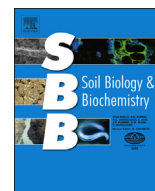




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Mineral-associated organic carbon and black carbon in restored wetlands

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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; Poepflau 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

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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

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