Effects of soil frost on growth, composition and respiration of the soil microbial decomposer community

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ABSTRACT

Most climate change scenarios predict that the variability of weather conditions will increase in coming decades. Hence, the frequency and intensity of freeze-thaw cycles in high-latitude regions are likely to increase, with concomitant effect on soil carbon biogeochemistry and associated microbial processes. To address this issue we sampled riparian soil from a Swedish boreal forest and applied treatments with variations in four factors related to soil freezing (temperature, treatment duration, soil water content and frequency of freeze-thaw cycles), at three levels in a laboratory experiment, using a Central Composite Face-centred (CCF) experimental design. We then measured bacterial (leucine incorporation) and fungal (acetate in ergosterol incorporation) growth, basal respiration, soil microbial phospholipid fatty acid (PLFA) composition, and concentration of dissolved organic carbon (DOC). Fungal growth was higher in soil exposed to freeze-thawing perturbations and freezing temperatures of $-6\,°C$ and $-12\,°C$, than under more constant conditions (steady $0\,°C$). The opposite pattern was found for bacteria, resulting in an increasing fungal-to-bacterial growth ratio following more intensive winter conditions. Soil respiration increased with water content, decreased with treatment duration and appeared to mainly be driven by treatment-induced changes in the DOC concentration. There was a clear shift in the PLFA composition at $0\,°C$, compared with the two lower temperatures, with PLFA markers associated with fungi as well as a number of unsaturated PLFAs being relatively more common at $0\,°C$. Shifts in the PLFA pattern were consistent with those expected for phenotypic plasticity of the cell membrane to low temperatures. There were small declines in PLFA concentrations after freeze-thawing and with longer durations. However, the number of freeze-thaw events had no effect on the microbiological variables. The findings suggest that the higher frequency of freeze-thaw events predicted to follow the global warming will likely have a limited impact on soil microorganisms.

1. Introduction

In addition to a general warming trend, most climate change models predict that the variability of weather fluctuations, including precipitation and temperature patterns, will increase in coming decades. At high-latitudes this is likely to be manifested in part by changes in the duration, timing and extent of snow-cover (IPCC, 2007). Consequently, predicted changes in temperature variability and associated perturbations, including increased frequencies of freeze-thaw cycles, may have more pronounced effects on the organisms of these systems than gradual changes in average temperature (Campbell et al., 2005; Kreyling, 2010). Any decrease in the insulating snow-layer on soil (Fitzhugh et al., 2001) may lead to enhanced soil freezing by cold weather, with lower temperatures reaching deeper layers. The intensity of freezing (Feng et al., 2007; Groffman et al., 2010; Haei et al., 2010a,b), as well as repeated freeze-thaw cycles (Henry, 2007; Koponen et al., 2006; Vestgarden and Austnes, 2009), affect the quantity, quality and turnover of soil organic matter. The freezing intensity also affects the availability of water to the microbial community (Jefferies et al., 2010; Öquist et al., 2009a). It follows that the three primary regulators of microbial activity in soil —substrate availability, temperature and moisture (Waksman and Gerretsen, 1931; Weintraub and Schimel, 2003)—are all strongly influenced by freeze-thaw cycles. Thus, any perturbations of freezing and freeze-thaw cycles may strongly influence the soil microbial community (Schimel et al., 2007).

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Although there has been recent progress towards understanding the influence of freeze-thaw cycles on the biogeochemical processes mediated by microbial communities, including C and N transformations (Matzner and Borken, 2008), few studies have focused directly on the communities, and the results of such studies have varied substantially. For instance, some studies have found that a large proportion of soil microbes (up to 50% by biomass) can be killed by a single freeze-thaw cycle (Jefferies et al., 2010), although this proportion appears to vary between soils, depending on the prior history of freeze-thaw events (Stres et al., 2010). In addition, several studies have shown that the composition of the microbial community can be affected by freeze-thaw cycles, but while some have found that fungi tolerate low water potentials, and in some cases dominate after prolonged freezing (Schadt et al., 2003; Schimel and Mikan, 2005, 2007), others have found fungi to be more sensitive to freeze-thaw cycles than bacteria (Feng et al., 2007; Matzner and Borken, 2008; Schimel et al., 2007; Schmitt et al., 2008). There have also been suggestions that the composition of fungal communities may be more sensitive to freeze-thaw perturbations than the composition of bacterial communities (Schmitt et al., 2008; Yergeau and Kowalchuk, 2008). The soil microbial community composition has also been shown to be affected by experimental freeze-thaw cycles to varying degrees (Koponen et al., 2006; Yergeau and Kowalchuk, 2008).

It should be noted that reported studies on effects of freeze-thaw cycles on microbial communities have not necessarily identified the actively growing soil communities, partly because they have usually been based on biomass-based assessments. Changes in levels of microbial biomass in the soil reflect changes in both biomass production and biomass destruction (e.g. due to predation or microbial death). Thus, any biomass estimate will only provide a snapshot of the combined effects of these processes on both active and non-active microorganisms. Consequently, concentrations of microbial biomass markers in soil may not be well correlated with the active contributions of the corresponding organisms to processes of interest, such as C-mineralization and nutrient cycling (Kemmitt et al., 2008; Rousk et al., 2009). A possible way of circumventing the problems of biomass-based approaches is to study microbial growth directly, for instance by measuring rates of leucine incorporation into bacteria (Bååth et al., 2001) and acetate incorporation into ergosterol (Bååth, 2001), as previously used to investigate microbial responses to another common form of soil perturbation, drying and rewetting (Bapiri et al., 2010).

In a previous study (Haei et al., 2010b) the effects of low temperatures, treatment duration, soil water content and frequency of freeze-thaw cycles on dissolved organic carbon (DOC) were studied in samples of riparian soil taken from a boreal forest of northern Sweden. In the work presented here, we investigated the effects of these factors on bacterial and fungal growth, basal respiration and the soil microbial phospholipid fatty acid (PLFA) composition in the same experiment. Riparian soils are the major sources of C received by streams in the study region (Öquist et al., 2009b; Seibert et al., 2009), thus any changes in processes affecting C contents of these soils may have profound implications for water quality (Ågren et al., 2010). Furthermore, it was recently demonstrated that soil frost intensity strongly affects both the concentrations (Haei et al., 2010a) and lability (Haei et al., 2010b) of DOC in soils of this area, as well as *in situ* soil respiration rates (Öquist and Laudon, 2008). Therefore, we also studied the responses of microbial (bacterial and fungal) growth and basal respiration to variations in levels of dissolved organic carbon (DOC) to evaluate the putative influence of the availability of C on the microbial community.

2. Materials and methods

2.1. Soil sampling and treatment

Soil was sampled from the riparian zone of a small first-order stream in the Krycklan catchment at Svartberget Long-term Ecological Research Forest (Svartberget LTER; 64°14'N, 19°46'E), 60 km northwest of Umeå, Sweden (Ågren et al., 2007; Buffam et al., 2007). The soils were predominantly ferric Podzols developed on glacial till, overlying gneissic bedrock at 5–10 m depth. In the riparian zone near the stream there were up to 1 m deep peat deposits (Seibert et al., 2009) with an increasing pH gradient of 4.0–5.2 downward in the soil profile (10–65 cm) (Cory et al., 2007). Stands of 100-year old Norway spruce (*Picea abies*) covered the experimental soils and blueberries (*Vaccinium myrtillus*) dominated the field layer (Köhler et al., 2008). The bottom layer was covered by moss-mats, consisting mainly of *Hylocomium splendens* and *Pleurozium schreberi* (Forsum et al., 2008). Samples from the upper 30 cm of the riparian soil, collected in early December 2007, were immediately homogenized and bulked. In the laboratory, roots and coarse material were removed and the soil was homogenized by sieving through 3 mm mesh. The organic content of the pooled soil sample was 18%. The water holding capacity (WHC) of the soil sample was measured after drainage by gravity of a 0.02 m high soil cylinder with saturated soil, as described by Ilstedt et al. (2000).

Our multi-factor experiment was based on a Central Composite Face-centred (CCF) design, generated using Modde 9.0 statistical package (Umetrics, Umeå, Sweden). CCF designs are commonly used to generate Response Surface Models (RSM) in order to study the integrated effects of several factors. In a CCF design, the experimental domain for three factors is represented by a cube, in which the axial points are located on the faces. The corner points in each dimension range between the lower and higher values of a factor. For each additional factor, a new dimension is added to the experimental domain. In addition to the corner points and axial points (central points of each face) in the cubic experimental area, replicated centre points (normally $n = 3$) are also included. Replicated centre points are based on the combination of central levels for all of the experimental factors (Box et al., 1978; Eriksson et al., 2008). Our CCF design was based on three levels of four freezing-related factors (incubation temperatures, soil water content, treatment duration and frequency of freeze-thaw cycles), giving a total of 27 samples, with three replicated centre points (Table 1). Considering the cubic experimental area for each incubation duration, the total of 27 samples was comprised of eight corner points as well as one centre point for the samples with incubation durations of 2 and 6 months, and six axial points as well as three identical centre points (combination of centre points of all the four factors) for the samples with 4 months incubation period (Table 1).

To adjust the water content of the soil samples to the three levels in the design (30%, 60% and 90% WHC; Table 1), the samples were air dried at 12 °C. The samples were then placed in 250 ml sealed polypropylene jars and kept at one of the three temperatures (−12 °C, −6 °C or 0 °C, all +/− 1 °C) using temperature controlled freezers and an ice-bath, respectively. The jars were kept at the above mentioned temperatures for 2, 4 or 6 months and exposed to varying numbers of freeze-thaw cycles (1, 3 or 7 cycles of thawing at 5 °C for 48 h and cooling to the initial temperature) at equal time intervals for each duration period (for more details see Haei et al., 2010b).

2.2. Laboratory analyses

At the end of each period (2, 4 or 6 months), the samples were thawed for 48 h at 5 °C, then stored in the dark at 20 °C for one day