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THE SOIL NITROGEN

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Nitrogen is one of the essential constituents of all living things. The total N-mass is of the order of 10^{23} g. Of this, 0.02 per cent, i.e., 2×10^{19} g is present in the biosphere, which includes the soil. The soil N is important for plant nourishment and belongs to the biological reaction system. Cyclic processes are one of Nature's magnificent designs and three such major processes in the transformation of N may be identified. Another cycle, called the internal cycle, is specifically related to the mineralisation-immobilisation of N.

An inventory of gains and losses of N in soil is of great interest, but only rough estimates are possible. Biological fixation of N is one of most fascinating ways of N-gain by soil. Microorganisms of different kinds, algae, grasses, weeds and plants have their own mechanism of N-fixation. In addition, there is chemical fixation by the soil colloids, both inorganic and organic. The soil losses are mainly effected by plant uptake, denitrification volatilisation and leaching. There is net gain of N in soil and the form in which N accumulates in soil is mostly organic. Having combined with soil humus, N is gradually stabilised to such an extent that its mean residence time may be of the order of 200-800 years. The degree of stabilisation is such that only a very small fraction is released, but a large part of this accumulated soil N gives rise to an enormous reservoir of N in soil. It is not known how this N can be released. The chemical nature of the soil N is still unknown. But it is known to be in the form of organic compounds. As such it is speculated that some strains of microorganisms, depending on carbon for energy, may be able to unlock the huge reserve of soil N.

Key words : Soil, Nitrogen, Biosphere, Biological Fixation

TOGETHER with C, H and O, N is one of the essential constituents of all living things. Of the latter, the plants meet their nitrogen need mainly from the soil. N is a component of chlorophyll, of amino acids, proteins, essential for carbohydrate utilisation, component of enzymes, vitamins, stimulates root activity and development and supports uptake of other nutrients. The main source of soil

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nitrogen is the atmosphere, which contains, together with oxygen and other gases, about 79 per cent N. In this connection a rough estimate of the distribution of global N given below is of great interest. The total N mass of the globe is of the order of 10^{23} g.

<i>N Pool</i>		<i>% of total N mass</i>
Atmosphere	...	1.96
Lithosphere	...	97.82
Biosphere	...	0.02

Approximately, 27,200 million tons of free N_2 is present above each acre of the earth. N is present in rocks mostly as nitrides of Ti, Fe and other metals, and also as NH_4^+ in silicates, and in some rocks as nitrates.

The biospheric nitrogen is present mostly in organic forms both on land, i.e., soil N, (40 per cent) and in the ocean bottom (47 per cent). 1–2 per cent mineralisation of this N supports plants and animals.

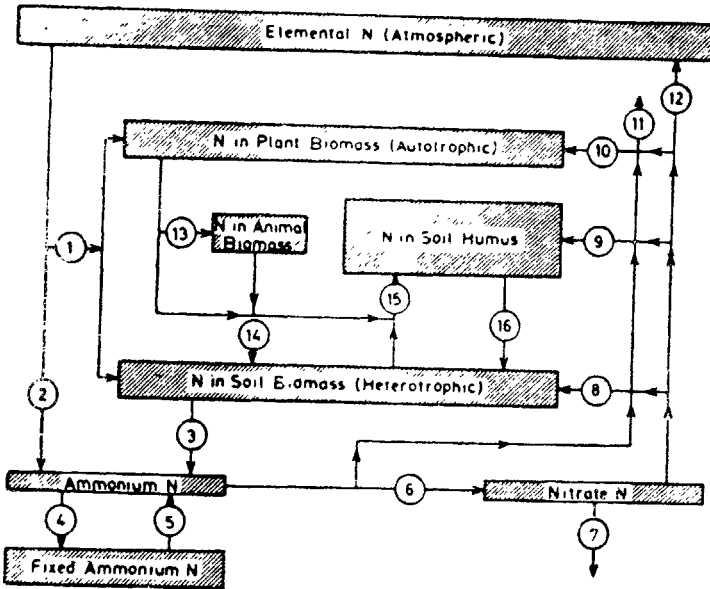
In spite of the fact that nitrogen is an inert gas, it is involved in a number of complex enzymic and chemical reactions in the soil, leading to the formation of a variety of compounds. The latter belong to amino acids, peptides, proteins, purines, pyrimidines, amides, amino sugars, nucleic acids, heterocyclic compounds derived from phenols, nitrogen-humus compound of unknown composition, and a good proportion of unidentified substances containing N. The proportions of these compounds, wherever and whenever present, vary according to the environmental conditions.

Along with P and S, N belongs predominantly to the biological reaction system, as distinct from the chemical reaction system to which other nutrients like K, Ca etc. belong. The latter are characterised by high energy of activation, obedience to law of mass action, dependence on temperature, and solution concentration etc., the last of which being altered by plant uptake. Unlike these nutrients, N requirement is not determined by plant uptake, and hence it becomes difficult to predict ability of soils to deliver N to plants. However, several transformation processes which have been identified in the soil provide some key to the information about plant need for N being satisfied. These transformation processes are fixation, nitrification, mineralisation, denitrification, immobilisation and stabilisation. These are mainly biochemical. But NH_4^+ fixation by clay colloids and NH_3 fixation by humus colloids are chemical in nature.

It is commonly observed that some of the biological as well as the chemical processes may occur simultaneously. In fact, at any point of time it is difficult, if not impossible, to determine the relative magnitude of each of the processes. Even if single transformations have been extensively studied in regard to soil environment mechanisms, intermediate and end products, there are many gaps in our complete understanding of these processes. The low content of soil N was usually explained by making denitrification, estimated by difference, responsible for it. Direct

measurements showed that denitrification is not so high as was made out to be. As a result, the nitrogen content of soil is not so low. But the high N-content could not also be easily explained. The story of this unexplained soil N is going to be focussed here.

Cyclic processes are said to be one of Nature's magnificent designs. The diagram given below represents the global cycle involving N. It is one of the most complex and comprehensive of the cyclic processes one finds in Nature, and show how variegated the routes of the cyclic processes are. One may identify three cyclic processes, viz., elemental, autotrophic and heterotrophic. These cyclic processes are demarcated in the diagram.



Pathways and pools in the cycling of soil nitrogen. (1) Nitrogen fixation, biochemical; (2) nitrogen fixation, industrial; (3) mineralization; (4) chemical NH₄ fixation; (5) NH₄ defixation; (6) nitrification; (7) leaching; (8) immobilization; (9) chemical fixation of NH₃ and oxidized forms of nitrogen; (10) plant uptake; (11) NH₃ evaporation; (12) denitrification; (13) animal consumption; (14) microbial consumption; (15) humus formation; (16) humus decomposition.

Pathways included in elemental cycle	Pathways included in autotrophic cycle	Pathways included in heterotrophic cycle
Nitrogen fixation (1)	Plant uptake (10)	Mineralization (3)
Animal consumption (13)	Animal consumption (13)	Nitrification (6)
Microbial consumption (14)	Microbial consumption (14)	Immobilization (8)
Mineralization (3)	Mineralization (3)	
Nitrification (6)	Nitrification (6)	
Denitrification (12)		

One can distinguished between the primary pool of N, the atmosphere, and the secondary pool, namely, animal biomass of dead organisms, humus from plant biomass, and $\text{NH}_4^+ - \text{N}$. The last one is derived from the primary pool by fixation through predominantly biological and to a small extent, chemical processes. $\text{NH}_4^+ - \text{N}$ may again be (i) immobilised by heterotrophic microflora; (ii) nitrified to be taken up by autotrophic plants; and (iii) react with soil colloids to get fixed and form the pool of unavailable N.

One can identify another cycle, called the internal cycle of N, in which mineralisation—immobilisation processes predominate. This can be enlarged by including another cycle, to account for loss of N by uptake, denitrification, leaching and humus formation.

The primary pool yields N, as already stated, to soil mainly by the process of biological fixation, which is generally recognised as nonsymbiotic and symbiotic fixation. Amongst the nonsymbiotic fixers are a host of microflora, such as BGA, photosynthetic bacteria, rhodospirillum, aerobic bacteria : azotobacter, Beijerinckia, Derxia and anaerobic bacteria : clostridium, actinomycete : Frankia in association with nonleguminous plants. The claims made by workers about the amount of N fixed by free-living bacteria vary considerably. Perhaps lower figures are nearer the actual values. But in addition to the above, about 100 organisms are recognised to be N-fixers, whose contribution to the N pool of soil may be appreciable. The rhizosphere of many grass sods has been found to be rich in N fixed from the atmosphere. Gains in soil N through BNF may be seen from the following data.

<i>Conditions</i>		<i>N gains in Kg/ha/yr</i>
Soil amended with crop residues	...	15-78
Field plots under sod like crop	...	14-56
Lysimetric studies	...	25-67
Stands of pines sp. or other monoculture nonnodulated trees	...	36-67

Amongst the higher plants the leguminosae family fixes in varying amounts atmospheric nitrogen in root nodules in symbiosis with the appropriate rhizobia. There are about 10-12 thousand plant species belonging to this family, out of which only 1200 species have been studied for nodulation. About 90 per cent of them produce nodules and nearly 100 species are utilised for food production. All of the thousands of plants, besides those cultivated, contribute substantially to N-content of the soil.

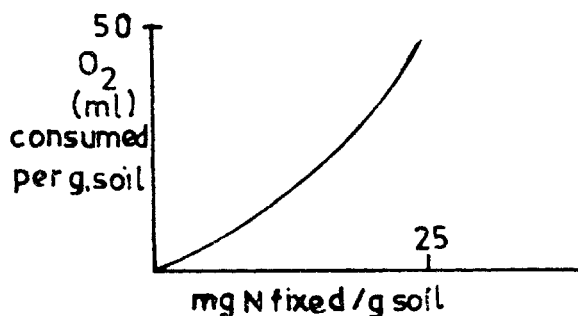
In the area of symbiotic BNF there is ample scope of genetic manipulation. For instance, development of N-fixing bacteria capable of living on the roots of such cereals as wheat; development of rhizobium strains that are insensitive to soil NH_4^+ and NO_3^- concentrations; development of legumes having high photo-

synthetic capability so that energy for N-fixation is provided to the bacteria in the nodules.

^{14}C and ^{15}N studies have shown that 11–17 per cent of biologically fixed N is taken up by plants, which have a preference for NO_3^- and to a small extent for NH_4^+ by some plants. The rest of N remains in soil as organic N. This may slowly become available, but not immediately. In course of time, the fixed N is converted into more stable forms, rendering availability of N very low. ^{15}N study has revealed that 10–40 per cent of applied N remains in soil. Plants remove 4–6 per cent of ^{15}N applied. After the second growing season 24 per cent ^{15}N remains behind, only 1.5 per cent of which becomes available to the plant, the remaining portion being in equilibrium with native soil N present as humus N. The mean residence time for the first year of added N is about 5 years. With the progress of humification of carbonaceous matter the mean residence time (MRT) may increase to 25 years. The MRT of N in native soil humus may vary from 200–800 years, and a small amount may remain unavailable for centuries.

All soil colloidal fractions which predominate in clay minerals, besides oxides of iron and aluminium and humic substances, are capable of reacting with NH_4^+ ion derived from the biologically fixed N or N-fertilisers. With the inorganic colloids the binding may be so strong that both leaching loss and biological availability are reduced considerably. Continuous extraction with K^+ may defix a major quantity of NH_4^+ and give an idea of the measure of fixation.

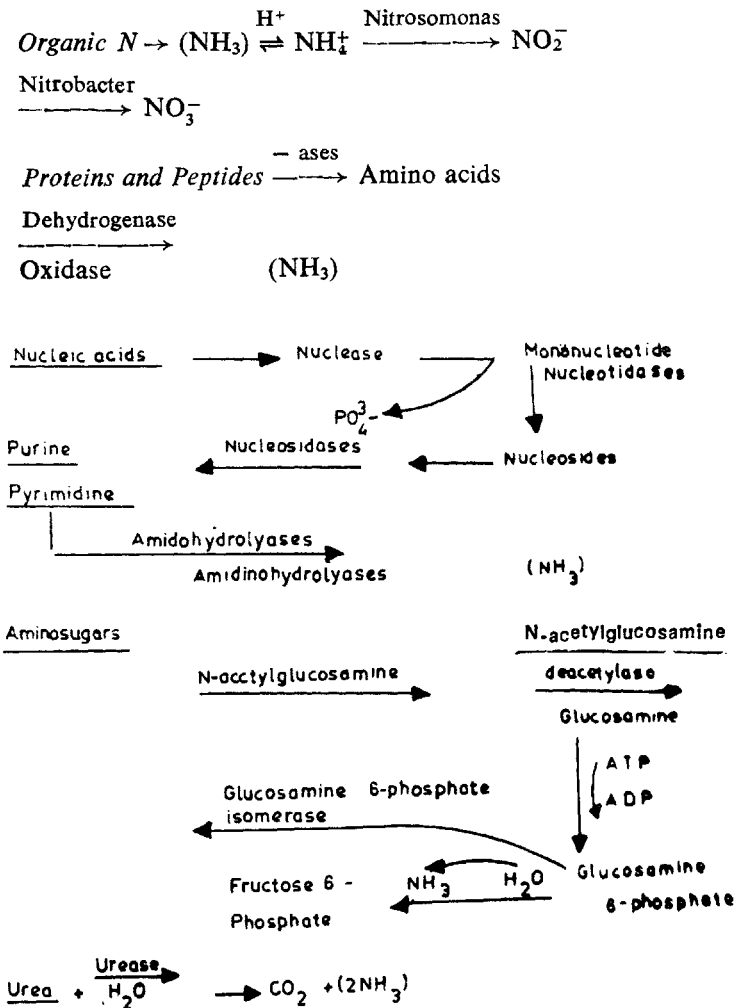
Organic colloids, particularly humus, react with NH_4^+ in an unknown manner. At high pH NH_4^+ gives rise to NH_3 which reacts with phenolic or their oxidation products leading to stabilisation of N. Fixation of NH_3 by organic matter is associated with oxygen uptake as shown by the figure.



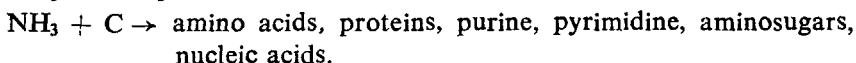
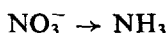
Reaction with phenols and formation of heterocyclic compounds, and nitrogen bridges are the other possible chemical reactions. Radio C dating has shown that soil fulvic and humic acids may have residence times varying from a few hundreds to a few thousands of years. In a dynamic soil system, at any point of time, three fractions of organic constituents could be identified:

Measurements on rhizosphere soil reveal that the volume of such soil may contain as high as 1,700kg/ha N.

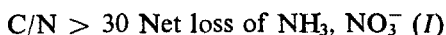
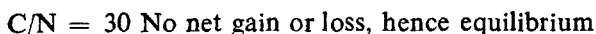
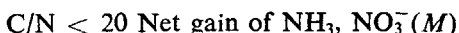
The key feature of the internal cycle of N in soil is immobilisation (organic N) \rightleftharpoons mineralisation (NH_3 - NO_3). Organic soil N, as already mentioned, is composed of a variety of substances, such as plant and animal residues, microbial biomass, including partially stabilised dead organic matter, and stable humus fraction. Once this last stage is attained, mineralisation stops but is compensated for by the formation of new humus material. Many of the organic compounds in soil are broken down by the appropriate enzyme systems into simpler compounds and finally to ammonia. This ammonia is then acted upon by specific microorganisms leading finally to NO_3^- . Some of these reactions are described below.



Some and often all of NH_4^+ and NO_3^- are simultaneously consumed by heterotrophic microflora and converted into microbial tissue. In this way the inorganic nitrogen forms are immobilised. The mechanism is the reverse of mineralisation.



Since mineralisation (M) and immobilisation (I) occur simultaneously one talks of net (M) and net (I). Energy for either of the processes is derived from carbonaceous sources, and hence a qualitative relation is observed between C/N ratio and net gain or loss of NH_3 and NO_3^- . Thus, it may be generally stated that

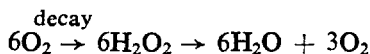
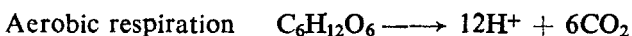
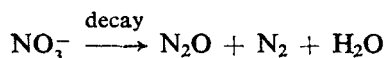
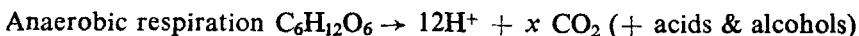


From the following data on C/N of some organic materials, one can easily judge whether they favour mineralisation or immobilisation.

<i>Material</i>	<i>C/N ratio</i>
Microbial tissue	6-12
Sewage sludge	5-14
Soil humus	10-12
Animal manures	13-25
Legume residues and green manure	13-25
Cereal residue and straw	60-80
Forest wastes	150-500

Soil gains in N take diverse ways. So also do soil losses in N. The following processes are generally ascribed to cause N loss in soil.

Anaerobic respiration corresponding to aerobic respiration is in fact bacterial denitrification.



Most of N of the atmosphere have passed at least once through this denitrification cycle.

For bacterial denitrification, organic compounds, reduced S compounds or H_2 act as electron donors; anaerobic or restricted O_2 condition; supply of NO_2^- or

NO_3^- to serve as terminal electron acceptors. Not all the heterotroph bacteria belonging to the *Agrobact*, *Bacillus* and *Pseudomonas* are capable of producing N_2 but some produce N_2O . The environmental condition should ensure poor drainage, temperature 25°C or higher, near neutral pH , good supply of decomposable organic matter.

In addition to denitrification, atmospheric N is enriched with NH_3 derived from volatilisation from various sources.

<i>Sources</i>		NH_3 loss (10^9 Kg/yr)
Wild animals	...	2-6
Domestic animals	...	20-35
Fuel combustion	...	4-12

Percentage losses of NH_3 from added fertilisers are of the following order :

<i>Fertiliser</i>		% loss
Urea	...	3.5-40
$(\text{NH}_4)_2\text{SO}_4$...	3-50
NH_4NO_3	...	17

The variations are due to pH , texture and organic matter content of soil.

After having looked into the ways by which the soil gains and loses N, it may be possible to make an inventory of N. But since the transformations and processes involving N are of a dynamic nature, it may not be possible to get the real picture of soil N unless measurements are made over long periods of time. On the other hand, it seems advisable to separate the soil organic components by chemical means, and find out if the N content of each fraction so extracted and chemical nature identified add up to the total. This simple procedure has revealed fractions that are not biodegradable to any appreciable extent and one

Soil N Distribution by Acid Hydrolysis

<i>Form</i>	<i>Method</i>	% soil N (usual range)
Acid insoluble N	Soil N remaining after 6N HCl hydrolysis (Usually by difference)	20-35
NH_3 -N	MgO distillation of hydrolysate	20-35
Amino acid N	Determined preferably by the ninhydrin - NH_3 method	30-45
Aminosugar-N	Steam distillation with phosphate-borate buffer (pH 11.2) corrected for NH_3 -N (Hexosamine N)	5-10
Hydrolysable Unknown N (HUN)	Not accounted for as NH_3 , amino acid or amino sugars. Part occurs as non α -amino N, arginine, histidine, lysine & proline	10-20

fraction at least that does not only biodegrade but defies chemical identification. This fraction has been labelled as the carrier of "unknown" nitrogen. The results of this kind of experiments are summarised below.

Acid insoluble N may comprise N as bridge linking quinone groups as described earlier. Amino acids attached to aromatic rings may not also be acid hydrolysable. Similar data on distribution of soil N collected from widely different climatic zones are as follows :

	Arctic (6)*	Cool Temperate (82)	Subtropical (6)	Tropical (10)
% Total soil N	0.02 — 0.16	0.02 — 1.06	0.03 — 0.30	0.24 — 1.61
Acid insoluble N**	13.9 ± 6.6	13.5 ± 6.4	15.8 ± 4.9	11.1 ± 3.8
NH ₃ -N	32.0 ± 8.0	27.5 ± 12.9	18.0 ± 4.0	24.0 ± 4.5
Amino acid—N	33.1 ± 9.3	35.9 ± 11.5	41.7 ± 6.8	40.7 ± 8.0
Aminosugar—N	4.5 ± 1.7	5.3 ± 2.1	7.4 ± 2.1	6.7 ± 1.2
HUN	16.5	17.8	17.1	17.6

*No. of samples examined

**This and the rest as % of total N.

Cropping has a tendency to increase HUN, apparently at the expense of amino acids as the following data show.

	Acid insoluble N (%)	NH ₃ -N (%)	Amino acid-N (%)	Amino- sugar-N (%)	HUN (%)
A. Virgin (10)	25.4	22.2	26.5	4.9	21.0
Cultivated (10)	24.0	24.7	23.4	5.4	22.5
B. Virgin (4)	20.8	19.8	44.3	7.3	7.8
Cultivated (4)	19.3	24.5	35.8	7.0	13.4

The percentage distribution of N is somewhat altered in the presence of added fertiliser N, as shown by the following data.

	Fertiliser derived organic N (%)	Native humus N (%)
Acid insoluble N	10.3	21.7
NH ₃ -N (organic)	10.6	18.1
Amino acid—N	59.0	36.0
Aminosugar N	9.9	8.0
HUN	10.9	16.2

The acid insoluble N, NH₃-N and HUN are comparatively large in native humus. It is likely that amino acid N which shows an appreciably high value in fertiliser derived organic N is converted into acid insoluble N, HUN and NH₃-N.

Both acid insoluble N and HUN are reservoirs of organic N of unidentified nature accumulated in soil over many many years. The acid insoluble part has been further extracted with 0.1 N NaOH solution and acidified to pH 2, and again extracted with 0.1 N NaOH and dialysed to give two fractions which contain about 15% or so of N. These fractions contain the locked-up N, called the 'unknown' N, but should be as thoroughly purified as possible in order to identify their N-compounds. It has been estimated that the total quantity of nitrogen so locked-up in the soil as unknown N is sufficient to supply the N requirement for crop production for 25-40 years at the present rate of demand. If this untapped source is made to yield N at an appropriate rate, the problem of N-supply will cease to exist. With the rising level of understanding of genetic engineering is it not pertinent to ask the question : Should it not be possible to produce by genetic manipulation strains of microorganisms which would unlock the N and release it from its hidden recess in the soil ? We will perhaps never unravel the exact nitrogen containing entities present in soil and the nature of N binding in them. In that case empirical trial and error methods may be the easiest and only way.

Our finger has been pointed to the mighty reservoir of N in the soil. Let us all search for the key that is going to unlock that reservoir.