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Inhibited enzyme activities in soil macroaggregates contribute to enhanced soil carbon sequestration under afforestation in central China

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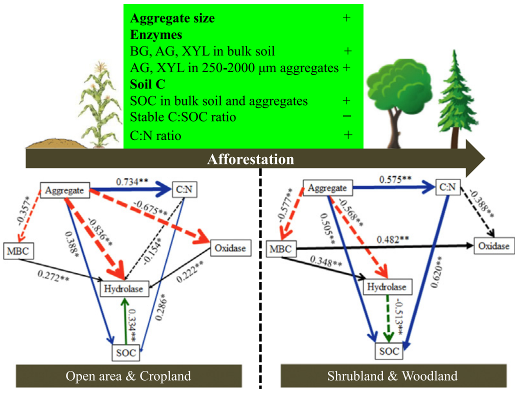
HIGHLIGHTS

- Hydrolases correlated negatively with SOC in aggregates under afforestation.
- Increased aggregate size negatively affected hydrolases in afforested soils.
- Inhibited enzymes in macroaggregates favored SOC sequestration under afforestation.

GRAPHICAL ABSTRACT



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ABSTRACT

The fate of soil organic carbon (SOC) sequestered by afforestation is crucial for the mitigation of the anthropogenic climate change but remains largely unclear. This lack of knowledge is particularly true for SOC turnover driven by enzyme activity. Here we measured hydrolase (including β-glucosidase, α-glucosidase, cellobiohydrolase and xylanase) and oxidase (including polyphenol oxidase and peroxidase) activities in soil aggregates following 30-year afforestation in central China. We also analyzed the relationships of enzyme activities with SOC concentrations, soil C:nitrogen (N) ratios and δ^{13}C values of soil organic pool (removing any carbonates by acid hydrolysis) and stable pool (NaOCl–resistant). Afforestation significantly enhanced soil β-glucosidase, α-glucosidase and xylanase activities in bulk soil, as well as SOC concentrations in bulk soil and all aggregate fractions compared to those in the open area and cropland. In particular, the woodland increased SOC concentration in >2000 μm macroaggregates by 4.2- and 3.2-fold, compared to the open area and cropland, respectively. Soil hydrolase activities were generally lower but SOC concentrations were higher in >2000 μm macroaggregates compared with those in other aggregate fractions following afforestation. Hydrolase activities were negatively correlated with SOC and C:N ratios in soil aggregate fractions following afforestation. Results of structural equation modeling indicated that the increasingly inhibited hydrolase activities with increasing soil aggregate size indirectly promoted SOC sequestration following afforestation. In addition, both hydrolase and oxidase were positively correlated with δ^{13}C values in the stable pool of the afforested soils, confirming the essential role of

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enzymes for SOC turnover in soil aggregates following afforestation. Overall, our results highlight the importance of unevenly distributed enzyme activities among soil aggregates in regulating SOC sequestration following afforestation.

1. Introduction

Soils store up to 2500 Pg carbon (C) to 1 m depth globally, more than three times the amount that is stored in the atmosphere (Lal, 2008). It has been postulated that a 5–15% increase in soil organic C (SOC) content to 2 m depth could potentially reduce atmospheric carbon dioxide (CO_2) by 16–30% (Baldock, 2007; Kell, 2011). These ideas have led to strong interest in practices that promote C sequestration in soils, including afforestation (Wang et al., 2016; Liu et al., 2017; Nath et al., 2018). Afforested soils have been shown as a sink for atmospheric CO_2 at the ecosystem, regional and global scales (Six et al., 2002a; Hernandez-Ramirez et al., 2011; Wang et al., 2016). However, the subsequent turnover of sequestered SOC due to processes such as decomposition has received less attention (Chomel et al., 2014); through decomposition, afforested soils can also act as a potential source of atmospheric CO_2. Thus, a better understanding of the underlying mechanisms of SOC dynamics is crucial for predicting the potential of afforestation to sequester C and mitigate the anthropogenic climate change.

The SOC decomposition process is predominantly catalyzed by a sequence of enzymes, including hydrolase and oxidase (Tabatabai and Dick, 2002; Sinsabaugh, 2010). Soil enzyme activities are sensitive to land-use changes and have been commonly used as indicators of C availability and soil quality (Trasar-Cepeda et al., 2008; Burns et al., 2013). However, despite extensive researches, the evidence for the impact of afforestation on soil enzymes remains uncertain (Trasar-Cepeda et al., 2008; Wallenius et al., 2011; Singh et al., 2012). This uncertainty can be attributed to changes in SOC quantity and quality (Six et al., 2002a; Smal and Olszewska, 2008), as well as changes in microbial biomass abundance and community structure following afforestation (Trasar-Cepeda et al., 2008; Zhang et al., 2016). In addition, soil enzyme activities are unevenly distributed in different physical locations within the soil structure (Ling et al., 2014; Bach and Hofmockel, 2016; Cenini et al., 2016). The various characteristics of soil aggregate fractions can induce differences in their responses to afforestation (Fansler et al., 2005) and hence contribute to contradictory responses of bulk soil enzyme activities to afforestation. The determination of enzyme activities in the bulk soil thus may not be sensitive enough to detect the response of SOC turnover to afforestation. Although some studies have evaluated changes in soil enzyme activities in the bulk soil under afforestation (Wallenius et al., 2011; Singh et al., 2012), how soil enzyme activities in aggregate fractions respond to afforestation remains largely unclear.

The decomposition of SOC via enzymes is tightly associated with the protection mechanisms of SOC compounds (Dungait et al., 2012; Cenini et al., 2016). SOC protected by different mechanisms, including by soil aggregation, mineral sorption and intrinsic molecular recalcitrance (Six et al., 2002a; Mikutta et al., 2006; Dungait et al., 2012; O'Brien and Jastrow, 2013), varies in their decomposition potentials and intrinsic decay rates (Bird et al., 2002; Mikutta et al., 2006). Several studies have indicated that the decomposition process is regulated more by the physical accessibility of SOC to enzymes than by the biochemical recalcitrance of SOC (Dungait et al., 2012; O'Brien and Jastrow, 2013). Soil chemical fractionation after the physical separation of SOC pool has been considered as an effective approach for evaluating the relative contributions of these different mechanisms, but has rarely been tested empirically (Dungait et al., 2012). Additionally, analysis of the natural abundance of ^{13}C offers an excellent approach to detect small shifts in SOC dynamics when land use change is accompanied by a shift between C_3 and C_4 plant species (e.g., Del Galdo et al., 2003; Cheng et al., 2013). SOC dynamics and their feedbacks following afforestation can thus be further elucidated if isotopic analyses are supplemented to quantify the overall SOC transformation rates (Guillaume et al., 2015).

In the Danjiangkou Reservoir area, a crucial water source for the Middle Route of the South-to-North Water Transfer Project in China, large areas of uncultivated land were converted to shrubland and woodland approximately 30-year ago to reduce soil erosion, water pollution and soil C depletion (Zhu et al., 2010). Our previous studies have reported that afforestation significantly increased SOC contents in bulk soil primarily due to increase in litter input and decrease in decay rate (Cheng et al., 2013; Deng et al., 2016). In this study, we measured the C-degrading enzyme activities (hydrolase and oxidase), C concentrations and $\delta^{13}C$ values of soil organic pool (removing any carbonates by acid hydrolysis) and stable (NaOCl–resistant) pool in soil aggregates across a land use change gradient (open area (uncultivated area), cropland, shrubland and woodland). The objective of this study is to reveal how the distribution of C-hydrolyzing enzyme activities in soil aggregate fractions affects the turnover and sequestration of SOC following afforestation. We hypothesized that 1) soil enzyme activities in bulk soil and aggregate fractions would increase following afforestation, reflecting the increases in organic substrates due to increasing litter inputs (Trasar-Cepeda et al., 2008); 2) soil enzymes would be lower and SOC concentration would be higher in macroaggregates compared to those in finer soil aggregate particles, because of the physical isolation of SOC to enzymes in macroaggregates (Dungait et al., 2012).

2. Materials and methods

2.1. Site description and experimental design

This study was conducted at the Wulongchi Experimental Station (32°45′N, 111°13′E) in the Danjiangkou Reservoir region (Cheng et al., 2013; Wu et al., 2016). Altitude at the station is approximately 280–400 m above sea level. This region has a subtropical monsoon climate, with marked annual variations in both precipitation and temperature. Mean annual precipitation is 749.3 mm, with 70–80% falls between April and October. Mean annual temperature is 15.7 °C, which ranges from approximately 4.2 °C in January to 27.3 °C in July. The soil is a loamy clay with 11% sand, 41% silt and 48% clay (Zhu et al., 2010), and is classified as Haplic luvisols according to Food and Agriculture Organization (1993). The soils are formed primarily from limestone and red sandstone, which have a pH range of 8.17–8.53 with strong alkali carbonate reaction (Zhang et al., 2016). Human activities, such as tillage and deforestation have led to soil erosion, water pollution and soil C and N depletion in this region (Zhu et al., 2010). In 1980s, large areas of uncultivated land (open area) around the reservoir were converted to woodland (*Platycladus orientalis* (*Linn.*) *Franco*) and shrubland plantations (*Sophora davidii* (*Franch.*) *Skeels* and *Amorpha fruticosa Linn.*) (Zhu et al., 2010). Maize and wheat were grown in rotation in cropland by conventional agricultural practices, including plowing to a 0.4 m depth and mineral fertilizations. The aboveground biomass of maize and wheat were removed through harvesting.

In this experiment, four land use types (woodland, shrubland, cropland and open area) were replicated four times (four blocks) using a randomized complete block design. Each block was approximately 3 ha (600 m × 50 m). The distance between two adjacent blocks was approximately 100 m. Comprehensive surveys of soil and vegetation were conducted in April 2017 to ensure the comparability (e.g., similar topographies and soil types) of the soil sampling plots among the four land types.