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NET NITROGEN MINERALIZATION FROM LIGHT- AND HEAVY-FRACTION FOREST SOIL ORGANIC MATTER

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Summary—Fine surface soil (< 2 mm) from four sites in Oregon and Washington and three in Costa Rica was separated by repeated flotation in NaI solution (sp. gr. < 1.2, 1.4, or 1.6 g cm^{-3}) into a light and a heavy fraction. Most organic matter in the light fractions consisted of partly-decomposed root fragments and other plant and microbial remnants and most in the heavy fractions was adsorbed or deposited on mineral surfaces or was protected within organo-mineral microaggregates. The light fraction had a consistently wider C:N ratio than the heavy, and net N mineralization during anaerobic incubation was greater from the heavy than from the light fraction in five of six soils for which both fractions were incubated. Net N mineralization was greater from the heavy fraction than from the whole soil of most sites perhaps because the light fraction immobilized N released from the heavy fraction when they were incubated together. Correlation between net N mineralization (as a proportion of total N) and C:N ratio was negative for the light fraction ($r^2 = 0.74$) but positive for the heavy fraction ($r^2 = 0.85$), suggesting that the C:N ratio does not control the extent to which heavy-fraction N is mineralizable.

INTRODUCTION

Organic matter in the soil can be divided into two broad categories: (1) mineral-free, partly-decomposed plant debris, and (2) organic matter adsorbed on mineral surfaces (or deposited on them by microflora) and sequestered within organo-mineral microaggregates. The mineral-free debris is lighter and can be separated from the whole soil by flotation, though as discussed here and elsewhere (Spycher *et al.*, 1983; Sollins *et al.*, 1983), the density of the separation medium is critical.

Although organic matter in the light fraction decomposes quickly (Greenland and Ford, 1964; Paul and Van Veen, 1978), it has a wide C:N ratio, and N mineralized during its decomposition tends to be reimmobilized by the microflora. Organic matter in the residual heavy fraction has a narrower C:N ratio (Satoh and Yamane, 1972; Young and Spycher, 1979; Spycher *et al.*, 1983), but decomposes more slowly than the mineral-free debris.

To help resolve the roles of the two fractions in below ground N dynamics, we compared net mineralizable N and total C and N in whole soil and heavy and light density fractions from four forest sites in the United States and three in Costa Rica. The sites spanned a wide range of climate and vegetation type but were restricted to volcaniclastic parent materials (Table 1). Paired plots at four of the seven sites, identical until the vegetation was modified, allowed us to examine effects of vegetation on soil composition and N availability. For example, red alder (*Alnus rubra* Bong.), a nitrogen fixer, had been planted on or removed from two of the Pacific Northwest (PNW) sites.

METHODS

Laboratory analyses

Aliquots of moist soil from all sites in Oregon and Washington except Waldo Lake were sieved (<2 mm) and assayed for NH_4^+ extractable with 1 N KCl. NH_4^+ in solution was measured with an HNU Systems ion selective electrode calibrated several times a day against four ammonium sulfate standards. One set of 14 extracts was also analyzed with a Scientific Instruments autoanalyzer (Technicon method 334-74A/A). Values obtained with the two methods correlated well ($r = 0.97$).

The remaining moist soil was air-dried and sieved (<2 mm), then roots > 1 mm dia were removed by hand. The plant debris was extracted by repeated flotation in NaI solution adjusted to sp. gr. 1.6 g cm^{-3}. The resulting light fraction contained some mineral material, mainly pumice, but there was little organic matter adsorbed on it (Spycher *et al.*, 1983). Surfactants were not used as they may adsorb onto soil particles and inflate values for C content (Turchenek and Oades, 1974). After being ground (<0.25 mm), whole soil and density fractions were analyzed for total C and N with a Leco WR 12 Automatic Carbon Analyzer and the semimicro Kjeldahl method.

Unground material (<2 mm) was used for incubations—10 g aliquots of whole soil and heavy fraction, 0.5 g aliquots of light-fraction material, which was more time consuming to extract. NaI was removed from the fractions before incubation by washing three times in distilled water or by percolating 200 ml of N-free Hoagland's solution through the samples. Material was incubated in 40 ml deionized water in closed glass bottles at 40°C for 7 days (Waring and Bremner, 1964), then mixed with 40 ml 1 N KCl, and allowed to equilibrate for 30 min. The supernatant was analyzed for NH_4^+. Incubations were performed in duplicate (or triplicate whenever sufficient material was available). Values denoted "net" are differences between the amount of NH_4^+-N extracted with KCl after incubation and the amount extracted from an unincubated aliquot of the same sample. It must be emphasized that the values are

Table 1. Location, vegetation, climate,

Site	Latitude (North)	Elevation (m)	Mean annual Precipitation (cm)	Mean annual Temperature (°C)	Parent material	Soil type
Wind River Experimental Forest, Carson, WA	45°49′	600	250	9	Andesite, basalt, breccia	Andic Haplumbrept
Cascade Head Experimental Forest, Otis, OR	45°3′	180	240	10	Interbedded basalt and tuffaceous siltstone	Typic Dystrandept
H. J. Andrews Experimental Forest (McRae Creek), Blue River, OR	44°16′	850	215	8	Andesite, breccia and volcanic ash	Andic Haplumbrept
Waldo Lake, OR	43°47′	1770	160	4	Volcanic pumice and Mt Mazama ash (~6600 yr old)	Entic Cryorthod
Monte Verde Costa Rica (near Pension Quetzal)	10°18′	1400	240	19	Rhyolite and volcanic ash (?)	Typic Dystrandept
La Selva, Costa Rica	10°25′	45	430	26	Alluvium derived from volcanic material	Typic Hydrandept
Turrialba Costa Rica	9°53′	650	270	24	Volcanic ash with some andesite fragments	Hydric Dystrandept

[a] At these sites only, each sample was a composite of 3–5 5-cm diameter cores.

also "net" in the sense that they represent the difference between gross mineralization and reimmobilization by the anaerobic microflora.

Soil samples from Waldo Lake were collected by R. D. Boone during a study of effects of tree death on levels of soil organic matter (R. D. Boone, unpublished M.S. thesis, Oregon State University, 1982). Boone found that light fractions of Waldo Lake soil separated at 1.4 or 1.6 g cm^{-3} contained much pumice; separation at 1.2 g cm^{-3} (NaCl solution) greatly reduced the amount of mineral material without markedly reducing the amount of organic matter.

To facilitate transport, we air-dried soils from Costa Rica after intact root systems had been hand picked from the samples. The Monte Verde soils were then sieved (<2 mm). The other Costa Rican soils (Hydrandepts and Hydric Dystrandepts) were ground with a mortar and pestle, and aggregates not readily fragmented (>2 mm) were discarded. A lighter NaI solution (1.4 g cm^{-3}) was used to fractionate Costa Rican soils, as it reduced filter clogging during removal of the light fraction from the supernatant.

Light fractions of the lowland Costa Rican soils and the Waldo Lake soil accounted for <1.5% of the total soil C, therefore we chose not to incubate them. Heavy-fraction material sufficient for incubation was readily isolated from all soils.

Effects of NaI treatment on N mineralization

To check whether NaI treatment killed the microflora responsible for net N mineralization under anaerobic conditions, we inoculated heavy- and light-fraction samples from Wind River and Cascade Head with 2 ml dilute suspension (~0.5 g l^{-1}) of the corresponding whole soil and inoculated Monte Verde light-fraction material and Waldo Lake heavy-fraction material with 0.5 ml dilute suspension of a Corvallis soil.

Inoculation had no consistent effect on net NH_4^+ mineralization from most soils. Adding the suspension to blanks did not increase their N content. Apparently, anaerobic ammonifiers survived the NaI treatment or rapidly recolonized after fractionation. Only the Waldo Lake heavy fractions, for reasons unknown, responded to inoculation. They were reinoculated by adding 0.5 ml dilute suspension prepared from local soil.

To determine whether Na and I remaining on exchange sites after density fractionation inhibited microbial activity, we incubated light-fraction material from the two Wind River stands after washing it with distilled water or N-free Hoagland's solution. Net NH_4^+ mineralization from conifer-stand light fractions was below detection limit regardless of treatment; NH_4^+ mineralized from the mixed alder–conifer stand samples averaged 55 μg g^{-1} (SE = 6) for those washed with distilled water and 73 μg g^{-1} (SE = 12) for those washed with N-free Hoagland's solution. We concluded that NaI on exchange sites did not greatly affect net NH_4^+ mineralization under anaerobic conditions.

Using soil from the Wind River and H. J. Andrews sites, we then measured the N mobilized from whole soil by the NaI wash and the change caused by the wash in the amount of NH_4^+ mineralized during subsequent anaerobic incubation. Rinsing with NaI and soils of the study sites

Vegetation type	Stand age (yr)	Sampling regime: Number of locations	Sampling regime: Depth (cm)	Sampling regime: Date	Reference
1. Douglas-fir	52	4	0–12	April 1981	Tarrant and Miller (1963)
2. Red alder/Douglas-fir	48/52	4	0–12		
1. Conifer	55	5	0–10	June 1981	Franklin *et al.* (1968)
2. Red alder	55	5	0–10		
Conifer	350–550	14	0–15	March 1981	—
Mountain hemlock	170–280	12	0–15	July 1981	R. D. Boone, (unpublished M.S. thesis, Oregan State University, 1982)
Tropical premontane wet forest	?	3	0–15	February 1982	—
1. Undisturbed tropical wet forest	Mixed	4	0–15	February 1982	Holdridge *et al.* (1971) Johnson *et al.* (1977, 1979)
2. Successional	2	3	0–15	February 1982	
1. Secondary tropical premontane wet forest	10–70	3[a]	0–15	February 1982	Ewel *et al.* (1981)
2. Devegetated since March 1979		3[a]			

solubilized 1.2% (SE = 0.2%) of the total N originally present in the whole soil (90 μg N g^{-1} dry mass) and decreased NH_4^+ mineralized during incubation by an average 70% (SE = 3%). Agitation in NaI solution (a treatment that should simulate the density fractionation procedure) solubilized an additional 1.0% of the total N (difference significant at $P < 0.05$) and decreased net NH_4^+ mineralization by an additional 53% (SE = 3%). We concluded that the N solubilized by NaI treatment is largely the same as that mineralized during incubation, therefore that less NH_4^+ was mineralized from the NaI-treated samples than from the unwashed samples.

RESULTS

C:N ratios of soil fractions and whole soil

The C:N ratio of the heavy fraction was narrower than that of the light fraction at all sites (Table 2). Ratios were narrowest at sites where N-fixing trees were or had been present. When the light-fraction C:N ratio was plotted against light-fraction C content (expressed as a percentage of whole soil), three clusters could be distinguished (Fig. 1): the lowland Costa Rican soils (little light-fraction C, narrow C:N ratio), the soils of the cool and highly productive Monte Verde and PNW alder sites (much light-fraction C, very narrow C:N ratio), and the conifer-dominated PNW soils (wide C:N ratio). Figure 1 also shows that dramatic changes that occurred in just a few years when vegetation was altered or removed.

Net NH_4^+ mineralization from soil density fractions

More N was mineralized as NH_4^+ from the heavy than from the light fraction of most soils (Table 3), especially where the light-fraction C:N ratio was >30. Only the light fractions from the Monte Verde site and the Cascade Head alder site, with C:N ratios <25, released a large amount of N into solution during incubation (Table 3).

Net NH_4^+ mineralization from the heavy fraction of soils from all 11 plots nearly equaled or exceeded mineralization from the corresponding whole soil, despite the fact that agitating whole soil in NaI solution reduced mineralization nearly 85%.

Expressed per gram dry mass, net N mineralization from both fractions and from whole soil correlated

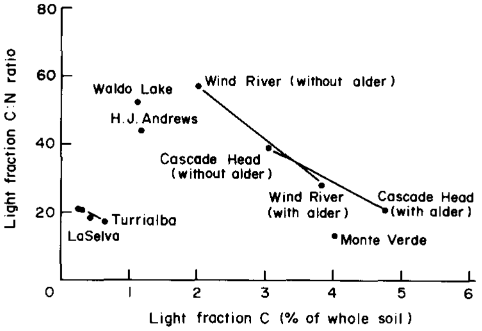

Fig. 1. Relation between the amount of light-fraction carbon and the C:N ratio of the light fraction. The two lines to the right extend from conifer dominated stands to adjacent stands, otherwise similar, in which conifers were replaced by red alder, a nitrogen fixer, 50 yr before sampling. The line to the left extends from the undisturbed second-growth stand at Turrialba, Costa Rica, to an adjacent site devegetated for 2 yr before sampling.

Table 2. Properties of fine whole surface soil (<2 mm) and soil fractions at forested sites in the Pacific Northwest and Costa Rica. SE in parentheses

Location, stand type	Light fraction (% of whole soil)	Carbon (% per unit dry mass)			Nitrogen (% per unit dry mass)			C:N ratio		
		Whole soil	Heavy fraction	Light fraction	Whole soil	Heavy fraction	Light fraction	Whole soil	Heavy fraction	Light fraction
Wind River										
Conifer	6.4 (1.2)	5.6 (0.3)	2.7 (0.2)	31.3 (1.4)	0.150 (0.009)	0.128 (0.010)	0.57 (0.02)	37.2 (1.3)	22.1 (3.2)	55.4 (3.1)
Alder/Conifer	13.6 (1.0)	9.7 (1.1)	4.5 (0.4)	30.3 (2.7)	0.380 (0.023)	0.278 (0.012)	1.11 (0.08)	25.6 (2.4	16.0 (0.9)	28.2 (4.4)
Cascade Head										
Conifer	9.3 (2.3)	12.5 (1.4)	10.4 (0.9)	32.7 (1.6)	0.589 (0.069)	0.446 (0.060)	0.87 (0.08)	21.3 (0.5)	23.6 (1.4)	38.6 (3.0)
Alder	14.7 (1.2)	15.2 (0.8)	10.0 (1.2)	32.4 (2.1)	0.868 (0.064)	0.630 (0.028)	1.53 (0.08)	17.7 (0.6)	17.5 (0.8)	21.4 (2.0)
H. J. Andrews	7.3 (1.9)	4.3 (0.4)	2.8 (0.1)	16.4 (2.0)	0.163 (0.010)	0.120 (0.005)	0.58 (0.13)	26.0 (1.4)	23.2 (0.7)	44.0 (7.8)
Waldo Lake[a]	11.1 (1.2)	2.4 (0.2)	1.2 (0.1)	10.2 (0.9)	0.083 (0.004)	0.052 (0.003)	0.20 (0.01)	27.5 (1.8)	23.5 (1.8)	52.2 (3.1)
Monte Verde	12.0 (0.5)	15.9 (0.7)	9.6 (1.8)	33.6 (1.8)	1.360 (0.129)	0.845 (0.196)	2.68 (0.22)	11.9 (0.8)	11.8 (0.9)	12.6 (0.4)
La Selva										
Control	0.79 (0.21)	4.6 (0.4)	4.4 (0.4)	31.3 (1.2)	0.394 (0.031)	0.381 (0.025)	1.53 (0.10)	11.8 (0.3)	11.5 (0.5)	20.7 (1.1)
Cutover	1.89 (0.39)	5.1 (0.6)	5.1 (0.5)	23.3 (2.6)	0.480 (0.051)	0.487 (0.041)	1.26 (0.04)	10.6 (0.3)	10.5 (0.2)	18.4 (1.5)
Turrialba										
Control	2.12 (0.67)	7.3 (0.5)	6.0 (0.7)	29.5 (2.3)	0.603 (0.052)	0.517 (0.037)	1.71 (0.09)	12.2 (0.3)	11.5 (0.5)	17.2 (0.5)
Devegetated	1.14 (0.25)	6.9 (0.3)	7.8 (1.0)	27.7 (2.2)	0.500 (0.021)	0.627 (0.127)	1.33 (0.07)	13.9 (0.1)	12.9 (0.9)	20.9 (0.8)

[a] Data provided by R. Boone, Oregon State University.

Table 3. Extractable NH_4^+ in whole soil and soil fractions before and after anaerobic incubation. SE in parentheses

Location	Stand type	Before incubation	After incubation ($\mu g\,N\,g^{-1}$ dry mass)			After incubation (% of total N)		
		Whole soil ($\mu g\,N\,g^{-1}$ dry mass)	Whole soil[a]	Heavy fraction	Light fraction	Whole soil[a]	Heavy fraction	Light fraction
Wind River	Conifer	6 (2)	18 (2)	30 (9)	< 16 (0)[b]	1.22 (0.15)	2.26 (0.63)	< 0.30 (0.00)[b]
	Alder/conifer	26 (4)	77 (18)	56 (5)	55 (6)	1.97 (0.38)	2.04 (0.26)	0.50 (0.04)
Cascade Head	Conifer	9 (1)	104 (7)	130 (24)	72 (12)	1.71 (0.16)	2.97 (0.49)	0.83 (0.13)
	Alder	2 (1)	110 (6)	163 (24)	155 (18)	1.30 (0.11)	2.63 (0.44)	1.03 (0.15)
H. J. Andrews	Conifer	8 (1)	26 (4)	40 (7)	13 (2)	1.54 (0.20)	3.52 (0.65)	0.34 (0.09)
Waldo Lake	Conifer	4 (1)	−3 (1)	11 (2)	—	−0.45 (0.15)	2.89 (0.46)	—
Monte Verde	Montane forest	2 (< 1)	93 (2)	79 (3)	545 (44)	0.69 (0.06)	1.10 (0.37)	2.06 (0.21)
La Selva	Undisturbed forest	11 (< 1)	45 (5)	37 (3)	—	1.18 (0.16)	0.96 (0.03)	—
	Successional vegetation	19 (5)	58 (12)	68 (3)	—	1.18 (0.12)	1.40 (0.07)	—
Turrialba	Second-growth forest	19 (1)	66 (10)	54 (3)	—	1.08 (0.08)	1.05 (0.02)	—
	Devegetated plot	5 (< 1)	19 (< 1)	59 (14)	—	0.38 (0.02)	0.93 (0.04)	—

[a] Net values (NH_4^+ extractable after incubation less amount extractable from aliquot of the same material before incubation).

[b] NH_4^+ levels were below detection limit for all samples from the Wind River conifer stand.

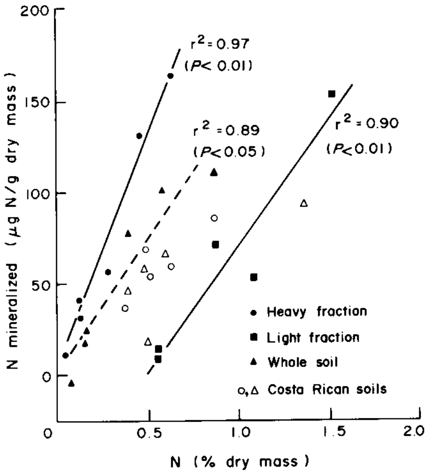

Fig. 2. Net N mineralized and % N in whole soil (<2 mm) and heavy and light soil fractions. The point for the Monte Verde light fraction is omitted and Costa Rican soils are not included in the regressions.

well with the proportions of N in the material, if Costa Rican soils were excluded (Fig. 2). Though N content in heavy fractions and whole soils varied widely across the PNW sites (0.08–0.87% and 0.05–0.63%, respectively), the proportion of total N mineralized varied little (2.0–3.5% and 1.2–2.0%, respectively); therefore the amount of N mineralized increased steadily with N content. When Costa Rican soils were added, the range in the proportion of N mineralized increased more than the range in total N content, and the correlation between N mineralized and N content became much weaker.

N mineralization from the light fraction, expressed as a proportion of total N in the fraction, correlated well with the N content of that fraction ($r^2 = 0.69$, $P < 0.05$), whereas N mineralization from the heavy fraction and whole soil showed no such correlation. When the proportion of total N mineralized was plotted against the C:N ratio, a clear pattern emerged that held for both fractions of the tropical and temperate soils (Fig. 3). The percentage of N mineralized from the light fraction decreased with a widening C:N ratio; the percentage mineralized from the heavy fraction increased. The proportion mineralized from whole soil showed a trend intermediate to these, peaking at a C:N ratio of about 25 and showing no correlation with C:N ratio ($r^2 = 0.01$).

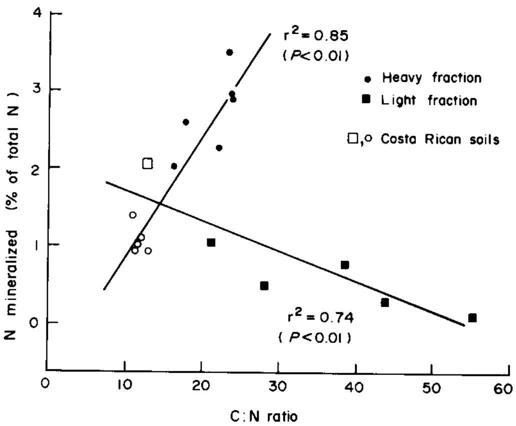

Fig. 3. Proportion of total N mineralized and the C:N ratio for heavy and light soil fractions. Costa Rican soils are included in the regressions.

DISCUSSION

Relation of N mineralization to C:N ratio

The contrasting relation between net N mineralization and the C:N ratio for the two density fractions (Fig. 3) was unexpected and apparently not yet reported. In a particularly comprehensive study of volcanic soils in Japan, Ohta and Kumada (1978) found, as we did, that net N mineralization from whole soil and the litter layer correlated poorly with the C:N ratio unless N mineralization was expressed as a proportion of total N (N_m:N_t ratio). N_m:N_t ratios from both studies peaked when the C:N ratio was 25–30. (The data for litter material may be comparable to ours for light-fraction material because both materials consisted of organic matter not associated with mineral particles.) Our results extend those of Ohta and Kumada by showing that net N mineralization from whole soil depends on the relative amounts of heavy- and light-fraction organic matter and on the C:N ratio of each. The pattern for whole soil is complex, in part because the light and heavy fractions show contrasting trends.

N_m:N_t ratios for most organic substrates increase with a narrowing C:N ratio because N is then present in excess of that required by the microflora. Light-fraction material (this study; Bernhard-Reversat, 1981), forest-floor material (Ohta and Kumada, 1978), and compost (Suzuki and Kumada, 1976) show this pattern.

N_m:N_t ratios for the heavy fractions increased with a widening C:N ratio. A first hypothesis regarding this is that heavy fractions of wide C:N ratio are richer than those of narrow C:N ratio in compounds that are readily decomposed (Parnas, 1975). As decomposition proceeds, compounds of wide C:N ratio, such as polysaccharides and fungal cell walls, are replaced by humic compounds richer in N but more difficult to decompose (Hayes and Swift, 1978). But this hypothesis does not explain the contrasting pattern between mineralization and C:N ratio for the two fractions.

A second hypothesis is that the degree of physical protection of heavy-fraction organic matter correlates with C:N ratio. After soil is dispersed ultrasonically, the C:N ratio of the resulting particles correlates inversely with their density (Satoh and Yamane, 1972; Spycher and Young, 1977; Turchenek and Oades, 1979; Young and Spycher, 1979). The lightest particles ($<1.6\ \mathrm{g\,cm^{-3}}$), mainly dead root fragments, have the widest C:N ratios. The particles of intermediate density (~ 1.6–$1.9\ \mathrm{g\,cm^{-3}}$) and intermediate C:N ratio may be situated before dispersion at locations accessible to microorgamisms, at or near the exterior of microaggregates or free in the soil (Young and Spycher, 1979). Microbially-produced carbohydrate should accumulate preferentially on such accessible particles, which would result in relatively wide C:N ratios, although nitrogenous compounds such as protein and amino acids would also accumulate (Burchill *et al.*, 1981; Tisdall and Oades, 1982). Accessible particles would have relatively large amounts of organic matter per unit mass of mineral material and thus intermediate densities.

The densest particles ($> 1.9\ \mathrm{g\,cm^{-3}}$) may be located before dispersion within microaggregates and therefore be inaccessible to the microflora. Such particles would not receive carbohydrate deposits directly, so their C:N ratio should be narrower than that of exterior particles. To accumulate on these inaccessible particles, organics would have to diffuse into aggregate interiors, presumably a slow process. The ratio of organic to mineral material would be low for interior particles and the density high. Because accessibility varies continuously with distance from the microaggregate exterior, density and C:N ratio of component particles should also vary continuously (Young and Spycher, 1979).

If this second hypothesis is correct, then heavy fractions from soils with extensive microaggregate structure should have narrower C:N ratios and less accessible organic matter than those with little microaggregate structure. Inaccessibility of the organic matter in some heavy fractions, rather than its chemical composition, would then explain the small proportion of N mineralized from the organic matter despite its narrow C:N ratio.

That more fertile soils (greater C and N content) mineralize a smaller proportion of total N is apparent in several sets of data and is to be expected because such soils also tend to be more highly aggregated. For example, an increase in organic-matter content and narrowing of the C:N ratio downward along a catena of Argiustolls was accompanied by a decrease in the N_m:N_t ratio (D. Schimel, unpublished Ph.D. thesis, Colorado State University, 1982). Campbell and Souster (1982) found that the N_m:N_t ratio tended to decrease with increasing C content in both virgin and cultivated Borolls and Boralfs. Similar trends can be seen in results of Jones and Parsons (1970) and Hiura *et al.* (1976). A relation between C:N and N_m:N_t ratios may not have been readily evident in these studies of whole soil (or in our study) because the light fractions, with their variable masses and C:N ratios, provided N sinks of widely varying strengths. Diverse mineralogies may also obscure patterns.

Heavy vs light fraction as a source of available N

Net N mineralization was greater from the heavy than from the light fraction (Fig. 2), but the pattern of gross mineralization may be different because microflora decomposing the wide C:N-ratio light fractions should reimmobilize N as it is mineralized. Net N mineralization was greater from the heavy fraction than from the whole soil (Table 3), probably because N released from the heavy fraction during incubation of whole soil is immobilized by microorganisms decomposing the light fraction. Trial calculations show, for example, that $200\text{–}500\ \mu\mathrm{g\,N\,g^{-1}}$ dry mass would be required to narrow the C:N ratio of the light fraction of each conifer soil to 25, an amount many times the difference between N mineralization from the heavy fraction and whole soil. It is unlikely that the large amount of net N mineralization from the heavy fraction was an artifact of the NaI treatment, since agitating the bulk soils in NaI solution markedly decreased N mineralization.

N mineralization from soil fractions has been measured infrequently. Bernhard-Reversat (1981) found that, during aerobic incubation, mineralization from the silt + clay size fraction of several Sahelian savannah soils was much greater than from a water-flotable fraction ($< 1.0\ \mathrm{g\,cm^{-3}}$).

Several studies have shown that net N mineralization increases with decreasing particle size, probably because the smaller particles have a narrower C:N ratio and higher surface-to-volume ratio than the coarser particles (Chichester, 1969; Ladd *et al.*, 1977; Cameron and Posner, 1979). This is consistent with our finding that net N mineralization is greatest from the heavy fractions ($> 1.6\ \mathrm{g\,cm^{-3}}$) because they contain most of the smallest (clay-size) particles after density fractionation. Median density of the clay-size fraction of soils dispersed in water without ultrasound ranged from $> 1.8\ \mathrm{g\,cm^{-3}}$ in two Andepts to $> 2.1\ \mathrm{g\,cm^{-3}}$ in an Argixeroll and a Haplohumult (Young and Spycher, 1979).

Tracer experiments by Paul and Juma (1981) suggested that "stabilized N" was the source of 40% of the inorganic N pool in a Boroll but provided little information about the mechanism of stabilization. Thus the relation between their results and ours is not clear.

Choice of fractionation density

In most previous applications of density fractionation, soils have been separated in water (e.g. Cameron and Posner, 1979; Bernhard-Reversat, 1981) or in an inorganic or organic medium of density $> 1.8\ \mathrm{g\,cm^{-3}}$ (Greenland and Ford, 1964; Turchenek and Oades, 1974, 1979; Paul and Van Veen, 1978; Young and Spycher, 1979). Separation at $1.3\text{–}1.6\ \mathrm{g\,cm^{-3}}$ rather than at $> 1.8\ \mathrm{g\,cm^{-3}}$ may be preferable because in many soils much organomineral material has a density of $1.7\text{–}2.0\ \mathrm{g\,cm^{-3}}$ (Satoh, 1976; Spycher *et al.*, 1983). Flotation in water can be inappropriate because much mineral-free organic matter has a density $> 1.0\ \mathrm{g\,cm^{-3}}$. Cameron and Posner (1979) separated mineral-free organic matter by "panning" the whole soil under water, a successful procedure because the soil consisted almost entirely of kaolinite, quartz, mineral-free organic matter, and aggregates that were easily dispersed by agitation in water. Because water is less active chemically than concentrated Na halide solution, flotation in water may be advantageous when isolating mineral-free organic matter for subsequent microbiological experiments.

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