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Influence of Liming on Microbial Degradation of Pine Needles. Differential Scanning Calorimetrical Investigations.

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Abstract

Combustion heat of needle litter decomposed by bacteria and fungi was measured by means of differential scanning calorimetry (DSC) in the context of a pine forest program. Correlating the evolved heat with weight loss and chemical determined cellulose concentration, energy contents of remaining and metabolized material could be calculated from DSC thermograms. Physical effects of liming (peak shift and enlarging of peak areas) and biological effects (reduced degradation of cellulose, accumulation of a lignin polymerization product) as seen in DSC thermograms are reported. The necessity of a combined DSC/DTG analysis was established.

1. INTRODUCTION

Forest decline, effects of air pollutants and acid rain, and possible actions to redevelop or ameliorate especially pine forests (*Pinus sylvestris*) in Berlin are investigated by the project "Forest Ecosystems Close to Conurbation". Microbiology participates in this program by investigating microbial activities in and degradation of acid and of especially limed needle litter and soil. Calorimetric experiments allow a fast insight into the state of the biological system. While calorimetry determines the actual metabolic heat flux of bacteria and fungi, differential scanning calorimetry (DSC) measures the heat of combustion of material not degradated by these microorganisms.

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2. MATERIAL AND METHODS

Fallen needle litter of Scots pine (*Pinus sylvestris*) was collected in forest in Berlin and exposed to decomposition from 0 up to 210 days under defined laboratory conditions (24 ± 1°C, 70% water content, for further details see [1]).

One part of the litter was treated with lime to raise the pH to 6.0 ("limed series") and a control part remained untreated (pH 3.6, "control series"). Alterations in the composition of pine litter during desintegration by populations of bacteria and fungi were determined by chemical analyses of the main components of the needles: holocellulose and lignin (for applied methods see [2,3]) and by differential scanning calorimetry (DSC, Dupont, type 910, series 99).

For DSC analysis small samples (3 mg) of dried and milled litter were heated up at a constant rate of 10°C per minute from 100°C to 600°C. Thus, the main components of litter burnt at their typical combustion temperatures. The amount of heat liberated by each detected component was determined as peak area in the thermograms. These characteristic peaks can be assigned to holocellulose or lignin and to their degradation products [4] . Under the simplified assumption that (i) the measured calorimetric curves are the sums of individual holocellulose and lignin contributions and that (ii) these curves follow a Gaussian distribution (compare with [5]) these areas were determined by an own iteration program for an Atari PC and expressed as percentages of the total amount of heat released (total area under the respective curve). Each DSC thermogram was three times subjected to the iteration program and the best fit was used to calculate the mean value of the three experiments.

3. RESULTS AND DISCUSSION

Control series

The main components of pine needles: holocellulose and lignin and their degradation products burnt at typical temperatures. The first peak in the calorimetric thermograms - assigned to holocellulose [2] - has a maximum at about 350°C (Figure 1). The second peak corresponds to lignin. Depending on the state of degradation, its maximum shifted from 460°C to 410°C after a decomposition period of 210 days. Two additional peaks appeared after 42 and 70 days of needle degradation, the first one (the third one in the thermogram) shifted from 480°C to 450°C, while the second one (the fourth one in the thermogram) assigned to a product of lignin polymerization stayed nearly constant at about 520°C.

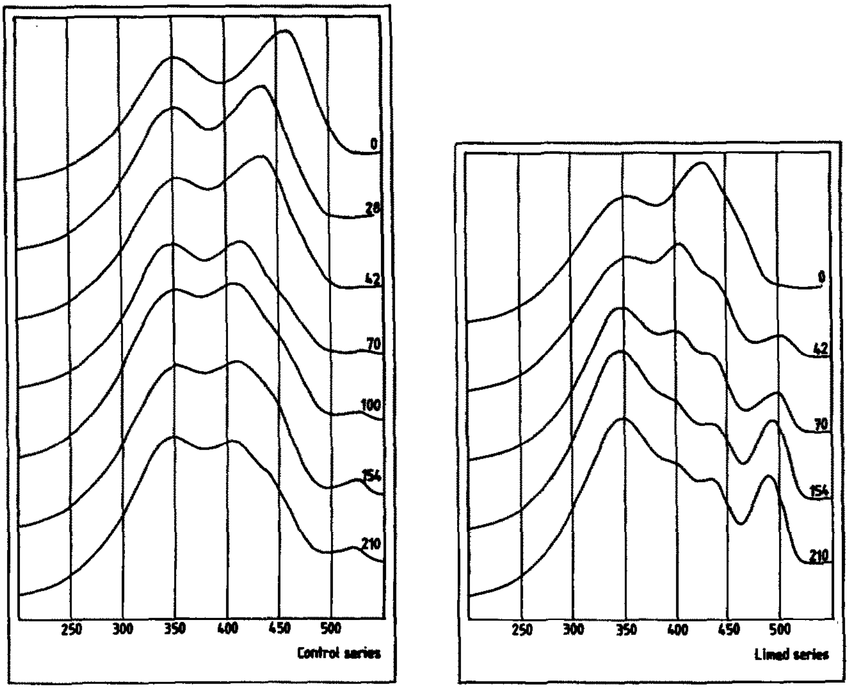
Limed series

Liming influences not only the composition of the microbial populations in the litter due to raising the pH value but also changes the shape of thermograms by physical actions (Figure 2).

Compared to the control series the peak maxima shifted to lower temperatures by 30°C. Moreover, the additional peaks appeared earlier, the third already at the beginning of the experiment (t = 0), not just at day 42 of decomposition (t = 42), the fourth peak - much more prominent than in control series - appeared at t = 42 instead of t = 70.

Total heat

It is well known, that the total heat observed in DSC experiments is not identically with that found in bomb calorimetry. Thus, only 50% of the expected 21 to 22 kJ/g organic dry matter could me measured. Nevertheless, the total heat as function of the degradation state of needle litter can be examinated. In control as well as in limed series the total area under the curves, corresponding to the released quantity of heat per gram dry matter, increased. But taking into account the weight loss during degradation the real amount of heat released decreased with time of degradation (Figure 3). The heat of combustion of the microbially *converted* material can be estimated from that decrease and from the weight loss. 15 to 17 kJ/g organic dry matter were obtained with slightly decreasing tendency for control and limed series over the whole period of investigation.



Figures 1, 2. Thermograms (heat flux versus temperature) of untreated (control series) and limed (limed series) pine needle litter (*Pinus sylvestris*) exposed to decomposition for 0 to 210 days. Time of degradation indicated at the graphs.

Heat of individual substances

The experimental thermograms in Figure 1 and 2 are composed of overlapping individual curves which can be separated from the original trace. The areas under these individual curves correspond to the evolved heat of combustion of the specific substance. The area of the first peak increased during decomposition of litter in control series as well as in the limed ones (Figures 1,2). But taking into account the weight loss, as mentioned above for the total heat, the liberated heat of holocellulose decreased with the time of needle decomposition. Two possible reasons might exist for this observation: (i) a real decrease of heat of combustion or (ii) a decrease of the amount of cellulose per gram organic matter.

Using the chemically determined cellulose concentration as well as the weight loss for calculating the evolved heat of combustion, values varying from 13.0 to 16.0 kJ/g dry cellulose can be estimated for control series during the whole time of investigation (Figure 4). Fiechter obtained a combustion heat of of 16.8 kJ/g organic dry matter, near to the figure found in these experiments [6].

Combustion heat of cellulose in limed series increased from 17.0 to 23 kJ/g organic dry matter with progressing needle degradation. A possible reason for this increase could be the preferred microbial degradation of hemicellulose and amorphous cellulose, which are both easily usable for microorganisms. Under such conditions the energy-rich crystalline cellulose accumulates in the decomposing litter. Contrary to this, cellulose degradation should be well-balanced in control series, because of a well-balanced undisturbed microbial population.

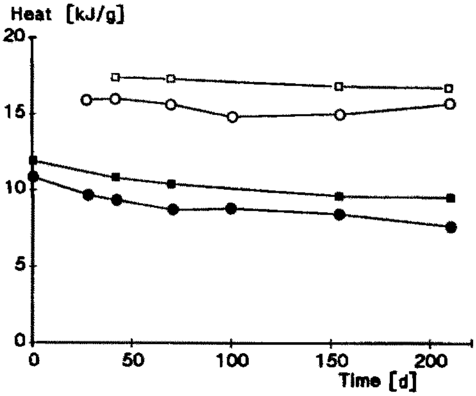


Figure 3. Evolved total heat in kJ/g organic dry matter of untreated and limed needle litter as function of degradation time in days.

—○— Control litter, microbially converted
—□— Limed litter, microbially converted
—●— Remaining control litter
—■— Remaining limed litter

In the course of degradation the area under the second peak (lignin) decreased, whereas the third and fourth peak areas increased even if weight losses are included. The reduction of the lignin peak was stronger in control series than in limed series. An exact calculation of the combustion heat of chemically determined Klason lignin was impossible in contrast to that of cellulose as this heat could not be clearly assigned to the differentiated lignin peaks (the second and the fourth peak). Only comparable thermograms obtained by thermogravimetry would permit exact calculations. Further investigations in this direction are in progress.

The third peak in thermograms of the limed series which is already detectable at at the begin of the experiment (t = 0) can be assigned to physical effects of liming. At the end of this investigation (t = 210) the fourth peak was most prominent in limed series. It was supposed that a polymerization product of lignin was accumulated due to the raised pH value. The enzyme laccase, responsible for this polymerization, was found in former investigations [7].

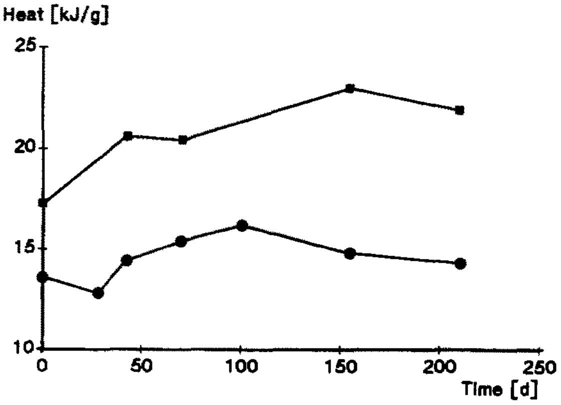


Figure 4 Evolved heat in kJ/g organic dry cellulose from untreated (control) and limed needle litter as a function of time in days.
–●– Cellulose from control litter
–■– Cellulose from limed litter

4. CONCLUSIONS

The results presented above show that for quantitative analyses of DSC thermograms DTG measurements have to be performed in parallel to distinguish between the effects of heat and/or mass alterations. Besides a strong increase of the pH value liming causes a clear inhibition of microbial decomposition of needle litter. Cellulose was less intensively degraded and lignin less modified. At the same time a hardly degradable polymerization product was accumulated. We conclude from such observations that liming is no useful means to redevelop forests of Scots pine.

5. ACKNOWLEDGMENT

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