Geoderma 156 (2010) 267–277

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Source determination of lipids in bulk soil and soil density fractions after four years of wheat cropping

G.L.B. Wiesenberg [a,*], M. Dorodnikov [b], Y. Kuzyakov [a]

[a] Department of Agroecosystem Research, BayCEER, University of Bayreuth, 95440 Bayreuth, Germany
[b] Institute for Botany and Landscape Ecology, Ernst Moritz Arndt University Greifswald, 17489 Greifswald, Germany

ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form 12 January 2010
Accepted 27 February 2010
Available online 30 March 2010

Keywords:
Fatty acids
Aliphatic hydrocarbons
Total lipids
Triticum aestivum
Soil density fractions
Soil organic matter sources

ABSTRACT

Preservation of soil organic matter (SOM) is strongly affected by occlusion within aggregates and by association of SOM with minerals. Protection of organic carbon (C) due to adsorption to mineral surfaces can be assessed by investigation of SOM in soil density fractions. Apart from the physical properties the preservation of SOM is affected by its chemical composition. While for bulk organic C this was demonstrated for numerous soils, SOM density fractions have been scarcely studied regarding their molecular composition. Lipids as a compound class that can derive from plants, microorganisms and contamination by products from incomplete combustion or fossil carbon were not investigated in density fractions so far. We hypothesized that molecular proxies deriving from lipid composition yield a large potential to elucidate the sources of organic matter entering soil, and in combination with density fractions they enable the identification of incorporation and preservation pathways of SOM.

We determined distribution patterns of aliphatic hydrocarbons and fatty acids as two representative groups for total lipids in soil density fractions. The fatty acids showed a predominant input of plant-derived poly unsaturated short chain and saturated long chain fatty acids in free particulate organic matter (fPOM). The microorganism-derived compounds such as unsaturated short chain fatty acids were largely abundant in fPOM and especially in occluded particulate organic matter (oPOM 1.6). The proportion of plant-derived components like long chain fatty acids increased with increasing density of the fractions, whereas the abundance of short chain fatty acids decreased in the same direction as indicated by the ratio of long chain vs. short chain fatty acids. The main portion of soil lipids (60% of total lipids) was recovered in the mineral (Min) fraction, which denotes the strongest protection of lipids adsorbed to mineral surfaces.

For the aliphatic hydrocarbons the contribution of plant- and microorganism-derived components was the largest in fPOM. Short chain alkanes as part of the aliphatic hydrocarbons showed contamination of soil by an incompletely burned plant biomass or fossil carbon. These contaminants were the most abundant in fPOM and subsequently attributed to particles with a low density, which derived probably from soot. However, a large contribution of fossil C was found in the Min fraction as well, which is thought to be attributed to degraded soot particles being adsorbed to minerals.

We demonstrated at the molecular level that the incorporation of individual C sources varies with the density fractions. It was found that microorganism-derived compounds were most abundant in fPOM and oPOM 1.6 fractions, whereas plant-derived long chain biopolymers were enriched in mineral dominated fractions (oPOM 2.0 and especially Min). Thus, preservation of plant-derived lipids in soil is strongly attributed to the association with minerals. Based on lipid composition in density fractions we evaluated several molecular proxies, which help to elucidate the sources of SOM.

1. Introduction

Physical fractionation techniques of soil organic matter (SOM) such as density fractionation have been applied to determine the association of SOM with primary particles and to quantify the amount of particulate organic matter (POM) between and within soil aggregates (Beare and Hendrix, 1994; Puget et al., 2000; John et al., 2005). Golchin et al. (1997) proposed a conceptual model linking POM decomposition and stabilization. They assumed that the protection of organic matter increases with the increasing density of SOM fractions, i.e. with the increasing association with mineral particles. Several recent studies supported this model showing several distinct trends within soil density fractions in the order free particulate organic matter (fPOM) > occluded particulate organic matter (oPOM) > mineral soil (Min) (e.g. John et al.,

* Corresponding author. Tel.: +49 921 552268; fax: +49 921 552315.
E-mail address: guido.wiesenberg@uni-bayreuth.de (G.L.B. Wiesenberg).

0016-7061/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.geoderma.2010.02.026

2005). In this direction contents of organic carbon (C) and nitrogen (N) as well as C:N ratios decrease within individual fractions, thus leaving minerals associated SOM rich in N. In the opposite direction the contribution of the individual fractions to bulk soil by weight strongly increases from fPOM towards Min. Hence, contribution to bulk SOM increases with increasing association to mineral particles. The mineral associated fractions have the largest potential for SOM stabilization, since they are (i) the largest fractions by weight and so, yield largest SOM portions, and (ii) contain the degraded chemically recalcitrant organic material bound to mineral surfaces (e.g. John et al., 2005).

While SOM fractions including density fractions have been characterized e.g. for their total and organic C and N contents, such investigations are limited for individual compound classes like soil lipids. Lipids are major organic components of fresh plant biomass and soils (e.g. Gregorich et al., 1996; Kögel-Knabner, 2002), which yield potential to be preserved in soils for decades (Wiesenberg et al., 2004a) until millennia (Huang et al., 1996). They play an important role for the incorporation and transformation of plant residues into soil and stabilization of soil organic matter (SOM). While the characterization of the lipid composition is available for particle-size separates of agricultural soils (Cayet and Lichtfouse, 2001; Quènèa et al., 2006; Wiesenberg et al., 2006) and forest soils (e.g. Marseille et al., 1999), this information is missing from aggregate and density fractions. Despite these studies describing the main storage of lipidic compounds associated with the silt and clay size fractions, it remains unclear, whether the lipids are predominantly bound to mineral surfaces or protected within soil aggregates. Furthermore, the documentation of lipid contribution to density fractions is currently not available. Especially due to the variety of sources for soil lipids including plant and microbial biomass (Harwood and Russell, 1984), or contamination by e.g. products from incomplete combustion processes (Wiesenberg et al., 2004a), lipids provide a potential to answer several questions related to the protection of the mineral associated SOM at the molecular level. We expected a predominant contribution of plant biomass-related lipids within the fPOM fraction and a large contribution of degraded plant- and microorganism-derived components to the Min fraction. The oPOM fractions are thought to show transformation stages of primary plant- and microorganism-derived lipids towards more resistant, i.e. long chain, biopolymers.

Soil lipids contain molecular proxies that are specific for source determination and taxonomic classification of plants and microorganisms (Gleixner et al., 2001). Plant-derived lipids are characterized by large proportions of long chain fatty acids, aliphatic hydrocarbons, and alcohols (e.g. van Bergen et al., 1998), whereas microorganism-derived lipids are characterized by large proportions of short chain fatty acids (Harwood and Russell, 1984). Previous soil lipid analyses on the ploughed horizons of agricultural soils have focused on the distribution patterns of individual lipid fractions like *n*-alkanes (Lichtfouse et al., 1994, 1997, 1998a; Wiesenberg et al., 2004a,b), fatty acids (Wiesenberg et al., 2004a,b), or bulk lipid extracts (e.g. van Bergen et al., 1998). Dynamics of soil lipids have been studied on field sites, where a monoculture practice results in a uniform biomass input over several years and a vegetation change resulted in a notable change in the biomass input into soil. These studies imply a change of plants with C_3-/C_4-photosynthesis for agricultural soils (Lichtfouse et al., 1994; Wiesenberg et al., 2004a,b) and for forest soil converted to C_4 vegetation (Quènèa et al., 2006). C_3- and C_4-grasses as well as forest trees are all characterized by a different qualitative contribution to SOM due to the differences in lipid biosynthesis and lipid composition of the individual functional plant groups (Bianchi and Bianchi, 1990; Maffei, 1996; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006). Hence, the modified plant biomass input resulted in soil lipid compositional changes that were detectable after a few years (Lichtfouse et al., 1994; Wiesenberg et al., 2004a,b). Alternative experiments for the investigation of soil lipid dynamics imply FACE (free air CO_2 enrichment) experiments, where plants have been kept under ambient and elevated atmospheric CO_2 concentrations (Wiesenberg et al., 2008a). In these experiments the added CO_2 carried a different isotopic ($\delta^{13}C$) label than natural air, which enabled turnover determination of plant-derived lipids in soils based on the compound-specific isotope values of plants and soils kept under natural and elevated CO_2 concentrations (Wiesenberg et al., 2008b). However, all these experiments implied a significant change in the quality of the incorporated biomass into soil (Bianchi and Bianchi, 1990; Maffei, 1996; Wiesenberg et al., 2004a; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006; Wiesenberg et al., 2008a) and subsequently a modification in the degradability of the incorporated biomass. Other experiments with a change from one to another C_3-grass, i.e. a change from grassland towards a wheat cropped soil, are expected to lack substantial changes in the quality of plant biomass input due to a similar lipid composition of the incorporated biomass (Maffei, 1996). However, the determination of molecular changes has not been tested for C_3-perennial grass/C_3-crop conversion experiments so far. We hypothesized that the molecular changes in soil after a vegetation change from a perennial C_3-grass towards wheat cropping are low due to their similar lipid composition (Maffei, 1996). Additionally, according to changes in bulk C we expected some changes in the lipid composition like a decrease in the total abundance due to the modification of the ploughing technique applied. Whereas grassland soil is not ploughed, wheat soil is ploughed annually. We check the composition of plant-derived changes in soil affected by the conversion from C_3-grassland towards C_3-monoculture cropping.

Based on the described potential of molecular proxies to follow C incorporation from various sources in soil during long term inputs, we evaluated 1) the applicability of lipids to trace short term changes by moderate modification of C input, and 2) the contribution of plant-, microorganism-, and contamination-derived lipidic components to soil density fractions.

2. Materials and methods

2.1. Samples

Soil and plant samples were collected from the experimental station 'Heidfeldhof' of the University of Hohenheim, Stuttgart (Germany). The soil was described as a Gleyic Cambisol (WRB, 1998) developed from Loess (Marhan et al., 2008). The uppermost 10 cm of the A_p horizon were sampled for this study. The site was treated as grassland until a monoculture of spring wheat (*Triticum aestivum* cv Triso) was introduced in 2002. Since then, soil was tilled in spring before crop sowing. Beginning in 2003, inorganic NPK fertilizers were applied (140 kg N, 60 kg K, and 30 kg P ha^{-1}) to each plot (Erbs and Fangmeier, 2006). Soil sampled in 2002 from the permanent grassland converted thereafter to wheat monoculture was available as a reference. From 2006 soil samples were taken after four years of wheat cropping. While for 2002 only one soil sample for the whole plot was available, for 2006 two replicate samples were taken representing five individual subsamples, each. The soil samples were air dried at room temperature and sieved through a 2 mm mesh. All visible root and plant remains were carefully removed with tweezers. Samples of wheat straw were collected in 2006 during harvest from the same two subplots where soil samples were taken. Straw samples were divided into leaf and stem tissues. All samples were air dried. Two replicates were analysed for each plant tissue.

2.2. Separation of density fractions

For the soil sample from 2002 only a low amount of soil was available which was not sufficient for density fractionation followed by lipid analyses. Hence, only soil samples of the year 2006 were separated for density fractions. The fractionation procedure by John et al. (2005) was used with some marginal modifications. 8 g of air dried soil were placed in a centrifugation tube and 35 ml of sodium