

EFFECT OF REPEATED DRYING AT DIFFERENT TEMPERATURES ON SOIL ORGANIC MATTER DECOMPOSITION AND CHARACTERISTICS, AND ON THE SOIL MICROFLORA

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Summary—Samples of a slightly acid sand soil were subjected to frequently repeated cycles of drying (85 or 30°C), moistening and incubation (29°C, 4 weeks). During about 60 cycles the loss of carbon, as CO₂, from the samples dried at 85°C, 30°C and the undried ones was found to be 31.2, 18.0 and 17.0% respectively. A complete depletion of degradable material was not achieved after 60 drying-wetting cycles. After 60 cycles the 85°C-samples still showed the highest CO₂-production.

The microbial population was reduced in numbers and species in all samples. The highest numbers of bacteria and fungi were found in the samples originally dried at 85°C. However, only a relative low proportion of these microorganisms could grow on less easily accessible substrates.

Data on changes in the pH and on the C/N ratio of the organic matter are discussed, together with possible ways in which organic matter becomes available during heating.

INTRODUCTION

Birch (1958) reported on the effect of successive cycles of drying (100°C), remoistening and incubation on the mineralization of soil organic matter. In acid soil (pH 4.8) the decomposable material was practically exhausted after 12 cycles, during which the soil lost 9.5 per cent of its organic matter. In a soil with a higher pH (6.4), the cycles could be repeated over 200 times before CO₂ production ceased; 63.5 per cent of the soil organic matter was then decomposed (Birch and Friend, 1961).

Here a report is given of experiments similar to those by Birch and Friend, but with a slightly acid sand soil (pH 5.2). Soil samples were dried at temperatures of 30 and 85°C, and compared with samples that had never been dried. The aim was to exhaust the soil in such a way that decomposition had practically ceased and to determine then the degradability of the remaining soil organic matter and the C/N ratio reached, and to get some information on changes in microbial life.

MATERIALS AND METHODS

The soil used was a Pleistocene sand soil used as arable land; pH (water) 5.2; C 3.13%; N 0.16%.

Drying at 85°C lasted 1 day; drying at 30°C lasted 3 days. After drying the samples were remoistened with freshly deionized water, homogenized by mixing and rubbing through a sieve (3 mm mesh), and placed into incubation tubes (initially 500 g dry soil; later 100 g in smaller tubes). The amount of water lost during drying

was replenished. The water content of the differently dried samples varied less than 1 per cent, so they received initially the same amount: 20 g/100 g dry soil (the relation between water content and CO₂ production of this soil is given by a rather flat curve in the range between 50 and 70 per cent of the water holding capacity). In the course of time less water was replenished, due to a decrease of w.h.c. mainly as a result of rapid loss of sorption capacity of soil colloids, especially in the samples dried at 85°C, and ultimately also due to loss of organic matter. The undried samples were also mixed and received water if necessary. The samples were aerated with CO₂-free, moist air. During the first 2 h of incubation the samples were aerated at increased speed to get rid of non-biologically produced CO₂. After that CO₂ evolved from the soil was trapped in an NaOH solution (1 N, or 0.1 N later) and determined titrimetrically (Birch, 1959).

In some short-term experiments the CO₂ evolution was recorded by a high-frequency technique (Bruins, 1963).

The incubation of the soil samples was carried out at 29°C for periods of 4 weeks.

Carbon in the samples was determined according to a standard dry combustion method (Spithost, 1932).

To determine the easily decomposable fraction of soil organic matter, small subsamples, less than 1 g, were suspended in a nutrient solution, adjusted to the soil's pH and subjected to forced aeration in a closed circuit. The CO₂ was removed from the air and determined (Bruins, 1963). Additional O₂ could be supplied if necessary. The nutrient solution contained: 2 g KH₂PO₄; 1 g MgSO₄.7 H₂O; 1 g NH₄NO₃; 0.1 g

CaSO₄; 0.1 g NaCl; 10 mg FeCl₃; 10 ml soil extract; and 1 litre tapwater. The subsamples were inoculated with identical, small amounts of a fresh soil suspension. A determination ran in two-fold and was repeated once.

The numbers of micro-organisms were estimated using a soil extract agar (Bunt and Rovira, 1955). Silica gel plates were prepared according to the method employed by Smith (1951), improved by Giambiagi (1965). The following media were used:

Soil extract agar 1 (s.e.a. 1). A modified Bunt and Rovira agar, containing: 1 g glucose; 0.4 g bacteriological peptone; 0.2 g yeast extract, 0.2 g meat extract and; 100 ml soil extract (Lochhead, 1940) instead of the original amounts.

Soil extract agar 2 (s.e.a. 2). According to Lochhead (1940).

Starch agar. Prepared with potato starch.

Cellulose agar. Prepared with hydro-cellulose according to Harmsen (1946), who used a modification of Scales' method (1916).

Calcium caseinate agar. Preparation. Solution A: 1 g casein is dissolved in 8.7 ml 0.1 N NaOH plus 25 ml water by heating gently; when dissolved 225 ml tap water is added. Solution B: 1 g glucose; 0.5 g KH₂PO₄; 0.2 g MgSO₄ · 7H₂O; trace FeSO₄; 5 ml of a 3% (w/v) CaCl₂ solution in tap water; 12 g agar and 750 ml tap water. The solutions are heated separately at 105°C for 20 min and after slight cooling mixed together, and sterilized at 110°C for 30 min.

Tannic acid agar. Tannic acid (3 g); 0.5 g glucose; 0.2 g yeast extract; 0.2 g meat extract; nutrient salts as in s.e.a. 1; 12 g agar; water 1 litre; bromothymolblue 20 mg; pH to 6.0 with KOH. Glucose, yeast and meat extract served as a starter for growth. Organisms enduring and decomposing tannic acid were estimated. Under and around well growing colonies the medium became more alkaline.

The pH of the media was brought to between 6.5 and 7.0 unless stated otherwise.

RESULTS

Loss of organic matter as CO₂

The average daily CO₂ production of each series of the samples at the start of the experiment is shown in Fig. 1. The samples dried at 85°C showed the highest activity, not only in a very marked initial flush but also in later stages when the activity of the micro-organisms reached a steady state. The samples dried at 30°C showed a similar pattern on a smaller scale, but the level at steady state proved often lower than that of the undried samples. The latter only showed a slightly increased activity at the beginning, due to mixing and agitating.

During the experiment the magnitude of the flushes, the steady state levels and consequently the total CO₂ production were decreasing. Decomposition curves of the samples are given in Fig. 2.

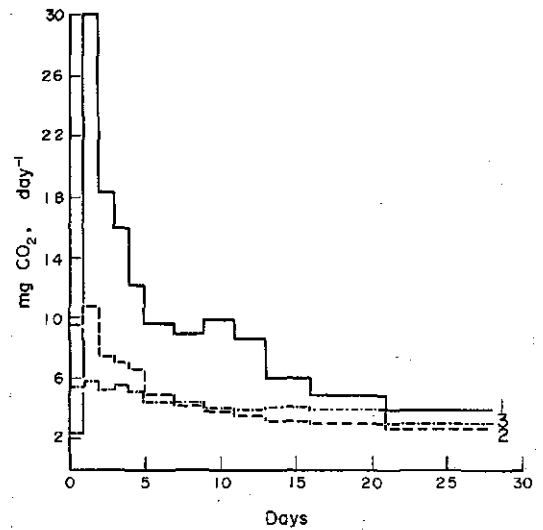


Fig. 1. Daily CO₂ production of soil samples (per 100 g, dry weight basis), dried at 85°C (1) and 30°C (2) for the first time, and undried (3).

After 60 repetitions the decomposition rate was still highest in the soil dried at 85°C, although the differences in CO₂ production with the other samples had become rather small.

The ultimate loss of organic matter—measured as CO₂—was highest in the samples dried at 85°C, namely, 977 mg C/100 g soil or 31.2 per cent. In the samples dried at 30°C and in the undried the carbon loss was 565 mg or 18.0 per cent and 532 mg or 17.0 per cent, respectively. The difference in C loss between the samples dried at 30°C and the undried samples is

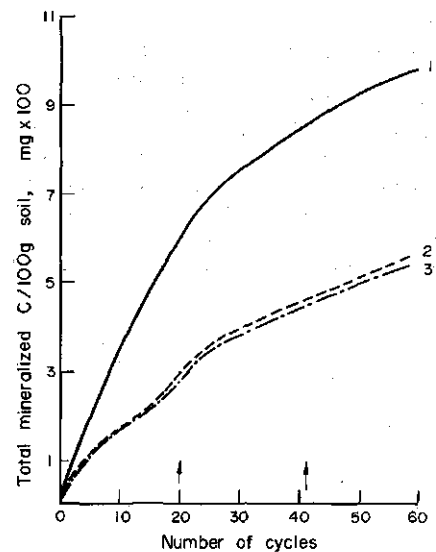


Fig. 2. Loss of carbon (as CO₂) from the samples dried at 85°C (1), 30°C (2) and undried (3). ↑pH adjustment.

Table 1. Organic carbon decomposed in samples from different treatments during a 2-week period of forced aeration ("easily decomposed carbon")

	C content (%)	C in sample (mg)	Mineralized C (mg)	(%)
Original sample*	3.13	626	35	5.6
Undried sample	2.23	435	11	2.5
30°C dried sample	2.03	429	7	1.6
85°C dried sample	1.28	286	14	4.9

* Air dried material from the original sample.

negligible. This feature was already known from a preceding, short experiment.

No correction is made for CO₂ losses during drying (on average 3.6 mg C/100 g dry soil in samples dried at 85°C for the first time; the loss decreased with continued dryings).

The carbon loss in the samples can be calculated from direct determinations of carbon by dry combustion. The carbon contents of the samples are given in Table 1, first column. According to this method the loss in the samples dried at 85 and 30°C and in the undried ones was 60, 35 and 29 per cent, respectively. These values are much higher than those measured at microbial decomposition (31, 18 and 17 per cent, respectively). The differences are in part due to accumulated losses of fine organic particles, adhering to the walls of the trays in which the samples were wetted and mixed, adhering to the sieves and disappearing as a fine dust when the samples were treated in a dry condition. The finer the soil organic matter was fragmented the more material was lost. Fragmentation was most pronounced in the samples dried at 85°C, where also a loss of volatile compounds could occur (aromatic aldehydes and acids). This is of minor importance for the total loss of C.

Accumulated losses of CO₂ occurring during drying contribute to the difference and also losses from the undried samples, which still mineralized at a lowered rate when the others were dried.

After the series of 60 cycles the samples were stored in a moist condition in cotton plugged flasks at 29°C for more than 1 year and at about 17°C for more than 3 years. Occasionally sterile distilled water was supplied to supplement evaporation loss. During and after these 4 years, studies were made as described below.

The flush

Freshly dried soil shows after remoistening a marked flush in its CO₂ production. The magnitude of the flush depends on the drying temperature (Fig. 1). In the course of the experiment the amount of CO₂ evolved in the flush decreased. At the end of the experiment we wondered if a flush still would exist. To demonstrate its presence the CO₂ evolved was measured (Bruins, 1963) and recorded. Samples always dried at 30 or 85°C were again dried at the corresponding temperature, remoistened and incubated (Fig. 3, A and B).

It is clear that a small flush still exists in both cases. In the samples dried at 30°C all CO₂ is produced during the flush. The samples dried at 85°C show a quickly formed flush followed by a continuing CO₂ production. However, the samples always dried at 30°C and now by exception dried at 85°C show a distinct flush and a further CO₂ production at a relatively high rate. Easily decomposable material, not available after drying at 30°C, is apparently formed in relatively large amounts by heating at 85°C.

Easily decomposable organic matter

It is to be expected that the sample which had the highest C loss would end up with the lowest content of easily decomposable material. To check this, small subsamples of the different treatments were suspended in a buffered nutrient solution and subjected to forced aeration. The CO₂ evolved during 2 weeks was used as a measure of the easily decomposable fraction of soil organic matter. The results of an experiment are given in Table 1. The organic matter present in the samples dried at 85°C still proved more susceptible to microbial attack than that of the samples dried at 30°C or the undried ones. The high degree of decomposability of the organic matter in samples which had already

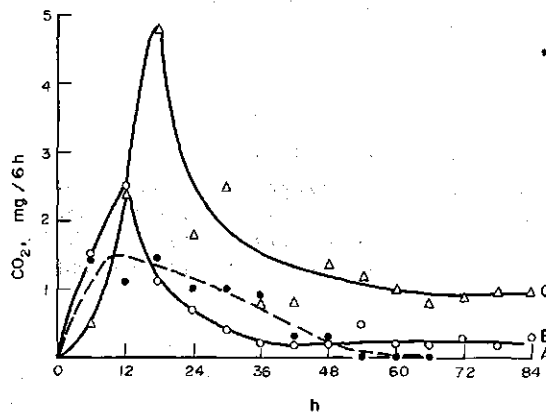


Fig. 3. CO₂ production rate of soil samples, measured at the end of the experiment. A: dried once more at 30°C (●); B: dried once more at 85°C (○); dried at 30°C, finally at 85°C (▲). Samples A and C contained about 110 g soil (dry weight basis), sample B nearly 100 g. Corrections for the difference in weight have not been made.

lost the most easily decomposable part of it, is rather unexpected. Apparently heat promotes continuously a transition of difficultly decomposable organic matter into relatively easily degradable organic substrates.

The data for "original sample" in Table 1 refer to part of the original sample kept in an air-dry state throughout the experiment. It is known (Birch, 1960; Jager, 1968), that dry storage of soil during long periods leads to a higher production of CO_2 when moistened and incubated than it would have done in a fresh condition. Nevertheless, the percentage of mineralized C is not much higher than for the 85°C dried sample.

pH Changes

pH changes occurred in the samples as a consequence of mineralization. The samples dried at 30°C and the undried samples showed a tendency to come to a lower pH (H_2O), while the samples dried at 85°C showed a rise. During the experiment the pH of the samples was measured at intervals, and if necessary, adjusted to about the initial pH (5.2) with a dilute $\text{Ca}(\text{OH})_2$ solution or with dilute H_2SO_4 , to avoid the influence of too large a pH deviation on the mineralization process.

Table 2. Shifts in pH (H_2O) in the samples after repeated drying, remoistening and incubation, pH at start 5.2 (or 3.9 in KCl)

	Not dried	Dried 30°C	Dried 85°C
After 1 incubation	4.9	5.0	5.2
After 20 cycles	4.4 (5.0)	4.8 (5.2)	5.3
After 44 cycles	4.8 (5.3)	5.0 (5.2)	6.1 (5.3)
After 59 cycles	4.8	4.7	5.9

Values in parenthesis show the pH after adjustment.

The changes in pH in the different samples and the pH values after adjustment are given in Table 2.

The rise in pH is due to accumulation of ammonium as nitrification was completely inhibited after drying at 85°C. This is a normal feature after treatment of soil by heat or chemicals whereby biomass is killed. The nitrifying bacteria are among the first organisms to be damaged or killed. In the non-dried samples and in those dried at 30°C nitrification occurred. The formation of nitrate leads to a lowered pH. In the samples dried at 30°C loss of nitrogen probably occurred from nitrite (Gerretsen and De Hoop, 1957; Jager 1968) giving a smaller reduction in pH values than in the undried samples.

C/N ratio of soil organic matter

The C/N ratio of soil organic matter decreased during the experiment from 19.6 in the original sample to 14.6 in the samples dried at 85°C. The samples dried at 30°C, however, had decreased less, i.e. to a ratio of 17.4, while the undried samples showed a value of 15.7. The divergent C/N ratios resulting from the different

treatments fit in with results of earlier experiments in which the C/N ratio of the net mineralization (C mineralized/N mineralized) was determined. In that case we observed the highest C/N ratio after drying at 85° or 105°C, while it was lowest after drying at 30°C; the mineralization of the undried samples showed an intermediate value (Table 3). These mineralization values explain the C/N ratios of the remaining organic matter as found in this experiment. (An enhanced C/N ratio of the mineralization ultimately leads to a lowered C/N ratio of the remaining organic matter; the reverse also holds.)

Table 3. Carbon and nitrogen mineralized per 100 g soil (dry weight basis) after drying at different temperatures, and the C/N ratio of the net mineralization

	mg C	mg N	C/N
(A)			
Undried	84.6	4.46	18.9
Dried at 30°C	88.0	5.17	17.0
Dried at "high temp."	148.4	7.06	21.0
Dried at "high temp." + Ca humate	177.8	7.77	22.9
(B)			
Undried	33.4	2.13	15.7
Dried at 30°C	28.6	2.80	10.2
Dried at 85°C	51.6	2.89	17.8

(A) Incubated during three periods of 2, 5 and 3 weeks, respectively. "High temperature" drying occurred at 30, 105 and 85°C, respectively. Calcium humate was administered at the second incubation.

(B) These samples were incubated for one period of 26 days. The carbon mineralization, and consequently the C/N ratio, is very low after drying at 30°C.

The cause of the different C/N ratios of the mineralization is the different way in which biomass and humus are influenced by heating at differently high temperatures. Drying at low temperatures exerts a relatively greater influence on biomass than on humus. The C/N ratio of the subsequent mineralization is lowered as a consequence of the extra amount of N mineralized from killed biomass (Jager, 1968; Jagnow, 1972). At high temperatures nearly all the biomass is killed but a relatively greater part of humus is made available, whereby the C/N ratio of mineralization is widened. This explanation is supported by the observation that addition of calcium humate in the experiments where the soil was dried at 85°C still further increased the C/N ratio of the mineralization (Table 3).

Micro-organisms

We supposed that the microflora in soil samples, in which decomposition had made so much progress would be able to mineralize more difficultly decomposable substrates, and would be present in relatively high numbers. To test this, suspensions of the different samples were mixed with nutrient salts and humic acids

Table 4. Growth of microbes from the different samples on tannic or humic acid in silica gel plates (numbers per gram of dry soil $\times 10^3$)

	Tannic acid	Humic acid
Undried	Mainly <i>Penicillia</i> and some <i>Phomopsis</i> sp. (100)	Some minute colonies (10)
Dried at 30°C	Few <i>Penicillia</i> (10)	More frequent growth of small and minute colonies (20-30)
Dried at 85°C	No growth	Few minute colonies (5-10)

(0.2 per cent) or tannic acid (0.5 per cent) in silica gel plates. Data about growth after incubation during 3 weeks are given in Table 4. The lowest numbers were observed on the plates inoculated with soil from samples dried at 85°C. The microflora of this sample thus had no greater ability to degrade difficultly decomposable substrates than the microflora of the other samples.

Four years afterwards the numbers of microorganisms were determined more elaborately to get information on the size of the microflora if grown on media of different composition and accessibility (Table 5).

The number of bacteria developing on s.e.a. 1 were for the undried samples and the samples dried at 30 and 85°C about 20, 40 and 75 per cent, respectively, of the number found in the soil at start. The soil was from a plot lying bare fallow for about 1 year and thus had relatively low numbers.

The number of fungal colonies was more reduced than the number of bacterial colonies. The high number of bacteria in the samples dried at 85°C seems to corroborate the unexpected result mentioned earlier (Table 1).

The number of bacteria developing on Lochhead's soil extract agar was not very different for the three treatments. The numbers of fungal colonies from the dried samples were not very different. The highest numbers were found in the undried samples. The bac-

terial numbers on both types of soil extract agar were identical for the undried samples, but the bacterial flora of the samples dried at 30°C and even more that of the samples dried at 85°C seemed better adapted to the relatively rich nutrient conditions of the modified Bunt and Rovira agar than to those of the rather poor Lochhead's soil extract agar.

The ability of the microflora of the undried samples to hydrolyze starch and casein seemed to be greater than that of the other samples.

The number of cellulose hydrolyzing bacteria and fungi was less than 100/g dry soil in all samples.

Fungi growing on tannic acid agar and showing features of its degradation were most abundant in the undried samples; half of this number was present in the samples dried at 30°C. Very low numbers, less than 100/g soil, if any, were present in the samples dried at 85°C.

The microbial potential to decompose less easily available substrates was lower in the samples dried at 85°C than in the other samples. In contrast, the number of bacteria growing on easily decomposable substances, as in s.e.a. 1, was higher than in the other samples and formed the majority in the bacterial population. This must be due to a better supply of easily decomposable substances.

Streptomycetes seemed to have disappeared from all samples; a few *Nocardias* were found but only in the undried samples. The fungal flora in the samples

Table 5. Numbers of bacterial and fungal colonies grown in different media per gram of dry soil

	Undried		Dried at 30°C		Dried at 85°C	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Soil extract agar 1	$3.2 \pm 1.1 \times 10^6$	36×10^3	$6.6 \pm 0.8 \times 10^6$	8×10^3	$11.9 \pm 1.0 \times 10^6$	6×10^3
Soil extract agar 2	$2.7 \pm 0.3 \times 10^6$	100×10^3	$3.5 \pm 0.9 \times 10^6$	6×10^3	$3.7 \pm 0.7 \times 10^6$	8×10^3
Starch agar	*	*	$1.6 \pm 0.4 \times 10^5$	4×10^3	$> 2.0 \times 10^5$	6×10^2
Cellulose agar	<100	<100	<100	<100	<100	<100
Casein agar	†	†	$> 4 \times 10^5$	6×10^2	$> 2.5 \times 10^5$	3×10^2
Tannin agar	0	8×10^3	0	4×10^3	0	<100

1: Modified Bunt and Rovira agar. 2: Lochhead's soil extract agar.

* Strongly dissolved; largest numbers.

† Strongly dissolved, especially by fungi.

The fresh soil at the start of the experiment contained 16.5×10^6 bacteria plus streptomycetes and 5.1×10^5 fungi per gram of soil (dry weight) on Bunt and Rovira's agar.

Table 6. Numbers of bacterial, fungal and streptomycetel colonies per gram of soil (dry weight) on modified Bunt and Rovira agar (after further storage of the different samples)

	Bacteria	Fungi	Streptomycetes
Undried	$97 \pm 13 \times 10^5$	0.5×10^5	$5.5 \pm 1.6 \times 10^5$
Dried at 30°C	$83 \pm 16 \times 10^5$	9.3×10^5	0.2×10^5
Dried at 85°C	$182 \pm 42 \times 10^5$	$37 \pm 7 \times 10^5$	$< 0.01 \times 10^5$

existed mainly of a few *Penicillium* species. It seemed most diverse in the undried samples.

For a further period of about 9 months the samples were stored in their flasks at about 17°C. They became gradually dry and were remoistened when dry for about 1 month. Three months afterwards the numbers were again estimated.

The highest numbers of bacteria and fungi were found in the samples originally dried at 85°C (Table 6). Streptomycetes were present in high numbers in the undried sample, in low numbers in the sample dried at 30°C and were not found in the sample dried at 85°C. A similar number of bacterial colonies on the plates was found in the undried sample and in that dried at 30°C; the latter showed a higher number of fungal colonies than the undried one. Fungi and streptomycetes consisted of abundantly sporulating species. The species composition was very poor, although from the samples dried at 30°C a slightly more varied picture was obtained.

Perhaps the conditions for growth and sporulation of fungi and streptomycetes were favourable during the period when the samples dried gradually (Jensen, 1943; Kouyeas, 1964). Bacilli were present in all samples, but never dominating.

When the numbers in Tables 5 and 6 are expressed per unit of soil carbon (100 mg)—which in fact is more reasonable than per gram of soil—the differences between the samples become greater. The multiplication factors are 4.5 for the undried sample and 4.9 and 7.8 for the sample dried at 30 and 85°C respectively.

DISCUSSION

Carbon loss

The results show, in agreement with those of Birch and Friend (1961), that only extended cycles give the low values of a strongly diminished organic matter decomposition. From the decomposition curve of the samples (Fig. 2) it is clear that the end point of decomposition had not yet been reached after 60 cycles.

The percentage loss of carbon, as CO₂, was much smaller than in Birch and Friend's experiments, most likely due to a different composition of soil organic matter, formed from other materials under other conditions, and owing to lower drying temperatures.

Soil samples aerated with CO₂-free air, absorb CO₂ when exposed to a normal atmosphere (during drying). When again aerated as before the absorbed CO₂ is desorbed. The undried samples and those dried at 30°C have a low pH (Table 2) and consequently are

hardly influenced by the CO₂-HCO₃⁻ balance. CO₂ present in the samples was lost during the first 2 hours of increased aeration. A greater influence of the CO₂-HCO₃⁻ balance can be expected in the samples dried at 85°C, as their pH was higher. Here some of the HCO₃⁻ might have contributed to the measured CO₂ production, but hardly or not influenced the large differences in CO₂ production between the 85°C dried samples and the others.

The flush

The flush in the CO₂ production was found to be still present at the end of the experiment (Fig. 3). Theories about the origin of the flush in the decomposition after partial sterilization are reviewed by Jenkinson (1966). The primary factor for an extra mineralization is an extra amount of easily decomposable food, made available in some way. Flushes reflect easily decomposable, mainly water soluble material (Birch, 1959; Jager 1968), derived from dead biomass and soil organic matter fractions transformed or made available by heat. The role of temperature is clearly seen in Fig. 3. Birch (1959) observed that the effect of temperature was more important than the effect of drying.

The mechanism involved, acting under dry and moist conditions, depends on the source, e.g., biomass and humus. Members of the biomass are differently sensitive to heat (Bollen, 1969). The flush after the use of fumigants depends on organic matter from killed or damaged biomass, while dead biomass plus changed humus material are responsible for the flush after heating (Jenkinson, 1966). Indirect evidence for the contribution of *killed biomass* was found in the enhanced mineralization of nitrogen and a lowered C/N ratio of the net mineralization after freezing, or drying at 30°C (Jager, 1968). The increased amounts of amino- and ammonium-N extractable from soil samples after partial sterilization also point to killing of the biomass and its contribution to nitrogen mineralization (Stevenson, 1956; Paul and Tu, 1965; Ivarson and Sowden 1966, 1970). Next to dead biomass, further substrates for the newly developing population of microorganisms are a part of the humus as normally available and an extra amount of humus made available by heat. In all probability the extra amounts of food result in a primed decomposition of soil organic matter.

The contribution of *dead soil organic matter (humus)* to the flush is caused by chemical alterations and by physical phenomena. Hydrolysis can play a role during the time the soil is heated under moist conditions, as Birch and Friend (1961) observed for organic phos-

phates. Hydrolysis, however, is in all probability not an important mechanism in making organic matter available during heating, as the population of soil micro-organisms itself has a high hydrolyzing potential, leaving only inaccessible material. Heating of dry soil contributes in some way—and not merely by killing a greater part of the biomass—to the amount of easily available substrates. The amounts of coloured compounds extractable with water and also the amount of aromatic compounds increase with rising temperatures.

The amount of soil organic matter made soluble during heating in a moist state depends on the water content of the samples (Jager, *et al.*, 1969). In addition to destructive chemical reactions a purely physical solubilization plays a role. Among others, desorption of adsorbed matter may occur during heating.

The differences in decomposition between the samples dried at 30°C and undried ones proved negligible in our experiments. This was due to the relatively long incubation periods, in which the influence of the flush was only small.

Micro-organisms

The picture we get from a population of micro-organisms by way of plate counting and determination of groups of organisms, however, is incomplete. Only the aerobic and mesophilic part of the population able to thrive on the agar used is present. Besides that the picture only has a temporary value, as the composition of the population changes rapidly due to changing external conditions.

Strongly reduced groups, the numbers of which are below the limit of the least diluted soil suspension, are not observed and often thought to be absent. Streptomycetes presumably were present in the samples when the counts of Table 5 were made, in numbers much lower than 10^3 /g soil. These numbers were probably not original but were introduced by a small soil infusate given after the last drying (Fig. 3). The amount of different spores entering each sample may be assumed to be about the same. Different were, however, the chemical, physical and biological conditions in the samples, and they determined, together with changing conditions, the chance of some introduced organism or spore to survive, to germinate and to grow or to be killed. Streptomycetes had the best chance in the undried sample; the sample dried at 30°C seemed to offer a slightly favourable environment for growth, while in the sample dried at 85°C little or no growth was possible (Table 6). A real difference seems to exist between the population of the sample dried at 85°C and the others.

The phenomena described apply only to the slightly acid sand soil used. Other soils may react quite differently as was found by Jagnow (1972), who compared the mineralization of C and N in more than 50 East African soils from very different habitats after they had been dried at 30°C and kept in a dry state for different periods.

REFERENCES

- BIRCH H. F. (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Pl. Soil* **10**, 9–32.
- BIRCH H. F. (1959) Further observations on humus decomposition and nitrification. *Pl. Soil* **11**, 262–287.
- BIRCH H. F. (1960) Nitrification in soils after different periods of dryness. *Pl. Soil* **12**, 81–97.
- BIRCH H. F. and FRIEND M. T. (1961) Resistance of humus to decomposition. *Nature, Lond.* **181**, 731–732.
- BOLLEN G. J. (1969) The selective effect of heat treatment on the microflora of a greenhouse soil. *Neth. J. Pl. Path.* **75**, 157–163.
- BRUINS E. H. (1963) Determination of carbon dioxide using a high frequency oscillator. *Analyt. Chem.* **35**, 934–936.
- BUNT J. S. and ROVIRA A. D. (1955) Microbial studies of some subantarctic soils. *J. Soil Sci.* **6**, 119–128.
- GERRETSEN F. C. and DE HOOP H. (1957) Nitrogen losses during nitrification in acid sandy soil. *Can. J. Microbiol.* **3**, 359–381.
- GIAMBIAGI N. (1965) Méthode rapide pour la préparation de silicagel stérile. *Biologie Sol* **3**, 24–28.
- HARMSSEN G. W. (1946) *Onderzoekingen over aërobie cellulose ontleding in den grond*. Thesis, Agricultural University, Wageningen.
- IVARSON K. C. and SOWDEN F. J. (1966) Effect of freezing on the free amino acids in soil. *Can. J. Soil Sci.* **46**, 115–120.
- IVARSON K. C. and SOWDEN F. J. (1970) Effect of frost action and storage of soil at freezing temperature on the free amino acids, free sugars and respiratory activity of soil. *Can. J. Soil Sci.* **50**, 191–198.
- JAGER G. (1968) The influence of drying and freezing of soil on its organic matter decomposition. *Stikstof* **12**, 75–88.
- JAGER G., VAN DER BOON J. and RAUW G. J. G. (1969) The influence of soil steaming on some properties of the soil and on the growth and heading of winter glasshouse lettuce—I: Changes in chemical and physical properties. *Neth. J. agric. Sci.* **17**, 143–152.
- JAGNOW G. (1972) Bodenatmung, Stickstoffmineralisation und Humusabbau ostafrikanischer Böden nach Trocknung und Wiederbefeuchtung. *Z. PflErnähr. Düng. Bodenk.* **131**, 56–66.
- JENKINSON D. S. (1966) Studies on the decomposition of plant material in soil—II: Partial sterilization of soil and soil biomass. *J. Soil Sci.* **17**, 280–302.
- JENSEN H. L. (1943) Observations on the vegetative growth of actinomycetes in the soil. *Proc. Linn. Soc. N.S.W.* **68**, 67–71.
- KOUYEAS V. (1964) An approach to the study of moisture relation of soil fungi. *Pl. Soil* **20**, 351–363.
- LOCHHEAD A. G. (1940) Qualitative studies of soil micro-organisms—III: Influence of plant growth on the character of the bacterial flora. *Can. J. Res. Ser. C* **18**, 42–53.
- PAUL E. A. and TU C. M. (1965) Alteration of microbial activities, mineral nitrogen and free amino acid constituents of soils by physical treatments. *Pl. Soil* **22**, 207–219.
- SCALES F. M. (1916) A new method of precipitating cellulose for cellulose agar. *Zentbl. Bakt. Abt. II* **44**, 661–663.
- SMITH W. K. (1951) Improvements in the ion-exchange method of preparing silica gels. *Proc. Soc. appl. Bact.* **14**, 139–147.
- SPITHOST C. (1932) De methodiek van de humusbepalingen. *Versl. landbouwk. Onderz. Ned.* **38B**, 65–103.
- STEVENSON I. L. (1956) Some observations on the microbial activity in remoistened air dried soils. *Pl. Soil* **3**, 170–182.