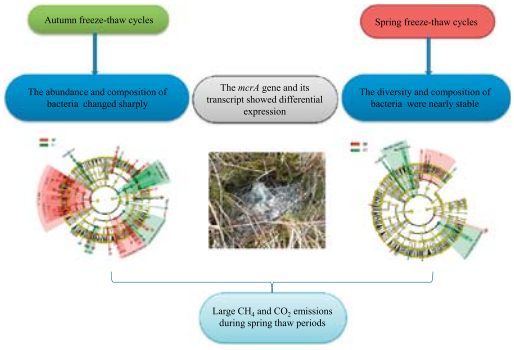
HIGHLIGHTS

- Spring and autumn FTCs conversely affect quantities of bacteria and methanogens.
- Bacterial diversity and abundance changed sharply upon autumn FTCs.
- Temperature and substrates mainly regulate bacterial abundance and composition.
- Spring large CH_4 emissions mainly came from autumn FTCs in seasonal frozen marsh.

GRAPHICAL ABSTRACT



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ABSTRACT

Diurnal freeze-thaw cycles (FTCs) occur in the spring and autumn in boreal wetlands as soil temperatures rise above freezing during the day and fall below freezing at night. A surge in methane emissions from these systems is frequently documented during spring FTCs, accounting for a large portion of annual emissions. In boreal wetlands, methane is produced as a result of syntrophic microbial processes, mediated by a consortium of fermenting bacteria and methanogenic archaea. Further research is needed to determine whether FTCs enhance microbial metabolism related to methane production through the cryogenic decomposition of soil organic matter. Previous studies observed large methane emissions during the spring thawed period in the Sanjiang seasonal frozen marsh of Northeast China. To investigate how FTCs impact the soil microbial community and methanogen abundance and activity, we collected soil cores from the Sanjiang marsh during the FTCs of autumn 2014 and spring 2015. Methanogens were investigated based on expression level of the methyl coenzyme reductase (*mcrA*) gene, and soil bacterial and archaeal community structures were assessed by 16S rRNA gene sequencing. The results show that a decrease in bacteria and methanogens followed autumns FTCs, whereas an increase in bacteria and methanogens was observed following spring FTCs. The bacterial community structure, including *Firmicutes* and certain *Deltaproteobacteria*, was changed following autumn FTCs. Temperature and substrate were the primary factors regulating the abundance and composition of the microbial communities during autumn FTCs, whereas no factors significantly contributing to spring FTCs were identified. Acetoclastic methanogens from order *Methanosarcinales* were the dominant group at the beginning and end of both the autumn and spring

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FTCs. Active methanogens were significantly more abundant during the diurnal thawed period, indicating that the increasing number of FTCs predicted to occur with global climate change could potentially promote CH_4 emissions in seasonal frozen marshes.

1. Introduction

Freeze-thaw cycles (FTCs) are a common phenomenon in soils of temperate and cold climates. It includes two physical process cycles: freezing and thawing, which can be divided into annual FTCs and multiple diurnal FTCs. The latter has been shown to play a significant role in greenhouse emissions in boreal wetlands (Wei and Wang, 2017; Yu et al., 2007). FTCs can promote the emission of methane (CH_4), a significant greenhouse gas possessing a radiative force 25 times greater than that of carbon dioxide (CO_2) (Change, 2014), and the frequency of diurnal FTCs is expected to increase under climate warming scenarios (Change, 2014; Wang et al., 2017a.

Large CH_4 emissions from boreal wetlands during spring FTCs have received increasing attention in recent years, and they appear to be a significant fraction of the annual CH_4 budget (Song et al., 2012; Tokida et al., 2007). This phenomenon has also been repeatedly observed in arctic wetlands, mires, and tundra, which accounted for 25%, 11% and 6% of the annual budget, respectively (Friborg et al., 1997; Hargreaves et al., 2001; Raz-Yaseef et al., 2017). However, the contribution of CH_4 emissions to the annual flux during FTCs is unclear. Some research found that CO_2 and CH_4 fluxes emitted during autumn and spring FTCs (Liptzin et al., 2009; Mastepanov et al., 2008; Tagesson et al., 2012; Wille et al., 2008), while the other studies found both CO_2 and CH_4 emissions have been observed during winter rather than autumn and spring FTCs in arctic tundra and grassland (Y.H. Wang et al., 2014; Zona et al., 2016). In wetland soils, methanogenesis is driven by a complex community of fermenting bacteria, syntrophic bacteria, and methanogenic archaea (Cicerone and Oremland, 1988). CH_4 release is the direct net result of methanogenesis and CH_4 oxidation processes. However, the microbial dynamics present during large spring CH_4 emissions associated with FTCs have not been well examined.

FTCs, a type of abiotic stress, can create anaerobic environment and increase substrate availability by physically destroying soil aggregates and soil microorganisms (Hentschel et al., 2008; Oztas and Fayetorbay, 2003; Yu et al., 2011). FTCs may also induce structural changes in soil microbial communities that regulate enzyme activity (Kumar et al., 2013; Sharma et al., 2006). However, the strength of the FTCs' effects is dependent upon the ecosystem in which they occur. For example, subtle effects on soil bacteria were observed during FTCs in grassland soil (Feng et al., 2007; Grogan et al., 2004), whereas FTCs significantly reduced soil microbial abundance and activity in arctic-alpine soil and arctic mud flat (Larsen et al., 2002; Sawicka et al., 2010).

Because FTCs experiments have primarily been conducted in the laboratory, realistic field conditions and actual shifts in soil microorganisms cannot be fully replicated (Henry, 2007). Additionally, previous FTCs experiments considered only either autumn FTCs (Groffman et al., 2006; Neilson et al., 2001; Teepe et al., 2001) or spring FTCs (Holst et al., 2008). The responses of soil microorganisms to FTCs have been studied in steppes and forests. But to our knowledge, the in situ process has not been investigated in boreal wetlands (Urakawa et al., 2014; Wang et al., 2017b).

The objective of the present study was to determine how autumn and spring FTCs influence the in situ soil microbial community in a seasonal frozen marsh, located in Sanjiang of Northeast China. To investigate the impact of FTCs on total bacterial abundance, methanogen activity and community structure, we utilized a combined approach of quantitative PCR and 16S rRNA gene sequencing. We made the following hypotheses: i) Spring and autumn FTCs have contrasting effects on the abundance of bacteria and methanogens. We expected that microbial abundance and activity would be less at the start of spring FTCs than at the start of autumnal FTCs due to limited growth during the spring frozen period, compared to the summer growing season leading up to autumnal FTCs. ii) Multiple FTCs will decrease the diversity of bacterial and archaeal communities in both spring and autumn. iii) Large spring CH_4 mainly come from the entire autumn FTCs period due to increased anaerobic conditions and substrate availability for methanogens.

2. Materials and methods

2.1. Site description and soil sampling

The sampling site was located at the Sanjiang Wetland Experimental Station, Chinese Academy of Sciences (47°35′N, 133°31′E). The Sanjiang Plain in Northeast China has an area of 10.89×10^4 km^2, and belongs to the seasonal frozen region (Song et al., 2003). The site experiences a mean annual air temperature of 1.9 °C. The coldest month is January, with a mean temperature of −19.8 °C, and the warmest month is July, with a mean temperature of 21.6 °C. The growing season normally lasted from early May to late September. *Carex lasiocarpa* is the dominant type of vegetation, and the mean water depth is 40 to 50 cm at the sampling site.

Multiple diurnal freezing and thawing periods occurs along the soil profile in this seasonal frozen marsh, and an entire freeze-thaw period includes autumn FTCs of one year and spring FTCs of the next year. In seasonal frozen areas, autumn initially freezes in the surface and gradually freezes in the deeper layer, while spring thawing starts in both the surface and subsoil and moves toward the middle. Based on soil temperatures in the 0–5 cm layer, we collected soil samples on four different dates representing the first frozen phase and the last thawed phase of each season. These occurred on December 15th and 31st of 2014, and April 16th and 19th of 2015, respectively. On each sampling date, we randomly selected four plots in an area wherein large CH_4 emissions were observed by Song et al. (2012). For each plot (a circle with a 1 m diameter), we sampled four soil cores using a sterile cylindrical stainless steel soil sampler with a 10-cm diameter. The cores were comprised of a brown and fibrous root layer, a spongy peat layer, and a pale yellow and sticky gley soil layer. Peat soil layers from 0–5 cm and 5–10 cm were collected, and the corresponding layers were mixed to produce a composite soil sample for each plot, resulting in a total of 32 composite samples from the four sampling dates. Sub-samples for DNA and RNA analysis were stored in a Dewar filled with liquid nitrogen. The remaining soil samples were kept in a sterile insulated can with ice bags for soil physicochemical analysis.

2.2. CH_4 and CO_2 flux measurements

Gas flux measurements were conducted by applying static dark chambers and gas chromatography techniques (Agilent 4890D) with a flame ionization detector (FID). The detailed method for placement of the static dark chamber and gas flux measurements were described in our previous work (Song et al., 2009; Song et al., 2012).

2.3. Physicochemical analysis

Soil and air temperatures were acquired from an in situ meteorological station. Soil moisture was measured by oven-drying at 105 °C for