Effects of tillage systems and cover crop managements on soil nematode community structure

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Abstract

The anthropogenic impacts on cropland caused by farming practices change the soil health and quality. However, the effects of farming practices on soil health and quality were not discussed. In particular, studies of soil food web structure were limited. We evaluate the soil food web structure, using the soil nematode community. Because of soil nematode has widely habitat and various behavior, soil nematode community provides a good indicator to soil food web structure. We examined the effects of three tillage systems, moldboard plow/rotary harrow (MP), rotary cultivator (RC), and no-tillage (NT); three winter cover crop managements (fallow, rye and hairy vetch); and two nitrogen (N) fertilization rates (0 and 100 kg N ha^{-1} for upland rice and 0 and 20 kg N ha^{-1} for soybean production) on changes in soil nematode community structure. The objectives of this study were (1) comparing the effects of tillage and cover cropping on nematode community composition and diversity, (2) to determine whether there is a relationship between nematode community structure and the degree of surface soil translocation (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, (3) to determine whether NT and cover cropping could prevent rice cyst nematode (*Heterodera elachista*) infection by enhancing the health of soil ecosystem under continuous upland rice cultivation and reduce nematode populations more rapidly than conventional cultivation methods after conversion to soybean, (4) comparing the effects of tillage and cover crops on nematode community structure and indices in long term, (5) to determine whether there is a relationship between nematode community indices and soil carbon (C) sequestration.

The abundance of total nematodes (ALL), bacterial feeders (BAC), predators (PRD), omnivores (OMN), and obligatory root feeders (ORF) were greater in NT than in MP and RC, but fungal feeders and facultative root feeders (FFR) was greater in RC. Cove crop also influenced on nematode community, rye and hairy vetch were always higher in ALL, BAC, FFR, ORF, and OMN than fallow. Tillage significantly influenced DTL. Overall, NT showed lower DTL than RC and MP. DTL significantly negatively correlated with nematode abundances for BAC, OMN, and ORF, and structure index (SI).

Densities of *H. elachista* markedly increased in MP and RC after 4 years of continuous upland rice cultivation, but not in NT. *H. elachista* were 52.8 individuals per 20 g soil in MP and 72.7 in RC, but 2.1 in NT in 2006. However, in the fifth year of continuous cultivation of upland rice, *H. elachista* densities increased in NT; therefore, no differences were observed between tillage systems. After conversion to soybean, *H. elachista*

densities decreased in all treatments, although NT showed a more rapid decrease than the other tillage systems. Cover cropping and N fertilization did not affect *H. elachista* densities during the 9 years, but cover cropping reduced the proportion of *H. elachista* to ALL and N fertilization reduced rice yield with increasing *H. elachista*.

In 2003-2011, ALL, BAC, PRD, OMN, and ORF were greater in NT, but FFR was great in RC. Cover crops markedly increased ALL, BAC, FFR, ORF, and OMN. Seasonal changes in nematode community were also significant, in particularly, as increase soil C, nematode densities were also increased. The relationship between nematode indices and soil C was significant only in NT, but not for MP and RC. In NT, as increase soil C, enrichment index and SI were positively significant. Seasonal difference of nematode community between summer and autumn was bigger in upland rice rotation, but that was small in soybean rotation. For 9 years experiment, SI increased not only for NT but also MP and RC. These results suggest that increase of soil C would have a great impact to develop the more diverse nematode community structure.

In this research, SI values increased in all plots after converting to soybean cultivation as a summer crop, suggesting soybean cropping showed more benefits to develop the nematode community structure. In this regards, the further research would be desirable to determine the effects of tillage system and cover cropping with the combination of summer crop on nematode community structure. In addition, 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.

This paper discusses the significance of nematode community indices to evaluate the agro-ecosystems and the effects of farming practices such as tillage systems and cover cropping on nematode community structure. Nematodes will be used as bio-indicators of soil health significantly because they are ubiquitous and have diverse feeding behaviors and life strategies. The information regarding soil nematode community structure and farming practices will be helpful to design the sustainable agriculture.

Abbreviations

^{137}Cs: Cesium-137
ANOVA: Analysis of variance
BAC: Bacterial feeder
BD: Bulk density
C: Carbon
CI: Channel index
Caccum: Carbon accumulation
Cp: Colonizer–persister
DM: Dry matter
DTL: Degree of surface soil translocation
EI: Enrichment index
F/(*F*+*B*): Abundance ratio of FFR to FFR + BAC
FDNPP: Fukushima Daiichi nuclear power plant
FFR: Fungal feeder and facultative root feeder
H': Shannon–Wiener's index
MI: Maturity index
MP: Moldboard plow/rotary harrow for seed bed preparation
N: Nitrogen
NT: No-tillage
Naccum: Nitrogen accumulation
OMN: Omnivores
ORF: Obligatory root feeders
PRD: Predators
RC: Rotary cultivator
S: Numbers of nematode species
SI: Structure index
SOC: Soil organic carbon
TAF: Triethanolamine formalin

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Chapter 1: Introduction

1.1 Sustainable agriculture and healthy soil

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 (Stirling, 2014).

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).

1.2 Soil tillage system and soil nematode community

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.

Tillage system too affects the population density of nematodes (Govaerts et al., 2007; Okada and Harada, 2007). NT system is an alternative that has been increasingly used for crop production in the U.S., Europe, South America, and Asia during the past decade, owing to its environmental advantages over moldboard plowing. Several studies of NT report its great potential to improve soil quality (Logsdon and Karlen, 2004; Robertson et al., 2000), and the combination of cover crops and NT practice confers environmental benefits such as N leaching reduction, soil organic matter increase, and soil biological diversity enhancement (Komatsuzaki and Ohta, 2007).

1.3 Cover crop management and soil nematode community

Cover cropping is well known as a control practice for pathogenic nematodes. Nonhost cover crops may release materials that are toxic to the targeted pest and reduce populations and proportions of pathogenic nematodes. Many studies have found relationships between cover cropping and nematode ecology. Oat, marigold (*Tagetes* sp.), guinea grass (*Panucum maximum*), and sunn hemp (*Crotalaria juncea*) exert particularly noticeable nematicidal effects, and when incorporated into soil, have been shown to suppress root-knot nematodes (*Meloidogyne arenaria* and *M. chitwoodi*) (Guertal et al., 1998).

1.4 Farming practice and parasitic nematode

Soil nematodes are commonly regarded as harmful to agricultural production, because of plant parasitic nematodes. They attack plants and cause crop losses throughout the world. Some estimates suggest they cause 77 billion dollars of damage worldwide each year (Veech and Dickson, 1987).

Rice, the most important food crop in the world, is the staple food for more than half of the world's population; it is predominant in Asia, where >90% of the world's rice is grown and consumed. Several genera and species of parasitic nematodes are associated with rice, but only some are known or suspected to cause yield loss (Bridge et al., 2005). The rice cyst nematode, *Heterodera elachista*, infests areas of upland rice (*Oryza sativa*) production in Japan (Ohshima, 1974), and causes severe yield loss in both upland and irrigated rice (Bridge et al. 2005). Recently, *H. elachista* is becoming a widespread pest in Asia (Ding et al., 2011) and European countries (De Luca et al., 2013). Host plants of *H. elachista* are rice, millet (*Echinochloa esculenta*), corn (*Zea mays*), wheat (*Triticum aestivum*), and oat (*Avena sativa*), but *H. elachista* is mainly parasitic on rice and rarely on other *Poaceae* (Okada, 1960).

Root exudates from rice plants caused hatching of *H. elachista* eggs during vegetative growth of the host but the activity declined with plant senescence (Shimizu, 1976). The population of juvenile *H. elachista* released in the soil increases from March to April but decreases from August to winter. After juvenile *H. elachista* invade host plants, they develop cysts for the next generation. *H. elachista* juveniles do not hatch from eggs contained in the cysts from previous seasons in response to lower temperatures and autumn rains. As the season progresses, the nematodes remain at the same infection site and begin to swell into characteristic white spheres. This process takes 6–8 weeks, and the nematodes remain in this form until the host plant begins to senesce (Shimizu, 1976). The females die and their cuticle hardens and turns brown to form a cyst. *H. elachista* has two or three generations per year (Shimizu, 1977). However, because each cyst contains several hundred eggs, populations can rapidly increase on susceptible cereals (Shimizu, 1976).

Many scientists have reported that *H. elachista* severely reduces upland rice production. Nishizawa (1978) reported that population densities of *H. elachista* increased for 4 years in experimental plots of monocultured upland rice established on newly cleared forest land but began declining in the fifth year. When paddy rice was grown under upland conditions in pots that had been inoculated with different population densities of juvenile *H. elachista*, significant reductions in grain weight were observed with increasing *H. elachista* population density. In particular, higher yield reduction was observed when the roots were invaded by the nematodes before the tilling stage (Shimizu, 1976).

Chemical and cultural control of the rice cyst nematode has been reported (Nishizawa et al., 1972). Preplant treatment with dichloropropane-dichloropropene mixture was most effective and increased the yield of a paddy rice variety by approximately 30%; however, this effect was not apparent in the next season, suggesting a rapid buildup of the cyst population at harvest time in the first year (Nishizawa et al., 1972). Cropping of soybeans or sweet potatoes markedly decreased the cyst population at the end of the first season, but even 3 years of successive cultivation of these nonhosts failed to reduce the population to zero (Nishizawa et al., 1972). However, chemical control of *H. elachista* often fails to confer economic advantage, owing to the low return of upland rice production. In addition, crop rotation is limited to producing staple foods.

Chen (2007) evaluated the long-term effects of tillage practices on the soybean cyst nematode (*H. glycines*). Soybean yields increased in a corn–soybean rotation suggested that tillage alone is not an option for managing population of *H. glycines* in a northern climate and soil conditions. Soybean was grown for 4 years on a Lexington silt loam in Tennessee. Cyst numbers were lower in NT than in conventional tillage and in 6 years of continuous NT were lower than conventional or short-term NT systems. However, there is little information about the effects of NT on *H. elachista* population densities under field conditions.

In general, soil-borne diseases, including pathogenic nematodes, are most damaging when soil conditions are poor, as a result of inadequate drainage, poor soil structure, low organic matter, low soil fertility, and high soil compaction. All the above mentioned cultural practices ameliorate these physical characteristics as well as increase the diversity of the soil biota. Implementation of these practices improves soil health and reduces disease incidence in a sustainable manner (Abawi and Widmer, 2000). However, there has been little discussion of winter cover crop management for *H. elachista* population control.

1.5 Soil carbon and nematode community

Acting mainly through the response and function of organisms in the soil food web, organic matter amendments enhance soil structure, nutrient status, physical conditions, soil biological activity, and crop production and generally contribute to soil health (Hulugalle et al., 1986; Kang et al., 1981; Magdoff, 2001). Soil organic carbon (SOC) as organic matter is derived primarily from plant residues as well as from microbial residues and exudates, which are considered secondary resources (Kögel-Knabner, 2002). Organic matter accumulating in the soil feeds soil microbes and other soil organisms, potentially making crop production reliant on ecosystem self-regulation rather than on artificial inputs (Altieri, 1991). In arable fields, maintaining a large soil microbial biomass with high microbial activity enhances soil biological processes, such as organic matter decomposition and nutrient mineralization. These processes make nutrients available for crop growth (Coleman et al., 2004) and feed soil organisms (Weil and Magdoff, 2004).

The physical structure of agricultural soil changes with increasing soil organic matter content (Hamza et al., 2005; Six et al., 2000). Kahlon et al. (2013) reported that long-term use of conservation tillage and crop residue application reduced bulk density (BD) and penetration resistance with increased soil carbon (C) sequestration. Particularly in forest soil, BD was strongly correlated with SOC (Nanko et al., 2014; Perie and Ouimet, 2008). In cropland, tillage inversion markedly reduces soil hardness and BD by mechanical disturbance, but severe soil hardness often occurs in NT systems (Mu et al., 2007; Raper et al., 2000). Continued NT system; however, reduces soil hardness and BD with increased soil organic matter and the introduction of cover crops (Logsdon and Karlen, 2004; Raper et al., 2000). NT or conservation tillage and cover crop management increase soil organic matter content after long-term management, and this increase brings about soil structure changes, such as in BD and soil aggregate stability (Higashi et al., 2014; Nakamoto et al., 2012).

These soil physical changes, in general, lead to changes in soil biological properties. Although many scientists have reported long-term changes in soil C and soil quality in cropland in response to tillage and cropping systems (Doran, 1987; Franzluebbers, 2002; Havlin et al., 1990; Higashi et al., 2014), little is known about changes in the soil food web structure associated with soil quality changes over a long term. The structures of the soil food web, including nematode and other soil communities in agro-ecosystems, are affected by anthropogenic disturbances such as tillage inversion, cropping patterns, and nutrient management.

Nematodes are very sensitive to the available soil water in the soil matrix. Elliott et al. (1980) noted that the limiting factor for nematode survival often hinges on the availability of soil pore necks, which enable movement between soil pores. Yeates et al. (2002) measured the movement, growth, and survival of three genera of BAC nematodes in undisturbed soil cores maintained on a soil pressure plate. Interestingly, the nematodes showed significant reproduction when the diameter of water-filled pores was approximately 1 μm, suggesting that soil nematode may be active over a narrow range of soil moisture tension than previously thought.

Changes in the occurrences and abundances of different trophic groups of nematodes are often associated with changes in crop host and soil management practices (Ettema and Bongers, 1993) and may reflect changes in the soil food web structure. Effects of host plants on the structures of nematode communities have been documented (Neher, 1999). Host plants support the quantity and quality of soil food structure to provide certain plant root conditions to enhance the parasitic nematode directly.

Recently, long-term nematode community changes associated with farming practices have attracted interest. Pan et al. (2010) reported the impact of long-term application of chemical fertilizer and manure in soybean field in China, resulting in a significant correlation between bacterivores and soil nutrient status. Li et al. (2010) investigated nematode community changes in association with chemical fertilizer and manure application under greenhouse conditions, finding that these applications reduced the MI and channel index (CI), although the enrichment index (EI) increased with soil nutrients. However, little is known about long-term changes in nematode communities in association with tillage systems and cover crop management.

Inversion tillage mixes crop residues with soil at greater depths. Leaving crop residue and preserving stable surface soil are promoted as management practices for enhancing soil ecosystems. Fu et al. (2000) showed that soil nematodes were more abundant in NT than in MP in the field. In particular, BAC responded to residue addition earlier than did FFR in both MP and NT regimes. Higher nematode abundances were reflected by vertical residue distributions (Fu et al., 2000). Cover crop introduction resulted in twofold enrichment of opportunist BAC, which are active participants in N mineralization (DuPont et al., 2009). Ito et al. (2014) reported that tillage inversion exerted stronger effects on nematode community structure than did cover-crop management or manure input, and with increasing soil disturbance, MI and structure index (SI) decreased.

1.6 General objective

Information associating the tillage disturbance and its effect over soil nematode communities in different tillage systems has not been thoroughly discussed, especially in Asian countries. We investigated the effect of three tillage systems, i.e., MP, RC, and NT, and three cover crop treatments (fallow, rye, and hairy vetch) with two manure applications (0 and 1 Mg ha^{-1}) on nematode communities. The objectives for this study were

(1) Comparing the effects of tillage and cover cropping on soil nematode community composition and diversity.

(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.

(3) To determine whether NT and cover crop treatment could prevent rice cyst nematode infection by enhancing the health of the soil ecosystem under continuous upland rice cultivation and reduce nematode populations more rapidly than conventional cultivation methods after conversion to soybean.

(4) Comparing the long-term effects of tillage and cover crops on soil nematode community composition and indices.

(5) To determine whether there is a relationship between nematode community indices and soil C sequestration.

1.7 Thesis layout

This work is divided in other 5 chapters. Chapter 2 describes the effects of tillage systems and cover crop managements on soil nematode community structure in organic soybean production. Chapter 3 gives the reduction of the rice cyst nematode abundance by NT cultivation in continuous upland rice and after conversion to soybean production. Chapter 4 covers the responses of soil nematode community structure to soil C changes due to different tillage systems and cover crop managements over a nine-year period. Chapter 5 covers general discussion, conclusions and further recommendations.

Chapter 2: Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production

2.1 Introduction

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.

2.2 Materials and methods

2.2.1 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 during 2010–2011 (Fig. 2.1), 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 (moldboard plow: 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} (Fig. 2.2).

2.2.2 Management

Cover crops were manually sown on October 28, 2009. Seeding rates were 100 kg ha^{-1} for rye and

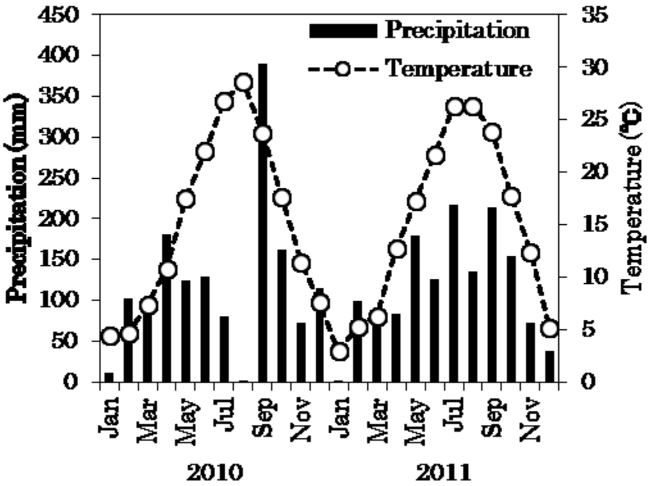

Fig. 2.1. Monthly temperature (line with markers) and precipitation (columns) at the study site (2010–2011).

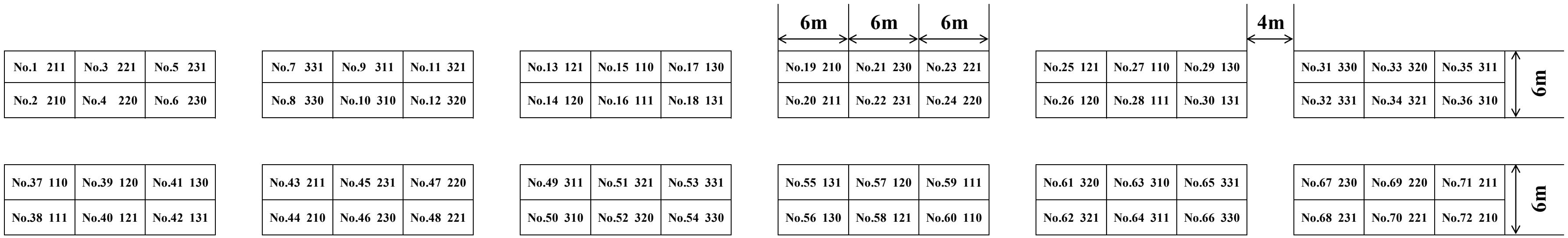

Fig. 2.2. The experimental plot design

Note: Numbers mean the plot number and the field treatments.

The hundred's digits show the tillage systems. 1, MP; 2, RC; 3, NT. The ten's digits show the cover crop managements. 1, Hairy vetch; 2, Rye; 3, Fallow.

The one's digits show the N fertilizer levels. 0, non-fertilized; 1, fertilized.

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.

2.2.3 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 C and N concentrations were quantified with a C/N coder (NC900, Sumika chemical analysis service, Ltd.).

2.2.4 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 (TAF), 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: CI, EI, and 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 *i*, 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 i = 3–5, BACi = abundance of BAC in *cp i*, FFRi = abundance of FFR in *cp i*, OMNi = abundance of OMN in *cp i*, PRDi = abundance of PRD in *cp i*, W_1 = 3.2, W_2 = 0.8, W_3 = 1.8, W_4 = 3.2, and W_5 = 5.0.

2.2.5 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 (GC4020, Canberra Industries Inc., 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) were used as reference standards.

2.2.6 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.

2.2.7 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.

2.3 Result

2.3.1 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 2.1). 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. Averaged soybean DM was 7.0 Mg ha^{-1} in 2010 and 6.3 Mg ha^{-1} in 2011, and soybean seed yield was 2.4 Mg ha^{-1} in 2010 and 1.7 Mg ha^{-1} in 2011 (data not shown).

2.3.2 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 2.2). 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 *cp*1 and FFR with *cp*2 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 2.3). The effects of cover

Table 2.1. Cover crop dry matter, carbon (C) and nitrogen (N) accumulation, and C/N ratio, compared among treatments

Treatment	2010				2011			
	DM (Mg ha^{-1})	C_{accum} (Mg ha^{-1})	N_{accum} (kg ha^{-1})	C/N ratio (%)	DM (Mg ha^{-1})	C_{accum} (Mg ha^{-1})	Naccum (kg ha^{-1})	C/N ratio (%)
Tillage								
NT	5.26	2.28	58.3	47.3	4.87 a	2.14 a	50.8	46.8
MP	5.63	2.45	75.8	53.7	3.37 b	1.46 b	40.9	40.2
RC	5.53	2.40	65.9	47.6	3.64 b	1.57 b	41.1	40.0
Cover crop								
Fallow	2.33 c	0.94 c	30.2 b	30.8 b	2.18 b	0.92 b	28.6 b	28.6 b
Hairy vetch	4.04 b	1.73 b	123.5 a	14.9 c	2.67 b	1.15 b	63.5 a	18.6 c
Rye	10.05 a	4.47 a	46.2 b	102.9 a	7.03 a	3.10 a	40.7 b	79.8 a
Manure								
1 Mg ha^{-1}	5.40	2.35	63.1	50.3	4.04	1.76	42.4	43.9
0 Mg ha^{-1}	5.54	2.40	70.2	48.8	3.88	1.69	46.1	40.8
F-value and significant effect (degree of freedom)								
Tillage (2)	0.228	0.233	2.090	1.441	4.681 *	5.077 **	1.372	2.661
Cover crop (2)	98.962 ***	104.655 ***	67.155 ***	246.030 ***	52.339 ***	55.148 ***	13.256 ***	190.077 ***
Manure (1)	0.092	0.058	1.023	0.195	0.132	0.118	0.423	1.276
Tillage × Cover crop (4)	2.095	2.186	6.549 ***	2.949 *	0.765	0.781	1.233	0.447
Tillage × Manure (2)	0.667	0.609	2.303	1.508	0.182	0.157	0.153	0.332
Cover crop × Manure (2)	1.598	1.406	1.100	0.313	2.070	1.849	0.534	1.399
Tillage × Cover crop × Manure (4)	0.500	0.522	2.658 *	1.241	1.350	1.224	0.361	0.377

Note: Data are F-values from repeated-measures ANOVA.

DM, whole plant dry matter; C_{accum}, carbon accumulation; N_{accum}, nitrogen accumulation; NT, no-tillage; MP, moldboard plow/rotary harrow; RC, rotary cultivator. Values within each column followed by a different letter (a–c) are significantly different at $P = 0.05$ based on a Tukey–Kramer test. (*), (**) and (***) represent: significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 2.2. Nematode taxa found in the study

Taxon	Family	cp score	2010		2011	
			Jun	Nov	Jun	Nov
BAC (bacterial feeder)						
Mesorhabditis -like	Rhabditidae	1	✓	✓	✓	✓
Diploscapter	Diploscapteridae	1	✓	✓	✓	✓
Bunonema	Bunonematidae	1	✓	✓	✓	✓
Pristionchus	Neodiplogasteridae	1	✓	✓	✓	✓
Geomonhystera	Monhysteridae	1	✓	✓	✓	✓
Monhystrella	Monhysteridae	1	✓	✓	✓	✓
Cephalobus	Cephalobidae	2	✓	✓	✓	✓
Heterocephalobus	Cephalobidae	2	✓	✓	✓	✓
Acrobeles, Celeborca	Cephalobidae	2	✓	✓	✓	✓
Acrobeloides	Cephalobidae	2	✓	✓	✓	✓
Cervidellus	Cephalobidae	2	✓	✓	✓	✓
Plectus s. str. spp.	Plectidae	2	✓	✓	✓	✓
Plectus (*Ceratoplectus*)	Plectidae	2	✓	✓	✓	✓
Tylocephalus	Plectidae	2	✓	×	×	✓
Wilsonema	Plectidae	2	✓	✓	✓	✓
Teratocephalus	Teratocepalidae	3	✓	×	×	×
Cylindrolaimus	Cylindrolaimidae	3	✓	✓	✓	✓
Rhabdolaimus	Rhabdolaimidae	3	✓	✓	✓	✓
Prodesmodora	Desmodoridae	3	✓	✓	✓	✓
Odontolaimus	Odontolaimidae	3	✓	✓	✓	✓
Bastiania	Bastianiidae	3	×	×	✓	✓
Prismatolaimus	Prismatolaimidae	3	✓	✓	✓	✓
Alaimus	Alaimidae	4	✓	✓	✓	✓
FFR (fungal feeder + facultative root feeder)						
Filenchus	Tylenchidae	2	✓	✓	✓	✓
Ditylenchus	Anguinidae	2	✓	✓	✓	✓
Safianema	Anguinidae	2	✓	✓	✓	✓
Aphelenchus	Aphelenchidae	2	✓	✓	✓	✓
Aphelenchoides	Aphelenchoididae	2	✓	✓	✓	✓
Diphtherophora	Diphtherophoridae	3	✓	✓	✓	✓
Tylencholaimus	Tylencholaimidae	4	✓	✓	✓	✓
PRD (predators)						
Ironus	Ironidae	4	✓	✓	✓	✓
Mylonchulus	Mononchidae	4	✓	✓	✓	✓
Solididens	Nygolaimidae	5	✓	✓	✓	✓
Discolaimus	Discolaimidae	5	✓	✓	✓	✓
OMN (omnivores)						
Amphidorylaimus	Dorylaimidae	4	✓	×	×	×
Mesodorylaimus	Dorylaimidae	4	✓	✓	✓	×
Eudorylaimus	Qudsianematidae	4	✓	✓	✓	✓
Microdorylaimus	Qudsianematidae	4	✓	✓	✓	✓
Dorylaimoides	Mydonomidae	4	✓	✓	✓	×
Opisthodorylaimus	Thornenematidae	5	✓	✓	✓	✓
Paraxonchium	Aporcelaimidae	5	✓	✓	✓	✓
Aporcelaimus	Aporcelaimidae	5	✓	✓	✓	✓
ORF (obligatory root feeder)						
Tylenchulus	Tylenchulidae	2	×	×	✓	×
Helicotylenchus	Hoplolaimidae	3	×	×	✓	×
Pratylenchus	Pratylenchidae	3	✓	✓	✓	✓
Heterodera (*Heteroderidae*)	Heteroderidae	3	✓	✓	✓	×
Criconemella	Criconematidae	3	✓	✓	✓	✓
Longidorella	Nordiidae	4	✓	×	✓	✓
Trichodorus	Trichodoridae	4	✓	✓	×	×
Dorylaimellus	Dorylaimellidae	5	✓	✓	✓	✓

Note. ✓ means observed taxa and × means non-observed taxa.

Table 2.3. Results of multiple comparison and summary of analysis of variance (ANOVA) for nematode density

Treatment	Density (Individuals per 20 g of soil)						ORF/non-ORF
	ALL	BAC	FFR	PRD	OMN	ORF	
Tillage							
NT	2673.7 a	1695.3 a	368.7 a	17.2 a	115.0 a	480.5 a	0.289 b
MP	987.8 c	471.7 c	176.3 b	11.3 b	31.5 c	297.8 b	0.509 a
RC	2230.7 b	1254.3 b	466.1 a	5.5 b	82.1 b	424.0 a	0.293 b
Cover crop							
Fallow	1640.5 b	922.4 b	273.8 b	8.5 b	79.8	357.3 b	0.390 ab
Hairy vetch	1985.3 ab	1100.2 ab	295.5 b	10.0 b	73.9	507.4 a	0.459 a
Rye	2106.3 a	1297.9 a	412.6 a	16.0 a	68.4	313.6 b	0.261 b
Manure							
1 Mg ha^{-1}	1960.4	1158.9	346.6	7.7 b	68.7	379.8	0.342
0 Mg ha^{-1}	1851.8	1040.8	301.3	16.2 a	80.8	415.2	0.411
Season							
Summer	2145.3 a	1283.8 a	368.6 a	14.8 a	83.7 a	397.3	0.350
Autumn	1679.6 b	929.6 b	284.5 b	8.1 b	64.4 b	393.6	0.394
F-value and significant effect (degree of freedom)							
Tillage (2)	48.905 ***	42.755 ***	19.032 ***	8.687 ***	27.065 ***	7.618 ***	8.865 ***
Cover crop (2)	6.226 **	6.339 **	6.976 **	3.183 *	0.214	8.111 ***	4.532 *
Manure (1)	1.178	1.939	1.624	13.970 ***	2.065	0.474	1.120
Season (1)	10.199 **	11.323 **	4.263 *	7.985 **	4.175 *	0.124	1.376
Tillage × Cover crop (4)	2.377	2.743 *	1.437	3.476 **	2.350	0.706	0.622
Tillage × Manure (2)	1.798	2.989	0.505	3.475 *	0.573	0.198	1.019
Tillage × Season (2)	4.492 *	4.397 *	3.879 *	0.430	2.194	4.250 *	1.822
Cover crop × Manure (2)	3.976 *	3.715 *	1.262	0.368	0.154	1.483	0.462
Cover crop × Season (2)	1.438	1.744	0.384	0.283	1.079	3.729 *	1.933
Manure × Season (1)	0.097	0.338	0.128	2.760	0.104	1.053	1.675
Tillage × Cover crop × Manure (4)	1.720	1.346	0.970	4.005 **	0.941	2.541 *	0.916
Tillage × Cover crop × Season (4)	1.570	1.687	0.322	0.565	1.563	1.660	3.794 **
Tillage × Manure × Season (2)	0.933	0.266	0.803	1.320	0.643	2.299	1.757
Cover crop × Manure × Season (2)	0.259	0.054	0.145	1.158	1.720	2.157	0.873
Tillage × Cover crop × Manure × Season (4)	0.436	0.143	0.182	0.658	2.401	3.011 *	0.761

Note: Data are F-values from repeated-measures ANOVA.

ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders + facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders; ORF/non-ORF, abundance ratio of ORF to non-ORF. Values within column followed by a different letter (a–c) are significantly different at $P = 0.05$ based on a Tukey–Kramer test. (*), (**), and (***) represent: significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

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. 2.3). 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 2.3). 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.

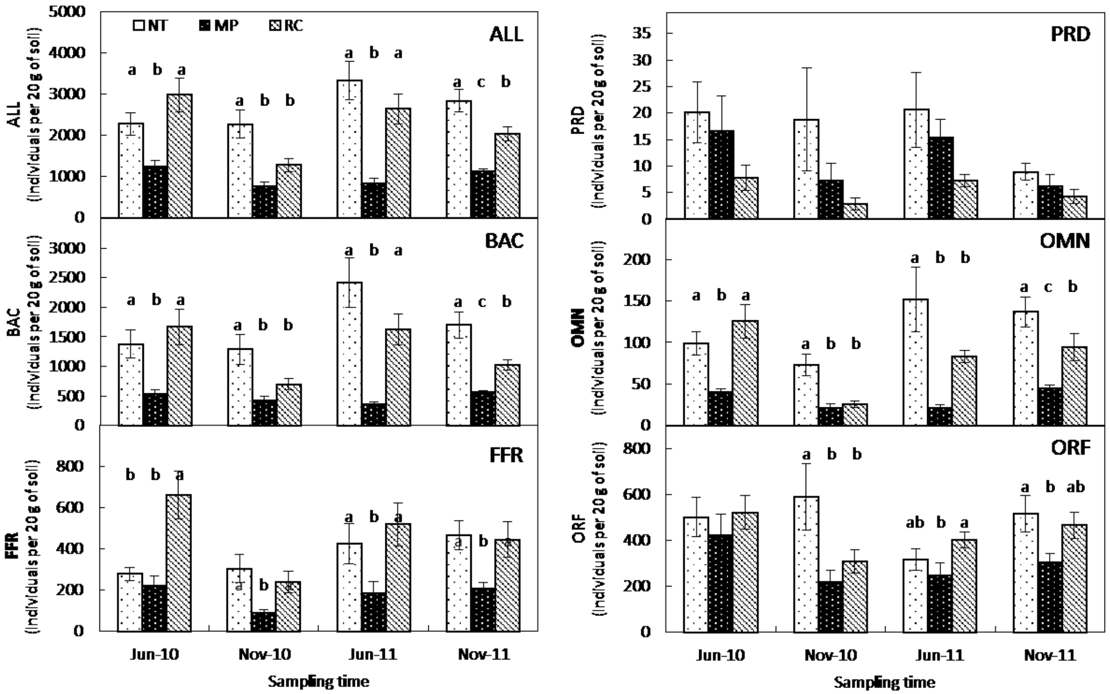

Fig. 2.3. Seasonal changes in population densities of each feeding group in no-tillage (NT), moldboard plow (MP), and rotary (RC) plots

Note. ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders + facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders. Different letters (a–c) are significantly different at $P = 0.05$ based on a Tukey–Kramer test.

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.

2.3.3 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 2.4). Overall, S, MI, and SI were higher in NT plots across tillage systems, whereas $F/(F + B)$ was lower in NT plots (Fig. 2.4). 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.

Table 2.4. Results of multiple comparison and summary of analysis of variance (ANOVA) for nematode diversity and community indices

Treatment	S	F/(F+B)	Community Indices			
			MI	CI	EI	SI
Tillage						
NT	27.1 a	0.19 b	1.93 a	27.5	57.4	69.3 a
MP	24.1 b	0.25 a	1.59 b	26.3	58.8	56.6 b
RC	25.3 b	0.26 a	1.67 b	30.3	57.0	47.3 c
Cover crop						
Fallow	25.1 b	0.22 b	1.71 b	28.6	54.3 b	56.8
Hairy vetch	24.4 b	0.21 b	1.61 b	26.1	58.4 ab	56.4
Rye	26.8 a	0.28 a	1.86 a	29.2	60.7 a	60.7
Manure						
1 Mg ha^{-1}	24.9 b	0.24	1.75	29.9	56.2 b	57.5
0 Mg ha^{-1}	26.1 a	0.23	1.70	25.3	59.9 a	58.5
Season						
Summer	26.0 a	0.24	1.80 a	25.6	60.8 a	62.8 a
Autumn	24.8 b	0.23	1.65 b	30.2	54.9 b	53.0 b
F-value and significant effect (degree of freedom)						
Tillage (2)	14.320 ***	7.603 ***	17.356 ***	0.952	0.226	32.368 ***
Cover crop (2)	6.931 **	5.343 **	7.352 ***	0.677	5.655 **	2.098
Manure (1)	7.743 **	0.015	0.372	2.803	4.032 *	0.400
Season (1)	6.818 *	0.372	8.816 **	3.077	9.722 **	19.021 ***
Tillage × Cover crop (4)	1.223	2.910 *	2.482 *	1.157	2.235	2.220
Tillage × Manure (2)	0.702	0.315	0.153	0.060	0.132	0.499
Tillage × Season (2)	1.340	3.373 *	5.828 **	0.913	1.301	4.007 *
Cover crop × Manure (2)	0.318	0.716	0.135	0.035	1.214	0.280
Cover crop × Season (2)	0.291	0.410	0.895	1.144	1.492	0.854
Manure × Season (1)	0.900	0.001	1.519	0.672	1.744	0.917
Tillage × Cover crop × Manure (4)	0.723	1.588	0.356	1.041	1.565	1.190
Tillage × Cover crop × Season (4)	0.111	0.652	3.785 **	0.203	0.246	0.233
Tillage × Manure × Season (2)	0.356	0.724	0.728	2.479	1.501	0.470
Cover crop × Manure × Season (2)	0.003	0.064	0.843	0.469	0.732	0.234
Tillage × Cover crop × Manure × Season (4)	0.361	0.522	1.322	0.683	0.362	0.333

Note. Data are F-values from repeated-measures ANOVA. S, species richness (number of taxa observed); $F/(F+B)$, abundance ratio of FFR to FFR + BAC; MI, maturity index; CI, channel index; EI, enrichment index; SI, structure index (Ferris et al., 2001). Values within each column followed by a different letter (a–c) are significantly different at $P = 0.05$ based on a Tukey–Kramer test. (*), (**), and (***) represent: significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

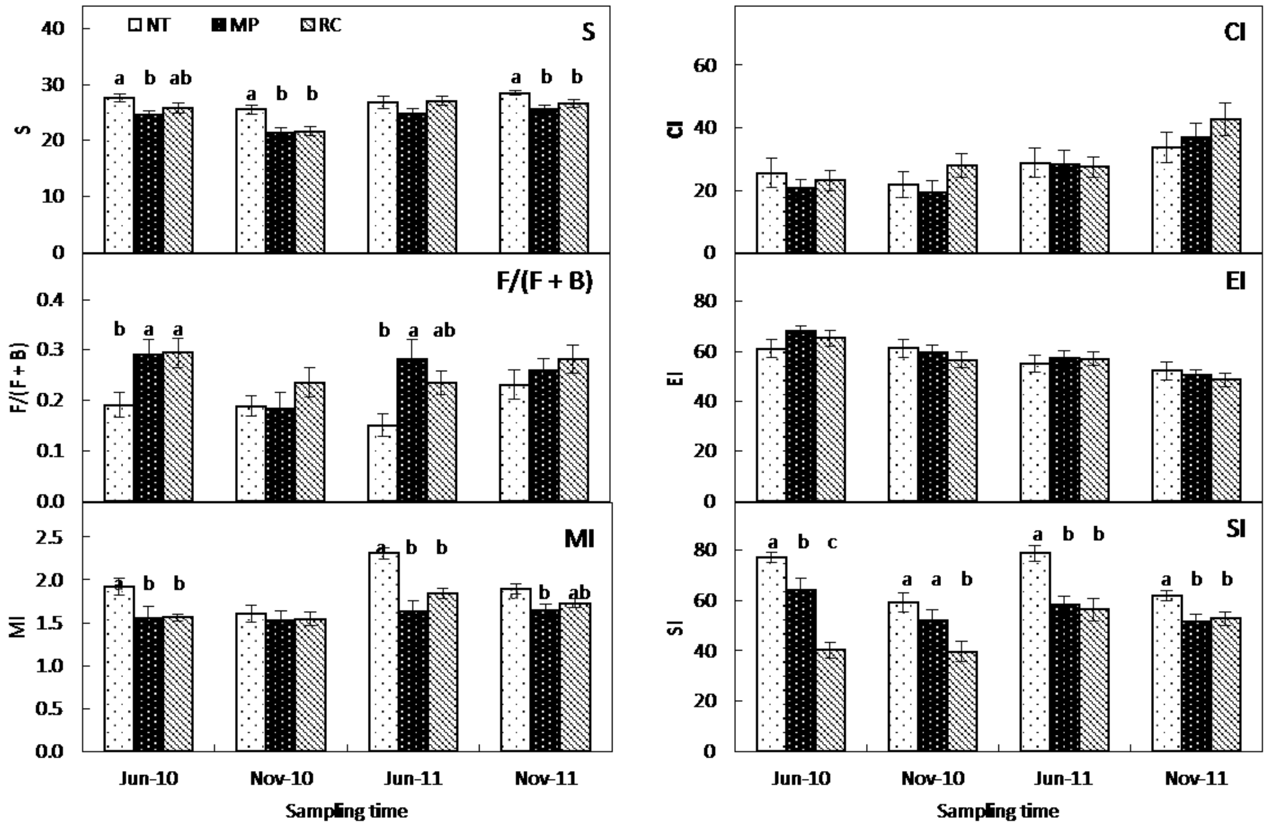

Fig. 2.4. Seasonal changes in nematode diversity and community indices in no-tillage (NT), moldboard plow (MP), and rotary (RC) plots

Note. S, species richness (number of taxa found); *F*/(*F* + *B*), abundance ratio of FFR to FFR + BAC; MI, maturity index; CI: channel index; EI, enrichment index; SI, structure index (Ferris et al., 2001). Different letters (a–c) are significantly different at $P = 0.05$ based on a Tukey–Kramer test.

2.3.4 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. 2.5). 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. 2.6).

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. 2.7). 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. 2.8).

2.3.5 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. 2.9).

2.4 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. 2.6 and 2.7).

In 2010 and 2011, nine years after converting the experimental plots to the specific tillage systems

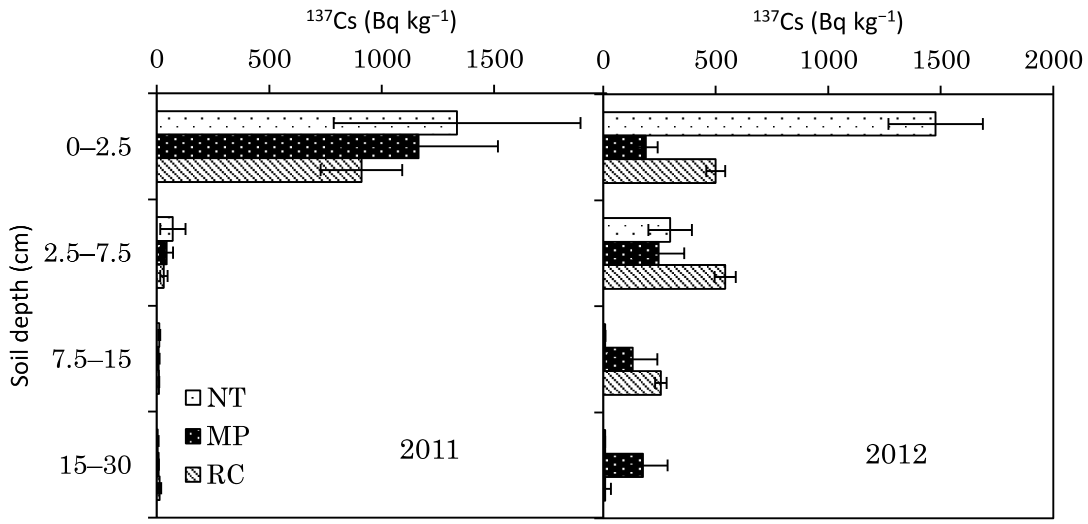

Fig. 2.5. Radioactive cesium distribution before (May 2011) and after (May 2012) tillage treatment across cover crop management.

Note. NT, no-tillage; MP, moldboard plow; RC, rotary. Horizontal bars indicate the standard error.

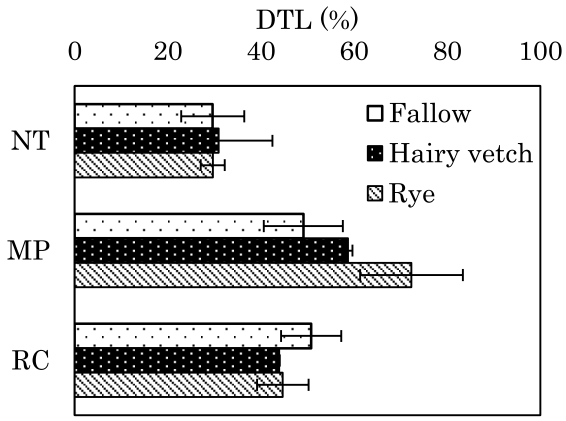

Fig. 2.6. The effects of tillage systems and cover crop treatments on the degree of soil translocation (DTL)
Note. NT, no-tillage; MP, moldboard plow; RC, rotary. Horizontal bars indicate the standard error.

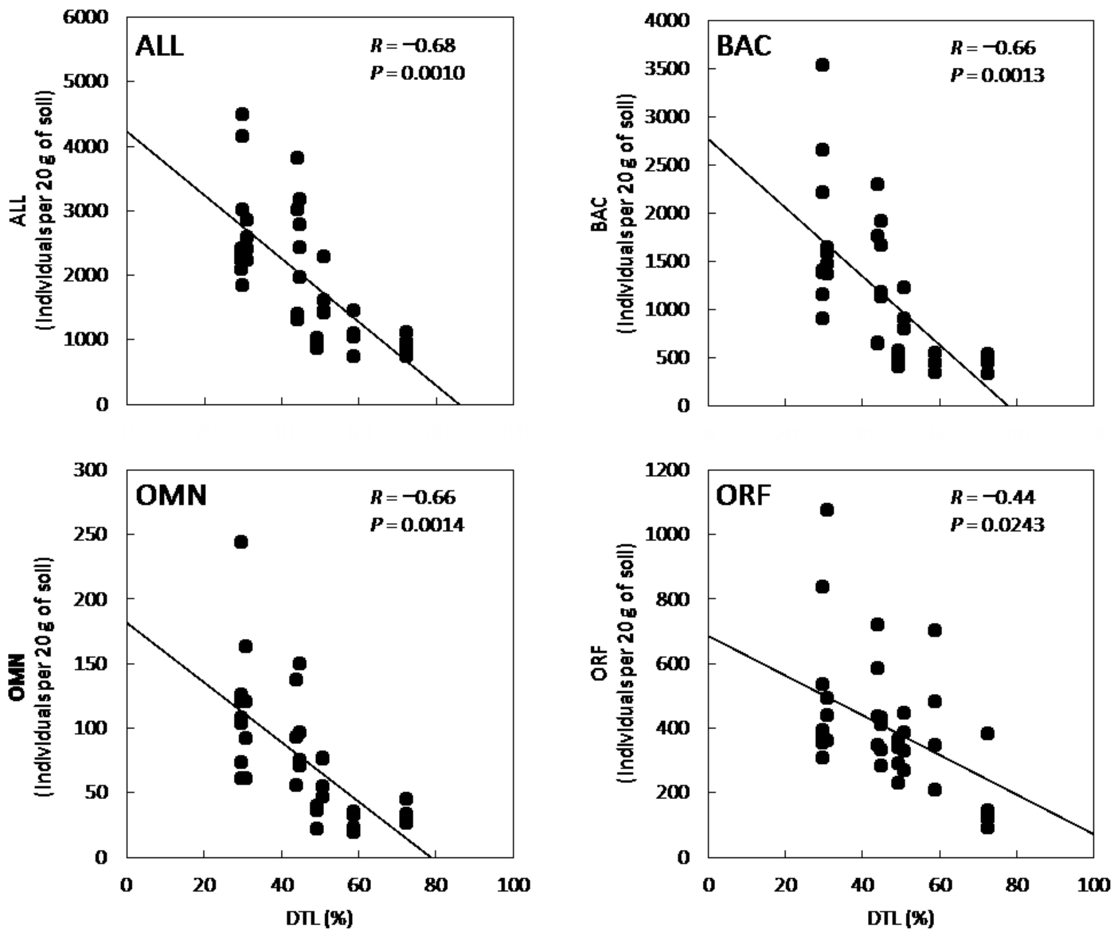

Fig. 2.7. Relationship between the degree of soil translocation (DTL) and total nematode abundance (ALL), bacterial feeders (BAC), omnivores (OMN), and obligatory root feeders (ORF)

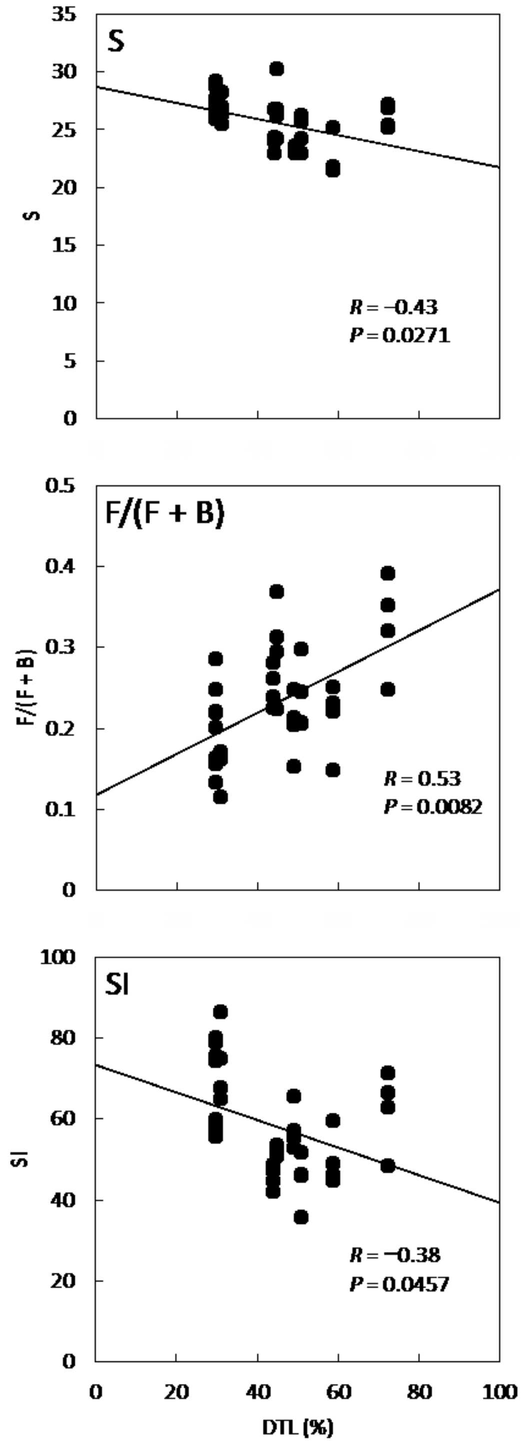

Fig. 2.8. Relationship between the degree of soil translocation (DTL) and total number of nematode species (*S*), abundance ratio of FFR to FFR + BAC [*F*/(*F* + *B*)], and structure index (SI)

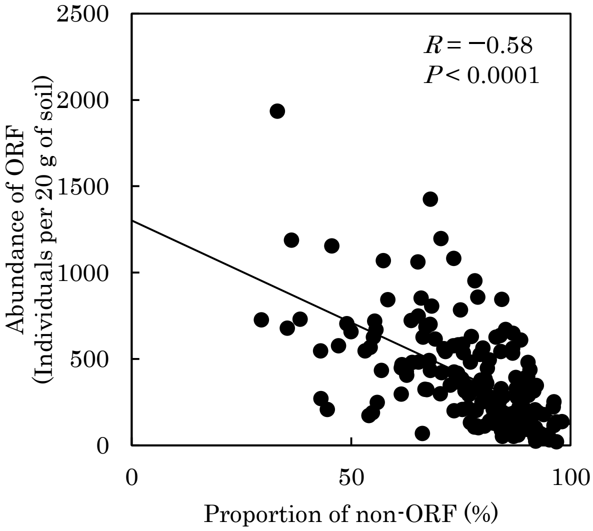

Fig. 2.9. Relationship between the abundance of obligatory root feeders (ORF) and the proportion of non-ORF

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 reflect 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 2.1) 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 2.3). In our experiment, the amount of manure applied was small compared to the amount of cover crop residue input (Table 2.1), 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 2.4). 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 FFR nematodes. 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. 2.7); 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.

2.5 Conclusion

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.

Chapter 3: No-tillage cultivation reduces the rice cyst nematode (*Heterodera elachista*) in continuous upland rice (*Oryza sativa*) and after conversion to soybean (*Glycine max*) in Kanto, Japan

3.1 Introduction

The rice cyst nematode *Heterodera elachista*, previously reported only in Japan, has recently been detected in Asia and Europe. Because control of *H. elachista* by fumigation is economically impractical, the control of *H. elachista* via farming practices in the form of tillage, cover cropping, and N fertilization is attractive. The objectives of this study were to determine whether NT and cover crop treatment could prevent rice cyst nematode infection by enhancing the health of the soil ecosystem under continuous upland rice cultivation and reduce nematode populations more rapidly than conventional cultivation methods after conversion to soybean. In particular, we focused on testing the following hypotheses.

1. NT would prevent an increase in *H. elachista* density and proportion in continuous cropping of upland rice.
2. Cover crop treatment would prevent an increase in *H. elachista* density and proportion in continuous cropping of upland rice.
3. Nematode diversity would be associated with NT and cover crop treatment and these would prevent the density and proportion of *H. elachista* from increasing.
4. Yield loss of upland rice would be reduced under NT and cover crop treatment via reduction of the population density of *H. elachista*.

3.2 Materials and methods

3.2.1 Study site and treatments

The study was conducted at the Field Science Center of Ibaraki University, Japan in 2002–2011 and is currently ongoing. Prior to 1950, this experiment field was a lowland forest; the site had been maintained as upland crop fields for >40 years and subjected to conventional tillage such as plowing and rotary cultivation. The climate is relatively humid and classified as Cfa (humid subtropical and hot summer) (Trewartha, 1968), and is suitable for double-crop rotation for main crops (in summer) and cover crops (in winter). The mean rainfall in the area in 2000–2002 was 1309 mm. Distribution of precipitation across the season is an important consideration. The highest rainfalls recorded in the period studied were in 2004–2005 (1554 mm) and in 2009–2010 (1560 mm) and the lowest rainfalls were recorded in 2003–2004 (1069 mm) and 2008–2009 (1051 mm).

The study covered 72 plots of 3 m × 6 m with 2 m wide aisles between plots. The soil was prepared using the respective tillage system: MP, RC, and NT. Cover crop treatments were hairy vetch, winter rye, and fallow since 2002. N levels were 0 and 100 kg N ha^{-1} for upland rice in 2003–2007 and 0 and 20 kg N ha^{-1} for soybean in 2008–2009. In 2010–2011, 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}. In a 4-replicate split–split experimental design, tillage system varied in the main plot and cover crop treatments and N input levels varied in subsubplots. In 2002–2007, the main summer crop was upland rice (*Oryza sativa* 'Yumenohatamochi'), and in 2008–2011, it was soybean (*Glycine max* 'Enrei' in 2008–2009 and 'Nattosyoryu' in 2010–2011).

Cover crops were sown by hand from late October to early November. Rye was sown at 100 kg ha^{-1} and hairy vetch at 50 kg ha^{-1}. No fertilization and shallow disking (approximately 3 cm depth) were applied after cover crop seeding. Cover crops and native weeds were grown until early April in the upland rice rotation or late May in the soybean rotation, and mowed by flail mower to return the cover crop residue to the soil. Under MP, the soil was plowed to a depth of 25–30 cm and the residues of cover crops incorporated into the soil. Under RC, cover crop residues were also incorporated by rotary cultivation to a depth of 0–15 cm. Under NT, cover crop residues were left on the soil surface. Upland rice and soybean were sown with a no-tillage seeder in late April for upland rice and late June for soybean. Seeding rates were 50 kg ha^{-1} for upland rice and 50 kg ha^{-1} for soybean. Phosphorus and potassium were applied at seeding time, with application rates of 100 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} for upland rice and soybean. N was applied only to subsubplots, at 100 kg N ha^{-1} for upland rice and 20 kg N ha^{-1} in 2008–2009 for soybean. In 2010–2011 bark with chicken manure was applied to subsubplots at 1 Mg ha^{-1} for soybean. Glyphosate isopropylammonium (1 L ha^{-1}, 41%) was applied before planting to all plots, after seeding of upland rice or soybean and hand weeding was performed two or three times during each growing period. Upland rice was harvested with a head-feeding combine and upland rice residue was returned to the soil. Soybeans were harvested with a walking-type binder similar to the local farmers; all of the soybean plants were harvested, moved into a greenhouse to dry, and threshed off the field. The resulting soybean residue was not returned to the soil.

3.2.2 Rice yield and grain-straw ratio analysis

Upland rice from each plot was threshed, and grain was separated per commercial standard and measured. Grain and straw (stems + leaves) were also measured. We calculated the grain:straw ratio as

$$\text{Grain:straw ratio (\%)} = \frac{\text{Dry grain yield (Mg ha}^{-1}\text{)}}{\text{Dry straw weight (Mg ha}^{-1}\text{)}} \times 100$$

3.2.3 Nematode sampling, extraction, identification, and diversity analysis

Soil nematode samples were collected from selected plots two times yearly from all treatments: after plowing (May 2003–2007 and June 2008–2011) and after harvest (October 2003 and November 2004–2011). Samples were collected with a steel trowel from the top 10 cm depth, excluding the uppermost soil layer.

Nematodes were extracted from the 20 g of fresh soil using Baermann funnels methods. The nematodes collected were heat killed (60 °C) and fixed with TAF, transferred to flamed slide glasses with approximately 1 ml of fixative, and observed under a microscope. Five hundred randomly chosen individual nematodes were identified to genus or family level to estimate density per 20 g of soil at a magnification of 1000×. Nematode taxa were assigned to feeding groups according to Yeates et al. (1993). In this research, we monitored the population density of *H. elachista* using Baermann funnels methods for 9 years long term nematode community changes. Minagawa (1998) reported cyst nematode extraction by Baermann funnels methods showed larger abundance than direct microscopic counts, therefore, our data of *H. elachista* density suggested the potential of *H. elachista* abundance in current soil condition.

We calculated Shannon–Wiener's index (H') a measure of diversity of nematodes. H' takes into account the species richness and proportion of each species and was calculated as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where S = total number of nematode species and p_i = proportion of total sample represented by species i.

3.2.4 Statistical analysis

Data were statistically analyzed by ANOVA or repeated-measures ANOVA (StatView, SAS Institute) for a split–split plot design, applying the Tukey–Kramer method (P <0.05). Regression analyses were also performed to evaluate the relationships between grain yield of upland rice, grain:straw ratio, *H. elachista* density, and H'.

3.3 Result

3.3.1 Population density and proportion of H. elachista

The population density of *H. elachista* was higher in May and June than in November (Table 3.1). In both continuous upland rice cultivation and soybean cultivation, the population density of *H. elachista* in MP and RC was equal to or greater than that in NT at most sampling times. *H. elachista* was not observed in our experimental field in May 2003 to May 2004. *H. elachista* was observed for the first time in November 2004, and mean population densities of *H. elachista* were 0.8 nematodes per 20 g fresh soil in NT, 0.3 in MP, and 0.8 in RC. In 2006, *H. elachista* was significantly ($P < 0.05$) affected by tillage system, being lower in NT than in RC (May 2006) and in MP (November 2006). *H. elachista* density in May 2007 was the highest density observed during the continuous cropping of upland rice (2003–2007). However, tillage system did not significantly influence the population density of *H. elachista*. The ranges of *H. elachista* densities were 0–758 nematodes per 20 g fresh soil in NT, 6–746 in MP, and 9–636 in RC (Fig. 3.1). In contrast to the trend in the other years, the population densities were significantly (P <0.05) higher in NT than in MP in November 2007. *H. elachista* in June 2008 showed the greatest abundance observed during 2003−2011, and densities varied widely among plots, particularly in NT. The density ranges were 3−1539 nematodes in NT, 106−1244 in MP, and 177−988 in RC. *H. elachista* density rapidly decreased after conversion to soybean. The density was significantly lower in NT than in MP and RC in June 2009 (P <0.05) and June 2010 (P <0.001). The population density continued to decrease and it became zero in all experimental plots in November 2011. Cover crop treatment did not significantly affect *H. elachista* density over the 9 years. However, population density was significantly influenced by N levels in November 2006 (P <0.05) and November 2007 (P <0.01). It was higher in the high N level than in the low N level.

The proportion of *H. elachista* was significantly affected by tillage system, similar to the population density, and was lower in NT than in MP and RC at most sampling times (Table 3.2). Moreover, the proportion was significantly affected by cover crop treatments. At most sampling times, the proportions of *H. elachista* in fallow plots were significantly higher than those in hairy vetch and rye plots. The proportion varied widely among plots, particularly in fallow. In June 2008, the range of *H. elachista* proportion was 5.9%–52.9% in fallow, 0.2%–31.4% in hairy vetch, and 0.1%–48.6% in rye. N levels significantly (P <0.05) affected the proportion of *H. elachista* only in November 2007; it was higher at the high N level than at the low N level.

Table 3.1. Effects of tillage system, cover crop treatment, and N level on *Heterodera elachista* population density in 2003–2011.

Treatment	2003		2004		2005		2006		2007		2008		2009		2010		2011	
	May	Oct	May	Nov	May	Nov	May	Nov	May	Nov	Jun	Nov	Jun	Nov	Jun	Nov	Jun	Nov
Tillage (T)																		
NT	0.0	0.0	0.0	0.8	3.9	1.7	2.1^{b}	3.6^{b}	198.4	47.9^{a}	262.1	3.8	8.2^{b}	0.0	1.1^{b}	0.0	0.3	0.0
MP	0.0	0.0	0.0	0.3	0.5	6.9	52.8^{ab}	21.6^{a}	221.4	11.9^{b}	419.6	0.9	35.1^{a}	0.6	10.4^{a}	0.1	0.6	0.0
RC	0.0	0.0	0.0	0.8	2.2	8.5	72.7^{a}	11.1^{ab}	249.0	23.9^{ab}	516.5	3.5	32.4^{a}	0.4	10.8^{a}	0.0	0.5	0.0
Cover crop (CC)																		
Fallow	0.0	0.0	0.0	0.6	4.6	10.6	73.5	11.4	285.8	24.4	372.7	2.6	22.4	0.1	7.4	0.0	0.9	0.0
Hairy vetch	0.0	0.0	0.0	0.1	0.1	2.9	19.9	11.5	222.6	20.7	423.9	1.6	28.1	0.5	6.9	0.0	0.1	0.0
Rye	0.0	0.0	0.0	1.2	1.7	4.4	35.1	14.9	158.4	37.2	394.4	3.7	26.0	0.4	8.3	0.1	0.5	0.0
N level (N)																		
High	0.0	0.0	0.0	0.7	1.3	5.5	40.1	17.0^{a}	255.4	39.4^{a}	487.1	3.1	29.6	0.5	8.3	0.0	0.5	0.0
Low	0.0	0.0	0.0	0.5	3.2	6.2	45.1	7.0^{b}	180.4	11.9^{b}	284.6	2.1	20.4	0.2	6.5	0.0	0.5	0.0
Significant effect																		
T	-	-	-	NS	NS	NS	*	*	NS	*	NS	NS	*	NS	***	NS	NS	-
C	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-
N	-	-	-	NS	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS	NS	-
T×CC	-	-	-	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	-
T×N	-	-	-	NS	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS	NS	-
CC×N	-	-	-	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	-
T×CC×N	-	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

Note: Unit of population density is individuals per 20 g fresh soil. NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. Numbers followed by the same letter within tillage system, cover crop treatment, and N level are not significantly different at $P<0.05$ by Tukey–Kramer test. *, **, and *** denote significance at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

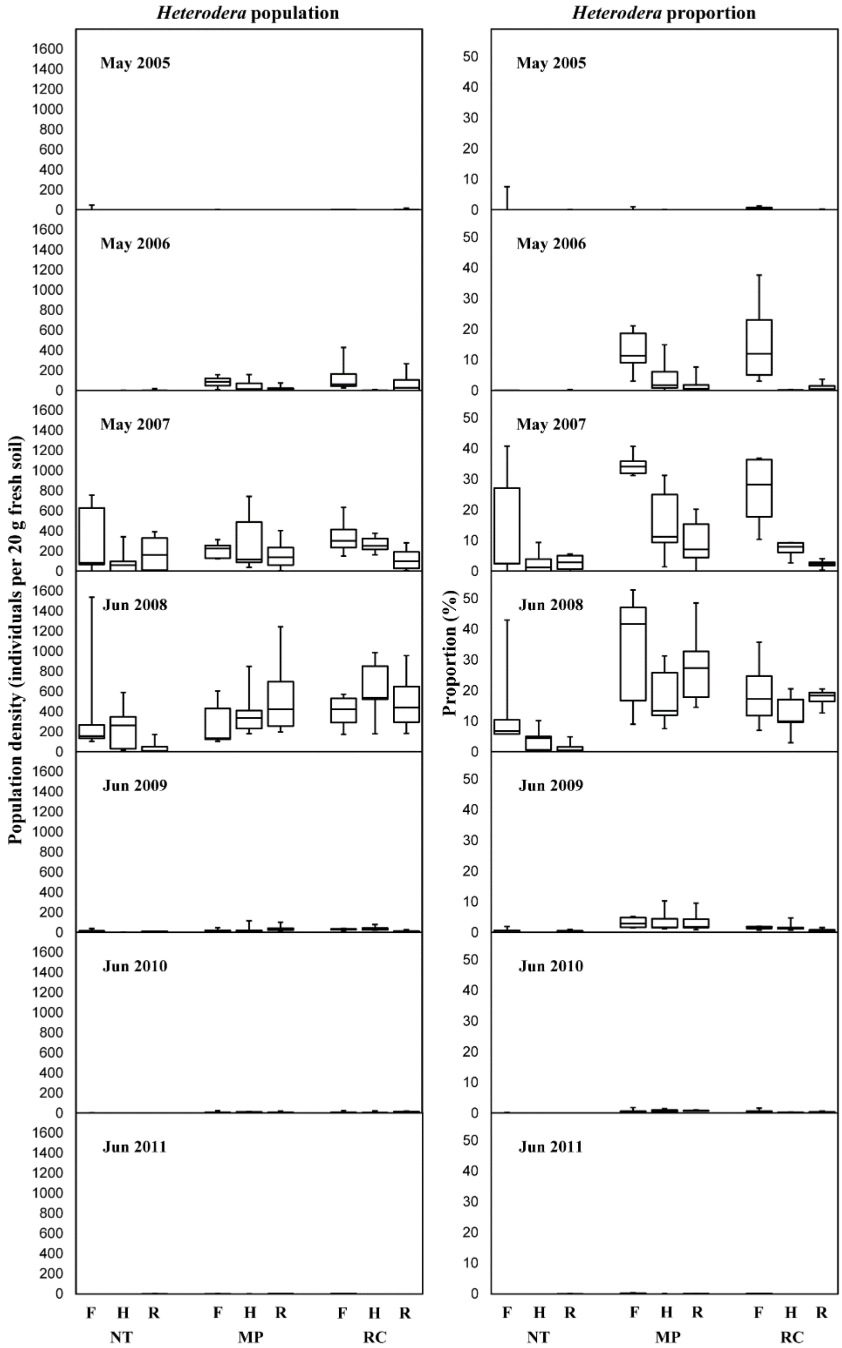

Fig. 3.1. ***Heterodera elachista*** **population densities and proportions in relation to tillage system and cover crop treatment.**

Note: Box plot shows variation of *H. elachista* population density in each tillage system and cover crop treatment in spring samples of 2005–2011. NT, no-tillage; MP, moldboard plow; RC, rotary cultivator; F, fallow; H, hairy vetch; R, rye. The line within the box represents the median, the box represents 50% of the data, and the whiskers represent maximum and minimum values of the data; $n = 4$.

Table 3.2. Effects of tillage system, cover crop treatment, and N level on *Heterodera elachista* proportion in 2003–2011.

Treatment	2003		2004		2005		2006		2007		2008		2009		2010		2011	
	May	Oct	May	Nov	May	Nov	May	Nov	May	Nov	Jun	Nov	Jun	Nov	Jun	Nov	Jun	Nov
Tillage (T)																		
NT	0.0	0.0	0.0	0.1	0.6	0.2	0.1^{b}	0.4^{b}	7.1^{b}	2.1	7.1^{b}	0.1	0.4^{b}	0.0	0.1^{c}	0.0	0.0	0.0
MP	0.0	0.0	0.0	0.1	0.1	2.1	6.2^{a}	4.4^{a}	19.3^{a}	1.8	26.6^{a}	0.1	3.6^{a}	0.1	0.8^{a}	0.0	0.1	0.0
RC	0.0	0.0	0.0	0.1	0.2	1.1	5.4^{ab}	1.9^{b}	11.5^{ab}	1.5	16.0^{ab}	0.2	1.5^{b}	0.0	0.4^{b}	0.0	0.0	0.0
Cover crop (CC)																		
Fallow	0.0	0.0	0.0	0.2	0.8	2.7	9.2^{a}	3.9^{a}	25.1^{a}	2.1	22.7	0.1	1.9	0.0	0.5	0.0	0.1	0.0
Hairy vetch	0.0	0.0	0.0	0.0	0.0	0.5	1.7^{b}	1.9^{b}	8.6^{b}	1.5	11.4	0.1	2.0	0.0	0.3	0.0	0.0	0.0
Rye	0.0	0.0	0.0	0.1	0.0	0.5	1.2^{b}	1.1^{b}	5.6^{b}	1.9	17.4	0.1	1.9	0.0	0.5	0.0	0.0	0.0
N level (N)																		
High	0.0	0.0	0.0	0.1	0.1	0.9	3.1	2.6	13.3	2.3^{a}	17.3	0.1	2.3	0.0	0.5	0.0	0.0	0.0
Low	0.0	0.0	0.0	0.2	0.5	1.6	5.0	1.9	12.6	1.2^{b}	16.7	0.1	1.4	0.0	0.4	0.0	0.0	0.0
Significant effect																		
T	-	-	-	NS	NS	NS	*	***	**	NS	**	NS	**	NS	***	NS	NS	-
C	-	-	-	NS	NS	NS	**	**	***	NS	NS	NS	NS	NS	NS	NS	NS	-
N	-	-	-	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	-
T×CC	-	-	-	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	-
T×N	-	-	-	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	-
CC×N	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-
T×CC×N	-	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

Note: Unit of proportion is percent. NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. Numbers followed by the same letter within tillage system, cover crop treatment, and N level are not significantly different at $P < 0.05$ by Tukey–Kramer test. *, **, and *** denote significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

3.3.2 Relationship between H. elachista density and nematode diversity

Tillage system significantly influenced *H′ at* most sampling times (Table 3.3). *H′* in NT was equal to or lower than that in MP and RC in the autumn of 2003–2007. In contrast to this trend, *H′* in NT was equal to or greater than that in MP and RC in 2008–2011. In addition, cover crop treatments affected *H′* at most sampling times. *H′* in hairy vetch was equal to or lower than those in fallow plots, and was higher in rye. *H′* was significantly influenced by N input levels at several sampling times. In October 2003, May 2004, November 2004, and June 2010, *H′* was lower at the high N level than at the low N level. In contrast, *H′* values in November 2006 and November 2011 were higher at the high N level than at the low N level.

A significant negative correlation was observed between the population densities of *H. elachista* and *H′* (P <0.0001, $R = -0.306$) in 2005–2011 (Fig. 3.2). In this experiment, the values of *H′* ranged from 0.18–2.64. Higher *H′* significantly reduced *H. elachista* population density. The relationship between *H′* and the proportion of *H. elachista* was also significant and negative (P <0.0001, $R = -0.280$). When *H′* was >2.0, the proportion was <20%.

3.3.3 Productivity of upland rice

The mean yield of upland rice was 2.03 Mg ha^{-1} in 2003–2007. Tillage system significantly (P <0.01) affected grain yield in 2006, although there were no significant differences and was higher in rye in other years between tillage systems (Table 3.4). Grain yields in 2006 were significantly lower in RC than in NT and MP. The mean grain yield was 1.50 Mg ha^{-1} in the RC plot, 29.0% and 28.0% lower than those in NT and MP, respectively. Cover crop treatment did not affect grain yield. In contrast, N input level significantly influenced yield at most sampling times. Grain yield was higher at the high N level than at the low N level in 2003–2006.

No significant differences between tillage systems in grain:straw ratio were observed. Cover crop treatment significantly (P <0.05) influenced grain:straw ratio in 2007, although these differences were not observed in other years. The grain:straw ratio was lower in hairy vetch than in fallow in 2007. N input level affected grain:straw ratio in 2003 and 2007. In 2003, the grain:straw ratio was significantly (P <0.05) higher at high N than at low N. In contrast in 2007, the grain:straw ratio was significantly (P <0.01) lower at the high N level than at the low N level.

3.3.4 Relationship between H. elachista and productivity of upland rice

In the fertilized plots (with high N level), the population density of *H. elachista* was

Table 3.3. Effects of tillage system, cover crop treatment, and N level on Shannon–Weaver index (*H′*) in 2003–2011.

Treatment	2003		2004		2005		2006		2007		2008		2009		2010		2011	
	May	Oct	May	Nov	May	Nov	May	Nov	May	Nov	Jun	Nov	Jun	Nov	Jun	Nov	Jun	Nov
Tillage (T)																		
NT	1.91	2.06^{b}	1.43	1.57^{b}	1.59	1.92^{b}	1.52	1.81^{b}	1.50	1.61^{ab}	1.40	1.47	1.66	1.36	1.73^{a}	1.63	1.84^{a}	1.87^{a}
MP	2.17	2.36^{a}	1.62	1.92^{a}	1.91	2.29^{a}	1.80	2.02^{a}	1.42	1.71^{a}	1.15	1.41	1.61	1.07	1.36^{b}	1.44	1.38^{b}	1.61^{b}
RC	1.91	2.28^{ab}	1.54	1.87^{a}	1.87	2.39^{a}	1.65	2.16^{a}	1.62	1.38^{b}	1.43	1.40	1.50	1.01	1.41^{b}	1.57	1.73^{ab}	1.74^{ab}
Cover crop (CC)																		
Fallow	2.16^{a}	2.31	1.57^{a}	1.70	1.92^{a}	2.17	1.86^{a}	1.99	1.44^{ab}	1.58	1.30	1.56	1.38^{b}	1.02	1.41^{b}	1.44	1.57^{b}	1.81^{a}
Hairy vetch	1.72^{b}	2.09	1.30^{b}	1.70	1.58^{b}	2.13	1.53^{b}	2.00	1.38^{b}	1.52	1.16	1.25	1.54^{ab}	1.14	1.30^{b}	1.55	1.36^{b}	1.50^{b}
Rye	2.11^{a}	2.29	1.72^{a}	1.95	1.89^{a}	2.31	1.61^{ab}	1.99	1.70^{a}	1.64	1.50	1.47	1.86^{a}	1.27	1.79^{a}	1.63	2.00^{a}	1.91^{a}
N level (N)																		
High	1.92	2.14^{b}	1.45^{b}	1.70^{b}	1.75	2.22	1.72	2.07^{a}	1.55	1.58	1.35	1.46	1.54	1.15	1.40^{b}	1.53	1.64	1.85^{a}
Low	2.13	2.40^{a}	1.67^{a}	1.93^{a}	1.84	2.18	1.59	1.89^{b}	1.45	1.57	1.28	1.38	1.66	1.14	1.61^{a}	1.56	1.63	1.58^{b}
Significant effect																		
T	NS	**	NS	**	NS	***	NS	**	NS	*	NS	NS	NS	NS	**	NS	**	*
C	**	NS	**	NS	*	NS	*	NS	*	NS	NS	NS	*	NS	**	NS	**	**
N	NS	**	*	*	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	*	NS	NS	**
T×CC	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T×N	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CC×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T×CC×N	-	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. Numbers followed by the same letter within tillage system, cover crop treatment, and N level are not significantly different at $P < 0.05$ by Tukey–Kramer test. *, **, and *** denote significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

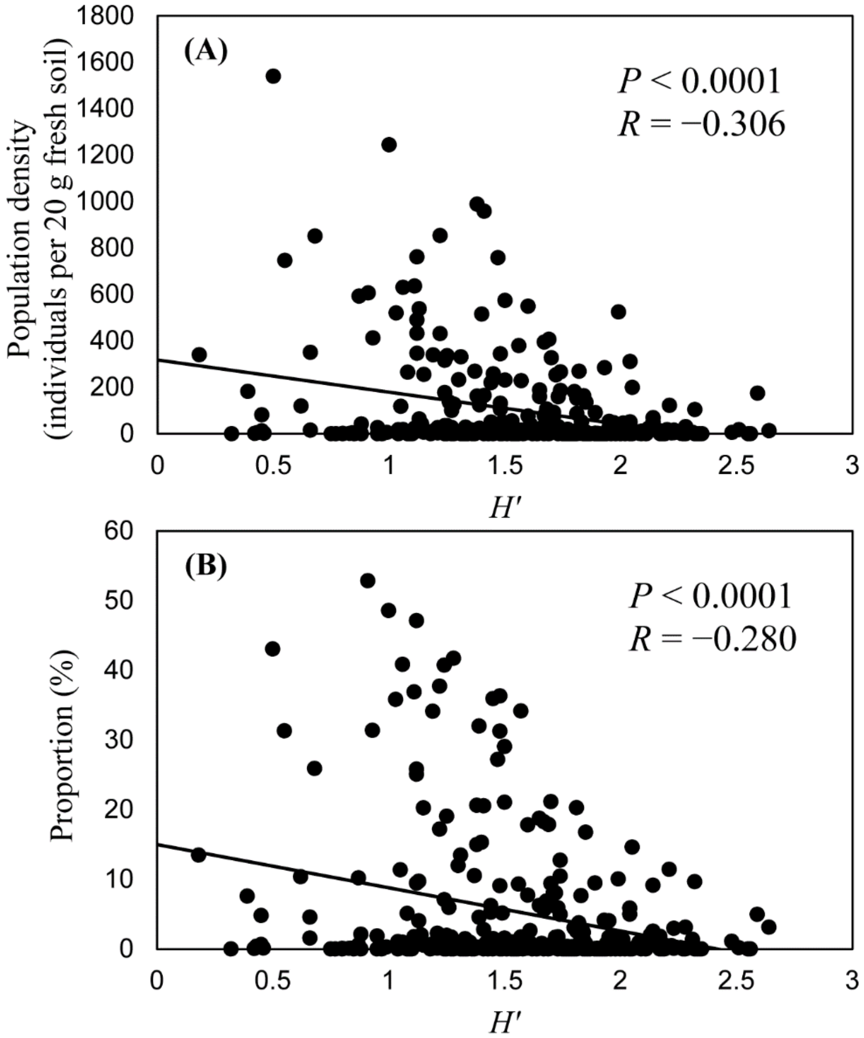

Fig. 3.2. Relationship between *H′* and *Heterodera elachista* population density and proportion.

Note: *H′* and *H. elachista* density and proportion were estimated in the spring sampling of 2005–2011. (A) Relationship between *H′* and *H. elachista* population density. (B) Relationship between *H′* and *H. elachista* proportion.

Table 3.4. Grain yield and grain:straw ratio of upland rice (*Oryza sativa*) in relation to tillage system, cover crop treatment, and N level in 2003–2007.

Treatment	2003		2004		2005		2006		2007	
	Grain (Mg ha^{-1})	Grain-straw ratio (%)	Grain (Mg ha^{-1})	Grain-straw ratio (%)	Grain (Mg ha^{-1})	Grain-straw ratio (%)	Grain (Mg ha^{-1})	Grain-straw ratio (%)	Grain (Mg ha^{-1})	Grain-straw ratio (%)
Tillage (T)										
NT	1.79	67.2	2.18	102.0	2.27	94.6	2.10^{a}	96.2	1.65	39.9
MP	1.72	69.5	2.61	97.5	2.53	97.8	2.07^{a}	87.1	1.72	51.2
RC	1.66	65.8	2.60	90.4	2.32	89.8	1.50^{b}	85.8	1.77	47.8
Cover crop (CC)										
Fallow	1.75	67.7	2.28	98.8	2.56	97.9	1.87	92.6	1.80	51.7^{a}
Hairy vetch	1.71	67.9	2.50	90.4	2.29	90.0	2.14	92.4	1.47	36.3^{b}
Rye	1.71	66.9	2.61	100.8	2.26	94.4	1.68	83.5	1.87	50.9^{ab}
N level (N)										
High	2.03^{a}	70.2^{a}	2.88^{a}	100.1	2.89^{a}	95.3	2.37^{a}	92.4	1.89	37.9^{b}
Low	1.42^{b}	64.8^{b}	2.05^{b}	93.2	1.85^{b}	92.9	1.40^{b}	86.8	1.53	54.8^{a}
Significant effect										
T	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
C	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
N	**	*	***	NS	***	NS	***	NS	NS	**
T×CC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CC×N	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
T×CC×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. Numbers followed by the same letter within tillage system, cover crop treatment, and N level are not significantly different at P <0.05 by Tukey–Kramer test. *, **, and *** denote significance at P <0.05, P <0.01, and P <0.001, respectively.

significantly negatively correlated with grain yield (*P* =0.0154, *R* = −0.222) and grain:straw ratio (*P* <0.0001, *R* = −0.491) (Fig. 3.3). In contrast, in the unfertilized plots (with low N level), the density was not correlated with grain yield but was significantly correlated with grain:straw ratio (*P* = 0.0035, *R* = −0.315).

Similarly to the population density, in the fertilized plots, the proportion of *H. elachista* was negatively correlated with grain yield (*P* = 0.0075, *R* = −0.244) and grain:straw ratio (*P* <0.0001, *R* = −0.390). In the unfertilized plots, no significant relationship was observed between proportion and grain yield. However, proportion was significantly negatively correlated with grain:straw ratio (*P* = 0.0057, *R* = −0.299).

3.4 Discussion

In this study, second-stage juveniles of *H. elachista* were observed after 2 years of continuous cropping of upland rice. Before this study, silage corn was cultivated in this field. Few *H. elachista* were expected to remain in the corn roots, and *H. elachista* increased after upland rice cultivation had started, as *H. elachista* are parasitic on corn (Okada, 1960). *H. elachista* continuously increased as long as upland rice cultivation continued until soybean cultivation had started, although seasonal changes were also observed. In particular, population density of *H. elachista* was high in spring because larvae emerged from cysts surviving the winter, produced by adult females, several of which died in autumn (Shimizu, 1976). These seasonal changes were well reflected in this experiment.

However, *H. elachista* population density significantly differed between tillage systems and N levels but not between cover crop treatments. Our 9-year field study revealed that NT reduced *H. elachista* population density during continuous cropping of upland rice. In a previous study (Shimizu, 1976), significant yield loss because of *H. elachista* occurred when its population density was >32 nematodes per 20 g fresh soil. Our results showed that NT can limit the increase of *H. elachista* population density to as high as five nematodes per 20 g soil for 4 years of rice continuous cropping, although the mean density of *H. elachista* in MP and RC increased to >50 nematodes. NT was expected to reduce *H. elachista* population growth when host plants were cultivated. However, *H. elachista* also increased under NT after 4 years of continuous rice cropping. One year after conversion to soybean, *H. elachista* density in NT rapidly decreased to <10 nematodes. The densities in MP and RC were reduced, but remained >32 nematodes even after 2 years of cultivation of the new crop. The patterns of density decrease showed a sharp contrast between tillage systems. In addition, *H. elachista* density was affected by N input levels. Increasing rice root biomass by N fertilization may have increased the population density of *H. elachista*.

Our results showed a significant negative relationship between *H. elachista* density and grain yield of upland rice and between *H. elachista* proportion and grain yield in an N-fertilized plot. Damage by *H. elachista* to growth of upland rice was severe at early seeding (Shimizu, 1976). Because

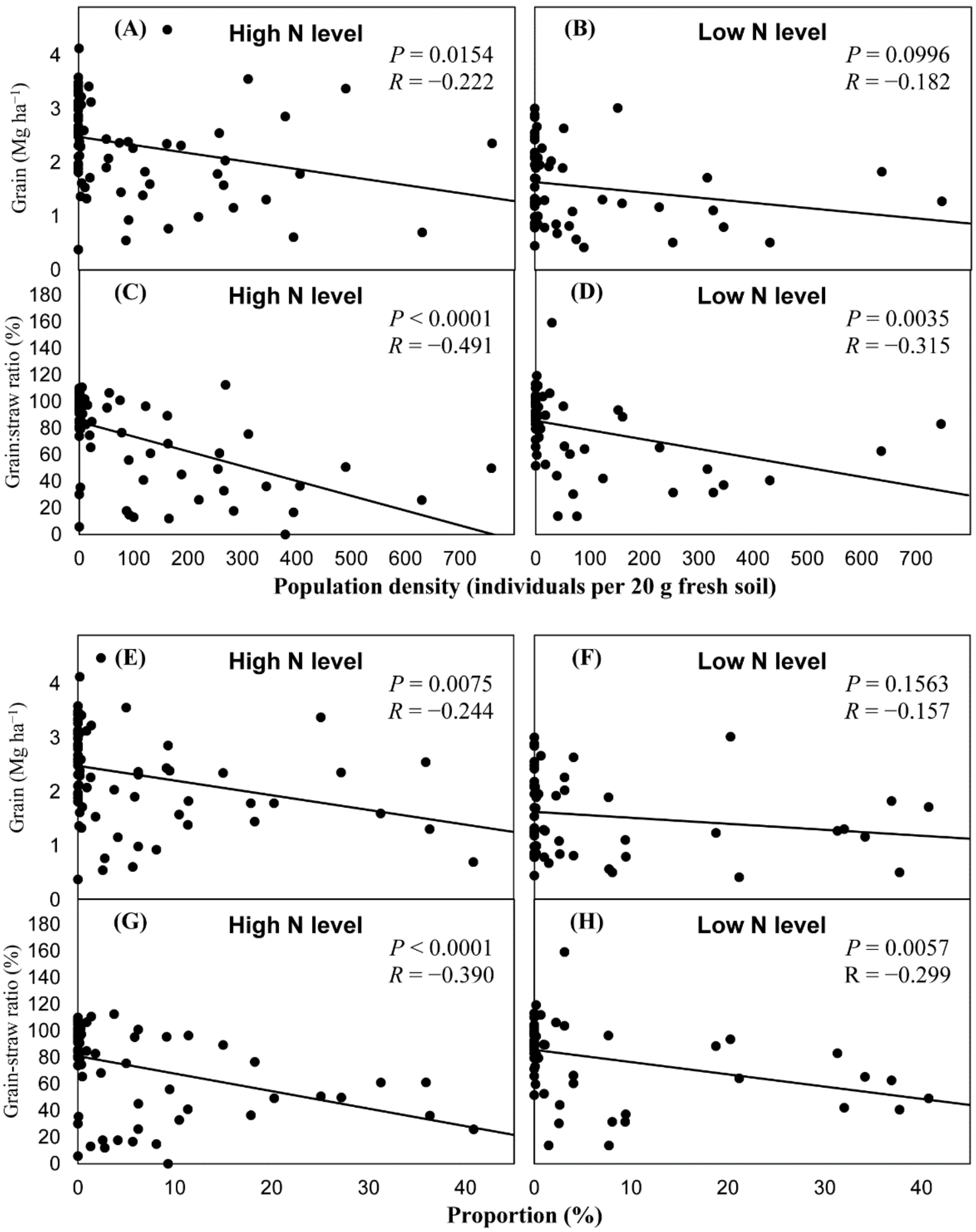

Fig. 3.3. Relationship between grain yield of upland rice and *Heterodera elachista* population density and proportion.

Note: The density and proportion of *H. elachista* were estimated in the spring sampling of 2005–2007. Relationship between grain yield and *H. elachista* population density at high N level (A) and low N level (B). Relationship between grain:straw ratio and *H. elachista* population density at high N level (C) and low N level (D). Relationship between grain yield and *H. elachista* proportion at high N level (E) and low N level (F). Relationship between grain-straw ratio and *H. elachista* proportion at high N level (G) and low N level (H).

seeding was performed at middle or late seeding times in the present study, the damage was expected to be lower than that in other studies, resulting in moderate damage to upland rice growth. However, there was no significant negative relationship between grain yield *and H. elachista* density or proportion in the unfertilized plot. In this plot, yield loss because of *H. elachista* was not clear, owing to the lower grain yield resulting from lack of fertilization. In addition, our data showed a significant negative relationship between *H. elachista* density and grain-straw ratio and between *H. elachista* proportion and grain-straw ratio in the fertilized plot. We observed an influence on grain:straw ratio by *H. elachista*. There was significant negative relationship between *H. elachista* density and grain:straw ratio in both of no and N-fertilization plot. This result suggests that *H. elachista* disturbed the translocation of the nutrient to the grain within the plant. In particular, in 2007, lower grain:straw ratio was caused by high *H. elachista* density.

Many studies have investigated the effect of tillage system on soybean cyst nematode population density, although with varying results (Chen et al., 2001; Edwards et al., 1988; Howard et al., 1998; Koenning et al., 1995; Noel and Wax, 2003; Tyler et al., 1983; Tyler et al., 1987; Workneh et al., 1999). Some studies have shown that NT prevented cyst nematode population increase (Edwards et al., 1988; Koenning et al., 1995; Tyler et al., 1983; Tyler et al., 1987; Workneh et al., 1999), suggesting that lower soil disturbance maintains low soil temperatures and enhances PRD population. Our study also found that NT reduced the population density of *H. elachista* compared with MP and RC at most sampling times.

H′ is a common indicator of diversity among soil nematode species. In general, *H′* values reflect rarer species of nematode abundance in total nematode. Because in our study, NT enhanced total nematode abundance, *H′* decreased during the first 5 years, although it increased 7 years after NT. Studies have shown contrasting results: NT increased *H′* (Okada and Harada, 2007) and decreased *H′* (Liu et al., 2013). Our data showed clearer results with respect to *H′* and tillage system: NT reduced *H′* in the short term and increased it in the long term. In addition, *H′* was a good predictor of soil nematode diversity, given that *H. elachista* decreased as *H′* increased. During upland rice cultivation, *H′* showed lower values in NT than in MP, although NT showed lower *H. elachista* density. This result suggests that *H′* would be a good indicator to predict *H. elachista* damages, but does not explain the effects of NT in reducing *H. elachista* density. Recently, it was reported that soil-borne fungi in NT can reduce cyst nematode density (Bao et al., 2011). In our field, fungal biomass was higher in NT than in MP or RC (Nakamoto et al., 2012; Zhaorigetu et al., 2008), suggesting that soil microbial conditions in NT affect *H. elachista* density. Further research will be necessary to determine the effects of NT on *H. elachista* density.

The use of cover crops has been widely implemented to reduce, both directly and indirectly, plant-parasitic nematodes in agricultural soils. The efficacy of cover crops frequently increases when they are incorporated into the soil as manures. Incorporating cover crop biomass helps to add nutrients into the soil and can improve soil quality by increasing organic matter and improving soil structure

(Allison, 1973; Magdoff and Van Es, 2000). In the present study, rye and hairy vetch cover crops did not directly affect *H. elachista*, but given that these cover crops enhanced total nematode abundance, *H. elachista* proportions were significantly reduced in rye and hairy vetch compared with that in fallow. Our data showed no significant effect of interaction between tillage system and cover crop treatment on *H. elachista* density. This interaction was significant only for *H. elachista* proportion in November 2006, suggesting that NT with cover cropping has the potential to reduce damage by *H. elachista* in upland rice cultivation.

N fertilization did not directly influence the density or proportion of *H. elachista*. N fertilization improved the growth of upland rice including roots and indirectly increased the population density of *H. elachista* by providing a host and habitat for *H. elachista*. This result suggested that the control of root biomass by no or reduced fertilization may reduce damage by *H. elachista*.

Upland rice cultivation will be a focus of attention in future studies because of the limitations of water resources in the next decade, particularly in Asia (Tuong et al., 2005). NT systems will be desired practices for developing sustainable rice production system because they reduce the energy needed for seedbed preparation and enhance soil C sequestration (Higashi et al., 2014) and soil biological diversity (Komatsuzaki and Ohta, 2007). Our results showed a significant effect of population control of *H. elachista* both in continuous rice cultivation and after a change to soybean cultivation. These results will contribute to the design of appropriate farming practices for upland rice production.

3.5 Conclusion

We observed trends of population change of *H. elachista* for 9 years in relation to tillage system, cover crop treatment, and N-fertilization level. In a field experiment, NT prevented increases in *H. elachista* population and proportion, both during continuous cropping system of upland rice and after change to soybean cultivation. Cover crop treatments of rye and hairy vetch did not directly affect *H. elachista* density. However, the proportions of *H. elachista* were significantly lower in cover crop treatments because the populations of other nematodes increased. N-fertilization level significantly affects the level of yield loss of upland rice because of *H. elachista*, given that higher N fertilization led to severe yield loss. Nematode diversity was significantly associated with population density of *H. elachista*, suggesting that a diverse soil ecosystem contributes to preventing population growth by *H. elachista*.

Chapter 4: Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop managements over a nine-year period in Kanto, Japan

4.1 Introduction

Response of soil food web structure due to soil quality changes through the long term anthropogenic disturbance due to farming practices were not well discussed. The objectives of this study were (1) to compare the long-term effects of tillage and cover crops on soil nematode community composition and indices, and (2) to determine whether there is a relationship between nematode community indices and soil C sequestration.

4.2 Materials and methods

4.2.1 Study site

This study was conducted as a part of a long-term experiment at the Field Science Center, Ibaraki University, Japan from 2002 to 2011. The climate is relatively humid and classified as Cfa (humid subtropical and hot summer) (Trewartha, 1968).

4.2.2 Farming practices

In a four replicated split–split experiment design, tillage systems were the variable in the main plot, cover crop managements were subplots, and manure applications were sub-subplots. The study covered a total of 72 plots, with each plot size 3 × 6 m. Soil preparation was performed according to the respective tillage system: MP, RC, and NT. Cover crops were winter rye, hairy vetch, and fallow. N levels were 0 and 100 kg N ha^{-1} for upland rice, and 0 and 20 kg N ha^{-1} for soybean. In the four-replicate split–split experimental design, tillage system varied in the main plot, cover crop varied in subplots, and N input level varied in sub-subplots. From 2002 to 2007, the main summer crop was upland rice and from 2008 to 2011 it was soybean.

The cover crop was sown by hand from late October to early November. Rye was sown at 100 kg ha^{-1} and hairy vetch at 50 kg ha^{-1}. No fertilization and shallow disking were applied after cover crop seeding. Cover crop and native weeds were grown until early April in the upland rice rotation or late May in the soybean rotation, and mowed by flail mower to return the cover crop residue to the soil. Under MP, cover crop residues were left on the soil surface and the soil was plowed to a depth of 25–30 cm and the residues incorporated into the soil. Under RC, cover crop residues were also incorporated by rotary cultivator to a depth of 0–15 cm. Under NT, cover crop residues were left on the soil surface. Upland rice and soybean were sown with a no-tillage seeder in late April for upland rice and late June for soybean. Seeding rates were 50 kg ha^{-1} for upland rice and 50 kg ha^{-1} for soybean. Phosphorus and potassium were applied at seeding time, with application rates of 100 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} for upland rice and soybean. Nitrogen was applied only to sub-subplots, at 100 kg N ha^{-1} for upland rice and 20 kg N ha^{-1} for soybean. Glyphosate isopropylammonium (1 L ha^{-1}, 41%) was applied to all plots before planting, and after seeding of upland rice or soybean, hand weeding was performed two or three times during each growing period. Upland rice was harvested with a head-feeding combine and upland rice residue was returned to the soil. Soybeans were harvested with a walking-type binder in the local farmer's manner. All soybean plants were harvested, moved into the greenhouse to dry, and threshed outside the field area, and the resulting soybean residue was not returned to the soil.

4.2.3 Soil sampling, measurement of soil carbon and bulk density

Samples were collected with a steel trowel from the top 10-cm depth (approximately 2.5–7.5 cm depth layer), excluding the uppermost soil layer. Each sample was separated from gravel and roots and hand-mixed. Two subsamples of 20 g from the 200-g soil sample were collected for nematode extraction.

Sampling was performed twice from each plot 1 or 2 weeks before nematode sampling. Four samples were taken from each depth layer including 0–2.5, 2.5–7.5, 7.5–15, and 15–30 cm. After sampling, soils were air dried for 1 week and then ground and sieved through a 2-mm mesh. Soil C was quantified with a C/N coder (NC900, Sumika chemical analysis service, Ltd.). Soil BD in the 0–2.5, 2.5–7.5, 7.5–15, and 15–30 cm soil layers was measured by hand with a steel cylinder of 5 cm diameter and 30 cm length (5887.5 cm^3) driven into the soil at the only summer sampling time. After removal of the cylinder, the soil core was divided at each depth with a hand knife, dried at 105°C, and weighed.

4.2.4 Nematode extraction, identification, and community analysis

Samples of 200 g soil were collected for nematode samples from each plot, twice a year, from all treatments: after tillage and after the harvest of upland rice or soybean. Samples were collected with a steel trowel from the top 10 cm depth, excluding the topmost layer of soil. Nematodes were extracted from 20 g of fresh soil by Baermann funnel extraction. Nematode samples collected in tubes were heat-killed and fixed with TAF, transferred to flamed slide glasses with approximately 1 ml of fixative, and observed. Five hundred randomly chosen individual nematodes were identified to genus or family to estimate numbers per 20 g of soil at a magnification of 1000×.

Nematode taxa were assigned to feeding groups based primarily on Yeates et al. (1993), and FFR nematodes were classified following Okada and Harada (2007). We used the following five feeding groups: BAC, FFR, PRD, OMN, and ORF. We refer to the feeding groups collectively as "ALL." We counted total nematode species (*S*) and calculated the $F/(F + B)$. Each nematode taxon was also assigned to a functional guild (Bongers and Bongers, 1998), which was defined using a combination of feeding group and life history traits expressed as *cp* scores from 1 (extremely *r*-strategist) to 5 (*K*-strategist) (Bongers, 1990). Nematodes of all feeding habits in *cp* classes 3–5 are considered to be indicators of soil ecosystem structure, and BAC in *cp* 1 and FFR in *cp* 2 are considered to be indicators of soil enrichment. Nematodes were classified into functional groups to calculate the following three indices: CI, EI, and SI (Ferris et al., 2001). CI, EI, and SI quantify the soil food web state as an indicator of the dominant decomposition pathways, a measure of opportunistic BAC and FFR nematodes, and an indicator of food web state affected by stress or disturbance, respectively. Indices were calculated as

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

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

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

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

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

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

where i = 3–5, BAC_i = abundance of BAC in *cp i*, FFR_i = abundance of FFR in *cp i*, OMN_i = abundance of OMN in *cp i*, PRD_i = abundance of PRD in *cp i*, $W_1 = 3.2$, $W_2 = 0.8$, $W_3 = 1.8$, $W_4 = 3.2$, and $W_5 = 5.0$.

4.2.5 Data analysis

Data were statistically analyzed by ANOVA or repeated-measures ANOVA (StatView, SAS Institute) for a split–split plot design, applying the Tukey–Kramer method with $P < 0.05$. Regression analysis was also performed to evaluate the relationship between soil C and BD and abundance of nematodes and nematode community indices, respectively.

4.3 Result

4.3.1 Soil nematode community structure changes

Nematode genus composition differed from year to year. Only a few genera were prominent throughout the years monitored. However, χ^2 tests showed that the genus composition was significantly affected by management treatment in all years. The genera that contributed most to the χ^2 values are listed in Table 4.1. From 2004 onwards, *Pristionchus*, *Prismatolaimus*, and *Pratylenchus* were the genera contributing most to the differences among management types.

Soil nematode community structure between 2005 and 2011 was significantly influenced by tillage system, cover crop, and soil N input, but also year differences were observed in nematode abundance and community structure indices (Table 4.2).

Among tillage systems, ALL, BAC, PRD, and ORF were highest in NT at most sampling times, although FFR was highest in RC (Fig. 4.1). In MP, total nematode abundance and trophic group abundances were lowest at most sampling times. This tendency was observed across years, and the abundances of ALL, BAC, and ORF were higher in spring than in autumn. For example, ALL was 3276 in NT in May 2004, but only 1738 in MP and 2710 in RC. This tendency was continued in June 2011, with 3269 in NT, 833 in MP, and 2638 in RC. Cover crop also influenced ALL at most sampling times. Hairy vetch showed highest ALL at the May sampling time during 2003 to 2008, and next was rye, with fallow showing the lowest values at most sampling time.

A similar pattern was obtained for BAC abundance, given that BAC comprised the major trophic group in the experiment. However, BAC occupation in ALL differed between tillage systems as the experiment continued. BAC abundance occupied 52% of ALL for NT, 55% for MP, and 61% for RC in May, 2003, but increased to 73% for NT in June 2011, although MP decreased to 44% and RC showed a similar value of 55%. For cover crop, rye and hairy vetch showed higher BAC than fallow

Table 4.1. Contribution (CONTR) of the five most important genera to total χ^2 for the frequency distribution of genera over management combination between tillage systems and cover crop managements in each of 9 years in the long term tillage system research project at Ibaraki, Japan.

Year	Month	Total χ^2	d.f.	Genus 1	CONTR (%)	Genus 2	CONTR (%)	Genus 3	CONTR (%)	Genus 4	CONTR (%)	Genus 5	CONTR (%)
2004	5	26498	304	Pristionchus	14.0	Pratylenchus	13.2	Mesorhabditis	10.3	Prismatolaimus	6.6	Safianema	6.2
	11	5320	296	Prismatolaimus	15.7	Pristionchus	13.5	Tylenchus	13.1	Acrobeloides	10.5	Pratylenchus	9.1
2005	5	21267	296	Pristionchus	19.0	Prismatolaimus	13.0	Tylenchus	9.4	Pratylenchus	7.6	Acrobeloides	6.9
	11	12460	320	Pristionchus	22.0	Prismatolaimus	20.8	Mesorhabditis	9.0	Rhabdolaimus	6.3	Tylenchus	5.6
2006	5	63344	360	Prismatolaimus	15.4	Heterodera	14.3	Pristionchus	12.8	Mesorhabditis	11.9	Pratylenchus	9.6
	11	11520	352	Tylenchus	20.5	Acrobeles	12.0	Prismatolaimus	9.9	Pristionchus	8.6	Pratylenchus	8.2
2007	5	43309	360	Heterodera	18.4	Pratylenchus	13.9	Pristionchus	13.0	Mesorhabditis	7.3	Prismatolaimus	7.0
	11	24520	400	Aphelenchus	15.1	Prismatolaimus	13.6	Other Anguinidae	12.9	Acrobeles	12.5	Mesorhabditis	8.9
2008	6	62366	344	Pratylenchus	19.4	Prismatolaimus	11.8	Heterodera	11.2	Panagrolaimus	10.7	Mylonchulus	8.8
	11	28756	312	Pristionchus	17.7	Prismatolaimus	11.6	Mylonchulus	8.7	Acrobeloides	8.4	Pratylenchus	8.4
2009	6	35843	456	Monhystrella	12.0	Pratylenchus	12.0	Other Anguinidae	6.9	Prismatolaimus	6.2	Mesorhabditis	5.7
	11	28052	376	Pristionchus	28.9	Other Anguinidae	11.8	Pratylenchus	9.4	Acrobeloides	8.0	Eucephalobus	3.8
2010	6	45146	368	Prismatolaimus	19.4	Pratylenchus	13.0	Pristionchus	8.6	Aphelenchus	7.2	Heterocephalobus	6.6
	11	12169	336	Acrobeles	12.5	Acrobeloides	12.4	Aphelenchoides	7.4	Pratylenchus	7.0	Pristionchus	7.0
2011	6	33848	368	Prismatolaimus	19.5	Pratylenchus	14.6	Aphelenchoides	8.5	Acrobeloides	6.0	Acrobeles	4.5
	11	18238	336	Safianema	17.1	Acrobeles	8.8	Pristionchus	8.3	Pratylenchus	7.6	Wilsonema	7.3

Table 4.2. Responses of soil nematode, soil carbon and bulk density to the effect of tillage, cover crop, nitrogen input and year in autumn.

Treatment	ALL	BAC	FFR	PRD	OMN	ORF	CI	EI	SI	Soil C	BD
Tillage	***	***	***	*	***	***	***	***	***	***	***
Cover crop	***	***	***	*	***	*	**	***	**	***	***
N input	***	***	***	NS	NS	NS	NS	NS	**	NS	—
Year	***	***	***	NS	***	***	***	***	***	***	***
Tillage × Cover crop	NS	NS	*	NS	NS	*	*	NS	NS	NS	***
Tillage × N input	***	***	**	NS	NS	NS	NS	NS	*	NS	—
Tillage × Year	NS	NS	NS	NS	**	***	***	***	**	NS	NS
Cover crop × N input	***	**	***	NS	*	NS	NS	NS	*	*	—
Cover crop × Year	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	***
N input × Year	NS	NS	NS	NS	NS	NS	NS	*	NS	—	—
Tillage × Cover crop × N input	***	**	**	NS	NS	*	NS	NS	NS	NS	—
Tillage × Cover crop × Year	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	***
Tillage × N input × Year	NS	NS	**	NS	NS	NS	*	NS	*	—	—
Cover crop × N input × Year	NS	NS	*	NS	NS	NS	NS	NS	NS	—	—
Tillage × Cover crop × N input × Year	NS	NS	*	NS	NS	**	NS	NS	NS	—	—

Note: ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders and facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders; CI, channel index; EI, enrichment index; SI, structure index; BD, bulk density. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. NS, no significant.

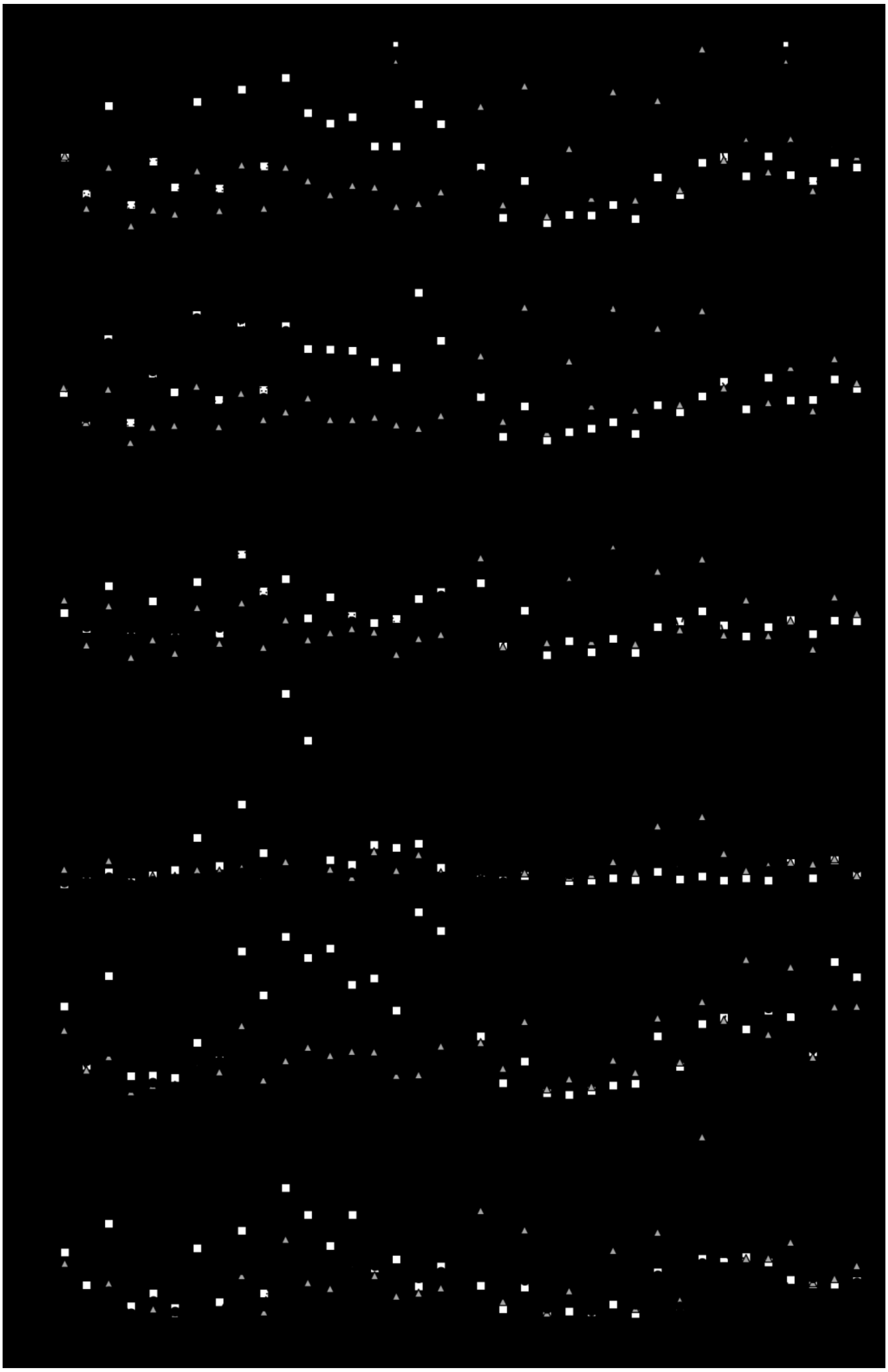

Fig. 4.1. Long-term effect of tillage and cover crop on the nematode densities of feeding groups from 2003 to 2011. *Note*: ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders and facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. *, significant at $P < 0.05$.

but the difference between rye and hairy vetch was small.

FFR was highest at RC (mean, 723) and 44% and 88% higher than NT and MP, respectively, in May 2003. Seasonal population change was also observed, with a difference of 95 individuals for NT, 273 for MP, and 547 for RC in 2003. This trend continued for most sampling times, but seasonal population changes of FFR were smaller in both 2010 and 2011 than in 2003–2009. Among cover crops, fallow showed lower FFR abundance than rye or hairy vetch at most sampling times.

PRD abundance was highest in NT at most sampling times, but the PRD occupation ratio was only 0–9.2% during the experiment period. Among cover crops, fallow showed lower PRD than rye or hairy vetch.

Soil nematode community indices changed significantly between tillage systems, cover crops, and years. The interactions of tillage × cover crop, tillage × year, cover crop × year, and tillage × N input × year for CI, tillage × year and N input × year for EI, and tillage × N input, tillage × year, cover crop × N input, and tillage × N input × year for SI were also significant.

Soil nematode community indices differed between 2003–2007 and 2008–2011. For CI, NT showed higher values than RC and MP from 2003 to 2007, but this difference disappeared from 2008 to 2011 (Fig. 4.2). EI values were lowest in NT than MP and RC from 2003 to 2007, but these differences also disappeared from 2008 to 2011. SI values were higher in NT than in MP and RC from 2005 to 2011, although the difference between NT and MP was small from 2003 to 2004. Cover crop management also showed significant effects on soil nematode community indices, with CI in fallow showing higher values than in rye and hairy vetch from 2003 to 2007, although the difference of CI between cover crops was moderate after 2008. In contrast, EI values were higher in rye and hairy vetch than in fallow at most sampling time. EI values were highest in rye at most sampling times, and as the study continued, SI values also increased.

4.3.2 Soil carbon and bulk density changes in relation to nematode community

For the soil properties, tillage system, cover crop, year, and cover crop × N input interaction significantly influenced soil C. For BD, tillage, cover crop, year, and tillage × cover crop and tillage × cover crop × year interactions were significant (Table 4.2).

Correlations between soil C and nematode abundance of each trophic group were significantly positive for each tillage system, and often significantly negative between nematode abundance of each trophic group and BD (Table 4.3). As soil C increased (Table 4.4), ALL increased in all tillage systems, but the proportionate increase of ALL due to soil C increase was greater in NT than in either MP or RC (Fig 4.3). This trend was also observed for BAC, OMN, and ORF, but not for FFR. In contrast, with decreasing BD (Table 4.5), the abundance of FFR and OMN increased significantly.

4.3.3 Changes in soil nematode community indices

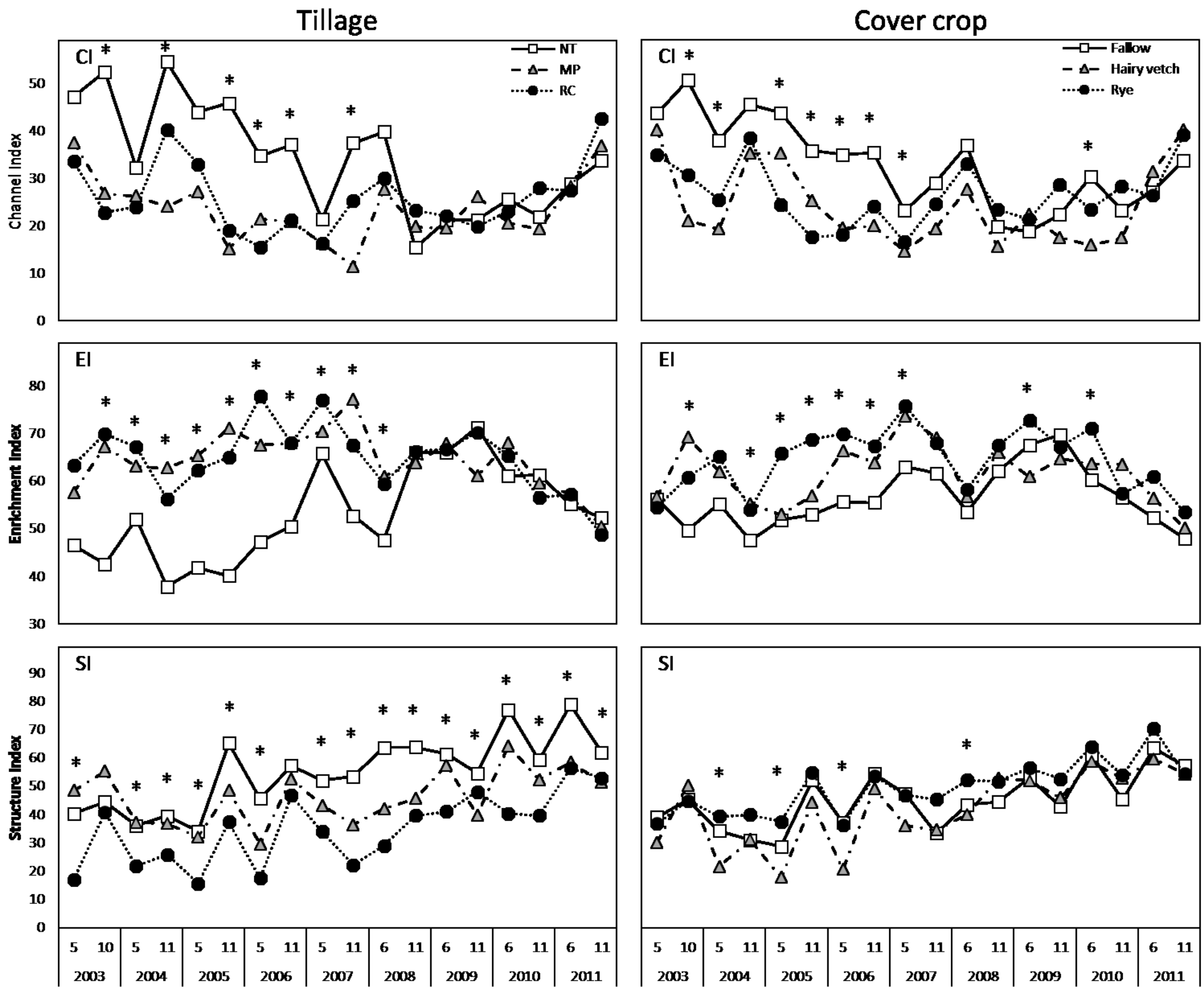

Fig. 4.2. Long-term effect of tillage and cover crop on the nematode indices from 2003 to 2011.

Note: CI, channel index; EI, enrichment index; SI, structure index; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. *, significant at $P < 0.05$.

Table 4.3. Correlation of nematode densities of feeding groups and community indices with soil carbon level and bulk density in autumn from 2003 to 2011.

Treatment	Population density (individuals per 20 g soil)						Community index		
	ALL	BAC	FFR	PRD	OMN	ORF	CI	EI	SI
Soil C									
NT	0.681***	0.724***	0.508*	0.074	0.555**	0.464*	-0.511**	0.487*	0.410*
MP	0.540**	0.425*	0.388	0.536**	0.500*	0.479*	0.178	-0.231	0.371
RC	0.664***	0.609**	0.567**	0.490*	0.641***	0.521**	0.132	-0.202	0.592**
BD									
NT	-0.396	-0.381	-0.416*	-0.204	-0.413*	-0.246	0.067	-0.119	0.011
MP	-0.331	-0.156	-0.464*	-0.324	-0.408*	-0.301	-0.291	0.05	0.059
RC	-0.278	-0.208	-0.427*	-0.219	-0.405*	-0.117	-0.191	-0.112	-0.045

Note: ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders and facultative root feeders; PRD, predators; OMN, omnivoress; ORF, obligatory root feeders; CI, channel index; EI, enrichment index; SI, structure index; BD, bulk density; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 4.4. Effects of tillage systems and cover crop managements on soil carbon contents.

Treatment	2003		2004		2005		2006		2007		2008		2009		2010		2011	
	May	Oct	May	Nov	May	Nov	May	Nov	May	Nov	Jun	Nov	Jun	Nov	Jun	Nov	Jun	Nov
Tillage (T)																		
NT	3.79	3.25	3.69	3.52	3.48	3.77^{a}	–	3.70^{a}	3.49	3.74^{a}	3.84^{a}	3.81^{a}	3.81^{a}	4.08^{a}	3.61^{a}	4.15^{a}	4.05^{a}	4.45^{a}
MP	3.68	3.35	3.66	3.25	3.28	3.45^{b}	–	3.39^{b}	3.35	3.45^{b}	3.62^{b}	3.59^{b}	3.56^{b}	3.70^{b}	3.19^{b}	3.77^{b}	3.64^{b}	3.73^{c}
RC	3.62	3.42	3.51	3.43	3.46	3.67^{ab}	–	3.57^{ab}	3.40	3.72^{a}	3.72^{ab}	3.73^{ab}	3.71^{ab}	3.87^{ab}	3.31^{ab}	4.03^{a}	3.97^{a}	4.07^{b}
Cover crop (CC)																		
Fallow	3.73	3.18	3.54	3.22	3.25^{b}	3.50	–	3.37^{b}	3.44	3.37^{b}	3.54^{b}	3.55^{b}	3.48^{b}	3.61^{b}	3.23	3.72^{b}	3.67^{b}	3.90^{b}
Hairy vetch	3.73	3.52	3.61	3.45	3.41^{ab}	3.68	–	3.52^{b}	3.55	3.66^{a}	3.77^{a}	3.77^{a}	3.78^{a}	3.99^{a}	3.39	4.06^{a}	3.87^{ab}	4.11^{ab}
Rye	3.64	3.32	3.71	3.54	3.56^{a}	3.71	–	3.77^{a}	3.25	3.88^{a}	3.85^{a}	3.79^{a}	3.80^{a}	4.00^{a}	3.46	4.14^{a}	4.06^{a}	4.18^{a}
T	NS	NS	NS	NS	NS	*	–	**	NS	*	*	*	*	**	*	**	***	***
C	NS	NS	NS	NS	*	NS	–	***	NS	***	**	*	**	**	NS	***	***	**
T × CC	NS	NS	NS	NS	NS	NS	–	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS

Note: Values with in each column followed by a different letter (a-c) are significantly different at $P = 0.05$ based on a Tukey-Kramer test.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

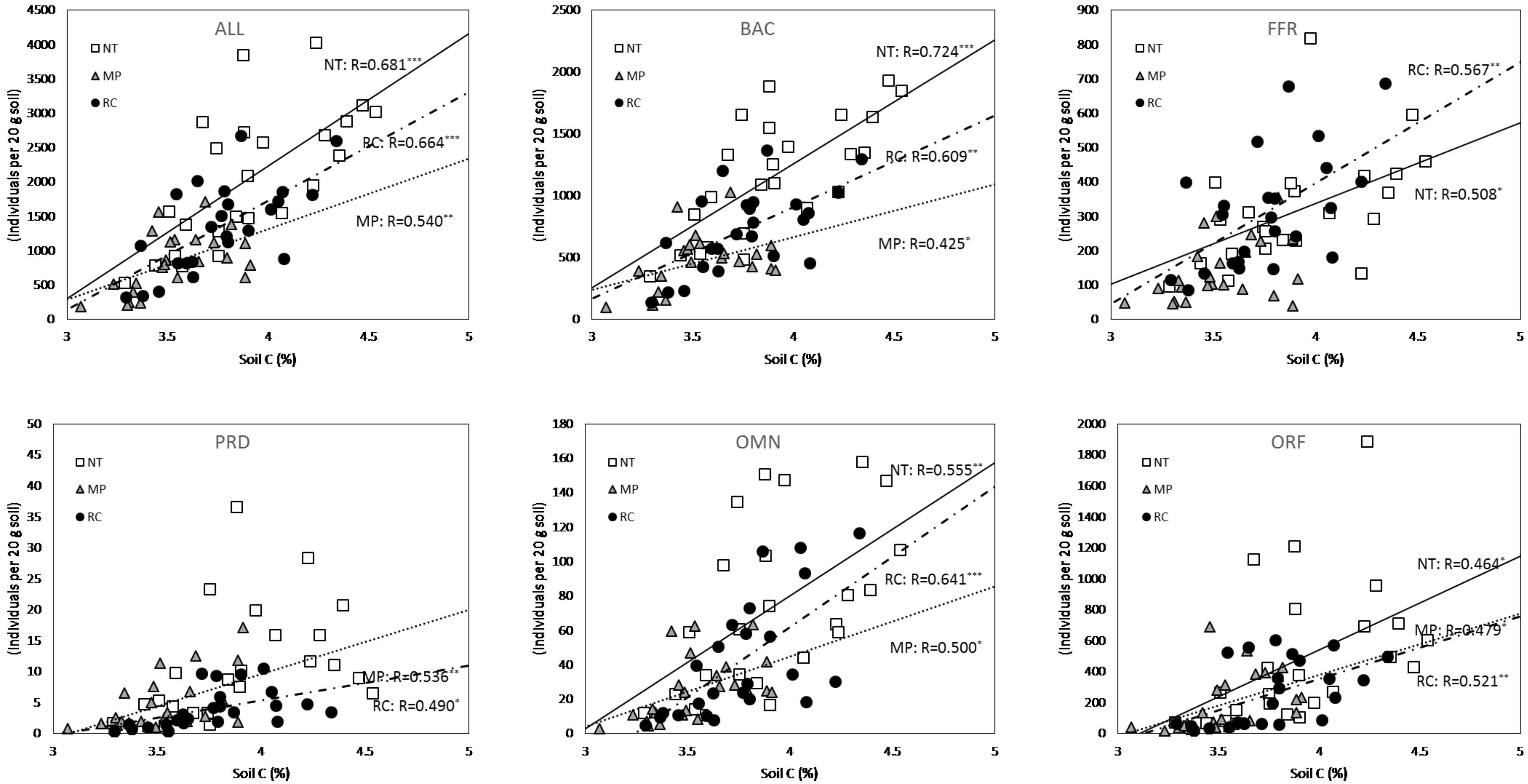

Fig. 4.3. Relationship between the population densities of feeding groups and soil carbon level in autumn from 2003 to 2011.

Note: ALL, total nematode abundance; BAC, bacterial feeders; FFR, fungal feeders and facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 4.5. Effects of tillage systems and cover crop managements on soil bulk densities.

Treatment	2003	2004	2005	2006	2007	2008	2009	2010	2011
	May	May	May	May	May	Jun	Jun	Jun	Jun
Tillage (T)									
NT	0.850[b]	0.740[a]	0.677[c]	0.681[a]	0.681[a]	0.681[a]	0.650[a]	0.721[a]	0.687[a]
MP	0.827[c]	0.602[b]	0.683[b]	0.623[b]	0.623[b]	0.623[b]	0.582[b]	0.665[b]	0.614[b]
RC	0.854[a]	0.569[c]	0.691[a]	0.601[c]	0.601[c]	0.601[c]	0.542[c]	0.661[c]	0.611[b]
Cover crop (CC)									
Fallow	0.822[c]	0.647	0.693[a]	0.636[b]	0.636[b]	0.636[b]	0.564[c]	0.709[a]	0.595[c]
Hairy vetch	0.867[a]	0.637	0.673[c]	0.683[a]	0.683[a]	0.683[a]	0.639[a]	0.726[a]	0.698[a]
Rye	0.841[b]	0.626	0.685[b]	0.587[c]	0.587[c]	0.587[c]	0.571[b]	0.611[c]	0.620[b]
T	***	***	***	***	***	***	***	***	***
C	***	NS	***	***	***	***	***	***	***
T × CC	***	.	***	***	***	***	***	***	.

Note: Values with in each column followed by a different letter (a-c) are significantly different at $P = 0.05$ based on a Tukey-Kramer test.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Tillage × year interaction significantly affected OMN and ORF abundance, and EI and SI indices. In contrast, the interaction between cover crop and year was significant only for CI (Table 4.2). For NT, CI was negatively correlated with contents of soil C, and EI and SI were significantly positive, but this relationship was not observed for MP and RC. With increasing soil C, CI decreased in NT, but this relationship was not repeated in MP and NT (Fig. 4.4). In contrast, EI showed a positive relationship with soil C in NT but not in MP or RC. SI also showed a positive relationship with soil C in NT and RC but not in MP.

A simplified food web structure on EI and SI trajectories were revealed the dogmatically changes over the year differences between the soil management practices, in particular tillage systems (Fig. 4.5). From 2004 to 2007, the trajectories in NT were located on lower EI and higher SI, from 2004 to 2007, but the trajectories converged on lower EI and higher SI in 2011.

4.4. Discussion

Berkelmans et al. (2003) reported that the nematode community responds quickly to a change in farming system and remains similar as long as the system is maintained. Our experimental findings agree with this principle. For example, in 2003, just after the long-term experiment was begun, nematode community change were observed between cover crops and tillage systems, but soil organic matter content did not differ between treatments. After the experiment was continued for nine years, the nematode community also changed with SOC owing to the maintenance of the same farming practices.

The effects of tillage system and crop residue incorporation on nematode community are controversial. Zhang et al. (2012) investigated the effects of tillage system (conventional tillage and no-tillage) and residue management (0, 50%, and 100% wheat residue incorporation) on the nematode community and reported that EI decreased in NT but that CI increased compared to conventional tillage, although soil nematodes were more sensitive to residue effects than to tillage effects after short-term practices. Pan et al. (2010) reported that bacterivore abundances were significantly correlated with SOC, although total nematodes showed no significant correlation. These results were similar to ours in some respects; however, our study showed clearer correlations between nematode community changes and soil C increases.

Most studies agree that soil biological properties are more strongly influenced by crop residue incorporation than by tillage system. Christensen et al. (2012) compared fungal biomass and fungivorous between conventional and organic fields in Denmark and reported that higher inputs of crop residue enhanced fungal biomass and fungivore abundance. Nakamoto et al. (2012) also reported that both rye and hairy vetch cover crops increased microbial activities and improved soil aggregate stability with increasing SOC.

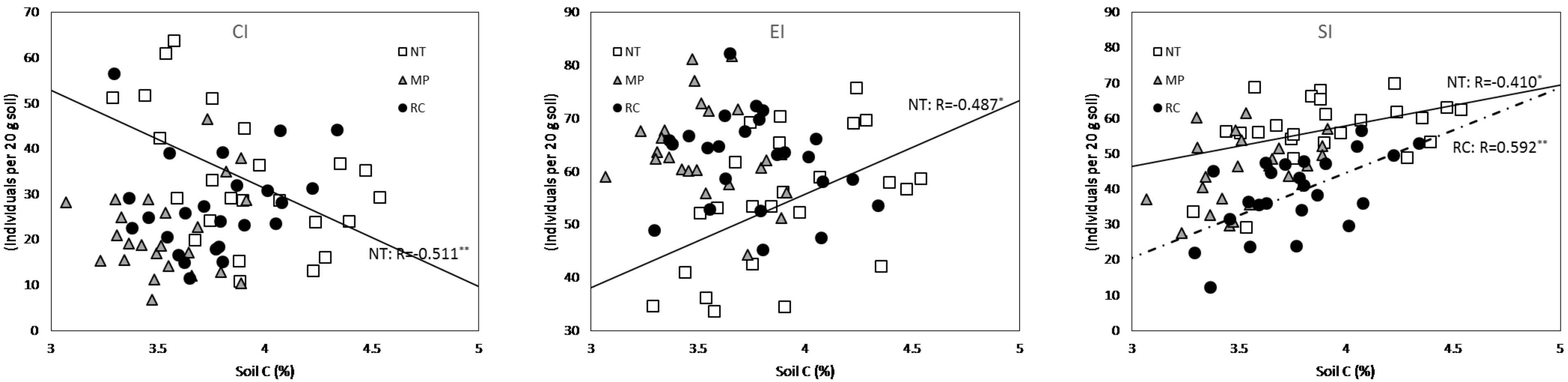

Fig. 4.4. Relationship between the nematode indices and soil carbon level in autumn from 2003 to 2011.

Note: CI, channel index; EI, enrichment index; SI, structure index; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

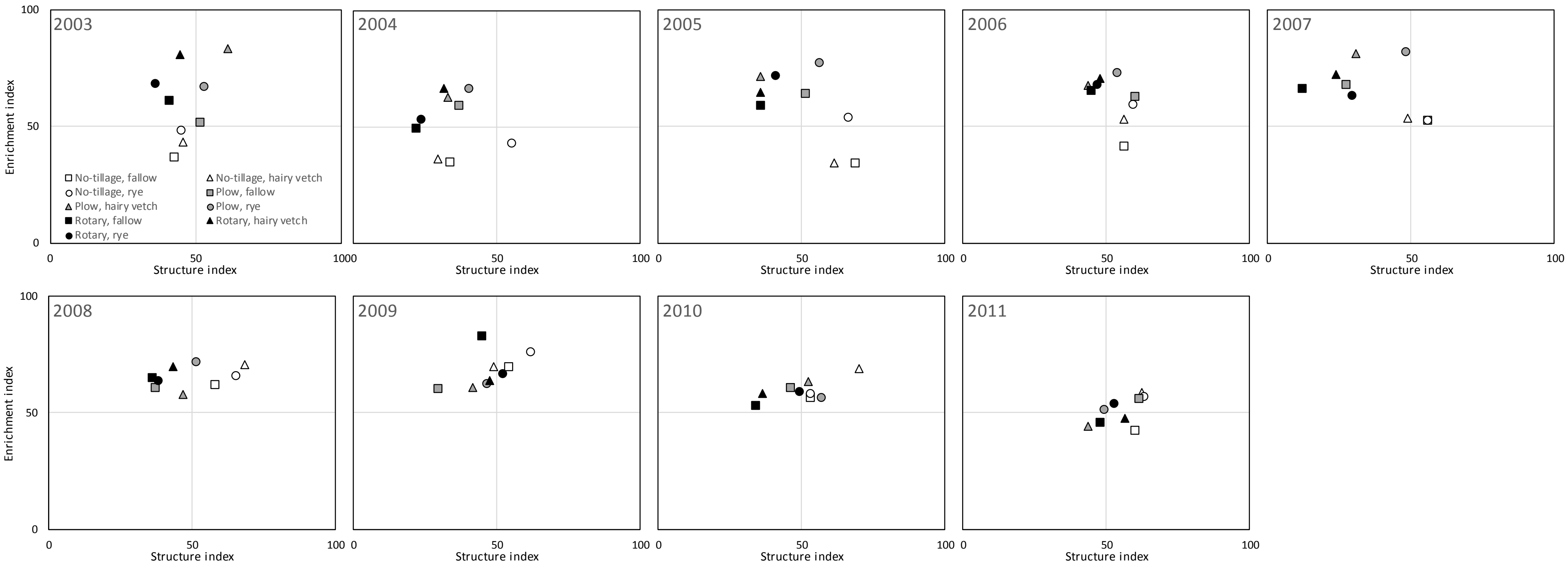

Fig. 4.5. Relationship between the SI and EI in the long-term tillage and cover crop treatments in autumn from 2003 to 2011.

Note: SI, structure index; EI, enrichment index; NT, no-tillage; MP, moldboard plow; RC, rotary cultivator.

Zhaorigetu et al. (2008) also reported that the use of a rye cover crop increased fungal biomass near the soil surface. This improvement by cover-crop residue incorporation or residue cover influences the fungal community more readily than does tillage system (Nishizawa et al., 2010).

Fungi and bacteria have different roles in the decomposition process, with mainly fungi being responsible for decomposition of recalcitrant compounds. Crop residue quality and incorporation of residues in the soil also affects the decomposition process. Nakamoto et al. (2012) determined the fungal-to-bacterial biomass ratio by a substrate-induced respiration-inhibition approach, suggesting that for a cover crop with a higher C:N ratio, such as rye, incorporation increased fungal biomass more than for a cover crop with a lower C:N, such as hairy vetch, and that crop residue cover on soil surfaces by NT also enhances fungal biomass more than does the incorporation of residue by tillage inversion. The relative abundance of fungivorous and bacterivorous nematodes theoretically provides information about fungal and bacterial contributions to decomposition of soil organic matter (Ferris et al., 2001; Vestergård, 2004).

Tillage systems, however, controlled the decomposition process of crop residue and no-till increased SOC in a long-term trial, owing to decline in the organic matter decomposition (Balesdent et al., 2000). Our long-term study revealed that tillage system exerted a stronger impact on the nematode community than did cover crop. Although the composition of nematode genera was different from year to year, there were significant management effects on genus composition in each year, with NT differing from MP and RC. In particular, as SOC increased, total nematode abundance and the abundance of each trophic group significantly increased in NT but not in MP or RC.

Ferris et al. (2012) investigated the abundance and metabolic footprints of soil nematode changes during long-term organic management, finding that the abundances of bacterivore and fungivore nematodes were enhanced by the annual use of winter cover crops but showed no relationship with the level of soil organic matter (Ferris et al., 2012). In our study, significant correlations were found between SOC and abundance of all nematodes, BAC, FFR, PRD, OMN, and ORF. However, the EI values were significantly correlated with SOC in NT but not in MP and RC. Ferris et al. (2012) reported a positive relationship between SOC and EI under NT. This observation was corroborated only in NT in our experiment, suggesting that a positive correlation between EI and SOC would be observed under lower soil disturbance, but that tillage inversion reduces these responses.

Nematode food web structure on EI and SI trajectories were very different between tillage systems during the upland rice cropping periods, but these differences in trajectories disappeared during soybean cropping periods. EI describes whether a soil ecosystem is nutrient-enriched (high-EI) or depleted (low-EI). In this study, EI values were higher in MP and RC than in NT during upland rice cultivation, but there was little difference between tillage systems after soybean cultivation, suggesting that the effects of summer crop management affect the nematode community structure more strongly than do tillage or winter cover crop management.

The SI represents an aggregation of functional guilds with *cp* values ranging from 3 to 5. The SI describes whether a soil ecosystem is structured/matured (high-SI) or disturbed/degraded (low-SI). In this study, SI values increased in all plots after conversion to soybean cultivation as a summer crop, suggesting that soybean cropping showed more benefits to the development of the nematode community structure. Further research to evaluate the effects of tillage system and cover crop in combination with a summer crop on nematode community structure would be desirable.

4.5. Conclusion

This study demonstrated that changing the summer crop type also changed the nematode community structure. The selected community indices reflected the differences in nematode communities well as long as the management systems were kept intact, but were too insensitive to expose residual effects of management systems. The χ^2 test was still able to detect large differences in nematode communities among management treatments at that time. These differences were caused not only by plant-parasitic but also by bacterivorous and fungivorous nematodes. Tillage system and cover crop strongly affected the nematode community structure during all experimental periods, and NT showed the highest total nematode abundance, BAC, and PRD. Cover-crop effects on nematode community did not differ between rye and hairy vetch, but fallow treatment resulted in the lowest abundances at all trophic levels. In general, the nematode community responds quickly (within a few years) to a changing cropping system and remains similar as long as that system is maintained. We identified this effect shortly after beginning the study, but we also observed a long-term change in nematode community structure, especially as soil C increased. However, we expect SOC to be only one of the factors changing nematode community structure, given that RC and MP conditions also developed the nematode community structure during the nine-year experiment period, suggesting that repeated identical tillage inversions applied to agro-ecosystems develop nematode community structure by creating appropriate soil environmental conditions.

Chapter 5: General discussion

5.1 Introduction

Soil health and soil quality are fundamental to the sustained productivity and viability of agricultural systems world-wide, however, soil managers became overdependent on chemical fertilizers to replace or enhance soil nutrients, which also degraded soil and water quality (Stamatiadis et al., 1999). As local environmental quality becomes increasingly degraded by agricultural practices, the importance of protecting and restoring soil resources is being recognized by the world community (Barford et al., 2001; Lal, 1998, 2001). Sustainable management of soil received strong support at the Rio Summit in 1992 as well as in Agenda 21 (UNCED, 1992), the United Nations Framework Convention on Climate Change (UNFCCC, 1992), articles 3.3 and 3.4 of the Kyoto Protocol (UNFCCC, 1997), and elsewhere. These conventions are indicative of the recognition by the world community of strong linkage between soil degradation and desertification on the one hand and loss of biodiversity, threats to food security, increases in poverty and risks of accelerated greenhouse effects and climate change on the other.

NT system is another alternative, that has been increasingly used for crop production in the US, Europe, South America, and Asia during the past decade due to their significant environmental advantage over moldboard plow. In East Asian countries, including Japan and China, just started to adopt NT farming, and the combination of cover crops and NT practice increased the environmental benefits such as N leaching reduction, soil organic matter increases, soil biological diversity improvements (Komatsuzaki and Ohta, 2007).

Healthy soil is defined as a stable system with resilience to stress, high biological diversity, and high levels of internal nutrient cycling (Van Bruggen and Semenov, 2000). The major activities of soil microbes include the decomposition of organic matter, mineralization of nutrients, N fixation, and suppression of crop pests and protection of roots. However, they can also be parasitic and cause injury to plants. Thus, there is a great need to assure that practices introduced in soil management to improve soil quality will also help to maintain the health of the soil. Managing cover crops and NT practice adoption in croplands may be able to contribute to the establishment of a healthy soil, but this depends in large part on understanding their basic biology and eco-system ecology.

This paper discusses the significance of nematode community indices to evaluate the agro ecosystems and the effects of farming practices such as tillage systems and cover crop managements on nematode community structure. This information will be helpful to design the sustainable agriculture in a Japanese condition.

5.2 Soil nematode community as a biological indicator

The soil food web has a primary role in keeping and improving soil health, especially enhancing soil nutrients. Bacteria and fungi dominate most mineralization of nutrients directly. The basal decomposer is affected by soil animals of higher trophic levels (e.g. protozoa, nematodes, mites, springtails, millipedes and earthworms) and these animals have indirect effects on soil nutrients.

Recently nematode community structure is paid more attention to evaluate the soil ecosystem and soil health. Nematode community structure reflect changes in the structure of soil food web (Bongers, 1990; Bongers and Ferris, 1999; Ferris et al., 2001; Ferris and Matute, 2003). Most ORF nematodes live in the soil and feed on plant roots, thereby reducing the plant's uptake of water and nutrients, and reducing tolerance to other stresses such as drought. Most species of nematodes are 'free-living', living in soil, and they have no directly effect on crop production. These feed on bacteria, fungi, protozoans and even other nematodes, and play a very important role in nutrient cycling and release of nutrients for plant growth (Fig. 5.1). Nematodes can be used as bio-indicators of soil health because they are ubiquitous and have diverse feeding behaviors and life strategies, ranging from colonizers to persisters. The combination of nematode feeding groups and *cp*-scaling into functional guilds has developed nematode faunal analysis into a more powerful tool which can be used as a bio-indicator of soil health and food web condition (Bongers and Ferris, 1999; Ferris et al., 2001; Ferris and Matute, 2003).

The evolution of a complex soil nematode community under agricultural practices can be monitored by the nematode community indices (Berkelmans et al., 2003; Bongers and Bongers, 1998; Ferris et al., 2001; Neher, 1999; Yeates and Bongers, 1999). The indices have been used successfully to distinguish well-functioning ecosystems from heavily disturbed or stressed system (Berkelmans et al., 2003; Neher, 1999; Yeates and Bongers, 1999), but also to detect more subtle differences among agricultural practices, including tillage systems and cover crop species. In particular, the effects of interaction of tillage systems and cover crop species on nematode community, particularly in Asia, have not been well studied.

5.3 Tillage system and nematode community

Soil tillage is aimed to improve soil structure and quality. 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 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.

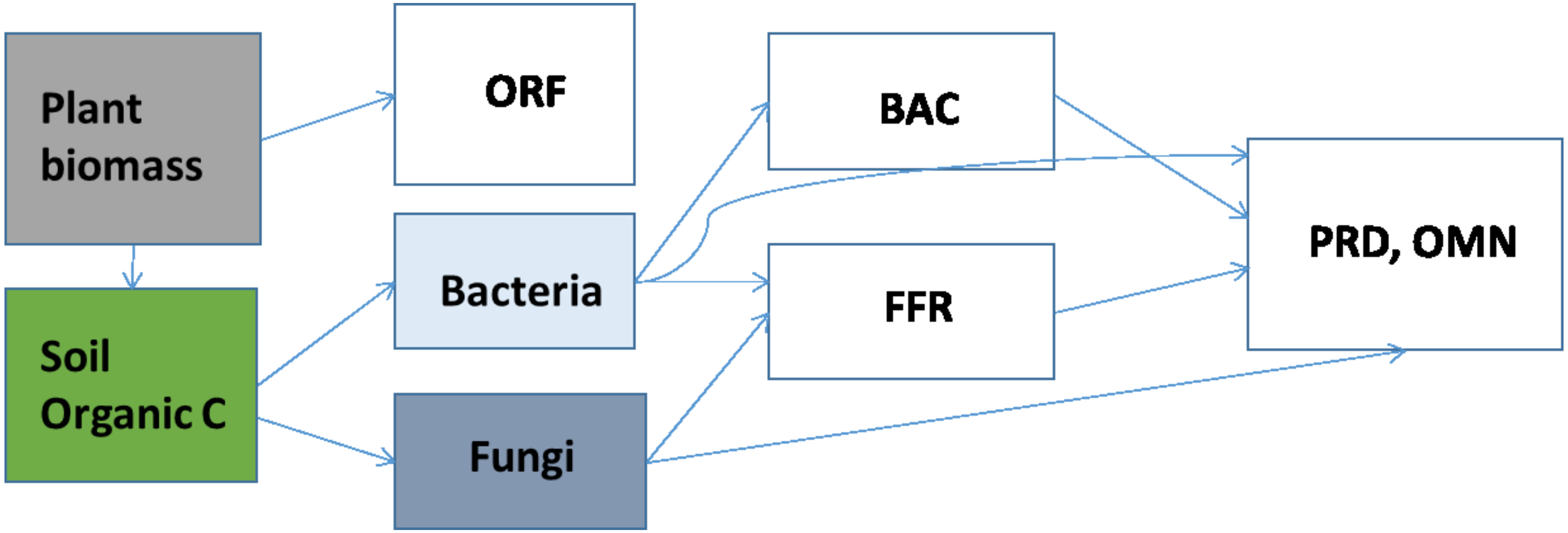

Fig. 5.1. Soil nematode community structure related with plant biomass, soil carbon, and microbial biomass.

Note: BAC, bacterial feeders; FFR, fungal feeders and facultative root feeders; PRD, predators; OMN, omnivores; ORF, obligatory root feeders.

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 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, BAC nematodes respond to the addition of crop residue faster than 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).

We used the 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.

Tillage significantly influenced DTL. 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 (Figure. 5.2). We measured the effect of the associated DTL on the nematode community composition. DTL significantly negatively correlated with nematode abundances for BAC, OMN, and ORF groups. Similarly, DTL significantly negatively correlated with SI (Ito et al., 2014). The SI represents an aggregation of functional guilds with *cp* values ranging from 3–5. SI describes whether a soil ecosystem is structured/matured (high SI) or disturbed/degraded (low SI).

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.

5.4 NT with cover crop can control the pathogen nematode abundance

Cover crop is intended to develop healthy soil; cover crops are a particularly beneficial ecosystem service in a cropland because they assist in supplying soil organic matter, adding biological fixed N, scavenging soil residual nutrients, suppressing weeds, and breaking pest cycles (Magdoff, 1993; Peet, 1996; Sarrantonio, 1998).The combinations between Cover crop and tillage system were strongly affects the nematode community. The rice cyst nematode (*Heterodera elachista*), previously reported only in Japan, has recently been detected in Asia and Europe. We evaluated the effects of three tillage systems (MP, RC, and NT); three winter cover crops (fallow, rye, and hairy vetch); and two rates of N fertilization (0 and 100 kg N ha^{-1} for upland rice, and 0 and 20 kg N ha^{-1} for soybean production) on population densities of *H. elachista* in 2003 - 2011. Population densities of *H. elachista* markedly

Fig. 5.2. The difference of the degree of surface soil translocation (DTL) between three tillage systems.

increased in MP and RC after 4 years of continuous upland rice cultivation, but not in NT. The densities of *H. elachist*a were 52.8 nematodes per 20 g fresh soil in MP and 72.7 in RC but 2.1 in NT in 2006. However, in the fifth year of continuous cultivation of upland rice, *H. elachista* densities markedly increased in NT; therefore, no differences were observed between tillage systems. After conversion to soybean, *H. elachista* densities decreased in all treatments, although NT showed a more rapid decrease than the other tillage systems. Cover cropping and N fertilization did not markedly affect *H. elachista* densities during the 9 years but cover cropping markedly reduced *H. elachista* proportion in total nematode abundance and N fertilization reduced rice yield with increasing *H. elachista*. Our results suggest that NT and crop rotation will be effective for controlling *H. elachista* densities, because NT reduced them and increased soil ecological diversity.

Upland rice cultivation will be a focus of attention in future studies because of the limitations of water resources in the next decade, particularly in Asia (Tuong et al., 2005). NT systems will be desired practices for developing sustainable rice production system because they reduce the energy needed for seedbed preparation and enhance soil C sequestration (Higashi et al., 2014) and soil biological diversity (Komatsuzaki and Ohta, 2007). Our results showed a significant effect of population control of *H. elachista* both in continuous rice cultivation and after a change to soybean cultivation. These results will contribute to the design of appropriate farming practices for upland rice production.

5.5 Long term effects of farming on nematode community

The effects of tillage system, cover crop treatment and N fertilization on the nematode community were assessed at the long term experimental site on the Kanto plain in Japan. We examined the effects of three tillage management systems (MP, RC, and NT); three winter cover crop types (fallow, rye, and hairy vetch); and two N fertilization rates (0 and 100 kg N ha^{-1} for upland rice and 0 and 20 kg N ha^{-1} for soybean production) on changes in nematode community structure. Sixty nine taxa were encountered, ALL, BAC, PRD, OMN, and ORF were greater in NT than in MP and RC, but FFR was great in RC than in NT and MP. Cove crop also influenced on nematode community structure, rye and hairy vetch were always higher in ALL, BAC, FFR, ORF, and OMN than fallow. Seasonal changes in nematode community structure were also significant, in particularly, as increase soil C, nematode abundance was also increased. The relationship between nematode indices and soil C was significant only in NT, but not for MP and RC. In NT, as increase soil C, EI and SI were positively significant. BD was also significantly negative correlated with FFR and ORM. Seasonal difference of nematode community between summer and autumn was bigger in upland rice rotation, but that was small in soybean rotation. For 9 years experiment, SI increased not only for NT but also MP and RC. These results suggest that increase of soil C would have a great impact to develop the more diverse nematode community structure.

EI and SI provide a quantitative estimate of the soil food web state. EI is a measure of soil N enrichment, and SI is an indicator of the food web state affected by stress or disturbance. In this research, SI values increased in all plots after converting to soybean cultivation as a summer crop, suggesting soybean cropping showed more benefits to develop the nematode community structure. In this regards, the further research would be desirable to determine the effects of tillage system and cover crop managements with the combination of summer crop on nematode community structure.

5.6 Conclusions

This paper discusses the significance of nematode community indices to evaluate the agro-ecosystems and the effects of farming practices such as tillage systems and cover crop managements on nematode community structure. Nematodes will be used as bio-indicators of soil health significantly because they are ubiquitous and have diverse feeding behaviors and life strategies, ranging from colonizers to persisters. The information regarding soil nematode community structure and farming practices will be helpful to design the sustainable agriculture.

5.7 Further research

We focused on nematode community structure in surface soil (0–10 cm), because, the nematode density is greater in surface than in subsoil (Ferris and McKenry, 1976). However, nematodes exist in the deeper soil layer, and tillage inversion may cause the translocation of nematodes from deeper soil to surface. Therefore there is need to have been clarified the effects of farming practices on nematode community in deeper soil.

Secondly, in our research, SI increased continuously not only in NT but also in MP and RC. This result suggests that MP and RC systems can also develop the soil ecosystem structure, however questions is raised about the maximum value of SI in each tillage systems.

Variation of vegetation managements combined with summer crops and cover crops would be another issues. They affect the nematode structure community strongly, especially ORF nematodes due to the host plants existence. Although we clarified the effects of cover crops on nematode community, we should evaluate the effects of whole vegetation management on nematode community to develop the sustainable soil managements.

Reference

Abawi, G.S., Widmer, T.L., 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology 15, 37-47.

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

Allison, F.E., 1973. Soil organic matter and its role in crop production. Elsevier.

Altieri, M.A., 1991. Traditional farming in Latin America. Ecologist (United Kingdom).

Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil and tillage research 53, 215-230.

Bao, Y., Neher, D.A., Chen, S., 2011. Effect of soil disturbance and biocides on nematode communities and extracellular enzyme activity in soybean cyst nematode suppressive soil. Nematology 13, 687-699.

Barford, C.C., Wofsy, S.C., Goulden, M.L., Munger, J.W., Pyle, E.H., Urbanski, S.P., Hutyra, L., Saleska, S.R., Fitzjarrald, D., Moore, K., 2001. Factors controlling long-and short-term sequestration of atmospheric CO2 in a mid-latitude forest. Science 294, 1688-1691.

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. Applied Soil Ecology 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. Applied Soil Ecology 10, 239-251.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14, 224-228.

Bridge, J., Plowright, R.A., Peng, D., 2005. 4 Nematode Parasites of Rice. Plant parasitic nematodes in subtropical and tropical agriculture, 87.

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. Applied Soil Ecology 21, 233-250.

Chen, S., 2007. Suppression of *Heterodera glycines* in soils from fields with long-term soybean monoculture. Biocontrol Science and Technology 17, 125-134.

Chen, S., Stienstra, W., Lueschen, W., Hoverstad, T., 2001. Response of *Heterodera glycines* and soybean cultivar to tillage and row spacing. Plant disease 85, 311-316.

Christensen, S., Borrego, E., Shim, W.-B., Isakeit, T., Kolomiets, M., 2012. Quantification of Fungal Colonization, Sporogenesis, and Production of Mycotoxins Using Kernel Bioassays. Journal of visualized experiments: JoVE.

Coleman, M.D., Isebrands, J., Tolsted, D.N., Tolbert, V.R., 2004. Comparing soil carbon of short rotation poplar plantations with agricultural crops and woodlots in North Central United States. Environmental Management 33, S299-S308.

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

De Luca, F., Vovlas, N., Lucarelli, G., Troccoli, A., Radicci, V., Fanelli, E., Cantalapiedra-Navarrete, C., Palomares-Rius, J., Castillo, P., 2013. *Heterodera elachista* the Japanese cyst nematode parasitizing corn in Northern Italy: integrative diagnosis and bionomics. Eur J Plant Pathol 136, 857-872.

Ding, Z., Namphueng, J., He, X.F., Peng, D.L., Huang, W.K., 2011. First Report of the Cyst Nematode (*Heterodera elachista*) on Rice in Hunan Province, China. Plant Disease 96, 151-151.

Doran, J., 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biology and Fertility of Soils 5, 68-75.

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. Applied Soil Ecology 41, 157-167.

Edwards, J., Thurlow, D., Eason, J., 1988. Influence of tillage and crop rotation on yields of corn, soybean, and wheat. Agronomy Journal 80, 76-80.

Elliott, E.T., Anderson, R., Coleman, D.C., Cole, C., 1980. Habitable pore space and microbial trophic interactions. Oikos, 327-335.

Ettema, C.H., Bongers, T., 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biology and Fertility of 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. Applied Soil Ecology 18, 13-29.

Ferris, H., Matute, M., 2003. Structural and functional succession in the nematode fauna of a soil food web. Applied Soil Ecology 23, 93-110.

Ferris, H., McKenry, M.V., 1976. Nematode Community Structure in a Vineyard Soil. Journal of Nematology 8, 131-137.

Ferris, H., Sanchez-Moreno, S., Brennan, E., 2012. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. Applied Soil Ecology 61, 16-25.

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. Applied Soil Ecology 22, 39-54.

Franzluebbers, A., 2002. Soil organic matter stratification ratio as an indicator of soil quality. Soil and Tillage Research 66, 95-106.

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 Biology and

Biochemistry 32, 1731-1741.

Govaerts, B., Fuentes, M., Mezzalama, M., Nicol, J.M., Deckers, J., Etchevers, J.D., Figueroa-Sandoval, B., Sayre, K.D., 2007. Infiltration, soil moisture, root rot and nematode populations after 12 years of different tillage, residue and crop rotation managements. Soil and Tillage Research 94, 209-219.

Guertal, E., Sikora, E., Hagan, A., Rodrıguez-Kábana, R., 1998. Effect of winter cover crops on populations of southern root-knot and reniform nematodes. Agriculture, ecosystems & environment 70, 1-6.

Hamza, O., Bengough, A.G., Bransby, M.F., Davies, M.C.R., Hallett, P.D., 2005. Applying geotechnical engineering to plant root biomechanical behaviour, Eco- and Ground Bio-engineering: The Use of Vegatation to Improve Slope Stability. Springer Science & Business Media, Kluwer, Dordrecht.

Havlin, J., Kissel, D., Maddux, L., Claassen, M., Long, J., 1990. Crop rotation and tillage effects on soil organic carbon and nitrogen. Soil Science Society of America Journal 54, 448-452.

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 and Tillage Research 138, 64-72.

Howard, D.D., Chambers, A., Lessman, G.M., 1998. Rotation and fertilization effects on corn and soybean yields and soybean cyst nematode populations in a no-tillage system. Agronomy journal 90, 518-522.

Hulugalle, N., Lal, R., Ter Kuile, C., 1986. Amelioration of soil physical properties by mucuna after mechanized land clearing of a tropical rain forest. Soil Science 141, 219-224.

Ito, T., Araki, M., Komatsuzaki, M., Kaneko, N., Ohta, H., 2014. Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production. Applied Soil Ecology 86, 137-147.

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

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139-162.

Kahlon, M.S., Lal, R., Ann-Varughese, M., 2013. Twenty two years of tillage and mulching impacts on soil physical characteristics and carbon sequestration in Central Ohio. Soil and Tillage Research 126, 151-158.

Kang, B.T., Sipkens, L., Wilson, G.F., Nangju, D., 1981. Leucaena (*Leucaena leucocephala* (Lam) de Wit) prunings as nitrogen source for maize (*Zea mays* L.). Fertilizer Research 2, 279-287.

Kawashima, M., Komori, S., 1962. On the Displacement of Soil by the Tillage machines (I) On the Expressions of the Displacement of Soil. Journal of the Japanese Society of Agricultural Machinery 24, 56-60.

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil and Tillage Research 61, 61-76.
Koenning, S., Schmitt, D., Barker, K., Gumpertz, M., 1995. Impact of crop rotation and tillage system on *Heterodera glycines* population density and soybean yield. Plant disease 79, 282-286.
Komatsuzaki, M., Ohta, H., 2007. Soil management practices for sustainable agro-ecosystems. Sustainability Science 2, 103-120.
Lal, R., 1998. Soil erosion impact on agronomic productivity and environment quality. Critical Reviews in Plant Sciences 17, 319-464.
Lal, R., 2001. World cropland soils as a source or sink for atmospheric carbon. Advances in Agronomy 71, 145-193.
Li, Q., Jiang, Y., Liang, W., Lou, Y., Zhang, E., Liang, C., 2010. Long-term effect of fertility management on the soil nematode community in vegetable production under greenhouse conditions. Applied soil ecology 46, 111-118.
Liu, F., Zhang, J., Wang, F., Liang, L., 2013. Response of soil nematode ecological and maturity indexes and faunal analysis to the conservation tillage. Journal of Food, Agriculture and Environment 11, 1111-1117.
Logsdon, S.D., Karlen, D.L., 2004. Bulk density as a soil quality indicator during conversion to no-tillage. Soil and Tillage Research 78, 143-149.
MAFF, 2014. Ecological farmers survey.
Magdoff, F., 1993. Building soils for better crops: organic matter management. Soil Science 156, 371.
Magdoff, F., 2001. Concept, components, and strategies of soil health in agroecosystems. Journal of nematology 33, 169.
Magdoff, F., Van Es, H., 2000. Building soils for better crops. Sustainable Agriculture Network Burlington.
McSorley, R., Dickson, D.W., 1989. Effects and dynamics of a nematode community on maize. Journal of nematology 21, 462.
MEXT, 2011. Extension Site of the Distribution Map for Radiation Dose. Ministry of Education, Culture, Sports, Science and Technology, 3-2-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-8959, Japan.
Minagawa, N., 1998. Separation efficiency of plant-parasitic nematodes by Baermann funnels, New technology of comprehensive agriculture. National Agriculture Research Center, pp. 168-171.
Minton, N.A., 1986. Impact of conservation tillage on nematode populations. Journal of nematology 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 KCl solution and analysis of soil-overturning by its method. Japanese Journal of Farm Work Research 30, 207-213.
Mu, Y., Komatsuzaki, M., Moriizumi, S., Gu, S., Araki, M., Araki, H., Hirata, S., 2007. Effects of

cover cropping and tillage system on soil physical character in upland rice cultivation. Japanese Journal of Farm Work Research 42, 11-20.

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. Applied Soil Ecology 34, 140-151.

Nakamoto, T., Komatsuzaki, M., Hirata, T., Araki, H., 2012. Effects of tillage and winter cover cropping on microbial substrate-induced respiration and soil aggregation in two Japanese fields. Soil Science and Plant Nutrition 58, 70-82.

Nanko, K., Ugawa, S., Hashimoto, S., Imaya, A., Kobayashi, M., Sakai, H., Ishizuka, S., Miura, S., Tanaka, N., Takahashi, M., 2014. A pedotransfer function for estimating bulk density of forest soil in Japan affected by volcanic ash. Geoderma 213, 36-45.

Neher, D.A., 1999. Nematode communities in organically and conventionally managed agricultural soils. Journal of Nematology 31, 142-154.

Nishizawa, T., 1978. Annual population changes of soil nematodes in the field of continuous cropping or rotation. Witthayāsān Kasētsāt 12, 3.

Nishizawa, T., Komatsuzaki, M., Sato, Y., Kaneko, N., Ohta, H., 2010. Molecular characterization of fungal communities in non-tilled, cover-cropped upland rice field soils. Microbes and environments 25, 204-210.

Nishizawa, T., Shimizu, K., Nagashima, T., 1972. Chemical and cultural control of the rice cyst nematode, *Heterodera oryzae* Luc *et* Berdon Brizuela, and hatching responses of the larvae to some root extracts. Japanese Journal of Nematology 2, 27-32.

Noel, G., Wax, L., 2003. Population dynamics of *Heterodera glycines* in conventional tillage and no-tillage soybean/corn cropping systems. Journal of nematology 35, 104.

Ohshima, Y., 1974. *Heterodera elachista* n. sp., an upland rice cyst nematode from Japan. Japanese Journal of Nematology 4, 51-56.

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

Okada, T., 1960. Nematode damage and method for nematode control. Agriculture and horticulture 35, 1475-1478.

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.

Pan, F., McLaughlin, N.B., Yu, Q., Xue, A.G., Xu, Y., Han, X., Li, C., Zhao, D., 2010. Responses of soil nematode community structure to different long-term fertilizer strategies in the soybean phase of a soybean–wheat–corn rotation. European Journal of Soil Biology 46, 105-111.

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

Perie, C., Ouimet, R., 2008. Organic carbon, organic matter and bulk density relationships in boreal forest soils. Canadian Journal of Soil Science 88, 315-325.

Raper, R., Reeves, D., Schwab, E., Burmester, C., 2000. Reducing soil compaction of Tennessee Valley soils in conservation tillage systems. J. Cotton Sci 4, 84-90.

Robertson, G.P., Paul, E.A., Harwood, R.R., 2000. Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. Science 289, 1922-1925.

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.

Shimizu, K., 1976. Influence of the upland rice cyst nematode, *Heterodera elachista*, on the yield of the upland-cultured paddy rice. Japanese Journal of Nematology 6, 1-6.

Shimizu, K., 1977. Seasonal fluctuation and rhizospherical distribution of population of the upland rice cyst nematode, *Heterodera elachista*. Japanese Journal of Nematology 7, 49-57.

Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099-2103.

Soil Survey Staff, 2014. Keys to Soil Taxonomy, in: Service, U.-N.R.C. (Ed.), 12th ed. ed, Washington, DC.

Stamatiadis, S., Werner, M., Buchanan, M., 1999. Field assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California). Applied Soil Ecology 12, 217-225.

Stirling, G.R., 2014. Biological Control of Plant-parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture, Biological Crop Protection Pty. Ltd, Australia.

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

Tuong, T.P., Bouman, B., Mortimer, M., 2005. More rice, less water-integrated approaches for increasing water productivity in irrigated rice-based systems in Asia. Plant Production Science 8, 231-241.

Tyler, D., Overton, J., Chambers, A., 1983. Tillage effects on soil properties, diseases, cyst nematodes, and soybean yields. Journal of soil and water conservation 38, 374-376.

Tyler, D.D., Chambers, A.Y., Young, L.D., 1987. No-Tillage Effects on Population Dynamics of Soybean Cyst Nematode1. Agron. J. 79, 799-802.

UNCED, 1992. Agenda 21, in: UNCED (Ed.), Conches, Switzerland.

UNFCCC, 1992. United Nations Framework Convention on Climate Change, in: Nations, U. (Ed.), New York.

UNFCCC, 1997. Kyoto Protocol to the United Nations Framework Convention on Climate Change, in: Nations, U. (Ed.).

Van Bruggen, A., Semenov, A., 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology 15, 13-24.

Veech, J.A., Dickson, D.W., 1987. Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists. Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists.

Vestergård, M., 2004. Nematode assemblages in the rhizosphere of spring barley (*Hordeum vulgare* L.) depended on fertilisation and plant growth phase. Pedobiologia 48, 257-265.

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.

Weil, R.R., Magdoff, F., 2004. Significance of soil organic matter to soil quality and health. Soil organic matter in sustainable agriculture. CRC Press, Boca Raton, FL, 1-43.

Workneh, F., Tylka, G., Yang, X., Faghihi, J., Ferris, J., 1999. Regional assessment of soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* using area-frame sampling: Prevalence and effects of tillage. Phytopathology 89, 204-211.

Yeates, G., Dando, J., Shepherd, T., 2002. Pressure plate studies to determine how moisture affects access of bacterial‐feeding nematodes to food in soil. European Journal of Soil Science 53, 355-365.

Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. Agriculture, Ecosystems & Environment 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. Journal of nematology 25, 315.

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

Zhang, X., Li, Q., Zhu, A., Liang, W., Zhang, J., Steinberger, Y., 2012. Effects of tillage and residue management on soil nematode communities in North China. Ecological Indicators 13, 75-81.

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 and Environments 23, 201-208.

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