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Functional Ecology

DOI: 10.1111/1365-2435.12377

Published: 12/02/2015

Publisher's PDF, also known as Version of record

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Priming of the decomposition of ageing soil organic matter: concentration dependence and microbial control

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Summary

1. The amount of carbon (C) stored in soil is an important regulator for the global climate and soil fertility and is the balance between formation and decomposition of soil organic matter (SOM). Decomposition of SOM can be powerfully affected by labile carbon (C) supplements in, for example, the rhizosphere. A stimulation of SOM mineralisation induced by labile C additions is termed ‘priming’, and the mechanisms for this phenomenon remain elusive.

2. The most widely held explanation assigns priming to successional dynamics in $r$- and $K$-selected groups within the microbial community; groups which have also been connected with fungal ($K$-selected) and bacterial ($r$-selected) decomposers. New evidence has also suggested that recently formed SOM is particularly sensitive to priming. We investigated (i) the labile C concentration dependence of SOM mineralisation, (ii) the susceptibility of differently aged SOM to priming and (iii) if priming is due to bacterial or fungal growth dynamics.

3. To create an age gradient of traceable SOM, we spiked a pasture soil using $^{14}$C glucose, and subsampled plots 1 day, 2 months, 5 months and 13 months after application (i.e. SOM aged 1 day – 13 months). Glucose (0–4000 µg C g$^{-1}$) was added in subsequent laboratory experiments, and respiration, SOM mineralisation ($^{14}$CO$_2$ evolution), bacterial growth rates (leucine incorporation) and fungal biomass (ergosterol) were tracked during ca. 1 week.

4. Mineralisation of SOM aged 2–13 months showed similar labile C concentration dependencies, and priming increased mineralisation of SOM systematically by up to 350%. The glucose treatments induced variable microbial growth responses for differently aged SOM, which were unrelated to the priming effect.

5. That successional dynamics in microbial $r$- and $K$-selected groups, or bacterial and fungal decomposers, respectively, underpinned priming was incompatible with the results obtained. An alternative explanation could be that SOM transformation by extracellular enzymes, for subsequent respiration, could be triggered by labile C. In conclusion, labile C primed the mineralisation of 2–13 months aged SOM, and the mechanism for this priming was unrelated to microbial growth dynamics.

Key-words: global change biology, microbial community dynamics, microbial decomposer ecology, microbial nutrient mining, priming effect, rhizosphere, soil carbon sequestration, soil organic carbon turnover

Introduction

Understanding the processes that govern the turnover of soil organic matter (SOM) is central to understanding the functioning of soil systems and to predicting plant nutrient supply (Waksman & Gerretsen 1931; Schimel 1995).

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these subcomponent pools are not interdependent. The realisation that the labile subcomponents of SOM, such as root exudate compounds, can modulate the decomposition rates of the other SOM subcomponents (Kuzyakov, Friedell & Stahr 2000; Kuzyakov 2010) challenges this assumption. A symptom of this interdependence, or coupling, between labile C input and soil C output is the now frequently noted priming effect (Kuzyakov, Friedell & Stahr 2000; Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010). The ‘priming effect’ is defined as an increase in decomposition of SOM in response to the input of easily available C sources (Kuzyakov, Friedell & Stahr 2000; Ostle et al. 2009; Garcia-Pausas & Paterson 2011).

Although reports of priming effects on SOM mineralisation are growing increasingly abundant in the literature (Kuzyakov 2010), the underlying mechanisms remain largely elusive, which currently limits their explicit incorporation into soil C models (Ostle et al. 2009; Perveen et al. 2014). Naturally, to some extent, the plant processes that generate an input of labile C into soil and the microbial processes that regulate the outflow of soil C are interdependent. Several possible explanations for the microbial underpinnings for the priming effects have been forwarded in recent years, including, for example, the nutrient mining hypothesis (see recent overviews in Dijkstra et al. 2013 and Chen et al. 2014). However, the most widely held explanation for the priming effect to date is that the addition of labile C stimulates the growth and metabolic contribution by microbial r-strategists, followed by the gradual increase in abundance of K-strategists whose metabolic performance causes the SOM decomposition (Fontaine & Barot 2005; Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010; Perveen et al. 2014). Although it is a gross generalisation and certainly exceptions abound, it has been proposed that the pioneering r-strategists are dominated by bacterial decomposers, while the effective K-strategist decomposers of SOM are more dominated by fungi (Fontaine & Barot 2005; De Graaff et al. 2010; Dungait et al. 2013; Reinsch et al. 2013). The proposed successional dynamics of the microbial community growing on the labile C additions is likely to characterise the decomposition of substantial C additions, such as the decomposition of cellulose in plant material additions (Rousk & Bååth 2007; De Graaff et al. 2010). However, it is less obvious how successional dynamics between r- and K-strategists can explain a modulated SOM decomposition in a system with a semi-continuous availability of low concentrations of labile C, such as that in the rhizosphere (Jones, Nguyen & Finlay 2009). Moreover, if changes in the growth rates of different groups within the microbial community would explain priming effects of SOM, the effects would only manifest with a time dynamic that allows for successional dynamics within microbial communities and thus responses in microbial growth rates (Rousk & Bååth 2011), and at rates of labile C additions that are sufficient to affect microbial growth rates (Blagodatskaya & Kuzyakov 2013; Reischke, Rousk & Bååth 2014).

Assessments of how SOM priming varies with the age of SOM have to date included very different time spans. For instance, Sullivan & Hart (2013) studied how an age gradient of 900–3M years responded to glucose additions, noting a range from positive priming in the younger end, gradually progressing to negative priming in the older end of the chronosequence. In contrast, within a different age-context based on a C3–C4 vegetation change, Blagodatskaya et al. (2014) found that CO2 release from SOM primed by glucose additions tended to be younger (< one decade) rather than older (> one decade). Along a glacial chronosequence (10, 70 and 120-year-old soil), priming of SOM decomposition by plant litter was found to be consistently undetectable (Guelland et al. 2013). However, a field experiment with long-term elevated atmospheric CO2 concentrations in a temperate forest resulted in priming of SOM as a consequence of increased root exudation (Phillips, Finzi & Bernhardt 2011). The primed C released from the SOM appeared to be due to accelerated turnover of ‘young’ microbiologically derived components of the SOM (Phillips et al. 2012), rather than older fractions. This emphasises the need to understand the hitherto understudied influence of priming on recently formed SOM, shortly after it passes through the microbial community.

Experimental designs to address SOM mineralisation priming have often used labelled glucose additions to unlabelled SOM (e.g. De Nobili et al. 2001; Tuomela et al. 2002). This approach is susceptible to the increasing difficulty of partitioning CO2 derived from the labile C or SOM of variable recalcitrance over time. An alternative approach, proposed by, for example, Kuzyakov (2010), is to use multiple C isotopes to partition the contribution of SOM from that of the labile C addition. The approach we utilised here can also be used to unequivocally assign the CO2 produced to SOM formed since the initial field application of 14C. Unlike 13C-based assignment of CO2 to sources, the negligible background signal of 14C removes the necessity of relying on mixing models to establish links. The strengths of this approach are that the age fraction of SOM studied is both well defined and from an unambiguous source. This approach is limited to the age fractions of SOM formed since the initiation of the experiment, but is thus, as such, well adapted to investigate recently formed SOM.

Our study had three main objectives: (i) to determine SOM mineralisation priming effects as a function of the concentration of the added labile C (glucose), (ii) to determine if the priming of SOM mineralisation depends on the age of SOM and (iii) if the SOM mineralisation priming effect could be assigned to microbial growth dynamics induced by labile C additions.

Materials and methods

SOIL, FIELD EXPERIMENT AND SAMPLING

The experimental site used was an agricultural pasture grassland soil from a hyper-oceanic climatic region in Abergwynnog, Gwynedd, UK (53°14’N, 4°1’W) and has been described in greater detail previously (Glanville et al. 2012; Rousk, Hill & Jones 2014). The vegetation at the site consists of perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.). The mean
annual soil temperature is 11 °C (10 cm depth), and the mean annual precipitation is 1300 mm. The sandy clay loam soil is derived from post-glacial alluvial deposits and is classified as a Eutric Cambisol (FAO) or a Dystric Eutrudult (USDA) with an organic matter content (loss-on-ignition, 16 h at 550 °C) of 7.7 ± 0.8% and a pH (1:1, water extraction) of 5.2 ± 0.2. The site is used as an intensively grazed pasture (5 ewe ha⁻¹) and has received regular fertilisation (120 kg N ha⁻¹ y⁻¹). Three randomly assigned 1 m² plots were established in August 2010, and large herbivores were excluded with cages (Rousk, Hill & Jones 2014). In August 2010, ¹⁴C-labelled glucose was evenly distributed to the three 1 m² plots. A dilution solution of glucose (64 MBq <1 µm in 5-L water per 1 m²; uniformly labelled, PerkinElmer, UK) was dispensed using watering pots. Pilot experiments showed that a 5-L water addition spread over 1 m² reached 5 cm depth within 1 h at the same soil moisture level. It has previously been shown that glucose uptake is entirely dominated by microbial processes in the studied soil, and that other putative sinks (e.g., abiotic binding) are negligible (Hill, Farrar & Jones 2008; Rousk & Jones 2010; Rousk et al. 2011). Further, it has been repeatedly shown that the microbial community quickly incorporates additions of low concentrations of labile C, such as glucose, and a majority of the ¹⁴C-labelled glucose added to the field plots likely was assimilated within minutes of addition (e.g., Hill, Farrar & Jones 2008; Rousk & Jones, 2010). Thus, the ¹⁴C-SOM age gradient generated spanned from microbial C (1 day after addition) via necromass to more stabilised SOM (Murugan et al. 2013).

The experimental plots were sampled on four separate occasions 1 day after addition (August 2010), about 2 months after addition (October 2010), about 5 months after addition (January 2011) and about 13 months after addition (September 2011). On each sampling occasion, 5 randomly positioned cores (5-cm diameter, 5-cm depth) from each plot were combined into composite samples. Sampled cores that were removed were replaced with similar soil cores from outside the plots and marked with sticks to avoid resampling. The sampled soil could be classified as rhizosphere soil due to the high root density (0.35 ± 0.02 kg m⁻² in the top 10 cm). All sampling dates were done in relatively mild conditions (soil temperatures at 10 cm on October 2010: 13 °C; January 2011: 8 °C; September 2011: 14 °C), and soil moisture (gravimetric, 24 h 105 °C) was similar (30-32%, 31-39% and 30-38% for October 2010, January 2011 and September 2011, respectively; Rousk, Hill & Jones 2014). On each sampling occasion, soil was brought into the laboratory within 1 h of sampling, and sieved through a 5-mm mesh, removing visible pebbles and roots, and homogenised and mixed by hand into one composite sample per plot. This level of sieving has previously been shown to have no significant effect on mineralisation in this soil (Jones & Willett 2006).

**¹⁴C-SOM MINERALISATION DETERMINATION**

To determine the glucose concentration dependence of the mineralisation of differently aged SOM, 8 concentrations of glucose were prepared for each SOM age time-point. Soil samples (5 g) were installed in 50-mL hermetically sealed microcosms, and the glucose solutions were added at a rate of 50 µL g⁻¹ soil to achieve additions of 0.0, 0.26, 1.26, 3.6, 12.0, 800 and 4000 µg glucose-C g⁻¹ soil. This corresponded to ca. 0.01–200 nm glucose-C in the soil solution. NaOH traps (1 mL 1 M NaOH in 6-mL scintillation vials) were used to capture the ¹⁴CO₂ produced from mineralised SOM and were changed frequently (5–8 times; See time series in Figs) during the ca. 150 h study periods conducted for the three experiments. ScintSafe scintillation cocktail (Fisher Scientific, Leicestershire, UK) was added to the NaOH, and the ¹⁴C content of the captured CO₂ was determined in a Wallac 1404 liquid scintillation counter (Wallac, Milton Keynes, UK).

**BACTERIAL GROWTH AND FUNGAL BIOMASS**

To determine the responses of bacterial and fungal communities to the 8 concentrations of glucose addition for each SOM age time-point, soil samples (10 g) were installed in identical hermetically sealed microcosms as those used for ¹⁴C-SOM mineralisation determination (above) and were subsampled for bacterial growth (leucine incorporation) and fungal biomass (ergosterol concentration) determinations over the approximately 150 h study periods (see time h in figs) with the study period length determined using leucine (‘Leu’; Kirchman, K’nees & Hodson 1985) incorporation in bacteria extracted from soil using the homogenisation/centrifugation technique (Bääh, Pettersson & Söderberg 2001; Rousk & Bääh 2011). Fungal biomass was assessed by estimating the ergosterol concentration (Joergensen 2000; Ruzicka et al. 2000; Rousk, Brookes & Bääh 2009).

**RESPIRATION AND MICROBIAL BIOSMASS**

For respiration measurements, we weighed 10.0 g of soil into 50-mL centrifugation tubes kept at 22 °C and connected to an automated multichannel infrared gas analyser (PP-systems Ltd, Hitchin, UK). These were monitored in parallel with the ¹⁴C-SOM mineralisation measurements (see above).

Microbial biomass was estimated using the substrate-induced respiration (SIR) method (Anderson & Domsh 1978) shortly after sampling the soils at the four SOM age time-points. Glucose (10 mg g⁻¹) was added to the soil samples (10.0 g in 50-mL polypropylene tubes) followed by mixing the sampled by shaking and stirring with a spatula. SIR rates used to estimate microbial biomass were the mean respiration rates after they stabilised (about 0.5 h after addition and for 7-8 h) using an automated multichannel infrared gas analyser (PP-systems Ltd, Hitchin, UK). SIR was converted to biomass using the relationship: 1 mg CO₂ h⁻¹ at 22 °C corresponds to 20 mg biomass-C (recalculated from the relationship 1 mL CO₂ h⁻¹ corresponds to 40-04 mg biomass-C at 22 °C; Anderson & Domsh 1978).

**CALCULATIONS AND STATISTICAL ANALYSES**

To estimate the rate of decay for the ¹⁴C-labelled SOM for each time-point, a single negative exponential function was fitted to the data obtained from the treatment without glucose addition (0 µg glucose-C g⁻¹ soil):

\[ Y = A + B \times e^{-k\times t} \]  

eqn 1

where \( Y \) is the rate of ¹⁴C-SOM mineralisation (dpm g⁻¹ h⁻¹) at time \( t \), \( A \) is an asymptote, \( B \) the pool size that is decaying, \( k \) is the specific rate of decay (h⁻¹) and \( t \) is the time-point (h). A relatively high value of \( k \) would signify a relatively reactive SOM, while relative lower values would indicate a SOM more resistant to degradation.

To stabilise variance (variance scaled with the mean), measured values were logarithmically transformed prior to statistical tests. To test for a glucose concentration dependence of the measured variables, linear regression analyses were used on the logarithmically transformed values. This analysis utilised the full power of 8 levels along a continual scale of the independent variable (the glucose concentration), unlike categorical tests, such as an ANOVA analysis. Both the immediate effects (within about 24 h after addition) and the cumulative effects during the entire study period (ca. 70–175 h, depending on SOM age time-point) were tested separately (Table 2). Type-II major axis regressions were used to describe the relationships between SOM mineralisation and respiration, cumulative bacterial growth and the ergosterol concentrations. For these tests, overall analysis was performed including all data-points from both the ca. 24-h and full 70- to 175-h study periods (depending on SOM age time-point). To exclude the possibility that only differences between time-points caused any observed trend, the relationships with each time-points were also investigated (i.e. for each SOM age time-point, both the 24-h and full study periods were tested separately).
Results

**Respiration, Microbial Biomass and Fungal Biomass**

The respiration rate measured at 22 °C varied over the SOM age time-points ($P < 0.01$). September 2011 was highest at 4.0 µg CO$_2$·C g$^{-1}$ h$^{-1}$, August 2010 was second highest at 3.0 µg CO$_2$·C g$^{-1}$ h$^{-1}$, while January 2011 and October 2010 were lower but indistinguishable at about 2.4 and 2.6 µg CO$_2$·C g$^{-1}$ h$^{-1}$, respectively. The microbial biomass concentration estimated using SIR did not vary significantly between the SOM age time-points (Table 1), but remained stable at 1.0 ± 0.1 (mean ± 1 SE) mg C g$^{-1}$.

**Respiration**

In the 1-day aged SOM treatments, sampled in August 2010, after addition of the different glucose rates (0–4000 µg C g$^{-1}$), the respiration rate predictably scaled with the amount of added glucose, both within 24 h and cumulatively over 164 h (both $P < 0.001$). While minimal differences could be resolved between 0 and 6.4 µg glucose-C g$^{-1}$, higher rates ranked according to addition level (Fig. 1a). An 8-fold difference between the lowest and highest additions could be resolved within 24 h, and after 164 h, about 3160 µg CO$_2$·C g$^{-1}$ had been respired in the highest addition, while only about 250 µg CO$_2$·C g$^{-1}$ had been produced in the lowest additions. The pattern was very similar in the 2–13 months SOM treatments, resulting in consistently strong concentration dependences of respiration both within about 24 h and cumulatively over 144–173 h (Table 2, Figs 2a, 3a, 4a).

**14C-SOM mineralisation**

The 14C-SOM decay for each SOM age time-point could be modelled by a negative exponential curve (equation 1), yielding $R^2$ values ranging between 0.94 and 0.99. The estimated rate of decomposition of SOM decreased with age. The specific decay rates ($k$; see material and methods) were 0.089, 0.029, 0.022 and 0.016 h$^{-1}$ for 1-day, 2-month, 5-month and 13-month aged SOM, respectively (Table 1).

After addition of glucose (0–4000 µg C g$^{-1}$), the 14CO$_2$ evolution derived from differently aged SOM (1 day–13 months) could be observed in all treatments. The 1-day aged SOM (Fig. 1b) did not show a significant instantaneous or longer-term concentration dependence on the added glucose (Table 2). Two months after labelling, the mineralisation of SOM did show a significant concentration dependence, where the amount of mineralised SOM increased with higher concentrations of glucose additions both immediately (i.e. within 22 h of addition) and cumulatively over the studied 148 h period ($P = 0.016$, and 0.021, respectively; Table 2). About 3-fold more SOM was respired at the highest glucose addition compared with the lower addition treatments (Fig. 2b). The pattern was similar for 5 months aged SOM, where a positive concentration dependence of SOM mineralisation was evidenced both immediately (within 23 h), as well as cumulatively over 144 h ($P = 0.013$, and 0.0054, respectively, Table 2). About 3.5-fold, more SOM was mineralised in the highest compared with the lowest glucose addition (Fig. 3b). The SOM that had been aged 13-month again showed a similar response to glucose additions, with a positive glucose dependence both within 28 h, and cumulatively over 173 h ($P = 0.0003$ and 0.001, respectively). The highest addition of glucose again induced an about 3-fold higher cumulative mineralisation of SOM than the lowest additions (Fig. 4b).

By relating the 14C-SOM mineralisation in the control treatment (Figs 1–4, without glucose addition) to the basal respiration rate at each time-point (Table 1), we estimated the change in participation of the 14C-labelled fraction of the SOM to total respiration. The first time-point, 1 day after glucose application in the field experiment, yielded an index of ca. 1.2 Bq 14C µg$^{-1}$ CO$_2$·C, which decreased to 0.50, 0.13 and 0.08 Bq 14C µg$^{-1}$ CO$_2$·C for the 2-month, 5-month and 13-month time-points, respectively. From this, we can observe that the contribution of the added 14C to total respiration decreased by ca. 93% between 1 days’ and 13 months’ ageing in the field.

Table 1. 14C-SOM decay rate, respiration rates and soil microbial biomass

<table>
<thead>
<tr>
<th>SOM age</th>
<th>Seasonal time-point</th>
<th>14C-SOM decay rate ($k$)</th>
<th>Basal respiration</th>
<th>Microbial biomass C (SIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>1 day</td>
<td>August 2010</td>
<td>0.089b 0.0035</td>
<td>3.0b 0.089</td>
<td>1.07 0.18</td>
</tr>
<tr>
<td>2 months</td>
<td>October 2010</td>
<td>0.029a 0.0043</td>
<td>2.6a 0.094</td>
<td>1.22 0.20</td>
</tr>
<tr>
<td>5 months</td>
<td>January 2011</td>
<td>0.022a 0.0075</td>
<td>2.4a 0.053</td>
<td>0.94 0.12</td>
</tr>
<tr>
<td>13 months</td>
<td>September 2011</td>
<td>0.016a 0.0031</td>
<td>4.0c 0.095</td>
<td>0.84 0.20</td>
</tr>
</tbody>
</table>

The 14C-SOM decay rate ($k$) is reported in h$^{-1}$, respiration is reported in µg CO$_2$·C h$^{-1}$ g$^{-1}$ and soil microbial biomass-C (measured using SIR) is reported as mg C g$^{-1}$ soil. Lower case letters indicate significantly different values between the three seasonal time-points (ANOVA with Tukey’s pair-wise comparisons at $\alpha = 0.05$).
Fig. 1. Cumulative respiration (panel a), cumulative soil organic matter (SOM) mineralisation (panel b), cumulative bacterial growth measured as leucine incorporation in extracted bacteria (panel c) and the fungal biomass concentration (panel d) during the ca. 1-week study period for the 1-day field aged SOM. The legend denotes the glucose concentrations (0–4000 μg C g⁻¹). Values are the mean and error bars ±1 SE.

Table 2. The glucose concentration dependence of measured variables as tested using a linear regression analysis on log-transformed data against the log-transformed glucose concentration (n = 24 for each test). Both the immediate (within about 1 day) and longer-term (during 70–170 h) glucose concentration dependence were separately tested. The specific time-points used are indicated in italics in the table.

<table>
<thead>
<tr>
<th>Age of SOM</th>
<th>1 day (August 2010)</th>
<th>2 months (October 2010)</th>
<th>5 months (January 2011)</th>
<th>13 months (September 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14C-SOM</strong> Time-points</td>
<td>22 h 69 h 22 h 148 h 23 h 144 h 28 h 173 h</td>
<td>0.006 &lt;0.0001 &lt;0.0001 0.002 &lt;0.0001 0.0009 &lt;0.0001* 0.0018*</td>
<td>0.22 0.56 &lt;0.0001 0.13 0.0033 0.85 0.022 0.23 0.0006</td>
<td>0.004 0.16</td>
</tr>
<tr>
<td>f-value</td>
<td>0.1 1.5 6.9 6.1</td>
<td>0.13 9.5 12.3 14.4</td>
<td>0.04 0.06 1.5 0.6</td>
<td>0.10 0.06</td>
</tr>
<tr>
<td>R²-value</td>
<td>0.06 0.24 0.22 0.25 0.3 0.36 0.39</td>
<td>0.22 0.66 0.65 0.71 0.56 0.97 0.82*</td>
<td>0.22 0.10 0.33 0.001 0.22 0.06 0.42</td>
<td>0.004 0.16</td>
</tr>
<tr>
<td>Respiration</td>
<td>24 h 164 h 24 h 120 h 24 h 145 h 24 h 169 h</td>
<td>55.8 9.9 27.1 26.1</td>
<td>34.5 17.8 203* 28.1*</td>
<td>0.72 0.58</td>
</tr>
<tr>
<td>f-value</td>
<td>&lt;0.0001 &lt;0.0001 &lt;0.0001 0.0002 &lt;0.0001 0.0009 &lt;0.0001* 0.0018*</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.22 0.16</td>
</tr>
<tr>
<td>R²-value</td>
<td>0.72 0.58 &lt;0.0001 0.13 0.0033 0.85 0.022 0.23 0.0006</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.22 0.16</td>
</tr>
<tr>
<td>Bacterial growth</td>
<td>21 h 163 h 24 h 145 h 26 h 144 h 28 h 170 h</td>
<td>6.3 27.7 2.5 10.9 0.04 6.1 1.5 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f-value</td>
<td>0.019 0.0001 0.013 0.0033 0.85 0.022 0.23 0.0006</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.71 0.56 0.97* 0.82*</td>
<td>0.22 0.16</td>
</tr>
<tr>
<td>R²-value</td>
<td>0.22 0.56 0.10 0.33</td>
<td>0.04 0.06 0.42</td>
<td>0.04 0.06 0.42</td>
<td>0.004 0.16</td>
</tr>
</tbody>
</table>

*The cumulative respiration measurements at the 13-month seasonal time-point were fitted separately for low and high ranges of glucose, due to problems with calibration. Reported values include 32–4000 μg glucose C g⁻¹ concentrations only.

BacteriAl GROWTh

In the 1-day-old SOM sampling (August 2010), bacterial growth showed a concentration dependence both within 21 h of glucose additions, and lasting throughout the 164-h period studied (P = 0.019 and <0.0001, respectively, Table 2). However, the glucose effect did not scale uniformly with the application rate. While the highest glucose addition of 4000 μg C g⁻¹ almost completely inhibited the bacterial growth rate during the initial 20 h after application, the 800 μg C g⁻¹ addition stimulated the highest level of bacterial growth within 24 h (Fig. 1c). The subsequent higher rate of increase in the cumulative bacterial growth in the 4000 compared to the 800 μg C g⁻¹ over the full study period of 164 h led to a convergence of these highest glucose addition levels towards a similar level, with the
Fungal biomass clearly increased between the first and second measurement points in the highest glucose addition treatments in the soil samples for the 1-day aged SOM assessment (Fig. 1d). After this quick increase, it remained high throughout the study period (until 164 h), although a tendency for a gradual decline from the peak concentration at about 70 h could be seen (Fig. 1d). The lower addition rates of glucose did not result in any significant changes in the fungal biomass, although we note that the 800 µg C g⁻¹ treatment consistently tended to be higher than the lower addition rates throughout the study period. This resulted in no detectable glucose concentration dependence of fungal biomass 21 h after glucose addition, but a marginally significant relationship after 164 h ($P = 0.053$; Table 2). The 5 and 13 months aged SOM collections did not reproduce this pattern. No concentration dependence was found for fungal biomass for the 5-month aged SOM collection. Thus, the indicated concentration dependence of fungal biomass at 170 h in the 13-month aged SOM collection ($P = 0.036$; Table 2) could not be backed up by trends set by previous time-point and therefore should be interpreted with caution.

SOM mineralisation dependence

A clear relationship could be shown between the overall SOM mineralisation in the full data set and respiration (slope estimate 2.32; $P = 0.0012$; Fig. 5a). However, we note that this relationship was at least partly driven by differences between seasonal time-points. When tested separately for each SOM age time-point, and both for the 24-h and full study periods, this pattern held mostly true. Only 800 µg C g⁻¹ glucose treatments resulted in 80% higher cumulative bacterial growth than the other treatments, which were indistinguishable after 145 h (Fig. 2c).

The pattern in the samples collected for the 5-month aged SOM assessment showed a nearly identical pattern to that of the 2-month aged SOM, resulting in identical treatment effects and similar effect sizes (Table 2, Fig. 3c). The samples for the 13-month aged SOM assessment, the bacterial growth showed a similar pattern to the 2 and 5 months aged SOM collections (Fig. 4c). No concentration dependence was detected within 28 h (Table 2), while dependence could be observed after 170 h ($P = 0.036$; Table 2). However, the rate of increase of cumulative bacterial growth induced by the 4000 µg C g⁻¹ treatment was faster at the end of the study period. This was similar to the samples collected for the 1-day aged SOM (Fig. 1c) and suggested that the highest rates of bacterial growth would have been found in the highest glucose concentration if the study period had been conducted for longer. Also, this suggested a transient early inhibition by the highest treatment that gradually lessened over time (similar to the pattern in Fig. 1c).

**Fungal biomass**

**SOM mineralisation dependence**

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excepting the 22–24 h assessments of the 1-day and 5-month aged SOM (Fig. 5a), positive relationships between SOM mineralisation and respiration were demonstrated (with mentioned exceptions, all \( P < 0.05 \)). However, the slopes estimated from the separate assessments (ranging from 0.38 to 0.79, with a mean ± SE of 0.58 ± 0.07) were consistently lower than those estimated for the overall relationship, thus probably estimating the effect remaining after any artefact that differences in \(^{14}\text{C}-\text{SOM} \) mineralisation rate between seasonal time-points was omitted. No patterns between the mineralisation of SOM and bacterial growth (Fig. 5b) or fungal biomass (Fig. 5c) could be found. However, we could observe that the mineralisation of 13 months aged SOM showed a positive dependence on both bacterial growth (estimated slopes 2.24 and 1.36 for 28 h and 173 h, respectively; Fig. 5b) and fungal biomass.
concentration (slope estimates 1-91 and 1-19 for 28 and 173 h, respectively; Fig. 5c). None of the other assessments indicated a connection between SOM mineralisation and bacterial growth or fungal biomass.

Discussion

PRIMING EFFECTS: SOM AGE AND GLUCOSE CONCENTRATION DEPENDENCES

While the uptake of low concentrations of labile C into microbial biomass is fast (Hill, Farrar & Jones 2008), occurring in minutes to hours, the subsequent turnover of the microbial biomass is slower. Bacterial turnover times in the rhizosphere have been estimated to be <3 days (Bäath & Johansson 1990; Christensen et al. 1995; Söderberg & Bäath 1998; Söderberg & Bäath 2004), while that for fungi is slower at 4–5 months in bulk soil (Rousk & Bäath 2007) or about 20–70 days on plant litter (Kuehn et al. 2000; Rousk & Bäath 2007). Estimates of microbial turnover based on tracing 14C additions of low concentration (nm levels) of glucose in grassland soils have resulted in turnover time estimates of about 20 days at field conditions (Glanville et al. 2012). Thus, it would reasonable to conclude that the glucose assimilated by the microbial community has turned over within a few days up to a few months. A number of processes contribute to the stabilisation of microbial necromass components (Schmidt et al. 2011), and microbial cell wall components alone (amino sugars) are estimated to compose a significant proportion of the SOM in grassland soils (Murugan et al. 2013). The SOM age gradient we sampled should thus first have been dominated by microbial biomass-C. Consequently, 14CO2 release from 1-day-old SOM would not be derived from the non-living SOM, but rather microbial turnover, and could therefore be termed ‘apparent priming’ (sensu Blagodatskaya & Kuzyakov 2008). The later time-points, however, were progressively more dominated by physically or chemically stabilised SOM (Schmidt et al. 2011) derived from microbial products, and 14CO2 release would be a result of ‘real priming’ (sensu Blagodatskaya & Kuzyakov 2008), stemming from progressively older and more recalcitrant fractions of the non-living SOM. This interpretation could also be strengthened with the verification by estimates of the specific decay rate of the differently aged 14C-SOM (Table 1), which showed increasing resistance to decomposition with age.

Excepting only the SOM aged 1 day (Fig. 1; the single time-point that should be regarded as microbial biomass-C; see above), the mineralisation of SOM increased with higher glucose application rates for 2-month aged SOM (Fig. 2), 5-month aged SOM (Fig. 3) and 13-month aged SOM (Fig. 4). The positive relationship between SOM mineralised and glucose addition concentration for 2–13 month aged SOM appeared to be consistent and gradually increased throughout the entire span of glucose concentrations (0.26–4000 μg C g−1), leading to 300–350% SOM priming effects at the highest concentration of glucose, relative to 14C-SOM respired in soil without glucose additions. The respiration responses matched these patterns in kind (Figs 2–4). When explicitly tested, the dependence of SOM mineralisation on respiration was consistent for SOM aged between 2 and 13 month, and only the 1-day-old SOM (i.e. microbial biomass turnover) diverged from this pattern (Fig. 5).

The results on the glucose concentration dependence of priming are similar to the single previously reported investigation of the glucose concentration dependence of SOM priming. Paterson & Sim (2013) used six increments of glucose concentrations from 0.05 to 1 mg 13C-labelled glucose (20–400 μg glucose-C) g−1 soil, to two agricultural soils (pH = 6 and SOM = 7.6%, and pH 5–6 and SOM = 4.7%, respectively) and one forest soil (pH = 3.1, SOM = 78.5%) and monitored contribution to respiration during about one week. The priming effect of SOM mineralisation consistently increased with higher glucose addition concentrations, matching our here reported results. However, in contrast with our results, the positive connection between glucose addition rates and the level of priming reported by Paterson & Sim (2013) appeared to saturate between 200
and 400 μg glucose-C g⁻¹. In SOM aged 2–13 months, we consistently saw a clear incremental increase in SOM priming all the way to the highest levels of addition (4000 μg glucose-C g⁻¹ soil). It is of course possible that the priming effect on recently formed SOM that we addressed in our experimental design had a different glucose concentration dependence than the natural SOM of variable age studied by Paterson & Sim (2013).

**THE MECHANISM OF PRIMING: THE SOIL MICROBIAL COMMUNITY?**

We found a glucose concentration-dependent priming effect of SOM aged 2–13 months. The most widely held explanation for the priming effect is that the addition of labile C stimulates the growth and metabolic contribution by microbial r-strategists, followed by the gradual increase in abundance of K-strategists whose metabolic performance causes the SOM decomposition (Fontaine & Barot 2005; Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010; Perveen et al. 2014). Further, it has been proposed that the pioneering r-strategists are dominated by bacterial decomposers while the effective K-strategist decomposers of SOM are more dominated by fungi (Fontaine & Barot 2005; De Graaff et al. 2010; Dungait et al. 2013; Reisch et al. 2013). Could our measurements of microbial growth induced by addition of glucose verify these suggested mechanisms?

In contrast with the consistently positive relationship between SOM priming and respiration induced by higher additions of glucose, the microbial growth responses were much less clear (Fig. 5). Bacterial growth tended to increase with higher glucose additions, but the positive relationship saturated, and sometimes even reversed to an inhibition, at the highest concentrations (e.g. Fig. 1c). During the ca 1-week long monitoring period for each SOM age time-point, the 800 μg glucose-C g⁻¹ addition of glucose resulted in similar (1 day, Fig. 1; 13 months, Fig. 4) or higher levels of bacterial growth than the 4000 μg glucose-C g⁻¹ addition. The level of fungal biomass formed was clearly stimulated by the highest glucose addition in the 1-day-old SOM time-point (with no clear evidence for SOM priming; Fig. 1d), while it remained unresponsive to the full range of glucose additions (0.26–4000 μg C g⁻¹) in SOM aged 2–13 months (Figs 2c and 4c). Since our assessment of fungi was based on biomass concentrations, unlike the assessment of bacteria, it is possible that intensive grazing could have obscured positive fungal responses (Clarholm 1981; Cotner et al. 1997; Rousk & Bäath 2007). However, the consistent SOM mineralisation priming between short term assessments (within 24 h), with minimal possibility for changes in growth, and over one week assessments, where substantial increases in fungal growth could have occurred, makes this an unlikely explanation. Thus, there was no evidence for a connection between the levels of priming of SOM aged 1 day–13 months and overall microbial growth.

It has been shown that positive changes in bacterial growth as a response to favourable growth conditions only manifest 6–10 h after the new conditions occur, while growth rate responses to inhibitory effects are immediate (reviewed by Rousk & Bäath 2011). Our results verify this, and the first time-point of bacterial growth measurements (ca. 5 h after glucose additions) for SOM age time-point shows similar rates for all glucose additions (Figs 1c, 2c, 3c, 4c), except the highest glucose concentration, which sometimes induced inhibitory effects (Fig. 1c). Although no changes in the microbial growth rates had occurred, the pattern for higher SOM priming with increasing glucose additions had already started to manifest (see insets to Figs 2b, 3b, 4b) within this timeframe (ca. 5 h after glucose additions).

A disconnect between the soil microbial biomass size and composition and SOM decomposition, where SOM decomposition is largely unaffected by experimental manipulations of the microbial community size, composition and diversity, has frequently been noted (Griffiths et al. 2000; Wertz et al. 2006; Kemmitt et al. 2008; Rousk et al. 2011). However, it has been suggested that the lack of association between SOM decomposition and the microbial community stems from experimental assessments of bulk soil sampled, with the expectation that a stronger connection exists in soil environments fuelled by labile C inputs, such as that in the rhizosphere (Garcia-Pausas & Paterson 2011). In line with this expectation, links between SOM priming effects and microbial community dynamics have recently been reported for a variety of situations where labile C was present (e.g. Garcia-Pausas & Paterson 2011; Blagodatskaya et al. 2011; reviewed by Kuzyakov 2010). This probably underlies the widely held rationale that successional dynamics among r- and K-strategists within the microbial community explain the SOM priming induced by labile C additions (see above). We universally find that labile C additions result in priming of SOM mineralisation shortly after labile C addition as well as during the course of 1 week after the additions, and that this occurs for SOM ages 2–13 months. However, this could not be assigned to dynamics in putative r- and K-strategists within the microbial community. Responses in microbial growth rates must logically occur for successional transitions within the microbial community to manifest. In the studied grassland soil, the detected priming effects were not related to microbial growth. Therefore, we can conclude that the mechanism for the observed priming of SOM mineralisation was not successional dynamics between microbial r- and K-strategists per se. This finding is consistent with one of the few other studies directed at testing the connection between microbial successional dynamics and priming (Reinsch et al. 2013), where differences in microbial use of added labile C (from r-selected bacteria to K-selected fungi and actinomycetes, as interpreted by the authors) in a Danish heath land did not translate into a change in the priming effect. This calls for the identification for mechanisms other than general
changes in microbial growth dynamics most often highlighted as a likely explanation for the priming of SOM (e.g. Kuyzakov 2010; Garcia-Pausas & Paterson 2011). Alternatively, an enhanced resolution of the microbial community beyond r- and K-strategists or bacterial and fungal groups (Pascual et al. 2013) or a consideration of changes in the resident microbial community’s physiology, affecting, for example the microbial C-use efficiency (Keiblinger et al. 2010), will be required.

ALTERNATIVE MECHANISMS FOR THE PRIMING OF SOM MINERALISATION

The rate-limiting step for SOM mineralisation in soil is thought to be the activity of oxidative enzymes including peroxidases (Osono 2007; Hofrichter et al. 2010; Drake et al. 2011) that, for example depolymerise SOM macromolecules and produce soluble substrates for microbial assimilation. In a secondary step, thought to be exclusively intracellular (Burns 1982; Burns & Dick 2002; Sinsabaugh & Follstad Shah 2012), the labile C substrates are metabolised, and CO2 is released. The availability of H2O2 will govern the activity of the rate-limiting SOM oxidising enzymes, and the H2O2 supply, in turn, is regulated by a range of different oxidases that use simple labile C sources, including, for example sugars such as glucose and substrate (Ander & Marzullo 1997; Hallwell & Gutteridge 1999). Thus, it has been forwarded that the underlying mechanism for the priming of SOM mineralisation by labile C addition could be due to the stimulation of H2O2 production, inducing an increased activity of, for example peroxidases that trigger the solubilisation (e.g. via depolymerisation with peroxidases or cell wall deconstruction with endoglucanase) and subsequent respiration of SOM (Bengtson, Barker & Grayston 2012). Further, it has recently been suggested that intracellular soil enzymes can be stabilised in soil and that an intact reaction chain from SOM to CO2 consequently can be maintained in soil, contributing to a substantial fraction of the CO2 evolved, even in the absence of metabolising cells (Maire et al. 2013). Extracellular enzymes are abundant in the soil matrix and numbers are relatively stable over time (Burns et al. 2013). Taken together, it is possible that the supply of H2O2 would show a glucose concentration dependence; if this indeed is the rate-limiting step for the transformation of SOM to monomer C substrates that immediately are oxidised to CO2 by the resident microbial community or by stabilised intracellular enzymes in the soil matrix (Maire et al. 2013), the lack of a link to simultaneous microbial growth rates could be explained. Rather, the historical conditions that have produced the stock of extracellular enzymes could determine the ability of glucose to prime the mineralisation of SOM. While this putative mechanism is consistent with the obtained results, it has yet to be experimentally tested. It should be noted that the hypothesised enzymatic stability of peroxidases in soil can be questioned (Sinsabaugh 2010) and their dependence on soil physicochemistry, including, for example mineralogy and pH, will need consideration. So far, we can conclude that microbial growth dynamics could not explain the priming of SOM aged 2–13 months in the studied soil.

Acknowledgements

This work was supported by grants from the Swedish Research Council (Vetenskapsrådet, grant no: 621-2011-5719), the Royal Physiographical Society of Lund (Kungliga Fysiografiska Sällskapet), and the Crafoord Foundation (grant no: 2012050). This research was also supported by the UK Natural Environment Research Council Grant no. NE/I012303/1.

Data accessibility

All data are included in the main article or can be found in referenced published articles.

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Received 3 July 2014; accepted 11 November 2014
Handling Editor: Carly Stevens