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Studies in soil bacteriology. II.

Ammonification in soils and in solutions.

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In an earlier article[1]) we have called attention to the fact that inoculation of soils into solutions gives no adequate criterion of the nitrifying powers of soils, owing to the fact that some nitrifying soils fail entirely to nitrify in solutions, and that when in other cases nitrification occurs to some extent in each medium, the amount of nitrification in solutions is not commensurate with the amount of nitrification by the same soils as soils. The question naturally arises whether a similar condition does not exist regarding the process of ammonification.

Ammonification for the present purpose may be defined as the conversion of organic nitrogen into ammoniacal nitrogen. It is probably the most important of all the nitrogen transformations, and accurate knowledge of the conditions which favor it is of permanent importance.

The studies of ammonification which have been made, and they are very numerous and important, have been conducted by isolating organisms and studying the chemical transformations induced by such pure cultures in solutions of artificial composition, or by throwing in various ways, complexes of species in the form of small amounts of soils, or of suspensions derived from soils, into solutions for the purpose of study.

In view of the importance of the subject and in view of the doubts cast upon the validity of results arrived at by present methods of studying ammonification, a series of experiments was undertaken to ascertain whether reliable conclusions regarding the ammonifying power of a soil can be had by study of its effects when inoculated into solutions.

The bacteriological methods were similar to those described in our earlier paper with exception as to the form of nitrogen used[2]) and may be so understood unless special description is given.

Chemical methods.

To 400 grams of soil were added 10 c. c. of chloroform and water sufficient with that already present in the soil to make 1200 c. c. and the mixture shaken for one hour. After settling somewhat the solution was filtered through a Pasteur-Chamberland filter. An aliquot part was taken. Nitrogen in the form of ammonia was liberated by magnesium oxide, caught in a standard acid solution and titrated with a standard alkaline solution.

When the amount of ammonia was very small is was Nesslerized instead of being titrated.

The amounts are expressed in terms of nitrogen.

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1) Stevens and Withers, Studies in soil soil bacteriology. I. Nitrification in soils and in solutions. (Centralbl. f. Bakt. Abt. II. Bd. XXIII. p. 355.

2) Stevens and Withers, l. c.

Experiments.

Experiment No. 62 was designed to test the relative rapidity of ammonification in soils as compared with solutions. 240 milligrams of nitrogen as peptone, asparagin and cottonseed meal respectively were placed in 500 c. c. Erlenmeyer flasks, 400 c. c. of tap water was added to each and the whole autoclaved at 115° for 15 minutes, then inoculated with 1 c. c. of soil suspension from experiment No. 32 [1]) and allowed to stand 9 days.

240 milligrams of nitrogen as peptone, asparagin and cottonseed meal respectively were also mixed with 400 grams of soil No. 1866. The mixture was autoclaved as above and inoculated with 10 c. c. of soil suspension from experiment No. 32 in such a way as to be about two thirds saturated after inoculation and then allowed to stand 12 days. Analyses at the end of the period gave results indicated in table 1.

Table 1. Experiment 62.
Medium 400 grams, N 240 mgs. Dec. 12, 1907.
Showing a different intensity of ammonification in soils and in solutions.

Conditions						Results	
Sample number	Time of incubation Days	Medium Kind	Inoculum Kind	Inoculum Quantity	Initial nitrogen Form	Ammoniacal nitrogen recovered Per cent	Ammoniacal nitrogen recovered Milligrams per 100 c. c. solution
1938	9	Water	Soil	1 c. c.	Peptone	72.5 [2])	43.5
1939	9	„	Suspension from Exp. 32	1 c. c.	Asparagin	100.0	60.0
1940	9	„	do.	1 c. c.	C. S. M. [3])	36.5	21.9
1955	12	(Sterile soil 1866)	do.	10 c. c.	Peptone	26.6	106.4
1956	12	do.	do.	10 c. c.	Asparagin	32.9	135.6
1957	12	do.	do.	10 c. c.	C. S. M.	23.1	92.4

It is seen here that though the soil culture received 10 times as large an inoculum, i. e. 10 times as many bacteria and stood 3 days longer, the ammonia produced in it from peptone was 36% of that produced in solution, 33% in the case of asparagin and 65% in the case of cottonseed meal.

While the experiment does not admit of definite conclusions, because of the lack of parallelism in duration of the experiment and amount of inoculum, it certainly indicates very strongly that there is a great difference in the ammonifying power of this soil under the two conditions, i. e. 1) when inoculated into solution, 2) when inoculated into soil itself.

Experiment No. 63. This experiment was precisely like experiment No. 62 except that the inoculum was greenhouse soil, No. 1866.

The results are shown in table 2.

1) Stevens and Withers l. c.
2) The ammonia originally present was determined and found to be of negligable quantity.
3) C. S. M. signifies cottonseed meal.

Table 2. Experiment 63.
Medium 400 grams, N 240 mgs., Dec. 7, 1907.
Inoculum: suspension of soil 1866.
Showing a different intensity of ammonification in soils and in solutions.

Conditions					Results	
Sample number	Time of incubation Days	Medium Kind	Inoculum Quantity	Initial nitrogen Form	Ammoniacal nitrogen recovered Per cent	Milligrams per 100 c. c. solution
1941	9	Water	1 gram	Peptone	79.1 [1])	47.5
1942	9	„	1 „	Asparagin	78.1	46.9
1943	9	„	1 „	Cottonseed meal	39.8	23.9
1958	12	St. soil 1866	10 grams	Peptone	22.4	86.9
1959	12	„ „ „	10 „	Asparagin	28.7	114.8
1960	12	„ „ „	10 „	Cottonseed meal	17.5	70.0

Conditions similar to those found in the previous experiment are noted. Based upon the percent of ammonia found; peptone and asparagin are ammonified much more vigorously in solutions than in soil cultures; cottonseed meal likewise, but not with so marked a difference, there being 28%, 36%, 44%, as much ammonia produced in soil as in solution with peptone, asparagin, cottonseed meal respectively.

Experiment No. 88. 120 milligrams of nitrogen as cottonseed meal were weighed into 300 c. c. Erlenmeyer flasks, 200 c. c. of tap water added and the whole sterilized.

120 milligrams of nitrogen as cottonseed meal were also mixed with 200 grams of each soil to be tested, flasked and sterilized.

100 grams of the soil to be tested, were well shaken with 200 c. c. of water and 1 c. c. of the suspension was added to each flask of the solution. 1 c. c. of the suspension was also added to 20 c. c. of sterile water, mixed, and this diluted suspension was poured into the sterile soils in the flasks and thoroughly mixed. Soils Nos. 1667, 1867, 1783, 1784, 1931 and 2069 were tested in this manner thereby bringing into 200 grams of solution the same number of organisms and the same species of organisms as were mixed with 200 grams of the soil culture. This arrangement also provided that the organisms in soil media were in soils of the same quality as the soils from which these organisms were derived (identically the same soil except for sterilization). The cultures were incubated 7 days, then analyzed.

The results are given in table 3.

(See Table 3 p. 779.)

Here again, basing upon the percent of ammonia produced, there is a great difference in the intensity of ammonification in soils and in solutions. In five of the six samples of soil tested, ammonification was again greater in the solution than in the soil cultures, while in one instance ammonification was greater in the soil culture.

Not only is there a difference in rapidity and absolute quantity of ammonification, but the rank of the soils as ammonifiers is different according to whether their ammonifying powers are measured in soils or in solutions.

1) Original ammonia was of negligible amount.

Table 3. Experiment 88.

Medium 200 grams, N 120 mgs. as C. S. M. Time 7 days.
Comparing six different soils regarding ammonification in soil and solution.

Sample number	Conditions: Medium Kind	Conditions: Inoculum Suspension of soil	Results: Ammoniacal Per cent	Results: Nitrogen recovered Milligrams per 100 c. c. solution
2152	Solution	1667	18.2 [1])	10.9
2153	Soil	1667	9.1	18.2
2154	Solution	1867	34.3	20.6
2155	Soil	1867	18.9	37.8
2156	Solution	1783	23.1	13.9
2157	Soil	1783	14.7	29.4
2158	Solution	1784	37.1	22.3
2159	Soil	1784	22.4	44.8
2160	Solution	1931	24.5	14.7
2161	Soil	1931	18.9	37.8
2162	Solution	2069	24.5	14.7
2163	Soil	2069	30.8	61.6

Compared in soil cultures their rank as ammonifiers is:	Soil No.	Compared in solution their rank is:
1.	2069	3.
2.	1784	1.
3.	1867	2.
3.	1931	3.
5.	1783	5.
6.	1667	6.

Experiment 82. To test relative ammonifying powers of various pure cultures in soil and in solution.

The tests above recited were made with soils, composites of bacterial species. It was deemed advisable to ascertain how far the conclusions indicated by these tests hold true when pure cultures of species are employed. To this end 15 pure cultures of ammonifying organisms were obtained from the most promising sources by plating from soils, cow manure, horse manure and from two labeled cultures from the laboratory stock. All of these pure cultures were inoculated into broth.

120 milligrams of nitrogen as cottonseed meal were weighed into each of sixteen 300 c. c. Erlenmeyer flasks and 200 c. c. of tap water added. An equal amount of cottonseed meal was mixed with 200 grams of soil in each of the sixteen flasks of the same size. All were autoclaved for one hour. Both soils and liquid media were inoculated with 1 c. c. of broth culture. To inoculate the soil flasks, tubes with 20 c. c. of sterile water were first inoculated with 1 c. c. of the broth culture and this dilution poured upon the soil and mixed with it. Ammonia was determined at the end of the fourth day. The results are given in table 4.

1) Original ammonia was of negligible amount.

Table 4. Experiment 82.

Medium 200 grams, inoculum 1 c. c., N 120 mgs. as C. S. M. Time 4 days. March 16, 1908.

Comparing ammonification of pure cultures in soil and in solution.

Conditions			Results			
			Ammoniacal nitrogen recovered			
Sample number	Medium Kind	Inoculum Kind	Per cent	Difference in favor of soil	Milligrams per 100 c. c. solut.	Difference in favor of soil
2077	Sterile 1867	Organism 1	12.6		25.2	
2078	Water	„ 1	24.0	— 11.4	14.4	10.8
2079	Sterile 1867	„ 2	7.0		14.0	
2080	Water	„ 2	12.1	— 5.1	7.3	6.7
2081	Sterile 1867	B. subtilis	25.2		50.4	
2082	Water	„ „	11.0	14.2	6.6	43.8
2083	Sterile 1867	Organism 4	1.4		2.8	
2084	Water	„ 4	0.0	1.4	0.0	2.8
2085	Sterile 1867	„ 5	37.2		74.4	
2086	Water	„ 5	19.6	17.6	11.8	62.6
2087	Sterile 1867	„ 6	23.8		47.6	
2088	Water	„ 6	18.7	5.1	11.2	36.4
2089	Sterile 1867	B. mycoides	2.8		5.6	
2090	Water	„ „	5.8	— 3.0	3.5	2.1
2091	Sterile 1867	Organism 8	22.4		44.8	
2092	Water	„ 8	15.9	6.5	9.5	35.3
2093	Sterile 1867	„ 9	18.2		36.4	
2094	Water	„ 9	15.6	2.6	9.4	27.0
2095	Sterile 1867	„ 10	23.8		47.6	
2096	Water	„ 10	28.7	— 4.9	17.2	30.4
2097	Sterile 1867	„ 11	34.3		68.6	
2098	Water	„ 11	28.5	5.8	17.1	51.5
2099	Sterile 1867	„ 12	3.5		7.0	
2100	Water	„ 12	3.5	0.0	2.1	4.9
2101	Sterile 1867	„ 13	32.9		65.8	
2102	Water	„ 13	19.6	13.3	11.8	54.0
2103	Sterile 1867	„ 14	21.7		43.4	
2104	Water	„ 14	18.9	2.8	11.3	32.1
2105	Sterile 1867	„ 15	9.8		19.6	
2106	Water	„ 15	3.0	6.8	1.8	17.8
2107	Sterile 1867	Soil susp. 1867	27.3		54.6	
2108	Water	„ „ 1867	16.8	10.5	10.1	44.5

Here, basing on percent of ammonia, ammonification was greater in soils than in solutions in the large majority of cases. In only three instances, was ammonification greater in solutions than in soils. Basing upon amount converted per cubic centimeter of solution, the ammonification was greater in soil in every instance. Basing upon percent converted the rank in ammonifying power is as follows:

Rank teste in soils		Rank teste in solution	Source of organism
1.	2085 (6)[1]	4.	Horse manure
2.	2097 (8)	2.	„ „
3.	2101 (2)	4.	Soil
4.	2107 (8)	8.	Cow manure
5.	2081 (2)	12.	Soil
6.	2087 (8)	7.	Cow manure
6.	2095 (6)	1.	Manure
8.	2091 (2)	9.	Soil
9.	2103 (4)	6.	„
10.	2093 (4)	10.	„
11.	2077 (8)	3.	B. subtilis-culture
12.	2105 (6)	15.	Soil
13.	2079 (80)	11.	B. mycoides-culture
14.	2099 (00)	14.	Horse manure
15.	2089 (90)	13.	Cow „
16.	2083 (4)	16.	„ „

(See Table 5 p. 782, 783.)

5 species hold the same rank in soil and solution; 2 have changed one rank, 2 two ranks, 4 three ranks, 1 five, 1 six and 1 seven ranks; some assuming a higher rank in soils, others higher in solutions.

It is seen from the tabulation that not only is the absolute ammonifying power different in the two media but also that the rank of the organisms as ammonifiers is greatly altered by the mode of test[2].

Experiment 105, May 25, 1908. To further test the same point, whether tests made in solutions are fair criteria by which to judge either the absolute or relative ammonifying power of soils, experiments Nos. 105, 200 and 201 were made.

In these experiments the cultures were all made in duplicate and with checks. Four soils were used and with each the ammonifying power was tested in three ways:

1. By inoculation of a suspension into a sterile mixture of cotton-seed meal and water (i. e. solution condition).

2. By inoculation of a suspension into sterile soils.

3. By adding the nitrogenous material directly to the live soil. The cultures were kept 10 days, then analyzed.

The results are given in table 5, and summarized in table 6.

(See Table 6 p. 783.)

It is seen here that at the end of 4 days soils Nos. 1931 and 2069 ammonified most vigorously in natural condition. next in sterile soil + suspension and least in the watery suspension: Nos. 1784 and 1667 most in watery suspension. It appears then that some soils will give a maximum test of ammonifying power in the liquid medium, others in the soil medium; that the test made in the liquid is not a fair criterion of ammonifying power. Though the discrepancies are not so great as in the case with the phenomenon of nitrification, they are sufficient to change the rank of soils in ammonifying power, as for example, in the above case where soil 1931 ammonified more than three times as rapidly in soil as in solution as did also soil 2069; while soil 1784 ammonified a

1) Parentheses show termination of number of corresponding soil tested in solution.

2) While the analyses of all samples were not completed on the same day, the analyses of all cultures inoculated with the same soil were made at the same time. Therefore while the actual rank of these cultures as ammonifiers is not given here, the general conclusion drawn is legitimate.

Table 5. Experiments 105, 201.

Medium 200 g, inoculum 1 c. c., N 120 mgs. as C. S. M. May 25, 1908.
Showing ammonification in soils and in solutions.

Conditions				Results			
				Ammoniacal nitrogen recovered			
Sample number	Time of incubat. days	Medium Kind	Inoculum Kind	Per cent	Net	Milligrams per 100 c. c. solution	Net
2285	10	Water	Suspension 1784	29.00		17.4	
2286	10	„	„ „	29.40		17.6	
aver.				29.20		17.5	
2287	10	„	—	2.34	26.86	1.4	16.1
2288	10	Sterile soil	Suspension 1784	21.01		84.0	
2289	10	„ „	„ „	22.40		89.6	
aver.				21.31		85.2	
2290	10	„ „	—	2.10	19.21	8.4	76.8
2291	10	Live soil 1784	—	22.40		89.6	
2292	10	„ „ „	—	23.10		92.4	
aver.				22.75		91.0	
2293	10	Sterile soil 1784	—	1.40	21.35	5.6	85.4
2294	10	Water	Suspension 1667	26.20		15.7	
2295	10	„	„ „	27.10		16.3	
aver.				26.65		16.0	
2287	10	„	—	2.34	24.31	1.4	14.6
2296	10	Sterile soil 1784	Suspension 1667	9.80		39.2	
2297	10	„ „ „	„ „	12.60		50.4	
aver.				11.20		44.8	
2298	10	„ „ „	—	0.00	11.20	0.0	44.8
2299	10	Live soil 1667	—	0.70		2.8	
2300	10	„ „ „	—	17.50		70.0	
aver.				9.10		36.4	
2301	10	Sterile soil 1667	—	0.00	9.10	0.0	36.4
2372	4	C. S. M. + H_2O	Suspension 2069	11.20			
2373	4	do.	„ „	9.10			
aver.				10.15	8.98	6.1	5.4
2374	4	do.	—	1.17		0.7	
2375	4	Sterile soil 1867	Suspension 2069	23.81			
2376	4	„ „ „	„ „	23.11			
aver.			—	23.46	18.56	46.9	37.1
2377	4	„ „ „	—	4.90		9.8	
2378	7	Live soil 2069	—	32.21			
2379	7	„ „ „	—	30.11			
aver.				31.16	27.66	72.0	63.9
2380	7	Sterile soil 2069	—	3.50		8.1	
2390	7	C. S. M. + H_2O	Suspension 2069	14.24			
2391	7	do.	„ „	23.57			
aver.			—	18.91	18.21	11.3	10.9
2392	7	do.	—	0.70		0.4	
2393	7	Sterile soil 1867	Suspension 2069	28.01			
2394	7	„ „ „	„ „	24.51			
aver.				26.26	23.46	52.5	46.9
2395	7	„ „ „	—	2.80		5.6	
2396	7	Live soil 2069	—	32.91		81.7	73.6
2397	7	„ „ „	—	37.81		8.1	
aver.				35.36	31.86		
2380	4	Sterile soil 2069	—	3.50			

Table 5 continued. Experiment 200. July 30, 1908.

Conditions				Results			
Sample number	Time of incubat. days	Medium Kind	Inoculum Kind	Ammoniacal nitrogen recovered Per cent	Net	Milligrams per 100 c. c. solution	Net
2363	4	C. S. M. + water	Soil suspension 1931	6.77			
2364	4	do.	„ „ „	9.10	7.24	4.8	4.3
aver.				7.94		0.5	
2365	4	do.	—	0.70			
2366	4	Sterile soil 1867	Soil suspension 1931	25.21			
2367	4	„ „ „	„ „ „	23.81	17.51	45.0	35.0
aver.				24.51		10.0	
2368	4	„ „ „	—	7.00			
2369	4	Live soil 1931	—	24.51			
2370	4	„ „ „	—	26.60	23.46	54.7	50.2
aver.				25.56		4.5	
2371	4	Sterile soil 1931	—	2.10			
2381	7	C. S. M. + water	Soil suspension 1931	4.43			
2382	7	do.	„ „ „	13.07	8.05	5.3	4.8
aver.				8.75		0.5	
2383	7	do.	—	0.70			
2384	7	Sterile soil 1867	Soil suspension 1931	28.70			
2385	7	„ „ „	„ „ „	28.70	23.10	57.4	46.2
aver.				28.70		11.2	
2386	7	„ „ „	—	5.60			
2387	7	Live soil 1931	—	24.50			
2388	7	„ „ „	—	22.41	22.06	50.2	47.2
aver.				23.46		3.0	
2389	7	Sterile soil 1931	—	1.40			

Table 6. Summary of table 5.

Nos. 105, 200, 201, N. 120 mgs as C. S. M.

Medium	Soil inoculum	Days	Net nitrogen as ammonia	Days	Net nitrogen as ammonia
CSM and H_2O	1931	4	7.24 %	7	8.05 %
Sterile soil 1867	1931	4	17.51 „	7	23.10 „
Live 1931		4	23.46 „	7	22.06 „
CSM and H_2O	2069	4	8.98 „	7	18.21 „
Sterile 1867	2069	4	18.56 „	7	23.46 „
Live 2069		4	27.66 „		31.86 „
CSM and H_2O	1784	10	26.86 „		
Sterile 1784	1784	10	19.21 „		
Live 1784		10	21.35 „		
CSM and H_2O	1667	10	24.32 „		
Sterile 1784	1667	10	11.20 „		
Live 1867			9.10 „		

trifle better in solution than in soil. It is apparent that a proper comparison of the actual field ammonifying power of these three soils could not be had by comparing their ammonifying power in solutions. It is to be noted also that in the original data from which these conclusions are drawn determinations were made in duplicate with control cultures and for the most part with excellent agreement between duplicates.

Experiment 209. Aug. 20. 1908. An experiment similar to the preceding was also made with pure culture of several laboratory species and of three "wild" forms, Nos. 5, 7 and 11 isolated from various sources.

To inoculate, 1 c. c. of a 48 hour old broth culture was added to 20 c. c. of sterile water and poured into the soil flask or into the liquid culture, all in strictly parallel manner. The results are presented in table 7.

Table 7.

Test of pure cultures for ammonification in soil and in solution. August 20, 1908. Time, 7 days. Initial nitrogen 120 mg as C. S. M. Inoculum 1 c. c. of 48 hour broth cultures.

Sample number	Conditions: Medium: Kind	Conditions: Medium: Amount in c. c.	Conditions: Inoculum: Kind	Results: Ammoniacal Nitrogen recovered: Per cent: Found	Per cent: Average	Excess in favor of soil	Excess in favor of solut.	Milligrams per 100 c.c. sol.: Found	Milligrams per 100 c.c. sol.: Average	Excess in favor of soil	Excess in favor of solut.
2437	Sterile 1867	200	B. subtilis	16.84	16,14			33.7	32.3		
2438	„ 1867	200	„ „	15.44		3.91		30.9		7.8	
2439	Water	60	„ „	11.41	12.23			22.8	24.5		
2440	„	60	„ „	13.05				26.1			
2441	Sterile 1867	200	B. mycoides	8.42	7.72			16.8	15.4		
2442	„ 1867	200	„ „	7.02		6.22		14.0		12.4	
2443	Water	60	„ „	0.52	1.50			1.0	3.0		
2444	„	60	„ „	2.48				5.0			
2445	Sterile 1867	200	B. megatherium	14.38	14.21			28.8	28.4		
2446	„ 1867	200	„ „	4.04		9.54		28.1		19.0	
2447	Water	60	„ „	4.21	4.68			8.4	9.4		
2448	„	60	„ „	5.14				10.3			
2449	Sterile 1867	200	Organism 5	8.03	7.26			16.1	14.5		
2450	„ 1867	200	„ 5	6.49		6.77		13.3		13.5	
2451	Water	60	„ 5	0.37	0.49			0.7	1.0		
2452	„	60	„ 5	0.61				1.2			
2453	Sterile 1867	200	„ 7	11.16	10.85			22.3	21.7		
2454	„ 1867	200	„ 7	10.53			14.87	21.1			29.7
2455	Water	60	„ 7	25.27	25.72				51.4		
2456	„	60	„ 7	26.16							
2457	Sterile 1867	200	„ 11	12.63	11.56			25.3	23.1		
2458	„ 1867	200	„ 11	10.49			13.15	21.0			26.3
2459	Water	60	„ 11	24.28	24.71			48.6	49.4		
2460	„	60	„ 11	25.13				50.3			

As with the composite inoculations of the last experiment some species are seen to ammonify more vigorously in solutions other species in soils.

Organism No. 5 produced less than 7 % as much ammonia in solution as in soil; B. megatherium 32 %; B. mycoides 19 %. While organism No. 7 produced only 42 % as much ammonia in soil as in solution organism No. 11 gave 46 %.

Ranking the organisms according to their ammonifying power in solution we have; No. 7, No. 11, subtilis, megatherium, mycoides,

No. 5; in soil, subtilis, megatherium, No. 11, No. 7, mycoides, No. 5.

Thus it is seen that neither the absolute nor the relative ammonifying power of these organisms for soils can be ascertained by testing them in solutions.

Summary and Conclusions.

1. Some bacterial soil complexes ammonify faster in solutions.
2. Some bacterial soil complexes ammonify faster in soils.
3. Some pure cultures of organisms ammonify faster in soils, others faster in solutions.
4. The rank of soils, bacterial soil complexes, or of pure cultures is different as measured in soils or in solutions.

The general conclusion stands out clearly as it did regarding nitrification that neither for soils nor pure cultures of organisms can the ammonifying power be adequately determined by testing in solutions; that not even the relative ammonifying power of two soils or two organisms can be determined with certainty by the method of testing in solutions. While there is not so great a discrepancy between results by the solution and the soil method as regards ammonification as obtains regarding nitrification still the discrepancy is sufficient to necessitate using soil as the medium in which to test ammonifying powers in cases where any degree of accuracy is desired and to check all determinations by soil cultures.

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