

Impact of poplar on soil organic matter quality and microbial communities in arable soils

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ABSTRACT

Poplars grown in short rotation coppice on agricultural land are a promising bioenergy crop. This study aimed to evaluate the soil organic matter (SOM) quality and viable microbial consortium under six-years-old poplar (*Populus maximowiczii*) and under wheat (*Triticum aestivum*) at a test site in central Germany. The SOM molecular composition and stability was determined by pyrolysis-field ionization mass spectrometry (Py-FIMS). The microbial consortium was assessed in terms of microbial phospholipid fatty acid (PLFA) profiles. Py-FIMS and the PLFAs agreed in showing crop-specific differences in the SOM quality and in the associated microbial communities. Higher proportions of carbohydrates, long-chained fatty acids, sterols and suberins at the expense of N-containing compounds under poplar than under wheat were associated with lower concentrations of microbial PLFAs in the organic matter. A higher ratio of total fungal to bacterial (f/b) PLFAs, a lower ratio of Gram-positive to Gram-negative bacterial PLFAs and lower biomass of arbuscular mycorrhizal fungi in the organic matter were revealed under poplar than under wheat. Lower N- and increased C-availability in the SOM promoted fungal vs. bacterial colonization, increased the SOM stability by a lower decomposability and caused SOM accumulation under poplar.

Keywords: bioenergy; soil carbon storage; phospholipid fatty acid profiles; *Populus*; short rotation coppice

Poplars (*Populus* spp.) grown as short rotation coppice (SRC) are promising candidates to produce biomass and they can also increase the soil organic matter (SOM) contents in arable soils (Kahle et al. 2010). The SOM quality is a leading control of its microbial decomposability (Leinweber et al. 2008) and thereby of its stability and the sustainability of C storage. However, little is known on the SOM quality under SRC so far. The use of lipids as biomarkers provided a first indica-

tion of a sustainable increase in C storage under SRC (Jandl et al. 2012). Crop-specific impacts on the SOM quality were proved for several SOM constituents by pyrolysis-field ionization mass spectrometry (Py-FIMS) (Schmidt et al. 2000). The specific structure of the microbial community can explain the microbial SOM turnover and the soil C storage (Jensen et al. 2005). The structure of the microbial community can be described by phospholipid fatty acid (PLFA) profiles, which

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use cell membrane lipids as biomarkers for specific groups of organisms (Zelles 1999). For poplar grown on arable soils, combined analyses of the SOM composition and microbial community structure in the soil and comparisons to annual arable crops are missing so far. We hypothesized that an increased total litter input from poplar may mutually alter the inter-linked molecular composition of SOM and the microbial community structure. Confirmation of this hypothesis would imply that changed SOM composition can be used as a predictor of sustainable increased C storage in the soil. The objectives of the present study were to describe the poplar-specific SOM quality at an arable site in comparison to that under an annual crop and to predict its stability.

MATERIAL AND METHODS

Test site and sampling. The test site Cahnsdorf is located in central Germany (51°85'N, 13°75'E). Its annual mean of temperature is 8.6°C and that of precipitation is 563 mm. Cambisol is the dominating soil type. The soil texture is 75% sand, 23% silt and 2% clay. An arable field previously cultivated with annual crops was partly planted with SRC in 2004, while in the other parts the annual arable use was continued (Landgraf et al. 2007). The plot size was 4.50 m × 4.20 m. Five replicates per treatment were established in a randomized block design. The planting of poplars was done in double rows with 1.50 m distance between and 0.75 m distance within the rows. Soil samples were taken with a corer (3 cm diameter) from the upper topsoil (0–10 cm soil depth) under the six-years-old SRC with poplar (*Populus maximowiczii* clone Japan 104) and under winter wheat (*Triticum aestivum*) in spring 2010 at each of the five replicated plots. The crop rotation at the arable site comprised: rape – winter wheat – winter wheat. At the arable site 120 kg N/ha were applied to the winter wheat. Poplar received no fertilisation. Spring sampling was chosen, since this is usually the best period to indicate land-use-specific effects on soil microbial properties (Alef 1991).

C and N concentrations and pH in the soil. Soil C and N concentrations were determined using a CN analyzer (Vario EL III, Elementar Analysensysteme, Hanau, Germany). As carbonate was absent all C was considered to be organic (C_{org}). The soil pH was measured electrometrically in 1 mol CaCl₂ solution (w/v = 1:2.5) using a glass

pH electrode. The total water content was determined from sub-samples which were oven-dried at 105°C for 24 h.

Pyrolysis-field ionization mass spectrometry (Py-FIMS). About 3 mg of the air-dried, 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 (San Jose, USA). The samples were heated in a vacuum of 10⁻⁴ Pa from 50°C to 700°C, in temperature steps of 10°C over a time period of 18 min. Between magnetic scans the emitter was flash heated to avoid residues of pyrolysis products. 65 spectra were recorded for the mass range 15 to 900 *m/z*. Ion intensities were referred to 1 mg of the sample. Marker signals (*m/z*) were assigned to relevant compound classes according to Leinweber et al. (2009).

Phospholipid fatty acids. Phospholipids were extracted and analyzed using the method of Forstegård et al. (1993). The total amount of phospholipid fatty acids (PLFAs) was used to indicate the total microbial biomass. The sum of PLFA considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1 ω 9, 16:1 ω 7t, i17:0, a17:0, 17:0, cy17:0, 18:1 ω 7, and cy19:0) was used as an index of bacterial biomass (Frostegård et al. 1997). Prefixes 'i' (iso) or 'a' (anteiso) describe the location of a methyl branch to one or two C-atoms, respectively, from the aliphatic end (e.g., i15:0). The prefix 'cy' refers to cyclopropane fatty acids. Monounsaturated fatty acids are generally indicative of Gram-negative (G⁻) and iso or anteiso branched chain fatty acids in soils typically indicate Gram-positive (G⁺) bacteria (Zelles 1999). The PLFA 18:2 ω 6 was used as indicator for fungal biomass (Ibekwe and Kennedy 1998), 16:1 ω 5 as indicator for biomass of arbuscular mycorrhizal fungi (Olsson et al. 1995) and 20:4 as indicator for biomass of protozoa (Frostegård et al. 1997).

Statistical analyses. Means and standard deviation (SD) were calculated for the indicators of the chemical composition of the SOM and for microbial PLFA concentrations under poplar and wheat and compared by Student *t*-test. Differences between the means were considered to be statistically significant at *P* < 0.05. Statistical analyses were computed by using Statistica (Statistica for Windows 1996, StatSoft, Tulsa, USA). Crop-specific differences in the composition of SOM and microbial PLFAs were analysed using the detrended correspondence analysis (DCA) in paleontological statistics software package (PAST) (Hammer et al. 2001).

RESULTS AND DISCUSSION

The C_{org} concentration was larger (18.6 g/kg) and the C/N ratio was higher (11.0) under poplar than under wheat (8.1 g/kg, 9.6). Increased C sequestration in arable soil under poplar in SRC agreed with Kahle et al. (2010). The soil pH ($CaCl_2$) was slightly lower under poplar (6.4) than under wheat (6.7). The SOM under poplar revealed about twice the ion intensity than under wheat in Py-FI mass spectra (Figure 1). The Py-FI mass

spectrum of SOM under poplar and under wheat were dominated by carbohydrates (m/z 60, 84, 96, 114, 126), lignin monomers (m/z 150, 178, 194, 208) and long-chained fatty acids (m/z 256, 280, 312, 340, 368, 396, 424, 452). Under both crops, palmitic acid (m/z 256) and linolenic acid (m/z 280) were dominating long-chained fatty acids in the spectrum of SOM. Higher proportions of long-chained fatty acids with the m/z signal 340 (behenic acid) and m/z signals 424 (C_{28}) and 452 (C_{30}) in the spectrum were specific for SOM under

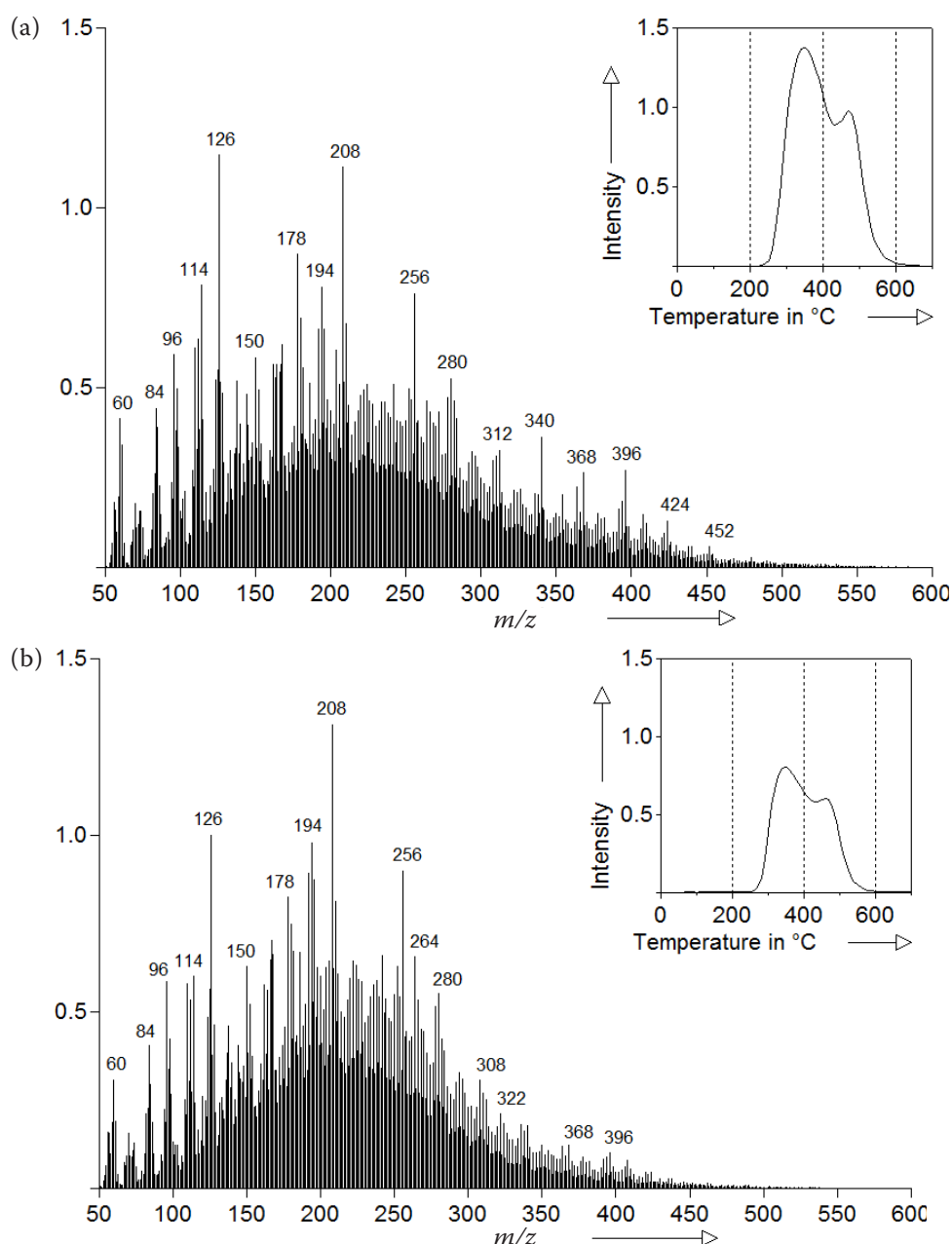


Figure 1. Thermograms of total ion intensity (upper right) and averaged pyrolysis-field ionization (Py-FI) mass spectra from soil organic matter under (a) poplar and (b) wheat at the arable site Cahnsdorf in April 2010

Table 1. Volatile matter and relative proportions (in % of total ion intensities) of important compound classes of soil organic matter at an arable site under poplar and wheat in spring 2010 ($n = 4$, standard deviation in parentheses)

Crop	VM	CARB	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPT	SUBE	FATTY
Poplar	6.4	6.8 ^b (0.12)	11.9 ^a (0.15)	2.7 ^a (0.14)	8.4 ^b (0.16)	10.0 ^a (0.15)	7.5 ^a (0.07)	2.0 ^b (0.16)	3.3 ^b (0.04)	0.2 ^b (0.00)	2.4 ^b (0.12)
Wheat	3.8	6.0 ^a (0.54)	12.9 ^b (0.21)	2.5 ^a (0.29)	7.4 ^a (0.39)	11.2 ^b (0.21)	7.9 ^b (0.15)	1.0 ^a (0.07)	2.7 ^a (0.31)	0.1 ^a (0.00)	2.0 ^a (0.18)

Different letters indicate significant crop-specific differences ($P < 0.05$). VM – volatile matter (%); CARB – carbohydrates; PHLM – phenols and lignin monomers; LDIM – lignin dimers; LIPID – alkane, alkene, n -alkylester, n -alkyldiester lipids; ALKY – alkylaromatics; NCOMP – N-containing compounds; STER – sterols; PEPT – peptides; SUBE – suberin; FATTY – free fatty acids n -C₁₆ to n -C₃₄

poplar. In contrast, higher proportions of nonadiene (m/z 264) and the alkenes C₂₂H₄₄ (m/z 308) and C₂₃H₄₆ (m/z 322) in the spectrum were specific for SOM under wheat.

For the first time, the present study showed an accumulation of specific long-chained fatty acids (m/z 340, 424, 452) in SOM under *P. maximowiczii*. These long-chained fatty acids are components of epicuticular waxes (Schulten et al. 1986) and hydrophobic. Their increased proportions can lead to SOM accumulation (Ostertag et al. 2008). Since wax production of poplar varies species-specific (Cameron et al. 2002), the present results are only referred to *P. maximowiczii*. However, epicuticular waxes have an important impact on stress resistance of poplar and were recommended as an additional selection criterion in poplar breeding (Cameron et al. 2002). Therefore, our results strengthen this recommendation by the indicated significance of waxes for the SOM stability.

The portions of compound classes of SOM under poplar and wheat differed significantly except for

lignin dimers (Table 1). SOM under poplar revealed higher portions of carbohydrates, lipids, sterols, peptides, suberin and free fatty acids, but lower portions of phenols and lignin monomers, alkylaromatics and N-containing compounds than SOM under wheat. Phenols and lignin monomers and alkylaromatics revealed the largest proportion of SOM under both crops. However, the third-largest proportion of SOM under poplar was accounted to lipids, but under wheat to N-containing compounds.

The total concentrations of microbial PLFAs in the organic matter (mmol/kg C_{org}) were lower under poplar than under wheat (Table 2). This was valid separately for the concentrations of bacterial, total fungal, arbuscular mycorrhiza fungal and protozoa PLFAs. The ratio of total fungal to bacterial (f/b) PLFAs was larger (0.10) and the ratio of Gram-positive to Gram-negative (G+/G-) bacterial PLFAs was smaller (0.75) under poplar than under wheat (0.08 and 1.00, respectively).

The comparably low f/b ratio under wheat measured in the present study agreed with the level observed

Table 2. Total amount (means and standard deviation in parentheses) of microbial phospholipid fatty acids (PLFAs), total of bacterial and fungal PLFAs, amount of PLFAs from Gram-positive (G+) bacteria, amount of PLFAs from Gram-negative (G-) bacteria, total amount of fungal PLFAs, amount of PLFAs from arbuscular fungi (AMF) and protozoa in the organic matter under poplar and wheat in April 2010 ($n = 5$)

Crop	PLFA						
	total microbial	total bacterial	total fungal	G+ bacteria	G- bacteria	AMF	protozoa
(mmol/kg C _{org})							
Poplar	4.5 ^a (0.54)	1.7 ^a (0.24)	0.2 ^a (0.01)	0.5 ^a (0.08)	0.7 ^a (0.08)	0.2 ^a (0.01)	0.1 ^a (0.02)
Wheat	9.9 ^b (0.52)	4.1 ^b (0.22)	0.3 ^b (0.08)	1.4 ^b (0.10)	1.4 ^b (0.08)	0.3 ^b (0.01)	0.2 ^b (0.02)

Different letters indicate significant crop-specific differences ($P < 0.05$)

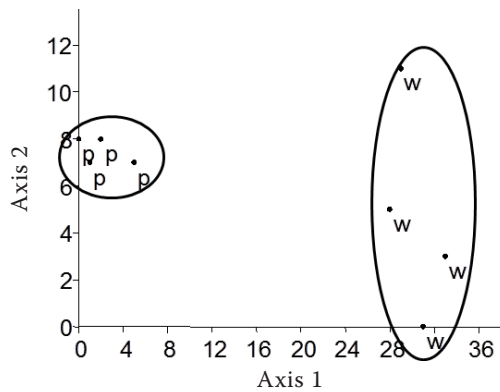


Figure 2. Ordination diagram of crop-specific chemical composition of the organic matter in the soil from DCA analysis of proportions of substances in the organic matter and phospholipid fatty acid (PLFA) concentrations (p – poplar; w – wheat) at the arable site Cahnsdorf in April 2010

also under wheat at another arable Cambisol in Germany (Ngosong et al. 2010). It seems most probable that besides the increased labile C pool, lack of tillage under poplar significantly contributed to the wider f/b ratio, since more intensive tillage, as for wheat, can decrease the f/b ratio (Bailey et al. 2002). The lower biomass of arbuscular mycorrhizal fungi under poplar than under wheat, might be explained by inter-fungal competition under the ectomycorrhizal host plant poplar. The lower colonization of SOM by protozoa under poplar points to a delayed nutrient release from the microbial biomass as labile nutrient pool, since protozoa feed bacteria and release their nutrients (Priha et al. 2001). In contrast to Pennanen et al. (1998), we found no increased colonization of G⁺ bacteria under poplar with the lower pH. This might be explained by the very slight difference in the soil pH (0.3). The increased f/b ratio under poplar fits to the results of Bailey et al. (2002), who found that dominance of fungal activity leads to increased C accumulation in soils.

As analyzed by DCA, the chemical quality of organic matter under poplar and wheat differed greatly (Figure 2). The first two DCA axes explained together 40% (29% and 11% from the first and second axis, respectively) of the total variability of the chemical composition (including concentrations of PLFAs) of the organic matter under the crops. In the present study, a molecular fingerprinting of bulk SOM by Py-FIMS was combined for the first time with a fingerprinting of microbial community structure by PLFA's to investigate how SRC with poplar affects the sustainability of C-storage in soil in comparison to a traditional annual crop.

The larger SOM contents in the upper 10 cm of the topsoil under the six-year-old stand of *P. maximowiczii* than under wheat also coincided with enrichments in carbohydrates, which belong to the rather labile C sources in soils. It is well known that poplar hosts ectomycorrhizal fungi, which significantly increase the assimilate transfer into the soil (Podila et al. 2009). Thus, higher carbohydrate proportions under poplar might be caused by the presence of ectomycorrhizal roots. The generally low microbial colonization of SOM under poplar agreed with results under other poplar clones in France (Moscatelli et al. 2005). Higher carbohydrate proportions in combination with lower N availability promote selectively fungal vs. bacterial growth and activity (Keiblinger et al. 2010) and thereby decomposition of lignin (Romani et al. 2006). This might explain the wider f/b ratio under poplar (0.10) than under wheat (0.08).

In conclusion, poplar-specific SOM properties comprised accumulation of hydrophobic compounds and carbohydrates and lower availability of organic nitrogen. These result in a lower microbial colonization with an increased ratio of fungal to bacterial biomass. Thus, poplar in SRC may be an approach to transfer additional C from atmosphere into biomass and store it in soil along with producing bioenergy. Concentrations of foliar waxes should be further tested as a criterion to select poplar genotypes with a sustainable increased C storage in the soil. Furthermore, the consequences of increased concentrations of carbohydrates for the SOM turnover after return to crop rotation should be assessed.

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