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Microbial assimilation of plant-derived carbon in soil traced by isotope analysis

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Abstract The flow of new and native plant-derived C in the rhizosphere of an agricultural field during one growing season was tracked, the ratios in different soil C pools were quantified, and the residence times (τs) were estimated. For this the natural differences in ^{13}C abundances of: (1) C_4 soil (with a history of C_4 plant, *Miscanthus sinensis*, cultivation), (2) C_3 soil (history of C_3 plant cultivation), and (3) $C_{4/3}$ soil (C_4 soil, planted with a C_3 plant, *Triticum aestivum*) were used. Total amounts and $\delta^{13}C$ values of total soil C, non-hydrolysable C, light fraction C, water-soluble C, microbial biomass C, and phospholipid fatty acids (PLFA) were determined. Using the $\delta^{13}C$ values of soil C in a mixing and a 1-box model enabled the quantification of relative contributions of C_3 plant and C_4 plant C to the total amount of the respective C pools in the $C_{4/3}$ soil and their τs. Compared to early spring (March), the percentage of C_3 plant C increased in all pools in June and August, showing the addition of new C to the different soil C fractions. In August the contribution of new C to microbial biomass C and water-soluble C reached 64 and 89%, respectively. The τs of these pools were 115 and 147 days. The $\delta^{13}C$ values of the dominant soil PLFA, 18:1ω7c, cy19:0, 18:1ω9c, 16:0, and 10Me16:0, showed wide ranges (−35.1 to −13.0‰) suggesting that the microbial community utilized different pools as C sources during the season. The $\delta^{13}C$ values of PLFA, therefore, enabled the analysis of the metabolically active populations. The majority of $\delta^{13}C$ values of PLFA from the $C_{4/3}$ soil were closely related to those of PLFA from the C_3 soil when *T. aestivum* biomass contributions to the soil were high in June and August. Specific populations reacted differently to changes in environmental conditions and supplies of C sources, which reflect the high functional diversity of soil microorganisms.

Keywords Carbon-13 · Carbon dynamics · *Miscanthus* · Soil carbon · Soil microbial biomass · PLFA

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Introduction

The measurement of $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) values is a powerful tool to establish pathways and rates of C exchange between plants and soil, and it relies on a different ^{13}C content of the current vegetation from that of the native soil C (Balesdent et al. 1987). In agriculture, C_3 plants ($\delta^{13}C$ ~−26‰) are usually planted in soils previously under C_4 vegetation ($\delta^{13}C$ ~−12‰) or vice versa, and the relative contribution of new C_3–C and native C_4–C can subsequently be quantified using a stable isotope mass balance. However, the dynamics of total soil C occurring over 1–5 years due to changes in soil management are difficult to measure because a large amount of background C corresponds to stabilized humus (Gregorich et al. 1994). In contrast, the dynamic soil C fractions, e.g. light fraction C, microbial biomass C, or water-soluble C, usually respond quickly to changes in C supply (Haynes 2000; Haynes and Beare 1997). The water-soluble C is the main C and energy source for soil microorganisms (Stevenson 1994) and with respect to turnover times it is considered as the most dynamic component of soil C (McGill et al. 1986).

The soil microbial biomass, a small fraction of the total soil C (Anderson and Domsch 1989), plays a crucial role because microorganisms are responsible to a great extent

for the regulation of soil C fluxes, e.g. assimilation, mineralization, and transformation of plant-derived C. The $\delta^{13}C$ values of microbial biomass from field samples reflect microbial C sources (Coffin et al. 1990; Pelz et al. 1998; Boschker et al. 1999). This concept is based on studies showing that $\delta^{13}C$ values of microbial biomass are closely related to that of the growth-supporting C substrate (Coffin et al. 1990; Pelz et al. 1998; Abraham et al. 1998). Little is known about isotopic effects of C transfer in soil through the microbial community. Recent studies revealed that $\delta^{13}C$ values of microbial biomass were ^{13}C enriched by about −2‰ relative to that of the corresponding total soil C, indicating that an isotope effect due to microbial degradation may also be of importance in soil (Angers et al. 1995; Santruckova et al. 2000). The authors concluded that this isotope effect could be induced by selective assimilation of different soil C pools or ^{13}C fractionation during microbial metabolism, in particular during respiration where the released CO_2 is depleted in ^{13}C.

It is extremely difficult to obtain microbial C from soil because of the likelihood of co-extracting material from indigenous soil C (Sparling et al. 1990), resulting in an inaccurate determination of $\delta^{13}C$ values. Phospholipids, however, can easily be extracted from soil. Due to their rapid degradation by phospholipases in dead cells they represent living cells and can be used for carbon flux studies. Therefore, phospholipid fatty acids (PLFA) can be used to identify microbial substrates in extracting microbial lipids from soil samples and to determine their $\delta^{13}C$ values (Waldrop and Firestone 2004).

To our knowledge no systematic investigation has been carried out in soil systems on ^{13}C signatures of soil microbial biomass C and microorganism-specific PLFA with regard to different pools of soil C. We have investigated the immediate microbial assimilation of the exported C from a C_3 plant (*Triticum aestivum*) grown for 1 year on soils previously under long-term C_4 vegetation (*Miscanthus sinensis*).

The goals of this study are: (1) the quantification of the C deposition from different vegetation types into the soil using the different isotopic signatures of C_4 and C_3 crops, (2) the determination of the residence times (τs) for the different soil C pools, and (3) the assessment of the differences in the microbial activity in the soils as a function of the vegetation cover.

Materials and methods

Field site experiment and sampling

The field site is located at Bachs in the northwest of the canton of Zurich, Switzerland, at about 400 m above sea level. The climate at this location is moderate (average annual temperature, 11°C) and humid (annual rainfall, 1,170 mm). The soil is a weakly humic loamy stagnic Gleysol and the perennial rhizomatous grass *Miscanthus sinensis* was planted in August 1993 at a density of 1 plant m^{-2} on an area of 625 m^2 (Kohli et al. 1999) and continuously cultivated in this soil. More characteristics of the soils are given in Table 1. On 15 March 2000, half of the *M. sinensis* plot was ploughed, left for 1 week to dry, and tilled.

A conventional agricultural field flanked the *M. sinensis* plot (Fig. 1). This soil was cultivated with a rotation of grassland (1992, 1993, 1994, and 1996) and annual C_3 crops, i.e. barley *Hordeum vulgare* (1991) and winter wheat *Triticum aestivum* (1995, 1998–2000), which was only interrupted for 1 year, when the C_4 plant, corn *Zea mays*, was grown (1997) (R. Meier, personal communication). The grassland was composed of a mixture of clover and various C_3 grasses. The agricultural soil was also ploughed and tilled along with the *Miscanthus* soil before they were simultaneously planted with *T. aestivum*, except for a 2-m-wide strip between the (previously) *M. sinensis* and the *T. aestivum* plots.

Throughout this manuscript the soil from the remaining *M. sinensis* plot is designated as "C_4 soil", that from the conventional grassland–annual crop rotation plot planted with *T. aestivum* as "C_3 soil", and the ploughed and tilled *Miscanthus* plot planted with *T. aestivum* as "$C_{4/3}$ soil".

The soils were sampled the same day as sowing (22 March 2000) and on additional sampling dates, covering the growing season: 9 May, 13 June, 21 August, and 21 October 2000. Since the precision of stable isotope analysis is usually 0.1 pro mille or better (Abraham et al. 1998) we

Table 1 Selected physicochemical and biological properties (means±SDs, n=4) of soils

Soil history	Sampling dates	pH	Water content (%)	n (%)	Total soil C and selected C pools (mg C g^{-1} soil dry weight)			
					Total soil C	Light fraction C	Water-soluble C	Microbial biomass C
C_4 Soil	22 March	6.6±0.1	21.2±0.3	0.21±0.01	20.5±1.6	1.56±0.36	0.09±0.02	0.56±0.10
	13 June	6.6±0.3	17.1±1.2	0.23±0.02	23.1±0.9	1.38±0.36	0.11±0.02	0.45±0.05
	21 August	6.7±0.0	20.0±1.0	0.22±0.01	20.6±0.5	1.91±0.35	0.07±0.01	0.55±0.04
$C_{4/3}$ Soil	22 March	6.6±0.1	19.6±1.1	0.21±0.01	20.0±1.1	1.66±0.12	0.06±0.02	0.47±0.12
	13 June	6.8±0.0	14.7±0.7	0.22±0.01	20.1±0.9	1.30±0.23	0.09±0.02	0.40±0.04
	21 August	6.9±0.3	19.9±0.5	0.21±0.01	19.6±0.7	1.80±0.40	0.07±0.01	0.47±0.05
C_3 Soil	22 March	5.5±0.4	19.0±1.9	0.23±0.02	21.2±1.7	1.19±0.34	0.05±0.00	0.39±0.20
	13 June	5.9±0.0	14.4±1.0	0.27±0.01	21.7±0.4	1.04±0.16	0.10±0.02	0.46±0.12
	21 August	5.9±0.1	21.0±1.0	0.24±0.01	21.7±0.4	1.43±0.31	0.04±0.01	0.65±0.18

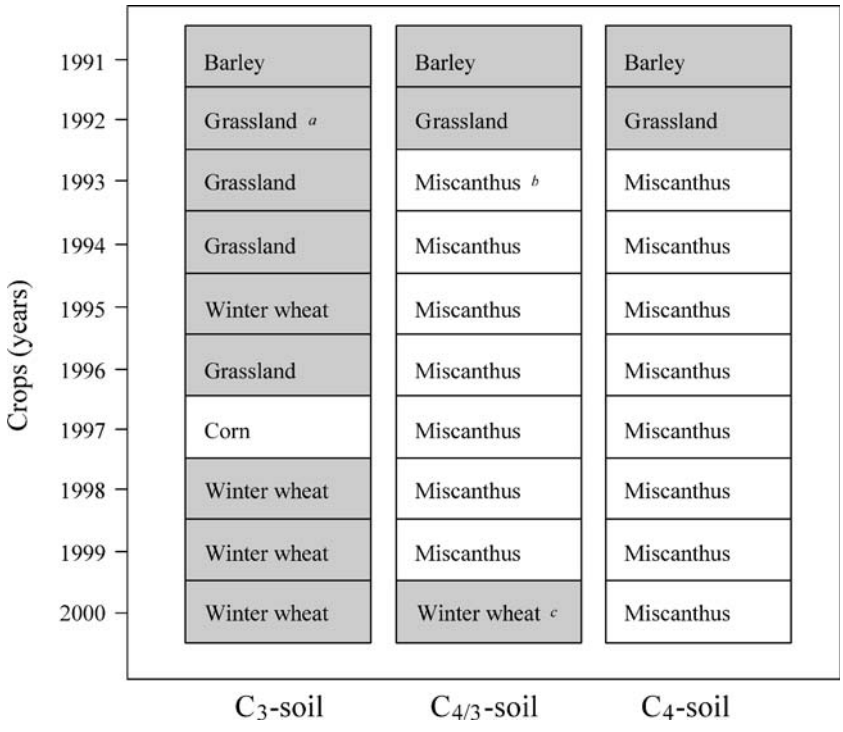

Fig. 1 Crop history of the designated soil types during the last 10 years. Periods with C_3 (*grey background*) and C_4 (*white background*) vegetation are shown. Grassland (*a*) was created by planting a mixture of clover and various C_3 grasses. In August 1993, the grassland was ploughed, tilled, and *Miscanthus sinensis* (*b*) planted (15 March). **c** The *M. sinensis* culture was ploughed, tilled and winter wheat *Triticum aestivum* (*c*) planted (22 March 2000)

expect that our observed $\delta^{13}C$ variance mainly reflects the soil heterogeneity. Twenty soil cores (2 cm diameter×10 cm depth) were randomly taken from the representative plots (usually at the rhizosphere) and bulked. The soil samples were crumbled, visible plant material and stones removed, and mixed. After sieving (<4 mm) 100-g portions (n=4) were frozen at −80°C until further analysis.

Separation and determination of selected soil C pools

Both total soil C and C pools were considered organic C because preliminary analyses revealed that soils of the plough layer at Bachs were free of carbonate (data not shown).

The non-hydrolysable C, which is mainly composed of stable humus (Stevenson and Cole 1999), was extracted by the hot 6N HCl hydrolysis method as described by Martel and Paul (1974). Briefly, air-dried soil was mixed with 6N HCl in a glass centrifuge tube (1/10, s/v) and then heated to 100°C for 18 h, and was vortexed about every 1 h. The tube was then cooled at room temperature and subsequently centrifuged (10 min, 4,000 *g*). The supernatant, which included the hydrolysable C pool (neither quantified nor $\delta^{13}C$ values measured), was discarded and the residue (the non-hydrolysable C) was rinsed with deionized water, dried at 50°C and analysed for its $\delta^{13}C$ values (this fraction was not quantified).

The light fraction C, which includes both undecomposed and partly decomposed plant and microbial residues (Cadisch et al. 1996; Gregorich et al. 1996), was isolated from sieved (<2 mm) and air-dried soil (10 g) by dispersion of 1 g soil in 40 ml NaI solution (adjusted to a density to 1.7 g cm^{-3}) (Gregorich and Ellert 1993). This solution was homogenized, centrifuged (10 min, 4,000 *g*) and after sedimentation the supernatant was transferred by suction to a filtration unit on a 20-μm glass fibre membrane. The soil sample was resuspended in NaI (40 ml) solution and the procedure repeated once to ensure the complete removal of the light fraction. The residue was washed with 10 mM $CaCl_2$ (40 ml) and distilled water, dried at 70°C, weighed and finely grounded.

The water-soluble C pool was obtained by suspending 10 g air-dried soil in 20 ml distilled water. This suspension was first centrifuged (10 min, 15,000 *g*) and the supernatant was then filtered through a 41-μm Millipore filter. The filtrates were freeze-dried and kept frozen until analyses of C content and $\delta^{13}C$ values.

Microbial biomass C was obtained from soil samples by a modified chloroform-fumigation–extraction method (Vance et al. 1987) as described by Horwath and Paul (1994). The amount of soluble C in the fumigated and unfumigated soil extracts was used to determine microbial biomass C from replicate soil samples (n=4) according to the following relationship:

$$\text{Microbial biomass C} = (C_f - C_{uf})/K_{ec}$$

where C_f is C in the fumigated extract, C_{uf} is C in the unfumigated extract and K_{ec} the value for the extraction efficiency for microbial biomass C (Vance et al. 1987) was considered to be 0.38. The $\delta^{13}C$ values of microbial biomass C were determined as described by Ryan and Aravena (1994).

Stable C isotope analysis

The $\delta^{13}C$ values of the various C pools (plant biomass, total soil C, non-hydrolysable C, light fraction C, water-soluble C, and microbial biomass C) were analysed using a Finnigan MAT DELTA-S isotope ratio mass spectrometer (IRMS) (Bremen), interfaced with an EA-1110 elemental analyser (Carlo Erba, Milan) via a variable open split interface (Conflo II; Finnigan, Bremen). The isotopic values of the pool were expressed in the standard delta notation:

$$\delta^{13}C = \left[\left(R_{sample}/R_{PDB}\right) - 1\right] \cdot 10^3$$

where R_{sample} and R_{PDB} are the $^{13}C/^{12}C$ isotope ratios corresponding to the pool and the international PeeDee Belemnite limestone (PDB) standard for C, respectively.

Quantification and determination of residence times of selected soil C pools

The different C pools of the $C_{4/3}$ soil were derived from both C_4 and C_3 plant C. The contribution of C_3 plant C, relative to the total C of the respective pool was estimated by modification of an existing simple mixing model (Kao 1997):

$$\delta_{C3} \cdot m_{C3} + \delta_{C4} \cdot m_{C4} = \delta_{C4/3} \cdot M$$

where δ_{C3} is the δ value of C_3 plant C pool (e.g. *T. aestivum*), δ_{C4} is the δ value of the C_4 plant C pool (*M. sinensis*), $\delta_{C4/3}$ is the δ value of C pool from the $C_{4/3}$ soil, m_{C3} is the C mass of C_3 plant pools, m_{C4} is the C mass of C_4 plant pools, and M is the total C mass of the particular pool. Since M of a particular pool is $m_{C3}+m_{C4}$ we can write:

$$\delta_{C3} \cdot m_{C3} + \delta_{C4} \cdot (M - m_{C3}) = \delta_{C4/3} \cdot M$$

Thus we derive the contribution of C_3 plant C (m_{C3}/M), relative to the total C in that pool from Eq. 4:

$$\delta_{C3} \cdot m_{C3}/M + \delta_{C4} \cdot (1 - m_{C3}/M) = \delta_{C4/3}$$

$$(\delta_{C3} - \delta_{C4}) \cdot m_{C3}/M + \delta_{C4} = \delta_{C4/3}$$

$$m_{C3}/M(\%) = \left[\left(\delta_{C4/3} - \delta_{C4}\right)/(\delta_{C3} - \delta_{C4})\right] \cdot 100$$

We applied the changes in $\delta^{13}C$ values of the different C pools from the $C_{4/3}$ soil (Fig. 2b–f) to a simple 1-box model (Eq. 8) (Schimel 1993) and estimated time courses of $\delta^{13}C$ in the $C_{4/3}$ C pools [$=\delta^{13}C_{C4/3}$ (t)]. For this model, we considered the C pool (e.g. microbial biomass C) in the $C_{4/3}$ soil to be well mixed and of constant size and the changes of $\delta^{13}C$ as due to the influx of C from the C_3 pool; thus the outward flux is of the same magnitude with the $\delta^{13}C$ value given by the actual (varying) value in the $C_{4/3}$ pool. Accordingly, there is an exponential decrease in the $\delta^{13}C$ value of the $C_{4/3}$ pool, starting from the value of March for $C_{4/3}$ and asymptotically approaching the lower boundary defined by the C_3 pool:

$$\delta^{13}C_{C3/4}(t) = \left(\delta^{13}C_{4/3}(t = \text{March}) - \delta^{13}C_{C3}\right) \cdot \exp\left(-t/\tau\right) + \delta^{13}C_{C3}$$

The mean τ indicates how fast the C in the pool is replaced by C_3–C, thus τ is inversely proportional to the turnover rate in the pool. The τ for the different pools is estimated by fitting the data set of Fig. 2b–f to the exponential equation given above.

PLFA analysis

Total lipids were extracted from replicate (n=4) soil samples (20 g wet weight) according to a modified Bligh–Dyer method (White et al. 1979) and further fractionated to neutral lipids, glyco- and phospholipids by column chromatography on silica gel (ICT, Basel) as described by Abraham et al. (1998). The phospholipids were dried, and fatty acid methyl esters were generated as referenced by Bossio and Scow (1998). The PLFA methyl esters were identified using gas chromatography (GC) and the MIDI peak identification software (MIDI, Newark, Del.), whereas peak identification was verified using mass spectrometry (MS).

The nomenclature of PLFA is in the form of A:BωC, where A designates the total number of Cs, B the number of double bonds, and C the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes "-c" for *cis* and "-t" for *trans* refer to geometric isomers. The prefixes "i-" and "a-" refer to iso- and anteisomethyl branching, and mid-chain methyl branches are designated by "Me-," preceded by the position of the branch from the acid end. A cyclopropyl ring is indicated as "cy-". In accordance with Zelles (1997), we considered the PLFA i15:0, a15:0, 16:1ω7c, 16:1ω5c, 10Me16:0, i17:0, a17:0, cy17:0, 18:1ω7c, 10Me18:0, cy19:0 to be predominantly of bacterial origin.

The $\delta^{13}C$ values and identities of PLFA were analysed on a dual mass spectrometer setup, using a GC for sample introduction to the MS and IRMS (GC/MS/IRMS) (Pelz et al. 1998). The GC/MS/IRMS system consisted of three parts: (1) a Hewlett Packard 5890 GC, (2) a GCQ Finnigen MAT ion trap MS, (3) and a 252 Finnigan MAT IRMS. The GC conditions for PLFA analysis were according to Abraham et al. (1998). As each component eluted from the column (Hewlett Packard Ultra 2), 10% of the effluent was carried through a 290°C transfer line into the MS for component identification and estimation of purity. The remaining 90% of the effluent passed through a 940°C combustion furnace into the IRMS where PLFA were transformed into CO_2 and its $\delta^{13}C$ values were measured as

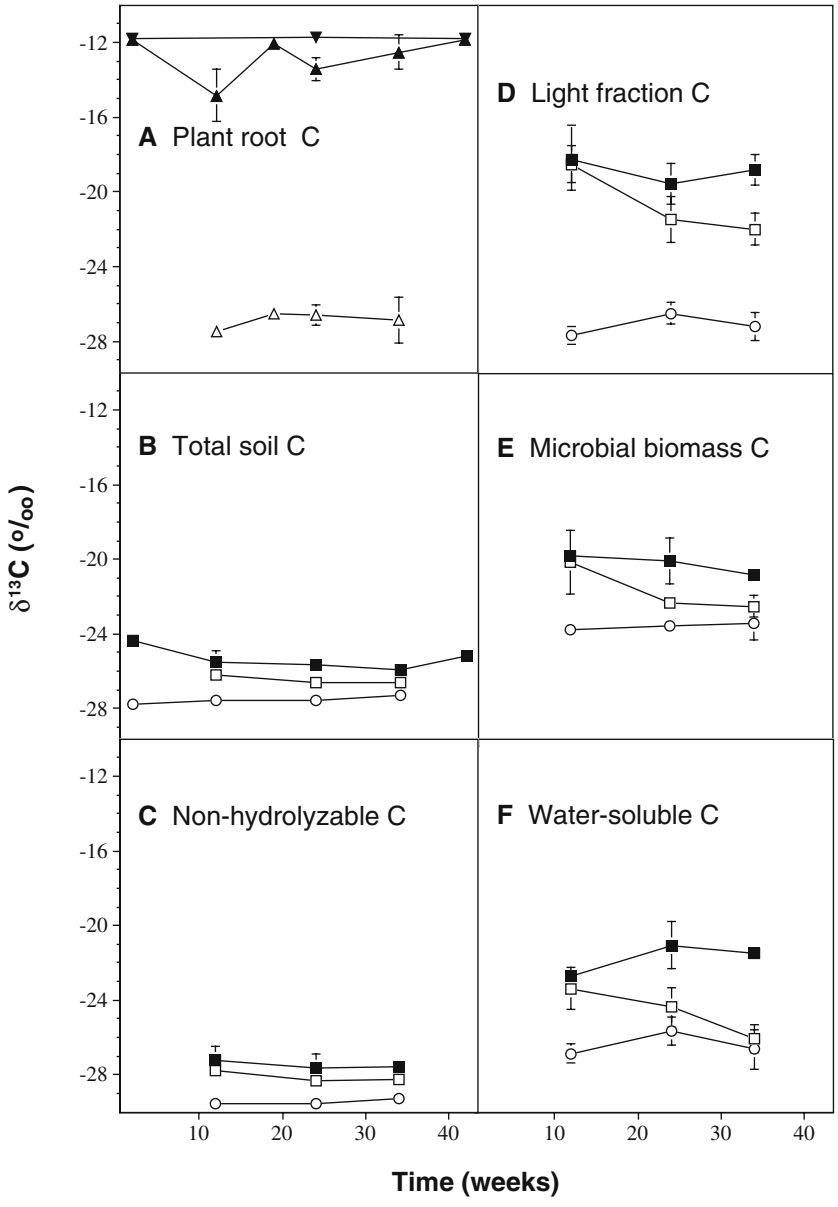

Fig. 2 Dynamics of $\delta^{13}C$ values (means±SDs) of **a** plant C from roots [*M. sinensis* (*filled triangle*), *T. aestivum* (*open triangle*)], and leaves [*Miscanthus* (*inverted filled triangle*)], **b** total soil C, **c** non-hydrolysable C, **d** light fraction C, **e** microbial biomass C, and **f** water-soluble C, respectively. In addition to the sampling dates (*x*-axis refers to weeks of the year) of 22 March, 13 June, and 21 August 2000, samples of plant and total soil C were also taken on 12 January, 9 May, and 21 October from the remaining *M. sinensis* plot (C_4 soil) (*filled square*), conventional grassland–annual crop rotation plot planted with *T. aestivum* (C_3 soil) (*open circle*), and ploughed and tilled *Miscanthus* plot planted with *T. aestivum* ($C_{4/3}$ soil) (*open square*)

described in Abraham et al. (1998). Reliable $\delta^{13}C$ measurements were obtained from individual PLFA, requiring a minimum amount of approximately 20 ng.

Statistical analysis

The data (e.g. total microbial biomass, $\delta^{13}C$ values) were generated using a combination of duplicate measurements from each of the four samples. Statistical analyses were performed using Student's *t*-test (one-tailed distribution, homoscedastic analysis, $P<0.05$). SDs are given in parentheses.

Results

Physicochemical soil properties

The soils were relatively dry at the selected sampling times in March, June, and August. The pH of the C_4 soil and the $C_{4/3}$ soil was significantly higher than that of the C_3 soil (Table 1).

The amounts of total soil C (Table 1) ranged from 1.96±0.07 to 2.31±0.09% of dry weight soil. The largest dynamic C pool was the light fraction C (5–10% of the total soil C), followed by microbial biomass C (1–4%), and water-soluble C (<1%). In all soils, the C concentrations of light fraction C and microbial biomass C seemed to increase slightly in August, whereas water-soluble C was slightly elevated in June (Table 1).

M. sinensis roots were statistically significantly, highly ^{13}C enriched in comparison to those of *T. aestivum* ($\delta^{13}C$,

−12.8 and −27.0‰, respectively, Fig. 2a). The $\delta^{13}C$ values of total soil C from the C_4 soil were distinct from those of the C_3 soil ($\delta^{13}C$, −25.3 and −27.4‰, respectively, Fig. 2b) as were the values of the non-hydrolysable C from the C_4 soil relative to those of the C_3 soil ($\delta^{13}C$, −27.4 and −29.4‰, respectively, Fig. 2c). The ^{13}C enrichments of the C_4 soils for the light fraction C, which are the water-soluble C, and microbial biomass C, were higher in comparison to those of total soil C and non-hydrolysable C (Fig. 2d–f).

After *T. aestivum* was planted in the $C_{4/3}$ soil in March 2000, the $\delta^{13}C$ measurements on 13 June and 21 August clearly revealed the contribution of the new C_3–C from *T. aestivum* roots to the native C in all pools of soil C (Fig. 2). The mixing model (Eq. 7) enabled us to quantify the relative contribution of C_3–C (from both newly added *Triticum* and C_3 plants cultivated in the past in this soil) to the total C content of the different pools from the $C_{4/3}$ soil. On 22 March 2000, 35% of the total soil C from the C_4 soil originated from old C_3–C (before *M. sinensis* was planted) (Table 2). In June and August, the relative contribution of C_3–C increased in all pools of the $C_{4/3}$ soil. Especially the water-soluble C pool which originated primarily from C_3–C (89% in August), whereas 64% of microbial biomass C (August 21) was derived from C_3–C.

Table 2 Quantification of the contribution of C_3–C[a], relative to that of total C, and estimated residence times[b] (τs) of the different soil C pools of the $C_{4/3}$ soil. *PLFA* Phospholipid fatty acids, *n.d.* not determined

Carbon pools	Relative contribution of C_3–C (%)			τ (days)
	March[c]	June	August	
Total soil C	35	51	53	353
Non-hydrolysable C	23	37	42	344
Light fraction C	3	28	38	278
Water-soluble C	15	72	89	147
Microbial biomass C	8	64	64	115
PLFA				
i15:0	57	68	58	n.d.
a15:0	45	68	41	n.d.
16:1ω5c	32	43	64	n.d.
16:1ω7c	30	79	77	n.d.
16:0[d]	23	78	82	n.d.
10Me16:0	41	84	31	n.d.
i17:0	84	68	92	n.d.
a17:0	100	47	43	n.d.
cy17:0	58	98	68	n.d.
18:0[d]	100	90	71	n.d.
10Me18:0	100	84	61	n.d.
cy19:0	64	77	43	n.d.

[a] m_{C3}/M, mixing model, where m_{C3} is the C mass of C_3 plant pools and M is $m_{C3}+m_{C4}$ (Eq. 7)
[b] 1-Box model (Eq. 8)
[c] After tillage
[d] Except 16:0 and 18:0 all PLFA are considered to be predominantly of microbial origin according to Zelles (1997)

The resulting values of the mean τs, which were estimated by the 1-box model, were 353, 344, 278, 147, and 115 days for total soil C, non-hydrolysable C, light fraction C, water-soluble C, and microbial biomass C, respectively.

Most soil PLFA are constituents of microbial origin (Zelles 1997) and were extracted on 22 March, 13 June, and 21 August from soil samples. The PLFA profiles from soil samples showed 15 major compounds, which accounted for 91% of the total amount of PLFA and they corresponded to: saturated chain (16:0, 18:0), saturated terminal-branched chain (i15:0, i17:0, a15:0, a17:0, which are characteristic for many Gram-positive bacteria), saturated mid-branched chain (10Me16:0, 10Me18:0), cyclopropyl (cy17:0, cy19:0), mono-unsaturated (16:1ω5c, 16:1ω7c, 18:1ω9c, 18:1ω7c), and polyunsaturated (18:2ω6c, characteristic for eukaryotes, e.g. fungi, Protozoae) PLFA. The profiles were always similar, i.e. differences between the soil types (C_4, C_3, and $C_{4/3}$ soil) and changes throughout the year were only small (data not shown). The dominant PLFA were always 18:1ω7c, cy19:0, 18:1ω9c, 16:0, and 10Me16:0 ranging from 19 to 22%, 10–14%, 10–11%, 8–11%, and 6–8% of the total amount of PLFA, respectively (data not shown). The total PLFA concentration varied from 6.8±0.6 μg g^{-1} dry weight soil (C_3 soil in June) to 13.5±1.5 μg g^{-1} dry weight soil (C_3 soil in August).

The $\delta^{13}C$ values of PLFA from the different soils varied from −35.7‰ (16:1ω7c, C_3 soil in March) to −13.0‰ (10Me18:0, C_4 soil in June) (Fig. 3). In comparison to $\delta^{13}C$ values of soil microbial biomass C, the PLFA values were usually ^{13}C depleted (with the exceptions of 18:0 and 10Me18:0 from the C_4 soil in June, and a17:0 from C_3 soil and $C_{4/3}$ soil in August). The PLFA obtained from C_4 soils were ^{13}C-enriched, relative to those from C_3 soils.

We were unable to determine reliable $\delta^{13}C$ values for 18:1ω9c and 18:1ω7c, because their $^{12}C^{16}O_2$ and $^{13}C^{16}O_2$ ion currents were not baseline separated under the chromatographic GC-IRMS conditions; additionally 18:1ω9c overlapped with 18:2ω6c. Minor peak overlaps with other compounds were also determined for 16:1ω7c and 10Me16:0 but we considered their measured $\delta^{13}C$ values reliable because the overlaps were relatively small (~5%).

The PLFA 16:1ω7c, 16:1ω5c, and 16:0 from C_3 soil samples in March were statistically significantly, highly ^{13}C depleted (−35.7±0.6, −33.7±0.5, and −32.6±0.4‰, respectively), in comparison to the corresponding $\delta^{13}C$ values of total soil C (−27.6±0.1‰) and microbial biomass C (−23.7±0.1‰) (Fig. 3a). However, the $\delta^{13}C$ values of these PLFA became ^{13}C enriched in June (−28.2±1.1, −25.6±1.4, and −26.1±0.4‰, respectively) (Fig. 3b) and August (−26.0±0.7, −28.2±0.7, and −27.3±1.0‰, respectively) (Fig. 3c).

Following the tillage of the $C_{4/3}$ soil on 15 March, $\delta^{13}C$ values of PLFA from this soil became ^{13}C depleted within 1 week in comparison to those from the original non-tilled C_4 soil. The calculations of the relative C contribution from C_3 plants to the total PLFA of the $C_{4/3}$ soil on 22 March revealed that a17:0, 18:0, and 10Me18:0 were completely derived from C_3–C (100%), whereas 16:1ω7c, 16:1ω5c, and 16:0 were to a substantially smaller ex-

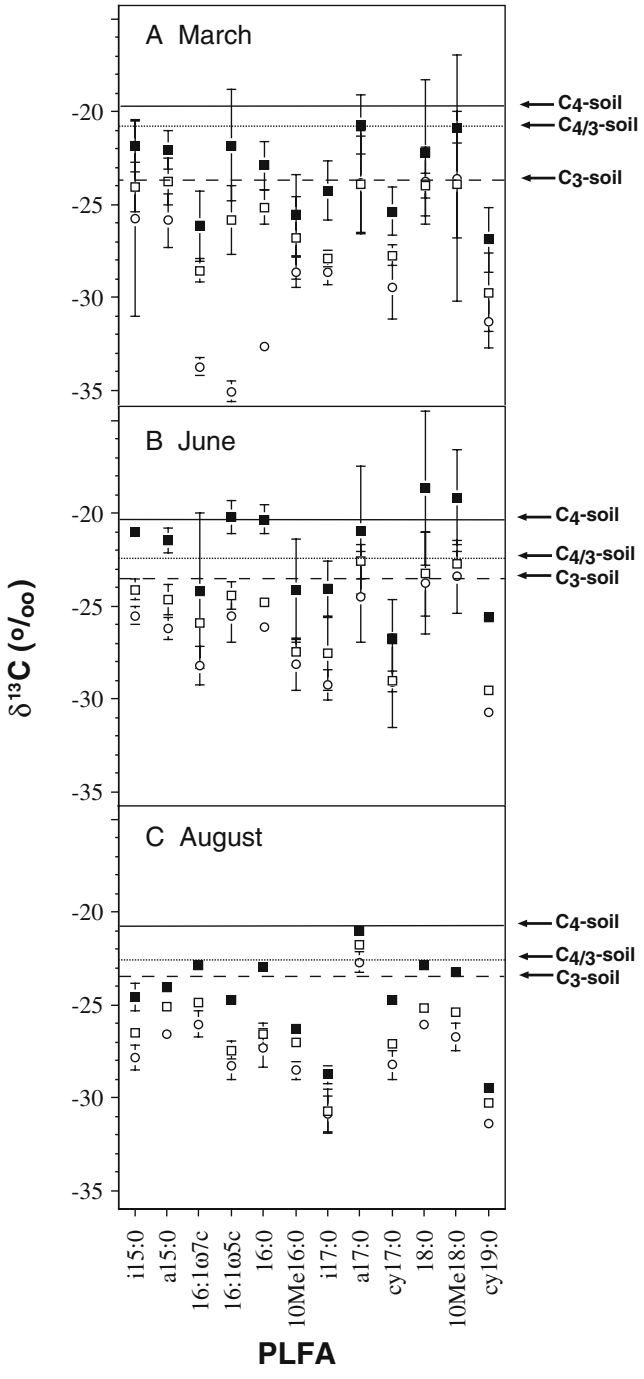

Fig. 3a–c Relationship of $\delta^{13}C$ values (means±SDs) of dominant soil phospholipid fatty acids (PLFA) to those of microbial biomass C from the C_4 soil [PLFA (*filled square*); microbial biomass C, *full lines*], C_3 soil [PLFA (*open square*); microbial biomass C, *dashed lines*], $C_{4/3}$ soil [PLFA (*filled circle*); microbial biomass C, *dotted lines*]. Samples were taken on **a** 22 March, **b** 13 June, and **c** 21 August 2000

tent derived from C_3–C (32, 30, and 23%, respectively) (Table 2).

When *T. aestivum* biomass production was high in June (approximately 60 cm plant height), the majority of $\delta^{13}C$ values of PLFA from the $C_{4/3}$ soil were closely related to those of PLFA from the C_3 soil (except for 16:1ω5c and a17:0). On 21 August, when *T. aestivum* had stopped growing and plants had started to die back, the majority of $\delta^{13}C$ values of $C_{4/3}$ soil PLFA were still closely related to those from the C_3 soil. Contrary to these PLFA, $\delta^{13}C$ values of a15:0, 10Me16:0, a17:0, and, cy19:0 shifted and became closer to those of the C_4 soil.

Discussion

The $\delta^{13}C$ value of *M. sinensis* biomass, determined in our study (−12.8‰), was similar to that given in the literature (−12.4‰) (Kao 1997). We expected that the C input from the perennial rhizomatous grass *M. sinensis* with a wide root system would be larger than that from grassland or annual crops, which would result in higher C inputs to soil than with the latter (Table 1). Indeed, after an 8-year cultivation period, the $\delta^{13}C$ value of total soil C from the C_4 soil was significantly different to that of the C_3 soil, revealing the contribution of *M. sinensis*-derived C to the total soil C of the C_4 soil (Fig. 2b). This difference was not only found in the total soil C but also in the stable humus fraction of the C_4 soil, reflecting a considerable ^{13}C enrichment of the non-hydrolysable C (Fig. 2c). The higher ^{13}C enrichments of light fraction C, water-soluble C, and microbial biomass C in comparison to those of total soil C and non-hydrolysable C showed that the C_4 plant C contributions were substantially higher in the dynamic pools (Fig. 2d–f). Our results confirmed the importance of these pools as sensitive indicators of management-induced changes in the fate of crop residues and the turnover of soil C constituents (Angers et al. 1995).

The $\delta^{13}C$ value of the light C fraction from the C_4 soil was always less negative than that of microbial biomass and the most ^{13}C depleted fraction, the water-soluble C, indicating a larger contribution of C_4–C, presumably from fragments of *M. sinensis* biomass (cf. Gregorich et al. 2000). Since the main C source to soil microorganisms is considered to be the water-soluble C (Stevenson 1994), the ^{13}C-enrichment of microbial biomass C, relative to water-soluble C indicated an isotope effect due to microbial assimilation as suggested by Angers et al. (1995) and Santruckova et al. (2000). Recent controlled laboratory studies revealed isotope effects in microbial C assimilation. During decomposition of organic matter, fungi showed a different ^{13}C fractionation between sugars derived from either C_3 or C_4 plants (Henn and Chapela 2000). This study showed that the assimilation of C_3 and C_4 plant-derived sugar was sensitive to the non-random ^{13}C-distribution in the molecule and proposed that a differential fractionation between C_3– and C_4–C also takes place in the field. These findings from field and laboratory studies raise the question about the validity of the assumption that biological C processing does not significantly alter $\delta^{13}C$ values during C transformation (Vander Zanden et al. 1999; Wedin et al. 1995). Yet these results could provide urgently needed data for a re-evaluation of ecosystem models describing the C transfer to microbial cells based on the variation of isotopic abundance. Another explanation might be that the water-

soluble fraction is partly formed from microbiologically derived products. During microbial turnover processes a substantial release of often simple and water-soluble organic products, such as amino acids from active and dead cells, takes place. As is well known, in many, enzymatic, biochemical processes heavy isotopes (e.g. ^{13}C) are discriminated against light isotopes (e.g. ^{12}C) the release of microbial products into the water-soluble fraction should result in a relative ^{13}C-enriched microbial biomass and a ^{13}C-depleted water-soluble fraction.

The $\delta^{13}C$ values of the C pools from the $C_{4/3}$ soil were always in between the values of the respective pools from the C_4 and C_3 soil, which showed in principle the applicability of the mixing model. The application of this model revealed a significant contribution of new C (from plant exudates of *T. aestivum* roots) to the native soil C in all pools between March and August (Table 2). At the end of the plant growth season, 18% of the total soil C in the rhizosphere comprised new C from the current vegetation period. In August, the water-soluble C originated primarily from new C (74%), whereas microbial biomass C was composed of only 56% new C, and the difference indicated that the soil microorganisms also utilized considerable amounts of older, C_4 plant-derived C sources.

The τs for total soil C, non-hydrolyzable C, light fraction C, water-soluble C, and microbial biomass C, respectively, estimated by the 1-box model, compared well with the amounts of C_3–C in the respective soil C pools obtained from the mixing model (Table 2). Here, water-soluble C and microbial biomass C were found to have the largest contribution from C_3–C after the growing season and the 1-box model indicated the lowest mean τ for these two pools. The linear modelling approach by Gregorich et al. (2000) predicts a higher turnover rate for the soluble organic C than the microbial biomass in a Canadian agricultural soil. The linear model, and the 1-box model have an advantage over the mixing model, as they should allow a prediction of the C turnover in the respective pool. The advantage of the mixing model is its time independence, i.e. the contribution of C_3–C can be determined at any time. The 1-box model enabled us to estimate the time until 95% of the C in a pool was replaced, which corresponds to 3τ. In accordance with our estimate, the mean τs of the dynamic C pools of soils from the US corn belt were 100 days (Collins et al. 2000) and those of microbial biomass C from Chinese karst soils were 105 days (Piao et al. 2000).

The mean τ of soil organic C in the top layers of different British Podzols, Brown Podzolic soils and Stagnohumic Gleysols was in the range of 2–50 years (Bol et al. 1999). Wedin et al. (1995) estimated that about 14% of the total soil C from soils of east-central Minnesota (USA) was new C derived from C_4 plants over 2 years. However, the mean τ of total soil C in our study is considerably less than previously reported (around 353 days). According to the 1-box model (Eq. 8) the soil should carry the isotopic signal of the C_4 plants within a few years of C_4 plant cultivation. However the $\delta^{13}C$ values (Fig. 2b–f) showed that the soil still carried a strong C_3 signal, even in the fast reactive pools such as, e.g. light fraction C, microbial biomass C, and water-soluble C. Furthermore, $\delta^{13}C$ values of the C_4 pools did not increase in the C_4 soils during the growing season investigated in this study (Fig. 2b, c).

However, one also must consider that microorganisms do not only assimilate the new organic material, which in our case would be the C_4 substrate, but also use to a large extent old organic material from C_3 plants, even after 8 years of C_4 plant cultivation (Fig. 2e). Another reason for this discrepancy between the calculated and measured values could be an overestimation of the rhizodeposition. Högberg et al. (2001) suggested that the respiratory C loss of the roots is higher than that of the microorganisms. The authors concluded that the rhizodeposition and its contribution to the soil formation is not as large as so far assumed. This agrees well with the findings of Gaudinski et al. (2000, 2001) who showed that the fine roots have a much longer life span than previously estimated (up to 11 years), suggesting a slower fine root turnover than so far anticipated. Slow root C turnover and its impact on soil C sequestration was also observed by Matamala et al. (2003) in two forest plantations in North Carolina. In this study, conducted in pine forest, 45% of the root C was replaced in 3 years. Based on our results we have to conclude that the seasonal course of the $\delta^{13}C$ in the soil cannot be simply extrapolated to predict its long-term fate.

Bulk C pools, e.g. water-soluble C and microbial biomass C can only be obtained from soil samples depending on the extraction methods, and hence are operationally defined. Therefore, these soil C pools are only to a limited extent indicative of their origin. The analysis of PLFA could overcome this drawback because PLFA have been used to determine the structure of complex microbial communities in situ (White 1983) and their $\delta^{13}C$ values can be used as indicators of microbial substrate usage (Abraham et al. 1998; Boschker et al. 1999). Still one must keep in mind that the physicochemical extraction process might bias the result to some degree and might lead to some deviation from the original signature. To take this possible bias into account, the use of $\delta^{13}C$ values of PLFA for the characterization of microbial biomass was calibrated by growing representatives of major bacterial and fungal species in the laboratory on isotopically defined C sources (Abraham et al. 1998). Thus the $\delta^{13}C$ values could be assigned to the respective microorganisms. The results of this study revealed that each substrate was individually processed, leading to a range of $\delta^{13}C$ values in microbial biomass and PLFA, whereas different strains showed their own pattern of variance within the $\delta^{13}C$ values of PLFA. In our study, the PLFA obtained from C_4 soils were ^{13}C enriched, relative to those from C_3 soils, revealing the influence of C_4 plant-derived C sources on $\delta^{13}C$ values of PLFA.

The high $\delta^{13}C$ variability of individual soil PLFA suggested that the microbial community simultaneously utilized different C sources. In the C_3 soil in early spring (March), members of the microbial community, comprising the highly ^{13}C-depleted PLFA 16:1ω7c, 16:1ω5c, and 16:0 (Fig. 2, $\delta^{13}C$, −35.7 to −32.6‰), were likely to use

CH_4 as a substrate because biogenic CH_4 has a depleted C isotopic signal of between −65 and −50‰ (Pelz et al. 2001). In June and August, populations which contained these PLFA, utilized substantial amounts of the new added C (from *T. aestivum*) (Table 2; 77–82%). At the end of the growing season the majority of PLFA from the $C_{4/3}$ soil comprised C_3–C from *T. aestivum*. In contrast, a15:0, 10Me16:0, a17:0, and cy19:0 were at this point substantially less composed of C_3–C from the plant, pointing to a different C source for the microorganisms producing them (Table 2; 31–43%).

Our data mirror the fact that PLFA extracted from the field originate from viable biomass (White 1983). The $\delta^{13}C$ values of PLFA therefore represented values of metabolically active populations within the microbial community, whereas the value of microbial biomass C reflected the entire soil microbial community, that are inactive or active microorganisms (Roszak and Colwell 1987; Stenström et al. 2001). Furthermore, our results showed that specific populations within the microbial community reacted differently to changes of environmental conditions and supplies of C sources, due to the high functional diversity of soil microorganisms, also evidenced by the *r* and *K* strategies of microbial C uptake in soils (Stenström et al. 2001). Selected PLFA are specific biomarkers for specific groups of microorganisms, e.g. i15:0 and a15:0 for Gram-positive bacteria, 10Me18:0 for actinomycetes, or 18:2ω6c for fungi and Protozoae (Zelles 1997). Respective $\delta^{13}C$ analyses of these compounds or any other biomarkers would enable us to link a specific population with their specific activity in the soil. Such an approach was successfully applied to study the microbial utilization of recalcitrant and simple C compounds in oak woodland and open grassland soils (Waldrop and Firestone 2004).

Tillage can promote the release and subsequent degradation of previously protected organic matter and at the same time induces rapid changes in microbial community structure (Adu and Oates 1978; Calderon et al. 2000). One week after tillage of the $C_{4/3}$ and C_3 soils, the $\delta^{13}C$ values of PLFA from the $C_{4/3}$ soil were ^{13}C depleted relative to those from the original, non-tilled C_4 soil and closely related to those of the C_3–C (Fig. 3). This indicated a change of microbial C sources and not a change of the microbial community because the PLFA profiles were very similar throughout the year (data not shown). In the $C_{4/3}$ soil in March after tillage, certain microbial populations, containing a17:0, 18:0 and 10Me18:0, utilized mainly C_3–C sources probably stored in the soil since at least 1993 (Table 2). Contrary to the $\delta^{13}C$ values of bulk soil C pools, only the values of PLFA were significantly influenced by tillage, demonstrating that $\delta^{13}C$ values of PLFA were extremely sensitive and fast indicators of environmental changes.

Conclusions

The 8-year cultivation of *M. siniensis* (C_4 plant), followed by a short growing period of *T. aestivum* (C_3 plant), offered a unique opportunity to study C transfer dynamics from plants to soil. Observing the transition from C_4 to C_3 soil we found a fast but relatively small change in the $\delta^{13}C$ signature of the total soil. The analysis of the different soil fractions, however, showed that the most overt changes in $\delta^{13}C$ were in the light soil fractions, the water-soluble soil fractions, and the microbial biomass (easily degradable fractions), whereas the total soil C and the non-hydrolysable fraction (stable fractions) showed only minor changes in $^{13}C/^{12}C$ ratio.

This indicates that:

1. The C deposition rate of the easily degradable fraction is much higher than that of the stable soil fraction. However, the τ of the easily degradable fraction is much shorter due to a much higher turnover rate. Thus, the contribution of the easily degradable fractions to the formation of the total soil layer is small due to their high turnover rate and the respiratory loss.
2. In contrast only a small amount of C is transferred to the soil in the form of the stable fraction while its turnover rate is much slower. These stable fractions represent a large part of the soil C. This result is supported by earlier findings showing that soil formation takes several years to decades, because the turnover rate of the fine roots, which contribute to a large extent to the belowground soil formation (besides litter), is slower than so far assumed.
3. The use of PLFA represents a very valuable tool for tracing the C transfer from plants to soil microorganisms and for determining microbial C sources (C_3 or C_4).
4. Soil disturbances like tillage or changes in the water or nutrient regime will change the C transfer rates to the soil very strongly and might eventually lead to a new equilibrium between the different soil C fractions.

This study clearly showed that the soil as a highly complex system is far from a homogeneous unit. A highly differentiated approach as demonstrated must be considered when analysing the soil C stocks for their growth dynamics and their C-sequestration potential.

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