



# Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils

E.G. Gregorich<sup>a,\*</sup>, B.C. Liang<sup>b</sup>, C.F. Drury<sup>c</sup>, A.F. Mackenzie<sup>d</sup>, W.B. McGill<sup>e</sup>

<sup>a</sup>Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Central Experimental Farm, Ottawa, Ont., Canada K1A 0C6

<sup>b</sup>Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, Sask., Canada S9H 3X2

<sup>c</sup>Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, Ont., Canada N0R 1G0

<sup>d</sup>Department of Natural Resource Science, Macdonald Campus of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Que., Canada H9X 3V9

<sup>e</sup>Department of Renewable Resources, University of Alberta, Edmonton, Alta., Canada T4L 1W1

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## Abstract

Understanding the dynamics of soil C is key to managing soil organic matter to enhance soil quality and ecosystem functioning, and reduce trace gas emissions from soils. Our objective was to determine the source and turnover of C pools in some agricultural soils in eastern Canada. Soils from five field experiments under continuous maize cropping for 4–37 yr were sampled, and the organic C content and stable C isotope (<sup>13</sup>C) composition of whole soil and water soluble and microbial biomass fractions determined. The <sup>13</sup>C results showed a clear distinction between the water soluble organic C and microbial biomass C, with the water soluble organic C more like the whole soil and the microbial biomass more like the maize residues. A simple linear model was used to explore the relationship among the soil organic constituents and evaluate the turnover of these carbon pools. Even though the water soluble organic C had a higher turnover rate than the microbial biomass C, the proportion of C<sub>4</sub>-derived C in the biomass was about 2.5 times greater than that in water soluble organic C. Apparently the large amount of native soil C, the small amount of water soluble organic C, and its equilibrium with the native soil C, cause humus to dominate the isotopic composition of water soluble organic C even though the water soluble C is very active. Our results suggest that the quantity, as well as the turnover rate, of soil organic matter constituents that are in equilibrium influence the isotopic composition of such constituents. Crown Copyright © 2000 Published by Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

A better understanding of how soil organic matter affects soil quality, ecosystem functioning, and atmospheric CO<sub>2</sub> concentrations would be gained through knowledge of its dynamics. Soil organic matter comprises different groups of constituents that vary in mass and rate of turnover. Its dynamics reflect the bio-

logical activity, soil properties (e.g. texture), and the quantity and quality of plant residues returned to the soil. Humus is the largest, most stable pool of carbon in soil, comprising mostly resistant material (Jenkinson, 1990). The soil microbial biomass is a source and sink of biologically mediated nutrients and is responsible for transforming organic matter and nutrients within soil. Water soluble organic C (WSOC) is not only a C source for microorganisms, but its production is also believed to be microbially mediated (e.g. Christ and David, 1996). McGill et al. (1986) suggested that the flow of C through water soluble components supplies substrate for microbial biomass turnover.

\* Corresponding author. Tel.: +1-613-759-1909; fax: +1-613-759-1926.

E-mail address: gregoriche@em.agr.ca (E.G. Gregorich).

Table 1  
Site and soil characteristics and amounts, isotopic composition and proportions of C<sub>4</sub>-derived C in whole soil C, water-soluble organic C (WSOC) and microbial biomass C (MBC) in some eastern Canadian soils

Soil and location	Crop	Time <sup>a</sup> (yr)	Soil texture (g kg <sup>-1</sup> )	Whole soil C <sup>b</sup>			WSOC			MBC		
				Amount (g kg <sup>-1</sup> )	δ <sup>13</sup> C (‰)	C <sub>4</sub> -C (%)	Amount (mg C kg <sup>-1</sup> )	δ <sup>13</sup> C (‰)	C <sub>4</sub> -C (%)	Amount (mg C kg <sup>-1</sup> )	δ <sup>13</sup> C (‰)	C <sub>4</sub> -C (%)
Ste. Rosalie clay 45°3'N73°6'W	grass	8	sand = 100	44.2(0.9) <sup>c</sup>	-27.0(0.1)	43(3)	-27.5(0.5)	446(19)	-24.8(0.9)	49	446(20)	-18.4(0.6)
	maize		clay = 710	29.5(3.0)	-24.9(0.3)	16(2)	-24.9(0.5)	446(20)	-18.4(0.6)			
Brookston clay loam 42°1'N82°4'W	grass	37	sand = 280	40.7(2.7)	-26.4(0.3)	60(4)	-27.5(0.5)	589(11)	-23.9(0.5)	75	248(6)	-14.8(0.5)
	fertilized maize		clay = 370	21.8(1.3)	-22.9(0.4)	45(1)	-22.9(0.3)	218(23)	-14.8(0.2)			
Chicot sandy clay loam 45°3'N73°6'W	nonfertilized maize	37	clay = 370	19.6(0.2)	-24.0(0.3)	49(1)	-23.4(0.2)	241(10)	-24.7(1.0)	50	194(26)	-18.2(1.4)
	grass		sand = 500	15.7(0.4)	-24.5(0.6)	35(6)	-24.5(0.6)	239(27)	-23.5(0.6)			
Brandon loam 45°3'N75°4'W	maize	11	clay = 280	15.2(1.5)	-22.1(0.6)	33(4)	-22.1(0.1)	170(11)	-17.5(1.8)	51	294(22)	-23.8(0.8)
	grass		sand = 420	19.1(0.3)	-25.8(0.2)	25(1)	-26.7(0.4)	278(4)	-16.6(0.8)			
Fox loamy sand 42°5'N80°3'W	maize	4	clay = 280	19.2(1.1)	-25.1(0.1)	18(2)	-25.1(0.5)	170(11)	-17.5(1.8)	51	294(22)	-23.8(0.8)
	grass		sand = 880	7.1(0.2)	-25.7(0.3)	22(3)	-24.9(0.4)	278(4)	-16.6(0.8)			
	maize	7	clay = 60	7.9(1.0)	-23.6(0.3)	15(2)	-20.5(0.6)	278(4)	-16.6(0.8)	60	278(4)	-16.6(0.8)

<sup>a</sup> Period under continuous maize cropping.

<sup>b</sup> Data from Gregorich et al. (1996) and Liang et al. (1998).

<sup>c</sup> Numbers in brackets are standard deviations.

Our objective was to analyze the organic C content and stable C isotope (<sup>13</sup>C) composition of whole soil and water soluble and microbial biomass fractions to determine the source of this C in some eastern Canadian agricultural soils. A simulation model was used to evaluate the turnover of these carbon pools to get a better understanding of the relationships among these soil organic constituents.

## 2. Materials and methods

We sampled five fields in eastern Canada in the spring of 1996. All had been under continuous maize cropping for 4–37 yr and had different textures and soil C contents. Relevant characteristics of the sites and soils are given in Table 1. The maize was grown using practices typical in the region. At each site, soils that had been maintained under grass adjacent to the maize plots were used as reference samples. Ten to fifteen subsamples of the surface layer (about 0–20 cm depth) were collected at each site and then combined (~1 kg total). Visible plant residues and roots were removed and the fresh soil sieved (5 mm) and then stored at 2°C until analyzed. Maize grain was removed from the plots and the residues were incorporated into the soil by moldboard plow each autumn on the Ste. Rosalie, Chicot, Brookston and Brandon soils and in the spring on the Fox soil. Other details on the history of these sites are given by Liang et al. (1998) and Gregorich et al. (1996).

Soil microbial biomass C (MBC) was determined by the fumigation-extraction method (Voroney et al., 1993). Ultrapure water (Compact Milli-Q and Milli-RQ Water Systems, Millipore, Bedford, MA) and 125 mM K<sub>2</sub>SO<sub>4</sub> were used to extract soluble organic C with or without fumigation. In brief, soluble C extracted with 125 mM K<sub>2</sub>SO<sub>4</sub> from fumigated and nonfumigated samples was obtained by shaking for 1 h on a mechanical shaker and filtering through Whatman No. 2 filters. For water extracts glass beads were used to help disperse the soil particles during the shaking period for the Ste Rosalie soil which had a high (71%) clay content. Soluble C in the ultrapure water extracts (i.e. WSOC) was obtained by centrifuging at 10,000 rpm for 10 min and filtering through a 0.45 μm glass fibre filter using a suction funnel under a pressure of -7 kPa. Microbial biomass C was calculated as the difference in soluble organic C between fumigated and nonfumigated samples divided by 0.35 (Sparling et al., 1990). Soluble organic C was determined on a total organic carbon analyzer (Shimadzu TOC-5050, Tokyo, Japan). After determining soluble C in the ultrapure water extract, the solution samples containing small amounts of inorganic C were acidified to pH 3 with 50 mM H<sub>2</sub>SO<sub>4</sub> and then freeze-dried. The stable C isotope

ratios were measured using a tracemass isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) interfaced to a Roboprep unit (Knight et al., 1994). The natural abundance of heavy isotopes was expressed as parts per thousand relative to the international standard PDB using delta units ( $\delta$ ). The  $\delta^{13}\text{C}$  value was calculated from the measured C isotope ratios of the sample and standard gases as:

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 10^3$$

where  $R$  is the  $^{13}\text{C}/^{12}\text{C}$  molar ratio calculated from measurements of mass 45/44 ratio of the sample or standard carbon dioxide gas.

The  $\delta^{13}\text{C}$  (‰) of MBC was estimated as the  $\delta^{13}\text{C}$  of the C extracted from the fumigated sample in excess of that extracted from the nonfumigated sample, as follows:

$$\delta^{13}\text{C} = (\delta^{13}\text{C}_f \times C_f - \delta^{13}\text{C}_{\text{nf}} \times C_{\text{nf}}) / (C_f - C_{\text{nf}})$$

where  $C_f$  and  $C_{\text{nf}}$  were the amounts of C extracted from the fumigated and nonfumigated samples ( $\text{mg C kg}^{-1}$ ) and  $\delta^{13}\text{C}_f$  and  $\delta^{13}\text{C}_{\text{nf}}$  were the  $^{13}\text{C}$  natural abundance of the fumigated and nonfumigated extracts (‰), respectively.

At each site the soil under grass was considered the reference and the proportion of  $\text{C}_4$ -derived C in WSOC and MBC was calculated as follows:

$$\text{C}_4 - \text{C} (\%) = (\delta - \delta_{\text{ref}}) / (\delta_{\text{mr}} - \delta_{\text{ref}}) \times 100$$

where  $\delta = \delta^{13}\text{C}$  value of WSOC or MBC extracted from the maize soil,  $\delta_{\text{mr}} = -11.8\text{‰}$ , the mean value of maize residue (Liang et al., 1998) and  $\delta_{\text{ref}} = \delta^{13}\text{C}$  value of WSOC or MBC extracted from the reference grass soil.

A simple linear model was constructed to simulate the dynamics of humus C, WSOC and MBC in these soils (Appendix A). WSOC is the smallest component, MBC is the second smallest, and humus C is the difference between the whole soil C and the sum of the WSOC and MBC. In the model, WSOC is in equilibrium with soil C as a whole and with MBC, which is in turn supplied with C from maize or WSOC. The turnover time of the WSOC is determined by flux from humus and the microbial biomass. The MBC respire most of the C it receives from the maize residues and thus has only a small proportion left to equilibrate with WSOC. Turnover of MBC is set to match input of maize C, and the whole system is set to steady state.

### 3. Results and discussion

The flush of C induced by fumigation (i.e. fumigated minus nonfumigated samples) and extracted by either

125 mM  $\text{K}_2\text{SO}_4$  or ultrapure water was similar (Fig. 1). The actual amounts of C extracted by the water and the salt solution were also about the same in the control soils and the fumigated soils (data not shown). The C flush, expressed in  $\text{mg C kg}^{-1}$  soil, for the water extraction accounted for  $75 \pm 10\%$  of the fumigated value, and for the  $\text{K}_2\text{SO}_4$  extraction this was  $76 \pm 8\%$ . These findings suggest that the extraction efficiency (i.e.  $K_{\text{EC}}$ ) was the same for both extractants, and that the organic C extracted by 125 mM  $\text{K}_2\text{SO}_4$  and ultrapure water in fumigated or nonfumigated soils probably was derived from the same fraction of soil organic matter. Besides easier laboratory procedures, advantages of using water extracts for soil WSOC and MBC are that the large quantities of salt (with a relatively small amount of organic C in the  $\text{K}_2\text{SO}_4$  extract) may hamper accurate mass spectrometric analysis, and freeze-dried water extracts allow for direct combustion and simultaneous measurement of  $\delta^{13}\text{C}$  (Knight et al., 1994). Lower concentrations of  $\text{K}_2\text{SO}_4$  have also been used to reduce the osmotic effect on lysing living cells during extraction of the control soils for microbial biomass and  $^{13}\text{C}$  measurements (Merckx and van der Linden, 1988; Bruulsema and Duxbury, 1996).

The amount of WSOC was substantially lower under continuous maize than under grass for all soils except the Chicot soil, for which amounts of WSOC under maize or grass were about the same (Table 1). The WSOC  $\delta^{13}\text{C}$  values for the maize soils were less negative than those for the grass soils (Table 1) due to the input of  $\text{C}_4$ -derived C. Most of the C in WSOC was derived from  $\text{C}_3$  vegetation; the proportion of  $\text{C}_4$ -

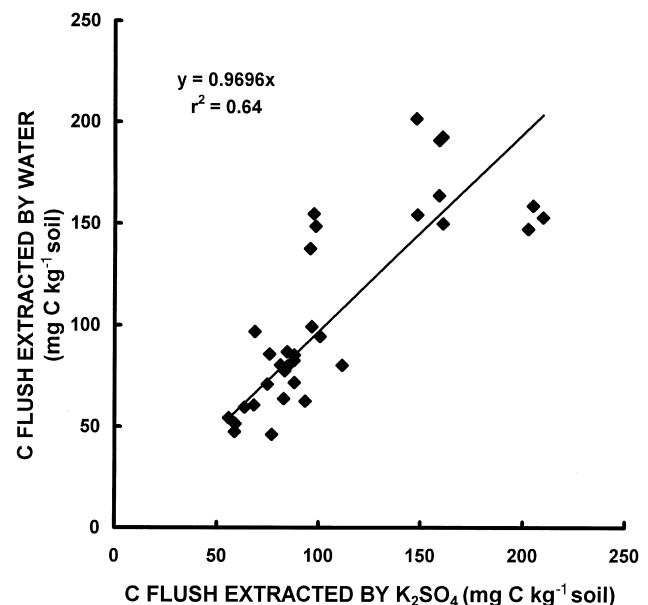


Fig. 1. Flush of C (fumigated C minus nonfumigated C in samples) extracted by water and 125 mM  $\text{K}_2\text{SO}_4$ .

derived C in the WSOC ranged from 10 to 34% across all of the sites. In the Fox soil the greater contribution of C<sub>4</sub>-derived C to the WSOC can be attributed to the soil's coarse texture and to spring plowing, which incorporated the residues from the previous year and thus would have enhanced residue decomposition shortly before soil sampling.

There was little difference in the amount of C<sub>4</sub>-derived C in either the WSOC or MBC in the fertilized and the nonfertilized treatments in the Brookston soil (Table 1). The long-term (i.e. 37 yr) fertilization had resulted in significantly greater amounts of soil C having a larger amount of C<sub>4</sub>-derived C relative to that in the nonfertilized soil. The MBC was significantly larger in the fertilized soil than in the nonfertilized soil ( $P < 0.05$ , Table 1) but was enriched in <sup>13</sup>C to the same extent. Most of the MBC was derived from maize residues even though more than 74% of soil C originated from C<sub>3</sub> plants.

Across all sites, the proportion of C<sub>4</sub>-derived C in soil C ranged from 4 to 26% (Table 1). The  $\delta^{13}\text{C}$  values of the WSOC were strongly correlated with those of the whole soil ( $r^2=0.79$ ,  $P = 0.002$ ). That a large proportion of variation in  $\delta^{13}\text{C}$  of the WSOC is explained by variations in the  $\delta^{13}\text{C}$  of the whole soil C suggests that the isotopic composition of WSOC is in equilibrium with that of the whole soil C.

The  $\delta^{13}\text{C}$  values for the microbial biomass were substantially less negative than those of the WSOC in soils under maize, reflecting the enrichment of <sup>13</sup>C and the greater contribution of maize residue C to the microbial biomass (Fig. 2). Ryan et al. (1995) reported about a 2-fold difference between the %C<sub>4</sub>-C in microbial and extractable C. Across all of the sites, 50–75% of the MBC was derived from maize C, even

though 74–96% of soil C originated from C<sub>3</sub> plants (Table 1). These data are consistent with those of Ryan and Aravena (1994), who reported that 55% of the MBC was derived from C<sub>4</sub>-C after 5 yr of continuous maize. Angers et al. (1995) found that up to 35% of MBC was C<sub>4</sub>-derived after 11 yr of continuous maize cropping.

A simple linear model was constructed to explore the relationships among the soil carbon pools and evaluate their turnover rates. Results of the simulations for each soil 1, 5 and 30 yr after the change-over to maize cropping are given in Table 2. The  $\delta^{13}\text{C}$  values of humus C change relatively little ( $\sim 1\text{--}2\text{‰}$ ) over 30 yr whereas the shift in  $\delta^{13}\text{C}$  values of the MBC is large ( $\sim 11\text{‰}$ ), and change very rapidly, reaching new steady state values within about 2 yr of the initiation of maize cropping (Fig. 3; Tables 1 and 2). The 5‰ shift in  $\delta^{13}\text{C}$  values of the WSOC over 30 yr is not as large as that of the MBC, but steady state values are reached within the same period as that reached for the MBC. The model also shows a clear distinction in the isotopic composition between WSOC and MBC. Most of the MBC comprises C<sub>4</sub>-derived C. After 30 yr, 10–23% of humus C and 30–50% of the WSOC is C<sub>4</sub>-derived C, whereas 82–92% of the C in MBC is C<sub>4</sub>-C. Even though the WSOC has a higher turnover rate than does MBC, the proportion of C<sub>4</sub>-C in the biomass is about 2.5 times greater than that in WSOC. Apparently the large amount of native soil C, the small amount of WSOC, and its equilibrium with the native soil C cause humus to dominate the isotopic composition of WSOC even though the WSOC is very active. Taken together with the C content, isotope data and modelling results suggest that the quantity, as well as the turnover rate of constituents that are in

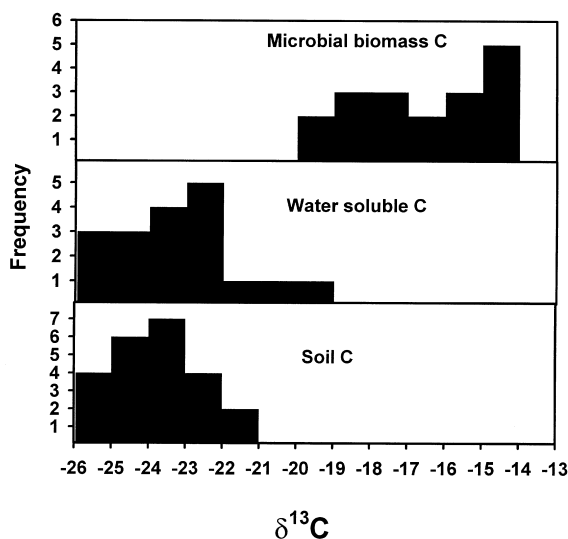


Fig. 2. Frequency histogram of  $\delta^{13}\text{C}$  for whole soil C, water soluble organic C and microbial biomass C.

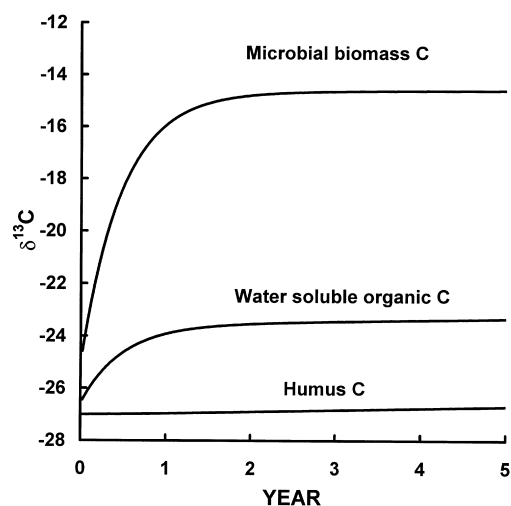


Fig. 3. Simulation of changes in the  $\delta^{13}\text{C}$  of humus C, water soluble organic C and microbial biomass C for the Ste. Rosalie clay soil.

Table 2

Simulated  $\delta^{13}\text{C}$  and %C<sub>4</sub>-C values for humus C, water soluble organic C (WSOC), microbial biomass C (MBC) and whole soil C (sum of humus C, WSOC and MBC) after 1, 5 and 30 yr

Soil	Time (yr)	$\delta^{13}\text{C}$				% C <sub>4</sub> -carbon			
		Humus	WSOC	MBC	Whole soil	Humus	WSOC	MBC	Whole soil
Ste. Rosalie clay	1	-27.0	-23.9	-15.9	-26.8	0.3	20.5	69.7	1.4
	5	-26.7	-23.3	-14.6	-26.5	2.1	24.4	80.0	3.3
	30	-25.1	-22.1	-14.3	-24.9	12.6	32.6	82.1	13.7
Brookston clay loam (fertilized)	1	-26.4	-23.6	-14.0	-26.2	0.3	25.1	83.6	1.3
	5	-26.1	-23.3	-13.6	-26.0	1.8	26.9	86.5	2.9
	30	-24.8	-22.3	-13.5	-24.7	11.1	33.7	87.7	12.0
Chicot sandy clay loam	1	-24.5	-21.7	-13.3	-24.3	0.3	22.1	90.1	1.5
	5	-24.2	-21.5	-13.1	-24.1	2.1	23.9	91.4	3.2
	30	-23.0	-20.5	-13.0	-22.8	12.4	31.9	92.3	13.4
Brandon loam	1	-25.8	-23.5	-13.2	-25.7	0.3	21.9	89.3	1.1
	5	-25.6	-23.3	-13.2	-25.5	1.6	23.0	90.0	2.4
	30	-24.5	-22.4	-13.1	-24.4	9.5	29.2	90.8	10.2
Fox loamy sand	1	-25.6	-20.0	-13.7	-25.2	0.6	38.1	85.3	3.7
	5	-25.2	-19.4	-13.2	-24.7	4.0	42.4	90.1	7.1
	30	-22.6	-18.0	-12.9	-22.2	22.7	53.6	92.1	25.3

equilibrium, influence the isotopic composition of such constituents.

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## Appendix A

### A.1. Basic concepts and structure of the model

The model is based on steady-state conditions. It comprises a series of first order transfers in which MBC links maize carbon (MC) with WSOC, and WSOC links MBC with soil humus carbon (HC) (Fig. A1). Production of CO<sub>2</sub> is proportional to microbial biomass under steady-state conditions, so there is no term for carbon utilization efficiency or growth yield. Transfer of C from microbial biomass to humus is

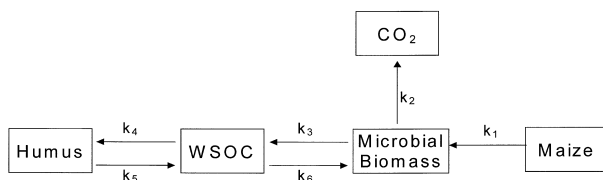


Fig. A1. Diagram of the model used to interpret  $\delta^{13}\text{C}$  data.

through the WSOC component, and the reciprocal transfer of C from humus to microbes is also through the WSOC component.

Time scale is important in using such a model. This model ignores short-term dynamics of C, which would require a more mechanistic treatment. Over long periods of constant management, however, highly dynamic components reach steady state. Once steady state is reached, there is no benefit to including short-term dynamics which have disappeared. Consequently, this model is best applied to periods that are long enough that a quasi-steady-state is reached and a steady-state approximation is not misleading. The purpose of the model is to elucidate the insights that these data yield about relationships among soil organic constituents.

### A.2. Constants, site-specific rates and state variables

The model is constructed using four rate constants and one constant value for  $\delta^{13}\text{C}$  (Table A1). All other first order rate constants became site specific and were derived from state variables in combination with the

Table A1

Constants and their values as used in the model to interpret  $\delta^{13}\text{C}$  data; constants correspond to the flows in Fig. A1

Constant	Value
Rate of maize decomposition ( $k_1$ ; yr <sup>-1</sup> )	1
Rate of microbial death ( $k_3$ ; yr <sup>-1</sup> )	2
Turnover time of soil humus ( $k_5$ ; yr <sup>-1</sup> )	0.02
Annual addition of maize C ( $A$ ; mg kg <sup>-1</sup> yr <sup>-1</sup> )	750
$\delta^{13}\text{C}$ of maize C ( $\delta\text{MC}$ ; ‰)	-12

constants ( $k_1$ ,  $k_3$ ,  $k_5$ ,  $A$  and  $\delta\text{MC}$ ) for steady-state conditions. Consequently the rate of turnover of WSOC and of microbial biomass; the microbial respiration rate; and  $\delta^{13}\text{C}$  values of humus, WSOC, microbial biomass become site-specific outputs from the model. State variables were taken from the observed site characteristics.

The values selected for the constants are generic. The turnover time of humus ( $k_5$ ) is consistent with the turnover time of humus in PHOENIX (McGill et al., 1981), and the rate of microbial death ( $k_3$ ), and of decomposition of maize ( $k_1$ ) are consistent with the data presented by McGill et al. (1981, 1986).

An advantage of steady-state conditions is that it reduces the number of independent variables that must be used to describe an integrated system fully. Consequently, the model was not tuned to each site; rather it was used to analyze differences among sites and among components at a site.

#### A.3. Calculation of rates and flows

Rates are designated by the letter  $k$ , with each rate numbered as in Fig. A1. Values of  $k_1$ ,  $k_3$  and  $k_5$  are fixed (Table A1). The value of all others are site-specific as follows:

$$k_2 = A \times k_1 / \text{MBC} \quad (\text{A.1})$$

$$k_4 = k_5 \times \text{HC} / \text{WSOC} \quad (\text{A.2})$$

$$k_6 = k_3 \times \text{MBC} / \text{WSOC} \quad (\text{A.3})$$

where  $k_1$  is the rate of decomposition of maize C ( $\text{yr}^{-1}$ ),  $k_2$  the rate of respiration by microbial biomass (as C;  $\text{yr}^{-1}$ ),  $k_3$  the rate of microbial death ( $\text{yr}^{-1}$ ),  $k_4$  the rate of humification of WSOC ( $\text{yr}^{-1}$ ),  $k_5$  the rate of depolymerization of humus C ( $\text{yr}^{-1}$ ),  $k_6$  the rate of microbial uptake of WSOC ( $\text{yr}^{-1}$ ),  $A$  the annual addition of maize C ( $\text{mg kg}^{-1} \text{yr}^{-1}$ ), MBC the microbial biomass C ( $\text{mg kg}^{-1}$ ), WSOC the water soluble organic C ( $\text{mg kg}^{-1}$ ) and HC the humus C ( $\text{mg kg}^{-1}$ ).

Flows are designated by the letter  $F$ , with the subscript corresponding to the subscript for  $k$ , such that  $F_1$  is the flow of C from maize to MBC, etc. (Fig. A1). Flows are calculated for each time step ( $dt$ ), which was set at 0.02 yr.

$$F_1 = \text{MC} \times k_1 \times dt \quad (\text{A.4})$$

$$F_2 = \text{MBC} \times k_2 \times dt \quad (\text{A.5})$$

$$F_3 = \text{MBC} \times k_3 \times dt \quad (\text{A.6})$$

$$F_4 = \text{WSOC} \times k_4 \times dt \quad (\text{A.7})$$

$$F_5 = \text{HC} \times k_5 \times dt \quad (\text{A.8})$$

$$F_6 = \text{WSOC} \times k_6 \times dt \quad (\text{A.9})$$

where MC is the maize C ( $\text{mg kg}^{-1}$ ) and all other variables are as previously defined.

Original values for the state variables (MBC, WSOC and HC) were taken from the observations at each site. Under steady-state conditions the values should not change over time. Values were, however, calculated after each time step by summing flows into, and subtracting flows out of, each component. This provided a simple check on conservation of mass in the spreadsheet calculations.

#### A.4. Calculation of $\delta^{13}\text{C}$

As the maize C was redistributed among soil C fractions, the  $\delta^{13}\text{C}$  value of each component changed. The  $\delta^{13}\text{C}$  values were calculated as follows:

$$\begin{aligned} \delta\text{MBC} = & [(\text{MBC}_{t-1} - F_2 - F_3) \times \delta\text{MBC}_{t-1} + F_1 \\ & \times \delta\text{MC} + F_6 \times \delta\text{WSOC}_{t-1}] / \text{MBC} \end{aligned} \quad (\text{A.10})$$

$$\begin{aligned} \delta\text{WSOC} = & [(\text{WSOC}_{t-1} - F_4 - F_6) \times \delta\text{WSOC}_{t-1} \\ & + F_3 \times \delta\text{MBC}_{t-1} + F_5 \\ & \times \delta\text{HC}_{t-1}] / \text{WSOC} \end{aligned} \quad (\text{A.11})$$

$$\begin{aligned} \delta\text{HC} = & [(\text{HC}_{t-1} - F_5) \times \delta\text{HC}_{t-1} + F_4 \\ & \times \delta\text{WSOC}_{t-1}] / \text{HC} \end{aligned} \quad (\text{A.12})$$

where  $\delta\text{MBC}$  is the  $\delta^{13}\text{C}$  of microbial biomass C (‰),  $\delta\text{WSOC}$  the  $\delta^{13}\text{C}$  of water soluble organic C (‰) and  $\delta\text{HC}$  the  $\delta^{13}\text{C}$  of humus C (‰). Subscripts  $t - 1$  denote the value at the end of the preceding time step. All other variables are as previously defined.

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