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RAPID STABILIZATION AND MOBILIZATION OF ^{15}N IN FOREST AND RANGE SOILS

TIMOTHY C. STRICKLAND,[1]* PHILLIP SOLLINS,[1] NATHAN RUDD[1] and DAVID S. SCHIMEL[2]†
[1]Department of Forest Science, Oregon State University, Corvallis, OR 97331 and
[2]Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, U.S.A.

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Summary—Effects of physical protection of soil organic matter (SOM) on rates of nitrogen (N) mineralization and immobilization were studied in five soils of contrasting mineralogy and physical structure from North and Central America. Whole soils were exposed for 60 days to ^{15}N NH_4Cl, then separated into a heavy and a light density fraction ($\gtrsim 1.70\ Mg\ m^{-3}$). Substantial ^{15}N was incorporated into the denser (heavy) fraction of each soil during the 60-day exposure indicating that organo–mineral complexes can form quite quickly. Next, sonicated and unsonicated heavy fraction (HF) material was slurry incubated for 5 days. (The effect of sonication was used as an operational measure of the degree of physical protection.) Most of the newly-formed organic N was non-labile. However, some ^{14}N and ^{15}N were released into solution during the slurry incubation, mainly as dissolved organic N (DON). NH_4^+ was also released and was more highly labelled than the DON, suggesting that the NH_4^+ was derived from a more active pool. We expected that the degree of labelling of the NH_4^+ and DON released during incubation would be lower for sonicated than for unsonicated HFs because the label would be diluted by release of physically-protected native ^{14}N. In fact, sonication had no effect on release of $^{14}NH_4^+$ or $^{15}NH_4^+$ during the slurry incubation but markedly increased release of ^{14}N and ^{15}N as DON. In addition, sonication increased the degree of labelling of the NH_4^+ and perhaps of the DON. Overall these results suggest that some ^{15}N was incorporated into the HFs as an active but protected ("active-protected") fraction. Only a small portion of this active-protected fraction was chloroform-labile suggesting that it was not microbial biomass.

INTRODUCTION

Researchers since the 1950s have thought of soil organic carbon and nitrogen as forming multiple compartments with differing mineralization kinetics. Jansson (1958), for example, distinguished active from inactive N pools on the basis of ^{15}N tracer kinetics. Martel and Paul (1974) used physical and chemical methods to isolate soil fractions with very different ^{14}C ages. Based on such studies, and on work with simulation models (e.g. Parton *et al.*, 1988), many researchers now refer to four pools of soil organic C and N: a debris pool including litter and roots, an active pool (microbial cells and metabolites), a medium-turnover pool (turnover time of decades to several centuries), and a stable pool. The medium-turnover and stable organic pools are especially important to ecosystem stability, providing nutrient reserves and increasing tilth and resistance to erosion. Better understanding of the mechanisms of soil organic matter (SOM) stabilization would thus aid in predicting system response to disturbance and management. The mechanisms by which C and N are incorporated into the slower-turnover pools (i.e. medium and stable) remain a subject of speculation (Tiessen *et al.*, 1984). The stable pool is thought to be primarily chemically stabilized, whereas the medium-turnover pool is thought to be both chemically stabilized and physically protected (Parton *et al.*, 1988). Chemical stabilization is thought to involve formation of heteropolycyclics whose tertiary structure hinders access to enzyme-reactive sites (Duxbury *et al.*, 1989). Physical protection is generally assumed to involve sequestration of organic matter within aggregates as well as formation of organo–mineral complexes on clay surfaces (Young and Spycher, 1979; Tisdall and Oades, 1982).

Unfortunately, methods for isolating the different SOM pools as fractions do not exist (Tiessen *et al.*, 1984). It has been suggested that fractions differing in density may approximate the mathematically defined pools (Young and Spycher, 1979; Spycher *et al.*, 1983). More specifically, Sollins *et al.* (1984) have suggested that the structural and active pools may equate roughly to a light density fraction, and the medium-turnover plus stable pools to the complementary heavy fraction.

Our objective was to examine effects of physical protection on N mineralization and immobilization rates. Effect of sonication, specifically the difference in release of dissolved organic N (DON) and subsequent mineralization to NH_4^+ between sonicated and unsonicated samples, was used as an operational

*Present address for correspondence: ManTech Environmental Technology Inc., c/o U.S. EPA, 200 SW 35th Street, Corvallis, OR 97333, U.S.A.
†Present address: UCAR, Box 3000, Boulder, CO 80307, U.S.A.

measure of the degree of physical protection. This definition gives a minimum estimate of the amount of physically protected substrate: it assumes that any N that is physically accessible will be mineralized whereas, in fact, organic N that is both chemically stabilized and physically protected will not be mineralized even if sonication renders it accessible.

Five soils were selected of contrasting texture and mineralogy, two variables thought to influence strongly the degree of physical protection. These two variables, texture and mineralogy, are also relatively static properties of a soil. If they could be linked to physical protection mechanisms, the extrapolation of models of soil and ecosystem processes to large areas would be facilitated, because static properties such as these are more readily sampled and mapped over large areas than dynamic variables such as SOM turnover (Burke *et al.*, 1990).

In the experiments described here, we first exposed whole soil to ^{15}N for 60 days and measured incorporation into a heavy fraction ($>1.7\ Mg\ m^{-3}$). We next removed and discarded the light fraction on the assumption that (1) the light fraction would have little tendency for physical protection, and that (2), because of its high C:N ratio, the light fraction would act as a sink for N released from the heavy fraction (Sollins *et al.*, 1984) thus obscuring any trends in degree of physical protection of the HF organic matter. The heavy fractions (HFs) were then slurry-incubated for 5 days and release of ^{14}N and ^{15}N as NH_4^+ and DON were measured.

In designing this experiment, we assumed that the following scenario would hold true. After the initial 60-day exposure of whole soils to ^{15}N, only a small amount of label would have been incorporated into the HFs. Most of the label would have been retained in the light fractions—plant debris and unattached microbial cells. A subsequent short (5 day) slurry incubation of the HFs would then release large amounts of ^{14}N as DON and NH_4^+. Sonication immediately before the slurry incubation would disrupt aggregates in these soils (Strickland *et al.*, 1988) and cause physically protected N to be released as NH_4^+ and DON, thus decreasing the degree of labelling of the NH_4^+ and DON. The difference in degree of labelling between the sonicated and unsonicated samples could then be used as an index of the amount of physically-protected N that was released.

MATERIALS AND METHODS

Single composite soil samples were collected from 0- to 15-cm depth of mineral soil at the sites indicated in Table 1 and stored field-moist (4°C) in our Oregon laboratory until needed. These soils, described in more detail by Strickland *et al.* (1988), span a range of texture, mineralogy and C content as well as size and stability of aggregates. The overall procedure consisted of four steps and is summarized in Fig. 1.

Step 1—labelling and isolation of heavy fraction

A subsample (200 g) of each of the five soils was placed in a 4-litre plastic bottle, and 20 ml of a $^{15}NH_4Cl$ solution (500 mg $^{15}N\ l^{-1}$, 99 atom%, ICON Services Inc., 19 Ox Bow Lane, Summit, N.J.) were added evenly over the soil surface ($50\ \mu g\ ^{15}N\ g^{-1}$ field-moist soil). The five containers were covered loosely with lids and kept at room temperature (25 ± 3°C) for 60 days. This exposure period was chosen to ensure that the label would cycle through the microflora, and was based on a study by Ladd *et al.* (1981) which showed that labelling of microbial cells peaked after 4–8 weeks. Twice weekly during the exposure period, samples were misted with deionized water (dH_2O) to replace moisture lost in evaporation, then stirred. After the exposure to ^{15}N, the light fraction was separated by flotation in a sodium iodide solution ($1.70\ Mg\ m^{-3}$) and discarded. The remaining HF was rinsed three times with 1.0 M NaCl to remove NaI and then three times with deionized H_2O (Strickland and Sollins, 1987).

Step 2—heavy fraction characterization

A 25-g subsample of each HF was dried at 90°C and used to determine total C (LECO combustion analyzer, Laboratory Equipment Corp., St Joseph, Mich.) and total N (as NH_4^+ after Kjeldahl digestion). A second 25 g subsample was placed in an evacuated chamber with 1.0 ml of chloroform for 5 days at room temperature to provide an estimate of the amount of microbial N present (Brookes *et al.*, 1985). After chloroform treatment, samples were shaken for 30 min (100 strokes min^{-1}) in 100 ml of 50 mM Na_2SO_4, then centrifuged. The supernatant solutions (termed "chloroform extracts" hereafter) were then gravity filtered (Whatman No. 1) and analysed for

Table 1. Soils examined

Site	Location	Soil type	Vegetation*
Waldo	Florida	Ultic haplaquod	Slash pine
La Selva	Costa Rica	Oxic dystropept†	Abandoned pasture
Konza	Kansas	Pachic argiustoll	Tallgrass prairie
Cascade Head	Oregon	Typic dystrandept	Douglas fir, red alder
H. J. Andrews	Oregon	Andic haplumbrept	Burned clearcut, formerly old-growth Douglas-fir and western hemlock

*Slash pine, *Pinus elliotii* Engelm; Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco; red alder, *Alnus rubra* Bong.; western hemlock, *Tsuga heterophylla* (Raf.) Sarg.
†Old alluvium, upper terrace soil of the Helechal consociation (Sollins *et al.*, 1992).

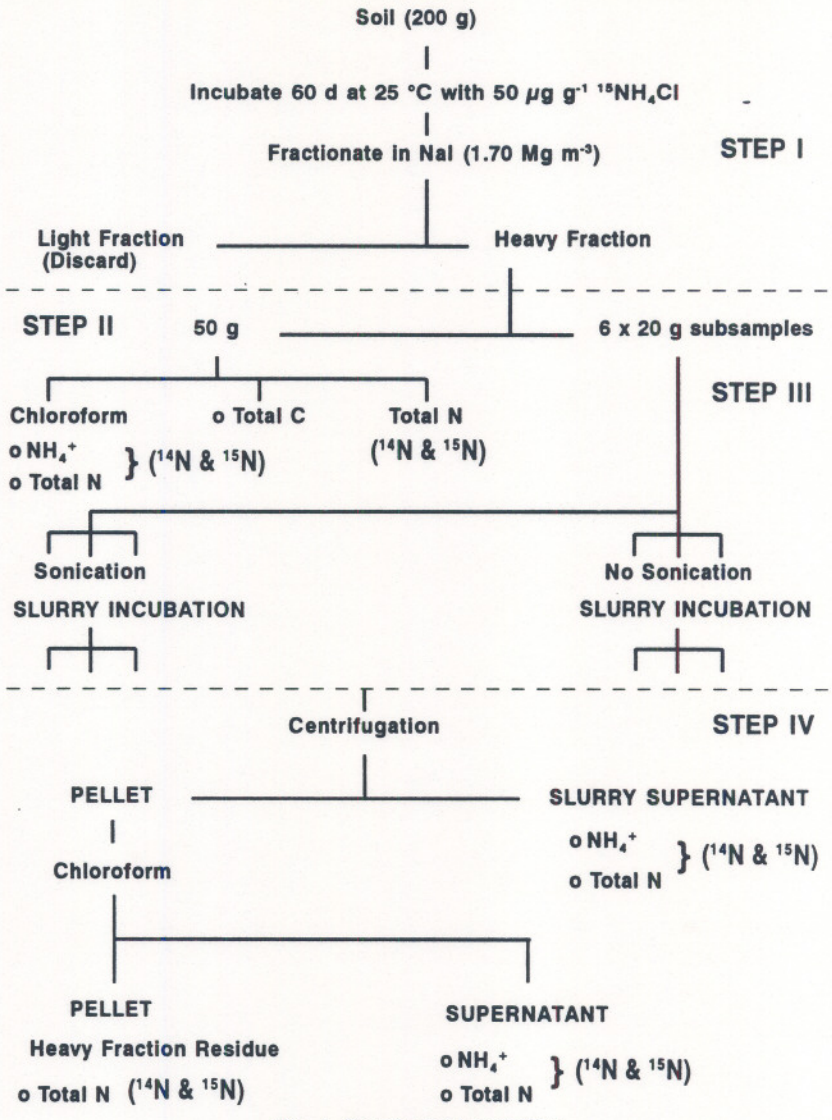

Fig. 1. Experimental procedure.

NO_3^- (Alpchem Rapid-flow Analyzer), and Kjeldahl digested and analysed for NH_4^+.

Step 3—aggregate dispersion and slurry incubation

Six 20-g subsamples of each HF were placed in 100-ml glass test tubes. 60 ml of a sterile, dilute, minimal-salts medium containing KH_2PO_4, K_2HPO_4, NaCl, NH_4Cl, $MgCl_2$, and Na_2SO_4 (150, 350, 50, 50, 15 and 50 mg l^{-1}, respectively) were added to each tube. Three subsamples of each HF were then sonicated for 5 min (Braun-Sonic 2000, Braun Scientific, Germany, set at 100 W and 20 KHz). This procedure had been shown to disperse from 70 to 97% of the aggregates in these soils (Strickland *et al.*, 1988). Tubes were kept submerged in an ice bath during sonication, which prevented any noticeable temperature rise. Because sonication can also disrupt microbial cell walls, the sonicated and unsonicated HF slurries were inoculated with 0.1 g of the corresponding whole soil, then kept at room temperature for 5 days. Air was bubbled constantly through the slurry to promote aeration.

Step 4—N fractions released during incubation

After the slurry incubation, the HF was separated from the minimal-salts solution by centrifugation (4068 *g* for 10 min). The supernatant was decanted, and the entrained solution was removed from the HF by vacuum filtration (Whatman No. 50, cellulose, 2.7-μm retention). The supernatant and entrained solutions were combined and analysed for NH_4^+, NO_3^-, and Kjeldahl N. The pelleted HF was divided and treated as described in Step 2 above (i.e. total C and N, and chloroform-labile N). In addition, the pellet remaining after the chloroform exposure and Na_2SO_4 extraction was oven dried, and analysed for total C and N (termed hereafter HF residue).

Table 2. Standards used to measure ^{15}N recovery during diffusion

Stock solution added (ml)*		^{15}N enrichment (%)	
$^{14}NH_4{}^{14}NO_3$	$^{15}NH_4Cl$	Calculated	Detected
1.0	0.50	4.76	4.12
2.0	1.00	4.76	4.22
3.0	1.50	4.76	4.17

*Concentration of stock solutions used: $^{14}NH_4{}^{14}NO_3$, 100.14 mg l^{-1} NH_4-N and NO_3-N; $^{15}NH_4Cl$, 10 mg l^{-1} NH_4-N.

^{15}N determination

Solutions were prepared for analysis by the method of O'Deen and Porter (1979), as modified by MacKown *et al.* (1987) for Kjeldahl digests. A 30 ml aliquot of each solution (leachate or Kjeldahl digest) was spiked with 100 μg of unlabelled NH_4-N (as NH_4^+Cl) to raise N content to a concentration adequate for ^{15}N analysis. The solutions were then brought to pH 13+ with 13.0 M NaOH, and the ammonia was trapped in an acid solution (0.1 M HCl). A second aliquot was treated with Devarda's alloy before diffusion in order to convert NO_3^- to NH_4^+. The acid solutions were oven dried (60°C) and analysed for ^{15}N by mass spectrometry (Isotope Services, Los Alamos, N.M.).

Three standards were included in each batch of 70 samples to determine the recovery of ^{15}N. The standards spanned a range of N concentrations at a constant ^{15}N enrichment [4.76% ^{15}N at 200, 250, and 300 μg N (14 + 15) as NH_4Cl]. Based on the measured recovery of these standards, all results were multiplied by 1.14 (Table 2). Results were also corrected by subtracting the amount of ^{15}N occurring naturally in the 100 μg N spike added to all samples before diffusion (0.38%, $n = 3$, SE = 0.01%).

Statistical analyses

All analyses used the PC SAS statistical package (SAS Institute, 1987). For analysis of incorporation of ^{15}N into the HFs (Step 1), laboratory replicates were used as data points. For all subsequent analyses (Steps 2–4), means of the laboratory replicates were used as the data points since trials had shown that variation among replicates was small relative to the overall experimental error. Data were log-transformed to ensure that residuals were normally distributed with constant variance. Fisher's protected LSD was used to compare treatment means for planned comparisons. The Tukey–Kramer test was used for unplanned comparisons and in cases where the F-value was not significant.

RESULTS AND DISCUSSION

Rapid labelling of the heavy fractions

Considerable label was incorporated into the HFs of all five soils during the initial 60-day exposure to ^{15}N (Table 3), suggesting rapid incorporation of the ^{15}N into organo–mineral forms. Other studies have shown that inorganic ^{15}N is incorporated rapidly into an unextractable fraction. McGill *et al.* (1975) reported substantial incorporation of ^{15}N into relatively unextractable forms within as little as 1 day. Kelley and Stevenson (1985) and He *et al.* (1988a) found that, after exposing soil to labelled $(NH_4)_2SO_4$ for as little as 1 week, most of the ^{15}N could not be recovered with mild chemical extractants. Furthermore, the recently added ^{15}N was only slightly more recoverable than the native ^{14}N (He *et al.*, 1988b).

Effects of slurry incubation

N was released from the HFs during the second (5-day slurry) incubation, but mainly as DON rather than NH_4^+ (Tables 4 and 5). No NO_3^- was detected at this or any other stage of the experiment. Neither the DON nor NH_4^+ are likely to have been present before the slurry incubation since we had leached the soil thoroughly with concentrated NaI and deionized water in the process of isolating the HFs. We have not located reports of other studies in which release of DON and NH_4^+ during incubation were compared.

For all soils, the NH_4^+ released was more highly labelled than the DON. It seems reasonable to assume that the NH_4^+ was produced by deamination of the DON. Given this assumption, the fact that the NH_4^+ was enriched relative to the DON suggests that at least two OM pools were present in the HFs, and that the mineralized NH_4^+ came from the more active of these two HF pools.

Table 3. Characteristics of heavy fractions (HF) isolated after 60 day incubation of whole soil with $^{15}NH_4Cl$

Site	^{15}N added* (mg kg^{-1} soil)	^{15}N retained† (mg kg^{-1} HF)	^{14}N g kg^{-1} soil	C g kg^{-1} soil	C:N
Waldo	58.9	0.7[a]	0.8	14.1	17.6
Konza	71.3	6.6[b]	2.9	29.5	10.2
Cascade Head	95.5	7.0[b]	5.3	85.3	16.1
H. J. Andrews	73.3	9.0[b]	2.5	32.0	12.8
La Selva	93.3	9.0[b]	5.5	46.9	8.5

^{15}N values followed by the same superscript letter are not significantly different ($P > 0.05$, Tukey's test on log-transformed data).

*^{15}N added to whole soil. Since ^{15}N was added to field-moist soils, the level of addition expressed on a dry weight basis varied considerably.

†^{15}N present in heavy fraction after light fraction removal. Values are geometric means of three laboratory replicates.

Table 4. Means and SEs of three laboratory replicates for soluble ammonium and organic nitrogen (DON) in supernatant after slurry incubation. ^{14}N and ^{15}N values are mg kg^{-1} heavy fraction. See Table 5 for statistical analysis

Soils and treatments	Ammonium			DON*		
	^{14}N	^{15}N	Atom % ^{15}N†	^{14}N	^{15}N	Atom % ^{15}N†
Waldo						
Unsonicated	1.03	0.03	2.93	10.25	0.06	0.59
SE	0.26	<0.01	0.59	0.22	0.02	0.18
Sonicated	0.52	0.03	7.38	29.49	0.29	0.99
SE	0.22	0.01	1.45	1.83	0.03	0.06
Konza						
Unsonicated	1.58	0.04	3.06	44.58	0.22	0.49
SE	0.43	<0.01	0.67	4.57	0.06	0.12
Sonicated	1.28	0.04	3.51	108.59	0.57	0.52
SE	0.32	0.01	0.45	7.38	0.09	0.07
Cascade Head						
Unsonicated	0.32	0.01	3.07	3.03	0.01	0.14
SE	0.00	<0.01	0.86	0.76	<0.01	0.10
Sonicated	0.28	0.01	4.84	12.16	0.05	0.38
SE	0.09	<0.01	0.96	0.75	0.01	0.02
H. J. Andrews						
Unsonicated	0.36	0.02	7.43	4.58	0.01	0.15
SE	0.06	<0.01	1.82	1.18	0.01	0.13
Sonicated	0.42	0.06	14.26	12.45	0.02	0.14
SE	0.00	<0.01	1.07	1.69	0.01	0.06
La Selva						
Unsonicated	0.70	0.01	1.72	1.83	0.01	0.35
SE	0.12	<0.01	0.15	0.44	0.01	0.32
Sonicated	0.61	0.01	2.75	5.02	0.03	0.51
SE	0.24	<0.01	0.64	0.49	0.01	0.05

All inorganic nitrogen was present as ammonium; nitrate was not detected.
*DON calculated as total Kjeldahl N minus ammonium.
†Atom % ^{15}N: $^{15}N/^{14}N \times 100$. Values are means of the quotients for three laboratory replicates.

Effects of sonication

Sonication increased the amount of both labelled and unlabelled DON released during the slurry incubation of the HFs (Table 5). The sonication may have exposed adsorbed organics to attack by microflora and extracellular enzymes, or it may have caused release of the DON into solution via abiotic processes. Given that the Waldo soil is the least aggregated and the La Selva soil the most aggregated of the five soils that we studied (Strickland *et al.*, 1988), we expected sonication to increase N release more for the La Selva than for the Waldo soil. In fact, the pattern was the opposite. The Waldo soil showed the second largest increase in release of DON plus NH_4^+ for both ^{14}N and ^{15}N (after Cascade Head), and the La Selva soil showed the smallest increase (based on data in Table 4).

Sonication did not significantly influence release of $^{14}NH_4^+$ and $^{15}NH_4^+$ from the HFs during the slurry incubation (Table 5). Other studies have shown large increases in release of NH_4^+ after physical disruption, but these used whole soil or particle size fractions rather than a heavy fraction, and used grinding rather than sonication to disrupt structure (e.g. Elliott, 1986). For the sonicated HFs, as for the unsonicated (see above), the NH_4^+ released during incubation was more highly labelled than the DON (Table 5) indicating that the NH_4^+ derived from a more active pool.

Contrary to expectations, we saw no indication that sonication caused dilution of the label, as would have occurred if a large pool of physically-protected native ^{14}N had been released into solution (see Introduction). On the contrary, sonication increased the degree of labelling of the NH_4^+ released during the slurry incubation for all five soils (Table 5) and probably also increased labelling of the DON ($P = 0.11$). This increase in labelling, as opposed to a dilution of the DON and NH_4^+, suggests that a portion of the newly formed HF N, although physically protected (i.e. released upon sonication), was

Table 5. Means for soluble ammonium (NH_4) and organic nitrogen (DON) present in the supernatant after slurry incubation (based on data in Table 4). ^{14}N and ^{15}N values are mg kg^{-1} heavy fraction

Sonication	^{14}N		^{15}N		Atom % ^{15}N	
	NH_4	DON	NH_4	DON	NH_4	DON
Unsonication	0.67^{1a}	6.50^{1b}	0.02^{1a}	0.02^{1a}	3.23^{1a}	0.30^{1b}
Sonicated	0.54^{1a}	18.94^{2b}	0.03^{1a}	0.08^{2a}	5.48^{2a}	0.43^{1b}

Numbers are geometric means of five soil HFs. Column and row means within ^{14}N, ^{15}N, and atom % ^{15}N were compared with separate ANOVAs (5 blocks × 2 treatments) on log-transformed data. Dissimilar superscript numbers and letters denote differences within columns and rows, respectively ($P < 0.05$).

Table 6. Means for Kjeldahl ^{14}N, ^{15}N, and atom % ^{15}N of the chloroformed samples. ^{14}N and ^{15}N values are mg kg^{-1} heavy fraction

		Chloroform labile	
Isotope	Sonication	T_0	T_5
^{14}N	Unsonicated	6.20[a]	21.70[b]
	Sonicated	—	26.50[b]
^{15}N	Unsonicated	0.041[a]	0.140[b]
	Sonicated	—	0.185[b]
Atom % ^{15}N	Unsonicated	0.64[a]	0.69[a]
	Sonicated	—	0.74[a]

Numbers are geometric means of five soil HFs. Means were compared by ANOVA on log-transformed data (5 blocks × 3 treatments) within ^{14}N, ^{15}N and atom % ^{15}N. Means with the same superscript letter are not significantly different ($P > 0.05$, Tukey's test).

quite chemically or biologically active (at least as active as the native N). We term this active but physically protected pool "active-protected".

Chloroform-labile fractions

Data on chloroform-labile fractions (Table 6) offer information on the nature of the "active-protected" N released during the slurry incubations. For example, Van Veen *et al.* (1987) have suggested that much of the physically protected soil N is contained in microbial cells. Table 6 shows, however, that the amount of chloroform-labile ^{14}N and ^{15}N present in the HFs before the slurry incubation (T_0) was small relative to the total amount of N present after the incubation (T_5), thus only a small portion of the active-protected N pool could have been microbial biomass N.

Heavy-fraction residues

The HF residues (pellets remaining after the chloroform treatment at time T_5) contained 5–10% less ^{14}N than did the HFs before the slurry incubation (Table 7). In contrast, the amount of ^{15}N in the residues decreased much less, and most of the label was still present at T_5, even in sonicated samples. Of the relatively large amount of label incorporated into the HFs during the initial 60-day exposure to ^{15}N (see Table 3), only a small portion was present in the HFs as a active-protected pool (compare results of slurry incubation in Table 6 with HF residue in Table 7). Instead, nearly all was in a non-active pool.

We recognize that the 5-day slurry incubaton may not have effected complete release of the label from the active pools. However, we suggest that if all of the recently-incorporated ^{15}N had been in an active-protected pool, the release of 5–10% of the total HF N should have reduced the atom% ^{15}N of the HF residue. In fact, however, the degree of labelling of the HF residues was no different from that of the HF before the slurry incubation (Table 7). This provides further evidence that the majority of the ^{15}N incorporated during the initial 60-day exposure to ^{15}N was in a non-active pool.

Implications about SOM pools

Physical protection does seem to be an early step in the stabilization of N within SOM. We did not expect much label to be incorporated into the HFs. Of what was incorporated, we expected the majority to enter the active unprotected pool, mainly as attached microbial biomass (see Introduction). In fact, substantial label was incorporated into the HFs, and most of it was stabilized into non-active OM.

Our experimental design, which physically separated heavy- and light-fraction material before the slurry incubation, may provide further insight into the nature of SOM pools. We found no evidence of a large native pool of HF active-protected N; in fact, results suggest that the pool of newly-formed organic N was of the same size, lability, and degree of physical protection as the native material. The small size of this active pool relative to the non-active pool implies that the main pool of active OM in soils is indeed the light fraction.

If we assume that the non-active HF N is either the "medium-turnover or stable" OM pools (Parton *et al.*, 1988), an interesting question then is: "How did material with a turnover time of decades to centuries get formed within 60 days?" One possibility is rapid N incorporation into existing SOM heteropolycyclics. In fact, uptake of ^{15}N within minutes into non-KCl extractable forms has been documented in soils that do not fix NH_4^+ within clay lattices (S. Hart, pers. commun., 1991). An additional possibility is rapid stabilization via a physical protection mechanism that is unaffected by sonication. This would seem to imply protection other than within soil microaggregates since we had found previously that 70–97% of the microaggregates in the soils that we studied were dispersed by sonication (Strickland *et al.*, 1988).

Conclusions

Based on results presented here, some recommendations for further research can be offered:

(1) Rapid incorporation of label into the HFs, and subsequent release from sonicated samples, indicates that N can be incorporated quickly into a

Table 7. Means for Kjeldahl ^{14}N, ^{15}N and atom % ^{15}N of the heavy fraction residue at time T_0 and T_5. ^{14}N and ^{15}N values are mg kg^{-1} heavy fraction

		Heavy fraction residue	
Isotope	Sonication	T_0	T_5
^{14}N	Unsonicated	2770[b]	2483[ab]
	Sonicated	—	2432[a]
^{15}N	Unsonicated	5.62[a]	5.16[a]
	Sonicated	—	5.06[a]
Atom % ^{15}N	Unsonicated	0.20[a]	0.20[a]
	Sonicated	—	0.21[a]

Numbers are geometric means of five soil HFs. Means were compared by ANOVA on log-transformed data (5 blocks × 3 treatments) within ^{14}N, ^{15}N and atom % ^{15}N. Means with the same superscript letter are not significantly different ($P > 0.10$, Tukey's test).

physically-protected pool. The mechanisms responsible for this rapid uptake deserve further study.

(2) Sonication increased release of DON more than NH_4^+. Release of DON during incubations has typically been ignored, and the mechanisms of DON release are largely unknown.

(3) Measures of aggregation did not correlate well with effects of sonication on N dynamics among the five soils that we studied. In fact, sonication caused the largest changes in the soil that we believed to be least aggregated. Thus, although physical protection may well be an important mechanism of N stabilization, a better understanding of mechanisms of N incorporation into and release from SOM may be needed before experiments on effects of physical protection can be conducted successfully. Moreover, the large differences among soils suggest caution in drawing conclusions about patterns of SOM dynamics and N transformations from studies of a single soil type.

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