



Little effects on soil organic matter chemistry of density fractions after seven years of forest soil warming



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ABSTRACT

Rising temperatures enhance microbial decomposition of soil organic matter (SOM) and thereby increase the soil CO₂ efflux. Elevated decomposition rates might differently affect distinct SOM pools, depending on their stability and accessibility. Soil fractions derived from density fractionation have been suggested to represent SOM pools with different turnover times and stability against microbial decomposition.

To investigate the effect of soil warming on functionally different soil organic matter pools, we here investigated the chemical and isotopic composition of bulk soil and three density fractions (free particulate organic matter, fPOM; occluded particulate organic matter, oPOM; and mineral associated organic matter, MaOM) of a C-rich soil from a long-term warming experiment in a spruce forest in the Austrian Alps. At the time of sampling, the soil in this experiment had been warmed during the snow-free period for seven consecutive years. During that time no thermal adaptation of the microbial community could be identified and CO₂ release from the soil continued to be elevated by the warming treatment. Our results, which included organic carbon content, total nitrogen content, $\delta^{13}\text{C}$, $\Delta^{14}\text{C}$, $\delta^{15}\text{N}$ and the chemical composition, identified by pyrolysis-GC/MS, showed no significant differences in bulk soil between warming treatment and control. Surprisingly, the differences in the three density fractions were mostly small and the direction of warming induced change was variable with fraction and soil depth. Warming led to reduced N content in topsoil oPOM and subsoil fPOM and to reduced relative abundance of N-bearing compounds in subsoil MaOM. Further, warming increased the $\delta^{13}\text{C}$ of MaOM at both sampling depths, reduced the relative abundance of carbohydrates while it increased the relative abundance of lignins in subsoil oPOM. As the size of the functionally different SOM pools did not significantly change, we assume that the few and small modifications in SOM chemistry result from an interplay of enhanced microbial decomposition of SOM and increased root litter input in the warmed plots. Overall, stable functional SOM pool sizes indicate that soil warming had similarly affected easily decomposable and stabilized SOM of this C-rich forest soil.

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1. Introduction

Mean annual temperatures in the alpine region are expected to increase by more than 4 °C by the end of this century (Gobiet et al., 2014). Higher temperatures lead to enhanced microbial activity and higher CO₂ release from soil, imposing the probability of warming induced reductions in soil C stocks (Cox et al., 2000; Lloyd and

Taylor, 1994). While increased CO₂ production can be detected in long-term soil warming experiments (Melillo et al., 2011; Reth et al., 2009; Schindlbacher et al., 2009), changes in the soil organic matter stocks and especially changes in soil organic matter chemistry, however, might not be readily visible. Changes in the chemical composition of SOM, e.g. a depletion of easily available carbon C-forms have been made responsible for a decline in the response of CO₂ production with warming (Kirschbaum, 2004; Melillo et al., 2002). Depletion of easily available C-forms and a higher microbial activity might also force the microbes to alter their substrate source utilization pattern towards more stable SOM pools

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(Frey et al., 2013). This might not only change the size of these pools but also the chemical composition. SOM chemistry of different pools might thus be a crucial component to understand and estimate the response of decomposition processes to warming. However, little is known about the effect of warming on different functional pools of SOM.

Soil fractions derived from density fractionation have been argued to represent different pools of SOM with varying turnover times, accessibility by microbes and thus stability (Crow et al., 2007; Schulze et al., 2009; von Lützwow et al., 2007). Plant litter enters the SOM pool as free particulate organic matter (fPOM). fPOM is characterized by fast turnover times and low stability. It usually consists of easily degradable plant components such as cellulose, starch and other carbohydrates (von Lützwow et al., 2007), with smaller contributions by lignin, cutin and other compounds that are less decomposable. Products of enzymatic decomposition of fPOM as well as soluble litter components can be taken up by microbes or can become part of the mineral associated organic matter (MaOM), along with the now microbially transformed compounds. Usually the largest percentage of SOM is mineral-associated; this fraction is considered to have the longest time of up to >1000 years, depending on the minerals present (Schulze et al., 2009). Mineral association also provides the greatest protection from microbial decomposition (Baisden et al., 2002; Poirier et al., 2005) and MaOM is sometimes even referred to as a passive pool (e.g. Gaudinski et al., 2000; Schulze et al., 2009). Both fPOM and MaOM can be incorporated in aggregates (Poirier et al., 2005). While in aggregates POM is only slowly transformed and it is older and more stable than fPOM (von Lützwow et al., 2007). Based on their properties with respect to stability and persistence, the size, turnover time, and chemical composition of these SOM pools, but also the transformations from one fraction to another might be affected by soil warming in different ways.

To study the effect of soil warming on the chemical composition of different soil organic matter fractions, we took advantage of a soil warming experiment in Achenkirch/Austria where soil had been warmed by 4 °C during the snow-free seasons from 2005 until the current study in 2012. In contrast to other long-term soil warming experiments, the positive response of soil respiration to warming (~40% increase) remained stable throughout the seven years of warming (Schindlbacher et al., 2012). The sustained enhancement of soil respiration suggests that substrate depletion did not play a major role in this C-rich soil so far. Additionally the microbial community in these soils did not show strong signs of physiological adaptation to the persisting higher temperatures (Schindlbacher et al., 2011, 2015; Kuffner et al., 2012).

Based on the suggested different properties of the three density fractions (fPOM, oPOM and MaOM), we hypothesized that soil warming led to (I) a reduction of plant derived compounds such as carbohydrates and lignin (Gleixner et al., 2002), especially in the fPOM fraction and (II) increased microbial transformation of the MaOM fraction indicated by lower C:N ratios, higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Six et al., 2001; Wallander et al., 2004), as well as increases in N-bearing compound contents (Gleixner et al., 2002).

To that end, we determined organic C (OC) and total N (TN) contents, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\Delta^{14}\text{C}$ values of the bulk soil and of the three individual density fractions, fPOM, oPOM and MaOM. In addition we analyzed the samples with pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) to determine the effect of warming on the macromolecular composition of the different SOM pools, including contents of lignin, carbohydrates and the contents of N-bearing compounds.

2. Material and methods

2.1. Site description and soil sampling

Soils for this study were sampled at a long-term soil warming experiment located near Achenkirch, Austria (47° 34' 50" N; 11° 38' 21" E). The experimental site is situated on a north-north-east slope of a mountain in the Northern Limestone Alps at 910 m a.s.l. The snow-free period in this area lasts from April/May to November/December. Local mean annual air temperature and precipitation were 6.9 °C and 1506 mm (1992–2012), respectively (Achenkirch village; ~7 km away at similar altitude; data from Zentralanstalt für Meteorologie und Geodynamik (ZAMG)). The ~130-years old forest is dominated by Norway spruce (*Picea abies*), with interspersed European beech (*Fagus sylvatica*) and silver fir (*Abies alba*). The soils are a mosaic of shallow Chromic Cambisols and Rendzic Leptosols. The bedrock is dolomite. Soils are characterized by high carbonate content and near neutral pH. Organic C stocks were estimated to be ~10 t ha⁻¹ in the organic layer and ~120 t ha⁻¹ in the mineral soil (Schindlbacher et al., 2009).

The warming experiment was set up in 2004 (Schindlbacher et al., 2009). Three pairs of control and warmed plots were established. Warmed plots are heated by resistance heating cables (0.4 cm diameter, TECUTE – 0.18 Ohm/m/UV, Etherma, Austria). The cables were buried in 3 cm deep slots and had a spacing of 7–8 cm. The soil temperature of each warmed subplot was kept 4 °C above that of the adjacent control subplot during the snow free-seasons, starting in spring 2005. At control plots (n = 3) cables were inserted but not heated.

Sampling took place in October 2012. Plots were sampled using a soil corer with a diameter of 5 cm. From each plot 10 cores were taken and bulked according to their sampling depth. Samples were air-dried at 60 °C before further analysis.

2.2. Density fractionation of soil

Sieved (<2 mm) and air-dried (60 °C) mineral soil samples from 0–10 and 10–20 cm of each plot were fractionated by density separation. For this purpose, the samples were dispersed in sodium polytungstate solution (SPT, Sometu, Berlin, Germany) at densities of 1.6 g cm⁻³ and 2.0 g cm⁻³ using a similar procedure as described by John et al. (2005). First, 10 g soil and 40 ml of SPT with a density of 1.6 g cm⁻³ were gently shaken. After sedimentation, the solution was centrifuged at 5085 g for 1 h (Varifuge 3.2RS). The supernatant was filtered through 0.45 µm pre-washed cellulose-acetate-filter (Schleicher & Schuell, Germany) and the fPOM fraction <1.6 g cm⁻³ was washed with 200 ml deionized water. Then the pellet was dispersed with 2.0 g cm⁻³ SPT and 10 glass beads and was shaken for 16 h at 60 rpm and centrifuged at 5085 g for 1 h. The supernatant with particles <2.0 g cm⁻³ (oPOM) was washed with 200 ml deionized water and filtered through 0.45 µm cellulose-acetate filters. The pellet contained the mineral associated organic matter fraction >2.0 g cm⁻³ (MaOM). To remove the SPT salt, the pellet was washed three times with deionized water. Thereafter, carbonate was removed from density fractions and bulk soil by stepwise addition of ultra-pure hydrochloric acid (HCl) until a constant pH value of 2.0 was reached during incubation at 60 °C over 2–3 weeks. Again, the soil samples were stepwise washed with deionized water to remove HCl down to an electric conductivity of 1 mS m⁻¹ in the supernatant. Removal of SPT salt by washing and treatment with HCl to remove carbonates caused a loss of dissolved organic carbon (DOC) in all density fractions, ranging between 2 and 8% of total bulk soil C. The fPOM, oPOM, MaOM fractions were freeze-dried and then finely ground with a ball mill for C, N, and isotope analyses.

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