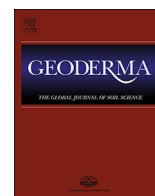




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Two-year responses of earthworm abundance, soil aggregates, and soil carbon to no-tillage and fertilization

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ABSTRACT

Agricultural practices often lead to a decrease in soil carbon (C), which often begins shortly after a natural system is converted to arable land. Conservation agriculture practices, such as no-till farming and weed mulching (NWM), may help maintain soil C. Increases in plant biomass through fertilization can also increase soil C. However, there is limited knowledge of the initial C dynamics following land conversion followed by conservation agriculture and fertilizer application, and of the soil biological and physical processes associated with these dynamics. Here, we studied the effects of two tillage practices (conventional tillage and NWM), fertilizer, and their interaction on earthworm abundance, soil aggregates, and soil C at two years after land conversion from grassland to agricultural land. We conducted a 2×2 factorial experiment (tillage/NWM and fertilized/not fertilized) in an experimental field in central Japan. We found that soil bulk densities and C concentrations at 0–5 cm depth were lower and higher, respectively, in the NWM plots than in the tillage plots at two years after the start of land conversion. After two years, earthworms were found only in the NWM plots. The percentage of macroaggregates (> 2 mm) was higher, and larger amounts of C were stored, in macroaggregates in NWM plots than in tillage plots. Amounts of crop biomass, roots, and plant residues were larger in the fertilized NWM plots. In the long term, such organic matter could accumulate in the form of macroaggregates, thereby further increasing soil C stock. Overall, we found that the soil biological and physical parameters that potentially control soil C responded to agricultural practices within a short period. The maintenance of biological activities by NWM and increased organic matter input by crop residues through fertilization can enhance soil C accumulation, which could have significant consequences for sustainable crop yields.

1. Introduction

Management of soil carbon (C) in agricultural land is important for sustainable crop production and the maintenance of soil ecosystem functions (Carter, 2002). Agricultural practices often lead to a decrease in soil C (Murty et al., 2002), which occurs mainly in the first ten years after the conversion of natural systems (e.g., forests and grasslands) to arable land (Luo et al., 2010). Recent studies suggest that conservation agriculture approaches, such as no-till farming, cover crops, and mulching with crop residues, can help to maintain or even increase soil C (Plaza-Bonilla et al., 2013; Higashi et al., 2014; Villamil and Nafziger, 2015). The unique dynamics of soil C under conservation agriculture practices are often explained by biological processes and/or formation of soil physical structures (e.g., formation of macroaggregates by earthworms; Arai et al., 2013) that are less common in conventional agriculture (Kladivko, 2001). However, there is limited knowledge of

the changes that occur in soil C soon after conversion of natural systems to arable land under conservation agriculture practices, and of the biological and physical processes associated with these initial C dynamics.

Soil aggregates are groups of organic and inorganic materials that bind to each other (Kemper and Rosenau, 1986; Bronick and Lal, 2005). The formation and disruption of soil aggregates often lead to significant consequences for soil C dynamics (Six et al., 2000). Aggregate formation can be facilitated by the activities of organisms (e.g., earthworms, plants, and microorganisms) and an input of organic materials (e.g., mulching, plant residues, and manure) (Six et al., 2004; Fonte et al., 2012). In particular, earthworms often increase macroaggregates through their feeding and casting activities (Ketterings et al., 1997; Bossuyt et al., 2005; Kawaguchi et al., 2011; Arai et al., 2013). Macroaggregates originating from earthworm casts have high water stability and enriched C concentration compared to the surrounding soil

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(Arai et al., 2013). Previous studies have shown that tillage practices often decrease earthworm density and/or biomass by causing physical injury and death (Boström, 1995; Johnson-Maynard et al., 2007; Briones and Schmidt, 2017).

In parts of Japan, conservation agriculture is being conducted based on no-till farming and weed mulching (NWM), combined with organic fertilizer and no-herbicide and no-pesticide farming (Arai et al., 2014; Yagioka et al., 2015). In this agricultural system, the aboveground parts of weeds are used for mulching after being mowed, whereas the roots are kept intact to function as living mulch. Arai et al. (2014) found that this type of organic matter input and reduced soil disturbance led to an increase in earthworm abundance and the amount of macro-aggregates. In contrast, although fertilizers are basically not used in this system, their application can potentially increase aboveground and belowground weed and crop biomass. The combination of NWM and fertilizer can thus lead to an increase in total organic matter input (i.e., mowed weeds, crop residues, and roots), which may in turn enhance soil C accumulation (Dumanski et al., 1998; West and Post, 2002). However, little is known about the interaction effects of NWM and fertilizer on the amount of organic matter input, or their possible consequences for earthworm abundance, soil aggregates, and soil C, especially soon after land conversion.

The aim of this study was to investigate the effects of two tillage practices (i.e., conventional tillage and NWM), fertilizer, and their interaction on earthworm abundance, soil aggregates, and soil C. The study was conducted during the first two years after conversion of grassland to an experimental field, when the soil C decrease is often most apparent (Luo et al., 2010). Our specific hypotheses were as follows: (1) earthworm abundance, soil aggregate size distribution, and soil C would differ between NWM and tillage plots, even shortly after land conversion, and (2) NWM and fertilizer would have interactive effects on these three variables. We measured the soil C of not only the bulk soil but also of different aggregate size fractions at different soil depths to infer the possible underlying biological and physical processes. We believe that a better understanding of initial C dynamics after land conversion will provide an important step toward predicting the long-term effects of conservation agriculture approaches on crop yield and soil ecosystem functions.

2. Materials and methods

2.1. Study site and experimental design

We conducted this study between 2010 and 2012 in an experimental field at Yokohama National University, central Japan (35° 28' 20"N, 139° 35' 20"E, 58 m elevation). The annual precipitation and annual average temperature during 2010–2014 were 1557–1998 mm and 15.8–16.5 °C, respectively. The soil type is classified as Andosol (IUSS Working Group WRB, 2015). The field had been grassland for > 30 years before the experiment started in 2010. Our study used a 2 × 2 factorial design with tillage/NWM and fertilized/non-fertilized treatments. The tillage treatment comprised a combination of tillage and weed removal by hand. The no-tillage treatment comprised NWM. In May 2010, we established four 6 m × 6 m blocks, which were further divided into four 3 m × 3 m plots. Four factorial combinations were randomly assigned to the four plots in each block: tillage with fertilization, tillage without fertilization, NWM with fertilization, and NWM without fertilization. Each plot consisted of three ridges.

Tillage and fertilization treatments, sowing, and harvesting were each conducted twice per year. The tillage plots were tilled using a tiller (Pianta FV200; Honda, Tokyo, Japan). We could not measure the depth to which the soil was tilled accurately, because the height of the ridges often decreased gradually over a period of a few days or weeks after tilling. We assume that the influence of tillage reached ca. 15–20 cm deep, given that the diameter of the circular tiller blades was 25 cm. In the tillage plots, weeds including roots were removed from the plot

before tilling and periodically during the growing period, when their height became taller than the sown plants. In the NWM plots, the aboveground parts of the weeds were periodically mown with a sickle when the weeds became taller than the sown plants. Mown weeds were left on the ground where they had been growing. The roots of the weeds were kept intact in the soil.

Tilling, sowing, fertilizer application, and harvesting were sequentially repeated (except for rye, which was sown in November 2010), from May 2010 to June 2012. Kidney beans were sown in May 2010 and harvested in July 2010. Japanese radish was sown in August 2010 and harvested in January 2011. Rye was sown in November 2010 without tilling or fertilizer, and harvested in May 2011. Soybean was sown in May 2011 and harvested in December 2011. Wheat was sown in November 2011 and harvested in June 2012. In the fertilized plots, 50 kg N ha⁻¹ fertilizer was applied after each sowing. We used an inorganic fertilizer (8-8-6 NPK) in May and August 2010 and a pellet organic (no chemicals) compound fertilizer (8-8-8 NPK) in May and November 2011. The amount of C added by the latter type of fertilizer was negligible (< 60 g C m⁻² yr⁻¹). We used neither herbicides nor pesticides at our study site. We refer to May 2010, May 2011, and June 2012 as before, one year after, and two years after the initiation of the experiment, respectively.

2.2. Earthworm measurement

We sampled the earthworms before and at two years after the initiation of the experiment. Earthworms were sampled by a combination of hand-sorting and mustard extraction (ISO, 2006; Muramoto and Werner, 2002). We first moved 50 cm × 50 cm of soil (depth: 15 cm) from each plot into separate boxes, and hand-sorted the earthworms. Then, while the soil was removed, we sampled the earthworms below a depth of 15 cm by pouring mustard solution on the soil. We poured 4.5 L of mustard solution, which was prepared by mixing 7 g of powdered mustard per 1 L of tap water, on each quadrat (0.25 m²) (Muramoto and Werner, 2002). The soil was returned to the plots after sampling. Earthworms collected by the two methods were pooled and treated as one sample for each plot. The numbers of individuals and wet weights for each species were recorded. Before the initiation of the experiment, earthworms were sampled only from the tillage plots in May 2010; this sampling was performed before tilling, to prevent soil disturbance in the NWM plots. Samples from the fertilized and non-fertilized plots were treated as separate samples (i.e., *n* = 8). Earthworms were sampled from all plots two years after the initiation of the experiment (i.e., *n* = 16) in June 2012. Sampling was conducted soon after the harvesting of wheat and before the next tilling. The measurements at two years after the initiation of the experiment were conducted on different ridges from those measured before treatment to minimize the possible influence of previous sampling practices.

2.3. Bulk soil analyses

We collected bulk soil samples before, one year after, and two years after the initiation of the experiment. Sampling in 2011 was conducted after rye was harvested, and sampling in 2012 was conducted after wheat was harvested. We measured the bulk density, water content, pH, C concentration, N concentration, and C stock of soil at 0–5 cm depth before treatment. Soil samples were collected before treatment in plots that were randomly selected in each block (i.e., *n* = 4) using a 100 cm³ core. We first measured the wet weight of each sample, and then air-dried the sample. Part of each sample was kept separate for water stable aggregate (WSA) analyses, to be described in Section 2.4. We measured the air-dried weights of the WSA subsamples and the remaining soil. Part of the remaining soil was dried in an oven at 105 °C for 24 h. Bulk density (Mg m⁻³) and water content (%) were calculated from the oven-dried weight and the wet weight. Soil pH of each sample (soil:water = 1:5 w/w) were measured after removing plant roots, plant

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