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Relationships between C and N availability, substrate age, and natural abundance ^{13}C and ^{15}N signatures of soil microbial biomass in a semiarid climate

Jeff S. Coyle [a], Paul Dijkstra [a,*], Richard R. Doucett [b], Egbert Schwartz [a], Stephen C. Hart [c,d,e], Bruce A. Hungate [a,d]

[a] Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA
[b] Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA
[c] School of Forestry, Northern Arizona University, P.O. Box 15018, Flagstaff, AZ 86011, USA
[d] Merriam-Powell Center for Environmental Research, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA
[e] School of Natural Sciences and Sierra Nevada Research Institute, University of California, Merced, CA 95344, USA

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ABSTRACT

Soil microbial organisms are central to carbon (C) and nitrogen (N) transformations in soils, yet not much is known about the stable isotope composition of these essential regulators of element cycles. We investigated the relationship between C and N availability and stable C and N isotope composition of soil microbial biomass across a three million year old semiarid substrate age gradient in northern Arizona. The $\delta^{15}N$ of soil microbial biomass was on average 7.2‰ higher than that of soil total N for all substrate ages and 1.6‰ higher than that of extractable N, but not significantly different for the youngest and oldest sites. Microbial ^{15}N enrichment relative to soil extractable and total N was low at the youngest site, increased to a maximum after 55,000 years, and then decreased slightly with age. The degree of ^{15}N enrichment of microbial biomass correlated negatively with the C:N mass ratio of the soil extractable pool. The $\delta^{13}C$ signature of soil microbial biomass was 1.4‰ and 4.6‰ enriched relative to that of soil total and extractable pools respectively and showed significant differences between sites. However, microbial ^{13}C enrichment was unrelated to measures of C and N availability. Our results confirm that ^{15}N, but not ^{13}C enrichment of soil microbial biomass reflects changes in C and N availability and N processing during long-term ecosystem development.

1. Introduction

Natural abundance stable carbon (C) and nitrogen (N) isotope measurements are widely used as a tool in ecological research (Nadelhoffer et al., 1996; Högberg, 1997; Evans, 2001; Robinson, 2001; Staddon, 2004; West et al., 2006). Soil and plant C and N pools differ in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios as a result of isotope fractionation during C and N transformations and isotope differences in the original substrates. However, there is almost no information on the stable isotope composition of soil microorganisms, even though this community is central to C and N cycling in soils. Knowing the processes that influence the microbial biomass isotope composition will increase our understanding of isotope differences in the products of microbial activities, specifically soil organic matter, respired CO_2 and inorganic N.

Heterotrophic organisms such as animals exhibit consistent but variable ^{15}N enrichments relative to their diet, while both depletions and enrichments in natural ^{13}C abundance isotope compositions are observed (Minagawa and Wada, 1984; Post, 2002; Vanderklift and Ponsard, 2003). We hypothesize that microbial C and N pools similarly exhibit significant but variable ^{15}N and ^{13}C enrichments relative to other soil C and N pools. Although some microorganisms are autotrophic, the majority of the soil microbial community is heterotrophic and is expected to exhibit patterns of isotope fractionation characteristic for other heterotrophic organisms.

Consistent enrichments of the natural ^{15}N abundance isotope composition of microbial biomass have been observed. Dijkstra et al. (2006a) reported that $\delta^{15}N$ of microbial N was (~3.5‰) higher than that of soil total and extractable N. Higher $\delta^{15}N$ values than their purported substrates were also reported for ectomycorrhizal and saprotrophic fungi (Gebauer and Taylor, 1999; Hobbie et al., 1999; Kohzu et al., 1999) and cultured microorganisms (Macko and Estep, 1984; Collins et al., 2008). Even some myco-heterotrophic

* Corresponding author. Tel.: +1 928 523 0432; fax: +1 928 523 7500.
E-mail address: paul.dijkstra@nau.edu (P. Dijkstra).

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plants are ^{15}N enriched relative to their fungal hosts (Gebauer and Meyer, 2003; Trudell et al., 2003).

Dijkstra et al. (2008) hypothesized that fractionation during N dissimilation and export (deamination and associated transaminations, and NH_4^+/NH_3 state change; Fig. 1) is the main cause for microbial ^{15}N enrichment. This fractionation preferentially removes the lighter ^{14}N from microbial cells during N mineralization. The resulting enrichment is to a variable degree balanced by opposite fractionation during N assimilation. Dependent on C availability, N dissimilation or N assimilation will dominate (Fig. 1). With low C availability, organic N will be utilized mostly as a source of C and energy, and excess N will be removed from the cell (net N mineralization). Under these conditions, $\delta^{15}N$ of microbial biomass will be high. With high C availability, organic N (and inorganic N) is used as a source for N. Under these conditions, fractionation is low and $\delta^{15}N$ of microbial biomass is close to that of the N substrates. This model of fractionation resembles models proposed for ecto- and ericoid mycorrhizal fungi (Hobbie and Hobbie, 2008). At high external inorganic N concentrations, fractionation during uptake and assimilation may result in ^{15}N-depleted biomass (Henn and Chapela, 2004). This is also found for plants (Evans, 2001). However, such high concentrations are likely rare in natural soils.

The factors that influence the microbial C isotope composition are less clear at this time and reflect the combined influences of substrate signatures and fractionation during CO_2 release (kinetic isotope fractionation during metabolic processes, intra-molecular isotope sorting in different metabolic pathways, and the influence of anaplerotic processes that incorporate ^{13}C enriched C into microbial biomass). Schmidt and Gleixner (1998) and Hobbie and Werner (2004) conclude that predicting the C isotope composition of whole organisms is difficult with current knowledge, especially because it is insufficiently understood which metabolic pathways are active in microbial organisms. The presence of multiple and unknown substrates in soil, with distinct isotope compositions adds to this complexity. For example, lignin and cellulose are clearly different in ^{13}C signatures (Bowling et al., 2008).

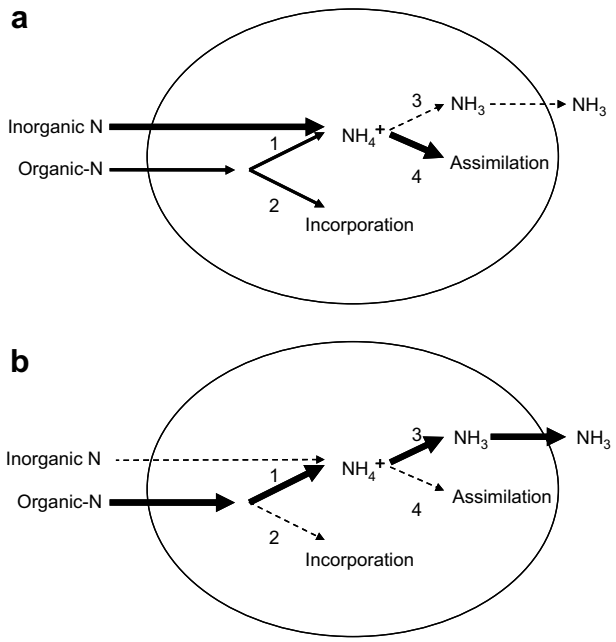

Fig. 1. Model for ^{15}N fractionation under high (a) and low (b) C availability. Circle represents cell membrane, and width of arrows indicates the relative rates. 1- kinetic isotope fractionation during deamination and associated transaminations, 2- direct incorporation, 3- equilibrium isotope fractionation during NH_4^+/NH_3 state transition, and 4- kinetic isotope fractionation during N assimilation (glutamine synthetase). Under low C availability, inorganic N uptake is low. During N mineralization, NH_3 is exported from the cell. NH_4^+/NH_3 state change is required to pass through hydrophobic barriers (membranes and channels). Fractionation during uptake is considered minimal as organic and inorganic substrate concentrations at the plasmalemma are low under normal soil conditions.

The few studies that have reported on the natural ^{13}C abundance of soil microbial biomass found higher $\delta^{13}C$ values for microbial C than for soil total C pool (Bruulsema and Duxbury, 1996; Šantrůčková et al., 2000; Potthoff et al., 2003; Dijkstra et al., 2006a; Murage and Voroney, 2007). In addition, ^{13}C-enriched fungal tissues were observed in field (Högberg et al., 1999; Kohzu et al., 1999) and laboratory studies (Gleixner et al., 1993; Henn and Chapela, 2000; Henn et al., 2002). In the literature there are conflicting results on the relationship between the isotope composition of respired CO_2 and microbial C: both depletions (Šantrůčková et al., 2000) and enrichments (Werth and Kuzyakov, 2009) are observed. Bowling et al. (2008) in a meta-analysis estimate that CO_2 produced from soil is on average 1‰ enriched relative to soil.

Here we report on relationships between C and N availability and the stable C and N isotope composition of soil microbial biomass and other soil pools across three million years of soil development using the semiarid Substrate Age Gradient of Arizona (Selmants and Hart, 2008). A substrate age gradient is an ideal tool for this study, as it covers a large range of C and N availabilities, stable isotope signatures, and ecosystem C and N cycling processes that have naturally evolved over long periods of time (Walker and Syers, 1976; Bormann and Sidle, 1990; Crews et al., 1995; Vitousek, 2004; Wardle et al., 2004; Lambers et al., 2008; Selmants and Hart, 2008). We tested the hypotheses that 1) soil microbial biomass is ^{13}C and ^{15}N enriched relative to other soil pools, but these enrichments are variable and change with substrate age; and 2) the degree of enrichment relative to other soil pools is negatively correlated with relative C and N availability for ^{15}N. Although the fractionating steps in C metabolism have been identified (Schmidt and Gleixner, 1998; Hobbie and Werner, 2004), it is still difficult to predict the C isotope composition of whole organisms (Hobbie and Werner, 2004). Therefore, we hypothesize that the C isotope composition of microbial biomass is not a function of C and N availability. Part of this study is included in a more comprehensive analysis of relationships between microbial N isotope composition, substrate availability and ecosystem processes (Dijkstra et al., 2008).

2. Materials and methods

The Substrate Age Gradient of Arizona is located in northern Arizona, U.S.A. within the San Francisco Volcanic Field, and is described in Selmants and Hart (2008). In short, four study sites were selected ranging in substrate age from 930 y (Sunset Crater; Typic Ustorthent), 55,000 y (O'Neill Crater; Typic Durustand), 750,000 y (Red Mountain; Typic Argiustoll), to 3,000,000 y (Cedar Mountain; Typic Haplustalf). All substrates were derived from volcanic cinder deposits. Sites experienced similar climate (mean annual precipitation 360 mm per year, mean annual air temperature 10 °C), and were located at a similar elevation (between 1900 and 2075 m above sea level) on stable land surfaces with slopes less than 1%. Soil pH ranged from 6.2 to 6.7. Vegetation at each site consisted of piñon pine (*Pinus edulis* Engelm.) and one-seed juniper (*Juniperus monosperma* Engelm.) trees, while intercanopy spaces were occupied by blue grama grass (*Bouteloua gracilis* (Wild. ex Kunth) Lag. ex Griffiths), except at the youngest site where grass was mostly absent. Additional details on the Substrate Age Gradient of Arizona are available in Selmants and Hart (2008).

Soil samples were taken on March 10, 2005. At each site, eight adjacent 100-m^2 plots were laid out in intercanopy spaces between trees. From each plot, a single composite soil sample was taken by

combining four individual soil samples (A horizon, 0–10 cm depth). Each sample was taken from grass-dominated areas, at least 3 m away from adjacent C_3 trees and avoiding occasional other C_3 plants. Soil samples were kept at 4 °C until processed the next day.

The next day, soil samples were sieved (2 mm mesh). Carbon and N concentrations of microbial biomass were determined using chloroform-fumigation extraction (Dijkstra et al., 2008). Each soil sample was divided into two equal subsamples. One soil subsample was immediately extracted with a 0.25 M K_2SO_4 solution, shaken for 1 h, and filtered over a Whatman no. 1 filter. The second soil subsample was similarly extracted with 0.25 M K_2SO_4 after exposure to chloroform for five days. Extract solutions were dried in a ventilated drying oven at 60 °C, and grinded to a fine powder in preparation for isotope analysis. Isotope and element composition of salts was determined by combustion in the presence of silver in an NC 2100 elemental analyzer (CE Instruments, Milan, Italy) connected to a Thermo-Finnigan Delta plus XL isotope ratio mass spectrometer (Thermo-Electron Corp., Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory (http://www.mpcer.nau.edu/isotopelab). Soil total C and N concentration and isotope composition were similarly determined on the EA-IRMS after drying at 105 °C and homogenization by grinding with mortar and pestle. Soil water content was determined gravimetrically by overnight drying at 105 °C.

The $\delta^{13}C$ and $\delta^{15}N$ values were calculated as $\delta = 1000 \times [(R_{sample}/R_{standard}) - 1]$ with R defined as the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. C and N isotope compositions were expressed in parts per thousand (‰) relative to VPDB and AIR respectively. Isotope composition of microbial biomass was calculated using mass balance, as

$$\delta^{13}C_{MB} = \left(\delta^{13}C_F \times C_F - \delta^{13}C_{NF} \times C_{NF}\right) / C_{MB} \quad (1)$$

$$\delta^{15}N_{MB} = \left(\delta^{15}N_F \times N_F - \delta^{15}N_{NF} \times N_{NF}\right) / N_{MB} \quad (2)$$

where F and NF are fumigated and non-fumigated (immediately extracted) samples respectively, and MB stands for microbial biomass. Estimates of microbial biomass C and N, calculated as the difference in element composition between fumigated and non-fumigated samples, were not corrected for extraction efficiency. We determined the ^{13}C and ^{15}N enrichment of microbial biomass relative to other soil C and N pools as:

$$\varepsilon^{13}C_{ME} = \delta^{13}C_{MB} - \delta^{13}C_{NF} \quad (3)$$

$$\varepsilon^{13}C_{MS} = \delta^{13}C_{MB} - \delta^{13}C_S \quad (4)$$

$$\varepsilon^{15}N_{ME} = \delta^{15}N_{MB} - \delta^{15}N_{NF} \quad (5)$$

$$\varepsilon^{15}N_{MS} = \delta^{15}N_{MB} - \delta^{15}N_S \quad (6)$$

where ME and MS indicate the difference between microbial and extractable (non-fumigated) and microbial and soil total C and N respectively. S represents soil total C and N pool. Microbial enrichment was determined separately for each soil sample.

Differences between sites were evaluated using one-way ANOVA. Fisher's Least-Significant-Difference Test was used to identify significant differences among multiple means. Linear regression was performed to calculate correlation coefficients between site means.

3. Results

C and N concentration of soil total, extractable, and microbial pools changed significantly across three million years of weathering and soil development (Fig. 2, Table 1). Soil total C and N concentration increased until 750,000 y, and declined thereafter. Microbial C and N concentrations followed the same pattern as soil total C and N with substrate age. There were small but significant site differences in C and N concentration of the extractable pool, with highest values reached after 55,000 y. The C:N (mass) ratios of soil total and extractable pools were highest for the youngest site and not significantly different between older sites (Table 2).

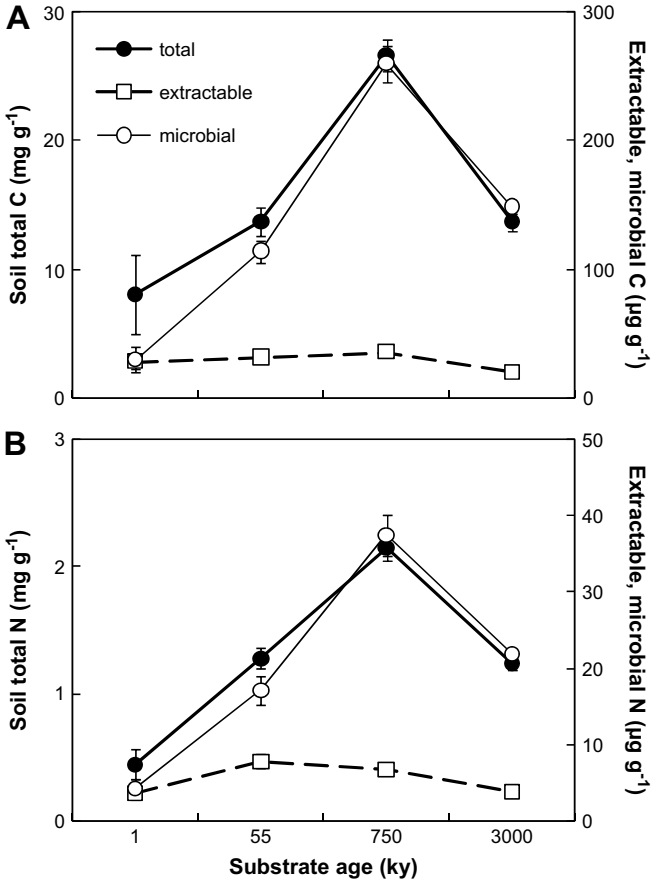

Fig. 2. Mean (±s.e.) carbon (A) and nitrogen (B) concentration of soil total, extractable, and microbial pools for four sites along the Substrate Age Gradient of Arizona.

Table 1
One-way ANOVA analysis of differences between four sites along the Substrate Age Gradient of Arizona. Soil C and N concentration as mg g^{-1} dry soil; soil extractable and microbial C and N content as µg g^{-1} dry soil; isotope values in ‰.

	df	F	*n*	*P*
Soil total C	3;27	19	7–8	<0.001
Soil total N	3;27	59	7–8	<0.001
Extractable C	3;28	4.1	8	<0.02
Extractable N	3;28	9.2	8	<0.001
Microbial C	3;28	96	8	<0.001
Microbial N	3;28	58	8	<0.001
C:N ratio total soil	3;27	4	7–8	<0.02
C:N ratio extractable pool	3;28	13	8	<0.001
C:N ratio microbial biomass	3;28	0.02	8	0.99
$\delta^{13}C$ of soil total C	3;27	220	7–8	<0.001
$\delta^{15}N$ of soil total N	3;27	36	7–8	<0.001
$\delta^{13}C$ of the extractable C	3;27	21	7–8	<0.001
$\delta^{15}N$ of the extractable N	3;28	1.0	8	0.39
$\delta^{13}C$ of microbial C	3;27	34	7–8	<0.001
$\delta^{15}N$ of microbial N	3;28	10	8	<0.001
$\varepsilon^{13}C_{ME}$	3;27	11	7–8	<0.001
$\varepsilon^{15}N_{ME}$	3;28	3	8	0.06
$\varepsilon^{13}C_{MS}$	3;26	1.6	7–8	0.22
$\varepsilon^{15}N_{MS}$	3;27	8	8	<0.001

Table 2
C:N (mass) ratio of soil total, extractable and microbial pools for four sites along the Substrate Age Gradient of Arizona. Values are means ± s.e. ($n = 8$, except * where $n = 7$).

Site	Age (year)	Total	Extractable	Microbial
Sunset Crater	930	15.3 ± 2.0	8.1 ± 0.5	7.1 ± 1.7
O'Neil Crater	55,000	10.7 ± 0.2	4.2 ± 0.3	7.1 ± 0.7
Red Mountain	750,000	12.4 ± 0.1	5.3 ± 0.4	7.0 ± 0.3
Cedar Mountain	3,100,000	11.0 ± 0.1*	5.4 ± 0.5	6.8 ± 0.1

Significant differences in C:N ratio of the microbial pool were absent.

The youngest site had a low $\delta^{13}C$ value of soil total C, likely caused by a low abundance of *B. gracilis*, a C_4 grass (Fig. 3). The coarse minerals and low water holding capacity at this site (Selmants and Hart, 2008) may have limited growth and establishment for these superficially rooted plants. In soils older than 55,000 y, there was a small but significant decline in soil total $\delta^{13}C$. This may reflect a shift in C input from C_4 grasses to C_3 trees, or long-term differences in soil organic matter processing and storage. The $\delta^{13}C$ of microbial biomass followed the same pattern with substrate age. Unexpectedly, extractable C had a much lower $\delta^{13}C$ value than the other soil C pools, except for the youngest site. Soil total $\delta^{15}N$ values decreased slightly as substrate aged from 930 to 55,000 y, but then increased steadily with time (Fig. 4). Extractable N was ^{15}N enriched relative to soil total N, but followed a similar pattern over time. The microbial N isotope signature was lowest at the youngest site (Fig. 4).

Microbial biomass was significantly ^{13}C enriched relative to soil total and extractable C at all sites. Microbial ^{13}C enrichment relative to soil total C was highest at the youngest site, declined to a minimum at 55,000 y and slightly increased again at older sites (Fig. 3). Microbial ^{13}C enrichment relative to the extractable pool showed large increases with substrate age (Fig. 3).

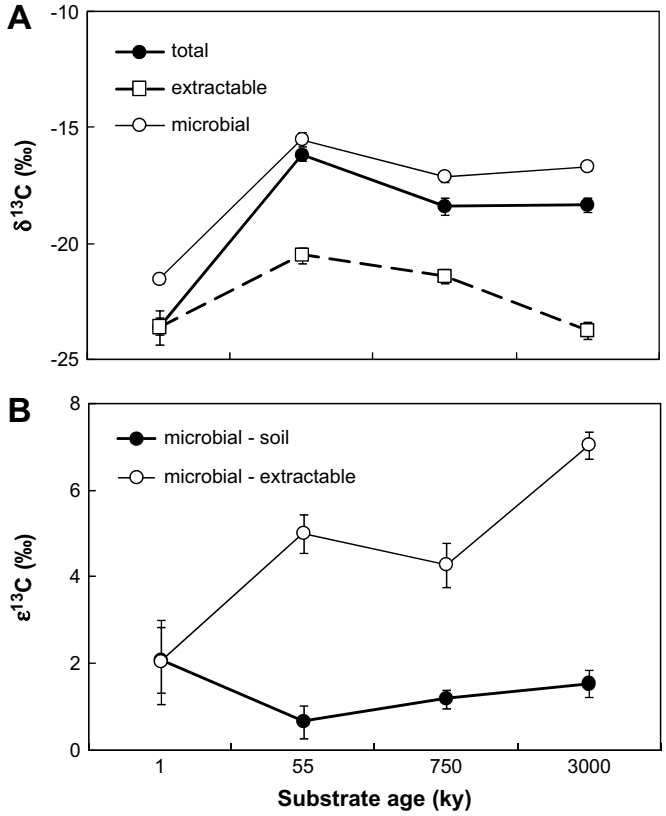

Fig. 3. Mean (±s.e.) $\delta^{13}C$ of soil total, extractable, and microbial pools (A) and $\varepsilon^{13}C_{ME}$ (microbial ^{13}C enrichment relative to extractable C) and $\varepsilon^{13}C_{MS}$ (microbial ^{13}C enrichment relative to soil total C) (B) for four sites along the Substrate Age Gradient of Arizona.

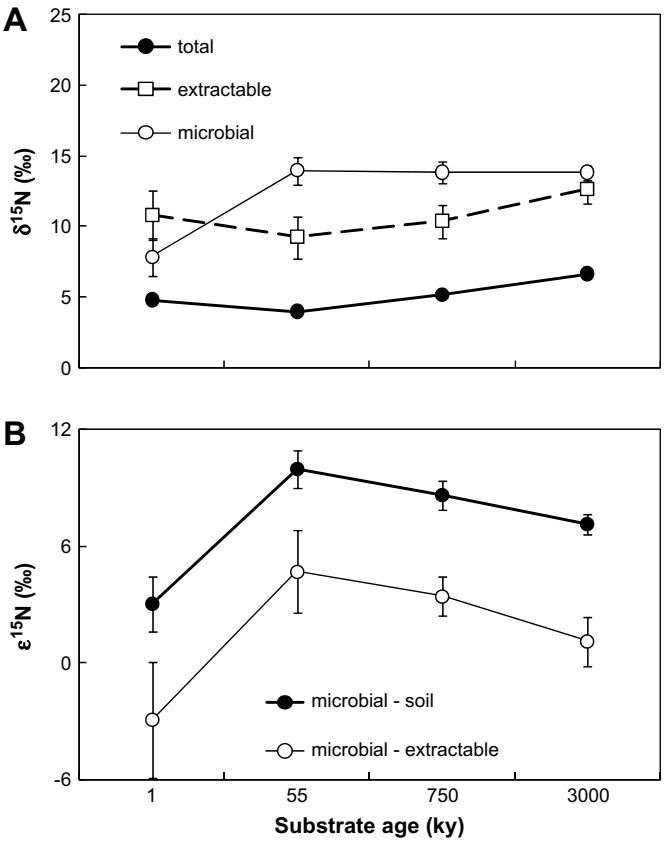

Fig. 4. Mean (±s.e.) $\delta^{15}N$ of soil total, extractable, and microbial pools (A) and $\varepsilon^{15}N_{ME}$ (microbial ^{15}N enrichment relative to extractable N) and $\varepsilon^{15}N_{MS}$ (microbial ^{15}N enrichment relative to soil total N) (B) for four sites along the Substrate Age Gradient of Arizona.

Microbial N was ^{15}N enriched relative to the total soil. This enrichment reached its maximum after 55,000 y and declined thereafter. Microbial biomass was also significantly enriched relative to the extractable N at 55,000 and 750,000 y of substrate development, but not significantly different from the extractable fraction at the youngest and oldest sites. However, the patterns of microbial ^{15}N enrichment relative to the extractable and total N pool were similar, showing an initial increase followed by a decrease with continued soil development (Fig. 4).

Microbial ^{13}C enrichment and C:N ratio of soil total and extractable pools were not significantly correlated (Fig. 5). As hypothesized, there was a negative relationship between microbial ^{15}N enrichment relative to soil extractable N ($P < 0.05$) and soil total N ($P = 0.11$) and C:N ratio (Fig. 6).

4. Discussion

4.1. C and N across three million years of soil development

We observed significant changes in total C and N content and isotope composition across three million years of soil development (Fig. 3, 4). Changes in total C and N content occur across other age gradients as well (Walker and Syers, 1976; Bormann and Sidle, 1990; Crews et al., 1995; Vitousek, 2004; Wardle et al., 2004) and reflect long-term effects of weathering of soil minerals, affecting the type

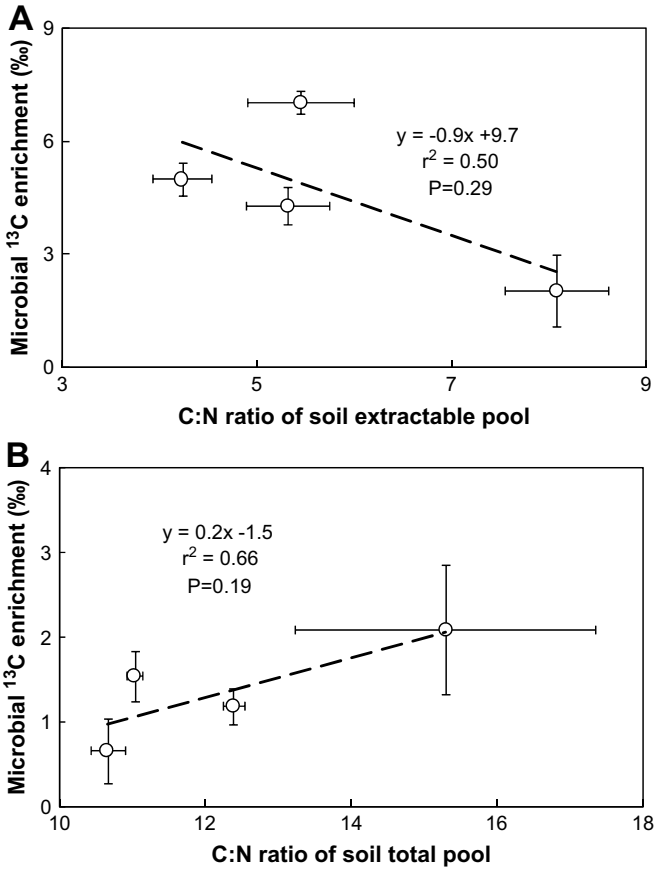

Fig. 5. Relationship between C:N ratio of the soil extractable pool and $\varepsilon^{13}C_{ME}$ (microbial ^{13}C enrichment relative to extractable C) (A) and between C:N ratio of the soil total pool and $\varepsilon^{13}C_{MS}$ (microbial ^{13}C enrichment relative to soil total C) (B) for four sites along the Substrate Age Gradient of Arizona. Each point represents the mean value (±s.e.) for a site.

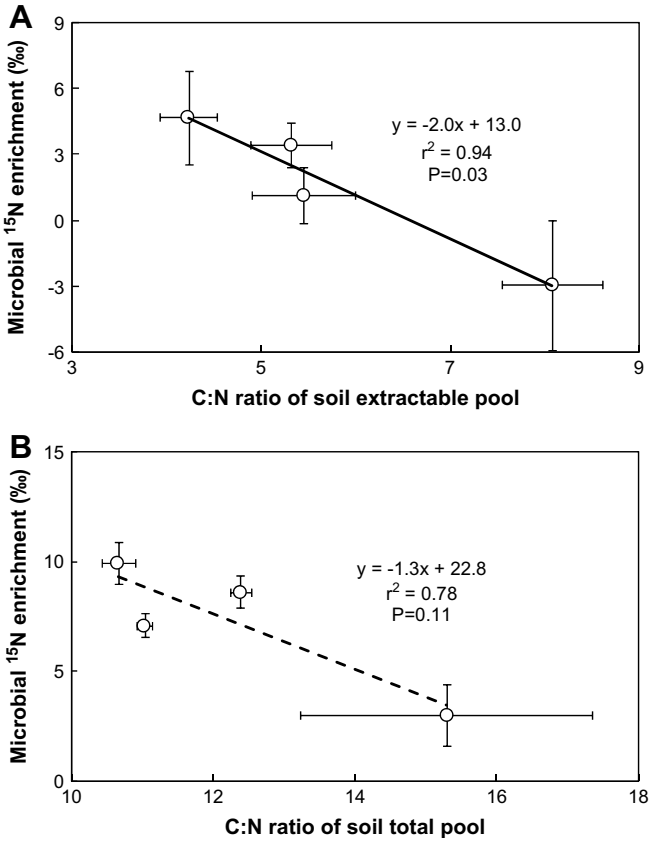

Fig. 6. Relationship between C:N ratio of the soil extractable pool and $\varepsilon^{15}N_{ME}$ (microbial ^{15}N enrichment relative to extractable N) (A) and between C:N ratio of the soil total pool and $\varepsilon^{15}N_{MS}$ (microbial ^{15}N enrichment relative to soil total N) (B) for four sites along the Substrate Age Gradient of Arizona. Each point represents the mean value (±s.e.) for a site.

and quantity of clay minerals and C and N storage (Torn et al., 1997; Powers and Schlesinger, 2002; Selmants and Hart, 2008).

There were also changes in soil C and N isotope composition with soil development. Although the sharp increase in δ^{13}C over the first 55,000 y (Fig. 3) is likely caused by increased abundance of C_4 grasses, the small decrease for the more weathered soils may be associated with altered soil organic matter processing and storage. In support of the latter idea, we observed a similar pattern of C isotope composition along the tropical mesic Long Substrate Age Gradient in Hawaii (Vitousek, 2004): an initial increase of δ^{13}C values of soil followed by small decreases at older sites, even though vegetation exhibited C_3 photosynthesis at all sites (Dijkstra, Hart, Schwartz, and Hungate unpublished).

Long-term changes in δ^{15}N of soil total N are the result of preferential removal of the light isotope through N-loss processes, such as nitrification, denitrification, and ammonia volatilization, and leaching of organic and inorganic N (Austin and Vitousek, 1998; Handley et al., 1999; Amundson and Baisden, 2000; Amundson et al., 2003; Houlton et al., 2006), and to an unknown but likely small degree by accumulation of ^{14}N in vegetation biomass (Gebauer and Schulze, 1991). Leaching is likely of minor importance in these soils, although transport from shallow to deeper soil layers may occur. Denitrification may occur sporadically after intense rain events, but nitrification activity is high in these aerated soils (Selmants and Hart, 2008). Our main focus in the present study is not on the long-term processes that are responsible for changes in the isotope composition, but on the relationships between the isotope composition of soil total C and N, the more dynamic microbial and extractable pools and C and N availability. For this reason, we focus on microbial isotope enrichment relative to other soil C and N pools, thus normalizing for long-term trends in C and N isotope composition.

4.2. Carbon and nitrogen isotope composition of soil microbial biomass

Based on previous studies, we expected a higher δ^{13}C and δ^{15}N for microbial biomass than for other soil C and N pools. These expectations were supported by this study. The mean ^{13}C enrichment relative to soil total C was 1.4‰ ($n = 30$, s.e. = 0.3), in the same range as observed previously for C_3 soils (1.7‰, Dijkstra et al., 2006a), in line with a 2.2‰ average enrichment reported by Šantrůčková et al. (2000), and a 1.7‰ enrichment for grass-dominated soils by Gregorich et al. (2000). Werth and Kuzyakov (2009) observed a 3.2‰ enrichment of microbial biomass relative to soil total C. The mean ^{15}N enrichment relative to extractable N (1.6‰ in this study; $n = 32$, s.e. = 1.1) was lower than reported for other soils (3.4‰; Dijkstra et al., 2006a). In contrast, the average ^{15}N enrichment of microbial N relative to soil total N was 7.2‰ ($n = 31$, s.e. = 0. 7), higher than previously observed (3.2‰, Dijkstra et al., 2006a).

During chloroform-fumigation extraction, only part of microbial cells is extracted and measured (Jenkinson et al., 2004). We assume that the isotope composition of the extracted C and N represents that of whole cells. At present, it has not been possible to ascertain that this is the case. However, several arguments give us confidence that

our measurements describe the isotope composition of the complete microbial biomass. First, similar to results presented here, in-vitro experiments show that microorganisms are often ^{13}C (Henn et al., 2002) and ^{15}N enriched relative to their substrates (Collins et al., 2008). Field-collected fungal tissues also indicate common ^{13}C and ^{15}N enrichments (Gebauer and Taylor, 1999; Hobbie et al., 1999; Högberg et al., 1999; Kohzu et al., 1999; Trudell et al., 2004). Second, consistent ^{13}C and ^{15}N enrichments of soil microbial biomass are observed in studies using different experimental methodologies (Šantrůčková et al., 2000; Potthoff et al., 2003; Dijkstra et al., 2006a,b, 2008; Engelking et al., 2007; Murage and Voroney, 2007). Third, although some compounds such as chitin are ^{15}N depleted and ^{13}C enriched (Gleixner et al., 1993; Taylor et al., 1997), and are not included in the chloroform-labile C and N that is extracted from soil, the concentration of these compounds is low so their effect on the isotope composition of whole cells and organisms is likely small. Finally, we observed a strong correlation between $\delta^{15}N$ of microbial biomass and that of DNA extracted from soil (Schwartz et al., 2007).

4.3. C and N availability and microbial isotope enrichments

Biological organisms have isotope signatures that reflect the isotope composition of their substrates and fractionating transformations that occur within those organisms (Fig. 1). The soil ecosystem is spatially and temporally complex, with many interacting soil C and N pools, each with potentially a different isotope composition. This makes soil a challenging and complex isotope puzzle (Högberg, 1997; Evans, 2001; Robinson, 2001). Our understanding of the processes that affect the C and N isotope composition of plants, soils, animals, and microorganisms is rapidly growing (e.g., Gleixner et al., 1993; Austin and Vitousek, 1998; Ehleringer et al., 2000; Evans, 2001; Robinson, 2001; Amundson et al., 2003; Hobbie and Werner, 2004; Collins et al., 2008; Dijkstra et al., 2008). This situation forces us to consider the question, which of the many potentially fractionating processes has the largest effect on the isotope composition of ecosystem C and N pools. Understanding the isotope composition of soil microbial biomass is crucial to answering this question.

We hypothesized that C and N availability affects ^{15}N enrichment of microbial biomass. Therefore we expect that at sites where the C:N ratio is high, microbial ^{15}N enrichment will be low, and that for sites where the C:N ratio is low, ^{15}N enrichment will be high (Fig. 1). This expectation is supported by in-vitro experiments where ^{15}N enrichment increased for media with low C:N ratio (Collins et al., 2008).

Microbial ^{15}N enrichment was especially low for the youngest site of the substrate age gradient. Low ^{15}N enrichment was also observed for the youngest site of the Long Substrate Age Gradient in Hawaii (Dijkstra et al., 2008), at high C availability along a dung deposition gradient (Dijkstra et al., 2006b) and at high values of C:N ratio across an Arizona elevation gradient (Dijkstra et al., 2008). Young volcanic soils, and other young substrates, are often N limited, have relatively high C:N ratios of extractable and soil total pools (Table 2), exhibit low rates of net and gross N mineralization (Yu et al., 1999; Hedin et al., 2003; Selmants and Hart, 2008), low vegetation biomass and productivity (Bormann and Sidle, 1990; Vitousek and Farrington, 1997; Vitousek, 2004; Wardle et al., 2004) and exhibit a strong response to N fertilization (Vitousek and Farrington, 1997).

Microbial ^{15}N enrichment relative to soil and extractable N pools correlated negatively with C:N ratio (Fig. 6), as also observed for a substrate age gradient in Hawaii and an elevation gradient in Arizona (Dijkstra et al., 2008). A similar but not-significant trend was observed between microbial enrichment relative to soil total N and C:N ratio. A negative relationship between microbial ^{15}N enrichment and C:N ratio was expected according to the model described in Fig. 1. Discrimination against the heavy N isotope resulted in ^{15}N-enriched microbial biomass at relatively low C availability.

There were also significant changes in ^{13}C enrichment of microbial biomass compared to soil total and extractable C with substrate age (Fig. 3). Fractionation during decomposition has been implicated in the increase of $\delta^{13}C$ during litter decomposition (Melillo et al., 1989; Gleixner et al., 1993; Ehleringer et al., 2000). The differences in ^{13}C enrichment were not related to C and N availability (Fig. 5), and thus require an alternative explanation. These small variations in ^{13}C enrichment may be related to microbial growth stages (Henn et al., 2002), or small differences in source signatures (for example induced by variable stomatal closure, Ehleringer et al., 2000). The enrichment of microbial biomass relative to extractable C was high, driven by low $\delta^{13}C$ values of the extractable pool (Fig. 3). This finding cannot be explained at this moment, but may be related to accumulation of residues from lignin decomposition in the extractable pool. Lignin is usually depleted and can have a C signature that is lower than that of the total plant by up to 4‰ (Bowling et al., 2008).

5. Conclusions

The present study shows that microbial ^{15}N enrichment relative to soil total and extractable N pools is variable and related to relative C and N availability (Dijkstra et al., 2006b, 2008, this study). Microbial biomass is also ^{13}C enriched relative to soil extractable and total C pools. This enrichment is also variable, but not related to C:N ratio. The small variations in microbial signatures are caused by substrate signatures and fractionating processes in soil. If we can understand these small isotope variations, we will have discovered an important tool in ecosystem sciences.

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