



## Vertical and seasonal variations of soil carbon pools in ginkgo agroforestry systems in eastern China

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### ABSTRACT

Agroforestry provides opportunities to decrease the levels of carbon dioxide (CO<sub>2</sub>) released into the atmosphere by increasing the carbon (C) stored in agricultural systems. In agroforestry systems, soil C pools serve as the most important and stable C sink, but there is limited information on the vertical and seasonal variations of soil C pools. In this study, the vertical and seasonal variations of soil organic C (SOC) and its labile pools were measured in five planting systems: a pure ginkgo (*Ginkgo biloba* L.) planting system, a pure wheat (*Triticum aestivum* L.) field, a pure metasequoia (*Metasequoia glyptostroboides* Hu et Cheng) seedling system, a ginkgo and wheat agroforestry system, and a ginkgo and metasequoia seedling agroforestry system. Among these systems, the ginkgo and wheat system had a significantly higher SOC content than the other systems throughout the year, particularly at depths of 0–10 cm and 10–20 cm. Additionally, the pure ginkgo and pure metasequoia systems had lower SOC contents than the other planting systems, and this decrease was attributed to the relatively limited tree litter input and lower fine root biomass. Microbial biomass C (MBC) and soil readily oxidizable C (ROC) exhibited similar vertical and seasonal variations and reached minimum values in winter. The highest MBC and ROC contents were observed in the ginkgo and wheat system at a depth of 0–10 cm, i.e., 127.3 mg kg<sup>-1</sup> and 4.49 g kg<sup>-1</sup>, respectively. The highest water-soluble organic carbon (WSOC) content was observed in summer at a depth of 0–10 cm, i.e., 472.2 mg kg<sup>-1</sup>. A Pearson correlation analysis indicated that soil properties were significantly correlated with SOC and labile C fractions. The results suggested that an agroforestry system resulted in a greater increase in the soil C sink; in particular, the ginkgo and wheat system achieved the best results. Basic soil properties played key roles in soil carbon formation. These results provide important information about SOC and labile C fraction dynamics resulting from planting systems and depth variations and strengthen our understanding of soil C sequestration in agroforestry systems.

### 1. Introduction

The global soil carbon (C) pools contain 2500 gigatons (Gt) of carbon, which is three times higher than the atmospheric carbon pool and 4.5 times higher than the biotic carbon pool (Lal, 2004). In the context of global warming, dramatic climate changes have been caused by greenhouse gas (GHG) emissions, especially carbon dioxide (CO<sub>2</sub>) emissions. Thus, the carbon sink capacity of soil has received extensive attention by international researchers because a higher soil C sequestration capacity results in more atmospheric CO<sub>2</sub> being taken up, soil fertility being enhanced, and soil quality being improved (Wiesmeier et al., 2014; Zhao et al., 2018). Forests store the majority of organic C in terrestrial ecosystems as a result of C sequestration, and soils store the majority of organic C in forest ecosystems (Lorenz and Lal, 2010; Scharlemann et al., 2014). Hence, forest soil carbon pools are important

C sinks that are crucial for ecosystem functioning and the global C budget.

However, various factors affect the stability of soil carbon pools; in particular the formation and decomposition of soil organic carbon (SOC) are key ecological processes (Hiltbrunner et al., 2013). For example, land use changes may increase the amounts of CO<sub>2</sub> released into the atmosphere by influencing SOC decomposition, and different types of vegetation usually provide litter with different physical and chemical properties by influencing SOC formation (Bárcena et al., 2014; Gosheva et al., 2017; Román-Sánchez et al., 2018). Moreover, climate, fertilization, and soil bulk density and aggregate ability strongly affect forest soil carbon pools (Brar et al., 2013; Franzluebbers, 2005; Miller et al., 2004). Thus, it is important to implement sustainable and adaptive forest management practices to better manage forests sustainably to cope with future climate change challenges. Agroforestry systems,

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which combine trees with agricultural crops and/or livestock, supply a large number of ecosystem services and environmental benefits (Hergoualch et al., 2012). Moreover, agroforestry can increase the amount of C stored in agricultural systems while still allowing the growth of food crops (Montagnini and Nair, 2004). For example, perennial trees in agroforestry systems can fix CO<sub>2</sub> through photosynthesis and can serve as important C sinks, and plant residues and dead branches that are retained on the soil surface are crucial for soil C input and improvements in soil aggregation (Chen et al., 2017; Oelbermann et al., 2006). Moreover, perennial trees in agroforestry systems can be deeply rooted, which is beneficial for SOC accumulation in deep soil layers (Cardinael et al., 2015).

In general, SOC pools can be divided into labile and recalcitrant C pools. The recalcitrant C pool is relatively stable and has a longer turnover time (Chen et al., 2016). In contrast, soil labile C pools, such as microbial biomass C (MBC), water-soluble organic C (WSOC), and readily oxidizable C (ROC), are more sensitive indicators of changes in land management practices because of their key roles in soil nutrient accumulation and energy supply in soil microbial activities (Shao et al., 2015; Yu et al., 2017). Crop residue incorporation is an important measure for improving air quality, mitigating climate change and increasing SOC input (Dikgwathle et al., 2014). Previous studies have demonstrated that crop residues with different properties produced various effects on the composition of soil C fractions and the stability of soil aggregates (Langenbruch et al., 2014). Perennial plants generally participate in SOC formation by providing organic C to soil via branch pruning, root turnover and exudation, and leaf litter (Khalid et al., 2007). Agroforestry systems, which consist of perennial and annual plants, can supply primary organic C to soil and can have a positive effect on the SOC content. Labile C pools are strongly time-sensitive, and seasonal changes may play a vital role in nutrient availability and microbial activity.

Ginkgo (*Ginkgo biloba* L.), a ‘living fossil’ that is native to China, is widespread around the world because of its beautiful appearance and strong adaptability (Major, 1967). In China, ginkgo is usually intercropped with crops, seedlings, and vegetables. In ginkgo agroforestry systems, ginkgo leaves, crop residues, and root exudation are the major sources of soil C input (Guo et al., 2018; Khalid et al., 2007). Previous studies focused mainly on the distribution of soil C in the topsoil (< 20 cm). However, information on the labile fraction dynamics is urgently needed for sub-soil layers. Thus, the primary aim of this study was to quantify the distribution of labile C fractions to a soil depth of 1 m in a ginkgo agroforestry system. Five typical planting systems were selected: a pure ginkgo planting system, a pure wheat (*Triticum aestivum* L.) field, a pure metasequoia (*Metasequoia glyptostroboides* Hu et Cheng) seedling system, a ginkgo and wheat agroforestry system, and a ginkgo and metasequoia seedling agroforestry system. Three objectives were established: (1) characterize the differences in the soil C pools among the various planting systems; (2) quantify the SOC contents and labile C fractions in different layers (0–10, 10–20, 20–40, 40–60, and 60–100 cm); and (3) analyze the correlation among soil labile C pools and basic soil properties.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in Yellow Sea Forest Park (32°33′–32°57′ N, 120°07′–120°53′ E), Dongtai City, Jiangsu Province. This site is located in the alluvial plain in the middle and lower reaches of the Yangtze River. The area has a subtropical warm humid monsoon climate that is hot and rainy in summer and cold and dry in winter. The annual average temperature is 15.6 °C, annual precipitation is 1044 mm, the annual average frost-free period is 237 days, and the annual sunshine duration is 2209 h. The local soil is characterized as alkaline sandy loam soil.

### 2.2. Experimental design and soil sampling

Through a preliminary investigation, five popular planting systems were selected for the experiment: a pure ginkgo planting system (G), a pure wheat field (W), a pure metasequoia seedling system (M), a ginkgo and wheat agroforestry system (GW), and a ginkgo and Metasequoia seedling agroforestry system (GM). These systems are referred to as G, W, GW, M, and GM based on the first letter. The ginkgo forest was planted in 2002, and the agroforestry systems were established in 2004 using a row and plant spacing of 2 × 8 m. A pure ginkgo planting system with a row and plant spacing of 2 × 8 m was gradually intercropped with an interrow crop. Adjacent pure ginkgo planting systems with a row and plant spacing of 3 × 3 m were selected in this study. Metasequoia seedlings were planted in 2010 with a row and plant spacing of 0.8 × 0.8 m. Maize was planted as a rotation crop after wheat. Based on information provided by local farmers, fertilizers are applied twice per year, i.e., in mid-May and mid-October. Compound fertilizer was applied, i.e., a total of approximately 0.9 t ha<sup>-1</sup>, divided equally into two applications.

Soil samples were collected in 2016 during four typical months representing the four seasons: March, July, October, and January. In each planting system, three plots with a dimension of 100 m<sup>2</sup> were randomly selected for soil sampling. On each sampling date, soil samples were collected at depths of 0–10, 10–20, 20–40, 40–60, and 60–100 cm. Soil drilling (4-cm diameter) was used to collect the soil samples from five selected locations in each plot, based on an S-shaped curve, and the samples were combined to form a composite sample according to the inquartering method. A total of 75 samples was collected on each sampling date; the samples were placed on ice in a cooler and then transported to the laboratory. The three cutting ring method was used to collect soil samples from each plot on the first sampling date to determine the bulk density.

### 2.3. Soil analysis

Soil bulk density was detected using a gravimetric method. Briefly, the original soil was retrieved with a cutting ring, and the dry soil weight was measured, after which the soil bulk density was calculated. Soil pH was measured using a pH meter at a 1:2.5 soil/water (w/v) ratio. Total N was determined using the Kjeldahl method, total P was determined using a molybdenum colorimetric method, and total K was determined using an acid solution and flame photometric method. Soil ammonium-N (AN, indigo blue colorimetric method) and nitrate-N (NN, dual band ultraviolet spectrophotometry) were extracted with 2 M KCl (w/v = 1:5, 180 r, 1 h) and were determined using UV spectrophotometry (Lu, 1999; Shibata et al., 2011). The basic soil properties are shown in Table 1.

SOC was determined using a modified Walkley and Black method. Briefly, 0.5 g of soil was digested using 5 mL of 0.8 M 1/6K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> at 175 °C for 10 min, after which the extracts were titrated with standardized FeSO<sub>4</sub>, and the SOC content was calculated according to potassium dichromate consumption (Gao et al., 2017).

MBC was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, fresh sampled soil (equivalent to 5 g of oven-dried soil) was fumigated with chloroform for 24 h at 25 °C after sieving (< 2 mm), followed by extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub> for 30 min on a shaker (180 r). Non-fumigated soil was also extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> for 30 min. MBC was determined using a liquid TOC analyzer (Elementar, Germany). MBC was calculated as 2.22 × (C extracted from fumigated soil - C extracted from non-fumigated soil) (Liu et al., 2012; Wu et al., 1990).

WSOC was determined by shaking fresh soil (equivalent to 5 g of oven-dried soil) at a soil/water (w/v) ratio of 1:5 on a shaker at room temperature for 30 min, followed by centrifuging at 3000 rpm for 10 min. The supernatant was filtered through a membrane filter (pore

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