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Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter

Urlakning och nedbrytning av vattenlöslig organisk substans från olika löv- och barrförnor.

by

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Introduction

The water-soluble organic substances in the soil are of great importance for soil formation, as well as for the formation of complex compounds containing many inorganic substances, which are thus available to plants (see *e.g.* SCHEFFER, ULRICH & HIESTERMANN 1957, KONONOVA & D'YAKONOVA 1960). Litter contains considerable amounts of water-soluble substances, which are more or less easily leached, according to the type of litter. The subject of the first part of this paper is a comparison between different types of litter with respect to leaching and decomposition of water-soluble organic substances. The values are taken from experiments with the different types of litter, reported earlier in *Oikos* (NYKVIST 1959 a and b, 1961 a and b, 1962). In the second part, an account is given of chemical analyses of some aliphatic acids, amino acids and sugars in different litter extracts.

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I. Factors Influencing the Leaching and Decomposition of Water-Soluble Organic Substances from Litter

The amount of water-soluble substances in litter from different deciduous and coniferous trees has been investigated previously by *e.g.* MELIN (1939), WITTICH (1943, 1944) and MATTSON & KOUTLER-ANDERSSON (1944). The factors influencing the leaching of these substances from different types of litter are, however, imperfectly known. One of the most important factors is the preparation of the litter. Thus, the amount of water-soluble organic substances leached from needle litter during one day increases from about 1 % to 10—12 % if the litter is ground beforehand (Table I). The corresponding increase is considerably smaller in leaf litter. The importance of

Table I. Amount of water-soluble organic and inorganic substances as a percentage of the dry weight of the litter, and amount of leached water-soluble inorganic substances as a percentage of total amount of inorganic substances in fresh litter. The litters were leached anaerobically for one day at 25° C.

Type of litter		Water-soluble organic substances in % of dry weight of litter	Water-soluble inorganic substances in % of dry weight of litter	Leached inorganic substances in % of total amount of inorganic substances in litter
Fraxinus excelsior	Unground	16.5	3.6	52
	Ground	20.8	3.8	55
Alnus glutinosa	Unground	12.0	1.3	25
	Ground	12.2	1.3	25
Betula verrucosa	Unground	10.7	1.5	33
	Ground	13.7	2.1	46
Quercus robur	Unground	7.1	0.9	15
	Ground	13.3	1.1	19
Fagus silvatica	Unground	3.8	1.1	17
	Ground	6.2	1.4	22
Picea abies	Unground	1.1	0.3	3
	Ground	12.5	1.1	12
Pinus silvestris	Unground	0.9	0.1	5
	Ground	10.2	0.5	19

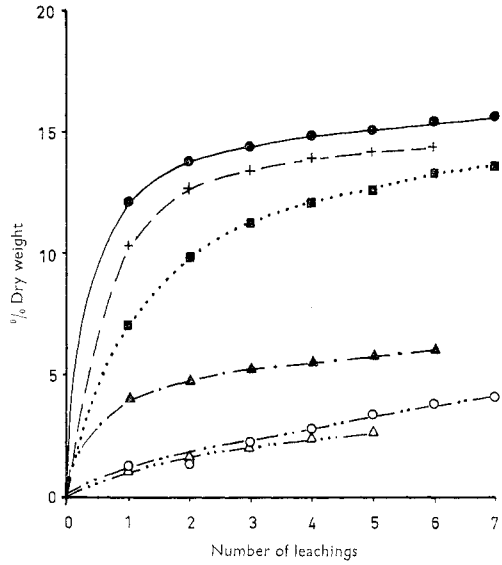
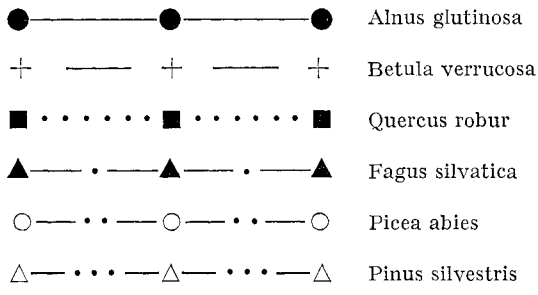


Fig. 1. Total amount of water-soluble organic substances obtained by repeated anaerobic leaching of the same litter at 25° C. Duration of each leaching one day. Dry weight of organic substances as percentage of dry weight of litter. Unground litter.



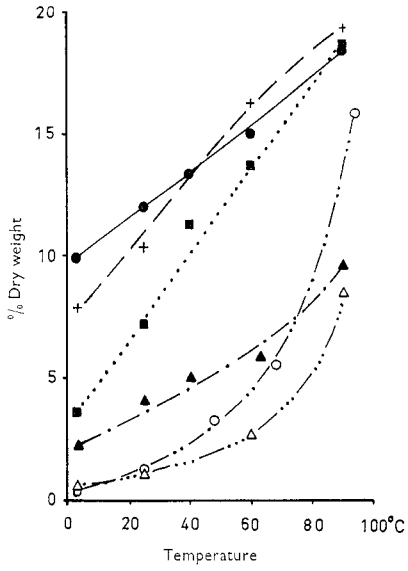


Fig. 2. Influence of temperature on amount of water-soluble organic substances leached anaerobically during one day. Otherwise as in Fig. 1.

the structure is also evident from Fig. 1. It is seen that the water-soluble substances are leached slowly from needle litter, but rapidly from leaf litter, especially that of alder and birch.

The leaching of litter under natural conditions in Sweden takes place at a lower temperature than that used, on practical grounds, in my experiments, *i.e.*, 25° C. The effect of temperature varies with the type of litter. Fig. 2 shows the amount of water-soluble organic substances leached during one day from different types of unground litter at varying temperature. As far as needle litter of spruce and pine is concerned, remarkably high values were recorded at 90° C. This is probably attributable to the fact that the outer layer of the epidermis, which consists of cutin, underwent a change at high temperature and became more permeable. This factor is of greater consequence in leaching of needle litter than in that of leaf litter, in view of the anatomic and morphologic differences between leaves and needles.

As pointed out above, the different litters contain a varying amount of water-soluble substances, that are leached more or less rapidly, depending on the structure of the litter. In needle litter, these substances are leached slowly, and therefore increase in quantity with a longer duration of leaching (Fig. 3). In leaf litter, on the contrary, an increase occurs only during the first days; thereafter, the amount remains constant or decreases. A decrease

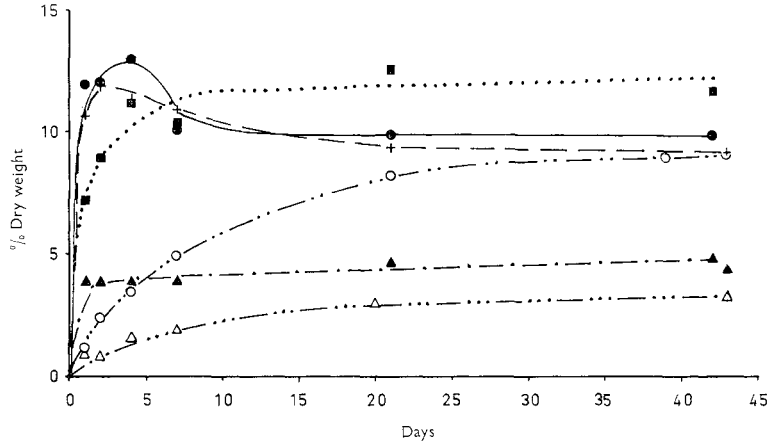


Fig. 3. Influence of duration of anaerobic leaching on amount of leached water-soluble organic substances. Temperature 25° C. Otherwise as in Fig. 1.

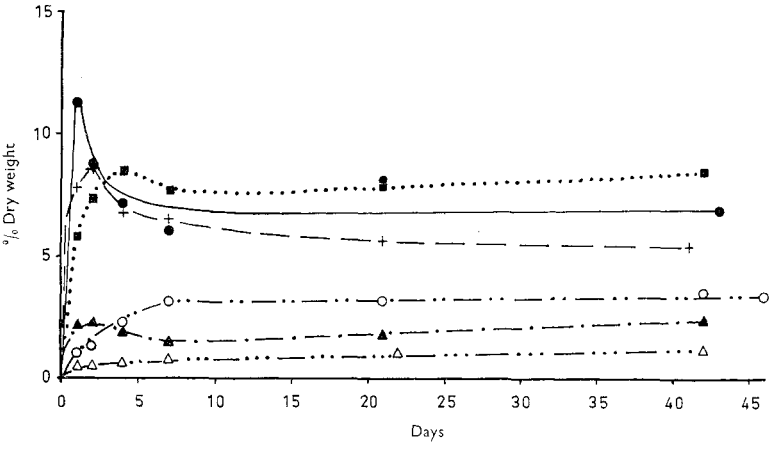


Fig. 4. Influence of duration of aerobic leaching on amount of leached water-soluble organic substances. Temperature 25° C. Otherwise as in Fig. 1.

takes place because the water-soluble organic substances are decomposed to a larger extent than they are leached. Since decomposition is greater under aerobic conditions than under anaerobic, the quantity of water-soluble substances is always less in leaching under the former conditions than under the latter (*cf.* Figs. 3 and 4).

Several investigations have shown that the amount of water-soluble substances decreases on decomposition of the litter (for literature, see Nykvist 1959 a, p. 202). With the exception of needle litter of pine, this also

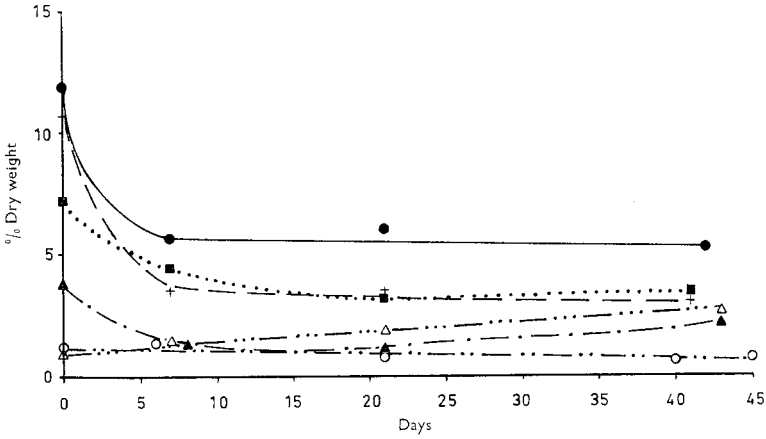


Fig. 5. Influence of decomposition of the litter on amount of water-soluble organic substances leached anaerobically during one day. Temperature 25° C. Abscissa: number of days of decomposition in water-saturated air at 20° C. Otherwise as in Fig. 1.

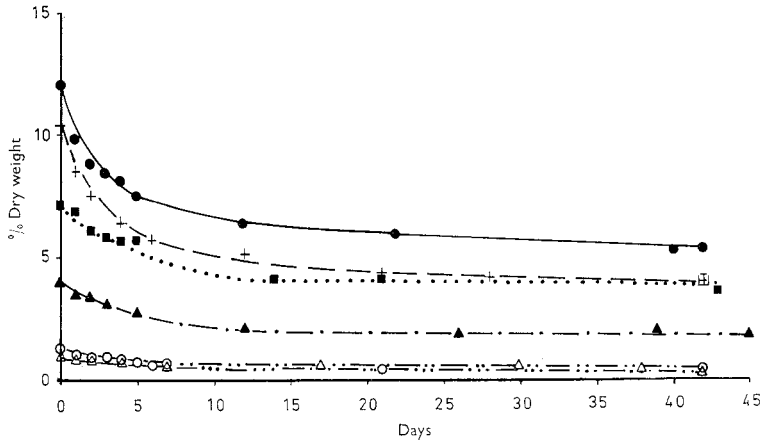


Fig. 6. Amount of water-soluble organic substances in litter extracts stored under aerobic conditions. Temperature 25° C. The litter extracts were obtained by anaerobic leaching of the litter during one day at 25° C. Otherwise as in Fig. 1.

applied in my experiments when the litter was decomposed in a water-saturated chamber at 20° C, after which it was leached for one day at 25° C, and its content of water-soluble organic substances determined (Fig. 5).

These experiments show that the water-soluble substances are decomposed within the litter as well, decomposition being greatest during the first week. Consequently, when investigations are to be made of the chemical composition and decomposition of litter, only fresh litter should be collected, *i.e.*, litter that has recently fallen to the ground (*cf.* also Tables IV—VIII).

To investigate the decomposition of the water-soluble organic substances, I made experiments on litter extracts obtained by leaching of litter for one day at 25° C, followed by separation of the water-soluble substances from the leached litter. The litter extract was kept both under anaerobic and under aerobic conditions, produced in the latter case by bubbling air through it. The content of water-soluble organic substances was then determined at fixed intervals. The results under aerobic conditions are shown in Fig. 6, from which it is seen that the organic substances decreased most during the first week. Although this applied under anaerobic conditions as well, the decrease—*i.e.*, decomposition—was greater under aerobic conditions, as can also be inferred from Table II.

Table II. Decomposition (weight decrease) of water-soluble substances, and amount of unfilterable substances in litter extract stored under both aerobic and anaerobic conditions. Values calculated as a percentage of dry weight of fresh litter extract. In anaerobic decomposition, each value represents one sample; in aerobic decomposition, the mean of two or three samples.

Litter extract	Decomposition of water-soluble substances				Unfilterable substances after 4 days' decomposition of litter extract	
	Aerobic		Anaerobic			
	4 days %	42 days %	4 days %	42 days %	Aerobic %	Anaerobic %
Fraxinus excelsior	22	39	13	24	13	2
Alnus glutinosa.....	29	51	11	24	13	0
Betula verrucosa.....	25	55	12	37	12	2
Quercus robur.....	30	42	5	17	13	2
Fagus silvatica	32	39	18	32	17	4
Picea abies.....	30	54	16	41	9	0
Pinus silvestris	22	53	9	40	7	0
Fraxinus excelsior autoclaved, sterile	3		0		1	2
Fraxinus excelsior autoclaved, inoculated	23				11	

Under aerobic conditions, the extract had a high bacterial content after one day, and after a few days small "clots" were found, consisting of bacteria and fungal hyphae in a granular substance. The "clots" increased in number and size during prolonged aerobic decomposition. Protozoa were also present, in some litter extracts as soon as after 4 days. The dry weight of the "clots" and other unfilterable substances was computed by deter-

mining the difference between the substances in 100 ml of an unfiltered extract and in 100 ml of a filtered portion. Even if this determination is not exact, it gives a rough estimate of the unfilterable substances (Table II). After four days, the weight of the unfilterable substances in litter extract of birch was 12.3 % of the original amount of fresh water-soluble substances. When the unfilterable substances were weighed after centrifugation and washing with distilled water, the corresponding value was 10.4 %.

Under anaerobic conditions as well, bacteria (mainly rod-shaped) were found in the extract after one day, and after a few days a "precipitate" consisting of bacteria appeared. On the other hand, neither fungal hyphae nor Protozoa were ever found in the extract under anaerobic conditions, nor did its colour undergo any change. Under aerobic conditions, on the contrary, the extracts of leaf litters became darker, and those of alder and ash were dark brown already after two days. The change in colour was not as marked in extracts from needle litter of pine and spruce (*cf.* LOSSAINT 1959).

Leaching of water-soluble substances from fresh litter is not influenced by microbial activity (NYKVIST 1959 a). Their decomposition seems, however, to be performed by microorganisms. This is apparent from an experiment with sterilized litter extract, performed as follows (Table II). Leaf litter of ash was leached anaerobically for one day at 25° C. The extract was separated from the litter, transferred to glass bottles, and sterilized by heating in an autoclave at 120° C. It then became turbid, and a small white "precipitate" settled after a few hours. In the aerobic series, two glass bottles with sterile extract were inoculated with unsterile litter extract, and two were kept sterile. Before the air was bubbled through the four bottles, it was forced through a wash bottle containing cottonwool saturated with an antiseptic solution (Biosept®; cetyl pyridinium chloride in 0.1 % aqueous solution). The sterile air thus obtained was purified by passing it through one wash bottle with pure cottonwool, and two with distilled water.—In the anaerobic series, a filled glass bottle with sterile litter extract was kept under anaerobic conditions for four days.

Under aerobic conditions, the sterilized litter extract inoculated with microorganisms changed from its original yellow-brownish colour to dark brown after a few days. In addition, a large number of clots appeared. Under the same conditions, the sterile extract was only slightly darker after four days.

KONONOVA (1961, p. 147) discussed the darkening of clover leaves after 3—4 days' decomposition, and stated that "this appears to be brought about by the action of oxidizing enzymes in the tissues and also by the activity of mould fungi which form a weft on the leaf surfaces". My experi-

ments have shown that the water-soluble substances are of importance for darkening of the litter during the initial stage of decomposition (*cf.* NYKVIST 1959 a, p. 200). Moreover, oxidizing enzymes are destroyed by heating to 120° C. Since the extract sterilized in an autoclave at 120° C, and subsequently inoculated with microorganisms became dark brown after a few days, this darkening must have been due to the activity of the microorganisms. The pH of the inoculated extract rose from 5.5 to 8.2 during four days, also as a result of microbial activity (Table III). In order to investigate the influence of pH on the darkening of the extracts, the following experiments were carried out. Sodium hydroxide was added to fresh extracts of ash, beech and birch until pH 8.6 was reached. The colour then turned to dark brown. When this extract was neutralized with hydrochloric acid, its colour became lighter, but was still somewhat darker than that of the initial extract. It is evident from these experiments that the darkening of litter extracts after a few days may, actually, be a result of the rise in pH during aerobic decomposition (*cf.* MIKOLA 1956, p. 12).

Table III. The pH of fresh litter extract and litter extracts stored under both aerobic and anaerobic conditions for four days.

Litter extract	Fresh litter extract	Litter extract stored under aerobic conditions for 4 days	Litter extract stored under anaerobic conditions for 4 days
Fraxinus excelsior	5.7	8.4	5.9
Alnus glutinosa.....	5.0	7.3	4.6
Betula verrucosa.....	5.4	7.7	4.7
Quercus robur.....	5.0	6.9	4.2
Fagus silvatica	5.6	7.5	5.1
Picea abies.....	4.5	6.5	4.2
Pinus silvestris	4.6	6.6	4.2
Fraxinus excelsior autoclaved, sterile	5.5	5.5	
Fraxinus excelsior autoclaved, inoculated.....	5.5	8.2	

II. Some Aliphatic Acids, Amino Acids and Sugars in Litter Extracts and their Changes during Aerobic and Anaerobic Decomposition

Experimental Methods

Preparation of litter extracts

About 2,000 ml of extract was obtained by anaerobic leaching of litter for one day at 25° C (*cf.* NYKVIST 1959 a). The litters were collected on the same sites as in previous experiments. Here and in the following, one day always denotes 24 hours. The leaf litter was unground, whereas the needle litter was ground, so that sufficient water-soluble substances would be obtained after one day's leaching. Some portions of the extract separated from the litter were stored during four days under anaerobic conditions, and others under aerobic, *i.e.*, air was forced through the extract (NYKVIST 1959 a, p. 197). The pH and the amount of organic and inorganic substances were determined in the fresh litter extract, as well as in the extracts stored under anaerobic and aerobic conditions. The decomposition of the water-soluble substances and the amount of unfilterable substances (see p. 9) formed under these conditions are shown in Table II, and the pH values in Table III.

For the chemical analyses, 100 – 400 ml of the extracts were used, the difference in volume depending on the amount of water-soluble substances in the different litters. Since, under aerobic conditions, the pH of the litter extract rises (see Table III), this may result in precipitation of some organic substances. In order to restore the pH to its original value and thus dissolve a possible precipitation, hydrochloric acid was added to the litter extract stored under aerobic conditions.

Before determining the aliphatic acids, amino acids and sugars in the litter extracts, the substances in question must be purified. This was done by the ion exchange technique. The filtered litter extract (100 – 400 ml) was first passed through a column of a cation exchange resin containing about 40 g of "Amberlite IR-120" (H^+) at a rate of 0.5 ml/minute. The column was washed with distilled water (300 – 400 ml) until all anions (organic and inorganic acids) and non-ionic substances (*e.g.* carbohydrates) were removed. The cations and amino acids were then eluted with 400 – 500 ml of *N* NH_3. The two samples were concentrated to dryness *in vacuo* at 40° C, and the residues dissolved in distilled water (100 ml/sample). Each sample then passed a column of an anion exchange resin containing about 40 g of "Amberlite IRA-410" (CO_3^{2-}) at a rate of 0.5 ml/minute. The columns were washed with 300 – 400 ml of distilled water and were then eluted with

400 – 500 ml of *N* HCl (BLOCK, DURRUM & ZWEIG 1958, p. 120; LINSKENS 1959).

The four samples thus obtained were concentrated to dryness *in vacuo* at 40° C, and the residues dissolved in distilled water (1 – 4 ml/sample). 1 – 30 µl portions of these concentrated samples were analyzed by paper chromatography. By concentrating the samples to dryness, a great deal of the dark-coloured substances in several litter extracts became insoluble. This procedure has, however, no effect on the sugar, aliphatic acid and amino acid content of the extracts.

Sample I, which passed through both the cation and the anion exchange column contained non-ionic material, such as carbohydrates. Sample II contained organic and inorganic acids and had passed through the cation exchange column, but was retained on the anion one, and then eluted by *N* HCl. Sample III was retained on the cation exchange column, and eluted by *N* NH_3. The sample was then concentrated to dryness, dissolved in distilled water, and passed through an anion exchange column. This sample contained cations and the amino acids arginine and lysine. Sample IV, containing other amino acids, was retained both on the cation resin and the anion resin, from which they were eluted by *N* NH_3 and *N* HCl, respectively. All four samples were frequently tested for sugars, aliphatic acids and amino acids, as a check on the method.

About 28 % of the water-soluble substances from leaf litter of ash (*Fraxinus excelsior*) was recovered in sample I, and 35 % in sample II. The corresponding values for samples III and IV were 4 % and 3 %. About 30 % was lost by the treatment; it was retained by the resins, and could not be eluted. An accumulation of dark-coloured material was observed at the top of the anion exchange column; when it had been used several times, most of the anion resin was dark-coloured. The dark colour became lighter when the column was eluted with hydrochloric acid, but darkened again when it was regenerated with sodium carbonate. Many tests showed that the dark-coloured anion resin still has the ability to retain anions. These dark-coloured substances seem to be retained in the anion resin by adsorption or precipitation, and not by ion exchange (LUTWICK & DELONG 1954, see also SCHEFFER & ULRICH 1960, p. 114). Owing to the original dark colour of the cation resin, it is not possible to see if such an accumulation occurs in this column.

The main object of my investigation was a qualitative chemical analysis of some aliphatic acids, amino acids and sugars in the different litter extracts. However, only the substances present in large amounts were determined. The volume to which the samples were concentrated was dependent on the amount of substances in the extract. Thus, samples from litter

extract of beech were concentrated four times more than those of ash, in order to obtain measurable amounts of sugars, amino acids and aliphatic acids. This implies that a substance present in the same concentration in leaf litter of ash and beech may not be found in extract of ash, but found as a trace in extract of beech.

Chromatographic technique

Descending paper chromatography was used in the analyses. In view of the complex mixture of amino acids and, to a certain degree, also of aliphatic acids, two-dimensional paper chromatography was necessary for separation of these substances.

The substances under investigation were determined in the extracts by chromatographing, on the same paper, standard solutions of known substances and small amounts (1—30 µl) of the concentrated samples of the litter extracts. Owing to contaminants in the samples, which influence the R_f values, it was, however, also necessary to apply on the same spot both the sample of litter extract and standard solutions of substances which, from previous chromatograms, could be expected to occur in the sample. By comparison with this chromatogram and a chromatogram of the sample, the unknown substances could be identified. As far as the sugars are concerned, the chemical determination was simplified by colour reactions with the various sugars.

In view of the great differences between the various types of litter with respect to their content of aliphatic acids and sugars, a rough quantitative analysis of these substances provides valuable information. The quantity of a substance of unknown concentration was estimated by visual comparison on the same chromatogram with a standard solution of known concentration (BLOCK, DURRUM & ZWEIG 1958, p. 85). Most values were, however, also determined by measurements of the total colour of the spots with a photoelectric densitometer (Spinco Analytrol; for details of the method, see BLOCK, DURRUM & ZWEIG 1958, pp. 75—78, 94). With this method, the determinations can be made with an accuracy of $\pm$ 5 %. Owing to dark-coloured contaminants in the samples and tailing of the spots, I could not, however, achieve better accuracy than $\pm$ 50 %.

The amino acids in litter extracts were separated by two-dimensional chromatography. The first solvent was *n*-butanol—acetic acid—water (4 : 1 : 5), and the second water-saturated phenol in an ammonia atmosphere (LINSKENS 1959, pp. 152—153). The addition of 0.1 % cupron (α-benzoinoxime) to the phenol retards its decomposition. The amino acids were detected by spraying the dried chromatogram with a 0.5 % solution of ninhydrin in water-saturated *n*-butanol containing acetic acid (7 %). The chromato-

gram was then dried at 70—80° C for about 10 minutes. Because of the two-dimensional technique, no quantitative analyses were made.

Samples of litter extracts containing sugars also contain a large amount of other substances, which interfere with the separation of the sugars. A running chromatogram (Durchlauf) with the solvents *n*-butanol—ethanol—water (4 : 1 : 5) was found to be the most convenient. The chromatogram was run for three days; after drying, it was sprayed with silver nitrate-ammonia solution or a solution of *p*-anisidine-phosphoric acid in ethanol (LINSKENS 1959, pp. 87 and 94). Chromatograms sprayed with aniline phthalate reagent were also used for quantitative estimation in the photo-electric densitometer.

Of the organic acids, I analyzed only the non-volatile aliphatic acids. For quantitative determination of those present most abundantly, the one-dimensional technique was used, with *n*-butanol—formic acid—water (4 : 1 : 5) as solvent. Since lactic and succinic acid have practically the same

Table IV. Amino acids in fresh and decomposed litter extracts. The extracts were obtained by anaerobic leaching of litter during one day at 25° C.

+ (present), Tr. (trace), — (absent)

Treatment of litter extract	Type of litter	Glutamic acid	Leucines	Valine	α-Alanine	Serine	Threonine	Glycine	Aspartic acid	Lysine	Arginine	Unidentified substance
Fresh litter extract	Fraxinus excelsior	+	+	+	+	+	+	Tr.	Tr.	Tr.	—	+
	Alnus glutinosa	+	+	+	+	+	Tr.	+	+	+	+	+
	Betula verrucosa	+	+	+	+	+	+	+	+	Tr.	—	+
	Quercus robur	+	+	+	+	+	+	Tr.	+	+	+	+
	Fagus silvatica	+	+	+	+	+	+	+	+	+	—	+
	Picea abies	+	+	+	+	+	+	+	+	+	+	+
	Pinus silvestris	+	+	+	+	+	+	+	+	+	+	+
Anaerobic decomposition of litter extract during 4 days	Fraxinus excelsior	+	—	—	+	—	—	—	—	—	—	+
	Alnus glutinosa	+	+	+	+	+	Tr.	+	+	+	+	+
	Betula verrucosa	+	—	+	+	+	+	+	+	—	—	+
	Quercus robur	+	+	+	+	+	+	Tr.	+	+	+	+
	Fagus silvatica	+	+	+	+	+	+	+	+	+	—	+
	Picea abies	+	+	+	+	+	+	+	+	+	+	+
	Pinus silvestris	+	+	+	+	+	+	+	+	+	+	+
Aerobic decomposition of litter extract during 4 days	Fraxinus excelsior	—	—	—	—	—	—	—	—	—	—	—
	Alnus glutinosa	—	—	—	—	—	—	—	—	—	—	—
	Betula verrucosa	—	—	—	—	—	—	—	—	—	—	—
	Quercus robur	—	—	—	—	—	—	—	—	—	—	—
	Fagus silvatica	—	—	—	—	—	—	—	—	—	—	—
	Picea abies	Tr.	—	—	Tr.	—	—	—	—	—	—	—
	Pinus silvestris	—	—	—	—	—	—	—	—	—	Tr.	—

R_f value in this solvent, no separate quantitative determination could be made.

For the qualitative determination of lactic and succinic acid and for some other acids occurring in small amounts, the two-dimensional technique must be used. Previous analyses were done on Whatman No. 1 filter paper, but for detection of the small amounts of different aliphatic acids on a two-dimensional chromatogram, it was necessary to use acid-washed filter paper (Macherey & Nagel 2261). The first solvent was ethanol—concentrated ammonia—water (80 : 5 : 15), and the second *n*-butanol—formic acid—water (4 : 1 : 5). The formic acid was removed by blowing steam over the chromatogram. Warm air from a fan prevented the paper from becoming overloaded with water. The chromatograms were sprayed with bromophenol blue, and the acids then appeared as yellow spots on a blue background.

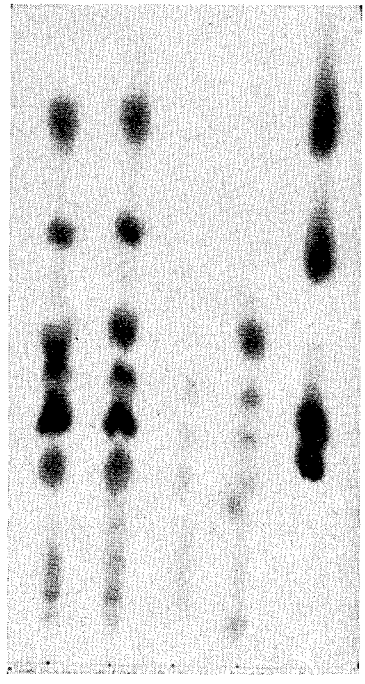
Results and Discussion

Amino acids

The different amino acids in the litter extracts are listed in Table IV. In the fresh extracts, 9 to 10 amino acids were found, and one unidentified substance, probably a peptide. Of the amino acids, glutamic acid was present in the greatest amount in all extracts. In litter extract of alder, no specially high concentration of amino acids was found, as might be expected from the high nitrogen content of the litter (*cf.* ARRHENIUS 1957). Anaerobic decomposition for 4 days at 25° C did not noticeably influence the amino acid content, except in extracts of ash litter. Under aerobic conditions, however, most amino acids had nearly completely disappeared after four days at 25° C, as a result of microbial activity (*cf.* 2 and 3 in Fig. 7).

KNAPP & LINSKENS (1954) analyzed the free amino acids in leaf litter of beech (*F. silvatica*), which had been stored for several months in moist condition, and had been decomposed by microorganisms. They found alanine, serine, cysteine and traces of glutamic acid, and probably also tryptophane. My results in Tables II and IV indicate that the original free amino acids in the litter were rapidly utilized by the microorganisms during decomposition of the litter. The amino acids found by KNAPP & LINSKENS seem, therefore, to be formed during the microbial decomposition of the proteins in the litter.

Free amino acids have been recovered from the soil by several investigators. PAYNE, ROUATT & KATZNELSON (1950) found that air-drying of a moist soil increased its free amino acid content. The method for concentrating the amino acids in soil leachates also seemed to be of importance for their determination in soil. Thus, these authors found free amino acids



1. 2. 3. 4. 5.

Fig. 7. Amino acids in litter extract of ash. The chromatogram was run with *n*-butanol—acetic acid—water and sprayed with ninhydrin.
1. Fresh litter extract.
2. Litter extract sterilized by heating in an autoclave and stored under aerobic conditions for four days at 25° C (see p. 9).
3. Litter extract sterilized by heating in an autoclave, inoculated with unsterile litter extract, and stored under aerobic conditions for four days at 25° C.
4. Litter extract (unsterile) stored under anaerobic conditions for four days at 25° C. The darkest spot is an unidentified substance, probably a peptide.
5. Standard solutions of leucine (highest R_f value), valine, α-alanine and glutamic acid (lowest R_f value).

in two soils when the soil leachates were concentrated by freeze-drying, whereas none were detected after concentration *in vacuo* at 40° C. The mild heat treatment probably results in a loss of amino acids owing to an interaction between simple sugars and amino acids in the soil leachate (GOTTSCHALK & PARTRIDGE 1950, LEA & HANNAN 1950). In my experiments, the samples were concentrated *in vacuo* at 40° C, but the sugars and organic acids were separated from the amino acids in a cation exchange column beforehand.

DADD, FOWDEN & PEARSALL (1953) investigated the free amino acids in mor, mull and peat. The most widely distributed were aspartic and glutamic acid, alanine, glycine and serine. In a black, amorphous and greasy mor under oak, they also found threonine, leucines, valine, γ-butyric

acid, asparagine and glutamine. Some of the results suggested that the total number and concentration of free amino acids are greater in February and April than in July.

Quantitative determination of the free amino acids in soils was performed by PUTNAM & SCHMIDT (1959). They found aspartic and glutamic acid, valine, leucine and lysine in each of the samples. By adding glucose and sodium nitrate to the soil, the amount and number of amino acids increased markedly. They also investigated the persistence of arginine, tryptophane and lysine added to three different soils. After 2 to 3 days, most of the amino acids added to the soil had disappeared in the free state. In sterile soils, however, there was no disappearance of the amino acids. Similar results were obtained by GREENWOOD & LEES (1956), and are in agreement with my analyses of amino acids in fresh and aerobically decomposed litter extract (*cf.* Table IV and Fig. 7).

Free amino acids have also been recovered from different types of soil by TOMBESI (1953) and SIMONARTS & PEETERS (1954), and in different types of peat by COULSON, DAVIES & KHAN (1959) and ALEKSANDROVA (1960).

Table V. Sugars in fresh and decomposed litter extracts. Amount of sugars calculated as a percentage of dry weight of fresh litter extract. The extracts were obtained by anaerobic leaching of litter during one day at 25° C.

+ (small amount), Tr. (trace), — (absent)

Treatment of litter extract	Type of litter	Glucose %	Fructose %	Xylose %	Sucrose %
Fresh litter extract	Fraxinus excelsior	4.5	1.1	—	—
	Alnus glutinosa	6.2	3.3	2.1	—
	Betula verrucosa	6.9	3.4	3.4	0.6
	Quercus robur	9.1	6.8	Tr.	Tr.
	Fagus silvatica	5.1	7.7	0.8	—
	Picea abies	6.1	1.8	Tr.	—
	Pinus silvestris	6.1	1.5	—	—
Anaerobic decomposition of litter extract during 4 days	Fraxinus excelsior	0.7	—	—	—
	Alnus glutinosa	0.8	0.8	0.7	—
	Betula verrucosa	1.3	0.6	2.5	0.5
	Quercus robur	5.7	4.5	Tr.	Tr.
	Fagus silvatica	+	—	0.5	—
	Picea abies	—	1.8	Tr.	—
	Pinus silvestris	2.3	1.1	—	—
Aerobic decomposition of litter extract during 4 days	Fraxinus excelsior	0.7	—	—	—
	Alnus glutinosa	0.8	Tr.	Tr.	—
	Betula verrucosa	0.5	+	—	—
	Quercus robur	Tr.	Tr.	—	—
	Fagus silvatica	+	Tr.	—	—
	Picea abies	Tr.	Tr.	—	—
	Pinus silvestris	—	—	—	—

Table VI. Sugars in fresh and decomposed litter extracts obtained by anaerobic leaching of unground litter during one day at 25° C. Amount of sugars calculated as a percentage of dry weight of the litter. Bracketed values: corresponding values calculated for ground litter.

+ (small amount), Tr. (trace), — (absent)

Treatment of litter extract	Type of litter	Glucose %	Fructose %	Xylose %	Sucrose %
Fresh litter extract	Fraxinus excelsior	0.9 (1.1)	0.2 (0.3)	—	—
	Alnus glutinosa	0.8 (0.8)	0.4 (0.4)	0.3 (0.3)	—
	Betula verrucosa	0.8 (1.1)	0.4 (0.5)	0.4 (0.5)	0.07 (0.09)
	Quercus robur	0.7 (1.3)	0.5 (1.0)	Tr.	Tr.
	Fagus silvatica	0.3 (0.4)	0.4 (0.6)	0.04(0.06)	—
	Picea abies	0.08 (0.8)	0.02 (0.2)	Tr.	—
	Pinus silvestris	0.06 (0.7)	0.02 (0.2)	—	—
Anaerobic decomposition of litter extract during 4 days	Fraxinus excelsior	0.1 (0.2)	—	—	—
	Alnus glutinosa	0.1 (0.1)	0.1 (0.1)	0.09 (0.09)	—
	Betula verrucosa	0.2 (0.2)	0.07 (0.09)	0.3 (0.4)	0.06 (0.08)
	Quercus robur	0.5 (0.8)	0.4 (0.6)	Tr.	Tr.
	Fagus silvatica	+	—	0.02 (0.04)	—
	Picea abies	—	0.02 (0.2)	Tr.	—
	Pinus silvestris	0.02 (0.2)	0.01 (0.1)	—	—
Aerobic decomposition of litter extract during 4 days	Fraxinus excelsior	0.1 (0.2)	—	—	—
	Alnus glutinosa	0.1 (0.1)	Tr.	Tr.	—
	Betula verrucosa	0.06 (0.08)	+	—	—
	Quercus robur	Tr.	Tr.	—	—
	Fagus silvatica	+	Tr.	—	—
	Picea abies	Tr.	Tr.	—	—
	Pinus silvestris	—	—	—	—

Sugars

Of the sugars, glucose and fructose appeared in the greatest amounts and were found in all fresh litter extracts. Calculated as a percentage of the amount of water-soluble substances in the extracts, the concentration of these sugars was highest in extract of oak, and lowest in that of ash (Table V).

From the values in Table V and the amount of water-soluble substances in the litter (Table I: sum of water-soluble organic and inorganic substances), the amount of water-soluble sugars was calculated as a percentage of the dry weight of the litter (Table VI). When the values were computed from the amount of water-soluble substances leached from *unground* litter during one day, great differences were present between needle litter and leaf litter, owing to the slow leaching of these substances from the former.

The total amount of water-soluble sugars in the different litters was calculated from the amount of water-soluble substances leached from *ground* litter during one day (Table VI: bracketed values). Of the litters investigated, leaf litter of oak and birch contained the largest amount of sugar, and

needle litter of pine the smallest. The difference between leaf litter of beech and needle litter of spruce and pine was, however, inappreciable.

The sugars in the litter extracts were easily decomposed by microorganisms, and, with the exception of ash litter, the decomposition was greater under aerobic conditions than under anaerobic (Fig. 8 and Table VI).

Free sugars are formed in small amounts during the microbial decomposition of other carbohydrates in the litter. Thus, NAGAR (1962) found free glucose in four different soils, and ALVSAKER & MICHELSEN (1957) free glucose, arabinose, fructose, xylose, galactose and ribose in cold-water extract from the uppermost layer of a pine forest soil.

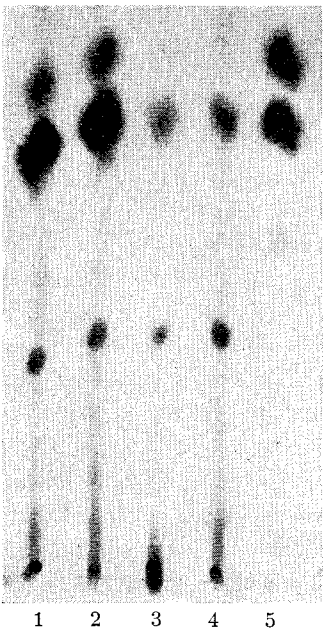
Several investigations have been made of the carbohydrates in different soils, and the sugars obtained after their hydrolysis. A review of the relevant literature has recently been given by GUPTA (1962). Soil organic matter has been found to contain about 10 % of carbohydrate, determined as sugar in soil hydrolysates. The main sugars obtained from hydrolysis of carbohydrates in the soil are xylose, arabinose, mannose, glucose and galactose, of which glucose is the most abundant (for literature, see GUPTA).

After hydrolysis of coniferous and deciduous litter, SOWDEN & IVARSSON (1962) identified the following sugars: rhamnose, ribose, xylose, arabinose, mannose, glucose and galactose. They also followed the decrease in these constituents of carbohydrates on decomposition of the litter (IVARSSON & SOWDEN 1962).

Aliphatic acids

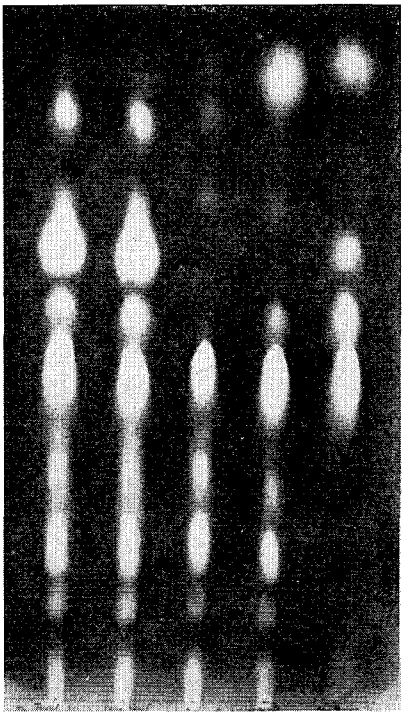
A large number of organic and inorganic acids are present in the various litter extracts. Only the most abundant, non-volatile aliphatic acids were determined. With the method applied, the aromatic acids were not separated. Unidentified acids with low R_f values in *n*-butanol—formic acid—water were observed in all litter extracts (Fig. 9, below phosphoric acid). A test with a special reagent for phosphoric acid (ammonium molybdate-perchloric acid; see WALDI 1955, p. 139) showed that these acids were due partly to tailing of the phosphoric acid. Probably other inorganic acids as well were to be found among these acids with low R_f values.

Certain aliphatic acids—such as oxalic acid—also have a low R_f value in both solvents (butanol—formic acid—water and ethanol—concentrated ammonia—water) and could not be determined in the litter extracts with the method in question, owing to contamination by inorganic acids. However, litter extracts always contain calcium ions (ARRHENIUS 1957, LOSSAINT 1959, NYKVIST 1959), and as calcium oxalate is a nearly insoluble compound, oxalic acid can be present in such extracts only in very small quantity.



1 2 3 4 5

Fig. 8



1 2 3 4 5

Fig. 9

Fig. 8. Sugars in litter extract of ash. Running chromatogram with *n*-butanol—ethanol—water, sprayed with silver nitrate—ammonia solution.
1. Fresh litter extract.
2. Litter extract sterilized by heating in an autoclave and stored under aerobic conditions for four days at 25° C (see p. 9).
3. Litter extract sterilized by heating in an autoclave, inoculated with unsterile litter extract, and stored under aerobic conditions for four days at 25° C.
4. Litter extract (unsterile) stored under anaerobic conditions for four days at 25° C.
5. Standard solutions of fructose (highest R_f value) and glucose.

The litter extracts (1—4) also have a spot halfway to the glucose spot. This spot is an unidentified substance, which gives no colour reaction with specific sugar reagents.

Fig. 9. Some aliphatic acids in litter extract of ash. The chromatogram was run with *n*-butanol—formic acid—water and sprayed with bromophenol blue.
1. Fresh litter extract.
2. Litter extract sterilized by heating in an autoclave and stored under aerobic conditions for four days at 25° C (see p. 9).
3. Litter extract sterilized by heating in an autoclave, inoculated with unsterile litter extract, and stored under aerobic conditions for four days at 25° C.
4. Litter extract (unsterile) stored under anaerobic conditions for four days at 25° C.
5. Standard solutions of lactic acid (highest R_f value), malic acid, citric acid and phosphoric acid.

Table VII. Some aliphatic acids and phosphoric acid in fresh and decomposed litter extracts. Amount of aliphatic acids calculated as a percentage of dry weight of fresh litter extract. The extracts were obtained by anaerobic leaching of litter during one day at 25° C.

+ (small amount), Tr. (trace), — (absent).

Treatment of litter extract	Type of litter	Malic acid %	Citric acid %	Malonic acid %	Lactic + succinic acid %	Fumaric acid %	Glycolic acid %	Phosphoric acid %
Fresh litter extract	Fraxinus excelsior	6.8	2.7	1.8	Tr.	—	0.9	2.7
	Alnus glutinosa	0.8	0.8	+	Tr.	—	Tr.	0.8
	Betula verrucosa	1.3	3.9	0.3	+	Tr.	Tr.	3.6
	Quercus robur	1.0	1.0	Tr.	Tr.	—	Tr.	
	Fagus silvatica	0.5	1.0	Tr.	Tr.	—	Tr.	9.3
	Picea abies	1.3	1.3	Tr.	Tr.	Tr.	Tr.	1.3
	Pinus silvestris	0.9	1.8	—	Tr.	Tr.	—	0.6
Anaerobic decomposition of litter extract during 4 days	Fraxinus excelsior	Tr.	1.6	0.4	8.6	—	0.4	2.7
	Alnus glutinosa	—	0.8	—	2.5	—	Tr.	0.8
	Betula verrucosa	Tr.	3.9	Tr.	5.2	—	Tr.	3.6
	Quercus robur	Tr.	1.0	Tr.	4.0	—	Tr.	
	Fagus silvatica	Tr.	0.3	—	2.5	—	Tr.	9.3
	Picea abies	Tr.	1.3	Tr.	2.2	Tr.	Tr.	1.3
	Pinus silvestris	+	1.8	—	3.0	—	—	0.6
Aerobic decomposition of litter extract during 4 days	Fraxinus excelsior	—	0.3	0.4	—	—	0.4	2.7
	Alnus glutinosa	—	—	—	—	—	—	<0.8
	Betula verrucosa	—	+	—	—	—	Tr.	3.6
	Quercus robur	—	—	—	—	—	—	
	Fagus silvatica	—	+	—	—	—	—	9.3
	Picea abies	—	—	—	—	—	—	1.3
	Pinus silvestris	Tr.	0.6	—	—	—	—	0.6

Tartaric acid was not found in any of the litter extracts. It is, however, difficult to separate it from phosphoric acid, and small amounts might therefore have been concealed by the large spot of phosphoric acid.

Most aliphatic acids of importance—oxalic and tartaric acid excepted —have a higher R_f value than phosphoric acid, and can be identified by the method applied (Table VII). The most abundant of the aliphatic acids in the litter extracts were calculated as a percentage of the amount of water-soluble substances in the fresh extracts. Although malic and citric acid were found in measurable quantities in all undecomposed extracts, they were present in much higher concentration in extract of ash than in any of the others. This difference was more pronounced when the amount of aliphatic acids leached from *unground* litter during one day was calculated as a percentage of the dry weight of the litter (see p. 18). In this case, the smallest amounts of malic and citric acid were found in leaf litter of beech

and needle litter of pine and spruce (Table VIII). When the values were calculated on the amount of water-soluble substances leached from *ground* litter, on the other hand, leaf litter of beech, oak and alder yielded smaller amounts of malic and citric acids than needle litter of pine and spruce (Table VIII: bracketed values). The calculated amount of aliphatic acids, obtained after leaching of *ground* litter permits an evaluation of the total amount of water-soluble aliphatic acids in the different litters (Table VIII: bracketed values).

Under anaerobic conditions, it was found that lactic and succinic acid were formed in the litter extracts. The amount of some other aliphatic acids, such as malic acid, decreased considerably. Citric acid, being more resistant under anaerobic conditions, decreased only in litter extracts of ash and beech.

Under aerobic conditions, the aliphatic acids were rapidly decomposed by microorganisms (Fig. 9 and Tables VII and VIII). Even after two days' aerobic decomposition of litter extract from birch, only traces of the aliphatic acids could be detected.

The phosphoric acid content of the litter extracts was also estimated, and considerable differences were found between the different types of litter. No decrease in phosphoric acid occurred under either anaerobic or aerobic conditions.

Investigating "water extracts from spruce needles, birch leaves and moss under various decomposition conditions, and also water extracts taken from forest litter", KAURICHEV & NOZDRUNOVA (1961) found that oxalic acid was always present, and "that citric acid and volatile acids have been detected". The method they used was a "chromatographic separation into silica gel".

A qualitative analysis of the non-volatile organic acids in peat was performed by ALEKSANDROVA (1960). The filtered solution, pressed from the peat, was acidified with sulphuric acid and purified by extraction with ether. The organic acids were separated on one-dimensional chromatograms with a solvent of *n*-butanol, formic acid and water. She found that "the spots of organic acids thus revealed, corresponded in location on the chromatogram to oxalic, malic, lactic, succinic, glutaric and adipic acids". The presence of lactic and succinic acid in peat is in good agreement with my finding that these acids were formed in litter extracts under anaerobic conditions. In view of my observations of the rapid decomposition of malic acid in litter extracts, under both aerobic and anaerobic conditions, it is nevertheless remarkable that malic acid was found in water extracts from peat.

MATTSON & KOUTLER-ANDERSSON (1941) calculated "the organic acids"

Table VIII. Some aliphatic acids and phosphoric acid in fresh and decomposed litter extracts obtained by anaerobic leaching of unground litter during one day at 25° C. Amount of aliphatic acids calculated as a percentage of dry weight of litter. Bracketed values: corresponding values calculated for ground litter.

+ (small amount), Tr. (trace), — (absent).

Treatment of litter extract	Type of litter	Malic acid %	Citric acid %	Malonic acid %	Lactic + succinic acid %	Fumaric acid %	Glycolic acid %	Phosphoric acid %
Fresh litter extract	Fraxinus excelsior	1.4 (1.7)	0.5 (0.7)	0.4 (0.4)	Tr.	—	0.2 (0.2)	0.5 (0.7)
	Alnus glutinosa	0.1 (0.1)	0.1 (0.1)	+	Tr.	—	Tr.	0.1 (0.1)
	Betula verrucosa	0.2 (0.2)	0.5 (0.6)	0.04 (0.05)	+	Tr.	Tr.	0.4 (0.6)
	Quercus robur	0.1 (0.1)	0.1 (0.1)	Tr.	Tr.	—	Tr.	
	Fagus silvatica	0.02 (0.03)	0.05 (0.08)	Tr.	Tr.	—	Tr.	0.5 (0.7)
	Picea abies	0.02 (0.2)	0.02 (0.2)	Tr.	Tr.	Tr.	Tr.	0.02 (0.2)
	Pinus silvestris	0.01 (0.1)	0.02 (0.2)	—	Tr.	Tr.	—	0.01 (0.1)
Anaerobic decomposition of litter extract during 4 days	Fraxinus excelsior	Tr.	0.3 (0.4)	0.1 (0.1)	1.7 (2.1)	—	0.1 (0.1)	0.5 (0.7)
	Alnus glutinosa	—	0.1 (0.1)	—	0.3 (0.3)	—	Tr.	0.1 (0.1)
	Betula verrucosa	Tr.	0.5 (0.6)	Tr.	0.6 (0.8)	—	Tr.	0.4 (0.6)
	Quercus robur	Tr.	0.1 (0.1)	Tr.	0.3 (0.6)	—	Tr.	
	Fagus silvatica	Tr.	0.01 (0.02)	—	0.1 (0.2)	—	Tr.	0.5 (0.7)
	Picea abies	Tr.	0.02 (0.2)	Tr.	0.03 (0.3)	Tr.	Tr.	0.02 (0.2)
	Pinus silvestris	+	0.02 (0.2)	—	0.03 (0.3)	—	—	0.01 (0.1)
Aerobic decomposition of litter extract during 4 days	Fraxinus excelsior	—	0.06 (0.07)	0.1 (0.1)	—	—	0.1 (0.1)	0.5 (0.7)
	Alnus glutinosa	—	—	—	—	—	—	< 0.1 (< 0.1)
	Betula verrucosa	—	+	—	—	—	Tr.	0.4 (0.6)
	Quercus robur	—	—	—	—	—	—	
	Fagus silvatica	—	+	—	—	—	—	0.5 (0.7)
	Picea abies	—	—	—	—	—	—	0.02 (0.2)
	Pinus silvestris	Tr.	0.01 (0.1)	—	—	—	—	0.01 (0.1)

in undecomposed and decomposed litters from values for the acidity, excess base and acidoids. The "organic acids" were put equal to the sum of the acidity and excess bases minus the acidoids. "The acidity was found by electrometric titration of the original material to pH 7" and the excess base "by a backward titration to methyl orange of the ash obtained by gentle ignition in the electric furnace. The acidoids were determined by electrometric titration of the completely electrodialysed material to pH 7". Compared with other litters investigated, leaf litter of ash (*F. excelsior*) and elm (*U. glabra*) contained large amounts of "organic acids" (calculated in milliequivalents/100 g dry matter). Despite the different method of determination of organic acids, a comparison between their investigation and mine also reveals—with the exception of oak litter—other similarities in the sequence between the different litters with respect to their organic acid content. The aforementioned authors also concluded that "the diffusible organic acids belong to the most easily decomposed components of the litter and that the water-soluble organic matter, consisting largely of organic acids, rapidly decreases during the first few months" (1941, p. 25, *cf.* WAKSMAN 1932, p. 409). I have found that the non-volatile aliphatic acids are easily decomposed by microorganisms under aerobic conditions; under anaerobic conditions, some aliphatic acids are formed and other decreased. However, the aliphatic acids constitute only a part of the water-soluble substances. In litter extract of ash, about 35 % of the water-soluble substances is retained by an anion resin, and some proportion is probably not retained by ion exchange (see p. 12).

It has been demonstrated in several laboratory and field investigations that the pH of litter rises during decomposition (for literature, see NYKVIST 1959 b, p. 219; 1962, pp. 242, 244). My earlier experiments have shown that the factors determining the rise in pH are to be found in the water-soluble substances in the fresh litters. The pH of litter extracts rises under aerobic conditions, but falls under anaerobic. MATTSON & KOUTLER-ANDERSSON (1941) found that the pH increases rapidly under aerobic conditions, as a result of decomposition of the "organic acids". My analyses of the aliphatic acids in different litters have shown that malic and citric acids are the most important aliphatic acids in the fresh litters, and that these substances are rapidly decomposed by microorganisms under aerobic conditions. It can be stressed that, under favourable conditions, these aliphatic acids were almost completely decomposed after a few days, with an accompanying rise in pH. Other investigations have shown an increase in pH during decomposition of the litter after several days or, as a rule, several weeks.

According to MATTSON & KOUTLER-ANDERSSON (1941), "the quantities

of organic acids in the original materials show a general relationship to the quantities of bases". When the organic acids in the litter extracts are decomposed, the presence of bases results in an increase in pH. The bases are, however, subsequently leached from the litter or taken up by plants or microorganisms, and the pH falls. Such a decrease in pH has been reported by MATTSON & KOUTLER-ANDERSSON (1941, 1954), MIKOLA (1954) and VIRO (1955). The low pH values found in many soils (HESSELMAN 1926) indicate, however, that organic acids are also produced during decomposition of the organic matter in the soil. It is a well established fact that many microorganisms produce organic acids during decomposition of organic substances (see *e.g.* RIPPEL-BALDES 1955, pp. 182—186; WAKSMAN 1932, p. 682).

Unknown substances in litter extracts

The aliphatic acids, sugars and amino acids constitute only 10 to 25 % of the amount of water-soluble substances. Considerable labour has been devoted to attempts to analyze the rest of the water-soluble substances. Investigations by HANDLEY (1954), LOSSAINT (1959) and KAURICHEV & NOZDRUNOVA (1961) have shown that aqueous extracts of leaves and litters contain substances similar to vegetable tannins, but no satisfactory method for the chemical analysis of these complex materials has yet been worked out. An investigation of the polyphenols of leaves, litter and humus from mull and mor sites was carried out by COULSON, DAVIES & LEWIS (1960), and showed a greater amount of simple polyphenols in fresh beech leaves from a mor site than those from a mull site. They found the greatest amount of simple polyphenols in fresh leaves, followed in order by litter and humus.

Summary

The subject of this paper is a comparison between seven different leaf and needle litters with respect to the leaching and decomposition of their water-soluble substances. The experiments led to the following results and conclusions:

1. The total amount of water-soluble organic and inorganic substances leached from ground litter during one day is determined, and expressed as a percentage of the dry weight of the litter. The values obtained after the first day of leaching are: ash 25 %, birch 16 %, oak 14 %, spruce 14 %, alder 13 %, pine 11 % and beech 8 %.

2. The water-soluble substances are easily leached from leaf litter, especially that of alder, ash and birch, and slowly from unground needle

litter of pine and spruce. Leaf litters of beech and oak are in an intermediate position in this respect.

3. The influence of temperature on the leaching of water-soluble substances is greatest in oak and spruce litter. Notably large amounts are leached from needle litter of pine and spruce at high temperatures.

4. The amount of water-soluble substances is greater when leaching takes place under anaerobic conditions than under aerobic. This is due to greater decomposition of water-soluble organic substances under aerobic conditions.

5. During the first week of decomposition, the amount of water-soluble substances in leaf litter decreases considerably. The water-soluble organic substances are easily decomposed inside the litter as well. Under natural conditions—when leaching and decomposition proceed simultaneously—these substances are decomposed to a greater extent inside needle litter than inside leaf litter, owing to the slower leaching from the former.

6. In the fresh litter extracts, about nine amino acids are found, glutamic acid being present in the greatest amount. Under aerobic conditions, the amino acids disappear almost completely in four days. Anaerobic decomposition for the same period does not noticeably influence the amino acids in litter extracts, those of ash litter excepted.

7. Glucose and fructose are found in all types of litter extract, xylose in five of seven analyzed, and sucrose in two. Oak and birch litter proves to contain the greatest amount of sugar (about 2 % of the dry weight of the litter). During decomposition of the litter extracts, the amount of sugar decreases and—with the exception of ash litter—this decrease is greater under aerobic conditions than under anaerobic.

8. The most important non-volatile aliphatic acids in the fresh litter extracts are malic and citric acid. Compared with the other litters investigated, ash litter contains appreciable amounts of aliphatic acids; especially malic acid (1.7 % of the dry weight of the litter). Needle litter of pine and spruce has a higher content of malic and citric acid (0.3—0.4 % of the dry weight of the litter) than leaf litter of beech, oak and alder, but these acids are not so easily leached from needle litter as from leaf litter. Under aerobic conditions, the aliphatic acids are rapidly decomposed by microorganisms, and have disappeared almost completely after a few days. Malic acid is also rapidly decomposed under anaerobic conditions. Citric acid is more resistant, and in most extracts no decrease is noted after four days. Comparatively great amounts of lactic and succinic acid are formed under anaerobic conditions.

9. The pH rises under aerobic conditions, but falls under anaerobic.

The influence of the aliphatic acids on the change in pH during decomposition of the litters is discussed.

10. Sugars and aliphatic acids constitute only a part of the water-soluble organic substances decomposed during four days.

11. The leaching of water-soluble substances from fresh litters is not of microbial origin, in contrast to the decomposition of these substances. Thus, when sterile litter extracts are stored under aerobic conditions for four days, there is no decrease in their content of water-soluble substances, sugars, organic acids or amino acids. Nor does any change occur in the pH under these circumstances.

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References

ALEKSANDROVA, I. V., 1960: (A method of studying the qualitative composition of organic matter in soil solutions.) Pochvovedeniye 1960, No. 11, 85—87. (Soviet Soil Science 1960, 1218—1221.)

ALVSAKER, E. & MICHELSEN, K., 1957: Carbohydrates in a cold water extract of a pine forest soil. Acta chem. Scand. *11*, 1794—1795.

ARRHENIUS, O., 1957: Plant-food from decayed leaves and conifer needles. Acta agric. Scand. *7:* 4, 321—340.

BLOCK, R. J., DURRUM, E. L. & ZWEIG, G., 1958: A manual of paper chromatography and paper electrophoresis. Acad. Press, New York. 710 pp.

COULSON, C. B., DAVIES, R. I. & KHAN, E. J. A., 1959: Chemical studies on upland peat in North Wales. J. Sci. Food Agric., 1959, No. 4, 209—217.

COULSON, C. B., DAVIES, R. I. & LEWIS, D. A., 1960: Polyphenols in plant, humus and soil. I. Polyphenols of leaves, litter, and superficial humus from mull and mor sites. J. Soil Sci. *11*, 20—29.

DADD, C. C., FOWDEN, L. & PEARSALL, W. H., 1953: An investigation of the free amino-acids in organic soil types using paper partition chromatography. J. Soil Sci. *4*, 69—71.

GOTTSCHALK, A. & PARTRIDGE, S. M., 1950: Interaction between simple sugars and amino-acids. Nature *165*, 684—685.

GREENWOOD, D. J. & LEES, H., 1956: Studies on the decomposition of amino acids in soils: I. Plant and Soil *7*, 253—262.

GUPTA, U. C., 1962: The carbohydrate fraction of soil organic matter. Soils and Fertilizers *25*, 255—257.

HANDLEY, W. R. C., 1954: Mull and mor formation in relation to forest soils. Forestry Commission Bull. No. 23. 115 pp.

HESSELMAN, H., 1926: Studier över barrskogens humustäcke, dess egenskaper och beroende av skogsvården. Medd. Skogsförsöksanst. Stockh. *22:* 5, 169—378.

IVARSON, K. C. & SOWDEN, F. J., 1962: Methods for the analysis of carbohydrate material in soil: I. Colorimetric determination of uronic acids, hexoses, and pentoses. Soil Sci. *94*, 245—250.

KAURICHEV, I. S. & NOZDRUNOVA, YE. M., 1961: (The role of components of water-soluble organic plant residue in forming available (mobile) iron-organic compounds.) Pochvovedeniye 1961, No. 10, 10—19. (Soviet Soil Science 1961, 1057—1064.)

KNAPP, R. & LINSKENS, H. F., 1954: Über Aminosäuren aus der Blattstreu einiger Pflanzenarten von Wäldern auf verschiedenen Böden. Naturwissenschaften *41*, 480—481.

KONONOVA, M. M., 1961: Soil organic matter, its nature, its role in soil formation and in soil fertility. Translated from the Russian by T. Z. Nowakowski and G. A. Greenwood.—Pergamon Press, Oxford. 450 pp.

KONONOVA, M. M., & D'YAKONOVA, K. V., 1960: (Soil organic matter and aspects of plant nutrition.) Pochvovedeniye 1960, No. 3, 1—11. (Soviet Soil Science 1960, 229—236.)

LEA, C. H. & HANNAN, R. S., 1950: Biochemical and nutritional significance of the reaction between proteins and reducing sugars. Nature *165*, 438—439.

LINSKENS, H. F., 1959: Papierchromatographie in der Botanik. Springer Verlag, Berlin. 392 pp.

LOSSAINT, P., 1959: Étude expérimentale de la mobilisation du fer des sols sous l'influence des litières forestières. Thès. Fac. Sci. Univ. Strasbourg. Ser. E. N° d'ordre *179*, 1—107.

LUTWICK, L. E. & DELONG, W. A., 1954: Leachates from decomposing leaves. II. Interaction with soil-forming materials. Canad. J. Agr. Sci. *34*, 203—213.

MATTSON, S. & KOUTLER-ANDERSSON, E., 1941: The acid-base condition in vegetation, litter and humus: I. Acids, acidoids and bases in relation to decomposition. Lantbr-Högsk. Ann. *9*, 1—26.

— 1954: The acid-base condition in vegetation, litter and humus: XI. Acid and base in decomposing litter. Ibid. *21*, 389—400.

MIKOLA, P., 1954: Kokeellisia tutkimuksia metsäkarikkeiden hajaatumisnopeudesta (Engl. summary: Experiments on the rate of decomposition of forest litter). Commun. Inst. for. Finl. *43:* 1, 1—50.

— 1956: Studies on the decomposition of forest litter by Basidiomycetes. Commun. Inst. for. Finl. *48:* 2, 1—22.

NAGAR, B. R., 1962: Free monosaccharides in soil organic matter. Nature *194*, 896—897.

NYKVIST, N., 1959 a: Leaching and decomposition of litter. I. Experiments on leaf litter of *Fraxinus excelsior*. Oikos *10*, 190—211.

— 1959 b: Leaching and decomposition of litter. II. Experiments on needle litter of *Pinus silvestris*. Ibid. *10*, 212—224.

— 1961 a: Leaching and decomposition of litter. III. Experiments on leaf litter of *Betula verrucosa*. Ibid. *12*, 249—263.

— 1961 b: Leaching and decomposition of litter. IV. Experiments on needle litter of *Picea abies*. Ibid. *12*, 264—279.

— 1962: Leaching and decomposition of litter. V. Experiments on leaf litter of *Alnus glutinosa*, *Fagus silvatica* and *Quercus robur*. Ibid. *13*, 232—248.

SCHEFFER, F. & ULRICH, B., 1960: Humus und Humusdüngung. Band I. Morphologie, Biologie, Chemie und Dynamik des Humus. Ferdinand Enke Verlag, Stuttgart. 238 pp.

SCHEFFER, F., ULRICH, B. & HIESTERMANN, P., 1957: Die Bedeutung der Chelatisierung in der Agriculturchemie und Bodenkunde. Z. PflErnähr. Düng. *76*, 146.

SIMONART, P. & PEETERS, F., 1954: Acides amines libres dans l'humus. Trans. 5th Int. Congr. Soil Sci. 3, 132—135.

SOWDEN, F. J. & IVARSON, K. C., 1962: Methods for the analysis of carbohydrate material in soil: 2. Use of cellulose column and paper chromatography for determination of the constituent sugars. Soil Sci. *94*, 340—344.

TOMBESI, L., 1953: The paper chromatography and the percolation method of Lees applied to the study of the transformations of the amino acids in the soil. Ann. sper. agrar. (Roma) *7*, 1219—1239.

VIRO, P. J., 1955: Investigations on forest litter. Commun. Inst. for. Finl. *45:* 6, 1—65.

WAKSMAN, S. A., 1932: Principles of soil microbiology. The Williams & Wilkins Company, Baltimore. 829 pp.

WALDI, D., 1955: Chromatography with particular consideration of paper chromatography. E. Merck AG, Darmstadt. 185 pp.

Sammanfattning

Urlakning och nedbrytning av vattenlöslig organisk substans från olika löv- och barrförnor.

Föreliggande undersökning är en jämförelse mellan sju olika löv- och barrförnor med avseende på urlakning och nedbrytning av vattenlöslig substans. Undersökningen har gett följande resultat:

1. Den totala halten vattenlöslig organisk och oorganisk substans har bestämts i procent av förnans torrvikt. Följande värden erhölls efter ett dygns anaerob urlakning av malen förna vid 25°C: ask 25 %, björk 16 %, ek 14 %, klibbal 13 %, tall 11 % och bok 8 %.

2. Den vattenlösliga substansen urlakas långsamt från omalen barrförna av gran och tall men relativt hastigt från lövförna, särskilt förna av al, ask och björk.

3. Temperaturens inverkan på urlakningen av vattenlöslig substans är störst för ek- och granförna. Vid hög temperatur urlakas anmärkningsvärt stor mängd vattenlöslig substans från omalen barrförna av gran och tall.

4. Mängden erhållen vattenlöslig substans är större, när urlakningen försiggår under anaeroba förhållanden än under aeroba. Detta beror på en större nedbrytning av vattenlöslig organisk substans under aeroba förhållanden.

5. Vid förnans nedbrytning minskar mängden urlakad substans på grund av den vattenlösliga organiska substansens mikrobiella nedbrytning inne i förnan. Under naturliga förhållanden, då urlakning och nedbrytning försiggår samtidigt, nedbryts dessa substanser i större utsträckning inne i barrförnan än inne i lövförnan, beroende på den långsammare urlakningen från barrförna.

6. Färskt förnaextrakt innehåller följande aminosyror: glutaminsyra, leucin, valin, α-alanin, serin, treonin, glycin, asparaginsyra, lysin samt i förnaextrakt av al, ek, gran och tall även arginin. Under aeroba förhållanden försvinner de fria aminosyrorna nästan fullständigt under fyra dygn. Anaerob nedbrytning under samma tid påverkar inte märkbart aminosyrorna i förnaextrakt av al, ek, bok, gran och tall, men en minskning av de fria aminosyrorna har konstaterats i förnaextrakt av ask och björk efter fyra dygns anaerob nedbrytning.

7. Glykos och fruktos förekommer i samtliga sju undersökta förnaextrakt, xylos i fem och sackaros i två. Ek- och björkförna innehåller den största mängden socker (cirka 2 % av förnans torrvikt). Vid förnaextraktens nedbrytning minskar sockerhalten på grund av en mikrobiell nedbrytning. Denna nedbrytning är i de flesta förnaextrakt större under aeroba förhållanden än under anaeroba.

8. Äppelsyra och citronsyra är de viktigaste icke flyktiga alifatiska syrorna i färskt förnaextrakt. Jämfört med de andra undersökta förnorna in-

nehåller askförna en stor mängd alifatiska syror, främst äppelsyra (1,7 % av förnans torrvikt). Barrförna av gran och tall innehåller mer äppelsyra och citronsyra (0,3—0,4 % av förnans torrvikt) än lövförna av bok, ek och al, men dessa syror urlakas inte så lätt från barrförna som från lövförna. Under aeroba förhållanden nedbryts de alifatiska syrorna snabbt av mikroorganismer och har efter några få dygn nästan fullständigt försvunnit. Äppelsyra nedbryts snabbt även under anaeroba förhållanden. Citronsyra nedbryts däremot med större svårighet, och i de flesta extrakten kunde någon minskning ej konstateras efter fyra dygns anaerob nedbrytning. Förhållandevis stora mängder mjölksyra och bärnstenssyra bildas under anaeroba förhållanden.

9. Förnaextraktens pH ökar under aeroba förhållanden men minskar under anaeroba beroende på bildningen av mjölksyra och bärnstenssyra. Ökningen av pH under aeroba förhållanden sammanhänger med den samtidiga mikrobiella nedbrytningen av alifatiska syror, huvudsakligen äppelsyra och citronsyra.

10. Socker och alifatiska syror utgör endast en del av de vattenlösliga organiska substanser, som nedbryts under fyra dygn.

11. Urlakningen av vattenlösliga substanser från färsk förna är ej av mikrobiell natur till skillnad från nedbrytningen av dessa substanser. Sålunda minskar inte mängden vattenlösliga substanser, sockerarter, alifatiska syror eller aminosyror, när sterilt förnaextrakt förvaras fyra dygn under aeroba förhållanden. Ej heller sker någon förändring av förnaextraktets pH.