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Prolonged drought changes the bacterial growth response to rewetting

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ABSTRACT

Rewetting a dry soil can result in two response patterns of bacterial growth and respiration. In type 1, bacterial growth starts to increase linearly immediately upon rewetting and respiration rates are highest immediately upon rewetting. In type 2, bacterial growth starts to increase exponentially after a lag period with a secondary increase in respiration occurring at the start of the exponential increase in growth. We previously observed that the type 1 response occurred after rewetting 4-day dried soil and type 2 for 1-year dried soil. Here we studied in detail how the duration of drought related to the two types of responses of bacterial growth and respiration to rewetting. Soil was air dried for different time periods from 4 days up to 48 weeks. Upon rewetting, bacterial growth and respiration was measured repeatedly at 17 °C during one week. Drought periods of ≤2 weeks resulted in a type 1 response whereas drought periods of ≥4 weeks resulted in a type 2 response. The lag period increased with drought duration and reached a maximum of ca. 18 h. The bacterial growth response was also affected by incubation of moist soil before drying–rewetting. The lag period increased with duration of moist soil incubation before the 4-day drying–rewetting event and reached also a maximum of ca. 18 h. The exponential growth increase in the type 2 response coincided with a secondary increase in respiration, which increased in magnitude with increasing drought duration. Cumulative respiration increased with drought duration and was ca. 4 times higher after 48 weeks of drought compared to 4 days. Thus, prolonged drought affected the response type of bacterial growth and respiration to rewetting, and also increased lag period, the magnitude of the secondary increase in respiration and total C release. The effect of drought was, however, modified by the lenght of the incubation period of moist soil before drought, suggesting that soil conditions before a drying–rewetting event need consideration when evaluating microbial responses.

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1. Introduction

Rewetting a dry soil will result in a pulse of CO_2 (Schimel et al., 2011; Kim et al., 2012; Placella et al., 2012). This phenomenon has been named the Birch effect after one of its first observers (Birch, 1958). The CO_2 pulse is large enough to be observed at field-scales when dry soil is moistened by rainfall events (Jenerette et al., 2008) and can contribute to a significant part of heterotrophic respiration in ecosystems (Yuste et al., 2005; Fan et al., 2015). The CO_2 pulse has been observed in many different ecosystems, including desert (Sponseller, 2007), agriculture (Priemé and Christensen, 2001), forest (Fierer and Schimel, 2002), and grassland soils (Warren, 2014). Respiration rates are often highest immediately upon rewetting, decreasing exponentially over time (Li et al., 2010; Kim et al., 2012; Meisner et al., 2013), but a secondary respiration increase has also been observed, with maximum rates reached around one day after rewetting (Göransson et al., 2013; Meisner et al., 2013). This secondary increase has been associated with more extensive drying (Meisner et al., 2013) or heating treatments (Haney et al., 2004) and may be involved in the increased release of CO_2 with more extensive drying (Chowdhury et al., 2011; Meisner et al., 2013; Barnard et al., 2015). As drying–rewetting events can affect soil C cycling, the microbial mechanisms that underlie the respiration response are of interest.

Two patterns of bacterial growth have been observed upon rewetting dry soil (Fig. 1). In the type 1 pattern, bacterial growth

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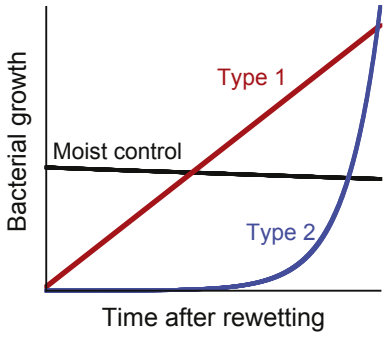

Fig. 1. Schematic overview of a type 1 and a type 2 response of bacterial growth upon rewetting a dry soil.

starts immediately upon rewetting and increases linearly with time (Iovieno and Bååth, 2008). This pattern has the highest respiration immediately after rewetting (Meisner et al., 2013), and thus microbial growth dynamics do not coincide with respiration. In the type 2 response (Fig. 1) the initial bacterial growth is very low after rewetting, and starts increasing exponentially only after a pronounced lag period. The exponential growth coincides with a secondary increase in respiration (Göransson et al., 2013). Bacterial growth, which previously was observed to have a type 1 response, was changed into a type 2 response after rewetting soils dried for one year instead of four days (Meisner et al., 2013). However, the question remains if the transition from the first to the second response type is gradual or occurs after a threshold time of drying.

Here we study how prolonged drought affects the transition from the type 1 to the type 2 pattern after rewetting to determine if there is a threshold of drought for this transition, and if the relationship saturates toward longer durations of drought. The aim was thus to determine how a gradient of drought durations influenced bacterial growth and the respiration responses in soil upon rewetting. We hypothesized that a longer drought period before rewetting (1) would change the bacterial growth from a linear growth increase upon rewetting (type 1) to an exponential growth increase after a lag period (type 2), (2) would increase the lag-period when a type 2 response was present, and (3) would increase the total CO_2 released from soil. In addition, we expected a secondary increase in respiration rate to coincide with the bacterial growth increase in type 2 responses, with increasing levels with increasing drought periods.

We performed a series of experiments where a soil, which initially had a type 1 response when rewetted after a 4-day period of drought, was dried for 4 days up to 48 weeks. We measured bacterial growth and respiration rates at high temporal resolution upon rewetting. During the study, it was found that the length of the incubation period of moist soil before the start of the drying period affected the microbial response. We thus also studied how the interaction between incubation time of moist soil and duration of drought affected respiration and bacterial growth responses after rewetting.

2. Materials and methods

2.1. Soil

Soil was collected from managed grassland in South Sweden in the autumn of 2012. The soil is classified as a sandy loamy brown earth soil (Cambisol, FAO; Inceptisol, USDA). This is a well-mixed soil without any conspicuous organic horizon and thus a composite sample was taken from approx. 0–20 cm depth. The soil had 15.6% soil organic matter (determined as loss on ignition at 600 °C) and a pH_{water} of 6.5. The soil was sieved fresh prior to the experiments to remove stones and roots, and the water content was adjusted to 50% of water holding capacity. This soil was used in previous experiments (Meisner et al., 2013), and fresh soil was shown to have a type 1 rewetting response after 4 days air-drying. Fresh soil was also sampled in autumn 2013 to verify that the type 1 response still remained in fresh soil.

2.2. Experiments

Moist soil was put into 500 ml microcosms containing lids to prevent water loss, and microcosms were incubated at room temperature (approx. 22 °C). They were regularly aerated and water was added to adjust to 50% WHC when needed. At different time points, the lid was removed and microcosms were put under a ventilator to dry (Fig. 2). They were then incubated dry without lids under the same conditions as microcosms with moist soil. The mean moisture content of dried soils before rewetting was 3.1 ± 0.1% WHC (mean ± SEM), and did not vary systematically with duration of drought. Rewetting was performed for all samples of an experiment at the same time. Therefore, the soils had not only different periods of drought, but also different periods of incubation in moist conditions before drying (Fig. 2). All treatments were replicated three times.

Three experiments were set up that ran for different periods. The results from the different experiments were combined to be able to analyze both (i) soils with different drought periods but constant incubation time with moist soil, and (ii) constant drought periods and different incubation times.

Experiment 1 was set up in autumn 2012 and ran for 19 weeks (Fig. 2). 18 microcosms were prepared with 120 g of soil in each. Microcosms were sampled two times. At the first sampling (Exp. 1a), soil had been air dried for 0 (continuously moist), 4 days, 1, 2, 4 and 8 weeks. At the second sampling (Exp. 1b), soil had been air dried for 9, 13 and 17 weeks.

Experiment 2 was set up in January 2013 and ran for 26 weeks (Fig. 2). 12 microcosms were prepared with 60 g of soil in each. Soil was air dried for 0 (continuously moist), 4 days, 4, 6 and 26 weeks before rewetting.

Experiment 3 was set up in autumn 2012 and ran for 48 weeks (Fig. 2). 15 microcosms were prepared with 60 g in each. Soil was air dried for 0 (continuously moist), 4 days, 8, 12, 24 and 48 weeks before sampling.

2.3. Rewetting

Responses of respiration and bacterial growth were measured after rewetting at a minimum of 10 time points during one week at 17 °C. Soil was divided into two sets for each replicate the day before rewetting and incubated in the dark at 17 °C (the expected summer soil temperature in the region). On the day of rewetting one set was rewetted up to 50% WHC in the morning and one in the evening. The two sets were used to allow response curves with high temporal resolution as was done previously (Meisner et al., 2013). The soil was rewetted with demineralized water using a pipette, after which the soil was mixed thoroughly with a spatula. The two sets per replicate are combined in the graphs.

2.4. Measurements

2.4.1. Respiration

For experiment 1, 3 g of soil was put in a 20 ml glass vial, purged with pressurized air, sealed and incubated at 17 °C. 4 ml air was sampled and stored in a 3 ml Exetainer® vial until analysis on a GC

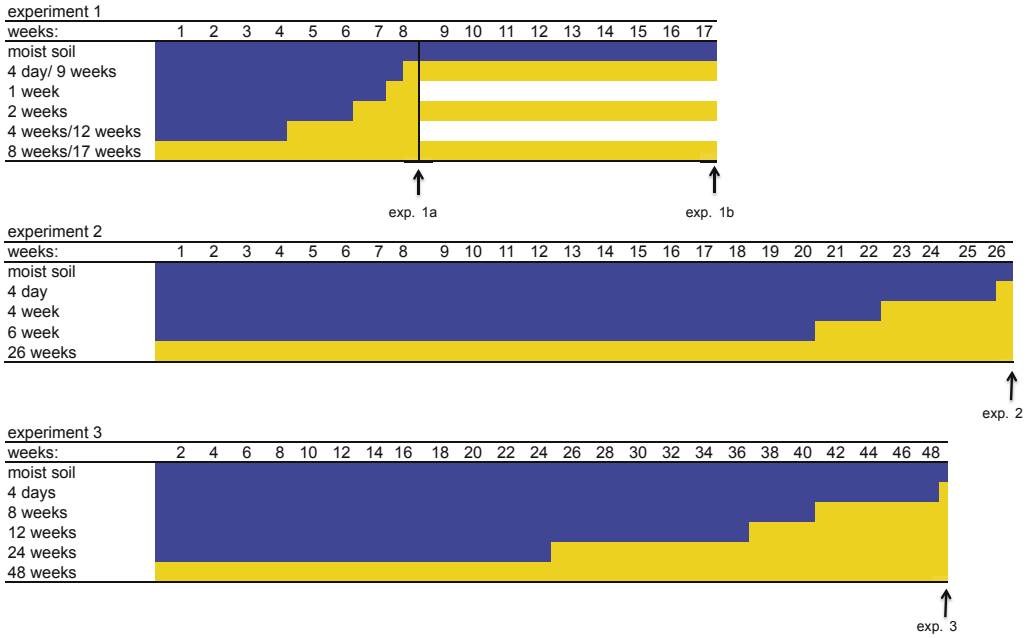

Fig. 2. Design of the study. Blue indicate incubation period of moist soil and yellow indicate the drying period of the soil. Arrows below the graphs indicate the time point when soil was sampled to be rewetted (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

equipped with a methanizer and a FID detector. For experiment 3, 1 g of soil was put in a 20 ml glass vial, purged with pressurized air, sealed and incubated at 17 °C. Incubation periods for the respiration measurements varied between 4 and 30 h, and data points were plotted at the arithmetic midpoint of the interval used. The CO_2 production was analyzed on the same GC as above, but directly with an autosampler. Respiration of experiment 2 was not measured.

2.4.2. Bacterial growth

Bacterial growth was measured by incorporation of ^{3}H-leucine (Leu) into extracted bacteria (Bååth et al., 2001). Briefly, at each time point after rewetting, soil was mixed with demineralized water by vortexing for 3 min. After a low speed centrifugation, bacterial Leu incorporation within the extracted bacteria was measured in the supernatant during 1 h at 17 °C by combining non-radioactive Leu and tritiated Leu ([^{3}H]Leu, 37 MBq ml^{-1}, 5.74 TBq mmol^{-1}, Perkin Elmer, USA) to yield a final concentration of 275 nM. Bacterial growth was expressed as the amount of Leu incorporated into extracted bacteria per g dry soil and h.

2.5. Modeling bacterial growth after rewetting

Growth of bacteria was modeled during the first 50 h after rewetting, because this period covered increased growth up to maximum values, after which the bacterial growth decreased again to the moist control situation (see also Meisner et al., 2013). A linear model was used for bacterial growth that started immediately upon rewetting (type 1 response, Fig. 1.), since the residuals showed that this model fitted the data best and this has been repeatedly shown to be an adequate model (Iovieno and Bååth, 2008; Meisner et al., 2013). The lag time was set to 0 h for this response type.

Bacterial growth was fitted with the Gompertz model (Gibson et al., 1988; Zwietering et al., 1990; Belda-Galbis et al., 2014) when growth had a lag period followed by an exponential increase in growth (type 2 response, Fig. 2.). The Gompertz model is a sigmoid model that has frequently been observed to describe growth of bacteria well (Zwietering et al., 1990) and is often used for bacterial growth in food sciences (Gibson et al., 1988; Belda-Galbis et al., 2014; Filannino et al., 2014) and also for describing plant growth (Paine et al., 2012). The Gompertz function expresses changes in growth as:

$$G_t = G_{t0} + A \times e^{-e^{b-ct}} \quad (1)$$

G_t is the logarithm of bacterial growth at time t, G_{t0} is the logarithm of the bacterial growth at t_0, A is the difference between the upper and lower asymptotes of the curve, that is initial growth and maximum growth, and b and c are fitted mathematical parameters.

The lag time was then calculated as:

$$Lag\ time = \frac{b-1}{c} \quad (2)$$

μ_{max} is the specific bacterial growth rate during the exponential growth phase and is calculated as:

$$\mu_{max} = \frac{A \times c}{e} \quad (3)$$

Maximum growth was calculated as:

$$Maxgrowth = G_{t0} + A \quad (4)$$

2.6. Cumulative respiration and growth

Cumulative respiration and bacterial growth was calculated for two time points. The 4 h time point was chosen to investigate the initial decoupling between growth and respiration shortly after rewetting; this was also the time point when respiration was first measured. The 50 h time point was chosen as a longer time-period, because respiration and growth had peaked approximately at this time, and this period included the most intensive sampling period.

2.7. Statistics

Curve fitting was done in Kaleidagraph 4.5.2 for Mac (Synergy Software) and regression statistics were done in R version 3.1.1 (RCoreTeam, 2014). One way ANOVA was performed if regression analysis was not possible. Post hoc Dunnet tests were done in the Multicomp package (Hothorn et al., 2008) by comparing treatments with the control.

3. Results

3.1. Bacterial growth

A prolonged drought before rewetting changed the type 1 pattern into type 2 (Fig. 3A), and the lag period increased with a longer duration of drought (Fig. 3B). A type 1 pattern was observed upon rewetting soil, which was freshly collected and dried for only 4 days, with bacterial growth increasing linearly with no lag period after rewetting (Fig. 3A, note that due to y-axis being log-transformed this model will appear curve-linear). A type 2 pattern was observed upon rewetting soils dried for longer time periods, with bacterial growth initially being lower than in 4 days dried soil and growth starting to increase exponentially after a lag period (data from the three different experiments, Fig. 3A). An 8 weeks drought had an 8 h lag period, increasing to 18 h after 48 weeks drought (Fig. 3B). The increased lag period with increasing drought duration was modeled with a logarithmic function.

The bacterial growth response changed from a type 1 to a type 2 response not only with a longer drought duration before rewetting, but also with a prolonged incubation of moist soil before drying (Fig. 4A). An exponential increase in growth started after a lag period upon rewetting of 4 day dried soils when the soils had been incubated moist for 26 and 48 weeks before drying, but not when soils were incubated 0 and 8 weeks before drying (Fig. 4B). In the two latter cases, a linear increase with no lag period in bacterial growth was found after rewetting (type 1).

The incubation period of moist soil before drying was only important when this period was longer than 8 weeks (Fig. 5). Shorter incubation periods resulted in a lag period aligning to the logarithmic function determined for soil without incubation (c.f. Fig. 3A). For example, the lag period of all drought periods in Exp. 1a, which lasted only for 8 weeks, were well modeled by the logarithmic function determined for non-incubated soils. In this experiment a lag period was found for soils dried for 4 or 8 weeks (4.5 h and 8 h, respectively), but not when the soil was dried for shorter periods. Thus, a transition from a type 1 to a type 2 response occurred after between 2 and 4 weeks of drought.

Specific bacterial growth rate (μ_{max}) during the exponential increase in growth in the type 2 response was on average 0.23 h^{-1} for all treatments that were fitted with the Gompertz function. There was no systematic variation due to the drought period or incubation time of moist soil. Maximum growth rate was always higher with prolonged drought than using only 4 days (Fig. 3A). Combining all data, maximum growth rate increased with increasing drought period (log (Max growth) = 1.53 + 0.38 × log(Weeks of drought), $R^2 = 0.75$, $P < 0.001$, data not shown).

3.2. Respiration

The constantly moist soil had low respiration rates, being higher after 8 weeks of incubation (Exp. 1a; Fig. 6A) than after 48 weeks of incubation (Exp. 3; Fig. 6B). A pulse in respiration was, as expected, observed upon rewetting dried soils. In Exp. 1a, the respiration responses could be modeled with a negative exponential function for the 4-day dried soil, with the highest respiration rates occurring immediately after rewetting (Fig. 6A). This was also the case for the 4-day dried soil in Exp. 3, when the soil was incubated moist for 48 weeks before drying (Fig. 6B).

The respiration response was more pronounced with longer drying periods and a secondary increase was observed with a maximum respiration rate around 35–40 h after rewetting. At this time point, respiration was around 2 times higher in soils with 8 weeks compared to 4 days drought in both experiments (Fig. 6A and B). An increasing drought period resulted in an even more conspicuous secondary increase in respiration, being 3 times higher for 24 weeks and 5 times higher for 48 weeks drought than respiration in the 4 days drought treatment (Fig. 6B).

3.3. Cumulative respiration and bacterial growth

Cumulative respiration was on average 20 μg C–CO_2 g^{-1} soil during 4 h after rewetting, which was around 4 times higher than the constantly moist control soil (Fig. 7A; $P < 0.001$). Duration of drought did not affect respiration during the first 4 h. Cumulative respiration increased linearly with prolonged drought over the first 50 h after rewetting (Fig. 7B), increasing from around 200 μg C–CO_2 g^{-1} soil with 4 days drying to almost 800 μg C–CO_2 g^{-1} soil after 48 weeks drought.

Cumulative bacterial growth was very different from the cumulative respiration during the first 4 h after rewetting, because

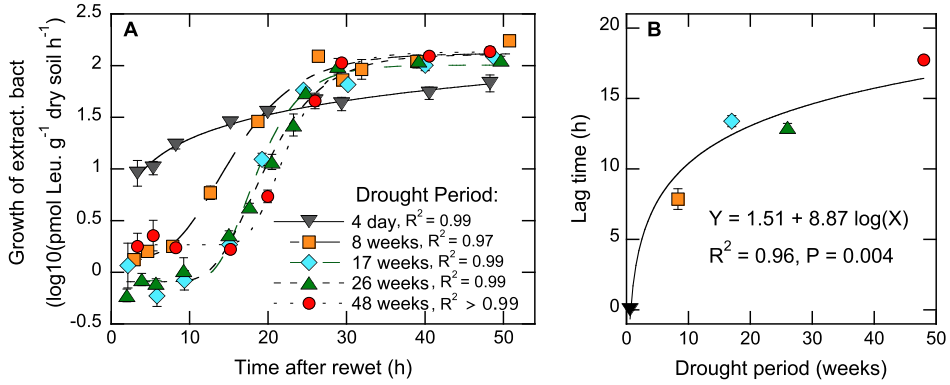

Fig. 3. Bacterial growth (panel A) and the lag period (panel B) in soil, which were not incubated before the drought period (see Fig. 1). Relationships between time after rewet and bacterial growth rates were fitted with a linear equation for the 4 day drought soil (fresh soil) and with the Gompertz equation for the 8 weeks (Exp. 1a), 17 weeks (Exp. 1b), 26 weeks (Exp. 2) and 48 weeks (Exp. 3) dried soils (panel A). The relationship between drought period and lag time was fitted with a logarithmic equation (panel B). Average values with SEM are presented (n = 3 microcosms per treatment).

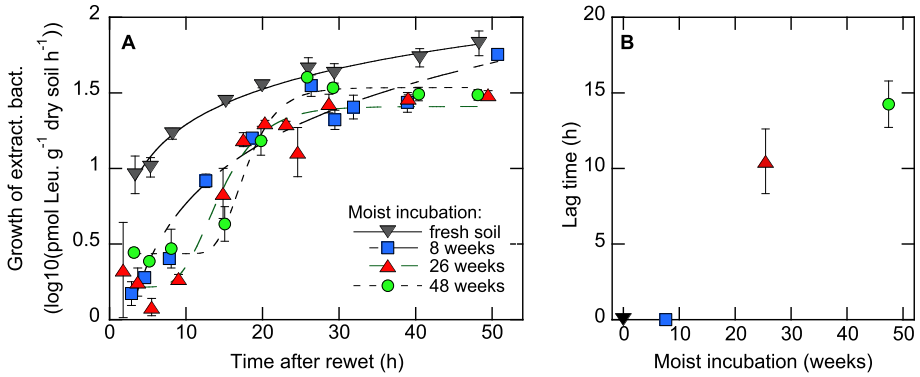

Fig. 4. Bacterial growth (panel A) and the lag period (panel B) upon rewetting 4 day dried soils, which were incubated moist before drying and rewetting. The relationship between time after rewet and bacterial growth rates were fitted with a linear function for 0 weeks (fresh soil) and 8 weeks (Exp. 1a) of moist incubation before drying and rewetting. 26 weeks (Exp. 2) and 48 weeks (Exp. 3) of moist incubation before drying and rewetting were fitted with the Gompertz equation (panel A). Average values with SEM (n = 3 microcosms per treatment).

growth in dried soils was on average 80% lower than in the constantly moist soils ($P < 0.001$, Fig. 7C). The lower cumulative bacterial growth and higher cumulative respiration 4 h after rewetting thus showed a clear decoupling between growth and respiration. There was no major effect of prolonged drought on this initial cumulative growth, except slightly higher values in 4-day dried soil from experiment 2. Bacterial growth increased with drought period over the first 50 h after rewetting, although there were large variations between different experiments (Fig. 7D). Incubation time of moist soil did not affect this relationship.

4. Discussion

4.1. Duration of drought and transition from type 1 to type 2

In line with our first hypothesis, prolonged drought caused a transition from a linear growth increase immediately upon rewetting (type 1 response) to exponential growth starting after a lag period of low growth (type 2 response). This transition was earlier shown to be present after 1 year of drought (Meisner et al., 2013), but we now show that a short lag period is already occurring after between 2 and 4 weeks of drought in this soil. This is well within the time frame of seasonal droughts present in areas with semi-arid or Mediterranean type climates (Jarvis et al., 2007; Vargas et al., 2012; Barnard et al., 2015), and similar drought periods are also occasionally found in northwestern Europe (Cienciala et al., 1997; Rebetez et al., 2006; Lund et al., 2012).

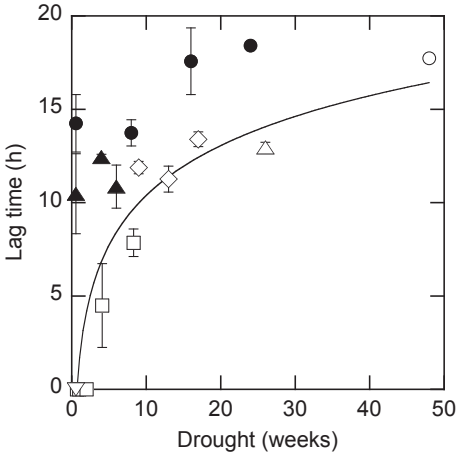

Fig. 5. Lag time as influenced by drought period. Squares are Exp. 1a, diamonds are Exp. 1b, up-facing triangles are Exp. 2, circles are Exp. 3 and the down-facing triangle is freshly collected soil. Open symbols are soils with ≤8 weeks moist soil incubation before drying and closed symbols are soils with >8 weeks moist soil incubation before drying. The line is the logarithmic model fitted through soils without moist incubation before drying (see Fig. 3B). Average values with SEM are presented (n = 3 microcosms per treatment).

The mechanism underlying the transition from a type 1 to a type 2 response was earlier suggested to be a combination of the size of the surviving microbial community after drying and the amount of available C released after rewetting (Göransson et al., 2013; Meisner et al., 2013). The type 2 response is characterized by two stages, a lag period with very slow bacterial growth and an exponential phase with an exponential increase in growth. We suggest that the size and physiological conditions of the surviving community after drought will be most important in determining the lag period, whereas the extent of the exponential growth phase depends on the amount of available C when growth starts (see 4.4.). It is well known that bacterial survival decreases with duration of desiccation, although species differences are profound (Chen and Alexander 1973; Nocker et al., 2012). Survival may indeed be lower when soils had a type 2 response, as suggested by using the initial bacterial growth rate as a proxy for survival. Soils with ≥4 weeks of drought all had lowest initial growth (Fig. 7C) and a type 2 response. Furthermore, earlier studies of soils with a type 2 response have all shown very low bacterial growth immediately after rewetting (Göransson et al., 2013; Meisner et al., 2013).

4.2. Lag period dependence on drought duration

Increasing the duration of drought resulted in longer lag periods (Fig. 3B), which is in accordance with our second hypothesis. The lag period was 18 h after a 48 week drought, which is only slightly higher than 14 h lag period for a one year drought in the same soil (Meisner et al., 2013). A 16 h lag period was reported for forest soils from the U.K. dried for 2 months (Göransson et al., 2013). Lag periods of 3 h and 48 h have been observed in plate and total counts after rewetting air-dried soil (Stevenson, 1956; Griffiths and Birch, 1961). Differences in the duration of the lag period between studies could partly be due to differences in incubation temperatures (Maienza and Bååth, 2014), because these differed from 17 °C (this study) to 30 °C (Stevenson, 1956). However, the duration of the lag period increased with duration of drought in our study conducted at the same standardized temperature.

Differences in the duration of the lag period within our study may be explained by a combination of a real and an apparent lag. A real lag period is caused by the adjustment of bacteria to new conditions, because they repair damaged parts, induce new enzymes necessary for future growth or awake from dormancy. An apparent lag period is

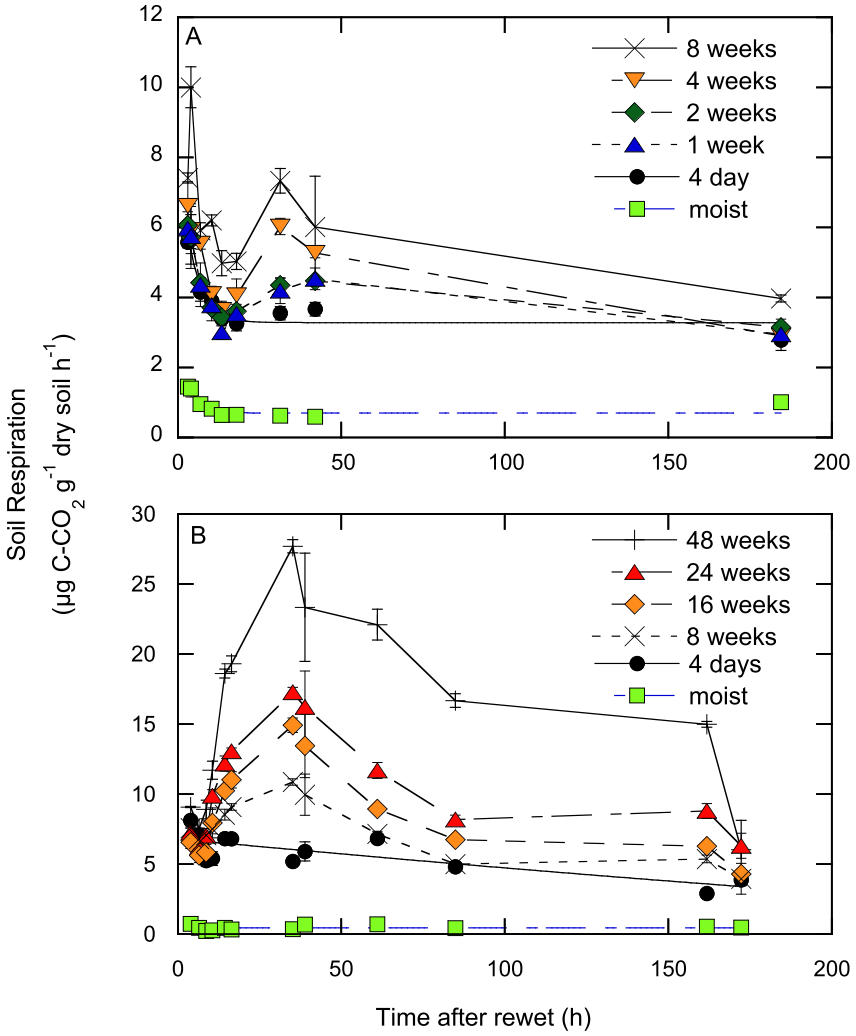

Fig. 6. Respiration rates after rewetting of soil dried for up to 8 weeks in Exp. 1a (panel A) and up to 48 weeks in Exp. 3 (panel B). Green squares denote the constantly moist soil. The respiration pulse in the 4 day dried soils were modeled by a negative exponential function ($R^2 = 0.91$ in panel A and $R^2 = 0.64$ in panel B). Average with SEM are presented (n = 3 microcosms per treatment).

observed when a small fraction of the bacteria increases their growth, but this will not be immediately detected, since a larger fraction of bacteria with unchanged growth will mask this. Apparent lag has been suggested as an explanation of the lag period in respiration and growth commonly found after adding glucose or other easily available substances (Stenström et al., 1998; Blagodatskaya et al., 2007; Reischke et al., 2014). However, a large fraction of the bacterial community is already growing in soil upon the addition of glucose, which may easily mask initial growth on glucose by a small fraction of the community. In contrast, the initial bacterial growth was very low in the dried soil with a type 2 response, which makes a small increase in growth more easily noticed. As such, the contribution by an apparent lag probably is smaller after rewetting dried soils than after the addition of glucose in soil.

An increase in real lag with prolonged drought may be caused by sub-lethal injuries, since stresses, such as drought and heat, will not only kill bacterial cells, but can also cause sub-lethal injuries in still viable cells (Mackey and Derrick, 1982, 1984; Nocker et al., 2012). Injuries such as damage to DNA, proteins, membranes and cell walls, are difficult to repair during drought as microbes are not active (Potts, 1994). Instead, repair mechanisms will immediately be initiated upon rewetting (Setlow and Setlow, 1996). Sub-lethal injuries will increase with duration of stress (Mackey and Derrick, 1982), leading to longer repair times before onset of growth after rewetting. Therefore a longer real lag period may be observed with increasing duration of drought.

The maximum lag period was ca. 18 h in our soil (incubated at 17 °C) even in the case of the longest drought periods (Fig. 5). One reason for a maximum lag period could be the presence of spore forming bacteria (Manzoni et al., 2014), including the genus *Bacillus*. Although extreme desiccation can induce injuries, like DNA-breaks, in dormant *Bacillus* spores (Dose et al., 1991), they will be much less affected by desiccation than growing cells. *Bacillus* spores will germinate rapidly (Levinson and Hyatt, 1956) and this genera is commonly described as fast growing under high substrate concentrations (Wipat and Harwood, 1999; Artursson and Jansson, 2003; Hery et al., 2005; Wolf et al., 2013) similar to the conditions in soil after rewetting dried soil. Increases in the number of DGGE bands of Firmicutes, including *Bacillus*, have also been observed after drying (Martí et al., 2012) and in soils that were stored dry for more than 150 years (archived soils from the Rothamsted Broadbalk experiment, Clark and Hirsch (2008)). Thus, we suggest that there will be a maximum duration of the lag period even after prolonged drought due to presence of spore formers in

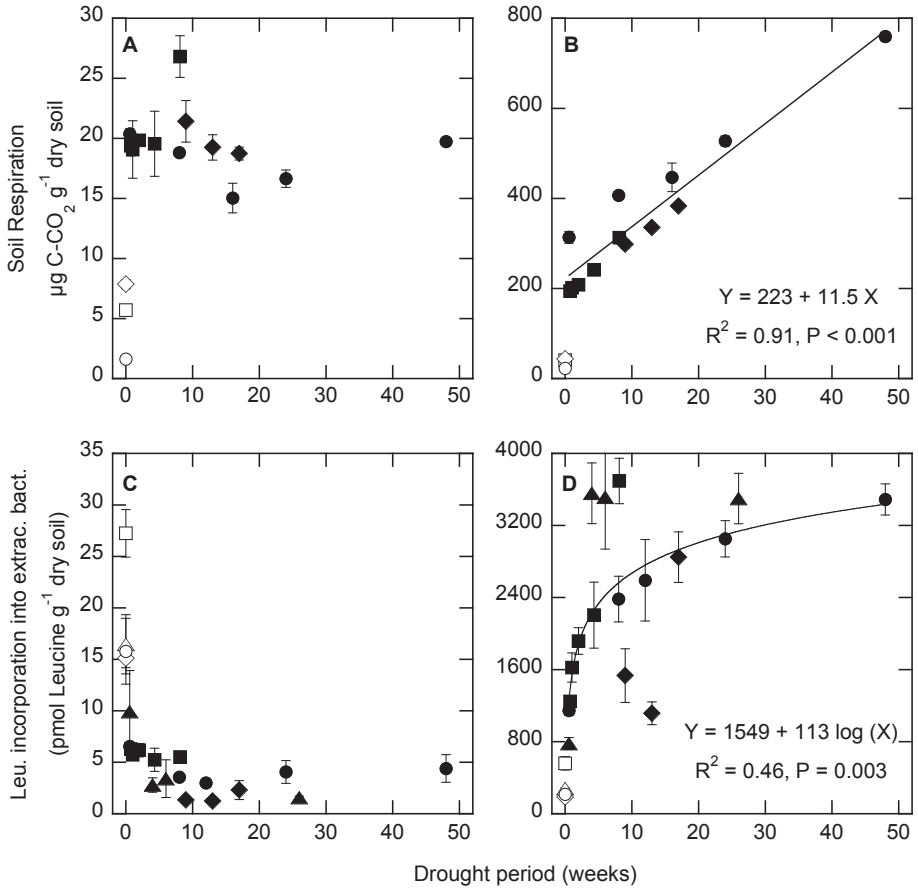

Fig. 7. Effect of drought period on cumulative respiration (panels A and B) and bacterial growth (panels C and D) during 4 h (panels A and C) or 50 h (panels B and D) after rewetting. Squares are Exp. 1a, diamonds are Exp. 1b, triangles are Exp. 2 and circles are Exp. 3. Average values with SEM are presented (n = 3 microcosms per treatment). Average values were used for the curve fits. Constantly moist soils (open symbols) were not used for the curve fits.

all soils, the maximum lag time set by the germination rate and start of exponential growth of surviving bacterial spores.

4.3. Lag period dependence on incubation duration

A transition from a type 1 to a type 2 response occurred also after rewetting 4-day dried soils that were incubated in moist conditions for more than 8 weeks (Figs. 4B and 5). The lag period in these moist incubated soils reached the same maximum duration of almost 20 h similar as the 48 week dried soil without moist incubation (see above). These results highlight the importance of parallel incubations of controls under the same conditions as the treatments to avoid artifacts in soil measurements.

The transition from a type 1 to a type 2 response induced by the length of the moist incubation period before drying may have been caused by a lower amount of microbial biomass since biomass (Ross et al., 1980) and activity (Fig. S1) decrease during incubation of moist soil. Soil drying also decreases the microbial biomass (Jensen et al., 2003; Wu and Brookes, 2005; Hueso et al., 2012). Thus, we suggest that both prolonged drying and incubation of moist soil will result in low amounts of surviving microbes, causing a transition to a type 2 response after rewetting. The mechanisms of the biomass decrease are, however, different. During drought, microbial biomass may decrease due to damage to microbial cells (Dose et al., 1991; Nocker et al., 2012). During moist incubation, carbon is lost from soil via respiration (Ross et al., 1980) and when carbon availability is lower in soil, microbial activity declines (Fig. S1) and biomass decreases. This suggests that drought decreases microbial biomass by directly affecting viability whereas prolonged incubation of soil decreases microbial biomass via a decrease in available resources.

4.4. The secondary increase in respiration

Longer drought periods resulted in higher cumulative soil respiration during 50 h after rewetting, which is in accordance with our third hypothesis (Fig. 7B). This shows that more C will be available with prolonged drought. The released C was, however, saturating microbial activity 4 h after rewetting (Fig. 7A). This saturating effect was earlier suggested to be similar as the saturating effect when adding high concentrations of glucose in the SIR method (Göransson et al., 2013).

If C released by drying–rewetting is not used during the initial lag period, then there will be a surplus of substrate left. This excess substrate is likely used during the exponential bacterial growth phase (see also 4.1), which coincides with the secondary increase in respiration. This is similar to the response of adding glucose to soil, since the exponential increase in bacterial growth coincides with a secondary increase in respiration after a lag period (Blagodatskaya et al., 2007; Reischke et al., 2014). The difference is that the resources after drying–rewetting come from a natural perturbation of the soil system, whereas a glucose treatment is an external source, added in an artificial situation.

There are three lines of evidence indicating that the secondary increase in respiration (Fig. 6) was caused by bacterial growth. First, the onset and duration of the secondary increase in respiration

coincided with the exponential increase in bacterial growth rates. Second, both peak respiration and maximum growth during this period increased with duration of drought, in a similar way as adding glucose at different concentrations increased both maximum growth and respiration (Reischke et al., 2014). Third, fungal growth was not likely important in explaining the secondary increase in respiration, because previous measurements have shown that the respiration increase and fungal growth rates do not coincide in the same soil studied here after 1 year of drought (Meisner et al., 2013).

4.5. Decoupling of respiration and growth

During the lag period, there is a pronounced decoupling between bacterial growth and respiration with low growth but high respiration. Fungal growth has been shown to be similarly disconnected from respiration during this period (Meisner et al., 2013). This decoupling between growth and respiration is consistent with previous results (Stevenson, 1956; Iovieno and Bååth, 2008; Göransson et al., 2013; Meisner et al., 2013; Blazewicz et al., 2014). The decoupling has been suggested to be due to non-growth activities of initially dormant cells (Blazewicz et al., 2014), for example spores that start respiring upon germination (Levinson and Hyatt, 1956). In addition, the fraction of dormant cells over active cells has been suggested to increase during drying (Manzoni et al., 2014). We find it unlikely, however, that a small population of spores or dormant cells could initiate such high respiration levels due to germination immediately after rewetting. Furthermore, respiration rates seem to be decreasing the first hours after rewetting (Fig. 6), which is not in accordance with respiration from germinating bacterial spores (Mandels et al., 1956). Thus, the initial respiration response cannot be solely explained by the awaking of dormant microbes.

An alternative explanation for the decoupling between growth and respiration could be the presence of oxidative enzymes (Maire et al., 2013). Damage to cells that occurs during drying is suggested to be difficult to repair, as fewer microbes are metabolically active during dry conditions (Potts, 1994; Vriezen et al., 2007). Still-functioning oxidative enzymes in these dead or damaged microbes may be responsible for the initial release of carbon dioxide after rewetting (Miller et al., 2005; Burns et al., 2013), even if the actual growth of viable cells is very low. Still-functioning enzymes can also explain consistent and significant levels of respiration during at least 3 months after all cells have been killed by irradiation (Ramsay and Bawden, 1983). A similar mechanism has furthermore been suggested for the initial decoupling between growth and respiration at higher than optimum growth temperatures (Pietikäinen et al., 2005).

4.6. Concluding remarks

Our results show that the response pattern of bacteria and respiration upon rewetting dry soil will depend on both the duration of drought and the duration of the incubation of moist soil before drying–rewetting. Both treatments resulted in a change in bacterial growth and respiration from an immediate increase in bacterial growth (type 1; Fig. 1) to an exponential increase in growth after a lag period (type 2). Although our study was made under laboratory conditions, our findings will have relevance for field situations. This is most obvious for the effect of drought, since extended drought periods can be observed in many habitats from arid to temperate ecosystems. However, the effects of incubation of moist soil before a drying–rewetting event suggest that the situation before a drought will also affect the response of the soil community to a drying–rewetting event. Fallow, for example, may be a similar treatment as our moist soil incubation, resulting in less available soil C for the microbes. Earlier studies on drought or precipitation legacy effects on the drying–rewetting response (Göransson et al., 2013; Evans et al., 2014) may also be confounded by changes in available C. Lower plant productivity during drought spells decreases the amount of available C (Ruehr et al., 2009; Fuchslueger et al., 2014). In contrast, the application of manure or incorporation of plant material into soil will increase the amount of C for soil microbes. Such management practices may also affect the drying–rewetting response of soil microbes. Our results therefore highlight a need for more studies on how the status of the microbial community before drying affects the rewetting response, and not only focusing on the actual drying–rewetting event *per se*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.06.002.

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