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Microbial response to the addition of glucose in low-fertility soils

F. C. Hoyle · D. V. Murphy · P. C. Brookes

Abstract Addition of soluble organic substrates to soil has been shown to either increase or restrict the rate of microbial CO_2–C evolution. This has been attributed to a priming effect resulting from accelerated or decreased turnover of the soil organic matter including the soil microflora. We investigated microbial responses to small glucose-C additions (10–50 μg C g^1 soil) in arable soils either amended or not with cellulose. An immediate CO_2–C release between 0 and 69 h (equivalent to 59% of glucose-C applied) was measured. However, only half of the CO_2–C respired could be attributed to the utilisation of glucose-C substrate, based on the percentage of ^{14}C–CO_2 evolved after the addition of a ^{14}C-labelled glucose tracer. Thus, although no evidence of an immediate release of 'extra' C above the rate applied as glucose-C was observed, the pattern of decomposition for ^{14}C-glucose suggested utilisation of an alternate C source. Based on this, a positive priming effect (1.5 to 4.3 times the amount CO_2–C evolved that was attributed to glucose-C

decomposition) was observed for at least 170 h in non-cellulose-amended soil and 612 h in cellulose-amended soil. Two further phases of microbial activity in cellulose-amended soils were attributed to either activation of different microbial populations or end-product inhibition of cellulase activity after glucose addition. During these subsequent phases, a negative priming effect of between 0.1 and 1.5 times was observed. Findings indicate that the response of the microbial community to small additions of soluble organic C substrate is not consistent and support the premise that microbial response varies in a yet to be predicted manner between soil type and ecosystems. We hypothesise that this is due to differences in the microbial community structure activated by the addition of organic C and the timing of soluble organic substrate addition with respect to the current dissolved organic C status of the soil.

Keywords Microbial respiration · Carbon · Cellulose · Trigger molecule theory

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Introduction

Although a significant quantity of organic matter is usually present in soil, the majority does not exist in a form that is readily available for microbial decomposition, nor does it provide sufficient maintenance energy for the soil microflora (Joergensen et al. 1990). In Western Australia, for example, rain-fed grain production systems are often typified by 7 months of active crop growth (average growing season rainfall 200–400 mm) and 5-month summer fallow (0–100 mm rainfall). Thus, annual organic matter inputs are typically small due to relatively low production levels (e.g. average wheat production 2.0 t ha^1) and are composed largely of animal and plant residues and

root exudates. Where the majority of the above-ground crop is removed, the readily available C inputs will be from root turnover and exudates. Organic-C uptake by the soil microbial community will therefore be primarily as low-molecular weight compounds including mono- and disaccharides (e.g. glucose, sucrose), amino acids and peptides.

Although previous studies have followed the fate of these compounds applied to soil, application rates have usually been high to ensure maximal CO_2 evolution without substrate limitations. The increase in CO_2 evolution resulting from large (1,000 or 5,000 µg glucose-C g^1 soil) additions of glucose to soil has been previously linked to either an accelerated turnover of the C in the microflora (Dalenberg and Jager 1989; Wu et al. 1993) or an accelerated mineralisation of non-living soil organic matter (Shen and Bartha 1996). However, with small additions (5–34 µg C g^1 soil) of soluble C substrates to soil, which better reflects the likely levels found in soil (Jones et al. 2004), previous studies have reported this to cause more CO_2–C to be evolved than was contained in the original substrate (De Nobili et al. 2001; Mondini et al. 2006). De Nobili et al. (2001) proposed that the additional CO_2–C evolution may result from an evolutionary strategy used by soil microorganisms to become 'metabolically alert' in response to trace amounts of substrate in soil solution, which indicate the front of a diffusion gradient for a forthcoming 'food event'. Thus, the food event would initially be detected as 'trigger molecules' diffusing through the soil solution to the waiting microorganisms. The microorganisms, by investing more energy than was contained in the original substrate (derived from endocellular reserves), are able to maintain a metabolically active state. The active microorganisms may therefore gain a benefit with respect to limited resource condition, compared to a survival strategy based on spore formation. This supports previous studies in which the soil microflora maintained an adenosine 5′-triphosphate (ATP) concentration and adenylate energy charge (AEC) ratio comparable to microorganisms growing exponentially in vitro (Brookes et al. 1983).

Although microbial assimilation of soluble C substrates such as glucose can be rapid, even under C-limited conditions (Bremer and Kuikman 1994; Jones and Murphy 2007), the proportion of substrate mineralised by microorganisms may be low due to 'metabolic arrest' (Morita 1988). For example, the availability of root exudates and/or readily decomposing compounds from plant residues is associated with greater mineralisation of added C substrates in non-C-limited soils (Swift et al. 1979), whereas under C-limited conditions, increased storage by microorganisms is observed (Nguyen and Guckert 2001). Thus, the utilisation efficiency of low-molecular-weight soluble C substrates such as glucose under C-limited conditions is

likely to differ from soil to soil (Bremer and Kuikman 1994; Bremer and van Kessel 1990; Nguyen and Guckert 2001). The aim of this study was therefore to determine whether a 'trigger molecule' response was apparent in low-fertility coarse-textured soils from Western Australia. Cellulose-treated soil was also studied to determine whether this response was altered in soils with a larger available pool of C.

Materials and methods

Soil description

Composite samples (0–5 cm) of three arable soils typically cropped under an annual spring wheat/legume rotation, were collected during the dry season (i.e. absence of growing plant), sieved (<2 mm) and stored for 21 days at 4°C. Soil 1 was a sand (5.9% gravimetric field moisture water content, 3.54% clay, pH$_{(CaCl2)}$ 5.54, 0.83% total C). Soil 2 was a sandy loam (5.8% gravimetric field moisture water content, 12.63% clay, pH 6.1, 1.45% total C). Soil 3 was a sandy loam (6.4% gravimetric field moisture water content, 11.57% clay, pH 5.1, 1.74% total C).

Experiment 1: $^{12}CO_2$

Soils were adjusted to 50% water-holding capacity (WHC), sub-sampled (25 g dry weight equivalent) and conditioned in air-tight 523-ml glass containers at 25°C for 4 days to overcome any pretreatment effects. Lids modified with gas septum ports were used to seal the containers. Soils were then either amended, or not, with 1 mg cellulose-C g^1 soil added as a finely ground dried powder and mixed thoroughly into soil to determine whether the response to glucose was more sustained in the presence of cellulose, and equilibrated at 25°C for a further 24 h at 100% relative humidity to reduce the effect of soil disturbance.

Organic-C solutions were prepared by adding C (glucose), N and P at a ratio of 10:1:1 as described by De Nobili et al. (2001) to each of three treatment replicates. The N and P were added to ensure that these nutrients did not limit microbial processes. The glucose-C was added to provide either 30 or 50 µg C g^1 soil in 1 ml aliquots. Appropriate control solutions containing inorganic N [applied as $(NH_4)_2SO_4$] and P (applied as KH_2PO_4), but no glucose-C were also applied to soil. On day 1, soils received a single application (1 ml applied as multiple droplets and mixed thoroughly through soil by hand using a sterile 2-mm metal probe) of either the glucose-C solution (at a rate of 30 or 50 µg C g^1 soil), or the control solution. Multiple application treatments were also applied to separate soil samples using the same total

amount and volume of glucose-C but in smaller aliquots (10 μg C g^1) on days 1, 7 and 16 (i.e. total of 30 μg g^{-1} soil) and days 1, 4, 7, 9 and 16 (i.e. total of 50 μg g^1 soil). All treatments were mixed at days 1, 4, 7, 9 and 16 to ensure breakdown of soil aggregates did not unduly influence soil organic matter breakdown between treatments. The final soil WHC in all treatments was 63%.

After the application of glucose-C or control solutions, soils were incubated at 25$\pm$2°C and soil CO_2–C evolution measured for 536 h using an infrared gas analyser (IRGA) at intervals ranging from 4–72 h (depending on the rate of buildup in the headspace), by extracting 1 ml of gas with a syringe after first homogenising the headspace gas. Treatments were analysed against different volumes of a CO_2 standard (4.95$\pm$0.10% CO_2 in helium, BOC). After each sampling, all containers were opened, and the headspace gas exchanged with fresh air. The effective volume of gas space in the container was adjusted on the basis of soil water content, CO_2 solubility at 25°C and soil volume. The CO_2 results are reported as either the average rate of CO_2–C evolved over a 24 h period, or as the difference in cumulative CO_2–C evolved between soils treated with glucose-C solution and control solutions either not amended, or amended with cellulose.

Experiment 2: $^{14}CO_2$

The most responsive soil from the previous experiment (Soil 3: sandy loam) was used to demonstrate the relative contribution of glucose-C to total soil CO_2–C evolution using uniformly ^{14}C-labelled D-glucose (specific activity 7,852 MBq mmol^1). Soil was collected field moist from a planted system and conditioned as described above. The soil was sub-sampled and weighed (25 g dry weight) into air-tight glass containers and rates of total CO_2–C and ^{14}C–CO_2 measured independently for each of three replicates. Non-amended and cellulose-amended soil samples were mixed with 1 ml of a solution (as multiple droplets) containing 30 μg of unlabelled glucose-C g^1 soil in addition to 0.01 μg ^{14}C-glucose g^1 soil to give 26 kBq ^{14}C-glucose g^1 soil (3.3 nmol glucose ml^1) and incubated for a total of 2,500 h (104 days). Appropriate control solutions (1 ml) containing inorganic N [applied as $(NH_4)_2SO_4$] and P (applied as KH_2PO_4) only were also prepared.

The experiments were performed in a controlled-temperature room kept at 25$\pm$2°C. The ^{14}C–CO_2 evolved was monitored for 2,500 h (104 days) by placing KOH traps (7.5 ml of 0.5 M KOH) inside each air-tight container. Traps were replaced at regular intervals (days 2, 4, 7, 14, 25, 36, 46 and 71) and stored in a CO_2-free environment until analysed. Headspace ^{14}C–CO_2 accumulation from a 1 ml aliquot of the KOH trapping solution and the activity of the initial ^{14}C-glucose solutions were measured by liquid

scintillation counting in 10 ml of 'Starscint' scintillation cocktail. Total CO_2–C evolution (μg CO_2–C g^1 soil day^1) was measured by IRGA as described above for a total of 2,326 h (approximately 97 days) and reported both as an average daily (24 h) rate of CO_2–C evolved and as the difference in cumulative CO_2–C evolved from soils treated with glucose-C and controls either not amended, or amended with cellulose. The ^{14}C activity is reported as the average number of disintegrations per minute (DPM) for each soil sample (25 g dry weight soil) in cellulose-amended or non-amended soil, and the cumulative activity of ^{14}C–CO_2 evolved reported as a percentage of total activity of the ^{14}C applied. The priming effect (P) resulting from the addition of glucose-C was calculated as follows: P=(unlabelled CO_2–C+^{14}C–CO_2 evolved from glucose amended soil) (unlabelled CO_2–C evolved from soil without added glucose).

Microbial biomass determination

Microbial biomass-C was determined by fumigation extraction (Wu et al. 1990) before addition of treatments. Non-fumigated and CHCl$_3$-fumigated soil (10 g dry weight soil) were extracted with 40 ml 0.5 M K_2SO_4 and extracts filtered (Whatman No. 42). Microbial biomass-C was analysed by measuring total oxidisable C (Shimadzu Model 5050) and adjusted by a factor of 2.22 (Wu et al. 1990). Fumigated and non-fumigated ^{14}C-labelled soil extracts were also analysed by LSC to determine K_2SO_4 extractable ^{14}C and microbial biomass-^{14}C at the completion of the experiment.

Statistical analyses

General analysis of variance (ANOVA) and repeat sampling ANOVA was used after testing for normal distribution to determine significant treatment effects on average daily CO_2–C evolution, cumulative CO_2–C evolution and ^{14}C activity measured in disintegrations per minute using GENSTAT 7th edition. Analytical data are given as mean$\pm$ least significant difference (LSD), or mean$\pm$standard error (n=3). Statistical tests were considered significant at the level $P\leq0.05$ unless otherwise stated.

Results

Experiment 1

Microbial biomass-C measured 71, 265 and 138 μg C g^1 in soil 1, 2 and 3, respectively, at the start of the experiment but had declined significantly (P<0.001, LSD=39) in soil 2 (173 μg C g^1 soil) and 3 (99 μg C g^1 soil) in non-cellulose-

amended soil at the end of the incubation. Microbial biomass-C was also lower in non-cellulose-amended soil 1 (75 μg C g^1 soil, $P<0.01$, LSD=22), soil 2 (152 μg C g^1 soil, $P<0.01$, LSD=12) and soil 3 (92 μg C g^1 soil, $P<0.02$, LSD=11), compared to cellulose-amended soil which measured 103, 181 and 109 μg C g^1 soil in soils 1, 2 and 3, respectively, at the end of the incubation.

Control soils evolved on average 5, 14 and 8 μg C g^1 soil day^1 (measured as CO_2–C) in soils 1, 2 and 3, respectively, whilst the rate of CO_2–C evolved from cellulose-amended soils was between three- to fourfold greater (16, 41 and 31 μg C g^1 soil day^1 in soils 1, 2 and 3, respectively). In all soil treatments, the cumulative CO_2–C evolved was signif-

icantly greater ($P\le0.05$) after the addition of a single pulse of glucose-C compared to the control (data not shown).

The cumulative difference in CO_2–C evolved between glucose-treated and control soils in non-cellulose-amended soil was the equivalent of between 21–51% of the glucose-C applied in Soil 1 (Fig. 1a), 60–71% in Soil 2 (Fig. 1b) and 77–128% in Soil 3 (Fig. 1c) after 536-h incubation. With the exception of one treatment (Soil 1, 50 μg C g^1 soil; Fig. 1a), multiple applications of glucose-C solution (either three or five applications of 10 μg C g^1 soil) generally caused between 6 and 30 μg CO_2–C g^1 soil less CO_2–C to be evolved compared to the equivalent single application of glucose-C (Fig. 1a,b,c).

Fig. 1 Cumulative CO_2–C (treatment minus control) evolved during incubation of non-cellulose-amended **a** Soil 1, **b** Soil 2 and **c** Soil 3 and cellulose-amended **d** Soil 1, **e** Soil 2 and **f** Soil 3 after a single addition of glucose-C solution at 30 μg C g^1 soil (*filled circle*) and 50 μg C g^1 soil (*filled diamond*), or after three applications of 10 μg C g^1 soil (*open circle*) at 24, 168, 384 h and five applications of 10 μg C g^1 soil (*open diamond*) at 24, 96, 168, 216, 384 h. *Capped bars* representing standard errors ($n=3$) are plotted for each sampling point, and may be smaller than symbols
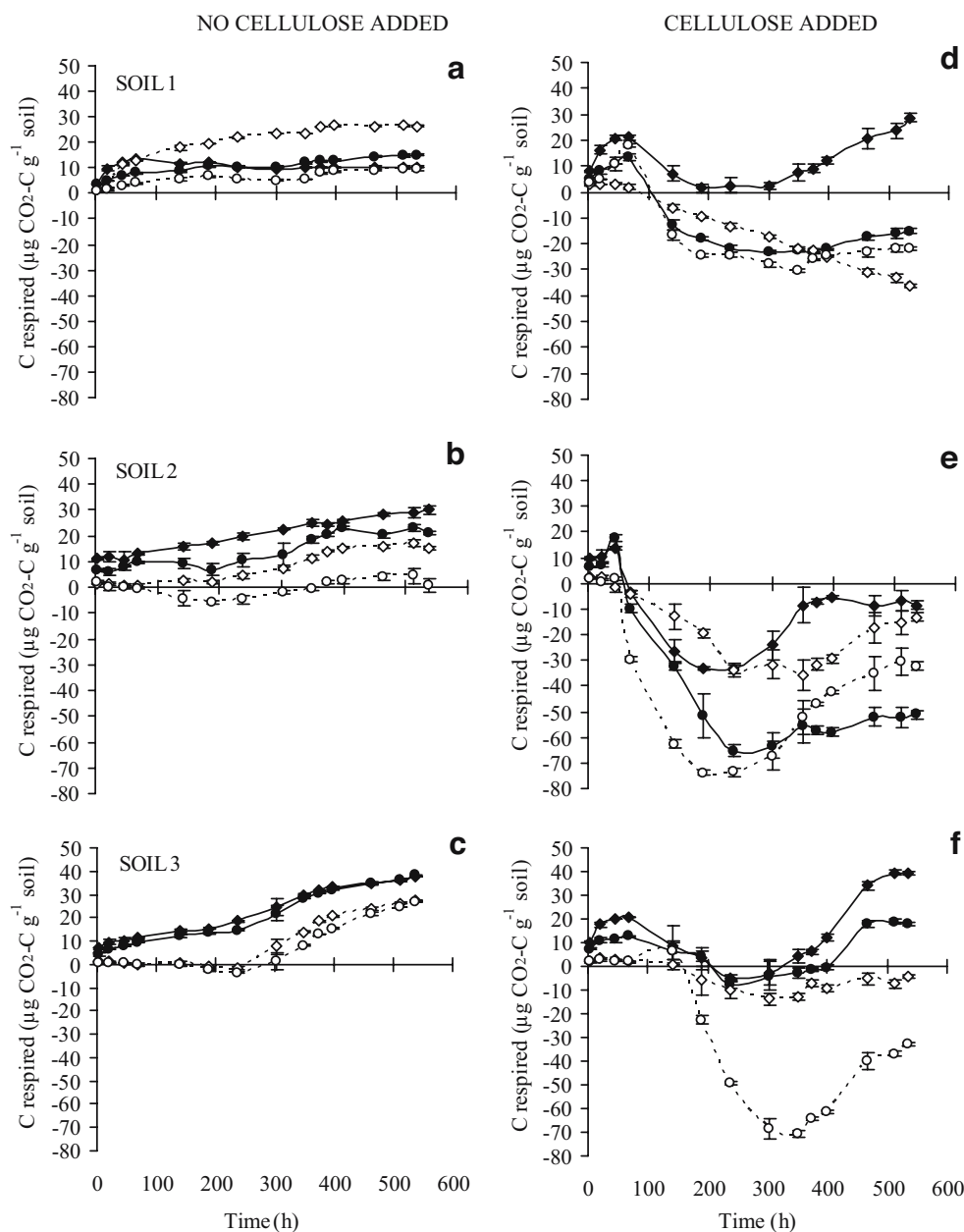
The rate of CO_2–C evolution in cellulose amended soils receiving a single addition of glucose also increased for up to 69 h after application (Fig. 1d,e,f). During this time, the equivalent of between 42–44% of the glucose-C applied in Soil 1 (Fig. 1d), 27–59% in Soil 2 (Fig. 1e) and 42–43% in Soil 3 (Fig. 1f) was evolved as CO_2–C. After this time, the rate of CO_2–C evolution in cellulose-amended soils receiving glucose then declined resulting in a negative net cumulative difference between treatments and control solutions for up to 536 h (Fig. 1d,e,f). A third, highly variable phase of CO_2–C accumulation was observed after 300 h with the rate of CO_2–C evolved either increasing or remaining close to that of the control treatment.

Experiment 2

The average daily rate of ^{14}C–CO_2 production from soil amended with 26 kBq ^{14}C-glucose g^1 soil (Fig. 2) showed a peak of activity within 43 h of application equivalent to 18% of the original ^{14}C-glucose addition and was consistent with the non-labelled glucose-C responses observed in Experiment 1. However, the response to application of ^{14}C-glucose was brief and within 170 h the average daily rate of activity had declined rapidly (Fig. 2). Another much smaller increase in ^{14}C–CO_2 evolution was observed between 170 and 331 h, after which ^{14}C activity gradually declined (Fig. 2). A total of approximately 59% of the applied ^{14}C-glucose was evolved as $^{14}CO_2$–C by 2,500 h
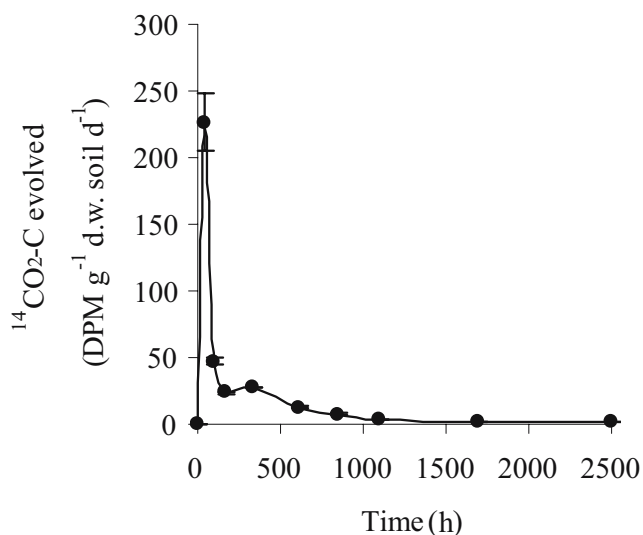
Fig. 2 Specific activity (activity day^1) measured in the number of DPM of ^{14}C–CO_2 in Soil 3 evolved after addition of 0.01 μg ^{14}C-glucose g^1 soil. Data is the average of cellulose- and non-cellulose-amended treatments. *Capped bars* representing LSD$_{DPM}$ ($P=0.05$) are plotted for each sampling point and may be smaller than symbols

(data not presented). Data is presented as the average DPM for non-cellulose- and cellulose-amended soils, as the pattern and rate of ^{14}C–CO_2 evolution was not significantly different ($P=0.05$) between these treatments.

Microbial biomass-C determined before incubation (121 μg g^1 soil, $P<0.05$, LSD=36) declined in non-cellulose-amended soil and measured 59 μg g^1 soil at the end of the incubation, significantly less than in soil amended with cellulose (121 μg g^1 soil, $P\leq0.05$, LSD=24). No measurable difference ($P=0.05$) in microbial biomass-C was observed between control and glucose-C treatments. This is reflected in low recovery (mean of 2.7%) of ^{14}C-glucose within the microbial biomass-C after incubation for 2,500 h. The remainder of the ^{14}C-glucose (38%) was assumed to have been retained within the soil organic matter pool of which 3.1% was recovered in 0.5 M K$_2$SO$_4$.

The rate of CO_2–C evolved (unlabelled) increased in non-cellulose-amended soil after amendment with glucose-C (within 4 h; Fig. 3a) and resulted in a cumulative difference in CO_2–C evolved of approximately 18% in glucose-C-treated soil (30 μg C g^1 soil) compared to the control (0 μg C g^1 soil) treatments (Fig. 3b). After 48 h the equivalent of 37% of the applied glucose-C had been respired, and the rate of CO_2–C evolution declined (Fig. 3a). After 7 days (185 h), the equivalent of approximately 50% of the added glucose-C had been respired. The cumulative difference in CO_2–C between 185 and 535 h then declined, until it reached a similar rate of CO_2–C evolution to the control soil, after which less CO_2–C was evolved from the glucose-treated soil than the control (Fig. 3b).

The response of cellulose-amended soils during the first 66 h after amendment with glucose-C (Fig. 3c) was similar to non-cellulose-amended soil (Fig. 3a). However, a second phase of rapid CO_2–C evolution in cellulose-amended soil was observed between 66 and 230 h in both glucose-amended and control soils (Fig. 3c). The resulting difference in cumulative CO_2–C evolved between glucose-treated and control soils (Fig. 3d) was equivalent to two times the amount of glucose-C added (30 μg C g^1 soil), but less than the total amount of C added as glucose and cellulose (1,030 μg C g^1 soil). In both non-cellulose- and cellulose-amended soil, the relative difference measured in cumulative CO_2–C evolved in glucose-C treatments (Fig. 3b,d) was less than 5% of the total basal CO_2–C evolved from soil organic matter decomposition during this experiment.

Although the cumulative amount of CO_2–C evolved was generally less than the total amount of glucose-C applied, a higher percentage (1.5 to 1.8 times) of CO_2–C was recovered within 43 h of glucose addition (30 μg C g^1 soil) than expected based on the percentage of microbial decomposition of ^{14}C-labelled glucose (Table 1). This positive priming effect continued for 170 h in non-cellulose-amended

Fig. 3 CO_2–C evolved in non-cellulose-amended soil shown as the **a** rate of CO_2–C evolved (μg CO_2–C g^1 soil day^1) for 0 (*open square*) and 30 μg glucose-C g^1 soil (*filled square*) and **b** the cumulative difference (*filled triangle*) between non-glucose- and glucose-amended treatments; and CO_2–C evolved in cellulose-amended soil shown as the **c** rate of CO_2–C evolved (μg CO_2–C g^1 soil day^1) for 0 (*open square*) and 30 μg glucose-C g^1 soil (*filled square*) treatments and **d** the cumulative difference (*filled triangle*) between non-glucose- and glucose-amended treatments at each sampling interval. *Capped bars* represent LSD (*P*=0.05)
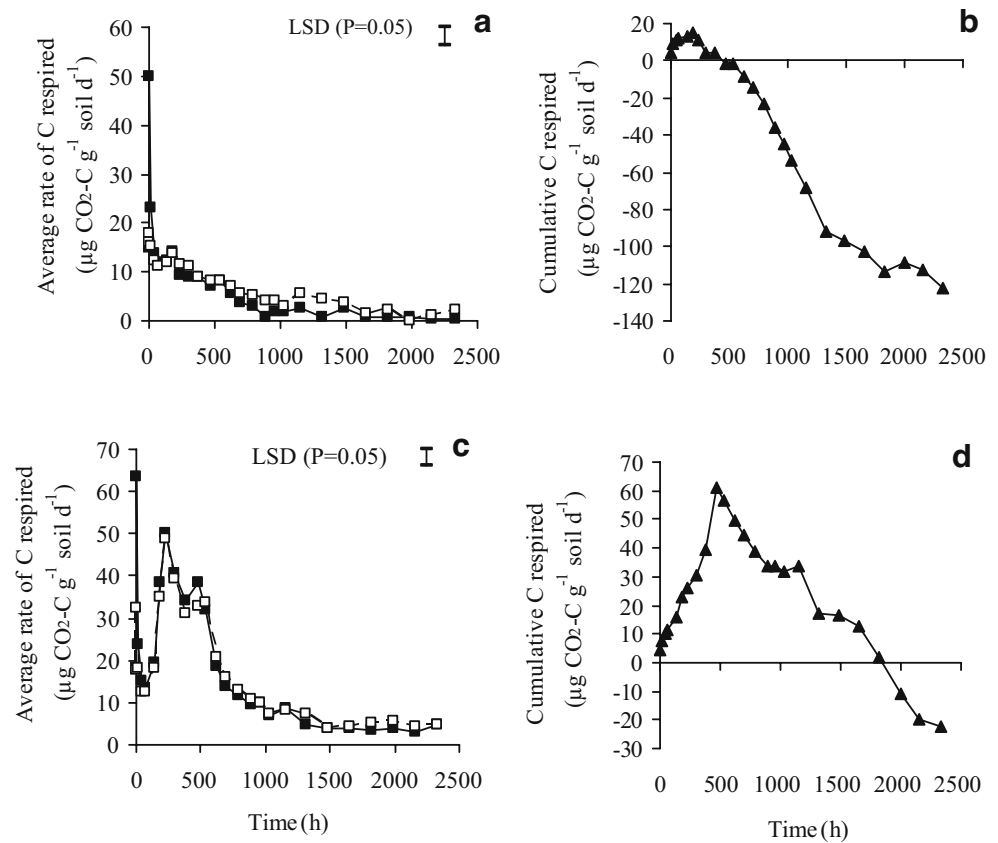
and 612 h in cellulose-amended soil. A negative priming response was then observed (0.1 to 1.5 times), which resulted in less CO_2–C than expected (Table 1). At the end of the incubation (1,692 h), the priming effects between the control soil and glucose-C-treated soil were negative in non-cellulose-amended, and only 30% of the expected respiration in cellulose-amended soil, indicating that the positive priming effect was associated with the initial experimental phase.

Table 1 Priming effect (*P*; μg glucose-C g^1 soil) resulting from the application of 30 μg C g^1 soil as glucose at eight sampling times in cellulose- and non-cellulose-amended soil

	Time (h)	Priming effect (*P*; μg C g^1 soil)	Evolution of ^{14}C–CO_2 (%)	Expected (*E*) amount of CO_2–C evolved (30 μg glucose-C × % ^{14}C–CO_2 evolved; μg C g^1 soil)	Additional CO_2–C (*PE*; μg C g^1 soil)	Ratio ((*P-E*)/*E*)
Non-cellulose amended	43	15.3	18.3	5.5	9.8	1.8
	97	18.2	22.1	6.6	11.6	1.7
	170	23.6	24.7	7.4	16.2	2.2
	331	7.4	32.6	9.8	2.4	0.2
	612	2.9	40.4	12.1	15.0	1.2
	852	6.8	44.9	13.5	20.2	1.5
	1,092	1.6	46.9	14.1	15.7	1.1
	1,692	6.7	51.0	15.3	22.0	1.4
Cellulose amended	43	14.2	19.1	5.7	8.5	1.5
	97	20.3	22.6	6.8	13.5	2.0
	170	34.6	24.8	7.4	27.2	3.7
	331	50.5	31.8	9.5	41.0	4.3
	612	53.4	39.3	11.8	41.6	3.5
	852	8.5	44.5	13.4	4.9	0.4
	1,092	12.6	47.2	14.2	1.6	0.1
	1,692	20.3	51.4	15.4	4.9	0.3

P=(unlabelled CO_2–C+labelled ^{14}C–CO_2 evolved from glucose amended soil) (unlabelled CO_2 evolved from soil without added glucose)

Discussion

The addition of a small amount of ^{14}C-glucose to soil (0.01 μg ^{14}C-glucose g^1 soil+30 μg glucose-C g^1 soil) caused a rapid peak of ^{14}C–CO_2 respiratory activity during the first 48 h in both cellulose- and non-cellulose-amended soil treatments as previously observed by Nguyen and Guckert (2001) within 15 min of ^{14}C-glucose application to soil. In our study, 21% of added ^{14}C-glucose was evolved as $^{14}CO_2$–C within 3 days. This is comparable to other studies, in which between 23 and 42% of glucose was evolved as CO_2–C within 3 days (Bremer and van Kessel 1990; Bremer and Kuikman 1994; Nguyen and Guckert 2001). In total, 50 to 66% (average 59%) of ^{14}C-glucose was evolved as ^{14}C–CO_2 during the incubation. This is typical of microbial decomposition of labile organic-C substrates, which usually results in 40–60% of the C being mineralised (Van Veen et al. 1985; Shen and Bartha 1996).

Because glucose is uncharged, it is not sorbed on to soil particles (Darrah 1991; Jones and Edwards 1998), and thus, the component of the ^{14}C-glucose (38%) not evolved as ^{14}C–CO_2 should either be immobilised into the soil microflora, be present in the soil as microbial metabolites or incorporated into the humified soil organic matter pool. In this study, a small percentage (3%) of ^{14}C-glucose was recovered after 2,300 h from soil solution, supporting previous studies which determined a low recovery (<1%) of ^{14}C-glucose from soil solution due to rapid microbial assimilation of glucose (Bremer and van Kessel 1990; Bremer and Kuikman 1994; Nguyen and Guckert 2001). Less than 3% of the ^{14}C-glucose was immobilised in the soil microflora, and thus, the remaining 35% of added glucose (after accounting for CO_2–C respiration) was assumed to have been retained within a non-labile (i.e. non-extractable in 0.5 M K_2SO_4) soil C fraction.

In Experiment 1, soils amended with glucose-C predominantly evolved less CO_2–C than was added in the glucose, indicating no additional release of CO_2–C from the soil microbial community during the first 374 h of incubation in non-cellulose-amended soil. This is in contrast to De Nobili et al. (2001), who measured a greater release of CO_2–C in excess of applied treatments (1.4 times the application of 11.3 μg C g^1 soil as glucose and 4.6 times the application of 11.3 μg C g^1 soil as root extract) in soil not amended with cellulose, 100–160 h after application. Although, in many cases, there was a lag phase of up to 100 h before more CO_2–C was evolved than was added, they attributed this response to endocellular utilisation of C. If the response observed by De Nobili et al. (2001) had resulted from the use of endocellular C, we might have anticipated 'extra' CO_2–C to have evolved within minutes or hours after glucose application. In contrast, Mondini et al. (2006) reported no lag phase when adding a wide range of substrates to soil including glucose, amino acids, compost extract and protein hydrolysates at concentrations ranging from 5 to 15 μg C g^1 soil.

Similarly, in cellulose-amended soils, the cumulative CO_2–C evolved (4–69 h) indicated no 'additional' immediate evolution of CO_2–C after application of glucose. Unexpectedly, in contrast to non-cellulose-amended soil, the rate of CO_2–C evolution in cellulose-amended soils receiving glucose then declined (relative to control CO_2–C evolution) indicating a decrease in microbial activity. One possible explanation is that the glucose inhibited β-glucosidase activity, the enzyme responsible for catalyzing the last reaction of cellulose degradation activity through end-product inhibition (Paul and Clark 1989), with recovery of enzyme activity linked to a decreasing concentration of glucose in soil. The response observed in cellulose-amended soil thus suggests a highly variable secondary phase of C mineralisation distinct from the initial glucose mineralisation phase. In all cases, no additional CO_2–C evolution occurred in cellulose-amended soils after addition of glucose-C.

The non-cellulose-amended soil, by comparison, lacked the secondary phase of glucose-C mineralisation observed in cellulose-amended soil suggesting a C limitation in these soils. The exception was Soil 3 where the addition of 30 μg glucose-C g^1 soil caused approximately 38 μg CO_2–C g^1 soil to be evolved after accounting for background CO_2–C (Fig. 1c). However, 'extra' CO_2–C was not evolved until after approximately 374 h, indicating a delayed response on application of glucose-C which could not be directly associated with the utilisation of endocellular C. Subsequent testing of Soil 3 (Experiment 2) also failed to obtain a similar response, and the amount of CO_2–C evolved was less than the amount applied as glucose-C. An alternative interpretation of our findings and those of De Nobili et al. (2001) suggest this delayed CO_2–C evolution is therefore more likely an indirect result of the applied glucose. For example, delayed CO_2–C evolution may result from an accelerated turnover of the soil microbial community, or the formation of a glucose-derived metabolite such as pyruvate or phosphenolpyruvate which may be more readily decomposed by a greater diversity of microorganisms.

Both De Nobili et al. (2001) and Mondini et al. (2006) found that repeated applications of small amounts of glucose-C caused more CO_2–C to be evolved, compared to a single application of the equivalent amount of glucose. This supports findings by Bremer and Kuikman (1994) where greater mineralisation of glucose-C was observed in soils where the microbial microflora had been 'activated' by a prior addition of glucose-C to soil. However, in C-limited soils, Morita (1988) suggested that although the assimilation of soluble C may be uninhibited, the stored substrate may not be immediately metabolised due to

metabolic arrest. Thus, in our study, a lower rate of assimilation and metabolism of glucose-C may be due to low C availability (Bremer and Kuikman, 1994) common in soils with low clay content. This is supported by variability in the response observed in Experiment 1 and 2 on the same soil (Soil 3) which was sampled during summer in the absence of growing plants for Experiment 1, and in Experiment 2 where soil was sampled in the presence of growing plants providing a labile C substrate suited to metabolism by the soil microflora.

Hamer and Marschner (2002) concluded that soil properties had no relationship to the direction and magnitude of response in CO_2–C evolution after addition of soluble organic substrates to soil and that the response could not be predicted. Instead, it is likely that the type of organic substrates applied and diversity of the active soil microbial community will have a large influence on the resulting response. This is in agreement with the results of both De Nobili et al. (2001) and Mondini et al. (2006) showing that complex organic substrates (e.g. root extracts) result in more 'extra' CO_2–C evolution; presumably due to activation of a larger component of the soil microbial community.

Priming effects from large substrate additions (>1,000 µg C g^1 soil; Dalenberg and Jager 1989; Wu et al. 1993) usually involve a change of between 10 and 20% in evolution of unlabelled CO_2–C, whereas trigger molecules may give up to five times more (De Nobili et al. 2001). In this study, the peak in activity associated with the addition of glucose-C to soil in small amounts was of relatively short duration in both cases and does not suggest enhanced mineralisation of soil organic matter or the use of endocellular C during the initial phases (0–250 h) of incubation. However, the total amount of CO_2–C anticipated due to the application of glucose-C, as determined by the pattern of $^{14}CO_2$-C evolved, indicates an additional release of CO_2–C (between two and four times the expected CO_2–C) from the micro-organisms that was not attributable to utilisation of the applied glucose-C substrate. Longer term, the application of glucose-C in non-cellulose-amended soil actually resulted in less CO_2–C than in soils with no glucose applied. Thus, this initial response may be associated with maintaining an internal concentration gradient against acquired substrates (Button 1991), as it is unlikely there would be sufficient substrate at these application levels to sustain growth (Nguyen and Guckert 2001).

Although findings from our study with low glucose-C applications suggested additional CO_2–C was evolved that could not be directly attributed to decomposition of the glucose-C substrate, it is not possible to determine whether this CO_2–C was derived from (1) endocellular utilisation of C, (2) increased microbial turnover or (3) enhanced mineralisation of soil organic matter (and in this study,

cellulose) resulting from stimulation of the native microflora. Because microbial biomass-C was not significantly influenced by glucose-C application to soil, this suggests that any loss in microbial biomass due to the application of glucose-C was either (1) too small to be measurable or (2) that increased CO_2–C evolution was not associated with increased microbial turnover and exhaustion of endocellular C reserves after triggering with glucose. Data from De Nobili et al. (2001) also showed no significant decline in microbial biomass after multiple additions of 'trigger molecules'. By comparison, amending soil with cellulose resulted in significantly greater ($P \le 0.05$) microbial biomass-C at the end of the incubation compared to non-cellulose-amended treatments, suggesting extra CO_2–C may primarily result from microbial growth and turnover. We propose that the prolonged secondary response observed in cellulose-amended soil resulted from microbial turnover and subsequent mineralisation of cellulose (a more complex substrate) as proposed by Dalenberg and Jager (1989). If the glucose-C addition had enhanced the mineralisation of soil organic matter, this would also have occurred in the non-cellulose-amended soil. Thus, differences observed in response patterns between the soils used here and those by De Nobili et al. (2001) and Mondini et al. (2006) suggest the mechanisms resulting in activation of the soil microflora are not universal.

Conclusions

In conclusion, the trigger molecule concept proposed by De Nobili et al. (2001) was not evident in these soils, and therefore cannot be considered as a universal microbial response. We propose, however, that any delayed or prolonged response (after the first 48 h) to the application of glucose-C as seen in this study and that of De Nobili et al. (2001) is more likely attributable to:

(1) differences in basal CO_2–C evolution measured between glucose-amended and non-amended soil due to the activation of different microbial populations on addition of glucose-C
(2) the formation of secondary metabolites from microbial transformation of added glucose-C which then stimulate CO_2–C evolution via a secondary response
(3) enhanced decomposition of cellulose and/or soil organic matter on application of a readily available substrate such as glucose.

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