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C and N availability affects the ^{15}N natural abundance of the soil microbial biomass across a cattle manure gradient

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Summary

The availability of C and N to the soil microbial biomass is an important determinant of the rates of soil N transformations. Here, we present evidence that changes in C and N availability affect the ^{15}N natural abundance of the microbial biomass relative to other soil N pools. We analysed the ^{15}N natural abundance signature of the chloroform-labile, extractable, NO_3^-, NH_4^+ and soil total N pools across a cattle manure gradient associated with a water reservoir in semiarid, high-desert grassland. High levels of C and N in soil total, extractable, NO_3^-, NH_4^+ and chloroform-labile fractions were found close to the reservoir. The $\delta^{15}N$ value of chloroform-labile N was similar to that of extractable (organic + inorganic) N and NO_3^- at greater C availability close to the reservoir, but was ^{15}N-enriched relative to these N-pools at lesser C availability farther away. Possible mechanisms for this variable ^{15}N-enrichment include isotope fractionation during N assimilation and dissimilation, and changes in substrate use from a less to a more ^{15}N-enriched substrate with decreasing C availability.

Introduction

The soil microbial biomass is the driving force behind decomposition of plant compounds and the cycling of nitrogen (N) and carbon (C). Microbial compounds are a source for soil organic matter (Kramer *et al*., 2003; Dieckow *et al*., 2005), while mineralized N is an important nutrient for plants, often limiting ecosystem productivity (Vitousek & Howarth, 1991; Chapin *et al*., 2002). Valuable information about soil N transformations can be gained by studying the ^{15}N natural abundance of elements of the N cycle (Högberg, 1997; Robinson, 2001; Staddon, 2004), but direct measurements of the ^{15}N natural abundance of the microbial biomass itself are lacking. We have previously reported that the natural abundance of the microbial biomass (determined using chloroform-extraction techniques, Brookes *et al*., 1985) is ^{15}N-enriched compared with the soil total (3.6‰) and salt-extractable N (4.1‰) for a broad range of climate, vegetation and soil types (Dijkstra *et al*., 2006). Although this ^{15}N-enrichment is analogous to that exhibited by most heterotrophic organisms relative to their substrates (e.g. DeNiro & Epstein, 1981; Macko & Estep, 1984; Minagawa & Wada, 1984; Högberg, 1997), food-web aspects cannot be distinguished from effects of the presence of different N-sources in the complex soil environment. Here we show that ^{15}N-enrichment of the microbial N-pool can vary significantly within one study and correlates with C and N concentrations in the soil. Studying the ^{15}N natural abundance of the microbial biomass is relevant for understanding changes in the ^{15}N natural abundance of the soil organic matter with depth and age and in ecosystems with open or closed N cycles (Tiessen *et al*., 1984; Högberg, 1997; Amundson *et al*., 2003).

We measured the ^{15}N natural abundance of the chloroform-labile fraction, a proxy for the soil microbial biomass, near a water reservoir in semiarid, high-desert grassland. Cattle caused a steep dung and urine gradient with greater concentrations of C and N close to the reservoir. We studied how the ^{15}N natural abundance of the chloroform-labile and other soil N pools responded to this change in C and N availability.

Materials and methods

The study area was located in a semiarid, high-desert grassland near Flagstaff, Arizona (35°34′20′N, 111°34′4′W, 1755 m above sea level, 230 mm rain annually). The soil is a cindery, mesic Typic Haplustoll (USDA Soil Taxonomic family;

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Taylor, 1983). This area is used for extensive winter grazing, and is dominated by perennial grasses (*Bouteloua eriopoda*, *B. gracilis*, *Sporobulus cryptandrus* and *Hilaria jamesii*) and occasional shrubs (*Chrysothamnus nauseosa* and *Gutierrezia sarothra*). We sampled surface soil along a transect 0–100 m distance from an artificial water reservoir. The soil immediately next to the reservoir (0 m) was strongly eroded and soil plus dried cow dung was present as a thin layer on top of an indurated, calcium carbonate-rich (calcic) soil layer. Vegetation was completely absent at the time of sampling (16 March 2003), up to at least 300 m away from the reservoir, caused by overgrazing and trampling.

We took four composite soil samples (0–10 cm depth, each of four subsamples) at 0 (within 20 cm), 5, 10, 25, 50 and 100 m distance from the edge of the water reservoir, avoiding recognizable dung deposits. The soil samples were processed the same day. We sieved the soil through a 2-mm aperture sieve. Soil moisture was determined gravimetrically (105°C). Soil pH was determined in a soil slurry using 1:2.5 (w/v) soil:-water ratio. Soil was dried at 105°C for at least 24 hours, ground and analysed for total C and N. Dung ($n = 8$), collected randomly from the transect, was dried at 70°C and ground to a fine powder before analysis.

Microbial biomass

Soil microbial biomass was determined as the chloroform-labile fraction using chloroform-fumigation-extraction techniques described by Brookes *et al.* (1985) and modified by Widmer *et al.* (1989) for soils with high soluble N contents. We added 100 ml 0.25 M K_2SO_4 to 50 g of soil followed by 1 hour of shaking. The extract was filtered through a Whatman No 1 filter paper and stored at −20°C until further processing. Filter plus remaining soil was placed in a desiccator and exposed to chloroform vapour for 24 hours, after which soil plus filter was extracted as described above. All K_2SO_4 extracts (organic + inorganic N) were placed in a ventilated oven (60°C) until dry, ground to a fine powder, and weighed for elemental and isotope analysis. Values of the chloroform-labile fraction were not corrected for extraction efficiencies.

NH_4^+ and NO_3^-

Soil NO_3^- and NH_4^+ were extracted from 10 g soil in 50 ml 1 M KCl solution. The solution was shaken for 1 hour, filtered through a Whatman No 1 filter paper and stored at −20°C until further processing. We used the NH_3-diffusion technique (Sigman *et al.*, 1997; Holmes *et al.*, 1998) to determine the ^{15}N natural abundance of NO_3^- and NH_4^+. Nitrate and NH_4^+ in the KCl extract were diffused onto a Whatman GF/A glass microfibre filter disc acidified with 20 µl 0.5 M $KHSO_4$ and sandwiched between two layers of Teflon (Stark & Hart, 1996). Ammonium was diffused as NH_3 after addition of MgO, while NO_3^- was reduced by adding Devarda's alloy and subsequently diffused as NH_3 onto a second filter. Each diffusion step was conducted at 35°C under continuous shaking for 7 days. Pure KCl (1 M) solution was added to make standard 90 ml solutions. Standard NH_4^+ and NO_3^- solutions were analysed using the same procedures and showed $\delta^{15}N$ values within 0.2‰ of their expected values. Holmes *et al.* (1998) showed similarly that for small volumes (less than 200 ml), diffusion of N standards was accurate.

Stable isotope analysis

Stable isotope ratios ($\delta^{15}N$), C and N concentration of soil, dung, fumigated and unfumigated K_2SO_4 extracts and glass fibre filters were analysed using a 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 the K_2SO_4 salts, silver wool was added to the end of the oxidation column of the elemental analyser. The ^{15}N natural abundance was expressed in standard notation ($\delta^{15}N$) in parts per thousand (‰) relative to atmospheric N_2, where $\delta = 1000*[(R_{sample}/R_{standard})\text{-}1]$, and R is the molar ratio $^{15}N/^{14}N$. Precisions were better than 0.2‰ for $\delta^{15}N$, 0.1% for N and 0.4% for C concentration using multiple international standards.

We calculated the difference between $\delta^{15}N$ of the chloroform-labile and that of other soil N pools using the following equations:

$$\Delta^{15}N_{MU} = \delta^{15}N_{MB} - \delta^{15}N_{UF} \quad (1)$$

$$\Delta^{15}N_{MS} = \delta^{15}N_{MB} - \delta^{15}N_{SN} \quad (2)$$

where $\Delta^{15}N_{MU}$ is the ^{15}N-enrichment of the microbial biomass (chloroform-labile fraction) relative to the unfumigated pool (UF or salt-extractable fraction), and $\Delta^{15}N_{MS}$ is the ^{15}N-enrichment of the microbial biomass (chloroform-labile fraction) relative to the soil total N pool (SN).

Statistics

To test whether $\delta^{15}N$ of the chloroform-labile fraction was significantly different from $\delta^{15}N$ of soil total or salt-extractable N, 95% confidence intervals were calculated for $\Delta^{15}N_{MU}$ and $\Delta^{15}N_{MS}$ and compared with zero. One-way ANOVA was used to test for differences between locations along the transect. To locate significance between multiple treatment means, the Duncan multiple range test (DMRT) was used. Correlation coefficients (r) were calculated to explore covariance between measured parameters using averaged values per distance. All differences were evaluated at $P < 0.05$.

Results

Soil characteristics and C and N concentrations across the gradient

Soil moisture content was significantly greater next to the water reservoir than at other locations (statistical information summarized in Table 1). We assume that this was related to leakage and spillage of water from the reservoir. The soil moisture contents at other locations were not significantly different from each other. Soil pH was typical for semiarid soils (pH 8.1–8.7) and did not vary significantly between locations (Table 1).

Soil total C and N, extractable C and N and chloroform-labile C and N increased significantly towards the water reservoir. Nitrate and NH_4^+ showed similar increases but, due to high variability, these were not significant (Figure 1, Table 1). Nitrate concentrations were always greater than NH_4^+. Large variability in these measurements was probably associated with patchy N-deposition. Chloroform-labile C and N showed significant correlations with soil C, extractable C and NO_3^- concentrations (Table 2). Extractable N concentration was correlated with NO_3^- but not with NH_4^+.

The soil C/N ratio increased gradually towards the reservoir, but became very large at location 0 m (Figure 1d, Table 1). The $\delta^{13}C$ value of the soil total C revealed the presence of calcium carbonates at that location ($\delta^{13}C$ of soil total C: -3.5 ± 0.18‰). At 0 m distance from the water reservoir, soil N, extractable and chloroform-labile C and N and inorganic N suddenly decreased. This decline was likely to be caused by the inclusion of the underlying calcic horizon with the soil sample. Increases of C/N ratio of the other soil fractions close to the reservoir were not significant (Figure 1d).

The increase in soil C and N close to the water reservoir (excluding location 0 m) was likely to be related to incorporation of relatively undecomposed dung into the soil. The C/N ratio of the net increase in C and N between location 100 m and 5 m (C/N ratio of 21) was close to that of undisturbed dung (25 ± 3.0). This increase in C/N ratio could not be attributed to inorganic C, as no corresponding rise in ^{13}C signature of the soil was observed. This was in contrast to location 0 m, where a large change in C/N was accompanied by a sharp rise in $\delta^{13}C$.

^{15}N natural abundance of N pools across the gradient

The $\delta^{15}N$ values of extractable, chloroform-labile and NO_3^-–N increased significantly towards the water reservoir (Figure 2, Table 1). The $\delta^{15}N$ value of NH_4^+ was very variable, and no trend with distance from the water reservoir was found. The concentrations and $\delta^{15}N$ of NO_3^- and extractable N-pool were strongly correlated (Tables 2 and 3), suggesting that NO_3^- was an important component of the extractable N-pool. The $\delta^{15}N$ of NH_4^+ was generally greater than that of NO_3^- (Figure 2b). The $\delta^{15}N$ value of soil total N showed only small changes across the manure gradient (Figure 2a). The $\delta^{15}N$ value of undisturbed dung was 5.0‰ ($\pm$ 0.8), in between the $\delta^{15}N$ values for the leaves of the dominant grass (*Bouteloua eriopoda*, 1.7‰ $\pm$ 0.5, P. Dijkstra & B. A. Hungate, unpublished data) and that of the soil (9.7‰ $\pm$ 0.8 averaged over entire transect).

Chloroform-labile N in relation to other N pools

The average difference between $\delta^{15}N$ of the chloroform-labile N and that of the extractable N ($\Delta^{15}N_{MU} = 4.45$‰, SE = 0.72, $n = 23$) and soil total N ($\Delta^{15}N_{MS} = 5.63$‰, SE = 0.50, $n = 22$) was significantly greater than zero. The ^{15}N-enrichment of the chloroform-labile fraction relative to the soil ($\Delta^{15}N_{MS}$) increased significantly towards the water reservoir (Figure 2, Table 1), reflecting mostly changes in $\delta^{15}N$ of the chloroform-labile fraction. ^{15}N-enrichment relative to the extractable N ($\Delta^{15}N_{MU}$) decreased from 7.5‰ (SE = 0.48, $n = 4$) at 100 m distance to -1.2‰ (SE = 0.44, $n = 4$) close to the reservoir (Figure 2b). The $\Delta^{15}N_{MU}$ value

Table 1 Statistical evaluations (one-way ANOVA) of the effect of distance on soil characteristics

	d.f.	*F*	*P*		d.f.	*F*	*P*
Soil moisture	5,17	29	< 0.001	Soil C/N	5,17	108	< 0.001
Soil pH	5,17	1.5	0.25	Extr C/N	5,17	2.1	0.11
Soil C	5,16	54	< 0.001	Micr C/N	5,17	2.3	0.09
Soil N	5,16	69	< 0.001	$\delta^{15}N$ soil N	5,16	4.5	< 0.01
Extr C	5,17	11	< 0.001	$\delta^{15}N$ extr N	5,17	26	< 0.001
Extr N	5,17	3.1	0.05	$\delta^{15}N$ NH_4^+	5,16	0.5	0.79
NO_3^-	5,17	2.3	0.10	$\delta^{15}N$ NO_3^-	5,17	6.4	< 0.001
NH_4^+	5,17	1.1	0.42	$\delta^{15}N$ micr N	5,17	5.3	< 0.001
Micr C	5,17	9.5	< 0.001	$\Delta^{15}N_{MU}$	5,17	29	< 0.001
Micr N	5,17	7.7	< 0.001	$\Delta^{15}N_{MS}$	5,17	6.9	< 0.01

Soil = soil total fraction, extr = extractable, and micr = chloroform-labile fraction, $\Delta^{15}N_{MU}\delta^{15}N$ of chloroform-labile N minus $\delta^{15}N$ extractable N,$\Delta^{15}N_{MS}\delta^{15}N$ of chloroform-labile N minus$\delta^{15}N$ soil total N (‰).

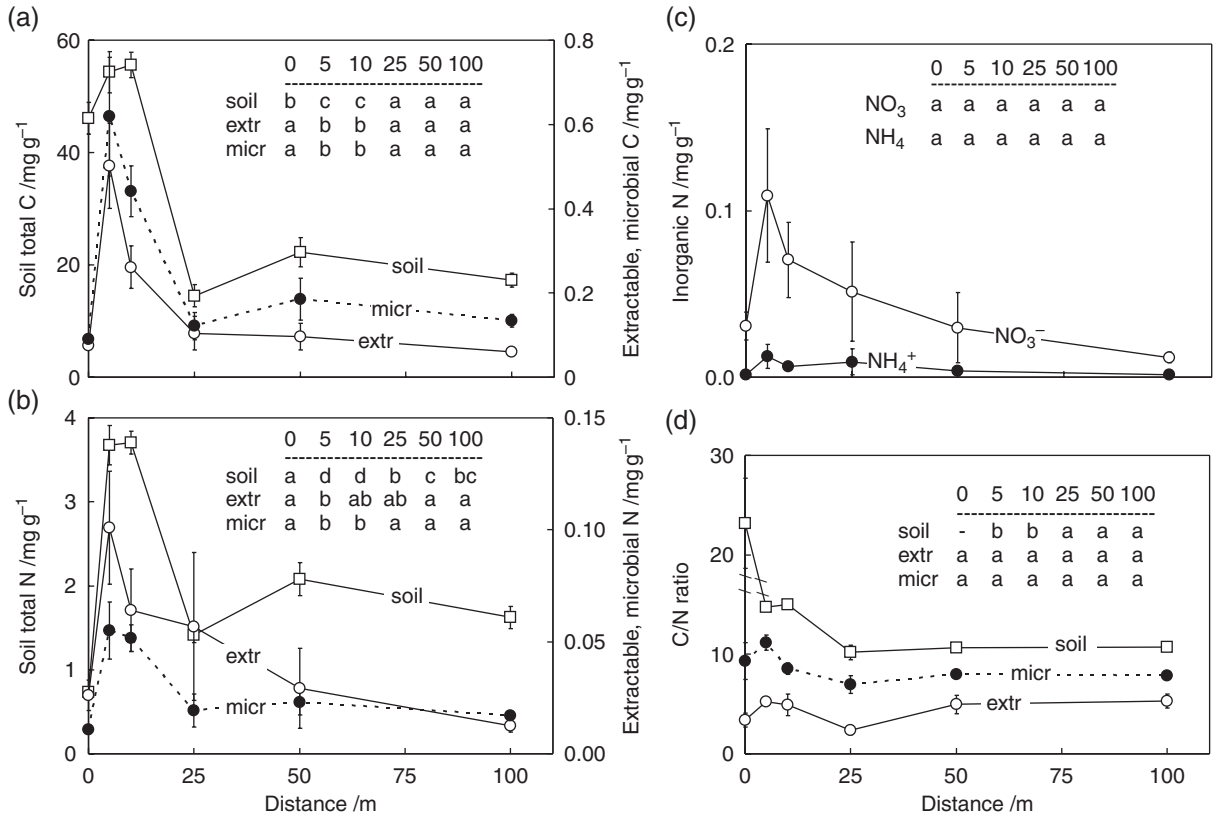

Figure 1 C (a), N (b) and inorganic N concentration (mg g^{-1} dry soil, c), and C/N ratio (d) in relation to distance from the water reservoir (means, $n = 3$–4, bars indicate ± one standard error). Soil total (soil), extractable (extr), chloroform-labile (micr). Different letters within a row indicate significant differences between means (DMRT) for that variable. For soil C/N ratio, location 0 m was excluded in calculation of DMRT. Figure 1(a) has an interrupted *y*-axis to accommodate the high C/N value at location 0 m.

at location 0 m was not significantly different from zero. A similar pattern was evident when $\delta^{15}N$ of the chloroform-labile N was compared with $\delta^{15}N$ of NO_3^- (data not shown). When the location close to the reservoir was excluded, $\Delta^{15}N_{MU}$ was correlated negatively with soil total C, extractable and chloroform-labile C and N and NO_3^- concentrations (Table 2, Figure 3). The inclusion of the underlying and distinct calcic horizon layer in the 0 m location sample diluted the concentrations of organic C and N, and NO_3^- per g soil, but did not affect their isotope signatures. As a result, the 0 m location did not fit the relationship described in Figure 3 and Table 2.

Table 2 Correlation coefficient (*r*) between soil total (soil), extractable (extr) and chloroform-labile (micr) C and N, NO_3, NH_4 (mg g^{-1} soil dry weight) and $\Delta^{15}N_{MU}$ ($\delta^{15}N$ of chloroform-labile N minus $\delta^{15}N$ extractable N, ‰) for values averaged per distance, excluding location 0 m ($n = 5$)

		Soil		Extr		Micr			
		C	N	C	N	C	N	NO_3^-	NH_4^+
Soil	N	1.00*							
Extr	C	0.86	0.86						
	N	0.75	0.73	0.92*					
Micr	C	0.95*	0.95*	0.97*	0.85				
	N	0.99*	0.99*	0.91*	0.83	0.97*			
NO_3^-		0.82	0.80	0.95*	0.99*	0.91*	0.88*		
NH_4^+		0.51	0.49	0.79	0.95*	0.67	0.62	0.91*	
$\Delta^{15}N_{MU}$		−0.89*	−0.87*	−0.98*	−0.94*	−0.96*	−0.94*	−0.97*	−0.81

*Indicates significance at $P < 0.05$.

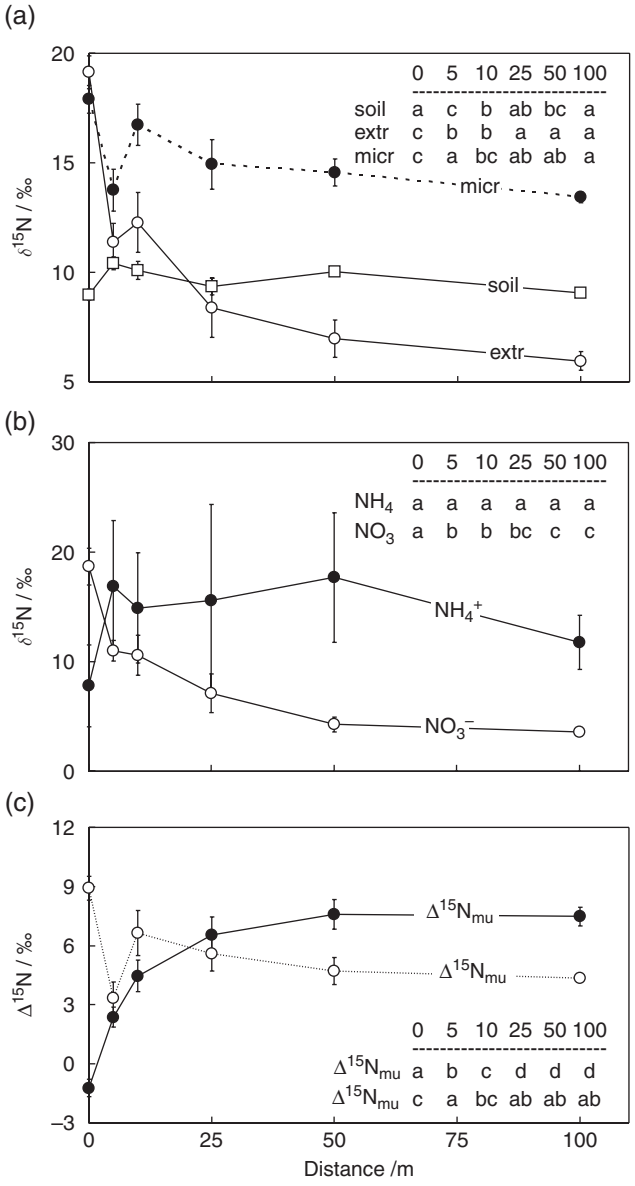

Figure 2 $\delta^{15}N$ (‰) of soil total (soil), extractable (extr) and chloroform-labile (micr) fractions (a), $\delta^{15}N$ (‰) of NO_3^- and NH_4^+ (b), $\Delta^{15}N_{MU}$ ($\delta^{15}N$ of chloroform-labile N minus $\delta^{15}N$ extractable N) and $\Delta^{15}N_{MS}$ ($\delta^{15}N$ of chloroform-labile N minus $\delta^{15}N$ soil total N,‰) (c) in relation to distance from the water reservoir (means, $n = 3$–4, bars indicate ± one standard error). Different letters within a row indicate significant differences between means (DMRT) for that variable.

Discussion

Processes across the cattle manure gradient

The presence of cattle resulted in greater values of soil total and extractable C and N and inorganic N close to the water reservoir (Figure 1). Dung and urine are high quality N-fertilizers; ammonia from urea is released quickly, while under optimal conditions the solid waste begins net N mineralization usually within a few weeks (Meyer *et al.*, 2002; Thomsen *et al.*, 2003). Net nitrification is probably rapid, as suggested from the large NO_3^- concentration. Dung is also a C source for microorganisms with a high C/N ratio and water-soluble organic matter concentration, and stimulates microbial respiration (Chantigny *et al.*, 2002; Thomsen *et al.*, 2003). Large extractable C concentration was associated with large microbial C and N content in this study (Figure 1). We conclude that rates of nitrification, mineralization and immobilization are likely to be increased with greater manure deposition. Under anaerobic conditions, large NO_3^- concentrations stimulate denitrification reactions (Meyer *et al.*, 2002), but these conditions are probably rare in this semiarid grassland.

^{15}N natural abundance of N pools

Much of the inorganic N is lost via NH_3 volatilization and N losses associated with nitrification and denitrification (Meyer *et al.*, 2002). Volatilization of ammonia can remove 30–50% of the N from urine patches in a few days (Carran *et al.*, 1982; Bedard-Haughn *et al.*, 2003), especially under conditions of high wind speed, low relative humidity and high soil pH (Bronson *et al.*, 1999), while losses in the form of N_2O up to a few per cent are not uncommon (Allen *et al.*, 1996; Flessa *et al.*, 1996). Volatilization, nitrification and denitrification discriminate strongly against the heavier ^{15}N isotope (Högberg, 1997; Bedard-Haughn *et al.*, 2003), and these processes result in large $\delta^{15}N$ values close to the water reservoir (Figure 2). Animal feedlots and manure storage facilities often exhibit large $\delta^{15}N$ values for NO_3^- (> 10‰, Kellman & Hillaire-Marcel, 2003). The $\delta^{15}N$ value of soil total N was not affected by the manure deposition. This is not surprising, as the soil total N pool turns over slowly and only slowly incorporates a new isotope signature. The variable NH_4^+ is most likely the result of patchy urine deposition, as ammonium from urea volatilizes very rapidly (thus causing a large standard error in both amount and $\delta^{15}N$ value).

What determines the variable ^{15}N-enrichment?

The ^{15}N natural abundance of an organism is determined by the $\delta^{15}N$ of its substrates or fractionation during N processing (Robinson, 2001). The $\delta^{15}N$ value of the microbial biomass (estimated from the chloroform-labile N) was greater than that of extractable N, NO_3^- and soil total N at small extractable C and N concentrations for locations away from the water reservoir (Figure 2). A similar ^{15}N-enrichment over the extractable and soil total N pool is reported elsewhere (Dijkstra *et al.*, 2006) and may be the rule, not the exception. However, close to the water reservoir, at large C and N concentrations, $\delta^{15}N$ of the chloroform-labile fraction converged with that of the extractable N and NO_3^- (Figure 2). The degree of

Table 3 Correlation coefficient (r) between $\delta^{15}N$ (‰) of chloroform-labile (micr), extractable (extr), NO_3^-, NH_4^+ and soil total (soil) N fractions for values averaged per distance ($n = 6$)

	Micr	Extr	NO_3^-	NH_4^+
Extr	0.84*			
NO_3^-	0.79	0.99*		
NH_4^+	−0.57	−0.62	−0.59	
Soil	−0.26	−0.16	−0.14	0.81

*Indicates significance at $P < 0.05$.

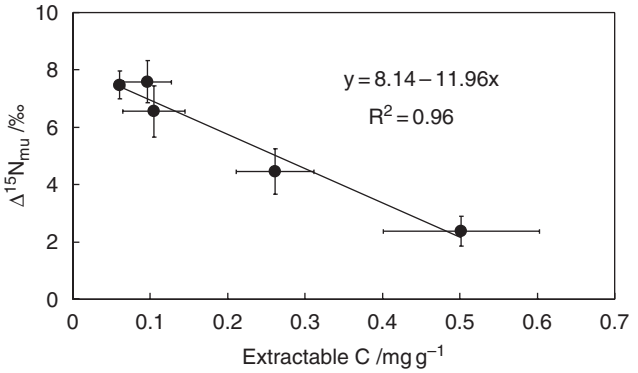

Figure 3 Relationship between $\Delta^{15}N_{MU}$ ($\delta^{15}N$ of chloroform-labile N minus $\delta^{15}N$ extractable N, ‰) and the extractable C concentration (mg g^{-1} dry soil, means, $n = 3$–4, bars indicate ± one standard error). Data excluding location 0 m.

^{15}N-enrichment (relative to the extractable N) correlated strongly with extractable C (Figure 3), N and NO_3^-, but not with NH_4^+ (Table 2).

The variable ^{15}N-enrichment of the chloroform-labile N versus other dynamic N pools across the manure gradient may be related to a change in the source of N utilized by the microbial biomass. Preferential use of NH_4^+ over NO_3^- was demonstrated by Jansson *et al.* (1955). Ammonium is often ^{15}N-enriched relative to NO_3^- (Nadelhoffer & Fry, 1994; this study). The $\delta^{15}N$ values of the chloroform-labile and NH_4^+ pools were similar at most distances. This suggests that the microorganisms used NH_4^+ as the source of N at low C availability (Jones & Richards, 1977). At high C availability close to the reservoir, the microbial biomass may have switched to NO_3^- as the dominant N source. This switch may have been caused by a decline in NH_4^+ (Figure 1c) or an increase in microbial N immobilization rate at high C availability. Thomsen *et al.* (2003) showed that fresh dung initially immobilizes NO_3^- from the soil environment. Although a gradual switch from NH_4^+ to NO_3^- would explain the decrease in ^{15}N-enrichment of the chloroform-labile relative to extractable N (Figure 2c), it is puzzling why the large variability in $\delta^{15}N$ of NH_4^+ is not reproduced in $\delta^{15}N$ of the chloroform-labile fraction. Moreover, even though preferential use of ammonium over nitrate was shown (Jansson *et al.*, 1955), Hart *et al.* (1994) found that NO_3^- and NH_4^+ immobilization occurred simultaneously during soil incubation. As an alternative explanation, microorganisms, foraging for C, may end up using older organic N compounds at low C availability. Older organic N, more closely associated with the soil minerals, has a greater $\delta^{15}N$ value than soil total N (Tiessen *et al.*, 1984; Kramer *et al.*, 2003). At high C availability, the microorganisms probably use the most available N source (in this case especially NO_3^-).

Alternatively, ^{15}N-enrichment of the chloroform-labile fraction may not be related to a specific N source, but may be the result of fractionation during N processing. Microbial-N transformations that result in the preferential export of ^{14}N from the cell will cause ^{15}N-enrichment. Nitrification, denitrification, N-dissimilation and export are such processes (Högberg, 1997). Nitrification and denitrification, increased under greater manure deposition, should result in considerable microbial ^{15}N-enrichment at the high NH_4^+ and NO_3^- concentrations close to the reservoir. This is in contrast to what was observed in this study (Figure 2). Under low C availability, N-dissimilation is a dominant process. Organic N is utilized as a C source and excess N leaves the cell, most likely as NH_3 (Schulten & Schnitzer, 1998). Isotope fractionation in this idea is associated with deamination and NH_4^+/NH_3 state change (Handley & Raven, 1992; Högberg, 1997). In contrast, under high C availability, net N immobilization occurs (Hart *et al.*, 1994), and the cell achieves the signature of its N source. The fractionation by N-dissimilation and export is partly balanced by fractionation occurring during N-assimilation (Werner & Schmidt, 2002). An analogous mechanism is proposed for mycorrhizal fungi, where fractionation occurs during N transfer to the host plant (e.g. Hobbie & Colpaert, 2003). However, mycorrhizal fungi were not involved here as vegetation was absent from this trampled and disturbed area.

We conclude that the ^{15}N-enrichment of the chloroform-labile fraction is related to C and N availability and possibly integrates changes in ecosystem processes of mineralization and immobilization. We postulate that under high C availability, the microbial biomass utilizes readily available N, while at the same time, isotope fractionation during processing is limited as only small amounts of N are removed from the cell. In contrast, at low C availability, selective uptake of a more ^{15}N-enriched N source, possibly NH_4^+ or older, more decomposed soil organic matter and isotope fractionation during deamination and N export result in large microbial ^{15}N-enrichments.

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