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^{13}C and ^{15}N natural abundance of the soil microbial biomass

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Abstract

Stable isotope analysis is a powerful tool in the study of soil organic matter formation. It is often observed that more decomposed soil organic matter is ^{13}C, and especially ^{15}N-enriched relative to fresh litter and recent organic matter. We investigated whether this shift in isotope composition relates to the isotope composition of the microbial biomass, an important source for soil organic matter. We developed a new approach to determine the natural abundance C and N isotope composition of the microbial biomass across a broad range of soil types, vegetation, and climates. We found consistently that the soil microbial biomass was ^{15}N-enriched relative to the total (3.2 ‰) and extractable N pools (3.7 ‰), and ^{13}C-enriched relative to the extractable C pool (2.5 ‰). The microbial biomass was also ^{13}C-enriched relative to total C for soils that exhibited a C3-plant signature (1.6 ‰), but ^{13}C-depleted for soils with a C4 signature (−1.1 ‰). The latter was probably associated with an increase of annual C3 forbs in C4 grasslands after an extreme drought. These findings are in agreement with the proposed contribution of microbial products to the stabilized soil organic matter and may help explain the shift in isotope composition during soil organic matter formation.

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1. Introduction

Although most soil organic matter (SOM) is plant-derived, only a small fraction of the yearly litter and root input becomes part of the stable organic matter pool, with most of it after repeated processing by soil microorganisms (Six et al., 2000, 2004). The natural abundance ^{13}C and ^{15}N content of the SOM is usually higher than that of the plant and fresh litter input (for example Lichtfouse et al., 1995; Handley et al., 1999; Amundson et al., 2003). Additionally, older, more decomposed SOM is ^{15}N- and, less consistently, ^{13}C-enriched compared to more recent organic compounds (Tiessen et al., 1984; Lichtfouse et al., 1995; Kramer et al., 2003). Fractionation during microbial processing may be responsible for these shifts in stable isotope composition (e.g. Nadelhoffer and Fry, 1994; Lichtfouse et al., 1995; Boutton, 1996; Kramer et al., 2003; Staddon, 2004). There are several possible mechanisms to explain this. Isotopically depleted materials may be used by microorganisms, while the leftover and isotopically enriched materials may end up in the SOM. Alternatively, the microbial biomass itself may be the origin of the isotopically enriched organic material. The latter possibility is in line with ^{13}C and especially ^{15}N shifts observed in food web studies (Koch et al., 1994; Michener and Schell, 1994; Neilson et al., 1998). Direct measurements of the natural abundance stable ^{13}C and ^{15}N composition of the soil microbial biomass are needed to distinguish between these mechanisms.

Few studies have measured the ^{13}C-composition of soil microbial biomass (reviewed by Potthoff et al., 2003), while we found none describing its ^{15}N composition. Sporocarps and hyphae of certain fungi show consistent ^{13}C and

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^{15}N-enrichments relative to substrate, host plants and SOM (for example Gleixner et al., 1993; Högberg et al., 1996, 1999a, b; Gebauer and Taylor, 1999; Hobbie et al., 1999; Kohzu et al., 1999; Hart et al., 2006). Fungi make up a substantial portion of the microbial community in the soil (Frey et al., 1999) and its isotope composition influences that of the total microbial biomass.

Here, we report on the natural abundance ^{13}C and ^{15}N composition of microbial biomass relative to other soil pools, using data from six ongoing experiments. These results may help explain the difference in isotope composition between litter, recent and stabilized SOM fractions.

2. Materials and methods

2.1. Soils

Stable isotope composition of microbial and other soil fractions was measured in six experiments covering a broad range of soil types (sandy to clay-loam), vegetation cover (grassland, shrubs, forests), climate (semi-arid, temperate, sub-tropical), and land-use practices (grazing, fire-managed and undisturbed natural vegetation; Tables 1 and 2).

Experiment #1: We sampled soil (A-horizon, 0–10 cm) from five grasslands along an elevation gradient near Flagstaff, Arizona, USA (Tables 1 and 2): open meadow in mixed conifer zone (2640 m above sea level), open meadow in ponderosa pine zone (2260 m), piñyon–juniper interspaces (1975 m), high desert grassland (1750 m) and Great Basin desert grassland (1380 m). At each site, four adjacent 100-m^2 plots were laid out, and one composite soil sample (of six subsamples) was taken per plot. We sampled soil in October 2002 and September 2003 (designated experiment 1a and b, Table 1).

Experiment #2: Soil (A-horizon, 0–10 cm) was collected from three locations along a 5 m slope near an ephemeral (spring) creek in May 2003 (2600 m, near Flagstaff, AZ). We sampled the recently dried creek bed, directly next to the creek and 2–3 m higher on the slope ($n = 4$). Vegetation was either absent (dried creek bed) or consisted of C3 grasses and Bebb willow (*Salix bebbiana* Sarg.) trees.

Experiment #3: Soil samples (A-horizon, 0–10 cm) were taken from semi-arid, high desert grassland (1750 m, near Flagstaff, AZ) in August 2004. Samples were collected from control ($n = 12$) and water amended (14 L m^{-2}, $n = 28$) plots over a 72 h period after wet-up.

Experiment #4: Soil (A-horizon, 0–10 cm) was sampled in March 2003 along a 100 m transect (100, 50, 25, 10, 5 and 0 m distance from a water reservoir) in grazed semi-arid, high desert grassland (1750 m, near Flagstaff, AZ; Dijkstra et al., 2006). The area was heavily trampled and eroded by cattle and devoid of vegetation at the time of soil collection.

Experiment #5: Soil samples were collected in July 1997 from two soil horizons (O and A) along a time-since-fire sequence (0.25, 3, 12 and 25 y after fire) of Florida scrub oak at the Kennedy Space Center, FL. We also sampled a mesic Hammock (at least 300 y old). The scrub-oak grew on very sandy acidic (pH 3.9–4.1) and well-drained soils, while the mesic Hammock vegetation was located on poorly drained soils and experienced anoxic conditions during part of the year (Schmalzer and Hinkle, 1996; Schmalzer et al., 2001).

Experiment #6*:* Soil (A-horizon, 0–15 cm) was collected in August 2001 from two adjacent ponderosa pine forest stands (2100 m, near Flagstaff, AZ): an unmanaged stand and a stand that was heavily thinned (1998–1999) and burned (spring 2000; Fulé et al., 2001). Circular subplots (0.59 m^2) were established within each of 10 plots per stand and either kept at natural rainfall levels (587 mm over 2 y) or amended with water (870 mm total over 2 y).

2.2. Analytical procedures

Soils were processed within 24 h or (experiment #6) undisturbed cores were kept at 4 °C up to 4 d. Soils were sieved (2 or 4 mm sieve) before processing. We

Table 1
Detailed description of methodology for individual experiments

Experiment # [a]	1a	1b	2	3	4	5	6
Procedure[b]	EFE	CFE	CFE	CFE	EFE	EFE	CFE
$CHCl_3$ fumigation	7 d	7 d	24 h	24 h	24 h	24 h	5 d
K_2SO_4 conc. (M)	0.50	0.25	0.25	0.25	0.25	0.50	0.50
Soil W:Vol	20:50	20:50	20:50	20:50	25:50	10:50	10:50
Preparation	Drying	Drying	Drying	Drying	Drying	Drying	Kjeldahl
Soil type[c]	1, 2, 4, 5, 7	1, 2, 4, 5, 7	8	2	3	9,10	6
Vegetation	Grasslands	Grasslands	Grasslands	Grasslands	Grasslands	Shrub and forest	Forest
Climate	Range[d]	Range[d]	Temperate	Semi-arid	Semi-arid	Sub-tropical	Intermediate

[a]See text for description of experiments.
[b]See text for description of EFE and CFE procedure.
[c]See Table 2 for soil types.
[d]Range from semi-arid to temperate climate.

Table 2
Soil types used in experiments (Table 1), MAT (mean annual temperature, °C), MAP (mean annual precipitation, mm), $\delta^{13}C$ and $\delta^{15}N$ (‰), % C and % N of total soil. Means, standard error (between brackets) and number of observations (*n*)

Soil #	Soil type	Description	MAT	MAP	Land-use	$\delta^{13}C$	$\delta^{15}N$	% C	% N	*n*
1	Lithic Camborthid	Great Basin desert	14	180	Range land	−3.0[b](0.2)	7.6 (0.2)	2.1(0.1)	0.04(0.01)	8
2	Typic Haplustoll	Desert grassland	12	230	Range land	−16.1(0.1)	7.7(0.1)	1.3(0.1)	0.12(0.01)	8
3	Typic Haplustoll[a]	Disturbed area near water reservoir	12	230	Range land	−14.8(0.2)	9.8(0.2)	3.4(0.5)	0.26(0.03)	18
4	Calcic Haplustand	Piñyon-juniper interspace	10	380	Limited grazing	−16.6(0.2)	5.7(0.1)	1.7(0.1)	0.16(0.01)	8
5	Mollic Eutroboralf	Ponderosa pine meadow	8	660	Limited grazing	−21.9(0.2)	3.8(0.1)	1.5(0.1)	0.11(0.01)	7
6	Typic Argiboroll	Ponderosa pine forest	8	660	No grazing	−23.0(0.1)	5.3(0.2)	2.6(0.1)	0.12(0.01)	36
7	Pachic Udic Argiboroll	Mixed conifer meadow	6	790	No grazing	−21.5(0.1)	5.7(0.1)	3.9(0.1)	0.35(0.01)	7
8	Pachic Udic Argiboroll	Riparian area	6	790	No grazing	−24.5(0.6)	4.4(0.1)	4.4(0.4)	0.48(0.06)	11
9	Arenic Haplohumods; Spodic Quartzipsamments	Florida coastal shrub	23	1310	Prescribed burn	ND	ND	ND	ND	—
10	Typic Argiaquoll	Florida mesic hammock	23	1310	No fire	ND	ND	ND	ND	—

[a]Gradient with large dung deposits.
[b]Presence of carbonates.

used two procedures for chloroform fumigation (Table 1). The first procedure was chloroform-fumigation-extraction (CFE; Brookes et al., 1985; Vance et al., 1987): a soil subsample was extracted immediately with a K_2SO_4 solution, while another subsample was first fumigated with chloroform and then extracted. The extracts were shaken for 1 h, filtered over a Whatman no. 1 filter and frozen at −20 °C until further processing. The alternative procedure was the extraction-fumigation-extraction (EFE; Widmer et al., 1989). Here a soil sample was first extracted with a K_2SO_4 solution, and subsequently fumigated with chloroform and re-extracted with a K_2SO_4 solution. The extracts were then treated as described for CFE. Some soils require longer exposure to chloroform to release all microbial C and N (Davidson et al., 1989; Haubensak et al., 2002). Therefore, we used different durations of chloroform exposure (24 h, 5 or 7 d, Table 1). We also varied the concentrations of the K_2SO_4 extraction solution (0.50 and 0.25 M); and soil weight to extract volume ratios (10:50; 20:50; 25:50 g:ml) in order to optimize the amount of C and N for IRMS analysis (Table 1). For most soils, the K_2SO_4 extracts were dried at 60 °C in a ventilated oven, and dried salts were ground with mortar and pestle to a fine powder. In experiment #6, fumigated and unfumigated extracts were digested using a modified micro-Kjeldahl procedure that excluded nitrate. A variable volume of digest (to achieve a mass of ~60 μg N) was diffused onto acidified paper disks, and dried for subsequent ^{15}N analysis (Stark and Hart, 1996; Holmes et al., 1998). Isotope signatures were corrected for N in blank samples and fractionation due to incomplete recoveries (Stark and Hart, 1996). Contamination of NH_4 signatures with N from organic compounds (Robinson, 2001) was not an issue after Kjeldahl digestion. Soils were ground to a fine powder using a roller mill and analyzed for total C and N.

The natural abundance ^{13}C and ^{15}N, and elemental C and N content of soil, dried extracts or paper filters were measured using an NC 2100 Elemental Analyzer interfaced with a Finnigan Delta Plus XL isotope ratio mass spectrometer at The Colorado Plateau Stable Isotope Laboratory (http://www4.nau.edu/cpsil/). For the analysis of ^{15}N of the K_2SO_4 salts, silver wool was added to the end of the oxidation column of the elemental analyzer. For analysis of ^{15}N of the paper filters (experiment #6), an in-line ascarite trap was used to trap excess CO_2. The ^{13}C and ^{15}N-isotope composition were expressed in standard notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand (‰) relative to NIST 1457 standard for C and atmospheric N_2 for N, where $\delta = 1000[(R_{sample}/R_{standard})-1]$, and R is the molar ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Precisions were better than 0.2‰ for $\delta^{15}N$, 0.1% for N-content, 0.1‰ for $\delta^{13}C$, and 0.4% for C-content based on multiple standards. For experiment #5, samples were analyzed on the Europa ANCA 20–20 CF-IRMS system at the University of California, Berkeley, California, USA.

2.3. Calculations and statistics

Calculation of the isotope signature of microbial biomass using CFE was done using mass balance, as follows:

$$\delta^{13}\mathrm{C_{MB}} = \left[\delta^{13}\mathrm{C}_F * \mathrm{C}_F - \delta^{13}\mathrm{C}_E * \mathrm{C}_E\right]/\mathrm{C_{MB}},$$

$$\delta^{15}\mathrm{N_{MB}} = \left[\delta^{15}\mathrm{N}_F * \mathrm{N}_F - \delta^{15}\mathrm{N}_E * \mathrm{N}_E\right]/\mathrm{N_{MB}},$$

where MB, *F* and *E* are microbial biomass ($CHCl_3$-labile), fumigated and extractable (unfumigated) fractions, respectively. For EFE, the C and N released after fumigation was a direct measurement of microbial biomass C and N (Widmer et al., 1989).

In addition to measuring ^{13}C and ^{15}N of the different soil fractions, we calculated (for individual soil samples) the difference between the microbial isotope composition and that of total and extractable soil C and N pools using the following equations:

$$\Delta^{13}\mathrm{C_{ME}} = \delta^{13}\mathrm{C_{MB}} - \delta^{13}\mathrm{C_E},$$

$$\Delta^{13}\mathrm{C_{MS}} = \delta^{13}\mathrm{C_{MB}} - \delta^{13}\mathrm{C_S},$$

$$\Delta^{15}\mathrm{N_{ME}} = \delta^{15}\mathrm{N_{MB}} - \delta^{15}\mathrm{N_E},$$

$$\Delta^{15}\mathrm{N_{MS}} = \delta^{15}\mathrm{N_{MB}} - \delta^{15}\mathrm{N_S},$$

where $\Delta^{13}\mathrm{C_{ME}}$ and $\Delta^{15}\mathrm{N_{ME}}$ are the ^{13}C and ^{15}N-enrichment of the microbial biomass (MB) relative to the extractable (*E*) fraction, and $\Delta^{13}\mathrm{C_{MS}}$ and $\Delta^{15}\mathrm{N_{MS}}$ are the ^{13}C and ^{15}N-enrichment of the microbial biomass relative to total soil fraction (*S*).

To test whether δ^{13}C and δ^{13}N of microbial biomass differed significantly from δ^{13}C and δ^{15}N of soil total and extractable C and N, averages and 95% confidence intervals were calculated for Δ^{13}C and Δ^{15}N. Differences between soils where C3-plant species were dominant (C3-soil) and where C4-plant species were dominant (C4-soil) were evaluated using *t*-test with unequal variances. All differences were tested at $P<0.05$.

3. Results

The sites in these experiments cover the range from soils dominated by C3 plant species and soils exhibiting the ^{13}C signature of C4 plants. Very high δ^{13}C values of the total soil were associated with the presence of calcium carbonate, a widespread phenomenon in semi-arid soils (Taylor, 1983). The presence of calcium carbonate was concluded from bubbling (CO_2) after addition of a diluted acid and decreases in the δ^{13}C of the total soil after acid fumigation (according to Midwood and Boutton, 1998; data not shown). The δ^{13}C values of the extractable and microbial C generally followed those of the total soil C pool (Fig. 1), except for carbonate-rich, semi-arid soils with high δ^{13}C values. In carbonate-rich soils, microbial and extractable C exhibited δ^{13}C values in line with the signature of the dominant vegetation (Fig. 1(A)). The average δ^{13}C value of microbial biomass (−18.9‰) was lower than that of total C (−17.8 ‰), or, in other words, $\Delta^{13}\mathrm{C_{MS}}$ was significantly lower than zero (Table 4). However, $\Delta^{13}\mathrm{C_{MS}}$ was not significantly different from zero for four out of five experiments (Table 3). Moreover, $\Delta^{13}\mathrm{C_{MS}}$ differed for C3, C4 and carbonate-rich soils (Table 4). The $\Delta^{13}\mathrm{C_{MS}}$ value was significantly greater than zero for C3-soils, but significantly lower than zero for C4 and carbonate-rich soils. Some of the δ^{13}C values of the microbial pool in C4-soils were close to those of C3 plant species. The δ^{13}C values of microbial biomass for carbonate-rich soils were in the range of C4 plants (Fig. 1(A)). The microbial biomass was significantly ^{13}C-enriched relative to the extractable C

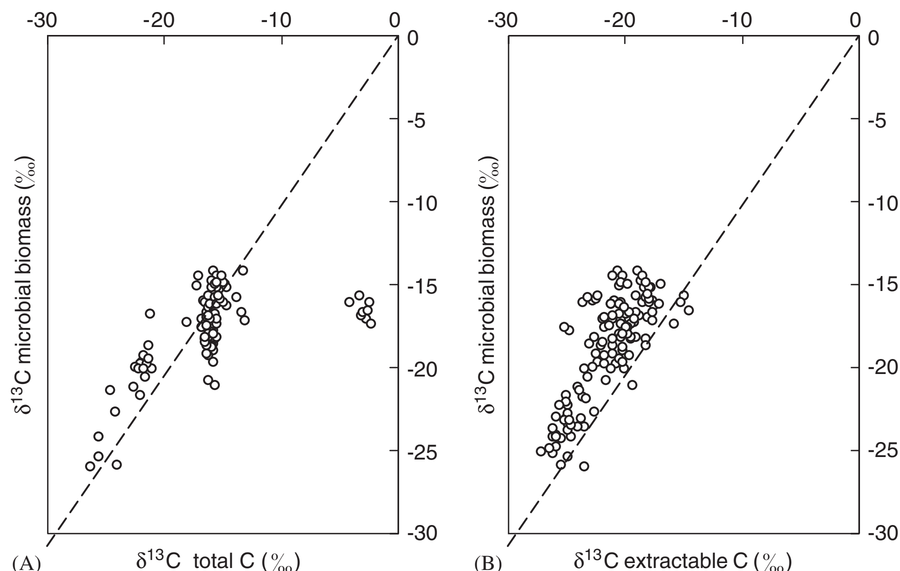

Fig. 1. Relationship of δ^{13}C of total (A) and extractable soil C (B) with δ^{13}C of microbial C (‰). Each point represents a single soil sample; number of observations and standard error information per experiment in Table 3. Line indicates 1:1 ratio.

Table 3
Mean, standard error and number of observations of soil (mg g^{-1} dry soil), extractable (extr; mg kg^{-1} dry soil) and microbial (micr; mg kg^{-1} dry soil) C and N, δ^{13}C (‰), δ^{15}N (‰), microbial enrichment relative to total soil ($\Delta^{13}C_{MS}$, $\Delta^{15}N_{MS}$) and extractable fractions ($\Delta^{13}C_{ME}$, and $\Delta^{15}N_{ME}$; ‰) for the different experiments described in Table 1

Experiment #		1a	1b	2	3	4	5	6
C	Soil	21.1 (2.2; 19)	21.0 (2.2; 19)	42.1 (3.3; 6)	12.7 (0.3; 38)	34.4 (4.6; 18)	ND	25.9 (1.4; 36)
	Extr	51.9 (4.6; 19)	29.5 (2.4; 19)	44.1 (2.7; 11)	18.4 (0.9; 40)	231 (49; 19)	243 (56; 20)	ND
	Micr	259 (22; 19)	116 (12; 19)	133 (17.6; 11)	56.0 (3.6; 40)	341 (61; 19)	950 (219; 20)	ND
N	Soil	1.51 (0.23;19)	1.58 (0.24; 19)	4.77 (0.61; 11)	1.20 (0.02; 39)	2.58 (0.25; 18)	ND	1.20 (0.06; 36)
	Extr	8.77 (0.68; 19)	7.94 (0.65; 19)	8.49 (0.98; 11)	4.74 (0.18; 40)	58.0 (12.0; 19)	24.0 (5.2; 20)	6.16 (0.49; 36)
	Micr	38.2 (4.3; 19)	18.6 (2.4; 19)	24.3 (3.0; 11)	6.71 (0.5; 40)	37.4 (5.5; 19)	111 (30; 20)	18.5 (1.3; 36)
δ^{13}C	Soil	−15.7 (1.6; 19)	−15.3 (1.6; 19)	−25.0 (0.4; 6)	−16.1 (0.1; 38)	−14.8 (0.2; 18)	ND	−23.0 (0.1; 36)
	Extr	−22.1 (0.5; 19)	−19.2 (0.5; 19)	−23.9 (0.2; 11)	−20.6 (0.2; 40)	−18.2 (0.2; 19)	−25.5 (0.2; 20)	ND
	Micr	−18.4 (0.4; 19)	−17.2 (0.5; 19)	−23.6 (0.5; 11)	−17.7 (0.2; 40)	−15.5 (0.2; 19)	−23.7 (0.2; 20)	ND
δ^{15}N	Soil	5.8 (0.3; 19)	6.3 (0.3; 19)	4.3 (0.13; 11)	7.76 (0.06; 39)	9.81 (0.17; 18)	ND	5.22 (0.16; 36)
	Extr	5.5 (0.4; 19)	6.5 (0.4; 19)	5.6 (0.6; 11)	7.7 (0.3; 40)	9.0 (0.7; 19)	3.6 (0.3; 20)	3.2 (0.2; 36)
	Micr	8.1 (0.4; 19)	11.2 (0.2; 19)	8.7 (0.8; 11)	11.1 (0.4; 40)	14.7 (0.4; 19)	7.1 (0.9; 20)	6.7 (0.4; 36)
	$\Delta^{13}C_{MS}$	−2.7 (1.4; 19)	−2.0 (1.5; 19)	0.7 (0.7; 6)	−1.7* (0.2; 38)	−0.7 (0.34; 18)	ND	ND
	$\Delta^{13}C_{ME}$	3.7* (0.4; 19)	1.9* (0.6; 19)	0.3 (0.4; 11)	2.9* (0.3; 40)	2.7* (0.3; 19)	1.8* (0.2; 20)	ND
	$\Delta^{15}N_{MS}$	2.2* (0.4; 19)	4.9* (0.4; 19)	4.4* (0.7; 11)	3.3* (0.4; 39)	4.9* (0.4; 18)	ND	1.4* (0.3; 36)
	$\Delta^{15}N_{ME}$	2.6* (0.5; 19)	4.6* (0.4; 19)	3.0* (1.2; 11)	3.5* (0.6; 40)	5.7* (0.6; 19)	3.5* (0.7; 20)	3.5* (0.4; 36)

*indicates microbial enrichments significantly different from zero ($P<0.05$), ND = not determined.

Table 4
Microbial ^{13}C-enrichment (‰) relative to total and extractable soil C

	Mean	se	n	P
All data				
$\Delta^{13}C_{MS}$	−1.6	0.4	100	<0.01
$\Delta^{13}C_{ME}$	2.6	0.2	100	<0.01
C3-soil				
$\Delta^{13}C_{MS}$	1.6	0.3	20	<0.01
$\Delta^{13}C_{ME}$	1.5	0.4	20	<0.01
C4-soil				
$\Delta^{13}C_{MS}$	−1.1	0.2	72	<0.01
$\Delta^{13}C_{ME}$	3.2	0.2	72	<0.01
Carbonate-rich soil				
$\Delta^{13}C_{MS}$	−13.7	0.3	8	<0.01
$\Delta^{13}C_{ME}$	0.4	0.7	8	NS

C3-soils (δ^{13}C soil less than −21‰), C4-soils (δ^{13}C between −13‰ and −17 ‰) and soils with carbonates (δ^{13}C greater than −5 ‰). Means, standard error (se) and number of observations (n). $P<0.01$ indicates significantly different from zero, NS = not significant.

for five of the six experiments and overall (Fig. 1(B), Table 3). Significant microbial ^{13}C-enrichments relative to extractable C were found for C3 and C4-soils, but not for carbonate-rich soils (Table 4).

The δ^{15}N value of microbial and extractable fractions followed δ^{15}N of the total soil N (Fig. 2). The average δ^{15}N of microbial N (9.7 ‰) was significantly higher than that of total (6.6 ‰) and extractable N (5.6 ‰, Table 5). The $\Delta^{15}N_{MS}$ and $\Delta^{15}N_{ME}$ values were significantly greater than zero for all experiments (Table 3), and for C3 and C4-soils separately (Table 5). Microbial biomass in carbonate-rich soils again showed only non-significant enrichments. Average microbial ^{15}N-enrichment was greater for C4- than for C3-soils ($\Delta^{15}N_{MS}$ $P<0.01$; $\Delta^{15}N_{ME}$ $P<0.01$). These conclusions were found irrespective of methodological differences in general methodology (CFE vs. EFE), chloroform exposure (1–7 d) and extraction conditions (Tables 1 and 3).

The δ^{13}C and δ^{15}N values of microbial biomass were positively correlated (Fig. 3). Similar correlations were observed for δ^{13}C and δ^{15}N of the extractable (Fig. 3) and total soil fractions (not shown).

4. Discussion

Most semi-arid ecosystems exhibit a dominance of C4-species and greater N-losses relative to temperate ecosystems, where C3 species are found and N-losses are low (Amundson et al., 2003). These differences affect the δ^{13}C and δ^{15}N of the SOM (Handley et al., 1999; Šantrůčková et al., 2000; Amundson et al., 2003) and as a result, the isotope composition of the microbial biomass (Figs. 1 and 2). We submit that the observed positive correlation between the ^{13}C and ^{15}N composition of microbial and other soil fractions (Fig. 3) is best explained by the

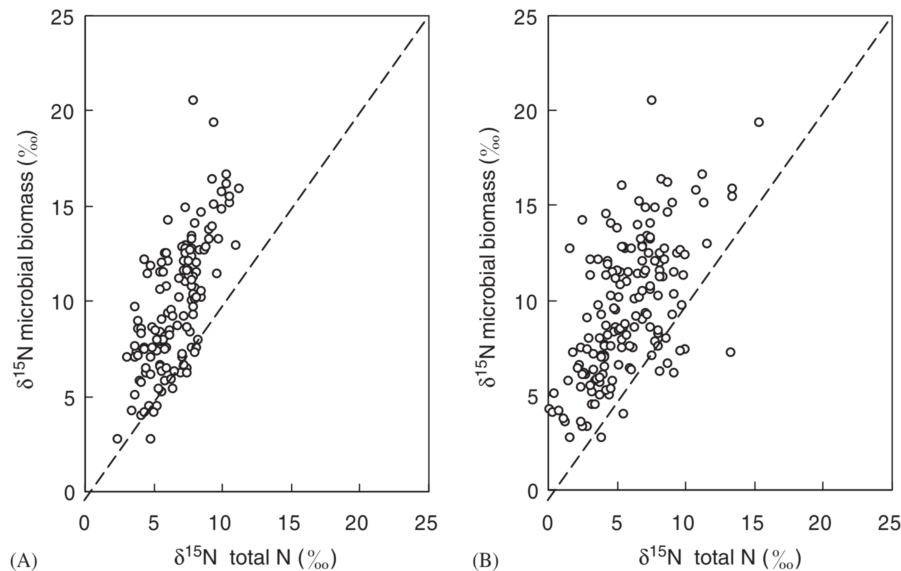

Fig. 2. Relationship of δ^{15}N of total (A) and extractable soil N (B) with δ^{15}N of microbial N (‰). Each point represents a single soil sample; number of observations and standard error information per experiment in Table 3. Line indicates 1:1 ratio.

Table 5
MMicrobial ^{15}N-enrichment (‰) relative to soil total and extractable N

	Mean	se	*n*	*P*
Total data				
$\Delta^{15}N_{MS}$	3.1	0.2	136	<0.01
$\Delta^{15}N_{ME}$	3.7	0.2	136	<0.01
C3-soil				
$\Delta^{15}N_{MS}$	2.4	0.3	56	<0.01
$\Delta^{15}N_{ME}$	3.2	0.3	56	<0.01
C4-soil				
$\Delta^{15}N_{MS}$	3.9	0.3	72	<0.01
$\Delta^{15}N_{ME}$	4.2	0.4	72	<0.01
Carbonate-rich soil				
$\Delta^{15}N_{MS}$	1.0	0.7	8	NS
$,^{15}N_{ME}$	1.8	1.0	8	NS

C3-soils (δ^{13}C soil less than −21‰), C4-soils (δ^{13}C between −13‰ and −17‰) and soils with carbonates (δ^{13}C greater than −5‰). Means, standard error (se) and number of observations (*n*). $P<0.01$ indicates significantly different from zero, NS = not significant.

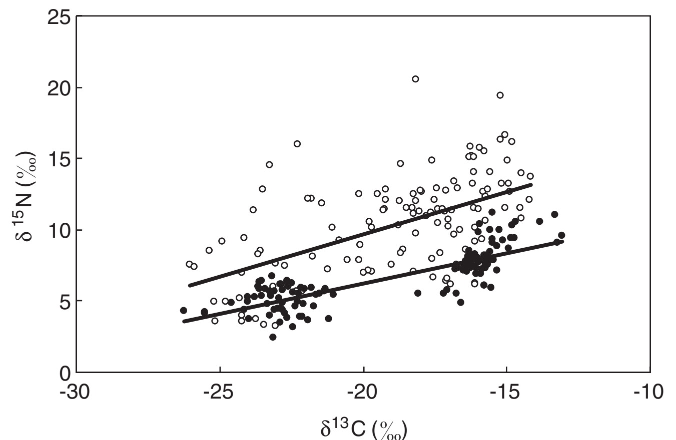

Fig. 3. Relationship between δ^{13}C and δ^{15}N (‰) for total SOM (closed circles) and microbial biomass (open circles). Total SOM: $\delta^{15}N = 0.42 \times \delta^{13}C + 14.71$ ($r = 0.82$, $n = 136$, $P<0.01$); microbial biomass: $\delta^{15}N = 0.60 \times \delta^{13}C + 21.60$ ($r = 0.55$, $n = 128$, $P<0.01$), and extractable fraction (not shown): $\delta^{15}N = 0.51 \times \delta^{13}C + 17.38$ ($r = 0.55$, $n = 128$, $P<0.01$).

co-occurring shifts in species composition and N-losses described above, and does not require an additional mechanistic explanation. However, microbial biomass exhibits small but significant deviations relative to extractable and total C and N pools (Figs. 1 and 2). We concur with Henn and Chapela (2001) that it is important, when studying live organisms, to focus on its isotope signatures *in relation to* that of other soil pools (Δ^{13}C, Δ^{15}N), instead on the absolute values of δ^{13}C and δ^{15}N. This is especially the case if a broad range of soils with different δ^{13}C and δ^{15}N values is compared.

4.1. Methodological considerations

We have determined the microbial isotope composition using the CFE technique (Brookes et al., 1985; Vance et al., 1987; Widmer et al., 1989). This method is widely used to estimate microbial C and N pool sizes (Jenkinson et al., 2004). We have adapted this method for measurement of δ^{13}C and δ^{15}N. However, since the fumigation-extraction technique has not been used before to measure natural abundance signatures of microbial biomass, we have to consider the appropriateness of this method for this purpose. The data presented in this paper is a first

evaluation of the use of this method for isotope analysis at natural abundance levels.

Although chloroform vapor quickly kills microorganisms in the soil, some soils require longer fumigation than other soils. For example, Davidson et al. (1989) found that a grassland soil required 7 d of chloroform exposure for maximum N release, while 1 d was sufficient for a forest soil. Similarly, Haubensak et al. (2002) reported that a minimum of 5 d was adequate for most of the tested soils. In this series of experiments, we have varied the fumigation duration and extraction conditions, but this did not affect the conclusions concerning isotope enrichment of the microbial biomass relative to other fractions (Tables 1 and 3).

Drying of the extract solutions in a ventilated oven can potentially result in the removal of volatile N compounds (e.g. ammonia), the loss of especially the lighter N-isotope (Handley and Raven, 1992; Högberg, 1997), and thus results in an increased ^{15}N of the extracts. We think that oven drying had only small effects on the measured isotope values and enrichments for the following three reasons. (1) Because N-loss is similar in fumigated and unfumigated oven-dried extracts, the $\delta^{15}N$ of microbial biomass, calculated as the difference between the two extracts, is not affected. Since $\delta^{15}N$ of the extractable soil pool increases by volatilization, but $\delta^{15}N$ of the microbial biomass is not affected, a low $\Delta^{15}N_{ME}$ is expected. This expectation can be contrasted with that using the micro-Kjeldahl technique (experiment #6). N-volatilization is absent with the micro-Kjeldahl technique since pH is low during sample destruction. However, the $\Delta^{15}N_{ME}$ value in experiment #6 was similar to that in experiments where the extract solutions were oven-dried (Table 3), demonstrating that oven drying had little effect. (2) Volatilization happens especially at high pH (Bronson et al., 1999) and desert soils are known for their high pH values (e.g. Dijkstra et al., 2006; soil # 1–3). However, ^{13}C and ^{15}N enrichments for the microbial biomass in these soils were not different from those of soils with medium (pH around 6, soil # 5–8) or low pH (4.0, soil # 9, 10). (3) A direct test whereby extracts, normally pH 5.5–6, are acidified to pH 2, did not significantly affect $\delta^{15}N$ of fumigated and unfumigated samples (data not shown).

It is well known that chloroform-labile C and N are less than the true total amount of C and N contained in the microbial biomass (Horwath and Paul, 1994). It is likely that the more recalcitrant portions of the cell, such as the cell wall, remain in the soil (Greenfield, 1995). It can be questioned whether the entire microbial cell or only the chloroform-labile compounds is ^{13}C and ^{15}N enriched relative to the total soil. Taylor et al. (1997) found that amino acids and proteins were ^{15}N-enriched relative to the total fungal cell. However, the difference was only 1.5‰, smaller than enrichments we found in this experiment (Tables 3 and 5). Similarly, although chitin was ^{13}C-depleted relative to the total microbial cell (Gleixner et al., 1993), both chitin and the total cell were ^{13}C-enriched relative to the cellulose substrate. Moreover, the finding of ^{13}C and ^{15}N-enrichments for whole cells and tissues (detailed below) suggests that microbial ^{13}C and ^{15}N-enrichments reported here are representative for the whole cell and not just the soluble part.

4.2. *Soil microbial biomass is ^{13}C-enriched*

The microbial biomass is ^{13}C-enriched (1.6‰) relative to the total C for C3-soils (Table 4). This enrichment is consistent with studies where the fumigation-incubation method has been used (Jenkinson and Powlson, 1976). Microbial C was ^{13}C-enriched with 1.7–5.6‰ relative to soil C (Rochette et al., 1999; Gregorich et al., 2000; Šantrůčková et al., 2000; Liang et al., 2002; Potthoff et al., 2003, but see Ryan and Aravena, 1994; Qian et al., 1997). Gleixner et al. (1993) observed a 4‰ ^{13}C-enrichment for Basidiomycetes growing on wood. Macko and Estep (1984) found on average 3.7‰ ^{13}C-enrichment for cultured *Vibrio harveyi*. The observed microbial ^{13}C-enrichments are also consistent with observations on fungal sporocarps. For example, mycorrhizal fungi were 2‰ enriched and saprophytic fungi are 4‰ ^{13}C-enriched relative to tree leaves (Högberg et al., 1999b; Hobbie et al., 1999). Kohzu et al. (1999) showed that ectomycorrhiza were 1.4‰ and wood-decaying fungi were 3.5‰ enriched relative to wood tissues.

The $\delta^{13}C$ value of the microbial fraction is lower than $\delta^{13}C$ of the total C for C4-soils (Fig. 1(A), Table 4), in contrast to the results for C3-soils. The ^{13}C-signature of these soils indicates that most of the SOM is derived from C4-plant species. However, since a record drought in 2002, annual C3 forbs have replaced much of the perennial C4 grasses (personal observation, Paul Dijkstra) at these sites. We propose that the low $\delta^{13}C$ values of microbial and extractable C for the C4-soil are the result of the presence of C3 litter. A greater ^{13}C-enrichment in C3-soils (2.4‰) than C4-soils (1.4‰) was also observed by Šantrůčková et al. (2000). Microbial isotope signatures responded within weeks to addition of C4 litter to a C3-soil (Gregorich et al., 2000; Potthoff et al., 2003; John et al., 2004). We did find significant increases of $\delta^{13}C$ of microbial C relative to extractable C for both C3 and C4-soils (Tables 3 and 4). Extractable and microbial fractions are both dynamic and will reflect the isotope composition of the most recent litter input.

4.3. *Soil microbial biomass is ^{15}N-enriched*

The microbial biomass is consistently ^{15}N-enriched relative to total and extractable soil fractions in our experiments (Fig. 2). This ^{15}N-enrichment is found in all experiments (Table 3) irrespective of methodological variations, vegetation, climate, and soil type. We did not find any literature data that could be directly compared with our dataset. The $\Delta^{15}N$ values are greater than values for $\Delta^{13}C$ (compare Tables 4 and 5). This is consistent with food web studies where the trophic-level increases in $\delta^{15}N$

are usually larger than in $\delta^{13}C$ (Koch et al., 1994; Michener and Schell, 1994). In contrast to our results, Henn and Chapela (2004), in a cell-culture experiment, found that fungi were ^{15}N-depleted relative to a diet rich in NH_4^+. However, this was explained by NH_3 diffusion into the cell, a phenomenon that is probably restricted to few natural soil niches. Macko and Estep (1984) showed ^{15}N-enrichment for *V. harveyi,* a marine bacterium, when fed with amino acids. Schmidt and Gleixner (2005) proposed that ^{15}N-enriched materials removed from soil by HF-treatment were derived from microorganisms. They estimated that this fraction was 1–4‰ ^{15}N-enriched relative to the soil. This corresponds well with our observations. Finally, our findings are also in agreement with reported ^{15}N-enrichments of fungal sporocarps relative to soils and plants (Högberg et al., 1996, 1999a; Gebauer and Taylor, 1999; Kohzu et al., 1999; Hobbie et al., 1999; Lilleskov et al., 2002).

4.4. Interpretation of ^{13}C and ^{15}N-enrichment of the soil microbial biomass

The ^{13}C and especially ^{15}N-enrichments of the microbial biomass observed in this study are consistent with findings from food web studies (Koch et al., 1994; Michener and Schell, 1994). Nonrandom intra-molecular isotope distributions (Roßmann et al., 1991; Hobbie and Werner, 2004) and fractionating biochemical steps during assimilation and dissimilation (Gleixner et al., 1993; Nadelhoffer and Fry, 1994; Högberg, 1997; Evans, 2001), including plant-fungus C and N transfer (Hobbie and Colpaert, 2003), are likely involved. However, in the complex environment of the soil, additional fractionating processes and impacts of multiple substrate pools with different isotope compositions need to be considered (Robinson, 2001).

It is well established that soil microorganisms have an important role in the formation of stabilized SOM (e.g. Kramer et al., 2003; Six et al., 2004). However, the role of the microbial biomass in determining the isotope composition of SOM fractions is less clear. Since microbial biomass is ^{15}N and in some cases ^{13}C-enriched relative to the total soil N and C, we postulate that the microbial compounds are directly involved in the isotope composition of the SOM. Our results do not support the idea that soil microorganisms preferentially utilize isotopically depleted substrates. If the microbial enrichments described in this study reflect the influence of fractionating processes, then $\delta^{13}C$ and $\delta^{15}N$ of the microbial products will gradually increase with repeated organic matter processing and humification. This expectation is partly confirmed by Kramer et al. (2003). They found a positive relationship between the degree of humification (aliphaticity) and $\delta^{15}N$ of the SOM. However, no such relationship was observed between $\delta^{13}C$ and aliphaticity. Similarly, Tiessen et al. (1984) observed that $\delta^{15}N$ of SOM associated with clay particles was higher than that associated with silt and sand.

On the other hand, the observed microbial enrichments may only reflect the isotope composition of the substrate used. It is well established that various soil C and N-pools have different isotope values (e.g. Tiessen et al. 1984; Nadelhoffer and Fry 1994; Kramer et al. 2003). Microorganisms that selectively utilize these compounds will exhibit a signature that is close to that of these substrate pools. As already mentioned, we believe that the ^{13}C-depletion of microbial biomass in C4 soils relative to total SOM (Fig. 1(A), Table 4) is related to the signature of the C-source (recent presence of C3-plants). This may also be the case for ^{15}N. Instead of causing ^{15}N-enrichment in stabilized organic matter, the high ^{15}N of the microbial biomass may be the consequence of consuming more-decomposed organic N-compounds, for example, during turnover of micro-aggregates (Six et al., 2004). More research is needed to evaluate which possibility best explains the microbial isotope compositions.

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