Mineralization of "non-metabolizable" glucose analogues in soil: potential chemosensory mimics of glucose

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Abstract

Glucose is widely used to study the dynamics of easily available organics in soil. Pure culture studies have revealed that many microorganisms can sense and respond to glucose through chemosensory mechanisms that are not directly reliant on energy catabolism. However, the rapid mineralization of glucose by microorganisms makes it difficult to disentangle its energy effects from such non-catabolic interactions. "Non-metabolizable" glucose analogues have proven useful in mechanistic studies of glucose in pure culture, but have never been applied to complex microbial communities in soil. We sought to determine how their mineralization in soil differs from that of glucose, and whether they have potential as a new approach for investigating chemosensory mechanisms in soil microbiology.

We incubated soil from an agricultural Haplic Luvisol under controlled conditions for 24 d and monitored CO_2 efflux after addition of (1) glucose, and three "non-metabolizable" glucose analogues: (2) 2-deoxyglucose (DG), (3) α-methylglucoside (αMG), and (4) 3-*O*-methyl-glucose (OMG), at three concentration levels, along with a control.

All three analogues did in fact produce a large increase in soil CO_2 efflux, but the dynamics of their mineralization differed from the rapid degradation seen for glucose. At medium and high concentrations, CO_2 efflux peaked between 2.5 and 4 d after amendment with DG and αMG, and was delayed by about one week for OMG.

The markedly different patterns of mineralization between glucose and OMG offer a new tool for investigating the behavior of glucose in soil. By using OMG as a glucose model, chemosensory mechanisms could be studied with limited interference from energy catabolism.

Key words: LMWOS / microbial metabolism / carbohydrate / soil sugars / soil respiration

Accepted December 08, 2016

1 Introduction

Glucose is widely used as a low molecular weight organic substance (LMWOS) to mimic increases in soil carbon (C) availability during natural processes such as root exudation or litter decomposition (*Schneckenberger* et al., 2008). Glucose is a major component of root exudates (*Derrien* et al., 2004) and a constituent of various organic polymers such as cellulose and hemicellulose (*Kögel-Knabner*, 2002; *Gunina* and *Kuzyakov*, 2015). Pure culture studies have established that glucose molecules interact with microbial chemoreceptors to stimulate various biochemical responses such as gene expression, catabolite repression, and chemotaxis (*Adler*, 1969; *Lengeler* and *Jahreis*, 2009). These are examples of chemosensory systems, forms of which have been found in all domains of life (*Kirby*, 2009). Such chemosensing mechanisms may play important roles for soil microbes responding to organic substances. However, it is difficult to use glucose to investigate these processes due to the confounding effects of its very rapid catabolism (*Schneckenberger* et al., 2008; *Fischer* et al., 2010).

"Non-metabolizable" glucose analogues have proven useful in mechanistic studies of glucose in pure cultures. These substances are chemically very similar to glucose, but with small differences blocking their microbial catabolism (see Fig. 1 for molecular structures) (*Koser* and *Saunders*, 1933; *Scarborough*, 1970; *Tyler* et al., 1967; *Tarshis* et al., 1976). These changes only involve one of the several functional groups of the molecule. Therefore, while biochemical reactivity is sharply reduced, relatively non-specific, abiotic processes, such as diffusion and adsorption, are unlikely to be greatly affected. The advantage is that the analogues show similar interactions with microbial glucose transport and regulatory systems. For example, uptake of glucose analogues by glucose transporters occurs in microbes as diverse as *E. coli* (*Halpern* and *Lupo*, 1966), algae (*Komor* and *Tanner*, 1971), ascomycetes (*Scarborough*, 1970), and purple sulfur bacteria (*Knaff* and *Whetstone*, 1980), among others. Glucose and 3-*O*-methyl-glucose trigger similar internal carbohydrate transformations in the fungus *Dendryphiella salina* (*McDermott* and *Jennings*,

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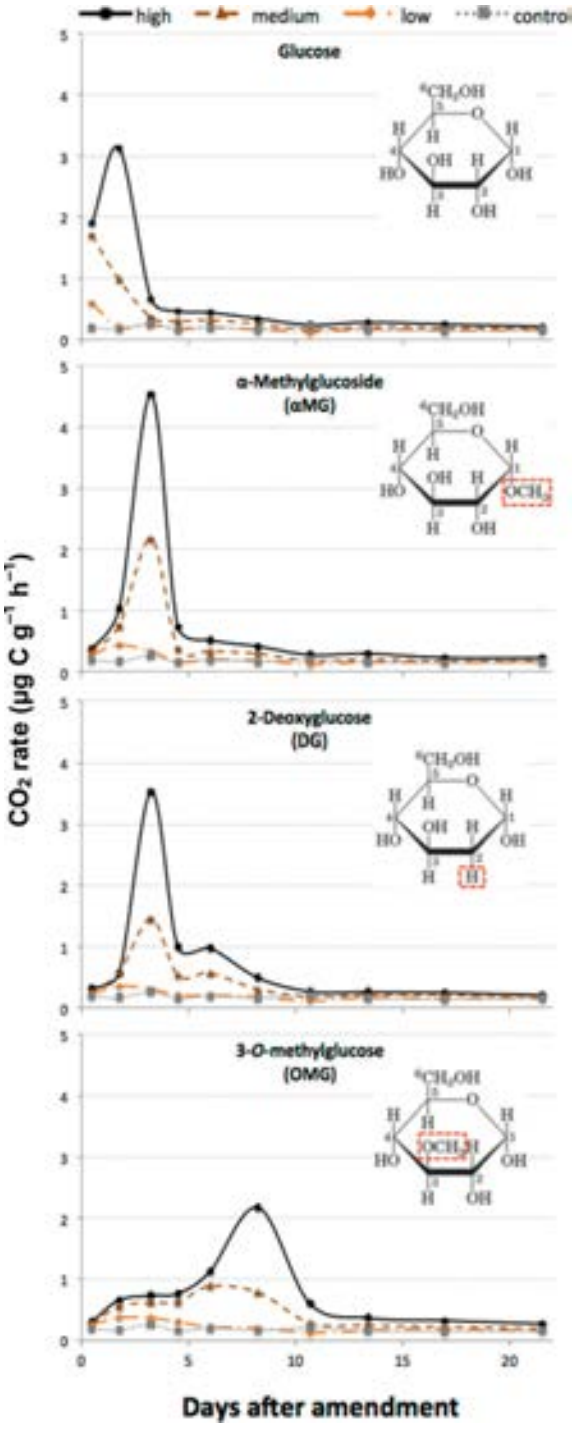

Figure 1: CO_2 efflux rates from soil after addition of glucose and the three glucose analogues α-D-methylglucoside (αMG), 2-deoxy-D-glucose (DG), and 3-*O*-methyl-D-glucose (OMG) at three concentrations (low = 35, medium = 175, and high = 351 µg glucose-C g^{-1} soil), with control (H_2O addition) shown for comparison. Points are plotted in the middle of their respective sampling periods. Error bars for standard errors of the mean were smaller than the symbol sizes.

1976) and, like glucose, α-methylglucoside and 2-deoxy-glucose stimulate chemotaxis in *Escherichia coli* (*Adler* and *Epstein*, 1974). Evidently, the molecular structures are similar enough to glucose to be recognized by transport and chemosensing proteins. Glucose analogues are therefore expected to share some, but not all, characteristics of glucose in soil (summarized in Tab. 1). These qualities allow non-reactive biochemical effects of glucose to be experimentally distinguished from the effects of its energy catabolism (*Adler* and *Epstein*, 1974; *McDermott* and *Jennings*, 1976).

To date, no studies have applied glucose analogues to investigate metabolism by complex microbial communities of soil. Based on the results of pure culture studies, we hypothesized that some glucose analogues might not be metabolizable by soil microorganisms. To test this, we compared the CO_2 efflux from control and glucose-amended soil with that from soil amended with three glucose analogues: α-D-methylglucoside (αMG), 2-deoxy-D-glucose (DG) or 3-*O*-methyl-D-glucose (OMG).

2 Material and methods

The experiment was performed with soil samples from the Ap horizon of a loamy agricultural Haplic Luvisol. The soil parameters and site conditions have been previously described in detail (*Kramer* et al., 2012; *Pausch* and *Kuzyakov*, 2012). Samples of air-dried and sieved soil (2 mm) were weighed into sealable glass jars (50 g dry weight), brought to 75% of water holding capacity, and preincubated for six weeks, with the jars opened regularly to maintain aerobic conditions. Vials of 1 M NaOH solution were placed inside the jars to capture the produced CO_2, which was subsequently determined by back-titration with 0.05 M HCl to a phenolpthalein endpoint, after carbonate precipitation with barium chloride.

Immediately prior to amendment, the moisture content of all samples was equalized to 64% of water holding capacity. Glucose and the analogues were added in 2-mL aliquots of aqueous solution at three concentrations for equimolar additions of 0.48, 2.4, and 4.9 µmol g^{-1} soil, corresponding to 35, 175, and 351 µg glucose-C g^{-1} soil. These amounts correspond to 10%, 50%, and 100% of MBC (Splettstoesser, 2016; pers. comm.), and were selected so that any variation with concentration would be observed. The same volume of water was added to the control samples. These additions returned the soil to 75% of water holding capacity. Four replicates were prepared for each treatment as well as control. The samples were incubated in the dark at 22°C for 24 d, with regular replacement of the NaOH vials.

Table 1: Expected characteristics of glucose analogues as compared to glucose.

Process	Comparison to glucose
diffusion	equivalent
adsorption	equivalent
microbial membrane transport	qualitatively similar
microbial chemosensory stimulation	qualitatively similar
metabolism	strongly suppressed

Trapped CO_2 was converted to CO_2 efflux rate for each sampling period. Tukey's HSD test was performed to identify pairwise significant differences among means. Differences were considered significant with p-value < 0.05. Statistical analysis was carried out in the R 3.2.0 software environment (*R Core Team*, 2015). Error bars represent standard errors of the mean.

3 Results

All three glucose analogues increased the CO_2 efflux above that of control soil. CO_2 efflux cumulated over 24 d was greatest for αMG and OMG at high concentration (Fig. 2). Since substrates were unlabeled, it is not possible to unambiguously assign the extra CO_2 to mineralization of the substrate. Nevertheless, the sum of control CO_2 efflux and added substrate carbon is indicated in Fig. 2 for comparison. This represents a hypothetical case of 100% mineralization of the substrate in combination with unaltered respiration of soil organic matter.

The timing of CO_2 release after analogue addition was very different to that for glucose-amended soil (Fig. 1). For glucose, CO_2 efflux peaked right after addition—during the first day for low and medium concentrations, but by 2.5 d at high concentration. CO_2 efflux from soil with analogues added at low concentration peaked within the first 2.5 d. In contrast, for medium and high concentrations of the analogues, these CO_2 peaks were delayed until between 2.5 and 4 d after amendment for DG and αMG, and were delayed by about one week for OMG.

At high concentrations, all analogues already increased CO_2 efflux during the first day. This was quantitatively similar for the three analogues, but much lower than the CO_2 efflux from glucose.

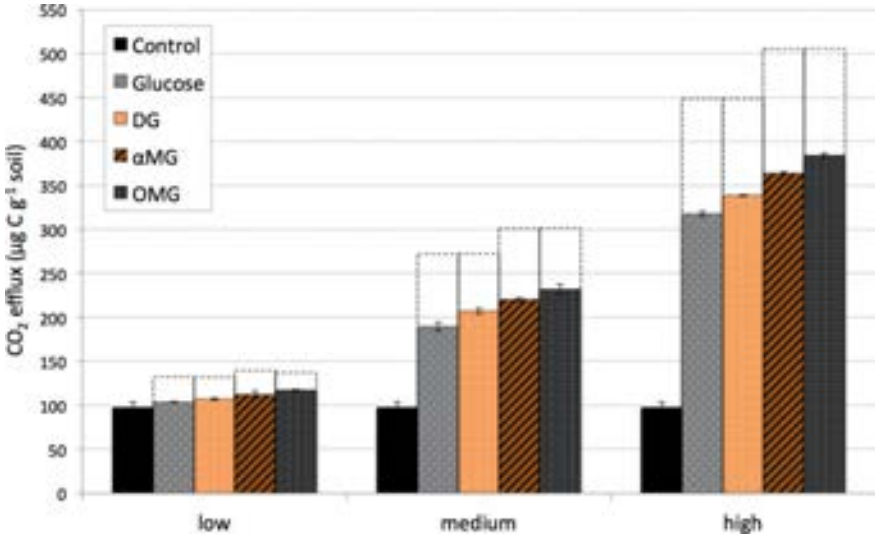

Figure 2: Cumulative CO_2 efflux over 24 days, after addition of water (control), glucose, and the three glucose analogues α-D-methylglucoside (αMG), 2-deoxy-D-glucose (DG), and 3-*O*-methyl-D-glucose (OMG) at three concentrations (low = 35, medium = 175, and high = 351 µg glucose-C g^{-1} soil). Error bars reflect standard errors of the mean. Dotted bars reflect the sum of total control CO_2 efflux and the added C, to indicate the hypothetical case of 100% substrate mineralization with unchanged SOM mineralization.

For glucose, the CO_2 efflux on the first day increased with the amount added, although not proportionally to the added amounts. In contrast, different concentrations of the analogues increased CO_2 efflux by similar amounts on the first day, with CO_2 efflux rates only diverging later.

4 Discussion

The CO_2 efflux greatly increased following the addition of all analogues. Most of the additional CO_2 originated from the added substances, since: (1) CO_2 efflux for glucose followed glucose mineralization dynamics (*Schneckenberger* et al., 2008), (2) the cumulative increase in CO_2 efflux (relative to control soil) was comparable for all analogues and glucose, which would be unlikely for completely different mechanisms of CO_2 stimulation, while (3) cumulative additional CO_2 efflux was somewhat higher for αMG and OMG, consistent with mineralization of the additional C atom of their methyl groups, and (4) the CO_2 evolved after high additions was close to or higher than the pre-existing biomass C, ruling out endogenous microbial C as a sole source of CO_2 efflux. Therefore, contrary to the initial hypothesis, "non-metabolizable" glucose analogues were in fact metabolized by soil microorganisms, and this hypothesis was rejected.

Although analogues stimulated higher cumulative CO_2 efflux than glucose, on the first day the three analogues caused much lower CO_2 efflux. CO_2 efflux on the first day was similar across increasing analogue concentrations. This pattern is consistent with a limited microbial catabolic potential that is saturated at these concentrations (*Anderson* and *Domsch*, 1978; *Anderson* and *Joergensen*, 1997; *Zyakun* and *Dilly*, 2005). The high rates of maximum CO_2 efflux show that all the analogues can be rapidly mineralized, but it took one (αMG), two (DG) or five (OMG) days to reach maximum decomposition rates. The activation of existing enzymes and the synthesis of new enzymes in response to stimuli usually occur on much shorter timescales (*Madigan* et al., 1997). The delayed catabolism is therefore most likely due to a lag phase in growth of those microorganisms that have the necessary catabolic abilities.

Although our hypothesis was rejected, metabolization of the analogues immediately after addition was much lower than that of glucose. Mineralization of OMG, in particular, was significantly suppressed and delayed relative to glucose. It remains to be confirmed whether OMG can mimic glucose in chemosensory and transport interactions in soil, as has been observed in pure culture studies. Such interactions are not dependent on glucose catabolism, and therefore could influence a wide range of microorganisms, even though they are not able to degrade it. In this case, OMG could provide a new

tool for investigating the details of processes with LMWOS (like glucose) in soil. For example, the activation of soil microorganisms by trace amounts of LMWOS (*De Nobili* et al., 2001) is hypothesized to involve microbial chemosensory mechanisms (*Blagodatskaya* and *Kuzyakov*, 2008). This mechanism could be supported by demonstrating an acceleration of microbial metabolism by OMG, despite minimal energy catabolism. There are also prospects for the study of longer-range biological carbohydrate transport, such as translocation in fungal hyphae (*Persson* et al., 2000).

5 Conclusions

The three "non-metabolizable" glucose analogues were in fact metabolized in soil, but the dynamics of their mineralization were different to the rapid degradation of glucose. Mineralization of OMG, in particular, was significantly suppressed and delayed relative to glucose, with peak mineralization occurring up to one week later. OMG therefore has the greatest potential for use as a glucose model compound with suppressed catabolism. If it can mimic glucose in chemosensing and membrane transport interactions, as has been previously demonstrated in pure culture, then it will provide a new tool for disentangling the effects of glucose on complex soil microbial communities.

Acknowledgments

We gratefully acknowledge the *Deutscher Akademischer Austauschdienst* (*DAAD*) for their financial support of Kyle Mason-Jones.

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