Relationship between Extracellular Enzyme Activity and Carbon and Nitrogen Availability in Soil

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Introduction

Most organic material entering the soil food web is comprised of relatively large polymers with limited solubility, such as cellulose, lignin, protein, or chitin. These molecules cannot be taken up directly by microorganisms.

Microorganisms first have to produce extracellular enzymes which break these molecules down into smaller, water soluble units.

Extracellular enzyme activity is therefore often seen as the rate limiting step of decomposition.

When the production of extracellular enzymes is induced by the substrate or repressed by a high level of products, enzyme activity may be well related to decomposition rate.

However, when enzymes are produced in response to a limited availability of their products (de-repression mechanism), an increase in enzyme activity may not result in an increase in decomposition.

The goal of the present study was to investigate the relationship between extracellular enzyme activity and C and N mineralization over time in soil samples to which C and N compounds with different availability and C to N ratios were added.

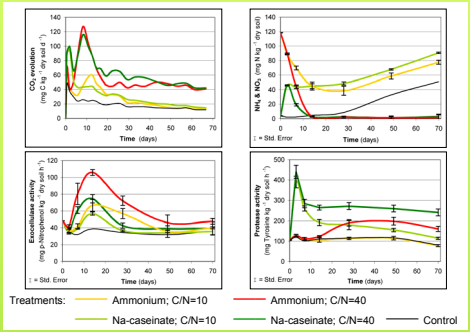
Material and Methods

For the main incubation, fresh soil samples were sieved and mixed with 0.1 mg N/g dry soil from ammonium or Na-caseinate. Cellulose was added to all the samples at two different rates such that the amendment C to N ratios were 10 and 40.

The samples were incubated at room temperature at a moisture content of 50% water holding capacity.

Samples were destructively analyzed for protease and exocellulase activity, as well as for ammonium and nitrate six times over a 70-day period. In addition CO_2 evolution was measured regularly.

Results



Exocellulase activity increased after three days of incubation and reached a maximum after 14 days regardless of N source and C to N ratio.

Exocellulase activity and CO_2 evolution were well correlated. However, after reaching a maximum, CO_2 production decreased faster than exocellulase activity, probably due to protection of the enzyme by organic and mineral soil components.

A peak in CO_2 evolution during the first seven days of incubation in the Na-caseinate amended treatments was likely due to the degradation of the protein, as it correlated very well with the activity of protease.

During the first seven days of incubation, protease activity increased in the Na-caseinate treatment, but not in the ammonium amended treatment.

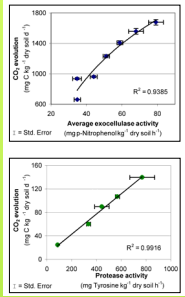
After 14 days of incubation, protease activity remained relatively stable. The activity was positively correlated with the C to N ratio and the amounts of carbon added.

The mineral N content in the ammonium amended soil samples decreased sharply during the first two weeks of incubation.

Despite the strong N immobilization potential of the soil, the mineral N content increased in the Na-caseinate amended samples during the first three days. When C from cellulose became available, the mineral N in the samples with a C to N ratio of 40 was almost completely immobilized.

Based on these results we formulated several hypotheses about the relationship between extracellular enzyme activity and C and N availability which we tested with additional experiments.

Hypothesis 1: Extracellular enzyme activity determines the rate of substrate degradation



Relationship between cumulative CO_2 evolution and extracellular enzymes.

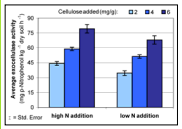
Top: Average exocellulase activity during a one-month incubation.

Bottom: Protease activity, measured two days after the addition of Na-caseinate.

- CO_2 evolution was used as a measure of cellulose and casein degradation. Both, exocellulase and protease activity were very well correlated with CO_2 evolution. The relationship was linear for protease activity and log-transformed exocellulase activity.

⇨Exocellulase and protease activity were useful key enzymes to assess cellulose and protein degradation, respectively, during the substrate induced phase of decomposition.

Hypothesis 2: Exocellulase activity is independent of nitrogen availability



Relationship between the average exocellulase activity and N availability in cellulose-amended soil samples during a one-month incubation. In addition, NH_4Cl was added to obtain C to N ratios of 10 (high N addition) and 40 (low N addition).

- A four-fold increase in ammonium addition resulted in a 18% increase in exocellulase activity.

⇨Exocellulase activity was not independent of N availability. An increase in N availability had a positive effect on exocellulase activity.

However, N availability did not affect the relationship between respiration rate and exocellulase activity. Therefore, the addition of ammonium increased the decomposition rate of the cellulose.

Acknowledgement

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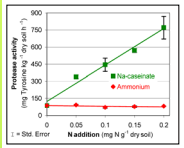
Hypothesis 3: During the initial days of the experiment, protease activity was substrate induced



Protease activity two days after the addition of different amounts of Na-caseinate and ammonium.

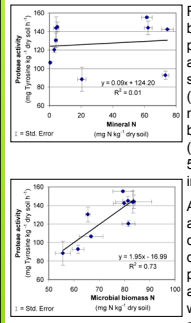
- Protease activity increased linearly with increasing Na-caseinate additions.

⇨Protease synthesis was induced by the presence of a substrate (Na-caseinate).

- The addition of ammonium had no effect on protease activity.

⇨Ammonium is a directly available N source, the microbes did not have to produce protease to gain access to the N.

Hypothesis 4: After the first month of the experiment, high mineral N availability repressed protease activity



Relationship between protease activity and mineral N in soil solution (top) and microbial biomass N (bottom) after 59 days of incubation.

A constant amount of cellulose and different rates of protein-N or ammonium-N were added to soil samples to reach C to N ratios of 10 and 40.

- The concentration of mineral N (ammonium and nitrate) had no direct effect on protease activity.

⇨A high mineral N availability did not repress protease activity.

- However, protease activity was very well correlated with microbial biomass, which in turn was related to the amount of C added.

⇨The correlation between protease activity and microbial biomass after 59 days of incubation may be due to constitutive synthesis of protease or related to the amount of proteins released by dead microorganisms. In the latter case protease activity would be related to microbial turnover.

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