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Soil Biology & Biochemistry 39 (2007) 2608–2620

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Quantification of priming and CO_2 emission sources following the application of different slurry particle size fractions to a grassland soil

D. Fangueiro[a,*], D. Chadwick[b], L. Dixon[b], R. Bol[b]

[a]*Departamento de Fitotecnia e Engenharia Rural, Universidade de Trás-os-Montes e Alto Douro (UTAD), Apartado 1013, 5001-801 Vila Real, Portugal*
[b]*Institute of Grassland and Environmental Research (IGER), North Wyke Research Station, Okehampton, Devon EX20 2SB, UK*

Received 22 December 2006; received in revised form 11 May 2007; accepted 12 May 2007
Available online 15 June 2007

Abstract

The highest emissions of CO_2 from soils and most pronounced priming effect (PE) from soils generally occur immediately after slurry application. However, the influence of different particle size slurry fractions on net soil C respiration dynamics and PE has not been studied. Therefore, a slurry separation technique based on particle sizes was used in the present study. Six distinct fractions (>2000, 425–2000, 250–425, 150–250, 45–150, <45 μm) were generated from two dairy slurries (one from cows fed a predominantly maize silage diet and the other from cows fed a grass silage diet) were applied to soil. During the first days of the 332 days experiment, all slurry fraction amendments significantly increased soil CO_2 effluxes (by 2–8 times) compared to the non-amended control. The increased CO_2 emission rates had a negative relationship with slurry particle size, but its duration was positively correlated with slurry particle size. The percentage of the cumulative CO_2 emitted was only higher in the first 8 days in the finest slurry particle sizes (<150 μm). The proportion of slurry-derived C emitted as CO_2 2 h after addition to soil varied between 29% and 100% of total emitted CO_2-C. Generally, the proportion of slurry-derived C emitted initially decreased rapidly in the <250 μm fractions, but decreased more slowly or even increased in the >250 μm fractions. The overall contribution of slurry C to total CO_2 emissions was higher in smaller slurry particle size treatments in the first days after application. The addition of the various slurry fractions to soil caused both significant positive and negative PEs on the soil organic matter mineralization. The timing and type (positive or negative) of PE depended on the slurry particle size. Clearly, farm based separation pre-treatment leading to two or more fractions with different particle sizes has also the potential to reduce or modify short-term CO_2 emissions immediately after slurry application to soil.

Keywords: Slurry; Carbon; Particle size fractionation; Soil respiration; CO_2 emissions; Priming effect

1. Introduction

Livestock slurry is applied to agricultural soils to return essential nutrients for plant growth and organic matter to maintain long-term fertility. Various factors, including animal diet and anaerobic decomposition during storage in the manure channels and slurry stores affect directly the particle size distribution and the chemical composition of cattle slurry applied to agricultural land (Moller et al., 2002). Indeed, during storage, anaerobic bacteria digest the dissolved particulate matter in liquid manure and hydrolyze suspended solids contributing to the transfer of nutrients (especially N and P) between different fractions and chemical forms in manure and leading to a decrease in total suspended solids (Henze et al., 1996; Zhu, 2000). Also, some dairy farmers have started to perform mechanical slurry separation, which results in a nutrient-rich solid fraction with particles >5–10 mm and a liquid fraction with particles <5–10 mm. It results in improved slurry management in terms of nutrients utilization, reducing costs related with slurry storage, and using the liquid fraction for fertigation.

However, application of animal slurries to agricultural soils can result in increased greenhouse gases emissions such as nitrous oxide (N_2O) (Dittert et al., 1998; Chadwick et al., 2000) or carbon dioxide (CO_2) (Bol et al., 2003) and methane (CH_4) (Gregorich et al., 1998; Rochette and

*Corresponding author. Tel.: +351 259 350 806; fax: +351 259 350 436.
E-mail address: dfangueiro@utad.pt (D. Fangueiro).

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doi:10.1016/j.soilbio.2007.05.012

Gregorich, 1998). Measurements have shown that the highest emissions of CO_2 and CH_4 from soils occur immediately after slurry application (Chadwick et al., 1998; Chantigny et al., 2001; Flessa and Beese, 2000). However, sequestration and losses of C from slurries applied to agricultural soils had been poorly studied (Martinez and Peu, 2000; Rochette et al., 2000; Chantigny et al., 2001). Bol et al. (2003) used the natural abundance ^{13}C tracer technique (see Balesdent and Mariotti, 1996; Boutton, 1996; Boutton et al., 1998) to quantify the native soil- and slurry-derived CO_2 efflux after slurry application to two grassland soils of different C contents. The study showed that the incorporated slurry-C was lost twice as fast as from the native soil C in both soils and that the rate of slurry-derived C emitted increased with increasing soil carbon content.

The amount of easily decomposable C and available N in soils is directly affected by the intensity of biomass activation by substrate addition (Kuzyakov et al., 2000). Earlier studies did indicate that the amount of labile C and N depends on the particle size (sand/silt/clay) composition, management type and history of the soils (Amelung et al., 1998; Patra et al., 1999). Therefore, addition of readily decomposable C present in slurry to soil may change the activity of soil microorganisms and hence decomposition of native soil organic matter (SOM), inducing a so-called priming effect (PE) (reviewed in Kuzyakov et al., 2000), as observed in grassland systems by Bol et al. (2003). However, to our knowledge, the influence of different particle size slurry fractions on net soil C respiration dynamics and PEs, the aim of the present work, has not yet been studied.

2. Material and methods

2.1. Soil sampling and preparation

Soil material was collected from a permanent grassland site (De Bathe Cross) located near North Wyke Research Station in Devon, Southwest England (50°45′N, 4°53′W). The mean annual temperature for this site is 10.5 °C and mean annual precipitation is 1035 mm. The dominant vegetation was perennial ryegrass (*Lolium perenne*) and the coarse sandy loam soil is a typical Brown Earth of the Crediton series (Findlay et al., 1984). The site was grazed by cattle or sheep. Three subsites were randomly selected for soil sampling, which took place on July 8, 2005. For sampling, intact soil cores (20 cm × 50 cm) were isolated from the top soil (0–10 cm). After removal of vegetation and bigger roots, the soil from the 3–10 cm depth was wet sieved (<4 mm) and the soil from the three sampling sites was pooled to provide a single soil sample. The upper 0–3 cm was not used in this experiment as it contained too many larger roots. The sieved soil from the 3–10 cm depth was stored in sampling bags at 4 °C for 1 month before the start of the experiment.

2.2. Slurry preparation and fractionation

Dairy slurry was collected from the slurry reception pits on two local commercial dairy farms with different feeding strategies. Cows were predominantly fed either with ryegrass (*L. perenne*, a C_3 plant) silage on one farm and with maize (*Zea mays*, a C_4 plant) silage on the second farm. Slurries were stored in plastic barrels for 3 months prior to particle fractionation. The slurry was successively passed through five large sieves in order to obtain six fractions: >2000, 425–2000, 250–425, 150–250, 45–150 and <45 μm. The following protocol was used: 500 ml of whole slurry was put on a 2000 μm sieve; in order to facilitate the fractionation, 2 l of deionized water were added. The sieve, now partially immersed in deionized water, was shaken horizontally to allow the smallest particles to pass through the 2000 μm sieve. Two more litres of deionized water were then added to the sieve on two more occasions (i.e. a total of 6 l of deionized water was used) to complete this stage of the size separation of the slurry particles. The solid slurry particles remaining on the 2000 μm sieve after this separation procedure were then carefully removed and used for the experiment. The remaining solution with slurry particles (now <2000 μm) were then successively passed through the 425, 250, 150 and 45 μm sieves. Each solid fraction was then carefully removed from the respective sieve and stored at 4 °C for 2 weeks.

2.3. Slurry and soil analysis

The moisture contents of the soil and slurries (whole slurry and fractions) were determined by weighing before and after drying in an oven at 85 °C overnight. Soil and slurries samples were then ground into a powder. Total C and N contents of soil and slurries samples were determined using a CHN auto-analyzer (Carlo Erba NA2000, Milan, Italy).

Neutral detergent fibre (NDF) of the whole slurry and fractions was determined with the Gerhardt Fibrecap detergent system (FOSS UK Ltd, Warrington, UK). Reagents were as described by Van Soest et al. (1991), with the following exceptions; sodium sulphite was omitted and Termamyl (NCBE Enzymes, Reading, UK) replaced α-amylase. Acid detergent fibre (ADF) of the whole slurry and fractions was determined with the Gerhardt Fibrecap detergent system (FOSS UK Ltd, Warrington, UK). Reagents were as described by Van Soest et al. (1991). NDF and ADF results were corrected for ash content. Acid detergent lignin of the whole slurry and fractions was determined using a method based on the method described by Robertson and van Soest (1981). The percentage of cellulose and hemi-cellulose were estimated according to the following equations (Goering and Van Soest, 1970): Cellulose = ADF−lignin and hemi-cellulose = NDF−ADF.

The $\delta^{13}C$ values of soil and slurry samples were analysed at IGER North Wyke using a continuous flow ANCA 20/20SL system (Europa, Crewe, UK). Natural abundance

of ^{13}C was expressed as $\delta^{13}C$ values, which represents the ratios of $^{13}C/^{12}C$ relative to the international Vienna PDB (Pee Dee Belemnite) standard. The $\delta^{13}C$ values (per mill) are defined as

$$\delta^{13}C = [(\text{atom\% } ^{13}C_{\text{sample}} - \text{atom\% } ^{13}C_{\text{VPDB}})/\text{atom\% } ^{13}C_{\text{VPDB}}] \times 1000$$

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

2.4. Experimental design

For all the amended treatments, 329 g of wet soil (equivalent to 6 g C) were weighed into 1.5 l Kilner jars to which ca. 11–160 g of a specific slurry fraction (equivalent to 2 g C) was applied and then water (between 41.5 and 66.7 ml) was added to obtain a moisture content in the soil of 60% field capacity. For the $<45\,\mu m$ fraction no water was added, since this fraction already contained a large amount of water, consequently the moisture content was higher than 60% field capacity. The slurry fraction was then incorporated into the soil using a spatula to obtain a homogenous mixture. The amount of slurry (whole slurry or fractions) applied to each plot was the equivalent of $91\,m^3\,ha^{-1}$ (or $2000\,kg\,C\,ha^{-1}$). The control comprised soil only (329 g of wet soil equivalent to 6 g C) and did not receive any slurry. However, water was also added to the controls to obtain moisture content in the soil of 60% field capacity. The samples were incubated at 25 °C to ensure good slurry degradation and biomass activation in soil. The water content was adjusted weekly to maintain moisture content in the soil at 60% field capacity. To avoid large water losses from the soil, but to allow air to circulate in the jars, the two septum seals (10 mm diameter) in the lids of the Kilner jars were (temporary) removed between sampling dates. The experiment included 14 treatments with four replicates of each, and continued for 332 days.

2.5. CO_2-C efflux

To determine the CO_2-C efflux in each kilner jar, the holes in the lid were closed with silicone septa and air samples (2 ml) were taken from the headspace using a syringe. The CO_2-C flux measurement was performed daily for the first week (days 0.12–8) and then on days 10, 12, 14, 16, 18, 22, 25, 28, 56, 97 and 332. Up to day 18, air samples were taken 2, 15, 30 and 45 min after closing the Kilner jar. But after day 18, the interval time between headspace measurements was increased to 2, 30, 60 and 90 min, since CO_2 emissions rates had reduced and consequently greater incubation times were required to accumulate enough CO_2 in the headspace for measurement. The headspace samples were analysed immediately using an infra-red gas analyser ADC type 225 MK3 (Hoddesdon, Herts, UK) for CO_2 concentration. Fluxes were calculated by determination of the linear increase in CO_2 concentration. The cumulative flux was calculated by summing up all daily CO_2 fluxes for the whole experiment and assuming that, where no daily data were available, the daily CO_2 fluxes changed linearly between the nearest sampling dates for which samples were available. To enable tabulation of the results, a mean value of the CO_2 flux was calculated for the 4–7, 8–12, 14–18 and 22–28-days periods, this was possible as the measured fluxes in each interval were very similar.

The CO_2 emissions from the whole slurry were compared with the sum of CO_2 emissions from the six size fractions called here "recombined slurry". For this, the CO_2 emissions of the recombined slurry was calculated, taking into account the proportion of each size fraction relative to the whole slurry and using the following formulae:

$$CE_T = (CE_1 \times Q_1) + (CE_2 \times Q_2) + \cdots + (CE_n \times Q_n)$$

with CE_T: CO_2 emissions from the recombined slury (sum of six individual fractions), CE_n: CO_2 emissions observed from the fraction n, Q_n: proportion of the fraction n relatively to the whole slurry (%).

Additional head space samples ($10\,cm^3$) were taken on days 0, 2, 4, 8, 14, 22, 28 and 56 using evacuated exetainers in order to determine the $\delta^{13}C$ value of the emitted CO_2 using a gas chromatography–isotope ratio mass spectrometry (GC–IRMS) continuous flow system (Europa, Crewe, UK). The resulting CO_2 concentration and isotopic content were corrected (similar to Bol et al., 2003) for a small amount of ambient air (set at 360 ppm and $\delta^{13}C$ of −8‰) present in the exetainer samples.

The difference in $\delta^{13}C$ values between emitted CO_2 from the C_4 and C_3 slurry treatments was used to quantify the proportions of slurry-derived versus soil-derived CO_2-C emitted from the soil (Bol et al., 2003). This calculation was performed as follows:

$$\%\text{Slurry-derived C in emitted } CO_2 = (\Delta C_4 - C_3)/(\Delta SIC_4 - SIC_3),$$

where $\Delta C_4 - C_3$ represents the difference in $\delta^{13}C$ values between emitted CO_2 from the C_4 and C_3 slurry treatments and ΔSIC_4–SIC_3 denotes the difference in initial $\delta^{13}C$ values of the material in the Kilner jars for each of the comparable C_4 and C_3 treatments of the whole or isolated slurry size fractions. The actual amount of CO_2 emitted from native soil in the C_4 treatments at a specific day can be calculated as follows:

$$\%\text{Soil-derived C} = ((100 - \%\text{Slurry-derived C})/100) \times (\text{Amount of } CO_2 \text{ emitted on that day for that specific } C_4 \text{ treatment})$$

The priming factor was then calculated as follows:

(Amount of CO_2 emitted from 'native' soil in the C_4 treatments)/(amount of CO_2 emitted from control)

Therefore, a value >1 suggested positive priming, 1 indicates the absence of priming, and <1 negative priming of soil-emitted CO_2 by the application of slurry.

For clarity, we indicated in Table 4 the significant ($P<0.05$) observations of positive priming in **bold** and those of negative priming in *italics*.

2.6. Statistical analysis

The whole experiment was performed with four replicates. The statistical analyses were conducted using the GENSTAT package software 8.11 for Windows XP (Lawes Agricultural Trust, Rothamsted Experimental Station, 2005). The statistical differences between the treatments over time were examined using two-way analysis of variance (ANOVA). Differences between the two slurry types were analysed using one-way ANOVA. To determine the statistical significance of the mean differences, least significant difference tests (LSD) were carried out based on a *t*-test. Statistical differences for which $P<0.05$ are referred to in the text as significant. The Genstat Procedure Library MULTMISSING procedure, an iterative regression technique for estimating missing values from units in multivariate dataset (Beale and Little, 1975), was used to estimate missing values in the $\delta^{13}CO_2$ dataset. The errors of observed and calculated values are presented as standard errors (SE) calculated as: $SE = SD/\sqrt{n}$; with SD: standard deviation of n replicates. The calculation of SD for differences between two means (a and b) was performed using the following Gaussian equation: $SD_{difference} = (SD_a{}^2 + SD_b{}^2)^{0.5}$. In figures, standard errors were indicated using error bars (negligible when not visible).

3. Results

3.1. Characteristics of the soil and slurry fractions used

The main characteristics of the soil and slurry fractions used in the present work are shown in Table 1. The relative proportions of the individual particle size fractions were similar in the C_3 and C_4 slurries. More than 50% of the slurry particles were smaller than 45 μm, whereas particles with sizes in the range 45–250 μm represented less than 10% of the slurry particles. The total C, total N and C:N in the soil were always much lower than in the whole slurry and slurry fractions. Total C in the whole C_4 slurry and its fractions were significantly higher than their respective C_3 slurry fractions, except in the >2000 μm fraction. The C content significantly decreased in the smaller particle size fractions of the slurry. The N content was similar in all C_3 and C_4 slurry fractions, except for the significantly higher N content in the >2000 μm C_3 slurry fraction. However, N content did significantly increase with decreasing slurry particle size. Indeed about 80% of total N was present in the <45 μm size fraction. The C:N ratio was always significantly higher in the C_3 slurry fractions than the C_4 slurry, but significantly decreased with particle size for both slurries ($P<0.05$).

Table 1
Main characteristics of the C_3 and C_4 slurry fractions, soil and whole C_3 and C_4 slurries (dry weight basis)

Fraction		Relative proportion	C (%)	N (%)	C:N	$\delta^{13}C$ (‰)	Moisture content (%)	Lignin (%)	Cellulose (%)	Hemi-cellulose (%)
>2000 μm	C_3	15.8 ± 1.0^{c}	$^{1}43.31\pm0.39^{bc\ 2}$	1.21 ± 0.07^{fg}	36.11 ± 1.95^{c}	-27.40 ± 0.33^{ab}	78.7 ± 1.0^{bc}	11.28 ± 0.42^{ef}	30.62 ± 1.53^{b}	27.25 ± 1.50^{a}
	C_4	11.5 ± 0.4^{e}	43.60 ± 1.06^{ab}	0.81 ± 0.03^{h}	54.27 ± 2.23^{a}	$-19.03\pm0.24^{\alpha}$	70.9 ± 2.1^{cde}	13.20 ± 0.54^{d}	35.42 ± 0.98^{a}	26.33 ± 0.78^{a}
425–2000 μm	C_3	23.9 ± 2.2^{b}	37.31 ± 2.74^{gh}	1.08 ± 0.08^{g}	34.51 ± 1.56^{c}	-26.92 ± 0.17^{a}	74.9 ± 0.9^{bcd}	18.39 ± 0.47^{a}	25.37 ± 1.28^{c}	17.81 ± 3.08^{d}
	C_4	27.7 ± 1.2^{b}	46.65 ± 0.32^{a}	1.10 ± 0.03^{g}	42.36 ± 0.86^{b}	$-20.98\pm0.33^{\beta}$	81.9 ± 0.4^{b}	18.10 ± 0.14^{abc}	33.18 ± 0.96^{a}	21.35 ± 0.89^{bcd}
250–425 μm	C_3	3.8 ± 0.4^{d}	39.68 ± 0.98^{h}	1.49 ± 0.06^{de}	26.66 ± 0.98^{d}	-27.28 ± 0.09^{a}	77.3 ± 1.2^{bc}	17.58 ± 0.24^{abc}	23.34 ± 0.32^{cd}	23.60 ± 1.08^{abc}
	C_4	2.1 ± 0.4^{d}	44.44 ± 0.29^{bcd}	1.35 ± 0.05^{ef}	33.11 ± 1.19^{c}	$-21.04\pm0.07^{\beta}$	66.8 ± 7.9^{e}	19.00 ± 0.17^{a}	29.06 ± 0.32^{b}	17.31 ± 2.12^{d}
150–250 μm	C_3	2.0 ± 0.4^{d}	36.33 ± 1.17^{h}	1.64 ± 0.08^{cd}	22.29 ± 0.51^{e}	-27.88 ± 0.07^{bc}	61.3 ± 7.4^{e}	16.28^{c}	19.44 ± 0.69^{f}	26.22 ± 2.79^{ab}
	C_4	1.3 ± 0.4^{d}	41.62 ± 1.04^{bcd}	1.61 ± 0.04^{d}	26.02 ± 1.25^{d}	$-21.64\pm0.09^{\alpha}$	65.8 ± 3.4^{de}	$(16.8)^{3}$	(48)	19.42 ± 1.47^{cd}
45–150 μm	C_3	4.1 ± 0.3^{d}	35.61 ± 0.85^{h}	1.91 ± 0.05^{b}	18.61 ± 0.14^{f}	-28.71 ± 0.14^{d}	78.5 ± 1.07^{bc}	18.21 ± 0.48^{ab}	21.80 ± 0.63^{def}	16.96 ± 0.83^{d}
	C_4	2.5 ± 0.2^{d}	40.46 ± 0.60^{cde}	1.83 ± 0.05^{bc}	22.21 ± 0.54^{e}	$-21.73\pm0.06^{\alpha}$	77.7 ± 0.94^{bc}	16.90 ± 0.58^{bc}	22.40 ± 0.47^{de}	19.44 ± 0.64^{c}
<45 μm	C_3	50.3 ± 1.9^{a}	32.35 ± 0.48^{i}	4.01 ± 0.13^{a}	8.11 ± 0.35^{g}	-28.34 ± 0.17^{cd}	95.8 ± 0.37^{a}	(5.31)	(1.19)	(12.39)
	C_4	54.8 ± 1.7^{a}	37.39 ± 0.71^{fgh}	4.21 ± 0.15^{a}	8.91 ± 0.15^{g}	$-22.02\pm0.05^{\chi}$	96.6 ± 0.21^{a}	(8.7)	(10)	(12.81)
Whole slurry	C_3		$38.10\pm0.91^{\gamma}$	$2.53\pm0.14^{\phi}$	$15.15\pm0.60^{\gamma}$	-27.39 ± 0.31^{ab}	$86.1\pm1.1^{\phi}$	$10.59\pm0.20^{\gamma}$	$13.67\pm1.18^{\gamma}$	$16.91\pm0.65^{\gamma}$
	C_4		$43.14\pm0.47^{\phi}$	$2.29\pm0.03^{\phi}$	$18.86\pm0.25^{\phi}$	$-21.54\pm0.10^{\chi}$	$85.1\pm0.7^{\phi}$	$12.23\pm0.22^{\phi}$	$20.03\pm0.24^{\phi}$	$19.96\pm0.62^{\gamma}$
Soil			2.09 ± 0.07	0.22 ± 0.01	9.48 ± 0.10	-29.21 ± 0.17	14.76 ± 0.09			

[1]Mean ± standard error ($n = 5$).
[2]Values marked by the same letter or symbol are not significantly different between specific slurry type or fraction ($P<0.05$) by LSD test.
[3]The values in parentheses were estimated considering the value of the parameter in the whole slurry and the relative proportion of each fraction.

The bulk $\delta^{13}C$ values of the two slurries partly reflected the origin of the animals' silage, i.e., a maize derived C_4 slurry ($\delta^{13}C = -21.5‰$) and a ryegrass-derived C_3 slurry ($\delta^{13}C = -27.4‰$). The highest $\delta^{13}C$ values were measured in the >2000 μm for both slurries. In the case of the C_3 slurry, the >2000 and 425–2000 μm fractions had similar $\delta^{13}C$ values. The $\delta^{13}C$ values decreased when the particle size decreased, hence the lowest $\delta^{13}C$ values in both slurries were in the <45 μm fractions. The bulk soil $\delta^{13}C$ was −29.2‰, reflecting a C_3 (grass) system.

The lignin, cellulose and hemi-cellulose content of the whole slurries were generally significantly lower than the fractions (Table 1). The cellulose and hemi-cellulose content generally decreased with decreasing particle size fraction of the slurry, whereas lignin only showed a significant difference between the >2000 μm and all other slurry fractions.

3.2. Daily and cumulative CO_2 fluxes

Fluxes of the CO_2 emitted from the soil after application of slurry particle size fractions and whole slurry are shown in Table 2. The rate of emitted CO_2 from the soil only (control) during the incubation period at 25 °C was relatively low and with minor variations (16 ± 2 mg C kg^{-1} soil day^{-1}). During the 332-days incubation period, no significant differences ($P > 0.05$) in CO_2 emission rates between the C_3 and C_4 slurry fractions were found, except on days 0.12, 2, 4 and 8 (Table 2). However, significant differences were found in the rates of CO_2 emitted from the whole slurry and the different particle size fractions (Table 2). The application of the whole slurry strongly increased the rate of emitted CO_2 following application to more than 20 times that of the control. The addition of the various slurry fractions significantly increased the CO_2 emissions to 20–82 mg C kg^{-1} soil day^{-1}, or 2–8 times that of the control, 3 h (0.12 day) after application. Furthermore, an inverse relationship was observed between the rate of CO_2 emitted and the slurry particle sizes (Table 2). The differences in emitted CO_2 between the control and the slurry treatments steadily decreased during the experimental period. During the first 2 days after application, the CO_2 emissions of the whole slurry treatments were significantly higher than the individual slurry fraction treatments. Higher CO_2 emissions were observed during the first week of incubation in the treatments with the smallest particles (i.e. <250 μm), but especially in the <45 and 45–150 μm fractions. From day 8 onwards, the opposite situation was observed with the highest CO_2 emissions coming from the coarse fractions (especially the >2000 μm). After 2 weeks, the CO_2 emissions of the control, the <45 and 45–150 μm treatments were similar. However, for larger slurry particle size fractions (especially the 425–2000 and >2000 μm) significantly higher CO_2 emissions than the control did still occur on day 97. On day 332 of incubation, there were no significant differences ($P > 0.05$) in CO_2 emissions between control and any of slurry fractions.

The calculated CO_2 flux over the whole 332-day incubation period was significantly higher in the >2000, 425–2000 and 250–425 μm fractions of the C_4 treatment than the control (soil only; Table 2). The total CO_2 flux over the whole 332-day incubation period of the two most coarse slurry fractions (425–2000 and >2000 μm) was significantly higher than the finest (<45 and 45–150 μm) fractions.

Comparing the CO_2 emissions from the whole slurry treatment to the recombined slurry, it appears that the most significant emissions ($P < 0.05$) occurred in the first 2 days following the whole slurry application compared to the individual slurry fractions (Table 2). Hence, the cumulative CO_2 emission from the whole slurry during the whole experiment was significantly greater ($P < 0.05$) than the recombined slurry.

Fig. 1 shows the cumulative CO_2 emissions expressed as percentage of initial total carbon content. The percentage of the cumulative released CO_2 emitted in the first 3 days increased as particle size decreased. Indeed on day 3, from

Table 2
Rates of CO_2 emitted from the top soil (mg C kg^{-1} soil day^{-1}) after C_4 slurry particle size fractions and whole C_4 slurry application and amounts of C lost as CO_2 after 332 days (g C kg^{-1} soil)

Time after slurry application (days)	0.12	1	2	3	*4–7	*8–12	*14–18	*22–28	56	97	332	∑0–332
Soil (control)	[1,2]11.5^{d}	10.2^{e}	14.6^{c}	8.3^{e}	17.8^{e}	19.6^{e}	21.2^{d}	22.0^{b}	23.6^{bc}	10.8^{b}	18.1^{a}	4.5^{c}
Whole slurry	^{A}257.1^{a}	A187.4^{a}	^{A}80.4^{a}	A38.8^{ab}	^{A}46.8^{a}	^{A}44.0^{a}	^{A}42.1^{a}	^{A}29.6^{a}	^{A}32.7^{ab}	^{B}17.0^{ab}	^{A}17.1^{a}	^{A}7.4^{a}
Recombined slurry[3]	^{B}32.1	^{B}49.2	^{A} 43.2	^{A}48.4	^{A}40.6	^{A} 39.8	^{A}36.8	^{A}34.5	^{A}34.5	^{A}27.8	^{A}23.5	^{B}5.8
>2000 μm	20.6^{d}	18.8^{cd}	26.1^{bc}	15.8^{de}	25.6^{cd}	36.9^{b}	35.0^{b}	26.8^{a}	26.0^{abc}	19.0^{a}	16.1^{a}	6.6^{a}
2000–425 μm	33.3^{cd}	20.2^{cd}	14.7^{c}	18.3^{d}	19.5^{c}	26.6^{c}	28.0^{c}	29.9^{a}	30.1^{abc}	18.9^{a}	14.5^{a}	6.6^{a}
425–250 μm	43.2^{cd}	40.7^{b}	29.6^{bc}	32.4^{bc}	32.9^{b}	29.3^{c}	24.7^{cd}	27.7^{a}	38.6^{a}	13.3^{ab}	11.5^{a}	6.2^{ab}
250–150 μm	41.0^{cd}	28.3^{c}	24.8^{bc}	24.2^{cd}	21.4^{de}	21.5^{de}	22.9^{d}	26.5^{a}	22.9^{bc}	10.6^{b}	13.8^{a}	5.4^{bc}
150–45 μm	67.9^{bc}	43.2^{b}	38.9^{b}	30.1^{bc}	26.0^{cd}	26.0^{cd}	23.8^{d}	21.4^{b}	19.3^{cd}	13.9^{ab}	15.4^{a}	5.0^{bc}
<45 μm	81.5^{b}	41.1^{b}	35.0^{bc}	41.5^{a}	30.2^{bc}	25.2^{cd}	21.5^{d}	19.1^{b}	18.9^{c}	16.2^{ab}	17.3^{a}	5.4^{bc}

*Mean value observed in the time interval.

[1]Mean ($n = 4$).

[2]For each sampling period the values marked by different letters are significantly different for a specific slurry type or fraction ($P < 0.05$) by LSD test. Capital letters used for comparison between the whole slurry and recombined slurry only.

[3]See Section 2.5 for information about calculation of the values presented for the recombined slurry.

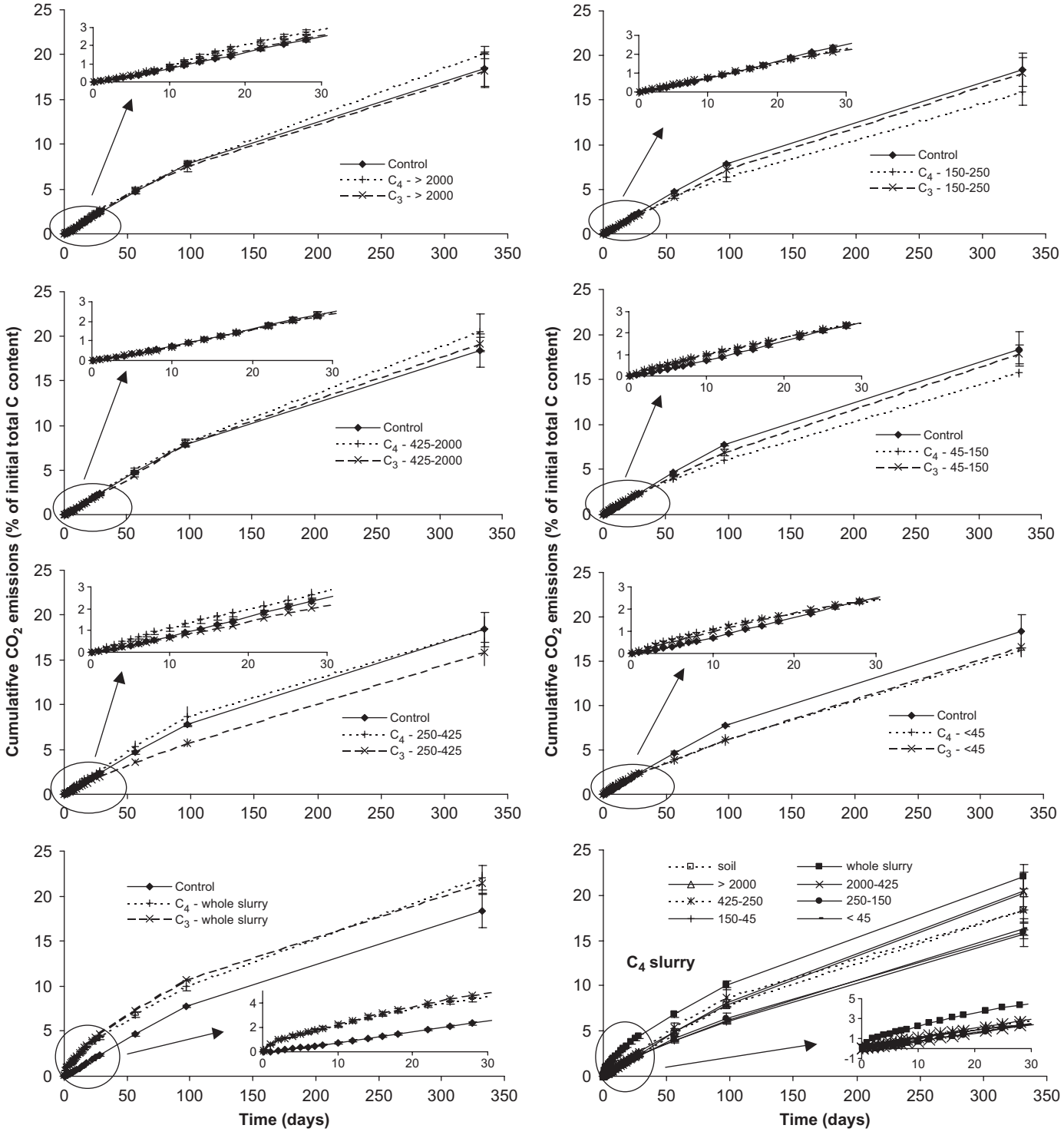

Fig. 1. Average cumulative CO_2 emissions after incorporation of different slurry fractions into soil (percentage of initial total C content) ($n = 4$).

0.19% to 0.22% of initial total C was lost from the >250 μm fractions, but from 0.26% to 0.44% was lost in the finer fractions (<150 μm). After 8 days, significantly more of the initial total C was lost as CO_2 from treatments with the finest applied slurry fractions (45–150 and <45 μm) (0.85%), when compared to other slurry treatments (0.64%) and the control (0.56%). The calculated CO_2 flux over the whole 332-day incubation period was significantly higher than the control (soil only) in the >2000 and 425–2000 μm fractions of both slurry types, as well as in the 250–425 μm fraction of the C_4 slurry and the 150–250 μm fraction of the C_3 slurry (Table 2).

3.3. $\delta^{13}C$ value of the emitted CO_2 and quantification of source of the CO_2 emissions

The $\delta^{13}C$ value of soil emitted CO_2 from the control (soil only) treatment was relatively constant (-23.8 ± 0.5‰) during the experimental period. The $\delta^{13}C$ values of the CO_2 emitted from the whole slurry treatments were initially (3 h after application) relatively high (-14.2‰ and -17.7‰); for the C_4 and C_3 treatments, respectively. Thereafter, the values decreased and remained relatively constant until day 28, at -19.1 ± 0.5‰ for the C_4 and -21.9 ± 0.5‰ for C_3 treatment. On day 56, both whole slurry treatments had comparable $\delta^{13}C$ values of the emitted CO_2 (-19.3‰ and -19.7‰; for the C_4 and C_3 treatments, respectively). During the first 28 days the $\delta^{13}CO_2$ from the >2000 and 425–2000 μm treatments were constant at -20.2 ± 0.5‰ and -21.2 ± 0.3‰ for the C_4 compared to -23.1 ± 0.2‰ and -23.1 ± 0.4‰ for C_3 slurry, respectively. However, on day 56, in both >2000 and 425–2000 μm fractions, C_4 and C_3 treatments had comparable $\delta^{13}C$ value of emitted CO_2 of -18.4 (±0.2)‰. The emitted CO_2 $\delta^{13}C$ observed for the 250–425 μm fractions remained relatively constant during the first 56 days of experiment, i.e. -21.4 ± 0.9‰ for the C_4 and -23.6 ± 0.5‰ for the C_3 treatment. The $\delta^{13}C$ values of emitted CO_2 of the 150–250 and 45–150 μm fractions for the C_3 treatment were -24.1 ± 0.5‰ and -23.7 ± 0.7‰, respectively, for the first 28 days, but decreased for both size fractions to -19.8 (±0.1)‰ on day 56. Emitted $\delta^{13}CO_2$ in the C_4 treatment of 150–250 and 45–150 μm slurry fractions was much higher (-15.1‰ and -14.4‰, respectively) in 2 h after their application, but then slowly decreased to around -24.2‰ and -22.6‰, respectively in the following 28 days. However, on day 56 in both C_4 treatments, it increased to -19.1‰ and -20.6‰, close that of their respective C_3 treatments. The CO_2 emitted from the <45 μm treatment had the lowest $\delta^{13}C$ values immediately after application (-8.2‰ for the C_4 and -9.2‰ for C_3 treatment). These values slowly decreased to reach a minimum value of -21.3 and -23.5 for the C_4 and C_3 treatment, respectively, on day 14 and then remained constant until day 56 (Table 3).

The $\delta^{13}C$ difference of the emitted CO_2 between the C_4 and C_3 treatments for the whole slurry and the isolated size fractions generally decreased with length of time after application, from 4.4 ± 1.0‰ (after 3 h) to 2.4 ± 0.2‰ (during period of days 1–28) to 0.1 ± 0.4‰ (day 56). Even if high values of $\delta^{13}C$ difference of the emitted CO_2 between the control (soil only) and C_4 and C_3 slurry amended treatments were observed in the few hours after application (mean values of 5.8 for C_4 and 10.2 for C_3), they became smaller in the following days. Indeed, between days 1 and 56 the $\delta^{13}C$ difference of the emitted CO_2 between the control (soil only) and C_4 and C_3 slurry amended treatments, was ca. 0.8 ± 0.4‰ for the C_3 treatments and 2.6 ± 0.4‰ for the C_4 treatments.

The proportion of slurry-derived C emitted shortly (3 h) after slurry incorporation varied between 29% and 100% of the total emitted CO_2-C (Fig. 2). Generally, during the first 56 days of incubation, the proportion of slurry-derived C initially decreased rapidly in the finer fractions (<250 μm), but decreased more slowly or even increased in the >250 μm fractions. For example, for the >2000 μm fraction the slurry-derived C contribution increased up to day 14, when it peaked at a value of 52% and then decreased slowly. In contrast, the slurry-derived C portions in the fractions 45–150 and 150–250 μm decreased from 100% to 41% and 91–44%, respectively, in the first 2 days. Two fractions exhibited a different behaviour of the proportion of slurry derived CO_2 emitted: (i) the <45 μm treatment was fairly constant (34 ± 4%) during the experimental period and (ii) in the 250–425 μm treatment it decreased from 100% to 12% during the first 22 days and then increased back to 61% on day 28. On day 56, all C emitted was generally soil derived in all treatments, except

Table 3
$\delta^{13}C$ (‰) of the air samples from the treatments studied

Treatment	0.1 day	2 days	4 days	7–8 days	14 days	22 days	28 days	56 days
C_3 soil	−25.9 (0.9)	−21.2 (0.3)	−23.2 (0.8)	−24.5 (0.5)	−24.4 (0.3)	−24.5 (1.1)	−24.7 (0.4)	−22.3 (0.8)
C_4 Whole slurry	−14.2 (1.1)	−17.4 (1.5)	−20.6 (0.7)	−20.0 (0.3)	−18.4 (1.2)	−18.6 (0.8)	−19.7 (0.9)	−19.6 (0.5)
C_3 Whole slurry	−17.7 (0.1)	−20.8 (1.1)	−22.4 (1.1)	−23.8 (0.2)	−22.7 (1.1)	−20.5 (1.2)	−21.5 (0.7)	−19.3 (0.6)
C_4 >2000 μm	−21.0 (0.8)	−19.3 (0.9)	−20.2 (1.2)	−19.4 (1.3)	−19.5 (1.3)	−20.9 (0.5)	−21.0 (0.6)	−18.5 (1.1)
C_3 >2000 μm	−23.5 (0.8)	−22.1 (0.7)	−23.4 (0.7)	−23.0 (1.1)	−23.8 (0.7)	−23.3 (0.3)	−22.7 (0.2)	−18.5 (1.2)
C_4 425–2000 μm	−19.7 (1.6)	−20.4 (0.5)	−21.3 (1.0)	−22.5 (0.4)	−21.5 (0.2)	−21.2 (0.4)	−21.9 (0.5)	−18.1 (0.4)
C_3 425–2000 μm	−22.4 (1.2)	−21.7 (0.5)	−24.5 (1.2)	−24.0 (0.2)	−23.0 (0.7)	−23.1 (0.1)	−22.8 (0.4)	−18.3 (1.2)
C_4 250–425 μm	−17.0 (1.4)	−18.8 (0.6)	−22.4 (0.2)	−23.4 (0.2)	−23.4 (0.7)	−23.0 (0.4)	−20.4 (0.9)	−22.6 (1.0)
C_3 250–425 μm	−23.7 (0.6)	−22.3 (0.4)	−24.7 (0.4)	−24.7 (0.5)	−24.7 (0.3)	−23.8 (0.5)	−24.1 (1.3)	−21.1 (1.3)
C_4 150–250 μm	−15.1 (2.4)	−18.8 (0.4)	−17.9 (3.8)	−22.0 (0.6)	−22.9 (0.3)	−20.9 (1.7)	−24.2 (1.0)	−19.1 (0.3)
C_3 150–250 μm	−23.6 (1.0)	−21.6 (0.7)	−25.4 (0.6)	−24.0 (0.4)	−25.2 (0.4)	−23.9 (0.5)	−25.1 (1.2)	−19.8 (1.3)
C_4 45–150 μm	−14.4 (1.2)	−19.5 (0.5)	−22.1 (0.8)	−22.6 (0.3)	−23.3 (0.1)	−23.1 (0.4)	−22.6 (0.5)	−20.6 (0.4)
C_3 45–150 μm	−20.5 (2.9)	−22.4 (0.6)	−24.9 (0.9)	−24.8 (0.8)	−24.7 (0.4)	−23.0 (1.7)	−25.4 (0.5)	−20.0 (0.4)
C_4 <45 μm	−8.2 (0.4)	−13.3 (0.7)	−16.6 (0.9)	−18.5 (0.5)	−21.3 (0.6)	−20.7 (1.2)	−21.6 (0.3)	−20.8 (0.9)
C_3 <45 μm	−9.2 (1.8)	−15.6 (0.2	−19.0 (1.0)	−21.2 (0.9)	−23.5 (1.1)	−22.9 (1.1)	−22.3 (1.7)	−23.1 (0.7)

Values are the mean of four replicates. Values in parentheses are one standard error of the mean.

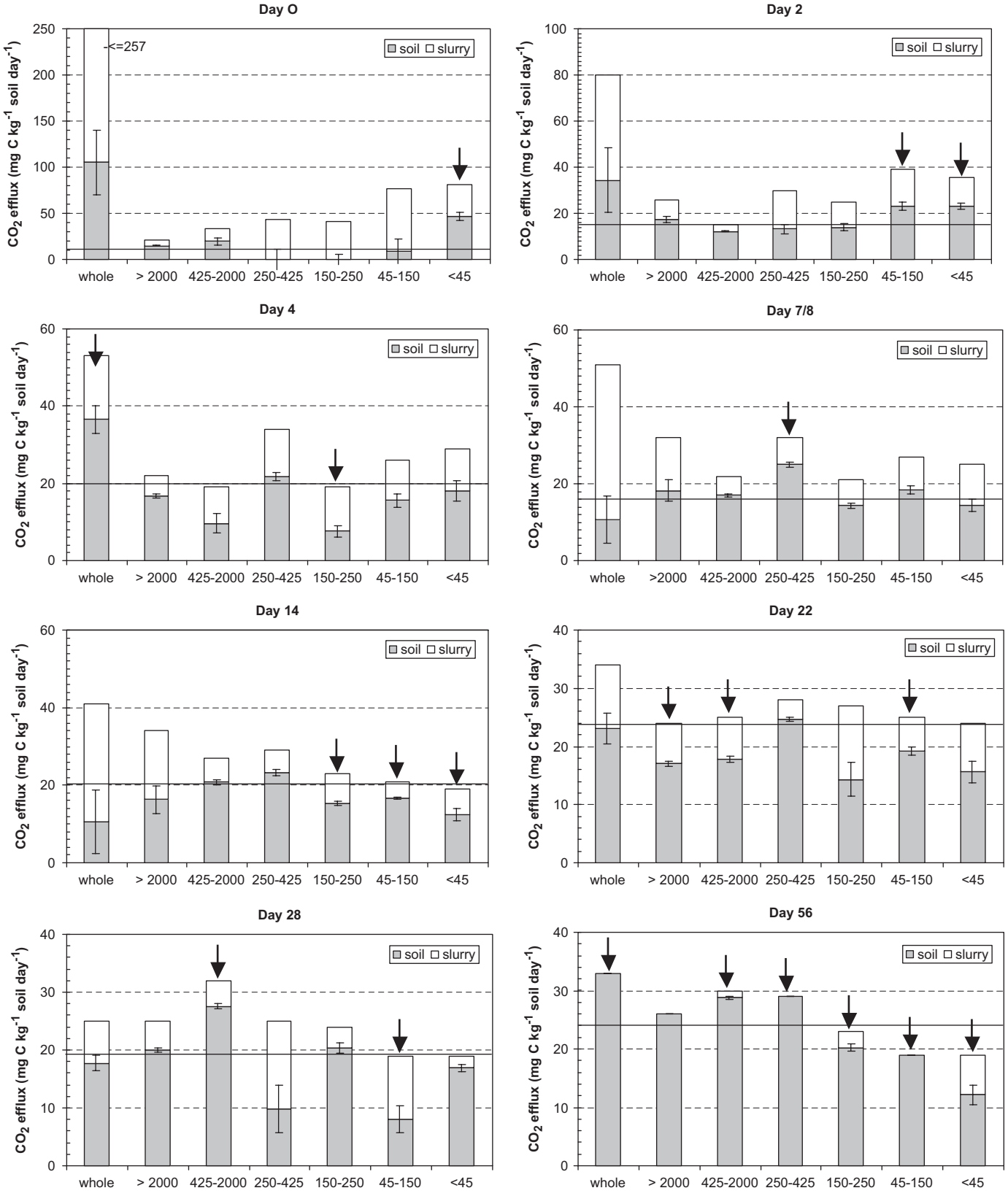

Fig. 2. Sources and rates of CO_2 emitted after slurry and slurry fractions application—the horizontal straight line represents the rates of CO_2 emitted from the non-amended soil. Mean values of four replicates. Black arrows indicate significant PE ($P<0.05$).

Table 4
Priming factor (soil derived/control) (values >1.00 indicate a positive PE and values <1.0 indicate a negative PE)—mean values of four replicates.

Time after slurry application (days)	Whole slurry	>2000 μm	425–2000 μm	250–425 μm	150–250 μm	45–150 μm	<45 μm
0.12	8.78	1.24	1.62	0.00	0.00	0.74	**3.92**
2	2.29	1.16	0.81	0.88	0.93	**1.53**	**1.54**
4	**1.83**	0.84	0.48	1.09	*0.38*	0.78	0.90
7/8	0.67	1.14	1.06	**1.56**	0.89	1.14	0.90
14	0.53	0.82	1.04	1.16	*0.77*	*0.83*	0.62
22	0.96	*0.71*	*0.74*	1.03	0.60	*0.80*	0.65
28	0.93	1.05	**1.45**	0.51	1.07	*0.42*	0.89
56	**1.38**	1.08	**1.20**	**1.21**	*0.84*	*0.79*	*0.51*

Bold numbers: significant positive priming ($P<0.05$).
Italic: significant negative priming ($P<0.05$).

in the <45 and 150–250 μm treatments (36% and 12% of slurry-derived emitted C, respectively).

3.4. PE on soil CO_2-C flux

The addition of the various slurry fractions to soil suggested positive ($\geqslant 1$) and negative ($\leqslant 1$) PEs on the native SOM mineralization (Table 4). The whole slurry induced a significant positive PE on days 4 and 56. The three smallest fractions (all <250 μm), all led to no or significant negative PE after day 2. However, the most important positive PE was induced by the finest fraction (<45 μm) on days 0.12 and day 2 (amounting to +35 and $+8\,mg\,C\,kg^{-1}\,soil\,day^{-1}$, respectively). It is worth noting that during the first 2 days a significant positive PE was observed only in the finest fractions (Table 4). In the fractions larger than 250 μm, no significant positive PE was observed in the first 4 days. Thereafter, the >2000 μm showed only a significant negative PE on day 22 whereas the 425–2000 μm fraction showed a significant negative PE on day 22, but a significant positive PE on days 28 and 56. Interestingly, on day 56 nearly all treatments led to a significant positive (whole slurry and fractions >250 μm) or negative PE (fractions <250 μm).

4. Discussion

4.1. Slurry fractions

The total N and total C contents, and C:N ratios of the two whole slurries used for the fractionation were typical of commercial dairy farm slurries (Chadwick et al., 2000; Bol et al., 2003). The slurry fractionation based on the particle size led to very distinct fractions which can be divided in two main groups: the smallest fractions (<250 μm) characterized by lower C:N ratios and the larger fractions (>250 μm) characterized by higher C:N ratios. The smallest fraction (<45 μm) with the lowest C:N ratio, due to a low C content and high N content, would be mainly composed of water soluble elements. Indeed, a major portion of the water added for slurry dilution, together with most of the ammoniacal-N, did end up in the <45 μm size fraction, thereby explaining why this fraction had a four times higher N content than the other fractions. Consequently, the <45 μm size fraction should be readily available for microbial utilization and be considered the most utilizable or readily decomposable. The largest fraction (>2000 μm) and the whole slurry did have significantly lower lignin contents than in the other particle size fractions. The low amount of lignin observed in the >2000 μm fraction may be due to it being mainly composed of only partly digested pieces of straw, maize and grass leaves.

According to Masse et al. (2005), the kinetics and efficiency of biological and physico-chemical degradation of slurry depends on its solid contents and particle size distribution. Hydrolysis rates and particle size are negatively correlated because smaller particles have a higher surface to volume ratio available for bacterial colonization and reaction (Masse et al., 2005). Furthermore, the smaller slurry-derived particles will percolate more easily through the soils and could reach deeper soil horizons. Therefore, the temporal degradation and flux trends in range of slurry fractions isolated in our experiment can be considered as a useful tool to help decipher and interpret the general trends observed in the literature for whole slurry applications to agricultural soils.

The $\delta^{13}C$ difference in signatures between the C_4 and the C_3 slurries (ΔSIC_4–$\Delta SIC_3 = 5.9‰$) was less than the maximum shift of ca. 14‰, probably resulting from some contributions of C_4-feed concentrate and maize silage to the predominantly fed grass silage on the farm. It is worth noting that for all the slurry fractions the value of (ΔSIC_4–ΔSIC_3) was higher than 5.9‰.

4.2. CO_2 fluxes

In agreement with previous studies (Chadwick et al., 1998; Rochette and Gregorich, 1998; Flessa and Beese, 2000; Rochette et al., 2000; Chantigny et al., 2001, 2002; Bol et al., 2003), the slurry application to soil was followed by a large, immediate increase in CO_2 emissions. Similarly, all slurry fraction amendments significantly increased the level of soil CO_2 emissions ($P<0.05$) but not to the same

extent. The evolution of CO_2 fluxes after applications of slurry fractions to soil can be divided into three phases: (i) an increase just after application, (ii) followed by a gradual decrease, to finally (iii) relatively constant values closed to those observed in the control. In the first 4 days, the emissions increased by a factor of 2–8 compared with the soil control. Considering the cumulative CO_2 emissions for the first 97 days of incubation, 15–35% of the cumulative CO_2 released by six slurry fractions and ca. 50% of the CO_2 released by the whole slurry was emitted in the first 3 days after slurry incorporation. Flessa and Beese (2000) attributed the high CO_2 emissions in the first hours after application to the release of CO_2 dissolved in slurry or CO_2 formation from HCO_3^- and CO_3^{2-} dissolved in slurry. According to Rochette et al. (2004), a strong CO_2 flush is often observed when anaerobically stored slurry are applied to acidic soils, as in our cases (pH = 5.2), due to the slurry ammonium carbonates dissociation which induced a CO_2 release. Similarly, Génermont (1996) reported very large carbonated-induced CO_2 emissions after cattle slurry application under field conditions. Such carbonated-derived CO_2 emissions will be characterized by highly enriched ^{13}C values (Angers et al., 2007) as discussed in Section 4.3. The largest CO_2 production rates in our study were initially observed in the treatment with the finest applied slurry particles (<45 μm). It could be that this related to the higher amount of applied N in this fraction, when compared with all other fractions (~5 times more than with the >2000 μm fraction). Furthermore, the surface to volume ratio of the smallest particles resulted in higher enhanced bacterial colonization and hence relatively more enhanced carbonate induced CO_2 emissions. It is well known that other parameter such as pH, ammonia:total N ratio, carbonate-C:total C ratio and volatile fatty acid:total C ratio influence C mineralization and CO_2 emissions from both the soil and slurry (Sommer and Husted, 1995; Sommer and Sherlock, 1996; Sorensen, 1998). Therefore, such parameters could be measured in future studies to help further explain the differences in CO_2 emissions among size fractions.

Following this initial release, CO_2 fluxes generally decreased in all amended treatments. The rapid decrease in CO_2 emissions, particularly in smaller particle size (<250 μm) treatments in the first 4 days of incubation, was partly caused by a shortage of easily respirable C following initial flush of the labile C from these slurry fractions directly after application. In the subsequent phase, the CO_2 emissions from the slurry fractions were more stable and still above that of the control soil, partly due to the consumption by the soil heterotrophs of the readily available C compounds in slurry (e.g. volatile fatty acids), which are abundant in anaerobically stored slurry (Kirchmann and Lundvall, 1993; Chantigny et al., 2001). In contrast to the first period, the CO_2 emissions were now more pronounced in the treatments with the larger particles, especially the >2000 μm fraction. According to Aita et al. (1997), the larger slurry particles had to be first colonized by soil microbes before significant decomposition occurred, which then led to an higher C availability and consequently higher CO_2 emissions.

In the last period between the 97 and 332 days after application, the microbial release of respirable C compounds in the soil only (control) and (soil + slurry fractions) treatments occurred at similar rates. Rochette et al. (2000) also observed different CO_2 fluxes from pig slurry amended soils and non-amended soils, but for only 1 month after slurry application and concluded that pig slurry application had no significant long-term effect on microbial biomass carbon nor on total C content of the soil.

The results of the current study did suggest a multi-stage decomposition of the slurry fractions, which is common for many components added to soil (Bol et al., 2003), as well as for soil itself (Kristiansen et al., 2004). For the whole slurry, the initial rapid decrease in emitted CO_2 was exponential, whereas in the slurry fractions such decrease was less severe. If, these temporal trends in CO_2 flux following different slurry applications are attributed to the decomposition of the labile C fraction of the slurry (Bol et al., 2003), then the isolated slurry particle size fractions did contain less labile C than the whole slurry, thereby explaining that their first stage of decomposition was less pronounced. Furthermore, the lignin content of the slurry fractions (except the >2000 μm fraction) was significantly higher than those of the whole slurry. Therefore, the C degradation of the slurry fractions in the second stage, due to the presence of more recalcitrant fraction (here lignin) should be slower than in the whole slurry and >2000 μm fraction. This would explain the differences in terms of CO_2 emissions between fractions and whole slurry after the first few days. After 97 days of incubation, less than 10% of the total initial C had been lost by CO_2 emissions. This value is low when compared with results obtained by Flessa and Beese (2000) who observed that more than 38% of total C from cattle slurry was lost during the same time period. However, the amount of C lost after 9 days (less than 1%) in our study was comparable to that measured over the same period in the study by Bol et al. (2003). Similarly, the C loss observed after 332 days (ca. 18% of total carbon) was in same range as observed by Kristiansen et al. (2004) after 224 days from soil incubated with maize-derived sheep faeces (15–20% of total initial carbon). The current study did indicate that the slurry application to soil had a pronounced but transient effect on CO_2 emissions, since the slightly higher respiratory activity in the first 4 days after application levelled off within a few weeks. Our study did indicate that the application of the six isolated fractions resulted in a lower CO_2 emission during the first 2 days after application when compared with the whole slurry application. Therefore, it can be considered that the slurry pre-treatment by solid separation leading to two or more fractions with different particle sizes has potential to reduce short-term CO_2 emissions immediately after slurry application to soil.

4.3. CO_2 emissions sources and PE

Slurry is a complex mixture of compounds that suffer degradation at different rates and should therefore be divided in separate (size) fractions, in order to assess how and when such isolated slurry components contribute to total CO_2 emissions, depending on their relative abundance and their availability to act as substrate for microbial decomposition and respiration. The contribution of slurry C to total CO_2 emissions was larger in the finest particle size fractions (<250 μm) during the first 8 days after soil amendment, but tended to be similar in all fractions later. Such results were not unexpected considering the fact that small particles are more reactive due to their high surface to volume ratio. Furthermore, an higher amount of total N and carbonate might have been introduced with these smallest particles since these fractions contains most of the water added for slurry size separation.

The $\delta^{13}C$ values of CO_2 produced in C_4 treatments of the whole slurry and its particle size fractions were generally higher than their respective C_3 treatments. This occurred especially in the first few hours after application, with mean ΔC_4–C_3 values in the range 1.0–8.5 against 0.1–3.0 in all treatments in the other days. Similar results were reported by Bol et al. (2003) with ΔC_4–C_3 values in the range 1.3–6.4. The intense relative ^{13}C enriched peaks of CO_2 production mentioned in the previous section in the first few hours following slurry or slurry fractions application were attributed to the rapid release of carbonates (Angers et al., 2007). We observed that contribution of the slurry derived C in the emitted CO_2 decreased quickly, in agreement with Rochette et al. (2000). In this first stage (lasting only a first few days), the slurry-derived CO_2 should be mainly due to the decomposition of labile C from small particles and release of carbonate-C. Thereafter, in the second phase, the decomposition process was much slower and might have included decomposition of readily available organic substrates and more recalcitrant material found in the coarser particles.

The $\delta^{13}C$ of CO_2 in the control was on average 5.4‰ higher than the value of the SOM from where it originated (−29.2‰). In a similar study, Angers et al. (2007) also reported a difference of 5.0‰. The differences between the $\delta^{13}CO_2$ from the amended treatments and the control in the present work are also in line with other studies, which also reported comparable high values during the few hours after application but very similar thereafter (Angers et al., 2007; Kristiansen et al., 2004).

The calculated priming factors in our study were generally lower than for the same soil under comparable experimental conditions obtained by Bol et al. (2003). Furthermore, Bol et al. (2003) only observed positive PE, whereas in the current study both positive and negative PE was observed. However, Bol et al.(2003) only studied the whole slurry and differences may also have been due to the different sampling periods of both soil and slurry, i.e. summer vs. winter. This may have influenced the soil and/or slurry quality, expressed through a decrease of the easily mineralizable C that in turn induced a decrease of their respiration rate. Our data showed that timing and type (positive or negative) of the PE depended on the slurry particle size.

Many mechanisms for PE have been proposed. However, generally, they remain controversial and hypothetical because the effects observed appear to be specific to the experimental conditions used (Kuzyakov and Bol, 2006). Recently, Kuzyakov and Bol (2006) showed that the dynamic of PE involves a chain of mechanisms. In a first step, microorganisms from soil switch from the decomposition of the hardly utilizable substrates like SOM to the freshly added substrate or part of this substrate that contain easily utilizable C source, such as some of the slurry fractions. This step is generally called "preferential substrate utilization". The second phase, called "microbial activation", corresponds to an increase of the microbial activity as a consequence of the first phase. After consumption of the most easily utilizable substrates, the activated microorganisms will use the remaining substrates, which are more utilizable than those normally present in the soil. Finally, the initial state of the soil will be reached as a consequence of the decline of microbial activity and biomass. Our data supported this theory. The C availability of the slurry fractions used in the present work depended on many factors and namely on the particle size which affected the decomposition rate and accessibility of the microorganism to these substrates.

The lack of many distinct PEs and increased CO_2 emission in the coarsest slurry fraction (>2000 μm) after its incorporation suggest that this fraction is less susceptible to priming and microbial decay. This fraction is mainly composed of large pieces of maize, grass or straw not digested by the cows, which had first needs to be colonized by soil microbes before significant mineralization and, therefore, requires more time to decompose (Aita et al., 1997). This fraction would normally remain on the soil surface after slurry application, with much reduced access for degradation by the soil microbial community (unless incorporated by machine or earthworms), hence would contribute less to the overall slurry-derived CO_2 emission. Negative PEs were mainly observed in the smallest slurry fractions. In the literature, negative PE are not described quite as often as positive PE (Kuzyakov et al., 2000). However, negative PE is of great significance to ecosystem organic matter dynamics. Indeed, the occurrence of negative PE implies that the addition of a substrate results in a reduced loss of native soil C from the substrate amended soil compared to the unamended soil. Reasons for negative PE for soil carbon after addition of different substances are reviewed by Kuzyakov et al. (2000), but they all remain hypothetical. Nevertheless, in our study, the most significant negative PE were observed in the finest (<250 μm) fraction after day 8. These fractions did have the lowest C:N ratio and induced the highest CO_2 emissions in the first few days after application to the soil.

Therefore, a possible decrease of the C:N ratio of the remaining slurry residue, after the initial labile respirable C losses from slurry fraction may explain the observed significant negative PE after day 8.

As could be seen in Fig. 2, the preferential substrate utilization step was most clearly observed shortly (day 0.12) after slurry application (3 h) in the finer (most easily available) fractions, especially the 150–250 and 45–150 μm fractions where most of the CO_2 emitted was slurry derived. In the larger fractions, namely the 425–2000 and >2000 μm fractions, the amount of slurry-derived CO_2 emissions were not so significant in the first day but increased in the following days. Kuzyakov et al. (2000) and Kuzyakov and Bol (2006) have reported that PEs occurred immediately or very shortly after the addition of a specific substance to the soil.

Therefore, it can be hypothesized that this step started earlier in the finest slurry particles size, as the most labile carbon was readily available but rapidly used. In contrast, in the large particles it continued longer due to the delay for microbes to colonize larger particles. As more carbon was initially available for priming in the finest fractions, a significant positive PE was observed in the <0.45 and 45–150 μm fractions during the first 2 days, whereas the positive PE started later in coarser fraction. The significant positive PE observed during the first 2 days in the treatments with the smallest fractions may also have been partly due to the higher amount of N (i.e. ammoniacal N) applied in these treatments. The microbial activation phase would also be influenced by the particle size. Indeed, as the relative extent of the enhanced CO_2 losses in each fraction was directly related to this phase, it is likely that this process took place first in the finest fraction and lasted up to day 4, whereas in the coarser fractions microbial activation was delayed. Finally, on day 56, the initial state of the soil was re-established in all treatments, except for the finest fraction where a part of the CO_2 released was still slurry derived.

Application of different slurry particle sizes could be useful for better optimization of slurry management considering that smallest particles have a relatively, quick and short effect whereas the coarser fractions will take more time to be degraded but their influence will last longer. Importantly, it was also shown that slurry application to soil could induce both an increase or decrease of the native soil C losses depending on the slurry particle size.

5. Conclusions

(1) Slurry fractionation based on particle size led to six distinct fractions in terms of carbon, nitrogen and dry matter. This particle size fractionation has the potential to reduce or modify short-term CO_2 emissions immediately after slurry application to soil.
(2) All slurry fraction amendments significantly increased soil CO_2 emissions on the first days of the 332 days experiment when compared to the unamended control. The increased CO_2 flux had a negative relationship with slurry particle size, but its duration was positively correlated with slurry particle size.
(3) The overall contribution of slurry C to total CO_2 emissions was higher in treatments where the smallest slurry particle sizes were applied to the soil.
(4) The PE depended on the slurry particle size. Positive PE appeared later in the treatment with coarser slurry particle fractions. Negative PE was observed mainly in the treatment with the smallest size slurry fractions.

Acknowledgements

The authors thank Pilar Flores for her technical assistance as well as the two reviewers for their excellent revision of the manuscript and constructive suggestions. IGER is grant aided by the Biotechnology and Biological Science Research Council (BBSRC).

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