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Short communication

Soil bacterial growth after a freezing/thawing event

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ABSTRACT

Bacterial growth after freezing/thawing was studied in two soils with a history of annual freezing/thawing events. Soil samples were frozen for 1 week at −3 °C or −18 °C, thawed at +4 °C, and respiration and bacterial growth (estimated using leucine incorporation) were compared with reference soils kept at +4 °C. There were no major differences between soils. A respiration pulse, peaking within 9 h, was found, but after 30–100 h respiration had decreased to that in the reference. Freezing at −18 °C resulted in 2.2–2.5 times higher cumulative respiration than the reference, while at −3 °C 1.6–1.8 times higher respiration was found. Bacterial growth rates immediately after thawing were 43–44% of the reference in the −3 °C and 23–26% in the −18 °C treatment. Growth rates then increased linearly, recovering after 36 h and around 50 h in the −3 °C and −18 °C freezing, respectively. Growth rates then increased further in the −18 °C, but remained lower or similar to the reference in the −3 °C treatment. The microbial response to freezing/thawing thus appeared similar to mild drying/rewetting (type 1 response *sensu* Meisner et al. (2015)).

Freezing/thawing is a common phenomenon in temperate and cold climat soils; a perturbation that may occur several times during the year. Freezing/thawing results in a pulse of respiration (Skogland et al., 1988; Schimel and Clein, 1996; Koponen et al., 2006; Henry, 2007; Kim et al., 2012) and a decrease in microbial biomass (Skogland et al., 1988; Henry, 2007; Yanai et al., 2004) indicating freezing/thawing being detrimental to microorganisms. The microbial community composition will also change after a freezing/thawing event (Yergeau and Kowalchuk, 2008; Männistö et al., 2009). These are similar effects as after drying/rewetting episodes (Skogland et al., 1988; Kim et al., 2012; Barnard et al., 2013). The actual mechanism may also be similar; freezing results in cells encountering altered osmotic potentials, eventually resulting in cells in a dry state. Thawing is thus a similar phenomenon as rewetting, although usually at much lower temperatures.

Two bacterial growth response patterns are found after rewetting dry soil. The type 1 response results in fairly high growth rates directly after rewetting. Bacterial growth then directly starts to increase linearly and recover rapidly to similar or slightly higher growth rates as in moist soil (Iovieno and Bååth, 2008). Respiration is highest within hours after rewetting, and then decreases exponentially in the type 1 response. The type 2 response initially has very low growth rates after rewetting, followed by a lag period and an exponential increase in growth, resulting in slower recovery of growth compared to the type 1 response, but eventually in much higher growth rates than in moist soils (Göransson et al., 2013). Type 2 often has a second peak following the initial respiration burst upon rewetting. The type of response is soil dependent, but also depends on the extent of drying, where harsher treatments (longer times) result in a type 2 and milder treatments in a type 1 response (Meisner et al., 2013, 2015).

Skogland et al. (1988) stated that the killing effect after drying/rewetting appeared stronger than after freezing/thawing. Thus, we hypothesized that freezing/thawing, presumably similar to a mild drying/rewetting, would result in a type 1 response of bacterial growth. Freezing temperatures affects the killing effect (Elliott and Henry, 2009). Therefore, we compared the effect of freezing at −18 °C and −3 °C, hypothesizing that −18 °C should result in a stronger killing effect and slower recovery of bacterial growth after thawing. We also predicted that −18 °C would result in higher respiration due to more dead bacteria and eventually higher bacterial growth than freezing at −3 °C.

Two agricultural soils from Finland were used. The mull soil is a histosol (28% organic matter, pH(H_2O) 5.7). The sandy soil is a medium textured dystric regosol (4.4% organic matter, pH 6.9).

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Mean annual temperature is 2–3 °C (Maljanen et al., 2009; with more background data). Frost can appear in December and the soils can remain frozen until April–June. Thus, the soils and their organisms have a history of freezing/thawing as a normal part of the annual cycle.

Twenty soil cores (5 cm diameter, 0–10 cm depth) were collected for each soil on 10th of October 2008, homogenized and kept at +4 °C (<2 months). Soil samples (25 g in 50 ml vials; 4 separate microcosms per soil and temperature) were frozen at −18 °C and −3 °C for seven days prior to thawing at +4 °C in a water bath. Soil samples at +4 °C were used as references. The use of a water bath resulted in fairly rapid thawing, within 2 h. The first sample was taken as frozen and bacteria immediately extract in +4 °C water (see below), but later samples were from thawed soil. Microbial activity was assessed by repeated sampling of the microcosms during a month (thus with n = 4 each time), but with emphasis on the first four days for bacterial growth. The first 12 h after thawing, samples were taken every 3 h, the next 3 days twice a day, and at later time points on an even longer time scale. Bacterial growth was measured using leucine incorporation (Bååth et al., 2001) for 2 h at +4 °C (4 h after 7 days). Growth estimated as leucine incorporation into extracted bacteria per hour and g of wet soil will henceforth be denoted bacterial growth rate.

Respiration was measured separately on a gas-chromatograph after thawing 20 g of soil in 550 ml bottles sealed with rubber septa using n = 3 separate microcosms per treatment. At each measurement occasion (up to 168 h after thawing, see Fig. 1) CO_2 concentration was measured in the beginning and at 3 times during 1.75 h periods. Respiration rate as CO_2 released per hour and g of wet soil at +4 °C was calculated. Cumulative respiration during the first 100 h was calculated using the trapezoid method.

The dynamics of microbial activities after thawing were similar for the two soils. Peak respiration was found after 9 h for the −3 °C freezing, and after 1–5 h for −18 °C (Fig. 1A, B). Maximum respiration was 6–9 times that of the +4 °C reference at −18 °C freezing, and 3–6 times for soils at −3 °C. Respiration became similar to the reference 30–100 h after thawing. Cumulative respiration for the −3 °C treatment calculated for the first 100 h after thawing was 1.6 and 1.8 times the +4 °C reference in the mull and sandy soil, respectively, with higher values in the −18 °C treatment (2.5 and 2.2 times the reference).

Bacterial growth after freezing/thawing was very different from respiration (Fig. 2). Lowest values were found immediately after thawing. Growth rates in the −3 °C treatment were 44 and 43% of the reference in the mull and sandy soil, respectively (Fig. 2C, D). Even lower values were found for the −18 °C treatment, 23 and 26%. The survival of the bacteria, estimated as initial growth rates directly after thawing or rewetting, was thus higher for freezing/thawing compared to drying/rewetting (<10%; Meisner et al., 2013). Cumulative respiration was also smaller (around 2 and 7 times the reference for freezing/thawing and drying/rewetting, respectively), also indicating less killing effects of freezing/thawing. Our results, especially after a −3 °C freezing, therefore corroborates the suggestion by Skogland et al. (1988), that freezing/thawing is less detrimental for soil microorganisms compared to drying/rewetting.

The bacterial growth rate increased linearly after thawing for both freezing temperatures and in both soils (Fig. 2C, D), with no indications of a long lag period. The dynamics of bacterial growth after freezing/thawing were thus similar to those found after drying/rewetting, resulting in a type 1 response (Iovieno and Bååth, 2008; Meisner et al., 2015). Noteworthy is that the earlier reported uncoupling between bacterial growth and respiration after drying/rewetting, here also for the first time is shown after freezing/thawing. Possible mechanisms explaining this uncoupling have been discussed earlier for drying/rewetting (Meisner et al., 2015) and it is likely that the same mechanism(s) will be true for freezing/thawing.

The rate of increase was similar in all cases (slope of the regression line varying between 0.15 and 0.16; Fig. 2C, D). Therefore, the recovery time, i.e. the time point when bacterial growth rates after thawing were the same as in the reference at +4 °C, differed with freezing temperature, being shorter after −3 °C freezing, 36 h in both soils, than after −18 °C (50 h and 48 h in the mull and sandy soil, respectively). Meisner et al. (2013) reported that it took only 13 h after rewetting to recover bacterial growth rate to that in the moist reference soil. However, bacterial growth and recovery time are temperature dependent. The study by Meisner et al. (2013) was made at +17 °C, while we use +4 °C. Maienza and Bååth (2014) found that a recovery time after drying/rewetting of a few hours at 25 °C, corresponded to a recovery time

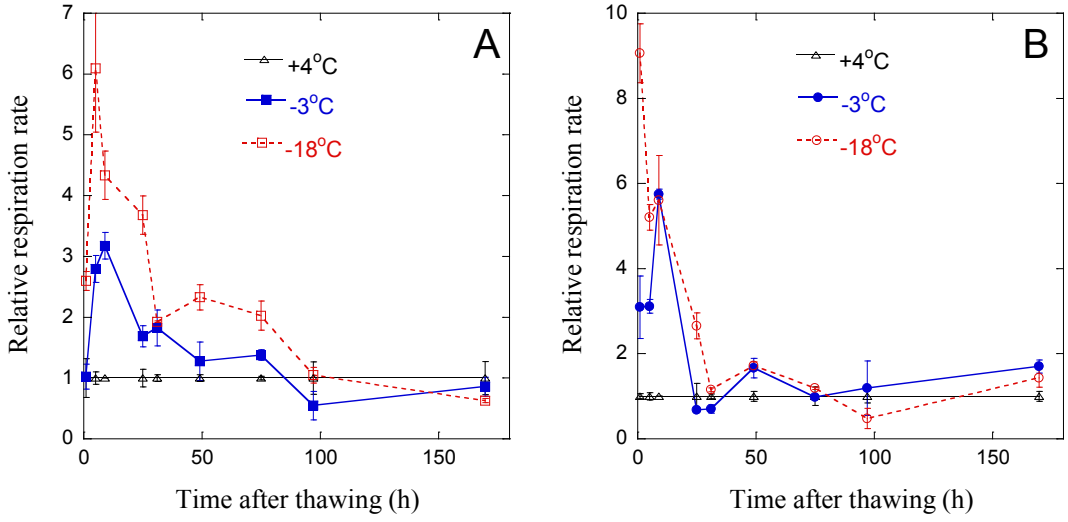

Fig. 1. Respiration rates at +4 °C after freezing/thawing A) a mull soil and B) a sandy soil. Filled blue symbols and thick line = frozen at −3 °C before thawing, open red symbols and stippled line = frozen at −18 °C before thawing, triangles and thin black line = soils kept at +4 °C as references. Data were standardized to 1 for the +4 °C reference for each soil and at every time point. Time indicates middle point of measurements. Bars indicate SE (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)