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Indicators for soil organic matter quality in no-till soils under perennial crops in Central Sweden

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ABSTRACT

Assessing the organic C (C_{org}) sequestration in no-till soils under perennial crops requires molecular-level quality indicators. Therefore, we investigated the quality of soil organic matter (SOM) in the topsoil under *Salix viminalis* L. and *Lolium perenne* L. at two test sites in Central Sweden. The willow *S. viminalis* (clone 78021) was grown in short rotation coppice, and the grass *L. perenne* in an adjacent meadow for 17 (site Ultuna) and 15 years (site Enköping), respectively. The concentrations of aliphatic lipids, determined by gas chromatography/mass spectrometry (GC/MS), as well as the molecular composition and thermal stability of the bulk SOM, determined by pyrolysis-field ionization mass spectrometry (Py-FIMS), were tested as indicators for the crop-specific SOM quality. Larger C_{org} concentrations (factor 1.4) in the topsoil (site Ultuna) under *S. viminalis* than under *L. perenne* corresponded to higher concentrations of summed aliphatic lipids (factor 1.6), mainly saturated *n*-alkanoic acids (factor 2.1) and *n*-alkanols (factor 1.5) in the GC/MS-analyses. Moreover, in the willow stand (site Ultuna) at soil depth of 0–10 cm disproportionally higher concentrations of saturated *n*-alkanoic acids (C_{17}–C_{36}) (factor 2.4) and *n*-alkanes (C_{21}–C_{36}) (factor 2.6) indicated a preferential sequestration of aliphatic C because the bulk C_{org} concentrations were only larger by factor 1.4. This crop-specific impact on SOM at soil depth of 0–10 cm was proven for both test sites. Furthermore, the Py-FIMS showed larger abundances of thermally stabile alkylaromatics (factor 1.4), and non-peptidic N-containing compounds (factor 1.3) in the *S. viminalis* plot (site Enköping), which supported a crop-specific C_{org}-sequestration of these compounds. Thus, in summary, accumulations in extracted long C-chain aliphatic lipids and the thermal stability of some substance classes indicated that the C_{org} sequestration by no-till may be more distinct in soils under *S. viminalis* than under *L. perenne*.

1. Introduction

Losses of soil organic matter (SOM) resulting from agricultural intensification and forest conversion to farmland contribute to the rise in atmospheric CO_2 (Lal, 2004). Hence, research focuses on mitigating the elevated CO_2 emissions by sequestering organic C (C_{org}) in soils (Lal, 2003, 2004). This C_{org} sequestration in soil requires an appropriate quality of the organic matter (Liu et al., 2006); that means it must be stabile and resist microbial decomposition. Although the persistence of SOM recently was described as an ecosystem property (Schmidt et al., 2011) the molecular composition may cause a stability as well (Leinweber et al., 2008).

Perennial crops on no-tilled soils can improve the SOM quality, *e.g.*, by accumulation of carbohydrates evoked by microbial activity that is higher than in tilled soils (Arshad et al., 2010). Promising results were obtained for the C_{org} storage in topsoil under short rotation coppice (SRC) on arable soils (*e.g.*, Makeschin, 1994; Hoosbeek and Scarascia-Mugnozza, 2009; Baum et al., 2009; Kahle et al., 2010). This positive effect was accompanied by a rapid biomass production with fast growing trees such as *Populus* and *Salix* spp. (Dimitriou et al., 2009). Also soils under other perennial crops, especially grasses, were described to have a high potential to store C_{org} (Janzen, 2004). In a ^{14}C pulse labeling experiment ryegrass (*Lolium perenne*) allocated about 48% of the total assimilated ^{14}C below the soil surface (Domanski et al., 2001). Despite many investigations on C_{org} sequestration (*e.g.*, Hoosbeek and Scarascia-Mugnozza, 2009; Baum et al., 2009; Kahle et al., 2010), the stability of such SOM enrichments is scarcely known. Furthermore, the C_{org} content strongly depends on crop-specific inputs to SOM, affecting its composition (Kahle et al., 2007). Changes in SOM were described quantitatively by the C_{org}

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concentrations (Baum et al., 2009) and enzyme activities (Kahle et al., 2007), but seldom qualitatively by, *e.g.*, concentrations of biomarkers or thermal properties of SOM compounds under SRC. However, biomarkers, such as phospho- and other lipids, can be highly suitable to forecast the C_{org} sequestration (Denef et al., 2007).

Soil lipids were determined in forest (Marseille et al., 1999) and agricultural soils (Wiesenberg et al., 2004; Jandl et al., 2012) to indicate the turnover of plant residues at a molecular level. The distribution pattern of aliphatic lipids is determined by gas chromatography/mass spectrometry (GC/MS), and it is influenced by the lipid inputs from plant biomass (van Bergen et al., 1997; Bull et al., 2000a; Jandl et al., 2007) and soil organisms (Jandl et al., 2005). Among these aliphatic lipids, long C-chains are more slowly decomposed than short C-chain ones (Bull et al., 1998). Consequently, a crop-specific SOM storage with concentrations of long C-chain aliphatic lipids disproportionally exceeding those of C_{org} may indicate a sequestration with slowly decomposing SOM as shown for SRC under willows and poplars (Jandl et al., 2012).

For annual arable crops, the SOM decomposability was related to the molecular composition and the thermal stability of compound classes (Leinweber et al., 2008) but such evidence is lacking for SOM under SRC or grassland. Especially non-targeted methods such as temperature-resolved pyrolysis-field ionization mass spectrometry (Py-FIMS) may detect all relevant substances which slowly decompose due to their chemical composition and/or stabile bonds to soil minerals (Leinweber et al., 2008). Therefore, it appears advantageous to use two independent methods for characterizing the SOM stabilization (i) GC/MS to quantify long C-chain aliphatic lipids that are slowly decomposed, and (ii) Py-FIMS to quantify especially thermally stabile compounds.

The objective of the present study was to test if long C-chain aliphatic lipid concentrations, slowly decomposable and thermally stabile SOM constituents indicate a no-till-initiated SOM stabilization under either SRC with willows or grassland grown with *Lolium perenne*.

2. Materials and methods

2.1. Test sites and soil sampling

The test sites are located in Uppsala (Ultuna, ULT; 59°47′N, 17°39′E) and in Enköping (ENK; 59°33′N, 17°00′E). General soil properties are summarized in Table 1. Further details of the experimental site ULT were given by Olsson and Samils (1984). Both test sites were established on former tilled arable sites with Vertic Cambisols (FAO classification) as the dominating soil type, each separated into two no-tilled treatments: SRC (with willows) and meadow (with grass) at the same time. Under the former arable tilled use, the upper horizon was homogenized by ploughing, under the no-till treatments a crop-specific vertical separation and C_{org} accumulation close to the soil surface (0–10 cm soil depth) was expected. In ULT *S. viminalis* clone 78021 was cultivated since spring 1993 and in ENK *S. viminalis* cv. Jorr since spring 1995, each as a 3-year rotation. At the adjacent meadow *L. perenne* was grown. No fertilizer or pesticides were applied since the cultivation was changed. Soil samples were taken from three replicated plots per treatment in a randomized test design. The samples were taken apart from desiccation cracks with a soil corer of 4 cm diameter after removing the litter layer in three soil depths (0–10 cm, 10–20 cm, 20–30 cm). Aboveground grass parts were completely cut to take the soil samples. All visible fine roots were removed before the samples were bulked per plot and sampling depth, air-dried and crushed for disaggregation, and sieved (<2 mm). These samples were milled with a mortar grinder RM 100 (Retsch, Haan, Germany) for elemental analyses and for the lipid extraction (GC/MS) and more finely ground by a pestle in an agate mortar for the Py-FIMS analysis.

Table 1
Location, soil type, composition of the particle-size fractions (%) at soil depth of 0–10 cm, average annual temperature (°C) and average annual precipitation (mm) of the test sites in ULT (Ultuna) and in ENK (Enköping).

	ULT	ENK
Location	59°47′N, 17°39′E	59°33′N, 17°00′E
Soil type	Vertic Cambisol	Vertic Cambisol
Clay (%)	58	39
Fine silt (%)	15	11
Medium silt (%)	12	11
Coarse silt (%)	6	18
Fine sand (%)	5	18
Medium sand (%)	2	2
Coarse sand (%)	2	1
Average annual temperature (°C)	5.2	5.2
Average annual precipitation (mm)	544	544

2.2. Elemental analyses

The concentrations of total C and total N were determined by Dumas dry combustion using a VARIO EL analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Since the samples had no $CaCO_3$, total C was considered to be C_{org}.

2.3. Determination of soil lipids

About 5 g of litter and 50 g of soil were Soxhlet-extracted for 24 h using 100 mL dichloromethane/acetone (9:1 by volume) (van Bergen et al., 1997). The extract was methylated by adding 50 μL of a 25% (by weight) tetramethylammonium hydroxide/methanol solution followed by ultrasonication in the bath RK 52H (Bandelin, Berlin, Germany) for 15 min (Jandl et al., 2002).

The individual components of the extracted lipids were separated on a Varian 3800 (Varian, USA) gas chromatograph (GC) equipped with a 25 m capillary column that was coated with a 0.25 μm film thickness of BPX 5 stationary phase (SGE, Australia) and with an inner diameter of 0.32 mm. For each GC/MS run, 1 μL of the derivatized extract was injected at 300 °C injector temperature. The carrier gas helium 5.0 was set up with a constant flow of 1 mL min^{-1}. Following split injection up to 45 s (splitless), the split ratio was 1:100 from 45 s up to 90 s and 1:5 from 90 s on. The temperature program for the GC started at 100 °C for 5 min, followed by heating at a rate of 5 °C min^{-1} to 280 °C for 40 min. The GC was connected to a double-focusing MAT 95 mass spectrometer (MS) (Finnigan, Bremen, Germany). Conditions for mass spectrometric detection in the electron impact mode were 4.7 kV accelerating voltage, 70 eV electron energy, 1.2 kV multiplier voltage, *m/z* 48–600 mass range, 0.3 s (mass decade)$^{-1}$ scan rate, and 0.6 s interscan time. An external standard mixture was used to quantify the lipid components (Promochem, Germany). Peaks were assigned by comparison of their mass spectra with the Wiley mass spectral library, software edition 6.0. The method was described in detail by Jandl et al. (2002).

2.4. Pyrolysis-field ionization mass spectrometry (Py-FIMS)

For Py-FIMS about 4 mg of the extra finely ground and homogenized samples were degraded by pyrolysis in the ion source (emitter: 4.7 kV, counter electrode −5.5 kV) of a double-focusing Finnigan MAT 95. The samples were heated in a vacuum of 10^{-4} Pa from 50 °C to 700 °C, in temperature steps of 10 °C. Between magnetic scans the emitter was flash heated to avoid residues of pyrolysis products. 65 spectra were recorded for the mass range *m/z* 15–900. Ion intensities were referred to 1 mg of the