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

Kyle Mason-Jones [a, *], Yakov Kuzyakov [a, b, c]

[a] Department of Agricultural Soil Science, Georg-August-University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[b] Department of Soil Science of Temperate Ecosystems, Georg-August-University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[c] Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia

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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

* Corresponding author.
E-mail address: kmasonj@gwdg.de (K. Mason-Jones).

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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.