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

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A B S T R A C T

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 14C-labeled 3-O-methyl-D-glucose (OMG) – a common “non-metabolizable” glucose analogue – on soil organic matter mineralization was compared to that of 14C-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 CO2 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.

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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 CO2 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
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.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
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<tbody>
<tr>
<td>Microbial triggering</td>
<td>Substrate stimulates acceleration of microbial metabolism, increasing endogenous C mineralization (substrate transformation not required)</td>
</tr>
<tr>
<td>Pool substitution</td>
<td>Substrate provides C that displaces endogenous C in the microbial biomass, which is released as CO2 (substrate transformed to utilize C)</td>
</tr>
<tr>
<td>N mining</td>
<td>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)</td>
</tr>
<tr>
<td>Energy-limited extracellular enzyme synthesis</td>
<td>Increase in available energy supports the synthesis of extracellular enzymes for SOM degradation (substrate transformed for energy)</td>
</tr>
<tr>
<td>Community dynamics</td>
<td>Substrate supports growth of some microbial species, shifting microbial community composition in favor of SOM decomposers (substrate transformed for C and energy)</td>
</tr>
<tr>
<td>Co-metabolism</td>
<td>Enzymes produced for decomposition of the added substrate also catalyze SOM degradation (substrate transformed for C and/or energy)</td>
</tr>
<tr>
<td>Preferential substrate utilization (negative priming)</td>
<td>Substrate provides a preferable source of energy and C for microorganisms, reducing SOM breakdown (substrate transformed for C and/or energy)</td>
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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 \([^{14}C]U\)-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 were coinciding with peak substrate mineralization. For OMG, the initial surge of priming after low and medium addition was followed by a delay. 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.

2.2. 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 unlabeled 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 \(^14\text{CO}_2\) 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: unlabelled 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
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 CO₂ efflux was positively correlated with substrate-derived CO₂ (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 ¹⁴C remaining by day 3 (<5% of added). For OMG, however, a high percentage of ¹⁴C remained in DOM until the mineralization peak had passed (maximum ¹⁴CO₂ 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 ¹⁴C-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), ¹⁴C-MBC values were similar to those of glucose on day 3. Microbial incorporation of ¹⁴C 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 CO₂ 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...
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
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 CO2, resulting in the same total CO2 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...
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...
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 14C activity of DOM (Fig. 4). Glucose was rapidly mineralized and disappeared quickly from the soil solution. Peak mineralization was delayed for OMG and 14C correspondingly persisted in the DOM until after the peak. Furthermore, despite the different timing of maximum CO2 efflux for OMG and glucose, 14C-DOM values after the respective maxima were closely comparable for the two substances.

The increases in 14C-MBC mirrored the decreases in 14C-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, 14C-MBC increased during the period of maximum CO2 efflux to reach the same levels as for those of glucose on day 3.

The differences between OMG and glucose are starker for 14C-DOM than for 14C-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 14C-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, 14C-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) 14C-DOM and 14C-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.

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