Microb Ecol (2014) 68:818–821
DOI 10.1007/s00248-014-0446-x

SOIL MICROBIOLOGY

Temperature Effects on Recovery Time of Bacterial Growth After Rewetting Dry Soil

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Received: 22 April 2014 / Accepted: 10 June 2014 / Published online: 22 June 2014

Abstract The effect of temperature on the recovery of bacterial growth after rewetting dry soil was measured in a soil that responded with bacterial growth increasing immediately upon rewetting in a linear fashion (type (i) response sensu Meisner et al. (Soil Biol Biochem 66: 188-192, 2013)). The soil was air-dried for 4 days and then rewetted at different temperatures. Bacterial growth over time was then estimated using the leucine incorporation method. At 25 °C, the recovery of bacterial growth to levels of a wet control soil was rapid, within 6 h, while at 15 °C, recovery time increased to around 60 h, becoming more than a week at 5 °C. The temperature dependency of the recovery time was well modeled by a square root function. Thus, temperature will not only directly affect growth rates but also affect length of transition periods, like resuscitation after a drying event. The temperature during the rewetting event thus has to be taken into consideration when analyzing the microbial response dynamics.

Introduction

Temperature and moisture content are two of the major environmental factors affecting microbial growth and activity in soils. The effect of temperature on microbial growth has been well studied in habitats ranging from low temperature Antarctic to high temperature desert soils [1, 2], showing that temperature sensitivity of growth in soil can be described by the square root model [3], where the square root of growth rate is linearly related to the temperature below the optimum temperature for growth. Moisture conditions are also well known to affect microbial growth and activity [4–7]. The importance of these factors has been emphasized during recent years due to the global change issues, where both temperature and soil moisture conditions, including drying/rewetting events, are predicted to change. Interactions between temperature and moisture on microbial activity in soil have therefore become an important area of study [8–10].

The recovery of bacterial growth after a drying/rewetting event has been studied earlier [6, 11–13], showing that two different patterns can be found. Meisner at al. [11] stated that pattern (i), originally described by Iovieno and Bååth [6], was characterized by recovery of bacterial growth starting more or less immediately after rewetting, increasing in a linear fashion over time. Respiration was highest directly after rewetting, followed by a gradual decrease. Pattern (ii), originally described by Göransson et al. [12], was characterized by very low bacterial growth immediately after rewetting, a pronounced lag period with low growth and finally an exponential increase in bacterial growth. Respiration for this pattern became immediately high, as for pattern (i), but remained high for a longer period, with often respiration increasing even further in a later stage.

Since bacterial growth is a temperature-sensitive process, it is hypothesized that the recovery time of growth after rewetting will also be temperature-dependent. The type (ii) pattern is similar to the microbial response to a shift-up in nutrients due to the addition of excess substrate [14–16], where the rate of growth during the exponential phase is expected to be affected by temperature in the same way as growth in soil under more stable conditions. The kinetics of the type (i) pattern is different, with a linear increase in growth starting immediately after water is added. That temperature is important also for this response type is indicated by the recovery time, which is the

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time to reach the level of growth in a constantly moist control soil, being 13 h at 17 °C [11], but only 8 h at 22 °C [6]. However, the temperature sensitivity of the recovery response over a large range of temperatures is not known, as is also the issue on the best model of temperature sensitivity of the recovery rate. This is essential to know if responses of drying/rewetting events are to be modeled in conditions of varying temperature. Here, we report on the effect of temperature on the recovery time of bacterial growth after rewetting a soil with a type (i) pattern. We used leucine incorporation as indicator of bacterial growth. Besides quantifying the temperature effect, we also compared bacterial growth in dry soil at different temperatures to elucidate if temperature affected the reliability of the leucine incorporation method in dry soils.

Materials and Methods

We used a soil, earlier studied by [6, 11] when studying rewetting, that repeatedly has resulted in a pattern (i) response. It is a managed grassland soil, originating from south Sweden, classified as a sandy loamy brown earth soil (Cambisol, FAO; Inceptisol, USDA). The soil had a pH in water of 6.6 and an organic matter content of 14.7 %. The soil was sampled in the spring of 2011, sieved (2.8 mm), and used within 2 weeks.

The rewetting experiment essentially followed the procedure by [11]. Soil was spread in a thin layer in a tray and air-dried at room temperature for 4 days, with a portion kept constantly wet as control (30 % gravimetric moisture, giving maximum bacterial growth rates). Rewetting was started by adding water to the air-dried soil to achieve 30 % moisture (three–four replicates), having two–three replicates for the moist control soil and one replicate for the dried soil. We compared three temperatures, 5, 15, and 25 °C. Initially, 5 and 15 °C was followed over 34 h and 25 °C over 8 h after rewetting. To achieve enough time resolution, a separate set of samples was rewetted in the morning and the afternoon for the 5 and 15 °C treatments (similar to the set-up of [11]). Since the time frame for the 5 and 15 °C treatment was too short to achieve complete recovery, the experiment was repeated with longer incubation times (up to more than a week, see Fig. 1). To increase the sensitivity in measuring low bacterial growth in the 5 °C treatment, the soil was also treated with 5 mg milled alfalfa per gram of soil 1 week before the drying started in the repeated experiment.

Leucine incorporation into bacteria extracted from soil was measured as a proxy for bacterial growth essentially according to Bååth et al. [17]. Extraction of the bacteria and subsequent measurements of leucine incorporation were made at the three temperatures, 5, 15, and 25 °C. The first measurement started 15 min after rewetting by weighing up 1 or 2 g of soil, adding 20 ml water with a subsequent 3 min vortexing to release bacteria, followed by a 10-min centrifugation step to isolate extracted bacteria in the supernatant. ^{3}H-leucine was then added to a 1.5-ml portion of the supernatant. The duration of

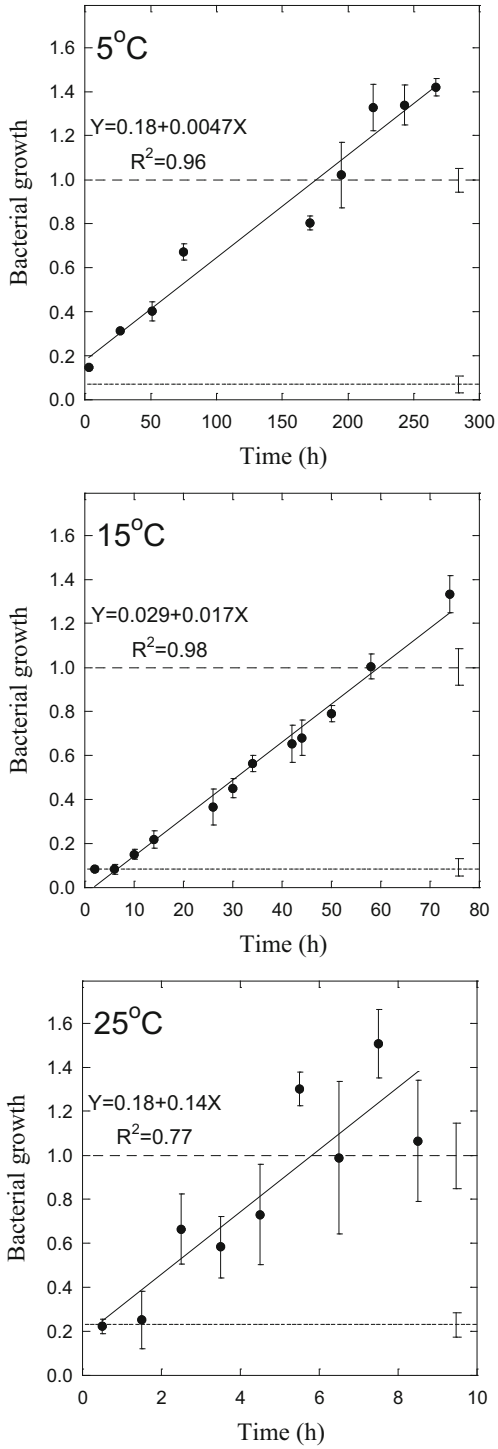

Fig. 1 Recovery of bacterial growth, measured using leucine incorporation, at 5, 15, and 25 °C after rewetting air-dried soil (*filled symbols* and *solid line*). Growth was standardized to one for the constantly wet soil (*dashed line*), and mean growth in dry soil is shown with a *dotted line*. *Bars* denote SE (n=3 or 4) for each measurement occasion for the rewetted soil, mean SE (n=2, mean for 9–12 measurement occasions) for the constantly wet soil, and SD (n=9–12 measurements over time) for the dry soil. The latter two bars are found to the right in the graph

incorporation with leucine varied with temperature being 1 h at 25 °C, 4 h at 15 °C, and 6 h at 5 °C. The leucine measurement was repeated at 9 to 12 sampling occasions during the incubation time. Also, the constantly wet soil and the air-dried soil were measured in the same way. For the latter, water had to be added to extract bacteria, but the period of vortexing and centrifugation before adding labeled leucine never exceeded 15 min.

The mean value of all measurements in the wet control soil was used to standardize the data for each temperature. Bacterial growth after rewetting versus the mean time of each incubation period with leucine was then fitted to a linear model [6], and the time taken for recovery to the bacterial growth in the wet control soil was estimated (Fig. 1). In the first trial for 5 and 15 °C, recovery time was estimated by extrapolation of the linear equation, since time was too short to achieve complete recovery. The recovery times were then square root transformed and a linear regression versus time was performed.

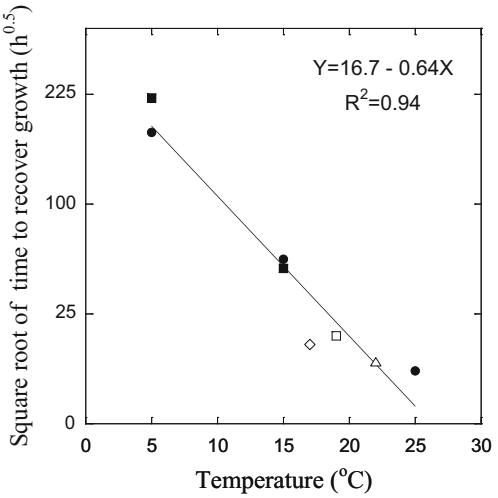

Fig. 2 Time to recover bacterial growth to that in wet soil after rewetting air-dried soil as a function of temperature. Recovery time was square root transformed, and a straight line was fitted. *Filled circles* indicate data from Fig. 1, *filled squares* indicate data from first test using extrapolation to calculate recovery time, *diamond* indicates data from Meisner et al. [11], *open square* indicates unpublished results (E. B.), and *triangle* indicates data from Iovieno and Bååth [6]. All the data were used for the regression

Results and Discussion

After rewetting, there was a linear increase in bacterial growth over time at all temperatures, eventually reaching the level found in the constantly wet soil (Fig. 1). Thus, this soil, as expected, showed the type (i) response throughout the temperature range studied. As hypothesized, the time to recovery varied with the incubation temperature. Recovery time was 175 h at 5 °C, 57 h at 15 °C, and 6 h at 25 °C, although the latter estimate is somewhat uncertain due to the rapid recovery and the short incubation time with leucine needed to be able to register the recovery.

Combining the data from Fig. 1 with data from earlier studies and from the present, where recovery time was estimated by extrapolation, showed that the temperature effect on recovery time could be modeled by a square root function (Fig. 2; R^2=0.94, p<0.001). The recovery time thus would be expected to vary between a few hours at temperatures around 25 to 30 °C, increasing to 1 week or more at 5 °C or lower.

Low bacterial growth was found in the dry soil, although significantly higher than zero (Fig. 1). However, we would expect bacterial growth in air-dried soil to be very close to zero, similar to respiration. We therefore think growth measured in the air-dried soil was overestimated. To measure leucine incorporation, the radioactive tracer has to be added in water, in slurry [18], or after extracting bacteria in a water solution (the method used in the present paper). Thus, even a dry soil by necessity has to be rewetted during the measurements. This is not a problem when it comes to altered nutrient conditions due to adding water, since bacterial growth will not react rapidly to a nutrient shift-up, and leucine incorporation raters will be stable for several hours [19]. However, adding water to a dry soil with the type (i) response will immediately result in increased bacterial growth. Even if the processing time before labeled leucine was made as short as possible, it still was around 15 min; this will be a period of resuscitation, increasing growth above that original present in the dry soil which we cannot measure with the leucine incorporation method. Furthermore, during the incubation with the labeled leucine, bacterial growth will also increase. This will be most problematic at high temperatures with more rapid recovery, while measurements at lower temperatures will be more correct. This was reflected in the dry soil at 25 °C having 22 % of the growth in wet soil, at 15 °C 9 %, and at 5 °C only 7 % (Fig. 1); the higher value for 25 °C was found even if the incubation time with leucine was only 1 h, while it was 6 h at 5 °C. Thus, with a soil with a type (i) response to rewetting, one should preferentially measure short times at low temperatures to achieve a more correct indications of the growth rate in the dry soil. With a type (ii) response, with a lag phase without little increased growth, there will probably be less of a problem.

In most cases, drying/rewetting events will be more common under higher temperature conditions, since drying of soil will be more pronounced in such situations. Our results therefore suggest that soil microbes will normally rapidly recover after a rewetting event in a soil with a type (i) pattern. However, there may be situations with drying/rewetting events at lower temperatures, for example, during winter or during night times in desert systems, where low temperatures can be found. Under these circumstances, longer recovery times are expected. The situation may be different in soils with a type (ii) pattern, with a lag period preceding an exponential growth.

The lag period for growth was around 12 h at 19 °C in a soil with the type (ii) pattern [12], which is the approximate time of complete recovery at the corresponding temperature in the soil studied here. Expecting the lag period to increase at lower temperatures, the effect of temperature on the recovery in a rewetting situation could thus be more important in the type (ii) than in the type (i) pattern, although this remains to be studied.

Acknowledgments This study was part of the Lund University Centre for Studies of Carbon Cycle and Climate Interactions (LUCCI).

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