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"Non-metabolizable" glucose analogue shines new light on priming mechanisms: Triggering of microbial metabolism

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ABSTRACT

Priming of soil organic matter decomposition has attracted much research interest, yet a conclusive mechanistic explanation of the phenomenon remains elusive. One proposal is that low molecular weight organic substances might "trigger" an acceleration of microbial metabolism. For the first time, we applied a glucose analogue to soil to demonstrate triggering of microbial metabolism, and to estimate its relative contribution to priming. "Non-metabolizable" glucose analogues have been widely used in pure culture studies to mimic glucose, but never in soil biochemistry. We hypothesized that analogue molecules will elicit a metabolic response in microorganisms despite limited catabolism, and thereby confirm the proposed triggering.

The effect of ^{14}C-labeled 3-*O*-methyl-D-glucose (OMG) – a common "non-metabolizable" glucose analogue – on soil organic matter mineralization was compared to that of ^{14}C-labeled D-glucose. OMG was mineralized, but its mineralization was initially impeded and substantially delayed, relative to glucose. OMG caused brief but strong priming in the first 24 h, increasing unlabeled CO_2 efflux by 173%, 89% and 36% above control for additions of 0.49, 2.4 and 4.9 μmol OMG g^{-1} soil, respectively. In contrast, glucose caused low or negative priming on the first day. On the first day after OMG addition, a negative correlation between priming and OMG mineralization indicated that triggering is a valid mechanism of microbial activation during a famine-feast transition, but is short-lived.

Glucose mineralization peaked on the second day for medium and high additions, coinciding with peaks in positive priming. Maximum substrate mineralization also coincided with peaks in priming for medium and high OMG levels, but these occurred 9 and 11 days after addition, respectively. This revealed non-triggering priming mechanisms, which contributed most to priming and were closely coupled to substrate mineralization. By separating energy- and substrate-dependent metabolic processes from triggering processes, the glucose analogue 3-*O*-methyl-D-glucose enabled triggering to be demonstrated, but triggering by glucose occurs without contributing greatly to priming.

1. Introduction

Addition of low molecular weight organic substances (LMWOS) to soil can change the mineralization rates of pre-existing soil organic matter (SOM), a phenomenon termed priming (Kuzyakov, 2010). Priming effects have attracted much research interest, yet a conclusive mechanistic explanation remains elusive (Rousk et al., 2015). In light of the roles that priming plays in the global C cycle and in plant nutrition, a better understanding of its drivers is urgently needed.

Various possible priming mechanisms have been proposed. These have been comprehensively reviewed elsewhere (Blagodatskaya and Kuzyakov, 2008) and are briefly summarized in Table 1.

The "microbial triggering" hypothesis holds that an increased LMWOS availability can be detected by microorganisms. They accelerate their metabolism and energy state in expectation of a "food event", increasing their CO_2 output (Blagodatskaya and Kuzyakov, 2008; De Nobili et al., 2001). Stimulation of short-term priming by very small additions of LMWOS has been explained by triggering (Mondini et al., 2006). Triggering is unique among the

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Table 1
Summary of proposed biotic mechanisms of priming effects with emphasis on the role of the added substrate.

Mechanism	Description
Microbial triggering (De Nobili et al., 2001)	Substrate stimulates acceleration of microbial metabolism, increasing endogenous C mineralization *(substrate transformation not required)*
Pool substitution (Blagodatskaya and Kuzyakov, 2008; Jenkinson et al., 1985)	Substrate provides C that displaces endogenous C in the microbial biomass, which is released as CO_2 *(substrate transformed to utilize C)*
N mining (Fontaine et al., 2011)	Increase in available C shifts nutrient limitation from C to nitrogen (N), causing microbial degradation of SOM to access N *(substrate transformed for C and/or energy)*
Energy-limited extracellular enzyme synthesis (Hamer and Marschner, 2005)	Increase in available energy supports the synthesis of extracellular enzymes for SOM degradation *(substrate transformed for energy)*
Community dynamics (Fontaine et al., 2003)	Substrate supports growth of some microbial species, shifting microbial community composition in favor of SOM decomposers *(substrate transformed for C and energy)*
Co-metabolism (Horvath, 1972)	Enzymes produced for decomposition of the added substrate also catalyze SOM degradation *(substrate transformed for C and/or energy)*
Preferential substrate utilization (negative priming) (Kuzyakov, 2002)	Substrate provides a preferable source of energy and C for microorganisms, reducing SOM breakdown *(substrate transformed for C and/or energy)*

proposed mechanisms, in that it does not necessarily require LMWOS to act as a C or energy source. In contrast, all the other proposed mechanisms require metabolic transformation of the substrate in order to stimulate priming (Table 1).

It is necessary to clearly distinguish between the physiological mechanism of triggering and the phenomenon of priming. A physiological mechanism might operate under various conditions but cause priming only in some cases. On the other hand, priming in a given situation might result from the operation of more than one physiological mechanism. Here we define "triggering" as an acceleration of microbial metabolic activity that is stimulated by an increase in LMWOS concentration, not by the energy or C that the LMWOS provides. Triggering is a metabolic "decision" based on food signals in the environment. When small amounts of LMWOS cause strong triggering, the increase in metabolic requirements will exceed the C and energy available from the LMWOS. In this case, microorganisms must mineralize endogenous resources, causing positive priming through either a loss of microbial biomass or through accelerated decomposition of SOM. Larger amounts of LMWOS could still stimulate triggering as defined above, but would also provide a larger source of readily available C. In this case, triggering could occur without priming, or even with negative priming. Therefore, triggering is a mechanism of microbial activation, and is not always associated with simultaneous priming, although it can explain it under some circumstances.

We postulated that triggering arises from chemosensory mechanisms that do not rely on substrate catabolism. Chemosensory systems are biological protein systems that interact with specific molecules and translate these interactions into intracellular regulation (Mauriello, 2013). The stimulating molecule could be outside the cell, within the cell membrane (e.g. when passing through a transporter) or inside the cell, depending on the location of the chemosensory system (Lengeler and Jahreis, 2009). This enables microorganisms to detect specific substances in their environment, or their intracellular chemistry, and respond appropriately. Such systems are known to be widespread in all domains of life (He and Bauer, 2014; Kirby, 2009). Chemotaxis in bacteria is a particularly well-studied example, but such systems are involved in regulation of various physiological processes (Kirby, 2009). Quorum sensing among bacteria is another well-known example (Duan et al., 2009).

Glucose is often used as a model LMWOS to mimic root exudates or decomposing litter (Schneckenberger et al., 2008), in which it also occurs naturally (Derrien et al., 2014; Gunina and Kuzyakov, 2015; Kögel-Knabner, 2002). Decoupling of non-enzymatic glucose-protein interactions (such as chemosensing and membrane transport) from the effects of glucose breakdown for C and energy can be achieved with "non-metabolizable" glucose analogues. This approach has been applied in pure culture to study carbohydrate membrane transport and chemotaxis (Adler, 1969; Henderson, 1990). Glucose analogues are chemically very similar to glucose and often show analogous interactions with microbial proteins, but are not easily degraded by common catabolic pathways such as glycolysis. The analogue 3-*O*-methyl-D-glucose (OMG, Fig. 1 inset) presents an opportunity to investigate the short-term effects of a glucose-like molecule in soil with limited interference from catabolism. Its uptake by the same transport systems as glucose has been demonstrated in various microorganisms (Beauclerk and Smith, 1978; Scarborough, 1970; Tarshis et al., 1976).

Our first objective was to find experimental evidence of triggering. We hypothesized that OMG would mimic glucose as a chemosensory stimulus, but its metabolism would be suppressed. In this case, OMG should cause a stronger short-term priming effect relative to its catabolism, and thus a higher priming-to-mineralization ratio than glucose, at least temporarily. Comparing priming and mineralization directly after OMG and glucose addition could therefore experimentally demonstrate a triggering mechanism. All other mechanisms proposed for priming require that the added LMWOS act as a C or energy source, and would therefore predict that suppressed metabolism of OMG would also limit its priming ability. Therefore, only a triggering mechanism could explain a greater priming-to-mineralization ratio for OMG.

Our second objective was to determine the extent to which non-triggering mechanisms contribute to priming. Glucose mineralization occurs very soon after its addition to soil (Schneckenberger et al., 2008), whereas mineralization of OMG should be markedly delayed. Since all proposed non-triggering mechanisms require the biochemical alteration of the substrate, priming by these mechanisms should be delayed for OMG relative to glucose, reflecting the delayed mineralization of OMG.

Finally, we examined the patterns of OMG and glucose mineralization and transformation in soil to determine whether comparisons between the two substances are credible. Such comparability is important for the potential use of LMWOS analogues in investigations of microbial processes in soil.

2. Materials and methods

2.1. Soil and experiment

An agricultural Haplic Luvisol was air dried and sieved (2 mm). Details of the soil properties and site conditions have been previously reported (Kramer et al., 2012; Pausch and Kuzyakov, 2012). Soil was weighed (50 g dry weight) into sealable glass jars and moistened. The samples were then pre-incubated in the dark at 22 ± 1 °C for two weeks, after which the temperature was reduced to 16 ± 1 °C, four days before addition of OMG or glucose.

Solutions of D-glucose and 3-*O*-methyl-D-glucose (Sigma Aldrich) were prepared to provide additions of 0.49, 2.4 and 4.9 μmol g^{-1} soil (equivalent to 35, 175 and 350 μg glucose-C g^{-1} soil). The solutions were labeled with 235 Bq g^{-1} soil of [^{14}C(U)]-D-glucose or [glucose-^{14}C(U)]-3-*O*-methyl-D-glucose (American Radiolabeled Chemicals). 1 mL aliquots of substrate solution were added dropwise onto the surface of each soil sample. These additions brought soil moisture content to 75% of water holding capacity. A set of control samples received the same volume of water.

A small vial containing 1 mL of 1 M NaOH was placed inside each jar to capture CO_2. The NaOH traps were changed regularly. Carbonate in an aliquot of each trap solution was precipitated with $BaCl_2$ and quantified by back-titration of the remaining NaOH with 0.025 M HCl, to a phenolphthalein endpoint. Another aliquot was mixed with Rotiszint Eco Plus scintillation cocktail (Carl Roth, Germany), along with an additional 0.3 mL NaOH to ensure high pH. The scintillation preparations were kept in the dark for at least 3 h for chemoluminescence to dissipate before quantification of ^{14}C activity with a Hidex 300 SL scintillation counter (Hidex, Finland).

Samples were removed for destructive sampling 3, 6, 13 and 40 days after substrate addition. Microbial biomass ^{14}C was determined by chloroform fumigation-extraction according to Vance et al. (1987) with modifications of Gunina et al. (2014), but with fumigation for 72 h. C extractable with 0.05 M K_2SO_4 from unfumigated soil was taken as dissolved organic matter (DOM). Extractable microbial biomass C (MBC) is reported without correction for extraction efficiency. DOM and MBC extracts were analyzed for ^{14}C in the same manner as the NaOH traps described above.

2.2. Calculations and statistics

Labeled CO_2 efflux was subtracted from the total CO_2 (labeled and unlabeled) to obtain unlabeled CO_2 efflux (from MBC and SOM). The labeled glucose ring of OMG was assumed to be mineralized with the unlabeled methyl group. Priming was calculated as the difference in unlabeled CO_2 efflux between soils with added substrate and control soils.

One-way ANOVA was performed for CO_2 efflux rates (total and unlabeled) on the first day, and for cumulative efflux rates at the end of the experiment (day 40), followed by Tukey HSD tests for differences among the means. Significance was taken at $p < 0.05$. All substrate-concentration combinations were considered together as distinct treatments. Differences in priming were tested as differences in unlabeled CO_2 efflux.

Error bars in plots reflect standard errors of the mean. For priming values, these were calculated from the standard deviations of unlabelled CO_2 efflux from control and treatment samples, using standard formulae for error propagation (Meyer, 1975).

For assessing the relationship between mineralization and priming, all substrate concentrations were pooled to provide a wide range of mineralization rates. For data after day 1, Spearman's rank correlation coefficient was computed to evaluate the relationship. This non-parametric approach avoided excessive influence by the extreme values of peak mineralization periods, which made standard linear regression inapplicable. Linear regression was separately applied to the maximum mineralization points of this data, as well as to the data from day 1.

3. Results

3.1. CO_2 efflux and substrate mineralization

Glucose-derived CO_2 efflux peaked within 1 day at low concentration and within 42 h at medium and high concentrations. Although OMG-derived CO_2 efflux began on the first day, this was 5–6 times lower than from glucose for all concentrations. Mineralization of OMG increased gradually, accelerating after the fifth day to peak on the ninth and eleventh days for medium and high concentrations, respectively. The lowest addition of OMG also produced a gradually increasing $^{14}CO_2$ efflux, reaching a maximum on the fourth day. Therefore, OMG mineralization at all concentrations was initially strongly impeded and substantially delayed, relative to that of glucose.

On the first day, total CO_2 (total of labelled and unlabelled CO_2) from OMG-amended soil was similar for all addition levels (Fig. 1). In contrast to total CO_2, the amount of CO_2 originating from OMG (labelled CO_2) increased with higher addition levels (Fig. 2a).

Cumulative mineralization of the two substances over the 40 days of incubation was comparable in magnitude (Fig. 2a). Mineralization was also concentration-dependent and followed the same pattern for both substances, with lowest values for the low levels of addition and similar values for medium and high levels of addition.

3.2. Priming effect

Glucose addition stimulated only slightly positive (low addition) or negative priming on the first day, which followed an inverse relationship to the rate of substrate mineralization and to the amount of added substrate (Fig. 3a). OMG caused positive priming on the first day, also inversely related to substrate mineralization: unlabeled CO_2 efflux was 173%, 89% and 36% above the control soil for low, medium and high concentrations of OMG, respectively. For low and medium concentrations, these were the highest rates of priming for the entire experimental period. The ratios of priming to substrate mineralization for glucose on the first day were 0.14, −0.07 and −0.15 for low, medium and high additions, respectively, while the corresponding values for OMG were 4.9, 1.0 and 0.25.

The medium glucose level caused positive priming on the second day, during the period of peak glucose mineralization. For the high glucose addition, negative priming on the first day was more than offset by strong positive priming on the second day, again coinciding with peak substrate mineralization. For OMG, the initial surge of priming after low and medium addition was followed by a

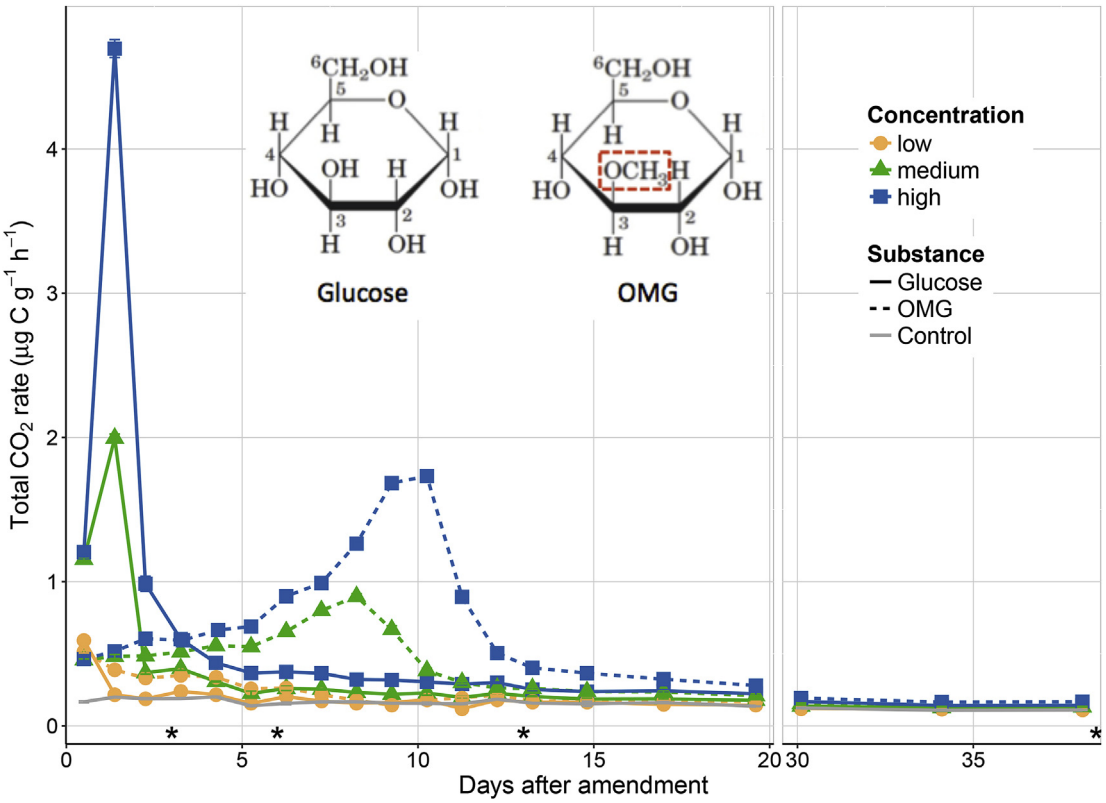

Fig. 1. Rate of total CO_2 efflux (labelled and unlabelled) after addition of glucose or OMG to soil (low, medium, high = 35, 175, 350 µg glucose-C g^{-1} soil, respectively). The molecular structures are inset. The times of ^{14}C-DOC and ^{14}C-MBC measurement are indicated with asterisks along the x-axis. Error bars represent ± standard error of the mean, but most are smaller than the symbol size.

period of positive, but relatively lower priming (Fig. 2b). For medium and high additions, priming then accelerated as the rates of substrate mineralization increased after the fifth day. Excluding the priming directly after addition (first 24 h), the highest priming rates for OMG at both medium and high additions coincided with the maximum rates of substrate mineralization.

The connection between substrate addition and SOM mineralization was examined using all samples and time-points of the experiment, except for day 1 (Fig. 3b). After the first day and considering all addition levels, unlabeled CO_2 efflux was positively correlated with substrate-derived CO_2 ($p < 0.001$) for both OMG and glucose, with Spearman's rank correlation coefficients of 0.58 and 0.46, respectively. The periods of peak mineralization, marked as large symbols in Fig. 3b, showed a strong linear increase of priming with substrate mineralization.

Cumulative priming over the entire period increased from low to high OMG additions, but this was not proportional to the added amounts (Fig. 2b). Cumulative priming by both glucose and OMG showed similar patterns of concentration dependence. Low and medium concentrations, with a 5-fold concentration difference, yielded approximately equal amounts of cumulative priming. Priming by high levels of addition was much stronger for both substances, despite these being only twice the medium concentrations.

3.3. DOM and MBC

Glucose was rapidly depleted from DOM, with little ^{14}C remaining by day 3 (<5% of added). For OMG, however, a high percentage of ^{14}C remained in DOM until the mineralization peak had passed (maximum $^{14}CO_2$ efflux: days 4, 9 and 11 for low, medium and high additions, respectively). The first DOM measurements after the glucose mineralization peaks were taken on day 3. Relative to the timing of the different mineralization peaks, these day 3 measurements for glucose are most comparable to the OMG measurements on day 6 (low addition) and 13 (medium and high additions). These corresponding peaks are marked "p" in Fig. 4. At these points after the OMG peaks, the ^{14}C-DOM from OMG had been reduced to the same or slightly lower levels than for glucose on day 3.

Three days after addition, incorporation of OMG into extractable MBC was much lower than for glucose (Fig. 5). After peak mineralization of OMG (day 6 for low- and day 13 for medium- and high additions), ^{14}C-MBC values were similar to those of glucose on day 3. Microbial incorporation of ^{14}C in relation to total MBC is presented as supplementary material.

4. Discussion

4.1. Objective 1: evidence of triggering

OMG mineralization was suppressed on the first day after addition, yet priming was positive over this period (Fig. 3a). This contrasted with very low or negative priming by glucose, despite much higher levels of mineralization. OMG therefore produced higher primed:labeled CO_2 ratios on the first day. Furthermore, the priming by both substances displayed a clear inverse relationship with the amount of substrate mineralized. These observations are consistent with the hypothesized chemosensory triggering mechanism, depicted in Fig. 6: microorganisms sense OMG or glucose, and respond by accelerating their metabolism in expectation of a 'food event' (De Nobili et al., 2001). This is mechanistically

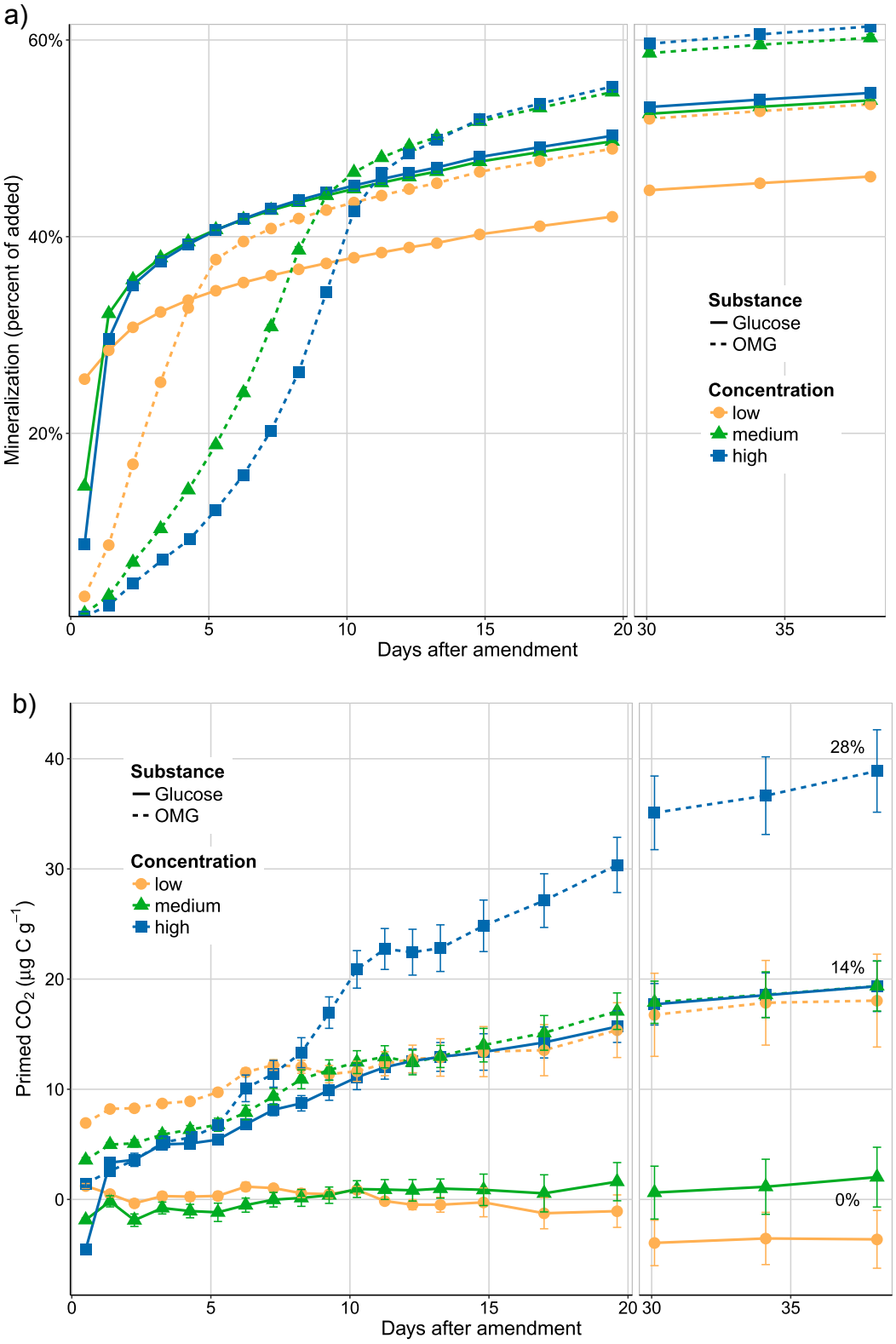

Fig. 2. (a) Cumulative $^{14}CO_2$ efflux by mineralization of glucose or OMG and (b) cumulative primed CO_2 efflux, with final cumulative priming reflected as percent of total control CO_2, at three concentration levels (low, medium, high = 35, 175, 350 µg glucose-C g^{-1} soil, respectively). Values are expressed as percent of the amount added. Error bars represent ± standard error of the mean, but most are smaller than the symbol size.

independent of catabolism of the added substrate, so it happens even if the microorganisms are unable to immediately consume OMG. In this case, unlabeled cellular resources must be mineralized to support the accelerated metabolism, causing an observable priming effect. However, when the added substrate can be partly utilized, this source of carbon and energy can help to meet

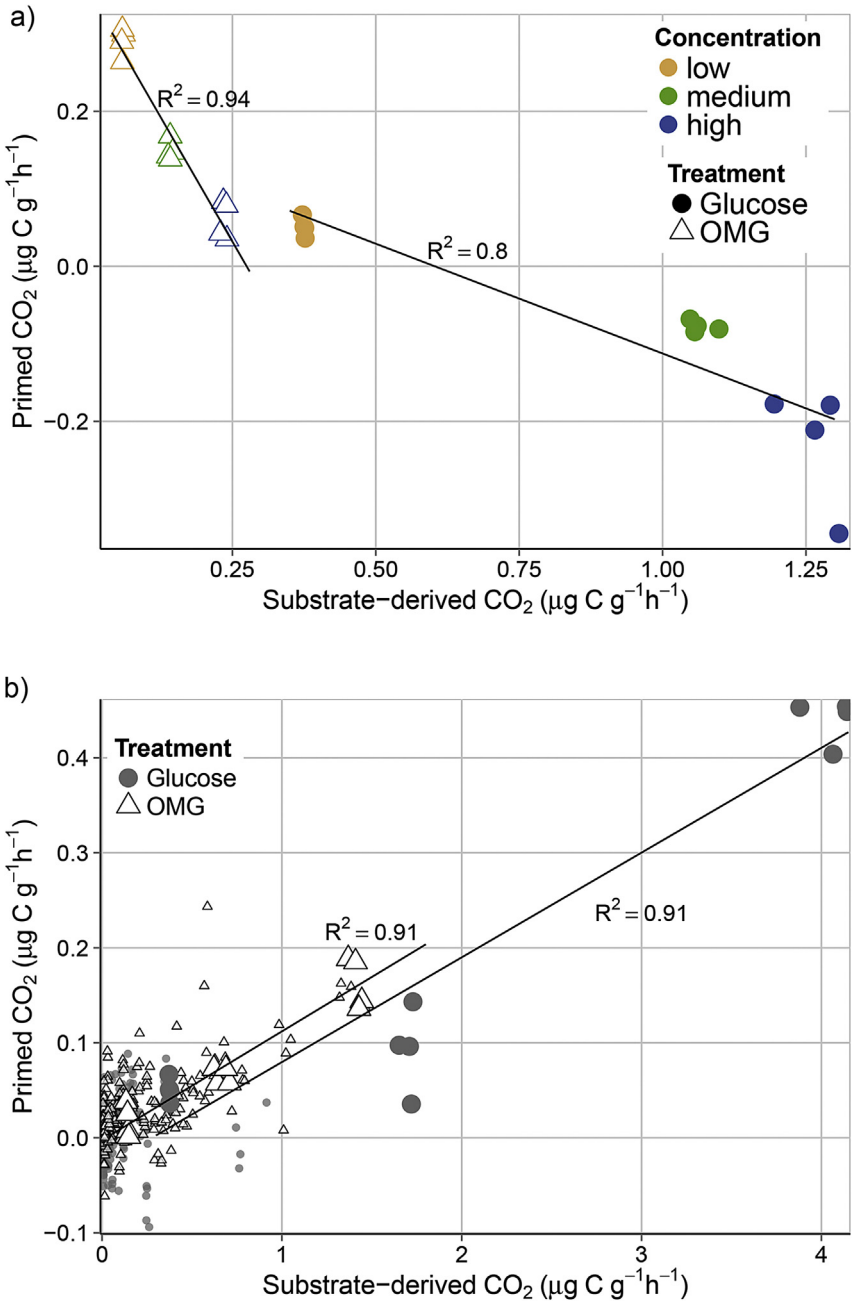

Fig. 3. Priming effect versus substrate mineralization rates for the first day (a), and all data points after the first day (b), for glucose or OMG at three concentration levels (low, medium, high = 35, 175, 350 µg glucose-C g^{-1} soil, respectively). Large symbols in (b) mark the period of maximum substrate mineralization for each treatment (including the first day for low glucose addition, although this was excluded in other cases). The regression lines in (b) are for these mineralization maxima alone. All measured data are shown.

increased metabolic requirements, which explains the inverse relationship between priming and mineralization. Hence, the priming decreases with higher OMG additions, closely matched by increases in OMG-derived CO_2, resulting in the same total CO_2 efflux (labelled plus unlabelled) on day 1 for all OMG levels. In the presence of readily metabolizable glucose, microorganisms could accelerate their metabolism without drawing on native C sources, with the higher additions reducing their reliance on SOM below control levels. Hence the triggering mechanism, operating across a range of substrate mineralization rates, is consistent with the observed phenomena of both positive and negative short-term priming on the first day after addition. Only triggering can explain this pattern of short-term priming by OMG and glucose, because all other hypothesized priming mechanisms (Table 1) would predict that reduced biochemical reactivity would lead to lower priming. We conclude that triggering is therefore a valid mechanism, but is short-lived and contributes little to priming by glucose.

4.2. Objective 2: delayed priming by OMG

Our second objective was to assess the importance of non-triggering mechanisms in priming by observing whether the delayed mineralization of OMG also induced a delayed priming effect. This delay was indeed observed: medium and high additions of OMG stimulated a second period of priming between days 5 and

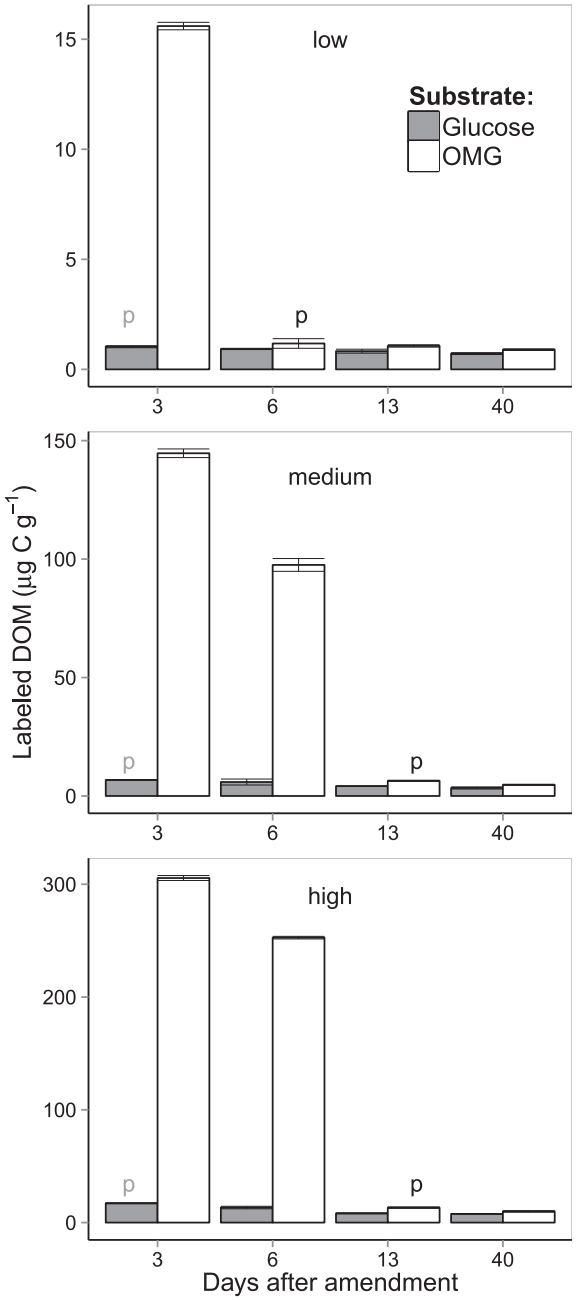

Fig. 4. ^{14}C in dissolved organic matter (DOM) extracted after glucose and OMG additions at three concentration levels (low, medium, high = 35, 175, 350 µg glucose-C g^{-1} soil, respectively). "p" indicates the first measurement after peak mineralization of glucose or OMG. These sampling times are comparable between glucose and OMG, in that they follow shortly after their respective mineralization peaks (see Figs. 1 and 2a). Note different scales of the y-axes. Error bars represent ± standard error of the mean.

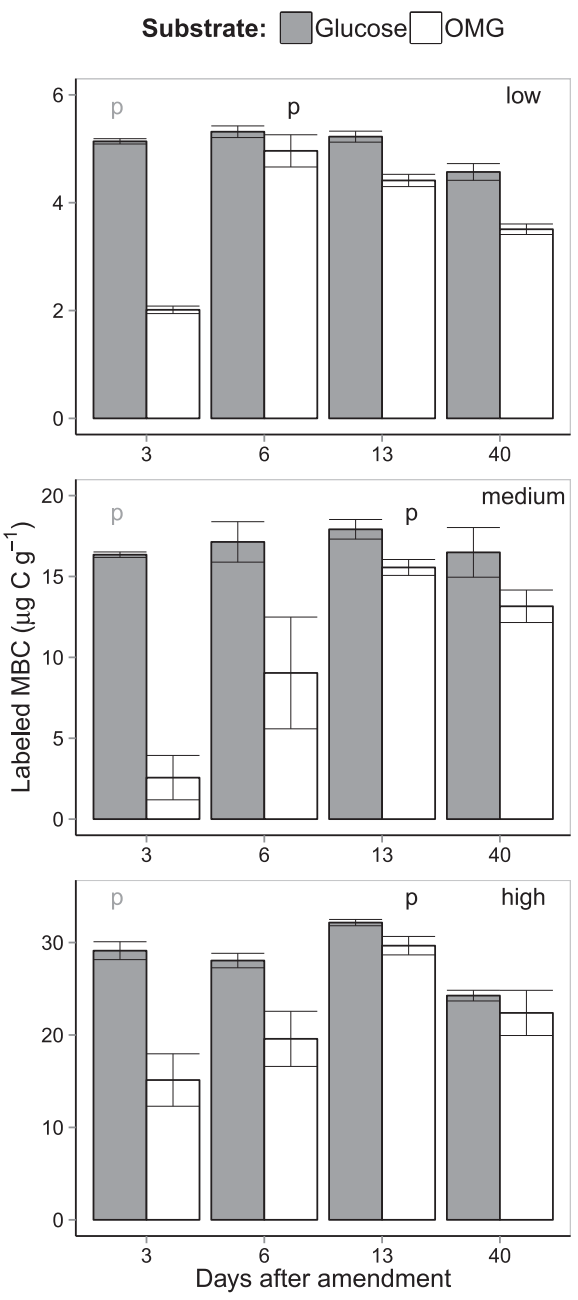

Fig. 5. ^{14}C incorporation into microbial biomass, extracted after glucose and OMG additions at three concentration levels (low, medium, high = 35, 175, 350 µg glucose-C g^{-1} soil, respectively). "p" indicates the first measurement after peak mineralization of glucose or OMG. These sampling times are comparable between glucose and OMG, in that they follow shortly after their respective mineralization peaks (see Figs. 1 and 2a). Note different scales of the y-axes. Error bars represent ± standard error of the mean.

12, in addition to the priming that occurred on the first day. Like the peaks of glucose priming on day 2, the highest rates of priming occurred at the same time as the mineralization maxima. A delayed priming effect, closely tied to the delay in mineralization, was therefore evident for OMG.

Substrate mineralization and priming were positively correlated for both substances over all times (excluding day 1). This is in stark contrast to the negative correlation on day 1, supporting the view that different mechanisms were responsible for priming at different times. The positive correlation after day 1 is consistent with non-triggering mechanisms that are reliant on C or energy from the substrate (Table 1). It is expected that glucose activates these non-triggering mechanisms very soon after addition, during its rapid mineralization, and so the resulting priming cannot be unambiguously distinguished from triggering. These two mechanisms were temporally separated for OMG, so that they could each be observed with relatively little interference from each other. For OMG, the relatively strong priming by triggering on the first day was short-lived, and the delayed non-triggering mechanisms accounted for the majority of primed C over the course of the experiment. In the case of glucose, triggering did not cause positive

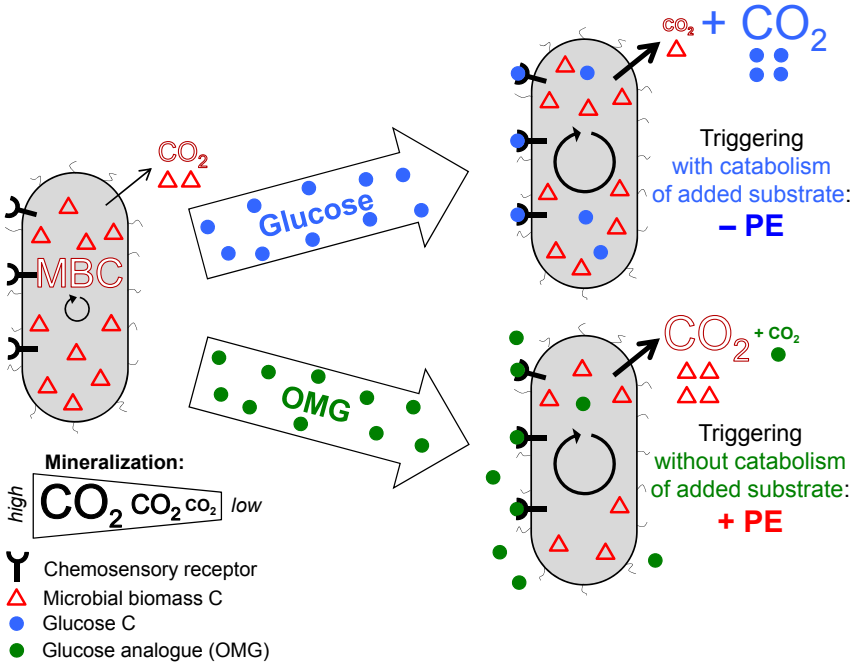

Fig. 6. Mechanism of triggering by glucose chemosensing. Both glucose and the glucose analogue (OMG) stimulate an acceleration of microbial metabolism. However, only glucose is readily metabolized. Microbes activated with OMG must therefore utilize their own internal resources to power their activation, producing a positive priming effect.

priming on the first day. Non-triggering mechanisms that are closely coupled to substrate mineralization are more important for priming by glucose.

4.3. Transformations of OMG and glucose

OMG mineralization was strongly suppressed shortly after addition and markedly delayed relative to glucose (Fig. 2a), but its mineralization over the full 40 days was within the same range. Mineralization of glucose increases with larger additions (Fischer et al., 2010; Schneckenberger et al., 2008), as partly seen here in the higher mineralization at medium and high, relative to low levels (Fig. 2a). Our results show that this relationship also applies to OMG.

The delay in OMG metabolism is also evident from the ^{14}C activity of DOM (Fig. 4). Glucose was rapidly mineralized and disappeared quickly from the soil solution. Peak mineralization was delayed for OMG and ^{14}C correspondingly persisted in the DOM until after the peak. Furthermore, despite the different timing of maximum CO_2 efflux for OMG and glucose, ^{14}C-DOM values after the respective maxima were closely comparable for the two substances.

The increases in ^{14}C-MBC mirrored the decreases in ^{14}C-DOM. Glucose incorporation into MBC was already near its maximum by the third day, just after the peak of glucose mineralization. OMG incorporation was much lower at this time but, for every level of substrate addition, ^{14}C-MBC increased during the period of maximum CO_2 efflux to reach the same levels as for those of glucose on day 3.

The differences between OMG and glucose are starker for ^{14}C-DOM than for ^{14}C-MBC, which can be explained as follows. OMG can be taken up by a variety of organisms without subsequent catabolism (Beauclerk and Smith, 1978; Scarborough, 1970; Tarshis et al., 1976), so it is plausible that biochemical processing of OMG in soil is delayed at the intracellular level. More OMG is taken up into cells than is metabolized, and so unmetabolized OMG accumulates, preventing further uptake and accounting for the sustained levels of ^{14}C-DOM (Fig. 4). This intracellular OMG is within the cell envelope, and therefore is detected as part of the chloroform-extractable MBC. As a result, ^{14}C-MBC from OMG increases earlier than mineralization (Fig. 5), but only reaches its maxima after peak mineralization, when most of the OMG has been biochemically transformed through microbial metabolic pathways. We cannot rule out the alternative possibility that slow membrane transport into microbial cells or some form of extracellular processing are actually responsible for the delay in mineralization. Chemosensory triggering would still be possible, since external chemoreceptors are widely distributed in microorganisms (Falke and Hazelbauer, 2001).

In summary, several similarities were observed between glucose- and OMG-treated soils:

i) A negative correlation between priming and mineralization for both substances was observed on the first day after addition;
ii) Priming maxima coincided with mineralization maxima;
iii) Priming and mineralization rates were positively correlated for time points after the first day;
iv) ^{14}C-DOM and ^{14}C-MBC where similar after the mineralization maxima; and
v) Percent substrate mineralization was comparable, and followed the same relationship to the amount added.

Taken together, these indicate that microbial metabolism of glucose and OMG are comparable, despite the considerable differences in the timing of peak mineralization. These observations therefore validate the comparisons and conclusions presented above, and demonstrate the potential of LMWOS analogues to disentangle soil microbial processes with different dependencies on substrate utilization.

5. Conclusions

Triggering of microbial metabolism is a valid mechanism of the famine-feast transition of soil microorganisms. However, triggering is short-lived and contributes little to priming in the presence of readily available LMWOS.

Non-triggering mechanisms are more significant drivers of priming, and are closely coupled to substrate utilization and prolonged stimulation of microbial biomass metabolism.

The new approach using "non-metabolizable" LMWOS analogues has demonstrated high potential to disentangle substrate C- and energy-dependent and independent microbial processes in soil.

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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.2016.12.015.

References

Adler, J., 1969. Chemoreceptors in bacteria. Science 166, 1588–1597.
Beauclerk, A.A.D., Smith, A.J., 1978. Transport of D-glucose and 3-O-methyl-glucose in the cyanobacteria aphanocapsa 6714 and nostoc strain mac. European Journal of Biochemistry 82, 187–197.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115–131. http://dx.doi.org/10.1007/s00374-008-0334-y.
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 and Biochemistry 33, 1163–1170. http://dx.doi.org/10.1016/S0038-0717(01)00020-7.
Derrien, D., Plain, C., Courty, P.E., Gelhaye, L., Moerdijk-Poortvliet, T.C.W., Thomas, F., Versini, A., Zeller, B., Koutika, L.S., Boschker, H.T.S., Epron, D., 2014. Does the addition of labile substrate destabilise old soil organic matter? Soil Biology and Biochemistry 76, 149–160. http://dx.doi.org/10.1016/j.soilbio.2014.04.030.
Duan, K., Sibley, C.D., Davidson, C.J., Surette, M.G., 2009. Chemical interactions between organisms in microbial communities. Karger, Basel. In: Collin, M., Schuch, R. (Eds.), Bacterial Sensing and Signaling, pp. 1–17.
Falke, J.J., Hazelbauer, G.L., 2001. Transmembrane signaling in bacterial chemoreceptors. Trends in Biochemical Sciences 26, 257–265.
Fischer, H., Ingwersen, J., Kuzyakov, Y., 2010. Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil. European Journal of Soil Science 61, 504–513. http://dx.doi.org/10.1111/j.1365-2389.2010.01244.x.
Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology and Biochemistry 43, 86–96. http://dx.doi.org/10.1016/j.soilbio.2010.09.017.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry 35, 837–843. http://dx.doi.org/10.1016/S0038-0717(03)00123-8.
Gunina, A., Dippold, M.A., Glaser, B., Kuzyakov, Y., 2014. Fate of low molecular weight organic substances in an arable soil: from microbial uptake to utilisation and stabilisation. Soil Biology and Biochemistry 77, 304–313. http://dx.doi.org/10.1016/j.soilbio.2014.06.029.
Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biology and Biochemistry 90, 87–100. http://dx.doi.org/10.1016/j.soilbio.2015.07.021.
Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology and Biochemistry 37, 445–454. http://dx.doi.org/10.1016/j.soilbio.2004.07.037.
He, K., Bauer, C.E., 2014. Chemosensory signaling systems that control bacterial survival. Trends in Microbiology 22, 389–398. http://dx.doi.org/10.1016/j.tim.2014.04.004.
Henderson, P.J.F., 1990. Proton-linked sugar transport systems in bacteria. Journal of Bioenergetics and Biomembranes 22, 525–569. http://dx.doi.org/10.1007/BF00762961.
Horvath, R.S., 1972. Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriological Reviews 36, 146–155.
Jenkinson, D.S., Fox, R.H., Rayner, J.H., 1985. Interactions between fertilizer nitrogen and soil nitrogen – the so-called "priming" effect. Journal of Soil Science 36, 425–444. http://dx.doi.org/10.1111/j.1365-2389.1985.tb00348.x.
Kirby, J.R., 2009. Chemotaxis-like regulatory systems: unique roles in diverse bacteria. Annual Review of Microbiology 63, 45–59. http://dx.doi.org/10.1146/annurev.micro.091208.073221.
Kögel-Knabner, I., 2002. The macromolecular organic composition of Plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139–162. http://dx.doi.org/10.1016/S0038-0717(01)00158-4.
Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55, 111–119. http://dx.doi.org/10.1016/j.pedobi.2011.12.001.
Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363–1371. http://dx.doi.org/10.1016/j.soilbio.2010.04.003.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382–396.
Lengeler, J.W., Jahreis, K., 2009. Bacterial PEP-dependent carbohydrate: phosphotransferase systems couple sensing and global control mechanisms. Karger, Basel. In: Collin, M., Schuch, R. (Eds.), Bacterial Sensing and Signalling. Contributions to Microbiology, vol. 16, pp. 65–87.
Mauriello, E.M.F., 2013. Cell biology of bacterial sensory modules. Frontiers in Bioscience 18, 928–943.
Meyer, S.L., 1975. Data Analysis for Scientists and Engineers. John Wiley and Sons, New York.
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. http://dx.doi.org/10.1007/s00374-005-0049-2.
Pausch, J., Kuzyakov, Y., 2012. Soil organic carbon decomposition from recently added and older sources estimated by?? 13C values of CO 2 and organic matter. Soil Biology and Biochemistry 55, 40–47. http://dx.doi.org/10.1016/j.soilbio.2012.06.007.
Rousk, J., Hill, P.W., Jones, D.L., 2015. Priming of the decomposition of ageing soil organic matter: concentration dependence and microbial control. Functional Ecology 29, 285–296. http://dx.doi.org/10.1111/1365-2435.12377.
Scarborough, G.A., 1970. Sugar transport in neurospora crassa. The Journal of Biological Chemistry 245, 1694–1698.
Schneckenberger, K., Demin, D., Stahr, K., Kuzyakov, Y., 2008. Microbial utilization and mineralization of [14C]glucose added in six orders of concentration to soil. Soil Biology and Biochemistry 40, 1981–1988. http://dx.doi.org/10.1016/j.soilbio.2008.02.020.
Tarshis, M.A., Bekkouzjin, A.G., Ladygina, V.G., Panchenko, L.F., 1976. Properties of the 3-O-methyl-D-glucose transport system in Acholeplasma laidlawii. Journal of Bacteriology 125, 1–7.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C 19, 703–707.