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Responses of soil microorganisms, carbon and nitrogen to freeze–thaw cycles in diverse land-use types

Cheng-Long Han[a], Yan-Jie Gu[a], Meng Kong[a], Ling-Wei Hu[a], Yu Jia[a,*], Feng-Min Li[a], Guo-Jun Sun[a], Kadambot H.M. Siddique[b]

[a] State Key Laboratory of Grassland Agroecosystem, Institute of Arid Agroecology, School of Life Sciences, Lanzhou University, 730000, China
[b] The UWA Institute of Agriculture and School of Agriculture & Environment, The University of Western Australia, LB 500 Perth, WA 6001, Australia

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ABSTRACT

Temperatures are predicted to increase in semi-arid regions, which will affect the frequency of some climatic events such as freeze–thaw cycles. To understand the impact of recurrent freezing and thawing on soil microorganisms and their environment, three soils from diverse land-use types (grassland, cut grassland and cropland) in the semi-arid area of the Loess Plateau were subjected to two different freeze–thaw cycles (FTC) for 48 days (either 4 days each at −2 °C and +3 °C for 6 FTC or 8 days each at −2 °C and +3 °C for 3 FTC). Nutrient content and mobilization in the soil, and microbial biomass and functional diversity were examined.

When subjected to these freeze–thaw cycles, soil respiration was low during the −2 °C phase and high during the +3 °C phase, decreasing in successive cycles, which indicates the rapid response of microbial activity even after long exposure to fluctuating temperatures. Soil respiration stabilized after 32 days in the three soils in the treatment with 3 FTC but continued to decrease in the treatment with 6 FTC. With each successive freeze–thaw cycle, microbial biomass C and N decreased gradually in all the tested soils. At the end of the experiment, the 6 FTC treatment had more microbial biomass C and N than the 3 FTC treatment in each soil type, indicating that microbial activity (respiration) was controlled mainly by the rapid freeze–thaw cycles rather than microbial biomass. Soil inorganic N concentration declined in all soils subjected to 6 FTC but increased when subjected to only 3 FTC, suggesting that the surviving microorganisms in the treatment with 6 FTC had more potential for using soil N to adapt to the freeze–thaw stress. The microbial functional diversity in the three tested soil types in response to the two freeze–thaw cycle treatments varied, suggesting differences in the native microbial communities in the soil from various land-use types.

1. Introduction

The Loess Plateau is an important agricultural region in Northwestern China. It has a temperate semi-arid climate, with temperature fluctuations, such as freeze–thaw cycles, especially in early spring (Grogan et al.; Hu et al., 2012). Predicted climate change may result in changes to soil freeze–thaw patterns as an indirect effect of global warming (Groffman et al., 1999). Turner et al. (2011) predicted that areas of the Loess Plateau would experience increases in mean temperature of 2.5–3.8 °C in the next 50 years, which will increase the frequency of freeze–thaw events (Sjursen et al., 2005).

Soil freeze–thaw events, a phase transition phenomenon of soil water, is a climate-driven pedoturbation (Yanai et al., 2004) which can impact microbial activity, soil nutrient cycles and fluxes of soil atmosphere trace gases (Grogan et al., 2004; Harris et al., 2006). Freezing and subsequent thawing of soils often result in a flush of microbial respiration (Burton and Beauchamp, 1994; Sjursen et al., 2005). Increased microbial activity following freeze–thaw events is related to the death of some soil microbes and the subsequent release of nutrients into the soil (Larsen et al., 2002; Yanai et al., 2004). Soil microbes use the released nutrients to survive and then produce a pulse respiration (CO_2) following the thaw (Schimel and Clein, 1996), which helps soil organisms to cope with these changes (Crowther et al., 2011; Dam et al., 2012; De Mesel et al., 2004; Koponen et al., 2006).

Freeze–thaw cycles in early spring are common, with substantial effects on soil microbial communities and nutrient cycling. If microbial communities decline then the metabolic activities and mobilization of nutrients tied up with microbial biomass may also decline (Schimel and Clein, 1996). Some authors have reported no observed effect on microbial biomass after freeze–thaw cycles (Grogan et al., 2004; Sharma

* Corresponding author at: State Key Laboratory of Grassland Agroecosystem, Institute of Arid Agroecology, School of Life Sciences, Lanzhou University, 730000, Gansu Province, China.
E-mail address: juyu@lzu.edu.cn (Y. Jia).

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et al., 2006). Different land-use types have different soil microbial populations with distinct morphologies, growth strategies, and ecological niches (Boer et al., 2005). Yanai et al. (2004) suggested that soil microorganisms may survive freeze–thaw cycles in soil high in organic matter content.

In the studies mentioned above, the long-time frozen soil was only thawed once and then analyzed for soil microorganisms and nutrients. The effect of the intensity of freeze–thaw temperature fluctuations on the response of microbes and nutrient cycles is largely unknown. Soil may be exposed to different frequencies of freeze–thaw cycles that could affect soil microbes differently. Therefore, we collected soil samples from three land-use types and subjected them to two different freeze–thaw cycles (rapid and slow) in the laboratory to measure soil microbial respiration rate, microbial biomass of C and N pools, soil inorganic N concentration, and microbial functional diversity. We tested the hypothesis that rapid freeze–thaw cycles will lower microbial biomass C and N, soil respiration and microbial functional diversity more than slow freeze–thaw cycles.

2. Materials and methods

2.1. Experimental setup

In October 2015, soil samples (0–20 cm depth) were extracted from grassland (GL), cut grassland (CG) and cropland (CL) at the Semi-arid Ecosystem Research Station of Loess Plateau (latitude 36° 02′ N; longitude 104° 25′ E, 2400 m above sea level) in Zhonglianchuan village of Yuzhong County, Gansu Province, China. The maximum distance between each of the two land-use types was about 600 m. Three plots (6 × 8 m) were randomly set in each of the three land-use types. The plots were considered true replicates given that the distance between each exceeded the spatial dependence (≥10 m). The soil was classified as Heima soil (Calcic Kastanozems, FAO Taxonomy) (Zhou et al., 2009). Table 1 shows the soil characteristics of the three sites.

The grassland, which had not been disturbed, grazed or harvested for the past 20 years, after being converted from cropland, contains plant species such as *Agropyron cristatum* (Linn.) Gaertn, *Stipa breviflora griseb* and *Artemisia frigida* Willd. The cut grassland had not been plowed for 15 years after being converted from cropland, and the aboveground biomass of seeded alfalfa (*Medicago sativa* L.) had been harvested twice a year (mid–July and mid–October). The cropland had been in a crop rotation system for the past 50 years with crops such as wheat (*Triticum aestivum*), maize (*Zea mays* L.) and potato (*Solanum tuberosum*) under local farming management practices. The climate is characterized as a semi-arid desert-grassland with a mean annual air temperature of 6.2 °C, mean monthly maximum air temperature of 19.0 °C (July) and mean monthly minimum air temperature of −8.0 °C (January). The area experiences frequent, repeated freeze–thaw events in early spring (Sjursen et al., 2005; Wang et al., 2005; Zhou et al., 2009). Air temperature records (2001–2012) from the China Meteorological Administration Yuzhong weather station show that the mean air temperature in winter (mid-November to mid-February) is about −5 °C, and ranges from −2 °C to +3 °C in early spring, with 3–6 naturally occurring freeze–thaw cycles (FTC) in March/April (Fig. 1a).

Table 1
Characteristics of the experimental soils at the beginning of the study. Mean values (n = 3) are displayed with standard errors (SE) in brackets.

Soil properties	Grassland	Cut grassland	Cropland
Soil organic carbon (g kg^{-1})	14.8 (0.030)	12.8 (0.378)	8.6 (0.155)
Soil nitrogen (g kg^{-1})	0.5 (0.007)	0.6 (0.020)	0.5 (0.003)
Inorganic N (mg kg^{-1})	0.7 (0.462)	4.8 (0.784)	6.4 (0.873)
Dissolve organic C (mg kg^{-1})	295 (5.3)	272 (9.2)	282 (7.2)
pH (H_2O)	8.3 (0.026)	8.4 (0.010)	8.4 (0.023)
C:N	30.9 (0.525)	22.1 (0.379)	18.6 (0.408)

The soil samples from the three land-use types were taken to the laboratory and sieved (2 mm) to remove roots and small animals. Each soil sample was divided into two parts: one was air-dried to determine soil organic carbon, total nitrogen and pH, while the other was used for laboratory incubation. The incubation soils were put in plastic containers (7.2 × 7.2 × 2.3 cm deep) and placed in climate chambers. The chambers were modified freezers with the base replaced by a 2 cm foam board. The chambers were equipped with a fan to ensure proper air mixing and to prevent the development of temperature gradients. The experiment had two factors: a) two freeze–thaw treatments (3 FTC and 6 FTC), and b) three soils each from a different land-use type (soil types). Three soil replicates per treatment were collected at 0, 16, 32, 48 days after the start of the treatment, corresponding to 0, 1, 2 and 3 FTC for the 3 FTC treatment and 0, 2, 4 and 6 FTC for the 6 FTC treatment. In addition, three mesocosms of each soil type per treatment were prepared to measure soil respiration.

The temperature of the climate chambers was initially set to −5 °C to imitate the natural scenario of mean air temperature in winter. Each soil sample was randomly placed in a climate chamber. Soil water content was maintained at 12 ± 1% (60% field water holding capacity) (Zhang et al., 2005). The containers were weighed weekly, with distilled water added throughout the experiment to replace any water loss. After 30 days, the temperature in the chambers was raised to +3 °C; the samples were divided into two treatments, half were placed in a chamber set to 3 FTC of 8 days at +3 °C and 8 days at −2 °C, and the other half were placed in a chamber set to 6 FTC of 4 days at +3 °C and 4 days at −2 °C (Fig. 1b).

2.2. Soil sample analyses

Soil organic carbon (SOC) content was determined using the Walkley and Black (1934) dichromate oxidation method. A factor of 1.33 was applied to make SOC comparable with that determined by dry combustion (Hai et al., 2010). Soil total nitrogen (TN) content was measured using the semi-micro Kjeldahl digestion procedure (Hai et al., 2010). Soil inorganic nitrogen (inorganic N) was extracted from fresh soil samples with a Smartchem Discrete Auto Analyzer (Yuan et al., 2016).

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined as the difference between C and N extracted with 0.5 M K_2SO_4 through chloroform-fumigation and non-fumigated soil samples. Since not all microbial constituents were released by fumigation, microbial biomass C and N were calculated using a K_{EC} factor of 0.45 and a K_{EN} factor of 0.57, respectively (Hu et al., 2012). Fumigation was with ethanol-free chloroform for 24 h at 25 °C in a darkened desiccation incubator. Organic C in extracts was measured using the TOC analyzer (multi-N/C UV3100, Analytikjena product, Germany) (Hu et al., 2012). The concentration of organic C in the non-fumigated samples was referred to as dissolved organic C (DOC) (Wallenstein et al., 2006).

2.3. Soil microbial respiration

The fresh soil samples from the three land-use types were sieved to 2 mm and put into glass jars (2.5 L) with three replicate containers per soil type in each treatment. Each soil sample was randomly placed in a climate chamber. Due to the absence of plant root residue, soil respiration was equalized with microbial respiration, measured at +3 °C and −2 °C in three replications for each soil type. Soil respiration (CO_2 concentration) was measured every two days for the duration of the experiment using a titration method (Feng et al., 2007; Zhang et al., 2005). To trap the respired CO_2, a beaker containing 1 M NaOH solution was kept inside the soil sample jar sealed with a rubber stopper for 24 h. Excess NaOH solution was determined by precipitation with $BaCl_2$ and titration with 0.25 M HCl using phenolphthalein as an indicator (Zhang et al., 2005). Microbial respiration was normalized to the dry