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N ISOTOPE FRACTIONATION AND MEASURES OF ORGANIC MATTER ALTERATION DURING DECOMPOSITION

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Abstract. Most soil organic matter (SOM) derives from plant material, but there are substantial gaps in our understanding of its transformation mechanisms. Alterations that occur as SOM decays and is stabilized have proved difficult to study owing largely to its diverse initial chemical composition and stable isotope values. We examined SOM stable isotope ratios in relation to composition using solid-state ¹³C Cross Polarization Magic Angle Spin (CPMAS) nuclear magnetic resonance (NMR) in sequentially deeper organic and mineral horizon soil samples collected from an unpolluted ecosystem in southeast Alaska, USA, dominated by C₃ vegetation. We found that, as humified carbon (C not respired as CO₂ during decomposition) increased in aliphaticity (ratio of unsubstituted aliphatics to carbohydrates), it also increased in ¹⁵N but showed no clear trend in ¹³C. These results imply that humification (and the concomitant stabilization of soil C) at our site resulted from microbial alteration of organics rather than from accumulation of recalcitrant compounds. The strong, and previously unreported, relation between ¹⁵N and SOM composition found at our study site suggests that degree of SOM humification may be correlated with an increase in δ¹⁵N SOM values relative to δ¹⁵N for fresh litter and other source material.

Key words: decomposition; humification; illuviation; isotope fractionation; natural ¹⁵N and ¹³C isotope abundance; soil organic carbon; soil organic matter dynamics; ¹³C solid-state nuclear magnetic resonance (NMR).

INTRODUCTION

Soil organic matter (SOM) is the largest terrestrial reservoir of fixed carbon, larger than the atmosphere and biosphere pools of carbon combined (Lal et al. 1998), and it exerts strong control on ecosystem dynamics (soil erosion rates, nutrient cycling, primary production). As a consequence of its large pool size, loss of soil carbon through decomposition represents potentially a large positive feedback to global warming. SOM is a heterogeneous mixture of organic materials, including some fresh or unaltered debris but mainly transformed products, or humic materials, that bear little morphological or compositional resemblance to the structures from which they derive. Such materials can include carbohydrates, lignin derivatives and other carboxylics, various alkyl and alkene compounds, and much material that cannot be well characterized chemically. The processes by which freshly deposited or-

ganic matter is converted to humic materials (referred to here as humification) have proven difficult to study, in part because of the diversity of compounds involved.

¹³C solid-state nuclear magnetic resonance (NMR) allows approximate quantification of three groups of compounds: O-alkyl (carbohydrate), alkyl, and aromatic (Baldock and Preston 1995). During humification, carbohydrates consistently decrease and alkyls increase, while aromatics behave variably; thus the ratio of alkyl to carbohydrate (aliphaticity) is the preferred index of SOM humification (Baldock et al. 1997). Humification is known to increase the aliphaticity and lignin : nitrogen ratio while decreasing the carbon : nitrogen ratio (Baldock et al. 1997, Nierop 1998, Schulden and Leinweber 2000).

SOM has also been widely observed to increase in ¹³C and ¹⁵N abundance with soil depth (Kendall 1998, Nadelhoffer and Fry 1988, Eshetu and Högberg 2000, Adams and Grierson 2001, Krull et al. 2002, Amundson et al. 2003). Increasing ¹³C and ¹⁵N with soil depth has often been ascribed to humification (Bauer et al. 2000, Brenner et al. 2001). For ¹³C the link is preferential

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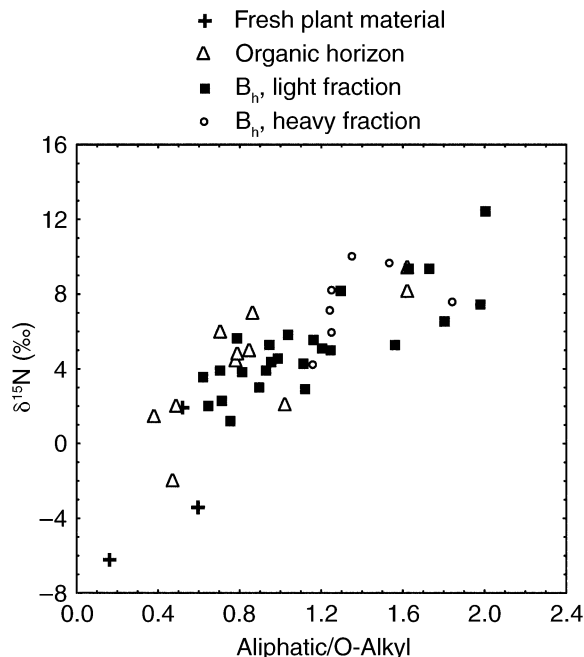


FIG. 1. $^{15}\text{N}_{\text{PDB}}$ abundance vs. aliphaticity ($R^2 = 0.68$, $n = 47$, $P < 0.0001$). Nuclear magnetic resonance (NMR) spectra were obtained for solid-state samples from fresh source materials and from sequentially deeper organic (O_{ica}) and mineral-soil (B_h) horizons, which were physically fractionated based on particle density at the University of Washington, Seattle, Washington, USA.

loss of the lighter ^{12}C via respiration, which concentrates ^{13}C in the remaining SOM. The mechanisms underlying ^{15}N accumulation, however, are not well understood (Adams and Grierson 2001), although thought to be the result of metabolism of microbial heterotrophs (Nadelhoffer and Fry 1994). Moreover, such trends in ^{13}C and ^{15}N abundance have rarely been related to trends in SOM composition as measured by solid-state NMR or other newly available techniques.

We examined relations between stable isotope ratios and SOM composition (^{13}C NMR) in sequentially deeper organic and mineral horizon soil samples collected from an ecosystem in southeast Alaska, USA, dominated by C_3 vegetation. Microbial processing is very slow in these unpolluted coastal temperate rainforest soils due to the wet, cool maritime climate, so decomposition is often incomplete. As a result, altered forms of organic matter persist in the soil for extended periods, and thick organic horizons develop (>40 cm). Unusually high rates of illuviation, the migration and subsequent immobilization of soluble and particulate organics through soil water movement, lead to large accumulations of carbon (>14% C by dry mass) in mineral horizons of these Spodosols.

METHODS

NMR spectra (300 MHz ^{13}C Cross Polarization Magic Angle Spin [CPMAS]; Bruker, Rheinstetten, Ger-

many) were obtained for solid-state samples from fresh source materials and from sequentially deeper organic (O_a) and mineral-soil (B_h) horizons, which were physically fractionated based on particle density (1.65 g/cm^3 Na polytungstate) at the University of Washington, Seattle, Washington, USA. For heavy fractions we could only obtain reliable NMR spectra (high signal : noise ratio) for the six samples with C content >14%. Aliphaticity was defined as the ratio of unsubstituted aliphatic carbon (0–45 parts per million [ppm]) to oxygenated carbon (carbohydrates; 45–110 ppm). Samples were then analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ with a 20/20 ANCA GSL continuous-flow isotope-ratio mass spectrometer (PDZ Europa, Cheshire, UK) at the Rosenthal School of Marine Science, University of Miami, Miami, Florida, USA.

RESULTS AND DISCUSSION

^{15}N abundance and aliphaticity

We found that $\delta^{15}\text{N}$ increased strongly (from -6 to $+13\text{‰}$) with increasing aliphaticity across all SOM pools examined (Fig. 1). Thus at our site, $\delta^{15}\text{N}$ appears to be a robust measure of organic-matter composition owing to strong preferential ^{15}N accumulation during humification. The mechanisms for our observed trend for ^{15}N with aliphaticity are unclear. Adams and Grierson (2001) point out that, despite ample empirical evidence for ^{15}N accumulation as a result of microbial processing, the mechanisms of N fractionation in soil are not yet well understood. This problem should be expected given that the few data on ^{15}N isotope discrimination during enzymatic reactions show isotope effects ranging from strongly negative to strongly pos-

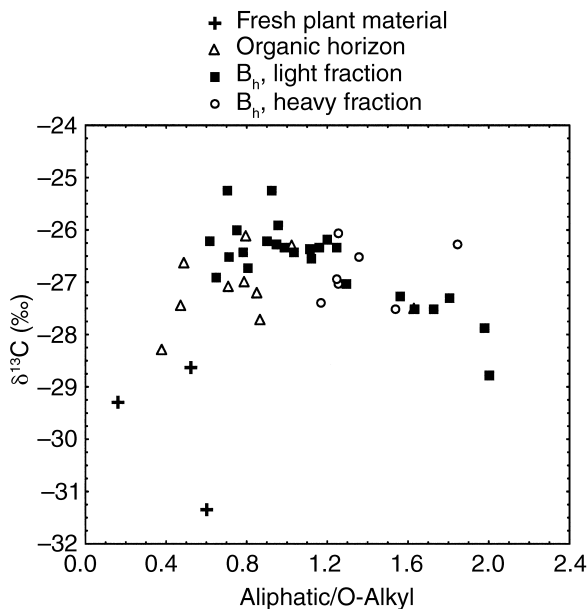


FIG. 2. $^{13}\text{C}_{\text{PDB}}$ abundance vs. aliphaticity ($R^2 = 0.003$, $n = 47$, $P = 0.91$).

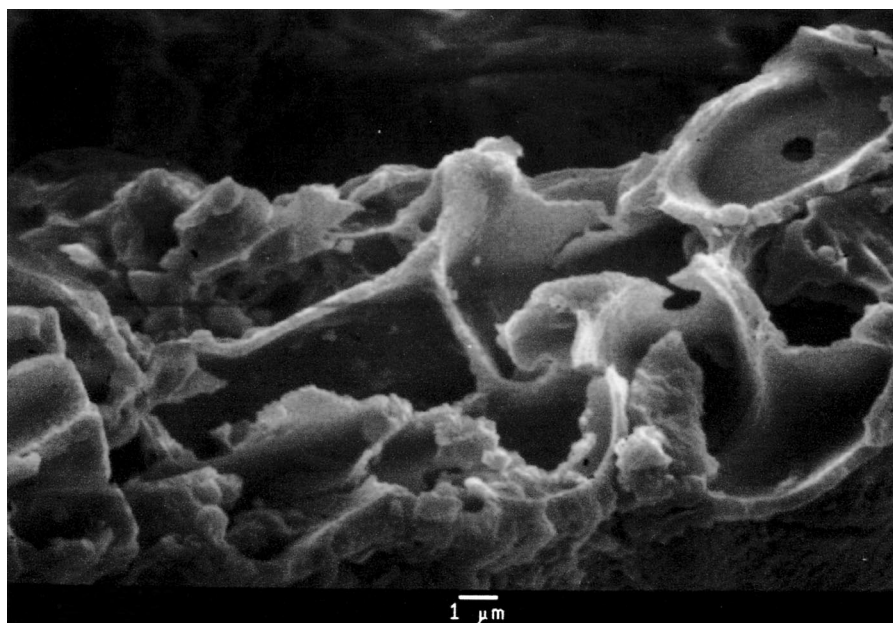


FIG. 3. Scanning electron microscope image of O-horizon material showing partially altered plant residues.

itive (e.g., Blair et al. 1985). Amundsen and Baisden (2000) have developed a two-compartment mass-balance model of soil and plant ^{14}N and ^{15}N in an attempt to describe effects of time and climate on N-isotope fractionation. Robinson et al. (1998) have developed a theoretical framework for modeling ^{15}N isotope effects mechanistically in plants but note that extending their framework to soil N dynamics is still a distant goal.

^{13}C abundance and aliphaticity

We found no trend in $\delta^{13}\text{C}$ with aliphaticity that was consistent across all SOM pools examined, and the range for ^{13}C was smaller than for ^{15}N (from -31 to -25% ; Fig. 2). Moreover, $\delta^{13}\text{C}$ values were low in both partially altered (carbohydrate-dominated) SOM from upper organic horizons and in strongly humified (highly aliphatic) light-fraction SOM from deeper illuvial mineral-soil horizons. We interpret the much stronger correlation of SOM composition with $\delta^{15}\text{N}$ than with $\delta^{13}\text{C}$ as resulting from low $\delta^{13}\text{C}$ values associated with illuvial material. Low $\delta^{13}\text{C}$ values have been widely observed in illuvial soil horizons (Nadelhoffer and Fry 1988, Högberg 1997) although the fractionation mechanism is unknown.

Examination of partially decomposed O-horizon material under a scanning electron microscope (SEM; AmRay, Bedford, Massachusetts, USA) at Oregon State University, Corvallis, Oregon, USA, showed the material contained partially altered plant residues (Fig. 3) and was mainly carbohydrate (O-alkyl carbon). By contrast, light-fraction material in B_h horizons consisted of amorphous precipitated organo-mineral material, as evidenced by dehydration cracks across an otherwise smooth surface (Fig. 4), and contained main-

ly aliphatic carbon. Thus, although we saw no trend at our sites, increasing $\delta^{13}\text{C}$ values may yet correlate directly with aliphaticity (and thus humification) in other ecosystems where illuviation is less pronounced.

^{13}C increases with soil depth are widely reported and thought to result from humification (Melillo et al. 1989, Schlesinger 1997), but the mechanisms of such C isotope fractionation are not yet entirely clear. Lipids and lignin tend to be ^{13}C depleted and are degraded more slowly, whereas cellulose and carbohydrate tend to be ^{13}C enriched and degrade more rapidly (Ehlinger et al. 2000, Adams and Grierson 2001). Preferential degradation alone should then cause decreasing ^{13}C abundance with humification (Ehlinger et al. 2000). Most soils, however, show increasing ^{13}C with depth, although some data suggest that this trend may stabilize or even reverse (Volkoff and Cerri 1987, Bauer et al. 2000, Garten et al. 2000, Krull et al. 2002) at depths greater than sampled in most studies. The initial increase in ^{13}C with depth is thus explained better by microbial resynthesis (processing) than by degradation of more labile plant and microbial residues. The strong increase in ^{15}N with increasing aliphaticity at our site strongly supports this interpretation.

The $\delta^{13}\text{C}$ values of light-fraction samples from B_h horizons that we identified as illuvial (all of which had aliphaticity >1) consistently decreased with increasing aliphaticity (Fig. 2). ^{13}C depletion was also reported by Fernandez et al. (2003) for soil plant residues of differing chemical quality during a 119-day decomposition experiment. One explanation is preferential degradation of isotopically heavier, more labile constituents, such as carbohydrates and proteins. However, the SEM imaging of the B_h horizon material suggests it

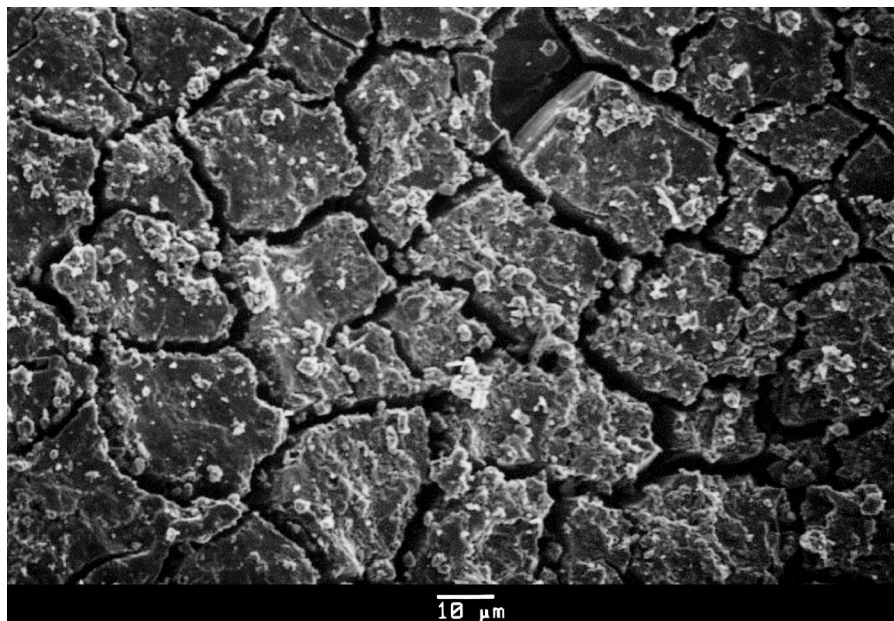


FIG. 4. Scanning electron microscope image of light-fraction material from a B_h horizon showing amorphous precipitated organo-mineral material as evidenced by dehydration cracks across an otherwise smooth surface.

is precipitated organo-mineral material, and NMR spectra indicate that it is aliphatic, and thus presumably less labile, than organic horizon (O_{1ca}) samples. Regardless of the cause, the unexpected pattern we observed is further evidence that mechanisms of C isotope fractionation in soil are far from completely understood.

CONCLUDING REMARKS

Soil organic matter humification (and resulting stabilization) remains an elusive yet important issue in ecosystem science research. It is an important mechanism influencing the global C cycle and may reduce erosion rates and strongly influence rates of nutrient cycling and primary production through changes in soil structure (aggregation) and ion retention capacity. The strong increase in $\delta^{15}\text{N}$ values and lack of consistently lighter $\delta^{13}\text{C}$ values with aliphaticity suggest that humification was due primarily to microbial processing, rather than to preferential degradation of labile constituents. Measurement of ^{15}N and ^{13}C ratios in relation to SOM composition in other ecosystems, in particular those in which illuviation is less pronounced, N is less limiting (open N systems), and microbial processing rates are faster, may help further explain mechanisms of N and C isotope fractionation in soil.

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