Original research article

Evaluation of root effects on soil organisms under different fertilization regimes by comparing rhizosphere and interrow soil in a wheat field

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Abstract: The aim of this study is to evaluate the effects of plant root on soil organisms. The response of soil organisms (microbes, nematodes, and microarthropods) to root and fertilization (four inorganic fertilization regimes and organic fertilization) was studied in a wheat field in Japan. Microbial substrate-induced respiration (SIR) and the population densities of nematodes and microarthropods in the rhizosphere and interrow soil were measured from April to June in 2004 and 2005. Application of inorganic NPK fertilizer had positive effects on the population densities of all three types of soil organism. Microbial SIR and the population density of bacterivorous nematodes were high in plots to which high levels of inorganic NPK fertilizer and/or ammonium nitrate for top dressing were applied. Application of ammonium nitrate increased the population density of microarthropods in 2005. Whereas organic fertilization regime had lower population density of nematodes and microarthropods than other inorganic fertilization regime having similar root biomass. Soil organisms in the rhizosphere and the interrow soil responded similarly to fertilization and, consequently, the rhizosphere/interrow ratio, defined by the ratio of the number of organisms per gram in rhizosphere soil to the number in interrow soil, was hardly affected by the fertilization regimes studied. The ratio indicated how much soil organisms were influenced by root. It was high for microarthropods population density and low for nematodes population density. Root biomass was strongly correlated with microbial SIR but it was not correlated with nematodes population density. With the results from the rhizosphere/interrow ratio and the correlations between soil organisms and root biomass, we suggested that fertilization affected microbial SIR and microarthropods population density through root. But the effect of root was not obvious to nematodes population density.

Keywords: Andosol, microarthropod, microorganism, nematode, rhizosphere/interrow ratio, wheat root

Abbreviations: CA, conventional inorganic fertilization with additional application of ammonium nitrate; CF, conventional inorganic fertilization; OF, organic fertilization; RA, reduced inorganic fertilization with additional application of ammonium nitrate; RF, reduced inorganic fertilization; SIR, substrate-induced respiration.

Introduction

Soil organisms play important roles in processes such as organic matter decomposition and nutrient cycling in arable fields. Fertilization is one of the major factors controlling the population densities and activity of soil organisms (Bünemann et al. 2006). Application of inorganic and organic fertilizers can indirectly but positively affect soil microbes and animals by increasing plant growth and stimulating root exudation, both of which lead to a greater input of organic substrates. Community structure and body size of soil organisms are also affected by fertilization (Mueller et al. 1993, Verschoor et al. 2001).

The rhizosphere is the zone of soil affected by plant roots, and rhizosphere soil differs from bulk (i.e., non-rhizosphere) soil in physical and chemical properties (Di Meo et al. 2003, Whalley et al. 2005). The rhizosphere is a hot spot of soil organisms: microbial activity is stimulated by organic carbon substrates supplied from the roots in the form of

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exudates and dead cells (Kuzyakov and Domanski 2000, Baudoin et al. 2001). The activity levels of microfauna and mesofauna are also high in the rhizosphere. Nematodes and protozoa are often concentrated in the rhizosphere because they migrate to the food sources and propagate there (Griffiths 1994). Ingham et al. (1985) showed that the rhizosphere, which accounts for 4-5% of total soil, included about 70% of the bacterivorous and fungivorous nematodes. However, little field research has been conducted on the effects of fertilization on soil biota in the rhizosphere.

The objectives of this study were to evaluate the effect of root on the soil bioactivity under different fertilization regimes and to characterize the difference between rhizosphere and interrow soil in an agricultural field.

Materials and Methods

The experiment was conducted in 2004 and 2005 at the University Farm of the University of Tokyo, Japan (35°43'N, 139°32'E). The soil was a dark, humus-rich volcanic ash of the Kanto Loam type (Humic Andosol).

Five fertilization regimes were tested. Three treatments, conventional fertilization (CF), reduced fertilization (RF), and organic fertilization (OF), had been maintained under a 1-year two-crop rotation system with winter wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) since 1993. Inorganic NPK fertilizer (12% N, 18% P_2O_5, 16% K_2O) was applied just before seeding at a rate of 700 kg ha^{-1} $year^{-1}$ in the CF plots and 350 kg ha^{-1} $year^{-1}$ in the RF plots. The OF plots were treated in late June every year with composted manure (cow manure and plant straw) that contained 69% water and 2.3% nitrogen on a dry weight basis, at a rate of 80 t ha^{-1} $year^{-1}$. At the start of the experiment in autumn 2003, the CF and RF plots were divided in half to create new plots, CA and RA, respectively. In these plots, ammonium nitrate (NH_4NO_3) was additionally applied to the soil surface at a rate of 137 kg ha^{-1}. Individual plots were 4 m × 5 m. There were three replications of each plot in 2004 and four in 2005. On 13 November 2003 and 25 November 2004, winter wheat (cv. Kinunonami) was sown after rotary tillage at a rate of 39 kg ha^{-1} with 18-cm row spacing. Additional NH_4NO_3 was applied on 8 March 2004 and 8 March 2005. No herbicide was applied during the experiment.

Soil samples were taken three times at 1-month intervals, in the middle of April, May, and June of 2004 and 2005. Rhizosphere soil was taken from directly below the cut wheat stem, and interrow soil, which contained few plant roots, was taken from between the plant rows. Using a boring sampler with a 4-cm diameter, soil from a depth of 0–10 cm was collected from four locations per plot and combined into a single composite sample for each plot. Soil samples for the measurements of soil bioactivity were passed through a 4-mm mesh, and roots were removed. Root biomass was determined from soil samples collected on 24 May 2004 and 24 May 2005.

The activity level of microorganisms was evaluated by means of the substrate-induced respiration (SIR) technique described by Nakamoto and Tsukamoto (2006). Soil equivalent to 10 g on a dry weight basis was mixed with 20 mg of glucose and incubated at 22°C. The CO_2 evolved between 2 and 4 h of incubation was measured using an infrared CO_2 analyzer (Model LX-720; Iijima, Japan).

Nematodes were extracted from 10 g of fresh soil over 48 h using a Baermann funnel, and counted. The collected nematodes were fixed in TAF, and 100 individuals per plot were randomly chosen, identified, and classified into four trophic groups (bacterivores, herbivores, fungivores, omnivores/predators) according to the nature of their anterior structures (Okada, 2002). Microarthropods were extracted over 48 h using a Tullgren funnel with a 2-mm mesh under fluorescent lamps. The collected samples were sorted into springtails (collembola) and three suborders of mites (oribatids, prostigmatids, mesostigmatids). The population densities of nematodes and microarthropods were expressed as the number per gram dry weight of soil.

The effects of fertilization treatments and root biomass were analyzed by means of one-way ANOVA using the StatView program (SAS Institute Inc., Cary, NC). The data on microbial SIR and the population densities of nematodes and microarthropods from three months were averaged because there were no obvious seasonal changes. The correlation coefficients were computed between root biomass and soil organisms.

Results

Root biomass was high in the plots to which a large amount of NPK fertilizer was applied and it was great in the rhizosphere soil of CA and OF. However it was significantly different in interrow soil only in 2004 (Table 1). Mean root biomass in all the five treatments was 2.27 and 2.07 mg cm^{-3} in the rhizosphere soil and 0.20 and 0.21 mg cm^{-3} in the interrow soil in 2004 and 2005, respectively; that is, the interrow soil contained about one-tenth the weight of roots recovered from the rhizosphere soil. The pH in the plots with inorganic NPK fertilizer was 5.7–6.0, about 0.2–0.3 lower than that in OF.

Microbial SIR was affected by fertilization (Table 2) and it was high in the plots to which a large amount

Table 1. Root biomass in rhizosphere and interrow soil (mg cm^{-3})

	2004		2005	
	Rhizosphere	Interrow	Rhizosphere	Interrow
CF	2.16 b†	0.23 ab	2.05 ab	0.21 ns
CA	2.51 a	0.32 a	2.38 a	0.26 ns
RF	1.76 c	0.10 b	1.68 b	0.14 ns
RA	2.09 bc	0.10 b	1.92 ab	0.22 ns
OF	2.78 a	0.24 ab	2.31 ab	0.21 ns
Mean	2.27	0.20	2.07	0.21

†Values followed by the same letter within a column are not significantly different ($P < 0.05$).

of NPK fertilizer was applied (CF, CA). Microbial SIR in OF was significantly higher than RF and RA to which inorganic NPK fertilizer was applied. Across the five fertilizer treatments, the response of microbial SIR in the rhizosphere soil was similar to that in the interrow soil.

The response of nematodes to inorganic fertilizer was similar to that of the microbial SIR. A large amount of inorganic NPK fertilizer applied at sowing (CA, CF) and/or NH_4NO_3 applied in early spring (CA, RA) elevated the population density of nematodes (Table 3). Bacterivores were the dominant trophic group: their population density was the highest in CA and the lowest in OF, in both the rhizosphere and the interrow soil. Fungivores were not affected by fertilization. In the rhizosphere samples gathered in 2004, the population density of herbivores was low in CF and OF, and that of omnivores/predators was high in CA and RA. The population density of each microarthropod group studied (collembola and three suborders of mites) showed no significant difference among the fertilization treatments in both years. However the total population density of microarthropods differed among treatments in 2005 and it was the highest in CA and the lowest in OF and RF in rhizosphere soil (Table 4).

Because each fertilization treatment affected the abundance of soil organisms in the rhizosphere and interrow soil in a similar way, the rhizosphere/ interrow ratio, defined by the ratio of the number of organisms per gram in rhizosphere soil to the number in interrow soil, hardly differed among fertilization treatments (Table 4). Rhizosphere/interrow ratio varied among different soil organisms. The ratio for nematodes population density was low and it was more than 2.5 for the population densities of microarthropods groups (Table 5).

We found a high positive correlation between root biomass and microbial SIR in rhizosphere soil (Table 6). There was no significant correlation between nematodes population density and root biomass in rhizosphere. The population densities of oribatid mites and prostigmatid mites partly had positive correlation with root biomass but total microarthropods population density was not significantly correlated with root biomass.

Discussion

Microbial SIR and nematodes population density (1×10^6 to 3×10^6 m^{-2}) in interrow soil were within the range found in the adjacent fields under different managements (Nakamoto and Tsukamoto 2006, Nakamoto et al. 2006). Usually nematodes population density ranges from 1×10^5 to 1×10^7 m^{-2} in temperate environments (Petersen and Luxton 1982, Robertson and Freckman 1995). The overall mean microarthropods population density observed in this study (5×10^3 to 1×10^4 m^{-2}) seemed to be smaller than previous two reports (1×10^4 to 1×10^5 m^{-2}) (Nakamoto and Tsukamoto 2006, Nakamoto et al. 2006). One possibility is that the collecting procedure was not efficient because we used sieved soil under study. Anyway, it was within the range (1×10^3 to 1×10^5 m^{-2}) observed in agricultural fields (Axelsen and Kristensen 2000, Doles et al. 2001, Coleman et al.

Table 2. Microbial substrate-induced respiration (ml CO_2 kg^{-1} dry soil h^{-1}) in rhizosphere and interrow soil

	2004			2005		
	Rhizosphere	Interrow	Rhizosphere/ interrow	Rhizosphere	Interrow	Rhizosphere/ interrow
CF	11.3 b†	7.2 a	1.6 ns	13.3 a	8.0 b	1.7 ns
CA	13.0 a	8.0 a	1.6 ns	15.0 a	9.1 a	1.6 ns
RF	8.5 c	6.0 b	1.4 ns	10.4 b	6.9 c	1.5 ns
RA	9.4 c	5.9 b	1.6 ns	11.4 b	8.1 b	1.4 ns
OF	11.8 ab	8.2 a	1.4 ns	14.7 a	9.2 a	1.6 ns

†Means of samples (collected three times from April to June) followed by the same letter within a column are not significantly different ($P < 0.05$).

Table 3. Relationship between nematode population density (individual g^{-1} dry soil) and fertilization in rhizosphere and interrow soil

	Rhizosphere					Interrow				
	Total	Bac	Her	Fun	Om/Pr	Total	Bac	Her	Fun	Om/Pr
2004										
CF	19.8 c†	14.2 bc	1.4 b	3.0 ns	1.2 b	16.0 c	11.2 bc	1.3 c	2.7 ns	0.7 ns
CA	35.7 a	25.0 a	4.5 a	3.9 ns	2.3 a	30.6 a	20.0 a	3.6 ab	5.2 ns	1.8 ns
RF	16.6 c	10.1 c	3.2 a	2.2 ns	1.1 bc	16.7 c	10.3 bc	2.5 bc	3.0 ns	1.0 ns
RA	25.3 b	15.7 b	3.2 a	4.4 ns	2.0 a	23.9 b	14.0 b	4.1 a	3.8 ns	2.0 ns
OF	16.1 c	10.1 c	1.9 b	3.5 ns	0.5 c	12.8 c	8.6 c	1.4 c	3.1 ns	0.3 ns
2005										
CF	21.2 ab	13.9 ab	3.0 ns	2.0 ns	2.3 ns	21.8 a	13.4 b	3.3 ns	2.8 ns	2.4 ns
CA	27.3 a	17.7 a	4.0 ns	2.3 ns	3.4 ns	24.0 a	18.0 a	2.4 ns	1.9 ns	1.7 ns
RF	19.4 bc	13.1 bc	2.5 ns	1.7 ns	2.1 ns	15.9 bc	9.0 c	2.9 ns	1.8 ns	2.2 ns
RA	22.4 ab	15.0 ab	2.6 ns	2.4 ns	2.4 ns	20.6 ab	13.0 b	3.3 ns	2.5 ns	1.8 ns
OF	14.6 c	9.1 c	2.0 ns	1.3 ns	2.2 ns	14.8 c	9.9 c	2.6 ns	1.0 ns	1.3 ns

†Means of samples (collected three times from April to June) followed by the same letter within a column are not significantly different ($P < 0.05$). Bac, bacterivores; Her, herbivores; Fun, fungivores; Om/Pr, omnivores/predators.

2004).

The application of nitrogen as inorganic salts in silvicultural practices often suppresses microbial respiration and biomass in forest soils (Smolander et al. 1994, Thirukkumaran and Parkinson 2000). In arable fields, however, the application of inorganic fertilizer can indirectly but positively affect microbial growth by increasing plant growth and stimulating root exudation (Bolan et al. 1996, Mahmood et al. 2005). In the present study, inorganic fertilization (i.e., initial NPK application and additional NH_4NO_3 application) increased microbial SIR in both the rhizosphere and the interrow soil. This finding can also be explained by better root growth through fertilization, which likely supplied a greater amount of organic matter from the roots to the soil.

Inorganic fertilization also has been shown to increase the numbers of free-living nematodes (Forge and Simard 2001, Vestergård 2004) and microarthropods (Sjursen et al. 2005). In the present study, higher population densities of nematodes and microarthropods in the inorganically fertilized plots appeared to be linked to higher microbial activities. It should be noted, however, that inorganic fertilization has a variable effect upon soil fauna. For instance, it may have toxic effects on microarthropods by changing the soil pH (Potter et al. 1985). Lindberg and Persson (2004) reported that the application of ammonium nitrate in solid form decreased the population density of mesostigmatid mites, whereas application in liquid form increased collembolan numbers.

Table 4. Relationship between microarthropod population density (individual g^{-1} dry soil) and fertilization in rhizosphere and interrow soil

	2004			2005		
	Rhizosphere	Interrow	Rhizosphere/ interrow	Rhizosphere	Interrow	Rhizosphere/ interrow
CF	0.20 ns	0.08 ns	2.5 ns	0.26 bc†	0.06 b	4.9 ns
CA	0.31 ns	0.13 ns	2.5 ns	0.41 a	0.12 a	3.3 ns
RF	0.24 ns	0.07 ns	3.6 ns	0.20 c	0.07 b	3.2 ns
RA	0.20 ns	0.09 ns	2.5 ns	0.31 b	0.12 a	2.6 ns
OF	0.29 ns	0.11 ns	2.7 ns	0.18 c	0.10 a	2.0 ns

†Means of samples (collected three times from April to June) followed by the same letter within a column are not significantly different ($P < 0.05$).

Table 5. The rhizosphere/interrow ratio for soil organisms

	2004 (n = 15)	2005 (n = 20)
Microorganism (SIR)	1.54 ± 0.04†	1.57 ± 0.05
Nematodes	1.15 ± 0.05	1.10 ± 0.05
Bacterivores	1.17 ± 0.05	1.12 ± 0.10
Herbivores	1.23 ± 0.11	1.10 ± 0.09
Fungivores	1.06 ± 0.13	1.07 ± 0.11
Omnivores/Predators	1.63 ± 0.17	1.47 ± 0.22
Microarthropods	2.75 ± 0.22	3.39 ± 0.46
Collembola	2.93 ± 0.58	2.98 ± 0.55
Oribatid mites	2.51 ± 0.37	5.88 ± 1.19
Prostigmatid mites	2.58 ± 0.17	3.04 ± 0.46
Mesostigmatid mites	3.43 ± 0.43	3.75 ± 0.32

†Data are means ± standard errors from three (2004) and four (2005) replications of all five fertilization treatments.

The application of organic matter generally increases the numbers of nematodes (Opperman et al. 1993, Verhoeven 2001) and microarthropods (Mueller et al. 1993, Miyazawa et al. 2002) by supplying various nutrients, enhancing plant growth, and altering soil conditions. In the present study, however, in the OF plots the population density of nematodes was small in spite of the high microbial SIR. The small total nematode population was due mainly to the decrease in the numbers of dominant bacterivorous nematodes compared with the numbers in plots that received inorganic fertilizer. Organic fertilization has been reported to have neutral or negative effects on nematodes (Bulluck et al. 2002), and Ferris et al. (1996) suggested that bacterial abundance may not limit the population of bacterivorous nematodes. Population density of microarthropods in the rhizosphere was lower in the OF plots than in the CA plots that had similar root biomass in 2005. One possibility is that our organic fertilization caused soil property changes that were unfavorable for microarthropods. The water content of the OF plots was usually 0.2–2.1% lower than that of the CA plots (31.4–38.3%), and the soil pH was 0.2–0.3 higher in the OF than in the CA plots. Humidity and pH changes are known to affect microarthropod populations (Kaneda and Kaneko 2002, Lindberg and Persson 2004).

The abundance and activity levels of organisms in the rhizosphere are distinct from those living in bulk soil. The rhizosphere effect is usually defined by the ratio of the number of organisms per gram in rhizosphere soil to the number in bulk (non-rhizosphere) soil. Fertilization can alter the rhizosphere effect because it changes the quality and availability of root exudates, which affects the diversity of the bacterial and fungal community structure in the rhizosphere (Baudoin et al. 2001, Gomes et al. 2003). In addition, Vestergård (2004) reported that the community structure of nematodes in rhizosphere soil of field-grown barley was affected by the application of inorganic fertilizer. Fertilization

Table 6. Correlation of root biomass with microbial SIR and population densities of nematodes and microarthropods

	2004 (n = 15)				2005 (n = 20)			
	Rhizosphere		Interrow		Rhizosphere		Interrow	
	r	P	r	P	r	P	r	P
Microorganism (SIR)	0.73	**	0.56	*	0.68	**	0.27	ns
Nematodes	0.19	ns	0.18	ns	0.06	ns	0.36	ns
Bacterivores	0.21	ns	0.28	ns	–0.05	ns	0.39	ns
Herbivores	0.02	ns	–0.07	ns	0.29	ns	0.13	ns
Fungivores	0.33	ns	0.25	ns	–0.12	ns	0.07	ns
Omnivores/Predators	0.23	ns	–0.05	ns	0.31	ns	0.05	ns
Microarthropods	0.46	ns	0.28	ns	0.37	ns	0.30	ns
Collembola	0.35	ns	0.23	ns	0.11	ns	0.25	ns
Oribatid mites	0.36	ns	0.29	ns	0.48	*	0.28	ns
Prostigmatid mites	0.62	**	0.29	ns	0.32	ns	0.27	ns
Mesostigmatid mites	0.16	ns	0.25	ns	0.35	ns	0.34	ns

The significance of the probability is as follows: * $P < 0.05$, ** $P < 0.01$.

changes nutrient availability (Lee et al. 1997) and level of decomposition of soil organic matter (Cheng et al. 2003) between the rhizosphere and bulk soil. Griffiths (1990) reported that the rhizosphere effects for nematodes and protozoa were more conspicuous in fertilized than in unfertilized soil, and Griffiths et al. (1992) reported a greater rhizosphere effect for bacterivorous nematodes under higher nitrate conditions. In our study, different organisms had different rhizosphere/interrow ratios, but, contrary to our expectations, the ratio was not affected by fertilization regimes. Pot experiments that compared rhizosphere and root-free soil showed that the rhizosphere/root-free ratio for the nematode population was 11–18 (Ingham et al. 1985) and 11.7–70.1 (Griffiths et al. 1992). Parmelee et al. (1993) reported that the ratio for nematodes and microarthropods was higher at greater root density in mineral soil.

This study showed that the rhizosphere/interrow ratio for soil organisms in a wheat field varied from 1.07 to 5.88. Because the interrow soil used in this study included some roots, it is not surprising to find that the rhizosphere/interrow ratio was low compared with the results from the pot experiments mentioned above. This ratio may be useful for investigating the relationship between roots and soil organisms. We used it to evaluate how much root affected soil bioactivity. Fertilization seemed to affect microbial SIR through root irrespective of the kinds of fertilizer. Because microbial SIR had a strong positive correlation with root biomass though rhizosphere/interrow ratio was not so high. It was suggested that nematodes were not dependent on root for the following two reasons. Firstly, rhizosphere/interrow ratio was about 1.1 in both years. It indicated that the response of nematodes was not dependent on rhizosphere. Secondly, we found no correlation between root biomass and nematodes population density. Microarthropods seemed to be affected by root because great rhizosphere/interrow ratio was observed. The difference in the ratio among soil organisms may reflect the difference in habitat preference of soil biota and plant specificity. It was supported by Griffiths et al. (1992) who reported that rhizosphere effects was differed among different kind of soil organisms and among different plant species.

In conclusion, fertilization greatly affected soil bioactivity and root biomass. It was suggested that the difference in soil bioactivity between rhizosphere and interrow soil was caused by root effect. We showed that the rhizosphere/interrow ratio and the correlation between soil bioactivity and root biomass provided information about the role of root. Root seemed to affect microbial SIR and microarthropods but it was not obvious to nematodes population density. It is suggested that the fertilization effects can be varied by the dependency of soil organisms on rhizosphere.

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