however that Nelson (1931) evaluated the glucose inhibition of both nitrite formation and nitrate formation in culture by adding glucose to actively nitrifying cultures, and found that 10% glucose inhibited the nitrification by only 4%. It is precisely this method of evaluating inhibition of nitrification, which seems the only valid one, that has been used in the present work.

SUMMARY

1. The effects of various organic materials on the oxidative nitrification process in soil have been tested by a new short-term technique.

2. None of the materials, all of which were tested at 0.1% concentration, markedly inhibited the process.

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The Immobilization of Mineral Nitrogen in Soils by Different Organic Materials

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When an organic carbon source, such as glucose, is percolated through soil together with a source of mineral N, such as nitrate, a heterotrophic population quickly builds up at the expense of both compounds, and the amount of mineral N in the percolate decreases. When the supply of one compound is used up, the heterotrophic proliferation stops and the population begins to decline. The lysis of the dead cells produces ammonium ions, which are nitrified by the soil to nitrate ions, and the nitrate-N concentration begins to rise again. Not all the N initially immobilized is released again, because a certain amount is lost, as a result of its conversion to gaseous nitrogen by the denitrifying bacteria, some of which can function under aerobic conditions (Meiklejohn, 1940).

This nitrogen immobilization cycle in soil has often been studied in pots or in the field, but there seems to have been no detailed study of the biochemical processes involved. The work here reported is a preliminary survey of these processes.

METHODS

The soil percolation technique (Lees & Quastel, 1944) has been used throughout.

Soils were percolated with either NaNO₃ solution alone, or with NaNO₃ solution plus the organic material under test, in the original soil percolation apparatus (Lees, 1946a). Ammonium, nitrite, and nitrate N were determined from time to time in 2 ml. samples of the percolates by the analytical methods already devised (Lees & Quastel, 1946). In fact the nitrite-N and ammonium-N concentrations in the percolates were never found to be more than a few µg./ml., and for simplicity they are excluded from the reported results. When it was necessary to use CO₂-free air, the modified percolation apparatus (Lees, 1946a) was employed, and air was supplied to it through two large KOH bubblers followed by a Sofnolite indicator tube. When extra CO₂ was necessary, it was introduced into the reservoir by means of a bubbler tube coupled to a Microid CO₂ generator. The CO₂ was supplied at the rate of approximately 1 bubble of CO₂/10 bubbles of entrant air.

Two soils have been used: (1) The garden soil (0.4% organic N, pH 7.8) used by Lees & Quastel (1946) (this soil is here described as the allotment soil); (2) Romney Marsh soil (0.2% organic N, pH 8.0) used by Lees (1946b).

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RESULTS

The results of investigations on nine different organic compounds, percolated through allotment soils, are given in Table 1. The pH of the percolates rose about 0.5 during the early stages of the experiment because the immobilization of the anionic NO_3^- released cationic Na^+ . In the later stages the pH again fell to normal as NO_3^- was generated. It will be seen that the initial nitrogen immobilization differed for different organic compounds. The final concentration of nitrate N, eventually built up in the percolate by lysis of the dead heterotrophs, was always less than the nitrate concentration of the nitrate-control percolate. The difference between the nitrate concentration of the control percolate and the nitrate concentration of the test percolate was presumably (at least towards the end of an experiment) some measure of denitrification loss. This loss also differed for different organic compounds. The constancy of the nitrate-N concentration of the control percolates indicated that there was no endogenous-nitrogen immobilization by compounds in the soil itself.

Table 1. *The immobilization of nitrate N by different organic compounds*

(Soil: allotment, 40 g. Percolate: 200 ml. 10^{-2}M-NaNO_3 plus the organic compound at 1.2 mg. C/ml. The minimum nitrate-N concentrations are italicized.)

Organic compound added	Nitrate N ($\mu\text{g./ml. percolate}$) after				
	4 days	8 days	12 days	20 days	24 days
None (control)	150	151	158	150	151
Glycerol	8	12	35	56	57
Glucose	17	20	80	93	104
Sucrose	23	26	81	86	103
K oleate	20	25	69	47	61
K stearate	12	20	32	50	106
None (control)	140	140	140	141	—
Na formate	102	79	116	135	—
Tristearin	135	130	134	129	—
Tributylin	19	50	68	79	—
Benzoic acid	70	60	77	125	—

The magnitudes of the temporary immobilization of mineral nitrogen, and eventual loss of total nitrogen, are not however solely dependent on the type of carbon source. Both increase as the amount

of soil in the soil-percolate system is decreased. Table 2 shows the results from an experiment in which various weights of a Romney Marsh soil were percolated with 200 ml. of 10^{-2}M-NaNO_3 plus glucose at 0.8 mg. C/ml. Marsh soil has good buffering properties, and no marked pH changes of the percolate were noted during the experiment. The same type of result has been obtained with allotment soil (Fig. 1) in the new percolation apparatus (Lees, 1947).

Fig. 1. The immobilization of nitrate N by various weights of allotment soil percolated with 100 ml. 10^{-2}M-NaNO_3 plus glucose at 0.4 mg. C/ml. Curve 1, 20 g. soil; curve 2, 10 g. soil; curve 3, 5 g. soil; curve 4, 2.5 g. soil.

The amount of nitrogen immobilized by a given amount of any one organic material seems to depend to some extent on the amount of free carbon dioxide in the soil percolate system (Table 3). Although the removal of carbon dioxide from the air entering the apparatus made no difference to the amount of nitrogen immobilized by a given amount of any one organic material (presumably because small amounts of carbon dioxide were in any case generated by the metabolic processes of the soil), the addition of carbon dioxide to the air entering the apparatus increased the amount of nitrogen immobilized.

A final point of interest is that the nitrogen-immobilizing mechanism appears to be poisoned by 10^{-3}M-azide , but not by similar concentrations of either iodoacetate or chlorate (Table 4). Un-

Table 2. *The effect of the change of soil weight on nitrogen immobilization and nitrogen loss in Romney Marsh soil percolated with nitrate and glucose*

(Percolate: 200 ml. 10^{-2}M-NaNO_3 + glucose at 0.8 mg. C/ml.)

Weight of soil (g.)	Nitrate N ($\mu\text{g./ml. percolate}$) after								
	1 day	2 days	3 days	4 days	5 days	6 days	9 days	13 days	16 days
2	121	92	97	100	90	71	52	32	43
10	130	114	83	65	68	49	63	59	77
20	138	95	82	75	87	85	87	91	107
40	134	94	80	80	112	108	126	126	144

Table 3. *The effect of CO₂ on nitrogen immobilization*(Weight of soil: 40 g. Percolate: 200 ml. 10⁻²M-NaNO₃ plus organic material at approx. 1.0 mg. C/ml.)

Soil	Organic material	Air supply	Nitrate N immobilized/mg. carbon supplied (μ g.)
Allotment	None	CO ₂ -free + 10% CO ₂	0
Allotment	Glucose	Ordinary	110
Allotment	Glucose	CO ₂ -free	99
Allotment	Glucose	CO ₂ -free + 10% CO ₂	134
Allotment	Lactic acid	Ordinary	108
Allotment	Lactic acid	CO ₂ -free	103
Allotment	Lactic acid	CO ₂ -free + 10% CO ₂	143
Marsh	None	CO ₂ -free + 10% CO ₂	0
Marsh	Lactic acid	Ordinary	67
Marsh	Lactic acid	CO ₂ -free	70, 100, 69
Marsh	Lactic acid	CO ₂ -free + 10% CO ₂	104, 143, 88
Marsh	Malic acid	CO ₂ -free	67, 48
Marsh	Malic acid	CO ₂ -free + 10% CO ₂	100, 67
Marsh	Fumaric acid	CO ₂ -free	61, 51
Marsh	Fumaric acid	CO ₂ -free + 10% CO ₂	67, 65
Marsh	Succinic acid	CO ₂ -free	57
Marsh	Succinic acid	CO ₂ -free + 10% CO ₂	69

Table 4. *The effects of sodium azide, iodoacetate, and chlorate on nitrogen immobilization in allotment soil*(Soil (40 g.) was percolated with 200 ml. 10⁻²M-NaNO₃ with glucose (equivalent to 1.2 mg. C/ml.) and 10⁻³M-azide, iodoacetate or chlorate. Control soil percolated with nitrate and glucose only. Negative control soil percolated with nitrate only.)

Poison added to percolate	Nitrate N (μ g./ml. percolate) after					
	1 day	4 days	6 days	8 days	12 days	15 days
Na azide	144	145	134	101	115	119
Na iodoacetate	107	0	15	10	45	32
Na chlorate	140	16	30	26	54	63
Control soil	110	24	50	56	72	76
Negative control soil	158	160	156	159	159	153

published results obtained by Dr J. H. Quastel and myself have suggested that 10⁻³M-iodoacetate or azide is toxic to nitrification in soil. Results showing the effect of 10⁻³M-chlorate on soil nitrification have already been published (Lees & Quastel, 1945).

DISCUSSION

The results are preliminary but suggest that the immobilization of soil nitrogen by various organic materials can be studied quantitatively by a soil percolation technique.

Both nitrogen immobilization and denitrification loss have been found to vary with the type of organic compound used to bring them about. Moreover, both increase as the percolate: soil ratio is increased. Lemmermann & Wickers (1914) found, by pot experiments, that denitrification in soil was increased as the fluid: soil ratio was increased. It is possible to explain these results on the assumption that a high water content in a soil favours anaerobiosis, and therefore favours denitrification; it is not possible similarly to explain the present results. In the percolation apparatus denitrification and nitrogen immobilization were found to be greatest where the smallest weight, i.e. the shortest column,

of soil was used; in other words, they were found to be greatest where anaerobiosis must have been minimal. One theory that might explain this result is that proliferation of the heterotrophs, denitrifying and other, takes place mainly in the percolate while lysis of the same heterotrophs is mainly accomplished by organisms or other agencies closely bound to the soil surface.

There are reasons for believing that nitrogen immobilization in soil may involve heavy-metal enzymes because the process is poisoned by azide. Either sulphhydryl enzymes do not play any important part in nitrogen immobilization, or iodoacetate is rapidly destroyed in the soil under the experimental conditions, because iodoacetate has, if anything, a stimulatory effect on nitrogen immobilization. The chlorate-sensitive enzyme system that was found in the nitrifying bacteria by Lees & Quastel (1945) does not appear to be involved.

The effect of CO₂ in increasing nitrogen immobilization in soil is interesting because, if the increase is due to CO₂ as CO₂, and not simply to a lowering of the pH at the soil surface (where micro-organisms in general congregate), then CO₂ must be considered as one possible source of organic carbon in soil. The

work of Krampitz, Woods & Werkman (1943) is germane to this point.

It is unfortunately not possible for me to continue this work at the moment. The results are presented as provisional ones that might provide some useful pointers in future investigations on the biochemistry of nitrogen immobilization by soil.

SUMMARY

1. The immobilization of nitrogen in soils by various organic materials has been studied by a soil-percolation technique.

2. The amount of nitrogen immobilization, and the amount of denitrification, induced by an organic compound differs from compound to compound.

3. The effects of enzyme poisons and of carbon dioxide on the immobilization process suggest that some heavy-metal enzyme and some carbon dioxide-assimilative process are involved.

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The Effects of Zinc and Copper on Soil Nitrification

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Although many papers have been published dealing with the effects of trace elements on plants, and on soil processes in the field, there appears to have been no exhaustive study of the effect of trace elements on the microbial processes of the soil. As these processes are an essential part of agriculture, the effect of trace elements is of practical as well as theoretical interest. Hence the work here reported was begun.

METHODS

The soil-percolation technique devised by Lees & Quastel (1946a) for the study of soil metabolism has been used throughout. The soil-percolation apparatus used was the simplified and improved form (Lees, 1947). The influence of either zinc or copper on soil nitrification was assessed by comparing the course of nitrification in a soil percolated with $(\text{NH}_4)_2\text{SO}_4$ solution with the course of nitrification in a soil percolated with a similar $(\text{NH}_4)_2\text{SO}_4$ solution to which a known concentration of either ZnSO_4 or CuSO_4 had been added.

Mineral nitrogen analyses were performed from time to time on samples of the percolates by methods similar to those already described (Lees & Quastel, 1946a). As it was

found early in the work that the conversion of ammonium N into nitrate N was always reasonably quantitative, ammonium-N estimations are not included in the results. Nitrite N was never found in quantities greater than a few $\mu\text{g./ml.}$; nitrite N is therefore bulked with nitrate in the reported nitrate-N figures. Percolate analyses have been converted into ' $\mu\text{g. N/g. soil}$ ' by the methods already described (Lees & Quastel, 1946a).

Copper was estimated in the percolates by the method of Sherman & McHargue (1942). Zinc was estimated spectrophotometrically.

Soils were stored damp in earthenware containers and turned from time to time. Samples of soil were taken from these containers, and air-dried and sieved (4.0–1.0 mm. mesh) before use.

All experiments were conducted with 10 g. soil percolated at 18–21° with 100 ml. of $2.5 \times 10^{-3} \text{M} \cdot (\text{NH}_4)_2\text{SO}_4$, with or without the addition of ZnSO_4 or CuSO_4 . Three soils were used: (1) an allotment soil (0.4% organic N, pH 6.8); (2) a Lincolnshire Fen soil (2.5% organic N, pH 7.0); (3) a Romney Marsh soil (0.2% organic N, pH 7.8).

Stimulated soils (Lees & Quastel, 1946b) were prepared as follows. The soils were first percolated with 100 ml. of $2.5 \times 10^{-3} \text{M} \cdot (\text{NH}_4)_2\text{SO}_4$ until nitrification was complete, washed out two or three times with 50 ml. lots of distilled water, and finally percolated with 100 ml. distilled water for 6 hr. This wash percolate was then discarded and the soils were ready for the experiment proper.

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