



Mineral-associated organic carbon and black carbon in restored wetlands



Qing Wang^{a,b}, Ping-Jiu Zhang^b, Min Liu^{a,*}, Zheng-Wei Deng^b

^a Department of Geography, Key Laboratory of Geo-information of the Ministry of Education, East China Normal University, Shanghai 200062, China

^b Anhui Key Laboratory of Natural Disasters Process and Prevention, College of Territorial Resource and Tourism, Anhui Normal University, Wuhu 241003, Anhui, China

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ABSTRACT

Stable soil organic carbon (SSOC) has a vital influence on soil's capacity for resisting disturbance and sequestering carbon. However, studies of the SSOC in restored wetlands are incomplete. We studied mineral-associated organic carbon (MOC) and black carbon (BC), two representatives of SSOC, in a wetland restoration chronosequence in Caizi Lake, East China. MOC was analyzed by both a physical method, involving dividing soil into size-fractions POM (>53 μm) and MOM (0.45–53 μm) and detecting OC in MOM (MOC_P), and a chemical method, acidifying soil with hydrofluoric acid (HF) and detecting OC loss (MOC_C). BC was analyzed by methods using dichromate (BC_{Cr}) and chemothermal oxidation (BC_{CTO}). MOC_C, BC_{Cr}, and BC_{CTO} were further determined in MOM for comparing results from different fractions and methods. We found an elevation of soil ecological function in wetlands restored from farmlands in Caizi Lake, which was supported by a general increase of MOC and BC with time after restoration at surface soil (0–6 cm). Comparison of methods showed that size fractionation method overestimated MOC by identifying all OC in MOM as MOC, since HF-soluble OC accounted for no more than 23.5% of the whole OC in MOM. As it may separate more components, e.g. charcoal, from BC continuum, the dichromate method was recommended as more meaningful in the study of BC recovery in restored wetlands with biomass-burning history. We presented the possible use of ratio (BC_{Cr}–BC_{CTO}):BC_{CTO} for BC source identification which produced a reasonable result similar to that of a commonly used method. The combination of physical and chemical fractionation methods revealed that the proportion of BC to TOC in POM was considerable, which indicated that POM was not invariably labile as was commonly understood. Finally, a detailed list of soil organic carbon components including MOC, BC and labile OC in each size fraction was represented by using sequential analysis and combination of fractionation methods.

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1. Introduction

Stable soil organic carbon (SSOC) after land use change has received considerable attention (Su et al., 2009; Saha et al., 2011; Poeplau and Don, 2013) for its fundamental role in affecting soil's capability to resist disturbance and sequester carbon (Lal, 2004; Dignac and Rumpel, 2012). SSOC has been shown to become a dominant component of total soil carbon relative to labile OC after land reclamation (Cambardella and Elliott, 1993; Lü and Liu, 2008; Song et al., 2012a). Those findings predicted a functional degradation under disturbance in the fragile soil lacking stable carbon. On

the other hand, soil carbon saturation has been observed in different soils where the increase of soil OC content was not proportional to the increase of OC input (Six et al., 2002a; Stewart et al., 2007, 2009). When the SSOC was saturated, the input carbon which accumulated in the labile pool was decomposed subsequently and did not enhance the carbon content remaining in the soil (Gulde et al., 2008).

Mechanisms of organic matter stabilization include chemically innate recalcitrance, protection through interaction with minerals, and occlusion in aggregates (Mikutta et al., 2006) or lack of microbial decomposers (Smernik and Skjemstad, 2009). Black carbon (BC) represents the first mechanism (Marschner et al., 2008), which gains stability from aromatic structure (Lehmann and Joseph, 2009). Mineral-associated organic carbon (MOC) is an example of the second mechanism, which obtains stability from physical

* Corresponding author. Tel.: +86 21 54341196; fax: +86 21 54345007.
E-mail address: mliu@geo.ecnu.edu.cn (M. Liu).

sorption to minerals and subsequently chemical bond with the surface (Kaiser et al., 2007).

Both BC (Brodowski et al., 2005; He and Zhang, 2006; Major et al., 2010; Knoblauch et al., 2011) and MOC (Piccolo et al., 2004; Plante et al., 2006; Yuan et al., 2008; Rumpel et al., 2010) have been studied globally in urban, cultivated, and forest soil. However, methods varied. One study (Hammes et al., 2007) compared seven methods to separate BC from soils and sediments, i.e. CTO-375, BPCA, Cr₂O₇, TOT/R, TG-DSC, NaClO, and UV, and summarized the advantages and disadvantages of each method. Method CTO-375 was believed to quantify exclusively the most condensed forms of BC (e.g., soot) and was found to have relatively high reproducibility (Hammes et al., 2007). It was the most widely used method to isolate BC from soils and sediments (Gustafsson et al., 1997, 2001; Nguyen et al., 2004). Method Cr₂O₇ was found to quantify both soot and relatively lower condensed char of BC continuum (Hammes et al., 2007) and was also widely used particularly in the studies taking charcoal into account (Lim and Cachier, 1996; Song et al., 2002; Rumpel et al., 2006a). Several methods separate MOC from soils, including size fractionation (Christensen, 2001), density fractionation (Rumpel et al., 2012), combination of size and density fractionation (Six et al., 2002b; Moni et al., 2012; Plaza et al., 2012), chemical fractionation (Eusterhues et al., 2003) and combination of physical and chemical fractionation (Plante et al., 2006; Lorenz et al., 2008; Lopez-Sangil and Rovira, 2013). Recently, analysis of MOC and recalcitrant OC were combined (von Lützow et al., 2007). For example, the chemical fraction was further separated from the mineral-associated fraction (Plante et al., 2005; Lorenz et al., 2008; Jagadamma and Lal, 2010). Meanwhile, a promising method of sequential chemical analysis of MOC and recalcitrant OC has emerged (Mikutta et al., 2006).

Different methods lead to different ecological meanings of SSOC components, and not all methods are applicable equally to all ecosystems. This paper covers a range of methods to measure SSOC and clarify the method-oriented uncertainty in restored wetlands. Approaches considered include: (1) size fractionation and HCl/HF treatment, which isolate MOC physically and chemically, (2) method CTO-375 and method Cr₂O₇, which analyze BC, (3) a combination of physical and chemical fractionation to measure MOC and BC in size fractions, and (4) a sequential chemical analysis of MOC and BC.

Converting reclaimed wetlands from agriculture is an important practice of wetlands restoration (Pan and Wang, 2009; De Steven and Gramling, 2012). The subsequent changes in ecological and biogeochemical processes in restored wetlands were studied widely (Lü and Liu, 2008; Vymazal, 2011; Inglett and Inglett, 2013). Enhancement of soil OC is an important indicator (Meyer et al., 2008) of ecological recovery in wetlands. However, most current studies focus on active OC (Stern et al., 2007; Höll et al., 2009; Wang et al., 2012; Song et al., 2012a), while SSOC change in restored wetlands has seldom been studied. By contrast, changes of stabilized OC have been studied recently in other restored ecosystems like pasture (Mosquera et al., 2012) and grassland (O'Brien and Jastrow, 2013).

The present study (1) describes the characteristics and change of BC and MOC in topsoil of lacustrine wetlands recovered from farmland for different restoration durations and (2) discusses ecological meanings of SSOC components analyzed by different methods in restored wetlands.

2. Materials and methods

2.1. Study area

Caizi Lake, located in Anhui Province, East China, is an important member of lacustrine wetlands located along the middle and lower

reaches of the Yangtze River. The lake is connected to the river and has an area of 242.30 km² in summer and 145.20 km² in winter. The mean annual temperature is 16.6 °C, and the mean annual precipitation is 1325 mm (Gao et al., 2011). Most of the wetlands at Caizi Lake were destroyed by extensive reclamation in the 1950's, but have been restored gradually since 1980's, resulting in a chronosequence of restored wetland. Rape (*Brassica campestris* L.) was the most popular crop sown in dry season before restoration whereas *Carex* grasses dominate currently. Burning crop straw was included in local farmland management for convenience and fertilizer. Burning grasses in restored and semi-pristine wetlands was common and black residues from biomass burning can be observed visually in soils of some sites. All sampling sites were far away from cities and industrial activities. The nearest industrial activity is from a petrochemical industrial park located 50 km south and the nearest big city is located about 100 km north of the sampling sites.

2.2. Sample collection

Sampling sites were chosen in triplicate with different duration of restoration (2, 5, 8, 10, 20 years) and each site had an area of 900 m² (30 m × 30 m). An intact lacustrine wetland was also chosen for comparison. Samples were collected by mixing five soils gained along an "S" curve from each site. Collected topsoil (0–16 cm) was divided into two layers (0–6 cm and 6–16 cm) according to the physical characteristics of the soil profile. Surface layer was brownish and soft whereas the layer below was yellowish and compact.

2.3. Analysis methods

Two representative analysis methods for each SSOC component were used. A combination of fractionation and a sequential chemical isolation were also considered. Fractionation and analysis scheme is illustrated in Fig. 1.

2.3.1. Physically isolated MOC (MOC_p)

MOC_p was determined based on Plante et al. (2006) and Zhang and He (2004). 40 g air-dried soil was passed through a 2-mm porosity sieve, and then added to a 250-mL bottle which was filled with distilled water containing 5 g/L sodium hexametaphosphate. The bottle was shaken for 18 h at 90 rpm. The suspension in the bottle was transferred to a 53-µm sieve, and rinsed with distilled water. The matter left on the sieve was considered as particulate organic matter (POM). The slurry that passed through the sieve was collected in a beaker simultaneously followed by filtering through a 0.45-µm membrane with a vacuum pump. The matter left on the membrane was considered as mineral-associated organic matter (MOM), and the solution that passed through the membrane was considered as containing dissolved organic matter (DOM). The POM and MOM were collected and dried at 60 °C, and then weighed before grinding and sieving through a 150-µm sieve. The volume of DOM solution was recorded and a drop of sulfuric acid was added to the solution. The solution was preserved at 4 °C. Organic carbon concentrations of POM, MOM and DOM (i.e. POC, MOC and DOC) were determined and expressed in g C/kg dry bulk soil. POC and DOC were used for balance calculations.

2.3.2. Chemically isolated MOC (MOC_c)

The carbon released by hydrofluoric acid (HF) treatment is associated with the mineral matrix (Eusterhues et al., 2003). The original MOC_c determination procedure (Eusterhues et al., 2003) was amended for this study. Based on the almost identical ¹³C CPMAS NMR spectra of HF treated and untreated mineral-free litter samples, they assumed that the HF treatment dissolved only soil

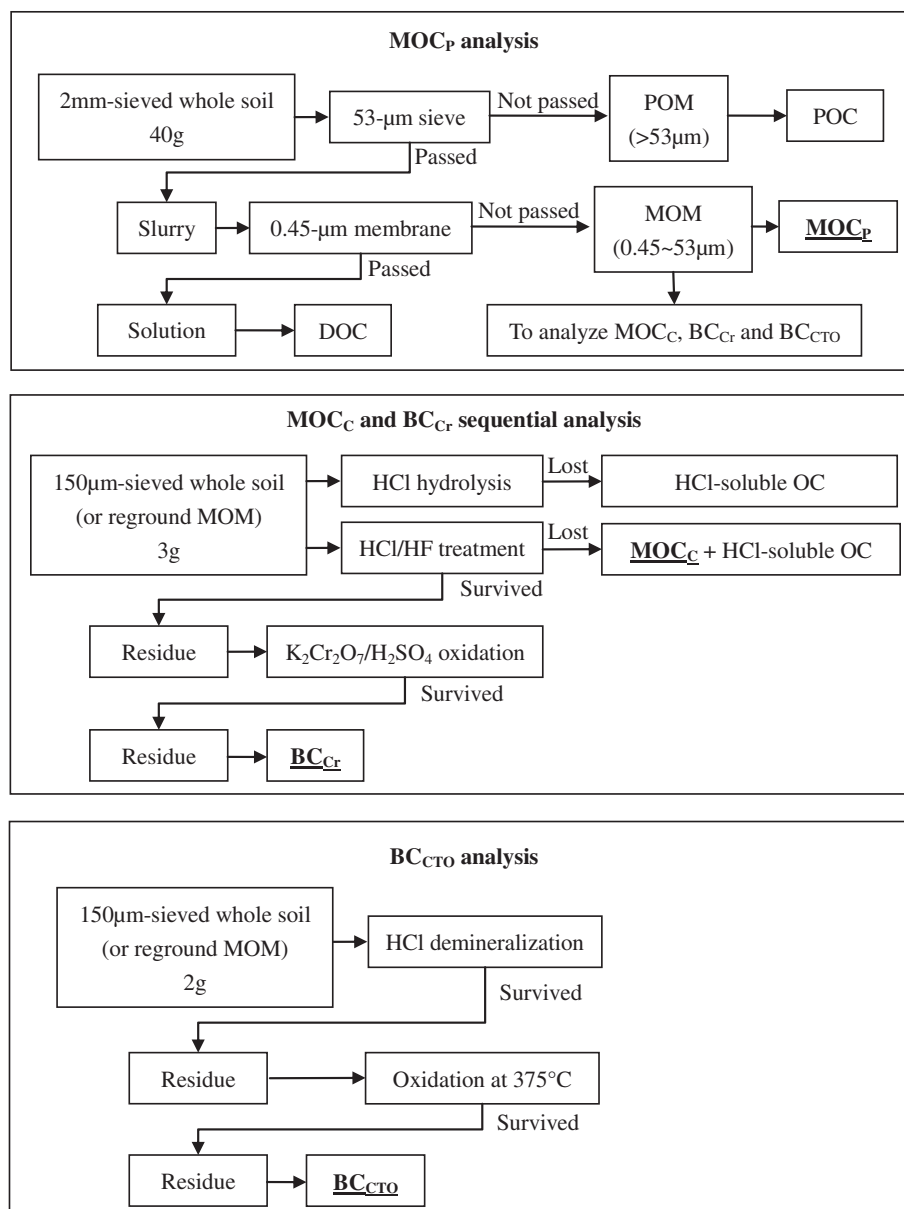


Fig. 1. Fractionation and analysis scheme used in this study. Definitions: MOC_p is physically isolated mineral-associated organic carbon. MOC_c is chemically isolated mineral-associated organic carbon. BC_{cr} is BC analyzed by the Cr₂O₇ method. BC_{cto} is BC analyzed by the CTO-375 method. POM (particulate organic matter) is the soil fraction with size of 53 μm–2 mm. MOM (mineral-associated organic matter) is the soil fraction with particle sizes of 0.45–53 μm. POC is TOC in POM. DOC is dissolved organic carbon in the water. MOC_p is quantified as TOC in MOM. MOC_c is quantified as HF-soluble OC, calculated by the difference between HCl-soluble OC and HCl/HF-soluble OC. OC loss in the HCl or HCl–HF treatments is the difference between the total OC in solid samples before and after acid treatment.

minerals and mineral-bound organic matter, while the non-bound soil organic matter remained nearly unaffected (Eusterhues et al., 2003). Hydrochloric acid (HCl) was used in this modified method for two reasons. First, HF reacts more quickly with carbonates than with silicates and HCl reacts more quickly with carbonates than HF. The presence of HCl prevents HF from reacting with carbonates and therefore avoids waste of expensive HF. Second and more importantly, calcium fluoride formed during the reaction of HF with silicates has a low solubility and tends to stop the reaction by depositing on the mineral surface. The presence of HCl, by keeping pH of the solution at a low level, enhances the solubility of calcium fluoride and therefore avoids both the ceasing of reaction and positive weighing error. A separate HCl hydrolysis experiment was conducted to exclude the OC loss by HCl hydrolysis from MOC. MOC_c is quantified as HF-soluble OC, calculated by difference between HCl-soluble OC and HCl/HF-soluble OC.

Air-dried soil (3 g) was ground to pass a 150-μm sieve, and added to a centrifugal tube. The tube was filled with 15 mL 3 M HCl and shaken for 15 min in an ultrasonicator. After a reaction time of 24 h, the soil in tube was rinsed 3–6 times with distilled water by shaking and centrifuging. The residue was allowed to react with 15 mL 10 M HF/1 M HCl (2:1, v:v) for 24 h. Then the cleaning procedure (rinse and centrifugation) was repeated until the solution pH rose to 4–5. Another 24-h reaction with 15 mL 10 M HCl was conducted, followed by the same cleaning procedure mentioned above. The residue was oven-dried, weighed and detected for OC. The OC loss (i.e. HCl/HF-soluble OC) was calculated.

In a separate experiment like the procedure in HCl/HF treatment, approximately 3 g soil was allowed to react with 15 mL 10 M HCl at room temperature (~10 °C). The reaction occurred on a horizontal shaker at 200 rpm for 24 h. The solution was then centrifuged and rinsed twice. The OC content in the dried residue

was analyzed by an element analyzer and the OC loss by HCl hydrolysis (i.e. HCl-soluble OC) was calculated.

2.3.3. BC analyzed by method Cr₂O₇ (BC_{Cr})

BC_{Cr} was determined following published procedures (Lim and Cachier, 1996; Song et al., 2002). Reground residue, remaining from acid treatment in MOC_C analysis, was spiked with 15 mL 0.1 M K₂Cr₂O₇: 2 M H₂SO₄ (1:1, v:v), and the reaction lasted 60 h at 55 °C. During the reaction, K₂Cr₂O₇ was added after the color changed after shaking to keep the oxidant in excess, and distilled water was added several times to maintain the solution volume. After the cleaning procedure, the residue was oven-dried and weighed. The organic carbon content in the residues was regarded as BC.

2.3.4. BC analyzed by method CTO-375 (BC_{CTO})

BC_{CTO} was determined based on Gustafsson et al. (1997, 2001). Ground whole soil samples were weighed and put into pre-weighed porcelain crucibles. After the soil was wetted with distilled water, 1 M HCl was added into each crucible twice during an interval of 1 h. Then the crucibles were dried at 60 °C in an oven. The acidification was repeated until effervescence upon acid addition ceased. The remains were ground again and oxidized in a muffle furnace at 375 °C for 24 h. The excess oxygen calculated by sample mass and the inner volume of furnace suggested the absence of charring. The organic carbon of the residue surviving after oxidation was considered as BC.

2.3.5. Combining physical and chemical fractionation

The distribution of SSOC in soil size fractions was studied using a combination of physical and chemical fractionation. MOC_C, BC_{Cr} and BC_{CTO} in MOM were determined further. The calculation of MOC_C in MOM did not include subtraction of DOC. OC concentrations in MOM were expressed in g C/kg MOM.

2.3.6. Sequential chemical analysis

The analysis of MOC_C and BC_{Cr} occurred in a successive and integrated experiment and was a sequential chemical analysis of SSOC in soil.

Contents of OC in soil and solution were determined in triplicate using wet oxidation with dichromate followed by titration with ferrous sulfate (Bao, 2000; Li et al., 2004). BC was quantified by a CHNS elemental analyzer (elementar™ Vario MICRO cube).

2.4. BC in size fractions

Combined fractionation analysis of SSOC components provides a chance to calculate, among the total content of BC in bulk soil, what percentage of it exists in MOM and in POM. The distribution of BC in fine fraction (MOM) and coarse fraction (POM) was calculated by the following equations:

$$D_M = BC_M f_M / BC_B * 100\% \quad (1)$$

$$D_P = 100\% - D_M \quad (2)$$

where D_M and D_P are the distribution of BC in MOM and POM, respectively (%); BC_M is BC content in MOM (g/kg MOM); f_M is the bulk soil mass fraction of MOM (%); BC_B is BC content in bulk soil (g/kg bulk soil).

Proportions of BC to TOC in MOM and POM were also considered. BC:TOC in MOM and POM were calculated by the following equations:

$$R_M = BC_M / TOC_M * 100\% \quad (3)$$

$$R_P = (BC_B D_P / f_P) / TOC_P * 100\% \quad (4)$$

where R_M and R_P are ratios of BC:TOC in MOM and POM, respectively (%); TOC_M and TOC_P are TOC in MOM and POM, respectively (g/kg MOM or POM); f_P is the bulk soil mass fraction of POM (%); BC_M , BC_B and D_P have the same meanings as in Equations (1) and (2).

2.5. Data processing

The relationships of SSOC with the duration of restoration and that with TOC were examined by linear regression, and the differences between SSOC components and SSOCs between 0–6 cm and 6–16 cm soils were compared by *t*-test.

3. Results and discussion

3.1. Mineral-associated organic carbon components

3.1.1. MOC_P

Change of MOC_P content with the duration of restoration is shown in Table 1. MOC_P content increased significantly with the duration of restoration at the 0–6 cm interval, but not at the 6–16 cm interval (Table 2). MOC_P was related significantly to TOC in both layers (Table 2). The average content of MOC_P at 0–6 cm was 8.61 ± 1.88 g/kg, for all sampling sites including the intact wetland. This value was statistically ($p < 0.01$) higher than that at 6–16 cm (5.50 ± 1.20 g/kg). The proportion of MOC_P to TOC was on average 59.0 ± 17.5%, and resembled the results from a long-term experiment paddy field where MOC, analyzed by a similar method, constituted 50.1%–74.0% of TOC at the 0–15 cm interval (Yuan et al., 2008).

MOC may be determined by subtracting POC from TOC (Ji et al., 2012) or detecting OC in the dried slurry that passed through the sieve (Piccolo et al., 2004; Yu et al., 2006). These methods were apparently based on a hypothesis that soil TOC is comprised exclusively of POC and MOC. However, DOC, a small but significant carbon component was ignored. In this research, 98.7 ± 3.4% recovery was obtained by calculated by dividing POM and MOM into bulk soil mass, but the ratio of the sum of MOC_P and POC to TOC, i.e. TOC recovery, was only 92.7 ± 8.3%. Including DOC in the calculation raised the recovery to 98.1 ± 9.5% (Table 1). Counting labile DOC in stable MOC would lead to an overestimate of MOC and a dubious conclusion, especially in the soil with high DOC concentration.

3.1.2. MOC_C

MOC_C content at a depth of 0–6 cm increased significantly with the duration of restoration (Table 2) while MOC_C content at a depth of 6–16 cm increased during the first 10 years after restoration and then decreased. MOC_C was related significantly to TOC at both layers (Table 2). MOC_C content at 0–6 cm, with an average of 3.70 ± 1.94 g/kg, was statistically ($p < 0.05$) higher than that at 6–16 cm with an average of 1.80 ± 0.64 g/kg. The proportion of MOC_C to TOC was on average 21.1 ± 8.4%. By comparison, the OC loss due to HF treatment was 19%–45% in forest soils of similar depth to our samples (Eusterhues et al., 2003). In a slash and burn agriculture land, 24%–33% of OC was lost after HF treatment (Rumpel et al., 2006a).

On average, 33% and 54% OC were lost after 10 M HCl hydrolysis and HCl/HF treatment, respectively. By subtracting the HCl-soluble component from the HCl/HF-soluble fraction, we estimate that HF-soluble OC is about 21% of TOC in restored wetlands. This value

Table 1
Contents of MOC_p and recoveries of TOC in MOC_p analysis procedures.

Sites	The duration of restoration/a	Depth/cm	TOC/g/kg	MOC _p /g/kg	POC/g/kg	DOC/g/kg	Recovery 1 of TOC ^a /%	Recovery 2 of TOC ^b /%
SX1	2	0–6	9.06 ± 0.61	6.54 ± 1.13	2.09 ± 0.06	1.12 ± 0.10	95.22	107.58
		6–16	6.57 ± 0.56	5.26 ± 0.53	1.26 ± 0.05	0.45 ± 0.00	99.17	105.95
XR2	5	0–6	12.30 ± 0.52	7.37 ± 0.66	4.42 ± 0.06	0.27 ± 0.02	95.55	97.73
		6–16	6.99 ± 0.31	5.15 ± 0.29	1.06 ± 0.03	0.18 ± 0.02	88.88	91.53
XR3	8	0–6	14.30 ± 0.30	6.46 ± 0.69	6.49 ± 0.07	0.43 ± 0.02	90.51	93.54
		6–16	7.32 ± 0.58	5.18 ± 0.03	0.96 ± 0.07	0.17 ± 0.02	83.87	86.18
XR4	10	0–6	15.50 ± 1.45	10.02 ± 0.62	6.67 ± 0.06	0.87 ± 0.02	107.78	113.41
		6–16	9.39 ± 0.10	5.87 ± 0.27	1.85 ± 0.01	0.34 ± 0.03	82.26	85.86
XZ1	20	0–6	29.90 ± 0.11	11.15 ± 0.75	12.87 ± 0.10	0.42 ± 0.04	80.34	81.72
		6–16	8.98 ± 0.19	7.71 ± 0.71	1.46 ± 0.03	1.08 ± 0.15	102.08	114.13
XZ2	20	0–6	24.00 ± 0.59	9.18 ± 0.24	12.96 ± 0.11	1.28 ± 0.07	92.29	97.63
		6–16	9.06 ± 0.18	6.54 ± 0.24	1.72 ± 0.05	0.45 ± 0.04	91.19	96.17
YZ1	20	0–6	28.30 ± 0.80	10.67 ± 0.04	15.44 ± 0.11	1.16 ± 0.11	92.30	96.41
		6–16	6.42 ± 0.27	4.16 ± 0.41	1.00 ± 0.00	0.88 ± 0.13	80.28	94.04
XR1	Intact wetland	0–6	18.40 ± 0.57	7.49 ± 0.20	10.79 ± 0.23	0.77 ± 0.02	99.38	103.57
		6–16	4.79 ± 0.16	4.11 ± 0.33	0.78 ± 0.04	0.07 ± 0.00	102.05	103.49

TOC is total organic carbon in bulk soil. MOC_p is defined as whole OC in MOM (fraction with size between 0.45 and 53 μm). POC is whole OC in POM (fraction with size between 53 μm and 2 mm). DOC is the dissolved organic carbon in the water.

^a (MOC_p + POC)/TOC.

^b (MOC_p + POC + DOC)/TOC.

seems to be a low limit of MOC_C since HCl can also release some mineral-associated OC by dissolving carbonate minerals.

OC that was adsorbed on and protected by minerals was believed to be released into solution when the surface of silicate matrix was dissolved by 10 M HF (Lim and Cachier, 1996). The HF treatment was assumed to only remove mineral-associated OC without affecting other form of OC as no apparent change was found in the isotopic signatures and the spectra of ¹³C CPMAS NMR and FTIR (Rumpel et al., 2006b). In addition to handling procedure effects, DOC may respond to the carbon loss (5%–10% of TOC) when the HF treatment is applied to mineral-free samples (Eusterhues et al., 2003). In this study, however, DOC was excluded via the difference of HCl-soluble and HCl/HF-soluble OC. Therefore, we assume that almost all of the HF-soluble OC in our analysis was mineral-bound.

3.1.3. Overestimate of MOC by using size fractionation method

The proportion of MOC_p to TOC was statistically ($p < 0.01$) higher than that of MOC_C, which was probably due to the fact that not all of the OC in MOM is chemically mineral-associated. This idea was confirmed by a low ratio of MOC_C to TOC in MOM. No more than 23.5 ± 10.5% of the whole OC in MOM was HF-soluble (Fig. 2). The HF/HCl treatment has proven efficient in removing mineral-associated OC by dissolving the surface of silicate matrix (Lim and Cachier, 1996) and Fe/Al oxide (Chen et al., 2011), to which the organic matter is bound (Schneider et al., 2010; Mikutta and Kaiser, 2011). Thus the mineral-associated organic carbon component was overestimated by the size fractionation method. The MOC_p content was on average 1.9 times higher than that of MOC_C in bulk soil. The overestimation was also inferred when a sequential chemical extraction was applied to mineral-associated organic matter (<20 μm) in four types of soils. Only 55%–75% of OC in MOM was explained by mineral protection (Lopez-Sangil and Rovira, 2013).

3.2. Black carbon components

3.2.1. BC_{Cr}

BC_{Cr} content at 0–6 cm depth increased significantly with the duration of restoration while no such relationship was found at 6–16 cm depth (Table 3). BC_{Cr} was significantly related to TOC at 0–6 cm. No significant relationship was observed at 6–16 cm (Table 3). BC_{Cr} content at 0–6 cm averaged 2.90 ± 1.09 g/kg and was

statistically ($p < 0.01$) higher than that at 6–16 cm, which averaged 1.38 ± 0.43 g/kg. The proportion of BC_{Cr} to TOC ranged from 10.9% to 29.9% and averaged 17.3 ± 5.0%. The ratio was comparable to published data, with BC estimated by HF-dichromate chemical oxidation. The BC:TOC ratio was on average 19% in soils (0–10 cm) in urban areas with few industrial effects such as park, campus, residential area, and vegetable garden (He and Zhang, 2006), and 11.7%–12.5% for forest soils (0–20 cm) (Yin et al., 2009a).

3.2.2. BC_{CTO}

BC_{CTO} content showed no significant relationship with the duration of restoration or with TOC at both 0–6 cm and 6–16 cm soils (Table 3). BC_{CTO} content at 0–6 cm, with an average of 1.29 ± 0.50 g/kg, was statistically ($p < 0.01$) higher than that at 6–16 cm with an average of 0.66 ± 0.04 g/kg. The proportion of BC_{CTO} to TOC was from 3.27% to 13.64% (8.52 ± 2.99% on average), and resembled the result of coastal wetlands in Shanghai (10.3 ± 4.5%) (Cheng, 2009).

3.2.3. Ratio of BC_{Cr} and BC_{CTO} and its possible use of identifying BC sources

There was no significant relationship between BC_{Cr} and BC_{CTO} content at 0–6 cm soil or at 6–16 cm soil (Table 3). BC_{Cr} content was statistically ($p < 0.01$) higher than BC_{CTO} content. The discrepancy can be explained by the difference of analytical methods. BC surviving from HF–Cr₂O₇ oxidation, i.e. BC_{Cr}, was believed to include charcoal and soot (Lim and Cachier, 1996) and even non-BC fractions when interfering materials existed (Hammes et al., 2007). On the other hand, soot was reported to be the only fraction among the BC continuum separated by method CTO-375 (Masiello et al., 2002), i.e. BC_{CTO}. Even some wood soot and grass soot were found to fail to be detected by method CTO-375, and only diesel- and hexane-derived soots could be effectively separated (Song et al., 2012b). The primary positive bias of method CTO-375 was charring (Masiello, 2004). However, charring could be avoided when the fine ground samples and sufficient oxygen were used (Gustafsson et al., 2001), as considered in our analysis.

The ratio (BC_{Cr}–BC_{CTO}):BC_{CTO} was tested for its possible use to identify BC sources by comparing the result with a commonly used method. The (BC_{Cr}–BC_{CTO}):BC_{CTO} approach is based on the published hypotheses that (1) the Cr₂O₇ method separated charcoal and soot (Lim and Cachier, 1996) and the CTO-375 method

Table 2Linear correlation coefficients (*r*) between the duration of restoration, TOC, MOC_p and MOC_c.

	The duration of restoration ^a	TOC	MOC _p	MOC _c
The duration of restoration	1	0.9733***	0.8332*	0.7499
TOC	0.4230	1	0.8399**	0.6366
MOC _p	0.3832	0.8386**	1	0.6997
MOC _c	0.4634	0.9054**	0.7091*	1

*** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$. TOC is total organic carbon in bulk soil. MOC_p is defined as whole OC in MOM (fraction with size between 0.45 μm and 53 μm). MOC_c is defined as the difference between HCl-soluble OC and HCl/HF-soluble OC.

^a N is 7 for the duration of restoration, and is 8 for others. The values in upper triangular matrix are for 0–6 cm soil and the values in lower triangular matrix are for 6–16 cm soil.

separated only soot that originated from high temperature burning (Song et al., 2012b); (2) soot was the main component of BC from fossil fuel burning while charcoal, plus a small quantity of soot, from biomass burning (Masiello, 2004); (3) soot with a long transport distance had a signal of background atmosphere and charcoal was a representative of local burning (Masiello, 2004). Therefore, a ratio $(\text{BC}_{\text{Cr}} - \text{BC}_{\text{CTO}}) : \text{BC}_{\text{CTO}}$ close to zero may infer that burning of fossil fuel was probably the main source of BC while a ratio more than 0.8 (in view of the possible source of soot from biomass burning and atmospheric transportation, 0.8 was used rather than 1.0) may infer that local biomass burning contributed to most of BC in soil.

The ratios of $(\text{BC}_{\text{Cr}} - \text{BC}_{\text{CTO}}) : \text{BC}_{\text{CTO}}$ of restored wetlands soils were greater than 0.8 except for site XR2 and the 6–16 cm depth of sites XR3 and SX1, from which we supposed biomass burning was the main source of BC. This result was confirmed by using method BC:TOC which has been applied successfully to identifying BC source in urban soils (Liu et al., 2011) and forest soils (Yin et al., 2009a). The method BC:TOC works based on a hypothesis that biomass burning and fossil fuel-caused aerosols had a typical BC/TOC ratio of 0.11 and 0.50, respectively (Novakov et al., 2000). BC defined in that method was similar to BC_{CTO} in this paper. $\text{BC}_{\text{CTO}} : \text{TOC}$ in restored wetlands varied from 0.03 to 0.14 with an average of 0.09, which suggested that biomass burning was the dominant source. Results of both BC:TOC and $(\text{BC}_{\text{Cr}} - \text{BC}_{\text{CTO}}) : \text{BC}_{\text{CTO}}$ were reasonable since the lacustrine wetlands of Caizi Lake had not been influenced directly by industrial activities.

The method $(\text{BC}_{\text{Cr}} - \text{BC}_{\text{CTO}}) : \text{BC}_{\text{CTO}}$ seemed not reliable when it was applied to fine fraction. Charcoal has a size of millimeter to

submicron (Masiello, 2004). It is so coarse that some may be excluded from fine fraction and thus fails to be detected. All ratios of $(\text{BC}_{\text{Cr}} - \text{BC}_{\text{CTO}}) : \text{BC}_{\text{CTO}}$ in MOM were less than 0.8 with an average of 0.36. Another uncertainty of this method was caused by the loss of BC after its entering wetland soil. Soot could be taken away by water and charcoal could be broken into smaller size or be oxidized, which may result in large variation of the ratio of charcoal to soot.

As biomass burning was the main source of BC in the wetlands, charcoal played an important role in soil SSOC change. Not considering charcoal would decrease the reliability of any conclusion about stable soil OC. However, charcoal was missing in analysis of BC_{CTO} . Furthermore, a low correlation existed between BC_{CTO} and TOC as well as the duration of restoration (Table 3). Therefore, we considered that BC_{Cr} was more meaningful than BC_{CTO} in the study of SSOC in restored wetlands, especially those with biomass-burning history.

3.3. Combination of fractionation methods

3.3.1. Distribution of BC in MOM and POM

In view of the performance of BC_{Cr} and BC_{CTO} demonstrated above, BC_{Cr} was chosen as an example, except special mention, for discussion in Sections 3.3 and 3.4.

According to the results calculated by Equations (1) and (2), the distribution of BC in MOM varied from 28.4% to 84.4% with an average of 55.5%, and that in POM varied from 15.6% to 71.6% with an average of 44.5% (Table 4).

If soot was solely taken into account, as revealed by results of BC_{CTO} , on average, 91.3% of soot was contributed by MOM, which

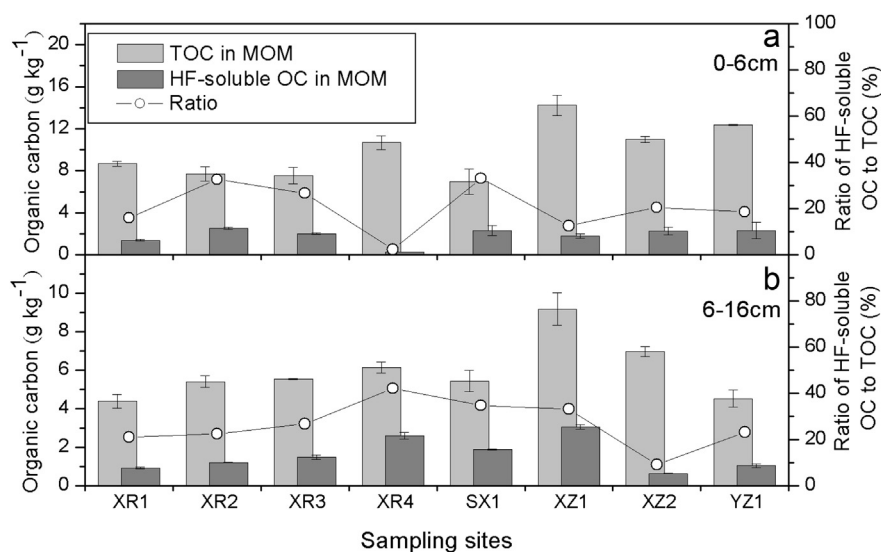


Fig. 2. Proportion of HF-soluble OC to TOC in MOM at depth of 0–6 cm (a) and 6–16 cm (b). The light gray bars are TOC in MOM, i.e. MOC_p; the dark gray bars are HF-soluble OC, i.e. MOC_c, in MOM. Error bars are standard deviations. All OC concentrations are expressed as g C/kg MOM.

Table 3
Linear correlation coefficients (*r*) between the duration of restoration, TOC, BC_{Cr} and BC_{CTO}.

	The duration of restoration ^a	TOC	BC _{Cr}	BC _{CTO}
The duration of restoration	1	0.9733***	0.9073**	-0.0547
TOC	0.4230	1	0.7399*	-0.0684
BC _{Cr}	0.3943	0.5350	1	0.5360
BC _{CTO}	-0.1860	0.4696	0.6769	1

*** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$. BC_{Cr} is BC analyzed by method dichromate. BC_{CTO} is BC analyzed by method CTO-375.

^a N is 7 for the duration of restoration, and is 8 for others. The values in upper triangular matrix are for 0–6 cm soil and the values in lower triangular matrix are for 6–16 cm soil.

indicated that soot existed mostly in silt and clay fractions while charcoal existed primarily in the coarse fraction.

3.3.2. Proportions of BC to TOC in MOM and POM

According to the results calculated by Equation (3), the average of BC:TOC in MOM was 15.6% (Table 4). As a ratio of recalcitrant carbon to the whole OC in fine fraction, this result was comparable to that of Plante et al. (2005) who found 86.8% of TOC in clay fraction lost after H₂O₂ treatment. It also resembled the results from a meadow surface soil where 14.2% and 9.4% OC survived in silt and clay fractions respectively after Na₂S₂O₈ oxidation (Lorenz et al., 2008). If it was expressed as g/kg bulk soil, BC content in MOM accounted for 9.2% of TOC in wetlands of Caizi Lake, which was slightly higher than the results of Jagadamma and Lal (2010) who gained a total of 3.1–7.5% of TOC remaining in silt and clay fractions after H₂O₂ treatment.

BC:TOC in POM was on average 30.6%. The value was 18.7% at 0–6 cm depth and 42.5% at 6–16 cm depth. The relative enrichment of BC in the coarse fraction at greater depth was also found in a German arable soil (Brodowski et al., 2007).

3.3.3. Reconsidering the role of POM in carbon saturation hypothesis

The unexpected high proportion of BC in POM challenged the common understanding that POM was labile. The paradoxical result has, actually, been reported indirectly by some studies where considerable BC was found in the fractions similar to POM such as light fraction or coarse fraction. The BC (analyzed by method dichromate) contributed 23.3% of TOC in light fraction (<1.8 g/cm³) at 0–20 cm depth of a forest soil (Yin et al., 2009b). At 0–10 cm depth of a German arable soil, the C-normalized BC (analyzed by method BPCA, an approach similar to method dichromate to detect charcoal

from BC continuum) concentration was 19.7% in the coarse fraction (63–250 μm). In deeper soil, the concentration increased up to 58%–72% (Brodowski et al., 2007). The restored wetlands in our study, the forest in Yin et al. (2009b) and the arable soil in Brodowski et al. (2007) all had histories of biomass burning. The residual charcoal may explain most of the BC in POM. In the German arable soil, a significant amount of charcoal has proven to exist in coarse fraction by using a scanning electron microscope (Brodowski et al., 2005). Therefore, carefulness is suggested when one regards POM as labile component, especially in the case that biomass burning is involved in land use or land management.

The high proportion of BC in POM also impacts the way carbon saturation hypothesis is applied. In the carbon saturation conceptual model they proposed, Six et al. (2002a,b) divided soil organic matter (SOM) into four pools: a biochemically-protected pool, a silt- and clay-protected pool, a microaggregate-protected pool, and an un-protected pool. They suggested that the former three pools' maximum protective capacity limits the increases in SOM with increased organic matter inputs. The carbon saturation hypothesis has been corroborated in many practices where those SOM pools were reified as measurable fractions (Chung et al., 2008; Gulde et al., 2008; Stewart et al., 2008, 2009). The unprotected pool was defined as POM or light fraction and the biochemically protected pool was isolated only from silt and clay fractions (e.g., Stewart et al., 2008). However, our research detected a high proportion of BC in POM. It is conceivable that biochemically stabilized carbon can exist in any kind of physically (size- or density-) isolated fractions. Therefore, the role of POM in fractionation scheme isolating the carbon pools needs to be reconsidered.

The distribution of BC changed with the duration of restoration (Fig. 3). At 0–6 cm depth, BC in bulk soil increased by 0.11 g/kg per year, of which 74.1% came from the increase of BC in POM. BC

Table 4
The distribution of BC in MOM and POM and percentages of BC to TOC in MOM and POM.^a

Sites	Depth/cm	Distribution of BC in MOM/%	Distribution of BC in POM/%	BC:TOC in MOM/%	BC:TOC in POM/%
SX1	0–6	81.18	18.82	17.85	12.97
	6–16	80.52	19.48	17.48	17.67
XR2	0–6	54.83	45.17	15.32	21.04
	6–16	71.80	28.20	13.14	24.99
XR3	0–6	48.52	51.48	15.70	16.60
	6–16	84.39	15.61	15.31	15.30
XR4	0–6	41.96	58.04	11.83	24.58
	6–16	45.25	54.75	17.84	68.35
XZ1	0–6	54.45	45.55	16.66	12.07
	6–16	57.80	42.20	11.83	45.59
XZ2	0–6	52.99	47.01	18.63	11.70
	6–16	71.76	28.24	17.94	26.77
YZ1	0–6	28.43	71.57	12.04	20.94
	6–16	50.50	49.50	17.00	69.53
XR1	0–6	30.95	69.05	18.93	29.31
	6–16	32.24	67.76	11.65	72.07 ^b
Average		55.47	44.52	15.57	30.59

^a BC_{Cr} is used as an example of BC; POM and MOM are the same meanings as in Table 1.

^b This is a single replicate value for the other replicate shows a meaningless value exceeding 100%.

content in POM was less than that of MOM at the beginning of restoration. However, attributing to a larger slope (i.e. increase rate), it caught up with the level of MOM in the 10th year after restoration, and it had surpassed the content in MOM and become the majority of BC in the following ten years. Both saturating pool and non-saturating pool were believed to show asymptotic relationships when SSOC accumulation dynamics was expressed over time (Stewart et al., 2007). Our research, however, produced linear lines in the plot of soil carbon content vs. time (Fig. 3). Given that asymptotic curves appear linear over short ranges, the large slope of BC in POM indicated the carbon accumulation in this pool was possibly in the early stage on a path to a “transient steady state” (O’Brien and Jastrow, 2013) or saturation level if it exists. The pool of BC in MOM nearly leveled off, indicating the carbon accrual in this pool was possibly approaching the steady state or saturation level. The results indicated BC was a saturating pool. In the other words, constantly inputted BC tended to accumulate in POM compared to MOM. Therefore, POM would contain more stable carbon in the long term in restored wetlands of Caizi Lake.

3.4. Sequential chemical analysis of SSOC

In light of the more meanings of MOC_C and BC_{Cr} in the wetlands (see 3.1.3 and 3.2.3), the sequential analysis of the two SSOCs would be of high value. Such a method has been developed and showed its prospects for better understanding of SSOC in soil (Mikutta et al., 2006). In contrast to Mikutta et al. (2006), we acidified samples prior to rather than after oxidation, and used $K_2Cr_2O_7$ instead of NaOCl as oxidant and a higher concentration of HF.

SSOC (MOC_C plus BC_{Cr}) varied from 1.57 g/kg to 9.02 g/kg with an average of 4.43 g/kg, accounting for 14.1%–54.8% (35.7% on average) of TOC. MOC_C contributed on average 52.5% to SSOC and BC_{Cr} contributed the rest 47.5%. The results were comparable to those in acid forest soils (Mikutta et al., 2006) where SSOC varied from 2.4 g/kg to 20.6 g/kg with an average of 9.56 g/kg, accounting for 49.0% of TOC.

The application of the sequential method to MOM gained an average of 3.05 g/kg SSOC, which accounted for 40.7% of the whole

OC in MOM. The larger percentage than that in bulk soil implied the enrichment of SSOC in MOM. The proportions of MOC_C and BC_{Cr} to SSOC were on average 59.4% and 40.6%, respectively.

Sequential chemical analysis, together with combination of fractionation methods, represented a detailed list of soil organic carbon components (Fig. 4). Fig. 4 illustrated three physical fractions i.e. DOM (<0.45 μm), MOM (0.45–53 μm) and POM (>53 μm). In MOM and POM, the whole OC was divided into MOC_C , BC and labile OC (LOC, calculated by subtracting MOC_C and BC from TOC) according to sequential chemical analysis.

On average, MOC_C accounted for 14% (10% in MOM and 4% in POM) of TOC in 0–6 cm soil, while BC accounted for 16% (7% in MOM and 9% in POM). The rest 70% was labile components and consisted of DOC (4%) and LOC in fractions (28% in MOM and 38% in POM). Noticeable differences in 6–16 cm soil were a larger proportion of OC in MOM which accounted for 77% of TOC, and less labile components which decreased by 15 percentage points to 55% (Fig. 4g,h).

Structure of OC components of wetland with 2-year duration of restoration (Fig. 4c,d) was apparently different from that of intact wetland (Fig. 4a,b). There were more OC in MOM (55% vs. 39%) and less SSOC (31% vs. 45%) two years after restoration. The less SSOC indicated the soil was relatively labile at the beginning of restoration. The structure of OC components was similar between 0–6 cm and 6–16 cm soils two years after restoration, which reflected the mix by tillage and implied that 2-year duration was not enough to eliminate agricultural effects on soil. However, longer duration (20 years, Fig. 4e,f) of restoration showed similar OC composition to those of intact wetland, which may indicate functional recovery of soil OC after 20-year restoration.

4. Conclusions

We studied the changes of MOC_C and BC by different methods in a wetland restoration chronosequence in Caizi Lake. A general increase of MOC_C and BC with the duration of restoration at surface soil (0–6 cm) of the restored wetlands suggested that restoring wetland from farmland enhanced soil ecological function. Comparison between different methods for MOC_C analysis showed that size fractionation method overestimated the content of MOC_C . The overestimate derived from including DOC in MOC_C and defining all OC in MOM as MOC_C . HF-soluble MOC_C accounted for no more than 23.5% of the whole OC in MOM. BC content analyzed by method HF- Cr_2O_7 was higher than that by method CTO-375 due to the methodological difference that charcoal and soot were isolated in the former method while only the soot was detected in the latter one. In the light of the function of charcoal in soil and the possible source of BC, the dichromate-based BC was more meaningful in the study of BC recovery in restored wetlands. The ratio ($BC_{Cr}-BC_{CTO}$): BC_{CTO} was tested for its possible use to identify BC sources by comparing its result with a commonly used method. Biomass burning was supposed to be the main source of BC according to the result of the ratio-based approach. It was reasonable since the wetlands had not been influenced directly by industrial activities. The combination of physical and chemical fractionation methods revealed the distribution of BC in MOM and POM and the proportion of BC in the size fractions. Proportion of BC to TOC in POM was considerable, which indicated that POC was not always as labile as was commonly understood. A sequential chemical analysis of SSOC was demonstrated and proven effective as judged by similar results between this method and an analogous method. By using combination of fractionation methods together with sequential chemical analysis, a detailed list of soil organic carbon components including MOC_C , BC and labile OC in each size fraction was represented.

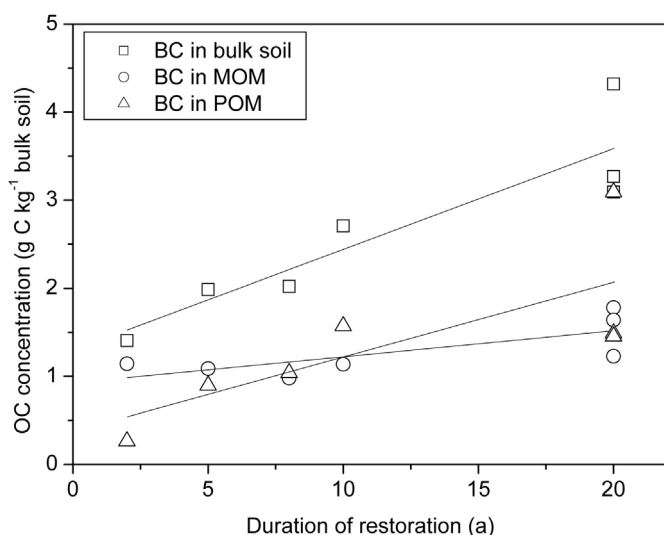


Fig. 3. BC in bulk soil, MOM and POM changed with the duration of restoration (0–6 cm). Note that all OCs are expressed as g/kg bulk soil. BC_{Cr} is used as an example of BC. BC in POM was determined by difference of that in bulk soil and MOM. Linear regression for BC in bulk soil (square): $y = 0.11x + 1.30$, $r = 0.91$, $P < 0.01$; for BC in MOM (circle): $y = 0.03x + 0.93$, $r = 0.76$, $P < 0.05$; for BC in POM (triangle): $y = 0.08x + 0.37$, $r = 0.75$, $P < 0.05$.

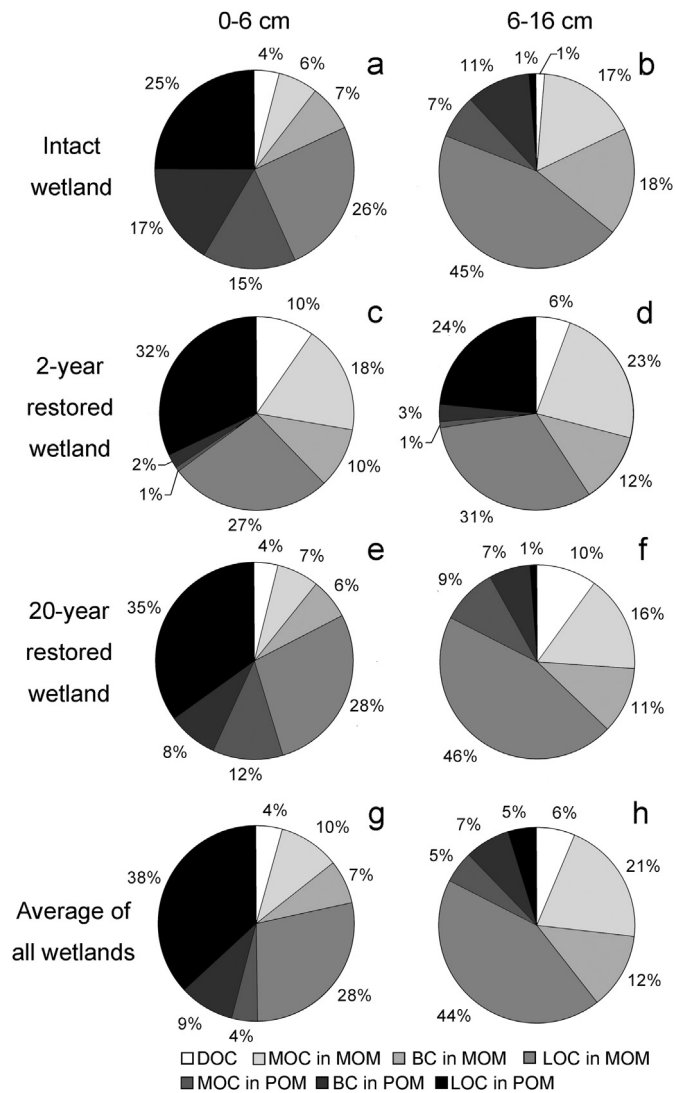


Fig. 4. Proportions of organic carbon components in 0–6 cm (a,c,e,g) and 6–16 cm (b,d,f,h) soils of restored wetlands. (a) and (b) are for intact wetland, (c) and (d) are for wetland with 2-year duration of restoration, (e) and (f) are for wetlands with 20-year duration of restoration, (g) and (h) are for the average of all wetlands. In clockwise direction from the top of each pie, sectors are DOC, MOC in MOM, BC in MOM, LOC in MOM, MOC in POM, BC in POM, LOC in POM, respectively. MOC and BC in POM is determined by difference of those in bulk soil and MOM. LOC is determined by subtracting MOC and BC from TOC. BC_C is used as an example of BC. MOC_C is used as an example of MOC.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2014.04.025>.

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