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On the Behavior of Nitrate in Paddy Soils.

BY

G. DAIKUHARA AND T. IMASEKI.

A great many manuring experiments carried out by different authors prove that under certain conditions nitrate nitrogen is superior to ammonia nitrogen, while under other conditions the reverse is true. It is to be regretted, however, that the different conditions, as nature of soil, its chemical composition and the character of the manure whether acidic or alkaline or neutral, have not always been stated, since this knowledge would have assisted much in recognising certain regularities.

For those tropical and subtropical countries, in which agricultural crops are raised in paddy soils or swamps, the question is also of vital importance, which of the two sources of nitrogen would be the more favorable. The conditions in the paddy soil for nitrification and denitrification are very different from those in the dry land soil. In the first place, the transformation of ammonia into nitrite by the nitroso-bacterium is very much depressed not only on account of less air penetrating into the soil, but also on account of much organic matter, which is by no means oxidized as easily as in dry land, and which according to Winogradsky, depresses the action of the nitrite-microbium; but also for the nitrate-microbium the conditions are very unfavorable, since its action is very much depressed by traces of ammonia. Loehnis¹⁾ however declared that only in the form of carbonate, ammonia is injurious for the nitrate-microbium not in the form of neutral salts. The paddy soil for rice culture in Japan has always been manured chiefly with excrements, fish manure, oil cakes, and frequently green manure; superphosphate was applied occasionally as a mere supplement to

1). Chem. Ztg. Repertorium 1925, I, 5.

those manures. These conditions being favorable for denitrification might lead to much loss, if nitrate were applied as a part of the nitrogen manure. Until recently however no experiments with plants cultivated in swamps have been made. It was Prof. M. Nagaoka¹⁾ who first carried out a number of careful experiments with plants cultivated by Japanese farmers in swamps as viz. rice, arrowhead (*Sagittaria sagittaeifolia*) and *Juncus effusus*. *Sagittaria* bulbs are used as food and *Juncus* is used for the manufacture of rugs. The Plants manured with nitrate remained pale in color and small in size. With rice the yield of the nitrate plants was only in one case a little above that of the control plants, in most cases, however, far below; in one case, where the soil received no lime and the ratio of 150 Kg N p. ha., the yield of the nitrate plants fell to about 2% of that of ammonia plants.

With *Juncus* the yield of the nitrate plants varied from 4.7-66% of the ammonia plants provided with equal doses of nitrogen. For *Sagittaria* the result was still more unfavorable. It is very remarkable that with *Juncus* the harvest of the nitrate plants diminished gradually with the increased applications of the nitrate.

Some experiments²⁾ carried out by one of us (Daikuhara) with rice plants in sand culture showed that the availability of nitrate is 42% of that of ammonia nitrogen. The average ratio for the manurial value of ammonia nitrogen to that of nitrate nitrogen calculated from different results of experiments carried out in our central and branch Stations during the last few years is 100 : 47.

As to the reason why the paddy plants cannot utilize nitrate nitrogen so well as ammonia nitrogen, Nagaoka³⁾ has proposed the two following factors :

1). Bull. College of Agric., Imp. University, Tokyo, Vol. VI., No. 3.

2). This Bulletin Vol. I., No. 1.

3). Nagaoka has also ascribed the pale yellowish color of the nitrate plants to the physiological influence of accumulated nitrate, but according to our observations the pale yellowish color appears in the first period of growth, 2-3 weeks after the application of nitrate, and recovers afterwards. It is very probable, therefore, due to the poisonous action of nitrite formed by the reducing action of certain bacteria.

1. Paddy plants do not accumulate a sufficient quantity of sugar in the leaves to convert all of the nitric acid absorbed into protein.

2. In paddy soils, denitrification and also formation of poisonous nitrites may take place.

As to the first we have instituted some tests. It might be assumed that an injurious accumulation of nitrate may take place in the leaves of the rice plants manured with nitrate but as to this there is no positive proof yet. But the result of our analysis shows practically no difference not only between the leaves of paddy and dry land rice, but also between those of the paddy rice applied with ammonium sulphate and sodium nitrate as shown in the following tables :

A. Sugar content in the leaves of the paddy and dry land rice.

Kind of rice plant.	Name of varieties.	Period of Growth.	Glucose %	Cane sugar %	Total sugar %
Paddy rice	Shin-shu ...	Before flowering	1.19	0.48	1.67
		Milky ripening	2.04	0.74	2.78
		Yellow ripening	1.94	0.24	2.18
	Suga-ippon.	Young leaves	0.89	0.48	1.37
		Before flowering	1.21	0.64	1.85
		Milky ripening	2.48	1.15	3.63
		Yellow ripening	2.47	0.55	3.02
	Dry land rice	Oiran ...	Before flowering	1.19	0.56
Milky ripening			2.02	0.85	2.87
Yellow ripening			2.16	0.56	2.72
Kiushu ...		Young leaves	0.89	0.52	1.41
		Before flowering	1.29	0.69	1.98
		Milky ripening	2.00	1.10	3.10
		Yellow ripening	2.59	0.46	3.05

B. Sugar content in the leaves of paddy rice with different manures.

Kind of Manures.	Period of Growth.	Glucose %	Cane sugar %	Total sugar %
Ammonium sulphate	Young leaves	0.99	0.33	1.32
	Before flowering	1.60	0.56	2.16
	Milky ripening	1.56	0.66	2.22
Sod'um Nitrate	Young leaves	0.87	0.47	1.34
	Before flowering	1.35	0.50	1.85
	Milky ripening	1.36	0.75	2.11

Thus we see that the first assumption by Nagaoka was not justified.

Some tests were made by Nagaoka with regard to the second cause, rendering however further observations desirable and we have therefore further studied the behavior of swamp plants towards nitrate, being certainly of considerable theoretical and practical importance.

With the denitrifying organisms in a wide sense the following groups may be distinguished after Jensen¹⁾:

- “1. Reduktion von Nitraten zu Nitriten und Ammoniak (Salpeterreduktion).
2. Reduktion von Nitraten zu Nitriten und niedrigeren, gasförmigen Stickstoff-Sauerstoffverbindungen (N_2O und NO).
3. Reduction von Nitraten und Nitriten unter Abspaltung elementaren Stickstoffes (Denitrifikation im engeren und eigentlichen Sinne).
4. Umbildung des Salpeterstickstoffes in organische Verbindungen (Salpeterassimilation).
5. Freiwerden von Stickstoff bei der Fäulnis organischer Stickstoffverbindungen.”

Denitrification will proceed much more energetically in moist land than in dry land and also nitrates will be reduced to nitrites more

1). Lafer, Handb. d. Techn. mycologie Band III., S. 182.

energetically by peculiar bacteria of the paddy soil, and these nitrites may persist for a time before they are further reduced to ammonia. Certain bacteria reduce nitrates very quickly to ammonia so that the intermediary step, the formation of nitrites, can not be recognized. Certain other bacteria, however, produce considerable quantities of nitrites from nitrates. Nitrites however show a strong poisonous character, since they act very powerfully on the amido-groups and hence will also attack the amido-groups of proteins and thus kill the living protoplasm.

In order to decide whether denitrification takes place extensively in paddy soil the following experiments were made :

LABORATORY EXPERIMENT. I.¹⁾

One hundred g of soil were placed in an Erlenmeyer's flask of 300 cc. capacity and added 150 cc. of a solution containing 2 g of sodium nitrate and 0,2 g of K_2HPO_4 which liquid kept the soil covered in a thin layer. A humy soil from our experimental field, a sandy loam soil from Kinai Branch Station and a heavy clayey soil from Kaga Province served for this experiment. The experiment was started on March 23, 1905; the flasks were shaken every day and tested from time to time for nitrite by the reaction of Griess. The room temperature during this experiment was 16–26°C. After 24 hours a very decisive reaction for nitrite was already observed with the humus soil, much less than in the sandy soil, and only a trace was found in the clayey soil. After 5 resp. 6 days, the maximum point of formation of nitrite (0,003 resp. 0,002% KNO_2)²⁾ was attained in the humy and the sandy soil, whereupon the nitrite decreased, disappearing after 3 resp. 4 weeks, much nitrate still remaining in the solution. In the clayey soil, however, the reduction of nitrate was very slow and the maximum point was reached (0,01% nitrite) after 4 weeks; a little nitrite was still present even after 5 months. From the start of the experiment ammonia was occasionally tested for and reactions obtained in all of the

1). Laboratory experiment I, II, III, IV, V and VI (d) were carried out by G. Daikuhara, and Laboratory expt. VI (a), (b) and (c) by T. Imaseki.

2). By colorimetric determination.

flasks, especially with the sandy soil, somewhat less so with the clayey soil and still weaker in the humus soil. With the humus and sandy soils a little sodium acetate was added to accelerate the bacterial action, after the nitrite reaction had disappeared. Thus again a gradual increase of the nitrite was observed and finally it reached a concentration of 0.1% resp. 0.05% nitrite 12 days after that addition, much foam being developed. No ammonia was observed in this case.

Further to ascertain whether the reduction of nitrate was due to bacteria, the mixture of soil and the nitrate solution mentioned above, was sterilized with steam, with chloroform and with mercuric chloride. Occasional testing for nitrite showed the absence of nitrite even after several weeks. This proves clearly that the reduction of nitrate in soils in the paddy state certainly is due to the action of denitrifying organisms.

LABORATORY EXPERIMENT. II.

Since in Japan much organic manure is commonly used in the paddy field, it is of vital importance to observe the influence of organic matter upon the reduction of nitrate in the paddy state. 150 cc. of the following nitrate solution was mixed with 100 g of air dry soil and kept at a temperature of 30–32°C. It was found that all the nitrate was reduced entirely after 48 hours.

Na-acetate (or glycerine)	0.5 %
NaNO ₃	0.2 %
K ₂ HPO ₄	0.2 %
MgSO ₄ + 7H ₂ O	0.02 %

On April 15, 1905, 100 g of the three different soils above mentioned were mixed with the same nitrate solution containing glycerine and kept at room temperature (13–26°C). The occasional tests for nitrite and ammonia gave the following results :

(a). Test for nitrite.¹⁾

From the start.	Humy clay soil.	Sandy loam soil.	Clay soil.
After 3 days	0.0013% KNO ₂	0.004% KNO ₂	0.002% KNO ₂
„ 4 „	0.0010% „	0.005% „	0.003% „
„ 6 „	0.0033% „	0.005% „	0.007% „
„ 9 „	trace	trace	0.0008% „
„ 12 „	0	0	trace

(b). Test for ammonia.

From the start.	Humy clay soil.	Sandy loam soil.	Clay soil.
After 3 days	little	moderate	moderate.
„ 4 „	„	„	„
„ 6 „	„	„	„
„ 9 „	„	„	„
„ 12 „	trace	trace	little

The above result shows that by the presence of a certain amount of organic matter the reduction of nitrate in the paddy soil is much accelerated and all the nitrate added (0.2%) reduced entirely after 9-12 days even at room temperature in spring and after 2 days when kept at 30-32°C.

LABORATORY EXPERIMENT. III.

Former experiments carried out by various investigators show that fresh stable manure favors denitrification more than a well rotten one, but in regard to fresh and rotten oil cakes no special observations seem to have been made, therefore we have made the following experiment:

(a). On Aug. 14, 1905, 100 g of soil from our Station at Nishigahara 2 g of rape cake (1) and 5 cc. of glycerine (2) and 150 cc. of the nitrate

1). The amount of nitrite was also here approximately determined colorimetrically by Griess test. A standard solution of pure KNO₂ served for the comparison.

solution of the following composition and kept at room temperature (20-32°C) with occasional testing for nitrite and nitrate :

NaNO ₃	2.00 g	} dissolved in 150 cc. H ₂ O.
K ₂ HPO ₄	0.20 g	
MgSO ₄ +7H ₂ O	0.02 g	

After 24 hours a stronger nitrite reaction was observed in (1) than in (2) (0.05% resp. 0.005% nitrite as KNO₂). The maximum amount of nitrite was attained after 3 days with (1) and after 5 days with (2) but after 4 resp. 7 weeks all the nitrate was reduced. Thus we see that fresh rape cake favors denitrification much more than glycerine.

(b). Some rape cake and soy been cake were left to putrefy in a warm place with a moderate supply of water and after 2 months rotting, samples were dried and finely pulverized. Two g of each, fresh cakes and the same amount of dry matter of the rotten cakes were mixed respectively with 100 g of air dry soil and 150 cc. of the following nitrate solution, and kept at 25°C.

NaNO ₃	1.75 g	} dissolved in 150 cc. of H ₂ O.
K ₂ HPO ₄	0.50 g	
MgSO ₄ +7H ₂ O	0.05 g	

The tests showed that with fresh rape cake all the nitrate was reduced after 2 weeks, but with rotten cake much nitrate was still present even after 10 weeks. With soy been cake which is poorer in carbohydrates and richer in protein the difference was much smaller and the nitrate was reduced entirely in the case of fresh and rotten cake after 10 and 12 days respectively.

Thus we see that the fresh rape cakes favor denitrification much more than the rotten.

LABORATORY EXPERIMENT. IV.

To see the effect of inoculation of denitrifying organisms, pure cultured, into the soil in the paddy state, the following 3 organisms were selected :

- a). Bact. denitrificans I. }
 b). „ „ „ II. }¹⁾
 c). „ nitrovorum.

One hundred g of soil from our Experiment Station were kept in an Erlenmeyer's flask of ca. 300 cc. capacity with 100 cc. of nitrate solution as in Expt. III, (a). After repeated sterilization the denitrifying organisms were inoculated on Aug. 16, 1905, and kept at room temperature. Of the supernatant solutions small doses were withdrawn from time to time with a sterilized pipette, and tested for nitrate, nitrite and ammonia with the following results :

(a). Bact. denitrificans I. Much nitrite was found after 24 hours and after 6 days the amount of nitrite reached the maximum (0.125% KNO_2). But even after 2 months later both nitrate and nitrite were still present.

(b). Bact. denitrificans II. The nitrite reaction was observed after 2 days from the start and after 9 days this reaction showed the maximum. All the nitrate and nitrite were reduced after 13 days from the start.

(c). Bact. nitrovorum. Nitrite was observed also after two days, the maximum point of the nitrite reaction after 6 days, and the disappearance of both nitrate and nitrite after 16 days from the start.

A moderate ammonia reaction was obtained in each mixture after 3 days, (a) yielded much, (b) and (c) a weak ammoniacal reaction after 5-10 days, and still a moderate reaction after 2 weeks.

Further with the flask (a) much pasty film was noticed on the surface of the soil, but with flasks (b) and (c) this was not the case; the liquids became gradually turbid and of a light reddish-brown color.

LABORATORY EXPERIMENT. V.

To observe the influence of starch, compost, straw and rape cake upon the reduction of nitrate in paddy soil and to determine the loss of nitrogen by denitrification the following experiment was made.

On Sept. 18, 1905, 28 Erlenmeyer's flasks of ca. 300 cc. capacity were

1). These two organisms were isolated from the soil in the field of this Station by S. Machida. Burri and Stutzer were the first to isolate denitrifying bacteria in pure culture.

filled with 200 g of humy soil from Nishigahara containing 30.53% H_2O and mixed with the following salts,¹⁾ dissolved in 250 cc. H_2O , and organic manures.¹⁾ As general nutrients 0.5 g di-potassium-phosphate and 0.05 g magnesium sulphate were added to each flask and kept at room temperature (15–26°C).

I.	Ammonium sulphate ²⁾	...	...	...	...	...	...	...	1.25 g.
II.	Sodium nitrate...	...	...	...	...	...	...	...	" "
III.	Ammonium nitrate	...	...	...	...	...	...	...	" "
IV.	{ Sodium nitrate	...	...	...	...	...	...	...	" "
	{ Starch	...	...	...	...	...	...	...	2.00 "
V.	{ Sodium nitrate	...	...	...	...	...	...	...	1.25 "
	{ Compost well rotten (a) sterilized	...	...	...	...	...	...	...	6.00 "
VI.	{ Sodium nitrate	...	...	...	...	...	...	...	1.25 "
	{ Compost well rotten (b) not sterilized	...	...	...	...	...	...	...	6.00 "
VII.	{ Sodium nitrate	...	...	...	...	...	...	...	1.25 "
	{ Rape cake	...	...	...	...	...	...	...	2.00 "
VIII.	{ Sodium nitrate	...	...	...	...	...	...	...	1.25 "
	{ Straw in powder	...	...	...	...	...	...	...	6.00 "
IX.	Starch	...	...	...	...	...	...	...	2.00 "
X.	Compost well rotten (a) sterilized...	...	...	...	...	...	...	...	6.00 "
XI.	Compost well rotten (b) sterilized...	...	...	...	...	...	...	...	6.00 "
XII.	Rape cake	...	...	...	...	...	...	...	2.00 "
XIII.	Straw in powder	...	...	...	...	...	...	...	6.00 "
XIV.	Control	...	...	...	...	...	...	...	—

The experiment was carried out in duplicate. While one series served for occasional testing the other served for the final determination. It was observed that flasks containing starch, rape cake and straw showed the strongest reaction for nitrate, and with the starch flask after 10 days from start,

1). Nitrogenous manures subjected to this experiment contained the following percentage of N :

a)	Ammonium sulphate...	...	...	...	...	20.50 % N
b)	Ammonium nitrate	...	...	...	...	34.80 " "
c)	Sodium nitrate...	...	...	...	...	15.55 " "
d)	Rape cake	...	...	...	...	5.70 " "
e)	Compost well rotten	...	...	...	...	1.41 " "
f)	Straw	...	...	...	...	0.57 " "

2). One g of precipitated magnesium carbonate was added to neutralize the acidity of sulfuric acid.

with the rape cake flask after two weeks and with the straw flask after four weeks, all the nitrate and nitrite were reduced. In the other flasks, however, to which the nitrate was added much nitrate was still present after 6 weeks and in the ammonium sulphate flask also strong reactions for nitrate and nitrite were noticed after three weeks. With Compost flasks (a) and (b) moderately strong nitrite reactions were observed after 2-3 days from the start but the reaction decreased gradually.

The flasks for the final determinations were kept for 45 days with frequent shaking until the nitrite reaction had entirely disappeared. 100 cc. of supernatant clear solution were withdrawn from each flask to determine the soluble nitrogenous compounds, and the remainder in the flask was evaporated to dryness after adding a little sulphuric acid. The total nitrogen, nitric and ammoniacal nitrogen were determined as usual with the following results :

No. of Flasks.	Total air dry soil.	In 100 parts of air dry soil.			In 100 part of the supernatant solution		Total amount of		
		Total N.	A'monia N.	Nitric N.	A'monia N.	Nitric N.	Total N. g	A'monia N. g	Nitric N. g
I.	144.4	0.456	0.098	0.005	0.043	0.004	0.705	0.184	0.011
II.	147.9	0.403	0.034	0.082	0.003	0.072	0.671	0.053	0.193
III.	147.0	0.495	0.053	0.094	6.043	0.072	0.843	0.120	0.210
IV.	142.3	0.311	0.015	0	0.004	0	0.447	0.025	0
V.	153.3	0.443	0.010	0.075	0.003	0.068	0.750	0.017	0.183
VI.	151.4	0.439	0.005	0.071	0.002	0.068	0.735	0.010	0.175
VII.	147.6	0.381	0.025	trace	0.006	trace	0.568	0.043	0
VIII.	152.9	0.348	0.005	0	0.001	0	0.533	0.009	0
IX.	143.0	0.307	0.020	0	0.001	0	0.440	0.030	0
X.	148.5	0.401	0.025	0	0.003	0	0.598	0.038	0
XI.	152.0	0.395	0.007	trace	0.003	trace	0.603	0.012	0
XII.	147.5	0.377	0.015	0	0.005	0	0.561	0.026	0
XIII.	153.5	0.340	0.004	0	0.002	0	0.524	0.007	0
XIV.	145.7	0.322	0.008	0.024	0.001	0.008	0.478	0.013	0.043

From the above figures the loss of nitrogen by the reduction of nitrate was calculated with the result shown in the following tables :

No. of flasks.	Kind of N-manures applied.	Remaining amount of soluble N applied.	Amount of soluble N applied.	percentage of soluble N remaining.	Loss of soluble N applied.
I.	$(\text{NH}_4)_2\text{SO}_4$	0.227 g.	0.256	88.7 %	11.3 %
II.	NaNO_3	0.193 "	0.194	99.5 "	0.5 "
III.	NH_4NO_3	0.365 "	0.435	83.9 "	16.1 "
IV.	NaNO_3 + starch	0.007 "	0.194	3.6 "	96.4 "
V.	" + compost a)	0.152 "	0.194	78.3 "	21.7 "
VI.	" + " b)	0.135 "	0.194	68.6 "	31.4 "
VII.	NaNO_3 + rape cake	0.007 "	0.194	3.6 "	96.4 "
VIII.	" + straw	0.009 "	0.194	4.6 "	95.4 "

This result confirms the observations made by occasional testing as above stated and clearly shows that the starch, rape cake and straw very much favor denitrification in the paddy soil, while well rotten compost especially when sterilized has far less influence upon denitrification. But little loss of nitrogen took place in flask II to which nitrate alone was applied, perhaps by the accidental absense of the denitrifying microbes proper.

LABORATORY EXPERIMENT VI.

To observe whether nitrate formation and denitrification¹⁾ take place in the dry land state, the following experiments were carried out.

A. Dry land top soil.

One hundred g of the following three samples of soil were placed in Erlenmeyer's flasks of ca. 300 cc. capacity with 2 g of sodium nitrate in solution and kept in a moderately dry condition, and in control flasks the same amount of distilled water was applied :

1). Nitrite formation is frequently the first step of denitrification with loss of nitrogen. In certain cases however the nitrite may be completely reduced to ammonia.

- 1). Humy soil of a field, from Nishigahara.
- 2). Clay soil from Kaga province.
- 3). Alluvial sandy loam soil from Arakawa, near Tokyo.

The experiment was begun April 11, 1905, and lasted 50 days, every day a portion of the contents of each flask was withdrawn to test for nitrite. The room temperature was 17-25°C. During 12 days from the start slight reaction for nitrite was observed in the soil (1) and (2) and then the reaction disappeared. In the soil (3) no trace of nitrite was found through the whole period. Thus we see that little or no nitrite formation takes place in the top soil of the dry land state, especially in the sandy soil and when nitrate alone was applied.

B. Top and Sub-soil in dry land state.

The same soils as in Experiment (A) just described served also for this experiment. They yielded the following chemical and physical data :

	(1) Humy soil.	(2) Clay soil.	(3) Sandy loam soil.
Hygroscopic water	12.53	1.81	2.90
Loss by ignition... ..	16.23	4.09	5.11
Nitrogen	0.35	0.22	0.19
Weight of $\left\{ \begin{array}{l} \text{in loose state ...} \\ \text{100 cc. in compact state.} \end{array} \right.$	$\left\{ \begin{array}{l} 60.40 \\ 105.55 \end{array} \right.$	$\left\{ \begin{array}{l} 93.50 \\ 146.50 \end{array} \right.$	$\left\{ \begin{array}{l} 98.25 \\ 150.15 \end{array} \right.$
Absorptive $\left\{ \begin{array}{l} \text{in loose state ...} \\ \text{power for H}_2\text{O in compost state.} \end{array} \right.$	$\left\{ \begin{array}{l} 106.67 \\ 70.25 \end{array} \right.$	$\left\{ \begin{array}{l} 49.77 \\ 31.81 \end{array} \right.$	$\left\{ \begin{array}{l} 48.62 \\ 31.28 \end{array} \right.$

450, 550 and 600 g of these soils resp. were mixed with 4 g of sodium nitrate in a moderate concentration and put in glass cylinders of 3,6 cm. diametre and 36 cm. height open at both ends, the upper half of the soil in a loose condition and the lower half in a compact condition, the lower end of the cylinder being closed with linen smeared with melted paraffin. Besides, the cylinders were covered with black paper, sun-light being allowed to reach only the surface of the soils.

The experiment was begun April 12, 1905, and every 2 days in the first period and every 5 days in the later, a portion was withdrawn from the upper and lower layers and tested for nitrate, nitrite and ammonia with the following result :

(1). In the humy soil to which sodium nitrate was added a little nitrite was observed after 2 weeks, increasing a little afterwards, and the reaction was always stronger in the sub-soil than in the top-soil. The reaction for ammonia was observed after one week and was always stronger in the top-soil.

In the control case, however, no nitrite was found through the whole period; after one month a trace of ammonia and after 50 days a trace of nitrate was observed.

(2). In the clay soil to which sodium nitrate was added, a little nitrite was observed after 2 days from the start, the reaction in the sub-soil increasing gradually, reaching the maximum after 2 weeks and remaining the same through the whole period, while in the top-soil no further increase was noticed. Much ammonia was found after two days from the start, the reaction of which was always stronger in the top-soil, just as with the humy soil (1). In the control case, neither nitrate nor nitrite was formed through the whole period, but a trace of ammonia was found after one month, a little more in the top-soil than in the sub-soil.

(3). In the sandy loam to which sodium nitrate was added, a trace of nitrite was observed in the sub-soil after 3 days from start, and the reaction increasing gradually, reached the maximum after 2 weeks, while in the top-soil only a slight reaction for nitrite was observed after 2 weeks. With regard to ammonia, only a trace of it was found after one week which remained constant through the whole period. In the control case, neither nitrate, nor nitrite was found through the whole period and not even a trace of ammonia was found.

The second experiment was carried out with the same soil and cylinders for a longer period viz. five months (June 26—October 28, 1905), the result of which exactly coincided with that of the former experiment above stated. Thus we see that denitrification takes place more or less in the

sub-soil in dry land state, while in the top-soil when kept in a loose condition almost no reduction of nitrate occurs if nitrate alone is applied to the field.

(C). The dry land state with application of some organic substance.

In order to determine the influence of the presence of some organic matter in the soil in dry land state upon denitrification, the following experiment was made with the three soils mentioned below :

- 1). Humy top-soil from a field of Nishigahara.
- 2). Clayey sub-soil „ „ „
- 3). Alluvial sand soil from Arakawa.

The cylinders of the diametre of 4 cm. and of the height of 55 cm. open at both ends, were filled with each soil in the air dry state, upper layer of 25 cm. deep in loose condition and lower layer of 30 cm. deep in compact condition. The soils of the first series were mixed with a solution of sodium nitrate and those for the second series with the same nitrate solution and starch, the ratio of both the nitrate and starch to the soil being 1 : 100. The soils were kept moderately moist and the lower open end of the cylinder was covered with filter paper and linnen and kept air tight with melted paraffin. The sides of the cylinders were covered with black paper.

The experiment was begun Oct. 5, 1905, and was finished Nov. 24, during the first period of which every two days, and later on every 10 days, a portion of the upper and lower layers of the soil was taken out by boring and tested for nitrate, nitrite and ammonia with the following result :

(1). In the humy top-soil to which both the nitrate and starch were applied, much nitrate was present in the upper layer during the whole period, while in the lower layer the nitrate decreased gradually and after 20 days only a trace of it was observed. The soil to which nitrate alone was applied showed a strong reaction for nitrate in both upper and lower layers until the end of the experiment. As to nitrite a trace of it was found in the lower layer during the first period in all cylinders which received nitrate and the reaction disappeared after 12 days in the cylinder to which the nitrate solution alone was applied, while in the cylinder to which both nitrate and

starch were applied the reaction became evident gradually, reaching the maximum after 10 days and then it gradually diminished to a trace after 20 days from start.

(2). In the clayey sub-soil a trace of nitrite was observed in the lower layer of all cylinders except that of the control case, and no further increase of nitrite was noticed in the cylinder to which nitrate alone was applied, while in the cylinder with nitrate and starch the reaction increased gradually reaching the maximum after three weeks from the start and afterwards decreasing gradually but showing the reaction until the end of the experiment.

(3). In the sandy soil with nitrate and starch the nitrate decreased remarkably after 10 days from the start in both upper and lower layers of the soil and after 20 days all the nitrate was reduced, while in the soil with nitrate alone, the nitrate reaction remained intense through the whole period. With nitrite there was no reaction in the control soil and the soil with nitrate alone, while in the soil with nitrate and starch a trace in the upper layer and much of it in the lower layer of soil were observed after 2-3 days from the start, reaching the maximum after 10 days and then decreasing gradually again.

The above results show that in the soil of the dry land state there is hardly any nitrite formation if the nitrate alone is applied. When, however, some organic matter is applied together with the nitrate some reduction will take place, especially in the sub-soil, where the access of air is insufficient, even all the nitrate here will eventually be reduced.

(D). Comparison of the effect of some organic manures upon the formation of nitrite and denitrification.

In order further to compare the effects of different organic matters, such as starch, rape cake, compost and straw, upon the degree of denitrification in the dry state of land, the following experiment was carried out :

Ten glass flasks of ca. 1 litre capacity with greased stoppers were filled with 700 g of fine humy soil from Nishigahara containing 19.15% of hygroscopic water. 150 cc. of a solution containing 3.5 g of NaNO_3 , 2 g of

K_2HPO_4 and 0.3 $MgSO_4$ were added to the flasks No. I-V, and the same amount of a solution containing only 2 g K_2HPO_4 and 0.3 g $MgSO_4$ without nitrate, to the flasks No. VI-X, after mixing the soil thoroughly with the organic matters¹⁾ mentioned :

I.	$NaNO_3$	3.5 g.
II.	$\left\{ \begin{array}{l} NaNO_3 \dots \dots \dots \\ Starch \dots \dots \dots \end{array} \right.$	$\left\{ \begin{array}{l} 3.5 \text{ ,,} \\ 5.0 \text{ ,,} \end{array} \right.$
III.	$\left\{ \begin{array}{l} NaNO_3 \dots \dots \dots \\ Compost, well rotten \dots \dots \dots \end{array} \right.$	$\left\{ \begin{array}{l} 3.5 \text{ ,,} \\ 21.0 \text{ ,,} \end{array} \right.$
IV.	$\left\{ \begin{array}{l} NaNO_3 \dots \dots \dots \\ Rape cake \dots \dots \dots \end{array} \right.$	$\left\{ \begin{array}{l} 3.5 \text{ ,,} \\ 5.0 \text{ ,,} \end{array} \right.$
V.	$\left\{ \begin{array}{l} NaNO_3 \dots \dots \dots \\ Straw \dots \dots \dots \end{array} \right.$	$\left\{ \begin{array}{l} 3.5 \text{ ,,} \\ 10.0 \text{ ,,} \end{array} \right.$
VI.	Starch (Control (a))	5.0 ,,
VII.	Compost well rotten (Control (b))... ..	21.0 ,,
VIII.	Rape cake (Control (c))	5.0 ,,
IX.	Straw (Control (d))... ..	10.0 ,,
X.	 (Control (e))	—

The experiment was begun Sept. 5, 1905, and every 10 days a certain portion was withdrawn from each vessel, the same amount of water added and tested for nitrite in the filtrate. After 10 days much nitrite was observed in the flasks II and IV, the amount of which as potassium salt approximately determined 0.35% resp. 0.088% KNO_2 . In flask III only a little nitrite and in all other flasks (except II and IV) only a trace or none of it was observed. After one month in all the flasks only traces of nitrite could be found.

The result shows that in top-soil of "dry" land only certain organic matters, such as starch or rape cake, favor nitrite formation to some extent, but well rotten compost and straw do not.²⁾

Increase of moisture will lead to an increase of nitrite and loss of nitrogen ; water was added after 33 days from the start at the ratio of 10 cc.

1). The contents of N in the nitrate and organic manures are the same as in Lab. Expt. V.

2). Stoklasa (Z. Landw. Vers. Wes. in Oesterreich, 1906, p. 844.) observed in Austrian soils serving for culture of sugar beets also denitrification did not take place to such an extent that it could be proved analytically.

H₂O to every 100 g original soil, thus keeping the soil in just a moderately moist condition. After 10 days nitrate reactions were obtained in the flasks II, IV and V. After two months from start the soil was dried after acidulating with a little H₂SO₄ and the total N, ammonia N and nitric N determined.

No. of flasks.	Kind of Manures.	In 100 pts dry soil.			N in total dry soil (g).		
		Total N.	Amm. N.	Nitric N.	Total N.	Amm. N.	Nitr. N.
I.	NaNO ₃	0.452	0.005	0.102	2.558	0.028	0.577
II.	NaNO ₃ + starch	0.397	0.005	0.018	2.145	0.028	0.102
III.	NaNO ₃ + compost	0.456	0.022	0.100	2.581	0.125	0.566
IV.	NaNO ₃ + rape cake	0.477	0.038	0.082	2.683	0.215	0.464
V.	NaNO ₃ + straw	0.432	0.016	0.082	2.445	0.091	0.464
VI.	starch	0.372	trace	0.006	2.105	—	0.034
VII.	compost	0.382	0.005	0.011	2.162	0.028	0.062
VIII.	rape cake	0.401	0.016	0.027	2.269	0.091	0.153
IX.	straw	0.354	0.011	0.027	2.003	0.062	0.153
X.		0.368	trace	0.004	2.083	—	0.023

From these figures the loss of nitrogen was calculated.

No. of flasks.	Kind of manures.	Soluble N applied (g).	Loss of N by denitrification.	
			g	%
I.	NaNO ₃	0.544	0.069	12.7
II.	NaNO ₃ + starch	0.544	0.504	92.6
III.	NaNO ₃ + compost	0.544	0.125	23.0
IV.	NaNO ₃ + rape cake	0.544	0.130	23.9
V.	NaNO ₃ + Straw	0.544	0.102	18.8

These results show that when common dry land top-soil becomes sufficiently moist, as by continuous rains, the presence of an easily putrefying organic matter favors denitrification to a considerable degree.¹⁾

VEGETATION EXPERIMENT I.²⁾

In order to ascertain the effect of the formation of nitrite and denitrification upon vegetation, the following experiments were made: Six zinc pots of ca. 30 cm. in diameter were filled with 15 Kg of humy soil from Nishigahara and six with alluvial soil from Arakawa. The following manures were applied per pot (in duplicate):

(A).	(NH ₄) ₂ SO ₄	5.0 g	(N in equivalent to (NH ₄) ₂ SO ₄)
	Na ₂ HPO ₄	5.0 "	
	K ₂ SO ₄	5.0 "	
(B).	NaNO ₃	6.7 "	
	Na ₂ HPO ₄	5.0 "	
	K ₂ SO ₄	5.0 "	

1). A considerable degree of denitrification was observed by Hugo Fisher after moderately liming the soil, but the amount of moisture was not stated.

2). All vegetation experiments were carried out by G. Daikuhara.

(C).	NaNO ₃ ...	...	...	...	...	...	6.7 g.
	Na ₂ HPO ₄	...	...	...	...	...	5.7 "
	Glycerine	...	...	...	...	...	10.0 cc.

Five young paddy rice plants (*var. Sekitori*) equal in size were trans-planted in each pot on July 5, 1905, and kept in paddy state. The plants in pot (A) was normal while those in pot (B) and (C), showed a yellowish green color in the first period of growth and much foam gathered on the surface of the soil especially in pot (C). After two weeks, however, the plants in pot (B) and (C) again acquired a green color but not the deep green as observed in pot (A).

The following table shows the result of observations on July 25 and on Sept. 5, 1905 :

A. Soil from Nishigahara.

Kind of Manures.	Length of plants (cm).				Number of stalks.			
	July 25		Sept. 5		July 25		Sept. 5	
	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.
(NH ₄) ₂ SO ₄	74.3	73.2	109.1	106.8	27	30	61	60
	72.1		104.5		33		58	
NaNO ₃	61.2	61.7	107.5	104.5	22	22	45	46
	62.1		101.5		22		46	
NaNO ₃ + Glycerine	56.1	56.1	89.4	91.8	17	17	20	20
	56.1		94.2		17		20	

B. Soil from Arakawa.

Kind of Manures.	Length of plants (cm).				Number of stalks.			
	July 25		Sept. 5		July 25		Sept. 5	
	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.
(NH ₄) ₂ SO ₄	75.8	72.5	104.9	103.5	26	29	54	55
	69.1		102.1		32		55	
NaNO ₃	60.6	60.0	108.4	104.7	19	17	34	33
	59.2		100.9		15		31	
NaNO ₃ + Glycerine	51.5	50.0	88.0	86.5	12	14	13	15
	48.5		85.0		15		17	

A photograph taken Sept. 2, 1905, and reproduced on Plate XVII shows clearly the differences in development. The plants were cut Oct. 25, 1905, and weighed in the air dry state with the following result :

A. Soil from Nishigahara.

Kind of Manures.	Weight of Grains (g).		Weight of Straw (g).		Total Yield (g).		Ratio of Total Yield.
	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.	
(NH ₄) ₂ SO ₄	67.13	61.31	129.38	128.44	196.50	189.75	100
	55.50		127.50		183.00		
NaNO ₃	57.75	56.81	85.50	87.94	143.25	144.75	76
	55.88		90.38		146.25		
NaNO ₃ + Glycerine	25.50	26.25	37.50	37.88	63.00	64.13	34
	27.00		38.25		65.25		

B. Soil from Arakawa.

Kind of Manures.	Weight of Grains (g).		Weight of Straw (g).		Total Yield (g).		Ratio of Total Yield.
	p. pot.	Average.	p. pot.	Average.	p. pot.	Average.	
(NH ₄) ₂ SO ₄	64.13	66.75	114.00	113.25	178.13	180.00	100
	69.37		112.50		181.88		
NaNO ₃	38.63	36.19	70.88	66.94	109.50	103.13	57
	33.75		63.00		96.75		
NaNO ₃ + Glycerine	18.75	17.81	22.13	22.13	40.88	39.94	22
	16.88		22.13		39.00		

. This result coincides exactly with that of the laboratory experiments stated above and shows that glycerine favors denitrification in paddy soil very much. The yellowish color of plants during the first period of growth is most probably due to the poisonous action of nitrite formed by the reduction of nitrate.

VEGETATION EXPERIMENT II.

36 zinc pots of ca. 25 cm. in diameter were filled with 11 Kg of humy soil from Nishigahara and the following manures applied, 3 pots serving for each trial.

No. of pots.	Kind of Manures.	Amount of Manures applied p. pot.
I.	(NH ₄) ₂ SO ₄	5.0 g.
II.	NaNO ₃	6.6 "
III.	{ (NH ₄) ₂ SO ₄	2.5 "
	{ NaNO ₃	3.3 "
IV.	{ NaNO ₃	6.6 "
	{ Starch	10.0 "

No of pots.	Kind of Manures.	Amount of Manures applied p. pot.
V.	{ NaNO ₃	6.6 g.
	{ Compost (a) sterilized ¹⁾	30.0 „
VI.	{ NaNO ₃	6.6 „
	{ Compost (b) not sterilized... ..	30.0 „
VII.	{ NaNO ₃	6.6 „
	{ Rape cake	10.0 „
VIII.	Starch	10.0 „
IX.	Compost (a) sterilized	30.0 „
X.	Compost (b) not sterilized	30.0 „
XI.	Rape cake	10.0 „
XII.	Control	—

As general manure 5 g of Na₂HPO₄ and of K₂SO₄ per pot were applied. Ten young paddy rice plants of equal size were transplanted into each pot on Sept. 24,²⁾ 1905, all pots were kept in the glass house until Nov. 20 and were then removed to a green house kept at 15–30°C. but even here the seeds did not ripen, probably on account of the development of numerous young shoots. The following observations were made Oct. 10 and Feb. 19 when the plants were cut.

1), The compost used in this experiment was well rotten.

2). The transplanting by some obstacle took place later than was desirable.

No. of pots.	Kind of Manures.	Average length of plants (cm.).		Average No. of stalks on Feb. 19.
		Oct. 10.	Feb. 19.	
I.	(NH ₄) ₂ SO ₄	34.0	66.7	32
II.	NaNO ₃	30.3	59.9	19
III.	(NH ₄) ₂ SO ₄ + NaNO ₃ ..	32.7	61.7	27
IV.	NaNO ₃ + starch ...	27.0	55.3	19
V.	NaNO ₃ + compost (a).	30.3	58.4	22
VI.	NaNO ₃ + compost (b).	31.5	55.0	20
VII.	NaNO ₃ + rape cake ...	31.2	54.3	22
VIII.	Starch only	28.2	50.7	17
IX.	Compost (a) only ...	27.9	52.0	17
X.	Compost (b) only ...	29.1	53.6	15
XI.	Rape cake... ..	30.9	55.5	21
XII.	Control	29.1	52.1	15

Plants in the nitrate pots showed the yellowish green color of the young growing leaves especially in those which received organic manures together with nitrate. The tests showed always much more nitrate (at least more than 3 times as much) in the yellowish leaves than in the normally green ones grown with (NH₄)₂SO₄ as a manure. But nitrate could not be observed by Griess' test in the yellowish green leaves.

The photograph taken Feb. 19, 1906, and reproduced on plate XVIII shows clearly the differences of growth. The plants weighed in the air dry state gave the following result :

No. of pots.	Kind of Manures.	Average weight p. pot in air dry state (g.).			Gain of yield p. pot by soluble N. ¹⁾	
		Grain(empty).	Straw.	Total.	g	Ratio.
I.	(NH ₄) ₂ SO ₄	2.570	54.430	57.00	31.50	100
II.	NaNO ₃	1.485	30.015	31.50	6.00	19
III.	(NH ₄) ₂ SO ₄ + NaNO ₃ ..	2.170	40.210	42.38	16.88	54

1). The gain of N by soluble N-salts was calculated by subtracting the yield of control pot from those of pot I, II, and III, the yields of VIII, IX, X and XI from those of IV, V, VI and VII respectively.

No. of pots.	Kind of Manures.	Average weight p. pot in air dry state (g).			Grain of yield p. pot by soluble N. ¹⁾	
		Grain (empty)	Straw.	Total.	g	Total.
IV.	NaNO ₃ + Starch ...	1.690	23.060	24.75	1.50	5
V.	NaNO ₃ + Compost (a)	2.560	25.940	28.50	6.00	19
VI.	NaNO ₃ + Compost (b)	1.690	23.810	25.50	4.12	14
VII.	NaNO ₃ + Rape cake ..	1.913	32.587	34.50	2.50	8
VIII.	Starch only	0.980	22.270	23.25	—	—
IX.	Compost (a) only ...	1.307	21.193	22.50	—	—
X.	Compost (b) only ...	1.190	20.190	21.38	—	—
XI.	Rape cake only ...	1.987	30.013	32.00	—	—
XII.	Control	1.263	24.237	25.50	—	—

The amounts of the total nitrogen in the air dry samples (after Kjeldahl determined), the gain of nitrogen by the application of ammonia and nitrate N were as follows :

No. of pots.	Kind of Manures.	Content of total N.		Gain of N p. pot by soluble N.	
		%	g	g	Ratio.
I.	(NH ₄) ₂ SO ₄	0.711	0.405	0.209	100
II.	NaNO ₃	0.782	0.246	0.050	24
III.	(NH ₄) ₂ SO ₄ + NaNO ₃ ..	0.743	0.315	0.119	57
IV.	NaNO ₃ + Starch ...	0.743	0.184	0.018	9
V.	NaNO ₃ + Compost (a)	0.705	0.201	0.070	33
VI.	NaNO ₃ + Compost (b)	0.662	0.169	0.044	21
VII.	NaNO ₃ + Rape cake ..	0.716	0.247	0.019	9
VIII.	Starch only	0.713	0.166	—	—
IX.	Compost (a) only ...	0.583	0.131	—	—
X.	Compost (b) only ...	0.585	0.125	—	—
XI.	Rape cake only ...	0.712	0.228	—	—
XII.	Control	0.768	0.196	—	—

These results show that the efficiency of nitrate nitrogen for the paddy rice is very unsatisfactory and that the simultaneous application of certain organic matters depresses the yield still further. Well rotten compost exerts a less depressing influence than fresh rape cake.

VEGETATION EXPERIMENT III.

In order to compare, now, dry land with paddy field in regard to the effect of their different degree of denitrification, the following experiment with buckwheat was made: 36 zinc pots of ca. 25 cm. in diameter were filled with 14 Kg humy soil from Nishigahara. The kind and the amount of manures applied were the same as in the vegetation experiment II. 31 seeds of buckwheat were sown Aug. 27, 1905, and 2 weeks after germination the young plants were reduced to 25 per pot all of equal size. The average height of plants measured Oct. 1, was as follows:

No. of pots.	Kind of Manures.	Average length of plants.
I.	$(\text{NH}_4)_2\text{SO}_4$	70.3 cm.
II.	NaNO_3	70.0 "
III.	$(\text{NH}_4)_2\text{SO}_4 + \text{NaNO}_3$	71.5 "
IV.	$\text{NaNO}_3 + \text{Starch}$	73.0 "
V.	$\text{NaNO}_3 + \text{Compost (a)}$	72.4 "
VI.	$\text{NaNO}_3 + \text{Compost (b)}$	76.4 "
VII.	$\text{NaNO}_3 + \text{Rape cake}$	74.8 "
VIII.	Starch only... ..	60.6 "
IX.	Compost (a) only	63.0 "
X.	Compost (b) only	64.8 "
XI.	Rape cake only	70.7 "
XII.	Control... ..	60.9 "

The plants were cut Oct. 25 and weighed in the air dry state with the following result:

No. of pots.	Kind of Manures.	Weight of seeds (g).		Weight of stalks (g).		Total yield (g).	
		p. pot.	Average.	p. pot.	Average.	p. pot.	Average.
I.	$(\text{NH}_4)_2\text{SO}_4$	a) 28.88	25.13	15.38	15.13	44.26	40.26
		b) 22.13		14.25		36.38	
		c) 24.38		15.75		40.13	
II.	NaNO_3	a) 25.88	26.51	16.50	16.25	42.38	42.76
		b) 27.01		16.13		43.14	
		c) 26.63		16.13		42.76	
III.	$(\text{NH}_4)_2\text{SO}_4 + \text{NaNO}_3$	a) 27.76	27.00	17.25	16.63	45.01	43.63
		b) 26.25		15.38		41.63	
		c) 27.01		17.25		44.26	
IV.	$\text{NaNO}_3 + \text{Starch}$	a) 18.73	20.26	13.50	14.50	32.25	34.76
		b) 20.25		15.38		35.63	
		c) 21.76		14.63		36.39	
V.	$\text{NaNO}_3 + \text{Compost (a)}$	a) 26.25	26.25	17.63	19.38	43.88	45.63
		b) 27.75		21.38		49.13	
		c) 24.75		19.13		43.88	
VI.	$\text{NaNO}_3 + \text{Compost (b)}$	a) 21.76	22.63	16.88	17.63	38.64	40.26
		b) 21.38		17.63		39.01	
		c) 24.76		18.38		43.14	
VII.	$\text{NaNO}_3 + \text{Rape cake}$	a) 27.00	27.50	18.00	17.75	45.50	45.25
		b) 28.51		18.00		46.51	
		c) 27.01		17.25		44.26	
VIII.	Starch only	a) 8.26	8.75	8.25	8.00	16.51	16.75
		b) 9.01		7.88		16.89	
		c) 9.00		7.88		16.88	

No. of pots.	Kind of Manures.	Weight of seeds (g).		Weight of stalks (g).		Total yield (g).	
		p. pot.	Average.	p. pot.	Average.	p. pot.	Average.
IX.	Compost (a) only	a) 12.01	11.76	10.13	10.38	22.14	22.14
		b) 12.38		10.88		23.26	
		c) 10.88		10.13		21.01	
X.	Compost (b) only	a) 13.88	12.75	11.25	11.13	25.13	23.88
		b) 12.00		10.88		22.88	
		c) 12.38		11.25		23.63	
XI.	Rape cake only	a) 23.25	21.75	13.88	13.38	37.13	35.13
		b) 21.00		12.38		33.38	
		c) 21.00		13.88		34.88	
XII.	Control	a) 12.01	11.88	9.75	10.00	21.76	21.88
		b) 11.63		10.13		21.76	
		c) 12.00		10.13		22.13	

To observe the effect of the several organic manures upon denitrification more clearly, a calculation was made by subtracting the yield of pot XII from those of I, II and III, and from the yields of IV, V, VI, VII, those of VIII, IX X and XI respectively with the following result :

No. of pots.	Kind of Manures.	Surplus yield by soluble N-salts.			
		Seeds.		Total yield.	
		g	Ratio.	g	Ratio.
I.	(NH ₄) ₂ SO ₄	13.25	90	18.38	88
II.	NaNO ₃	14.63	100	20.88	100
III.	(NH ₄) ₂ SO ₄ + NaNO ₃ ..	15.12	103	21.75	104
IV.	NaNO ₃ + Starch	11.51	78	18.01	86
V.	NaNO ₃ + Compost (a)	14.49	99	23.49	112
VI.	NaNO ₃ + Compost (b)	9.89	67	16.38	78
VII.	NaNO ₃ + Rape cake..	5.75	39	10.12	48

This result shows again that the well rotten compost when sterilized has no depressing effect upon the efficiency of nitrate-N, while when not sterilized it depresses the yield very much, owing certainly to the importation of denitrifying organisms into the soil. The efficiency of nitrate-N is also much depressed by the simultaneous application of rape cake.

CONCLUSIONS.

(1). When nitrate is applied to the paddy soil it is reduced to some extent first to nitrite and then to ammonia and to elementary N, the loss of which varies according to the species of denitrifying organisms and the amount of soluble organic compounds present originally in the soil.

(2). When nitrate is applied to the paddy soil together with much organic matter in easily available form for microbes such as glycerine, Na-acetate, starch, fresh oil cakes and straw, it is destroyed extensively by denitrification, the most part of its nitrogen being lost as free N, while only a certain portion of it remains in the soil, being partly assimilated by microbes and partly absorbed as ammonia by the soil or plants.

(3). The question why nitrate is not a favorable manure for plants grown in paddy land can be answered as follows :

- a). The *loss of N by denitrification* is larger in paddy soil than in dry land.
- b). More of the *poisonous nitrites* are formed there than in dry land.¹⁾
- c). *Loss of nitrate* takes place easily by the system of *irrigation*, practiced with paddy plants, being inevitable in the farmers practice.

(4). Dry land surface soil when no organic manures are applied along with nitrate,²⁾ does not favor denitrification nor nitrite-formation while in the subsoil reduction occurs to some extent. In very moist conditions, however, as in the rainy season and especially when much organic manure is applied along with nitrate, some denitrification takes place even in top-

1). Young rice plants placed in a potassium nitrite solution of 0.1% died after 5 days.

2). According to *Amphola* calcium nitrate is less attacked by denitrifying microbes than sodium or potassium nitrate, but in most soils calcium nitrate added will surely be changed by alkali-salts and nitrates of sodium or potassium be formed.

soil and the reduction can proceed so energetically in the sub-soil that all the nitrate applied may be reduced within a few weeks.

(5). Organic matters easily available to microbes favor denitrification to a large extent; further, straw or fresh rape cakes have more influence upon the reduction of nitrate than the same materials well rotten, which agrees with former observations on stable manure.

PRACTICAL SUGGESTIONS.

(1). The Chili-saltpetre is no suitable fertilizer for plants grown in paddy soil.

(2). When Chili-saltpetre is to be used for paddy plants, organic manures should not be applied along with it, hence artificial mixed fertilizers composed of Chili-saltpetre and some organic substances are to be avoided for paddy fields.

(3) When however an organic fertilizer is unavoidably to be used along with Chili-saltpetre for paddy plants, the former should be used only in a well rotten state.

In conclusion, the authors express their hearty thanks for valuable suggestions and advice given by the Director Prof. Y. Kozai and Prof. Dr. O. Loew and for the analytical service given by Assistant Mr. C. Matsuoka.

A. Soil from Nishigahara.

(3) NaNO_3 + glycerine. (2) NaNO_3 . (1) $(\text{NH}_4)_2\text{SO}_4$.

B. Soil from Arakawa.

(3) NaNO_3 + glycerine. (2) NaNO_3 . (1) $(\text{NH}_4)_2\text{SO}_4$.

Vegetation Experiment II (a).

Vegetation Experiment II (b).

No. 1. = $(\text{NH}_4)_2\text{SO}_4$ No. 2. = NaNO_3 .No. 3. = $(\text{NH}_4)_2\text{SO}_4 + \text{NaNO}_3$.No. 4. = $\text{NaNO}_3 + \text{Starch}$.No. 5. = $\text{NaNO}_3 + \text{Compost (a)}$.No. 6. = $\text{NaNO}_3 + \text{Compost (b)}$.No. 7. = $\text{NaNO}_3 + \text{Rape cake}$.

No. 8. = Rape cake only.

No. 9. = Compost (a) only.

No. 10. = Compost (b) only.

No. 11. = Starch only.

No. 12. = Control.

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