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Microbial growth responses upon rewetting soil dried for four days or one year

Annelein Meisner, Erland Bååth, Johannes Rousk*

Microbial Ecology, Department of Biology, Lund University, Ecology Building, SE-223 62 Lund, Sweden

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ABSTRACT

A pulse of respiration is induced by rewetting dry soil. Here we study the microbial responses underlying this pulse of respiration when rewetting soil dried for 4-days or 1-year. In the 4-days dried soil, respiration increased to a maximum rate immediately upon rewetting after which it decreased exponentially. In the 1-year dried soil, respiration also increased immediately, but then remained high for 16 h, after which it increased further, exponentially, with a peak rate after 20 h. The level of bacterial growth was initially lower in rewetted than in constantly moist soil, but started to increase linearly immediately upon rewetting 4-days dried soil. In 1-year dried soil, bacterial growth started only after a 16 h lag period of zero growth, and then increased exponentially to a peak after 30 h, at rates superseding those in continually moist soil. Fungal growth started to increase immediately upon rewetting, and reached the rate of the control soil after 2 days for the 4-days dried soil, and after a week for the 1-year dried soil. Thus, prolonged drying altered the pattern of bacterial and fungal growth after rewetting. Our results suggest that both fungal and bacterial growth are uncoupled from the initial respiration pulse and that growth responses and microbial C-use efficiency can be affected by prolonged drying.

1. Introduction

Rewetting dry soil is known to result in a pulse of respiration (Kim et al., 2012). This phenomenon was first noted over 60 years ago (Birch, 1958). Respiration rates during the first 24 h after rewetting have been found to increase by 100–4400% compared with those of moist soils in cropland, forest and grassland (Kim et al., 2012), with even higher values in rewetted desert soil (Sponseller, 2007). Most frequently, respiration is highest during the first day after rewetting, after which it decreases during subsequent days. Studies analysing the respiration response after rewetting at a high time resolution (measurements every few hours or shorter) usually report highest respiration rates even within an hour after rewetting (Borken et al., 2003; Lee et al., 2004; Sponseller, 2007; Iovieno and Bååth, 2008; Unger et al., 2010; Kim et al., 2012; Placella et al., 2012), followed by decreasing rates. The decrease in respiration has been modelled with a negative exponential function (Li et al., 2010). There are also reports where respiration shows a very rapid and pronounced initial increase, to a rate that remains stable for hours, and subsequently is followed by a further increase in respiration (Griffiths and Birch, 1961; Haney et al., 2004; Chowdhury et al., 2011; Göransson et al., 2013). This secondary increase in respiration appeared to be exponential when measured using a high time resolution (around 1 h; Göransson et al., 2013).

Bacterial growth has been observed to be uncoupled from the respiration pulse via two patterns. (i) Growth starts immediately after rewetting, and increases linearly from low values to the levels found in a constantly moist soil (Iovieno and Bååth, 2008). This pattern for bacterial growth coincided with a soil respiration rate that was highest within an hour after rewetting and then decreased exponentially over time (the most frequently reported respiration pattern, see above). (ii) Bacterial growth only starts after a clear lag period, after which it increases exponentially to rates higher than in a constantly moist soil (Göransson et al., 2013). This pattern coincided with soil respiration rates that were elevated immediately upon rewetting, remained elevated for hours, and then increased further in an exponential way (the less frequently reported pattern; see above). One explanation for the difference in bacterial growth and respiration responses connected with the two patterns may be related to the length of drying prior to rewetting, ranging from a few days (Iovieno and Bååth, 2008) to two months (Göransson et al., 2013). Consequently, it needs to be resolved whether an

* Corresponding author. Tel.: +46 46 2223745.
E-mail addresses: anneleinmeisner@gmail.com (A. Meisner), erland.baath@biol.lu.se (E. Bååth), johannes.rousk@biol.lu.se (J. Rousk).

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extended drought period can change the bacterial growth responses from pattern (i) to pattern (ii) within the same soil.

Although bacterial growth initially is uncoupled from respiration, other microbial decomposers, such as fungi, may be involved in the respiration pulse induced by rewetting dry soil. Reports of fungal responses upon rewetting a dry soil have been inconsistent to date. Fungal biomass has been observed to both increase and decrease after the rewetting of air-dried soil (Gordon et al., 2008; Scheu and Parkinson, 1994) and fungal growth has been observed to be unaffected after one or multiple drying-rewetting events (Bapiri et al., 2010). However, assessments of fungal growth responses at sufficient temporal resolution to address their role during the respiration pulse after rewetting dry soil are lacking thus far.

Here we studied the bacterial and fungal growth and respiration responses upon rewetting soil dried for 4 days or for 1 year to answer the questions: (1) Does prolonged drying change the microbial growth and associated soil respiration responses from pattern (i) to pattern (ii)? (2) Can fungal growth explain the respiration pulse induced by rewetting dried soil?

2. Materials and methods

Two separate experiments were conducted to measure either bacterial or fungal growth as well as the respiration pulse upon rewetting of soils dried 4 days or for 1 year (henceforth "4-days dried" and "1-year dried", respectively). Soil from a managed grassland in south Sweden, classified as a sandy loamy brown earth soil (Cambisol, FAO; Inceptisol, USDA), was collected in the autumn of 2012 (wet-sieved using 2.8 mm mesh-size; $pH_{water} = 6.6$; SOM by loss on ignition (600 °C overnight) = 13.5%). This soil sample was used for the moist control and 4-days dried soil. The same soil was also sampled in the autumn of 2011 ($pH_{water} = 6.2$; SOM = 15.2%), wet-sieved and air-dried for about one year, until the experiments were performed.

The soil collected in 2012 was first adjusted to 50% water-holding capacity (WHC). Moist soil for the constantly moist control and 4-days dried treatment was then weighed (equivalent to 14.35 g dry weight) into microcosms (100 ml plastic containers with lids). The same amount of 1-year dried soil was also weighed into separate microcosms. Microcosms were then placed at room temperature (22 °C) under a ventilator. Lids were left open for the 4-days and 1-year dried soils, while lids were closed for the moist control soil. After 4 days of drying, the moisture stabilised and the soil thus was fully air-dried. At each experiment, three series with two replicate microcosms were prepared per treatment. The three series of microcosms from the dried soils (4-days and 1-year) were rewet to 50% WHC at three different time points (morning, afternoon, evening the same day) and incubated in dark conditions in a temperature controlled room (at 17 ± 1 °C, the expected summer soil temperature in the region). They were then sampled several times. By rewetting at different time points our design achieved response curves with a high temporal resolution during the time frame with rapid changes in microbial activity (the first 50 h after rewetting). The different series are combined in the graphs.

Bacterial growth was measured using ^{3}H-leucine incorporation into extracted bacteria (Bååth et al., 2001), which uses an estimate for protein synthesis as an index for *in situ* bacterial growth rate. Briefly, soil was mixed with water by vortexing and subjected to a low speed centrifugation. The leucine incorporation of the extracted bacteria in the supernatant was then measured during 1 h at 17 °C. The amount of Leu incorporated into extracted bacteria per h and g dry soil was used as a measure of bacterial growth.

Fungal growth was measured using ^{14}C-acetate incorporation into ergosterol (Bååth, 2001), which uses an estimate of ergosterol synthesis as an index for *in situ* fungal growth rate. Briefly, soil was incubated with ^{14}C-acetate for 2 h at 17 °C. Ergosterol was then extracted, separated and quantified using HPLC and the incorporated radioactivity in the collected ergosterol fraction was then determined. The amount of acetate incorporated into ergosterol per h and g dry soil was used as a measure of fungal growth.

Soil respiration at 17 °C was measured at several time points during both experiments. One-gram subsamples of soil were placed into a 20 ml glass vials. After purging the head space atmosphere with pressurized air, the vials were sealed and the CO_2 production was analysed after 2 h at 17 °C with a gas chromatograph equipped with a thermal conductivity detector.

Regression analyses (linear, exponential, and power curves) were used to describe the temporal dynamics of respiration and growth rates. Curves were fitted using Kaleidagraph 4.1 (Synergy Software 2010). Our default model to describe changes in microbial growth rates over time were exponential (Brock, 1971). This was also used for respiration during the exponential growth phase and during declining respiration after rewetting (Li et al., 2010). However, by inspecting the residual to the fitted curve, we found instances where exponential models gave poor fits. For these, we chose the model least prone to show systematic patterns in the residuals to fitted curves. Cumulative respiration and bacterial and fungal growth during 125 h after rewetting were estimated by determining the area under the fitted curves, and values were normalised to unity for the control.

3. Results

The respiration rate in the constantly moist control soils decreased slowly over time following a negative exponential function (Fig. 1). The respiration rate in the 4-days dried soil increased immediately to levels ~5 times higher than in moist control soil, followed by an exponential decrease over time. The 1-year dried soil

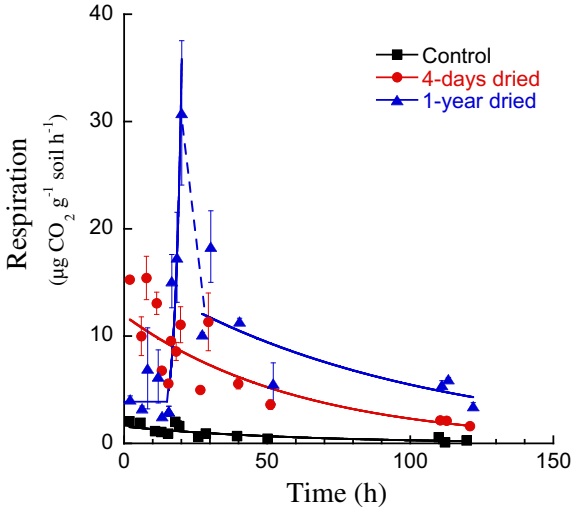

Fig. 1. Soil respiration after rewetting dried soil (in the experiment where also bacterial growth was measured, see Fig. 2). The respiration rate in the constantly moist control and the 4-days dried soil were modelled with a negative exponential function ($R^2 = 0.70$ and 0.84, respectively). For the 1-year dried soil different equations were used for different time intervals. During the first 16 h a straight line was used, between 16 and 20 h an exponential model was used ($R^2 = 0.77$). The rapid decrease after peak respiration was indicated by a dashed line, and the slower decrease after 27 h was modelled by a negative exponential equation ($R^2 = 0.68$). Error bars denote SEs ($n = 2$) and are smaller than the symbol when not seen.