



PRIFYSGOL
BANGOR
UNIVERSITY

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

Rousk, J.; Hill, P.W.; Jones, D.L.

Functional Ecology

DOI:
[10.1111/1365-2435.12377](https://doi.org/10.1111/1365-2435.12377)

Published: 12/02/2015

Publisher's PDF, also known as Version of record

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Rousk, J., Hill, P. W., & Jones, D. L. (2015). Priming of the decomposition of ageing soil organic matter: concentration dependence and microbial control. *Functional Ecology*, 29(2), 285-296. <https://doi.org/10.1111/1365-2435.12377>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

Johannes Rousk^{*1}, Paul W. Hill² and Davey L. Jones²

¹Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden; and ²School of Environment, Natural Resources and Geography, Bangor University, Bangor, UK

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 ¹⁴C 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 (¹⁴CO₂ 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).

Moreover, at a size two or three fold that of the atmosphere (Le Quéré *et al.* 2009), the carbon (C) stored in soil represents the largest pool held in terrestrial ecosystems. Most efforts directed at describing the exchange processes of the soil C pool rely on dividing SOM into a number of subcomponents, whose individual decomposition rates occur according to first-order kinetics (Manzoni & Porporato 2009; Xu *et al.* 2014). It is thus assumed that the turnover rates of

*Correspondence author. E-mail: johannes.rousok@biol.lu.se

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, Friedlel & 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, Friedlel & 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, Friedlel & 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 dynamic 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' microbially 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 Abergwyngregyn, 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 rye grass (*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 Eutrudept (USDA) with an organic matter content (loss-on-ignition, 16 h at 550 °C) of $7.7 \pm 0.8\%$ and a pH (1 : 5, 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 diluted solution of glucose (64 MBq < 1 nM 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 (30.2%, 31.9% and 30.8% 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, 1.28, 6.4, 32, 160, 800 and 4000 µg glucose-C g⁻¹ soil. This corresponded to ca. 0.01–200 mM 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 samplings. 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 series in figures). Bacterial growth was estimated using leucine ('Leu'; Kirchman, K'nees & Hodson 1985) incorporation in bacteria extracted from soil using the homogenisation/centrifugation technique (Bååth, Pettersson & Söderberg 2001; Rousk & Bååth 2011). Fungal biomass was assessed by estimating the ergosterol concentration (Joergensen 2000; Ruzicka *et al.* 2000; Rousk, Brookes & Bååth 2009).

RESPIRATION AND MICROBIAL BIOMASS

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 & Domsch 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 & Domsch 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):

$$Y = A + B \times e^{-k \times t} \quad \text{eqn1}$$

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 concentration. 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 within 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 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$, August 2010 was second highest at 3.0 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$, while January 2011 and October 2010 were lower but indistinguishable at about 2.4 and 2.6 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$, 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 \pm 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 $\mu\text{g C g}^{-1}$), 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 $\mu\text{g glucose-C g}^{-1}$, 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 $\mu\text{g CO}_2\text{-C g}^{-1}$ had been respired in the highest addition, while only about 250 $\mu\text{g CO}_2\text{-C g}^{-1}$ 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).

^{14}C -SOM MINERALISATION

The ^{14}C -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 $\mu\text{g C g}^{-1}$), the ^{14}C 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 ^{14}C -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 ^{14}C -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 $\text{Bq } ^{14}\text{C } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$, which decreased to 0.50, 0.13 and 0.08 $\text{Bq } ^{14}\text{C } \mu\text{g}^{-1} \text{ CO}_2\text{-C}$ for the 2-month, 5-month and 13-month time-points, respectively. From this, we can observe that the contribution of the added ^{14}C to total respiration decreased by ca. 93% between 1 days' and 13 months' ageing in the field.

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

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

The ^{14}C -SOM decay rate (k) is reported in h^{-1} , respiration is reported in $\mu\text{g CO}_2\text{-C h}^{-1} \text{ g}^{-1}$ 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 $\alpha = 0.05$).

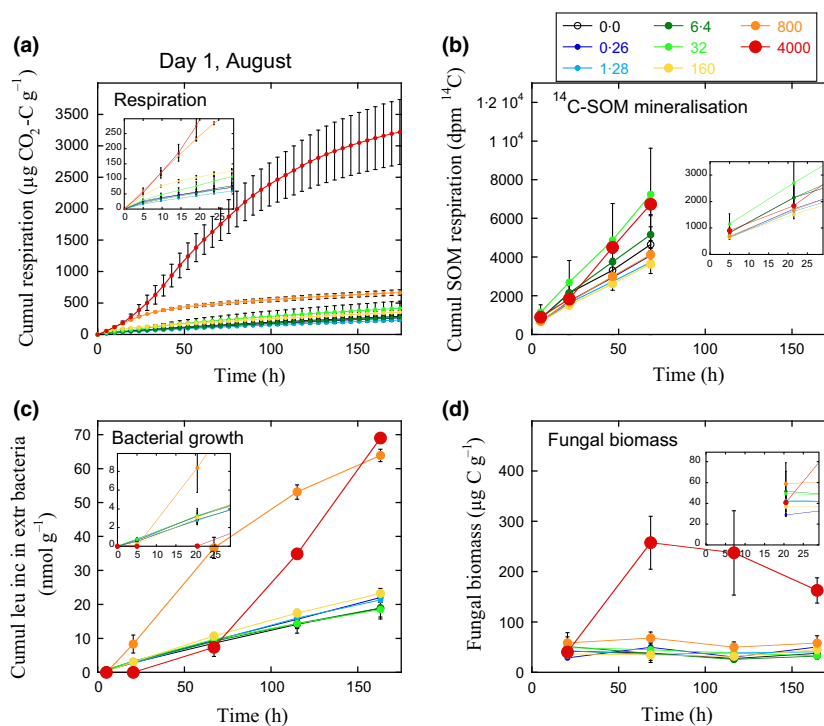


Fig. 1. Cumulative respiration (panel a), cumulative soil organic matter (SOM) mineralisation (panel b), cumulative bacterial growth measured as leucine incorporation in extracted bacteria (panel c) and the fungal biomass concentration (panel d) during the ca. 1-week study period for the 1-day field aged SOM. The legend denotes the glucose concentrations (0–4000 $\mu\text{g C g}^{-1}$). 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.

		Age of SOM							
		1 day (August 2010)		2 months (October 2010)		5 months (January 2011)		13 months (September 2011)	
$^{14}\text{C-SOM}$	<i>Time-points</i>	22 h	69 h	22 h	148 h	23 h	144 h	28 h	173 h
	<i>f</i> -value	0.1	1.5	6.9	6.1	7.4	9.5	12.3	14.4
	<i>P</i> -value	0.73	0.22	0.016	0.021	0.013	0.0054	0.0003	0.001
	<i>R</i> ² -value	0.006	0.06	0.24	0.22	0.25	0.3	0.36	0.39
Respiration	<i>Time-points</i>	24 h	164 h	24 h	120 h	24 h	145 h	24 h	169 h
	<i>f</i> -value	55.8	9.9	27.1	26.1	34.5	17.8	203*	28.1*
	<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0009	<0.0001*	0.0018*
	<i>R</i> ² -value	0.72	0.58	0.66	0.65	0.71	0.56	0.97*	0.82*
Bacterial growth	<i>Time-points</i>	21 h	163 h	24 h	145 h	26 h	144 h	28 h	170 h
	<i>f</i> -value	6.3	27.7	2.5	10.9	0.04	6.1	1.5	16.0
	<i>P</i> -value	0.019	<0.0001	0.13	0.0033	0.85	0.022	0.23	0.0006
	<i>R</i> ² -value	0.22	0.56	0.10	0.33	0.001	0.22	0.06	0.42
Fungal biomass	<i>Time-points</i>	21 h	164 h			28 h	147 h	28 h	170 h
	<i>f</i> -value	0.1	4.2			1.7	1.4	0.9	5.0
	<i>P</i> -value	0.77	0.053			0.2	0.24	0.35	0.036
	<i>R</i> ² -value	0.004	0.16			0.07	0.06	0.04	0.19

*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 $\mu\text{g glucose C g}^{-1}$ 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 $\mu\text{g C g}^{-1}$ almost completely inhibited the bacterial growth rate during the initial 20 h after application, the 800 $\mu\text{g C g}^{-1}$ 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 $\mu\text{g C g}^{-1}$ over the full study period of 164 h led to a convergence of these highest glucose addition levels towards a similar level, with the

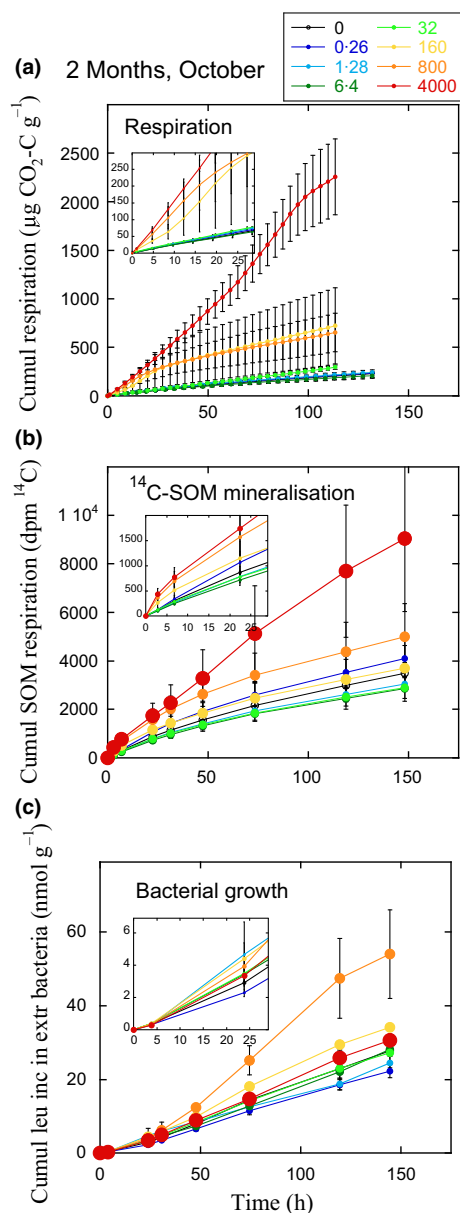


Fig. 2. Cumulative respiration (panel a), cumulative soil organic matter (SOM) mineralisation (panel b), and the cumulative bacterial growth measured as leucine incorporation into extracted bacteria (panel c) during the ca. 1-week study period for the 2-month field aged SOM. The fungal biomass concentration could not be determined due to analytical equipment failure. The legend denotes the glucose concentrations (0–4000 $\mu\text{g C g}^{-1}$). Values are the mean and error bars ± 1 SE.

lower addition treatments about 3-fold lower (Fig. 1c). The samples collected for the 2 months aged SOM (sampled in October 2010) showed a similar but not identical pattern of bacterial growth (Fig. 2c), lacking both the initial inhibition induced by the highest glucose concentration and a higher rate of increase in 4000 compared with 800 $\mu\text{g C g}^{-1}$ glucose additions. No concentration dependence was observed within 24 h of glucose treatments, while this changed into a clear glucose concentration dependence within 145 h ($P = 0.0033$; Table 2). The

800 $\mu\text{g C g}^{-1}$ 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 $\mu\text{g C g}^{-1}$ 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

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 $\mu\text{g C g}^{-1}$ 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

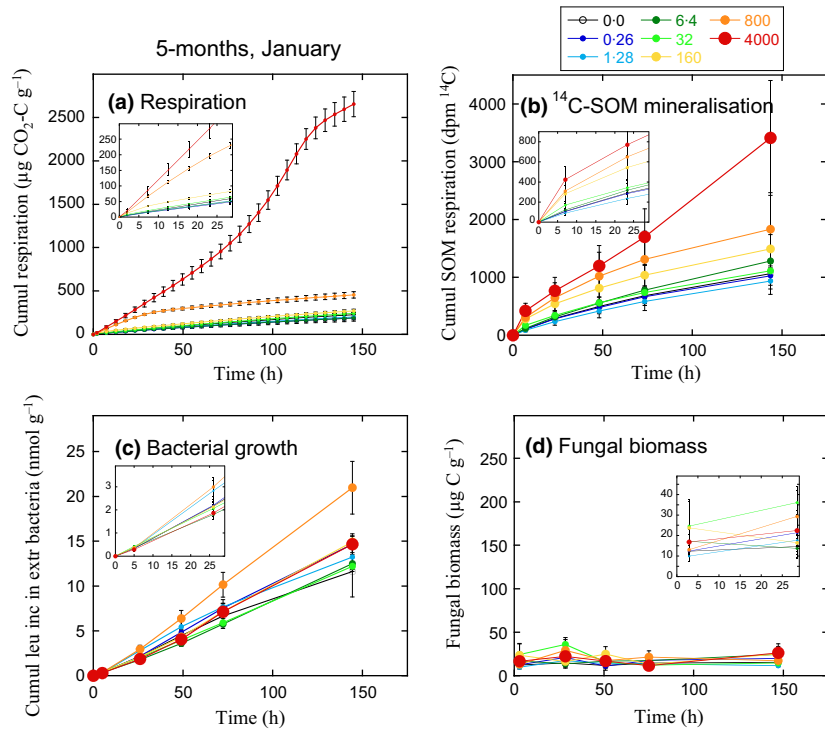


Fig. 3. Cumulative respiration (panel a), cumulative soil organic matter (SOM) mineralisation (panel b) and cumulative bacterial growth measured as leucine incorporation into extracted bacteria (panel c) and the fungal biomass concentration (panel d) during the ca. 1 week study period for the 5-month field aged SOM. The legend denotes the glucose concentrations (0–4000 $\mu\text{g C g}^{-1}$). Values are the mean and error bars ± 1 SE.

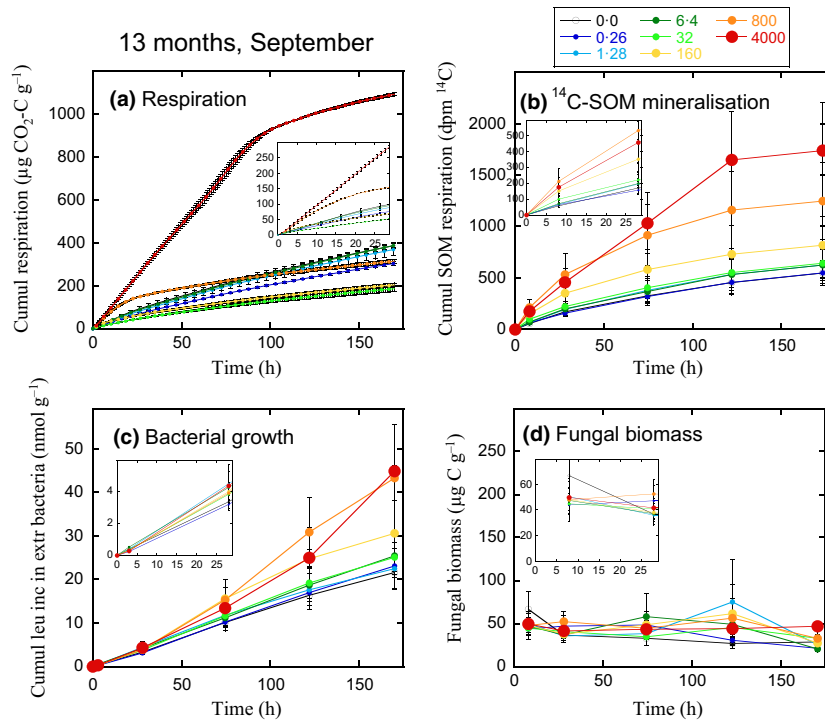


Fig. 4. Cumulative respiration (panel a), cumulative soil organic matter (SOM) mineralisation (panel b), cumulative bacterial growth measured as leucine incorporation into extracted bacteria (panel c) and the fungal biomass concentration (panel d) during the ca. 1-week study period for the 13-month field aged SOM. The legend denotes the glucose concentrations (0–4000 $\mu\text{g C g}^{-1}$). Values are the mean and error bars ± 1 SE.

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 \pm 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 $^{14}\text{C-SOM}$ 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

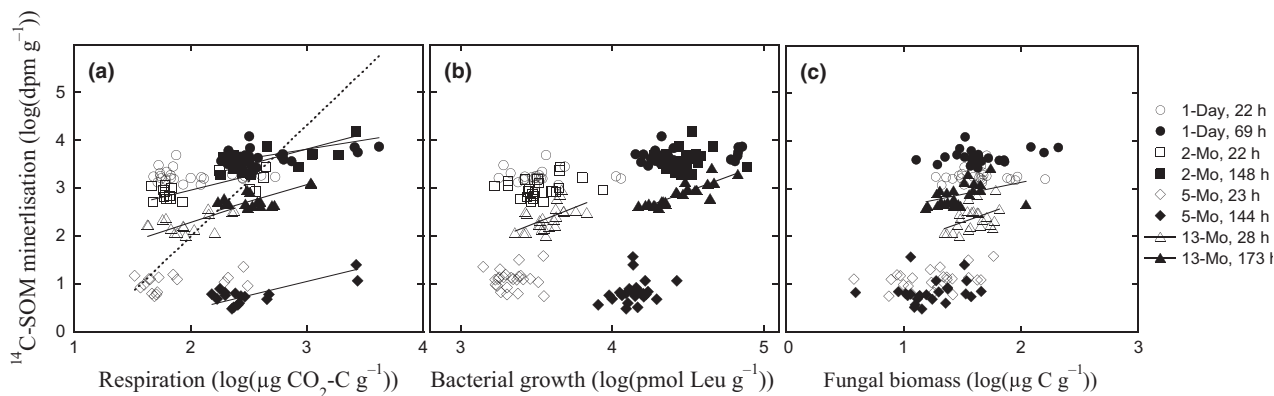


Fig. 5. The dependency of soil organic matter (SOM) mineralisation on respiration (panel a), bacterial growth (panel b) and fungal biomass (panel c). A type-II major axis linear regression was used to test the relationships between logarithmically transformed values. The relationship was tested overall, and for the 1-day and 7-day study periods for each SOM age time-point, separately (see legend for symbol definitions). Only statistically significant relationships ($P < 0.05$) are presented (see Results section).

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 (Bååth & Johansson 1990; Christensen *et al.* 1995; Söderberg & Bååth 1998; Söderberg & Bååth 2004), while that for fungi is slower at 4–5 months in bulk soil (Rousk & Bååth 2007) or about 20–70 days on plant litter (Kuehn *et al.* 2000; Rousk & Bååth 2007). Estimates of microbial turnover based on tracing ^{14}C 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 be 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, $^{14}\text{CO}_2$ 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 $^{14}\text{CO}_2$ 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 ^{14}C -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 $\mu\text{g C g}^{-1}$), leading to 300–350% SOM priming effects at the highest concentration of glucose, relative to ^{14}C -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 additions from 0.05 to 1 mg ^{13}C -labelled glucose (20–400 $\mu\text{g glucose-C g}^{-1}$ 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; Reinsch *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 & Bååth 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 & Bååth 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. Kuzyakov 2010; Garcia-Pausas & Paterson 2011). Alternatively, an enhanced resolution of the microbial community beyond *r*- and *K*-strategists or bacterial and fungal groups (Pascault *et al.* 2013) or a consideration of changes in the resident microbial community's physiology, affecting, for example the microbial C-use efficiency (Keibinger *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; Halliwell & 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 is 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: 20120550). 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.

References

- Ander, P. & Marzullo, L. (1997) Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation. *Journal of Biotechnology*, **53**, 115–131.
- Anderson, J.P.E. & Domsch, K.H. (1978) Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, **10**, 215–221.
- Bååth, E. & Johansson, T. (1990) Measurement of bacterial growth rates on the rhizoplane using ³H-thymidine incorporation into DNA. *Plant and Soil*, **126**, 133–139.
- Bååth, E., Pettersson, M. & Söderberg, K.H. (2001) Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. *Soil Biology and Biochemistry*, **33**, 1571–1574.
- Bengtson, P., Barker, J. & Grayston, S.J. (2012) Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. *Ecology and Evolution*, **2**, 1843–1852.
- Blagodatskaya, E. & Kuzyakov, Y. (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils*, **45**, 115–131.
- Blagodatskaya, E. & Kuzyakov, Y. (2013) Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology and Biochemistry*, **67**, 192–211.
- Blagodatskaya, E., Yuyukina, T., Blagodatsky, S. & Kuzyakov, Y. (2011) Three-source-partitioning of microbial biomass and of CO₂ efflux from soil to evaluate mechanisms of priming effects. *Soil Biology and Biochemistry*, **43**, 778–786.
- Blagodatskaya, E., Khomyakov, N., Myachina, O., Bogomolova, I., Blagodatsky, S. & Kuzyakov, Y. (2014) Microbial interactions affect sources of priming induced by cellulose. *Soil Biology and Biochemistry*, **74**, 39–49.
- Burns, R.G. (1982) Enzyme-activity in soil – location and a possible role in microbial ecology. *Soil Biology and Biochemistry*, **14**, 423–427.
- Burns, R.G. & Dick, R.P. (2002) *Enzymes in the environment: Activity, Ecology and Applications, Books in Soils, Plants, and the Environment Series 86*. CRC Press, New York, NY.
- Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.W., Wallenstein, M.D. *et al.* (2013) Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*, **58**, 216–234.
- Chen, R., Mehmet, S., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X.G. *et al.* (2014) Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology*, **20**, 2356–2367.
- Christensen, H., Rønn, R., Ekelund, F. & Christensen, S. (1995) Bacterial production determined by [³H]thymidine incorporation in field rhizospheres as evaluated by comparison to rhizodeposition. *Soil Biology and Biochemistry*, **27**, 93–99.
- Clarholm, M. (1981) Protozoan grazing of bacteria in soil – impact and importance. *Microbial Ecology*, **7**, 343–350.

- Cotner, J.B., Ammerman, J.W., Peele, E.R. & Bentzen, E. (1997) Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquatic Microbial Ecology*, **13**, 141–149.
- De Graaff, M.A., Classen, A.T., Castro, H.F. & Schadt, C.W. (2010) Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytologist*, **188**, 1055–1064.
- De Nobili, M., Contin, M., Mondini, C. & Brookes, P.C. (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology and Biochemistry*, **33**, 1163–1170.
- Dijkstra, F.A., Carillo, Y., Pendall, E. & Morgan, J.A. (2013) Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology*, **4**, 1–8.
- Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhart, E.S., Billings, S.A., Jackson, R.B. *et al.* (2011) Increases in the flux of carbon below-ground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecology Letters*, **14**, 349–357.
- Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S.L., Wen, Q., Brookes, P.C. *et al.* (2013) The variable response of soil microorganisms to trace concentrations of low molecular weight organic substrates of increasing complexity. *Soil Biology and Biochemistry*, **64**, 57–64.
- Fontaine, S. & Barot, S. (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecology Letters*, **8**, 1075–1087.
- Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B. & Rumpel, C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, **450**, 277–U10.
- Garcia-Pausas, J. & Paterson, E. (2011) Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. *Soil Biology and Biochemistry*, **43**, 1705–1713.
- Glanville, H., Rousk, J., Golyshin, P. & Jones, D.L. (2012) Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biology and Biochemistry*, **48**, 88–95.
- Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F. *et al.* (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. *Oikos*, **90**, 279–294.
- Guelland, K., Esperschütz, J., Bornhauser, D., Bernasconi, S.M., Kretzschmar, R. & Gagedorn, F. (2013) Mineralisation and leaching of C from ¹³C labelled plant litter along an initial soil chronosequence of a glacier forefield. *Soil Biology and Biochemistry*, **57**, 237–247.
- Halliwel, B. & Gutteridge, J. (1999) *Free Radicals in Biology and Medicine*, 3rd edn. Oxford University Press, Oxford, UK. 936 pp.
- Hill, P.W., Farrar, J.F. & Jones, D.L. (2008) Decoupling of microbial glucose uptake and mineralization in soil. *Soil Biology and Biochemistry*, **40**, 616–624.
- Hofrichter, M., Ullrich, R., Pecyna, M., Liers, C. & Lundell, T. (2010) New and classic families of secreted fungal heme peroxidases. *Applied Microbiology and Biotechnology*, **87**, 871–897.
- Joergensen, R.G. (2000) Ergosterol and microbial biomass in the rhizosphere of grassland soils. *Soil Biology and Biochemistry*, **32**, 647–652.
- Jones, D.L., Nguyen, C. & Finlay, R.D. (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant and Soil*, **321**, 5–33.
- Jones, D.L. & Willett, V.B. (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, **38**, 991–999.
- Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Eilersdorfer, G. *et al.* (2010) The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. *FEMS Microbiology Ecology*, **73**, 430–440.
- Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R.A. *et al.* (2008) Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective. *Soil Biology and Biochemistry*, **40**, 61–73.
- Kirchman, D., K'Neas, E. & Hodson, R. (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology*, **49**, 599–607.
- Kuehn, K.A., Lemke, M.J., Suberkropp, K. & Wetzell, R.G. (2000) Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology and Oceanography*, **45**, 862–870.
- Kuzakov, Y. (2010) Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry*, **42**, 54–60.
- Kuzakov, Y., Friedle, J.K. & Stahr, K. (2000) Review of mechanism and quantification of priming effects. *Soil Biology and Biochemistry*, **32**, 1485–1498.
- Le Quéré, C., Raupach, M.R., Canadell, J.G., Marland, G., Laurent, B., Ciais, P. *et al.* (2009) Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, **2**, 831–836.
- Maire, V., Alvarez, G., Colombet, J., Comby, A., Despinasse, R., Dubreucq, E. *et al.* (2013) An unknown oxidative metabolism substantially contributes to soil CO₂ emissions. *Biogeosciences*, **10**, 1155–1167.
- Manzoni, S. & Porporato, A. (2009) Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biology and Biochemistry*, **41**, 1355–1379.
- Murugan, R., Loges, R., Taube, F. & Joergensen, R.G. (2013) Specific response of fungal and bacterial residues to one-season tillage and repeated slurry application in a permanent grassland soil. *Applied Soil Ecology*, **72**, 31–40.
- Osono, T. (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological Research*, **22**, 955–974.
- Ostle, N.J., Smith, P., Fisher, R., Woodward, F.I., Fisher, J.B., Smith, J.U. *et al.* (2009) Integrating plant-soil interactions into global carbon cycle models. *Journal of Ecology*, **97**, 851–863.
- Pascual, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S. *et al.* (2013) Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems*, **16**, 810–822.
- Paterson, E. & Sim, A. (2013) Soil-specific response functions of organic matter mineralization to the availability of labile carbon. *Global Change Biology*, **19**, 1562–1571.
- Perveen, N., Barot, S., Alvarez, G., Klumpp, K., Martin, R., Rapaport, A. *et al.* (2014) Priming effect and microbial diversity in ecosystem functioning and response to global change: a modelling approach using the SYMPHONY model. *Global Change Biology*, **20**, 1174–1190.
- Phillips, R.P., Finzi, A.C. & Bernhardt, E.S. (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation. *Ecology Letters*, **14**, 187–194.
- Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K. & Finzi, A.C. (2012) Root and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO₂. *Ecology Letters*, **15**, 1042–1049.
- Reinsch, S., Ambus, P., Thornton, B. & Paterson, E. (2013) Impact of future climatic conditions on the potential for soil organic matter priming. *Soil Biology and Biochemistry*, **65**, 133–140.
- Reischke, S., Rousk, J. & Bååth, E. (2014) The effects of glucose loading rates on bacterial and fungal growth in soil. *Soil Biology and Biochemistry*, **70**, 88–95.
- Rousk, J. & Bååth, E. (2007) Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiology Ecology*, **62**, 258–267.
- Rousk, J. & Bååth, E. (2011) Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology*, **78**, 17–30.
- Rousk, J. & Jones, D.L. (2010) Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H₂O and 0.5 M K₂SO₄ soil extracts. *Soil Biology & Biochemistry*, **42**, 2331–2335.
- Rousk, J., Brookes, P.C. & Bååth, E. (2009) Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralisation. *Applied and Environmental Microbiology*, **75**, 1589–1596.
- Rousk, J., Hill, P.W. & Jones, D.L. (2014) Using the concentration-dependence of respiration arising from glucose addition to estimate *in situ* concentrations of labile carbon in grassland soil. *Soil Biology and Biochemistry*, **77**, 81–88.
- Rousk, J., Brookes, P.C., Glanville, H.C. & Jones, D.L. (2011) Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. *Applied and Environmental Microbiology*, **77**, 2791–2795.
- Ruzicka, S., Edgerton, D., Norman, M. & Hill, T. (2000) The utility of ergosterol as a bioindicator for fungi in temperate soils. *Soil Biology and Biochemistry*, **32**, 989–1005.
- Söderberg, K.H. & Bååth, E. (1998) Bacterial activity along a young barley root measured by the thymidine and leucine incorporation techniques. *Soil Biology & Biochemistry*, **30**, 1259–1268.
- Söderberg, K.H. & Bååth, E. (2004) The influence of nitrogen fertilisation on bacterial activity in the rhizosphere of barley. *Soil Biology & Biochemistry*, **36**, 195–198.

- Schimel, D.S. (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology*, **1**, 77–91.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A. *et al.* (2011) Persistence of soil organic matter as ecosystem property. *Nature*, **478**, 49–56.
- Sinsabaugh, R.L. (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry*, **42**, 391–404.
- Sinsabaugh, R.L. & Follstad Shah, J.J. (2012) Ecoenzymatic stoichiometry and ecological theory. *Annual Review of Ecology, Evolution and Systematics*, **43**, 313–343.
- Sullivan, B.W. & Hart, S.C. (2013) Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient. *Soil Biology and Biochemistry*, **58**, 293–301.
- Tuomela, M., Hatakka, A., Karjomaa, S. & Itavaara, M. (2002) Priming effect as determined by adding ¹⁴C-glucose to modified controlled composting tests. *Biodegradation*, **13**, 131–140.
- Waksman, S.A. & Gerretsen, F.C. (1931) Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. *Ecology*, **12**, 33–60.
- Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T. *et al.* (2006) Maintenance of soil functioning following erosion of microbial diversity by extractable lipid phosphate. *Environmental Microbiology*, **8**, 2162–2169.
- Xu, X., Schimel, J.P., Thornton, P.E., Song, X., Yuan, F. & Goswami, S. (2014) Substrate and environmental controls on microbial assimilation of soil organic carbon: a framework for Earth system models. *Ecology Letters*, **17**, 547–555.

Received 3 July 2014; accepted 11 November 2014

Handling Editor: Carly Stevens