Response of water extractable organic matter and its fluorescence fractions to organic farming and tree species in poplar and robinia-based alley cropping agroforestry systems

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Abstract

Organic farming and agroforestry both have the potential to develop sustainable and environmental-friendly agroecosystems and to sequester more soil organic C (SOC). In a long-term field trial, we evaluated the effect of 21-year organic farming and 4-year agroforestry (Robinia and Poplar-based alley cropping system) on water extractable organic matter (WEOM). The technique combining excitation emission matrix (EEM) spectra with parallel factor analysis (PARAFAC) was used to reveal the components of WEOM. In addition, WEOM was characterized by UV absorbance and fluorescence spectra. Organic farming generally increased SOC and total N contents but decreased the WEOM content as well as the WEOM components indicated by the maximum fluorescence intensity (F_{max}). Specific UV absorbance (SUVA) and humification index (HIX) of WEOM in organic farming implied WEOM in the organic farming had more components with aromatic structure but less humified. Higher fluorescence (FI) and freshness indices (BIX) of WEOM in organic farming system indicated that a higher percentage of WEOM was microbial-derived in the organic than in the integrated farming system. Robinia showed positive effect on SOC and total N contents in comparison with poplar and had stronger effects on the WEOM components, although the WEOM content did not differ between the two tree species. The significant farming × trees interactions on SOC and water extractable organic carbon (WEOC) indicated that the robinia effects were more pronounced in the organic farming system. Thus, the change of SOC was the result of interactive effect of farming and hedgerow trees in an agroforestry system. The low-input organic farming and robinia tended to result in change of quality of WEOM and led to enrichment of substances of high stability in WEOM. From above, the combination of organic farming and robinia trees is an important means for developing sustainable agricultural systems and soil carbon sequestration.

1. Introduction

Soil WEOM is an active, mobile and complex fraction of soil organic matter (SOM) and is sensitive to land use and management practices (Dong et al., 2009; Mao et al., 2012; Xu et al., 2013). WEOM participates in multiple soil processes, such as SOM translocation and mineralization, denitrification and trace gas production, solubility, transportation and toxicity of organic contaminants (Chantigny, 2003; Stark et al., 2007; Xu et al., 2013). It was also reported that WEOM modulates soil microbial community as a feedback to the production of WEOM from microbial activities (Bausenwein et al., 2008). Moreover, land-use and management practices were repeatedly reported to influence the dynamics of WEOM and biodegradability by affecting soil biochemical properties and structure and WEOM composition (Chantigny, 2003; Marschner and Kalbitz, 2003; Xu et al., 2013).

Agroforestry systems combine agriculture and forestry into a production system and are recognized as integrated approaches for sustainable land use aside from their contribution to climate change adaptation and mitigation (Ramachandran Nair et al., 2009; Lorenz and Lal, 2014). Agroforestry promotes C sequestration in tropical and temperate regions (Montagnini and Nair, 2004; Nair et al., 2010), depending on tree species and management of the agroforestry system (Lorenz and Lal, 2014). Trees, especially broadleaf trees having deep and extensive root systems and high belowground to aboveground biomass ratios, enhancing the potential for soil C sequestration (Laganiere et al., 2010; Lorenz and Lal, 2014). However, positive, neutral, and negative effects of trees on SOC pool have been observed in the meta-analysis of Laganiere et al. (2010). They reported that positive effects of planting conifer trees other than *Pinus* spp. on SOC pools may be negligible. In contrast, the planting of trees with N-fixers symbiosis for afforestation can increase the SOC pool as indicated by the > 30% increase in SOC pools (Johnson and Curtis, 2001; Lorenz and Lal, 2014). Moreover, compared with studies on SOC stocks, research on the effects of agroforestry systems on SOM quality and composition, for example on labile WEOM is scarce.

Many studies showed the positive effects of organic farming on SOC stocks (Gattinger et al., 2012) and the biomass and diversity of soil microorganisms (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008). The application of cattle farmyard manure (Heinze et al., 2010) and a more diverse crop rotation strengthens nutrient cycling in organic farming. However, no information exists whether the combination of organic farming and agroforestry results in an additive positive impact. The current agroforestry trial offers the unique possibility to investigate the effects of tree species (poplar and robinia) and organic farming on WEOM, investigating the following hypotheses: (1) Organic farming increases SOC and WEOM contents, especially that of the components of WEOM revealed by EEM-PARAFAC technique. (2) N_2-fixing robinia has stronger positive effects on SOC and WEOM than poplar, especially close to the hedgerow.

2. Materials and methods

2.1. Field experiment

Filed experiments have been carried out conducted in Scheyern Research Farm (TERENO site) located 40 km north of Munich (Germany) (48.50°N, 11.45°E) since 1992. The altitude of the farm ranges between 445 and 500 m (a.s.l.). The mean annual precipitation is 803 mm and mean annual temperature is 7.4 °C (Schröder et al., 2002). The central part of the research station was divided into two parts (in 1992): organic and integrated farming system, each striving for ecological and economical sustainability. Moreover, detailed studies on management-induced changes were carried out in plots sub-divided in integrated and organic farming (Schröder et al., 2002). The soil types are sandy to loamy Cambisols, derived from tertiary sediments and partly covered by loess and most of the soils have loamy texture (Flessa et al., 2002; Kölbl and Kögel-Knabner, 2004). The soils of current organic and integrated alley-cropping farming (agroforestry) field are comparable and have a soil texture of silty loam (USDA) and the soil particle distribution of top soil is 27% sand, 54% silt and 19% clay for organic field and 27% sand, 50% silt and 23% clay for integrated field.

In 2009, agroforestry parcels were incorporated into fields under both integrated and organic farming systems. Three swaths of trees, each comprised of several different species were planted in an alley cropping for the purpose of bioenergy production (30 m length for each tree species) and leaving 30 m wide arable soil for crop production (Fig. 1). The poplar (*Populus maximowiczii* × *P. nigra*) and Robinia (*Robinia pseudoacacia* L.) strip systems were chosen for this study in both organic and integrated systems as they are commonly used tree species in German agroforestry systems. The experiment consisted of four treatments with three replications: organic farming + poplar (O-pop), organic farming + robinia (O-rob), integrated farming + poplar (I-pop), and integrated farming + robinia (I-rob). The plot for each treatment is 30 × 30 m in size. The treed portions of both systems do not receive fertilizer either in manure or mineral form and do not receive weed control via mechanical means or pesticide. The tree density of poplar and robinia are the same.

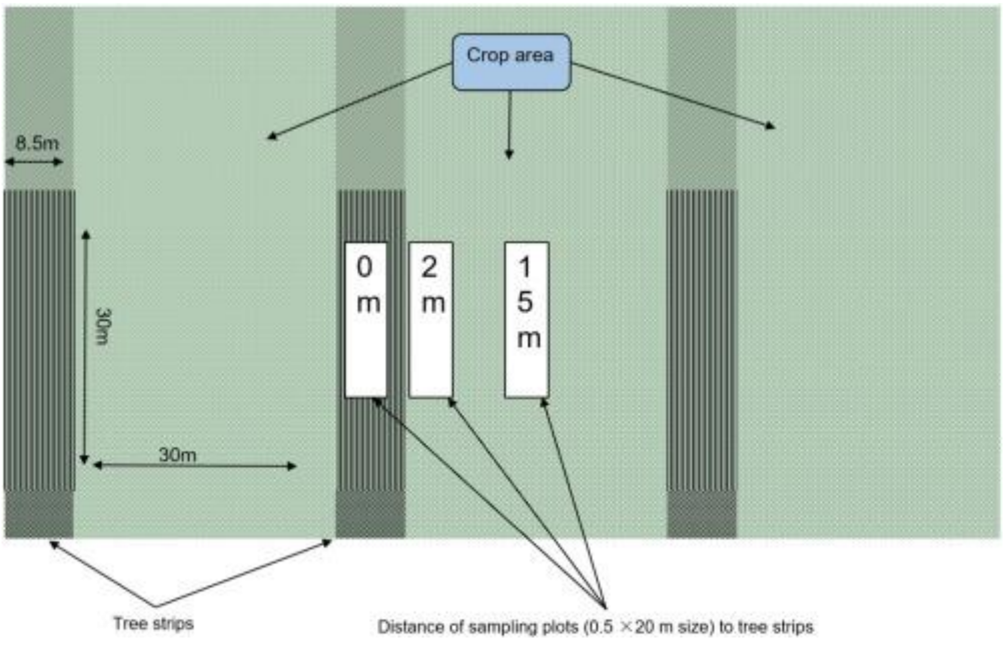

Fig. 1. Experimental layout of alley cropping agroforestry systems and sampling sites (0 m, 2 m and 15 m).

The organic farming system is low-input, utilizing nitrogen fixing cover crops instead of mineral nitrogen or synthetic inputs as green manures. Also no pesticide or herbicide was applied. Soils were tilled with moldboard. A seven-field crop rotation was run in the organic farming system: (1) Grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potatoes (*Solanum tuberosum* L.) + mustard (*Sinapis alba* L.) as cover crop, (3) winter wheat (*Triticum* aestivum L.), (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. In the integrated farming system, soils were tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil. Pesticides were completely forbidden in organic farming system while in necessity it is applicable in the integrated farming system. A four-field crop rotation with cover crops was run in the integrated farming system: (1) winter wheat; (2) potatoes; (3) winter wheat; and (4) maize (*Zea mays* L.).

2.2. Sampling and soil properties

Sampling plots were randomly set in the tree row (0 m), transition area (2 m from the tree row: 2 m) and middle of crop area (15 m from the tree row: 15 m) corresponding to poplar and robinia strips in organic and integrated farming system (Fig. 1). In May 2013, before vegetation developed after a special long winter time, three replicate composite samples were collected at a depth of 0–25 cm. SOC and total N was determined by a CN analyzer (EA3000 Eurovector) with an aliquot air-dried soil samples. Soil texture was determined by wet sieving and pipet method (Gee and Bauder, 1986). Briefly, sand and silt fractions ≥ 20 μm were measured by sieving after treatment with H_2O_2, silt and clay < 20 μm by a pipette procedure.

2.3. WEOM extraction and spectroscopic characteristics

Soil WEOM was extracted according to the method of Embacher et al. (2007). Briefly, 2 mm mesh sieved air-dried soil samples were shaken with 0.01 M $CaCl_2$ at a ratio of 1 to 2 (soil to volume) by using an overhead shaker for 10 min. After centrifugation (3000*g* 10 min), the supernatant was filtered through 0.4 μm polycarbonate membrane filters (Whatman). WEOC concentrations were quantified using catalytic high temperature combustion (680 °C) with a Shimadzu® TOC 5050A analyzer and expressed as mg kg^{-1} dry matter. Dissolved total N, NO_3-N, and NH_4-N of the extracts were quantified photometrically with an automated continuous flow analyzer (Skalar®), and WEON was quantified by subtracting inorganic N from dissolved total N.

Specific UV absorption (SUVA), obtained by dividing the absorption at 254 nm by WEOC concentration, provides the information about the aromatic structures (aromaticity) of WEOM components. Absorption was determined using 1 cm quartz cells with a Varian Cary 50 Bio UV–visible spectrophotometer (Corvasce et al., 2006).

Both pH and molecular concentrations can influence fluorescence (Zsolnay, 2003; Zsolnay et al., 1999). Therefore, to avoid concentration artifacts, dilution was made to have WEOC absorbance < 0.1 cm^{-1} at 254 nm (Zsolnay et al., 1999), and then WEOC extracts were acidified to reach a constant standard pH of 2 with 2 M HCl (Embacher et al., 2007, 2008). The pH has been used for fluorescence spectra (Embacher et al., 2007; Embacher et al., 2008; McKnight et al., 2001). Moreover, at low pH, most metal complexes dissociate, which should minimize the quenching of fluorescence due to metal complexation (McKnight et al., 2001). PARAFAC analysis were also used to decompose the EEMs measurement at a pH range of 2 to 12.5 (Chen and Kenny, 2007; Cuss et al., 2014; Fellman et al., 2008; Yang and Hur, 2014). In present study, EEMs spectra and fluorescence spectra for humification index calculation were measured separately and a round of whole measurement procedure including pH adjustment was finished within half an hour and no precipitation was observed in the samples. All the fluorescence measurements for humification index were done with a Varian Cary Eclispe fluorescence spectrophotometer using 1 cm quartz cells at 254 nm excitation. The fluorescence spectra were recorded (300 to 480 nm with 2 nm increment) at 21 ± 1 °C. Even though the optical density was adjusted by dilution in advance, the fluorescence emission spectra were corrected for “inner filter effect” (Gauthier et al., 1986) according to Zsolnay et al. (1999) with a simplified formula that measured fluorescence emission multiplies e^A, where A is the absorbance in cm^{-1} at the 254 nm excitation wavelength (Akagi and Zsoinay, 2008; Embacher et al., 2007). Humification index (HIX) indicating the complexity and condensation (H/C ratios) of WEOM was calculated as the ratio of integrated fluorescence emission peak at longer wavelength region (435–480 nm) over shorter wavelength region (300–345 nm) at 254 nm excitation wavelength (Zsolnay et al., 1999).

EEMs were obtained by scanning over excitation wavelength from 250 nm to 450 nm with an increment of 5 nm and emission wavelength from 300 nm to 600 nm with an increment of 5 nm. The slit wide was set as excitation slit 10 nm and emission slit 20 nm. Fluorescence data were corrected for inner-filter effect with absorption data as suggested by Lakowicz (2006):

where I_{corr} and I_{obs} are corrected and uncorrected fluorescence intensities, and A_{ex} and A_{em} are the absorbance values at the excitation and emission wavelength of the fluorescence intensity value. The correction is based on the assumption that the average path length of excitation and emission light is 50% of the cuvette width, respectively. Although the correction formula for EEMs and for fluorescence spectra for humification index calculation were different, this will not affect the results in a comparison study. Firstly, EEMs spectra and fluorescence spectra for humification index calculation were measured separately. Meanwhile, the correction for HIX calculation was done according to the published method, and EEM correction was also done followed the commonly reported method. Thus, the correction of fluorescence spectra for each parameter were done in the same way, respectively. Secondly, the two correction formulas are both simplified from the same original formula and base on the same theory as reported by Gauthier et al. (1986).

The fluorescence index (FI), strongly correlated with the structural conjugation and aromaticity and used to differentiate source of DOM, was calculated as the ratio of emission intensity at 470 and 520 nm at fixed excitation wavelength of 370 nm (McKnight et al., 2001). Biological/freshness index (BIX) or β/α index, an indicator of the relative contribution of recently microbially produced DOM, was calculated as the ratio of emission intensity at 380 nm (β) to the maximum emission intensity observed between 420 and 435 nm (α) for an excitation wavelength of 310 nm (Huguet et al., 2009; Wilson and Xenopoulos, 2009).

2.4. PARAFAC modeling

PARAFAC provides a way to decompose a dataset of EEM into individual fluorescence components (Bro, 1997; Andersen and Bro, 2003). An EEM can be reduced to trilinear terms and a residual array by

where, for EEM fluorescence data X_{ijk} is the fluorescence intensity of the *i*th sample at the *k*th excitation and *j*th emission wavelength. a_{in} is directly proportional to the concentration (here defined as scores) of the *n*th fluorophore in the *i*th sample. b_{jn} and c_{kn} are estimates of emission and excitation spectra (loadings) of *n*th fluorophore at wavelength *j* and *k*, respectively. F is the number of components and ε_{ijk} the residual matrix of the model which represents unexplained variability by the model. The full description about PARAFAC can be referred to Bro (1997). Components extracted by PARAFAC can be ascribed to specific species of organic matter present in liquid samples, but they are more likely represent groups of organic compounds having similar fluorescence properties. The PARAFAC model identifies the number of components as well quantifies the scores for each component that is directly proportional to the component's (fluorophore) concentration in the sample. This concentration can be converted into actual concentration if specific absorption coefficient or quantum yield associated with excitation and emission of each fluorophore is known (Singh et al., 2010). The concentration scores of the PARAFAC components were expressed as maximum fluorescence intensity (F_{max}) (R.U.) for each modeled component in current study (Murphy et al., 2013). F_{max} gives estimates of the relative concentrations of each component; however, direct comparison of relative concentrations between different components depends on the magnitude of their quantum efficiencies as well as on their individual responses to quenching effects (Baghoth et al., 2011).

Before the application of PARAFAC model, zero emission intensities were assigned for excitation wavelengths (λex) greater than emission wavelengths (λem). Raleigh scattering was minimized according to (Andersen and Bro, 2003; Borisover et al., 2012). An EEM of the 0.01 M $CaCl_2$ solution was obtained and subtracted from the EEM of each sample in order to removed most of the Raman scatter peaks, the any negative values produced by the sub traction were converted to missing value and the fluorescence was normalized by dividing the integrated area (Ex: 350 nm, Em: 371– 428 nm) under Raman scatter peak of the corresponding Milli-Q water of each set of measurement. This calibration procedure is universal and the Raman signal of water can be used irrespective of sample solvent and matrix and no spectral changes will occur from applying this method (Lawaetz and Stedmon, 2009) and the fluorescence intensities was reported in Raman units (R.U.). In total, the dataset contained 36 corrected and standardized EEMs. After an initial exploratory analysis (calculate its leverage using DOMFluor), one an outlier was identified and removed from the dataset. Therefore, 35 EEMs were performed with PARAFAC analysis in the end. Split-half analysis and examination of residual error plots were applied to get the appropriate number of components. A series of PARAFAC models were generated with Matlab (2009a) (The MathWorks, Natick, MA) by using DOMfluor toolbox specifically developed for PARAFAC analysis of DOM fluorescence (Stedmon and Bro, 2008).

2.5. Statistics

The results presented are geometric means and expressed on dry weight basis. The significance of treatment effects was tested by a two-way ANOVA using tree species and farming managements as independent factors and sampling distance as a repeated measure. ANOVA and Pearson correlation coefficients were calculated using the vegan package (Oksanen et al., 2015) in R (R Core Team, 2015).

3. Results

3.1. Soil organic matter and water extractable organic matter

Mean SOC and total N contents as well as the SOC/total N ratio were significantly higher in the organic than in the integrated farming system, whereas the WEOC, WEON, NO_3-N and NH_4-N contents as well as the WEO-C/N ratio were significantly lower (Table 1). Mean WEOC and WEON contents as well as their ratio were not affected by tree species, whereas the SOC, total N, NO_3-N, and NH_4-N contents as well as the SOC/total N ratio were significantly higher in the robinia than in the polar agroforestry system (Table 1). The effects on SOC were stronger in the organic farming system (Fig. 2), as shown by significant farming × tree interactions (Table 1). Mean SOC, total N and WEON contents at 15 m distance from the tree strips were significantly higher than at 0 m and 2 m distance, whereas the mean NO_3-N content was significantly lowest at 15 m distance (Table 1), especially in the organic farming system, leading to significant farming × distance interactions effect on SOC (Fig. 2) and WEON (Fig. 3). Mean WEOC, and NH_4-N contents as well as mean SOC/total N and WEO-C/N ratios were not affected by any sampling distance from the tree strip (Table 1).

Table 1. Effect of farming practices (1992–2013) and tree species (2009–2013) on SOC, total N and water extractable organic matter in poplar and robinia-based alley cropping systems.

Main effects	**SOC**	**Total N**	**SOC/total N**	**WEOC**	**WEON**	**WEO-C/N**	**NO_3-N**	**NH_4-N**
	(mg g^{-1} soil)			**(mg g^{-1} soil)**			**(µg g^{-1} soil)**	
Organic farming	13.0	1.42	9.1	53	1.2	45	5.5	0.50
Integrated	12.3	1.37	9.0	70	1.4	53	11.2	0.55
Poplar	11.3	1.27	8.9	62	1.3	50	7.5	0.48
Robinia	14.0	1.52	9.2	61	1.3	49	9.1	0.56
0 m distance	12.5	1.38	9.0	68.	1.3	53	9.1	0.52
2 m	12.1	1.34	9.0	56	1.2	49	8.2	0.54
15 m	13.3	1.46	9.1	60	1.3	46	7.6	0.50
Probability values								
Farming	< 0.01	0.01	0.01	0.01	0.05	NS	< 0.01	< 0.01
Tree species	< 0.01	< 0.01	0.04	NS	NS	NS	< 0.01	< 0.01
Sampling distance	< 0.01	< 0.01	NS	NS	0.07	NS	0.02	NS
Farming × tree	< 0.01	< 0.01	0.02	0.01	NS	0.03	NS	NS
Farming × distance	< 0.01	< 0.01	0.03	NS	0.08	NS	< 0.01	0.03
Tree × distance	0.02	< 0.01	NS	NS	0.06	0.04	NS	NS
CV (±%)	2	2	2	17	10	20	13	9

CV = mean coefficient of variation between replicate plots (n = 3); SOC: soil organic C; WEOC: water extractable organic carbon; WEON: water extractable organic N; NS: not significant.

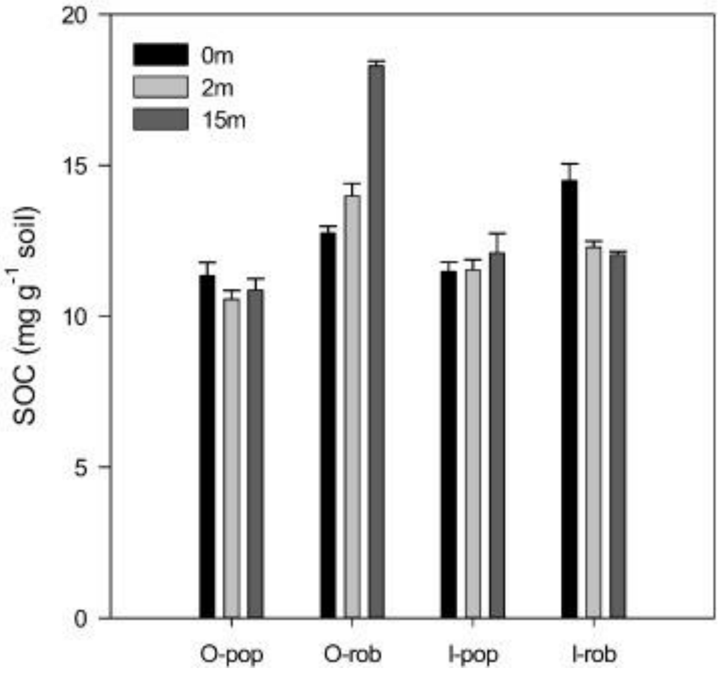

Fig. 2. The soil organic carbon (SOC) content of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

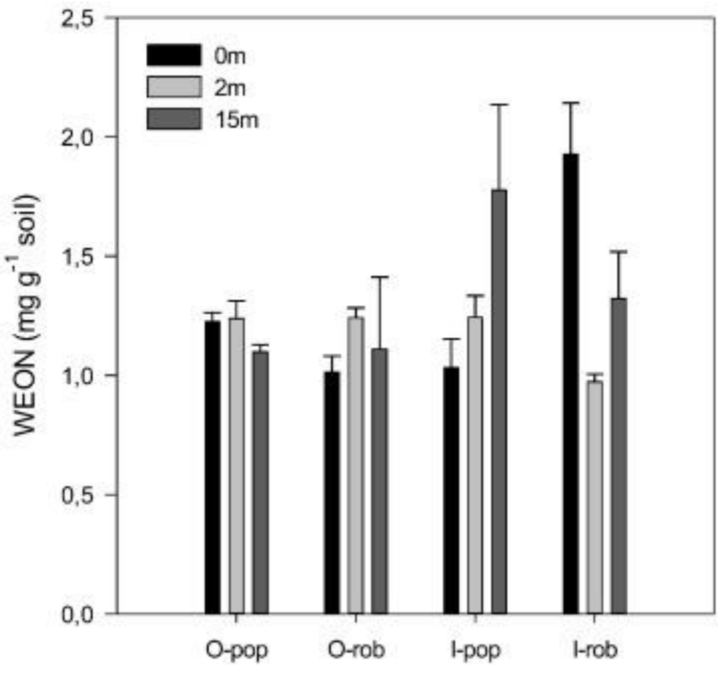

Fig. 3. The water extractable organic N (WEON) content of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

3.2. Spectroscopic properties of WEOM

Mean SUVA, FI, and BIX values were significantly higher in the organic than in the integrated farming system whereas the mean HIX value was lower (Table 2). Robinia significantly increased mean SUVA values in comparison with poplar but did not affect FI and HIX. However, this increase was solely observed in the organic farming system (Fig. 4), leading to significant farming × tree interactions (Table 2). In contrast, the significant farming × tree interactions on the HIX values were caused by significantly higher values in the integrated poplar agroforestry system (Fig. 5). The difference of SUVA and HIX between sampling distance was significant (Table 2). Mean SUVA and HIX values increased in the order 0 m < 2 m < 15 m distance. For the HIX values, this order was mainly caused by the robinia agroforestry system as indicated by the significant tree × distance interactions (Table 2, Fig. 5).

Table 2. Effects of farming practices (1992–2013) and tree species (2009–2013) on spectral characteristics and spectrally identified components of water extractable organic matter in poplar and robinia-based alley cropping systems.

Main effects	SUVA	HIX	Fmax (R.U.)				FI	BIX
			C1	C2	C3	C4		
Organic farming	1.07	2.8	1.00	1.57	0.89	0.32	1.61	0.77
Integrated	0.81	3.3	1.15	1.78	0.84	0.39	1.52	0.74
Poplar	0.85	3.1	1.00	1.57	0.82	0.32	1.55	0.76
Robinia	1.04	3.0	1.15	1.78	0.91	0.39	1.57	0.75
0 m distance	0.85	2.7	1.06	1.57	0.91	0.34	1.55	0.76
2 m	0.98	3.1	1.02	1.62	0.81	0.33	1.59	0.75
15 m	0.99	3.3	1.12	1.83	0.87	0.40	1.54	0.75
Probability values								
Farming	< 0.01	< 0.01	< 0.01	0.01	NS	< 0.01	< 0.01	0.02
Tree species	< 0.01	NS	< 0.01	< 0.01	0.02	< 0.01	NS	NS
Sampling distance	0.05	0.02	NS	0.03	NS	NS	NS	NS
Farming × tree	< 0.01	< 0.01	0.03	0.01	0.01	NS	< 0.01	NS
Farming × distance	NS	NS	NS	NS	NS	NS	0.02	NS
Tree × distance	NS	0.03	NS	0.04	NS	0.03	0.09	NS
CV (±%)	10	6	9	8	11	13	2	2

CV = mean coefficient of variation between replicate plots (n = 3); SUVA = specific UV absorption; HIX = humification index; FI = fluorescence index; BIX = freshness index; C1, C2, C4: fulvic/humic-like substances; C3: tryptophan-like substances; NS: not significant.

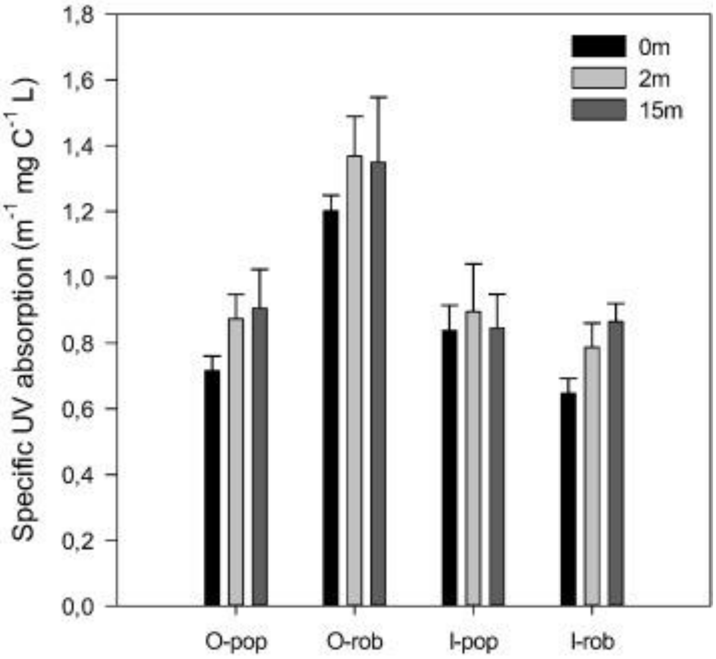

Fig. 4. The specific UV absorbance (SUVA) of water extractable organic matter (WEOM) of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

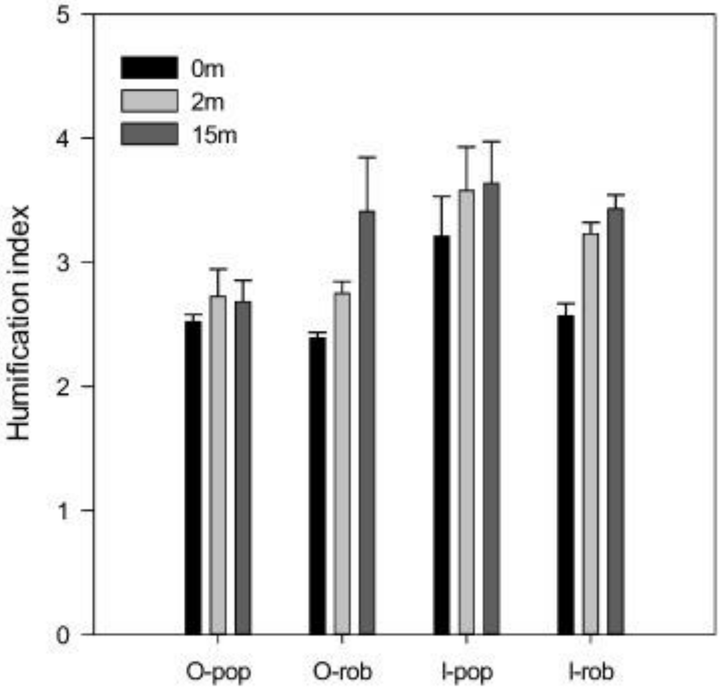

Fig. 5. The humification index (HIX) of water extractable organic matter (WEOM) of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

3.3. PARAFAC components of WEOM

After PARAFAC and split-half analysis of the excitation and emission matrix, four EEM-PARAFAC components were extracted (Fig. 6). The four components C1 to C4 explained 99.7% variability of samples. C1 has one excitation maxima at 325 nm and one emission maxima at 420 nm, resembling the humic-like PARAFAC-derived C1 in soil WEOM (Ohno and Bro, 2006; Borisover et al., 2012). C2 had one excitation maxima at ≤ 250 nm and one emission maxima at 435 nm. This component is similar to the fulvic-like DOM of boreal soils (Olefeldt et al., 2013) and fulvic-like C9 of marine DOM (Murphy et al., 2008). C3 with an excitation peaks at 260 nm and an emission peak at 360 nm resembled the tryptophan-like PARAFAC-derived component in soil WEOM (Borisover et al., 2012; Ohno and Bro, 2006). C4 in had two excitation maxima at 270 nm and 380 nm, and one emission maximum at 475 nm. This PARAFAC-derived component is similar to the humic-like substance, identified in soil WEOM (Borisover et al., 2012).

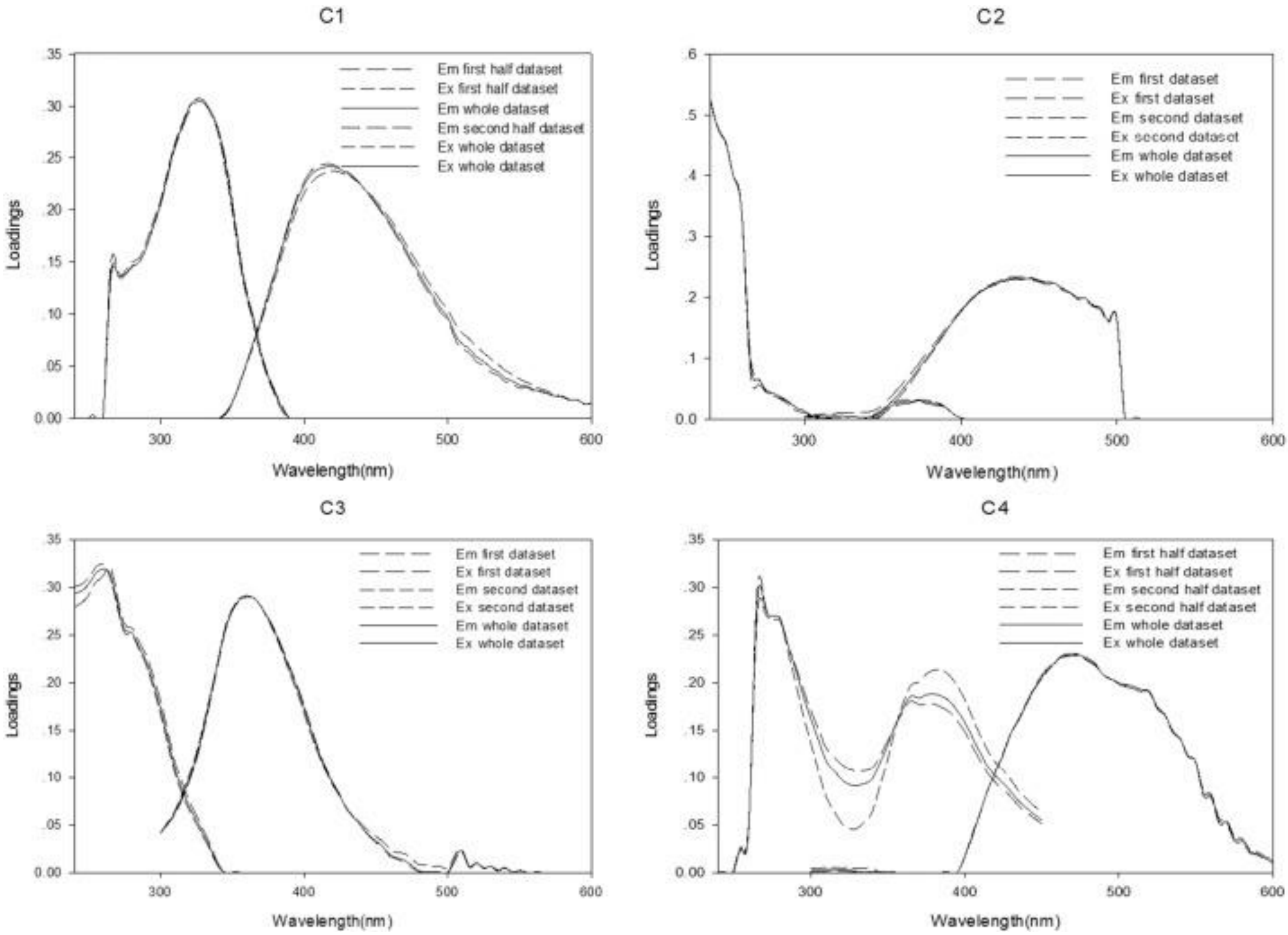

Fig. 6. Split-half analysis of the three components of WEOM extracted by PARAFAC model for all treatments. C1: humic-like substance; C2: fulvic-like substance; C3: tryptophan-like substance; C4 : humic-like substance.

The F_{max} of the PARAFAC-derived components followed an order of C2 (fulvic-like) > C1 (humic-like) > C3 (tryptophan-like) > C4 (humic-like) (Table 2). C1, C2 and C4 values were significantly lower in the organic than in the integrated farming system, whereas C3 did not differ between the two farming system. All four components were significantly larger in the robinia than in the poplar agroforestry system. Only the C2 values were significantly affected by the sampling distance, following the 0 m < 2 m < 15 m. The C1, C2 and C4 values were significantly correlated with the HIX values and negatively correlated with the FI and BIX values (Table 3). The C3 values were only significantly correlated with SUVA.

Table 3. Correlations between PARAFAC components of water extractable organic matter and soil spectral indices.

	SUVA	HIX	BIX	FI
C1	0.02	0.42*	− 0.54**	− 0.58**
C2	0.14	0.54**	− 0.55**	− 0.58**
C3	0.34*	− 0.25	− 0.12	− 0.27
C4	− 0.13	0.51**	− 0.57**	− 0.51**

C1, C2, C4: fulvic/humic-like substances; C3: tryptophan-like substances; SUVA: specific UV absorbance; HIX: humification index; BIX: freshness index; FI: fluorescence index.

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

4.1. Farming practice

Organic farming had positive effects on SOC and total N contents. This is in agreement with our first hypothesis and with previous studies, which reported organic farming tends to retained more SOM, especially in the upper soil (Pulleman et al., 2003; Marriott and Wander, 2006; Gomiero et al., 2011; Gattinger et al., 2012). This can be explained by the quality of the organic input as the inclusion of legume-based and more diverse crop rotation in the organic farming in comparison with the integrated system and agrees with Marriott and Wander (2006) that the legume-based organic management is as capable of building SOM as systems receiving manure or compost.

Long-term organic farming decreased the WEOC and WEON contents in the current study, contrasting our first hypothesis and previous studies (Chantigny, 2003; Xu et al., 2013). However, they observed only immediate effects, directly after application of crop residues and manure (Chantigny, 2003) or alfalfa (Xu et al., 2013). The lower WEOM contents in the organic farming system might be caused by a larger consumption than production rate of WEOM, since the degradation of organic matter is the main source of WEOM in organic farming system and the organic farming system is low-input and no external organic input was added. Moreover, strong seasonal effects on WEOM were repeatedly reported by others (Bausenwein et al., 2008; Embacher et al., 2007; Liang et al., 2011).

The increased SUVA, FI and BIX indicated WEOM in organic farming contained more aromatic components and more new WEOM was produced in the organic farming system. The increased spectral indices also indirectly reflected the high activities of microorganisms in the organic farming system. The decreased F_{max} of fulvic- and humic-like components contrast our first hypothesis and the results of Zhang et al. (2011) who observed that manure addition increased the humic-like component identified by EEM-PARAFAC at surface soil.

The difference might attribute to the sources of WEOM in the current organic farming system were more diverse and of more easily degradable due to the presence of legumes in the crop rotation. Whereas in the study of Zhang et al. (2011), the crop rotation is a simple corn/cotton rye rotation. Moreover, inorganic N seems to promote the formation of water-soluble, brown and recalcitrant compounds (Fog, 1988), which is in line with the increase in humic/fulvic-like substances of the current integrated farming system. The SUVA indicates the content of aromatic compounds, while according to Zsolnay et al. (1999), HIX shows the condensation (H/C ratio) of organic matter. Organic farming showed opposite effects on SUVA and HIX of WEOM, although the two indices both reflect the aromaticity of WEOM. Since HIX is a ratio of the upper quarter (435–480 nm) to lower quarter (300–345 nm) of the emission spectra, the existence of protein-like components might interfere the HIX as the corresponding peak for protein-like compounds lies in the lower quarter of emission spectra (Cuss and Guéguen, 2015) could potentially explain the opposite effects. The SUVA and HIX values might also indicate WEOM in organic farming contains more aromatic components but less condensed.

4.2. Tree species

Robinia had general positive effects on SOC and total N contents at 0–25 cm depth as well as on SUVA values and PARAFAC components in comparison with poplar, confirming our second hypothesis. Similarly, Nii-Annang et al. (2009) observed higher SOC contents at 0–3 cm depth in a robinia than in poplar stands of an alley cropping system, re-cultivating quaternary deposits for 9 years. Also Medinski et al. (2015) observed significantly higher SOC stocks at 0–10 cm depth in robinia than in poplar agroforestry systems. The higher residue input indicated by the biomass of robinia in previous years (Supp.-Table 1), may be one reason for the higher SOM content in the robinia agroforestry system. Another reason might be the higher N and lower lignin contents of robinia leaf litter and root residues in comparison with poplar (Kaleeem Abbasi et al., 2015; Mafongoya et al., 1998; Thippayarugs et al., 2008). This leads to an increased formation of microbial residues and in the long-term to higher SOM contents and points to the demand of sufficient N supply for soil C sequestration (Khan et al., 2016). This view is in line with the current observation that robinia had stronger effects on the PARAFAC components of WEOM than on the WEOC and WEON contents.

4.3. Sampling distance

SOC was not significantly accumulated in hedgerow of the current alley cropping system in comparison with the sampling points 2 and 15 m apart. This contrasts our second hypothesis and the results of Nii-Annang et al. (2009) and Medinski et al. (2015), but is in agreement with Udawatta et al. (2014) who did not observe significant differences in SOC and total N between hedgerows and crop areas, 21 years after establishing an alley cropping agroforestry system. They attributed the results to the maturity of their system. In the current case, the short-term establishment of hedgerows for 4 years may explain the absence of effects on SOC and total N contents between the sampling points. The disturbance of soil during tree planting may cause SOC loss (Six et al., 1998) and the rooting behavior of tree is different to crops, i.e. a larger volume is less densely rooted by robinia and poplar in comparison whit arable crops. This might also explain the lower SUVA and HIX values of WEOM in the hedgerow in comparison with the sampling points 2 and 15 m apart. In addition, the agronomic performance, for example of winter wheat was worse than that at 15 m distance during the sampling periods.

4.4. The relationship between WEOM components revealed by EEM-PARAFAC and spectroscopic indices of WEOM

SUVA, HIX, BIX and FI characterize WEOM from various aspects. SUVA indicates the compounds with aromatic structure in WEOM (Akagi et al., 2007); HIX describes the condensation of WEOM reflected by the H/C ratio (Zsolnay et al., 1999); FI strongly correlates with the structural conjugation and aromaticity of WEOM and can be used to differentiate the source of substance in WEOM, terrestrial-derived (higher FI value) or microbially-derived (low FI value) (Johnson et al., 2011), and BIX is an indicator of the relative contribution of recently produced WEOM through microbial activities (Huguet et al., 2009). Since the fulvic/humic-like substances are more chemically condensed and complex than tryptophan-like substances and have complex structure (Hudson et al., 2007). The positive correlation between SUVA and C3 (tryptophan-like substances) implied the C3 component mainly contributed to the SUVA of WEOM in current study. The positive correlation between C1, C2, C4 and the fluorescence-derived indices indicated the fulvic/humic-like substances contribute positively to the humification index of WEOM. This partially supports humification is a process decompose organic materials to structurally condensed substances. The negative correlation between BIX, FI and C1, C2, C4 indicated the higher fulvic/humic like substance in WEOM under natural conditions, the more WEOM might be microbially-derived and microbial process of organic materials leads to condensation of organic matter. Microbial activities and microorganism per se are important source of WEOM. From above, the WEOM components revealed by EEM-PARAFAC can also be used to judge the quality of WEOM.

5. Conclusions

Our data showed that long-term low-input organic farming had positive effects on SOC and total N as well as general negative effects on WEOM and its PARAFAC components. The spectral indices indicated the higher presence of more fresh and microbially-derived organic components of WEOM in the organic farming than in the integrated farming system. Robinia as hedgerow tree for 4 years showed positive effect on SOC and total N contents in comparison with poplar and had stronger effects on the PARAFAC components of WEOM, although the WEOM content did not differ between the two tree species. The robinia effects were more pronounced in the organic than in the integrated farming system. The correlation analysis suggested the WEOM component revealed by EEM-PARAFAC can be used to judge the quality of WEOM directly. Consequently, low-input organic farming and robinia tended to result in change of quality of WEOM and led to enrichment of WEOM of high stability. The alley-cropping agroforestry system combining robinia with organic farming has a great potential for sequestering SOC and developing a sustainable agroecosystem.

Table 1. Total above dry biomass (t ha -1) of Poplar and Robinia in the previous years (2009–2012)

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The following are the supplementary data related to this article.

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