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The influence of drying and freezing of soil on its organic-matter decomposition

Summary

Drying and freezing of soil affect the soil organic matter and especially its most reactive part, the biomass, as both influences are lethal to a smaller or larger part. The lethality depends on the temperature and on the duration of the treatment involved; both factors also influence the non-living organic matter, especially drying. Immediately after the treatment a greater amount of NH_4-N is extractable from the soil, originating from the killed biomass and likely also from humified organic material.

After restoration of normal favourable conditions, the surviving microbes find in their environment an amount of well-decomposable food, which leads to a rapid multiplication and an enhanced mineralization of organic matter becoming apparent by the formation of CO_2 and mineral N. The fact that the C/N ratio of the mineralized matter after drying or freezing is lower than in case of untreated, points to a preferential mineralization of nitrogen-rich substrates, including killed biomass. Secondary processes may lead to a loss in mineral nitrogen.

The magnitude of the effect of the treatments involved depends for different soils on their amount of decomposable organic matter and consequently on biomass.

Introduction

Drying and freezing are natural factors influencing the soil and the decomposition of its organic matter. A part of the living matter may be killed by it, the degree depending on the severity of the factors and circumstances involved. Drying of soil leads as a rule to a temporarily, more or less accelerated decomposition of soil organic matter [6, 7, 8, 12, 13, 25, 42, 61, 76, 83].

The effect of freezing on the mineralization of soil-organic matter is less clear. Some invesigators report positive results [15, 49, 50, 72], but others found no or only insignificant effects [22, 25, 62]. Harding and Ross [30] claim, that the effect of freezing may depend on the organic matter and the moisture content of the soil.

In this paper is reported about drying experiments, performed to study the reactions of soils from a temperate and humid climate after drying and remoistening with regard to the mineralization of their organic matter. To get an insight in the processes involved, extreme temperatures are sometimes applied.

Trying to find an explanation for the contradictory results mentioned in the literature, the effect of prolonged freezing and that of repeated freezing and thawing on the mineralization of soil organic matter is studied and compared with the effect of air drying at low temperatures. The decomposition in fresh soil was also determined, serving as a blank.

Materials and methods

Some characteristics of the soils used are presented in Table I.

When not otherwise stated, the sandy soil is used. The pine wood soil material consists of a mixture of the F and H layers of the A_0 horizon and the upper part of the A_1 horizon.

The soil was carefully homogenized by passing it repeatedly through a sieve (3 mm mesh) and divided into equivalent subsamples with the aid of a rotating sectorial sample divider.

Table I. **Characteristics of soils used.**

	pH (water)	Org. matter	Total N	C/N org. matter
Pleistocene sandy soil, arable land	5.2	6.2%	0.20%	15.0
Young marsh silt soil arable land	7.5	2.9%	0.15%	9.3
Soil material from a pine wood on pleistocene sand	3.8	52.0%	0.99%	29 approx.

After being kept at room temperature for about a week, the samples were used for the experiments. This was done to avoid the interference of the accelerating effect of mixing and agitating on the decomposition [36, 63].
In most cases we used homogenized soil, dried and stored at a relatively low temperature (10-15 °C). Before use the soil is rewetted with a soil infusate and further treated as a fresh soil. For at least one week the soil is left at room temperature before it is used, to overcome the effect of drying and storage. The incubations are carried out at 29 °C with samples of 100 or 500 g (dry weight basis), with water brought to optimal structure conditions, and aerated with carbondioxide free, moist air [36].
Evolved CO_2 is generally absorbed in an alcaline solution and determined titrimetrically by a method identical to that employed by Birch [6]. In short term experiments the CO_2 production is recorded by a high-frequency oscillator technique [10].
NO_3-*N* is determined by a modified method of Alten et al. [3], the 'method Maastricht' which proved to be reliable [29]. Hereby nitro-xylenol is formed from 3,4-xylenol and the nitrate in the soil extract; the intensity of its yellow colour is measured. For NH_4-*N*, the method of Growther and Large [26] is used in the more recent experiments. According to this method NH_4-N contributes to the formation of chlorophenolblue, whose colour intensity is measured. In the earlier experiments, however, a diffusion technique was employed, which was not quite satisfactory. For both determinations the samples were extracted in accordance to Bremner and Shaw [9].
The number of micro-organisms is determined using a soil extract agar [11].
The results are expressed on the basis of dry soil (105 °C).

The carbondioxide production of soil is used as a measure for the mineralization of soil organic matter; it may provide us also with a rough measure for the bruto nitrogen mineralization if the C/N ratio of the mineralized matter is not subject to marked changes. The netto nitrogen mineralization, which is commonly measured, is the bruto mineralization minus the amount assimilated by a growing microbe population or (eventually) fixated again in the organic residue and losses during nitrification; so it is the amount of mineral nitrogen accumulated during treatment and incubation.

RESULTS

Drying experiments

A typical example of the influence of drying and remoistening on the daily CO_2-production and the numbers of microbes during the incubation period is given in Fig. 1. It is clear, that drying enhances the mineralization of soil organic matter and does so the more, the higher the drying temperature was. This enhanced CO_2-production after partial sterilization was already observed by many workers. The flush of the CO_2 production reached its peak when the

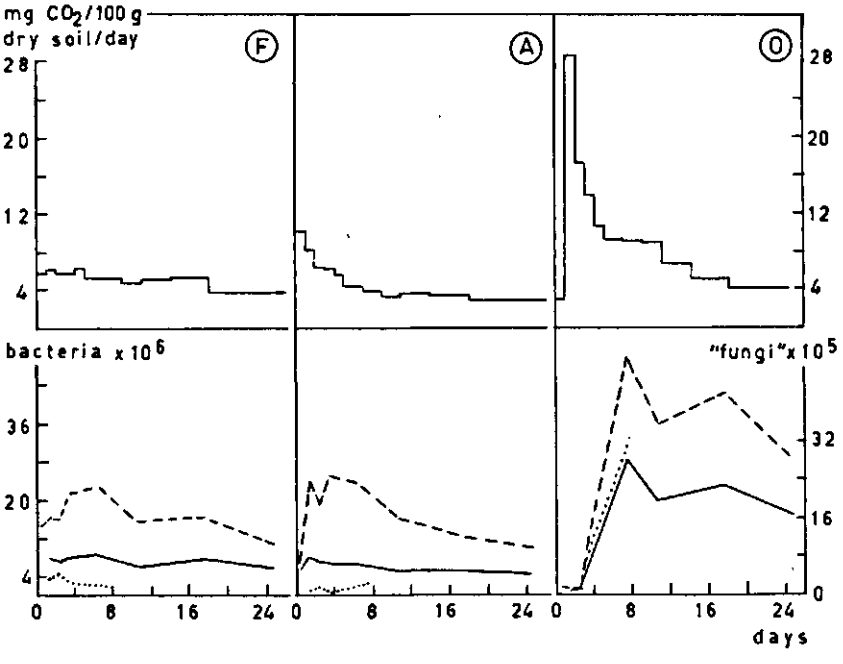

Fig. 1. Sandy soil; CO_2-production and numbers of microbes (plate counts) in:
F = fresh soil;
A = rewetted air-dried soil (30°C, 3 days);
O = rewetted, oven-dried soil (85°C, 1 day).
Germs per gram of soil (dry weight):
——— bacteria after three days of incubation;
- - - - - ibidem, after 7 days;
..... fungi.

numbers of the microbes still were at a rather low level (viable counts). This is most evident in the soil dried at 85 °C. The air-dried soil also shows that effect, although less pronounced. The group of fast growing microbes, being the most important for CO_2 formation, showed scarcely any flush in the fresh soil after filling the soil into the containers. In the air-dried soil their number was slightly reduced by the drying procedure, but increased slightly after remoistening. Drying at 85 °C caused a severe decrease in microbial numbers, followed by a violent revival of the decimated and partly introduced population after remoistening. This flush is attributed to the fact that drying of soil makes well decomposable material available for the microbes [7, 27, 42, 76, 82, 83, 84]. The high number of micro-organisms persisted for a long time and even the steady state level op the CO_2-production adjusted itself at a higher level than in fresh or air-dried soil. In the latter case it often decreased to a level below that of the fresh soil, which gives the impression that air-drying only temporarily enhances the availability of about the same amount of decomposable material.

A part of the *easily decomposable material*, made available by drying, *proved soluble in water*. If extracted from soil and added to pure quartz sand it gave rise to a distinct CO_2 peak during incubation, while the extracted soil nearly lost its initial peak (Fig. 2). In sand the extract hardly showed any nitrogen mineralization, notwithstanding the fact, that the C/N ratio of its organic matter was fairly low, viz. 11.6. Apparently a large part of this nitrogen is immobilized and another part is in the form of compounds fairly resistant towards microbial decomposition. Extracts of air-dried and fresh samples showed the same pecularities. This is in agreement with the results of Birch [7]. A large part of the easily-decomposable nitrogen-containing material, like amino acids or peptides, is hardly extracted with water [58]. Vlassak [79] determined the nitrogen fractions in water extracts of different soils. As an average he found, that 49% of the soluble nitrogen was organic. From this fraction 30% was free amino nitrogen and another 53—70% was bound in an hydrolisable form. In a sligthly acid sandy soil (arable land) 4.4 p.p.m. water-soluble organic nitrogen was found, an amount in the same order of magnitude as we determined in our sandy soil (3.2 and 6.6 p.p.m. in fresh and air-dried soil, respectively).
Krasilnikov [45, pp. 169-173] states, that microbial cells are adsorbed for the greater part by the soil. Dead microbial cells and other products of killed biomass with cationic groups will be mainly mineralized in adsorbed state and there contribute to the nitrogen mineralization.

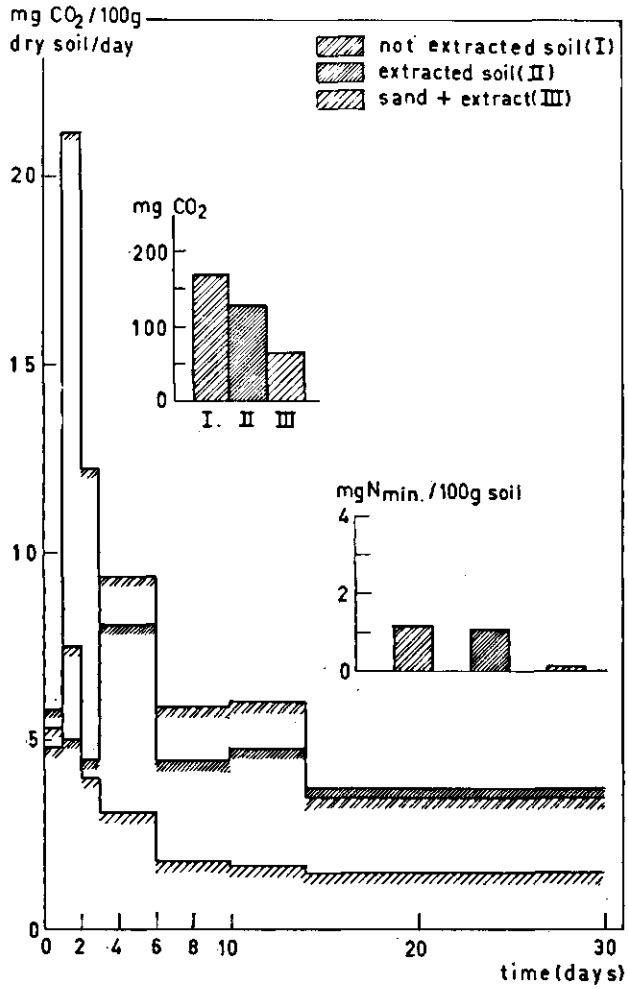

Fig. 2. Sandy soil; dried at 85°C (1 day). Pattern of CO_2-production of an oven-dried and remoistened soil extracted with water or not, and of the extract added to pure quartz sand. The total CO_2 produced during the experiment and the nitrogen mineralized are inserted.

The nitrogen mineralization is as a rule enhanced when the soil has been dried. After a three weeks' incubation period air-dried and oven-dried samples showed a nitrogen mineralization 6.3 p.p.m. and 14.0 p.p.m., respectively, higher than in the fresh soil. In oven-dried soil all mineral nitrogen formed during incubation was ammonia nitrogen, as the nitrifiers were killed by the temperature treatment. Not in all cases, however, air drying leads to an enhanced nitrogen

mineralization. Fig. 3 represents the nett nitrogen mineralization of fresh air-dried and oven-dried samples of this sligthly acid soil in course of time. The starting point of each curve of the dried samples is taken just after drying and remoistening. The differences with fresh soil are brought about by the temperature treatment, whereby a part of the biomass is killed and changes take place in the dead soil organic matter, leading to a higher quantity of extractable NH_4-N [68, 72]. From the NH_4-N curves it is clear, that the nitrogen mineralization showed a similar 'flush' as the carbon mineralization and that this flush is dependent on the temperature of drying [48].
In the fresh soil the amount of NH_4-N decreased as soon the quantity of NO_3-N showed a rize; thereafter the NH_4-N remained on a low and constant level, while the amount of NO_3-N gradually increased. The small increase in NH_4-N in the fresh soil during the first days may be due to the well-known effect of mixing and agitating the soil.
The samples dried at 85 °C show at the beginning a distinct flush in NH_4-N, followed by a more gradual and steady increase. The amount of NO_3-N was already reduced by the temperature treatment and during the first days of incubation it decreased further. But a small rest of the NO_3-N remained unconsumed.
The air-dried soil showed in the beginning a small flush in the amount of NH_4-N, followed by gradual decrease. From the moment the amount of NH_4-N started decreasing, there was a gap of about ten days before the beginning of the increase of NO_3-N. During this gap a loss of mineral nitrogen took place, due to the fact that nitratation did not follow immediately the nitritation, apparently because *Nitrobacter* is more sensitive towards unfavourable conditions than the ammonia-oxidizing *Nitrosomonas* [64]. This led to a production of such high concentrations of nitrite that a loss of mineral nitrogen via this compound occurred [86]. The fact, that slightly acid sandy soils often show marked nitrogen losses during nitrification was already noted by Gerretsen and De Hoop [24]. Gasser [23] using soils with a pH level of 6.3 or higher got with rewetted air-dried samples during incubation first a flush in the nitrogen mineralization, followed later by a more gradual increase.

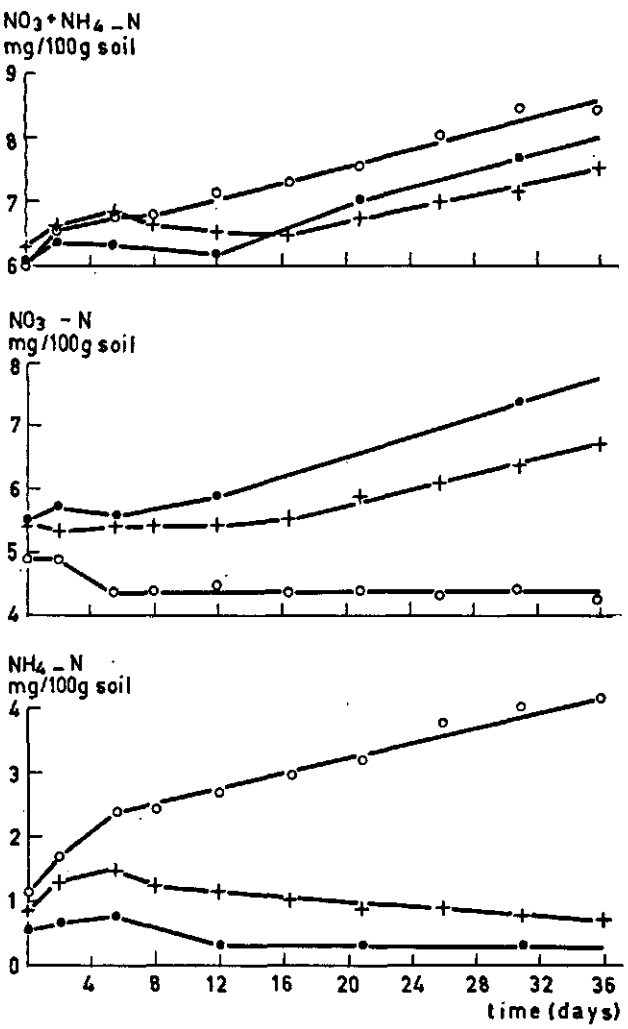

Fig. 3. Sandy soil. Mineralization of nitrogen in fresh, air-dried and oven-dried soil samples in course of time.
• fresh soil; + soil dried at 30°C; O soil dried at 85°C.

The *period of time* during which a soil is kept in a *dry state*, at drying temperature or below, is of influence on the mineralization of organic matter after rewetting [8, 83].
This phenomenon could now be confirmed, but proved to be important only at high drying temperatures. Fig. 4 shows the relative decomposition of organic matter in samples of three different experiments, dried at 30, 60 or 85 °C. The soil samples used were taken in different years and seasons from the same plot. They were stored either constantly at the drying temperatures or kept at that temperature only at daytime, cooling them down to 10-15 °C during the nights. The main conclusion from these relatively short-term experiments may be, that the effect of storage of the dry samples stimulated the CO_2-production most markedly during the first days of storage. A prolonged storage during long periods, may, depending on the storage temperature, further enhance the CO_2-production. This also appears from Fig. 6 and 7, in which the CO_2-production is shown of samples dried and stored at 10-15 °C for periods up to 2 years; the effect appears to be considerable. Consequently our results do not deviate from those of Waksman and

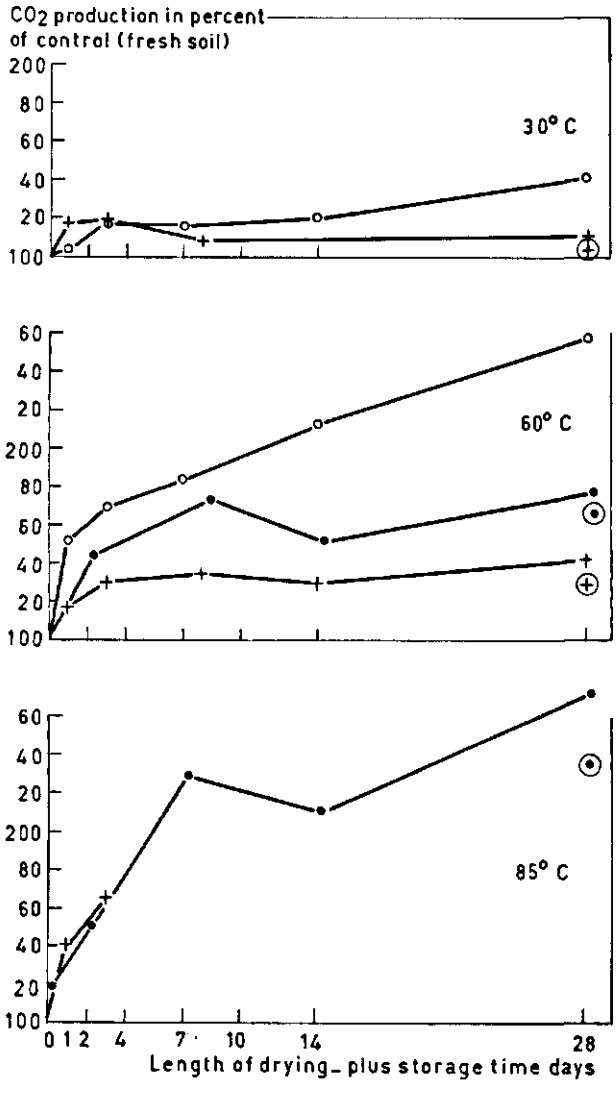

Fig. 4 Sandy soil. Relative CO_2-production of soil samples of different series of experiments, dried at 30, 60 and 85°C, respectively. Storage was continuously at drying temperature (——) in a few cases, however, during the nights at 10-15°C ⊙.

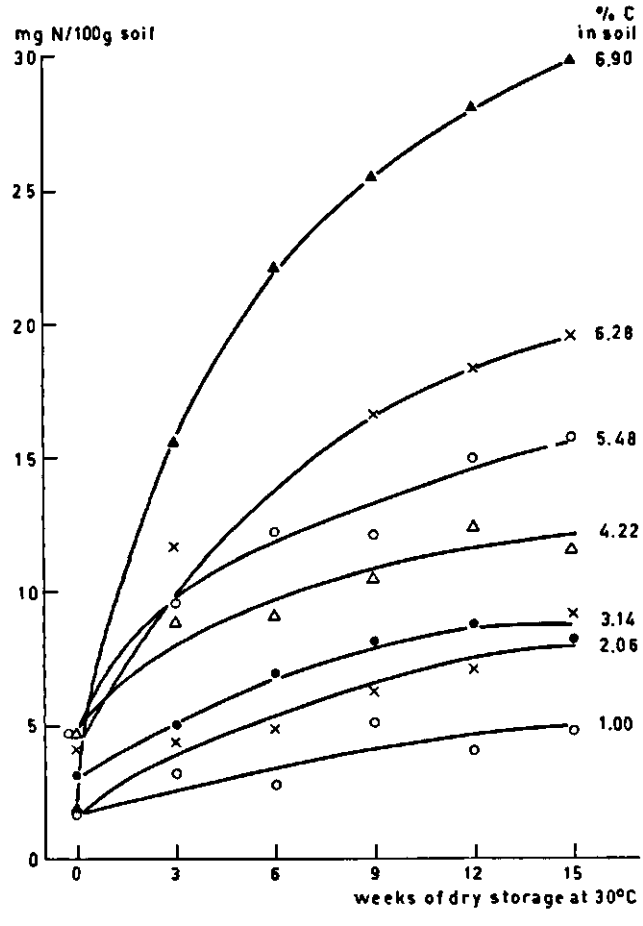

Fig. 5. Nitrogen mineralization in soils with different contents of carbon in relation to the length of the storage period. Data from Birch [8].

Starkey [83] and Birch [8], although the results presented in Fig. 4 are not clear for the samples dried and stored at 30° C.

At low temperatures only a part of the biomass is killed and made available for desintegration during desiccation. The higher the temperature, the more of the biomass will be killed, but then also the reactive part of the dead organic matter (humus) becomes more influenced; this effect of the temperature increases even beyond the lethal point of the biomass. It is still an open question how the higher temperatures of drying affect the dead organic matter in the soil. The unexpected effect of the prolonged storage of the dry samples is a similar problem.

Storage of the samples at night at a lower temperature — as in the field — leads to a lower mineralization of organic matter.

The nitrogen mineralization in these experiments showed during incubation, like the CO_2-production, a large increase after a short period of drying and storage. The effect of longer periods of storage in these experiments was irregular, giving only small or no further gains in mineral nitrogen and at 85 °C even a decrease.

Birch [8] on the contrary found in his tropical soils with different carbon contents (1-7%) and pH values ranging from 5.8 to 6.4 a high increase in nitrogen mineralization after a three-week's storage (30 °C), gradually levelling off at a prolongation of the storage period to 6, 9, 12 and 15 weeks. His results are plotted in Fig. 5, which shows a regular relation between nitrogen mineralization and the duration of dry storage, depending on the carbon content of the soil. The difference in behaviour between Birch's soils and ours presumably depends on the amount of reactive organic matter involved and its properties (digestibility, C/N ratio, pH). The pH of the soil has a significant influence on the nitrification and on eventual nitrogen losses during this process [24]. Harpstead and Brage [35] got as an average result of ten soils a decreasing effect of dry storage on the nitrate accumulation during the first 3 weeks of storage; from that time onward an increase started, which after 12 weeks reached the level of the fresh soil and proceeded to increase up to 50 weeks. Gasser [23] stated, that the nitrogen mineralization of soils after air-drying and storing is irregular during the first 12-16 weeks of dry storage; later mineral-nitrogen accumulation tends to increase. Livens [48] reported a relatively high increase in nitrogen mineralization after five days of dry storage below the drying temperature (45 °C). When longer stored, up to 270 days, only small increases in nitrogen mineralization were gained, maximally 10%. (Livens used three-days' incubation periods.) Cornfield [14] got with the soils used variable results in the nitrogen mineralization after one or twelve weeks air-dry storage.

Table II. **Increase in NH_4-N (p.p.m.) in two soil samples after drying at different temperatures and different storage periods**

	Length of the drying period	Dried at 30°C	Dried at 60°C	Dried at 85°C
Sample 1	Until constancy of weight	1.7		2.0
	plus 2 days	2.5		9.9
	plus 7 days	2.9		9.2
	plus 14 days	2.8		19.1
	plus 28 days	4.4		23.9
	plus 28 days*	5.4		33.9
Sample 2	1 day	0.9	1.8	
	3 days	1.7	2.9	
	7 days	3.2	5.5	
	14 days	4.1	5.8	
	28 days	10.0	7.0	

* From about 8.00 a.m. to 5.30 p.m. at drying temperature; during evening and night at 10-15°C.

Changes taking place in the soil organic matter during the storage period may be responsible for the differences in the decomposition during the incubation. An increase of NH_4-N during dry storage takes place, dependent on the length of the storage period and on the drying and storage temperature. Table II presents some results. These changes point, together with the evolution of small quantities of CO_2 by the dry samples, to changes occurring in the soil organic matter during storage, which may be responsible for the observed differences during incubation. Bunt and Rovira [11] observed a CO_2-production by dried and by sterilized soil at 30 °C, which is attributed to chemical oxidations or decarboxilations. Beckmann and Scharpenseel [4] also attribute the CO_2-evolution by limed (pH 6.5 and higher) and sterilized soil to decarboxilations. Nevo and Hagin [55] found, with regard to nitrification, that the changes taken place in air-dried soil during storage, were independent of the fact whether the biomass still was living or not and of the presence of oxygen. We observed that the CO_2-production of the dry soils at 30 °C was dependent on the temperature at which the soils were dried previously (85° > 60° > 30°).

Samples of the sandy soil taken from a field, lying bare fallow during the dry and warm summer of 1959, showed when incubated, a normal CO_2-production but hardly or no nitrogen mineralization. The soil had a high nitrate content (over 200 p.p.m. N) but lost a part of it during incubation, which was about balanced by a gain in NH_4-N. Considering, as shown in Table II, that after drying, prolonged at 85 °C the sample contained 23.9 p.p.m. NH_4-N, and that after incubation this amount increased to no more than 26 p.p.m., then it must be concluded that the newly developing population of micro-organisms immobilizes much nitrogen, and that the soil organic matter is changed in such a way that its organic nitrogen is made less available. The C/N ratios* of the mineralized organic matter of samples dried and stored at different temperatures for different periods are presented in Table III. The C/N ratio of the mineralized matter in the samples, dried at 30 °C, decreases gradually during storage. This is in agreement with the results of Winsor and Pollard [85] and Birch [8]. At the low drying temperature, the biomass will be affected the more the longer the storage period is, contributing more to the nitrogen mineralization. Besides this an increasing influence of the non-biomass organic matter may be expected. When dried at higher temperatures, however, the C/N ratio increases during storage. At the higher drying temperature the total biomass will be killed after a relatively short period and so made available for mineralization. At prolonged exposure to high temperatures, however, proteins are subject to irreversible changes, which make them less well decomposable. Van Schreven [67] found the nitrogen

* CO_2-C/N-mineral, accumulated during incubation.

Table III. **The C/N ratio of the mineralized organic matter in dependence of the drying temperature and the length of the storage period. Sandy soil. The samples were taken in different years and seasons.**

	Length of the drying period	Dried at 30°C	60°C	85°C
Sample 1	Until constancy of weight	24.3	12.8	—
	plus 2 days	—	13.8	11.6
	plus 7 days	28.1	15.0	16.1
	plus 14 days	11.3	20.1	9.3
	plus 28 days	15.8	25.8	19.6
	fresh soil	30.0	17.0	—
Sample 2	1 day	12.7	12.1	11.4
	3 days	15.5	17.4	21.1
	7 days	—	18.7	—
	14 days	8.8	—	—
	28 days	9.5	—	—

mineralization in dried plant material delayed in comparison with fresh, which in part was thought to be due to a diminished digestibility caused by the drying.

Anticipating on the next section it may be mentioned here that the effect of *drying* a soil at a temperature *below the freezing point*, as may happen in cold winters in the absence of a snow cover, proved to be rather small with respect to the carbon mineralization (Fig. 6). In this figure the C and N mineralization during incubation of samples dried for many weeks at 10-15 °C, dried during 23 days in a vacuum dessiccator under slight vacuum at – 10 °C, frozen in wet condition for the same period at the same temperature, and samples of fresh soil are compared. The dried samples show an accelerated decomposition during the first days of incubation, whereas the wet frozen and the fresh soil samples had a constant and nearly equal rate of CO_2-production. The C/N ratio of the mineralized organic matter is lowered by all treatments; drying causes the largest effects, freezing is less effective.

Drying of the soil not only proved to increase the solubility of organic matter, but also of mineral components, making them more available for plants. It improves also the structure of heavy soils. Drying of soils thus has to be considered as a favourable factor in soil fertility [1, 6, 8, 27, 42, 47, 61].

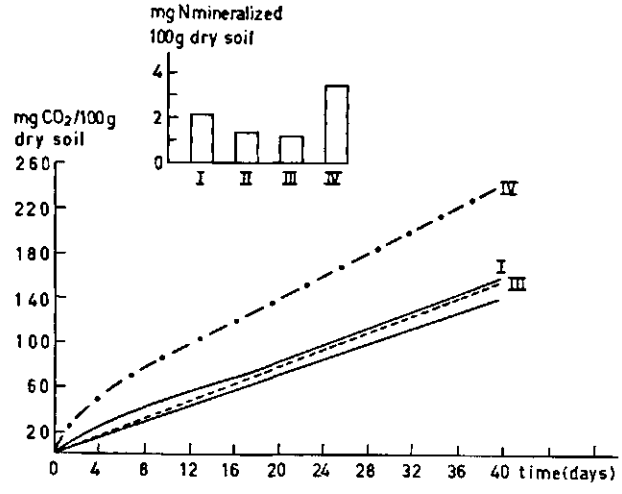

Fig. 6. Sandy soil. CO_2-production and N-mineralization of fresh, differently dried and frozen samples.
I: dried at -10°C (23 days).
II: frozen at -10°C (23 days).
III: fresh.
IV: dried at 10-15°C (many weeks).

Freezing experiments

Ehrhardt [15] and Harding and Ross [30] claimed an action of freezing similar to that of drying in their work. Ehrhardt studied the effect using a forest soil under spruce, rich in humus and determined the C and N mineralization; Harding and Ross determined the N mineralization after freezing at –20 °C, and found positive results under grass-clover pasture but hardly any effect in soil under tobacco plants. Soulides and Allison [72] reported a positive effect of freezing (–22 °C) on the organic-matter decomposition in soils with carbon contents ranging from 1.4 to 3.6%. From our experiments with two arable

Table IV. **The influence of different freezing temperatures and freezing periods on the subsequent carbon and nitrogen mineralization**

Freezing	Duration	Incubation period	Mineralization per 100 g soil (mg)		Difference with untreated soil (mg)		
°C	(days)	(days)	C	N	C	N	
-7°	0	14		1.0			Young marsh silt soil, arable land
	1	14		1.2		+ 0.2	
	3	14		0.8		— 0.2	
	7	14		1.1		+ 0.1	
	10	14		0.2		— 0.8	
	15	14		1.3		+ 0.3	
	21	14		0.8		— 0.2	
-7°	0	30	24.3	1.6			Pleistocene sandy soil arable land
	3	30	25.1	—	+ 0.8		
	7	30	27.1	1.5	+ 2.8	— 0.1	
-20 to -25°	7	30	24.0	2.0	— 0.3	+ 0.4	
-20 to -25°	0	30	23.5	1.7			Pleistocene sandy soil arable land
	7	30	23.2	1.8	— 0.3	+ 0.1	
	0	30	30.0	2.3			
	28	30	28.6	2.7	— 1.4	+ 0.5	

soils, rather low in organic matter, it is evident that freezing at –7 °C had scarcely any effect on the mineralization of C and N. If the samples were frozen at –20 to –25 °C, for 7 or 28 days a small increase in nitrogen mineralization perhaps may be detected during incubation (Table IV). Earlier is reported (Fig. 6) that freezing slightly enhanced the nitrogen mineralization.

The effect of *repeated freezing and thawing* was thought to be more lethal to the soil microbes than uninterrupted freezing [72, 77]. The sandy soil is used to study the effect of repeated freezing and thawing on the CO_2-production in soil. The samples were daily frozen at –8 °C and thawed at room temperature for about four hours. The recorded results are presented in Fig. 7.

Originally the soil was dried at 10-15 °C and kept stored at this temperature for about two years. After remoistening and inoculation with a soil infusate a distinct CO_2 flush appeared, characteristic for dried and remoistened soil (curve 1). The flush lasts about two days whereafter the curve becomes gradually linear. Curve 2 shows the CO_2-production in the same sample measured from the 6th day of incubation onward, which can be considered to represent a fresh, not treated, sample. The curves 3 to 8 show the CO_2-production in samples subjected to 5, 10, 14, 20 and 27 times repeated freezing and thawing. A decrease of the CO_2-production below the level of the fresh soil is apparent in this series until after 14 repetitions, when the CO_2-production seems to be stabilized on

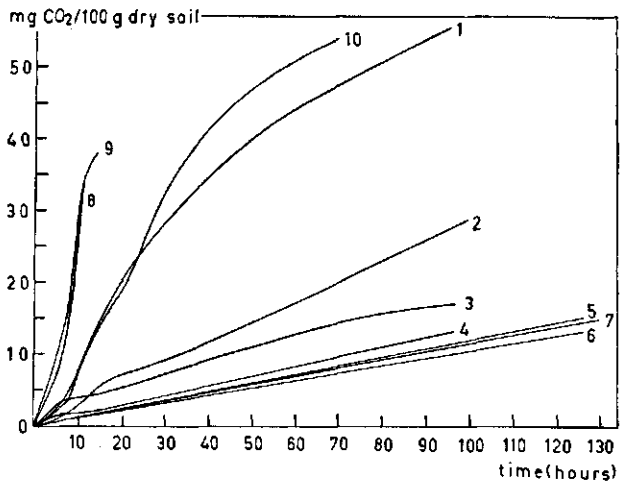

Fig. 7. Sandy soil. Effect of repeated freezing-thawing and drying on the CO_2-production of incubated soil samples. The curves represent:

1. Soil originally dried at 10-15°C, kept at this temperature for about two years, and then remoistened with a soil infusate.
2. As before, but the CO_2-production measured, beginning after an incubation period of 5 days.
3. After 5 times freezing-thawing.
4. After 10 times freezing-thawing.
5. After 14 times freezing-thawing.
6. After 20 times freezing-thawing.
7. After 27 times freezing-thawing.
8. After 32 times freezing-thawing with 200 mg glucose and 50 mg casamino acids added.
9. After 33 times freezing-thawing with 100 mg glucose and 10 mg casamino acids added.
10. After 34 times freezing-thawing, followed by 24 hours drying at 105°C.

a constant level. The number of microbes proved reduced by the treatments, but no revival occurred during incubation. After addition of easily decomposable carbon and nitrogen sources, however, an immediate and vigorous evolution of carbondioxide appeared (curves 8 and 9). This proves that the microbes in the soil are able to multiply and to resume a high metabolic activity as soon as decomposable substrates are present. This suggests that the reduced activity has to be attributed to the organic matter, becoming more resistant to microbial desintegration by repeated freezing. This increased resistance proved, however, reversible, as oven drying of the soil after 34 times freezing-thawing brought about a normal pattern of CO_2-production for a dried and remoistened soil (curve 10).

These investigations seem to justify the conclusion that the effect of repeated freezing-thawing of a soil, although causing a partial sterilization, is — with regard to the CO_2-production — opposite to that of drying remoistening.

Further experiments with the same soil, but now enriched with fragments of fresh plant material, were performed about three months after the addition. A similar pattern, but at a higher level was found. The addition of a mere carbon source proved to be sufficient to show the potential microbial activity. This type of experiments was further extended by comparing the *C and N mineralization* in *repeatedly frozen and thawed* samples, *continuously frozen* samples, *air-dried* samples and *fresh* samples of pine wood soil material, rich in organic matter and a young marsh silt soil from an arable plot poor in organic matter. The samples were incubated for four weeks.

For the *pine-wood soil material*, the results, expressed per 100 g soil, are brought together in Fig. 8.

The C mineralization seems to be suppressed by repeated freezing and thawing, which has been most pronounced during the first three days of incubation. A long period of uninterrupted freezing tends slightly to increase the CO_2-production, but air-drying has the largest effect. Statistical analysis revealed, however, that only the CO_2-production of the air-dried samples differed significantly from the fresh samples. These scarcely significant differences are mainly due to the unfortunately large variations between the samples of the fresh soil.

The treatments as such (before incubation) may exert a direct influence on the amounts of NH_4-N extractable from the soil, a fact earlier noted by Soulides and Allison [72] and by Allen and Grimshaw [2].

The nitrogen mineralization during incubation, including the effect of the treatments, was strongly stimulated by repeated freezing-thawing, also by freezing for long periods, but most of all by drying. With the exception of those frozen only once, all samples differ significantly from the fresh soil (1-0.1% level). With one exception the C/N ratio of the mineralized matter is lower than that of the fresh soil, indicating that due to the treatments, an extra mineralization of a nitrogen-rich substrate took place. This substrate, a part of the biomass, includes in this soil material relatively large amounts of micro- and mesofauna.

Bacteria and fungi were found to be reduced by all

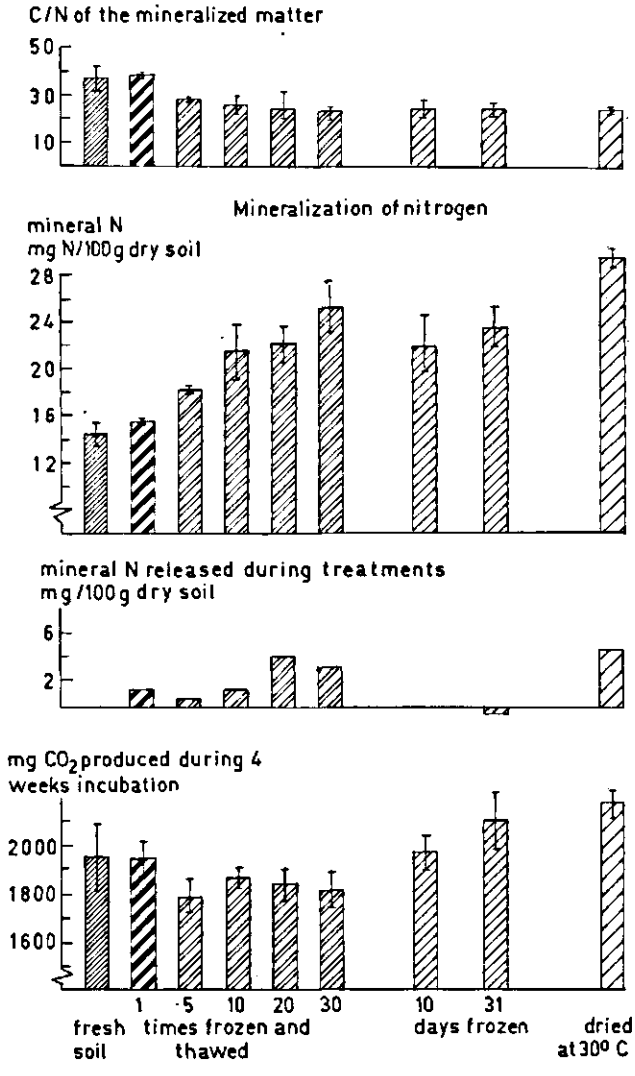

Fig. 8. Pine-wood soil material. C- and N-mineralization after different treatments and the influence of these treatments on the C/N ratio of the mineralized matter. Standard error:

$$s = \sqrt{\frac{(\bar{x}-x)^2}{n-1}}$$

treatments. However, overgrowing of the plates by the numerous *Trichoderma* and *Penicillium* colonies made correct counts impossible. An increase of bacterial numbers during incubation was evident in the dried samples. The number of fungal colonies was increased during incubation in all samples, but mainly in the dried ones.

The *young marsh silt soil*, treated in the same way as the pine-wood soil material, showed a different behaviour. The results, expressed here per kilogram soil, are presented in Fig. 9.

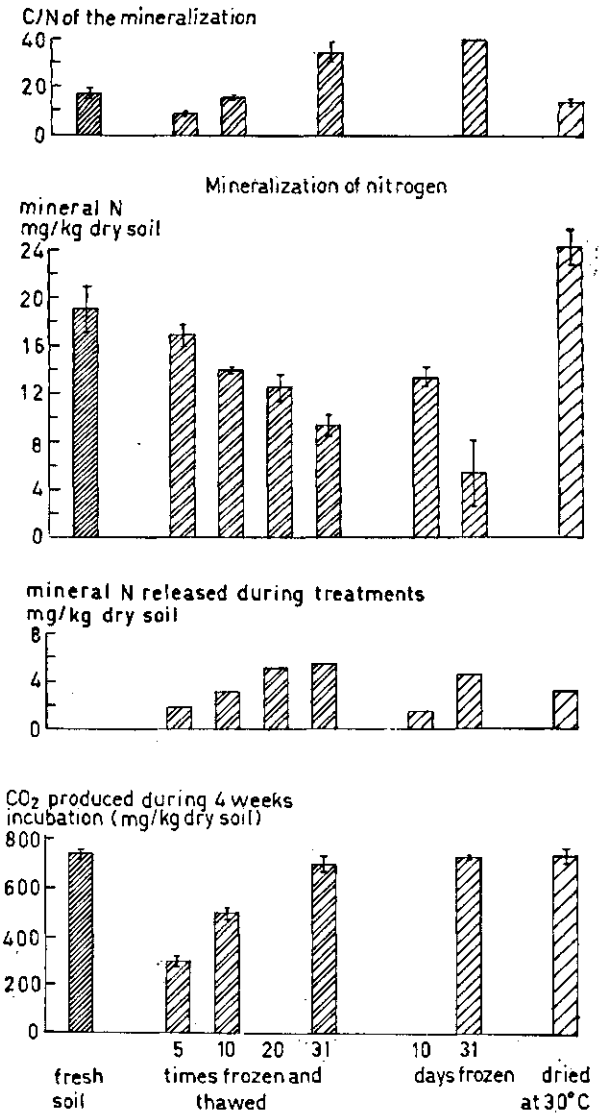

Fig. 9. Young marsh silt soil. See caption of Fig. 8.

Neither drying nor prolonged freezing had a significant effect on the CO_2-production. Only repeated freezing and thawing depressed it, however, with a gradual revival during the consecutive treatments.

The treatments had only o small influence on the liberation of NH_4-N. Small increases in NO_3-N were observed only in the repeatedly frozen and thawed samples.

As well repeated freezing-thawing, as the length of uninterrupted freezing depressed the nett mineralization of nitrogen. The differences with the fresh soil are all significant (1-0.1% level). Drying on the contrary brought about a significantly higher nitrogen mineralization (1-2% level).

The C/N ratio of the mineralized matter of fresh samples exceeded those of the 5 and 10 times frozen and thawed and of the dried samples, due to the decomposition of a substrate relatively rich in nitrogen in the latter. But a further continuation of the freezing-thawing treatment and a longer uninterrupted freezing stimulated the C mineralization and suppressed that of N to such an extent, that the C/N ratio of the mineralized matter surpassed that of the fresh soil.

All treatments reduced bacterial numbers, drying being most effective.

The differences in the C and N mineralization between the young marsh silt soil on the one side, and the pine-wood soil material and, so far as studied, the sandy soil on the other side are not clear. Presumably these differences are not only due to organic-matter content, its qualities and the pH, but also to the fact that one soil is rich in clay minerals (mainly illite) and the others are not. In the silt soil sorption processes may play a role and change the composition of the substrate available for decomposition.

Discussion

The accelerating effect of desiccation and remoistening on the decomposition of soil organic matter, already known from other investigations, has been confirmed in our experiments. The extent of the influence of drying depends on the temperature; the higher the latter the more lethal it is for the biomass and also the more the 'dead' organic matter is affected. Both contribute to the source of easily decomposable material which permits the development of surviving or introduced microbes. A high metabolic activity is shown during their early stage of growth, leading to a CO_2 peak in the decomposition pattern. The CO_2 peak precedes the peak in microbe numbers, a phenomenon often observed, which is generally explained by considering the bacteria in the lag and the early logarithmic phase of development pro cell and pro unit of weight more active than in the later stages of development [20, 80, 87]. Addition of or-

ganic material to soil shows a similar picture [42, 78]. Fungi proved to belong to the vigorously growing organisms in the dried and remoistened sandy soil. They contribute to the CO_2-evolution in the first and second day after rewetting. This strongly enhanced activity is possible only in general, when for the population of newly developing micro-organisms a source of easily decomposable food is available. During the phase of high metabolic activity, when the ability to produce adaptive enzymes is also highest the occurrence of a priming effect seems very probable. Waksman and Starkey [82, 83, 84] considered the enhanced availability of food as the principal factor of enhanced activity after drying. Skyring and Thompson [71] proved with an anaerobic incubation technique that denitrification in dried soil was more active than in fresh, due to increased amounts of well-decomposable substances. McGarity [53] got similar results also after freezing.

The decreased C/N ratio of the mineralized part of the organic matter after drying, and in many cases also after freezing treatments [8, 65] points to a preferential decomposition of a substrate rich in nitrogen. This enrichement mainly consists of biomass killed during the treatment. Factors lethal to the biomass tend to increase the amount of amino acids extractable from the soil. Stevenson [76] found that after air drying increased amounts of amino acids were extractable. Paul and Tu [59] isolated more free amino acids from air-dried, oven-dried and steamed than from fresh soil. In their experiments freezing had no effect, except after stimulation of microbial life by addition of glucose plus nitrate.

Extraction of fresh soil with water in the presence of CCl_4 increases the amount of extractable amino acids 25-100 fold over an extraction with water only. The amino acids are supposed to be derived from killed biomass or other colloidal material [73]. Ivarson and Sowden [37] stated that freezing caused an increase in the total amount of free amino acids; an effect which was higher in soils where the decomposition of soil organic matter was more complete. (Similar results with regard to the free sugars in soil are obtained by Ivarson and Gupta [38].) The effect of freezing was presumed to be a fragmentation or an enhanced porosity of organic gels which so contributed to an increased decomposibility. They consequently give the same explanation for the mechanism of freezing as was done by Birch [7] for drying, ignoring the killed biomass as a source of mineralizable C and N. From Table II and the Fig. 8 and 9 it is clear that the amount of NH_4-N is increased after each treatment adversely affecting the biomass. Allen and Grimshaw [2] made already this statement, ascribing it to freezing, and also found that addition of glycerol, which prevents crystal formation, also prevented the frost effect with regard to extractable NH_4-N. Crystal formation may lead to fragmentation of aggregates, mechanical desruption of living cells and too high electrolyte concentration [21, 51, 60].

Seifert [69] reports an enhanced nitrification when soil aggregates $>$ 2 mm were frozen, but no influence after freezing of aggregates $<$ 0.5 mm.

Jenkinson's [39] experiments too support the idea that killed biomass plays an important role as a substrate in the decomposition process in soil after partial sterilization. With the aid of a labelled biomass, grown in soil from added ^{14}C tagged ryegrass, he was able to calculate approximately the amount of biomass-C in the soil, which was found to be 2.3-3.5% of the soil organic carbon.

In the CO_2 produced during incubation, the highest specific activity is measured after a treatment affecting the biomass only (chloroform and methylbromide vapour). Air-drying, oven-drying and autoclaving, which also affect the non-living organic matter, caused a lower specific activity in the CO_2 produced during incubation, due to an increased decomposition of original non-labelled, soil organic matter. Besides this, an increased decomposition took place of labelled, more or less humified, rests of the added rye grass.

Chase and Gray [12] consider the decomposition of organic matter after partial sterilization as the result of two superimposed reactions: the decomposition of easily degradable material (the flush) and the slow decomposition of a relatively resistant substrate, which would proceed at a constant rate. Fig. 1 may give an idea of this principle.

When assuming the validity of Chase and Gray's hypothesis, that chloroform vapour does not influence non-biomass material, and that a priming action does not occur during the flush, it is possible to approximate the contribution of the biomass in the flush in Jenkinson's experiments. The value for the untreated soil is taken from Jenkinson's table 9 (the second 10 days' incubation period of the in a frozen state stored samples is chosen as the best approximate for the fresh soil). The samples denoted in Jenkinson's table 4 as 'no treatment' are consequently called here 'frozen'. For the assessment of the flush, the values of the untreated sample are subtracted from those of the other samples. From these data the percentage of labelled C in the flush CO_2-C is calculated for the chloroform-treated soil (20.7%). This value is used to calculate the amounts of non-labelled carbon in the biomass of the other treatments. In case of oven-drying and autoclaving the labelled C value of the chloroform-treated samples is used, because the former values are too high due to an increased decomposition of labelled non-biomass material. Presumably this occurs also after air-drying, but it is not possible with the available data to get a correct value. The value for the contribution of the killed biomass in the CO_2 flush is too high after air drying. The results are presented in Table V. The figures given are to be regarded at rather rough approximations. The contribution of the killed biomass as a source of carbon appears to be important; even more important is the biomass as a source of nitrogen due to its low C/N ratio: bacteria 4-6, fungi about 10 [74]; soil animals have even a lower C/N ratio.

Table V. **Approximation of the contribution of the biomass in the decomposition flush after different treatments. Figures are derived from Jenkinson's work [39]**

Treatment	Total decomposition CO_2-C mg per 100 g soil		'Decomposed in the flush'					
			CO_2-C mg per 100 g soil		unlabelled biomass mg C	total biomass mg C	total CO_2-C mg	biomass-C in flush %
	C*	C	C*	C				
None	0.65	9.5						
Freezing (-15°C)	1.47	14.6	0.82	5.1	3.14	3.96	5.92	67
Air-drying (20°C)	2.32	19.5	1.67	10.0	6.40	8.07	11.67	69
CCl_4 vapour	4.94	25.9	4.29	16.4	16.43	20.72	20.69	100
Oven-drying (80°C)	5.46	34.7	4.81	25.2	16.43*	20.72*	30.01	69
Autoclaving (120°C)	5.86	52.4	5.21	42.9	16.43*	20.72*	48.11	43

* See text.

Drying of soil as a factor in soil fertility may play a role in the temperate climatic zone of western Europe especially during spring when the seed beds are prepared and a crop canopy does not yet prevent the insolation of the soil surface. In this period drying of soil may show the well-known effects. Dark coloured soils will be most affected, as the temperature rises highest there. For the same reason a bare soil will be more affected then when a crop is present. In the latter case rain has a complex effect. Except remoistening the soil, it rinses organic substances down from the overground parts of the plant [54], which contribute to the total decomposition in the soil. Next to this, the roots in the dry soil region may give off an extra amount of simple organic compounds if they were forced to an osmotic adaption under the dry conditions [41]. The effect of drying will be more pronounced when the soil is used as grassland than as arable land, as the former has a higher content of decomposable organic matter and biomass (rhizosphere).

As the killed biomass is of importance for the decomposition after drying or freezing, it is not surprising that a soil reacts differently if sampled in different seasons [70], as crop-residues, rhizosphere exudates [56], application of manure and many other factors influence the number of micro-organisms [44] and generally the living part of soil organic matter, which must be reflected in the reaction on drying and freezing.

The beneficial effect of cold winters on arable land will, according to our results, not principally depend on an accelerated mineralization of organic matter due to partial sterilization during freezing, but to the preserving effect of a cold and dry period, during which a leaching of soluble plant nutrients (nitrate) does not occur [33, 34, 43] and the decomposition of organic matter is retarded until the next vegetation period. In heavy soils besides that the structure is improved by freezing.

The killed biomass is partly mineralized, partly used for the synthesis of new microbial material and contributes for another part to the formation of humus. Jensen [40] observed that 19-60% of the killed and air-dried fungal mycelia and bacterial cells were mineralized during a 60-days' incubation period; a small increase in 'α-humus' was found. Jenkinson [39] observed a 30% decomposition of bacterial cells *(Nitrosomonas)* in 10 days. Harmsen [31] underlines the great importance of the frequent killing of the biomass in the soil by factors as drying (insolation) and freezing with regard to humus formation, basing himself on the theory of Enders [16, 17, 18] and Enders and Sigurdsson [19]. Nitrogenous products from autolyzing cells may, for the formation of humuslike products, react with other soil constituents: with lignin or its decomposition products according to Waksman and Iyer [81] and Mattson and Koutler-Anderson [50a], with methylglyoxal according to Enders, and — as observed by Haider et al. [28] — with some phenols above pH 6.5. A formation of humus-like pigments by (autolyzing) micro-organisms in pure culture is often reported [e.g. 5, 46, 66, 75]. The above-described reactions, however, depend on the conditions prevailing in the soil, the concentrations of the compounds involved and the competition for these compounds with the newly developing population of micro-organisms, which in turn depends on several physical factors.

Much work still has to be done for elucidation of what really happens with living and non-living soil organic matter during drying and freezing.

The relation between the real number of microbes and their activity (CO_2-production) during the flush should be studied more closely.

The role of biomass as the agent for decomposition

and as a supplier of decomposable matter should be more extensively studied in relation to what happens in the field under changing weather conditions, as the living part of the soil organic matter is the most sensitive, reactive and dynamic. It is not known if, and in how far, quantitative and qualitative changes in soil biomass play a role in the periodic fluctuations of soil fertility as described by Van der Paauw [57].

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