Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production

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1. Introduction

Healthy, thriving ecosystems are generally highly diverse with numerous taxa. Soil ecosystems are known to comprise complex food webs, including a wide range of organisms, from single-celled bacteria, algae, and protozoa to multicellular mites, earthworms, collembolans, and nematodes. Nematodes vary widely in life strategies and fulfill various functions in soil food webs (Berkelmans et al., 2003; Bongers and Bongers, 1998). Soil fauna communities in agro-ecosystems, including nematodes, are strongly influenced by anthropogenic disturbances such as tillage inversion, cropping patterns, and nutrient management.

Maintaining a healthy soil ecosystem function is fundamental to ensure sustainability and viability of agricultural systems worldwide. Recent Japanese legislation has been introduced to promote the development of more environmentally friendly farming practices associated with the growing awareness of the importance of waste reduction. This policy is leading the spread of organic farming and farming practices oriented to environmental conservation in the region. According to recent data, 216,341 farms in Japan have engaged in environmental conservation farming, accounting for 21.5% of the total cropping area in the country (MAFF, 2014). Under conservation management, traditional agronomic methods are combined with modern farming techniques, and conventional inputs such as synthetic pesticides and fertilizers have been excluded or reduced. Instead of synthetic inputs, organic materials are used to build soil fertility. In addition, cover cropping and manure management are intended to promote soil health. Cover crops provide a particularly beneficial ecosystem service, by assisting in supplying soil organic matter, adding biologically fixed nitrogen (N), scavenging soil residual nutrients, suppressing weeds, and breaking pest cycles (Higashi et al., 2014; Magdoff, 1993; Peet, 1996; Sarrantonio, 1998).

Soil tillage is aimed to improve soil structure and quality. Moldboard plow/rotary harrow for seed bed preparation (MP), a conventional tillage system, turns the surface soil into the deep soil layer and thoroughly incorporates surface crop residues into the lower layers of the tilled area, removing crop residues from the soil surface. In Japan, more than 80% of cultivated cropland is tilled using rotary cultivators (RC) (Moriizumi et al., 1995). Soil is tilled with a rotary blade and crop residues are mixed with the soil, although not completely turned into the soil. This system is simple and easy to use by farmers, particularly for small to medium-scale Asian farms, enhancing the seedbed while reducing weed occurrence. However, intensive tillage, including MP and RC, is also associated to great disturbances to soil ecosystems.

Tillage strongly influences the location and level of fragmentation of crop residue and soils. Crop residue and surface soil remain on the soil surface in systems with no tillage (NT); on the other hand, they are fully incorporated into the soil in MP systems, whereas they are partially fragmented and incorporated into the soil in RC systems. Leaving crop residue and preserving a stable surface soil are promoted as a management to maintain the natural stability of soil ecosystems.

Fu et al. (2000) showed that soil nematodes are more abundant in NT than in MP. In particular, bacterial feeder (BAC) nematodes respond to the addition of crop residue faster than fungal feeder and facultative root feeder (FFR) nematodes under both MP and NT. The vertical distribution of crop residue has been shown to influence nematode abundance and community structure (Fu et al., 2000). Cover cropping has been shown to increase BAC abundance by two fold, which actively influences N mineralization (DuPont et al., 2009).

In general, larger organisms appear to be more sensitive to tillage operations than smaller ones, mostly because of the level of physical disruption of the soil, the burial of crop residue, and changes in soil moisture and temperature (Kladivko, 2001). The vertical distribution of crop residue in the soil due to tillage inversion is a key factor affecting soil ecosystems. Soil micro- and macro-organisms are mostly observed in the surface soil layer, in particular, Ou et al. (2005) observed highest nematode abundance in the 0–5-cm soil layer, and fungal biomass is shown to be higher in the surface soil than in subsoil layers (Zhaorigetu et al., 2008). Soil tillage inversion leads to direct habitat disturbance and promotes vertical translocation of organisms. In addition, it indirectly leads to changes in soil physical properties and the translocation of crop residue as a food source for organisms (Kladivko, 2001). The degree of surface soil translocation (DTL) is also known to decrease with the decrease in tillage depth. Different tillage tools also enhance soil nematode community structure by minimizing soil disturbance.

The evolution of complex soil nematode communities in agro-ecosystems can be monitored using the maturity index (MI) (Berkelmans et al., 2003; Bongers and Bongers, 1998; Neher, 1999; Yeates and Bongers, 1999). Maturity and diversity indices have been used successfully to distinguish well-functioning ecosystems from heavily disturbed or stressed systems (Berkelmans et al., 2003; Neher, 1999; Yeates and Bongers, 1999), and also to detect subtle differences among agriculture, including tillage systems and cover cropping. Despite the strong influence that tillage is known to have over soil ecosystems, the effect and interaction of different tillage systems and cover cropping on nematode community structure, particularly in Asia, has not been comprehensively assessed.

The objectives for this study were (1) to compare the effects of tillage and cover cropping on soil nematode community composition and diversity and (2) to determine whether there is a relationship between nematode community composition and structure and DTL. In addition, cover cropping and manure application may also influence nematode community structure. Thus, we hypothesized that DTL and cover cropping can synergistically affect soil nematode community structure.

2. Materials and methods

2.1. Study site

This study was conducted as a part of a long-term experiment at the Field Science Center, Ibaraki University, Japan from 2009 to 2011. The climate is relatively humid and classified Cfa (humid subtropical and hot summer) (Trewartha, 1968). The study site (N 36°1′57.7″, E 140°12′43.6″) is 170 km south from the Fukushima Daiichi nuclear power plant (FDNPP). Mean monthly temperature and precipitation ranged from 2.9 to 28.6 °C and 0.5 mm to 389.0 mm (1423 mm $year^{-1}$) during 2010–2011 (Fig. 1), respectively. The soil was an Epi-humic Wet Andosols (Typic Endoaquands) (Soil Survey

Staff, 2014), with a loam layer of 0–20-cm depth, a clay loam layer between 20–63-cm depth, and a light clay layer 63–100-cm depth. Soil chemical properties of the surface soil (0–30 cm) varied among treatments within the following ranges: pH, 5.9–6.3; EC, 67.8–112 μS cm^{-1}; CaO, 233.4–337.8 mg 100 g^{-1}; MgO, 26.0–38.1 mg 100 g^{-1}; and K_2O, 47.2–129.5 mg 100 g^{-1}.

In the four replicated split–split experimental design, tillage systems were considered as the main variable, with cover cropping and manure application as variables in the sub-subplots. The study covered 72 plots, and each plot was 3 m × 6 m with 2 m wide aisles between plots. The soil was prepared using the respective tillage system: MP (25–30-cm deep, rotary harrow, and sowing), RC (15-cm deep and sowing), and NT (no-tillage sowing). Cover crop treatments were hairy vetch (*Vicia villosa* "Mamesuke"), winter rye (*Secale cereal* "Ryokusyun"), and fallow (native weeds). Bark with chicken manure applications (N: 0.6%, P_2O_5: 0.5%, K_2O: 0.5%, C/N ratio: 20.0, and water content: 66.8%) were 0 and 1 Mg ha^{-1}.

2.2. Management

Cover crops were manually sown on October 28, 2009. Seeding rates were 100 kg ha^{-1} for rye and 50 kg ha^{-1} for hairy vetch. Cover crops were grown until late May and mowed using a flail mower. The residues were returned to the soil on June 7, 2010. Cover crop residues were left on the soil surface, and tillage was performed on June 14, 2010. In MP, the soil was tilled to a depth of 25–30 cm with the subsequent incorporation of the crop residue to the soil. In RC, cover crop residues were also incorporated to the soil using a rotary cultivator to a depth of 0–15 cm. In NT, cover crop residue was left on the soil surface. Soybean (*Glycine max* "Natto Syouryu") was sown with a no-tillage seeder (MJSE18-6, Mitsubishi, six rows, 1.8 m wide) on July 5, 2010. The seeding rate was 50 kg ha^{-1} for soybean. Manure was applied only in sub–sub plots at 1 Mg ha^{-1} for soybean. After seeding, weeds were removed manually two or three times during each growing period. Soybean was harvested with a binding machine on November 8, 2010 and soybean residues were removed at harvest. After the summer crop harvest, cover crop seeds were manually sown and the soil was disc-harrowed to the top 3cm soil surface layer in all plots to ensure all seeds where covered with soil.

Cover crops were sown on November 10 and disked down to 3 cm. All cover crops and native weeds remained in the area during the FDNPP accident on March 15 and 21, 2011. Radioactive cesium fallout in this area, measured by airborne monitoring in 2011, reached 78,000 Bq m^{-2} (MEXT, 2011). Cover crops were mowed on June 16, 2011. Tillage treatments were again applied on June 20 and soybean seeds were sown on July 4 the same year. The same farming practices were applied in 2011 and soybean was finally harvested on November 4, 2011.

2.3. Sampling

Soil samples for radioactive cesium measurement were collected by hand with a 5-cm diameter and 30-cm long steel cylinder (5887.5 cm^3) on May 31, 2011 and May 25, 2012. Two soil core samples were collected from the center of each plot. Each soil core was divided into four subsamples by depth: 0–2.5 cm, 2.5–7.5 cm, 7.5–15 cm, and 15–30 cm. The two samples from each depth were combined before further assessment.

Samples were collected over 200 g soil for nematode samples from each plots, twice a year, from all treatments: after tillage (June 28, 2010 and June 27, 2011) and after soybean harvest (November 15, 2010 and November 14, 2011). Samples were collected with a steel trowel from the top 10-cm depth, excluding the uppermost soil layer. Each sample was removed gravel and roots, and then hand-mixed. Two subsamples which were weighed 20 g soil from 200 g soil sample were made for nematode extraction.

Cover crop biomass was estimated from data collected in late May from the center of each plot using a 0.25 m^2 quadrat. Biomass was calculated by weighing oven-dried (60 °C for 72 h) subsamples. Cover crop carbon (C) and N concentrations were quantified with a C/N coder (Sumika chemical analysis service, Ltd. NC900).

2.4. Nematode extraction, identification, and community analysis

Nematodes were extracted from subsamples using Baermann funnel method (Japanese Nematological Society, 2004). 20 g of fresh soil was weighed on a Kimwipe™ tissue (Kimberly-Clark), and then placed samples on a stainless steel mesh screen on a glass funnel 120 mm in diameter. The funnel was filled with water to a level that is slightly over the mesh screen prior to placing the Kimwipe™ tissue containing soil on the mesh screen. Soil samples were immersed for 72 h at room temperature (approximately 25 °C), before collecting the nematodes, which actively moved to the bottom of the funnel. The nematodes collected were heat killed (60 °C) and fixed with triethanolamine formalin, transferred to flamed slide glasses with approximately 1 ml of fixative, and observed under a microscope. The first 500 nematodes encountered were identified to genus or family level to estimate density per 20 g of soil at a magnification of ×1000. After the identification of nematode, we calculated the mean nematode densities in two extracted subsamples from same plot.

Nematode taxa were assigned to feeding groups according to the descriptions by Yeates et al. (1993), and FFR nematodes were classified following Okada and Harada (2007). We used the following five feeding groups: BAC, FFR, predators (PRD), omnivores (OMN), and obligatory root feeders (ORF). We refer to the feeding groups collectively as “ALL.” We counted total nematode species (S) and calculated the abundance ratio of FFR to FFR + BAC [F/(F + B)]. Each nematode taxon was also assigned to a functional guild (Ferris et al., 2001), defined on the combination of feeding group and life history traits expressed as colonizer–persister (cp) scores from 1 (extremely *r*-strategist) to 5 (*K*-strategist) (Bongers, 1990). Nematodes of all feeding habits with a cp score 3–5 are considered to be indicators of soil ecosystem structure; BAC with a cp score 1 and FFR with a cp score 2 are considered to be indicators of soil enrichment. MI was calculated from the cp scores (Bongers, 1990). The following three indices: channel index (CI), enrichment index (EI), and structure index (SI) were calculated using population densities of functional groups as described by Ferris et al. (2001). MI, as the weighted mean frequency for all free-living taxa, may be considered as a measure of disturbance, with smaller values being indicative of a more disturbed environment and larger values characteristic of a less disturbed environment. CI, EI, and SI provide a quantitative estimate of the soil food web state, CI is an indicator of the dominant decomposition pathways, EI is a measure of opportunistic BAC and FFR nematodes, and SI is an indicator of the food web state affected by stress or disturbance, respectively. These indices were calculated as:

$$\mathrm{MI} = \sum \frac{v_i \times f_i}{n}$$

where v_i = cp score assigned to family , f_i = frequency of family i in a sample , and n = total number of individuals in a sample.

$$\mathrm{CI} = \frac{FFR_2 \times W_2}{BAC_1 \times W_1 + FFR_2 \times W_2} \times 100$$

$$\mathrm{EI} = \frac{e}{e + b} \times 100$$

$$\mathrm{SI} = \frac{s}{s + b} \times 100$$

$$b = (BAC_2 + FFR_2) \times W_2$$

$$e = (BAC_1 \times W_1) + (FFR_2 \times W_2)$$

$$s = (BAC_i \times W_i) + (FFR_i \times W_i) + (OMN_i \times W_i) + (PRD_i \times W_i)$$

where FFR_i = abundance of FFR in cp i , BAC_i = abundance of BAC in cp i , $W_1 = 3.2$, $W_2 = 0.8$, $W_3 = 1.8$, $W_4 = 3.2$, $W_5 = 5.0$.

2.5. Radioactive cesium measurements

A section of each soil sample was dried until reaching a constant weight at 105 °C (12–36 h) and coarse organic matter was removed by hand. The soil samples were subsequently pulverized in a blender (701BUJ, Asone Co. Ltd.) and 100 g of this soil was transferred to a 127-ml U-8 polystyrene cylindrical bottle (external size: 5-cm diameter × 6.8-cm height). Cesium-137 (^{137}Cs) concentrations were determined with a Ge-semiconductor detector (CANBERRA GC4020: Energy resolution at 1.33 MeV is less than 2.0 keV). The gamma spectra obtained were analyzed with a Gamma Explorer (Canberra Industries Inc.). A true coincidence summing correction considering the container geometry was applied. Gamma-ray emission at 661.64 keV for ^{137}Cs was measured for 1800–7200 s to secure 10 Bq (kg dry soil)$^{-1}$ as the quantitative limit for ^{137}Cs, which was calculated using the method reported by Cooper (1970). Nine nuclide mixed activity standard volume sources in alumina (Japan Radioisotope Association, Tokyo, Japan) were used as reference standards.

2.6. Soil vertical translocation analysis

We calculated DTL using the following formula by Kawashima and Komori (1962) on the basis of the ^{137}Cs concentrations in soil:

$$\mathrm{DTL} = \sum_{i=1}^{n} \frac{100a_i\,(2i - 1)}{2mn}$$

where m = total radioactive cesium concentration (Bq m^{-2}) in all soil layers before tillage, n = total number of soil layers, a_i = radioactive cesium concentration in the i^{th} soil layer.

2.7. Data analysis

Data were statistically analyzed by analysis of variance (ANOVA) or repeated-measures ANOVA (StatView, SAS Institute) for a split–split plot design, applying Tukey–Kramer test with P <0.05. Regression analyses were also conducted to evaluate the relationship between DTL and nematode abundance and community indices.

3. Results

3.1. Cover crop dry matter (DM), C and N accumulation, and soybean yields

The DM of cover crops and native weeds were significantly influenced by cover crop treatments in both years and by tillage systems in 2011 (Table 1). Significant differences were observed in DM and C accumulations in 2011 between tillage systems, although these differences were not observed in 2010. In 2011, NT showed a significantly higher DM and C accumulation than MP and RC. For cover crops, the highest DM and C accumulations were measured for rye plots, which also showed the highest C/N ratio among all cover crop treatments. Hairy vetch showed the highest N accumulation, resulting in the lowest C/N ratio. Rye showed the highest DM accumulation, which was 149% and 331% higher than hairy vetch and fallow, respectively. Manure application did not significantly influence cover crop growth. The interaction between tillage and cover crop was significant for C/N ratio and N accumulation in 2010. N accumulation was highest in MP hairy vetch crops, but this trend was not observed in NT and RC crops.

Differences in soybean biomass and seed yield were not significant between tillage systems, cover crops, and manure applications. Soybean DM was 7.0 Mg ha^{-1} in 2010 and 6.3 Mg ha^{-1} in 2011. Soybean seed yield was 2.4 Mg ha^{-1} in 2010 and 1.7 Mg ha^{-1} in 2011 (data not shown).

3.2. Nematode density and soil management

In field plots, 46 and 47 nematode taxa were observed in summer sampling in 2010 and 2011, respectively; however, the number of taxa was reduced to 43 and 42 in autumn in 2010 and 2011, respectively (Table S1). Most species were observed throughout all sampling periods. In relation to the functional guilds, defined as the combination of feeding group and cp score, BAC with cp1 and FFR with cp2 contained six and five taxa, respectively, and these guilds were observed throughout all sampling periods. In contrast, the number of nematode taxa of all feeding habits in cp 3–5 decreased from 27 and 26 taxa in June 2010 and 2011 to 24 and 22 taxa in November 2010 and 2011, respectively.

Tillage system significantly influenced nematode abundance. Thus, the abundance of all feeding groups was significantly higher in NT than in MP and RC plots (Table 2). The effects of cover cropping were significant on the abundance of BAC, FFR, PRD, and ORF but such influence was not observed for OMN nematodes. Manure application significantly influenced PRD nematode abundance. Seasons significantly affected nematode abundances, except for ORF. Nematode abundances changed seasonally, and consequently, the overall abundance trend was unclear (Fig. 2). Among tillage systems, the highest nematode abundance was found in NT plots. In one instance, the total nematode population density was 2673 individuals per 20 g soil in NT, 171% and 20% higher than those observed in MP and RC, respectively. The BAC group showed a large population density that was significantly affected by tillage system, cover cropping, and season. Across cover crops, manure applications and seasons, the population density of BAC in NT plots was 1695 individuals, which was 259% and 35% higher than in MP and RC plots, respectively. BAC population density in rye cover crop was significantly higher than in fallow, whereas BAC population density was higher in summer than in autumn. FFR population density was also significantly influenced by tillage system, cover cropping, and season, and it was lower in MP than in NT and RC plots. FFR abundance was higher in rye cover crops than in fallow and hairy vetch. FFR was 130% higher in summer than in autumn. Tillage system, cover cropping, manure application, and season significantly influenced PRD population density. PRD population density was 52% and 313% higher in NT than in MP and RC plots, respectively. PRD population density was higher in rye cover crop plots than in fallow and hairy vetch plots. PRD population density was 16 individuals with no manure application, 110% higher than with 1 Mg ha^{-1}. PRD was 1.8% higher in summer than in autumn. Tillage system and season significantly influenced OMN population density. OMN population density was 115 individuals in NT plots, 256% and 40% higher than for MP and RC plots, respectively. ORF population density was significantly affected by tillage systems and cover crops but not by manure application and seasons. ORF density in NT and RC was significantly higher than that in MP. Hairy vetch significantly increased ORF. In contrast to the ORF, the ratio of ORF to non-ORF was significantly higher in MP than in NT and RC, and that was higher in hairy vetch than in rye.

The interaction between tillage system and cover cropping was significant for BAC and PRD nematodes (Table 2). No differences in BAC density were observed between cover crops in MP plots; however, for NT and RC plots, BAC population densities were higher in rye plots than those in hairy vetch or fallow plots. On the other hand, only small differences were found in PRD population density between cover crops, although MP showed higher PRD population density in rye than in hairy vetch and fallow plots. The interaction between tillage system and manure application was significant for PRD population density. PRD was higher in the no manure-input plot than in that with manure application. However, NT with no manure application showed a 3.1-fold higher nematode density than NT plots with 1 Mg ha^{-1} manure application. The interaction between tillage system and season was significant for the population densities of ALL, BAC, FFR, and ORF guilds. ALL showed the same result as BAC, probably because BAC was overrepresented in the total nematode population. In BAC, seasonal changes were observed in NT and RC plots but not in MP. BAC densities were higher in NT and RC in summer than in autumn. Almost no seasonal changes were observed in FFR in

NT and MP; however, FFR in RC were significantly higher in summer than in autumn. ORF in MP and RC plots were higher in summer than in autumn. In contrast, in NT plots, ORF was higher in autumn than in summer. ORF population density also varied among cover crops and seasons. In addition, ORF seasonal variation differed among cover crops. ORF did not vary seasonally in fallow plots. ORF in hairy vetch plots was higher in summer than in autumn, although ORF in rye plots in summer was lower than in autumn.

3.3. Nematode species and community indices, and soil management

Tillage system, cover crop treatment, and manure application influenced nematode community indices, but the significant effects varied depending on the index (Table 3). Overall, S, MI, and SI were higher in NT plots across tillage systems, whereas F/(F + B) was lower in NT plots (Fig. 3). Tillage system, cover crop treatment, manure application, and season influenced S. For cover crops, S in rye was higher than those in hairy vetch and fallow. S in no manure application plots was higher than in plots treated with 1 Mg ha^{-1} manure application. S was also higher in summer than in autumn. F/(F + B) was influenced by tillage systems and cover crop treatments; therefore, F/(F + B) was significantly lower in NT plots than in the other tillage systems and was higher in rye plots than in the other cover crops. MI was affected by tillage systems, cover crops, and seasons. MI was higher in NT plots than in any other tillage system and higher in rye plots than in any other cover crops. In addition, MI was higher in summer than in autumn. EI was affected by cover cropping, manure application, and season. EI in rye plots was the highest among cover crop treatments, and EI under no manure application was higher than that under 1 Mg ha^{-1}. EI was higher in summer than in autumn. SI was influenced by tillage system and season. SI values were in descending order of NT, MP, and RC and were higher in summer than in autumn.

The interaction between tillage system and cover crop treatment was also significant for F/(F + B) and MI. F/(F + B) in MP and RC plots was higher with rye than with fallow or hairy vetch, although it was higher with fallow than with hairy vetch or rye in NT plots. In contrast, there was no difference in MI between cover crops in NT and RC. However, the effect of MP on MI was 25% and 36% higher with rye than with fallow and hairy vetch, respectively. The interaction between tillage system and season was significant for F/(F + B), MI, and SI. F/(F + B) differed between seasons. In NT, F/(F + B) was higher in autumn than in summer. In contrast, F/(F + B) in MP was higher in summer than in autumn. In addition, no seasonal change in F/(F + B) was observed for

RC. MI and SI in NT plots were higher in summer than in autumn, although these differences were not observed for MP and RC.

3.4. Soil vertical translocation and nematode community

Radioactive cesium contaminations were significantly higher in the 0–2.5 cm surface soil layer before tillage treatment in 2011 in all plots. In fact, over 85% of the total radioactive cesium was deposited in the 0–2.5 cm soil layer. (Fig. S1). After tillage, MP enhanced the mixing of the surface soil into deeper soil layers, with 21% of the surface soil mixed within the 2.5–7.5 cm soil layer, 19% within the 7.5–15 cm layer, and 52% within the 15–30 cm layer. RC also incorporated a 43% of the surface soil within the 2.5–7.5 cm layer, a 35% within the 7.5–15 cm layer, although NT did not change the soil distribution between before and after tillage treatment. Tillage significantly influenced DTL ($P = 0.002$). Overall, NT showed a lower DTL than RC and MP. DTL were 29.6%–30.9% for NT plots, 49.1%–72.3% for MP plots, and 43.9%–50.8% for RC plots; however, DTL did not significantly differ among cover crop treatments (Fig. 4).

DTL significantly negatively correlated with nematode abundances for ALL ($R = -0.68$, $P = 0.0010$), BAC ($R = -0.66$, $P = 0.0013$), OMN ($R = -0.66$, $P = 0.0014$), and ORF ($R = -0.44$, $P = 0.0243$) groups (Fig. 5). Similarly, DTL significantly negatively correlated with S ($R = -0.43$, $P = 0.0271$) and SI ($R = -0.38$, $P = 0.0457$). DTL was significantly positively correlated with F/(F + B) ($R = 0.53$, $P = 0.0082$) (Fig. 6).

3.5. Relationship between plant parasitic and non-plant parasitic nematodes

Across all treatments and sampling times, the proportions of total non-ORF nematodes were negatively correlated with the abundance of ORF ($R = -0.58, P < 0.0001$) (Fig. 7).

4. Discussion

Inversion tillage mixes crop residues with soil at greater depths and the type of tillage tool greatly influences the eventual location of aboveground residues within the soil profile. We used DTL as a proxy to measure the degree of soil disturbance by tillage and its effect on soil nematode community structure. We used ^{137}Cs fallout from the FDNPP accident as a tracer to detect the level of soil translocation, however, as our DTL results agreed with previous studies determined by the small pieces of chalk

(Kawashima and Komori, 1962) and rock fragments (Zhang et al., 2004) as a tracer.

In this research, sampling soil depths of between DTL determination and nematode extractions were not exactly same because the layer of the top 10-cm depth, excluding the uppermost soil layer was represented the nematode community rather than other soil depth layer (Japanese Nematological Society, 2004). However, DTL will be a good indicator to compare the degree of soil disturbance due to different tillage inversion to the soil ecosystem (Fig. 5 and 6).

In 2010 and 2011, nine years after converting the experimental plots to the specific tillage systems and cover crops, we measured the effect of the associated DTL on the nematode community composition. Nematode communities stabilized and were essentially identical in 2010 and 2011, although seasonal variations remained. BAC were more prevalent in NT than in RT and MP plots and in rye than in fallow plots, as previously reported (Fu et al., 2000). This can be primarily attributed to the greater crop residue left on the soil surface by cover crops, resulting in consistently higher microbial biomass in NT plots (Zhaorigetu et al., 2008).

Changes in the occurrence and abundance of different nematode feeding groups are often associated with changes in crop species and soil management practices (Ettema and Bongers, 1993) and may ref」：lect changes in the soil food web structure. The direct and indirect effects of the plant community on the structures of nematode communities have been previously documented (Neher, 1999). Hairy vetch, which increased ORF abundance in this study, is known to be a good host of an ORF, *Pratylenchus* (McSorley and Dickson, 1989). The larger DM accumulation with rye than in fallow or hairy vetch (Table 1) ensures the abundance of BAC, FFR, and PRD. This result agrees with the observation by Nahar et al. (2006) that the abundance of all feeding groups increased with the increase in organic matter input.

However, tillage systems directly affect ORF abundance by translocation of nematodes across soil layers, which can indirectly alter soil properties due to the differences in crop residue decomposition process. Nahar et al. (2006) reported that NT enhanced and MP reduced nematode abundance of all feeding groups, although Minton (1986) and Okada and Harada (2007) did not observe such differences in ORF and *Pratylenchus* between NT and RC. Our results agree with previous reports stating that ORF abundances in NT were equal to those in RC. A possible explanation is that *Pratylenchus* can survive in fragmented plant roots, and thereby is able to maintain its population in RC (Alby et al., 1983; Okada and Harada, 2007). In contrast, MP disturbs nematode surface soil habitat and transport fragments of plant roots to deeper soil layers, reducing the abundance of total nematodes in MP compared with NT.

Compared to tillage systems and cover crop treatments, the effect of manure application on nematode communities was limited. Okada and Harada (2007) reported that manure application increases nematode abundance, although Nahar et al. (2006) showed that the difference is not significant for PRD. Our results did not agree with those previous results, as most nematode abundances did not change or decreased after manure application (Table 2). In our experiment, the amount of manure applied was small compared to the amount of cover crop residue input (Table 1), suggesting that a greater amount would be required to produce an effect.

In this study, we used six indices of nematode diversity and community. Our results agree with those of Okada and Harada (2007), who observed higher values for S, MI, and SI in NT than in RC. In NT, as the abundance of *K*-strategists (cp scores 3–5) increased, SI values increased. On the other hand, F/(F + B) was higher in MP and RC than in NT, reflecting the greater abundance of BAC compared with that of FFR (Table 3). Okada and Harada (2007) reported that CI in NT is equal to or greater than that in RC, although F/(F + B) is less sensitive in detecting differences between NT and RC. This insensitivity of CI is probably caused by the stationary nature and low abundance of *r*-strategy fungal feeders. Several authors have suggested that EI can adequately detect the increase in soil fertility associated to the application of organic mulch or fertilizer in the US, Canada, and Japan (Bulluck III et al., 2002; Forge et al., 2003; Okada and Harada, 2007; Wang et al., 2006). In contrast, in this study, EI was decreased with manure application (1 Mg ha^{-1}). We speculated that manure application was extremely low for detection by EI.

A minimum level of soil disturbance by tillage inversion is expected to increase nematode abundances. Our results revealed that as soil disturbance increased, S and SI decreased (Fig. 6); however, cover crop and manure application did not significantly influence SI in the same way as tillage, suggesting that tillage has a stronger impact on the soil ecosystem than cover crop treatment and manure application. DTL showed a significant negative correlation with SI, suggesting that DTL could be useful to evaluate the level of ecosystem disturbance not only regarding soil translocation but also in relation to soil ecosystem development.

Our results agree with the observation by Nahar et al (2006) that there was a strong negative relationship between the proportion of non-ORF and the abundance of ORF. Both of NT and RC with rye cover crop increased non-ORF and lowered the ratio of ORF to non-ORF, possibly due to antagonistic effects of microbial community. Further research will be needed to be investigated the relationship between antagonistic effects of microbial community on ORF and soil managements.

5. Conclusions

This study showed that soil nematode community immediately responds to changes in DTL due to tillage inversion. NT effectively increased nematode abundance under the humid subtropical conditions prevailing in Kanto, Japan. Two years of field observations also revealed that tillage inversion can exert a stronger influence on nematode community and the structure of soil ecosystems than cover crop treatment and manure application. DTL can be used as a quantitative indicator of soil ecosystem due to tillage inversion; however, our research results are limited to Andosols under Japanese climatic conditions.

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References

Alby, T., Ferris, J.M., Ferris, V.R., 1983. Dispersion and distribution of Pratylenchus scribneri and Hoplolaimus galeatus in soybean fields. J. nematol. 15, 418.

Berkelmans, R., Ferris, H., Tenuta, M., van Bruggen, A.H.C., 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. Appl. Soil Ecol. 23, 223-235.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10, 239-251.

Bulluck III , L.R., Barker, K.R., Ristaino, J.B., 2002. Influences of organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomatoes. Appl. Soil Ecol. 21, 233-250.

Cooper, J.A., 1970. Factors determining the ultimate detection sensitivity of Ge (Li) gamma-ray spectrometers. Nucl. Instrum. Methods 82, 273-277.

DuPont, S.T., Ferris, H., Van Horn, M., 2009. Effects of cover crop quality and quantity on nematode-based soil food webs and nutrient cycling. Appl. Soil Ecol. 41, 157-167.

Ettema, C.H., Bongers, T., 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fertil. Soils 16, 79-85.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Forge, T.A., Hogue, E., Neilsen, G., Neilsen, D., 2003. Effects of organic mulches on soil microfauna in the root zone of apple: implications for nutrient fluxes and functional diversity of the soil food web. Appl. Soil Ecol. 22, 39-54.

Fu, S., Coleman, D.C., Hendrix, P.F., Crossley Jr, D.A., 2000. Responses of trophic groups of soil nematodes to residue application under conventional tillage and no-till regimes. Soil Biol. Biochem. 32, 1731-1741.

Higashi, T., Yunghui, M., Komatsuzaki, M., Miura, S., Hirata, T., Araki, H., Kaneko, N., Ohta, H., 2014. Tillage and cover crop species affect soil organic carbon in Andosol, Kanto, Japan. Soil Tillage Res. 138, 64-72.

Japanese Nematological Society, 2004. Nematology Experimental Method. Japanese Nematological Society.

Kawashima, M., Komori, S., 1962. On the Displacement of Soil by the Tillage machines (I) On the Expressions of the Displacement of Soil. J. Jpn. Soc. Agric. Mach. 24, 56-60.

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Tillage Res. 61, 61-76.

MAFF, 2014. Ecological farmers survey.

Magdoff, F., 1993. Building soils for better crops: organic matter management. Soil Sci. 156, 371.

McSorley, R., Dickson, D.W., 1989. Effects and dynamics of a nematode community on maize. J. nematol. 21, 462.

MEXT, 2011. Extension Site of the Distribution Map for Radiation Dose. Ministry of Education, Culture, Sports, Science and Technology, Tokyo 100-8959, Japan.

Minton, N.A., 1986. Impact of conservation tillage on nematode populations. J. nematol. 18, 135.

Moriizumi, S., Hayashi, N., Takahashi, M., Ikeda, K., Matsui, K., Kojima, K., 1995. Studies on the Soil Displacement by Rotary Tillage. IV. A method of investigation using KC1 solution and analysis of soil-overturning by its method. Jpn. J. Farm Work Res. 30, 207-213.

Nahar, M.S., Grewal, P.S., Miller, S.A., Stinner, D., Stinner, B.R., Kleinhenz, M.D., Wszelaki, A., Doohan, D., 2006. Differential effects of raw and composted manure on nematode community, and its indicative value for soil microbial, physical and chemical properties. Appl. Soil Ecol. 34, 140-151.

Neher, D.A., 1999. Nematode communities in organically and conventionally managed agricultural soils. J. nematol. 31, 142-154.

Okada, H., Harada, H., 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Appl. Soil Ecol. 35, 582-598.

Ou, W., Liang, W., Jiang, Y., Li, Q., Wen, D., 2005. Vertical distribution of soil nematodes under different land use types in an aquic brown soil. Pedobiologia 49, 139-148.

Peet, M., 1996. Soil management. In: Peet M (ed), Sustainable Practices for Vegetable Production in the South. Focus Publishing/R. Pullins Co., Newburyport, Massachusetts.

Sarrantonio, M., 1998. Building soil fertility and tilth with cover crops. In: Clark A (ed), Managing cover crops profitably, 2 ed. Sustainable Agriculture Network,, Beltsville, Maryland.

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Trewartha, G.T., 1968. An introduction to climate, 4th ed. New York.

Wang, K.H., McSorley, R., Kokalis-Burelle, N., 2006. Effects of Cover Cropping, Solarization, and Soil Fumigation on Nematode Communities. Plant Soil 286, 229-243.

Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. Agric. Ecosyst. Environ. 74, 113-135.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. J. nematol. 25, 315.

Zhang, J.H., Lobb, D.A., Li, Y., Liu, G.C., 2004. Assessment of tillage translocation and tillage erosion by hoeing on the steep land in hilly areas of Sichuan, China. Soil Tillage Res. 75, 99-107.

Zhaorigetu, Komatsuzaki, M., Sato, Y., Ohta, H., 2008. Relationships between Fungal Biomass and Nitrous Oxide Emission in Upland Rice Soils under No Tillage and Cover Cropping Systems. Microbes Environ. 23, 201-208.