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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

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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

would be no problems with other limiting nutrients. There was also no difference in threshold concentration for bacterial growth with or without adding extra N and P (experiments under 2.2. and 2.4.).

In the first experiment, 1 g soil was weighed into 50 ml centrifuge tubes or respiration vials and the different glucose-C concentrations were added as a solution. Bacterial growth during 2 h was then measured after 1 and 2 days, whereas respiration was measured during the first 24 h after glucose addition. In the repeated experiment only bacterial growth measurement was performed for 2 h one day after the addition of glucose.

2.5. Bacterial growth

To estimate bacterial growth, leucine (Leu) incorporation into bacteria extracted from soil using the homogenization/centrifugation techniques was used (Kirchman et al., 1985; Bååth, 1994; Bååth et al., 2001). Briefly, 1 g of soil and 20 ml distilled water was added to centrifugation tubes, which were vortexed for 3 min. After a centrifugation at 1000 × *g* for 10 min, 1.5 ml of the supernatant (the bacterial suspension) was transferred into a 2 ml micro centrifugation tube. A mixture of radio-labelled Leu, [^{3}H]Leu (37 MBq ml^{-1}, 5.74 TBq mmol^{-1}, Perkin Elmer, USA) and non-labelled Leu was added to each tube, resulting in 275 nM Leu in the bacterial suspension. After 2 h incubation at 20 °C the growth was terminated by adding 75 µl 100% trichloroacetic acid (TCA). Removal of non-incorporated Leu through different centrifugation steps was made according to Bååth et al. (2001). The amount of incorporated radioactivity was determined by using a liquid scintillator counter. The incorporation of ^{3}H-leucine, expressed as pmol Leu incorporated in extracted bacteria g^{-1} soil h^{-1}, was used as a proxy for bacterial growth.

2.6. Respiration

One gram of soil was added to a 20 ml respiration vial. The vial, including the soil, was purged with pressurized air. The vial was then closed with a crimp lid and incubated for 2 h or 24 h at 20 °C. The CO_2 concentration was analysed using a gas chromatograph.

2.7. Statistical analysis

An ANOVA, followed by a Dunnett test, was used to determine the glucose-C concentration, which initiate additional bacterial growth (significance: $P < 0.05$) compared to the control with no glucose addition.

3. Results

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

We measured respiration over 24 h in soils amended with 7 different glucose-C concentrations to establish a glucose-C concentration that gave a constantly high respiration during the lag phase, but did not result in a subsequent exponential increase in respiration (Fig. 1). This was the case with the 1000 µg glucose-C g^{-1} soil treatment, where respiration increased around 10 times compared to the unamended control and stayed at the same level until 12 h after the glucose addition. At lower concentrations of C addition, respiration started to decrease at an earlier time stage. At the lowest addition (100 and 200 µg glucose-C), the initial respiration was lower then for the 1000 µg treatment, while for the other treatments the initial respiration after adding glucose appeared to be similar to the 1000 µg treatment. After 24 h the respiration rate in the 1000 µg treatment was still 5 times higher compared to the unamended control. Respiration rate of all other treatments, except 2000 µg glucose-C, were closer to the unamended control rate, but still up to 3 times higher. Adding 2000 µg glucose-C resulted in highest respiration after 24 h (Fig. 1).

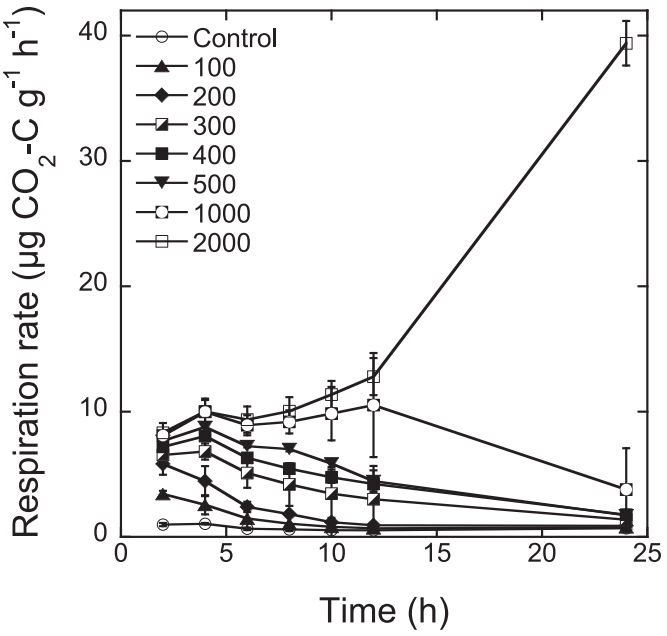

Fig. 1. Respiration rate after adding different glucose-C concentrations (µg C g^{-1} soil) at 20 °C. Respiration rate ($n = 2$) was measured during 2 h, where each time point on the *x*-axis indicates the end of the 2 h incubation. Bars indicate SE.

Bacterial growth was measured 24 h after the glucose amendment. Bacterial growth increased significantly using glucose-C concentration of 200 µg and higher (data not shown). Only an addition of 100 µg glucose-C did not resulted in any stimulation of bacterial growth.

Thus, extra growth of bacteria on the added glucose could be found even without any exponential increase in respiration. Since a clear stimulation of bacterial growth was especially seen after the addition of 500 and 1000 µg glucose-C, the development of respiration and bacterial growth over 86 h after glucose addition at these concentrations of glucose-C was studied in more detail. Respiration and bacterial growth clearly showed a diverging relationship over time (Fig. 2). At the starting point of the measurements (8 h after adding glucose) respiration had the highest rate, while bacterial growth was lowest. After around 12 h, respiration decreased, eventually reaching the rate of the control. The 500 µg glucose-C treatment reached this point earlier than the 1000 µg treatment (Fig. 2A). Bacterial growth, on the other hand, increased for both glucose-C concentrations after around 12 h. The increase in bacterial growth was highest in the 1000 µg glucose-C treatment, approximately 5 times higher than the unamended control, and 2 times higher compared to the 500 µg treatment. In both cases there was no or little further increase in bacterial growth after 24 h. After around 40 h, bacterial growth rate declined for the 1000 µg treatment (Fig. 2A), whereas in the 500 µg treatment bacterial a decrease was found after 60 h (Fig. 2B).

3.2. Threshold concentrations of glucose for inducing bacterial growth

Respiration during 24 h after adding up to 700 µg glucose-C g^{-1} soil increased almost linearly with increasing C concentration, with significant differences from the control already at 50 µg glucose-

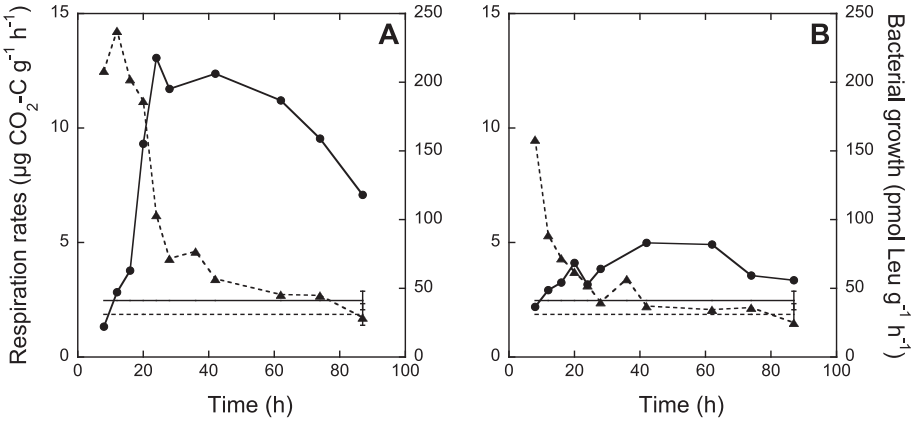

Fig. 2. Respiration (▲) and bacterial growth rate (●) during 2 h periods for two different glucose-C concentrations, 1000 µg g^{-1} soil (A) and 500 µg g^{-1} soil (B) at 20 °C. Each time point on the *x*-axis indicates the end of the 2 h incubation. Horizontal lines indicate mean values for the non-amended control over the entire incubation period for respiration (dashed line) and bacterial growth (full line), with bars indicating SD ($n = 11$).

C g^{-1} soil (Fig. 3). However, the bacterial growth rate only increased significantly at a concentration around 200 µg glucose-C g^{-1} soil both after 1 and 2 days incubation. Above this concentration, bacterial growth increased with increasing loading rates of glucose. Thus, there was a threshold concentration of glucose below which there was an increased respiration, but without any additional bacterial growth.

The threshold concentration was also determined in an additional experiment, where the glucose additions were narrowed down and the number of replicates increased. Adding up to 200 µg glucose-C g^{-1} resulted in soil bacterial growth rates not significantly different from the unamended control (Fig. 3). Bacterial growth after adding 250 µg glucose-C was significant higher compared to all other concentrations (0–200 µg).

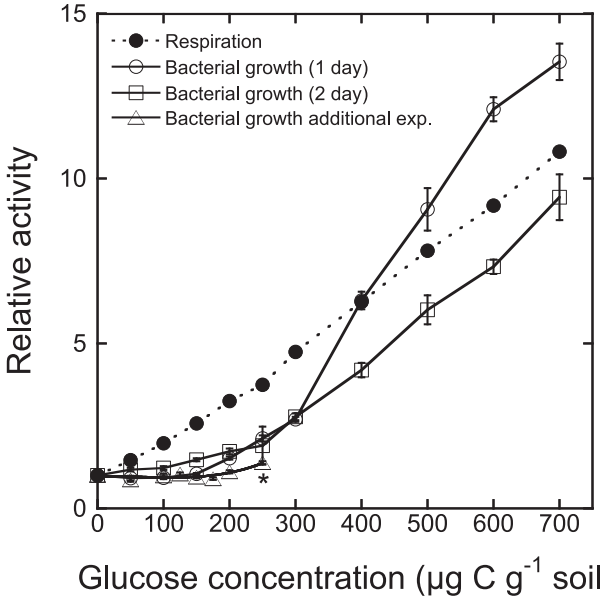

Fig. 3. Respiration during 24 h at 20 °C after adding different concentrations of glucose-C (●) and bacterial growth rates after 1 (○) and 2 (□) days measured during a 2 h period. The data were standardized to 1 for the unamended control. Bars indicate SE ($n = 3$). An additional experiment (△), measured after 24 h is also included, where bars indicate SE ($n = 6$) and * indicates a significant difference ($P < 0.05$) from all other glucose-C concentrations.

4. Discussion

Our first main finding was that an exponential increase in respiration after the initial lag phase is not needed to achieve additional growth on glucose of the bacterial community. Bacterial growth on the added glucose therefore also occurs with zero-order types of respiration responses (*sensu* Stenström et al., 2001). Thus, although only concentrations of 2 mg glucose-C per g^{-1} soil and higher resulted in a concomitant exponential phase in respiration (Fig. 1A; Reischke et al., 2014), bacterial growth on glucose will be found at lower concentrations. Our finding was in contrast to the study of Anderson and Domsch (1985, 2010), where they assume that a steady rate in respiration during the lag period reflects the energy demand for maintaining the metabolic active biomass, but does not result in additional growth on the added glucose. Nevertheless, they also stated that growth could appear after the "plateau phase" (Anderson and Domsch, 1985), which was shown to be the case in our study.

Furthermore, after adding both 500 and 1000 µg glucose-C g^{-1} soil, the development of respiration and bacterial growth after the lag phase was different; respiration rate decreased while bacterial growth increased. This emphasises that respiration and growth rate can be uncoupled, not only during the lag phase, but also later, and that respiration rate cannot be used as an indicator of bacterial growth in situations of changing substrate availability. This also suggests that it will be problematic to calculate C-use efficiencies during these periods.

Anderson and Domsch (1985) found that in the zero-order type of respiration response, the stable respiration started to decrease approximately when glucose in the soil solution became exhausted. Since the decrease in respiration rates was found before bacterial growth peaked, this suggests that glucose was taken up early by the bacteria and then stored in some form until used for growth later on. The incorporation of added glucose into storage compounds conforms to earlier proposed models of glucose utilization in soil (Bremer and Kuikman, 1994; Nguyen and Guckert, 2001). Other studies have also suggested that glucose initially can be stored rather than used directly for synthesis of structural components (growth; Blagodatsky et al., 2000; Kaštovská et al., 2010).

Our second main finding was that a glucose-C concentration of 200–250 µg g^{-1} soil and above will induce bacterial growth; concentrations below this did not and the bacterial community was thus not able to use the provided substrate for additional growth. It could be argued that using 24 h after addition as the time point of

measuring bacterial growth would be unreliable. However, the time point was chosen due to earlier findings by Reischke et al. (2014), where peak growth occurred around 24 h after the addition of glucose. This finding was corroborated by our results, in that increasing the time frame to 48 h did not increase bacterial growth rates or substantially alter the substrate concentration threshold of bacterial growth. However, the time point of peak growth will be affected by the incubation temperature used (S. Reischke and E. Bååth, unpublished results) and might be different for other substrates and different soils, and thus may need to be modified in further studies (see below).

The length of the lag phase in the present study (around 12 h with 500 and 1000 μg glucose-C g^{-1}) was similar to that found in an earlier study with this soil at 2000 μg glucose-C g^{-1} (Reischke et al., 2014). Although we cannot exclude that the lag period changed at lower concentrations, similar growth rates measured after 24 and 48 h suggest that our results are robust (Fig. 3). Furthermore, the lag period will be very difficult to estimate when peak rates will be close to that with no glucose addition, which will be the case at substrate additions below 300 μg glucose-C g^{-1}. Increasing the loading rates to very high concentrations, on the other hand, can increase the length of the lag phase significantly (Reischke et al., 2014).

Previous studies on the response of the microbial community as affected by glucose additions have found that the concentration of the available substrate may affect the ratio of respiration to assimilation of substrate C (Bremer and van Kessel, 1990). An increase in the glucose concentration will result in an increasing ratio of respiration to assimilation of glucose (Bremer and Kuikman, 1994; Tsai et al., 1997; Marstorp and Witter, 1999; Sawada et al., 2008). Bremer and Kuikman (1994) suggested that soil microbes would only incorporate the acquired glucose into structural compounds (growth) if a sufficient glucose concentration is available for growth. In case of an insufficient glucose supply, microbes will only use the glucose to incorporate it into storage compounds, which requires less energy through respiration than that needed for incorporation into structural compounds. Structural compounds for a growing community will thus be synthesised only if a threshold concentration is exceeded (Sawada et al., 2008). These suggestions are corroborated by our findings that, at additions below 200–250 μg C g^{-1} soil, no growth (synthesises of structural components) was found. However, Sawada et al. (2008) suggested that the threshold concentration between growth and no growth on the added substrate would be when the respiration pattern changed from a zero-time to a growth associated one (with exponential increase in respiration), similar to the suggestion by Anderson and Domsch (1985). We have shown (as discussed above) that bacterial growth on added substrate will also be present at concentrations far below this threshold. Our threshold concentration for bacterial growth was around 5 times lower than the highest concentration giving a zero-time respiration response (200 μg C g^{-1} soil compared to 1000 μg C g^{-1} soil).

Our threshold concentration for growth is still fairly high, however, compared to studies adding trace amounts of organic substance (5–15 μg C g^{-1} soil) triggering an increase in the activity of the microbial biomass (De Nobili et al., 2001; Mondini et al., 2006), or that found to result in production of microbial PLFA (15 μg C g^{-1} soil of different substrates; Dungait et al., 2013). This may be due to the soil studied by us, which is rich in organic matter with a high microbial biomass-C around 1 mg g^{-1} soil (unpublished results). Anderson and Domsch (1985) found a correlation between the amount of glucose-C resulting in zero-order responses, with no exponential phase in respiration, and the microbial biomass in soil. In soils with the lowest microbial biomass (<0.5 mg biomass-C g^{-1} soil) only 100 μg glucose-C g^{-1} was needed. Since we found that the threshold concentration for additional growth was around 5 times lower then the glucose-C concentration resulting in zero-order respiration, we suggest that, depending on the size of the microbial biomass, there could be threshold values for bacterial growth as low as around 20 μg glucose-C g^{-1} in some soils.

Further studies are, however, required to determine threshold concentrations for bacterial growth in different soils. Will there be a similar correlation between the amount of glucose needed to trigger increased bacterial growth and the microbial biomass as found by Anderson and Domsch (1985) between microbial biomass and the amount of glucose giving zero-order respiration? Or is the activity of the biomass the important variable? The type of substrate should also be considered in further investigations, from simple to complex ones, since different substrates may have different threshold concentrations for bacterial growth. Mixtures of substrates also need to be studied in this context.

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References

Aldén, L., Demoling, F., Bååth, E., 2001. Rapid method of determining factors limiting bacterial growth in soil. Applied and Environmental Microbiology 67, 1830–1838.
Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215–221.
Anderson, T.-H., Martens, R., 2013. DNA determinations during growth of soil microbial biomasses. Soil Biology & Biochemistry 57, 487–495.
Anderson, T.-H., Domsch, K.H., 1985. Maintenance carbon requirements of actively-metabolizing microbial-populations under in situ conditions. Soil Biology & Biochemistry 17, 197–203.
Anderson, T.-H., Domsch, K.H., 2010. Soil microbial biomass: the eco-physiological approach. Soil Biology & Biochemistry 42, 2039–2043.
Bååth, E., 1994. Measurement of protein-synthesis by soil bacterial assemblages with the leucine incorporation technique. Biology and Fertility of Soils 17, 147–153.
Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology & Biochemistry 33, 1571–1574.
Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.-H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Applied Soil Ecology 37, 95–105.
Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.-H., Kuzyakov, Y., 2009. Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. European Journal of Soil Science 60, 186–197.
Blagodatsky, S.A., Heinemeyer, O., Richter, J., 2000. Estimating the active and total soil microbial biomass by kinetic respiration analysis. Biology and Fertility of Soils 32, 73–81.
Bremer, E., van Kessel, C., 1990. Extractability of microbial ^{14}C and ^{15}N following additions of variable rates of labbelled glucose and $(NH_4)_2SO_4$ to soil. Soil Biology & Biochemistry 22, 707–713.
Bremer, E., Kuikman, P., 1994. Microbial utilization of ^{14}C[U]glucose in soil is affected by the amount and timing of glucose additions. Soil Biology & Biochemistry 26, 511–517.
De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biology & Biochemistry 33, 1163–1170.
Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology & Biochemistry 39, 2485–2495.
Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P., 2013. The variable response of soil microorganisms to trace concentrations of low molecular weight organic substrates of increasing complexity. Soil Biology & Biochemistry 64, 57–64.
Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N., 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology & Biochemistry 42, 896–903.
Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biology & Biochemistry 31, 145–153.

Hill, P.W., Farrar, J.F., Jones, D.L., 2008. Decoupling of microbial glucose uptake and mineralization in soil. Soil Biology & Biochemistry 40, 616–624.
Hoyle, F.C., Murphy, D.V., Brookes, P.C., 2008. Microbial response to the addition of glucose in low-fertility soils. Biology and Fertility of Soils 44, 571–579.
Joergensen, R.G., Scheu, S., 1999. Response of soil microorganisms to the addition of carbon, nitrogen and phosphorus in a forest Rendzina. Soil Biology & Biochemistry 31, 859–866.
Kirchman, D., Knees, E., Hodson, R., 1985. Leucine incorporation and its potential as a measure of protein-synthesis by bacteria in natural aquatic systems. Applied and Environmental Microbiology 49, 599–607.
Kaštovská, E., Šantrucková, H., Picek, T., Vašková, M., Edwards, K.R., 2010. Direct effect of fertilization on microbial carbon transformation in grassland soils in dependence on the substrate quality. Journal of Plant Nutrition and Soil Science 173, 706–714.
Marstorp, H., Witter, E., 1999. Extractable dsDNA and product formation as measures of microbial growth in soil upon substrate addition. Soil Biology & Biochemistry 31, 1443–1453.
Meidute, S., Demoling, F., Bååth, E., 2008. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biology & Biochemistry 40, 2334–2343.
Mondini, C., Cayuela, M.L., Sanchez-Monedero, M.A., Roig, A., Brookes, P.C., 2006. Soil microbial biomass activation by trace amounts of readily available substrate. Biology and Fertility of Soils 42, 542–549.
Nannipieri, P., Johnson, R.L., Paul, E.A., 1978. Criteria for measurement of microbial growth and activity in soil. Soil Biology & Biochemistry 10, 223–229.
Nguyen, C., Guckert, A., 2001. Short-term utilisation of ^{14}C[U]glucose by soil microorganisms in relation to carbon availability. Soil Biology & Biochemistry 33, 53–60.
Pennanen, T., Caul, S., Daniell, T.J., Griffiths, B.S., Ritz, K., Wheatley, R.E., 2004. Community-level responses of metabolically-active soil microorganisms to the quantity and quality of substrate inputs. Soil Biology & Biochemistry 36, 841–848.
Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biology & Biochemistry 70, 88–95.
Sawada, K., Funakawa, S., Kosak, T., 2008. Soil microorganisms have a threshold concentration of glucose to increase the ratio of respiration to assimilation. Soil Science and Plant Nutrition 54, 216–223.
Sparling, G.P., Ord, B.G., Vaughan, D., 1981. Microbial biomass and activity in soils amended with glucose. Soil Biology & Biochemistry 13, 99–104.
Stenström, J., Svensson, K., Johansson, M., 2001. Reversible transition between active and dormant microbial states in soil. FEMS Microbiology Ecology 36, 93–104.
Stotzky, G., Norman, A.G., 1961. Factors limiting microbial activities in soil 1. Level of substrate, nitrogen, and phosphorus. Archiv für Mikrobiologie 40, 341–369.
Tsai, C.S., Killham, K., Cresser, M.S., 1997. Dynamic response of microbial biomass, respiration rate and ATP to glucose additions. Soil Biology & Biochemistry 29, 1249–1256.
van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., Lundström, U.S., 2005. The carbon we do not see – the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37, 1–13.
Ziegler, S.E., White, P.M., Wolf, D.C., Thoma, G.J., 2005. Tracking the fate and recycling of ^{13}C-labeled glucose in soil. Soil Science 170, 767–778.