

The influence of the oil on nitrification of ammonium sulfate (Table X) was even more depressive. While a marked depression occurred with the unextracted grounds, the oil-free grounds showed no interference with nitrification at the end of 30 days. Reacted coffee grounds had essentially no effect on nitrification of the soil and none of their nitrogen appeared to be nitrified.

Coffee oil added to wheat straw and to dextrose retarded decomposition of these materials. Total carbon dioxide evolution at 30 days was less from the combinations, including 2000 p.p.m. of carbon from each source, than the sums from corresponding individual treatments.

Nevertheless, unextracted grounds gave more carbon dioxide than the extracted grounds. As the oil alone decomposed rapidly, its presence must have made significant contributions to the over-all carbon dioxide evolution from the unextracted grounds, both raw and reacted.

While the effect of raw grounds in reducing ammonification and nitrification is due to their oil content, the oil does not retard decomposition of the grounds.

Conclusions

Reacted sawdust, bagasse, and coffee grounds are more resistant to general decomposition in the soil than the raw materials. The increased resistance was most pronounced qualitatively with sawdust, while quantitatively it was greatest with bagasse. The increased resistance is desirable not only to decrease nitrogen demand but also to prolong persistence of soil-conditioning effects. Nitrogen in the reacted products was more than sufficient to provide an excess over the requirements for microbial decomposition, and the excess should become more or less rapidly available for plant growth. Spent coffee grounds, because of their high nitrogen content, appear to be a promising material to process into a reacted product.

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NITROGEN AVAILABILITY IN SOILS

Rapid Procedure for Estimating Potentially Available Soil Nitrogen under Greenhouse Conditions

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A rapid technique for determining a fraction of the more readily hydrolyzable nitrogen content of soils is described. Data are presented to support the thesis that nitrogen from this fraction was utilized by wheat grown under greenhouse conditions on seven soils of varying organic matter and total nitrogen contents.

CONSIDERABLE EFFORT has been expended to find a procedure for estimating the nitrogen-supplying power of soils, and various biological and chemical methods (7-12) have been proposed. However, most of these procedures are too time-consuming or unreliable for general use. In the absence of a suitable test, nitrogen fertilizer recommendations are arrived at by indirect and often erroneous means, with little or no consideration being given to the appreciable variation in the ability of different soils to supply nitrogen from their organic reserves. An accurate, rapid technique for estimating the potentially available nitrogen content of soils would therefore be useful.

The bulk of soil nitrogen is thought to be combined in organic substances that are highly resistant to decomposition.

but as much as a third of the nitrogen in some soils is in protein form and may be liberated by acid or alkaline hydrolysis (1, 4, 8). As only traces of amino acids have been detected in soils (7), it appears reasonable to assume that proteins are the basic source of the soil nitrogen released by microbial decomposition.

Although plant proteins are known to vary in amino acid composition, most of the protein nitrogen present in soils has lost its original identity as a result of refabrication by soil microbes. Bremner (7) found the amino acid composition of the protein fraction from 10 soils to be remarkably constant, while other investigators (2) have reported only minor variation in the composition of proteins from podsol and prairie soils.

If the protein fraction of soils is of fairly constant composition, and proteins constitute the principal reservoir from which nitrogen is released in soils, a rapid technique for estimating a comparable fraction of the protein content of soils would be useful. Furthermore, the ease of hydrolysis of the protein fraction of soils by chemical means should afford a measure of its rate of hydrolysis by biological means. A procedure is presented for estimating the more readily hydrolyzable nitrogen fraction in soils, and testing the hypothesis that this is the source of soil nitrogen utilized by plants.

Materials and Methods

Preliminary studies indicated that soil may be differentiated on the basis of the amounts of ammonia released by

hydrolysis in either acid or alkali, and that the percentage of total soil nitrogen released by such treatment is dependent upon concentration of the hydrolyzing solution, temperature, and time permitted for hydrolysis. Alkali treatment of soils resulted in the extraction of organic substances that interfered with subsequent colorimetric determination of ammonia. For this reason, alkaline hydrolysis was eliminated from further consideration.

Solutions of hydrochloric and sulfuric acids of various strengths were tested for varying periods of hydrolysis at several temperatures. Since most of the procedures tried resulted in placing soils in the same relative categories to one another, on the basis of the amounts of ammonia released, attention was centered on the development of a simple, rapid technique that would give reproducible results that would reflect the available nitrogen status of soils. The details of the procedure finally adopted are presented below.

Reagents. Sulfuric Acid Solution. Dilute 2.0 ml. of concentrated reagent grade H_2SO_4 to 1000 ml. with distilled water.

Nessler's Reagent. Dissolve 50 grams of KI in 50 ml. of distilled water without heating. Add slowly a saturated solution of $HgCl_2$ until a slight red precipitate persists. Add 400 ml. of clear 36% NaOH solution and dilute to 1000 ml. with distilled water. Let stand overnight and decant into a brown, glass-stoppered bottle.

Procedure. Pass air-dried soil through a 1.0-mm. screen.

Place 1.0 gram of soil in a 100-ml. beaker, add 2.0 ml. of H_2SO_4 solution,

and bring to dryness on a steam bath. Beakers should remain on the steam bath for at least 15 minutes after the soil appears to be dry.

Add 50 ml. of distilled water and 1 drop of 5% $Na_3PO_4 \cdot 12H_2O$, stir thoroughly, and filter through a Whatman No. 2 filter paper that has been washed free of ammonia. (All the filter paper tested has been found to contain ammonia in amounts varying from 0.25 to 0.75 mg. in an 11-cm. sheet.) Remove ammonia by washing the paper twice with 0.5% NaCl solution prior to filtering the soil suspension.

Place 5 ml. of the filtered solution in a test tube, add 0.2 ml. of Nessler's reagent, then shake and let stand for 20 minutes.

Determine transmittance at 420 $m\mu$ in a colorimeter and obtain the NH_4-N content from the standard curve prepared from solutions containing 1, 2, 3, 4, and 5 p.p.m. of NH_4-N , respectively.

This procedure has several advantages. The period of hydrolysis is limited to the time required to evaporate 2 ml. of the acid solution to dryness on a steam bath, and the reaction is effectively stopped at this point. Interference by iron and aluminum in the test for ammonia is kept to a minimum because of low acid concentration employed in hydrolysis and the addition of $Na_3PO_4 \cdot 12H_2O$. Sample size and dilution factors are such that colorimetric determinations for ammonia may be run directly on the extracts. The amounts of nitrogen released as ammonia from soils vary from 25 to 150 p.p.m., a range believed to be well within the limits of probability.

Calibration Studies

Seven representative New Jersey soils,

varying in organic matter content from 1.61 to 7.13%, were selected for correlation studies to determine the relationship between soil nitrogen released by hydrolysis in acid solution and soil nitrogen released to plants during the growth of a crop. The soils were analyzed for organic matter by a modified chromic acid procedure (6), and for total nitrogen by the procedure of Prince (5). Potentially available nitrogen (PAN) contents of soils were determined by the proposed method. Rates of nitrification were estimated after incubation of soils in beakers at 72° F. for 30 days (5). The results obtained are presented in Table I, in which the soils are listed in order of decreasing organic matter and total nitrogen contents.

Greenhouse Studies with Wheat

In order to classify the soils according to their rates of nitrogen release to growing plants, a greenhouse study employing Pennoll wheat as the test crop was conducted. Soils were placed in 6-inch plastic pots (4 pounds per pot) and the pH was adjusted to within the range of 6.5 to 7.0 with dolomitic limestone. All soils received 1.0 gram each

Table I. Pertinent Chemical Characteristics of Soils

Soil	Organic Matter, %	Total N, %	NO_3-N (30 Days), P.P.M.	PAN, ^a P.P.M.
Dutchess silt loam	7.13	0.28	64	96
Washington silt loam	4.60	0.19	99	78
Penn silty clay loam	4.08	0.17	63	65
Nixon loam	2.57	0.10	51	72
Collington sandy loam	2.02	0.08	3	59
Sassafras sandy loam	1.98	0.08	21	48
Lakewood sand	1.61	0.02	1	33

^a Obtained by hydrolysis.

Table II. Yield and Nitrogen Content of First Wheat Crop and PAN Levels of Soils Prior to Seeding

Soil	PAN, P.P.M.	Without Applied N ^a		100 Mg. Applied N ^a	
		Yield, g.	N removed by wheat, mg.	Yield, g.	N removed by wheat, mg.
Dutchess	96	7.5	115	9.5	178
Washington	78	5.7	102	8.1	149
Nixon	72	5.4	72	7.7	132
Penn	65	5.5	68	8.2	135
Collington	59	4.7	51	7.0	113
Sassafras	48	2.9	29	6.1	60
Lakewood	33	1.6	17	4.8	54

^a Average total values per pot from 2 clippings.

Table III. Correlation Coefficients between Soil Nitrogen Fractions and Yield and Nitrogen Content of First Wheat Crop

Soil N Fraction	Wheat	Correlation Coefficient
Organic matter	Yield	0.849 ^a
	N removed by wheat	0.898 ^b
Total N	Yield	0.903 ^b
	N removed by wheat	0.927 ^b
NO_3-N (30 days)	Yield	0.722
	N removed by wheat	0.846 ^a
PAN	Yield	0.978 ^b
	N removed by wheat	0.976 ^b

^a Significant at 0.05 level.

^b Significant at 0.01 level.

Table IV. Yield and N Content of Third Wheat Crop and PAN in Soils before and after Cropping

Soil	PAN, P.P.M.		Yield, G.	N Removed by Wheat, Mg.
	Before	After		
Dutchess	44	37	4.0	41
Nixon	41	46	2.8	27
Collington	37	33	2.1	18
Washington	34	40	3.1	30
Sassafras	34	37	2.4	25
Penn	32	41	2.8	28
Lakewood	30	32	1.0	5

of phosphorus and potassium from KH_2PO_4 and KCl , with half of each soil type receiving 100 mg. of nitrogen from a 3 to 1 mixture of NaNO_3 and $(\text{NH}_4)_2\text{SO}_4$. Each treatment was replicated four times with each soil.

Wheat was planted on August 26, 1957, and thinned to 10 plants to the pot after germination. Wheat was harvested by clipping to approximately 1-inch height on October 25, 1957, and again on January 21, 1958. In order to deplete their nitrogen levels further, second and third plantings of wheat were made on August 27, 1958, and September 15, 1959, on the soils that had received no nitrogen treatment for the first crop. All soils received phosphorus and potassium treatments for second and third crops, but no nitrogen was applied. The second and third crops were harvested in single clippings on January 15, 1959, and February 2, 1960, respectively. Plant samples were dried, weighed, and analyzed for total nitrogen content. Soil samples were taken from the pots after the second and third crops of wheat were removed and analyzed again for potentially available nitrogen content by the proposed procedure. The results obtained from the first and third crops of wheat are shown in Tables II and IV, along with the PAN contents of the soils prior to seeding.

Correlation coefficients between the various soil nitrogen fractions and the yield and nitrogen content of the first planting of wheat were calculated by the procedure described by Snedecor (9) and are shown in Table III.

Discussion of Results

The data of Table I show that the organic matter, total nitrogen, and potentially available nitrogen contents of the soils studied are closely related. Correlation coefficients for any pair of these fractions were significant at the 1% level. Rate of nitrification was less closely related, correlation coefficients between this and the three other soil nitrogen fractions being significant at the 5% level. The average nitrogen content of the organic matter in all soils was 3.82% and the PAN contents averaged 4.88% of the total nitrogen contents. Since organic matter content and total nitrogen content are currently the most generally accepted criteria for estimating the nitrogen-supplying power of soils, the close correlation between these two fractions and

PAN (determined by a more rapid procedure) appears to support the value of the latter determination.

According to Gortner and Holm (3), the ammonia released during the first 4 hours of acid hydrolysis of proteins is derived entirely from acid-amide linkages. Since the period of hydrolysis employed is less than a half hour, the ammonia released includes the small amount normally present in soils, the remainder probably coming from amide nitrogen. When subjected to hydrolysis by the proposed procedure, measurable amounts of ammonia were released from asparagine and urea, but not from aspartic acid, glutamic acid, glycine, or proline, which contain no amide nitrogen.

The nitrogen removed from the soils by the first wheat crop was significantly correlated with all four soil nitrogen fractions considered (Table III), with rate of nitrification having the poorest correlation. The only variables associated with yields that gave correlation coefficients significant at the 1% level were PAN and total nitrogen. In the 15 possible comparisons of pairs of soils having differences in PAN levels of 10 p.p.m. or more (Table II), both yield and amount of nitrogen removed were higher at the greater PAN level.

The application of 100 mg. of nitrogen per pot to the soils resulted in percentage-wise increases in yield of wheat that were in line with the PAN values of the soils (Table II). Applied nitrogen resulted in a 26.6% increase in yield on the Dutchess soil, and a 200% increase on the Lakewood sand. Percentage-wise increases in nitrogen content of the wheat crop, due to applied nitrogen, were somewhat higher than yield increases, and were also generally in line with PAN values.

The average yield per pot from the second wheat crop on all soils was 24% less, and the average amount of nitrogen removed per pot was 65% less than that of the first crop. Since PAN was not determined on the soils prior to planting the second crop, these data will not be discussed further.

Data on the yield and nitrogen content of the third wheat crop, along with the PAN values of the soils prior to seeding and after harvest, are shown in Table IV. Compared to similar values for the first wheat crop, average PAN values of soils were reduced by 44%, average wheat yield by 45%, and average nitrogen content of harvested wheat by 63%.

Wheat plants of the third crop developed symptoms of nitrogen deficiency within 2 weeks of emergence on all soils and these symptoms became more severe with time. This was expected from the uniformly low PAN levels of all soils prior to the seeding of the crop. The fact that the PAN levels of the soils were not changed appreciably by cropping indicates that wheat is unable to obtain sufficient nitrogen for normal growth from soils of such low levels, and is therefore unable to reduce the PAN levels further.

It was not expected that the small differences in PAN levels of the soils, prior to planting of the third wheat crop, would be reflected in differences in yield. However, in the six possible pairings of soils having differences in PAN levels of 10 p.p.m. or more (Table IV), both yield and amount of nitrogen removed were higher at the greater PAN level in every instance.

The data presented indicate that the nitrogen released by the described method of hydrolysis affords a measure of the nitrogen-supplying power of soils that is as reliable as the more time-consuming determinations of total nitrogen or organic matter content. Studies are currently in progress to determine the usefulness of the method in predicting the nitrogen fertilizer requirements of soils under field conditions.

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