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Threshold concentration of glucose for bacterial growth in soil

Stephanie Reischke [a, *], Manoj G.K. Kumar [a, b], Erland Bååth [a]

[a] Section of Microbial Ecology, Department of Biology, Lund University, Ecology Building, SE-223 62 Lund, Sweden
[b] Faculty of Mathematics and Natural Science, Microbial Ecology – Centre for Ecological and Evolutionary Studies, University of Groningen, 9747 AG Groningen, The Netherlands

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ABSTRACT

The activity of heterotrophic soil microorganisms is usually limited by the availability and quality of carbon (C). Adding organic substances will thus trigger a microbial response. We studied the response in bacterial growth and respiration after the addition of low amounts of glucose. First we determined if additions of glucose, at concentrations which did not result in an exponential increase in respiration after the lag phase, still stimulated bacterial growth. The second aim was to determine the threshold concentration of glucose needed to induce bacterial growth. Adding glucose-C at 1000 μg g^{-1} soil resulted in an increased respiration rate, which was stable during 12 h, and then decreased without showing any exponential increase in respiration. Bacterial growth, determined as leucine incorporation, did not change compared to an unamended control during the first 12 h, but then increased to levels 5 times higher than in the control. Thus, after the lag phase, a period with increasing bacterial growth, but at the same time decreasing respiration rates, was found. Similar results, but with a more modest increase in bacterial growth, were found using 500 μg glucose-C g^{-1} soil. Adding 50–700 μg glucose-C g^{-1} resulted in increased respiration during 24 h correlating with the addition rate. In contrast, bacterial growth after 24 h was only stimulated by glucose additions >200 μg C g^{-1} soil. Thus, there was a threshold concentration of added substrate for inducing bacterial growth. Below the threshold concentration growth and respiration appear to be uncoupled.

1. Introduction

Availability and quality of nutrients influence the activity of heterotrophic microorganisms in soil, where especially carbon (C) limitation has been considered to be common (Joergensen and Scheu, 1999; Aldén et al., 2001; Demoling et al., 2007). Therefore, many studies focused on adding an easy available C source, like glucose, to study the reaction of the microbial community to changes in substrate concentrations in soil (e.g. Stotzky and Norman, 1961; Tsai et al., 1997; Pennanen et al., 2004; Eilers et al., 2010). In a recent study, fungal and bacterial growth after adding different concentrations of glucose to soil was studied (Reischke et al., 2014). They showed that after adding glucose, bacterial growth did not increase during the lag phase (equivalent to the phase of stable respiration used for estimating substrate induced respiration, SIR; Anderson and Domsch, 1978). However, when respiration started to increase exponentially, bacterial, but not fungal, growth increased in parallel suggesting that bacteria were the main agent for the respiration response, but also that both the exponential increase in respiration and growth could be used to estimate the intrinsic bacterial growth rate, μ, on the added glucose. Most of the studies on effects of glucose additions use fairly high C concentrations, often more than 1 mg C g^{-1} soil (Nannipieri et al., 1978; Sparling et al., 1981; Griffiths et al., 1999). However, the concentration of easily available carbon in soil is much lower (usually <30 μg C g^{-1} soil; van Hees et al., 2005; Hill et al., 2008; Blagodatskaya et al., 2009), and studies adding few hundred μg glucose-C g^{-1} soil or lower has become more common (Bremer and Kuikman, 1994; De Nobili et al., 2001; Hoyle et al., 2008; Sawada et al., 2008; Dungait et al., 2013).

At high, C-saturated, concentrations of glucose addition, respiration and bacterial growth will change in a predictable pattern over time. An initial lag phase, with no extra growth on the added substrate and stable, high, respiration (the SIR level), will be followed by an exponential increase in both growth and respiration until the substrate becomes exhausted, where after respiration will decrease rapidly again. At ~20 °C the lag period typically is around 4–15 h, with peak activity after the exponential phase after around

* Corresponding author.
E-mail addresses: Stephanie.Reischke@gmail.com (S. Reischke), m.k.gopala.krishna@rug.nl (M.G.K. Kumar), Erland.Baath@biol.lu.se (E. Bååth).

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14–48 h (Anderson and Domsch, 1978; Blagodatskaya et al., 2007, 2009; Anderson and Martens, 2013; Reischke et al., 2014). Adding lower concentrations gives a different respiration response (Anderson and Domsch, 1985, 2010; Stenström et al., 2001; Sawada et al., 2008). At intermediate concentrations of glucose addition, there will not be any exponential phase in respiration. Instead there will be a period of stable respiration at the SIR level, followed by a decreased respiration. This respiration response was classified as a zero-order type by Stenström et al. (2001). The period with constant respiration at the SIR level will become shorter with decreasing loading rates of glucose. At even lower rates, respiration never reaches the SIR level, and has highest levels shortly after addition, followed by a rapid decrease (Anderson and Domsch, 1985). This response with decreasing respiration was classified as a first-order type by Stenström et al. (2001).

Anderson and Domsch (1985) estimated the highest addition rate giving a zero-order type of respiration response, that is where glucose addition gave a stable SIR response for several hours and then decreased. In the three soils studied most intensely this concentration varied between 60 and 1200 μg glucose-C g^{-1} soil. They assumed that this steady rate of respiration reflected the energy demand for maintaining the active microbial biomass, that is, the maintenance energy of the biomass with no associated additional growth on glucose (see also Anderson and Domsch, 2010). If this is true even quite large additions of an easily available C source like glucose will result in no net growth of the microbial community. However, direct measurements of growth have not been made after adding an available C source at concentrations resulting in zero-order type of respiration response in order to evaluate the assumption of no additional growth on the added glucose.

The respiration rate, however, does not always correlate with microbial growth (Meidute et al., 2008; Reischke et al., 2014). Therefore, it is possible that respiration measurements cannot be used to determine the threshold concentration of glucose for microbial growth in soil. One indication of this is that even very low amounts of carbon, so called trace amounts (5–15 μg C g^{-1} soil), have the ability to trigger the activity of the soil microbial biomass (De Nobili et al., 2001; Mondini et al., 2006). These studies measured the CO_2 evolution after the addition and not the direct growth response of the microbial community. However, more direct methods of detecting growth, incorporation of ^{13}C-labelled substrate into microbial phospholipid fatty acids, have also found indications of growth at low substrate-C additions (Ziegler et al., 2005; Dungait et al., 2013).

In the present study we have investigated the effect of low to medium concentrations of added glucose (50–1000 μg glucose-C g^{-1} soil) on bacterial growth in soil; concentrations that did not result in an exponential increase in respiration that is indicative of C-saturated concentrations. We only measured the bacterial growth response, using the leucine incorporation method, because the fungal contribution to glucose degradation was earlier shown to be minor in this soil at glucose concentrations ≤4000 μg glucose-C g^{-1} soil (Reischke et al., 2014). We aimed to address two questions. First, can additions of glucose at concentrations lower then those inducing an exponential growth phase in respiration still result in increased bacterial growth? And second, at what concentration threshold of added glucose will the bacterial community start growing on the added substrate?

2. Material and methods

2.1. Soil

Soil, classified as sandy loamy brown earth soil (Cambisol, FAO; Inceptisol, USDA), from a managed grassland from south-eastern Sweden was sieved (2.8 mm) and stored at 4 °C until use (not more than 2 weeks). Each experiment was performed on a new batch of soil. Soil organic matter content was 13.3% ± 1.2%, water content 26.2% ± 3.5% and pH(H_2O) 6.7 ± 0.3.

2.2. Glucose concentrations giving no exponential respiration phase

Initially different glucose concentrations were tested to find a concentration that increased respiration to the SIR level, but did not result in an exponential increase after the lag phase. In this experiment 30 g soil was weighed into 50 ml reaction tubes and different glucose-C concentrations were added as a solution. The concentrations used were 0, 100, 200, 300, 400, 500, 1000 and 2000 μg glucose-C per g of wet soil, since earlier results (Reischke et al., 2014) have found that 2000 μg glucose-C resulted in a clear exponential respiration phase. To avoid nutrient deficiency, NH_4NO_3 and KH_2PO_4 were added at a final concentration of 0.05 mg N or P per mg C added to the soil as glucose. To each reaction tube 1 ml of glucose solution was added in 0.25 ml batches, with mixing of the soil in between additions to ensure homogeneous incorporation of the substrate into the soil. The soil was then incubated at 20 °C for 24 h. Respiration was measured during 2-h periods until 12 h after the addition (approximately the time of the lag phase) and after 24 h (earlier shown to be approximately peak respiration after the exponential phase; Reischke et al., 2014), and bacterial growth was measured during 2 h after 24 h. In the experiments described below both longer and shorter incubation times were used, showing that 24 h was a suitable time frame to discover growth effects. At each time point 1 g of soil from the reaction tubes was used for respiration and growth measurements.

2.3. Bacterial growth at glucose concentrations giving no exponential respiration phase

Since the addition of 1000 μg glucose-C g^{-1} soil resulted in stable respiration for 12 h without any indication of an exponential phase, that is, a respiration pattern suggested to reflect maintenance and not growth (Anderson and Domsch, 1985, 2010), this concentration was used to test bacterial growth with a higher time resolution. A control with no glucose and a lower concentration, 500 μg glucose-C g^{-1} soil, was also included. Specifically, 100 g soil was weighed into 180 ml containers and amended with 0, 500 or 1000 μg glucose-C per g soil. NH_4NO_3 and KH_2PO_4 were added at a final concentration of 0.05 mg N or P per mg C added to the soil as glucose. To each container 2 ml of water with glucose was added, followed by homogenization by shaking for 1 min and mixing with a spatula for 30 s. The soil was then incubated at 20 °C for 86 h. Respiration and bacterial growth was measured regularly during 2 h-periods. Since we were not specifically interested in the lag phase, the first measurement was made towards the end of this phase, after 8 h. At each time point 1 g of soil from the containers was used for respiration and growth measurements.

2.4. Threshold concentrations of glucose for inducing bacterial growth

To investigate the minimum glucose-C concentration required to initiate additional growth of bacteria, respiration and bacterial growth were measured in triplicates using a range of amendments from 0 to 700 μg g^{-1} glucose-C in 50–100 μg increments. This was repeated in a final experiment where we added glucose-C in a range from 0 to 250 μg g^{-1}, with 25–50 μg increments, using 6 replicates per glucose level. After glucose addition, samples where incubated at 20 °C. No extra N or P was added here, since it was assumed that at low C additions in this nutrient rich soil, there