

125. THE NITROGEN CYCLE IN BIOLOGICAL SYSTEMS

3. AEROBIC DENITRIFICATION IN SOILS

BY ALEXANDER STEVEN CORBET

Department of Biochemistry, University College, London

AND WALTER REGINALD WOOLDRIDGE

Department of Biochemistry, London School of Hygiene and Tropical Medicine

(Received 29 April 1940)

ALTHOUGH it was formerly considered that denitrification in soil was largely an anaerobic process, considerable losses of N from tropical soils, under aerobic conditions, have been reported by, e.g., Meggitt [1923], Annett *et al.* [1928], Wilshaw [1934] and Diamond [1937]. Shutt [1910] showed that large losses of N followed cultivation of Canadian prairie soils, and Buswell & Neave [1927] give a detailed bibliography of the literature on the subject of N losses from soil as a result of denitrification. It can scarcely be doubted that, under suitable conditions, aerobic losses of N₂ from soils in temperate regions occur.

Various explanations have been advanced to account for the large N losses which may take place in tropical soils, particularly when primeval forest is felled and the soil is left exposed to the sun. In these latter circumstances, it is usually supposed that the increased temperature consequent upon the removal of shade results in an acceleration of the normal biological processes involving the decomposition of soil organic matter. There is little evidence to support this explanation, however, and in Wilshaw's pot experiments, the N recovered as nitrate or ammonium salts by leaching with water was less than 13% of the total lost, and the author considered it probable that enormous losses of N₂ occurred. There appears to be no experimental support for the view that soluble nitrogenous material may be leached from the soil in other than insignificant amount.

Losses due to physical changes. We obtained no appreciable losses of N from soil samples submitted to the following experimental conditions:

(1) Exposure of soil to a relatively high temperature in an atmosphere saturated with water vapour did not result in the formation of measurable amounts of soluble N compounds (inorganic or organic) which could be removed by leaching. Over a period of 201 days, the percentage of N changed from 0.174 to 0.165 at 4° and from 0.174 to 0.166 at 32°. (The standard error in all cases was 0.001.) Washing with water failed to effect any significant alteration in the N content of either sample.

(2) Samples of soil were washed with distilled water twice weekly and allowed to dry out at 30–55° between the washings. After 44 days the total N content of the series changed from 0.210 (s.e. 0.001) to 0.197 (s.e. 0.001) %, and 0.004 % of N was recovered in the leachings.

(3) Four samples of fresh soil were packed in glass cylinders and two of them were aerated by an upward current of air for 8 hr. daily over a period of 4 weeks. Each evening both the aerated and the unaerated soils were moistened with

ammonia-free water in amounts just sufficient to ensure that no water ran through the cylinders. On a "dry" basis, the change in total N content was from 0.129 to 0.128 and 0.123 % with the aerated soils, and from 0.129 to 0.128 and 0.126 % with the unaerated soils.

(4) For several reasons, it appeared possible that aerobic losses of N from recently cleared soil in tropical countries might be due to some effect of ultra-violet light. Although both a loss and a gain of N were found in the course of some preliminary experiments in which soil was exposed to a mercury vapour lamp [Corbet, 1935, 1], further work showed that it was rare for an alteration in the total N content of soil to be effected by such a method. We were unable to prove, in fact, that any change was the certain outcome of the action of ultra-violet light, other than the photochemical formation of small amounts of nitrite. Dhar [1935, 1] has reported N fixation by exposure to light of mixtures of cane sugar and sterilized soil in quartz vessels.

Losses due to chemical changes. It has been suggested frequently [e.g. Dhar, 1935, 2], that the chemical disruption of any ammonium nitrite formed in soil may be responsible for the release of N₂, but the studies of Fowler & Kotwal [1924] have shown that losses of N by this means are probably negligible. Jenkins [1933] also considered that there was no experimental evidence to support the view that denitrification results from the chemical decomposition of ammonium nitrite under natural conditions. No N losses occurred when mixtures of sterilized soil (5.0 g.) and aqueous solutions of ammonium sulphate or potassium nitrite (100.0 ml.), contained in a series of flasks, were aerated continuously for 7 days under conditions of sterility, although large losses followed the aeration of similar unsterilized mixtures (Table 1). In these

Table 1. *N losses from soil with added ammonium sulphate and potassium nitrite, during aeration, under sterile and non-sterile conditions*

The figures refer to mg. N contained in the flasks and each is the mean of four results.

(a) Under conditions of sterility								
Before aeration				After aeration				
Kjeldahl-N	Added NH ₃ -N	Added NO ₂ -N	Total N	Kjeldahl-N	NH ₃ -N in liquid	NO ₂ -N in liquid	Total N	
10.3	11.6	8.6	30.5	10.5	11.2	8.6	30.3	
On 4 flasks: Loss of N							0.9	
N liberated as NH ₃							0.8	
Difference							0.1	
(b) Under non-sterile conditions								
Before aeration				After aeration				
Kjeldahl-N	Added NH ₃ -N	Added NO ₂ -N	Total N	Kjeldahl-N	NH ₃ -N in liquid	NO ₂ -N in liquid	NO ₃ -N in liquid	Total N
8.0	12.6	8.6	29.2	11.6	8.0	4.6	0.0	24.2
On 4 flasks: Loss of N							19.9	
N liberated as NH ₃							4.3	
N presumed lost as gas							15.6	

The difference between the NO₂-N content of the 4 flasks before and after aeration was 15.8 mg.

experiments the pH value of the supernatant liquid was 7.1-7.2 at the beginning of the experiment; tests showed that sterile conditions were maintained throughout the experiment with sterilized mixtures. The air entering the system was

ammonia-free (passage through sulphuric acid) and the ammonia liberated from the flasks and carried in the air current was collected and estimated.

Losses due to biological changes. It has been pointed out in the two preceding papers [Corbet & Wooldridge, 1940; Wooldridge & Corbet, 1940], that, in sewage and similar biological systems, the presence of organic matter readily available for the energy needs and growth requirements of heterotrophic micro-organisms leads to synthetic changes accompanied by a proportionate immobilization of ammonia-N. In general, it is only when this process of protein synthesis is completed that nitrification of ammonia-N, if any remains, sets in. In the absence of ammonia-N, however, the conversion of readily available organic matter into more complex forms containing N can be effected at the expense of any nitrite or nitrate present. It was shown that such "organization" of nitrite- or nitrate-N occurs concurrently with losses of N_2 , which may be considerable.

By analogy, the necessary conditions for N losses to occur in soil would appear to be the presence of

- (1) the necessary micro-organisms or their appropriate enzymes,
- (2) organic compounds suitable for synthesis and energy purposes, and
- (3) nitrite and/or nitrate as the most readily available N source.

From the physical and chemical experiments already described, the indications are that the presence of micro-organisms is essential for N losses to occur from soil and that their appropriate enzymes are responsible for this loss.

It was found with fresh samples of two different types of soil that, while the micro-flora present effected the conversion of the ammonia-N in sterilized Reader's medium (25.0 ml.) into organic form without N loss, the substitution of nitrate for the ammonia-N was followed by significant losses (Table 2).

Table 2. *Losses of N from a glucose medium containing ammonium sulphate or potassium nitrate and inoculated with soil extract*

The flasks were incubated at 34° for about 5 weeks.

The figures refer to mg. N in the flasks and each in the mean of 4 experiments.

	Initial N content	Final N content	Significance of difference between initial and final N values		
			<i>n</i>	<i>t</i>	<i>P</i>
(a) With $(NH_4)_2SO_4$					
Forest soil (Sussex)	11.3	11.2	6	1.55	Between 0.2 and 0.1
Calcareous soil (Bucks)	11.3	11.3	6	—	—
(b) With KNO_3					
Forest soil (Sussex)	7.6	5.5	6	3.12	Between 0.02 and 0.01
Calcareous soil (Bucks)	7.6	7.1	6	2.74	Between 0.05 and 0.02

The organic matter in soil in a state of biotic equilibrium consists essentially of ligno-proteins [Norman, 1937], so that there is little or no organic matter readily available for bacterial synthesis: the addition of nitrite or nitrate is not, therefore, accompanied by N losses. The experiments set out in Table 3 show no appreciable N losses when a garden soil (5.0 g.) was aerated continuously for 21 days with added solutions of nitrite or nitrate (100.0 ml.). On the other hand in a similar experiment, when a "special" soil of high organic matter content¹

¹ A "special" soil was prepared by aeration of 250 g. of a similar soil to that used in the experiment detailed in Table 3, in the presence of water, with 25 g. glucose added in 5 biweekly instalments. The supernatant liquid contained no glucose after 2½ weeks when the solid was separated, dried and sieved, and inoculated with fresh soil.

was aerated for 27 days in the presence of added inorganic nitrogenous salts no N loss occurred in the flasks with additional ammonium sulphate, but significant losses accompanied the aeration of the soil samples with additional nitrite or nitrate (Table 4). In the experiments of Tables 3 and 4 no ammonia-N was liberated during the aeration periods.

It will be observed that the carbon/nitrogen ratios are different in the experiments of Tables 3 and 4, but from the preceding papers it will be evident that the C/N ratio is of itself not the factor controlling the N losses, although, in a general way this ratio reflects the proportions of biologically active C and N compounds present. A high ratio in soil probably betokens the presence of a large amount of easily decomposable organic matter of low N content, although such a state of affairs does not normally persist.

Table 3. *N losses during aeration of a normal soil, in presence of added potassium nitrite or potassium nitrate*

The figures refer to mg. N contained in the flasks, and each is the mean of results from duplicate flasks, except those in the second row of "after aeration" results in (A) which refer to a single experiment. (A) refers to the experiment in which N was added in the form of potassium nitrite and (B) to the experiment with added potassium nitrate.

	Before aeration					After aeration					Loss of N
	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	Total N	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	Total N	
(A)	0.0	0.0	0.0	5.6	5.6	0.0	0.0	0.0	5.6	5.6	0.0
	0.0	2.7	0.0	5.6	8.3	0.0	2.6	0.1	5.6	8.3	0.0
	0.0	5.4	0.0	5.6	11.0	0.0	2.5	2.3	6.2	11.0	0.0
	0.0	10.8	0.0	5.6	16.4	0.0	9.8	0.1	6.1	16.0	0.4
(B)	0.0	0.0	0.0	5.6	5.6	0.0	0.0	0.0	5.7	5.7	0.1 (gain)
	0.0	0.0	2.8	5.6	8.4	0.0	0.0	2.5	5.9	8.4	0.0
	0.0	0.0	5.5	5.6	11.1	0.1	0.0	4.2	6.0	10.3	0.8
	0.0	0.0	11.0	5.6	16.6	0.0	0.0	10.5	5.8	16.3	0.3

C/N ratio of soil before aeration in experiments (A) and (B) = 10.6.

Table 4. *N losses during aeration of a "special" soil, in presence of added inorganic nitrogenous salts*

The figures refer to mg. N contained in the flasks and each is the mean of results from duplicate flasks.

N compound added	Before aeration					After aeration					Loss of N
	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	Total N	NH ₃ -N	NO ₂ -N	NO ₃ -N	Organic N	Total N	
No addition	0.0	0.0	0.0	9.9	9.9	0.0	0.0	0.0	10.1	10.1	0.2 (gain)
(NH ₄) ₂ SO ₄	3.2	0.0	0.0	9.9	13.1	0.6	0.0	0.0	12.7	13.3	0.2 (gain)
KNO ₂	0.0	3.4	0.0	9.9	13.3	0.0	0.0	0.0	10.6	10.6	2.7
KNO ₃	0.0	0.0	3.3	9.9	13.2	0.3	0.0	0.0	10.6	10.9	2.3

C/N ratio of soil before aeration in the above experiments = 13.9.

It is possible that such conditions are found in soils in the equatorial belt which are suddenly bereft of forest shade and left exposed to the sun and rain. If, as is claimed, there exists in tropical and subtropical countries either a photochemical [Sarkaria & Fazal-ud-Din, 1933; Dhar & Mukherji, 1935] or a physico-chemical [de Rossi, 1933; 1935] mechanism able to convert organic N into nitrite N and leaving a soil residue of high C/N with excess carbonaceous matter in a form readily available to the micro-flora, the necessary conditions for N liberation would be fulfilled. The process of N impoverishment would be continuous, the nitrite produced during the day being decomposed biologically

during the night with N liberation, until checked by the establishment of vegetation.

We are strongly of the opinion that research into this important problem of N losses by biochemical erosion in soils should be continued by workers in tropical countries and we have put forward our own views at this juncture as we are unable to pursue the matter further.

The analytical procedure followed in this work was the same as that detailed previously by Corbet [1935, 2]. The total N in soil was estimated by Bal's modification of the Gunning-Kjeldahl method, using selenium as a catalyst [Ashton, 1936; 1937]. Removal of nitrite, when present in measurable amount, was found to be a necessary prelude to estimation of the total N, although small amounts of nitrate were "fixed" by the use of reduced iron (Schering-Kahlbaum) as recommended by Ashton.

SUMMARY

It is shown that a biological mechanism exists in soils which is able to effect aerobic losses of N_2 in the presence of

(1) a supply of carbonaceous material suitable for the synthetic and energy requirements of microbial growth, and

(2) nitrite and/or nitrate as the most readily available source of N.

The investigation reported in this and the two preceding papers was carried out as part of the programme of research of the Water Pollution Research Board of the Department of Scientific and Industrial Research, and the results are published by permission of the Department. We wish to express our indebtedness to Prof. J. C. Drummond for his continued interest.

Our thanks are due to the Borough Engineer of Finchley for the regular supplies of sewage used in this work and to Dr W. B. Haines for a supply of soil samples from the Malay Peninsula.

REFERENCES

- Annett, Aiyer & Kayasth (1928). *Mem. Dep. Agric. India, Chem.*, **9**, 155.
 Ashton (1936). *J. agric. Sci.* **26**, 239.
 — (1937). *J. Soc. chem. Ind., Lond.*, **56**, 101 r.
 Buswell & Neave (1927). *Soil Sci.* **24**, 285.
 Corbet (1935, 1). *Biological Processes in Tropical Soils*, p. 104. Cambridge.
 — (1935, 2). *Biochem. J.* **29**, 1094.
 — & Wooldridge (1940). *Biochem. J.* **34**, 1015.
 Dhar (1935, 1). *Acad. Sci. Pres. Address, Allahabad*, p. 10.
 — (1935, 2). *J. Indian chem. Soc.* **12**, 127.
 — & Mukherji (1935). *J. Indian chem. Soc.* **12**, 436.
 Diamond (1937). *Emp. J. exp. Agric.* **5**, 264.
 Fowler & Kotwal (1924). *J. Indian chem. Soc.* **7**, 29.
 Jenkins (1933). *Biochem. J.* **27**, 255.
 Meggitt (1923). *Mem. Dep. Agric. India, Chem.*, **7**, 31.
 Norman (1937). *Biochemistry of Cellulose, the Polyuronides, Lignin, etc.*, p. 181. Oxford.
 de Rossi (1933). *Boll. Sez. ital. Soc. int. Microbiol.* **5**, 132.
 — (1935). *Trans. Third Int. Congr. Soil Sci.* **1**, 135.
 Sarkaria & Fazal-ud-Din (1933). *Indian J. agric. Sci.* **3**, 1057.
 Shutt (1910). *J. agric. Sci.* **3**, 335.
 Wilshaw (1934). *Malay. agric. J.* **22**, 4.
 Wooldridge & Corbet (1940). *Biochem. J.* **34**, 1026.