

Quantification of priming and CO₂ respiration sources following slurry-C incorporation into two grassland soils with different C content[†]

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The fate of incorporated slurry-C was examined in a laboratory experiment using two UK grassland soils, i.e. a Pelostagnogley (5.1 %C) and a Brown Earth (2.3 %C). C₃ and C₄ slurries were incorporated into these two wet-sieved (C₃) soils (from 4–10 cm depth). Gas samples were collected 0.2, 1, 2, 3, 4, 6, 9, 20, 30 and 40 days after slurry application and analyzed for CO₂ concentration and δ¹³C content. Slurry incorporation into the soil strongly increased soil CO₂ respiration compared with the unamended soil. Total (40 day) cumulative CO₂ flux was higher for the Pelostagnogley than the Brown Earth. The ¹³C natural abundance tracer technique enabled quantification of the sources of respired CO₂ and priming effects (days 0–9). Proportionally more slurry-derived C was respired from the Pelostagnogley (46%) than the Brown Earth (36%). The incorporated slurry-C was lost twice as fast as the native soil C in both soils. Slurry incorporation induced a priming effect, i.e. additional release of soil-derived C, most pronounced in the Pelostagnogley (highest C content). The majority of respired soil-derived C (>70%) was primed C. The study indicated that potential reductions in ammonia volatilisation following slurry injection to grasslands might be negated by enhanced loss of primed soil C (i.e. pollution swapping). Copyright © 2003 John Wiley & Sons, Ltd.

Intensive application of animal slurries to grassland results in increased greenhouse gas emissions, ammonia volatilisation, and nitrate and phosphorus leaching into surface and groundwater.^{1,2} However, slurry injection into the soil can reduce ammonia volatilisation by up to 50%. This altered way of slurry application to agricultural land can potentially also influence soil CO₂ respiration. Unfortunately, less information is available concerning sequestration and losses of C from slurries applied to agricultural soils.^{3–5} Moreover, none of these studies achieved an accurate quantification of slurry-derived C sequestered by or lost from the soil, because no distinction could be made between the 'native' soil C and 'slurry-derived' C.

The ¹³C natural abundance tracer technique exploits the natural difference in stable isotope ¹³C content between C₃ and C₄ vegetation. It has been used to quantify the origin and turnover of 'native' and 'new' soil C and its fractions after C₃/C₄ vegetation change.^{6–8} It was used to trace added (C₄)

dung-C entering 'native' soil C pools and isolated particle-size fractions in C₃ grasslands.^{9,10} Slurry generally disappears faster from the soil surface than dung, due to its liquid nature and volatilisation of many dissolved compounds.¹¹ This fact is also exemplified in our previous grassland study where no slurry was present at the land surface after 4 weeks,¹² whereas, after 10 weeks, dung was still visible.¹⁰ Indeed, 78–92% of the C in pig slurry can be readily decomposed within 4 weeks,¹³ although for cattle slurry lower mineralisation rates have been reported.¹⁴ Glaser *et al.*¹² used the ¹³C natural abundance tracer technique to quantify the fate of slurry-C from C₄-fed livestock in C₃ grassland soil. They observed that the C loss occurred in two phases. In the first 48 h after application, slurry-C from the more liquid phase percolated rapidly into the upper soil and (temporarily) accumulated there, whereas, beyond 48 h, slurry-derived C from the decomposing solid phase was incorporated into the soil. Similar two-phase models have also been proposed for the decomposition of pig slurry applied to arable soils.^{4,5} Also, gas measurements have shown that the highest losses of CO₂ (and CH₄) from slurry occur immediately after application;^{5,14,15} however, these studies did not quantify the proportion(s) of slurry-derived C remaining in the various soil pools and the fraction that is completely decomposed and lost from the soil as CO₂.

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Table 1. Properties of the field sites and slurries used in the experiment

| | Rowden Moor | | De Bathe | |
|--|--|----------------|---|-----------------------|
| Management history | Permanent pasture 40 + years | | Permanent pasture 10 years | |
| Dominant vegetation | Perennial ryegrass | | Perennial ryegrass | |
| Fertiliser application | None since 1982 | | None since 1995 | |
| Grazed | Yes, cattle | | Yes, cattle or sheep | |
| Location | North Wyke, Devon, UK | | North Wyke, Devon, UK | |
| Soil Type | | | | |
| British classification | Pelo-stagnogley (Hallsworth series) | | Typical Brown Earth (Credition series) | |
| FAO classification | Dystric Gleysol | | Eutric Cambisol | |
| USDA classification | Typic Haplaquept | | Dystric Eutrocrept | |
| Chemical properties | Soil (4–10 cm) | Soil (4–10 cm) | C ₃ slurry | C ₄ slurry |
| C (%) | 5.09 ± 0.76 | 2.33 ± 0.99 | 37.4 ± 0.6 | 46.3 ± 0.5 |
| N (%) | 0.63 ± 0.07 | 0.30 ± 0.01 | 2.50 ± 0.10 | 2.16 ± 0.12 |
| C/N | 8.0 | 7.7 | 15.0 | 21.5 |
| Original soil δ ¹³ C (‰) | −28.6 ± 0.1 | −26.9 ± 0.1 | −30.7 ± 0.1 | −21.3 ± 0.2 |
| Original soil + C ₄ slurry | −26.8 ± 0.1 | −25.5 ± 0.2 | nd | nd |
| Incubated soil + C ₄ slurry | −26.6 ± 0.3 | −25.5 ± 0.1 | nd | nd |
| pH (CaCl ₂) | 4.7 | 4.7 | nd | nd |
| pH (H ₂ O) | 5.2 | 5.2 | nd | nd |
| Field capacity | 58 | 39 | — | — |
| Soil texture | | | | |
| % sand | 27 | 55 | — | — |
| % silt | 39 | 29 | — | — |
| % clay | 34 | 16 | — | — |

nd = not determined; — = not applicable.

The addition of readily decomposable slurry-C to the soil may increase the activity of soil microorganisms and can result in the increased decomposition of soil organic matter, frequently called the positive priming effect.^{16,17} Priming has also been observed with plant residues, rhizodeposits, mineral fertilisers, low molecular organic substances (e.g. glucose) and other additives as additional substrates.^{18–24} When positive priming occurs, the addition of slurry to soils may not necessarily result in a net C sequestration. This depends on the ratio between the degree of slurry utilisation and the degree of associated microbial biomass activation for soil C decomposition. The intensity of biomass activation by substance addition depends on the amount of easily decomposable C and available N in the soil.¹⁷ Earlier studies indicated that management type and history²⁵ and particle size (sand/silt/clay) composition²⁶ did modify the amount of labile C and N in grassland soils. Hence, in our experiment, where the two investigated soils had different total C content, total N content, soil texture and management history (Table 1), we also expected a difference in biomass activation to occur, which could result in a different soil organic matter (SOM) mineralisation and CO₂ efflux dynamics.

In the present study we examined whether the δ¹³C natural abundance tracer technique could quantify the short-term release of slurry-derived C in respired CO₂. Particular attention was given to any differential effect of the applied slurry-C on the priming action of native-C in both grassland soils.

EXPERIMENTAL

Field site details

Soil material was collected from two grassland sites, with differences in management history, soil type, chemical properties and soil texture. The two sites, Rowden Moor and De Bathe, are both located near North Wyke, Devon, southwest England (50°45' N, 4°53' W; grid reference SS675012). Mean annual temperature at both sites is 10.5°C and mean annual precipitation is 1035 mm, of which two-thirds falls between October and March.²⁷ The details of the sites and their soils^{27–30} are given in Table 1.

Soil sampling and preparation

At each site, three subsites were randomly selected for soil sampling, which took place on January 19, 2001. For sampling we isolated intact soil cores (20 × 25 cm²) from the topsoil (0–10 cm) which were stored at 5°C. Although the weather was sunny, the Pelostagnogley soil was still especially wet due to heavy rain in days before sampling. After removal of vegetation and bigger roots the soil from the 4–10 cm depth was wet sieved (<7 mm). The upper 0–4 cm was not used in this experiment as it contained too many larger roots. For the experiment we therefore used only material from the 4–10 cm depth interval. We weighed 118 g of the Pelostagnogley soil (C content = 5.09%; Table 1) and 257 g of the Brown Earth (C content = 2.33%; Table 1) into Kilner jars. This yielded a similar amount of soil C (i.e. 6 g C or equivalent to 6000 kg C ha^{−1}), the amount required for a

true comparison of C respiration dynamics between the two soil types in response to the slurry addition. The soils were then stored again at 5°C until further use.

Slurry preparation and application

Cows were fed with either ryegrass (*Lolium perenne*, a C₃ plant) or maize (*Zea mays*, a C₄ plant) silage. Two types of slurry were produced in this manner, i.e. a maize-derived C₄ slurry ($\delta^{13}\text{C} = -21.3\text{‰}$) and a ryegrass-derived C₃ slurry ($\delta^{13}\text{C} = -30.7\text{‰}$). The difference in $\delta^{13}\text{C}$ signatures between the C₄ and the C₃ slurries ($\Delta\text{SIC}_4 - \text{SIC}_3 = 9.4\text{‰}$) was less than the maximum shift of ca 14‰,⁶ probably resulting from C₃-concentrated feed which entered into the C₄ slurry. The initial C₄ and C₃ slurries contained 7.3 and 4.6% dry matter (DM). However, before application, the C₄ slurry was diluted with water to obtain a slurry with a 4.6% DM content, leading to similar DM contents of the C₃ and C₄ slurries. A slurry equivalent of 2 g C was applied to a soil equivalent of 6 g C and stirred to incorporate the slurry. The amount of slurry applied to each plot was 91 m³ ha⁻¹ (or 2000 kg C ha⁻¹). This value lies at the top of allowed/recommended application quantity for UK grassland systems, to maximise our tracer capacity. The mixture was filled in Kilner jars (ca. 10 × 10 × 15 cm = 1500 mL) for incubation. The control soils did not receive any slurry, but water was added equal to the amount present in the slurries. The samples were incubated at 27°C and the water content was adjusted every 3–5 days to maintain moisture content in the soil at 70% field capacity.

Slurry and soil analysis

The moisture contents of the soils and slurries were determined by weighing before and after drying in an oven at 85°C. Soil and slurry samples were then ground in a pestle and mortar to pass through a 600-µm sieve. Total C and N contents of soil and slurry samples were determined using a CHN auto-analyzer (Carlo Erba NA2000, Milan, Italy). The $\delta^{13}\text{C}$ values of soil and slurry samples were analysed at IGER North Wyke using a continuous flow ANCA 20/20 SL system (Europa, Crewe, UK). Natural abundances of ¹³C were expressed as $\delta^{13}\text{C}$ values which represents the ratios of ¹³C/¹²C relative to the VPDB standard. The $\delta^{13}\text{C}$ values are defined as:

$$\left[\frac{(\text{atom}\%^{13}\text{C}_{\text{sample}} - \text{atom}\%^{13}\text{C}_{\text{VPDB}})}{\text{atom}\%^{13}\text{C}_{\text{VPDB}}} \right] \times 1000.$$

The analytical precision of the $\delta^{13}\text{C}$ measurements was 0.1‰.

C respiration and statistical analysis

Periodically (on days 0.2, 1, 2, 3, 4, 6, 9, 13, 20, 30, 40 after slurry application) the Kilner jars were sealed with a greased rubber ring and a lid with a septum for a needle. 5, 15 and 45 min after closing the Kilner jar, air samples (12 mL) were taken from the headspace using evacuated exetainers. The collected samples in the exetainers were then analysed for their CO₂ concentration and $\delta^{13}\text{C}$ value by an independent analytical company (Iso-Analytical, Crewe, UK).

The resulting CO₂ concentration and isotopic content were corrected for a small amount of ambient air (set at 360 ppm

and $\delta^{13}\text{C}$ of -8‰) present in the exetainer samples. The $\delta^{13}\text{C}$ of the respired CO₂ was then calculated using the measured linear increase in CO₂ concentration and associated change in isotopic content from the gas samples taken after 5, 15 and 45 min, and expressed on a mg C kg soil⁻¹ day⁻¹ basis. This also corrects the C respiration data for the different amounts of two soils weighed into the Kilner jars (as discussed in the section 'Soil sampling and preparation').

The difference in $\delta^{13}\text{C}$ values between the respired CO₂ from the C₄ and C₃ slurry treatment (denoted as $\Delta\text{C}_4 - \text{C}_3$ in Table 2) was used to quantify the proportions of slurry- versus soil-derived CO₂-C emitted from the soil. This calculation was performed as follows: %slurry-derived C in respired CO₂ = $(\Delta\text{C}_4 - \text{C}_3) / (\Delta\text{SIC}_4 - \text{SIC}_3)$, where $\Delta\text{SIC}_4 - \text{SIC}_3$ denotes the difference in $\delta^{13}\text{C}$ values between both slurry types incorporated into the soil. The method assumes that any occurring fractionation in ¹³C between slurry C and the associated C respired from that slurry is similar for the C₃ and C₄ slurries. This underlying assumption forms the basis in all soil studies using the ¹³C natural abundance tracer technique.^{6,7}

This simple method of estimating the contribution of % slurry-derived C in respired CO₂ is only truly valid when the daily respiration rates from the C₃ and C₄ slurries are identical. Hence, for the samples taken after 20, 30 and 40 days, when the C respiration rates between the C₄ and C₃ treatments differed significantly, the estimates given are only tentative. Hence, the amount of primed C and the priming factor were not calculated for samples from days 20–40. Similarly, the cumulative respired CO₂ (see below) was only calculated for the first 9 days of the experiment.

The priming factor was calculated as follows: amount of CO₂ respired from the native soil C in the C₄ slurry treatment/amount CO₂ respired from the control. Therefore, a value >1 indicates positive priming, 1 indicates the absence of priming, and <1 negative priming of soil-respired CO₂ by the application of slurry (Table 2).

The cumulative respired CO₂ for the control, C₃-slurry, C₄-slurry, slurry-derived, SOM-derived and primed C were calculated by: (1) summing up all daily respired CO₂ for the first 9 days of the experiment, and (2) assuming that, where no daily samples were available, the amount of daily respired CO₂ changed linearly between the nearest sampling dates for which samples were available.

We also calculated % C loss from the various soil C sources as follows: % C loss = $(\text{C}_{\text{cum}} / \text{A}_\text{C}) \times 100$, where C_{cum} denotes the cumulative C respiration data (expressed in mg C kg⁻¹ soil day⁻¹) and A_C the actual C present in 1 kg soil for each source of C in the soil. The A_C values were for the Pelostagnogley 67.8 g (soil + incorporated slurry), of which 16.9 g was incorporated slurry and 50.9 g was native soil C. The value for the soil + incorporated slurry in the Brown Earth was 31.1 g, of which 7.8 g was incorporated slurry and 23.4 g was native soil C. The A_C value for the primed soil pool was assumed to be the same as the native soil C value for each soil type.

The statistical differences between experimental treatments over time were examined using two-way analysis of variance (ANOVA). Differences between the two soil types were analysed using one-way ANOVA.

Table 2. Sources and amount of CO₂ respired from the two topsoils (4–10 cm) after slurry application

| Time after slurry application (Days) | Respired CO ₂ | | | ΔC ₄ –C ₃ δ ¹³ C (‰) | Respired CO ₂ in C ₄ slurry treatment | | | Priming factor Soil derived/ control |
|--------------------------------------|---|-----------------------|-----------------------|--|---|--------------|------------|--|
| | Control | C ₃ slurry | C ₄ slurry | | Slurry-derived | Soil-derived | Primed | |
| | (mg C kg ⁻¹ soil day ⁻¹) | | | | (mg C kg ⁻¹ soil day ⁻¹) | | | |
| Rowden site (Pelostagnogley) | | | | | | | | |
| 0.2 | 12 ± 5* | 873 ± 130 | 874 ± 123 | 4.6 ± 0.7 | 427 ± 62 | 450 ± 40 | 438 ± 40 | 37.5 ± 3.4 |
| 1 | 20 ± 12 | 237 ± 18 | 257 ± 10 | 1.3 ± 0.2 | 36 ± 6 | 225 ± 6 | 204 ± 14 | 11.0 ± 0.9 |
| 2 | 41 ± 8 | 225 ± 34 | 247 ± 26 | 1.4 ± 0.3 | 37 ± 9 | 215 ± 17 | 174 ± 19 | 5.2 ± 0.5 |
| 3 | 13 ± 4 | 230 ± 6 | 196 ± 12 | 3.6 ± 0.6 | 74 ± 12 | 123 ± 14 | 110 ± 14 | 9.1 ± 1.1 |
| 4 | 12 ± 7 | 170 ± 7 | 176 ± 20 | 6.1 ± 0.8 | 113 ± 15 | 64 ± 15 | 52 ± 17 | 5.2 ± 1.5 |
| 6 | 60 ± 10 | 170 ± 19 | 187 ± 6 | 5.4 ± 0.9 | 107 ± 17 | 81 ± 16 | 21 ± 19 | 1.3 ± 0.4 |
| 9 | 51 ± 33 | 139 ± 9 | 223 ± 35 | 6.4 ± 0.7 | 152 ± 18 | 85 ± 22 | 34 ± 24 | 1.7 ± 0.8 |
| 20 | 19 ± 2 | 100 ± 24 | 174 ± 25 | 2.9 ± 0.4 | 54 ± 8 | 120 ± 23 | — | — |
| 30 | 35 ± 4 | 33 ± 7 | 110 ± 37 | 3.1 ± 0.7 | 36 ± 9 | 76 ± 16 | — | — |
| 40 | 15 ± 7 | 43 ± 7 | 93 ± 7 | 2.2 ± 0.7 | 22 ± 7 | 71 ± 3 | — | — |
| Σ0–9 | 358 ± 141 | 2523 ± 217 | 2752 ± 255 | | 1278 ± 172 | 1481 ± 247 | 1122 ± 490 | 4.1 ± 0.2 |
| De Bathe site (Brown Earth) | | | | | | | | |
| 0.2 | 31 ± 10 | 211 ± 4 | 251 ± 8 | 5.2 ± 0.3 | 139 ± 7 | 112 ± 7 | 79 ± 13 | 3.6 ± 0.4 |
| 1 | 14 ± 5 | 80 ± 1 | 112 ± 9 | 1.6 ± 0.3 | 19 ± 4 | 95 ± 5 | 81 ± 7 | 6.8 ± 0.5 |
| 2 | 12 ± 3 | 74 ± 1 | 85 ± 5 | 1.7 ± 0.3 | 15 ± 3 | 72 ± 5 | 60 ± 5 | 6.0 ± 0.5 |
| 3 | 36 ± 5 | 83 ± 13 | 89 ± 17 | 2.0 ± 0.4 | 18 ± 3 | 68 ± 3 | 32 ± 16 | 1.9 ± 0.2 |
| 4 | 18 ± 4 | 66 ± 3 | 61 ± 5 | 1.4 ± 0.3 | 9 ± 2 | 52 ± 3 | 34 ± 5 | 2.9 ± 0.3 |
| 6 | 15 ± 6 | 74 ± 1 | 86 ± 1 | 4.8 ± 1.0 | 44 ± 9 | 43 ± 11 | 28 ± 13 | 2.9 ± 0.8 |
| 9 | 9 ± 4 | 38 ± 3 | 49 ± 3 | 4.5 ± 1.1 | 23 ± 6 | 33 ± 7 | 24 ± 8 | 3.7 ± 0.9 |
| 20 | 8 ± 2 | 21 ± 8 | 67 ± 9 | 1.4 ± 0.4 | 10 ± 3 | 57 ± 12 | — | — |
| 30 | 17 ± 5 | 20 ± 5 | 60 ± 14 | 6.0 ± 0.2 | 39 ± 7 | 21 ± 4 | — | — |
| 40 | 3 ± 1 | 12 ± 1 | 24 ± 2 | 3.1 ± 0.5 | 8 ± 1 | 16 ± 1 | — | — |
| Σ0–9 | 176 ± 56 | 807 ± 35 | 943 ± 66 | | 341 ± 58 | 599 ± 84 | 426 ± 120 | 3.4 ± 0.2 |

*Mean ± Standard Error.

For the samples taken after 20, 30 and 40 days, when the C respiration rates between the C₄ and C₃ treatments differed significantly, the estimates given are only tentative.

RESULTS AND DISCUSSION

The incorporation of the slurry into the soil strongly increased ($p < 0.001$) the amount of respired C in both soil types during the 40-day experiment when compared with the unamended control (Table 2). The relative increase of soil CO₂ emission from the 4–10 cm depth samples was higher from the incubated Pelostagnogley soil (C content = 5.1%) than from the Brown Earth (C content = 2.3%). The increase in respired C was most pronounced in the first few days after the incorporation of the slurry into the soil (Table 2). The CO₂ emission generally decreased from all treatments beyond day 6 in the experiment ($p < 0.001$; Table 2). Similar trends in CO₂ fluxes have been obtained after application of slurry to agricultural soils.^{4,5,13–15,31} The results suggest a two-stage decomposition of slurry, which is common for many components added to soil as well as for substances which are readily adsorbed. For example, Rochette *et al.*⁴ proposed a two-stage decomposition process of slurry applied to land to explain CO₂ emission trends. In their specific study ca. 50% of the total CO₂ emission occurred in the first week after (C₃) slurry application. The rapid decrease in emitted CO₂ was exponential and was attributed to the decomposition of the labile fraction of the slurry C. The second phase was linear and much slower and involved decomposition of more recalcitrant C material. In our 40-day experiment, between 35 and 50% of the cumulatively released CO₂ was emitted in the first

10 days after slurry incorporation (Table 2). Significantly more C was respired from the C₄ than from the C₃ slurry treatment after 20 days in both soils ($p < 0.01$; Table 2). This limited the validity of the calculation of the quantification of the soil CO₂ respiration sources with our method to days 0–9 of the experiment. It also implied that the slurries were not totally identical in their composition. Nevertheless, the CO₂ efflux during the first week of incubation was very similar, suggesting that differences in slurry decomposition patterns are attributable to the second stage of the decomposition (slowly decomposable substances), whereas the first stage of the decomposition is similar for both types of slurry.

The ¹³C natural abundance tracer technique allowed us, during the first 9 days of the experiment, to assign the released CO₂ to its sources, i.e. the proportion of CO₂ that is derived from soil organic matter (SOM) and that derived from slurry mineralisation. Most of the C respired from the Brown Earth originated from the soil and not from the slurry, except for the very beginning of the experiment (Table 2). During the first 9 days of the experiment the proportion of slurry-derived C varied between 15 and 55% of the total C respired from the C₄ treatment of the Brown Earth, and for the combined 9-day experimental period the soil contributed 36 ± 9% (Table 2). However, for the Pelostagnogley, we observed that more of the respired C was derived from the slurry from days 4–9 in the experiment, i.e. 57–64%. This was in contrast to days 1–3 where 38–12% of the respired C was

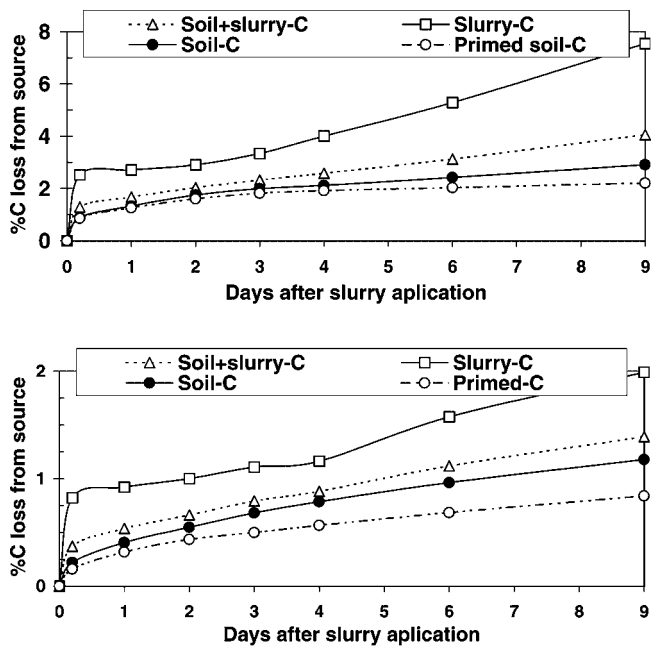


Figure 1. Cumulative percentage C loss from various soil C source pools (1) soil + incorporated slurry, (2) incorporated slurry only, (3) native soil only, and (4) primed native soil for the Pelostagnogley (top) and the Brown Earth (bottom).

derived from the slurry. Overall, for the 9-day period the soil contributed $46 \pm 11\%$ of the cumulative C respired from the Pelostagnogley (Table 2). The cumulative amount of C respired after 9 days in the C_4 slurry treatment was higher for the Pelostagnogley than for the Brown Earth, amounting to 2752 and 943 mg C kg⁻¹ soil, respectively (Table 2). These losses were equivalent to 4.1 and 1.4% of the C present in both soils (i.e. native soil C + incorporated slurry C; Fig. 1). Hence, the rate of C loss from the Pelostagnogley was nearly three times that from the Brown Earth. In both soils the observed rate of C loss derived from the incorporated slurry-C was generally twice that from the native soil C pool, i.e. 2.1 ± 0.2 and $2.0 \pm 0.3\%$ for the Pelostagnogley and the Brown Earth, respectively (Fig. 1).

Fleesa and Beese¹⁴ found that, after 9 weeks of incubation, only 38% of cumulatively released C originated from the applied cattle slurry, regardless of whether the slurry was injected or spread onto the soil surface. However, their incubation study was performed at 14°C, a lower temperature than the 27°C used in our study. Dendooven *et al.*^{13,32} suggested that between 5 and 62% of slurry C applied to a loamy soil is lost within 15 or 28 days incubation at 25°C, respectively. Clearly, our observed C losses for both soils fell well within these possible ranges. The faster rate of C loss from the newly incorporated slurry than from the native soil C is in line with general observations. The fact that the fresh slurry C input is lost twice as fast as the native soil C can assist in testing and improving agricultural SOM-C models.

Compared with the control, slurry addition caused a significant priming of SOM mineralisation. This primed amount of C decreased rapidly from an initial maximum 438 and 79 mg C kg⁻¹ soil day⁻¹ (Pelostagnogley and Brown Earth, respectively) to around 20 after 9 days (Table 2). Hence,

the high proportions of SOM-derived C found in the initially released CO₂ were promoted by the slurry addition. Similarly, we observed a rapid temporal decrease in the priming factor (i.e. the ratio of the daily soil-derived C emitted from the C_4 slurry treatment and that emitted from the control). The decrease in the priming factor was significantly more pronounced for the C-rich Pelostagnogley than for the Brown Earth with lower C content. At the Pelostagnogley, the priming factor decreased from 37.5 ± 3.4 (a clear indication of priming) for several hours to 1.3 ± 0.4 (no priming) at 6 days after slurry incorporation. Furthermore, most of the cumulative C loss from the native soil was primed C, i.e. 89 ± 3 and $74 \pm 1\%$ for the Pelostagnogley and the Brown Earth, respectively (Fig. 1).

The initially enhanced C flux from the native soil supports our hypothesis that the added slurry promotes the microbial decomposition of the native SOM in the first few days after the incorporation. A comparable effect was observed earlier for application of sewage sludge to soil.¹⁶ The rapid loss of positive priming is in agreement with other studies, which also reported that priming is a relative short-term phenomenon.^{17,21} We suggest that the activation of the microbial community involved in SOM mineralisation does not prevail for longer periods of time, because easily decomposable substrates within the slurry are almost completely degraded during this period.¹² Nevertheless, this study also shows that the degree of priming also depends on the nature and concentration of SOM. The Pelostagnogley soil probably exhibited a stronger priming effect because more labile C is found at higher C contents than in the Brown Earth.

However, our observation that slurry incorporation can induce significant positive priming indicates that calculation of losses of applied slurry C based on comparisons between CO₂ fluxes from slurry amended treatments and unamended controls may not be reliable. Such calculations, if not based on isotope labelling, may seriously overestimate the amount of C that is derived from the slurry and, hence, significantly underestimate the residence time of slurry in environment.

CONCLUSIONS

The natural ¹³C abundance tracer technique allowed a differentiation of CO₂ released from soil-inherent and added C, i.e. the technique previously used for solid and liquid samples^{10,12} is sensitive enough to trace C fluxes also of the gas phase. Our results showed clearly that grassland soils with higher C content are more prone to enhanced 'basal' SOM respiration and 'induced' priming losses of 'native' soil C after slurry incorporation than are soils with lower C contents.

Future studies might also focus on the pollution 'swapping' issues caused by slurry injection in grassland soils. Specifically, with respect to greenhouse gas budgets, studies into whether the benefits of reduced ammonia volatilisation outweigh the enhanced respiration loss of 'primed' native soil C (as observed in our study), and the increased N₂O emissions reported in the literature (e.g. Misselbrook *et al.*³³), are required. Particular attention should be given to those grasslands with soils saturated with water during much of the year and those that have a relatively high soil C content.

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