

## Stability and composition of soil organic matter control respiration and soil enzyme activities

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### Abstract

Relationships between soil organic matter (SOM) molecular composition, thermal stability and decomposability by soil enzymes and microbes are largely unknown. We incubated soils from unfertilized and NPK-fertilized neighboring field plots of a long-term rye (*Secale cereale*) monoculture experiment and investigated relationships between changes in the molecular-chemical composition of SOM, the CO<sub>2</sub> flux and the activities of enzymes. Pyrolysis-field ionization mass spectrometry (Py-FIMS) showed larger ion intensities in the NPK-fertilized than in the unfertilized soil at start of the incubation, only small changes in composition and thermal stability in the unfertilized soil, and a preferential reduction in thermally stable components as well as general shifts towards lower pyrolysis temperature after three weeks of incubation in the NPK-treatment. We found evidence that thermally labile and stable proportions of various compound classes were differently susceptible to decomposition, depending on the fertilization history of the soil. Irrespective of fertilization treatment, peaks in xylanase activity after 7 days of incubation followed by decreasing values were reflected by the ratio of xylan ( $m/z$  114) to xylose ( $m/z$  132) marker signals in the Py-FI mass spectra. Thus, the study proved that (1) SOM composition was changed due to long-term rye cropping without and with NPK-fertilization, (2) the modified SOM composition affected the decomposability and microbial parameters under optimized conditions and (3) the thermal properties of individual compound classes derived from Py-FI mass spectra can be sensitive predictors of microbial decomposition.

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### 1. Introduction

Total content and the chemical composition of soil organic matter (SOM) depend on the input from vegetation residues, anthropogenic inputs such as compost, manure (Leinweber and Reuter, 1992; Schulten and Leinweber, 1991), sewage (Leinweber et al., 1996) and air-borne dusts (Rumpel, 1999; Schmidt et al., 1996), and the soil management such as agricultural tillage, grazing and forest cutting (Magdoff and Weil, 2004). Whereas these inputs tend to increase SOM contents, tillage and aeration favor organic matter decomposition, and thus tend to decrease SOM contents. However, stabilization reactions prevent parts of the SOM from microbial attack and maintain SOM levels

in soil. Since the SOM is linked directly to the atmospheric CO<sub>2</sub> and, thus, to the climate change (Schlesinger and Andrews, 2000; Lal, 2004), great research efforts are currently undertaken to elucidate mechanisms of SOM stabilization (e.g. Kögel-Knabner et al., 2008; Lützwow et al., 2008; Marschner et al., 2008).

The stability of organic matter in soils is defined as resistance to microbial decomposition and can be studied by aerobic incubation experiments. This approach was widely applied to study the decomposability of organic matter from primary sources (Klimanek, 1990; Jensen et al., 2005), SOM from whole soil samples and physical soil fractions (Christensen, 1987; Gregorich et al., 1989; Leinweber, 1995; Schulten and Leinweber, 1999), and dissolved organic matter which enters soils (Kalbitz et al., 2003). The decomposability of crop residues in soil depends on the chemical composition such as lignin contents or

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lignin to plant nitrogen (N) ratio (Klimanek, 1990; Vanlauwe et al., 1996). Holocellulose carbon (C) and neutral detergent soluble plant N were found to be better predictors of C and N mineralization than lignin-related parameters (Jensen et al., 2005). Nevertheless, up to now the relationships between the chemical composition of SOM, its decomposability and the component fluxes released are not fully understood (Ryan and Law, 2005). Pyrolysis-field ionization mass spectrometry (Py-FIMS) provided evidence for relationships between the chemical composition and stability of dissolved organic matter and its decomposition in an incubation experiment (Kalbitz et al., 2003). Long-term monoculture cropping and the fertilization modified the chemical composition of SOM (Schmidt et al., 2000), but the consequences for stability and turnover are unknown.

Since microbial decomposition of SOM is mediated by soil enzymes, detailed studies of these enzymes are central in understanding the decomposition of organic matter (Sinsabaugh et al., 1994; Paul, 2007). For example, crop rotations, fertilization and tillage influenced the activity of various enzymes involved in the SOM turnover (Kandeler et al., 1999a,b,c; Vepsäläinen et al., 2004). A few studies tried to link the activity of enzymes involved in C cycling with the chemical composition of the vegetation litter or soils under long-term monoculture (Magid et al., 1997; Luxhøi et al., 2002). In an early state of decomposition,  $\beta$ -glucosidase activity was a very good predictor of release of low molecular weight organic compounds from the litter into the adjacent soil (Poll et al., 2006). In the later phase of decomposition, chemical and physical properties of litter from *Miscanthus* controlled its stability. Whereas brown material retained its rigid structure at sub-cellular level over long time, green material was quickly invaded and transformed into an amorphous tissue architecture by microorganisms (Luxhøi et al., 2002).

Although causal relationships between the chemical composition of litter and SOM as important substrates for microbial growth on the one hand and activities of enzymes involved in the turnover of these substrates on the other appear highly probable and plausible, in-depth understanding of these relationships is still lacking. Methodological reasons may be the insensitivity of analytical methods for probing the chemical composition of SOM, the indirect approaches to enzyme activity determinations and interference of decomposition reactions with neoformation of metabolites in one sample.

Therefore, the objectives of the present study were:

- (1) to investigate how long-term monoculture of rye under zero and NPK-fertilization altered the chemical composition of SOM in a Haplic Phaeozem developed in a sandy loess;
- (2) to study the decomposability of whole SOM and SOM fractions, differing in chemical composition and thermal stability, during short-term aerobic incubation under optimal conditions; and

- (3) to ascertain if and how the molecular-chemical composition of SOM and the activities of soil enzymes involved in the C-, N- and P-cycling reflected the different decomposability of SOM in fertilized and unfertilized soils.

## 2. Materials and methods

### 2.1. Field experiment and soil samples

Field moist soil samples were taken in fall 2000 from plots of the 'Eternal Rye Cultivation' experiment at Halle, Saxony-Anhalt, Germany. This field experiment on a Haplic Phaeozem was established in 1878 (Merbach et al., 2000). The plots sampled were uniformly cropped with rye (*Secale cereale* L.). We sampled the Ap horizon (0–20 cm depth) of the treatments 'unfertilized' (U, without any fertilization since 1878) and mineral fertilization (NPK). Mineral N fertilization was increased during the experimental period from 40 kg N (ha yr)<sup>-1</sup> (1878–1990) to 60 kg N (ha yr)<sup>-1</sup> (since 1991). The P (24 kg (ha yr)<sup>-1</sup>) and K (75 kg (ha yr)<sup>-1</sup>) applications remained constant for the whole experimental period.

For a general chemical characterization sub-samples were air-dried and sieved <2 mm. The soils contained about 10% clay, 18% silt and 72% sand (sandy loam). The contents of organic C and total N were 9.3 g kg<sup>-1</sup> (U) and 11.5 g kg<sup>-1</sup> (NPK), and 0.7 g kg<sup>-1</sup> (U) and 0.9 g kg<sup>-1</sup> (NPK), respectively. The pH value was lower in the unfertilized (pH: 5.3) than in the fertilized soil (pH: 6.0). No lime was applied at these plots. For the aerobic incubation experiment and the subsequent analyses of incubated samples (see below), the soil material was stored frozen (–20 °C) and thawed in a fridge immediately prior to the experiment.

### 2.2. Aerobic incubation experiment

A soil weight of 200 g from each treatment, moistened to 60% of water holding capacity, was incubated at 25 °C under aerobic conditions. The incubation experiments started with 10 (treatment U) and 16 (treatment NPK) replicates for the soil samples collected from the rye plots to enable destructive sampling after certain incubation periods. Samples from the unfertilized soil received a P fertilization equivalent to the concentration of DL-P in the treatment NPK. This was done to prevent P limitation because the decomposition of organic C and N compounds was to be studied. Prior to the incubated samples ambient air was sucked through an entrance filter filled with 400 mL of 10% potassium hydroxide solution to remove CO<sub>2</sub>. The CO<sub>2</sub>-free gas purged the CO<sub>2</sub> formed during incubation and pumped subsequently to the analyzer. The delivery rate of the pump was 200 L h<sup>-1</sup>. The CO<sub>2</sub> was determined with the infrared CO<sub>2</sub> analyzer BERLINA (MSA-AUER, Berlin, Germany). In the treatment U each three replicates

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