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# MINERALIZATION AND IMMOBILIZATION OF SOIL NITROGEN BY MICROORGANISMS

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## Abstract

The effects of environmental factors and differences in plant constituents on the microbial mineralization and immobilization of nitrogen were reasonably well known for individual soils by 1935. During the further study of these processes, it was found that ammonium is preferentially utilized by microorganisms, and that tracers and mathematical equations could be used to measure and describe the net mineralization-immobilization rates. The concept of an active-N and a passive-N phase was also documented, and extensive work was conducted to determine the size of biomass-N and active-N fractions, the fate of fertilizer N, and the identification of labelled and unlabelled N compounds in soil.

We measured the size and turnover of microbial biomass and other N fractions, using tracer and computer simulation techniques to gain a better understanding of the effect of microbial growth on N mineralization-immobilization interactions. Mathematical description of the processes predicted that, during a 12-week laboratory incubation of a previously labelled chernozemic soil, the initial biomass-$^{15}N$ which accounted for approximately 30% of the $^{15}N$ left in the soil (72 ng $g^{-1}$) immobilized 56 ng $g^{-1}$. A total of 75 ng $g^{-1}$ was transferred out of the biomass resulting in a final size of 53 ng $g^{-1}$. The initial total microbial N of 167µg $g^{-1}$ soil accounted for 4% of the total soil N. It immobilized 163 µg N $g^{-1}$ soil, and 187 µg $g^{-1}$ were transferred out of the biomass during the 12-week incubation, resulting in a final biomass of 143 µg N $g^{-1}$ soil.

The experimental values of labelled and total biomass and other fractions were similar to the simulated values. The model showed that the relative contribution of organic-N fractions to the mineral-N pool were: biomass (24%), metabolite-N (4%), active-N (32%) and stabilized-N (40%). These data show that the concept of a single active fraction cannot be justified and that the stabilized organic-N with a half-life of 27 years in the field contributes a major portion of the N mineralized.

## Introduction

The general relationships between microbial growth and the interchange of mineral and organic N were established by, for example Waksman & Tenney (1927) and Jensen (1929). The C/N ratio required for degradation without net immobilization was known to be approximately 25:1 and the effects of environmental factors and the differential rates of plant constituent decomposition had been studied in some detail (Waksman & Gerretsen, 1931: Pieters & McKee, 1938). In addition, the possibility of soil population turnover with subsequent release of N on decomposition had been postulated (Richards & Norman, 1931). In summarizing the early work, Harmsen & van Schreven (1955) wrote:

"... the study of the general course of the mineralization of organic nitrogen in soil was practically completed before 1935. It is surprising that many of the modern publications still consider it worthwhile to mention parenthetically observations dealing with these entirely solved problems." These authors, however, went on to point out that the relationships between C and N and the effects of environmental factors had to be determined on each soil type, indicating that the underlying principles were not understood.

Normal methods for studying residue and soil C and N transformations could not differentiate the source of C and N nor determine the effects of microbial growth. Therefore, Norman (1946) wrote that "the availability of the stable nitrogen isotope $^{15}N$ and the carbon isotope $^{13}C$ will make it possible to verify quantitatively the various nitrogen transformations in relation to the carbon cycle and should aid greatly in establishing the forms of nitrogen present in soil."

The review by Winsor (1958) noted the following points: the preferential utilization of ammonium by microorganisms (Winsor & Pollard, 1956); the usefulness of tracers to measure net mineralization-immobilization rates (Broadbent & Norman, 1946); and the feasibility of using mathematical equations to describe mineralization-immobilization processes (Kirkham & Bartholomew, 1955).

Jansson (1958) brought together many of the concepts in what is now a classical study of mineralization-immobilization of soil N. The further proof that ammonium rather than nitrate is central to microbial growth and decomposition reactions, and confirmation of an earlier suggestion (Gainey, 1936) that the soil is composed of an active and a passive fraction, were combined with a mathematical description of the reactions involving the soil biomass and the active fraction of soil organic matter (Fig. 1).

The literature also documents the extensive work done on determining the size of the active organic phase and the fate of immobilized tracer N. The ratio of $^{14}N$ to $^{15}N$ mineralized during incubation (availability ratio) was shown to decline progressively with time (Broadbent & Nakashima, 1965, 1967; Legg *et al.*, 1971), generally approaching the value 2. Attempts to characterize the active organic phase by autoclaving, acid hydrolysis and other techniques were unsuccessful since the extractability ratio was either higher or lower than the availability ratio (Chichester, 1969; Stanford, 1968, 1969; Stanford & DeMar, 1968; Smith *et al.*, 1978; Van Praag & Brigode, 1973).

Further work indicated that the immobilized N was primarily amino acid (Cheng & Kurtz, 1963; Stewart *et al.*, 1963; Knowles & Chu, 1969), amino sugar (Isirimah & Keeney, 1973; McGill, 1971), and a significant amount of unidentified N (Ladd & Paul, 1973). A great deal of work has been conducted on the mineralization-immobilization relationships of both $^{14}N$ and $^{15}N$ under a variety of conditions: the effect of temperature (Kai *et al.*, 1969; Stanford *et al.*, 1975), the effect of residue particle size (Sims & Frederick, 1970), the effects of such treatments as wetting and drying, and grinding (van Schreven, 1967; Sørensen, 1977), the influence of waterlogging and anaerobiosis (Tusneem & Patrick, 1971; Yoneyama & Yoshida, 1977), as well as the influence of living plants (Huntjens, 1971).



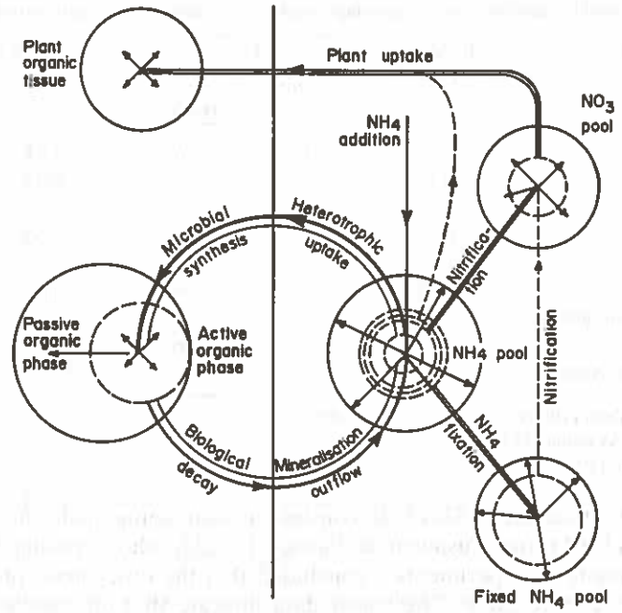


**Figure 1.** Relationship between internal nitrogen cycle and addition of mineral N to soil (Jansson, 1958).

The interaction of C and N has also been documented for a number of non-agricultural soils, such as meadows (Ulehlova *et al.*, 1970), deserts (West & Skujins, 1977), forests (Overrein, 1972), and tundra (Van Cleve, 1974). The study of systems other than cultivated sites has coincided with attempts to measure the soil biomass. Direct microscopy and chemical techniques, such as ATP measurement and chloroform-fumigation-incubation, have resulted in much larger estimates for the biomass than previously considered realistic (Anderson & Domsch, 1978; Van Veen & Paul, 1979; Jenkinson & Ladd, 1980).

The use of normal tracer techniques for $^{13}C$, $^{14}C$ and $^{15}N$ allowed the determination of the dynamics of inorganic and organic N and organic C added to the system. In addition, carbon dating using naturally occurring $^{14}C$ made it possible to characterize the passive fraction separated by hydrolysis, by classical techniques involving humic and fulvic acids, or as soil separates (Scharpenseel & Schiffman, 1977; Stout *et al.*, 1980).

Oades & Ladd (1977) recently completed a detailed review of the biochemical properties of soil constituents and the C and N metabolism of soil microorganisms. Paul & Van Veen (1978), in their review of the use of tracers in soil organic matter and microbial growth research, summarized the various tracer experiments and concluded that enough is known about the kinetics of the reactions for temperature and moisture curves to interrelate the reaction rates under a wide variety of field and laboratory conditions (Table 1). In their mathematical analysis of the available data, they stressed the fact that microorganisms in soil

**Table 1.** Effect of environment on the decomposition rate of plant residues added to the soil.

| Environmental condition | Relative rate | $T_{1/2}$ | | k ($day^{-1}$) | |
|---|---|---|---|---|---|
| | | Uncorr. | Corr. (60%) | Uncorr. | Corr. (60%) |
| Laboratory[1] | 1 | 17 | 9 | 0.04 | 0.08 |
| Rye straw Nigeria[2] | 0.5 | 28 | 17 | 0.02 | 0.04 |
| Rye straw, England[2] | 0.125 | 125 | 75 | 0.006 | 0.01 |
| Wheat straw, Saskatchewan, summer[3] | 0.125 | 125 | 75 | 0.006 | 0.008 |
| Wheat straw, Saskatchewan, year[3] | 0.05 | 230 | 160 | 0.002 | 0.003 |

[1] Paul & Van Veen (1978).
[2] Jenkinson & Ayanaba (1977).
[3] Shields & Paul (1973).

grow at high efficiencies. This is in contrast to statements made, for example, by Alexander (1961) and Tusneem & Patrick (1971), who – basing their observations on long-term experiments – concluded that the efficiencies of bacteria and fungi rarely reached 20%. The tracer data indicate that efficiencies reach 60% during the period of active growth. The concepts of maintenance energy developed for continuous cultures (Veldkamp, 1968) cannot be effectively applied at present to the soil system because of the large and unknown portions of resting compared to active cells within the microbial populations.

Calculations of decomposition rates from mineralization curves for plant residues and microbial products must take into account new microbial growth (Fig. 2). The need for the estimation of the soil biomass and the fact that an understanding of mineralization-immobilization reactions can only really be obtained on the basis of gross rather than net rates have been stressed (Paul & Van Veen, 1978). Tracer studies, together with mathematical descriptions of the processes occurring within the soil system, have generally described net rates of transfer between the biomass and specific fractions, such as the biomass, amino acids, amino sugars, microbial metabolites, cell-wall constituents, humic and fulvic fractions, and the resistant and active phases. It should now be possible to construct more meaningful models that allow testing of the actual processes involved, i.e., the determination of actual or gross transfer rates.

In conducting the experimental work to obtain the necessary parameters for a microbial growth-N transformations model as in Fig. 3, for example, it has been necessary to re-examine in detail the size of the fractions and the transfer rates involved. Past work on the turnover of the active fraction in general (Jansson, 1963; Smith *et al.*, 1978), and on specific components, such as amino acids (Ladd & Paul, 1973), amino sugars (Isirimah & Keeney, 1973) and cell-wall constituents (Kai *et al.*, 1973), show that these materials persist in the soil with a half-life ranging from 5 to 40 years. The resistant N associated with the 50% of the C that is non-hydrolyzable and that has a mean residence time greater than 1000 years does not have a significant effect on short-term N cycling.



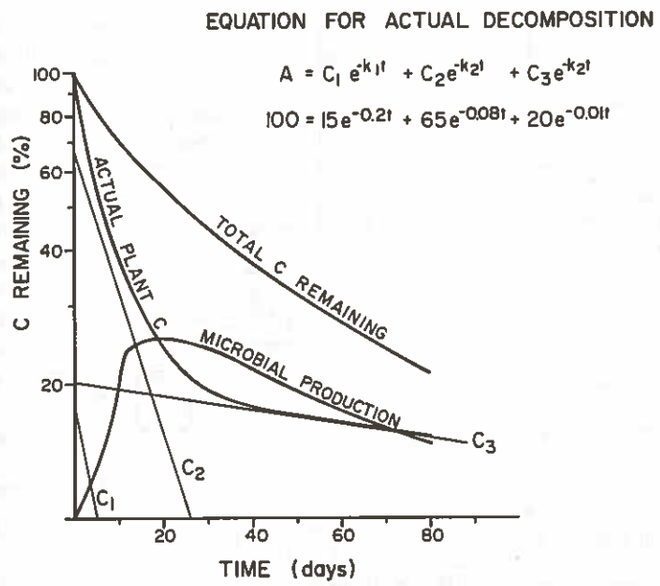


**Figure 2.** Decomposition of straw-C in the laboratory, plotted as a series of first-order reactions after correction for microbial production. The actual plant-C remaining is comprised of proteins and solubles ($C_1$), cellulose and hemicellulose ($C_2$), and lignin ($C_3$).

The determination of the sizes of the pools involved and estimates of their turnover rate require further experimental work. In this paper we have used a mathematical description of the mineralization-immobilization process to obtain further information on the size and turnover of the microbial biomass. Information on N transfers was obtained by combining a series of field and laboratory experiments involving $^{15}N$.

## Experimental Procedures

### Microplots

A field experiment was conducted on a Weirdale loam, a grey-black chernozemic soil with a 25-cm A horizon, to determine the $^{15}N$ utilization by spring wheat, and to obtain enriched $^{15}N$ soil samples. The surface horizon had a total N content of 0.42% and the pH was 7.4 (1:2.5 soil-water suspension). Metal cylinders (20 cm internal diameter, 60 cm long) were sunk into the soil and 10 $cm^3$ of $^{15}NH_4OH$ (180 mg N $cylinder^{-1}$), equivalent to 56 kg $ha^{-1}$, were injected into the soil in spring at a depth of 10 cm. The degree of labelling of $NH_4OH$ was 5.6% excess $^{15}N$. Phosphate fertilizer was added at seeding time at the rate of 20 kg $P_2O_5$ $ha^{-1}$. The fertilizer treatment was replicated four times.

At the end of the season, the wheat crop was harvested, the cylinders were removed, and soil cores underneath the lysimeters were taken. The cylinders were cut open and the soil was sectioned into A, AB and B horizons and air-dried. Soil subsamples were ground to 100 mesh and, along with the grain

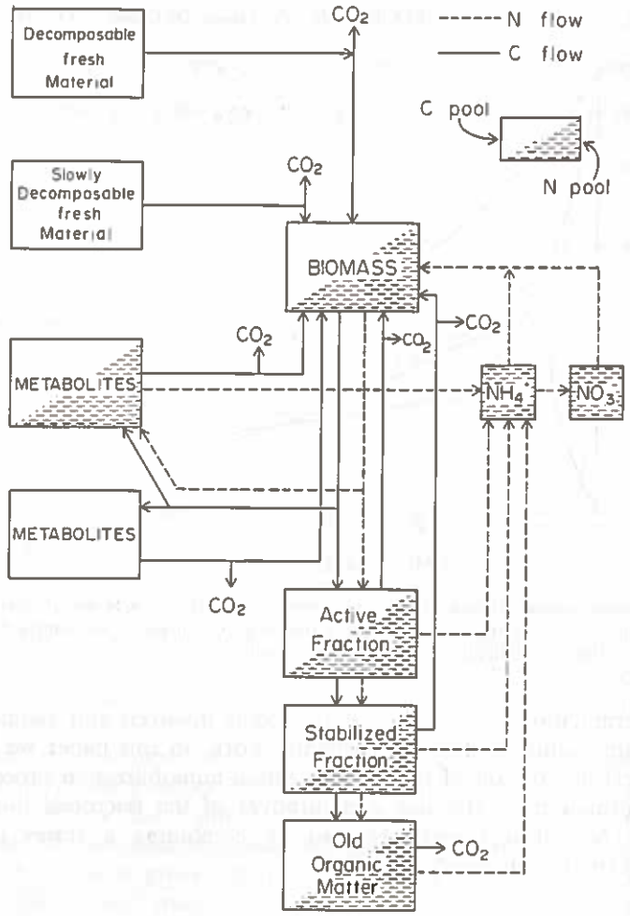


**Figure 3.** Flow chart for transfers of C and N in soil.

and straw samples, analysed for total N, including $NO_3^-$ – and $NO_2^-$ -N (Rennie & Paul, 1971). Isotopic ratio analysis was performed on an Atlas GD150 spectrometer.

### Incubation experiments

An incubation-leaching experiment was carried out with the enriched surface soil samples to determine the net mineralization of immobilized fertilizer N. Triplicate moist-soil samples (25 g oven dry basis), pre-incubated for three weeks, were mixed with equal amounts of sand and transferred to Büchner funnels lined with fibre-glass filter paper. The soil samples were leached with 200 cm³ of 0.01 M $CaCl_2$ solution, followed by 50 cm³ of nutrient minus N solution (Stanford & Smith, 1972). Excess moisture was removed by suction and the soil was incubated for 7 days at 28 ± 1° C. After each incubation period, the



samples were leached and reincubated. Duplicate 50 cm³ aliquots of leachates were distilled with MgO and Devarda's alloy for the determination of inorganic N and its $^{15}N$ abundance.

In a parallel experiment, 5 kg soil were moistened to field capacity and pre-incubated at 28 ± 1° C for 3 weeks. At regular intervals, 50 g soil (ODB) were used for biomass-N determination (Jenkinson & Powlson, 1976) as follows: the samples were exposed to ethanol-free chloroform vapours for 24 h in a desiccator, followed by evacuation of these vapours and subsequent incubation. At the end of the 10-day incubation, the soil was extracted with 100 cm³ of 2 N KCl and analysed for $NH_4^+$ – and $NO_3^-$ -N and their corresponding $^{15}N$ abundances. Biomass-N was calculated by dividing the amount of $NH_4^+$ -N accumulated during 10 days' incubation by a factor of 0.25 (R.P. Voroney, personal communication).

## Results

### Total recovery of $^{15}N$-fertilizer

The total recovery in grain, straw and soil of spring-applied $^{15}N$-aqua ammonia was 81.2%. The grain utilized 30.7% of the fertilizer, while the straw accounted for 11.7%. About 38.8% of the fertilizer N remained in the soil and was distributed as follows: A horizon, 26.9%; AB horizon, 11.4%; and B horizon, 0.5%. About 19% of the applied N could not be accounted for. Leaching losses were not likely as a very small portion of the fertilizer N was recovered in the B horizon.

The fertilizer-N remaining in the surface soil sample was mainly in organic forms since less than 1% was found in the non-exchangeable $NH_4^+$-N fraction and as mineral N. These samples were used in subsequent experiments to study the mineralization-immobilization relationships in soil.

### Net mineralization of total organic $^{15}N$ in soil

The mineralization of total organic $^{15}N$ in soil was measured in an incubation-leaching experiment. Initially, 241 ng $g^{-1}$ soil of total organic $^{15}N$ were present. The abundance of total N of the enriched soil was 0.37862% $^{15}N$, while the atom % $^{15}N$ abundance of mineralized N during incubation was ~ 0.4093% and showed a steady decline during incubation (Table 2). The higher atom % abund-

**Table 2.** Amount of N mineralized and atom % abundance of $NO_3^-$-N accumulated during incubation and $NH_4^+$-N accumulated after fumigation and 10-day incubation (biomass N).

| Incubation period (weeks) | N mineralized (μg $g^{-1}$ soil · $week^{-1}$) | Atom % abundance | |
|---|---|---|---|
| | | $NO_3^-$-N | Biomass |
| 0–1 | 8.8 | 0.40934 | 0.41314 |
| 3–4 | 16.1 | 0.40854 | 0.40312 |
| 9–10 | 8.9 | 0.40956 | 0.40778 |
| 11–12 | 6.2 | 0.40572 | 0.40364 |

ance of mineralized N than that of total N indicated that the $^{15}N$ remaining in soil after one season in the field was present in labile N fractions (active phase). Similar results were also reported by Legg *et al.* (1971) and Jansson (1958).

The cumulative $^{15}N$ mineralized showed a curvilinear relationship with incubation time. The amount of total organic $^{15}N$ remaining in the soil was calculated by subtracting cumulative $^{15}N$ mineralized from the initial amount present (241 ng $g^{-1}$ soil). Mathematical analysis of the curve for total organic $^{15}N$ remaining in soil showed that the mineralization of total organic N followed first-order kinetics (Fig. 4) and could be described as:

$$\text{Total organic } ^{15}N \text{ remaining in soil} = 239\, e^{-0.0129t}\ (r = 0.99^{**}) \quad (1)$$
$$t_{1/2} = 53.6 \text{ weeks at } 28 \pm 1°C \text{ and field capacity moisture content.}$$

The first-order rate constant in the above equation is a net mineralization rate constant, and the regression value for the original $^{15}N$ present, e.g., 239 ng, can be compared to the actual level of 241 ng $g^{-1}$ soil.

### Net mineralization of biomass-$^{15}N$

The biomass-N is an intregral part of the active organic phase. Its $^{15}N$ abundance was similar to the mineralized-N during incubation (Table 2). This implies that the nitrogen being mineralized has passed through the biomass. The gradual

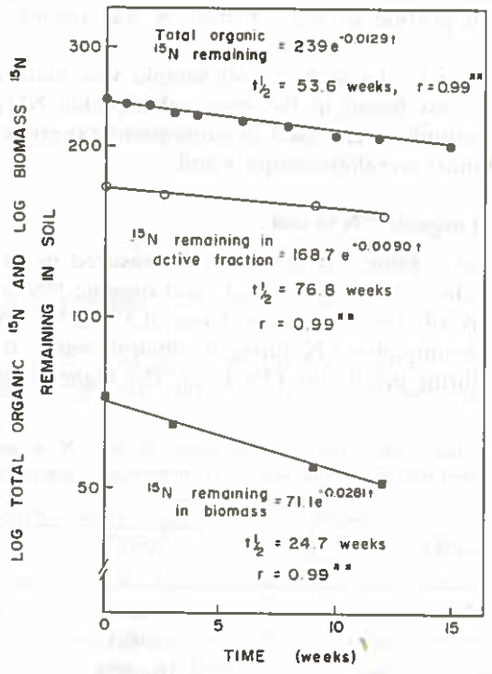


**Figure 4.** Total organic-$^{15}N$ and biomass-$^{15}N$ during incubation. The active N pool size and rate constant were calculated by the use of curve peeling techniques.



drop in atom % abundance also indicates that the biomass is turning over and is involved directly in the internal cycle of N. Mathematical analysis showed that the biomass-$^{15}N$ was mineralized at first-order rate kinetics and could be described as:

$$^{15}N \text{ remaining in biomass} = 71.1\, e^{-0.0281t}\ (r = 0.99^{**}) \quad (2)$$
$$t_{1/2} = 24.7 \text{ weeks at field capacity moisture content and } 28 \pm 1°C.$$

The biomass-N accounted for 30% of the total $^{15}N$ and had a much shorter half-life than the total organic $^{15}N$. Consequently, the non-biomass $^{15}N$ should be in other fractions which would be turning over more slowly.

### Calculation of the size of active $^{15}N$ fraction and its net mineralization rate

The $^{15}N$ remaining in the soil at the end of the season would mainly be present in the active organic phase. We have identified a part of this phase by measuring the size of the biomass-N. The size of the active $^{15}N$ fraction was then calculated by subtracting the size of biomass-N from the total organic $^{15}N$ remaining in soil. This approach is justified since the biomass-N measurement is independent of the measurement of total organic $^{15}N$ remaining in soil. Mathematical analysis using curve-peeling techniques showed that the active N forming the other part of the active organic phase mineralized at first-order rate kinetics and could be described as:

$$^{15}N \text{ remaining as active N} = 168.7\, e^{-0.0090t}\ (r = 0.99^{**}) \quad (3)$$
$$t_{1/2} = 76.8 \text{ weeks at field capacity moisture content and } 28 \pm 1°C.$$

The active N is mineralized at a much slower rate than the biomass N ($t_{1/2}$ = 24.8 weeks). The total organic $^{15}N$ remaining in soil can be described as the summation of the biomass-N and the active-N. Its dynamics are described by the following equation:

$$\begin{aligned} \text{Total organic } ^{15}N \text{ remaining in soil} &= {}^{15}N \text{ remaining in biomass} + {}^{15}N \text{ remaining as active-N} \quad (4) \\ &= 71.1\, e^{-0.0281t} + 168.7\, e^{-0.0090t} \end{aligned}$$

This equation, which expands equation (1), is valid in the short term since almost all the $^{15}N$ remaining in soil would be in the active organic phase.

### Measurement, calculation and estimation of sizes of $^{14}N$ fractions

The biomass $^{14}N$ size was measured by the chloroform-fumigation-incubation technique and accounted for 4% of the total N. The size of the active $^{14}N$ pool was calculated by using isotopic dilution principles on the assumption that its $^{15}N$ enrichment was similar to that of the biomass N as follows:

$$\text{Active organic } ^{14}N = \text{active organic } ^{15}N/\text{atom\% } ^{15}N \text{ excess of biomass N}$$

It accounted for 10% of the total-N in soil. The other $^{14}N$ was divided into two pools: the old-N with a half-life of 600 years accounted for ~50% of the

total N while the remainder ~36% was in the stabilized fraction with a half-life of 27 years in the field. The pool sizes and techniques used for their determination are summarized in Table 3.

**Table 3.** Initial values of N pools and techniques used for pool-size determination.

| Pool | $^{14}N$ | $^{15}N$ | Technique |
|---|---|---|---|
| | ($\mu g\ g^{-1}$ soil) | | |
| Biomass | 167 | 0.072 | Fumigation-incubation |
| Active fraction and metabolites | 389 | 0.169 | Isotopic dilution and curve-peeling |
| Old fraction | 2073 | 0 | Associated with old carbon (carbon dating) |
| Stabilized fraction | 1496 | 0 | By difference |

### Description of mineralization-immobilization of soil N using simulation

The transformations of C, $^{14}N$ and $^{15}N$ were described by first-order rate kinetics in a simulation model. The pool sizes and decay or transfer rate constants used (Table 4) yielded outputs which were similar to the experimental data (Table 5). The values of total organic $^{15}N$ remaining in soil and the cumulative $CO_2$-C evolved as predicted by the model were slightly higher than the experimental values; the mineral-$^{15}N$ level predicted was lower than the actual value. The other outputs were similar, indicating that the gross transfer-rate constants and pool sizes used (Table 4) were of the proper magnitude.

The gross transfer rates of material flowing into and out of various pools can be determined through the use of computer simulation models. The dynamics of various labelled und unlabelled N fractions in soil is shown in Table 6. During the 12-week incubation when no outside carbon was supplied, the initial biomass

**Table 4.** Initial values and decay or transfer rate constants used to describe mineralization-immobilization of C and N in soils.

| Pool | C | $^{14}N$ | $^{15}N$ | Decay rate (or transfer) constant | | Efficiency of utilization of C | C/N ratio |
|---|---|---|---|---|---|---|---|
| | ($\mu g\ g^{-1}$ soil) | | ($ng\ g^{-1}$ soil) | ($day^{-1}$) | | (%) | |
| Biomass | 1 000 | 167 | 72 | 0.0143 | | — | 6 |
| Active fraction | 2 302 | 384 | 167 | 0.0037 | $(0.5 \cdot 10^{-3})^1$ | 40 | 6 |
| Metabolite-C | 15 | — | — | 1.0 | | 60 | — |
| Metabolite-C + N | 15 | 5 | 2 | 1.0 | | 60 | 3 |
| Stabilized-C + N | 17 542 | 1 496 | 0 | $6.0 \cdot 10^{-4}$ | $(3.0 \cdot 10^{-6})^2$ | 40 | 11.7 |
| Old C + N | 24 455 | 2 073 | 0 | $3.0 \cdot 10^{-6}$ | | — | 11.8 |
| Decomposable C | 2 | — | — | 1.0 | | 60 | — |
| Slowly decomposable C | 50 | — | — | 0.1 | | 60 | — |
| Mineral N | — | 21 | 9 | — | | — | — |

[1] Transfer rate of active mineral to stabilized fraction.
[2] Transfer rate of stabilized fraction to old fraction.



of 72 ng $^{15}N\ g^{-1}$ soil grew (input) by a total of 56 ng $g^{-1}$ soil. The cumulative decay (output) value after 12 weeks was 76 ng $g^{-1}$, resulting in a biomass $^{15}N$ of 53 ng $g^{-1}$. The decay (output) products of biomass-N entered the metabolite-N (proteins and other nitrogenous compounds) and active-N pools in equal proportions. The metabolite pool is small at any time because it mineralizes rapidly. The active-$^{15}N$ pool increased by 38 ng $g^{-1}$ from 166 ng $n^{-1}$. The output of active $^{15}N$ was 54 ng, resulting in a final size of 150 ng $g^{-1}$. The predicted biomass-$^{15}N$, active-$^{15}N$ and metabolite-$^{15}N$ values at the end of the incubation period were similar to the experimental values.

**Table 5.** Comparison of experimental data with simulation model outputs at the end of 12 weeks' incubation.

| Pool | Experimental | Simulated |
|---|---|---|
| | ($\mu g\ g^{-1}$ soil) | |
| Total organic $^{15}N$ remaining in soil | 0.203 | 0.211 |
| Biomass-$^{15}N$ | 0.052 | 0.053 |
| Active fraction + metabolite-$^{15}N$ | 0.152 | 0.150 |
| Stabilized-$^{15}N$ fraction | ? | 0.007 |
| Mineral-$^{15}N$ | 0.045 | 0.039 |
| Mineral-$^{14}N$ | 115 | 121 |
| Cumulative $CO_2$-C evolved | 938 | 1153 |

**Table 6.** Dynamics of various N fractions in soil during 12 weeks' incubation.

| Fraction | Simulated values | | | | Experimental value at end of 12 weeks |
|---|---|---|---|---|---|
| | Initial size | Inputs | Outputs | Size at end of 12 weeks | |
| | | $^{15}N$ ($ng\ g^{-1}$ soil) | | | |
| Biomass-N | 72 | 56 | 76 | 53 | 52 |
| Metabolite-N | 2 | 38 | 39 | 1 | |
| Active-N | 166 | 38 | 54 | 150 | 152 |
| Stabilized-N | 0 | 7 | 0.1 | 7 | n.d. |
| Old-N | 0 | 0 | 0 | 0 | n.d. |
| Mineral-N | 9 | 86 | 56 | 39 | 45 |
| | | $^{14}N$ ($\mu g\ g^{-1}$ soil) | | | |
| Biomass-N | 167 | 163 | 187 | 143 | 152 |
| Metabolite-N | 5 | 94 | 98 | 1.0 | ? |
| Active-N | 384 | 94 | 125 | 352 | ? |
| Stabilized-N | 1496 | 15 | 55 | 1456 | n.d. |
| Old-N | 2073 | 0.1 | 0.4 | 2073 | n.d. |
| Mineral-N | 21 | 263 | 163 | 121 | 115 |

n.d. = not determined

The initial sizes of stabilized- and old-$^{15}N$ were set to zero as the $^{15}N$ remaining in soil was assumed to be in the active phase. At the end of 12 weeks incubation, 7 ng $g^{-1}$ soil had accumulated in the stabilized-N fraction, and 0.3 ng $g^{-1}$ in the old-N fraction. The $^{15}N$ in stabilized- and old-N cannot be determined experimentally.

Gross mineralization of $^{15}N$ from various fractions was 86 ng $g^{-1}$ soil, while the gross immobilization was 56 ng $g^{-1}$ soil, resulting in a net mineralization of 30 ng $g^{-1}$ and a final mineral-$^{15}N$ pool size of 39 ng $g^{-1}$. The net mineralization determined experimentally was 36 ng $g^{-1}$, with a final pool size of 45 ng $g^{-1}$ soil.

The biomass-$^{14}N$ grew by 163 μg $g^{-1}$ soil and decayed by 187 μg $g^{-1}$ soil during the 12-week incubation, resulting in a decline of 24 μg N $g^{-1}$ soil. The metabolite-N and active-N equal inputs also declined. The predicted value of active-$^{14}N$ was 352 while that of metabolite-N was 1 μg N $g^{-1}$ soil. The stabilized-N showed a net mineralization of 40 μg $g^{-1}$ soil, but there was no change in the old-N. Gross mineralization was 263 μg $g^{-1}$, while the gross immobilization was 163 μg $g^{-1}$, resulting in a net mineralization of 100 μg $g^{-1}$ and a final pool size of 121 μg $g^{-1}$ soil. The relative contribution of N fractions to the mineral-N pool were: biomass, 24%; metabolites, 4%; active-N, 32%; and stabilized N, 40%. It is not yet possible to determine the sizes of metabolite-, active-, stabilized- and old-N fractions.

Many attempts have been made to measure the size of biomass-, active- and metabolite-N. Jansson (1958) used isotopic dilution techniques to measure the size of the active organic phase (biomass- + active- + metabolite-N) but the values obtained showed an initial decline and a subsequent upward trend. It is now possible to measure experimentally the biomass-N and its net mineralization rate, but it is still not possible to measure the size of active-$^{14}N$. During initial periods of incubation, the active-$^{15}N$ can be estimated as the difference between total organic $^{15}N$ remaining in soil and the biomass-$^{15}N$. However, it is difficult to determine the atom% $^{15}N$ enrichment of the active-N pool. The biomass and active-N are closely associated as the microbial decay products enter the active-N. In addition, the decomposition of active-N results in $NH_4^+$, which is either reimmobilized or nitrified. Thus, the atom% $^{15}N$ abundance of $NO_3^-$-N or biomass-N should be similar to the $^{15}N$ abundance of active-N. However, a slightly higher $^{15}N$ enrichment of the active-N would be expected since the net mineralization rate constant of biomass-$^{15}N$ is about three times that of active-$^{15}N$, and the biomass incorporates mineral-N which has been diluted by N mineralized from soil organic fractions. Therefore, the atom% $^{15}N$ abundance of biomass-N and $NO_3^-$-N drops at a higher rate than that of active-N. Consequently, an increasing trend in the size of active $^{14}N$ is calculated as:

$$\text{active-}^{14}N = \text{active-}^{15}N/\text{atom\% } ^{15}N \text{ excess of biomass-N or } NO_3^-\text{-N}.$$

## Conclusions

The techniques for the use of tracers and simple models as employed in this study are readily applicable. Because of the large number of interacting pools, the measurement of gross and net transformation rates, however, is fairly difficult to determine and may be more difficult to comprehend. The summary for



$^{15}N$ biomass growth and decay (Fig. 5) helps to explain the movement of N through this fraction. The model predicts a gross growth of 56 ng $g^{-1}$ from the initial 72 ng $g^{-1}$. At the same time, microbial decay accounted for 76 ng $g^{-1}$ for a slight decrease in the biomass during incubation. The simulation data was in agreement with the actual measured value of biomass throughout the experiment.

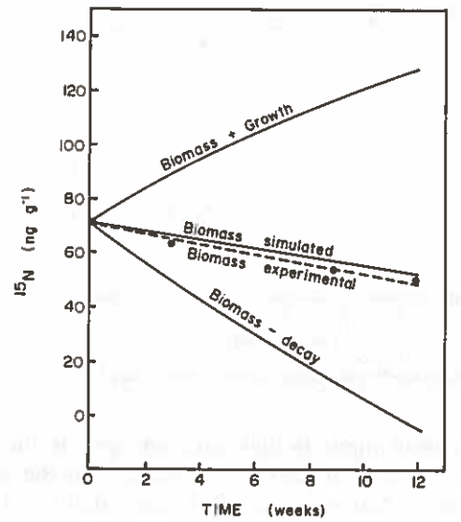


**Figure 5.** Biomass-$^{15}N$: cumulative growth and decay rates.

The initial total microbial-N of 167 μg $g^{-1}$ soil accounted for 4% of the total soil N. It immobilized 163 μg N $g^{-1}$ soil, and 187 μg $g^{-1}$ were transferred out of the soil biomass during the 12-week incubation, resulting in a final value of 143 μg N $g^{-1}$ soil. The experimental values of total microbial biomass were similar to the simulated results (Fig 6).

The fact that the growth of the biomass $^{14}N$ was equal to the original amount with a small drop in its $^{15}N$ content during the 12-week incubation period had two implications: (1) a portion of the biomass turned over more than once, while some of the $^{15}N$ in a resting portion was not affected, and (2) the mineralized N had a similar abundance as the biomass, therefore, during growth the biomass would reincorporate the labelled mineral N. This labelled N would be diluted owing to mineralization of older organic $^{14}N$ pools. Estimates of decay and transfer rates were therefore only attainable by simulating the transformations of all the N fractions. This indicated that of the N mineralized, 24% came from the biomass, 4% from metabolites, 32% from an active N pool other than the above, and the remainder (40%) from mineralization of a stabilized N pool.

In most terrestrial systems, the flow of carbon and nitrogen is closely interrelated. Generally, mineral-N does not accumulate in undisturbed grassland

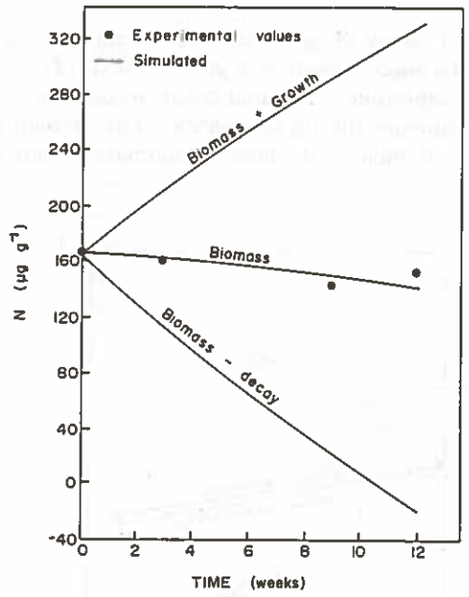


**Figure 6.** Total microbial biomass-N: cumulative growth and decay rates.

or forest sites since carbon input is high and nitrogen is the element limiting decomposition. Therefore, it is difficult to measure even the net mineralization and immobilization rates. Nitrogen transfers are usually estimated from the amount of nitrogen present in harvested plant materials. This does not take into account internal cycling, and grossly underestimates the movement of nitrogen through various components in the system. Nitrate accumulates in soils disturbed by cultivation, clear-cutting and sometimes even after a fire. It is then possible to measure net mineralization rates.

Under field conditions, some measure of the nitrogen transfers is obtainable from the carbon movement since carbon transformations involve the production of easily measured $CO_2$. The carbon utilized by the microorganisms should be equal to the $CO_2$ evolved if microbial growth efficiency approaches 50%. The C:N ratio of microorganisms under most conditions ranges from 5:1 to 15:1, depending on nitrogen availability and the types of microorganisms involved in the immobilization. Therefore, an estimate of gross immobilization of nitrogen can be obtained from the growth estimates obtained by measuring microbial $CO_2$ production.

The methods described in this paper for measurement of growth and net mineralization of N involved the separation of soil into biologically meaningful fractions and the measurement of the soil biomass. First-order kinetics appear to describe meaningfully the rates of the various transformations involved, and isotope dilution and computer simulation were shown to be useful techniques in unravelling the relationships between mineralization and immobilzation.

A further example of the usefulness of these techniques can be obtained by examining the carbon flow data. The biomass of 1000 μg C evolved 1000 μg



$CO_2$-C, therefore approximately 2000 μg $g^{-1}$ of available carbon were utilized for growth. Since C and N are closely interlinked and no exogenous carbon was added, the source of the carbon should be similar to that of the N, and gross immobilization rates were found to be equal to those shown in the earlier data.

The data in this paper are in agreement with available field data that indicate a large microbial biomass existing on only small concentrations of available substrate relative to the size of the biomass. The whole biomass cannot be envisioned as turning over once during each growing season. The degradation of soil metabolites, the active fraction and the stabilized fraction would result in the mineralization rates shown in this study. In the absence of an exogenous carbon supply, the large proportion of the population therefore would have to be considered to be in a resting state, with very low decay constants which neither affect the mineralization nor immobilization. This view of a living stabilized biomass with very low turnover rates does not coincide with the laboratory information available from liquid laboratory cultures. It is in agreement, however, with all of the data obtained in the numerous field investigations of both cultivated and natural ecosystems conducted in various parts of the globe.

Einstein said that "nature hides her secrets through intrinsic grandeur but not through deceit". Further insight into this grandeur will be attained when the movement of N through the various fractions is followed with tracers, supplemented with adequate and discerning mathematical analyses. The coupling of the N data with similar measurements of the carbon driving force should aid in acquiring further understanding of the nature of microbial growth and soil organic-matter turnover.

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**Rapporteur's comments:**

## Mineralization and Immobilization of Soil Nitrogen by Microorganisms

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Although some indications on mineralization-immobilization dynamics had already been provided before the establishment of tracer techniques, the apprehension of these two opposite microbial processes is by and large a result of tracer investigations.

The general impression is, however, that interest in performing investigations further to clarify the mineralization-immobilization turnover has been fairly weak during the past two decades. New research openings have not been effectively utilized. Greater interest has been devoted to more or less fugitive side-tracks of the processes, for example the so-called priming action or priming effect.

Dr E. A. Paul and his co-workers at the University of Saskatchewan constitute a research group, however, that has dealt with the central mineralization-immobilization problems. This has been done with considerable success. The present paper, Paul & Juma (1981), is an interesting contribution in this field.

The paper can be characterized as an exponent of what may be called functional microbiology. This kind of research treats the soil microflora as a working unit responsible for certain pools and pathways of the nitrogen cycle instead of studying microbial species, numbers and successions. In the application of soil microbiology – for example, in soil fertility – this functional approach has proved very fruitful.

My purpose in now to relate the material given by Paul & Juma (1981) to the general terrestrial nitrogen cycle.

Fig. 1 illustrates the universal cycle – an additional variant to those treated by Clark (1981) at this workshop. In the figure the branching of the universal cycle into three partial cycles is stressed.

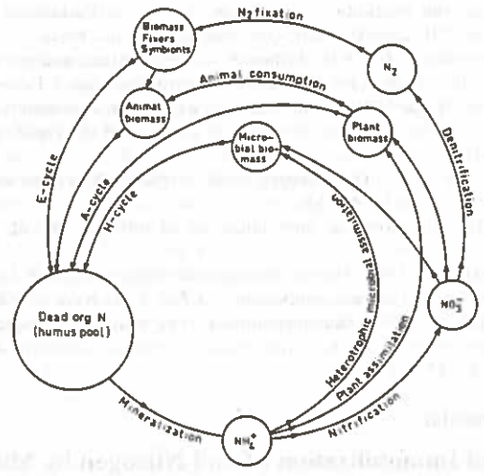


**Figure 1.** The universal nitrogen cycle divided into its three partial cycles, the elemental (E), the autotrophic (A) and the heterotrophic (H).

Fig. 2 illustrates the nitrogen cycle of the initial field experiment performed by Paul & Juma (1981). The elemental cycle was not operating (legumes were not grown; denitrification was unintentionally proceeding). Tagged ammonium fertilizer was introduced. The microbial biomass became tagged, and the dead organic pool was loaded with energy material in the form of crop residues.



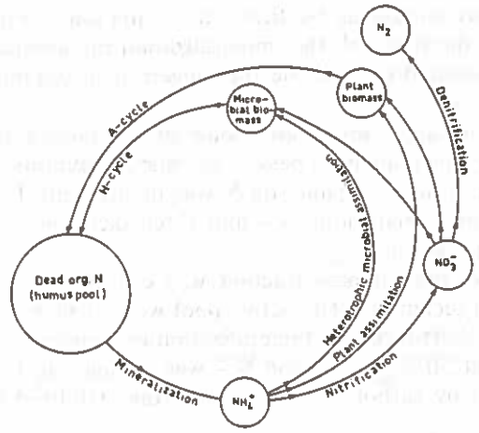


**Figure 2.** The nitrogen cycle operating in the field experiment of Paul & Juma (1982).

In the following incubation experiments the system was simplified to comprise the heterotrophic mineralization-immibilization cycle. This is shown in Fig. 3. The surplus net mineralization constituting the $NH_4^+$ and $NO_3^-$ pools was intermittently leached out, and its content of tagged N determined. This gave data for turnover calculations.

In addition, the size of the biomass pool was estimated by killing the biomass by fumigation and thereby transferring it into the dead active pool; from this, the increase in net mineralization gave an estimate of the biomass (according to the method of D. S. Jenkinson). Carbon mineralization measurements and determinations of biomass carbon were also performed, although detailed pertinent data are not given in the paper.

The pool of dead organic N was determined as the difference between total tagged organic N and the biomass pool. From the experimental $^{15}N$ data it was concluded that the nitrogen pathways constituting the heterotrophic partial cycle

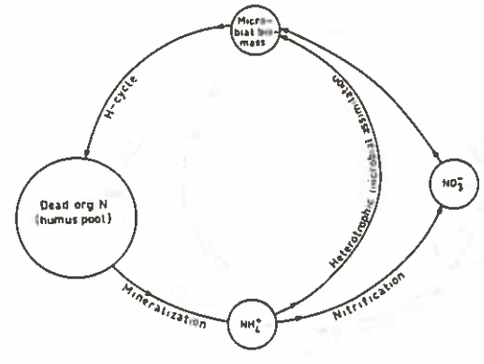


**Figure 3.** The heterotrophic mineralization-immobilization nitrogen cycle isolated in the incubation experiment of Paul & Juma (1981).

can be mathematically simulated by first-order equations. With regard to the comparatively short duration of the mineralization-immobilization incubation experiment, it was assumed that all the the tagged dead organic N remained in an active state.

However, this did not apply to the great bulk of non-tagged dead organic N in the soil. This bulk must include more passive organic N fractions.

As indicated in Fig. 4, total organic soil N was divided into four fractions, one living fraction – the microbial biomass – and three dead ones – the active, the stabilized, and the old fraction.

As indicated above, the biomass fraction was estimated by the chloroform-fumigation incubation technique. The active pool was calculated by using isotope dilution principles in short-term net mineralization experiments. The old fraction – accounting for about 50% of total soil N – was assumed to be associated with old carbon, estimated by carbon dating. Finally, the stabilized fraction was obtained by difference.

In my opinion, this fractionation – hitherto more or less hypothetical and tentative – is in great need of further research. In order to support functional microbiology a new, biologically founded analytical technique is urgently wanted. Conventional humus chemistry, dealing with the extraction and purification of fulvic and humic acids, has proved inadequate in this respect.

The paper by Paul & Juma (1981) ends up in a simulation model describing the gross mineralization and immobilization transformations between the different fractions (partial pools) of soil organic N with regard to tagged as well as untagged N. The model is founded on transformation equations described by first-order rate kinetics. The simulation model is not given in full detail but it yielded outputs which were compatible with the experimental data.

The simulation results are given a fairly detailed description and discussion. This discussion is competent and interesting, but it should be kept in mind that the underlying data are produced by a model in which comparatively few points are based on direct experimental evidence.

Generally, I am not too impressed by the rapid building of simulation models now going on in many sections of biological and ecological research. There is always the risk that the simulation results will be treated as truth and reality.

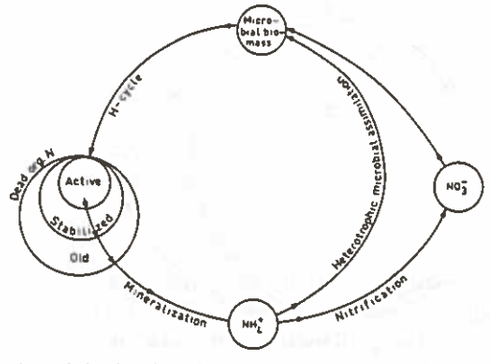


**Figure 4.** Illustration of the fractionation of the dead organic nitrogen pool put forward by Paul & Juma (1981).



In my opinion, production of direct experimental evidence is still more urgent and valuable in soil microbiology research than is simulation modelling. To build up a solid basis of experimental evidence through step-by-step tracer investigations is still a fundamental need in research on microbial mineralization-immobilization dynamics. Dr Paul and his co-workers are eminently qualified to continue and develop this research.

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