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^{13}C signature of CO_2 evolved from incubated maize residues and maize-derived sheep faeces

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Abstract

Analyses of the spatial and temporal variations in the natural abundance of ^{13}C are frequently employed to study transformations of plant residues and soil organic matter turnover on sites where long continued vegetation with the C_3-type photosynthesis pathway has been replaced with a C_4-type vegetation (or vice versa). One controversial issue associated with such analyses is the significance of isotopic fractionation during the microbial turnovers of C in complex substrates. To evaluate this issue, C_3-soil and quartz sand were amended with maize residues and with faeces from sheep feed exclusively on maize silage. The samples were incubated at 15 °C for 117 days (maize residues) or 224 days (sheep faeces). CO_2 evolved during incubation was trapped in NaOH and analysed for C isotopic contents. At the end of incubation, 63 and 50% of the maize C was evolved as CO_2 in the soil and sand, respectively, while 32% of the faeces C incubated with soil and with sand was recovered as CO_2. Maize and faeces showed a similar decomposition pattern but maize decomposed twice as fast as faeces. The $\delta^{13}C$ of faeces was 0.3‰ lower than that of the maize residue ($\delta^{13}C$ −13.4‰), while the $\delta^{13}C$ of the C_3-soil used for incubation was −31.6‰. The $\delta^{13}C$ value of the CO_2 recovered from unamended C_3-soil was similar or slightly lower (up to −1.5‰) than that of the C_3-soil itself except for an initial flush of ^{13}C enriched CO_2. The $\delta^{13}C$ values of the CO_2 from sand-based incubations typically ranged −15‰ to −17‰, i.e. around −3‰ lower than the $\delta^{13}C$ measured for maize and faeces. Our study clearly demonstrates that the decomposition of complex substrates is associated with isotopic fractionation, causing evolved CO_2 to be depleted in ^{13}C relative to substrates. Consequently the microbial products retained in the soil must be enriched in ^{13}C.

Keywords: $\delta^{13}C$; CO_2; Incubation; Isotopic discrimination; Maize; Sheep faeces

1. Introduction

The discrimination against the ^{13}C isotope associated with photosynthesis results in plant biomass that is depleted in ^{13}C relative to atmospheric CO_2 (O'Leary, 1988), the degree of depletion reflecting the type of photosynthetic pathway. Plants with the C_3-type (Calvin cycle) pathway become distinctly lower in ^{13}C than plants with the C_4-type (Hatch-Slack) pathway. This difference between C_3 and C_4 plants in ^{13}C abundance provides a unique tool in studies on C turnover in soils.

The decomposition of plant residues results in the formation of decomposer biomass, CO_2 and soil organic matter (SOM) with a ^{13}C signature that reflects that of the decomposing residue. On sites where long continued C_3 vegetation is replaced by C_4 plants (or vice versa), the new vegetation introduces a shift in the isotopic composition of the decomposers and the various SOM pools, and measurements of the spatial and temporal variations in the $^{13}C/^{12}C$ ratio have been widely used to delineate pools and turnover rates of SOM (Balesdent et al., 1987; Bonde et al., 1992; Besnard et al., 1996; Six et al., 2000; Roscoe et al., 2001).

The ^{13}C signature of CO_2 evolved during decomposition has been employed to trace the shorter-term decay pattern of plant residues and various other substrates added to soil (Mary et al., 1992; Rochette et al., 1999; Schweizer et al., 1999; Ekblad and Högberg, 2000) and to separate soil CO_2 evolution into contributions from root and decomposer respiration (Robinson and Scrimgeour, 1995; Cheng, 1996; Rochette and Flanagan, 1997; Andrews et al., 1999).

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Key issues in the application of natural ^{13}C abundance to study C turnover following addition of complex substrates to soil are the degree of isotopic homogeneity of the substrate and the degree of isotopic fractionation during the decomposition processes. It is well documented that different chemically defined plant components differ in their ^{13}C signature, the most notable feature being the depletion in ^{13}C of the lignin fraction (Benner et al., 1987; Wedin et al., 1995; Schweizer et al., 1999). Moreover, different plant components are known to decompose at different rates in the soil, lignin being more resistant than polysaccharides and proteinaceous material.

The significance of isotopic fractionation during microbial decomposition remains a controversial issue. It has been reported that ^{13}C is preferentially retained in microbial biomass (and thus in the soil) during decomposition, suggesting that the evolved CO_2 is depleted in ^{13}C compared with the initial substrate (Blair et al., 1985; Mary et al., 1992; Schweizer et al., 1999; Henn and Chapela, 2000). However, other studies suggest that isotopic discrimination may be negligible (Cheng, 1996; Ekblad and Högberg, 2000; Ekblad et al., 2002). Collins et al. (2000) reported no isotopic discrimination when maize residues were incubated with sand for 50 days, the $\delta^{13}C$ of the evolved CO_2 remained nearly constant at 12.2‰ (similar to that of the residue itself). Evidently, conflicting evidence is abundant, especially with regard to the quantitative significance of isotopic discrimination associated with turnover of complex substrates, although authoritative reviews generally suggest isotopic discrimination to be of little importance (Wedin et al., 1995; Boutton, 1996; Balesdent and Mariotti, 1996; Ehleringer et al., 2000). However, a preferential retention of ^{13}C over ^{12}C in the soil profile is commonly reported (Balesdent et al., 1993; Gregorich et al., 1995; Roscoe et al., 2001). Various mechanisms affecting the isotopic behaviour during C turnover in soil have been discussed by Amundson and Baisden (2000) and Ehleringer et al. (2000).

The significance of isotopic heterogeneity of complex substrates and of isotopic fractionation during microbial decomposition in soil is crucially important in studies where variations in the natural abundance of ^{13}C are applied to evaluate short- as well as long-term C turnover. In this study, we examined the ^{13}C signature of CO_2 evolved from maize (C_4-plant) residues and maize-derived sheep faeces incubated at 15 °C for 117 and 224 days, respectively, with quartz sand and with soil previously under C_3 crop plants. A novel aspect of this study is the inclusion of faeces along with the maize residue from which it originated. During the passage through the sheep's digestive tract, the more labile fractions of the maize residue will be decomposed and the faeces thus represents a partially decomposed substrate.

2. Materials and methods

2.1. Soils and substrates

The soil was from the 0–15 cm of a loamy sand with 7% clay (<2 μm), 10% silt (2–20 μm), 43% fine sand (20–200 μm), 39% coarse sand (200–2000 μm), 1.4% C and a pH ($CaCl_2$) of 5.8. The soil had been under C_3 crops (cereal dominated crop rotations given mineral fertilizers) for as long as records have been kept. The soil was sampled by coring to provide one large bulk sample corresponding to ca. 11 kg dry soil. After sampling, the soil was air-dried, sieved to <2 mm, wetted to 150 g water kg^{-1} soil (corresponding to 54% of soil water-holding capacity), and pre-incubated at 10 °C for 100 days. Before used in incubation studies, the soil was rewetted to 150 g kg^{-1} soil.

Pure quartz sand was wetted to 150 g water kg^{-1} sand (~70% of the sand water-holding capacity) with a multi-nutrient solution (Gahoonia and Nielsen, 1992) enriched with $NaNO_3$ (corresponding to 24 mg N kg^{-1} sand).

Maize (*Zea mays*, L.) was grown at Askov Experimental Station (55°28′N, 09°07′E) to growth stage BBCH 75 (Lancashire et al., 1991) and whole-crop harvested for silage. At this growth stage, the maize crop typically holds 30% dry matter and the kernels in the middle of the cob have become yellowish-white with a milky content. The maize crop was coarsely chopped, dried to constant weight at 80 °C and kernels removed. The dried maize residue was then chopped to ~2 cm, mixed and stored.

Adult domestic sheep were fed exclusively with whole-crop maize silage for 20 days to allow equilibrium in the ^{13}C content of the sheep faeces (Coates et al., 1991). During the following 10 days, faeces were collected on a daily basis and frozen until required for experimentation.

2.2. Incubation

Portions of moist soil or sand (300 g) were weighed into polyethylene (PE) containers and packed to a density of 1.4 g cm^{-3}. Air-dry maize residue (4 g dry matter, DM) or sheep faeces (6 g DM) was distributed on the surface and another 300 g soil or sand placed on top of the organic amendments. Each PE container was then transferred to a 3-l airtight glass jar containing a CO_2 trap (10 ml of 2 M NaOH) and a beaker with water to maintain air humidity. The jars were incubated at 15 °C for 117 days (maize residue) or 224 days (sheep faeces). Reference treatments with soil but no organic amendments and blanks without soil and organic amendments were included. Thus, the following treatments were established: maize residue sandwiched in soil (M_{soil}, three replicates), sheep faeces in soil (F_{soil}, three replicates), reference soil without amendments (Reference soil, three replicates), maize residue in sand (M_{sand}, six replicates), and sheep faeces in sand (Fsand, six replicates).

CO_2 evolution was determined with decreasing frequency, initially each day and finally every week. At each

sampling, the CO_2 trap was removed and replaced by a fresh one, and water was added to the beaker as necessary. In a closed incubation system with frequent replacement of the NaOH, the efficiency of the CO_2 trap is close to 100% and isotopic fractionation will remain insignificant (Boutton, 1991).

2.3. Analyses

The maize residue and sheep faeces used for incubations were analysed for contents of total C and N, lignin, acid detergent fibres (ADF), cellulose and hemicellulose (Van Soest, 1963). The $^{13}C/^{12}C$ ratio of the soil, the maize residue and the sheep faeces was determined by isotope-ratio mass spectrometry following dry combustion of ball-milled dry samples (ANCA.SL System, Europa Scientific Ltd, Crewe, UK). Total CO_2 evolution was determined by titration of excess NaOH in the CO_2 trap with 50 mm HCl and the CO_2 trapped in NaOH was then precipitated with $CaCl_2$ (4 ml 2 M NaOH + 4 ml 2 M $CaCl_2$). The NaOH was first adjusted to pH 10 with HCl to avoid precipitation of $Ca(OH)_2$. After addition of $CaCl_2$, the mixture was centrifuged at 4 °C and 30,000*g*. The supernatant was discharged and the pellet stirred in 5 ml of water and centrifuged again. This was repeated three times before freeze-drying the pellet containing the precipitated CO_2. Five milligrams of the dried pellet was used to measure the $^{13}C/^{12}C$ ratio on the mass spectrometer.

2.4. Calculations

By convention, the natural abundance of ^{13}C is expressed as

$$\delta^{13}C\ (‰) = \frac{{}^{13}C/{}^{12}C_{sample} - {}^{13}C/{}^{12}C_{PDB}}{{}^{13}C/{}^{12}C_{PDB}} \times 1000,$$

where $^{13}C/^{12}C_{sample}$ is the $^{13}C/^{12}C$ isotope ratio of the sample and $^{13}C/^{12}C_{PDB}$ is the isotope ratio of the international PDB (Pee Dee Belemnite) standard.

The fraction (f) of enrichment of the sheep faeces relative to the original maize residue was calculated as

$$f\ (\%) = \frac{\delta^{13}C_{faeces} - \delta^{13}C_{soil}}{\delta^{13}C_{maize} - \delta^{13}C_{soil}} \times 100,$$

where $\delta^{13}C_{faeces}$ is the isotopic ratio of the sheep faeces and $\delta^{13}C_{maize}$ is the isotope ratio of the maize residue.

The CO_2-C derived from faeces or maize residues was determined as the difference between CO_2 produced from the reference soil and amended soils.

Statistical analyses were mixed variance analysed on differences between subsequent sampling dates to allow a temporal comparison of isotopic compositions of the evolved CO_2, for $\delta^{13}C$ and t-tests for paired difference of means from the samples of organic material. Both were calculated with the statistical package SAS 6.11 (SAS Systems, Cary, NC). Standard errors of means are denoted SEM.

3. Results

The soil used for incubation showed a $\delta^{13}C$ of −31.6‰, a signature typical for organic matter in a soil that has been under C_3 vegetation for very long periods. Table 1 shows chemical characteristics of the maize residue and the sheep faeces. The faeces were higher than the maize in N, lignin and ADF. The C/N ratios of the faeces and its water-soluble fraction were substantially lower than the maize residue equivalents, but no differences were found for the lignin/N ratio. The $\delta^{13}C$ values of maize and faeces were −13.4 and −13.7‰, respectively. Assuming a negligible isotopic fractionation during the passage of maize through the sheep digestive tract and that the sheep had been raised on a C_3-diet before the experiment, it was estimated that 98.0% of the faeces was derived from the maize forage and 2.0% originated from sheep tissue and exudates.

Fig. 1 shows the CO_2 evolution rates during incubation at 15 °C. For the reference soil without organic amendments,

Table 1
Characteristics of maize residues and maize-derived sheep faeces used in the experiment (mg g^{-1} dry matter)

Material	C (mg g^{-1})	N (mg g^{-1})	Lignin (mg g^{-1})	ADF[a] (mg g^{-1})	Cellulose + hemi-cellulose (mg g^{-1})	Water-soluble C[b] (mg g^{-1})	C/N		Lignin/N	$\delta^{13}C$[c] (‰)
							Whole material	Water soluble		
Maize	38.3	0.72	2.0	25.8	23.9	27.4	53	40	2.8	−13.39
SEM	0.6	0.02	0.3	0.2	0.1	ND	ND	ND	ND	0.06
Faeces	36.0	2.19	6.1	31.8	25.7	25.5	16	8	2.8	−13.74
SEM	0.5	0.02	0.2	0.2	0.0	ND	ND	ND	ND	0.04
Probability	*	***	***	**	*	ND	ND	ND	ND	**

Significance levels: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. ND = not determined. SEM = Standard error of means, n = 4 or 5.
[a] Acid detergent fibres.
[b] Soluble in water at 23 °C for 48 h.
[c] $\delta^{13}C = (R_{sample} - R_{reference})/R_{reference} \times 1000$, where $R = {}^{13}C/{}^{12}C$.

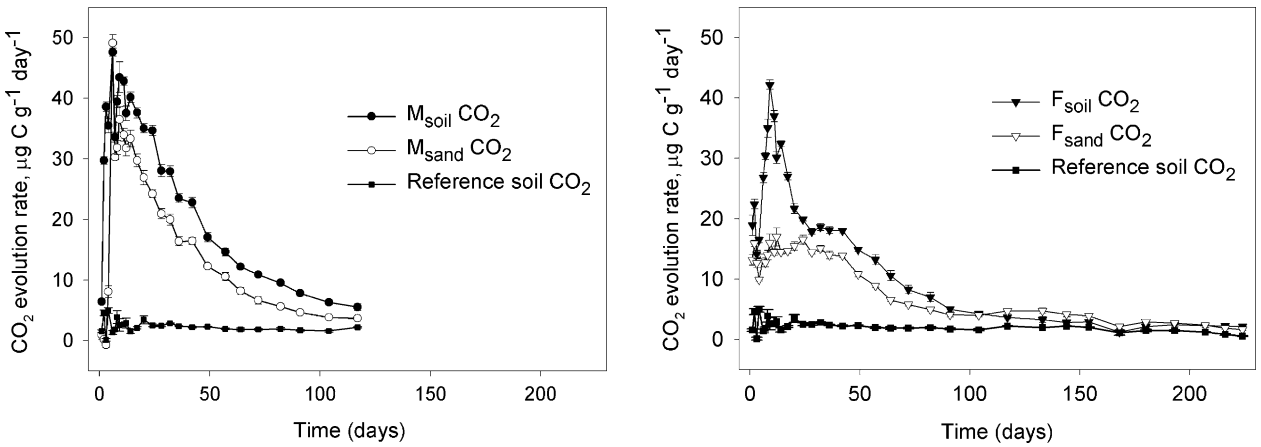

Fig. 1. The CO_2 evolution rate from soil and sand incubated with maize residues (a; M_{soil} CO_2 and M_{sand} CO_2) and maize-derived sheep faeces (b; F_{soil} CO_2 and F_{sand} CO_2) for 117 and 224 days, respectively. Soil without organic amendments (Reference soil CO_2) was included. Bars indicate ±1 SEM, $n = 3$ or 6.

the respiration was relatively low and constant except for the initial phase of the incubation. The organic amendments caused a substantially higher CO_2 evolution rate during the first 120 days of incubation; the rate peaked at day 12 then decreased. Generally, the CO_2 evolution rate was higher from amended soils than from sand-based incubations. During the initial 3–4 weeks, the difference between soil- and sand-based incubations was larger for faeces than for maize residues.

After 117 days of incubation, 63 and 50% of the C added in maize residues had been recovered as CO_2 in soil- and sand-based incubations, respectively (Fig. 2). The corresponding values for incubated faeces were 20 and 15%. After 224 days at 15 °C, 32% of the faeces C had been collected as CO_2 in soil- as well as sand-based treatments. Thus, maize residue decomposed more readily than sheep faeces, and decomposition was more rapid in soil than in pure quartz sand. For both incubation media, maize residues were decomposed twice as fast as sheep faeces.

Fig. 3 shows the $\delta^{13}C$ values associated with the CO_2 collected during incubation. The initial CO_2 flux from the reference soil was more enriched in ^{13}C than its soil C pool, but from day 6 onwards, the $\delta^{13}C$ value associated with CO_2 remains around −32‰, a value close to that of the reference soil C pool ($\delta^{13}C = -31.6$‰). The difference in $\delta^{13}C$ values between two subsequent sampling dates averages 1.0‰ (SEM = 0.3‰). Excluding the first sampling date (day 3), the average difference in $\delta^{13}C$ of the CO_2 from the reference soil is reduced to 0.4‰ (SEM = 0.2‰).

For incubations with maize residues, the CO_2 was depleted in ^{13}C by 1–7 $\delta^{13}C$ units relative to the maize residues itself ($\delta^{13}C = -13.4$‰). Excluding the first sampling date, the average difference in $\delta^{13}C$ between two subsequent sampling dates became 1.2‰ (SEM = 0.4‰) for CO_2 evolved from maize residues incubated with sand. The $\delta^{13}C$ of the CO_2 remained about three units lower than the $\delta^{13}C$ of maize whereas the $\delta^{13}C$ of CO_2 from maize incubated with C_3-soil decreased steadily during the incubation period.

For sheep faeces ($\delta^{13}C = -13.7$‰), the CO_2 evolved during the initial 3-week period was significantly depleted in ^{13}C (up to 11 $\delta^{13}C$ units). Subsequently, the $\delta^{13}C$ of CO_2 from faeces incubated with sand increased until day 104 and then decreased again. During the period where CO_2 evolution was dominated by contributions from faeces (Fig. 1), the $\delta^{13}C$ of CO_2 from soil- and sand-based incubations was similar. Later when the CO_2 derived from the organic amendment levelled off, the CO_2 from incubations with soil became depleted in ^{13}C compared to CO_2 from sand-based incubations.

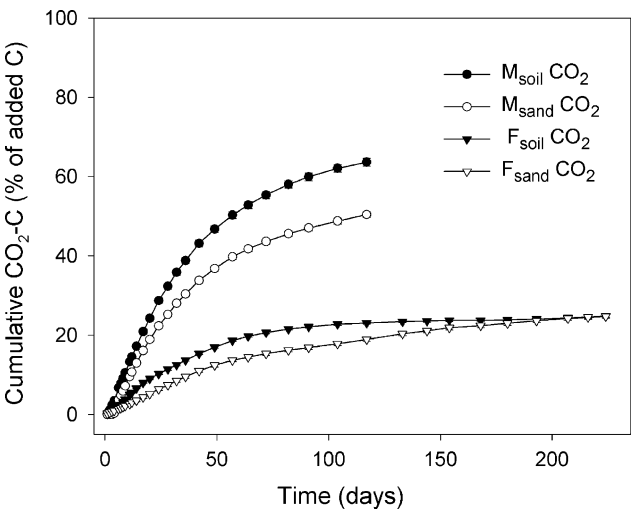

Fig. 2. The cumulative CO_2 evolution from soil and sand incubated with maize residues (M_{soil} CO_2 and M_{sand} CO_2) and maize-derived sheep faeces (F_{soil} CO_2 and F_{sand} CO_2) for 117 and 224 days, respectively. Bars indicate ±1 SEM, $n = 3$ or 6.

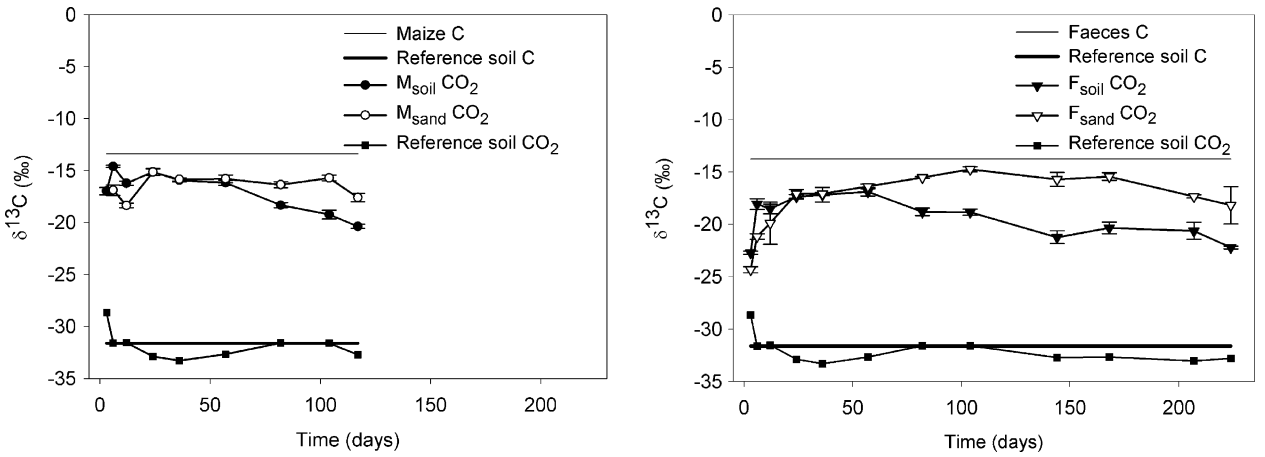

Fig. 3. The ^{13}C signatures ($\delta^{13}C$ ‰) of the CO_2 evolved from C_3-soil and sand incubated with maize residues and maize-derived sheep faeces, and from a C_3-soil without organic amendments (Reference soil). Horizontal lines indicates the $\delta^{13}C$ ‰ of the maize residues (Maize C), the sheep faeces (Faeces C), and C_3-soil (Reference soil C) at the start of the incubation. Bars indicate ± 1 SEM, $n = 3$ or 6.

4. Discussion

The unamended reference soil showed a transient increase in respiration during the first few days of incubation. This initial flush of CO_2 is ascribed to decomposition of remains from soil organisms that were killed during the sample preparations that preceded incubation. This CO_2 was enriched in ^{13}C, suggesting that the microbial tissues and metabolites which served as substrate for this microbial activity were enriched in ^{13}C. This deduction accords with previous reports (Blair et al., 1985; Mary et al., 1992; Henn and Chapela, 2000) showing that CO_2 evolved during decomposition of various simple and uniformly labelled substrates is depleted in ^{13}C relative to the substrates. Since the evolved CO_2 is typically depleted in ^{13}C, a preferential retention of ^{13}C over ^{12}C most probably occurred in the microbial biomass and the metabolites of the reference soil.

During most of the subsequent incubation period, the CO_2 evolved from the reference soil remained similar or slightly lower in $\delta^{13}C$ than the soil C pool, substantiating that microbial turnover of C may be associated with a slight isotopic discrimination. This results in ^{13}C being retained in the SOM that accumulates in soil profiles (Balesdent et al., 1993; Gregorich et al., 1995; Roscoe et al., 2001).

The maize residue incubated with pure sand for 117 days at 15 °C lost half of its C as CO_2. The CO_2 evolved throughout this phase of maize residue decomposition remained depleted in ^{13}C, the $\delta^{13}C$ of the CO_2 being around 3‰ lower than that of the maize substrate. Similar observations have been reported for easily decomposable substrates incubated with sand (Mary et al., 1992; Schweizer et al., 1999) or incubated in liquid batch cultures (Blair et al., 1985; Henn and Chapela, 2000), confirming that decomposition of simple substrates may involve significant isotopic fractionation. In contrast, Ekblad and Högberg (2000) and Ekblad et al. (2002) reported insignificant ^{13}C discrimination during microbial respiration of simple sugars added to soil material, and Collins et al. (2000) recorded no fractionation in sand-based incubations of maize residues.

During the period where maize decomposition was most intense, the CO_2 from sand- and soil-based incubations showed similar ^{13}C signatures, despite a major difference in their CO_2 evolution rates. Subsequently, when the production of CO_2 from maize levelled off, the CO_2 from soil-based incubations gradually decreased in $\delta^{13}C$. As incubation proceeds, ^{13}C depleted CO_2 originating from the C_3-soil accounts for an increasingly larger proportion of the total CO_2 and provides a dilution of the more ^{13}C-enriched CO_2 associated with the continued decomposition of the remaining and biologically more resistant maize residue.

In accordance with Coates et al. (1991), the passage of the maize through the digestive tract of the sheep caused only a small decrease in $\delta^{13}C$ value (<0.5‰). The main differences in the chemical properties of maize and faeces were higher contents of lignin, ADF and N in the faeces. However, the level of lignin in both substrates was low, reflecting that the maize crop had been harvested well before physiological maturity.

The faeces C was generally less available to decomposition than maize C. For sand- as well as soil-based incubations, the CO_2 evolved during the initial decomposition phase was significantly depleted in ^{13}C. Ruminant faecal C is composed of C remaining in undigested maize residue, of C residing in microbial products and microorganisms from the rumen, the intestine and the hindgut, and of C originating from the digestive tract itself (secretions, mucus, and dead tissue). It was estimated that some 2.0% of the faecal C might have derived from the animal itself. The sheep had been raised on a C_3-plant diet before the experiment started, and most likely the fraction of the faeces C originating from the animals themselves carries

a ^{13}C signature that reflects the previous C_3-based diet. The sheep-derived faecal C is considered to be the dominant source of CO_2 very early in the incubation. The ^{13}C-depleted nature of this source of CO_2 explains the reduced $\delta^{13}C$ values recorded during this period. Subsequently, the $\delta^{13}C$ of the CO_2 from faeces incubated with sand increases gradually as contributions of CO_2 from maize-derived faecal C become quantitatively more important than contributions from sheep-derived faecal C. After 100 days of incubation, the CO_2 from faeces and maize showed similar ^{13}C signatures. The incubation of maize residues was terminated after 117 days while sheep faeces was incubated for 224 days. Following day 57, the CO_2 from soil-based incubations showed a declining $\delta^{13}C$ value for both substrates reflecting the increased contribution from soil-derived and ^{13}C-depleted CO_2. For faeces incubated with sand, the CO_2 evolved after 168 days of incubation showed a slight decrease with respect to $\delta^{13}C$. At this stage of decomposition, the respiration activity was very low and almost similar to that of the reference soil. The reduced ^{13}C abundance in the CO_2 may signify microbial decomposition of the more recalcitrant fraction of the faeces (e.g. lignin-type materials) depleted in ^{13}C relative to whole-faeces material.

We conclude that the decomposition of complex substrates (maize residues and sheep faeces) is associated with a significant discrimination against ^{13}C, causing the evolved CO_2 to be depleted in ^{13}C and the microbial biomass to be enriched in ^{13}C. The microbially mediated isotopic fractionation during turnover of organic amendments to soil has to be considered more closely, particularly in short-term studies of C turnover.

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