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ALTERATION OF MICROBIAL ACTIVITIES, MINERAL NITROGEN AND FREE AMINO ACID CONSTITUENTS OF SOILS BY PHYSICAL TREATMENT

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INTRODUCTION

The reactions involved in the microbial transformation of soil organic matter are significantly affected by the previous physical treatments such as drying, heating, or storage, that the soil has undergone. The occurrence after heat treatment, of an initial sharp decrease in microbial numbers and often a toxicity to plants followed by a flush of microbial activity and increased fertility has been well documented [11 13 18]. It has also been demonstrated that milder treatments such as air-drying [1 2 3], freezing [7 12] burning of litter, and sunlight [10], application of pesticides [20 22], and even irradiation affect the soluble organic and inorganic nitrogenous compounds present in soils.

Birch [3 4], examining the effects of drying on East African soils has indicated that drying results in increased exposure of the surface area of the soil colloids resulting in a greater availability of nutrients. Soulides and Allison [21] concluded that freezing of soils results in less pronounced but similar physical, chemical and biological changes as those brought about by drying. They indicated that the increased decomposition of soil organic matter following intermittent drying or freezing is due to a release of nutrients, especially energy sources that can be rapidly oxidized by an active soil population. Chase and Gray [5] have supported the theory that readily decomposable water-soluble soil organic, matter accumulates during air drying, whereas Jenkinson [9], utilizing tracer techniques, indicated that the biomass of the soil is the fraction of soil organic matter influenced by these treatments.

The advent of biochemical techniques for the isolation of nitrogenous organic constituents and microbial intermediates, and the application of tracer techniques to the soil system, now makes it possible to investigate the effects of treatments, such as partial sterilization, on the soil biomass and on the organic matter constituents rather than on merely the final products of microbial degradation such as evolved CO_2 and mineralized nitrogen.

Biochemical techniques tend to be time consuming, requiring that all or a portion of a soil sample be stored for a period of time before analysis. It is imperative that the extent of biochemical alterations which may occur in a soil during storage be known.

The present investigation of the extent of solubilization of free amino acids, organic amino compounds and ammonia was designed to further ascertain the importance of these constituents in the soil system. Measurement of changes in the concentration of these constituents brought about by physical treatment was deemed important in assessing the alteration of soil components during storage and the interpretation of the basic physical and chemical changes that cause the well known flush of microbial activity after physical treatment.

EXPERIMENTAL METHODS

The soils used throughout the experiment were Chernozemic Black soils of medium texture, having a total nitrogen content of 0.22 to 0.25 per cent and pH values of 7.5. One of the samples (Hoey) was obtained from a summerfallow field in June, the other (Oxbow), had just grown a crop of wheat in the growth chamber and therefore represented a sample affected by plant roots. Samples of the Hoey soil were heated by free flowing steam and in an oven at 105°C for 2 hours. Other treatments included air drying for one week and 24 weeks and storage at 25% moisture in polyethylene bags at −16°C. Portions of the Oxbow sample were air dried for 12 weeks, others were frozen at a moisture content of 18%. Another sample was frozen at −79°C, then freeze dried and stored for 12 weeks.

Carbon dioxide evolution from soils incubated at 27°C was determined in an aeration train, utilizing 100 g of soil exposed to physical treatments such as drying, heating or storage conditions, plus 1 g of fresh soil. Microbial numbers were estimated by plate counts – the fungi on streptomycin rose-bengal agar and the bacteria on soil extract agar. Nitrate nitrogen was determined by the phenoldisulphonic acid method and exchangeable ammonia by steam distillation of an NaCl extract [8].

Free amino acids were extracted by leaching with 10 ml of 1 N ammonium acetate per gram of soil. Fifty grams of the steamed or oven-dried soil samples and 150 g of the soil subjected to milder treatments such as air drying were utilized. The leachate was concentrated *in vacuo* and the ammonium acetate removed by vacuum sublimation [16]. The residue, after sublimation was dissolved in a small amount of water, transferred to a beaker and the pH adjusted to 2.2 with 3 N H_2SO_4. Addition of isopropyl alcohol equivalent in volume to the solution containing the amino acids (8 ml) was utilized for desalting of the amino acids. The mixture was placed in the freezer for one hour, the precipitated salts removed by filtration, using fibre glass filter paper, and washed with 40 ml of cold 60% isopropyl alcohol. The amino acids in the filtrate were dried *in vacuo* and redissolved in 2 ml of pH 2.2. buffer.

The Moore and Stein procedure [14] was used for resolution of the individual amino acid components, Aminex MS cation-exchange resin ($56 \pm 9\ \mu$ size) being used for resolution. Contents of alternate tubes obtained from the fraction collector were used for ninhydrin analyses. The identity of individual amino acid peaks was verified by desalting [6] followed by paper chromatography in phenol–water, and butanol–acetic acid–water systems, and by specific spot tests.

RESULTS AND DISCUSSION

Alteration of free amino acid content by physical treatment

The recoveries of added amino acid from soils with 0.5 N ammonium acetate are quantitative if large amounts of amino acids are added to the soil system, however, at the low concentration normally present in soils, the recoveries are usually low with a fair degree of variability [16]. The use of 1 N rather than 0.5 N ammonium acetate as an extracting agent in conjunction with the desalting technique described, resulted in recoveries of amino acids that were less variable and more quantitative than previously reported.

The quantities of free amino acids extracted from an untreated Black Chernozemic silty clay loam from Saskatchewan (Table 1) were similar to those reported for a Brunizemic silt loam from Minnesota [17]. The total free amino acids approximated 5 μg/g with the individual amino acids being present in concentrations less than 1 μg/g of soil. Air-drying for one week caused a sharp increase in the quantities of methionine sulfoxide, glutamic acid,

TABLE 1

Free amino acid content (μg/g) of the summerfallow Hoey soil sample before and after physical treatment

Amino acid	Un-treated	Air dried 1 week	Air dried 24 weeks	Frozen 1 week	Frozen 24 weeks	Steamed 2 hours	Oven dried 2 hours
Methionine sulfoxide.	0.57	1.89	1.12	0.62	1.56	2.74	7.73
Aspartic acid	0.38	0.57	0.25	*	0.42	3.14	2.84
Threonine	0.29	0.29	0.26	0.39	0.12	2.52	1.56
Serine	0.08	0.45	0.29	0.33	0.13	3.34	1.55
Asparagine	0.22	0.74	0.23	0.32	0.19	2.36	1.82
Glutamic acid. . . .	0.94	3.31	2.39	0.47	0.39	9.80	9.76
Glycine.	0.28	0.39	0.26	0.23	0.24	1.57	2.80
Alanine	0.43	0.76	0.32	0.35	0.11	6.79	3.34
Valine	0.24	*	*	0.66	*	5.12	3.15
Methionine	0.66	0.51	0.46	0.38	*	3.84	3.57
Isoleucine	0.38	0.31	0.18	0.15	*	3.39	3.07
Leucine	0.30	0.48	0.17	0.27	0.25	6.88	2.74
Beta-alanine	0.25	0.77	0.18	0.26	*	0.80	1.62
Tryosine	*	0.45	0.45	0.59	0.76	1.39	2.83
Phenylalaline	*	0.48	0.21	*	0.43	2.24	3.07
γ-NH_2-Butyric acid .	*	0.44	*	0.29	*	3.49	1.81
Lysine	*	1.74	0.80	*	*	4.42	3.29
Histidine.	0.41	*	0.36	0.60	0.47	0.75	2.32
Total.	5.43	13.60	7.91	5.90	5.07	64.57	58.46

* Not present in measurable concentrations.

and lysine. On extended drying, the concentrations of these amino acids dropped somewhat but were still above those found in the untreated sample. Freezing caused some shift in the concentrations of individual amino acids present, but did not cause any overall change in the total soil free amino acid content. A number of peptides emerged with arginine from the ion exchange column. This amino acid which has been found to be present in similar concentrations as the other free amino acids [17] thus could not be measured in this study.

Steaming and oven-drying raised the total free amino acid content of the soils by a factor of 10, but the different environmental conditions during the heating period caused some differences in the concentrations of the individual amino acids, the soil which was oven-dried contained a much greater concentration of methionine sulfoxide, whereas the steamed sample had larger amounts of alanine and leucine.

In summerfallowed soil (Hoey) the readily decomposable materials presumably had already been metabolised. The effect of milder storage treatments was therefore, investigated on a somewhat similar soil which had just grown a crop of wheat under controlled conditions in the growth chamber (Table 2). The concentrations of aspartic acid, asparagine and glutamic acid were much higher in this soil, indicating the effect of rhizosphere conditions. This is in accord with other data showing the rhizosphere condition under soybeans [17] and alfalfa * which indicated that plant roots increase the concentration of asparagine, aspartic acid, glutamic acid and sometimes leucine without greatly affecting the concentrations of the other amino acids.

TABLE 2

Effect of storage on the free amino acid content ($\mu g/g$) of the rhizosphere Oxbow soil

Amino acid	Untreated	Air-dried 12 weeks	Frozen 12 weeks	12 weeks storage after freeze-drying	Liquid-nitrogen frozen
Methionine sulfoxide	0.17	0.27	0.17	0.65	0.37
Aspartic acid . . .	2.28	2.78	3.03	9.69	1.71
Threonine.	0.54	0.53	0.43	1.25	0.44
Asparagine	4.91	0.61	1.56	4.54	1.79
Glutamic acid . . .	2.39	3.97	1.17	12.71	0.88
Glycine.	0.15	0.37	0.38	0.65	0.33
Alanine.	0.73	0.87	0.65	2.42	0.79
Valine	0.34	0.72	0.46	1.47	0.64
Methionine	0.25	0.53	*	1.37	*
Isoleucine.	0.49	0.50	0.41	1.52	1.01
Leucine.	0.63	0.76	0.62	1.94	1.34
Tyrosine	0.56	0.47	0.69	0.72	0.89
Phenylalanine. . .	0.41	1.07	0.48	2.63	1.13
γ-NH_2-butyric acid.	0.17	0.20	0.14	0.61	—
Histidine	0.12	0.97	0.47	0.78	0.37
Total	14.14	14.62	10.66	42.95	11.69

Storage of the rhizosphere sample showed similar trends to those previously noted for the summerfallow soils; 12 weeks air-drying increased the amino acid concentration slightly whereas freezing caused a drop. Freeze-drying of a soil and storage in this

* Unpublished data. University of Saskatchewan.

condition caused a uniform significant rise in the concentration of all the amino acids, indicating that this treatment cannot be used for storage of soil to be used for microbiological or biochemical analysis.

A portion of each sample that had been stored in the frozen condition was thawed and analysed for amino acids, another portion was refrozen by dropping it into liquid nitrogen. The data in the last column on Table 2 indicate that there were some alterations in the concentrations of individual amino acids but that the total sums remained relatively equal.

Effect of amino nitrogen solubilization on microbial activity

The effect of treatment on three forms of available soil nitrogen and on two indices of microbial activity during subsequent incubation is shown in Table 3. Extensive plate counts of the effect of physical treatment on microbial numbers were conducted. However, since data such as these are of little significance in explaining subsequent mineralization rates the results are not shown in tabular form.

TABLE 3

The production of soluble nitrogenous compounds (μg/g) by physical treatment and its effect on nitrification and respiration (μg/g)

Treatment	Initial concentration of specific nitrogen compounds			Indices of microbial activity		
	Initial free amino acid-N	Ammonium-N	Soluble amino-N	Inorganic nitrogen after incubation	CO_2-C evolution 1 wk	CO_2-C evolution 5 wks
Oxbow soils						
Untreated	1.7	5.73	3.2	40.1	160	370
Air dried.	1.6	8.57	1.9	42.7	100	460
Frozen – 12 wks . . .	1.3	6.70	1.8	45.5	130	480
12 wks after freeze drying	4.9	8.0	5.1	43.2	320	640
Liquid nitrogen frozen	1.3	13.1	3.2			
Hoey soils						
Untreated	0.1	6.6	3.0	28.6	120	560
Streamed	7.7	29.8	11.9	87.0	360	1140
Oven dried.	7.0	25.0	11.7	79.7	480	1190
Air Dried – 1 wk . . .	1.7	10.2	3.4	37.9	160	930
Frozen – 1 wk	0.8	8.2	2.9	43.5	280	970
Air Dried – 24 wks . .	1.0	4.6	3.2	21.9	320	640
Frozen – 24 wks . . .	0.6	6.1	2.7	16.1	200	710

The population of actinomycetes, bacteria, and fungi, were greatly reduced by the heating and drying treatment. The freezing of both soil samples, especially for extended periods, caused a rise in the plate count of fungi. Reinoculation with fresh soil and incubation tended to equalize the population of all but the steamed soil samples where a long term increase in microbial numbers was noted.

The ammonia nitrogen shown in Table 3, was measured by alkali distillation of an acidified NaCl extract. The quantity of free amino acid nitrogen was calculated from the amino acid data in the previous tables. The chromatograms of the ammonium acetate leachates of soil always showed two peaks in addition to those corresponding to the amino acids reported in Tables 1 and 2. One group of ninhydrin positive compounds tentatively identified as sugar-amino compounds emerged from the ion-exchange resin before aspartic acid, another was retained by the cation-exchange resin and emerged in the vicinity of arginine.

The compounds emerging with and after arginine, had not previously been reported as a constituent of the free amino fraction of soil and no adequate R_F values were obtained without hydrolysis prior to paper chromatography. Tryptophan, histidine, arginine, and ammonia were found after hydrolysis. Arginine normally comes out in this range and may not have been a peptide constituent. Values reported in the column entitled 'Soluble amino nitrogen' (Table 3) therefore represent a summation of the nitrogen in the free amino acids plus the alpha amino nitrogen reacting with ninhydrin in the compounds emerging at either end of the chromatogram.

In the Oxbow soil, the CO_2 evolution was initially depressed by the drying treatment but then enhanced during longer incubation. Freeze-drying which resulted in an enhanced production of amino acids and a slight increase in the ammonia nitrogen did not affect the production of peptides measured in the amino-N fraction. Similarily the mineralization of nitrogen was not significantly affected, but the evolution of CO_2 was strongly enhanced.

After 5 weeks incubation the summerfallowed soil, which had been steam heated, released 45 μg of ammonium nitrogen per gram of soil during steam distillation. The net nitrification of this sample therefore amounted to only 42 μg/g. The available nitrogen of all other samples, including the oven dried was nitrified with only very small concentrations of constituents which released 5 to 9 μg of ammonium nitrogen during distillation with NaOH being present. All treatments except extended air-drying and freezing resulted in enhanced mineralization of nitrogen. Carbon dioxide evolution was increased by all treatments with oven-drying producing greater concentrations of CO_2 than steaming.

TABLE 4

Correlation between the amounts of organic and inorganic nitrogen released with microbial activity produced by physical treatment

Nitrogen compound	Correlation coefficients		
	Nitrogen mineralization	CO_2 – 5 wks	CO_2 – 1 wk
Ammonium-N	0.92 **	0.75 **	0.67 *
Soluble amino-N	0.85 **	0.74 **	0.81 **
Free amino Acid-N	0.89 **	0.58	0.74 **

* $P = .05$ ** $P = .01$

The data showing the correlation between the amount of organic and inorganic nitrogen released and microbial activity produced by physical treatment (Table 4) indicate that of the three nitrogenous constituents measured, the ammonium nitrogen values give the best indications of the extent of subsequent nitrogen mineralization. The lower correlations between CO_2 evolution and the measured nitrogenous constituents indicate that physical treatment also result in the production of non nitrogenous, energy-rich constituents.

Ninhydrin analyses of alpha amino groups in soil leachates

The determination of individual free amino acids in the soil system is a laborious procedure. The results from this study together with previously published data [16 17] have shown that the whole spectrum of free amino acids is usually uniformly affected by either physical treatment or microbial growth. An indication of total free amino nitrogen not requiring separation by ion-exchange techniques should prove a valuable addition to the tools utilized in microbiological research.

Quantitative recovery of added amino acids such as arginine, aspartic acids, and leucine by leaching the soil with salt solutions

TABLE 5

Recovery of added ammonium and amino acid nitrogen ($\mu g/g$) from soil with acidified 1 N NaCl and ninhydrin analyses

Nitrogen source added	Nitrogen added	Nitrogen Measured by Ninhydrin	Recovery %
Control.	None	24.9	—
$(NH_4)_2SO_4$	50	74.9	100
$(NH_4)_2SO_4$	100	99.6	74.7
Arginine	50	74.8	99
$(NH_4)_2SO_4$ + Arginine	25 + 25	74.1	98
$(NH_4)_2SO_4$ + Arginine	50 + 50	102.3	77.4

such as barium nitrate and sodium acetate and subsequent ninhydrin analyses has been previously reported [16]. The results in Table 5 indicate that ninhydrin analyses can be used directly on a soil leachate obtained with acidified NaCl as used for the determination of ammonium. One millilitre of ninhydrin added to the 1 ml soil leachate containing from 0.05 to 0.2 μM alpha amino nitrogen or ammonia, diluted with 5 ml of a 50% ethanol: water mixture will give a reading on a colorimeter at 570 μ which can be compared to appropriate standards [15]. In the data reported, the concentration of ammonium nitrogen and free amino nitrogen occurring in the unamended soil was exceptionally high (24.9 $\mu g/g$); still, recoveries of added nitrogen as $(NH_4)_2SO_4$ and arginine were quantitative where 50 μg nitrogen per gram of soil were added. Higher quantities of nitrogen in this form are only rarely found in soils.

The data in Table 3 indicate that the ammonium nitrogen, extracted and measured by alkali distillation, after physical treatment was always considerably greater than the amino nitrogen. The free amino acid nitrogen accounted for a still smaller proportion of the readily available nitrogen. Table 6 shows the results of studies utilizing the ninhydrin technique to measure the ammonia and the alpha amino nitrogen extracted from soil with acidified NaCl. Oxbow loam was amended with 1% glucose and 0.15% KNO_3 and then incubated for 3 days. The amino nitrogen values for the non-amended soil were very similar to those previously found

TABLE 6

Measurement of ninhydrin reacting compounds (alpha amino + ammonium) and steam-distilled amonia extracted from Oxbow Loam incubated with and without 1% glucose and 0.15% KNO_3

(Results expressed in μgN per gram of soil)

Treatment	Ninhydrin analysis		Steam distillation			
			NaOH		$Na_2B_4O_7 10H_2O$	
	Glucose + KNO_3	Non amended	Glucose + KNO_3	Non amended	Glucose + KNO_3	Non amended
Freezing . . .	8.5	2.8	8.25	5.0	9.0	5.4
Air drying . .	14.8	4.5	12.0	5.65	10.4	6.1
Steaming. . .	22.2	16.6	25.35	20.55	18.0	13.3

after ion exchange analyses of untreated or frozen soils, (Table 3) with approximately 3 μg/g being extracted. Microbial activity increased both the amino and ammonium nitrogen values for all treatments indicating that the biomass contributes to both forms of readily extractable nitrogen. The greatest effect attributable to the presence of an active microflora however was in the mildly treated samples where a three-fold increase in ninhydrin positive amino nitrogen was found, with a much smaller increase being noted for the nitrogen measured by distillation. The ammonium nitrogen values determined by steam distillation utilizing NaOH or a pH 10 $Na_2B_4O_7 . 10H_2O$ buffer were similar to the ninhydrin values in the amended samples, but considerably higher for the non-amended.

Ninhydrin reacts with ammonia and other free alpha-amino groups. The data presented would indicate that the amino nitrogen other than ammonia, accounts for the great majority of the nitrogen reacting with ninhydrin. The high ammonium nitrogen values obtained when utilizing NaOH to release the ammonium during steam distillation (Table 3) can be attributed to hydrolysis, for this nitrogen was not measured when the non-hydrolyzing ninhydrin procedure was used on a NaCl extract (Table 6).

Organic nitrogenous microbial intermediates, even when of a transient nature such as the free amino acids, can be measured in the soil system and their role in soil biochemical reactions investigated. The results from the amino acid analyses indicate that these compounds play a role in the enhanced mineralization of nitrogen after physical treatment. In the Chernozemic soils at least, therefore, the postulation [4] that soluble compounds affect CO_2 production but not nitrogen mineralization does not hold. The fairly uniform spectrum of amino acids produced by these different treatments indicates a common source. These compounds have been previously said [17] to result primarily from the micro-organisms in the soil and the present data confirms this.

In addition to the effect on the biomass, there is also, however, an effect of physical treatment on the soil organic nitrogen. The ammonium nitrogen measured by steam distillation was correlated to the highest degree with subsequent mineralization of nitrogen. This ammonium which was produced by hydrolysis during the distillation procedure, (probably from the amino sugar fraction of the soil humus) was not as greatly affected by microbial activity as are the free amino acids.

This investigation into the underlying principles behind the widely noted effects of physical treatment, such as air drying, heating and freezing on the soil by measuring organic nitrogenous intermediates in the soil system indicates that there is no single simple reaction in the soil resulting from such treatments. Physical treatments affect both the soil biomass or microbial population, and the soil organic matter. The soil biomass is affected by the autolyses of microbial cells and production of microbial intermediates such as the free amino acids, the soil organic matter is affected by an increased susceptibility to attack.

SUMMARY

The effect of physical treatments such as heating, air drying, and freezing on three types of nitrogenous intermediates, free amino acids, soluble alpha amino nitrogen, and ammonia produced during steam distillation was measured.

To further understand the chemical reactions involved in the alteration of the soil during physical treatments and storage the effect of treatments such as heating, air drying, and freezing on three types of available nitrogenous soil constituents were studied. The production of free amino acids, soluble alpha amino nitrogen and ammonium as measured by steam distillation was correlated with indices of microbial growth such as microbial numbers, CO_2 evolution and mineralization of soil nitrogen.

The indigenous total free amino acid content of a fallow and rhizosphere soil were approximately 5 and 14 $\mu g/g$ respectively. The concentration of

individual specific amino acids did not exceed 1 $\mu g/g$ of soil in the fallowed soil or 5 $\mu g/g$ in the rhizosphere sample.

Various methods of treatment before analysis caused a generally uniform shift in the concentration of all amino acids, with a maximum 10-fold increase being noted for the steamed and oven-dried samples. Individual amino acid concentrations were altered by air drying which increased the concentrations of methionine sulfoxide, glutamic acid, and lysine. The different physical conditions involved in steam heating and oven drying resulted in large differences in the concentration of methionine sulfoxide, leucine, and alanine. Freeze drying also resulted in the production of significant concentrations of free amino acids and ammonia. No storage technique is available which will not result in some alteration of either the soil biomass or available organic matter. For biochemical analysis freezing appears the least detrimental.

The free amino acid nitrogen and soluble alpha amino nitrogen were present in concentrations of only one third the ammonium measured by alkali distillation. The production of all three components during storage treatments was correlated to a high degree with the subsequent mineralization of nitrogen but could account for only a small portion of increase in mineral nitrogen produced after treatment. Carbon dioxide produced during incubation was not correlated with the nitrogen measured after storage treatments.

Ninhydrin can be used to estimate the total exchangeable ammonium and alpha amino nitrogen in soil leachate without prior separation of the individual components by such techniques as ion-exchange analysis. The organic amino compounds accounted for the majority of the nitrogen thus measured. The high ammonium concentrations found by distillation resulted from hydrolysis of the soil organic constituents. Physical treatments made this more susceptible to hydrolysis. Physical treatments therefore, were found to effect the biomass resulting in a release of amino acids and the soil organic-matter constituents making them more susceptible to decomposition.

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