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HAWAII AGRICULTURAL EXPERIMENT STATION,
E. V. WILCOX, Special Agent in Charge.

Issued April 25, 1914.

33
Bulletin No. 33.

THE ORGANIC NITROGEN OF HAWAIIAN SOILS.

BY

W. P. KELLEY,
Chemist,

AND

ALICE R. THOMPSON,
Assistant Chemist.

UNDER THE SUPERVISION OF

OFFICE OF EXPERIMENT STATIONS,

U. S. DEPARTMENT OF AGRICULTURE.

WASHINGTON:
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HAWAII AGRICULTURAL EXPERIMENT STATION, HONOLULU.

[Under the supervision of A. C. TRUE, Director of the Office of Experiment Stations, United States Department of Agriculture.]

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LETTER OF TRANSMITTAL.

HONOLULU, HAWAII, *August 4, 1913.*

SIR: I have the honor to submit herewith, and to recommend for publication as Bulletin 33 of the Hawaii Experiment Station, a paper on The Organic Nitrogen of Hawaiian Soils, by W. P. Kelley, chemist, and Alice R. Thompson, assistant chemist. On account of the great importance of nitrogen to growing plants it is highly desirable to know more about the nature of the nitrogenous substances in soils. In the research which served as a basis for the present bulletin an attempt was made to determine, so far as possible, the forms in which nitrogen occurs in the soils, and the relative percentages of the various nitrogenous products. These studies furnish an important contribution to the subject, which will later be supplemented by a study of the products of various vegetable proteids when acted upon by bacteria.

Respectfully,

E. V. WILCOX,
Special Agent in Charge.

Dr. A. C. TRUE,
*Director Office of Experiment Stations,
U. S. Department of Agriculture, Washington, D. C.*

Publication recommended.

A. C. TRUE, *Director.*

Publication authorized.

D. F. HOUSTON, *Secretary of Agriculture.*

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THE ORGANIC NITROGEN OF HAWAIIAN SOILS.

INTRODUCTION.

The greater part of soil nitrogen may reasonably be assumed to have been bound up at one time or another in protein combinations, since the nitrogen in the main has been derived from vegetable sources. Limited amounts of other nitrogen bodies, such as alkaloids, etc., also find their way into soils, but the nitrogen from such compounds could hardly be expected to amount to more than a small percentage of the total nitrogen present. The chemistry of soil nitrogen and the changes that it undergoes, therefore, must be largely those of plant proteins, brought about under complex and indeed extremely variable conditions. A great host of organisms inhabiting soils are associated with the transformations of the organic nitrogen bodies, and the conditions and environment in which the organisms function not only materially alter the rates of their action but also determine largely what the end products shall be. The presence of various chemical substances, both organic and inorganic, the acidity or alkalinity, and the degree of porosity of the soil, all exert important influences on the activity of soil organisms.

During the past few years considerable study has been devoted to the nitrogen compounds of the soil. In 1905-6, Shorey,¹ while chemist at this station, applied to a coffee soil from the island of Hawaii the methods formerly used in the study of protein, and thus determined the amounts of basic, nonbasic, ammonia nitrogen, etc., split off by means of boiling acids. In connection with his studies a pyridin derivative, picolin carboxylic acid, was isolated and identified, this being the first definite organic nitrogen compound to be isolated from a soil. Recently a number of other studies on soil nitrogen have been reported.²

The researches previously made on this subject naturally divide themselves into two classes, as indicated by the work of Shorey. First, a study of the individual compounds that occur in natural soils; second, a study of the products formed by acid hydrolysis. The extensive researches of Schreiner, Shorey,³ and their associates

¹ Hawaii Sta. Rpt. 1906, pp. 37-59.

² Jodidi, Michigan Sta. Tech. Bul. 4 (1909); Iowa Sta. Research Buls. 1 and 3 (1911); Robinson, Michigan Sta. Tech. Bul. 7 (1911); Lathrop and Brown, Pennsylvania Sta. Rpt. 1910, pp. 118-129; Jour. Indus. and Engin. Chem., 3 (1911), pp. 657-660.

³ U. S. Dept. Agr., Bur. Soils Buls. 47, 53, 74, 80, 87, 88, 89.

in the Bureau of Soils, conducted mainly with reference to the individual compounds actually occurring in soils, have brought to light much important information. Likewise the work of Jodidi,¹ Robinson,¹ and others, is of interest in its bearing on the hydrolytic products split off by means of acids. From the investigations on the hydrolytic products it has been shown that soils vary considerably in regard to the relative percentages of the several groups of nitrogen compounds split off in the treatment, and although only a comparatively small number of soils have been studied, these, in the main, have been found to yield the greatest amount of nitrogen in the form of monamino acids. Approximately 25 per cent of the nitrogen split off was in the form of amids, while the diamino nitrogen has been found in still smaller amounts, usually not more than 10 per cent of the total nitrogen dissolved.

It is not necessary to discuss in detail the studies previously made on this subject. It is sufficient to say that too much importance can hardly be given to the nitrogen of soils. The element lies at the very foundation of plant growth. The use of nitrogenous fertilizers has assumed enormous proportions throughout the world. In Hawaii extremely heavy applications have been made for many years, and the tendency during the past few years has been toward even greater applications. Many of the soils, however, contain a relatively high percentage of nitrogen. In some instances, even where very heavy applications of nitrogenous fertilizers are made, the soils contain 0.5 per cent or more of nitrogen.

Investigations on nitrification and ammonification in different Hawaiian soils have been under way in this laboratory for some time, and the results obtained have been of such nature as to emphasize the need for a better understanding of the chemical nature of the nitrogen bodies contained in these soils. Studies have accordingly been undertaken on this subject, employing the process of acid hydrolysis. In this work the nitrogen as a whole has been studied by subjecting to hydrolysis weighed portions of the original soils. On account of the great importance generally attached to humus, and the limited state of knowledge concerning the chemistry of this material, some study was devoted to the alkali soluble nitrogen bodies.

Since the substances to be investigated originally came from a number of plants, the protein of which has not been sufficiently studied, and have probably already undergone much change through the action of bacteria, there are no definitely understood materials to start with. The hydrolytic products obtained, therefore, leave much room for speculation. Just how far the cleavages had already gone in the soils previous to treatment remains a matter for much further work before definite conclusions can be drawn.

¹ Loc. cit.

THE NITROGEN OF HAWAIIAN SOILS.

The soils used in this investigation belong to the laterite class common to the islands. Sample No. 379 is a silty loam, containing considerable amounts of organic matter. It was taken from old pasture land in the Kula district of Maui, where semiarid conditions prevail.

Sample No. 428 is a highly organic sandy soil from Glenwood, in the Hilo district of Hawaii, where the rainfall is very heavy, but good drainage prevails. The sample came from land recently cleared of a tropical jungle and may be considered virgin fern forest soil.

Sample No. 447 is a silty loam from the Kunia district of Oahu, where semiarid conditions prevail, and is now being used for pine-apples, but formerly was devoted to pasture for many years.

Sample No. 292 taken from the lands previously used for rice experiments by this station, represents a loam soil containing considerable gravel, and has been devoted to aquatic agriculture for many years.

Samples Nos. 343 and 345 are each rice soils of a silty character, having been taken from the Kaneohe district on the windward side of Oahu. These lands have been devoted to continuous rice culture for from 30 to 40 years.

Sample No. 347 is a highly humus soil, taken from the Punuluu district of Oahu, and likewise has been continuously cultivated in rice for thirty or more years.

Samples Nos. 405 and 406 are silty soils taken from the Kalihi district of Oahu, and have been devoted to aquatic agriculture for many years, the former to rice and the latter to taro.

NITRATE AND AMMONIA.

It was deemed of interest to determine the amounts of nitrate and ammonia present in the soil preliminary to a study of the organic constituents. Nitrate was determined from water solutions, by the use of the phenol-disulphonic acid method, while the ammonia was determined by the direct distillation of separate portions of the soil with magnesium oxid. The results calculated to the water-free basis are given in the following table:

Total nitrate and ammonia nitrogen in Hawaiian soils.

Soil No.	Total nitrogen, per cent.	Nitrate nitrogen.		Ammonia nitrogen.	
		Parts per million.	Per cent of total nitrogen.	Parts per million.	Per cent of total nitrogen.
379.....	0.592	10	0.169	10	0.169
428.....	.770	45	.584	220	2.857
447.....	.354	62	1.751	10	.282
292.....	.122	4	.328	10	.819
343.....	.220	0	.000	22	1.000
345.....	.218	0	.000	32	1.468
347.....	1.241	70	.564	130	1.048
405.....	.195	1	.050	50	2.564
406.....	.456	15	.329	60	1.316

The above data show that the soils of Hawaii are similar to soils elsewhere in that the nitrogen present as nitrate and ammonia constitutes but a small percentage of the total nitrogen. In contrast to ordinary soils the ammonia content in most instances was considerably greater than the nitrate.¹ The nitrogen of these soils exists, therefore, very largely in organic combinations.

ORGANIC NITROGEN.

When a study is made of the organic nitrogen greater difficulties are at once encountered. The isolation of the various individual nitrogen compounds occurring in soils must necessarily be a tedious undertaking. It has been shown, however, that by means of the hydrolytic method as used in the study of protein chemistry, some conception can be obtained regarding the make-up of the nitrogen bodies of the soil. By the use of this method the amounts of nitrogen split off in the form of amids, diamino, and monamino acids are relatively easily determined.

Partly on account of the readiness with which these determinations can be made, and partly for the reason that the soil nitrogen may reasonably be supposed to have originated largely from vegetable proteins, many of which are known to be susceptible to complete hydrolysis, use has been made of the process of hydrolysis in this work. In addition it seems probable that the action of bacteria on soil nitrogen is progressive and of a hydrolytic nature.

On the other hand, the work of Osborne² and others show that the hydrolytic products vary widely with the different proteins and indicate that the results obtained in soil studies by the use of hydrolytic agents must be of the most general nature. Nevertheless, it is believed that much valuable information can be obtained in this way.

In the work reported in this bulletin the Osborne-Harris³ modification of the Hausmann⁴ method, as outlined by Jodidi⁵ in his studies on Iowa soils, has been used. The hydrolysis was conducted by heating to boiling under a reflux condenser for 10 hours 50-gram portions of the air-dried soils with 750 cubic centimeters strong hydrochloric acid, filtering, and making the filtrate to 1 liter. Aliquots of the solution thus obtained were used for the determination of the amid, basic, and nonbasic nitrogen. The amid nitrogen, determined as ammonia by the direct distillation of the solutions after making alkaline with magnesium oxid, would also contain the ammonia orig-

¹ The occurrence of large quantities of ammonium compounds is a phenomenon common to many Hawaiian soils.

² *The Vegetable Proteins*. London and New York, 1909.

³ *Jour. Amer. Chem. Soc.*, 25 (1903), p. 323.

⁴ *Ztschr. Physiol. Chem.*, 27 (1899), p. 95.

⁵ *Iowa Sta. Research Bul.* 1 (1911).

inally present in the soil. This was deducted from the amounts found.

The basic nitrogen was determined by precipitation with phosphotungstic acid in water solutions obtained by filtering the residue left after the distillation with magnesium oxid. As shown by previous investigators,¹ the nitrogen thus obtained in this precipitate occurs largely in the form of diamino acids. Purins, alkaloids, etc., may also be precipitated by phosphotungstic acid, and, therefore, if present, would be contained in this group. The nonbasic nitrogen was determined by subtracting the sum of the other two groups from the total nitrogen dissolved. While this group is now referred to by some investigators as monamino acids, Jodidi and Robinson have each brought forth evidence that it is not made up wholly of monamino acids. Just what other bodies occur in this group is not yet known. The results obtained, calculated as in all other cases in this bulletin on the water-free basis, are given in the following table:

Organic nitrogen of Hawaiian soils.

[Soluble in hydrochloric acid.]

Soil No.	Per cent of total nitrogen.	Per cent of the soil.				Per cent of nitrogen in solution.		
		Amid.	Basic nitrogen.	Nonbasic nitrogen.	Total.	Amid.	Basic.	Nonbasic.
379.....	71.96	0.095	0.030	0.300	0.426	22.30	7.04	70.42
428.....	72.59	.141	.028	.368	.559	24.79	5.01	66.27
447.....	67.51	.074	.033	.131	.239	30.96	13.81	54.82
292.....	91.80	.024	.012	.075	.112	21.43	10.71	66.96
343.....	90.91	.054	.020	.124	.200	27.00	10.00	62.00
345.....	88.52	.042	.017	.131	.193	21.76	8.81	67.88
347.....	80.42	.225	.097	.663	.998	22.55	9.72	66.43
405.....	84.62	.042	.019	.099	.165	25.45	11.51	60.00
406.....	91.22	.079	.055	.276	.416	18.99	13.22	66.35
Average.....						23.91	9.98	64.57

The above table shows that there is considerable variation in the amounts of soluble nitrogen in different soils. In soil No. 447 only 67.51 per cent of the total nitrogen was dissolved, while No. 292 yielded 91.80 per cent. Concerning the insoluble nitrogen very little is known.

AMIDS.

Upon distilling the solutions after making them alkaline with magnesium oxid approximately 25 per cent of the nitrogen in solution was obtained in the form of ammonia, here referred to as amid nitrogen, and all the soils studied, with the exception of Nos. 447, 343, and 406, yielded approximately the same relative amounts of nitrogen as ammonia. It is of interest to note that the amids constitute a

¹ Jodidi, loc. cit.; Robinson, loc. cit.

considerably higher percentage of the nitrogen of soils than are reported to occur in vegetable proteins. For example, the investigations of Osborne show that, on an average, the seed proteins contained 11.6 per cent of their nitrogen as amids. In a few cases, however, as, for example, gliadin from wheat and rye and hordein from barley, the amids comprised more than 20 per cent of the total nitrogen. On the other hand, a large number of proteins studied were found to contain considerably less than 10 per cent of their nitrogen in amid form.

A direct comparison of the amid nitrogen of the above soils with that found in soils elsewhere is possible in a few cases only, for the reason that the strengths of the acid, and the lengths of the time of digestion, used in the investigations on this subject vary so greatly. With the results obtained by Jodidi, however, our data are comparable, and from his work on Michigan peat and Iowa soils the amounts of amid nitrogen found were approximately the same as those found in Hawaiian soils.

BASIC NITROGEN.

The percentage of nitrogen precipitated by phosphotungstic acid was found to vary considerably in the different soils studied, but on the average to be similar to the amounts reported by Jodidi. In these studies no attempt was made to prove the nature of these nitrogen compounds, but from the work of others it seems permissible to consider them as being composed principally of diamino acids. It is noteworthy that the percentages of basic nitrogen in soils fall far below the percentages found in the majority of vegetable proteins. With the exception of glutenin from wheat, gliadin from wheat and rye, hordein from barley, and zein from maize, the basic nitrogen comprises more than 20 per cent of the total nitrogen in the vegetable proteins previously studied, and in a number of instances even more than 30 per cent of it. The basic nitrogen compounds of Hawaiian soils comprise only about 10 per cent of the total nitrogen.

Since the principal diamino acids that occur in vegetable proteins are arginin, histidin, and lysin, each of which can be precipitated from dilute solutions by phosphotungstic acid, it may be assumed that these compounds contain the principal diamino nitrogen split off in the hydrolysis of soil organic matter. The amounts found vary considerably. This may be accounted for in part by the fact that the phosphotungstic acid method, in order to give reliable results, must be conducted under as definite conditions as possible. In view of the presence in the solution of various inorganic salts dissolved in the hydrochloric acid digestion, it is hardly to be supposed that the conditions of this precipitate were indetical with the different soils. The precipitate is slightly soluble in the solutions employed, and

at the same time somewhat difficult to separate entirely from the nonbasic nitrogen in the solution. For these reasons some variation in the results is to be expected.

NONBASIC NITROGEN.

The percentages of nonbasic nitrogen, with the exception of that from soil No. 447, were found to be remarkably similar in every instance, amounting to about two-thirds of the nitrogen dissolved by boiling hydrochloric acid. In this respect the soluble nitrogen of soils is quite similar to that of vegetable proteins. The nonbasic nitrogen of soils is looked upon as being composed largely, but not entirely, of monamino acids, and probably such as are obtained in the hydrolysis of protein. Robinson,¹ for example, isolated leucin and isoleucin from hydrochloric-acid solutions of Michigan peat. Doubtless other monamino acids occur in the solutions. It is claimed, however, that a considerable portion of the nonbasic nitrogen of soils occurs in forms other than as monamino acids. Robinson, by the use of the Van Slyke nitrous acid method for the determination of monamino acids, found considerably less monamino acid in solution than was necessary to account for the nonbasic group, while Jodidi arrived at similar conclusions by the use of the formaldehyde titration method. Osborne,² has presented data supporting the idea that the nonbasic nitrogen obtained from vegetable proteins actually occurred as monamino acids. As yet no explanation of this difference between the nitrogen of soils and that of vegetable proteins has been proposed. The soluble nonbasic nitrogen in Hawaiian soils approximates the amounts found in soils elsewhere.

EFFECTS OF AERATION ON SOIL NITROGEN.

Some references have already been made to the fact that a wide range in the degree of aeration prevails in different Hawaiian soils, and that some of the soils studied in this investigation represent extremes in this respect. By reference to the previous description of the soils it is seen that soils Nos. 379, 428, and 447 represent aerated soils, 379 and 447 particularly so, since they are taken from well aerated land in sections where semiarid conditions have prevailed for many years. The remaining soils studied represent anaerobic conditions, since they have been used in aquatic agriculture a large part of the time for many years. So far as known no nitrogenous fertilizers of any sort have been applied to any of these soils.

It is generally held that the production of ammonia from organic nitrogen is necessary before its nitrification can take place, and that ammonia can be formed by a wide range of soil organisms. Some of these

¹ Loc. cit.

² *The Vegetable Proteins*. London and New York, 1909.

are aerobic, some anaerobic, while still others are able to act under either of the two conditions. It has also been shown at this station¹ that ammonification actually takes place in soils during the time of submergence. The relative amounts of amid, basic, and nonbasic nitrogen occurring in soils which had long been subjected to extreme conditions of aeration were determined to obtain evidence as to the nature of the chemical changes induced by the organisms when operating under the two sets of conditions.

By again referring to the table (p. 9) it will be seen that the percentages of the total nitrogen dissolved by hydrochloric acid were greater in every instance in the soils long subjected to anaerobic conditions. On an average 70.69 per cent of the total nitrogen was dissolved from aerated soils, while 87.93 per cent was rendered soluble in the unaerated soils. These data indicate that the putrefactive decay, which evidently predominates in submerged soils, leaves the nitrogen in a form more easily dissolved by hydrochloric acid than the process of *eremacausis*, that takes place under aerated conditions.

The relative amounts of the different groups obtained from the soils representing the two classes of conditions, however, were found to be quite similar in most instances. The table showing the nitrate and ammonia present (p. 7) indicates that with the exception of soils Nos. 347 and 406, those representing unaerated conditions contained next to no nitrate. The nitrate found in the remaining unaerated soils was formed almost entirely during the time of drying out in the laboratory. These samples were taken from the field in a wet state and then contained practically no nitrate. In fact, nitrification scarcely takes place at all in submerged Hawaiian soils. The data, therefore, fail to give any indication of a fundamental difference in the nature of the hydrolyses which take place under aerobic and anaerobic conditions.

HUMUS NITROGEN.

The alkali soluble organic matter of soils, usually known as humus, is generally considered to be of special importance. Only a part of the organic matter present in soils occurs as humus, and generally very little attention is paid to the remaining. For this reason some study has been given to the nitrogen bodies contained in it. In this investigation it was hoped to learn something regarding the chemical make-up of these bodies by determining the amounts of the different nitrogen groups actually present. Some light was also sought on the question whether or not the alkali soluble nitrogen bodies are really different from the organic nitrogen of soils as a whole. The soils used

¹ Hawaii Sta. Bul. 31.

in this phase of the work were the same as those employed in the studies reported in the preceding pages.

**NITROGEN DISSOLVED IN THE PRELIMINARY 1 PER CENT
HYDROCHLORIC ACID EXTRACTION.**

Calcium and magnesium are generally combined to some extent with the humus bodies in such a way as to render the organic matter less soluble in dilute alkalis. In order to break up such combinations the soils are treated with 1 per cent hydrochloric acid until no further amounts of calcium and magnesium are dissolved. It is customary in humus determinations, then, to dissolve the humus bodies in 4 per cent ammonia solution. In brief investigations¹ carried on in this laboratory it was observed that the dilute hydrochloric acid extracts obtained in the preliminary treatment contained considerable organic matter. In one instance the solution was darkly colored and found to contain a notable amount of organic matter. Usually such solutions are discarded. It has been shown, however, that considerable amounts of nitrogen are dissolved from certain soils² in this preliminary acid extraction. In the work here reported the soils were first extracted with 1 per cent hydrochloric acid, then filtered and washed to neutrality. The solutions thus obtained should contain the ammonia originally present. The solutions were evaporated to a small volume and the nitrogen in them was determined by the Kjeldahl method, with the following results:

Nitrogen of soils soluble in cold 1 per cent hydrochloric acid.

Soil No.	Per cent of soil.	Per cent of total nitrogen.	Soil No.	Per cent of soil.	Per cent of total nitrogen.
379.....	0.019	3.21	345.....	0.007	3.21
428.....	.041	5.33	347.....	.029	2.34
447.....	.012	3.39	405.....	.009	4.61
292.....	.004	3.28	406.....	.012	2.63
343.....	.004	1.82			

By comparing these data with those given in the first table it will be seen that in every instance the soils contained only about one-half as much ammonia nitrogen as was dissolved by 1 per cent hydrochloric acid, while in a number of instances still greater amounts of nitrogen were dissolved. Some organic nitrogen, therefore, was thus dissolved, although the amounts were small.

In preparing the humus solutions for studies on the nitrogen bodies a 3 per cent solution of sodium hydrate was employed. With Hawaiian soils sodium hydrate solution has a special advantage of

¹ Hawaii Sta. Press Bul. 33.

² Rimbach, Jour. Amer. Chem. Soc., 22 (1900), p. 695.

causing much less deflocculation of the clay, so that by ordinary filtration the solutions can be freed from all but traces of clay. Forty grams, after extracting with dilute hydrochloric acid, were treated with 2,000 cubic centimeters of 3 per cent sodium hydrate solution for a period of two days, with occasional shaking during the first day. The solutions were siphoned off and aliquot portions used in the studies.

SEPARATION OF DIFFERENT FORMS OF NITROGEN IN HUMUS.

A part of the humus can be precipitated from the alkali solutions by acids, and this method has been used for obtaining so-called pure humus. The amounts precipitated, however, vary with the amount of acid used. Shorey¹ has shown that after filtering out the precipitate obtained by acidifying the humus solution a still further precipitate can be obtained by carefully neutralizing the filtrate, and that of the precipitates thus obtained each contains nitrogen. The humus extract made with a 2 per cent sodium hydrate solution was found to contain 0.0399 gram nitrogen per 100 cubic centimeters of solution. The hydrochloric acid filtrate he found to contain 0.0251 gram of nitrogen per 100 cubic centimeters of the original solution, and on neutralizing this filtrate with caustic soda the precipitate formed was found to contain 0.0168 gram of nitrogen.

In the work here reported hydrochloric acid was carefully added to 1,000 cubic centimeter portions of the humus solution (corresponding to 20 grams of soil) to apparent neutrality to litmus paper, then 20 cubic centimeters of 1 per cent hydrochloric acid was added, the precipitate formed was collected on a filter and washed. In this way the humus matter was roughly separated into two parts. The precipitates thus obtained were afterwards subjected to acid hydrolysis by boiling with 400 cubic centimeters strong hydrochloric acid for a period of 10 hours, and then filtering and washing the residue. The amid and basic nitrogen contained in the original humus solutions, in the filtrates obtained from precipitating the solutions with dilute hydrochloric acid, and in those obtained upon hydrolyzing the humus precipitates, have been determined.

The following table shows the total nitrogen contained in the original humus solutions, and that in the portions obtained by the various separations:

¹ Hawaii Sta. Rpt. 1906.

The nitrogen of humus.

Soil No.	Total nitrogen in soil. Per cent of soil.	Humus nitrogen precipitated by HCl.		Humus nitrogen not precipitated by HCl. (c). Per cent of soil.	Total humus nitrogen by addition (a+b+c). Per cent of soil.	Total humus nitrogen determined directly. Per cent of soil.	Humus nitrogen in per cent of soil nitrogen.	Humus nitrogen not precipitated by HCl. Per cent of total humus nitrogen.	Humus nitrogen precipitated by HCl.	
		Hydrolyzable (a). Per cent of soil.	Non-hydrolyzable (b). Per cent of soil.						Hydrolyzable. Per cent of humus nitrogen.	Non-hydrolyzable. Per cent of humus nitrogen.
347.....	1.241	0.362	0.125	0.315	0.802	0.774	62.37	39.28	45.14	15.59
428.....	.770	.289	.051	.247	.587	.590	76.62	42.08	49.23	8.69
379.....	.592	.270	.041	.129	.440	.439	74.16	29.32	61.36	9.32
406.....	.456	.100	.027	.117	.244	.226	49.56	47.96	40.98	11.06
447.....	.354	.094	.026	.105	.225	.215	60.79	46.67	41.78	11.56
345.....	.218	.066	.019	.070	.155	.147	67.43	45.16	42.58	12.26
343.....	.220	.061	.012	.069	.142	.127	57.73	48.59	42.96	8.45
405.....	.195	.044	.012	.067	.123	.123	63.08	54.47	35.77	9.76
292.....	.122	.031	.012	.015	.058	.058	67.54	25.86	53.45	20.69
Average.....							64.36	42.15	45.92	11.93

The above data show that the humus nitrogen varied with different soils, but averaged 64.36 per cent of the total nitrogen. In every instance, except two, more than one-half of the nitrogen was dissolved by dilute alkali, while in two instances practically three-fourths of it was thus extracted. The bodies precipitated with dilute hydrochloric acid also contained nitrogen in varying amounts. The nitrogen bodies precipitated by hydrochloric acid upon subsequent hydrolysis yielded by far the greater portion of their nitrogen to the solutions, the insoluble residues having been found to contain 11.93 per cent of the humus nitrogen. By these methods, therefore, the nitrogen of soils can be separated into fractional parts.

AMID NITROGEN.

The amid nitrogen in the original humus solutions was first determined by evaporating the solutions on the water bath, after slightly acidifying with hydrochloric acid, then making alkaline with magnesium oxid and distilling. The relatively high percentages of ammonia thus obtained suggested that some hydrolysis had taken place during the time of the evaporation on the water bath, possibly through the action of the hydrochloric acid present. In order to eliminate this possibility, separate portions were distilled directly with magnesium oxid, after having been slightly acidified with hydrochloric acid. In like manner the solutions, obtained after filtering out the humus matter precipitated by hydrochloric acid, were distilled with magnesium oxid, and also the solutions obtained

by hydrolyzing the humus precipitate. The results are shown in the following table:

Amid nitrogen in humus.

Soil No.	Solutions slightly acidified and evaporated before distillation. Per cent of soil.	Solutions slightly acidified. No evaporation. Per cent of soil.	Not precipitated by HCl. (a). Per cent of soil.	Precipitated by HCl. (b). Per cent of soil.	Total.		
					a+b.	Per cent of total soil nitrogen.	Per cent of humus nitrogen.
347.	0.100	0.087	0.095	0.073	0.168	13.54	21.70
428.	.073	.073	.074	.056	.130	16.88	22.03
379.	.059	.052	.056	.061	.117	19.76	26.65
406.	.039	.028	.040	.020	.060	13.15	26.55
447.	.033	.026	.031	.027	.058	16.38	26.98
345.	.025	.018	.022	.016	.038	17.43	25.85
343.	.020	.020	.025	.018	.043	19.54	33.86
405.	.026	.017	.014	.023	.037	18.97	42.53
292.	.009	.012	.008	.011	.019	15.57	32.76
Average						16.80	28.77

The relatively high percentages of amid nitrogen obtained from the original humus solutions is noteworthy, as is also the fact that the nitrogen bodies not precipitated by hydrochloric acid contained practically all of the amid nitrogen existing as such in the humus solution. On an average, the hydrochloric acid precipitate yielded upon hydrolysis practically the same amounts of amid nitrogen as were contained in the original humus solutions. The total amid nitrogen contained in the humus, when calculated to the percentages of the total soil nitrogen, presents some variation, and on the average amounted to 16.80 per cent of the total soil nitrogen. When such data were calculated to the basis of the humus nitrogen it was found that the relative amounts of amid nitrogen contained increased with a decrease in the humus nitrogen. In other words, relatively greater amounts of amids occurred in soils which contain a low percentage of humus nitrogen. This may be purely a coincidence, but is probably due to the fact that either in the preparation of the humus solution, or in the process of determining the amid nitrogen in it, a certain amount of hydrolysis took place which would tend to markedly increase the relative amounts of amid obtained from those humus solutions containing the smallest amounts of nitrogen. On an average, 28.77 per cent of the humus nitrogen was found to be present as amids. It will be recalled that the amid nitrogen obtained upon hydrolyzing the soils as a whole amounted to considerably smaller percentages of the nitrogen dissolved.

BASIC NITROGEN.

The basic nitrogen bodies in humus were determined by the photungstic acid method, as already outlined. The results are recorded in the following table:

Basic nitrogen in humus.

Soil No.	In the original humus solution. Per cent of soil.	Not precipitated by HCl. (a). Per cent of soil.	Precipitated by HCl. (b). Per cent of soil.	Total.		
				a + b. Per cent of soil.	Per cent of total soil nitrogen.	Per cent of total humus nitrogen.
347.....	0.028	0.024	0.010	0.034	2.74	4.39
428.....	.027	.025	.010	.035	4.81	5.93
379.....	.017	.021	.007	.028	4.73	6.38
406.....	.015	.014	.009	.023	5.05	10.18
447.....	.021	.019	.006	.025	7.06	11.63
345.....	¹ .003	.017	.017	.034	15.59	23.13
343.....	¹ .004	.017	.008	.025	11.36	19.68
405.....	.011	.009	.008	.017	8.71	19.54
292.....	.014	.009	.017	.026	21.31	44.83

¹ There is apparently some error in these determinations.

It was found that the basic nitrogen amounted to practically the same percentages when determined directly from the original humus solutions as were present in the filtrates from the hydrochloric acid precipitate. The amounts of basic nitrogen split off in the hydrolysis of the precipitates were relatively smaller, as compared with the amid nitrogen, than were obtained in the filtrates. The total basic nitrogen, calculated to percentages of the total soil nitrogen, increased with a decrease in the amount of nitrogen in the soil, and when calculated to percentages of the total humus nitrogen the same relationships are even more marked. Soil No. 347, containing 0.77 per cent humus nitrogen, yielded only 4.39 per cent of it as basic nitrogen, while soil No. 292, containing 0.058 per cent humus nitrogen, yielded 44.83 per cent of it as basic nitrogen.

In view of the analytical error involved in the determination of the basic nitrogen, it is unsafe to generalize concerning the relatively great increases in the basic nitrogen of humus in passing from soils with large to soils of smaller humus nitrogen content. It seems probable, however, that hydrolysis took place during the alkali extraction process.

NONBASIC NITROGEN.

It is obviously not permissible to consider the difference between the total nitrogen in humus and the amounts of amid and basic nitrogen that occur in the original humus solutions as nonbasic nitrogen, for the reason that these solutions can not be considered as having been completely hydrolyzed. It is well known, for example, that various proteins are quite soluble in alkalis without the proteins undergoing any particular hydrolysis, as they can be precipitated, in a more or less unaltered condition, from such solutions by the addition of acid. On the other hand, the nitrogen compounds in the filtrates obtained from the humus precipitated by dilute hydrochloric

acid, and also those split off in the hydrolysis of the humus precipitate, may reasonably be considered as being made up of amid, basic, and nonbasic nitrogen compounds. We have, therefore, calculated the amounts of nonbasic nitrogen in these portions of humus. The results are shown in the following table:

Nonbasic nitrogen in humus.

Soil No.	In filtrates from HCl precipitates.			In hydrolyzed humus precipitates.			Total.	
	Per cent of soil.	Per cent of nitrogen in filtrate.	Per cent of humus nitrogen.	Per cent of soil.	Per cent of nitrogen in solution.	Per cent of humus nitrogen.	Per cent of soil.	Per cent of humus nitrogen.
347.....	0.196	62.22	25.32	0.249	68.78	32.17	0.445	57.49
428.....	.148	59.92	25.08	.213	73.70	36.10	.361	61.18
379.....	.052	40.31	11.84	.203	75.18	46.24	.255	58.08
406.....	.063	53.84	27.87	.071	71.00	30.97	.134	58.84
447.....	.055	52.38	25.58	.061	64.89	28.36	.116	53.94
345.....	.031	44.28	21.09	.033	50.00	22.45	.064	43.54
343.....	.027	39.13	21.26	.035	57.37	27.56	.062	48.82
405.....	.046	68.65	37.39	.011	¹ 25.00	¹ 8.78	.057	45.17
292.....				.003	¹ 9.67	¹ 5.18		
Average.....		52.59	25.05		64.84	31.98		53.38

¹ Not included in averages.

These data show the relatively large amounts of nonbasic nitrogen contained in humus. On the average about 25 per cent of the humus nitrogen occurred in the original humus solutions as nonbasic nitrogen compounds, or 52.59 per cent when calculated to the percentage of humus nitrogen soluble in dilute hydrochloric acid. The solutions obtained upon hydrolyzing the humus precipitated by dilute hydrochloric acid yielded a still greater amount of nonbasic nitrogen. On an average 64.84 per cent of this nitrogen occurred as nonbasic, which, when calculated to percentages of the total humus nitrogen, amounts to 31.98 per cent. By adding the nonbasic nitrogen in these two portions of humus it is found that 53.38 per cent of the humus nitrogen is made up of nonbasic nitrogen compounds. By referring to the data previously presented (p. 9) it will be seen that the relative amounts of nonbasic nitrogen in humus are somewhat less than the amounts of nonbasic nitrogen obtained in the hydrolysis of the soil as a whole.

Considering the different groups of nitrogen compounds as obtained from the different portions of humus, the preceding data show that the humus contained slightly less amid, basic, and nonbasic nitrogen than were split off upon hydrolyzing the soil nitrogen as a whole, but, on the other hand, the humus nitrogen bodies as such are made up of relatively more amid and basic nitrogen than the soil nitrogen as a whole. In other words, the nitrogen of soils soluble in 3 per cent sodium hydrate is bound up in bodies differing somewhat from the

nitrogen bodies not soluble in the solvent. In view of the relatively large amounts of amid, basic, and nonbasic nitrogen contained in the original humus solutions, it is believed that considerable hydrolysis of the proteins occurring in soils has taken place through the action of bacteria, and that the humus nitrogen is probably of more immediate value as a source of available nitrogen than is the nonhumus nitrogen. It seems justifiable to believe, therefore, that the humification process is really one of importance in soils as a step toward the production of available nitrogen compounds.

DETERMINATION OF HUMUS NITROGEN.

In view of the large amounts of amid nitrogen obtained in the original humus solutions some study was directed to the question of methods for the determination of total humus nitrogen. The data previously submitted, showing the total humus nitrogen, were obtained by first evaporating the solutions after acidification with hydrochloric acid, then subjecting the residues to nitrogen determination by the use of the regular Kjeldahl method. The determination of humus nitrogen is frequently made from ammonia solutions of humus after expelling the free and combined ammonia present by distilling the magnesium oxid.¹ But on account of the relatively large amounts of amid nitrogen found in the sodium hydrate solutions, which, if present in corresponding amounts in ammonia solutions of humus, would be lost in the magnesium oxid distillation, there is brought into comparison the nitrogen of these soils as found in both the sodium hydrate and ammonia solutions. The results are recorded in the following table:

Total humus nitrogen by different methods.

Soil No.	Amid nitrogen in NaOH solution. Per cent of soil.	Total humus nitrogen in NaOH solution. Per cent of soil.	Total humus nitrogen in am- monia solution. Per cent of soil	Nitrogen absorbed from am- monia solutions. Per cent of soil.	Soil No.	Amid nitrogen in NaOH solution. Per cent of soil.	Total humus nitrogen in NaOH solution. Per cent of soil.	Total humus nitrogen in am- monia solution. Per cent of soil.	Nitrogen absorbed from am- monia solutions. Per cent of soil.
347.....	0.100	0.774	0.657	0.502	345.....	0.025	0.147	0.140	0.085
428.....	.073	.590	.609	.753	343.....	.020	.127	.117	.175
379.....	.059	.439	.284	.523	405.....	.026	.087	.099	.139
406.....	.039	.226	.179	.264	292.....	.009	.058	.067	.072
447.....	.033	.215	.218	.147					

In some instances much higher percentages of nitrogen were found in the sodium hydrate solutions than in the ammonia solutions, and in some instances this difference about equals the amid nitrogen con-

¹ In determining the nitrogen in the ammonia solutions of humus it was found advantageous to evaporate to dryness two portions of the solution. In one the combined ammonia only was determined, which amounts were subtracted from the total nitrogen found in the other without distilling with MgO.

tained in the sodium hydrate solutions. Rimbach ¹ has shown that the humus nitrogen as determined in sodium hydrate and ammonia solutions, respectively, occurs in greater amounts in the former solutions, from which he concluded that sodium hydrate dissolves more nitrogen than ammonia. Similar observations have also been made by others. From work done in this laboratory it is doubtful whether sodium hydrate actually dissolves more nitrogen than ammonia, but rather that the increased amounts found represent amid nitrogen, which is lost in the methods employed in the determination of total humus nitrogen from ammonia solutions.

While the amounts of ammonia absorbed by the residues left after evaporating to dryness ammonia solutions of humus bear no definite relation to the amounts of humus nitrogen present, the absorption of ammonia by humus took place to a considerable extent, and correction should be made for this in humus determinations, as has been pointed out by Emery ² and others.

PERCENTAGE OF NITROGEN IN HUMUS OF HAWAIIAN SOILS.

Hilgard ³ has shown that humus from arid regions contains a higher percentage of nitrogen than humus from humid sections. He found that humus from arid regions contained on the average 15.23 per cent nitrogen, while the humus from humid regions contained only 4.23 per cent. In the work on soils at this station many humus and humus nitrogen determinations have been made and some of the results obtained are submitted in the following table:

Total humus and humus nitrogen in Hawaiian soils.

Soil No.	Humus. Per cent of soil.	Humus ash. Per cent of soil.	Humus nitrogen. Per cent of humus.	Soil No.	Humus. Per cent of soil.	Humus ash. Per cent of soil.	Humus nitrogen. Per cent of humus.
347.....	14.81	2.28	5.16	235.....	2.03	0.63	9.85
423.....	14.31	3.89	4.12	286.....	3.15	.94	10.35
379.....	8.20	1.91	5.35	287.....	3.64	1.38	7.31
406.....	3.08	1.64	7.33	288.....	3.06	1.48	5.88
447.....	3.61	1.54	5.94	312.....	5.26	1.19	5.27
345.....	1.74	1.13	6.83	313.....	4.14	1.44	6.85
343.....	2.45	1.73	5.21	314.....	3.94	1.17	6.81
405.....	1.74	1.07	4.91	315.....	4.33	.79	6.36
292.....	1.80	1.87	3.21	316.....	3.39	.81	6.22
282.....	2.77	1.04	7.87	317.....	5.12	1.18	6.32
253.....	1.78	.79	6.40				
254.....	2.72	.70	5.62	Average.....			5.83

These data show that the humus of Hawaiian soils contains nitrogen in amounts similar to those of humid soils elsewhere. Some of the soils used in this investigation (Nos. 379 and 447) came from sections which have been designated as arid, but the arid conditions which now prevail in these sections have probably not existed as such for

¹ Loc. cit.

³ Soils. New York and London, 1907, pp. 136, 137.

² Jour. Amer. Chem. Soc., 22 (1900), p. 255.

many generations, perhaps not more than 75 years, and the humus has been formed largely under humid or semihumid conditions.

It is probable that oxidation takes place more actively in arid than in humid soils, which oxidation probably results in a greater degree of decomposition of the nonnitrogen constituents, thus leaving a humus residue richer in nitrogen. Also, the humus may be considered as being older than that occurring in humid soils, for the reason that greater amounts of plant residues are continually becoming incorporated with the soils under conditions that are more favorable for plant growth, such as are offered by a more abundant moisture supply. For these reasons (perhaps others) it is to be expected that the humus of arid soils would be more largely composed of nitrogen constituents than that of humid soils.

SUMMARY.

(1) The nitrate and ammonia content of Hawaiian soils constitutes only a small percentage of the total soil nitrogen.

(2) Upon boiling different soils with strong hydrochloric acid, the amounts of nitrogen dissolved ranged from 67.51 per cent to 91.88 per cent of the total nitrogen. With two exceptions, the relative percentages of amid nitrogen, split off in the hydrolysis, were approximately the same, amounting on the average to 23.91 per cent of the nitrogen dissolved. Basic nitrogen occurred in the solutions in variable amounts, the average being 9.98 per cent of the soluble nitrogen. The percentages of nonbasic nitrogen, determined by difference, proved to be quite concordant in most of the soils, amounting on the average to 64.57 per cent of the soluble nitrogen.

(3) The relative percentages of amid and basic nitrogen, split off in the hydrolysis of Hawaiian soils, stand in the reverse order to that in which they occur in the vegetable proteins; while the percentage of nonbasic nitrogen practically equals that found in the vegetable proteins. It had been suggested that soil bacteria attack the nitrogen bodies in such way as to split off the basic nitrogen compounds, and that these then become ammonified, or otherwise lose their identity as diamino acid compounds, possibly being partially converted into amid forms.

(4) Anaerobic conditions predominate in Hawaiian soils, and under such conditions the nitrogen is more soluble than in well aerated soils, but the relative percentages of the different groups of organic nitrogen compounds seemed not to be affected by the predominance of one or the other of these conditions.

(5) The amount of nitrogen soluble in 1 per cent hydrochloric acid was about twice as large as that of ammonia originally occurring in the soils.

(6) The solubility in 3 per cent sodium hydrate varied from 49.56 per cent to 76.62 per cent of the total nitrogen. Of the nitrogen thus dissolved, 57.85 per cent was precipitated by dilute hydrochloric acid, of which 11.93 per cent (expressed in percentage of the humus nitrogen) remained insoluble after boiling in strong hydrochloric acid for 10 hours. Amids comprised 28.77 per cent of the humus nitrogen, of which about one-half existed as amid in the original humus solutions, and which remained in solution upon acidifying with hydrochloric acid. The remaining half was split off when the humus, precipitated by hydrochloric acid, was subjected to acid hydrolysis. The basic nitrogen ranged from 4.39 per cent to 44.83 per cent of the humus nitrogen, increasing as the total nitrogen of the humus decreased. Nonbasic nitrogen was found to constitute 53.38 per cent of the humus nitrogen, of which 25.05 per cent existed as such in the original humus solutions.

(7) The amounts of amid and basic nitrogen in humus expressed as percentages of the humus nitrogen were found to be higher than the amounts obtained by subjecting the original soil to hydrolysis.

(8) In view of the large amounts of amid occurring in humus solutions, it was found better to use sodium hydrate as the solvent for extracting humus that is to be used for total humus nitrogen determinations.

(9) The humus of Hawaiian soils contains a small percentage of nitrogen (5.88 per cent as an average of 22 samples), in which respect the humus of these soils closely resembles that found in humid soils in the States.

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