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Changes in soil aggregate carbon dynamics under no-tillage with respect to earthworm biomass revealed by radiocarbon analysis

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ABSTRACT

Soil aggregates are known to increase under no-tillage management (NT). We hypothesized that earthworm activity increase soil carbon (C), and investigated the effect of earthworm activity on soil aggregate size (>1 mm: L, <1 mm: S) and C accumulation patterns under NT by analyzing the water stable aggregate (WSA) and radiocarbon contained in surface soil (0–2 cm). The study was conducted at two NT sites (Andosols), at Ami town, Ibaraki, Japan, which were both converted from conventional tillage: one with a winter cover crop and no earthworms (NT control for 6 years: NC) and the other with no weeding and a high earthworm density (natural farming for 11 years: NF) in 2009. Total C concentrations at NC and NF were 5.0% and 8.1%, respectively. The L fraction was more abundant at NF than that at NC. Total C concentrations in both the L and S fractions were significantly higher at NF than at NC. WSA (>1 mm) composed approximately 80% of the total soil, reflecting NT and accumulation of earthworm casts (>1 mm). The winter cover crop *Secale cereale* L. at NC and earthworms and weeds *Digitaria ciliaris* (Retz.) Koel at NF had Δ^{14}C values of 30.1‰, 35.3‰, and 41.9‰, respectively. Earthworms incorporated C recently fixed by plants, whereas all soil samples showed negative Δ^{14}C values (<0‰), indicating that a significant part of soil C was composed of "pre-bomb" C (before the 1960s). The earthworm community in NF was dominated by epigeic Magascolecidae, which feed on both soil and plant residue. Earthworms were estimated to translocate at least 2.8 and 1.3 t C ha^{-1} from S and plant residues to L, respectively, within 11 years, and they consumed S soil and plant residues at a weight ratio of 9:1. Epigeic earthworm increased soil C by incorporating fresh C fractions in WSA.

1. Introduction

Management of soil organic matter (SOM) is crucial to improve soil quality and to attain agricultural sustainability (Lal and Kimble, 1997). SOM influences soil physical structure (Carter, 2002) and enhances nutrient recycling, water availability, and biodiversity while reducing erosion (Bronick and Lal, 2005). Among conservation management strategies, a no-tillage management (NT) is defined as one without human alteration to the soil except seeding (Derpsch, 2000). NT tend to have higher soil carbon (C) levels than conventional tillage systems, due to 1.5 times slower C turnover (Six et al., 2002b).

Under an NT, groups of primary particles that cohere to each other more strongly than to other surrounding soil particles, or soil aggregates (Kemper and Rosenau, 1986), accumulate, which increases the soil C pool (John et al., 2005; Balabane and Plante, 2004; Six et al., 2004a). The water stable aggregate (WSA) is an index of aggregate stability during wet conditions, and tensile strength is an index of aggregate stability during dry conditions (Schrader and Zhang, 1997). Tillage type influences both aggregate stability and aggregate chemical composition (Kasper et al., 2009). Pinheiro et al. (2004) reported that the aggregate distribution index is larger for NT than for conventional tillage. In addition, they showed that C concentration is significantly higher under NT than under conventional tillage in both the whole soil and aggregate fractions.

Earthworms enhance the formation of soil aggregate (Six et al., 2002b). Earthworms are ecosystem engineers that impact soil structure and nutrient cycling by their feeding, burrowing, and casting activities, which affect microorganisms and SOM decomposition (Brown et al., 2000; Lavelle et al., 1997; Jones et al., 1994). Studies have reported positive relationships between earthworms

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and soil C (Fonte et al., 2007; Bossuyt et al., 2005; Schrader and Zhang, 1997; Martin and Marinissen, 1993). Soil and plant residues ingested by earthworms are mixed in their guts and excreted as casts (Uchida et al., 2004). Plant residue C is rapidly protected by microaggregates within larger macroaggregates (Bossuyt et al., 2005). Earthworm activity becomes prominent under NT, where the number of earthworms is almost always higher than under conventional tillage (Wardle, 1995). The WSA (>1 mm) increases when earthworms are present (Ketterings et al., 1997). Furthermore, earthworm casts in loam soil are significantly more stable in terms of tensile strength than natural aggregates (Schrader and Zhang, 1997). Fonte et al. (2009a) suggested that handling plant residues and associated management practices are important for both the earthworm population and SOM storage. Natural farming in Japan is a unique method based on four major principles: no cultivation, no fertilizer, no weeding, and no pesticides (Fukuoka, 1987). Introducing perennial plants into the rotation particularly for continuous cropping with a fallow field results in significant accumulation of soil C (Luo et al., 2010). NT and no-weeding are beneficial for earthworms, because there is less disturbance and more food for the earthworms. Earthworms and soil aggregates increase under natural farming management in Japan (Miura et al., 2010).

SOM exists in various forms, with different decomposition characteristics and mean residence times. Natural isotopic tracers, including ^{14}C concentrations and C stable isotope ratios ($\delta^{13}C$), have been used to estimate ecosystem C dynamics (Hahn et al., 2006; Trumbore, 2006a, 2000b, 1993; Keeling, 1961a, 1958). The radiocarbon "bomb spike" caused by aboveground nuclear weapons tests (1959–1963) led to significantly increased levels of ^{14}C–CO_2 in the atmosphere. Thus, the "post-maximum-bomb" ^{14}C activities (1964–present) provide a natural isotopic signature (Goh et al., 1976). Hyodo et al. (2006) and Toyota et al. (2010) defined diet age as the time elapsed since C in the diet of consumers was fixed from atmospheric CO_2 by photosynthesis; it is estimated by comparing the radiocarbon content of these organisms to atmospheric $^{14}CO_2$ records. We hypothesized that earthworm activity and weed management rapidly increase soil C by incorporating fresh C into soil aggregates under NT. In this study, we used WSA and radiocarbon signature analyses to investigate how soil aggregates (>1 mm, <1 mm) were formed by earthworms under NT. We compared two different plots; both were converted from conventional tillage management into NT, but they had different earthworm densities and weed management prior to conversion.

2. Materials and methods

2.1. Site description

We investigated two sites at Ami town, Ibaraki, Japan: NT with rye winter cover crop (NT control [NC], $n = 4$; *Secale cereale* L.) management, at the Field Science Center of Ibaraki University, College of Agriculture (36°01′N, 140°12′S), and NT and no-weeding (natural farming [NF], $n = 4$; 36°00′N, 140°16′S, 346 m^2). The NF plot was located about 7.4 km northwest of the NC plot. Abundance and biomass of earthworms were high at NF (Miura et al., 2010), whereas we found no earthworm activity at NC, probably because it has been managed as conventional cropping agricultural land. The soils at both sites were humic allophane soils (Haludands and Haplic Andosols) (Nishizawa et al., 2010). Annual precipitation and mean air temperature were 992 mm and 14.3 °C, respectively.

The NC study plot, as the NT management reference, was assessed beginning in October 2003. *S. cereale* L. has been planted every October since 2003, and *Glycine max* has been planted with a drill in April of each year. The chemical fertilizer input was N: 0 kg ha^{-1} $year^{-1}$, P: 22 kg ha^{-1} $year^{-1}$, and K_2O_5: 100 kg ha^{-1} $year^{-1}$. The soil contained about 3% clay, 52% silt, and 45% sand. Soil pH (H_2O), electrical conductivity (EC), cation exchange capacity (CEC), and the levels of CaO, MgO, and K_2O were 6.6, 72 mS cm^{-1}, 234 mrq kg^{-1}, 315 mg kg^{-1}, 34 mg kg^{-1}, and 119 mg kg^{-1}, respectively. Detailed information on the experimental plots has been provided previously (Nishizawa et al., 2010; Zhaorigetu et al., 2008).

Chemical materials and tillage treatment had not been applied for 11 years at the NF site, and vegetable crops were grown with minimum control of weeds, which included mostly annual herbaceous plants such as *Digitaria ciliaris* (Retz.) Koel, *Setaria faberi* Herrm., and *Persicaria longiseta* (De Bruyn) Kitag. Crops such as *Raphanus sativus* var. *sativus*, *Brassica campestris* L., *Brassica rapa* var. *perviridis*, *Colocasia esculenta*, *Smallanthus sonchifolius*, *G. max*, and *Abelmoschus esculentus* were cultivated from April to December 2009 (Miura et al., 2010). A small amount of bean curd residue was applied as fertilizer. Aboveground weeds were cut (15 cm × 15 cm area) before seeding. The field was tilled, and cow manure was used as a fertilizer before the start of NT and no-weed management. The soil contained about 9% clay, 31% silt, and 60% sand. Soil pH (H_2O), EC, CEC, CaO, MgO, and K_2O were 6.2, 152 mS cm^{-1}, 43 mrq kg^{-1}, 382 mg kg^{-1}, 80 mg kg^{-1}, and 278 mg kg^{-1}, respectively. Field management data were collected at the site through observation and farmer interviews.

2.2. Sample collection and preparation for radiocarbon analysis

Soil, earthworm, and plant (*S. cereal* and *D. ciliaris*) samples were collected in July 2009. Earthworms were collected by hand sorting at soil depths of 0–10 cm (50 cm × 50 cm, four replicates) at NF for ^{14}C analysis and biomass measurements (ISO, 2006). Earthworm biomass and abundance were 34.1 g earthworm FW m^{-2} and 31 individuals m^{-2}, respectively, at a depth of 0–10 cm on July 29, 2009. We collected soil samples at a depth of 0–2 cm ($n = 4$), and the samples were separated into soil aggregates of >1 mm (L) and <1 mm (S) fractions by dry sieving with a 1 mm mesh. Litter and stones were removed. As aboveground *S. cereale* from NC and *D. ciliaris* from NF fix atmospheric CO_2, they were collected in 2009 and oven dried at 60 °C for isotope analysis. After 72 h, the specimens were ground into a fine powder using a ball mill. These plants samples were pooled into one sample for the ^{14}C analysis. Each soil sample was treated with 0.5 M HCl to remove carbonate C prior to isotope analysis. Organic C and nitrogen (N) concentrations in the soil and litter were analyzed using an N–C analyzer (Sumigraph NC-95A, Shimadzu, Kyoto, Japan). We assumed that fraction sizes >1 mm included earthworm casts, following Ketterings et al. (1997), who found that adding earthworms increased the >1 mm aggregate fraction in a field.

We collected earthworms at NF to identify the possible effects of earthworms on incorporated C using the bomb-^{14}C signature. Earthworms were weighed after rinsing with distilled water to remove soil and feces and then kept in a glass plate container at 20 °C to empty their gut contents. After 12 h, they were rinsed with distilled water and weighed, kept in glass containers at −30 °C for 24 h, and dried in a vacuum dryer for 24 h. The specimens were ground into a fine powder without gut contents using an agate mortar. Epigeic Megascolecidae dominated at NF, where *Amynthas agrestis* (Goto and Hatai, 1899), *Amynthas micronarius* (Goto and Hatai, 1898), *Metaphire hilgendorifi* (Michaelsen, 1892), *Pheretima irregularis* (Beddard, 1892), and *Amynthas vittatus* (Goto and Hatai, 1899) were collected in 2009