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Effect of Winter-Flooding and Organic Farming on Density of Aquatic Oligochaetes in Ricefields: Case Study in Miyagi Prefecture, Northeastern Japan

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Abstract

Although aquatic earthworms (oligochaetes) in paddy fields have various functions for rice cultivation such as enhancement of nitrogen mineralization and imbedding of weed seeds through bioturbation, factors influencing their density have not been sufficiently understood. We investigated the effect of winter-flooding and organic farming on density of aquatic oligochaetes in ricefields of Miyagi prefecture, northeastern Japan. Aquatic oligochaete community in the winter-flooded organic fields was composed of four nauid species, ie, *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, *Bothrioneurum vej dovskyanum* and *Ilyodrilus templetoni*, of which *L. hoffmeisteri* was the most abundant. It became clear that winter-flooding treatment and organic fertilizer application were effective in increasing aquatic oligochaetes in organic ricefields.

Introduction

Aquatic earthworms (Oligochaete) occur in various kinds of freshwaters. They are abundantly found in paddy fields, and have important function for rice cultivation as a conveyor belt type feeder (Rhoads, 1974). The worms burrow into paddy soil and feed on and excrete them into the soil surface. Such soil disturbance (bioturbation) brought about by oligo-

chaetes caused a vertical redistribution of the soil particles and buried weed seeds 3-5 cm under the soil surface (Kikuchi and Kurihara 1977), resulting in suppressing the development and growth of weeds. The weeding effect of aquatic oligochaetes was suggested by the results of field study that weed densities were lower in the plots with higher density of oligochaetes than the plots with fewer oligochaetes (Kikuchi et al., 1975).

Aquatic oligochaetes enhance nitrogen mineralization and phosphorus transformation into labile form in paddy soils through stimulation of organic matter decomposition, and also increase the release of ammonium nitrogen and soluble phosphorus into the floodwater from the paddy soil (Kikuchi and Kurihara, 1977; Kikuchi and Kurihara, 1982; Ito and Hara, 2010). Aquatic oligochaetes burrow, and feed on and excrete soils onto soil surface. In flooded paddy fields, oxidized layer is formed on the soil surface and suppresses the diffusion of soluble nitrogen and phosphorus into floodwater from soils. Such a soil disturbance breaks the oxidized soil layer and enhances soil-water interface area, resulting in accelerating the nutrient diffusion from soil into overlying water. The enhanced release rates of inorganic nitrogen and phosphorus cause increases of phytoplankton and zooplankton in the overlying water. It became clear

that the presence of aquatic oligochaetes increased populations of microalgae, macrophytic algae, floating macrophyte and also zooplankton (Cladocera and Ostracoda) in the floodwater (Kikuchi and Kurihara, 1982). Consequently, aquatic oligochaetes influence nutrient dynamics in the soil-floodwater interface and impact the ecosystem in paddy floodwater.

Bioavailable nutrients (N and P) in submerged paddy soils and floodwater increased in proportion to the densities of aquatic oligochaetes (Ito and Hara, 2010). These functions will benefit organic rice farming with no chemical fertilizers and pesticides. Aquatic oligochaetes are projected to contribute to the increase of rice production and biological enrichment in organic ricefields. Thereafter, estimation of oligochaete density is very important for evaluating the functions in organic ricefields.

It has been clarified that aquatic oligochaetes showed higher density in the ricefields with greater contents of soil organic matter and soil moisture (Simpson *et al.*, 1993a; Kikuchi *et al.*, 1975), and application of organic matter increased oligochaetes in the ricefields (Simpson *et al.*, 1993b; Kikuchi *et al.*, 1975). It is considered that organic matters are substrate for soil bacteria and represent potential food source for the aquatic oligochaetes (Simpson *et al.*, 1993a). Information about pesticide impacts on aquatic oligochaetes in ricefields is still scarce and the impacts varied with dependence on kinds of pesticides. No pesticide impacts (Simpson *et al.*, 1993a; Rossaro *et al.*, 2012) or positive impacts (Mesléard *et al.*, 2005) on oligochaete density in ricefields have been reported. On the other hand, an insecticide, imidacloprid significantly decreased oligochaete density in the paddy mesocosm experiments (Hayasaka *et al.*, 2012). Taking the results into consideration, organic rice farming is presumed to increase the oligochaete density due to application of organic matters and no use of pesticides that are essential to organic farming systems. However, densities of aquatic oligochaetes have been measured only in organically managed ricefields by several researchers (Simpson *et al.*, 1993a; Suhling *et al.*, 2000; Mesléard *et al.*, 2005; Wilson *et al.*, 2008).

Most of the rice paddies had been converted from natural wetlands. The ricefields are often located in areas that historically provided important waterfowl habitat, such as the Ebro Delta in Spain and the Sacramento Valley in California (Fasola and Ruíz, 1996 ;

Elphick, 2000). It has been clarified that winter-flooded fields potentially serve as an important substitute habitat for many species of waterbirds in California (Elphick and Oring, 1998; Elphick, 2000) and in Japan (Kurechi, 2007). Furthermore, it is known that winter-flooding provides benefit for rice production through decreasing weed density and increasing rice straw decomposition (Bird *et al.*, 2000; Groenigen *et al.*, 2003). Takada *et al.* (2014) verified that both the density and species richness of spiders, ubiquitous predators in rice paddies, were significantly higher in the winter-flooded ricefields than in the conventional ricefields. However, little information is available for aquatic oligochaetes in the winter-flooded organic ricefields except for the result reported by Yachi *et al.* (2012).

The objective of the present study was to investigate the effect of winter-flooding and organic farming on abundance of aquatic oligochaetes in ricefields as case study in Miyagi prefecture, northeastern Japan.

Materials and methods

Surveyed fields

We surveyed three sets of adjoining ricefields located in Osaki City and Ishinomaki City of Miyagi Prefecture, northeastern Japan (Table 1). Tajiri A and B soils belonged to Fine-textured Gley soil, and Kanan soil belonged to Muck soil according to the classification of cultivated soils in Japan (Nat. Inst. Agric. Sci., 1983). Each set of ricefield has been managed by the same farmer. For Tajiri A fields, all three fields have been conventionally managed with conventional use of chemical fertilizers and pesticides till 2003 and have been organically managed with winter-flooding since 2004. For Tajiri B fields, the conventional and winter-flooding ricefields have been continuously cultivated by each farming method since 2004. In Japanese organic rice farming, organic fertilizer and rice bran are usually used for supply of nutrients to rice plants and weed control (Nozoe *et al.*, 2012), respectively. However, in Kanan fields, neither organic fertilizer nor rice bran were applied to the organically managed ricefields. For Kanan fields, low pesticide treatment without fungicide and insecticide was surveyed as the reference treatment.

According to the weather data of Furukawa and Ishinomaki observing stations (Japan Meteorological Agency), the average of mean annual air temperature in 2005 and 2006 were 11.0 and 11.5 °C for Tajiri and

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Table 1. Agricultural management conducted at the surveyed ricefields in 2005-2006.

Field name	Tajiri A (Oosaki City, Miyagi)		Tajiri B (Oosaki City, Miyagi)		Kanan (Ishinomaki City, Miyagi)	
	Winter-flooding + organic	Organic*1	Conventional	Winter-flooding + organic	Conventional	Winter-flooding + organic
Treatments						
The first year converting from conventional management to organic management with winter-flooding	2004	2004	2004	2004	-*2	2004
The year converting from organic management with winter-flooding to each treatment	-	2005	2005	-	-	-
Surveyed year	2005, 2006	2005	2005, 2006	2006	2006	2005, 2006
Period of winter flooding	January to mid March	-	-	January to mid March	-	December to spring flooding
Starting time of spring flooding	Beginning of May	Mid March	Mid March	Mid March	Mid March	Late April
Mid season drainage	-	-	7 days in late July	-	7 days in late July	-
Fertilizer	Organic fertilizer (4.2, 5.2 g m ⁻² as N) in one month before transplanting*3	Organic fertilizer (4.2 g m ⁻² as N) at transplanting	Compound fertilizer (5 g m ⁻² as N)	Organic fertilizer (3.7 g m ⁻² as N) in one month before transplanting	Compound fertilizer (5.2 g m ⁻² as N)	Compound fertilizer (6 g m ⁻² as N)
Organic materials for weed control	30, 20 g m ⁻² for rice bran and 30 g m ⁻² for waste soybean*3	30 g m ⁻² for rice bran and 30 g m ⁻² for waste soybean	-	60 g m ⁻² for rice bran and 45 g m ⁻² for waste soybean	-	-
Pesticides	-	-	Fungicide, insecticide, herbicide*4	-	Fungicide, insecticide, herbicide*4	Herbicide*5
Rice variety	Hitomebore	Hitomebore	Hitomebore	Hitomebore	Hitomebore	Sasanishiki
Transplanting time	5/22, 5/21	5/22	5/22, 5/21	5/23	5/23	6/7, 6/10

*1 : Organic management without winter-flooding.

*2 : Hyphen indicates no operation or no application.

*3 : The first and second figures indicate the values for 2005 and 2006.

*4 : Fungicide (probenazole), insecticide (fipronil, dinotefuran) and herbicide (bensulfuron-methyl, clomeprop, oxaziclomefone) were applied with standard quantity.

*5 : Herbicides (pretilachlor, simetryne, molinate, MCPB) were applied with half of standard quantity.

Kanan sites, respectively.

Measurement of aquatic oligochaete density

In order to estimate the density of aquatic oligochaetes in the ricefields studied, we randomly collected plow layer soil of 10 cm depth with 4 replications from each ricefield using core sampler with a diameter of 7 cm. We sieved the soil sample with a net of 0.5 mm mesh and moved the residues retained on sieve into sorting trays with tap water. We picked up oligochaete worms by hand-sorting and counted the individual number visually, according to Yachi (2012). Species composition was examined for the specimens from the winter-flooded organic fields collected on Jun 29 in 2006. Brinkhurst and Jamieson (1971) was used for oligochaete identification.

Soil characteristics

Three soils researched in this study were alluvial soils derived from river deposit. Total carbon and nitrogen contents of soils were measured by dry combustion method using NC-analyzer (Sumigraph model NC-80). Texture was measured by the pipette method (Wada, 1986), after pretreatment with H₂O₂ and ultrasonic dispersion.

Statistical analysis

We tested for significant differences in the oligochaete densities between three or two treatments using Tukey's HSD or student's t-test, respectively with significance at $P < 0.05$. All analyses were conducted using JMP v4.0.5.J (SAS Institute, 2001).

Results

Four species of aquatic oligochaetes, ie., *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, *Bothrioneurum vej dovskyanum* and *Ilyodrilus templetoni*. all of which belonging to the family Naididae sensu (Erséus et al., 2008) were recognized in the specimens from winter-flooded organic fields. Among them, *L. hoffmeisteri* was the most abundant, accounting for more than 60 % of the total oligochaetes in all three sites studied.

Winter-flooded ricefields had higher oligochaete densities compared to organic ricefields without winter-flooding or conventional ricefields. The densities of aquatic oligochaetes increased from spring to summer, especially in the conventional ricefields and the ricefields with low application of pesticide (low

pesticide ricefields) (Figures 1 and 2). In Tajiri A site, averaged values of the oligochaete densities during study period were 22,275, 9,427 and 3,702 m⁻² for winter-flooded organic field, organic field and conventional field, respectively. They were 5,785, 3,066 and 657 for winter-flooded organic field, organic field and conventional field with low application of pesticide in Kanan site in 2005, respectively. Winter-flooded organic field and conventional field in Tajiri A site had averaged densities of 49,289 and 10,025 m⁻², respectively in 2006. The average densities in winter-flooded organic field and conventional field were 16,435 and 3,316 m⁻² in Tajiri B site in 2006. Those of winter-flooded organic field, organic field and low pesticide field were 25,471, 7,866 and 9,092 m⁻² in Kanan site in 2006, respectively.

The oligochaete densities were higher in the ricefields with winter-flooded organic farming than those of the conventional or low pesticide ricefields for Tajiri A in 2005, Tajiri B in 2006 and Kanan in both years (Figures 1 and 2). Organically managed ricefields without winter-flooding had significantly higher density than the conventional ricefield and had lower density than the winter-flooded organic ricefield with significant difference in Tajiri A (Figure 1A). On the other hand, densities of aquatic oligochaetes showed no significant difference between the organic ricefield and the low pesticide ricefield in Kanan site in both years (Figures 1B and 2C). The oligochaete densities in the winter-flooded organic ricefield showed higher in Tajiri A site than Kanan site in both years, and showed higher in Tajiri A site than in Tajiri B site in 2006 (Figures 1 and 2).

Discussion

Density of aquatic oligochaetes in winter-flooded organically managed paddy

Average of oligochaete densities during summer three months ranged from 16,435 to 49,289 m⁻² for winter-flooded organically managed ricefields in Tajiri and Kanan sites, except Kanan in 2005 with fewer number of observations. These values were greater than the highest abundance of 5,836 m⁻² obtained in the winter-flooded organically managed ricefields of Kamakura City, Kanagawa Prefecture, Japan (Yachi et al., 2012) and also higher than mean densities in conventional ricefields of the Laguna Province, Philippines (4,700-10,400 m⁻²) (Simpson et al., 1993a). The oligochaete densities in winter-flooded organical-

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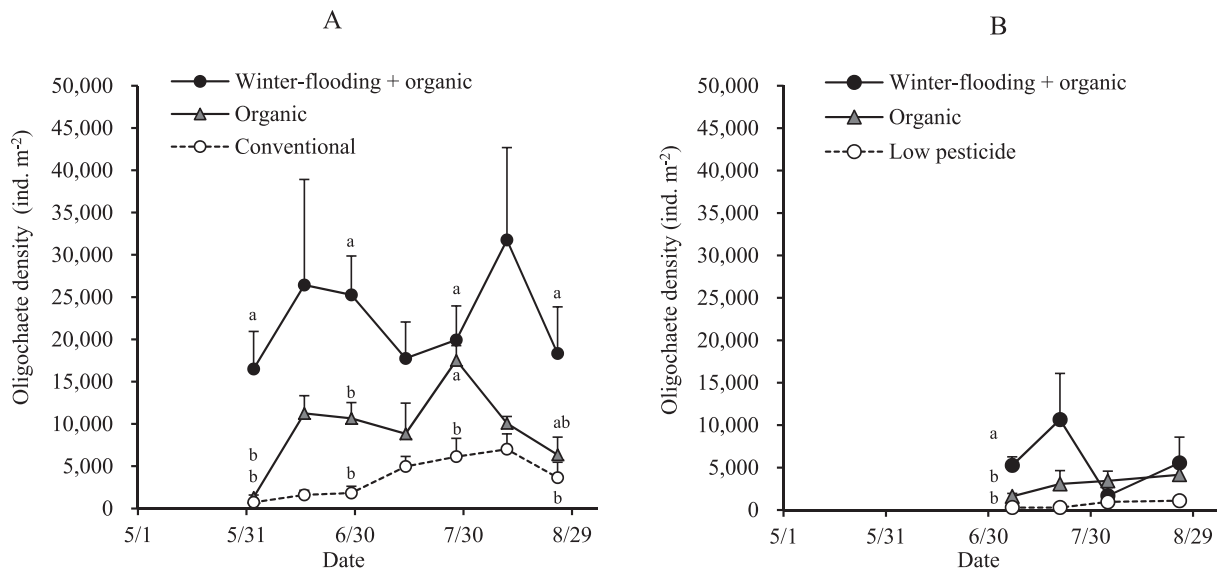


Figure 1. Aquatic oligochaete densities in the paddy fields of Tajiri A (A) and Kanan (B) in 2005. Vertical lines show upper standard errors (n = 4). Different alphabet shows significant difference with P value < 0.05 between oligochaete densities obtained at the same research date.

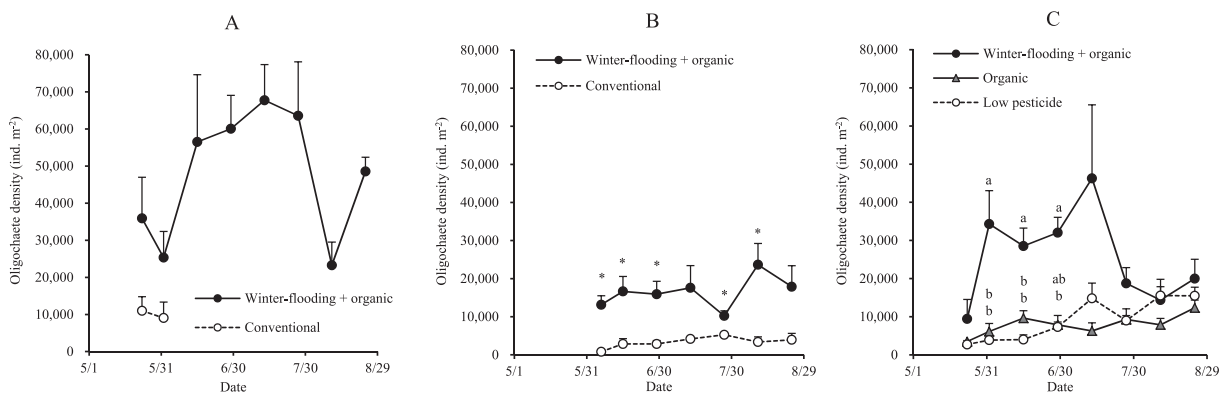


Figure 2. Aquatic oligochaete densities in the paddy fields of Tajiri A (A), Tajiri B (B) and Kanan (C) in 2006. Vertical lines show upper standard errors (n = 4). Different alphabet shows significant difference with P value < 0.05 between oligochaete densities obtained at the same research date.

ly managed ricefields can be affected by the duration period of flooding, application of organic fertilizer and organic matter content of paddy soil.

Oligochaete densities of winter-flooded organically managed ricefields were compared between Tajiri A and Tajiri B sites for the purpose of analyzing the effect of soil organic matter on aquatic oligochaetes. The two places were close each other and had same duration of winter-flooding and organic management. The oligochaete density in winter-flooded organic ricefields was about 3 times higher in Tajiri A than those in Tajiri B (Figures 2A and 2B). Plow layer soils had higher contents of organic matter for Tajiri A than Tajiri B, as shown by total C and N contents

(Table 2). The result suggests that high content of soil organic matter contributes to growth of aquatic oligochaetes. Soil organic matter could be substrate for soil bacteria and represent potential food source for the aquatic oligochaetes, as shown by former researchers (Simpson et al., 1993a). It is possible that larger application rate of organic fertilizer also contributed to higher density of aquatic oligochaetes in Tajiri A field as shown in Table 1.

We compared the oligochaete densities of winter-flooded organically managed ricefields between Tajiri A and Kanan fields for estimating the influence of organic fertilizer application on oligochaete growth. In spite of lower soil organic matter in Tajiri A than

Table 2. Some properties of the surveyed ricefields.

Field name	Tajiri A (Oosaki City, Miyagi)			Tajiri B (Oosaki City, Miyagi)		Kanan (Ishinomaki City, Miyagi)		
	Winter-flooding + organic	Organic	Conventional	Winter-flooding + organic	Conventional	Winter-flooding + organic	Organic	Low pesticide
Total C g kg ⁻¹	19	-* ¹	19	14	15	27	26	28
Total N g kg ⁻¹	1.7	-	1.8	1.3	1.4	2.4	2.3	2.5
Texture	CL* ²	CL	CL	-	-	-	-	-

*1 : Not determined

*2 : Clay loam

Kanan (Table 2), the oligochaete densities were higher in Tajiri A than in Kanan in both of 2005 and 2006 (Figures 1 and 2). The difference suggests that application of organic fertilizer and organic materials (rice bran, waste soybean) stimulated aquatic oligochaete reproduction. Previous researchers also reported the application of organic matter increased oligochaete density in ricefields (Simpson *et al.*, 1993b; Kikuchi *et al.*, 1975).

Effect of winter-flooding on aquatic oligochaete density

Combination of winter-flooding and organic farming practice significantly increased oligochaete densities compared to the organic management without winter-flooding for Tajiri A in 2005 and Kanan in 2005 and 2006. In order to estimate winter-flooding effect on oligochaete densities, we compared the increasing ratio of oligochaete density from 2005 to 2006 between winter-flooded organic ricefield and organic ricefield. The ratios of oligochaete densities averaged over July and August (averages of four sampling times) in 2006 to those in 2005 were greater in the winter-flooded organic ricefield (4.3) than in the organic ricefield (2.9). It can be concluded that winter-flooding is effective in increasing aquatic oligochaetes in organically managed ricefields.

Conclusions

From the foregoing results, it is concluded that the winter-flooding is effective in enhancing aquatic oligochaete density in organically managed ricefields, and greater amount of soil organic matter and larger application of organic fertilizer also contribute to increasing aquatic oligochaetes.

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