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9. ABSTRACT

An examination of the effect of alternate drying-rewetting cycles on nitrogen and carbon mineralization in five Hawaiian soils, three of which were silty clays. Results showed that the temperature of drying as well as drying-rewetting cycles enhanced N and C mineralization in practically all soils. There was generally greater N release when incubation after drying was included than when it was omitted. In all unincubated soils, save the Akaka (a silty clay), air-drying caused greater N release than heating at 60C, regardless of the number of drying-rewetting cycles. The organic-matter-rich Akaka soil showed increased N mineralization with increased temperature of drying. Mineralization of C was greater in samples incubated after drying-rewetting cycles than those subjected in incubation only. A highly significant correlation was found between rates of N and C mineralization per cycle. A significant correlation was also noted between C/N ratio of the soils and N released during drying and rewetting followed by incubation. It is proposed that in addition to microbial stimulation through drying, heat was directly responsible for the major amount of N and C release in unincubated samples by chemical alteration of otherwise unavailable organic matter and by killing off of organisms. When incubation followed the drying and heating treatment, the direct effect of heat, together with increased microbial activity and associated changes during incubation, accounted for the N and C release.

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Soil Nitrogen and Carbon Mineralization as Affected by Drying-Rewetting Cycles[1]

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ABSTRACT

The temperature of drying as well as drying-rewetting cycles enhanced N and C mineralization in practically all soils. There was generally greater N release when incubation after drying was included than when omitted. In all unincubated soils, save the Akaka, air-drying caused greater N release than heating at 60C, regardless of the number of drying-rewetting cycles. The organic matter-rich Akaka soil showed increased N mineralization with increased temperature of drying. Mineralization of C was greater in samples incubated after drying-rewetting cycles than those subjected to incubation only. A highly significant correlation existed between rates of N and C mineralization per cycle. Significant correlation was also noted between C/N ratio of the soils and N released during drying and rewetting followed by incubation. It is proposed that in addition to microbial stimulation through drying, heat was directly responsible for the major amount of N and C release in unincubated samples by chemical alteration of otherwise unavailable organic matter and by killing off of organisms. When incubation followed the drying and heating treatment, the direct effect of heat together with increased microbial activity and associated changes during incubation accounted for N and C release.

Additional Key Words for Indexing: ammonification, "birch effect."

DRYING and rewetting treatments affect a number of soil constituents, especially nitrogen and carbon compounds. Alternate drying and rewetting temporarily stimulates microbial activity and enhances mineralization of N and C besides other nutrient elements (2, 11, 15, 19, 21). Heating at high temperatures enhances the effect of drying-rewetting with reference to N (3, 5, 9) and C (3, 5). Broadbent et al. (7) showed that drying soil at 100C and rewetting stimulated mineralization of N and C in both allophanic and nonallophanic soils of New Zealand. Recently Singh reported that prolonged heat treatment of Hawaiian soils resulted in increase of NH_4-N release without changing NO_3-N level (B. R. Singh, 1968. Studies on nitrogen transformation and nitrate adsorption in soils. *Ph.D. Diss., University of Hawaii, Honolulu*). This work was undertaken to elucidate the effect of alternate drying-rewetting cycles on N and C mineralization in Hawaiian soils.

MATERIALS AND METHODS

Five Hawaiian soils, Wahiawa silty clay (Tropeptic Eutrotthox), Paaloa silty clay (Humoxic Tropohumult), Koko silty clay loam (Ustollic Eutrandept), Lualualei clay (Typic Chromustert), and Akaka silty clay (Typic Hydrandept) were included in this study. The soils differ widely in physical, chemical, and mineralogical properties because of differences in their parent material and the environment under which they have developed. Their sites of collection and properties have been described in an earlier paper (18) and some properties are given in Table 1.

Surface samples of the aforementioned soils were collected and passed through an 8-mesh sieve, except the Akaka which was screened through a 6-mesh sieve because of its very high moisture content. Total N and organic C were determined by the standard Kjeldahl and dry combustion methods respectively. The soil pH was measured using an Expandomatic Beckman pH meter in a 1:2.5 soil-water mixture that was allowed to stand for 12 hr with occasional stirring.

To study the effect of drying and rewetting on mineralization, 50 g (on oven-dry basis) of the above five soils were used. For nitrogen release study, the soil was dried in one of three ways: (a) air-dried, (b) oven-dried at 60C, or (c) oven-dried at 100C. For the air-drying treatment, the soil was spread out in 15 × 10 × 3-cm paper boxes and left at room temperature (27C) for 48 hr per drying cycle. In case of oven-drying treatments, the soil was put in a 500-ml Erlenmeyer flask and oven-dried at 60C or 100C for 24 hr. After any of the three drying treatments, the soil was analyzed either (i) immediately with no incubation period or (ii) after 8 days incubation at room temperature for each cycle of drying. Where more than one drying period was required, the wetting (brought back up to moisture equivalent) and drying cycle was repeated. Up to three such cycles were carried out. Incubation, when called for, was carried out in a 500-ml Erlenmeyer flask at the moisture equivalent level. The flask was stoppered with cork which was enclosed in polyethylene film. The stopper was opened at regular intervals (first, second, fifth, and eighth day in each incubation cycle) to replenish the oxygen supply in the flask. Soils were extracted with 2N KCl and the soil extracts were analyzed for NH_4- and NO_3-N by the micro-Kjeldahl distillation procedure of Bremner (6). NO_2-N was not included in this study because only insignificant accumulation of this form of nitrogen was found in the soils. NH_4- and NO_3-N contents in all five soils were measured before the soils were subjected to any drying and rewetting treatment, for both unincubated and incubated (8 days) conditions. In effect, these nitrogen values at this stage were "zero cycle" values. All treatments were replicated two times.

Carbon dioxide production in incubated samples was measured by the procedure outlined by Agarwal and Kanehiro (1). In essence, the procedure consists of trapping the evolved CO_2 in 5 ml of standard 1N NaOH solution placed in a glass vial which was suspended from a cork used to stopper a 500-ml Erlenmeyer flask containing the soil sample. Each flask was tightly capped with polyethylene film. This system was checked to ensure that there was no significant loss of CO_2. At regular intervals (first, second, fifth, and eighth day in each incubation cycle), the alkali was quantitatively transferred (using CO_2-free distilled water) to a 250-ml Erlenmeyer flask. A fresh supply of 5 ml NaOH solution was placed in the vial for the next interval. Ten milliliters of 1N $BaCl_2$ were added to the 250-ml flask to precipitate the carbonate formed by the reaction of CO_2 with NaOH in the vial. The excess alkali was titrated with standard 0.5N HCl, using six drops of 1% phenolphthalein indicator (prepared in 95% ethyl alcohol). Determinations at regular intervals were necessary to find out the rate of CO_2 production and also to replenish the oxygen supply in the

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Table 1—Some properties of the experimental soils

Soil type	Moisture equivalent* %	pH	Total N* %	C* %	C/N ratio	Origin and mineralogy
Wahiawa silty clay	30	4.9	0.174	1.44	8.2	Developed from basalt. Kaolin, Fe oxides
Paaloa silty clay	30	3.7	0.252	3.26	12.9	Developed from basalt. Kaolin, Fe and Al oxides, mica.
Koko silty clay loam	33	7.2	0.288	3.12	10.8	Developed from volcanic ash. Kaolin, montmorillonite.
Lualualei clay	43	7.6	0.099	1.06	10.7	Developed from alluvium. Montmorillonite.
Akaka silty clay	176	4.4	0.812	14.60	18.0	Developed from volcanic ash. Allophane, hydrated Fe and Al oxides.

* Values expressed on oven-dry weight basis.

flask. At the end of 8 days incubation, the whole process of drying, rewetting, and incubation was repeated for the second and third cycles. CO_2 production in control incubated samples (without drying and rewetting) was also measured at the end of 8-, 16-, and 24-day periods to give comparable control values corresponding to drying and rewetting treatment values at the end of one, two, and three cycles, respectively.

RESULTS AND DISCUSSION

Mineralization of Nitrogen and Carbon

Figures 1 to 5 depict the effect of drying-rewetting cycles on the release of available N in the experimental soils. It should be noted that the zero cycle values were subtracted from the others before plotting these curves. The magnitude of mineralized N increased with increasing number of drying-rewetting cycles, except for the Paaloa soil oven-dried at 100C (Fig. 2) where there was a decrease in available N. This decrease was presumably due to fixation of released NH_4^+ in the mica fraction of the soil. The listing of mineralogical properties in Table 1 shows that the Paaloa soil is the only soil that contains appreciable

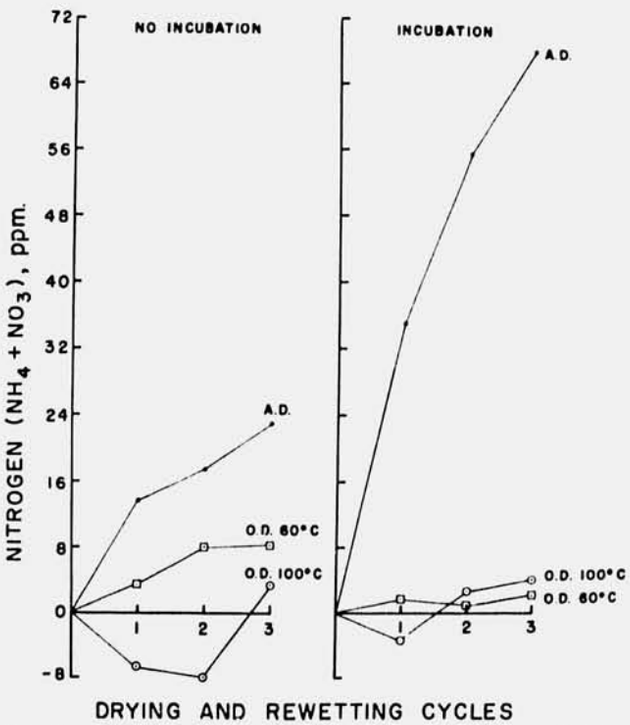

Fig. 2—Effect of drying-rewetting cycles on N mineralization in unincubated and incubated Paaloa soil.

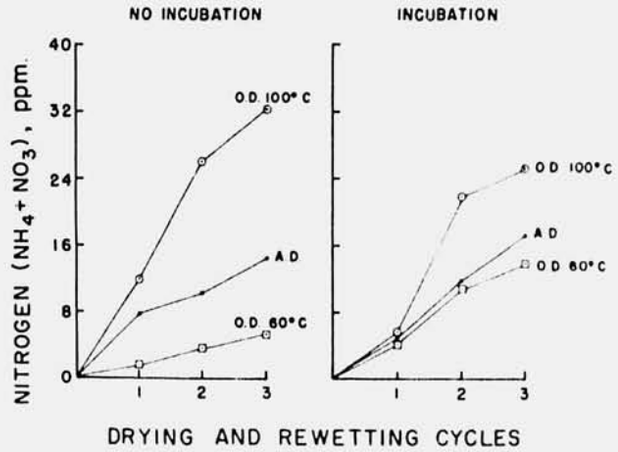

Fig. 1—Effect of drying-rewetting cycles on N mineralization in unincubated and incubated Wahiawa soil.

amounts of mica in the five soils studied. Previous work by Mikami and Kanehiro (16) has shown that a highly significant relationship exists between fixed ammonium and percent mica in Hawaiian soils. Work in the past (Y. Kanehiro, unpublished data) has also shown that ammonium is fixed to a greater extent in these soils at 100C than at room temperature. It appears from Fig. 2 that heating at 60C was not as effective as heating at 100C in fixing released NH_4-N. Although the Lualualei soil contains large amounts of montmorillonite, an expanding layer silicate mineral capable of ammonium fixation, no negative available nitrogen values were obtained with oven-drying at 100C (see Fig. 4) as in the Paaloa soil. Some ammonium fixation of released NH_4-N probably occurred (as suggested by lower available nitrogen values in the unincubated 100C oven-drying treatment as compared to the air-drying treatment) but not to the extent as in the micaceous Paaloa soil.

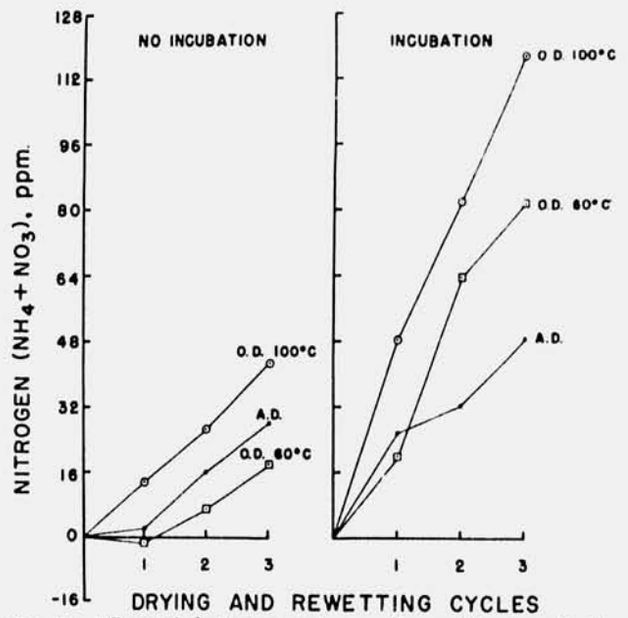

Fig. 3—Effect of drying-rewetting cycles on N mineralization in unincubated and incubated Koko soil.

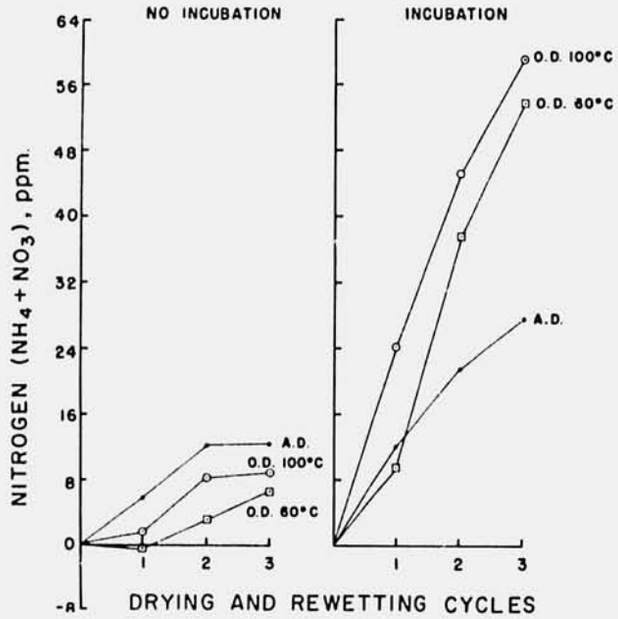

Fig. 4—Effect of drying-rewetting cycles on N mineralization in unincubated and incubated Lualualei soil.

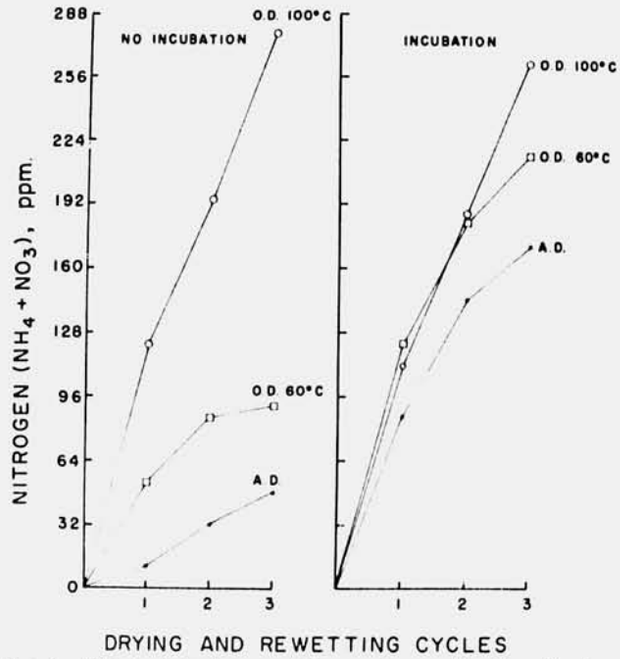

Fig. 5—Effect of drying-rewetting cycles on N mineralization in unincubated and incubated Akaka soil.

The statistical analysis, following a factorial completely randomized design, showed significant "F" values for cycles and temperatures, as well as for their interaction in all cases except for the Koko soil. This indicates real differences in available N release brought about by cycles and temperatures under both unincubated and incubated conditions. Interestingly, the coefficient of variation values were less than 6%, signifying good agreement between the two replicates.

The data on the influence of drying-rewetting cycles on cumulative CO_2 production in incubated soils are included in Table 2. In general, C mineralization was greater in samples incubated after a drying-rewetting cycle than those subjected to incubation only. Like N, the mineralization of C was enhanced by repeating the drying-rewetting cycles. The mineralization of C also generally increased with the temperature of drying in soils; however, there were exceptions, e.g., the Akaka oven-dried at 100C, where there was a large decrease at the highest temperature. The greatest C release for a particular drying-rewetting cycle was in the Akaka soil. By far, the major portion of increase in CO_2 production as a result of drying-rewetting has been ascribed to a stimulation of microbial activity (2, 3, 20) because of partial sterilization of soil accomplished through drying. Ko and Lockwood (14) also reported an increased fungal spore germination which was associated with increased soluble carbohydrates and amino acids resulting from soil sterilization. Nevertheless, it has also been stressed that some CO_2 after heat treatment may be evolved by purely chemical processes (8). The direct effect of heat on N release may be accompanied by an increase in available C. Here also, as with N, the release could be brought about by chemical alteration of otherwise unavailable organic matter or by killing off of microorganisms.

Table 2—Effect of drying-rewetting cycles on cumulative CO_2 production in incubated soils (Control values subtracted)

Soil	Treatment	Air drying	Oven drying 60C	Oven drying 100C
		mg C/50 g soil		
Wahiawa	One cycle	1	2	4
	Two cycles	2	3	11
	Three cycles	3	6	19
Paalua	One cycle	6	3	1
	Two cycles	10	6	3
	Three cycles	12	9	13
Koko	One cycle	7	7	20
	Two cycles	10	15	38
	Three cycles	14	22	57
Lualualei	One cycle	3	1	4
	Two cycles	5	6	12
	Three cycles	7	10	18
Akaka	One cycle	20	22	14
	Two cycles	36	40	8
	Three cycles	41	61	11

The increased mineralization of N and C at higher temperatures has been substantiated by others on similar observations pertaining to the release of N (3, 5, 9) and C (3, 5). The effect of drying in relation to N and C release was cumulative as was also observed by Birch (2) and Broadbent et al. (7). Multiple drying gave a cumulative effect which was in agreement with the findings of Souldies and Allison (19).

A highly significant correlation existed between rates of N and C mineralized per cycle for each drying treatment. In computing these correlations, soil effect was disregarded. The following "r" values were obtained.

Drying Treatment	"r" (d.f. 13)
Air-drying	0.816**
Oven-drying 60C	0.801**
Oven-drying 100C	0.709**

** Significant at the 1% level.

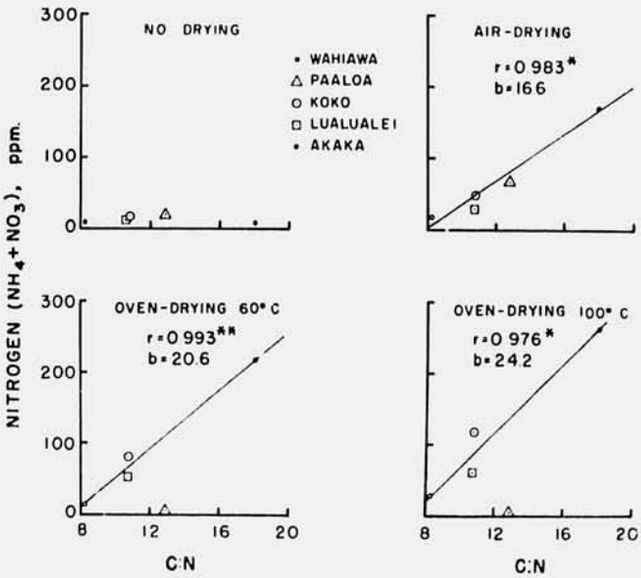

Fig. 6—Relationship between C/N ratio and N mineralization in soils as affected by the temperature of drying.

Figure 6 illustrates the relationship between C/N (organic carbon to total nitrogen) ratio of the experimental soils and the amount of N mineralized in three drying and rewetting cycles. With no heat applied, the slope of the line, or "b", is essentially zero. With an increase in the intensity of drying, the slope of the line increased from 16.6 for air-drying, to 20.6 for oven-drying at 60C, to 24.2 for oven-drying at 100C. It is pertinent to mention that for computing the "r" and regression coefficient "b" in Fig. 6, the Paaloa soil was ignored so that an exceptional property of NH_4^+ fixation by a micaceous soil at higher temperatures was eliminated from general consideration.

The C/N ratio seemingly influenced the N and C mineralization capacity of the experimental soils. In four soils (excluding Paaloa) the C/N ratio (Table 1) decreased in the order, Akaka > Koko > Lualualei > Wahiawa. Available N in three cycles of drying and rewetting including incubation (Fig. 1, 3, 4, and 5) followed the same order of decrease. Existence of such a relationship is evidently supported by significant correlations between C/N ratio of the soils and N mineralized in three cycles (Fig. 6). In the air-drying treatment, where not much NH_4^+ fixation is expected, the Paaloa soil occupied the position that it would be expected to according to its C/N ratio, i.e., between the Akaka and Koko soils. The marked increase in mineralized N and C in the case of the Akaka soil was probably due to its high C/N ratio, as well as to its high C and N contents.

Increased N mineralization in the experimental soils can be attributed to microbial stimulation through drying but this cannot be the sole factor. It was demonstrated (Fig. 1–5) that although increased N mineralization generally occurred under incubated conditions, a substantial amount of it was contributed by drying alone. Under incubated conditions, the release of N can be attributed to microbial stimulation (2), decomposition of dead organisms and fresh surfaces exposed on organic colloids during the preceding drying (4), and additional amounts of soil organic matter rendered available by processes other than killing of organisms (13). On the other hand, release of N in unincubated samples can probably be attributed to death of cells and the splitting off of NH_4^+ from the proteinaceous part of organic matter. The killing off of *Nitrosomonas* at 53–55C and *Nitrobacter* at 56–58C as reported by Gibbs (10) must have contributed to the observed mineralization of N in oven-drying treatments through death of cells. In contrast to most microbiological transformations, ammonification has been noted to proceed vigorously in the temperature range, 50–70C (12). However, moisture may have been limiting for the thermophiles at 60C and 100C, since dryings were done in an unsaturated atmosphere in the oven. Therefore, significant contribution of thermophilic mineralization to the observed N release is not very likely. In addition to limiting moisture, the short (three 24-hr cycles period of oven-dryings may be considered inadequate for appreciable contribution by thermophiles. Whether or not the postulated splitting off of NH_4^+ can be regarded as a part of additional amounts of organic matter rendered available for decomposition (13) is a matter of speculation. Some justification to this may be found in the work of Payne et al. (17) and Stevenson (20). These workers reported that air-drying increased the amino acid content of the soil, indicating a small degree of decomposition of the nitrogenous constituents of soil organic matter.

Comparison of Nitrogen Mineralization and Nitrification Under Unincubated and Incubated Conditions

Figures 1 to 5 illustrate the comparative net available N values released under unincubated and incubated conditions. There was generally greater available N release when incubation was included. However, there were exceptions, the most noticeable being in the Wahiawa (Fig. 1) and Akaka (Fig. 5) soils oven-dried at 100C. In these two soils, the magnitude of mineralized N was greater in unincubated samples for each drying-rewetting cycle. In the Paaloa soil (Fig. 2) the effect of heating, especially at 100C, was to lower N release presumably due to fixation of released NH_4^+ as discussed previously. In all unincubated soils, except the Akaka, air-drying caused greater N release than heating at 60C, regardless of the number of drying-rewetting cycles; however, results for 100C were inconsistent.

The data on NH_4- and NO_3-N under unincubated and incubated conditions for three drying-rewetting cycles at different temperatures of drying are included in Table 3. In general, the mineralization of NH_4-N increased in all soils following incubation. The NO_3-N data for the Koko and Lualualei soils showed a definite influence of incubation on nitrification. Nitrification was virtually complete in these two soils when subjected to air-drying. An appreciable amount of NH_4-N was also nitrified in some of the treatments at 60C with incubation. On the other hand, most of the initial and released NH_4-N remained unnitrified in unincubated soils, even in the Koko and Lualualei soils.

Table 3—Effect of drying-rewetting cycles, without and with incubation, on NH_4- and NO_3-N (ppm N)

Soil	Drying rewetting cycle	Air-drying				Oven-drying 60C				Oven-drying 100C			
		No incubation		Incubation		No incubation		Incubation		No incubation		Incubation	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Wahiawa	0	16	33	15	34	16	33	15	34	16	33	15	34
	1	24	33	18	35	18	33	21	31	30	32	26	28
	2	27	33	25	35	21	32	28	31	44	32	40	31
	3	31	33	31	35	22	33	32	31	49	33	45	29
Paolou	0	61	121	48	111	61	121	48	111	61	121	48	111
	1	71	125	84	110	63	123	50	110	83	92	66	89
	2	76	124	105	109	67	123	51	108	101	73	93	68
	3	80	126	118	108	69	121	54	106	120	68	110	52
Koko	0	49	121	15	77	49	121	15	77	49	121	15	77
	1	51	120	3	115	49	119	2	111	60	123	66	74
	2	67	119	0	125	56	120	40	116	72	123	104	70
	3	74	123	1	140	64	124	60	114	88	124	136	74
Lualualei	0	14	13	12	14	14	13	12	14	14	13	12	14
	1	17	15	3	35	12	14	1	34	15	13	48	13
	2	20	19	1	46	16	14	29	34	20	14	60	11
	3	16	22	1	52	18	15	50	30	21	14	74	11
Akaka	0	9	6	2	4	9	6	2	4	9	6	2	4
	1	24	1	92	0	64	3	125	3	132	4	111	7
	2	45	1	180	0	97	3	182	6	203	5	189	4
	3	60	3	[illegible]	0	104	1	217	5	265	27	263	5

At the higher temperature of 100C, there was a trend for the accumulation of NH_4-N in all soils. The repeated drying-rewetting cycles increased NH_4-N mineralization in all soils. However, this was not true for air-dried incubated Koko and Lualualei soils where repeated cycles favored nitrification of released NH_4-N.

In view of the foregoing discussion, it is proposed that heat was directly responsible for the major amount of N and C release in unincubated samples by chemical alterations of otherwise unavailable organic matter, and by killing off of organisms. When an incubation period followed the drying and rewetting treatment, the direct effect of heat together with increased microbial activity and associated changes during incubation accounted for N and C release.

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