

LETTER

¹⁵N enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function

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Abstract

Organic carbon (C) and nitrogen (N) are essential for heterotrophic soil microorganisms, and their bioavailability strongly influences ecosystem C and N cycling. We show here that the natural ¹⁵N abundance of the soil microbial biomass is affected by both the availability of C and N and ecosystem N processing. Microbial ¹⁵N enrichment correlated negatively with the C : N ratio of the soil soluble fraction and positively with net N mineralization for ecosystems spanning semiarid, temperate and tropical climates, grassland and forests, and over four million years of ecosystem development. In addition, during soil incubation, large increases in microbial ¹⁵N enrichment corresponded to high net N mineralization rates. These results support the idea that the N isotope composition of an organism is determined by the balance between N assimilation and dissimilation. Thus, ¹⁵N enrichment of the soil microbial biomass integrates the effects of C and N availability on microbial metabolism and ecosystem processes.

Keywords

$\delta^{15}\text{N}$, carbon and nitrogen availability, ecosystem function, N cycling, N mineralization, resource availability, soil microbial biomass, stable isotopes.

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INTRODUCTION

The activity of soil microorganisms is controlled by the availability of organic carbon (C) and nitrogen (N), and has a large influence on ecosystem processes such as productivity, decomposition rates, soil respiration, N mineralization, and ecosystem N losses (Hart *et al.* 1994; Hart & Stark 1997; Lovett *et al.* 2002; Schimel & Bennett 2004). Pivotal in the relationship between microbial metabolism and ecosystem processes is the utilization of organic N compounds: when soil microbes utilize organic N as a source of C and energy, they have to export excess N, which becomes available to plants and other microorganisms. Nitrogen released by soil microorganisms and, in diminishing importance, by organisms from higher trophic levels, is a rate-limiting step in the N-cycle, controlling net primary productivity in many natural terrestrial ecosystems (Vitousek & Howarth 1991). It is therefore imperative that we understand the

relationship between resource availability, microbial metabolism, and ecosystem processes.

The natural abundance ¹⁵N composition of heterotrophic organisms is typically higher than that of the source of N. For example, animals exhibit ¹⁵N enrichments relative to their diets (Post 2002; Vanderklift & Ponsard 2003), and ectomycorrhizal fungi are ¹⁵N enriched relative to the soil organic matter and their plant host (Högberg 1997; Taylor *et al.* 1997; Hobbie *et al.* 1999; Kohzu *et al.* 1999; Hart *et al.* 2006). Similarly, the soil microbial biomass is ¹⁵N enriched relative to the soil soluble and total N (Dijkstra *et al.* 2006a,b; Pörtl *et al.* 2007). It is hypothesized that the ¹⁵N enrichment of animals and mycorrhizal fungi is caused by discrimination against the heavy ¹⁵N isotope during N assimilation, dissimilation (Macko & Estep 1984; Minagawa & Wada 1984; Macko *et al.* 1987; Högberg 1997; Hobbie *et al.* 1999; Ponsard & Averbuch 1999; Collins *et al.* 2008) and export (Fig. 1). In this paper, we apply this

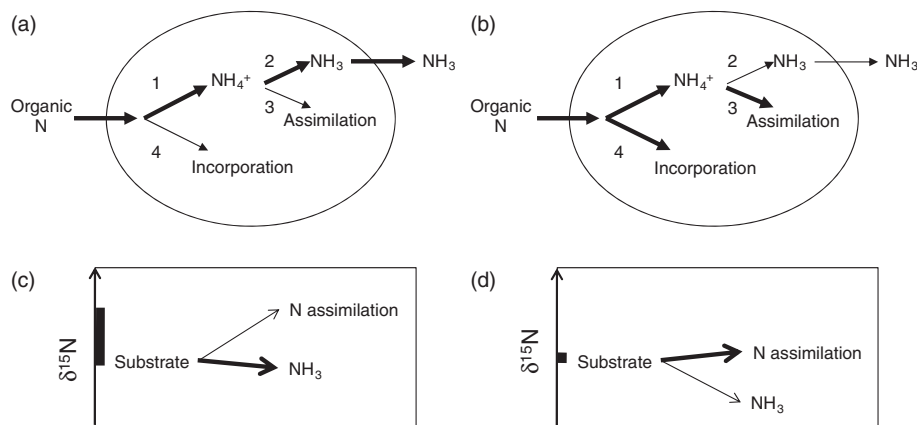


Figure 1 A conceptual model exploring the relationship between ^{15}N fractionation and N assimilation and dissimilation in soil microbial cells under conditions of (a) low C availability and (b) high C availability. A schematic representation of fractionation shows how under low C availability (c) microbial biomass will exhibit high ^{15}N enrichment, while under high C availability (d) low ^{15}N enrichments are expected. Nitrogen assimilation is stimulated by high C availability, while N dissimilation is stimulated by low C availability. Ellipses represent cell membranes and width of arrows indicates relative process rates. Nitrogen dissimilation (deamination and associated transaminations; arrow 1) discriminate against ^{15}N (Macko *et al.* 1987; Hobson *et al.* 1993; Högberg 1997). After deamination, excess N appears in the cell as NH_4^+ , but is lost as NH_3 passing through the hydrophobic membrane or channels (Jahn *et al.* 2004). Due to equilibrium isotope effects during $\text{NH}_4^+/\text{NH}_3$ state change (Handley & Raven 1992; Högberg 1997; arrow 2), the $\delta^{15}\text{N}$ value of NH_3 is reduced relative to that of NH_4^+ . Discrimination against ^{15}N also occurs during N assimilation (glutamine synthetase and associated transaminations, arrow 3) and reduces ^{15}N enrichment caused by N dissimilation. Direct incorporation of the organic-N substrate is not fractionating (arrow 4). The vertical position relative to the y-axis (c, d) reflects the relative $\delta^{15}\text{N}$ value of the products of assimilation and dissimilation. The thickened bar of the y-axis represents the microbial ^{15}N enrichment relative to the substrate pool. Under steady state conditions, it is assumed that net N assimilation equals N release upon cell death.

model of isotope fractionation to soil microorganisms, and predict and test relationships between isotope composition, microbial metabolism, resource availability and ecosystem processes.

We have recently shown that the ^{15}N enrichment of the soil microbial biomass relative to the soil soluble N increased with a decrease in C content across a cattle dung deposition gradient (Dijkstra *et al.* 2006b). We hypothesize that this increase in ^{15}N enrichment reflects a shift from N assimilation to N dissimilation, driven by lower C availability. When the relative availability of C is low, organic N is primarily used as a source of C and energy, and excess N is removed from cells (Fig. 1a). Fractionation during N dissimilation and export results in preferential loss of the light ^{14}N isotope, resulting in high ^{15}N enrichment of the cell relative to its N source (Fig. 1c). Conversely, when relative C availability is high, it is expected that N assimilation activity is high and fractionation during N assimilation will compensate for fractionation during N dissimilation, resulting in low ^{15}N enrichment relative to the N source (Fig. 1b,d). Because N export is determined by the balance between N assimilation and dissimilation, high ^{15}N enrichment should correlate positively with high net N mineralization rate.

To investigate the significance of changes in microbial ^{15}N enrichment, and its relationship with metabolic and ecosystem processes, we sampled A-horizon soil from sites

along the C. Hart Merriam Elevation Gradient in Arizona, the Substrate Age Gradient of Arizona (Selmants & Hart 2008) and the Long Substrate Age Gradient in Hawaii (Vitousek 2004). These experimental gradients cover a broad range of climates, soil types, substrate age, species composition, soil organic matter content, productivity, and N mineralization rates. We tested the following four hypotheses that follow directly from the conceptual model (Fig. 1): (1) soil microbial biomass is often ^{15}N enriched relative to the soil total and soluble N; (2) this enrichment varies between sites within gradients; (3) ^{15}N enrichment is negatively correlated with relative C and N availability; and (4) ^{15}N enrichment is positively correlated with net N mineralization.

MATERIALS AND METHODS

The Long Substrate Age Gradient in Hawaii is described in detail elsewhere (Crews *et al.* 1995; Vitousek 2004). Sites are located on the island of Hawaii (Thurston – 300 year, Laupahoehoe – 20 000 year, and Kohala – 150 000 year old), Molokai (Kolekole – 1 400 000 year old) and Kauai (Koke'e – 4 100 000 yr old). Mean air temperature is 16 °C and annual precipitation is 2500 mm. The vegetation is a mesic tropical montane forest, dominated by *Metrosideros polymorpha*. Soil (A horizon, 0–10 cm depth) was collected

near *M. polymorpha* trees in October 2004. Number of replicates was four (Thurston, Koke'e), six (Laupahoehoe) or eight (Kohala, Kolekole).

The Substrate Age Gradient of Arizona is located near Flagstaff on the San Francisco Volcanic Field (Selmants & Hart 2008) and consists of four sites (Sunset Crater – 930 year, O'Neil Crater – 55 000 year, Red Mountain – 750 000 year and Cedar Mountain – 3 000 000 year old). Mean air temperature is 11 °C and annual precipitation is 340 mm for all four sites. Soil samples (A horizon, 0–10 cm depth) were collected in March 2005 from interspaces in semi-arid piñon-juniper woodlands that were either bare (youngest site) or sparsely covered with *Bouteloua gracilis*. Number of replicates is eight for each site.

Soils and vegetation (open grasslands and meadows) associated with the C. Hart Merriam Elevation Gradient are described in Dijkstra *et al.* (2006a). Sites were located in the Great Basin desert (mean annual temperature 14 °C, annual precipitation 180 mm, elevation above sea level 1380 m), desert grassland (12 °C, 230 mm, 1750 m), piñon-juniper woodland (10 °C, 380 mm, 1975 m), ponderosa pine forest (8 °C, 660 mm, 2260 m), and mixed conifer forest (6 °C, 790 mm, 2640 m). Soil samples (A-horizon, 0–10 cm depth, $n = 4$) were taken in October 2002 and September 2003 from meadows in forests, grassy interspaces in piñon-juniper woodland or open grasslands.

Methodology for determining the natural ¹⁵N abundance of the soil microbial biomass is based on the chloroform-fumigation-extraction procedure (Brookes *et al.* 1985) and described and discussed in Dijkstra *et al.* (2006a). Briefly, soil was homogenized and sieved (2 mm mesh). One portion (20 g) was immediately extracted with 50 mL 0.25 M K₂SO₄, while a second portion (20 g) was first fumigated with chloroform for 5 days at field moisture followed by K₂SO₄ extraction. The extract solutions were dried in a ventilated oven at 60 °C, the salts ground to a fine powder and analysed on an NC 2100 Elemental Analyzer (CE Instruments, Milan, Italy) interfaced with 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 N and C content was determined by drying soil at 105 °C in a ventilated oven, followed by grinding in a mortar and analysis on the mass spectrometer. Microbial C and N content was determined by subtraction as

$$N_{mb} = N_f - N_e, \quad (1)$$

where N_{mb} is the nitrogen content of the microbial biomass, N_f is the nitrogen in the K₂SO₄ extract after 5 day fumigation with chloroform, while N_e is the nitrogen measured after immediate extraction with K₂SO₄, all

expressed per g of dry soil weight. The N isotope composition of the microbial biomass was calculated as

$$\delta^{15}N_{mb} = (\delta^{15}N_f * N_f - \delta^{15}N_e * N_e) / (N_f - N_e). \quad (2)$$

We calculated the microbial ¹⁵N enrichment relative to the soluble N pool as,

$$\Delta^{15}N = \delta^{15}N_{mb} - \delta^{15}N_e. \quad (3)$$

Determination of net N mineralization in soil from sites along the C. Hart Merriam Elevation Gradient was done in March 2002. Soil was air-dried and sieved before incubation at 60% of water holding capacity and 25 °C in a controlled environment cabinet. Net N mineralization rates were determined from changes in NH₄⁺ and NO₃⁻ concentrations over 48 days (day 35–83 after start incubation, $n = 8$). Additionally, a 31-day soil incubation experiment was conducted in June 2006. Soil (A horizon, 0–10 cm depth, $n = 8$) was collected from three sites along the C. Hart Merriam Elevation Gradient (Great Basin desert, piñon-juniper woodland and mixed conifer forest) and incubated in the lab at moisture contents representing spring conditions. At the start and at the end of the incubation, the N isotope composition of the soil microbial biomass was determined as described above. Soil NH₄⁺ and NO₃⁻ concentrations were determined after extraction with 0.25 M K₂SO₄ at the start and the end of the incubation using a Lachat Quickchem FIA+8000 autoanalyzer (Lachat, Loveland, CO, USA). Concentrations were used to calculate net N mineralization. Net N mineralization rates for sites along the Substrate Age Gradient of Arizona were determined in September 2006 by incubating 10 g of soil at 23 °C and –22 kPa matrix potential for 28 days ($n = 6$). Net N mineralization rates of mineral soil for the sites along the Long Substrate Gradient in Hawaii were published by Hedin *et al.* (2003) using a 7–10 day lab incubation.

Statistical analysis was done using ONEWAY or ANOVA, with Least Significant Difference (LSD) to distinguish between multiple sites within each gradient. Regression analysis was done to evaluate the relationships between net N mineralization and $\Delta^{15}N$, and between C : N ratio and $\Delta^{15}N$. As pointed out by D. Robinson (pers. comm.), the latter relationship may produce a spurious correlation, as N_e is present on both axes (eqns 2 and 3). We followed the procedure outlined by Brett (2004) to determine whether a spurious correlation existed. We randomly resampled $\delta^{15}N_f$, $\delta^{15}N_e$, N_f , N_e , and C_e where C_e is the C content in non-fumigated extract (see equations above), and recalculated the regression of $\Delta^{15}N$ on C : N ratio using the same sample size. After repeating this 1000 times, we calculated the average value of r . We compared this with the average r obtained after randomly resampling (1000 times) the input variables as a group.

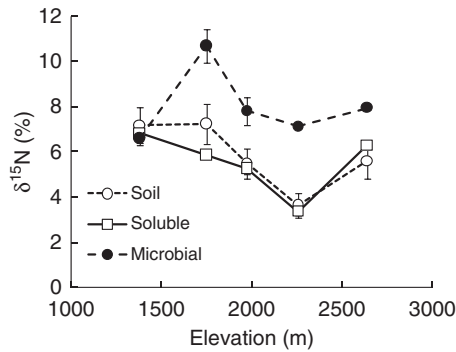


Figure 2 Natural abundance $\delta^{15}\text{N}$ (%) of the soil total, soluble and microbial N for five sites along the C. Hart Merriam Elevation Gradient in 2002. Symbols are mean values per site (\pm SE). Number of replicates for 2002 is 4, except for the mixed conifer site (2640 m), where $n = 3$.

RESULTS AND DISCUSSION

The natural abundance $\delta^{15}\text{N}$ signature of soil microbial biomass, averaged across all sites along the C. Hart Merriam Elevation Gradient, was significantly higher than the $\delta^{15}\text{N}$ values of the soil total and soluble N (Fig. 2; $P < 0.001$). A high $\delta^{15}\text{N}$ value of the microbial biomass has been reported for other soils (Dijkstra *et al.* 2006a,b; Pörtl *et al.* 2007). However, there were significant differences between sites ($P < 0.01$) and years ($P < 0.01$) in microbial ^{15}N enrichment relative to the soluble fraction (Fig. 3; site*year interaction $P = 0.082$) and relative to the soil total N (site*year interaction $P < 0.05$). For example, there was no significant difference between $\delta^{15}\text{N}$ of the microbial, soluble, and total N pool in Great Basin desert soil (1380 m above sea level), but large differences in desert grassland soil (1750 m above sea level; Fig. 2).

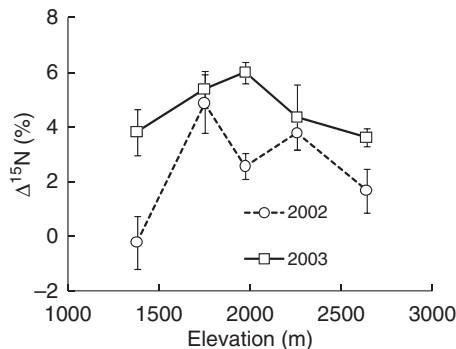


Figure 3 ^{15}N enrichment of the microbial biomass ($\Delta^{15}\text{N} = \delta^{15}\text{N}$ of soil microbial biomass $- \delta^{15}\text{N}$ of soil soluble N; %) for five sites along the C. Hart Merriam Elevation Gradient in 2002 and 2003. Symbols are mean values per site (\pm SE). Number of replicates for 2003 is 4, except for the ponderosa pine site (2260 m), where $n = 3$.

Differences in microbial ^{15}N enrichment relative to the soluble N were also observed along the substrate age gradients in Arizona ($P = 0.06$) and Hawaii ($P < 0.01$, Fig. 4a,b). Sites along each gradient are similar with respect

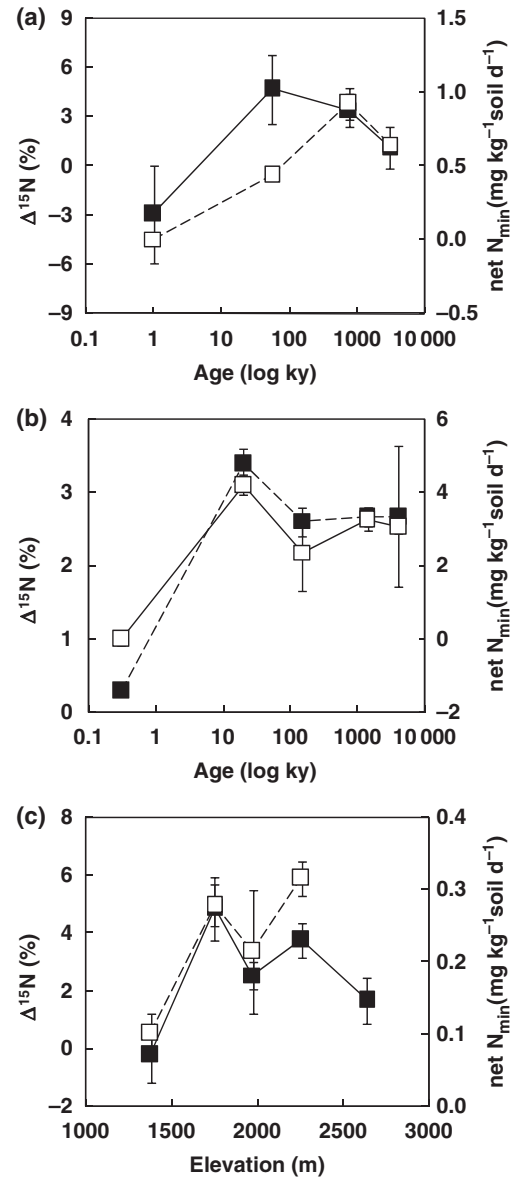


Figure 4 ^{15}N enrichment of the microbial biomass ($\Delta^{15}\text{N} = \delta^{15}\text{N}$ of soil microbial biomass $- \delta^{15}\text{N}$ of soil soluble N; %, closed symbols) and net N mineralization (open symbols, mg kg^{-1} soil day^{-1}) with substrate age (a, Substrate Age Gradient of Arizona; b, Long Substrate Age Gradient-Hawaii) and elevation (c, C. Hart Merriam Elevation Gradient-Arizona). Symbols are mean values per site (\pm SE). There was a significant positive correlation between net N mineralization and microbial ^{15}N enrichment for the substrate age gradient in Hawaii ($r = 0.98$, $P < 0.01$), and a positive association for the substrate age gradient in Arizona ($r = 0.71$, $P = 0.29$) and elevation gradient ($r = 0.93$, $P = 0.067$).

to climate, topography, vegetation cover, and derived from the same parent materials. The only difference between the sites is the time the parent materials were deposited (Vitousek 2004; Selmants & Hart 2008). The microbial biomass at the youngest sites on both substrate age gradients exhibited low ^{15}N enrichment (not significantly different from zero). With increasing age, enrichment first increased and then decreased slightly. The pattern of change in ^{15}N enrichment with ecosystem development over millions of years is remarkably similar, despite large differences in climate (semiarid vs. tropical), vegetation (open woodland vs. tropical rainforest) and soil type (Crews *et al.* 1995; Chadwick *et al.* 1999; Selmants & Hart 2008) between Hawaii and Arizona.

We hypothesized an association between high ^{15}N enrichment and low relative C availability, and low ^{15}N enrichment and high relative C availability (Fig. 1). The results from the substrate age gradients in Arizona and Hawaii support this hypothesis. Plant growth on young volcanic soils is strongly N-limited (Vitousek 2004; G. Newman, K. Hess & S.C. Hart, unpublished data), while net N mineralization rates (Hedin *et al.* 2003; Fig. 4) and N_2O and NO production (Hall & Matson 1999) are low in younger soils relative to older sites. Sites of intermediate age exhibit high standing biomass, productivity and net N mineralization as these soils become less N-limited and soil organic matter accumulates (Hedin *et al.* 2003; Vitousek 2004). We therefore assume that the microbial biomass is strongly N-limited in the young soils and becomes more C-limited with age. The finding of low ^{15}N enrichment for the microbial biomass in young soils and high enrichment as C becomes more limited (Fig. 4a,b) supports our conceptual model (Fig. 1).

The decrease in ^{15}N enrichment with further substrate and ecosystem development is likely associated with the occurrence of P-limitation. Decreasing P availability at the older sites is evident from fertilizer experiments (Vitousek & Farrington 1997) and soil analysis (Crews *et al.* 1995; Chadwick *et al.* 1999; Hall & Matson 2003; P. Selmants & S.C. Hart unpublished data). Although the N : P ratio in litter is constant (Vitousek 1998) or shows relatively small increases with age (Hobbie & Vitousek 2000), increases in the N : P ratio of the soil and soluble fractions are large (Neff *et al.* 2000; Olander & Vitousek 2000). From this, we can conclude that the decrease in ^{15}N enrichment at the older sites is not the result of a reemerging N limitation as was observed for the youngest sites. Further evidence against N limitation for older sites is found in the very high N-losses as NO and N_2O (Hall & Matson 2003), and organic and inorganic N losses in leachate (Hedin *et al.* 2003). Instead, we think that due to P limitation, the soil microbial biomass has to solubilize large amounts of organic matter in order to satisfy its P requirements. The high

availability of C, even when N is present, will shift the balance between N dissimilation and assimilation towards N assimilation compared to a situation where C is limited (Fig. 1). A similar phenomenon was observed across a cattle dung deposition gradient (Dijkstra *et al.* 2006b) where ^{15}N enrichment of the soil microbial biomass decreased when C but also N availability increased.

More generally, we expect a negative correlation between the microbial ^{15}N enrichment and the relative activity of the N assimilation and dissimilation pathways; the latter is a function of relative C and N availability. We observed negative relationships between ^{15}N enrichment and the C : N ratio of the soluble fraction (Fig. 5). This relationship was significant for two of the three gradients ($r = -0.97$, $P < 0.05$ for the Substrate Age Gradient in Arizona; $r = -0.87$, $P < 0.01$ for the C. Hart Merriam Elevation Gradient; $r = -0.58$, $P = 0.30$ for the Long Substrate Age Gradient in Hawaii). High ^{15}N enrichments were associated with low C : N ratios, suggesting a high N dissimilation activity. Conversely, when C : N ratio was high, suggesting increased N assimilation, low microbial ^{15}N enrichment was observed. We confirmed that the relationship in Fig. 5 was not due to spurious correlation between two non-independent variables using a randomized resampling approach (Brett 2004, see Materials and methods). Average r of the regression of $\Delta^{15}\text{N}$ on C : N ratio when all input variables were randomly and independently sampled was $r = 0.06$

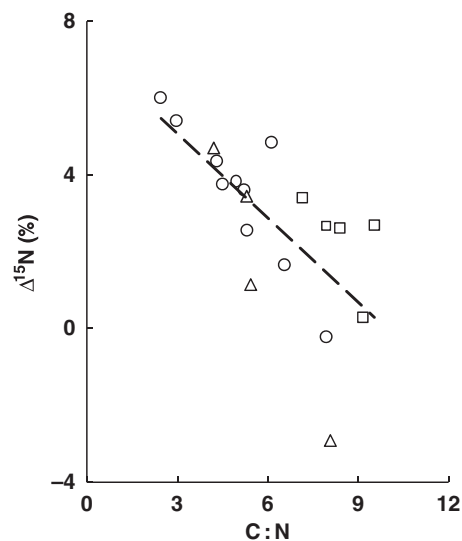


Figure 5 Relationship between C : N ratio of the soluble fraction and ^{15}N enrichment ($\Delta^{15}\text{N} = \delta^{15}\text{N}$ of soil microbial biomass $- \delta^{15}\text{N}$ of soil soluble N; %). C. Hart Merriam Elevation Gradient in Arizona (open circles), Substrate Age Gradient of Arizona (open triangles), and Long Substrate Age Gradient in Hawaii (open squares). Combined regression for all soils (line; $r = -0.69$, $P < 0.01$, $y = 7.25 - 0.73x$). Symbols are mean values per site.

(SD = 0.26, 95% percentile interval -0.46 to 0.58). When the $\Delta^{15}\text{N}$ and C:N ratio were similarly resampled, r equalled -0.71 (SD = 0.13, 95% percentile interval = -0.93 to -0.42). These results show that we were justified to statistically test our observations vs. the null-hypothesis of $r = 0$.

As predicted by the model (Fig. 1), we did observe positive associations between net N mineralization rates and ^{15}N enrichment of the soil microbial biomass for all three soil gradients (Fig. 4). Combined data from the three gradients yielded a significant correlation [expressed as relative values of ^{15}N enrichment and net N mineralization, calculated for each gradient as $100 * (\text{observed} - \text{lowest}) / (\text{highest} - \text{lowest})$; $r = 0.87$, $P < 0.01$, $y = 5.45 + 0.86x$, with $y = \text{net N mineralization rate}$ and $x = \Delta^{15}\text{N}$]. A short-term soil incubation experiment provided further evidence for the positive correlation between net N mineralization rate and microbial ^{15}N enrichment. At the start of the incubation, $\delta^{15}\text{N}$ of the soil microbial biomass was enriched relative to the soluble N, but the enrichment was not significantly different between sites ($P = 0.59$; Fig. 6). At the end of the incubation, microbial ^{15}N enrichment had increased, with the highest increases observed for soil from piñon-juniper woodlands ($P < 0.001$), concomitant with the highest net N mineralization rate ($P < 0.01$).

In the above discussion, we assume that the $\delta^{15}\text{N}$ of the microbial materials extracted using the chloroform fumigation-extraction method is representative for the whole microbial cell. However, it is clear that only portions of the microbial cells are extractable after fumigation (Jenkinson *et al.* 2004). Cell wall materials for example do not become solubilized. So is there a reason to assume that the $\delta^{15}\text{N}$ of cell wall materials differ from that of the extracted materials, likely dominated by proteins? Taylor *et al.* (1997) analysed the isotope composition of ectomycorrhizal fungi, and

separated fruiting bodies into protein, amino acids, and chitin. The average $\delta^{15}\text{N}$ of the total N was 6.3%, the protein-N was 7.8%, amino acid-N was 7.4%, while chitin-N had a signature that was -2.1% . These results prove two points: 1- chitin-N is depleted relative to the rest of the tissue, and 2- chitin has only minimal effects on the N isotope composition of the total tissue. Taylor estimated that chitin only made up 10% of the total N in the mycorrhizal fruiting bodies. We argue that not extracting chitin has only minimal effects on the isotope ratios, a point already made by Dijkstra *et al.* (2006a). Protein content on the other hand does determine the total N content of the cell and is closely related to its N isotope signature. Furthermore, it is likely that the fraction remaining in the soil after extraction contains proteins as well. There is no reason to assume that the protein content associated with cell wall fractions has a different signature than protein that becomes solubilized. Unfortunately, the microorganisms are closely associated with the soil matrix, making a clean separation impossible. However, we can compare the results presented above with observations on other organisms or with *in vitro* studies (e.g. Collins *et al.* 2008).

It is clear from these results that microbial ^{15}N enrichment is related to the relative C and N availability and net N mineralization, a key regulator of many ecosystem processes (Schimel & Bennett 2004). Trees in the Hawaiian tropical forests do not support ectomycorrhizal fungi (Treseder & Vitousek 2001) and while piñon pine trees in the piñon-juniper woodlands have ectomycorrhizal fungi (Hart *et al.* 2006), grass species exhibit symbiosis mostly with arbuscular mycorrhiza (Read & Perez-Moreno 2003). At this moment, we can only speculate whether similar results will be obtained in areas where ectomycorrhiza are present. However, an indirect comparison can be made between our results and ^{15}N analyses of mycorrhizal and saprotrophic fruiting bodies. Differences in $\delta^{15}\text{N}$ values between fungal species are widely observed (Taylor *et al.* 2003; Trudell *et al.* 2004). Most of these differences would be predicted by our model. For example, saprotrophic fungi growing on C-rich N-poor wood are less ^{15}N enriched than ectomycorrhizal fungi involved in N export to the host plant (Kohzu *et al.* 1999). Litter decaying saprotrophic fungi exhibit higher $\delta^{15}\text{N}$ values than wood decaying fungi (Hobbie *et al.* 2001), reflecting the much greater C availability for latter organisms. A positive correlation between net N mineralization and ^{15}N enrichment has also been observed for mycorrhizal fungi across an N deposition gradient (Lilleskov *et al.* 2002), similar to what we observed for the soil microbial biomass (Fig. 4). In addition to valid arguments presented by Lilleskov *et al.* (2002), we can add our own explanation for this phenomenon: with increased N deposition, less C is available for the fungus (either from the plant host or the soil environment), resulting in an increased

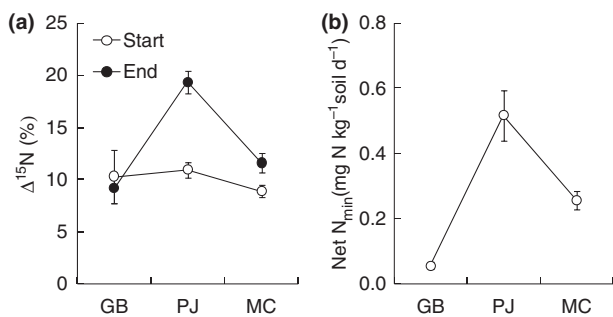


Figure 6 Microbial ^{15}N enrichment (a, $\Delta^{15}\text{N} = \delta^{15}\text{N}$ of soil microbial biomass $- \delta^{15}\text{N}$ of soil soluble N; %) at the start and end of a 31-day soil incubation, and net N mineralization rate (b, $\text{mg N kg}^{-1} \text{ soil day}^{-1}$) during incubation of soil from the Great Basin desert (GB), piñon-juniper woodland (PJ) and mixed conifer (MC).

N dissimilation and export (either to the plant or into the soil environment), thus causing higher $\delta^{15}\text{N}$ values. As the mycorrhizal fungi make up a substantial proportion of the soil microbial biomass, more work is required to quantify the contribution of (ecto) mycorrhizal hyphae to the overall ^{15}N signature of the soil microbial biomass.

Our results show consistent patterns in ^{15}N enrichment of microbial biomass across N immobilization-mineralization gradients (Fig. 4a,b). This is surprising as the microbial organisms have access to a broad suite of N sources (NO_3^- , NH_4^+ , and a myriad of soluble and insoluble organic N compounds) with markedly different isotope signatures (Högberg 1997; Robinson 2001). Moreover, many processes that impact the $\delta^{15}\text{N}$ values of inorganic N pools, such as NH_3 volatilization, nitrification and denitrification, have fractionation constants as high or higher than those associated with deamination and $\text{NH}_4^+/\text{NH}_3$ state change (Handley & Raven 1992; Högberg 1997; Robinson 2001). The ^{15}N enrichment of soil microbial biomass relative to soil total and soluble N in environments that stimulate denitrification (Hawaii) or NH_3 volatilization and nitrification (Arizona) suggests that fractionation associated with these processes is quantitatively less important than fractionation associated with N dissimilation and export.

We submit that changes in $\delta^{15}\text{N}$ values of the microbial biomass during short-term incubation (Fig. 6) are directly linked to small increases in $\delta^{15}\text{N}$ of the total soil organic matter observed in very long-term incubations (Nadelhoffer & Fry 1988). Older soil organic matter has higher $\delta^{15}\text{N}$ values but lower C : N ratios than more recent materials (Kramer *et al.* 2003; Liao *et al.* 2006a,b; Sollins *et al.* 2006). We propose that during soil organic matter formation, relative C availability continuously decreases causing the release of inorganic N by microorganisms. As a consequence, more and more of the light ^{14}N isotope is removed from materials that eventually end up as highly ^{15}N enriched stable soil organic matter.

In agreement with suggestions by Robinson (2001), our results strongly support the idea that $\delta^{15}\text{N}$ functions as an integrator of N transformations. In addition, we believe that these N transformations are predictably associated with relative C and N availabilities to the soil microbial community. For other elements, such as C, oxygen and hydrogen, relations between isotope composition, metabolic processes, and ecosystem properties have been thoroughly established (West *et al.* 2006). We submit that N may be added to this list: ^{15}N enrichment integrates the influence of C and N availability over N assimilation, dissimilation and export, and is directly related to N mineralization and C and N cycling, plant-soil relations, soil organic matter stabilization, and ecosystem development.

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REFERENCES

- Brett, M.T. (2004). When is a correlation between non-independent variables "spurious". *Oikos*, 105, 647–656.
- Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.*, 17, 837–842.
- Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J. & Hedin, L.O. (1999). Changing sources of nutrients during four million years of ecosystem development. *Nature*, 397, 491–497.
- Collins, J., Dijkstra, P., Hart, S.C., Hungate, B.A., Flood, N. & Schwartz, E. (2008). Nitrogen source influences natural abundance ^{15}N enrichment of *E. Coli*. *FEMS Microb. Lett.*, in press.
- Crews, T.E., Kitayama, K., Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller-Dombois, D. *et al.* (1995). Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology*, 76, 1407–1424.
- Dijkstra, P., Ishizu, A., Doucett, R.R., Hart, S.C., Schwartz, E., Menyailo, O.V. *et al.* (2006a). ^{13}C and ^{15}N natural abundance of the soil microbial biomass. *Soil Biol. Biochem.*, 38, 3257–3266.
- Dijkstra, P., Menyailo, O.V., Doucett, R.R., Hart, S.C., Schwartz, E. & Hungate, B.A. (2006b). C and N availability affects the ^{15}N natural abundance of the soil microbial biomass across a cattle manure gradient. *Eur. J. Soil Sci.*, 57, 468–475.
- Hall, S.J. & Matson, P.A. (1999). Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature*, 400, 152–155.
- Hall, S.J. & Matson, P.A. (2003). Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecol. Monogr.*, 73, 107–129.

- Handley, L.L. & Raven, J.A. (1992). The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ.*, 15, 965–985.
- Hart, S.C. & Stark, J.M. (1997). Nitrogen limitation of the microbial biomass in an old-growth forest soil. *Ecoscience*, 4, 91–98.
- Hart, S.C., Nason, G.E. & Myrold, D.D. (1994). Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. *Ecology*, 75, 880–891.
- Hart, S.C., Gehring, C.A., Selmants, P.C. & Deckert, R.J. (2006). Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forest of the south-western USA. *Funct. Ecol.*, 20, 42–51.
- Hedin, L.O., Vitousek, P.M. & Matson, P.A. (2003). Nutrient losses over four million years of tropical forest development. *Ecology*, 84, 2231–2255.
- Hobbie, S.E. & Vitousek, P.M. (2000). Nutrient limitation of decomposition in Hawaiian forests. *Ecology*, 81, 1867–1877.
- Hobbie, E.A., Macko, S.A. & Shugart, H.H. (1999). Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia*, 118, 353–360.
- Hobbie, E.A., Weber, N.S. & Trappe, J.M. (2001). Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytol.*, 150, 601–610.
- Hobson, K.E., Alisauskas, R.T. & Clark, R.G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. *Condor*, 95, 388–394.
- Högberg, P. (1997). ^{15}N natural abundance in soil-plant systems. *New Phytol.*, 137, 179–203.
- Jahn, T.P., Möller, A.L.B., Zeuthen, T., Holm, L.M., Klærke, D.A., Mohsin, B. *et al.* (2004). Aquaporin homologues in plants and animals transport ammonia. *FEBS Lett.*, 574, 31–36.
- Jenkinson, D.S., Brookes, P.C. & Powlson, D.S. (2004). Measuring microbial biomass. *Soil Biol. Biochem.*, 36, 5–7.
- Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K. & Wada, E. (1999). Natural ^{13}C and ^{15}N abundance of field-collected fungi and their ecological implications. *New Phytol.*, 144, 323–330.
- Kramer, M., Sollins, P., Sletten, R.S. & Swart, P.K.N. (2003). N isotope fractionation and measures of organic matter alteration during decomposition. *Ecology*, 84, 2021–2025.
- Liao, J.D., Boutton, T.W. & Jastrow, J.D. (2006a). Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biol. Biochem.*, 38, 3184–3196.
- Liao, J.D., Boutton, T.W. & Jastrow, J.D. (2006b). Organic matter turnover in soil physical fractions following woody plant invasion of grassland: evidence from natural ^{13}C and ^{15}N . *Soil Biol. Biochem.*, 38, 3197–3210.
- Lilleskov, E.A., Hobbie, E.A. & Fahey, T.J. (2002). Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytol.*, 154, 210–231.
- Lovett, G.M., Weathers, K.C. & Arthur, M.A. (2002). Control of nitrogen loss from forested watersheds by soil carbon:nitrogen ratio and tree species composition. *Ecosyst.*, 5, 712–718.
- Macko, S.A. & Estep, M.L.F. (1984). Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Org. Geochem.*, 6, 787–790.
- Macko, S.A., Fogel, M.L., Hare, P.E. & Hoering, T.C. (1987). Isotope fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chem. Geol.*, 65, 79–92.
- Minagawa, M. & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta*, 48, 1135–1140.
- Nadelhoffer, K.J. & Fry, B. (1988). Controls on natural abundance nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Sci. Soc. Am. J.*, 52, 1633–1640.
- Neff, J.S., Hobbie, S.E. & Vitousek, P.M. (2000). Nutrient and mineralogical control on dissolved organic C, N and P fluxes and stoichiometry in Hawaiian soils. *Biogeochemistry*, 51, 283–302.
- Olander, L.P. & Vitousek, P.M. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49, 175–190.
- Ponsard, S. & Averbuch, P. (1999). Should growing and adult animals fed on the same diet show different $\delta^{15}\text{N}$ values? *Rapid Commun. Mass Spectrom.*, 13, 1305–1310.
- Pörtl, K., Zechmeister-Boltenstern, S., Wanek, W., Ambus, P. & Berger, T.W. (2007). Natural ^{15}N abundance of soil N pools and N_2O reflect the nitrogen dynamics of forest soils. *Plant Soil*, 295, 79–94.
- Post, D.M. (2002). Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology*, 83, 703–718.
- Read, D.J. & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytol.*, 157, 475–492.
- Robinson, D. (2001). $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends Ecol. Evol.*, 16, 153–162.
- Schimel, J.P. & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, 85, 591–602.
- Selmants, P. & Hart, S.C. (2008). Substrate age mediates the influence of tree islands on carbon and nitrogen dynamics in semiarid woodland soils. *Glob. Biogeochem. Cycles*, (in press).
- Sollins, P., Swanston, C., Kleber, M., Filley, T., Kramer, M., Crow, S. *et al.* (2006). Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. *Soil Biol. Biochem.*, 38, 3313–3324.
- Taylor, A.F.S., Högbom, L., Högbom, M., Lyon, A.J.E., Näsholm, T. & Högbom, P. (1997). Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytol.*, 136, 713–720.
- Taylor, A.F.S., Fransson, P.M., Högbom, P., Högbom, M. & Plamboeck, A.H. (2003). Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. *New Phytol.*, 159, 757–774.
- Treseder, K.K. & Vitousek, P.M. (2001). Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology*, 82, 946–954.
- Trudell, S.A., Rygielwicz, P.T. & Edmonds, R.L. (2004). Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth conifer forests. *New Phytol.*, 164, 317–335.
- Vanderklift, M. & Ponsard, S. (2003). Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia*, 136, 169–182.

- Vitousek, P.M. (1998). Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian *Metrosideros polymorpha*. *Ecosyst.*, 1, 401–407.
- Vitousek, P.M. (2004). *Nutrient Cycling and Limitation Hawaii as a Model System*. Princeton Univ Press, Princeton, NJ.
- Vitousek, P.M. & Farrington, H. (1997). Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry*, 37, 63–75.
- Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, 13, 87–115.
- West, J.B., Bowen, G.J., Cerling, T.E. & Ehleringer, J.R. (2006). Stable isotopes as one of nature's ecological recorders. *Trends Ecol. Evol.*, 21, 408–414.

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