Effects of bio-based nutrient on the growth of rice plants in relation to abundance of aboveground arthropods in organic paddy fields with different histories of management

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Title:

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General Introduction

Chapter 1:

The effects of different histories of organic paddy fields on the aquatic and terrestrial community structures

Chapter 2:

Indirect effects of bio-based nutrient on the abundance of terrestrial arthropods through the growth of rice plants in organic paddy fields

Chapter 3:

Effects of soil organism on the abundance of aboveground arthropods in organic paddy fields

General Discussion

Summary

Reference

General Introduction

More than half of the world's population depends on rice, suggesting that the rice production should be fulfilled the concept of sustainability. Sustainable of rice production should be achieved through the farming management which maintains or enhances the quality of the environment, and conserves or enhances the natural resources. The characteristic of sustainability of rice production is the agricultural practices that utilize microorganisms and invertebrates. Rice fields have unique characteristics that make them has a rich biodiversity. However, rice fields constitute man-made ecosystems that dynamic and rapidly changing as consequences of the agricultural development.

Modern agriculture practice, it can lead to a higher yield, due to its extensive use of fertilizers, herbicides and pesticides, and thus it contributed to eliminating hunger. However, the intensive practices and chemical inputs in the modern paddy fields would cause several ecological problems (Pimentel et al. 1992). Considering the advantages and disadvantages of modern agriculture, it is of high importance to reassess all agricultural practices (Li 2001). In the assessment process, it is found that the organic paddy fields can be a good alternative to achieve the sustainable of rice production. Developing new agricultural technologies for improving the rice production that does not depend strongly on the use of fertilizers and agrochemicals is essential.

Organic paddy field uses no chemical fertilizers or pesticides, making it the most promising method for eliminating the negative effects of modern farming. In organic paddy fields, some farmers use bio-based nutrients from aquatic organisms instead of chemical or organic fertilizers to build the soil. Several studies have suggested that the use of bio-based nutrients instead of synthetic fertilizers can improve the production as fewer pest insects would be present (Altieri and Nicholls 2003; Butler et al. 2012; Facknath and Lalljee 2005; Stafford et al. 2012). However, as few studies have examined these hypothesized advantages of organic paddy fields, investigations are necessary to determine the effects of long term of organic methods on rice production through the interaction among organism in aquatic and terrestrial ecosystem.

In addition, although understanding of the differential effects on agricultural ecosystem due to organic and conventional farming has been paying much attention, it is still under debate by ecologists. In general, organic farming, which has limited use of chemical fertilizer and pesticide suggests the increase of species richness and abundance of organisms involved in a community, although the effects on ecosystem services in agricultural environment of organic farming still remain largely unexplored (Bengtsson & Weibull, 2005; Gabriel et al., 2006; Rundlof & Smith, 2006; Winquist, 2011). The facts that plants play an important role in term of management of farming system are well known for arthropod community is conditioned by those plants as bottom up effects. Therefore, understanding of interactions between and/or among organisms in agro-ecosystem is also important in order to evaluate the consequences of management practice in ecosystem services and functions in an agricultural environment (Hole et al., 2005, Ponce et al., 2011).

Biodiversity is the one of the important cornerstones to develop sustainable agriculture. Understanding of unit of agricultural activity which includes living and nonliving components in agro-ecosystem is a key to determine the effective farming systems. The system in sustainable agriculture would ideally keep a diverse and active plant community (Mader et al., 2002). In agricultural system, the ecological factor such as soil fertility has influenced the composition of the plant communities, while the functions of the plant communities would in turn affect the nutrient status in soil at ecosystems (Mader et al., 2002; Bambaradeniya & Amarasinghe, 2003). As biodiversity performs ecosystem services such as recycling of nutrients, regulation of local hydrological processes, regulation of the abundance of undesirable organisms and detoxification of noxious chemicals (Altieri and Nicholls, 1994;

Leveque & Mounolo, 2001), various options of agro-ecosystem management and design that enhance the functions of biodiversity in crop fields would have to be precisely decided. The study of biodiversity associated with agro-ecosystems such as rice fields might help to understand not only maintenance of the biological diversity in ecosystems, but also the practice of sustainable agriculture (Pimental et al., 1992).

In general, biotic communities in an agricultural ecosystem would react as a process of recovery from various disturbances including chemical inputs (Bambaradeniya, 2000). Biodiversity in agro-ecosystem including the diversity of plant and animal species is affected by agriculture at which different type of land could provide the different diversity in an ecosystem (Parris, 2001). Previous studies on the biodiversity of rice fields deal mainly with agronomic aspects, where the rice pests, natural enemies, and weeds have been surveyed extensively. However, comprehensive studies on biodiversity in paddy fields in term of ecological aspects are scanty.

Aquatic organisms inhabit the soil-floodwater ecosystem of paddy fields and are an important component of paddy field fertility. Aquatic organisms have played an important role in organic matter composition and nutrient translocation which are considered key components of soil fertility (Roger et al. 1987; Simpson et al., 1992). Some experiments have showed that aquatic organisms like aquatic oligochaetes gave a positive effect on plant productivity through food-web effects due to nutrient release (Coleman et al., 1984, 1989; Ingham et al., 1985; Seta¨la¨ & Huhta, 1991; Simpson et al., 1992; De Ruiter et al., 1993; Chen & Ferris, 2000; Schroter et al., 2003; Blouin et al., 2005; Hines et al., 2006). On the other hand, the researches in terms of terrestrial organisms also have revealed the importance of terrestrial organisms in community structure and ecosystem processes (Putten et al., 2009). Primary producers in aboveground ecosystem are the main source of organic carbon. While, aquatic organisms that inhabit the soil-floodwater ecosystem of paddy fields contribute to nutrient cycling in several ways. For instance, microcrustaceans, insect larvae, mollusks, oligochaetaes which release native minerals from soil play prominent roles in decomposition process of photosynthetic aquatic biomass (Simpson and Roger, 1993). In general, the organisms involved in aboveground and belowground ecosystems may interact each other, and these interactions would influence on performance of the related individuals. However, as most ecologists have tended to study the interactions of related organisms in each ecosystem separately, there have been few studies which have revealed the interactions among organisms through ecosystems (Holz et al., 2000; Kardol and Wardle, 2010).

One of the most important issues in ecology is to understand the direct and indirect interactions between and/or among organisms involved in ecological communities (Strauss, 1991; Wootton, 1994; Polis & Winemiller, 1996; Ohgushi, 2008). Food web which depicts feeding connections in an ecological community can be reticulated and provide a basic framework for understanding the organization of a community (Holz et al, 2000; Ohgushi, 2008). Nutrient transfer activities in food web express the presence nutrient or energy flow through trophic levels in a community (Holz et al., 2000). For example, plants provide nutrients for supporting the growth and development of phytophagous arthropods which in turn would be supplied as essential food for carnivores (McNeill and Southwood, 1978; Mattson, 1980; Greenfield et al., 1987, Amwack and Leather, 2002). Therefore, to understand the dynamic interactions in food web which is illustrated by bottom-up and top-down effects has been paid much attention by ecologists, however, our understanding of the dynamic interactions is not complete.

Long-term application of organic fertilizer indeed results in an increase in the carbon content of soils (Fließbach et al., 2007). This, in turn, likely affects the soil fauna and alters the decomposition of crop residues and hence the availability of nutrients (Lundquist et al., 1999). The effect of allochthonous resource input on soil fauna may further influence aboveground animal communities as increased carbon availability may cascade up to higher trophic levels (Halaj and Wise, 2002). Surface-active predators may benefit from increased prey availability from the belowground subsystem (Oksanen et al., 1997), either improving (Scheu, 2001) or reducing (Birkhofer et al., 2008a) pest suppression by natural enemies. As mineral (NPK) fertilizer application may reduce (Ryan, 1999). Considering the increased attention that the sustainability of farming practices receives the lack of thorough studies on the effects of different histories on below and aboveground biota is unfortunate.

Sustainable rice production in organic paddy fields is the goal of this study. Organic paddy fields with different histories were used to clarify the effects of organic farming on the paddy field ecosystems. **In chapter 1**, the study were to examine (1) how the different histories in term of the period as an organic paddy field on the community structure of aquatic communities and (2) how those effects associated on the community structure of terrestrial communities through plant-mediated interactions. Next, in **chapter 2**, were to understand: (1) how the interaction of aquatic organisms influences on bio-based nutrient release in organic paddy fields (2) how those interaction effects directly and indirectly on the abundance of terrestrial arthropods through mediated rice plant performance and nutrient. In addition, **chapter 3** were to reveal the effects of soil organisms on the abundance of aboveground arthropods by measuring the soil organism activity, nutrient availability, and abundance of the arthropods. Finally, the effects of bio-based nutrient on the growth of rice plants in relation to abundance of aboveground arthropods in organic paddy fields with different histories of management will be discussed in **chapter 4**.

Chapter 1

The effects of different histories of organic paddy fields on the aquatic and terrestrial community structures

Introduction

In agriculture, modern farming practices often lead to changes in food web structure and communities dominated by a few common species, which together contribute to pest outbreaks (Crowder et al. 2010). Several studies reported that organic farming used no chemical fertilizers or pesticides, making it the most promising method for eliminating these ecological damage by increasing the biodiversity (Altieri and Nicholls 2003; Butler et al. 2012; Facknath and Lalljee 2005; Stafford et al. 2012). Some researchers have argued that organic farming and sustainable agriculture are synonymous (Rigby and Careras 2001). Mannion (1995) noted that organic farming is a holistic view of agriculture that aims to reflect the profound interrelationship between agriculture biota, its production and the overall environment.

Organic farming increased organic matter and active soil biology, which exhibit good soil fertility as well as complex food webs and beneficial organisms (Altieri and Nicholls 2003). The effects of high resource input in the soil may further influence on aboveground communities (Halaj and Wise, 2002). The field, which has a long history of organic farming potentially develop valuable and sustainable of belowground and aboveground ecosystems with natural cycles (Rigby and Careras 2001; Oehl et al. 2004; Birkhofer et al. 2008). However, little is known about the influence of different histories of organic farming on the functions of agro-ecosystems particularly in paddy fields.

As a wetland in agro-ecosystem, organic paddy field is important for biological conservation because they support a wide variety of organisms and great biodiversity (Kano et al 2010, Maltchik et al. 2010). Aquatic organisms are an important factor for maintaining the sustainability in an organic paddy field, and it's contributed to nutrient cycling in several ways. In organic paddy fields, some farmers use bio-based nutrients from aquatic organisms to build the soil fertility. For instance, microcrustaceans, aquatic insects and aquatic oligochaetaes play prominent roles in the decomposition process of photosynthetic aquatic biomass (Simpson and Roger, 1993). However, bio-based nutrients release more slowly than synthetic fertilizers and rice plants take up the bio-based nutrients gradually. Consequently, these rice plants contain less nutrients and may be less suitable for pest insects (Hsu et al. 2009; Staley et al. 2010). Several studies have suggested that the use of bio-based nutrients can improve plant production as fewer pest insects would be present (Altieri and Nicholls 2003; Butler et al. 2012; Facknath and Lalljee 2005; Stafford et al. 2012). This study in this chapter tried to reveal the effect of different history of organic paddy fields on the community structure of aquatic and terrestrial communities.

Therefore, the aims of the study are to examine (1) how the different histories in term of the period as an organic paddy field effects on the community structure of aquatic communities and (2) how those effects associated on the community structure of terrestrial communities through plant-mediated interactions. We hypothesized that the organic paddy fields would increase sustainable and valuable of aquatic and terrestrial community structure gradually through the year. So that history of organic farming is important as an indicator of the agro-ecosystem function.

Materials and Methods

Study area and experimental design

The field survey was conducted in the area surrounding Horinouchi at Sakata city, Yamagata, Japan (38° 55′ N, 139° 50′ E), during the growing season of the rice plants in 2012 to 2014. The study area is characterized by Dfa (humid continental climate) according to the Köppen climate classification (Pidwirny 2011). A humid continental climate is typified by large seasonal temperature differences with very warm summers and cool winters. During the study year from 2012 to 2014, the mean annual precipitation was approximately 2014 mm and the mean monthly temperature from May to September was approximately 21.8 °C (Japan Meteorological Agency 2014). The terrain is flat lowland covering approximately 2 km from east to west and 5-7 km from north to south, and the area primarily paddy field cultivation. A typical paddy field covers approximately 3000 m^2. In addition, in the area, organic paddy fields were distributed among conventionally managed paddy fields.

To compare the effects of the different histories of organic paddy fields on the aquatic and terrestrial community structure, field survey was conducted in the organic paddy fields with different periods of time as organic fields such as 5, 10, and 20 years. Three replicate paddy fields from each of organic paddy fields with different histories were selected in 2012. Based on the result in 2012, organic paddy fields were classified into low and high biodiversity. The field survey was continued in the paddy field with three pairs of 5 and 20 years of organic paddy fields in 2013 and 2014. All fields had been cultivated without any fertilizer (organic and inorganic) and agrochemicals (insecticides, herbicides, and fungicides). Rice seedlings variety used was "*Koshihikari*" which is a popular variety planted in Japan, transplanted in May of each year.

Sampling of aquatic communities

Aquatic community samples were collected randomly at nine 1 × 1 m plots laid out in a cross pattern in each field. To estimate the abundance of aquatic macro-invertebrates, a field survey was conducted approximately every 2 weeks during the growth period of rice plants under water flooded conditions from May to June in 2012 - 2014. On each sampling occasion, box samplings (0.25 m^2 acrylic boxes) were deployed in each plot, and the aquatic macro-invertebrates were captured by a sweeping net (0.45 × 0.2-m, 0.5-mm mesh) until no additional aquatic macro-invertebrates were captured in three consecutive sweeps. The samples were transiently kept in the plastic bag and brought to the laboratory. In the laboratory, samples were washed through 0.5-mm sieve, and placed into the white tray where live aquatic macro-invertebrates were separated manually from plant materials and soils. The plant materials and soils were then placed in tullgren gauze to extract macro-invertebrates living in the soil.

To investigate the potential factors affecting aquatic macro-invertebrate communities, macrophytics and algal biomass were measured in each plot of organic paddy field with different histories. Macrophytics were sampled manually at 50 × 50-cm-area in each plot. These macrophytics were oven-dried at 70°C for 72 h and their biomass was measured. Samples of algae were collected by taking about a liter of the paddy field water in each plot. On return to the laboratory, water samples were filtered through glass microfiber filter papers (Whatman GF/C; 110 mm Ø). The materials retained by the filter paper may have contained algae, zooplankton and/or other organic and inorganic particulates. These materials were determined the biomass by adding 7 ml of solvent (ethanol 96%), and stored in the dark at room temperature through overnight. Thereafter, the samples were centrifuged at 4000 cycles per minute for 10 minutes, and measured the chlorophyll on spectrophotometer at 665 nm (maximum absorption of chlorophyll a), and at 750 nm (minimum absorption of chlorophyll a). The algal biomass was calculated according to the following equation: $((Abs_{665} - Abs_{750}) \times$ vol. of solvent $\times$ 10 000) / (absorption coefficient for ethanol $\times$ vol. of water filtered $\times$ length of the cuvette in the spectrophotometer). In addition, detritus samples were also taken using a hand trowel in approximately 20-cm^2-areas and 3-cm-deep for each plot. These detritus samples were determined the nutrition concentration in terms of nitrogen (N) and carbon (C) concentrations. To analyze the N and C concentrations, the detritus were oven-dried at 70°C for 72 h. The dried detritus was ground into powder and then analyzed with an automatic NC determination unit (Sumigraph NC-220F; Sumika Chemical Analysis Service, Osaka, Japan).

Sampling of terrestrial communities

Terrestrial arthropods were also surveyed in each field approximately every 2 weeks from July to September in 2012 - 2014, by sampling randomly at five 3 × 1-m plots laid out in a cross pattern in each field. At each sample time, 20 sweeps with a 36-cm-diameter insect-net were performed on each plot. The samples were sprayed with ethanol and brought to the laboratory. In the laboratory, each sample was sorted into different taxa and counted. The insects and spiders collected from the fields were identified and classified into the smallest possible taxa using available keys and guides for the different taxa.

As indices of habitat complexity in terrestrial communities, weed biomass was determined and rice plant growth was measured. Weeds were collected manually at nine 50 × 50-cm-plots in each field. These weeds were oven-dried at 70°C for 72 h and their biomass was measured. To measure the rice plant growth, 30 rice plants were randomly selected in each field, and height and number of tiller/hill was measured every month. In addition, to estimate the rice plant nutrient, another 24 plants in a 1 × 1-m plot was sampled at three random locations in each field on August. The shoots of the rice plant were dried in an oven at 70°C for 72 h, and the plant biomass/m^2 was recorded before chemical analysis. To analyze the N and C concentrations in rice plants, the dried shoots were ground into powder and then analyzed with an automatic NC determination unit (Sumigraph NC-220F; Sumika Chemical Analysis Service, Osaka, Japan).

Results

Community structure of aquatic macro-invertebrate communities

Twenty-seven taxonomical groups of aquatic macro-invertebrates were identified in total, mainly at the family level in the study fields (Table 1). Based on the functional feeding group, the aquatic macro-invertebrates founded in the study fields were categorized into three groups, scarper, collectors-gatherer, and predator (Table 1). Mud snail (*Cipangopaludina chinensis malleata*) was only found in 20 years of organic paddy fields (Table 1). The total abundance of aquatic macro-invertebrates were higher in 20 years than 5 years organic paddy fields and increase by the year (Figure 1). Collector-gatherers were most abundant group in aquatic community, both in 20 and 5 years organic paddy fields (Figure 1). The abundance of scrapers increased in 20 years or 10 years than 5 years organic paddy fields, and resulted the same pattern in each year (Figure 2). Mud snails and pond shell snails were high in 20 years organic paddy fields, while planorbid and small freshwater snails were low (Figure 2). Mainly the abundance of each species of collector-gatherers was no significant differences between 20, 10, and 5 years in all year (Figure 3). The abundance of aquatic macro-invertebrate predators were varied in each species in each year (Figure 4). However, the important aquatic predators such as dragonfly larvae, small hydorphilid, and small diving beetles were greater in 20 years than 5 years organic paddy fields in all year (Figure 4).

Principal component analysis (PCA) in 2012 showed that the community structure of 10 years organic paddy fields not significantly different from 20 and 5 years organic paddy fields (Figure 5a). In 2013 showed that the community structure of aquatic macro-invertebrates were significantly different between 20 and 5 years organic paddy fields, it's also depicts the aquatic macro-invertebrates that contributed to the vector of the 1st component scores or 2nd ones, showing that mud snail, punkies, crane fly larvae, tadpole and dragonfly larvae characterized the aquatic community structure (Figure 5b). However, PCA analysis in 2014 resulted that community structure of aquatic macro-invertebrate in 20 and 5 years was no significant difference (Figure 5c).

Resources analysis of aquatic communities

The N, C concentration and C/N ratio of detritus and microphytics biomass were significantly higher in 20 and 10 years than 5 years of organic paddy fields (Table 3). However, algal biomass was only marginally significant in 20 years than that in 5 years organic paddy fields.

Association between resources factor and aquatic macro-invertebrates

The N concentration of detritus and algal biomass (chlorophyll a concentration) were significantly positively correlated with abundance of aquatic macro-invertebrates (N concentration of detritus: $df = 4$, $t = -4.17$, $p < 0.001$; algal biomass: $df = 4$, $t = -5.59$, $p < 0.001$, Figure 6). The results showed that there is significant indirect and direct correlation among the aquatic organisms and their resources (Figure 7).

Community structure of terrestrial communities

A total of twenty-eight species or families of terrestrial arthropods were observed (Table 2). The terrestrial arthropods were classified into three groups, herbivores, predators, and neutral insects. All arthropods were found in all histories of organic paddy fields. Moreover, the total abundance of terrestrial arthropods was not significantly different among the history organic paddy fields. Almost the abundance of each species of herbivores, predators, neutral insects in terrestrial community also showed no significant difference between the different histories of organic paddy fields (Figure 4-6). However, the abundance of Tetragnatha spiders and Chironomid in 2013 and 2014 indicated a significantly higher in 20 years than 5 years of organic paddy fields (Figure 10 and 11).

The principal component analysis showed that the community structure of terrestrial community structure among 5, 10, and 20 years organic paddy fields were not significantly different (Figure 12). However, there is a significant direct interaction between the species of herbivores, predators and neutral insects in terrestrial communities (Figure 13).

Recourse analysis of terrestrial communities

The N, C concentration and C/N ratio in the rice plants in 2012 and 2013 was marginally significant between the histories of organic paddy fields, while in 2014 they were significantly higher in 20 years than 5 years organic paddy fields (Table 4). The weed biomass showed also increased in 20 years organic paddy fields (Table 4). In addition, the significant difference in weed biomass was similar to differences in rice plant growth and production (Figure 14 and 15).

Discussion

This study demonstrated that the period of paddy fields cultivated with organic methods affected the aquatic community structure, although has no effect on the terrestrial community structure. The results also confirmed that long term of organic paddy field increased the nutrition concentration in detritus, and these may effect on the abundance of the aquatic macro-invertebrate and their interactions. However, nutrient concentration in the rice plant was not highly changed in the long term of organic paddy fields and low effect on the abundance of terrestrial arthropods. In addition, the rice plants in 20 years organic paddy fields had greater productivity than those in 5 years organic paddy fields.

Long term of organic farming can greatly enhance the basal resources such as detritus and algae due to increasing organic input every growing season (Entry et al. 1997; Kandeler et al., 1999; Garcı´a-Gil et al., 2000). Present study resulted that N and C concentration of detritus were higher in 20 years and 10 years of organic paddy fields which has a longer term as organic field compared to 5 years organic paddy fields. However, the algal biomass was only tended to be higher in 20 years than 5 years of organic paddy fields. Detritus typically includes the bodies or fragments of dead plant and animal as well as fecal material, and important resource for organisms living in paddy fields (Anderson 1979; Lawler and Dirtz 2005). Moreover, the algal biomass would respond positively to increasing of nutrient in detritus (Lawler and Dirtz 2005). Our study supported these reviews.

Organic farming is an advantageous method for aquatic organisms (Yamazaki et al. 2004). Although many factors would effects on the abundance of aquatic organisms, history of organic farming is one of the important factors influence on their existence (Birkhofer et al. 2008). Long period of bio-based nutrient management in the organic fields could foster the abundance of aquatic organisms (Chinnadurai et al. 2014). Our study also resulted that the abundance of aquatic macro-invertebrate in total was higher in 20 years than 5 years organic paddy field, although several macro-invertebrate species were greater in 5 years organic paddy fields. Moreover, our study provides the evidence that the community structure of aquatic macro-invertebrate were affected by the different histories of organic paddy fields. In addition, the nutrition of detritus and algae biomass also had positively correlated with the abundance of aquatic macroinvertebrates. Many studies have shown that aquatic macroinvertebrate distribution has been related to the basal resources in aquatic ecosystems (Pavluk et al., 2000). However, each macro-invertebrates play fundamental roles in aquatic ecosystems, being consumers at intermediate trophic levels and thus serving as channels by which bottom-up and top-down forces are transmitted (Wallace et al., 1999). The epilithic algae that grows on the surfaces of substrates consumed by scrapers, the fine detritus, deposited on the substrate consumed by gatherers and finally, live animals consumed by predators.

The relative abundance of scrapers is an indication of the availability of fine detritus, and scraper increase with increasing algae abundance (Takizawa et al. 2009). Our results showed that the abundance of scrapers, detritus, and algae were greater in 20 years of organic paddy fields, suggesting that the appearance of the scraper in paddy fields were affected by the long term histories of organic paddy fields. Hawkins et al. (1982) also mentioned that high abundance of the scraper correlated with increasing the light level because the high percentage of lightening stimulates the growth of algae as a source of scarper food. In addition, our study showed that in 5 years organic fields could not find mud snail, but have a high abundance of small freshwater snail. Species of snail plays an important role as an indicator of soil fertility in paddies (Simpson et al. 1994). It indicated that long term organic farming can maintain the suitable habitat for growth and development of mud snail, but decreasing abundance of small fresh water snail due to food competition with mud snail.

The input of bio-based nutrient every growing season in organic fields could increase the carbon resources and may further influence the abundance of aquatic macro-invertebrates particularly the collector-gatherers (Yamazaki et al. 2001). Previous study noted that the collector-gatherers are the predominant groups at most sites (Uwadiae 2010). In our study fields, collector gatherers are the dominant macro-invertebrate, although their abundance had low affected by the different histories of organic paddy field. However, several species of collector gatherers such as Chironomid, Sludge worm, and other Diptera larvae are important prey for aquatic predator. Present study resulted that the abundance of important species of aquatic predators in paddy fields such as dragonfly larvae and diving beetle, were increased in 20 years organic paddy fields. Dragonfly and diving beetle are the bio-indicator organisms for functional agrobiodiveristy in aquatic ecosystem of paddy fields (Tanaka et al. 2011). However, other predator species were also high in 5 years and their abundance was not affected by the histories of organic paddy fields. Interaction between multiple predators can result in facilitation, interference, or neutral relationship (Crowder et al. 1997, Schmitz 2007). Predation is a key factor structuring communities and drives food web dynamics (Carey and Wahl 2010). Moreover, aquatic predators have a strong consumptive effect in terrestrial ecosystems (Knight et al. 2005).

Use of bio-based nutrient for several years indeed results in an increase in the carbon content of soils (Fließbach et al., 2007). Soil with a high C content prevents nutrient leaching and holds a greater proportion of nutrients important to plants (Batjes 1996; Leu 2007). This, in turn, likely affects the terrestrial arthropod communities (Halaj and Wise, 2002). Based on the C–nutrient balance hypothesis (Phelan et al. 1995), plants with a high C concentration might have high resistance to insect attack due to the increased partitioning of foliar phenolic glycosides (Mattson et al. 2005). In addition, bio-based nutrients also release N slowly and rice plants take up the bio-based N gradually. Consequently, these rice plants contain less N may be less suitable for terrestrial arthropods (Hsu et al. 2009; Staley et al. 2010). Our study showed that all organic paddy fields with different histories have similar N concentration, C concentration and C/N ratio in rice plants. The results also indicated that generally the abundance and community structure of terrestrial arthropods were no significant differences between the histories of organic paddy fields in all year. It's suggested that organic fields could control the excessive nutrient in plants and in turn decrease the population of terrestrial arthropods, although the organic paddy fields have long histories as organic ones.

Even though the abundance of Tetragnatha spider and chironomid were significantly higher in 20 years than that in the 5 years organic paddy fields, and they were positively correlated. Tetragnatha spiders are one of the bio indicator organisms of biodiversity in terrestrial ecosystem (Tanaka et al. 2012). These spiders increased with the increasing abundance of chironomid. Previous studies have suggested that chironomid insects are frequently consumed by the spiders in paddy fields (Ishijima et al. 2006; Tahir and Butt 2009). Chironomids larvae are collector gatherers in aquatic ecosystems, and their adults are neutral insects in terrestrial ecosystems. Their existence in paddy fields was correlated with the habitat complexity in the fields such as weeds and rice plant abundance. Our results showed that the weed biomass and the rice plant growth were increased in 20 years organic paddy fields. These results reveal that long term of organic paddy fields enhances the population of spiders through indirect correlation with habitat complexity and organic input.

Many studies have shown that organic paddy fields can provide suitable habitat for biota (Rigby and Caceres 2001; Birkhofer et al. 2008; Crowder et al. 2010). The present study suggests that long term of organic paddy fields enhance the availability of bio-based nutrient in the fields and in turn affects the community structure in aquatic ecosystem through increasing the abundance of macro-invertebrates and their interactions. Moreover, the long term application of bio-based nutrients in organic paddy fields could control the population of terrestrial arthropods particularly the pest insects but increase the growth and production of rice plants. Our comprehensive study indicates that long cultivation of organic paddy fields improves the biotic interactions within and between aquatic and terrestrial ecosystem thereby might achieve sustainability of the paddy field system and rice production.

Table 1. List of aquatic macro-invertebrate found in the study area. The (●) indicates that the aquatic macro-invertebrate was found in that history of organic paddy field.

Taxa	**Common name**	**Japanese name**	**Functional feeding group**	**20 years**	**5 years**
Nematoda	Round worm	センチュウ	P	●	●
Oligochaeta					
Stylaria lacustris	Stylaria		C-g	●	
Tubifex tubifex	Sludge worm	イトミミズ	C-g	●	●
Hirudinea					
Glossiphoniidae sp.	Leech	シマイシビル	P	●	●
Richardsonianidae sp.	Leech	ヒル	P	●	●
Mollusca					
Cipangopaludina chinensis laeta	Mud snail	マルタニシ	Scr	●	
Gyraulus chinensis	Planorbid snail	ヒラマキミズマイマイ	Scr	●	●
Radix auricularia japonica	Small fresh water	モノアラガイ	Scr	●	●
Sphaerium japonicum	Pond shell	ドブシジミ	Scr	●	●
Amphibi					
Hyla japonica (L)	Tadpole	オタマジャクシ	C-g	●	●
Rana japonica (L)	Tadpole	オタマジャクシ	C-g	●	●
Crustacea					
Laevicaudata spp.	Brine shrimp	タマカイエビ	C-f	●	●
Daphnia spp.	Water flea	ミジンコ	C-f	●	●
Arachnida					
Hydrachnidiae sp.	Water mite	ミズダニ	P	●	●
Odonata					
Sympetrum frequens (L)	Dragonfly larvae	アキアカネ	P	●	●
Sympetrum infuscatum (L)	Dragonfly larvae	ノシメトンボ	P	●	●
Coleoptera					
Berosus punctipennis (L & Ad)	Small Hydrophilid	ゴマフガムシ	P	●	●
Hydroglyphus japonicus L & Ad)	Small Diving beetle	チビゲンゴロウ	P	●	●
Sternolophus rufipes (L & Ad)	Medium Diving beetle	ヒメゲンゴロウ	P	●	●
Diptera					
Chironomidae spp. (L)	Chironomid larvae	ユスリカ	C-g	●	●
Culicidae spp. (L)	Mosquito larvae	ボウフラ	C-g	●	●
Certopogidae sp. (L)	Punkies	ヌカカ	P	●	●
Tabanidae spp. (L)	March fly larvae	アブ科	P	●	●
Tipulidae spp. (L)	Crane Fly larvae	ガガンボ	P	●	●
Ephemeroptera (L)	May fly larvae	カゲロウ	C-g	●	●
Hemiptera					
Notonecta triguttata	Backswimmers	マツモムシ	C-g	●	●
Aquarius paludum	Pond skater	アメンボ	P	●	●

Abbreviation: P: predators; C-g: collectors-gatherers; Scr: scrapers; L: larvae; Ad: adults

Table 2. List of terrestrial arthropods found in the study area. The (●) indicates that the terrestrial arthropod was found in that history of organic paddy field

Taxa	Common name	Japanese name	Functional feeding group	20 years	5 years
Hemiptera					
Sogatella furcifera	White backed planthopper	ヒメトビウンカ	H	●	●
Laodelphax striatellus	Small brown planthopper	トビイロウンカ	H	●	●
Nephotettix spp.	Leafhopper	ヨコバイ	H	●	●
Aphididae sp.	Aphid	アブラムシ	H	●	●
Stenotus rubrovittatus	Mirid bug	アカスジカスミカメ	H	●	●
Erythraeidae sp.	Mite	ダニ	P	●	●
Coleoptera					
Lissorhoptrus oryzophilus	Rice water weevil	イネ水ゾウムシ	H	●	●
Oulema melanopus	Rice leaf beetle	ハムシ	H	●	●
Scymnus sp.	Dusky Lady Beetle	ヒメテントウ	P	●	●
Lepidoptera					
Naranga Aenescens	Green semilooper	イネツロムシ	H	●	●
Pelopidas mathias	Rice skipper		H	●	●
Orthoptera					
Oxya japonica	Rice grasshopper	ハネナガイナゴ	H	●	●
Tetigonidae spp.	Bushcricket	キリギリス科	P	●	●
Diptera					
Shyrphidae sp.	Hoverfly	ヒラタアブ	P	●	●
Culicidae sp.	Mosquito	カ科	N	●	●
Chironomidae sp.	Chironomid	ユスリカ	N	●	●
Nematocera spp.	Fly	カ亜目	N	●	●
Tipulidae sp.	Crane fly	ガガンボ	P	●	●
Tabanidae sp.	March fly	アブ科	P	●	●
Hymenoptera					
Ichneumonidae sp.	Ichneumon wasps	ヒメバチ	P	●	●
Dryinidae spp.	Dryinid wasp	キアシカマバチ	P	●	●
Neuroptera					
Chrysopidae sp.	Lacewing	クサカゲロウ	P	●	●
Araneae					
Tetragnatha caudicula	Tetragnatha spider	アシナガグモ	P	●	●
T. praedonia	Tetragnatha spider	アシナガグモ	P	●	●
T. maxila	Tetragnatha spider	アシナガグモ	P	●	●
Pachygnatha quadrimaculata	Pachygnatha spider	アシナガグモ	P	●	●
Neoscona adianta	Neoscona spider	ドヨウオニグモ	P	●	●
Linyphiidae	Linypidae spider	サラグモ	P	●	●

Abbreviation: P: predators; H: Herbivores; N: Neutral insects

Table 3. The resource factors of aquatic communities (mean ± SE) in 5 years and 20 years organic paddy fields

Resource factors of aquatic communities	20 years	10 years	5 years	df	χ^2	*p*
2012						
N content of detritus (%)	0.39 ± 0.007 a	0.30 ±0.01 b	0.22 ± 0.008 c	5	22.48	<.0001
C content of detritus (%)	3.96 ± 0.06 a	2.83 ± 0.12 b	2.12 ± 0.07 c	5	28.21	<.0001
C/N ratio of detritus (%)	10.17 ± 0.11	9.36 ± 0.11	9.66 ± 0.22	5	2.84	>0.05
2013						
N content of detritus (%)	0.40 ± 0.004	—	0.24 ± 0.01	1	4.12	<0.05
C content of detritus (%)	4.71 ± 0.07	—	2.48 ± 0.12	1	10.38	<0.01
C/N ratio of detritus (%)	11.08 ± 0.13	—	10.18 ± 0.07	1	11.26	<0.001
2014						
N content of detritus (%)	0.36 ± 0.005	—	0.22 ± 0.16	1	10.58	<0.01
C content of detritus (%)	4.00 ± 0.05	—	2.31 ± 0.18	1	11.56	<0.001
C/N ratio of detritus (%)	11.44 ± 0.09	—	10.20 ± 0.17	1	5.33	<0.05
Microhytics biomass (g)	21.13 ± 2.4	—	8.43 ± 0.86	1	-3.67	<0.001
Algal biomass (chlorophyll a, µg/l)	98.44 ± 5.23	—	67.18 ± 12.54	1	-1.77	0.09

Means of N and C concentration in 2012 followed by a different latter are significantly different by a generalized linear mixed model (GLMM) with a field as a random effect using the identity link function (lme4 package; maximum likelihood estimation).

Table 4. The resource factors of terrestrial communities (mean ± SE) in 5 years and 20 years organic paddy fields

Resource factors of terrestrial communities	20 years	10 years	5 years	df	χ2	p
2012						
N content of rice plants (%)	0.80 ±0.01	0.72 ± 0.02	0.68 ± 0.02	5	4.68	0.09
C content of rice plants (%)	38.10 ± 0.15	37.24 ± 0.37	36.65 ± 0.16	5	4.75	0.09
C/N ratio of rice plants (%)	45.59 ± 0.79 b	52.06 ± 1.90 ab	55.96 ± 1.50 a	5	7.07	<0.05
2013						
N content of rice plants (%)	0.83 ±0.01	—	0.77 ± 0.01	1	4.54	>0.05
C content of rice plants (%)	38.56 ± 0.10	—	36.92 ± 0.11	1	4.83	0.053
C/N ratio of rice plants (%)	46.03 ± 0.79 b	—	48.87 ± 0.45 a	1	5.89	<0.05
2014						
N content of rice plants (%)	1.11 ± 0.02	—	0.85 ± 0.01	1	-8.57	<0.001
C content of rice plants (%)	38.66 ± 0.19	—	37.28 ± 0.09	1	-6.74	<0.001
C/N ratio of rice plants (%)	34.81 ± 0.82	—	44.02 ± 0.61	1	8.86	<0.001
Weed biomass (g)	26.60 ± 2.67	—	41.76 ± 3.94	1	8.02	<0.001

Means of C/N ration of rice plant in 2012 followed by a different latter are significantly different by a generalized linear mixed model (GLMM) with a field as a random effect using the identity link function (lme4 package; maximum likelihood estimation).

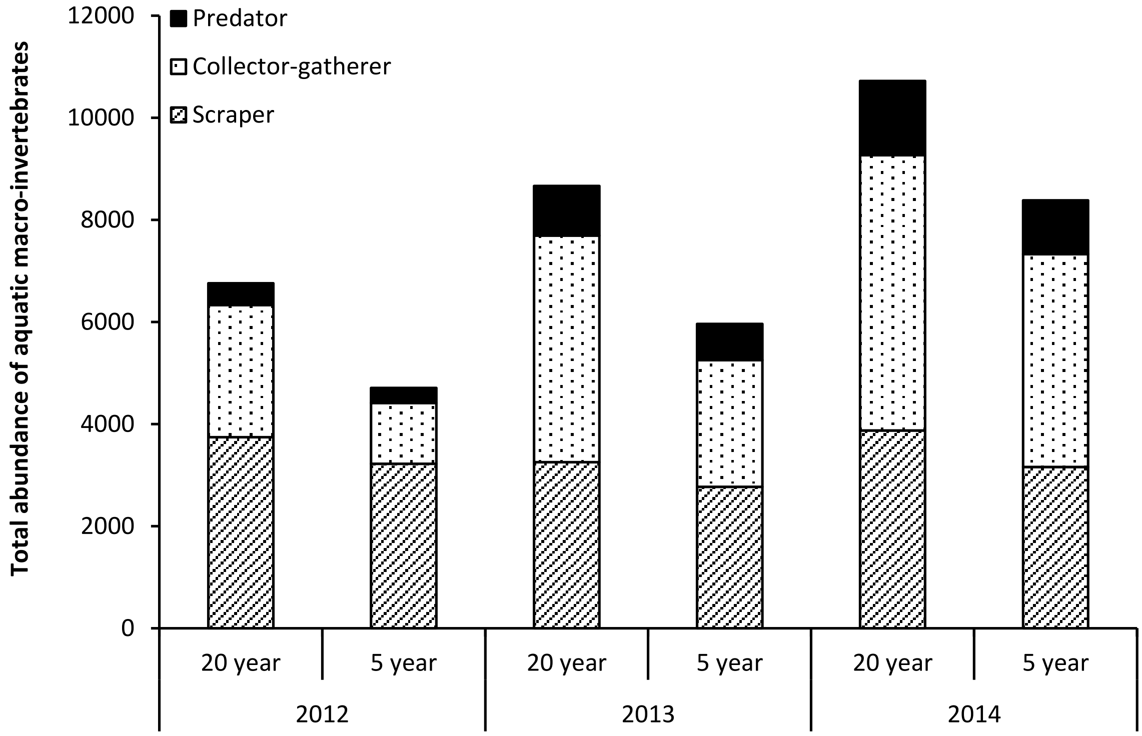

Figure 1. Total abundance of scrapers, collector-gatherers, and predators in aquatic ecosystem by 5 and 20 years organic paddy fields in 2012-2014.

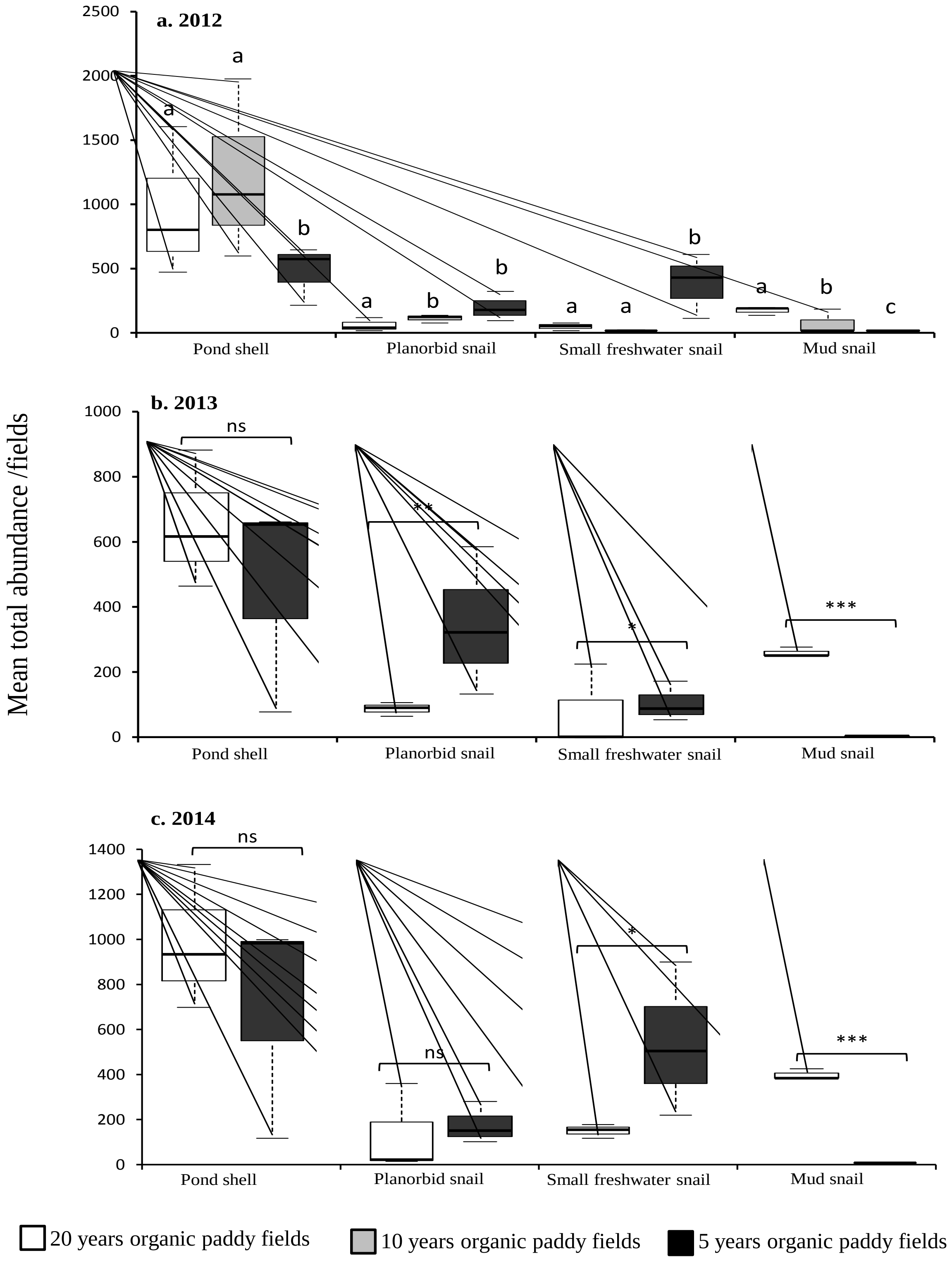

Figure 2. Mean total abundance (± SE) of aquatic scrapers in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [***p < 0.001, **p < 0.01, *p < 0.05, ns (not significant, p ≥ 0.05)]

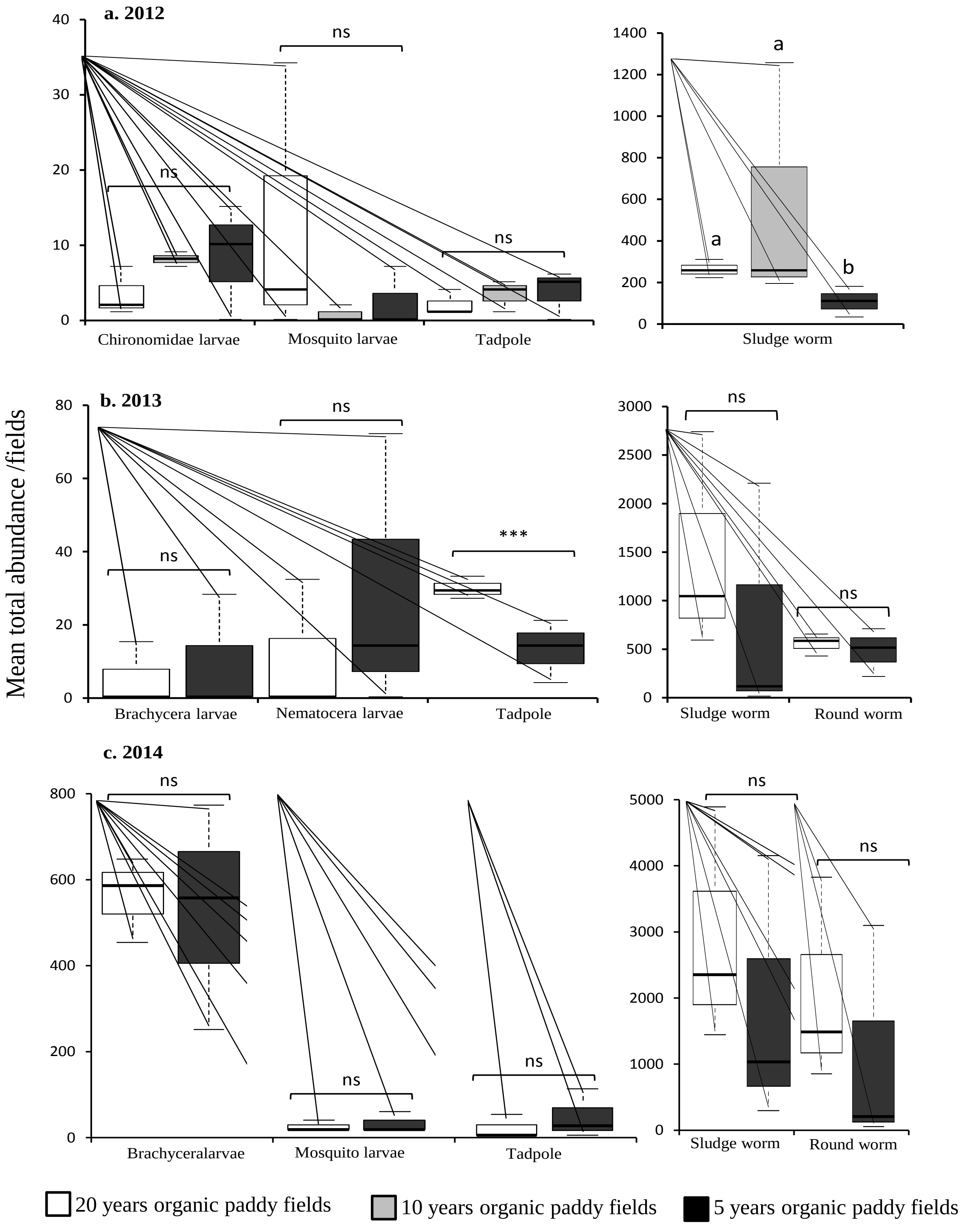

Figure 3. Mean total abundance (± SE) of aquatic collector-gatherer in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. . Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [ns (not significant, $p \geq 0.05$)]

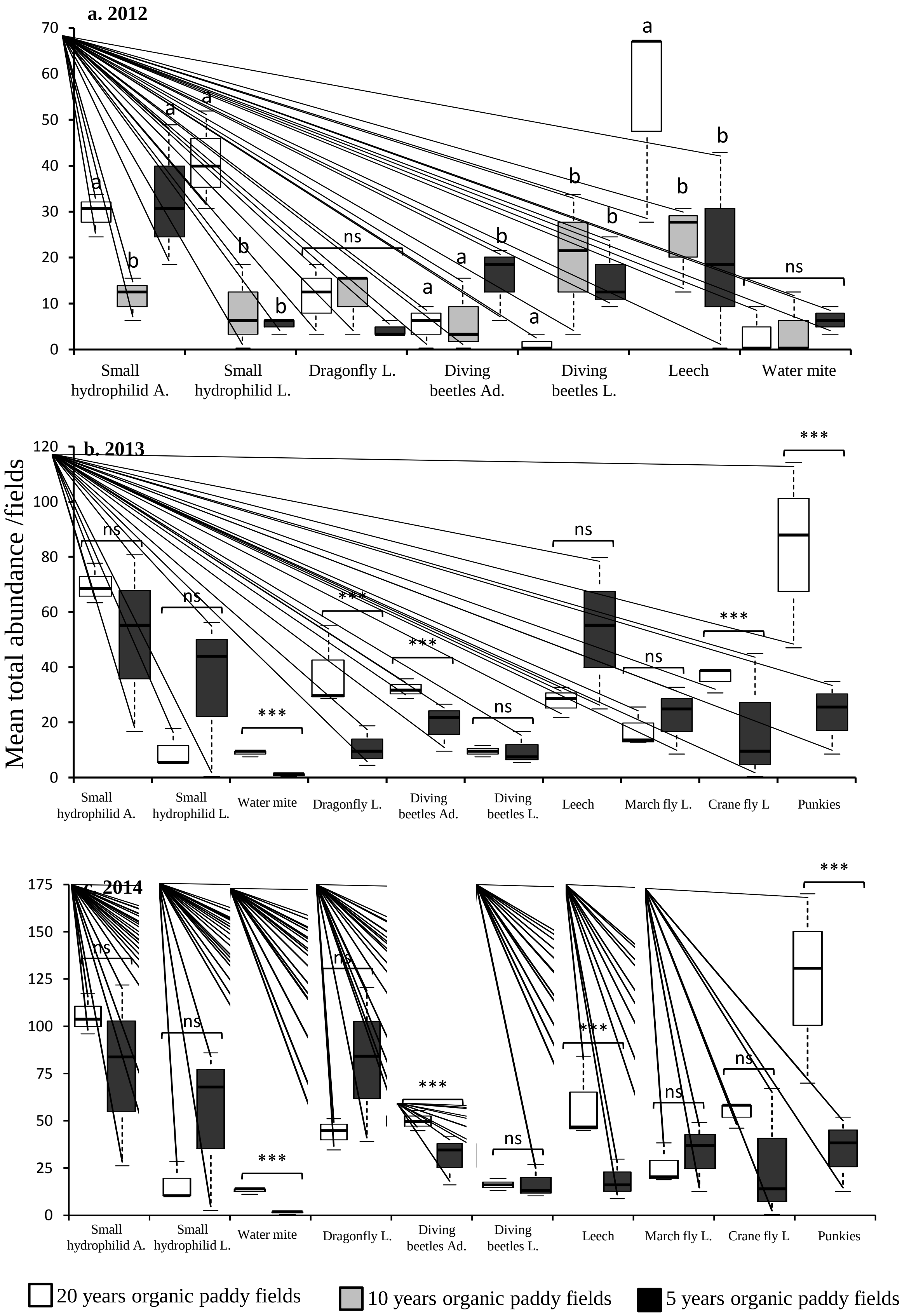

Figure 4. Mean total abundance (± SE) of aquatic predators in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [***$p < 0.001$, ns (not significant, $p \geq 0.05$)]

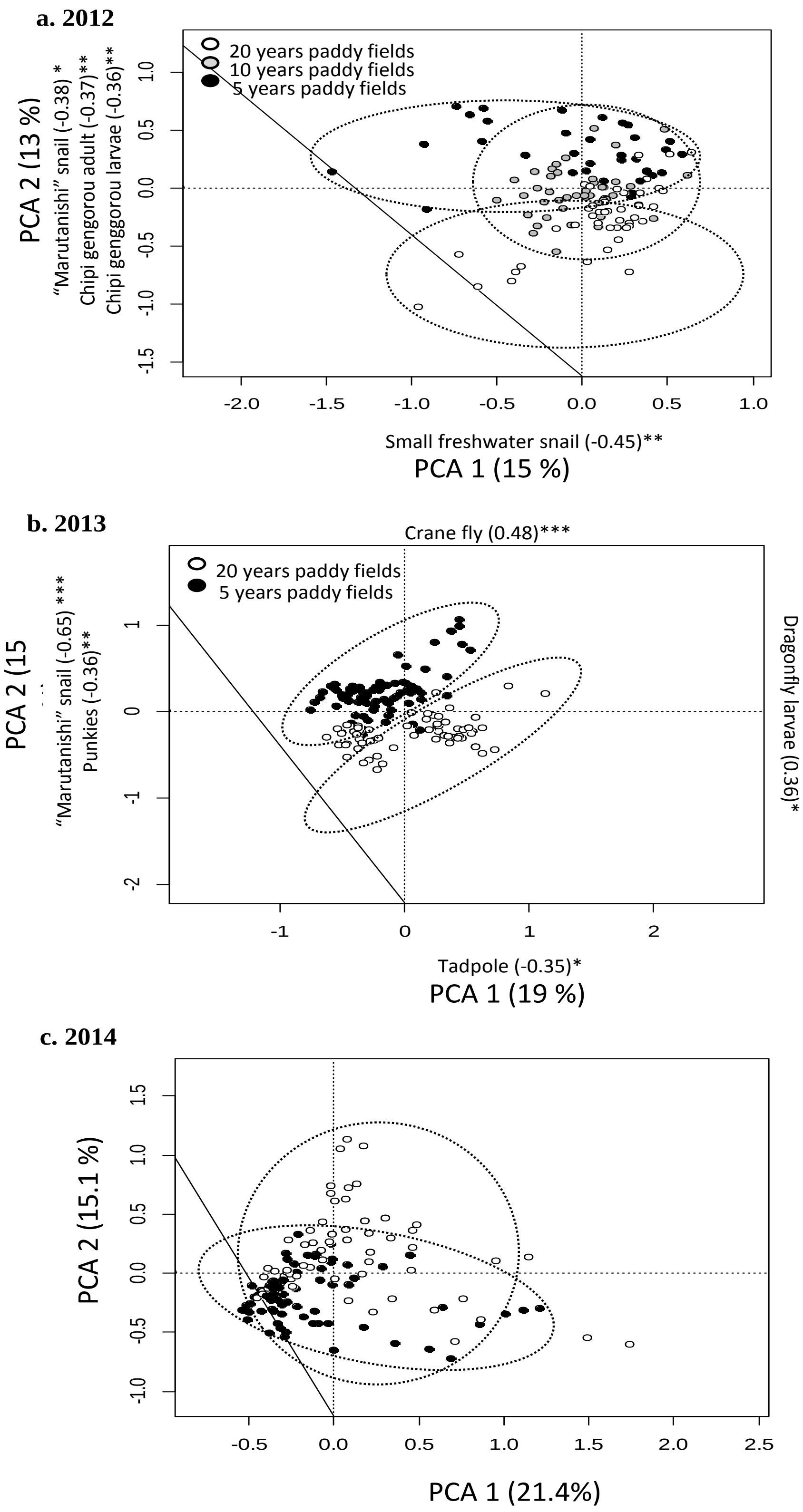

Figure 5. Principal component analysis (PCA) of the community structure of aquatic macro-invertebrates in 5, 10, and 20 years organic paddy fields in 2012, 2013, and 2014. Figures with % indicate the percentage contributed by the 1st principal component analysis or 2nd one. Names of aquatic macro-invertebrates refers to those that contributed to the respective vectors. Figures in parentheses indicate factor loadings (*p < 0.05, **p < 0.01, ***p<0.001).

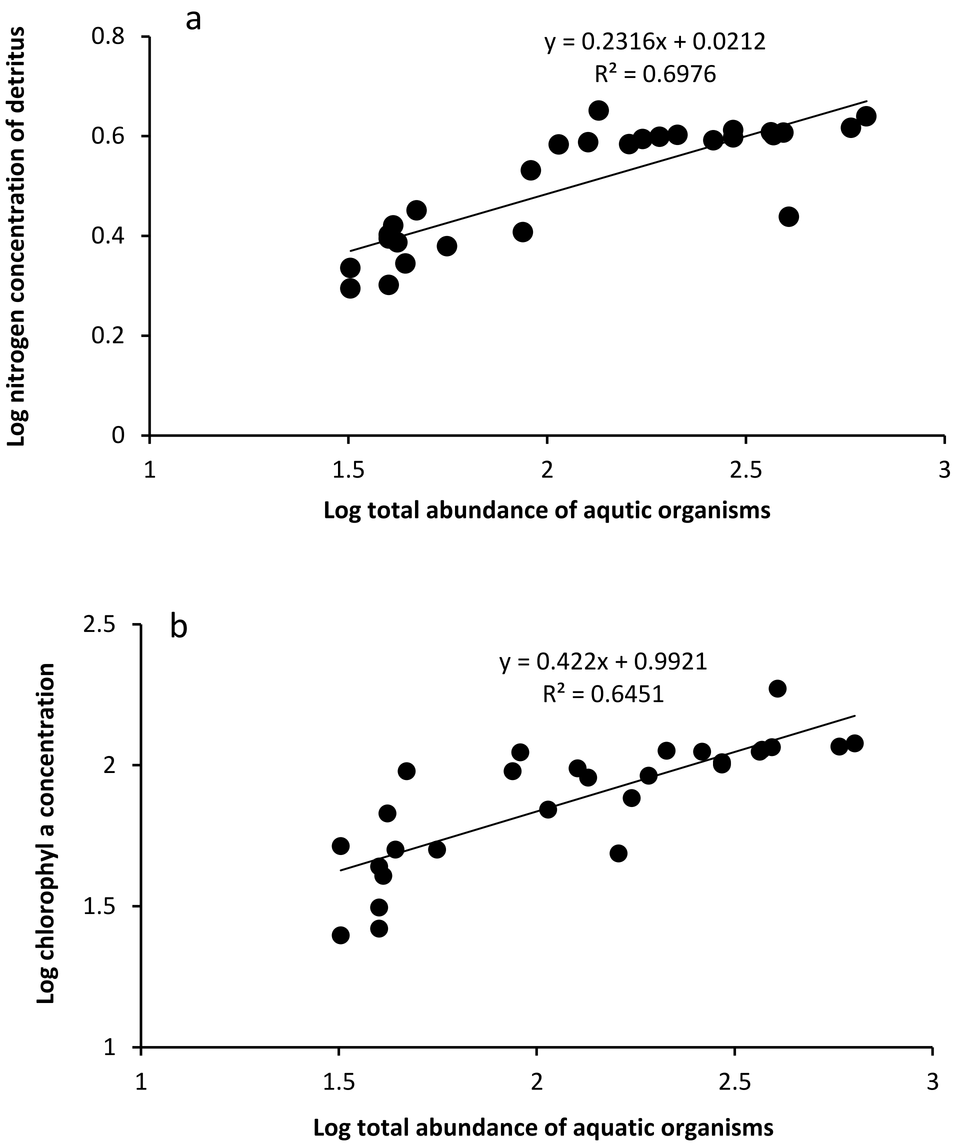

Figure 6. The relationship between log total abundance of aquatic marco-invertebrates and a) log chlorophyl a concentration ($p<0.001$) and b) log nitrogen concentration of detritus ($p<0.001$) in organic paddy fields.

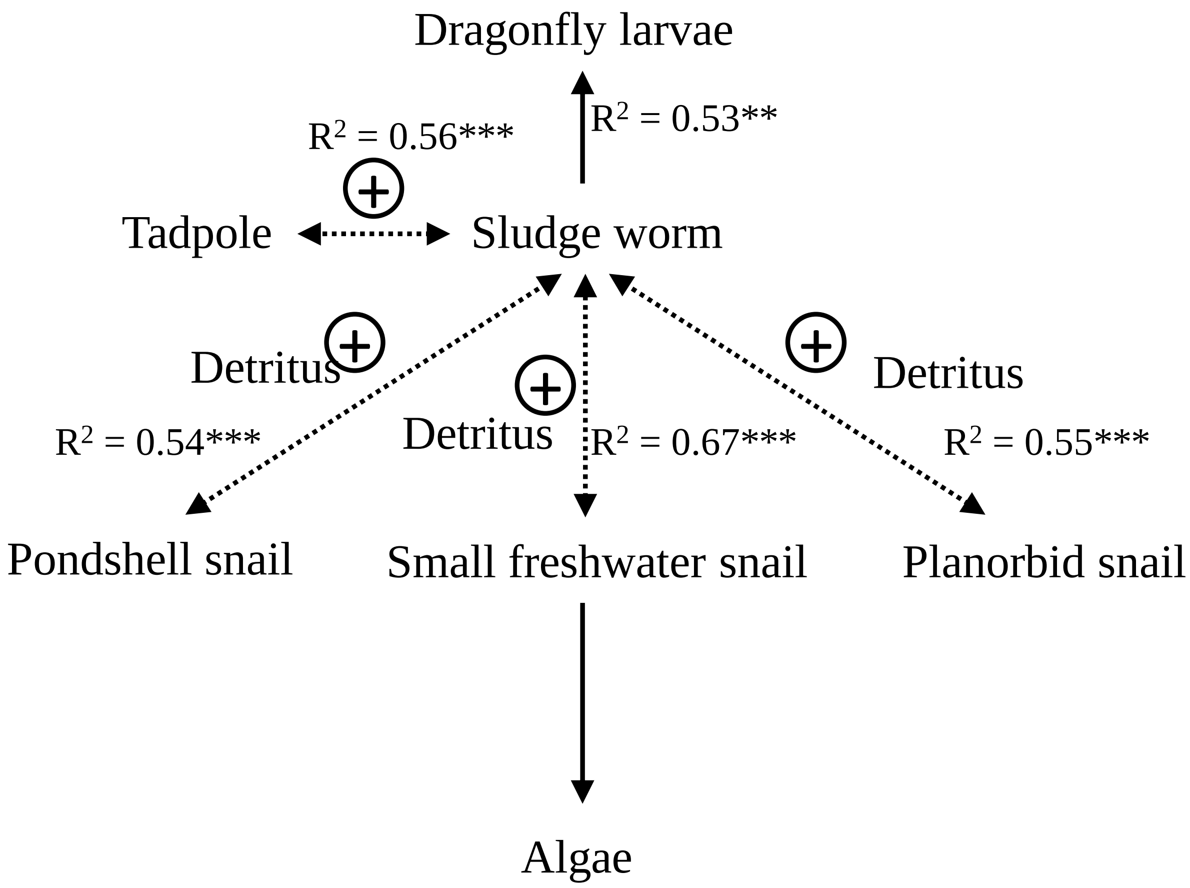

Figure 7. The interaction between scrapper (pondshell snail, small freshwater snail, and planorbid snail), collector-gatherers (sluge worm, tadpole), predator (dragonfly larvae), and their resources (algal, detritus) in 20 years of organic paddy fields. Soild arrows indicate direct interactions, broken arrows indicate the indirect interaction. R donate the Pearson correlation coefficients (**$p < 0.01$, ***$p<0.001$)

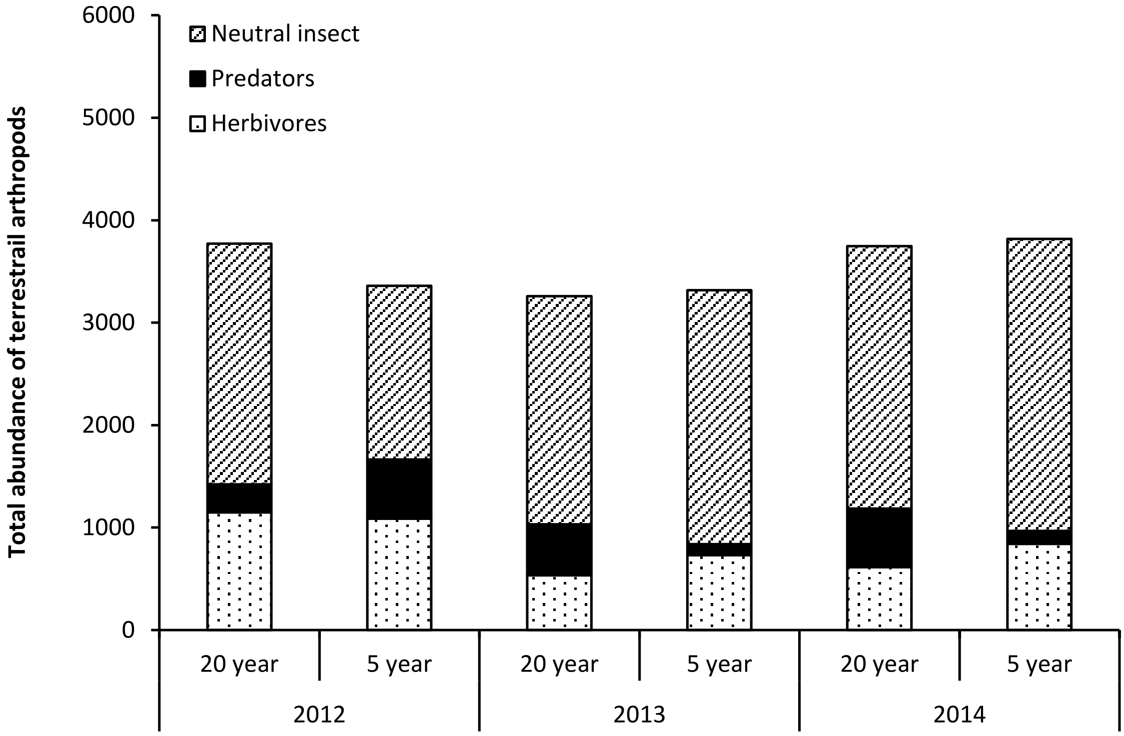

Figure 8. Total abundance of herbivores, predators, and neutral insects in terrestrial ecosystem by 5 and 20 years organic paddy fields in 2012-2014.

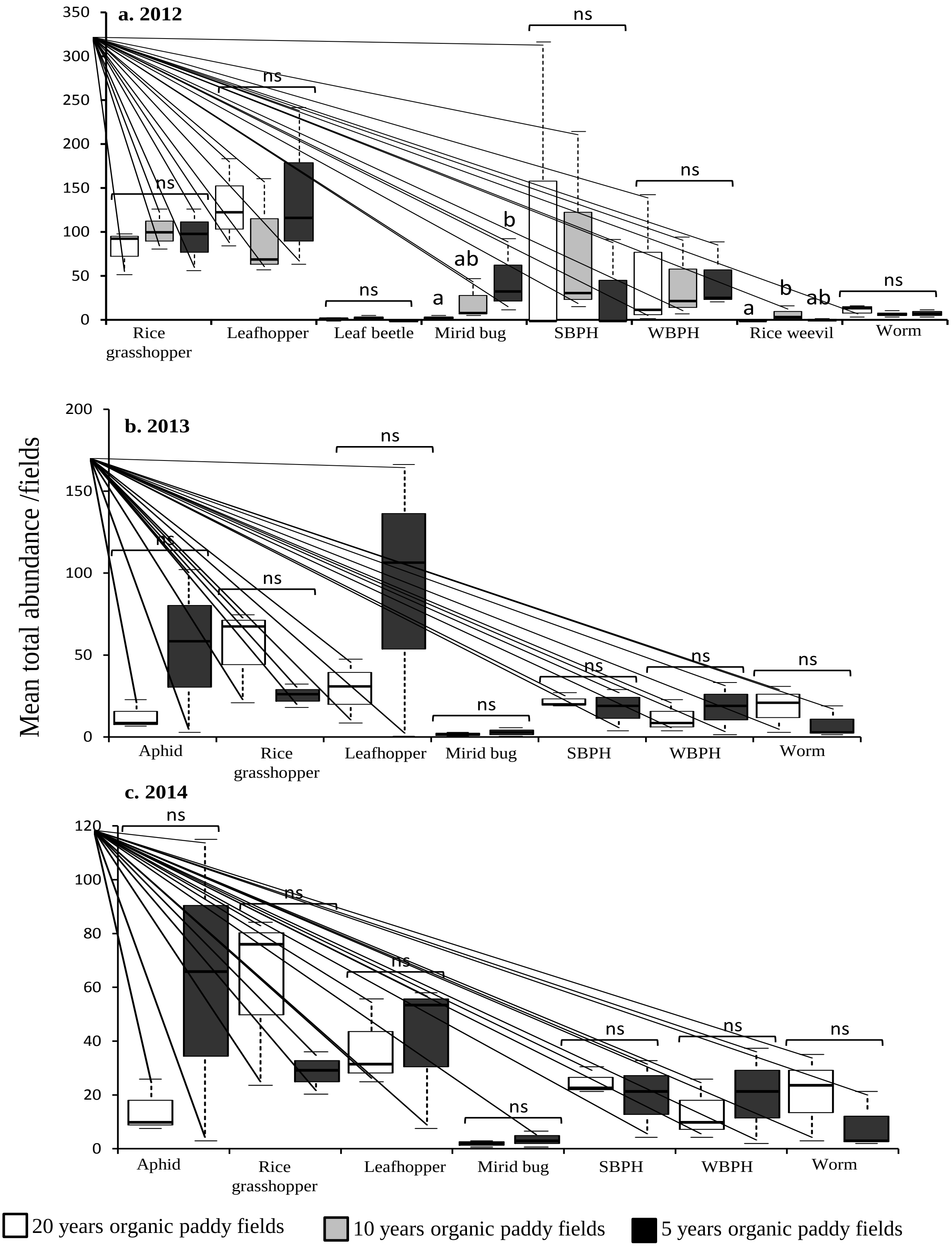

Figure 9. Mean total abundance (± SE) of terrestrial herbivores in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [ns (not significant, $p \geq 0.05$)]

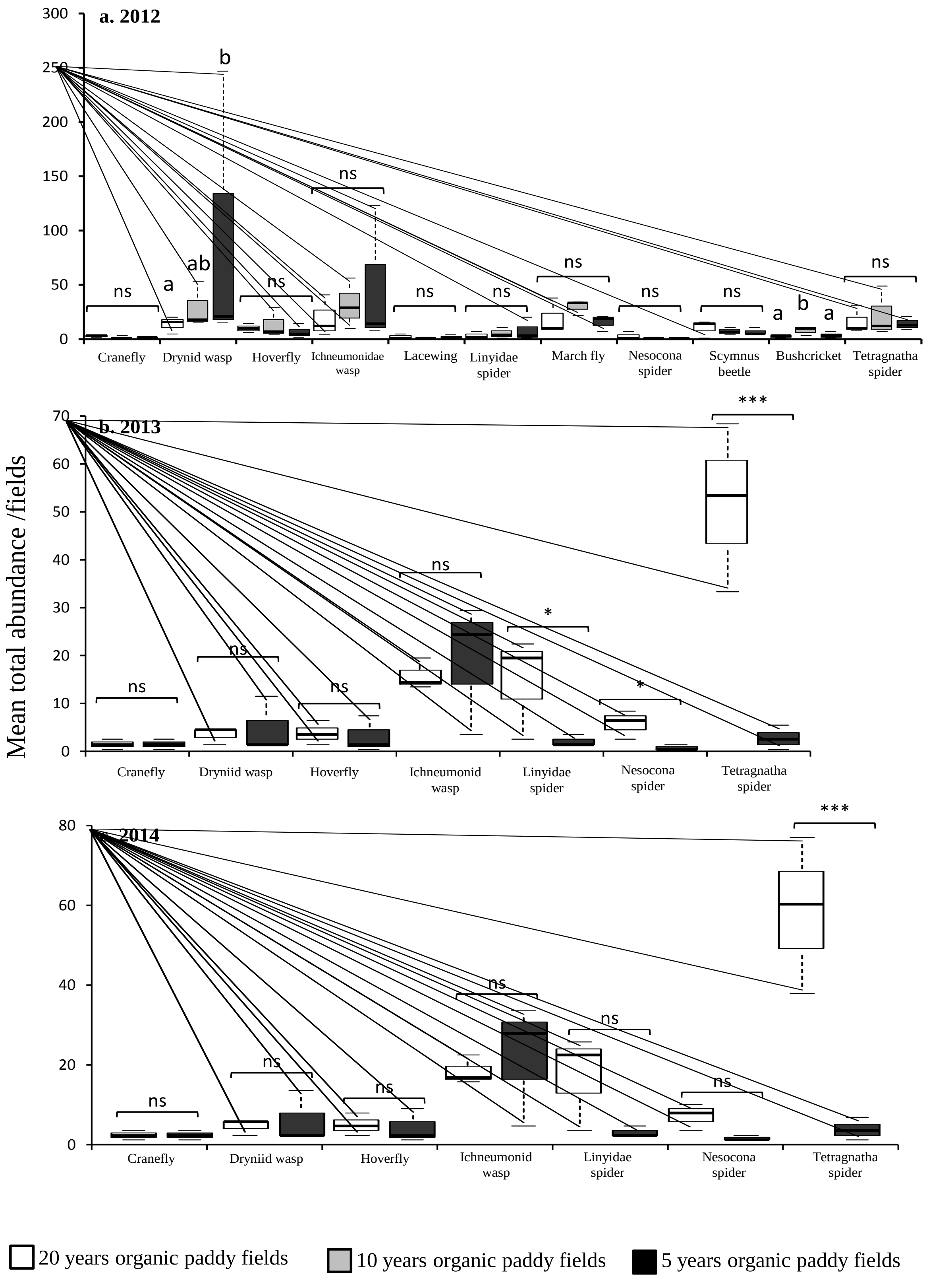

Figure 10. Mean total abundance (± SE) of terrestrial predators in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [***p < 0.001, *p < 0.05, ns (not significant, p ≥ 0.05)]

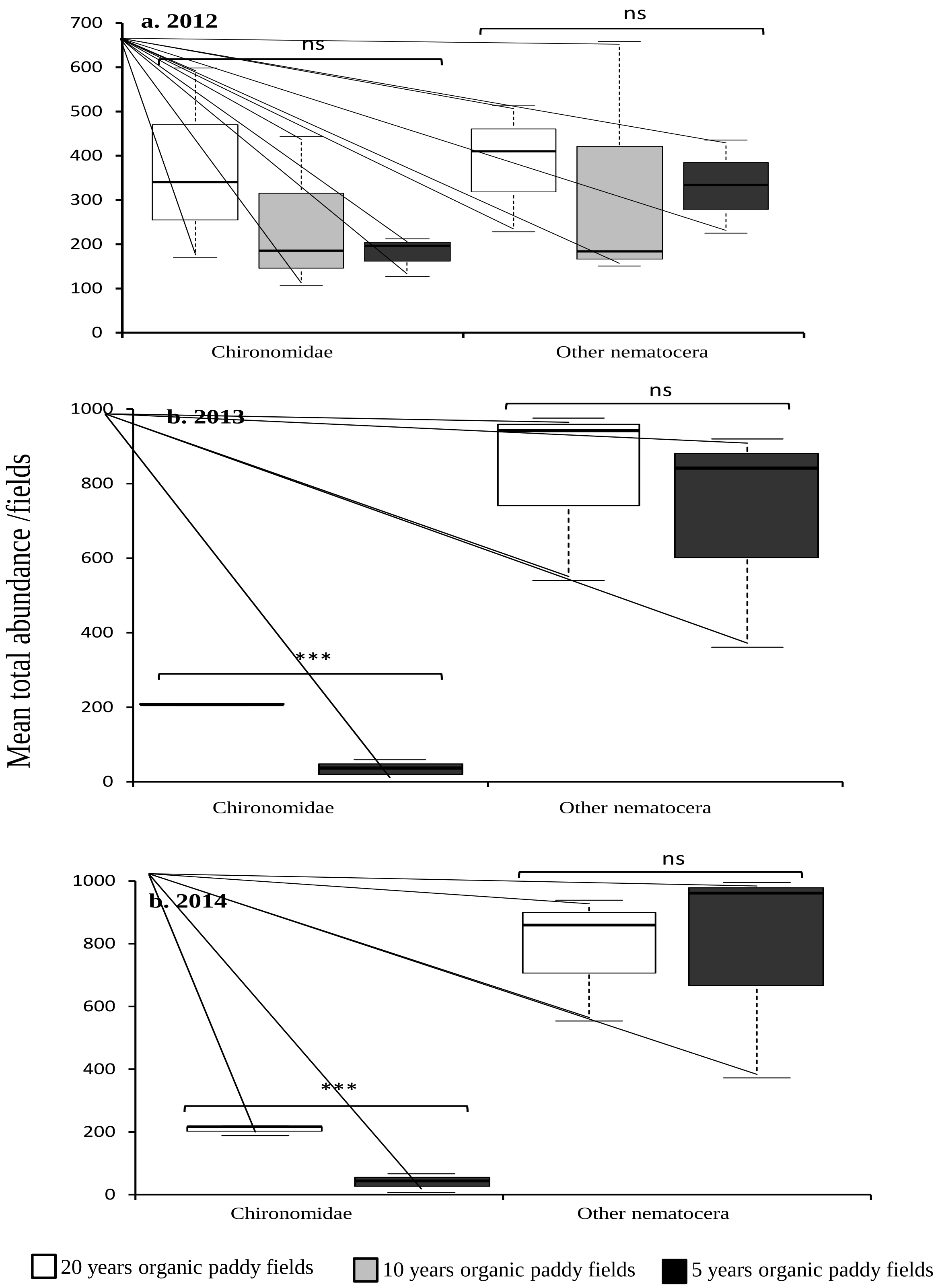

Figure 11. Mean total abundance (± SE) of terrestrial neutral insects in 5, 10, and 20 years of organic paddy fields in 2012, 2013, and 2014. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [***$p < 0.001$, ns (not significant, $p \geq 0.05$)]

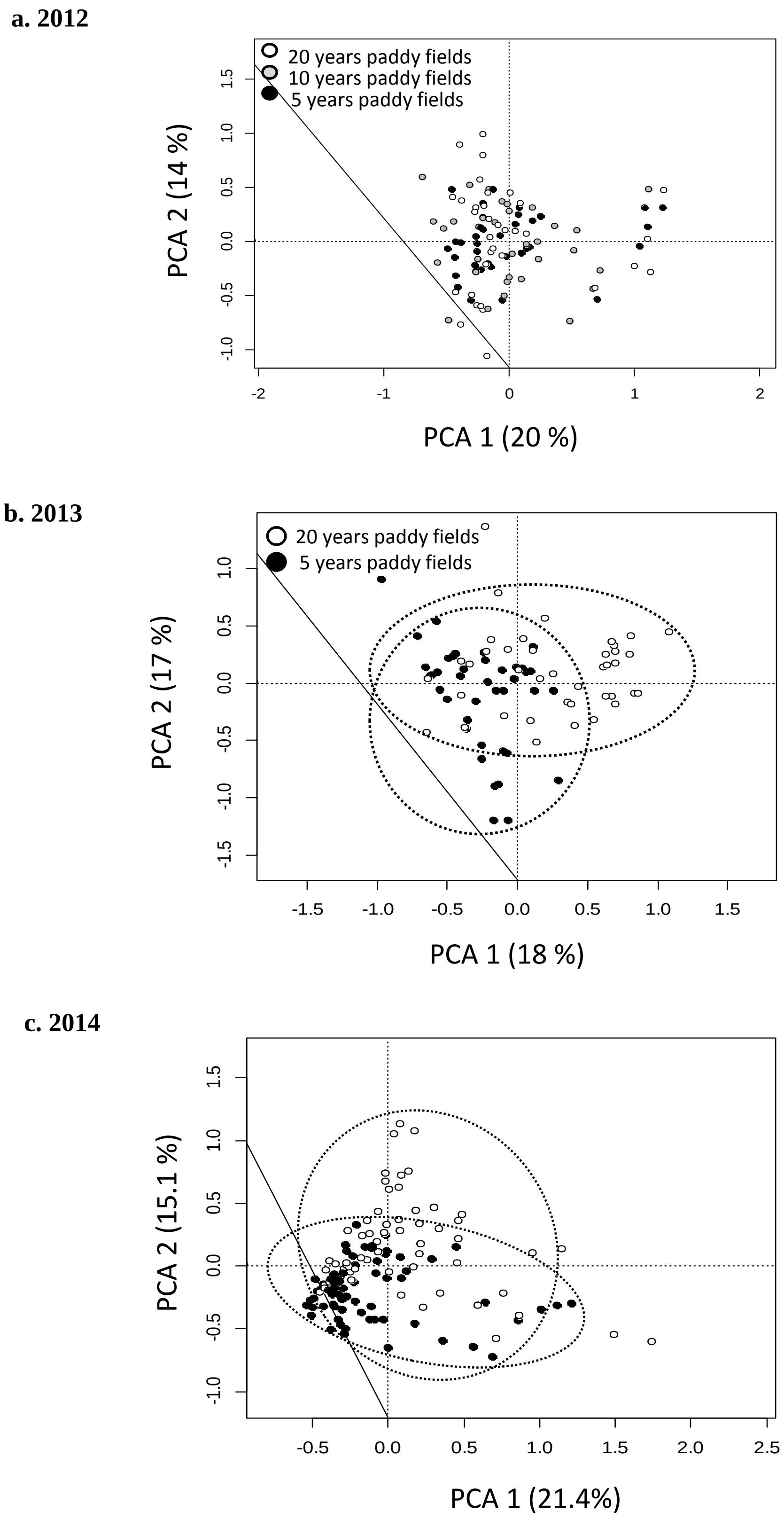

Figure 12. Principal component analysis (PCA) of the community structure of terrestrial arthropods in 5, 10, and 20 years organic paddy fields in 2012, 2013, and 2014. Figures with % indicate the percentage contributed by the 1st principal component analysis or 2nd one.

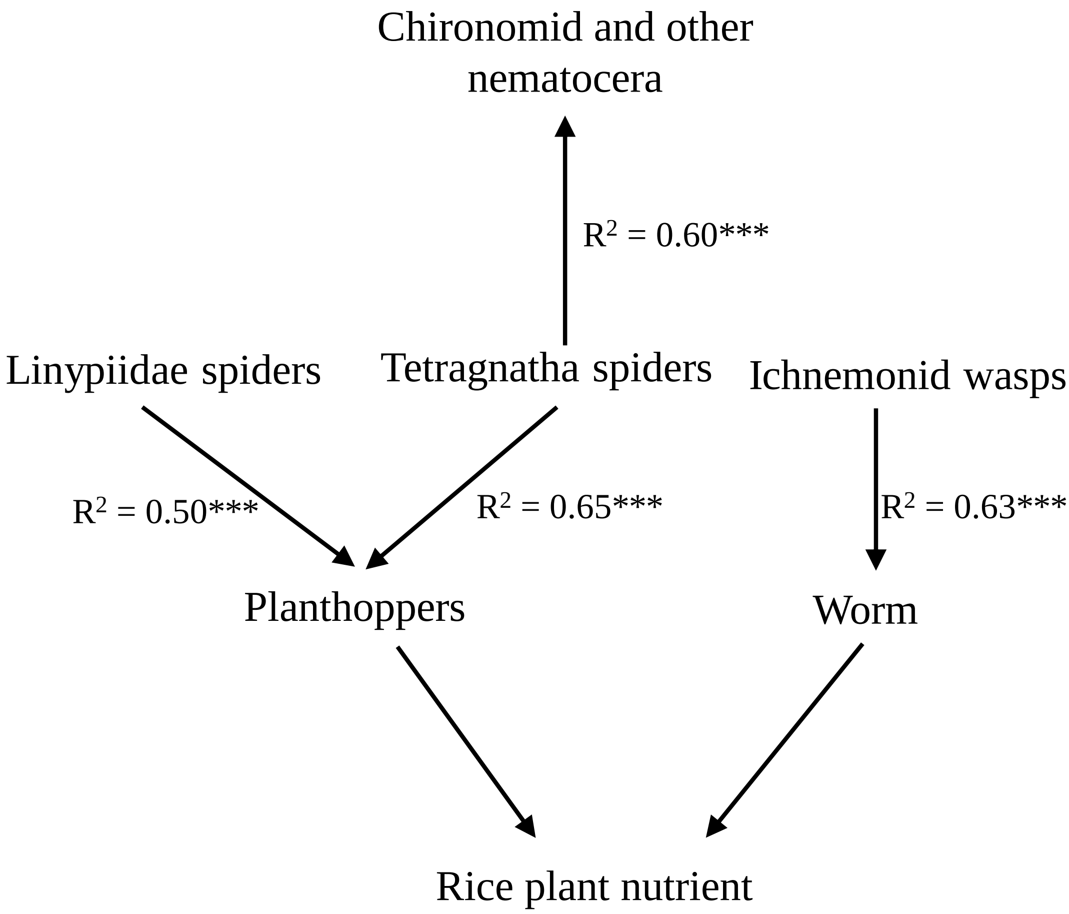

Figure 13. The interaction between herbivores (planthoppers and worm), predators (tetragnatha spiders, linypiidae spiders, and Ichneumonid wasps), neutral insect (chironomid and other nematocera) organic paddy fields. Soild arrows indicate predators and prey interactions. R donate the Pearson correlation coefficients (***p<0.001)

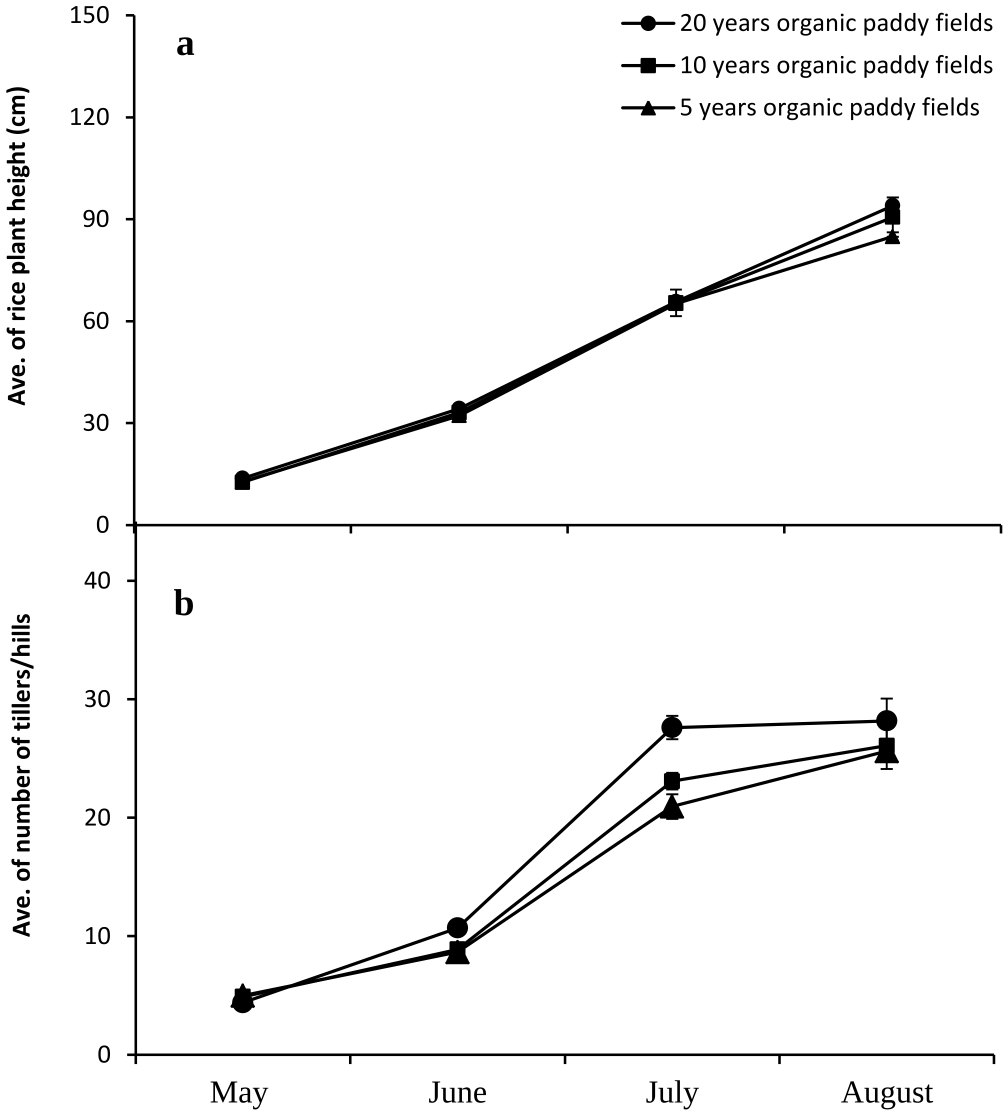

Figure 14. a. Rice plant height. **b** Number of tillers of rice plant in 5, 10, and 20 years organic paddy fields during the growing season from May to August. The data were collected from 2012-2013 of each field except 10 years organic paddy fields.

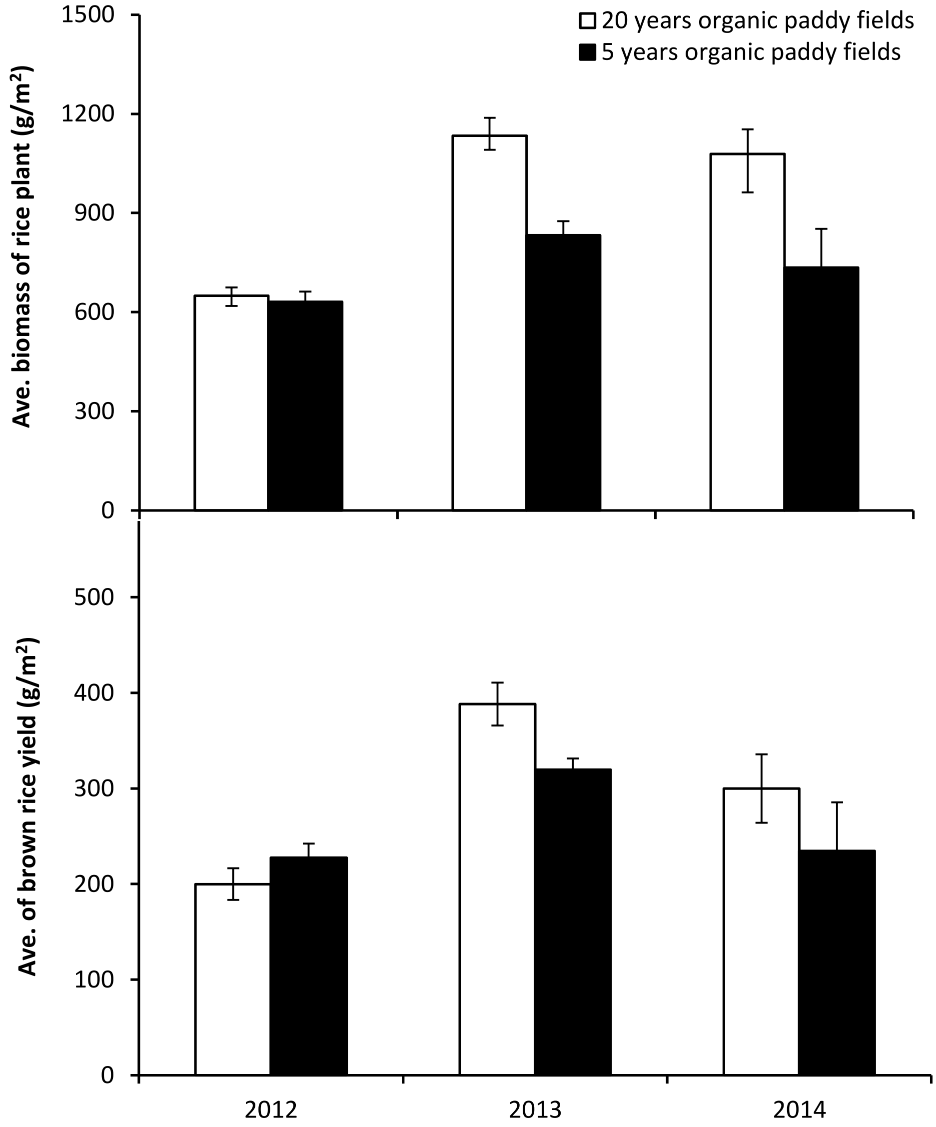

Figure 15. a. Rice plant biomass. **B.** brown rice yields of 5 and 20 years organic paddy fields in 2012-2014.

Chapter 2:

Indirect effects of bio-based nutrient on the abundance of terrestrial arthropods through the growth of rice plants in organic paddy fields

Introduction

As one of the world's most important food production sites, paddy fields are also highly important agro-ecosystems for biological conservation (Dahms 2005; Malthick et al. 2010). These wetland ecosystems often support a high biodiversity of aquatic flora and fauna, which hold key roles in ecosystem processes including nutrient cycling and pest control (Lawler 2001; Katano et al. 2003; Wilson et al. 2008; Kano et al. 2010; Katayama et al. 2011). However, modern agriculture which relies heavily on synthetic fertilizer, pesticide, and herbicide can lead to several ecological problems including the loss of biodiversity and corresponding decline ecosystem service and function (Wilson et al. 2008; Ponce et al. 2011).

Organic farming is considered an environmentally friendly method of cultivation and the most promising way to overcome the negative effects of modern farming (Ponce et al. 2011). Organic farming makes limited use of chemical fertilizer and pesticide, and exploits natural resources as nutrients. Bio-based nutrients use the function of aquatic organisms can build the soil fertility in organic paddy fields. In addition, it preserves the species richness and abundance of organisms involved in the ecological community (Winquist et al. 2011). Previous studies suggested that organic farming has lower leaching of nutrients and higher carbon storage, and it may be able to increase the biodiversity of aquatic biota and their bio-based nutrient release in the fields (Yamazaki et al. 2004; Bengtsson et al. 2005; Dham 2005).

Aquatic organisms inhabit the soil-floodwater ecosystem of paddy fields and are an important component of paddy fields (Simpson and Roger, 1993). Bio-based nutrient was released by aquatic organisms involve the flow of nutrients in aquatic ecosystems and across to other ecosystem with their bottom-up effect (Nakano and Murakami 2001, Sabo and Power 2002, Baxter et al. 2004). Transfer nutrients between ecosystem can affect the abundance of organisms, community structure (e.g. predation and competition), and ecosystem processes (Polis et al., 1997; Lundberg &Moberg, 2003). Deposition of aquatic insects on field bottom-up effects on the detritivore communities and further affects plants and plant–insect interactions (Bultman et al. 2014). For instance, the larval stages of many aquatic organisms are detritus feeder and also vulnerable to a suite of aquatic predators, whereas the adult stages are important consumers in the terrestrial habitat (Knight et al 2005). However, as few studies have examined these studies in organic paddy fields, investigations are necessary to determine how the bio-based nutrient subsidies effect on the relationship between aquatic and terrestrial ecosystem.

The broad aims of this study were to understand: (1) how the interaction of aquatic organisms influences on bio-based nutrient release on organic paddy fields (2) how these interactions effect directly or indirectly on the abundance of terrestrial arthropods through mediated rice plant performance and nutrient. To address the aim of this study, a factorial experiment in the fields was conducted by modifying the abundance of two different functional feeding groups of aquatic organisms. In addition, a laboratory experiment was also conducted to understand the behavior of aquatic organism’s interaction. The hypothesis of this study was the different abundance of aquatic organism may effects on their interaction and bio-based nutrient release, it subsequently influences on the abundance of terrestrial arthropods.

Materials and Methods

Study organisms

Two aquatic macro-invertebrate species with different functional feeding groups, a mud snail, *Cipangopaludina chinensis laeta* (Martens) (Caenogastropoda: Viviparidae), and a loach, *Misgurnus anguillicaudatus* (Cantor) (Cypriniformes: Cobitidae), were manipulated in this experiment. Both species occur in paddy fields in Japan, although populations of the mud snail and the loach have decreased dramatically in recent years because of modern farming practices. The mud snail plays an important role as a soil decontaminator in paddy fields and feeds non-selectively on organic and inorganic bottom materials as well as benthic and epiphytic algae, mostly by grazing (Jokinen 1982; Simpson et al. 1994). The loach typically lives in ditches around paddy fields and migrates into the paddy field to lay eggs (Tanaka 1999). The diet is composed mostly of small aquatic invertebrates and detritus. Chironomids, tubifex worms, small gastropods, cladocerans, and amphipods are the primary prey of the loach (Tabor et al. 2001; Schmidt and Schmidt 2014).

Field experiments

Study area and experimental design

The field experiment was conducted on an experimental paddy field of Yamgata University Research Farm, Tsuruoka, Yamagata Prefecture, Japan (38° 43′ N, 139° 49′ E) in 2014. In the experimental paddy fields, soil is classified as Gleyic Fluvisols (FAO, ISRIC, and ISSS, 2006). Irrigation water was supplied through an irrigation channel that was connected to a river flowing from a mountainous area, thus the water did not contain drainage water from other paddy fields. During the study year, the mean annual precipitation was approximately

1382.5 mm and the mean air temperature was 25.9°C from May through September. The paddy fields were cultivated without any fertilizer (organic and inorganic) or agrochemicals (insecticides, herbicides, and fungicides). The paddling was implemented using a puddling tractor a week prior to transplanting. Rice seedlings were transplanted in 30 May at a spacing of 30 × 15-cm and variety used was "Sasanishiki" which is a local variety planted in Shonai area.

A field experiment was done to understand the effects of the mud snail and the loach on the abundance of aboveground arthropods via rice plant growth. Twenty five 2.5 × 2.5-m plots were laid out in two 7 × 20-m paddy fields. Each plot was designed using eight 60 cm × 1.2-m levee wave plate sheets separated by 1-m-wide paths. The two sides of each plot have a 60 × 20-cm water channel window made from iron mesh. Water flow was maintained approximately 10-cm-deep in all plots during the experiment. The experiment design involves five replications of each of five treatments: low (S50) and high (S100) densities of the mud snail (n = 50 individuals/plot and n = 100 individuals/plot, respectively), low (L50) and high (L100) densities of loach (n = 50 individuals/plot and n = 100 individuals/plot, respectively), and the mud snail and loach together (M50), each at the low density (n = 50 mud snail + 50 loach = 100 individuals/plot). Mud snails were collected from biotope nearby organic paddy fields in Sakata city, Yamagata Prefecture. And individuals of loach were ordered from the fish store. Before using in the experiment, the mud snail and loach were maintained in the container using paddy fields water in the laboratory under a 16L: 8D photoperiod at 23 ± 1°C for one week. Then, we selected individuals of the mud snail (size: approximately 20 mm) and loach (size: approximately 5cm) for the field experiment to determine the effects of these species on the abundance of aboveground arthropods through rice plant growth.

Field sampling

To determine the effects of the mud snail and loach on the abundance of other aquatic macro-invertebrates including the juvenile of mud snail and loach, a box sampling (0.1 m^2 acrylic boxes) was placed in each plot and the aquatic macro-invertebrates were captured by a sweeping net (0.2 m × 0.15 m, 0.5-mm mesh) in three consecutive sweeps. The samples were transiently kept in the plastic bag and brought to the laboratory. In the laboratory, samples were washed through 0.5-mm sieve, and placed into the white tray where live aquatic macro-invertebrates were separated manually from plant materials and soils. The aquatic macro-invertebrates were then identified under a digital microscope and counted. The aquatic macro-invertebrates were collected three times during the period of the experiments.

Algal biomass were determined by taking about a liter of paddy field water in each plot. In the laboratory, the algal biomass were determined as same as method of algal biomass calculation used in chapter 1. Emergence of insects was sampled continuously for 4 days during three sampling periods using a plastic cylindrical cage (30 cm in diameter × 30 cm in height) with a yellow sticky trap at the top. These traps were nailed to the ground at the center of each plot. Insects will emerge from the below ground and fly or crawl toward to the top, ending up at the yellow sticky trap. At the beginning in August, to determine the abundance of terrestrial arthropods in each plot, a sweeping net was used as same as in chapter 1. The sweeping sampling is the most appropriate ways to capture terrestrial arthropods in paddy fields. To measure the rice plant growth, rice plant and soil nutrient, and rice productivity, we used the method as same as used in chapter 1.

Laboratory experiments

To clarify the interaction between the mud snail and the loach, a factorial experiment in the laboratory was conducted under a 16L: 8D photoperiod at 23 ± 1°C. The experimental design includes ten replications of five treatments: single and double of the mud snail, single and double of the loach, single combined mud and loach. The experimental units were maintained in 1-L capacity plastic containers during 2 weeks. One week prior to the start of the experiment, plastic containers were filled with water taken from a biotope. A 1-mm mesh screen was placed over each plastic container to prevent emigration or immigration of organisms. The interspecific and intraspecific interaction between mud snail and loach were assessed by visual observation of their behavior and mortality. At the end of the experiment, algal biomass were calculated using the same equations used in the field experiments.

Results

Fields experiment

Response of aquatic organisms to the presence of mud snail and loach

The abundance of juvenile mud snail was higher in the treatment of low density than in the high density and in the treatment of snail and loach together after 30 days (Figure 1a). However, after 60 days the abundance of juvenile mud snail was dramatically decreased in the low density treatment, and other treatments were increased (Figure 1a). The juvenile loach was found after 60 days in all treatment, and it was higher in the treatment of high density compared to other two treatments (Figure 1b).

The other aquatic organisms collected were classified based on their feeding function, namely scrapers, gatherers, and predators. Back swimmer and mayfly larvae were the most founded scrapers in experimental fields, and their abundance were higher in mud snail treatment either in the low density or in the high density (Figure 2a). Other scrapers abundance were low and no significant differences among the treatments (Figure 2a). The abundance of gatherers also was significantly higher in the snail treatments compared to the loach and mixed species treatment (Figure 2b). In addition, the all predators collected were no significantly different among the treatments (Figure 2c).

Nutrition concentration of detritus and algal biomass

The N, C concentration, and C/N ratio showed no significantly different among the treatment (Table 1). However, the algal biomass was significantly higher when the loach present in the treatments, including low and high density of loach, and mixed of snail and loach (Table 1). In addition, the mud snails and loach were negatively correlated (Figure 3).

Response of terrestrial organisms to the presence of mud snail and loach

The abundance of insect emergences such as Chironomid, Culicidae, and Ceratopongidae were significantly higher in the treatment of high density of mud snail compared to other treatments particularly after 50 days (Figure 4). The abundance of leaf hoppers in 60 and 90 days was significantly higher in low and high density of mud snail than other treatments, and the abundance of aphid in 30 days also showed the same significant difference and pattern (Figure 5). However, other herbivores showed no significant differences in 30, 60, and 90 days after the treatment (Figure 5). The abundance of neutral insects was fluctuated but they were tended to be higher in the snail treatments either in the low and high density compared to the loach treatments and mixed species treatment (Figure 6). The spider abundance also showed same differences (Figure 7).

Growth, biomass, yields, and nutrition concentration of rice plants

Number of tillers of rice plants were significantly higher in the treatment of high density of mud snail than other treatments (Figure 8a). However, the height, biomass, yields, and nutrition concentration (including C, N, and C/N ratio) were no significant difference among the treatments, although they were tended to be higher in the high density of mud snail treatments (Table 2).

Laboratory experiments

In the laboratory experiment, the algal biomass was significantly greater in the single and double loach and combined of single loach and snail, compared to the treatment of single and double snail (Figure 9). In addition, the mortality of mud snail and loach were 100% and 20%, respectively, when they were combined as a treatment (Figure 10). However, the mortality of the mud snail and the loach were increased in the double individual compared single individual (Figure 10).

Discussion

This study showed that density of mud snail and loach, and their interaction may alter the bio-based nutrient concentration in term of N and C and indirectly influenced the abundance of terrestrial arthropods through the growth and nutrition of rice plant. The presence of loach as an aquatic predator decreased the abundance of other aquatic organisms such as dipteran larvae, this in turn reduced the available prey for terrestrial predators such as spider. In contrast, mud snail improved bio-based nutrient concentration, especially in the high density, and it increased the abundance of emergence insect and their terrestrial predators.

Density effects occur when the population growth rates are regulated by the density a population (Crey and Wahl. 2010). The interaction between organism will effects on their growth. Our results showed that the density of mud snail effects on the reproduction rate. The mud snail with low density reproduce the juvenile higher than the mud snail of higher density in the 30 days. However, after 60 days the mud snail with the high density reproduce the juveniles higher that than in the low density. Our study suggested that there is an interaction between individual mud snail in term of food, therefore in the high density of mud snail the reproduction of juveniles will be appear later than the lower density. However, the loach reproduction resulted that the loach with high density reproduce higher than loach of low density after 60 days. The present study also suggested that the interaction between the mud snail and loach decreased the reproduction of their juveniles. This results showed that there is an interaction between different feeding functional of aquatic species, and it effects on their performance.

The interaction between aquatic organism could affect the availability of resources such as detritus and algae (Entry et al. 1997; Kandeler et al., 1999; Garcı´a-Gil et al., 2000). Detritus typically includes the bodies or fragments of dead plant and animal as well as fecal material, and important resource for organisms living in paddy fields (Anderson 1979; Lawler and Dirtz 2005). Present study resulted that N and C concentration of detritus tended to be in the high density of mud snail compared to low density and other treatments. The mud snails also had a higher detritus resource than the loach. However, the algal biomass was significantly higher when the loach is present because loach is the one of the aquatic predators which feed in the small aquatic organism. The algal biomass would respond positively to increasing of nutrient in detritus (Lawler and Dirtz 2005). However, our study showed the different results. Our laboratory experiments showed that the loach decreased the mortality of snails and their interaction was negatively correlated. It suggested that the behavior of loach which often habit on the inside of soil may interfere the snail which existed on the surface of the soil as a scraper.

Aquatic organisms are an ecologically important due to their interaction and its influence on the community structure of the aquatic ecosystem (Gilber, 2006). Our results showed that the mud snail and the loach had negative relation. The mud snail and loach also had effects on other aquatic organisms. The loach as predators decrease the abundance of other small insects which potentially as a prey such as dipteran larvae. Moreover, the behavior of the laoch also affects negatively on other scrapers. The results also suggested that the abundance of loach in the fields could decrease the emergence of insects, and it in turn influence on the availability of potential prey for predators. The abundance of the spiders in the loach plot was decreased due to the low of prey such as chironimdae. Chironomids are one of the important prey for the spiders. The present results indicated that the loach could decrease the abundance of chironomids adults and subsequent influence on the abundance of the spiders.

Species of snail plays an important role as an indicator of soil fertility in paddies (Simpson et al. 1994). Our study supports this review. The mud snail with the high density could increase the gartherers which is play important as a detritus decomposer. The high number of mud snail increased the bio-based nutrient for rice plant. The growth and the productivity of rice plant tended to be increased, and it indirectly influences on the abundance of herbivorous and their predators. In addition, mud snail also increased the emergence of insects which potentially as a prey of the predator such as spiders.

The present study suggests that the density of the mud snail and the loach changed the community structure of aquatic and terrestrial communities through their function in paddy field ecosystems. The mud snails increase the bio-based nutrients in organic paddy fields and in turn could effect on the population of terrestrial arthropods. However, the loach as predators decreased the population of the terrestrial arthropods. Although the relationship between the laoch and the snail were negatively correlated, our comprehensive study indicates that each species of aquatic with different functional feeding give different impact on the community structure organic paddy fields. To better understand the functions and benefits of each aquatic organism in the organic paddy fields, more studies should be done to reveal the relationships each aquatic organism.

Table 1. Algal biomass and nutrition concentration of detritus in each treatment

Treatments	Algal biomass (chlorophyll a) (mean ± SE, µg/l)	Nutrition concentration of detritus (mean ± SE, %)		
		Nitrogen	Carbon	C/N ratio
L50	81.23 ± 5.27 a	0.17 ± 0.007	1.93 ± 0.08	11.43 ± 0.04
L100	94.96 ± 6.48 a	0.14 ± 0.008	1.68 ± 0.08	11.71 ± 0.08
S50	46.12 ± 7.33 b	0.13 ± 0.011	1.62 ± 0.11	11.79 ± 0.09
S100	33.29 ± 3.75 b	0.15 ± 0.007	1.83 ± 0.06	12.06 ± 0.47
M50	80.86 ± 5.42 a	0.14 ± 0.011	1.70 ± 0.12	11.73 ± 0.23
df	7	7	7	7
χ^2	118.09	7.80	7.82	3.92
p	< 0.0001	0.09	0.09	> 0.05

Table 2. Rice plant biomass, brown rice yields, and nutrition concentration of rice plants in each treatment

Treatments	Rice plant biomass (mean ± SE, g/m^2)	Brown rice yields (mean ± SE, g/m^2)	Nutrition concentration of rice plant (mean ± SE, %)		
			Nitrogen (N)	Carbon (C)	C/N ratio
L50	459.2 ± 29.16	167.2 ± 9.68	1.08 ± 0.045	38.08 ± 0.23	35.46 ± 1.24
L100	463.2 ± 37.68	168.4 ± 14.66	1.02 ± 0.03	38.16 ± 0.18	37.37 ± 1.16
S50	478 ± 38.92	175.6 ± 15.58	1.02 ± 0.02	38.46 ± 0.15	37.90 ± 0.75
S100	494.8 ± 32.60	177.6 ± 12.92	1.03 ± 0.02	38.86 ± 0.17	37.69 ± 0.81
M50	443.8 ± 42.50	152 ± 21.49	1.08 ± 0.04	38.08± 0.23	35.47 ± 1.25
df	7	7	7	7	7
χ^2	-142.72	-121.10	5.80	5.27	9.14
p	> 0.05	> 0.05	> 0.05	> 0.05	0.057

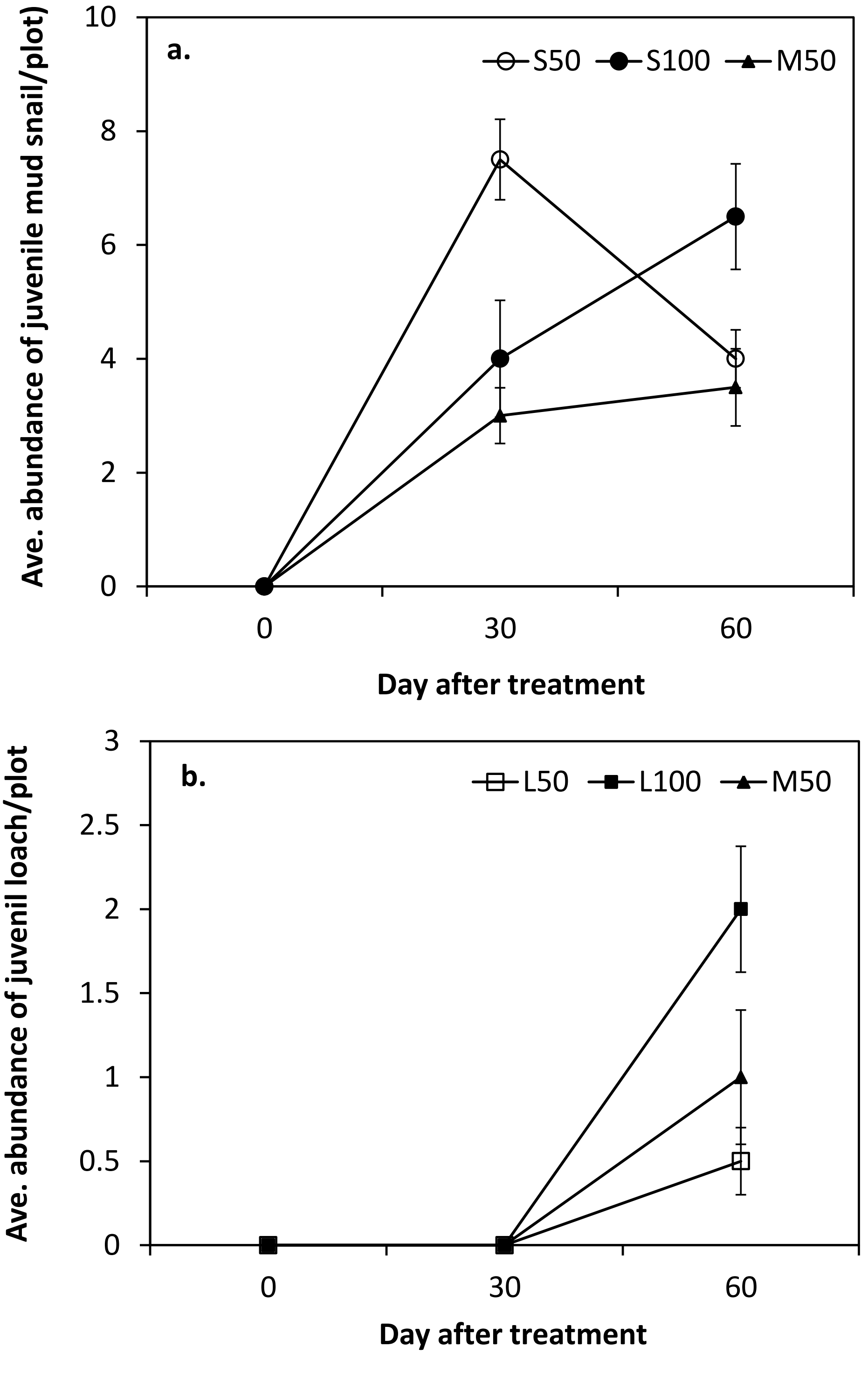

Figure 1. The abundance of juvenile (a) mud snail and (b) loach in response to the interaction within and between mud snail and loach in 0, 30, and 60 days after the treatment.

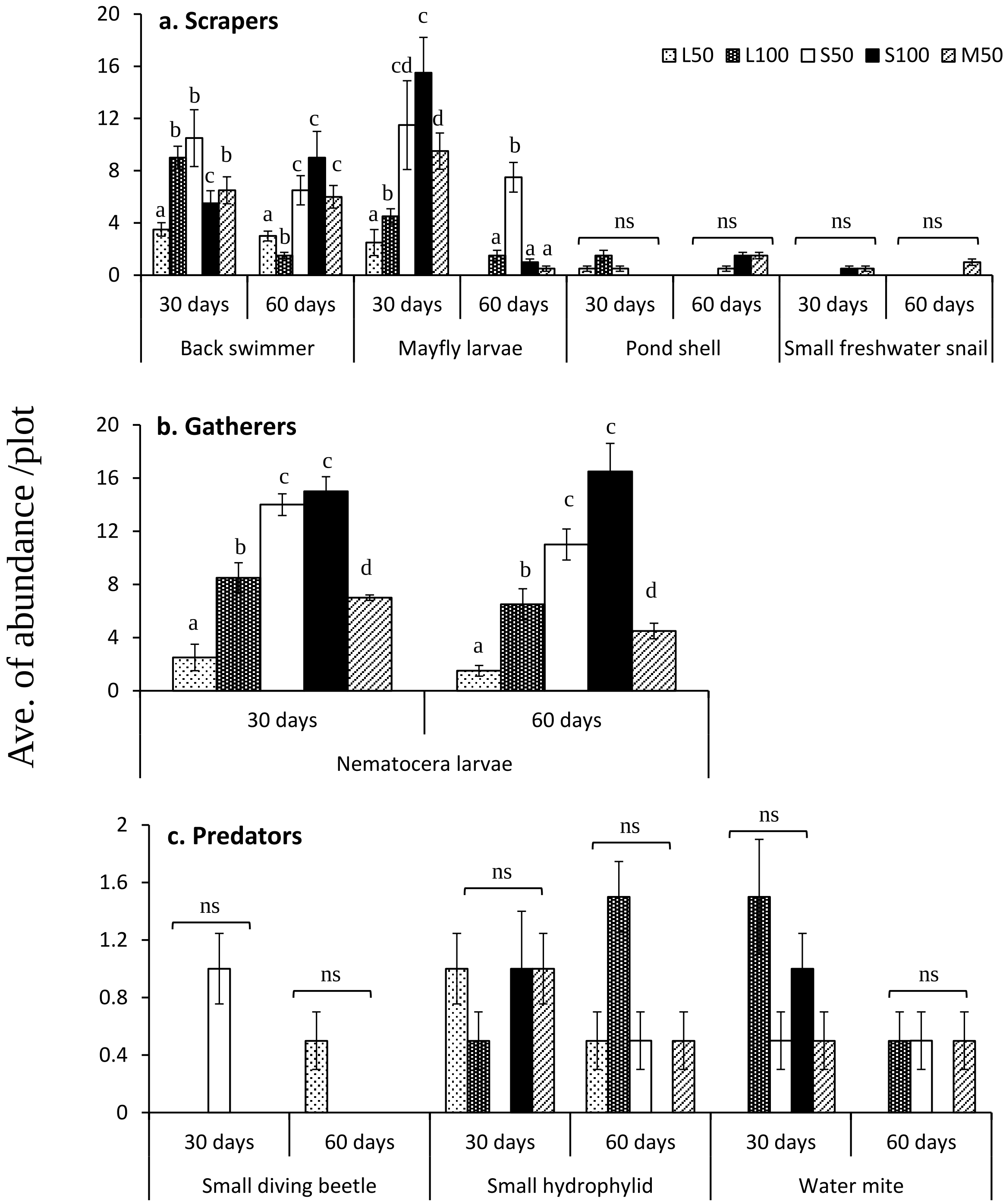

Figure 2. Abundance (mean ± SE) of (**a**) scrapers, (**b)** gatherers, and (**c**) predators in response of the mud snail and loach interaction in 30 days and 60 days after the treatment. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

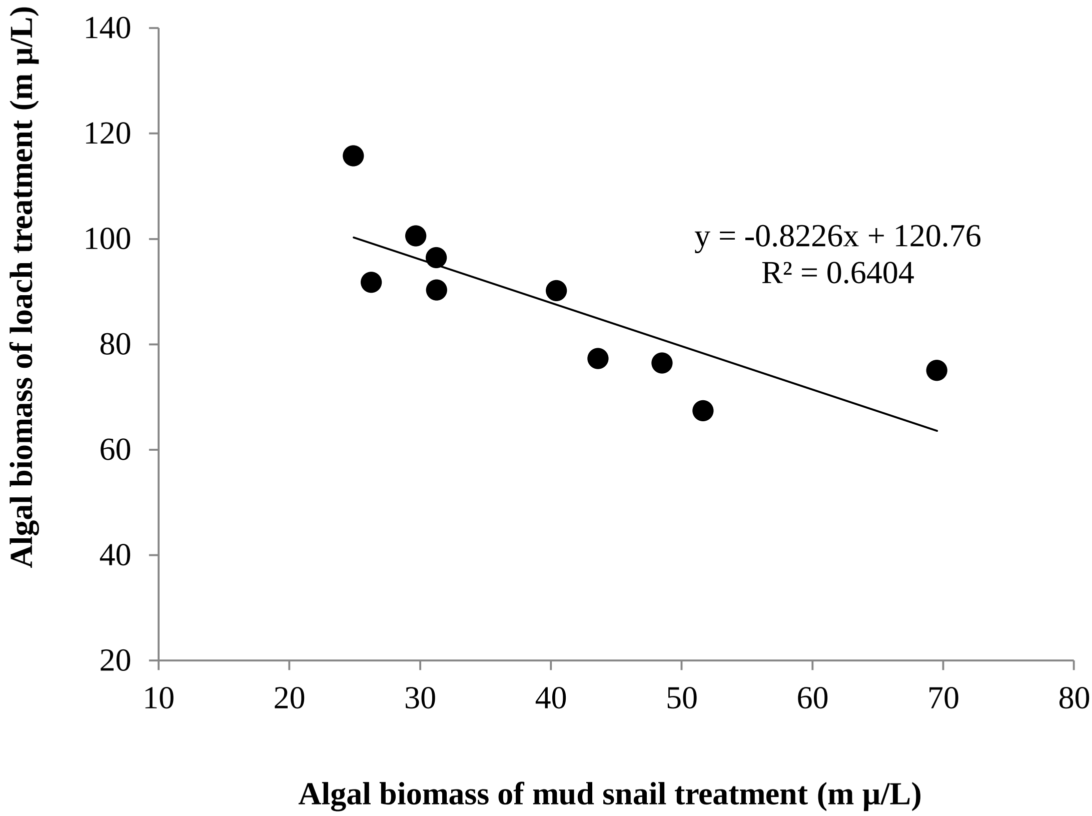

Figure 3. The relationship of between mud snail and loach used the of algal biomass ($p<0.001$)

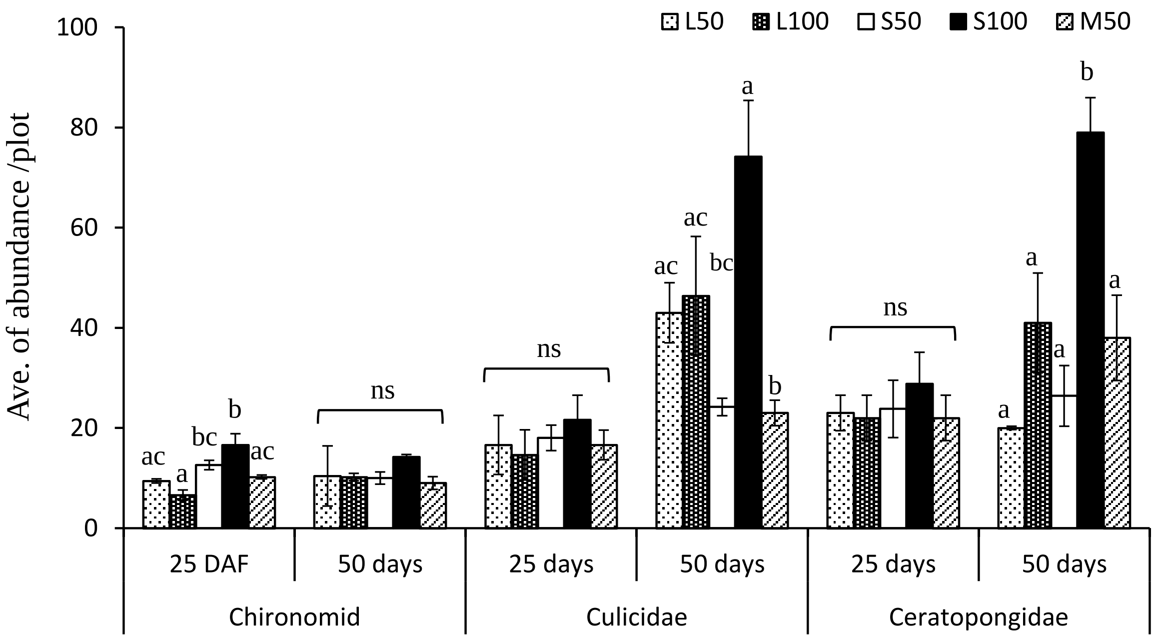

Figure 4. Abundance (mean ± SE) of insect emergence in response of the mud snail and loach interaction in 25 days and 50 days after the treatment. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

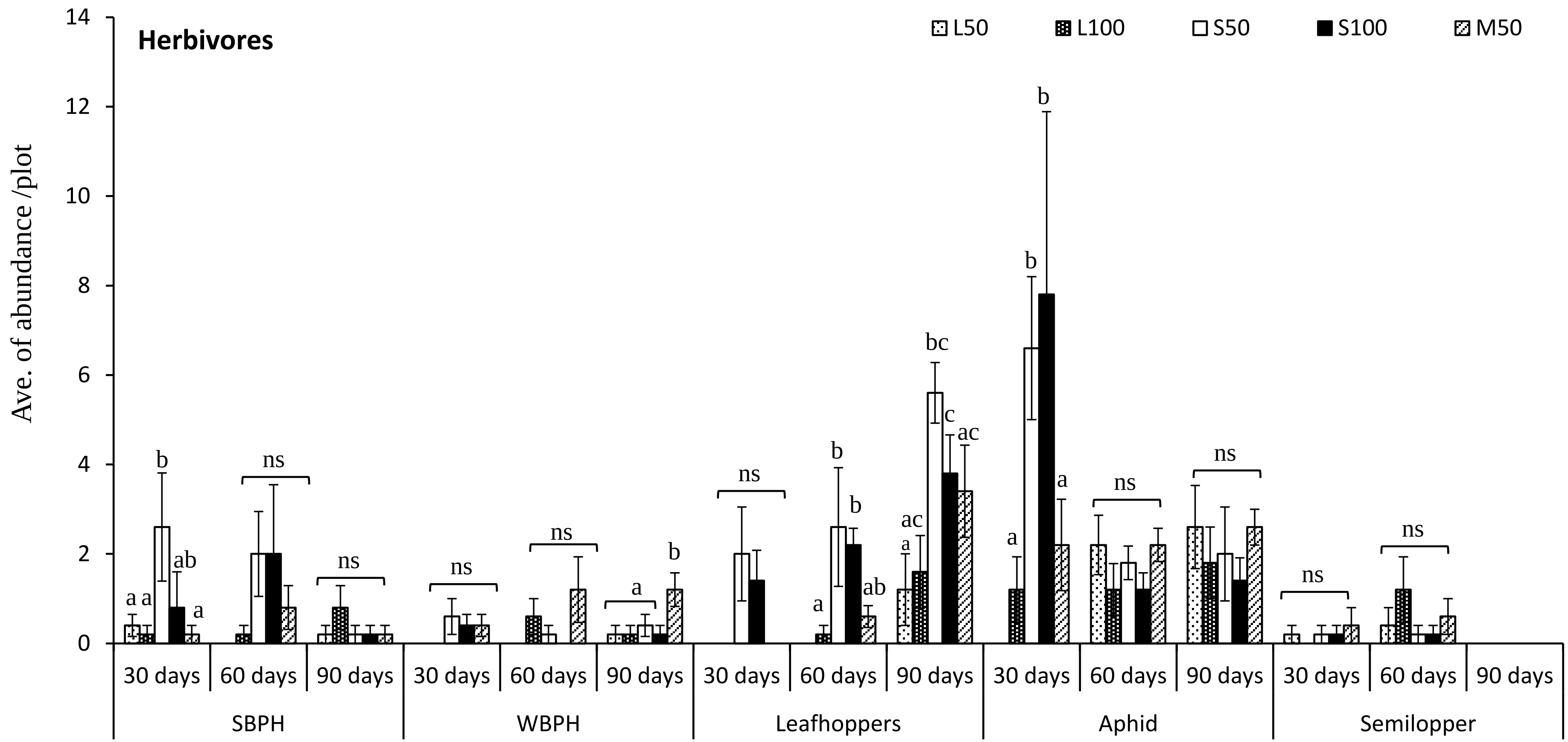

Figure 5. Abundance (mean ± SE) of terrestrial herbivores in response of the mud snail and loach interaction in 30 days and 60 days after the treatment. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

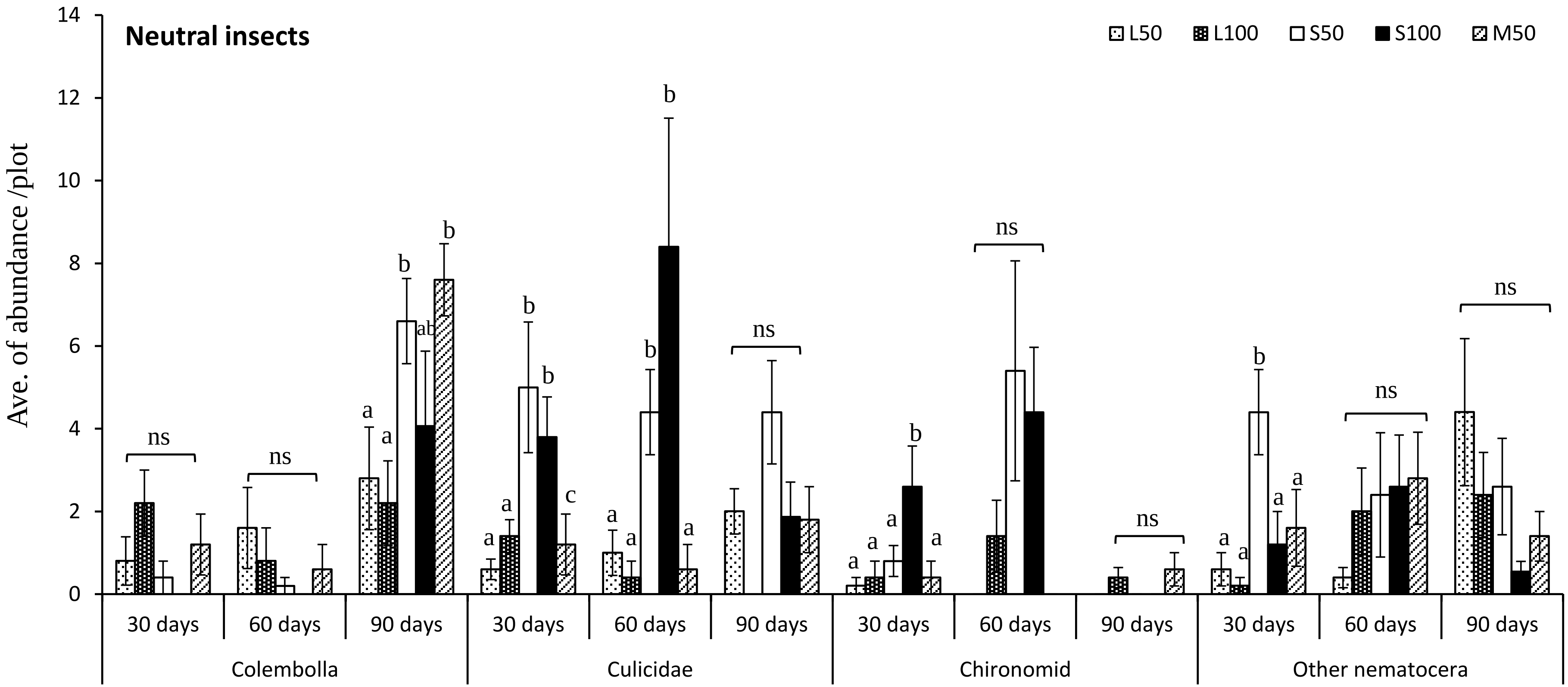

Figure 6. Abundance (mean ± SE) of terrestrial neutral insects in response of the mud snail and loach interaction in 30 days and 60 days after the treatment. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

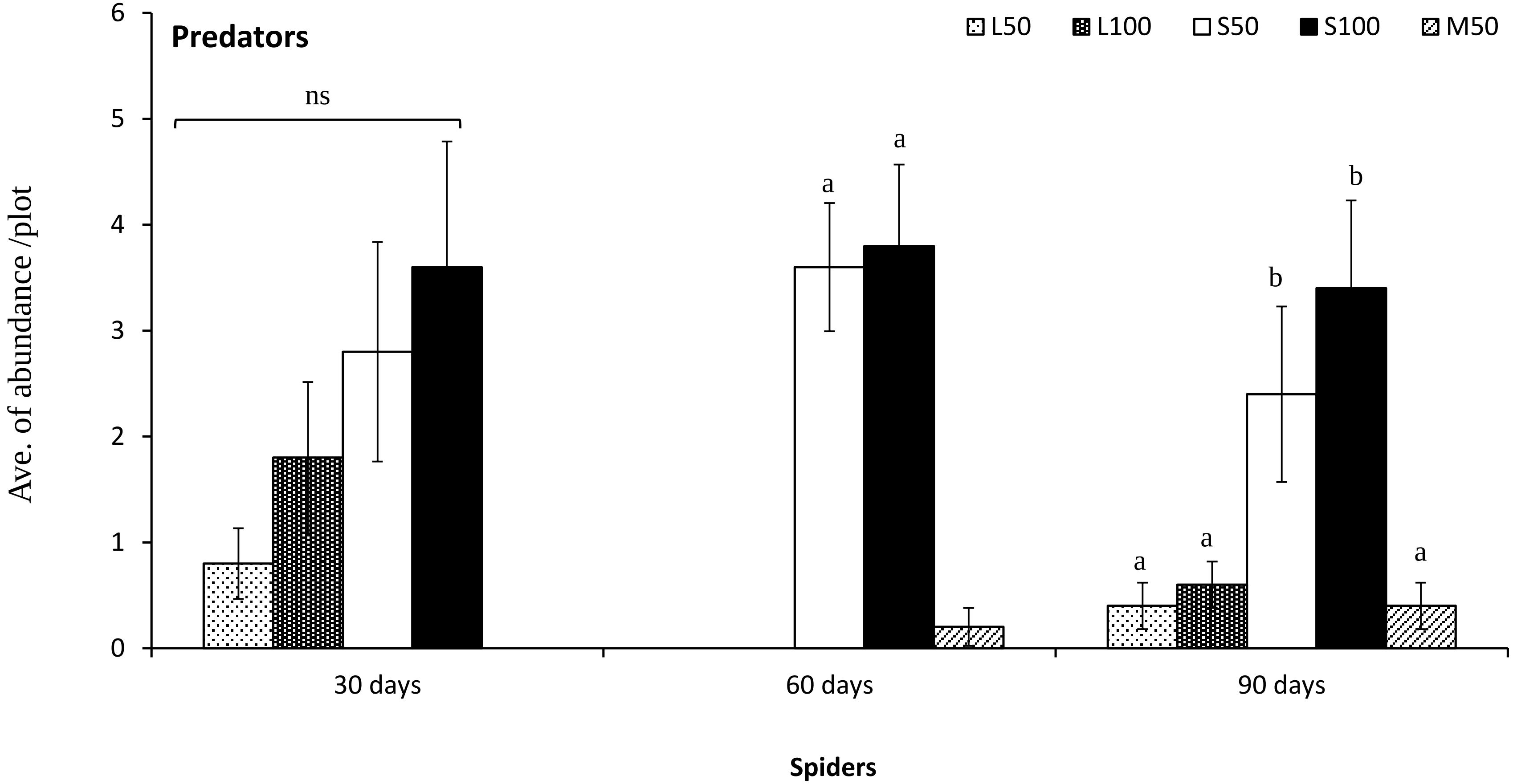

Figure 7. Abundance (mean ± SE) of terrestrial predators in response of the mud snail and loach interaction in 30 days and 60 days after the treatment. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

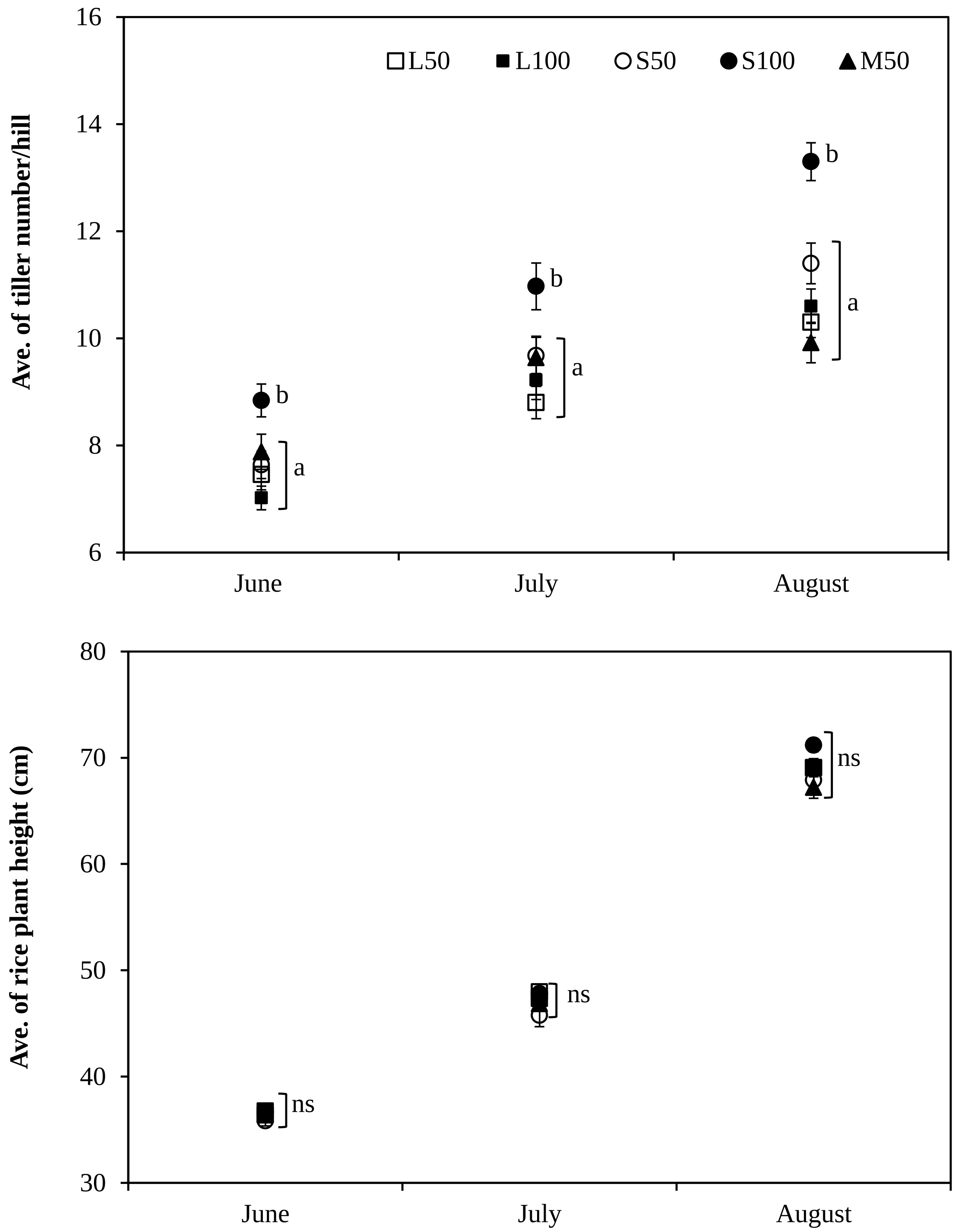

Figure 8. **a.** Number of tiller of rice plants and **b**. Rice plant height in response of the mud snail and loach interaction in June, July,and August. Value markers followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and plot as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

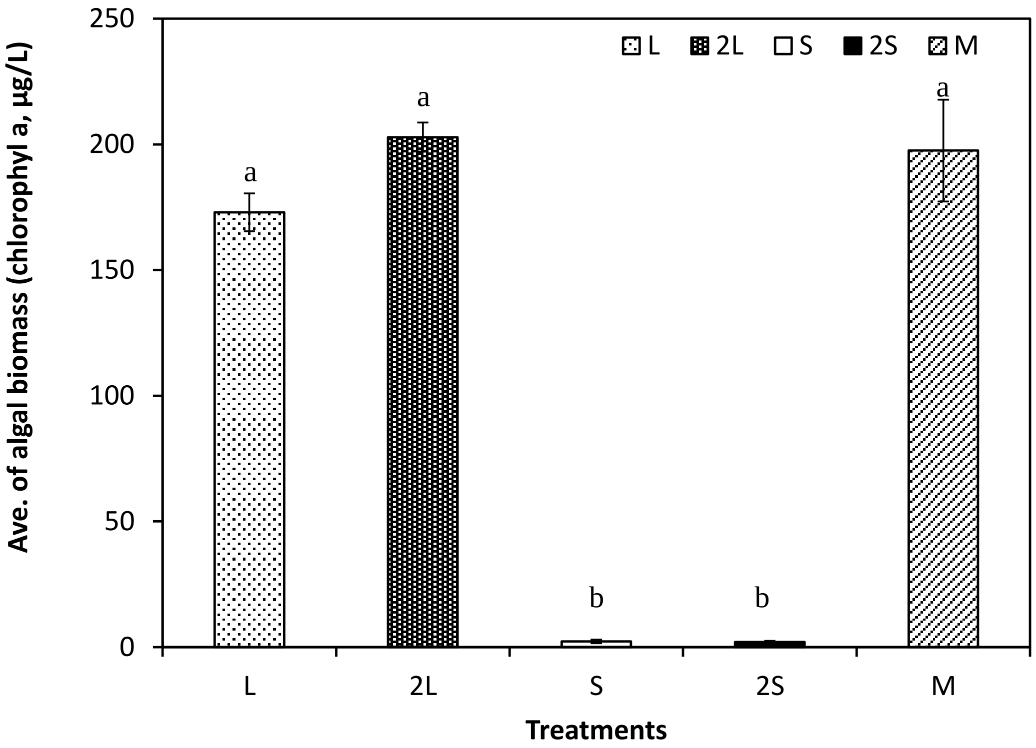

Figure 9. Algal biomass in the response of interaction between the mud snail and loach in laboratory experiments. Value bars followed by a different latter are significantly different by a generalized linear mixed model (GLMM) including parameters with gaussian distribution and replication as a random effect [$p < 0.05$, ns (not significant, $p \geq 0.05$)]

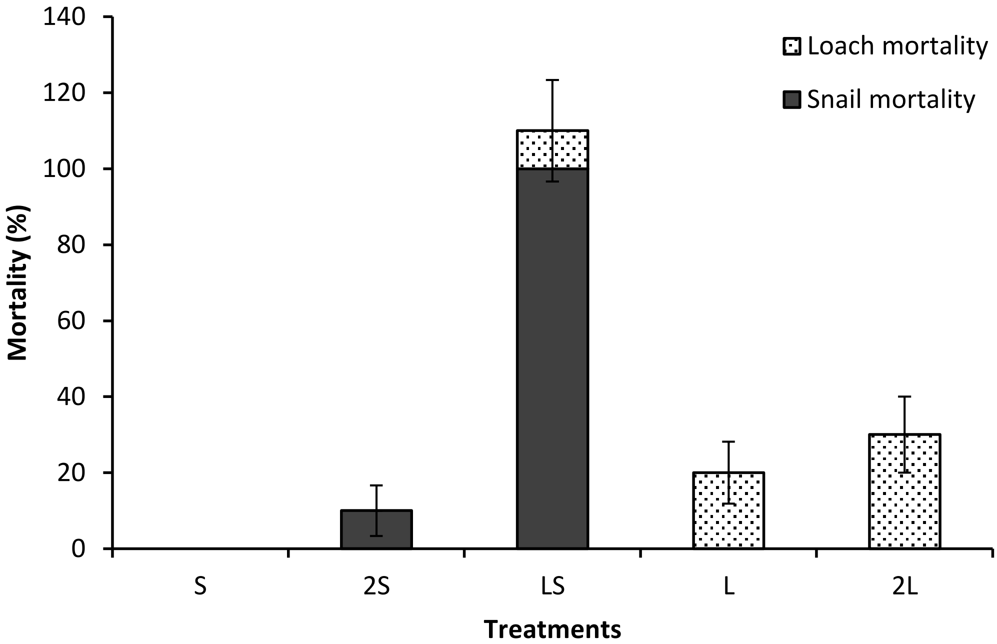

Figure 10. Mortality (± SE) of mud snail and loach in the response of their interaction in laboratory experiments.

Chapter 3:

Effects of soil organism on the abundance of aboveground arthropods in organic paddy fields

Introduction

Soil organisms play a fundamental role in agricultural ecosystems. They are integrated to the cycling of organic matter and associated nutrients, and physically alter the soil structure through their movement (Belnap 2001, Jouquet et al 2006, Wolfe 2005). Soil organism can adversely affect soil the fertility, ultimately altering the availability and composition of nutrients within the soil (Ehrenfeld, Jouquet et al 2006, Lavelle 1996, Belnap 2001). Soil organisms also contribute to the succession of above ground communities through supplying of nutrients as well as selective consumption of available resources (Deyn 2003). Thus, the conservation of soil organisms biodiversity is beneficial to biotic communities in aboveground and belowground ecosystems. The function of soil organisms in their communities, and their effects upon the environment, are often decreased due to lack of their abundance (Lavelle 1996). Loss of diversity of soil organisms may have a large effect upon on an agricultural ecosystem.

Soil communities are complexly related to the aboveground communities. Soil organisms decompose and utilize organic matter and compounds deposited by aboveground organisms. Aboveground communities subsidize the organic matter and compound, which could in turn impact soil organisms diversity, and vise versa (Wolfe 2005). Interactions between aboveground organisms and the soil community can have beneficial (Goverde et al. 2000,Bonkowski et al. 2001), negative (Chapman 1997a), or null effects (Barbosa and

Krischik 1991, Setala et al. 1998) on agricultural ecosystems. Soil organisms activity has a potential interactions with herbivore populations that could be mediated through changes in plant growth and nutrition. Soil organisms mineralize organic matter, thereby releasing nutrients such as nitrogen to other trophic levels (Newell and Porter 2000) and enhancing plant productivity (Bonkowski et al. 2001).

In response to the relationship between below and above ground communities, there is growing interest to understand the potential impact of soil organism communities on the aboveground communities such as herbivorous insects and their arthropod predators (Adams and Wall 2000, Hooper et al. 2000, van der Putten et al. 2001, Bardgett and Wardle 2003). Complex community interactions have been examined separately in belowground communities (Mikola and Setala 1998, Laakso and Setala 1999, Sundareshwar et al. 2003, Blum et al. 2004) and aboveground communities (Silliman and Bertness 2002, Denno et al. 2003, Pennings and Silliman 2005), but very few studies have examined the relationship between belowground and aboveground interactions particullary in organic paddy fields.

The aims of the study are to reveal the effects of soil organisms on the abundance of aboveground arthropods by measuring the soil organism activity, nutrient availability, and abundance of the arthropods. We predict that subsidies of organic input in the organic paddy fields should increase microbial N immobilization and denitrification, effectively increase the N available for arthropod abundance.

Materials and methods

Experiments 1: Effects of soil nutrient of herbivorous insects

Laboratory experiment:

Insects

The rice grasshopper *Oxya japonica* (Thunberg) (Orthoptera: Acrididae) is the most abundant herbivore in paddy fields in northern Japan. This study investigated the effects of soil nutrients associated on rice plant quality, and the growth and performance of *O. japonica* in paddy fields. Young *O. japonica* nymphs occur in June and adults appear in late July or August. We collected 1st instar nymphs from the Yamagata University Research Station, Tsuruoka, Yamagata (38°43N, 139°49E) in early June. Approximately 50 nymphs were maintained in an insect cage (34 × 26 × 34 cm) in the laboratory under a 16L: 8D photoperiod at 23 ± 1°C and were provided with suitable amounts of rice plant seedlings (*Oryza sativa* L.) every day for 2 weeks. We identified their sex under a microscope at high magnification (Stereo Zoom; Olympus, Tokyo, Japan) and found that most of the individuals were female. Second instar females were starved for 24 h before the experiment. Then, we selected individual female 2nd instar nymphs with similar body weights and lengths for the bioassay to clarify the effects of soil nutrients on their growth and performance.

Plants

The rice variety used was *Sasanishiki*, which is a local variety planted in the Shonai area. From mid-April to early June, rice seeds were soaked in water for 24 h every week and sown in a box filled with standard soil as a rice nursery. One month later, the rice seedlings were used for the laboratory experiments.

Soil treatments

The soil used for the experiments was collected from the organic and conventional paddy fields surveyed in the field experiments, and dried in a greenhouse (22 ± 2°C) for 3 weeks. Then, the soil was ground and strained through a 0.5-mm sieve. Next, 9.5-cm-diameter, 12-cm-high plastic pots were filled with 250 g of soil, and one rice seedling was planted in each pot. The plants were replaced with new plants for each period of plant biomass measurement. Thirty plastic pots with rice plants were prepared for each organic and conventional soil treatment and used for three measurements. First, 10 plastic pots with rice plants for each treatment were set in a greenhouse, and their positions were randomized between organic and conventional soil treatments. Bioassays were performed after 2 weeks. The remaining 20 plastic pots were subsequently used for the second and third measurements. A suitable amount of water was given to each plant every day during the experiment.

Growth and performance of *O. japonica*

To assess the effects of soil nutrients on the growth and performance of *O. japonica* via rice plant quality, we measured the body weight, body length, development time, survival, and leaf area consumption of *O. japonica*. One 2nd instar female *O. japonica* was released into each plastic pot, which was placed within a plastic cylindrical insect cage (9 cm in diameter × 30 cm in height) with nylon gauze at the top. The development time of the nymphs was determined daily by checking their molt. To measure body weight and length, nymphs were transferred from the cages and anesthetized with CO_2 gas to facilitate the measurements. The plants were also removed from the pots, and the leaf that had been consumed by *O. japonica* was cut. To estimate the leaf area consumed, individual leaves were photographed using a digital microscope, and the leaf area consumed was calculated by applying the polygon formula (Fig. 1). After measuring body weight and length, the *O. japonica* nymphs were returned to the cages with new plants. Each nymph was measured three times over a 1-month period.

Plant biomass and chemical analysis

The N and C contents of the experimental rice plants were analyzed. Plants damaged by *O. japonica* during the experiment were harvested. These plants were oven-dried at 70°C for 72 h and their biomass was measured. The dried plant shoots were ground into powder and analyzed with an automatic NC determination unit (Sumigraph NC-220F).

Experiment 2: Effects of soil nutrient on aboveground arthropods in organic paddy fields

Field experiment:

Study area

The experiment was conducted in 2013 to 2014, in the area surrounding Horinouchi at Sakata city, Yamagata, Japan (38° 55′ N, 139° 50′ E). The mean annual precipitation was approximately 2062.7 mm, and the mean monthly temperature in May to September during the same period was approximately 21.6 °C (Japan Meteorological Agency 2014). In this area, organic paddies were distributed among conventionally managed paddies and a typical size of paddy fields is approximately 3000 m^2. Most paddy fields are conventional fields and less than 1% comprises organic fields. Three pairs of 5 years and 20 years organic paddy fields were use for field experiments.

Sampling of soil communities

To determine community structure of the soil organism in 5 and 20 years of organic paddy fields. Soil were took in nine 20 × 20-cm plots and 20-cm-deep in each field. Soil samples were transferred to the plastic bag and brought to the laboratory. Then, the soil was strained through a 0.5-mm sieve, and the soil organisms were visually identified and counted. On the other hand, other soil samples were collected using cylindrical cores (15-cm-diameter and 10-cm-deep) in nine plots each field to assess the microbial activity and soil nutrient. We measured net potential N mineralization (ammonification and nitrification) and carbon mineralization (net potential CO_2 production) during 30-days aerobic incubation. Microbial N and DON (dissolved organic N) were determined by chloroform fumigation/extraction followed by persulfate digestion (Cabrera and Beare 1993). In all cases, inorganic soil N was determined after extraction with 30 mL of 2.0 mol/L KC1 (Sims et al. 1995). To measure soil nutrients in terms of the N and carbon (C) concentrations. The air dried soil was ground into powder and then analyzed with an automatic NC determination unit (Sumigraph NC-220F; Sumika Chemical Analysis Service, Osaka, Japan).

Sampling of aboveground arthropods

Arthropods were sampled using a beating methods in nine 50 × 50-cm plots in 5 ad 20 years of organic paddy fields. In the laboratory, each sample was sorted into different taxa and counted. The insects and spiders collected from the fields were identified and classified into the smallest possible taxa using available keys and guides for the different taxa.

Statistical analysis

Statistical analyses were performed with R (ver. 3.1.0 for Windows; R Foundation for Statistical Computing, Vienna, Austria). We examined the effects of 5 and 20 years organic paddy fields in the field experiments on the abundance of soil and aboveground arthropods, N and C concentrations of the plants and soil, and plant biomass by applying a generalized linear mixed model (GLMM) with field as a random effect using the identity link function (lme4 package; maximum likelihood estimation). For the laboratory experiment, to identify the difference in the effects of organic and conventional soil quality on growth (body weight and length) and duration of development of *O. japonica*, as well as C and N of plants, we also used a GLMM (lmer function in the lme4 package; maximum likelihood estimation) with individual grasshopper as a random effect. The information on the N and C concentrations, plant biomass, and growth of *O. japonica* was assessed using the lmer function, while data on the abundance and duration of development of *O. japonica* were analyzed using the glmer function. To analyze the effects of organic and conventional soil on plant biomass and leaf area consumed, we use a generalized linear model (GLM) with the glm function because no random effects were involved in these measurements. The significance of the fixed effect for all analyses was assessed using likelihood ratio tests (Zuur et al. 2009).

Results

Laboratory experiment

Growth and performance of *O. japonica*

The *O. japonica* were heavier when individuals fed on rice plants grown in conventional soil on June 23 (df = 1, $\chi2 = 11.55$, $p < 0.001$) and July 2 (df = 1, $\chi2 = 5.67$, $p < 0.05$) (Fig. 1a), but did not differ significantly between treatments on July 10 (df = 1, $\chi2 = 2.34$, $p > 0.05$; Fig. 1a). A similar trend was observed for body length (June 23: df = 1, $\chi2 = 3.11$, $p > 0.05$; July 2: df = 1, $\chi2 = 4.82$, $p < 0.05$; July 10: df = 1, $\chi2 = 12.55$, $p < 0.001$; Fig. 1b). The duration of development of *O. japonica* that fed on plants grown in organic soil was longer than that for those fed on plants grown in conventional soil at the 2nd instar (df = 1, $\chi2 = 8.01$, $p < 0.01$; Fig. 1c), but did not differ significantly between soil treatments at the 3rd, 4th, and 5th instars (3rd instar: df = 1, $\chi2 = 2.35$, $p > 0.05$; 4th instar: df = 1, $\chi2 = 0.51$, $p > 0.05$; 5th instar: df = 1, $\chi2 = 3.20$, $p > 0.05$; Fig. 1c). *O. japonica* consumed a significantly larger area of leaves on plants grown in conventional versus organic soil (June 23: df = 1, t = 7.87, $p < 0.001$; July 2: df =1, t = 3.74, $p < 0.01$; Fig. 2a). The rice plant biomass was significantly higher in conventional versus organic soil on June 23 (df = 1, t = –2.471, $p < 0.05$) and July 2 (df =1, t = –2.75, $p < 0.05$), but not on July 10 (df =1, t = 0.28, $p > 0.05$; Fig. 2b).

Chemical analysis of plants

The N concentration in the rice plants did not differ between plants grown in organic and conventional soil (Table 1). However, the C concentration and C/N ratio were significantly higher in plants grown in organic soil (Table 1).

Field experiment

Abundance of soil organisms

The abundance of soil organisms, including dipteran larvae (Nematocera and Brathycera) and oligochaeta (Enchytraeidae sp. and Lumbricidae sp.) were significantly higher in organic than conventional paddy field (Nematocera larvae: df = 1, $\chi^2 = 6.47$, $p < 0.05$; Brachycera larvae: df = 1, $\chi^2 = 4.61$, $p < 0.05$; Enchytraeidae sp. : df = 1, $\chi^2 = 10.17$, $p < 0.01$; Figure. 3).

Soil microbial activity and soil nutrient

Microbial biomass C and N were significantly greater in organic than conventional paddy fields (Biomass C: df = 1, $\chi^2 = 9.41$, $p < 0.01$; biomass N: df = 1, $\chi^2 = 12.39$, $p < 0.001$, Figure 4). Ammonium and decomposition carbon were also affected by farming methods in paddy fields (Ammonium: df = 1, $\chi^2 = 12.29$, $p < 0.001$; decomposition C: df = 1, $\chi^2 = 6.37$, p

< 0.05, Figure 5b-c), while the nitrate was only marginally significant different between organic and conventional paddy fields (df = 1, χ^2 = 2.86, p = 0.09, Figure 5b). Moreover,the N, C concentration,and C/N ratio were also significantly higher organic than conventional paddy fields (N concentration: df = 1, χ^2 = 10.34, p < 0.01; C concentration: df = 1, χ^2 = 12.48, p < 0.01; C/N ratio: df = 1, χ^2 = 15.11, p < 0.001, Figure 6a-c).

Abundance of aboveground arthropods

The abundance of *O. japonica,* which the most abundance herbivores was significantly higher in the conventional paddy fields than the organic fields (df = 5, Z = –4.04, p < 0.001; Fig. 7). However, the abundance of other aboveground arthropods were generally shown no significant difference between farming paddy fields (df = 1, χ^2 < 2, p > 0.05, Figure 7) and only the abundance of Collembolla was significantly higher in organic than in cenventional paddy fields (df = 1, χ^2 = 7.51, p < 0.001, Figure 7d).

The relation between soil organism and aboveground arthropods through the soil activity. The abundance of soil organisms was significantly positively correlated to the microbial biomass C (df = 5, χ^2 = 3.30, p < 0.01, Figure 8a). However, the abundance of aboveground arthropods was positively correlated to the N concentration of soil (df = 5, χ^2 = 6.47, p < 0.05, Figure 8b).

Discussion

This study demonstrated that nutrients in organic and conventional paddy field soils affected rice plant quality. The results also confirmed that soil nutrients in conventional fields caused higher growth, development, and leaf consumption in *O. japonica* than those in organic fields via changes in the nutrient contents of rice plants. In addition, the long term of organic paddy fields could change the activity of microbial soil and soil nutrient, which in turn tended to be influence on the abundance of aboveground arthropods.

Altieri and Nicholls (2003) noted that the soil from organic fields had a low N content arising only from living organisms, while soil in conventional fields was supplied excessive N from synthetic fertilizers. Our study supported these reviews. In contrast, however, we observed a higher C content in organic fields than in conventional fields, although no significant difference was observed in the C/N ratio. Soil with a high C content prevents nutrient leaching and holds a great proportion of nutrients important to plants (Batjes 1996; Leu 2007). Conventional farming has caused a massive decline in soil C and nutrient loss due to the overuse of synthetic fertilizers (Altieri and Nicholls 2003; Leu 2007).

Leaf nutrient content is an important component in host plant acceptance and suitability for herbivorous insects (Facknath and Lalljee 2005), and total N is the most critical nutritional factor affecting the interactions between plants and herbivorous insects (Mattson 1980; Scriber 1984). This study indicated that the N concentration of rice plants was higher in conventional fields than in organic fields, as shown in several other studies (Facknath and Lalljee 2005; Hsu et al. 2009; Staley et al. 2010, 2011). The N concentration of rice plants in the laboratory experiment did not differ significantly between plants grown in organic and conventional soils, perhaps because we collected soil for the laboratory experiment before chemical fertilizers were applied in the conventional paddy fields. A previous study reported that the soil of conventional fields had high nutrient loss, which influenced the N concentration in plants (Hansen et al. 2000). In addition, as shown here, the C content and C/N ratio, which is also an important factor for plant–herbivore interactions, was higher in plants grown in organic soil. Based on the C–nutrient balance hypothesis (Phelan et al. 1995), plants with a high C concentration might have high resistance to herbivorous insect attack due to increased partitioning of foliar phenolic glycosides (Mattson et al. 2005). Therefore, our results suggest that the soil of organic fields reduces rice plant quality and suitability for herbivorous insects, including rice grasshoppers.

The quality of a host plant due to differences in the soil nutrient content can also influence insect feeding and growth, and plant quality has been shown to affect the growth and development of grasshoppers (Joern et al. 1997; Huis et al. 2008). In our study, the rice plants grown in organic soil lowered nymph performance, survival, and food consumption of *O. japonica*. In addition, plants with rich nutrients grown with synthetic fertilizers were nutritionally more favorable for herbivorous insects than unfertilized plants and hence supported larger insects and showed faster growth (Hsu et al. 2009). This study revealed that rice plants grown in organic soil prolonged the development time of *O. japonica*. Dixon (2000) suggested that the developmental time depends on food quality, with more nutritious food supporting shorter developmental times. Consequently, nutrient availability in the soil might indirectly affect the growth and feeding of *O. japonica* via plant nutritional quality.

The activity of soil organism is also affected by farming methods (Altieri and Nicholls 2003). The input of bio-based nutrient every growing season in organic fields could increase the carbon resources and may further influence the abundance of soil organisms (Yamazaki et al. 2001). We also found fewer soil organism in 5 organic fields than in 20 years of organic paddy fields. The activity of the soil organism also increased by the long term of organic paddy fields. However, the abundance of the aboveground was no significantly effect between 5 and 20 years of organic paddy fields, although in 20 years was tended to be has higher aboveground arthropods This study provided evidence that the bio-based nutrient input in the organic paddy fields fields enhanced the soil organism activity and soil nutrient, which in turn influence on the aboveground arthropods.

A few studies have demonstrated that plants grown in organic soil are less suitable for herbivorous insects than plants grown in conventional soils (Butler et al. 2012). Our study specifically relates the soil nutrition of organic and conventional fields to differences in foliar chemical content, which in turn influence the growth and performance of *O. japonica*. We found that the N concentration was higher in the soil and plants of conventional fields, while the C concentration and C/N ratio were higher in organic fields. However, in organic paddy fields with the different histories, the abundance of aboveground was only tended to be higher in 20 years than 5 years. Although the soil organisms and nutrients were significantly higher in 20 years than 5 years organic paddy fields. For understanding the functions and benefits of long term organic farming, more studies should be done to reveal the relationships among soil organisms and aboveground arthropod in organic paddy fields.

Table 3 Nitrogen (N) concentration, carbon (C) concentration, and C/N ratio of rice plants planted in organic and conventional soil

Soil treatment	Nitrogen concentration (mean ± SE, mg/g)	Carbon concentration (mean ± SE, mg/g)	C/N ratio (mean ± SE)
Organic	2.79 ± 0.12	42.27 ± 0.14	15.41 ± 0.64
Conventional	3.20 ± 0.19	40.57 ± 0.70	13.15 ± 0.91
df	1	1	1
t	-1.77	2.34	2.02
p	0.11	< 0.05	<0.05

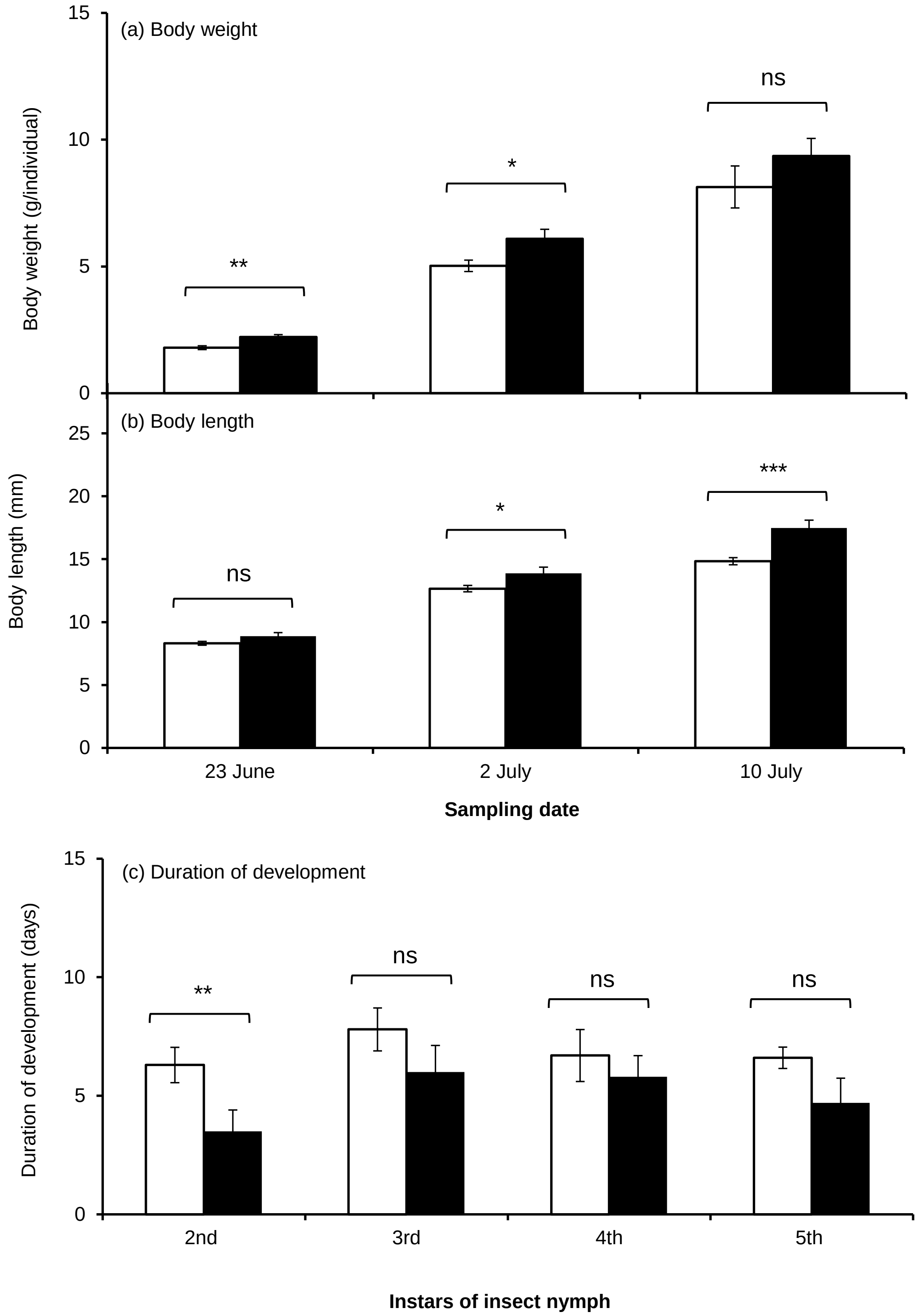

Fig. 1 a Body weight, **b** body length, and **c** duration of development (mean ± SE) of *Oxya japonica* feeding on rice plants planted in organic (open bars) and conventional (solid bars) soil. Significant differences according to a generalized linear mixed model (GLMM) including parameters with individual *O. japonica* as a random effect [***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns (not significant, $p \geq 0.05$); $n = 10$]

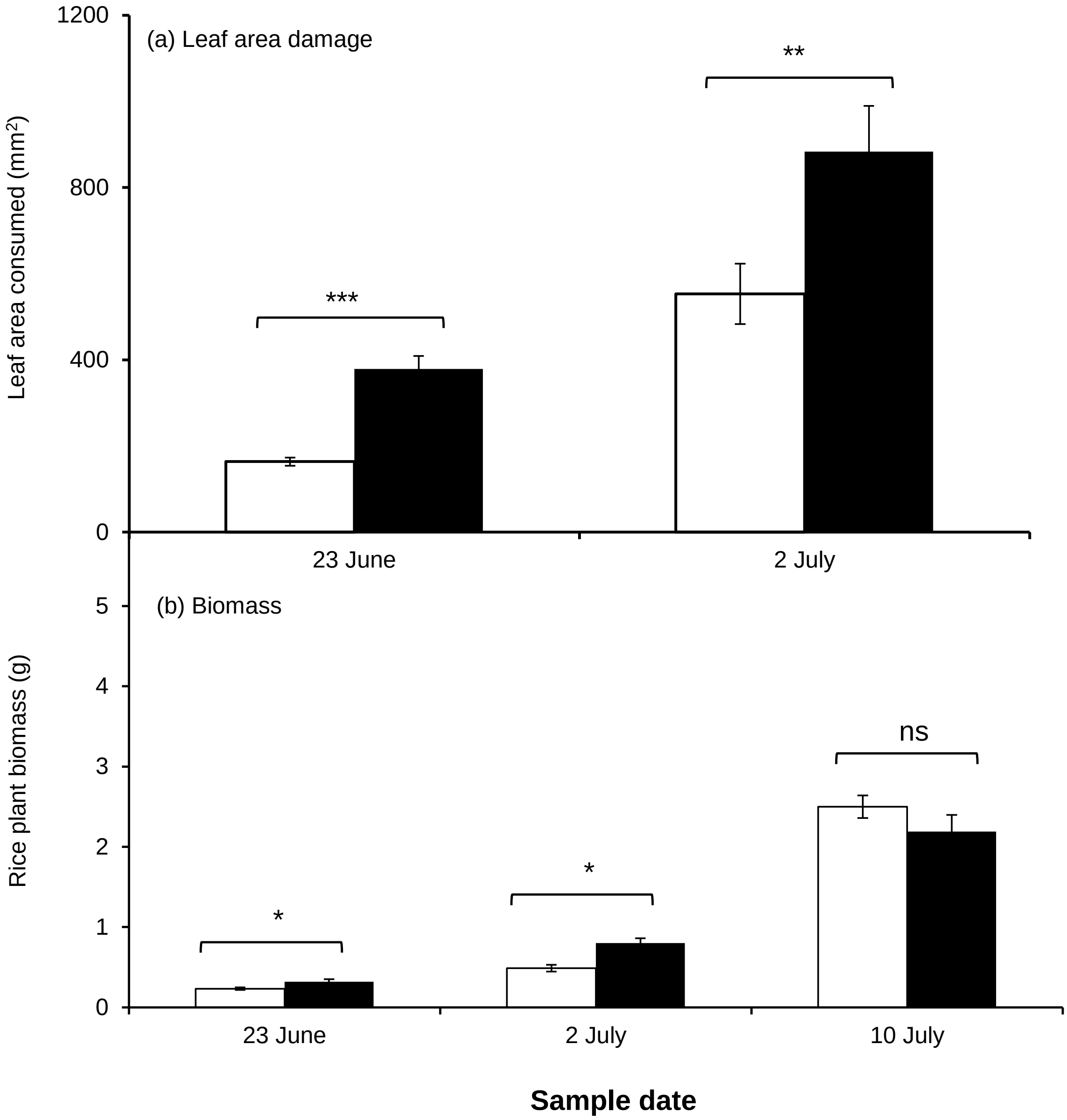

Fig. 2 a Leaf area consumed by *Oxya japonica*. **b** Biomass of rice plants planted in organic (open bars) and conventional (solid bars) soil. Significance difference according to a generalized linear model (GLM) including parameters: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns (not significant, $p \geq 0.05$, $n = 10$). Leaf area damage was not measured on 10 July because damage to conventional plants was very heavy and therefore difficult to measure

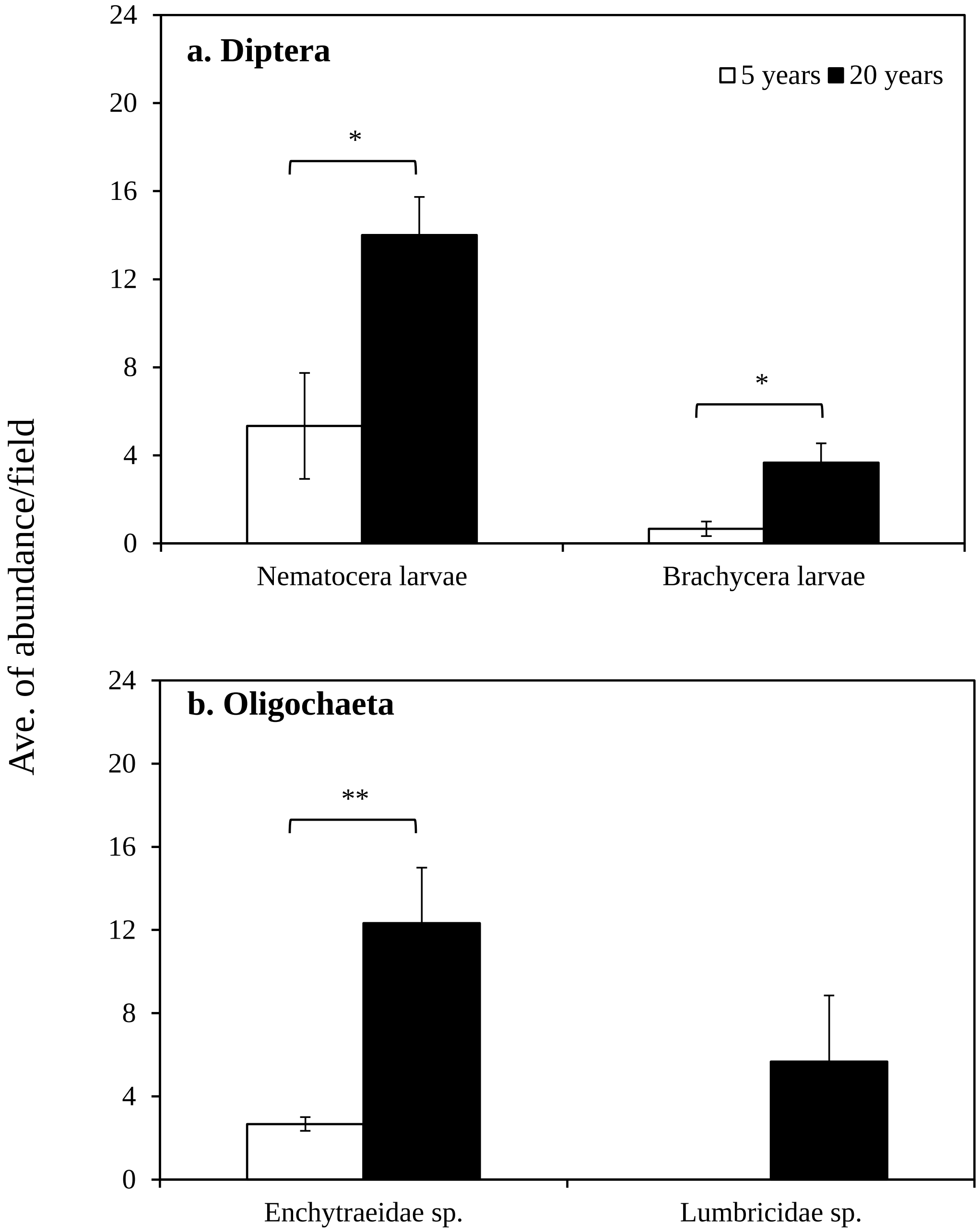

Figure 3. Abundance of dipteran larvae (a) and oligochaeta (b) in soil of 5 and 20 years organic paddy fields. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect (**$p < 0.01$, *$p < 0.05$)

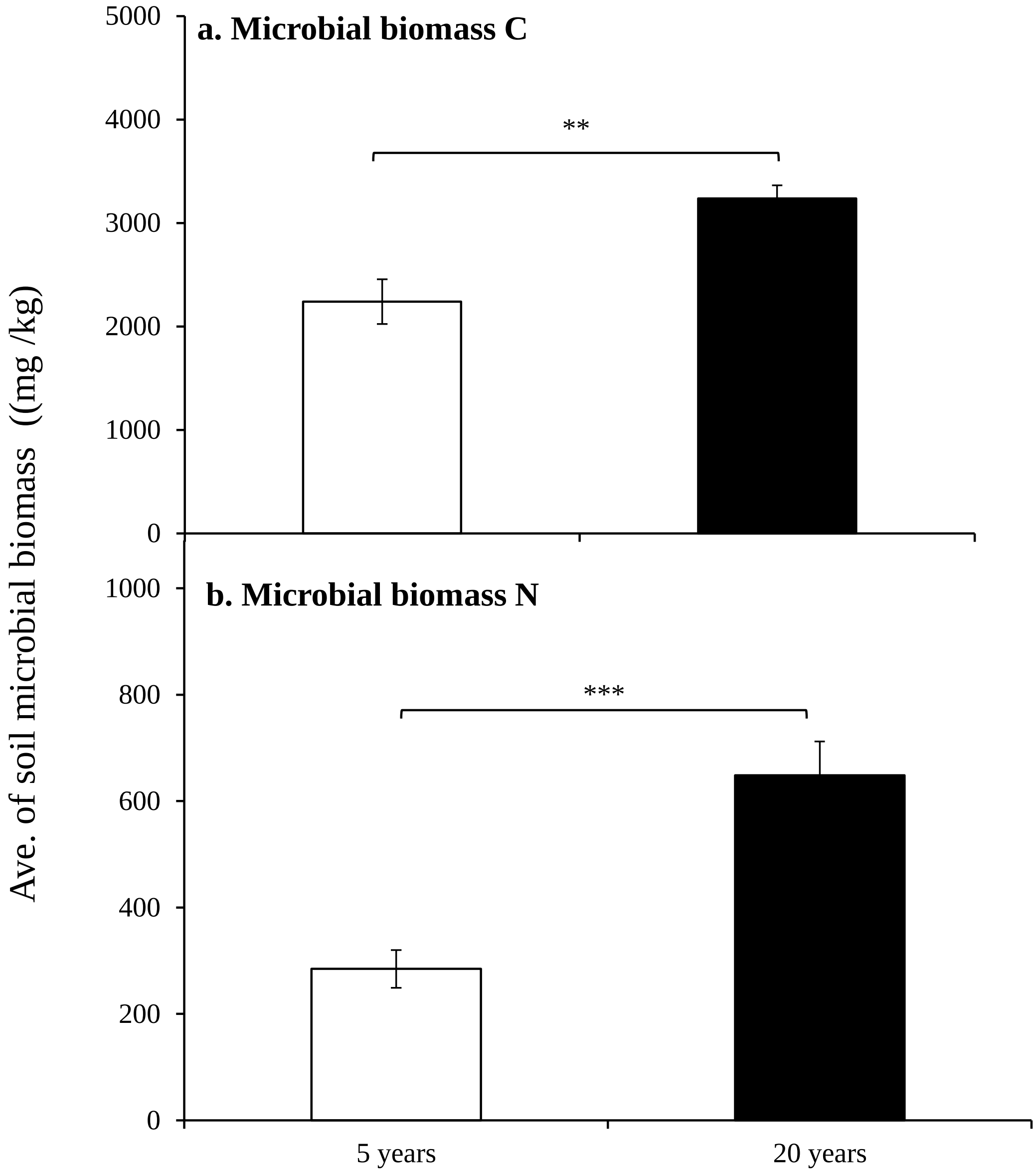

Figure 4. a. Microbial biomass C and **b**. Microbial biomass N in soil of 5 and 20 years organic paddy fields. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Gaussian error distribution and field as a random effect (***$p < 0.001$, **$p < 0.01$)

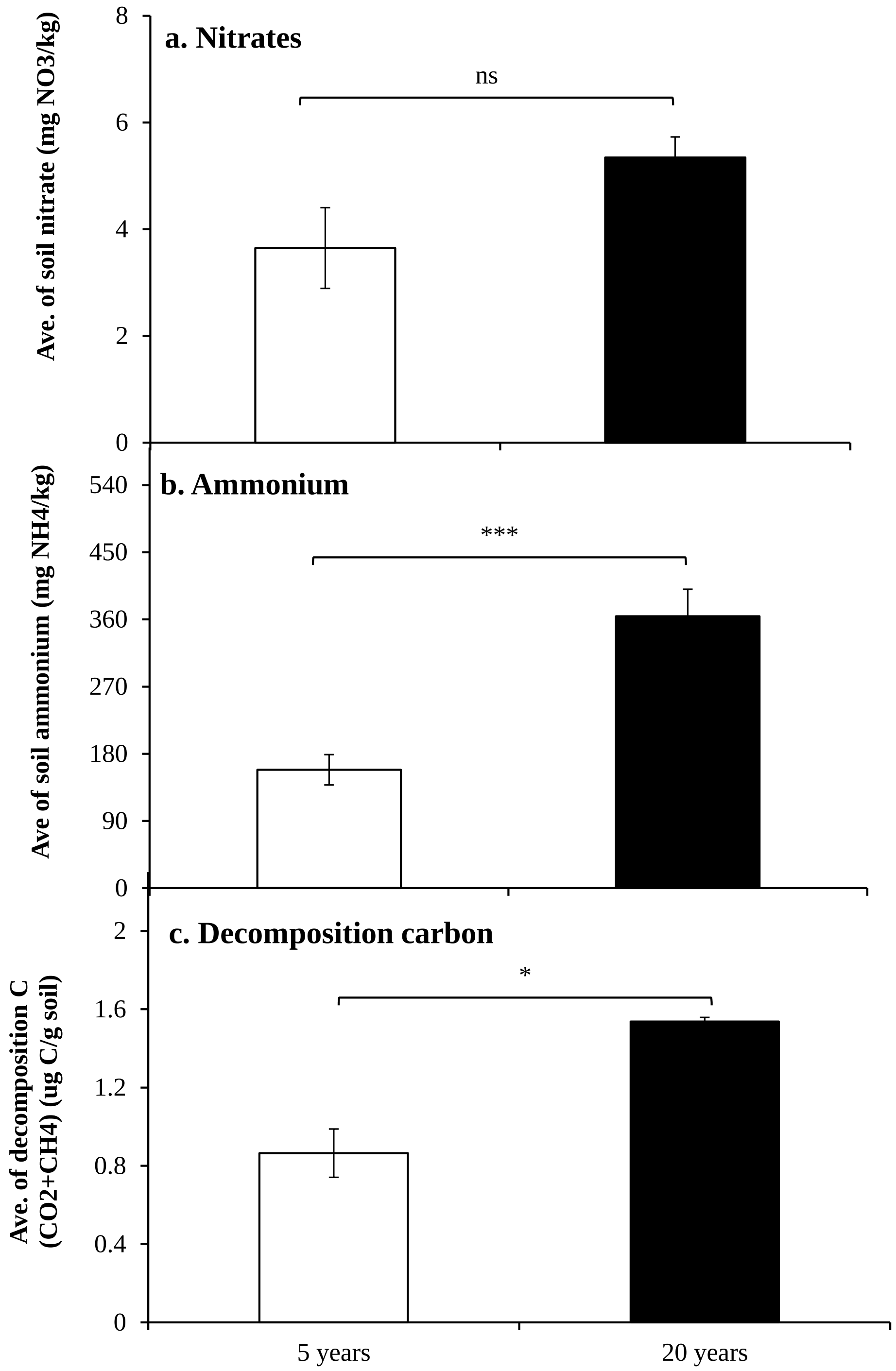

Figure 5. a. Nitrates **b**. Ammonium, and **c**. Decomposition carbon in soil of 5 and 20 years organic paddy fields. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Gaussian error distribution and field as a random effect [***$p < 0.001$, *$p < 0.05$, ns (not significant, $p \geq 0.05$)]

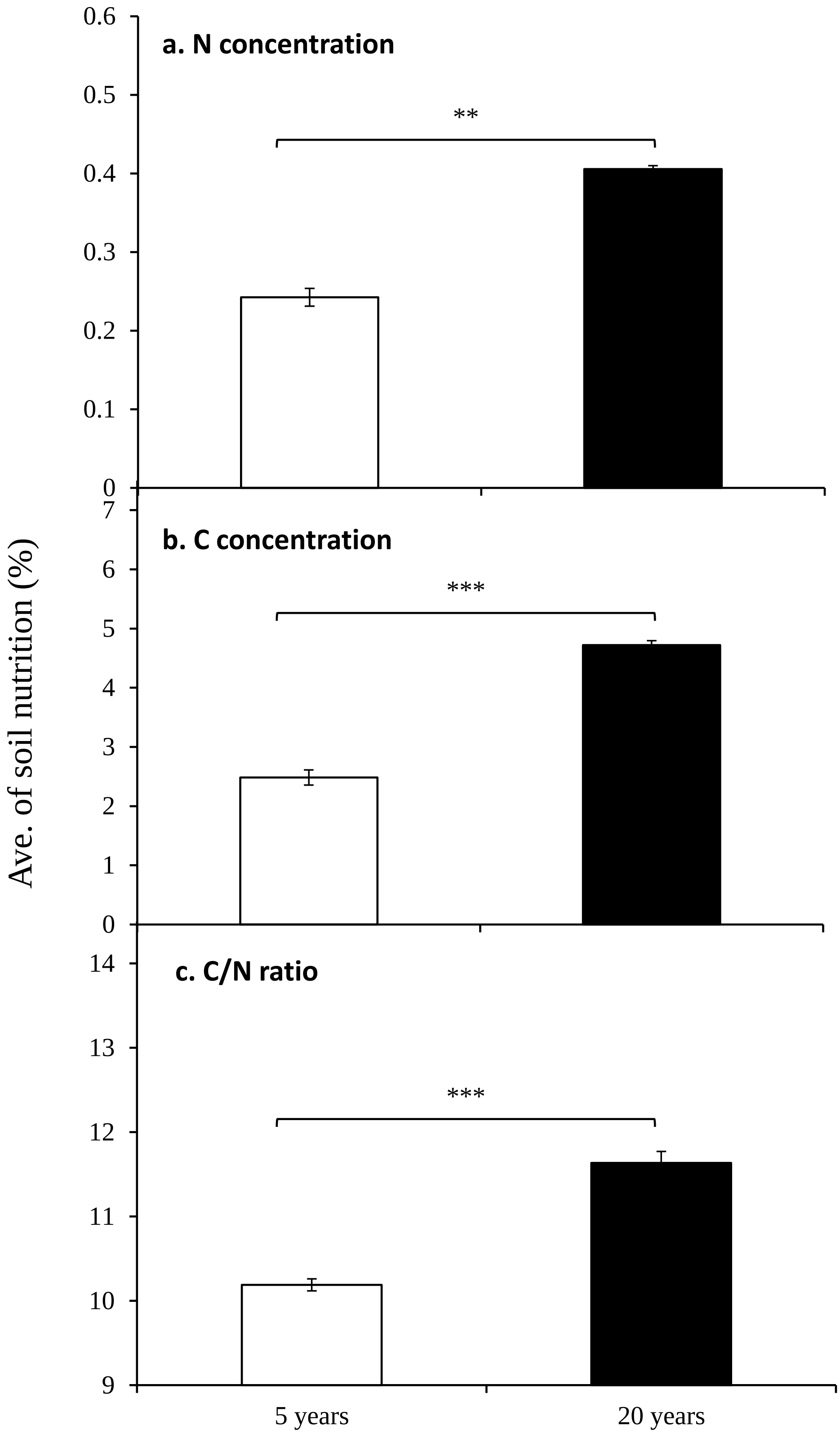

Figure 6. a. N concentration **b**. C concentration, and **c**. C/N ratio of 5 and 20 years organic paddy fields soil. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Gaussian error distribution and field as a random effect (***p < 0.001, **p < 0.01).

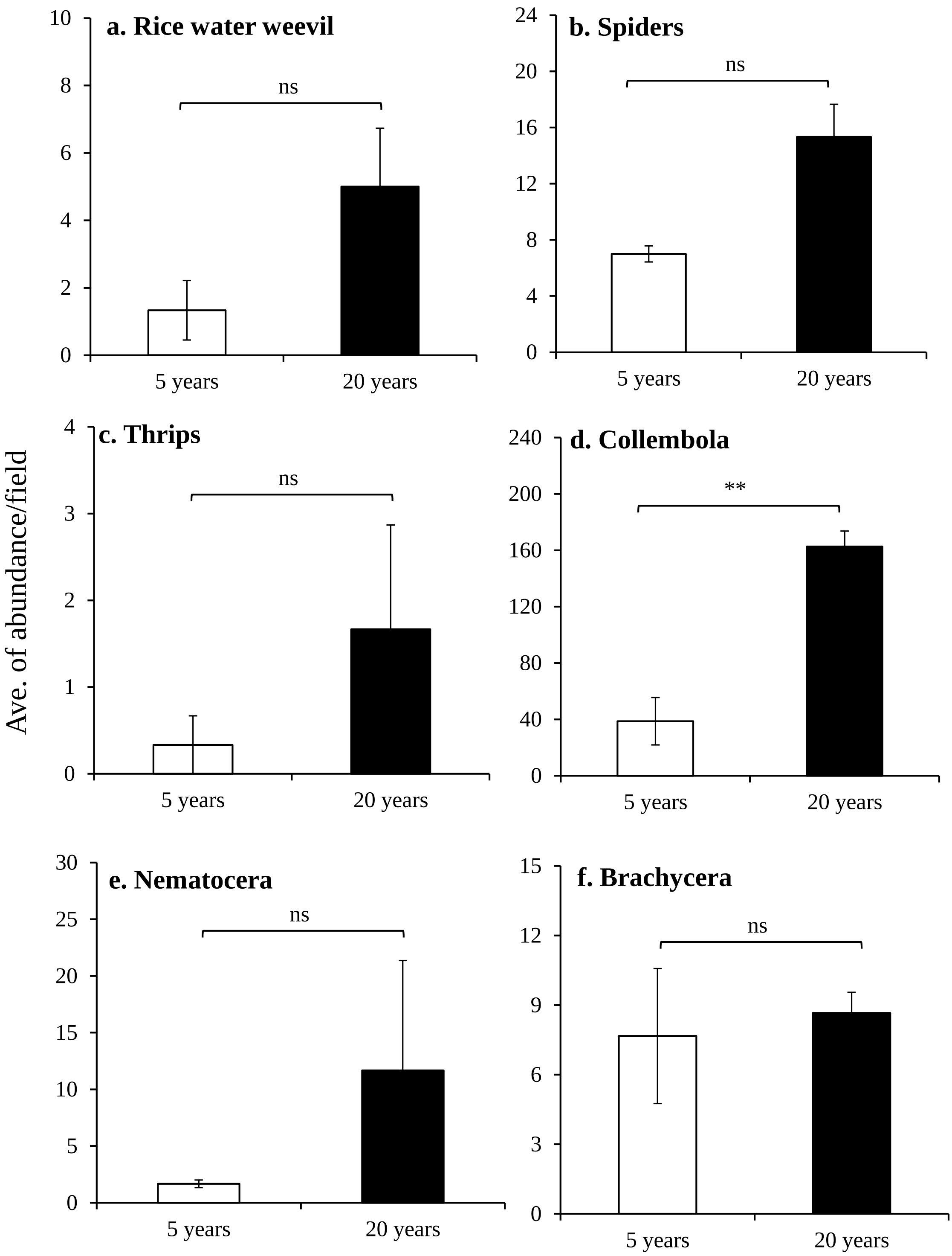

Figure 7. Abundance of rice water weevil (a), spiders (b), thrips (c), collembolla, Nematocera (e), and Bractycera (f) in aboveground ecosystem of 5 and 20 years organic paddy fields. Significant differences according to a generalized linear mixed model (GLMM) including parameters with Poisson error distribution and field as a random effect [**$p < 0.01$, ns (not significant, $p \geq 0.05$)]

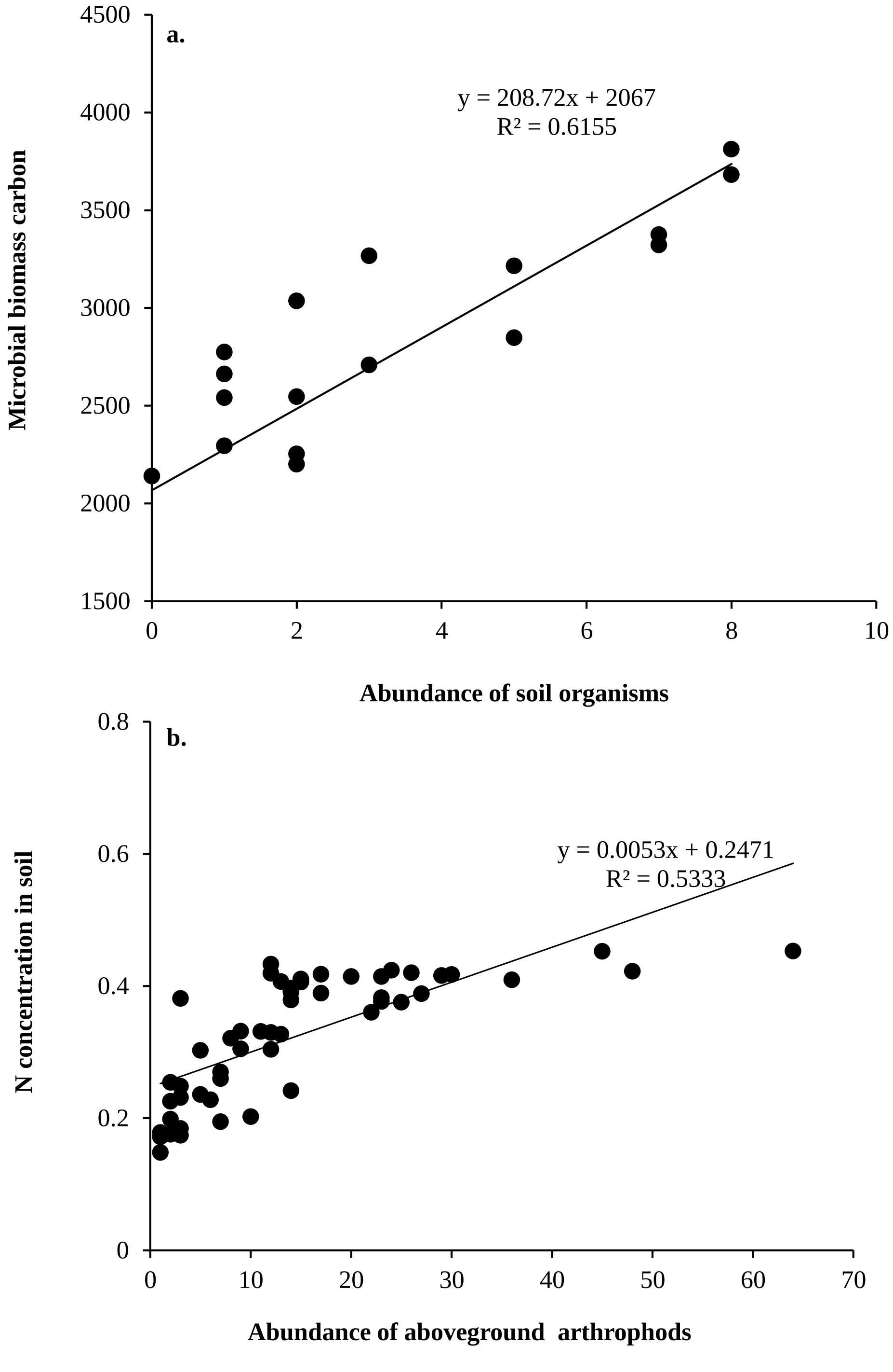

Figure 8. The relationship between total abundance of soil organisms and microbial biomass carbon ($p<0.01$) (a) and between total abundance of arthropods and N concentration of soil ($p<0.05$) in organic paddy fields.

General Discussion

Understanding the effects of bio-based nutrient on the growth of rice plants in relation to the abundance of aboveground arthropods in organic paddy fields with different histories of management is an important issue in order to achieve the sustainable rice production in organic paddy fields. Applications of organic matter in organic farming usually increase primary production and organism consumers. Green manure incorporation produces decomposition products may also affect on aquatic organisms and indirect effect on the terrestrial arthropods (Roger et al. 1991). These processes can modify the structure of the aquatic and terrestrial community in paddy field. However, considerable spatial, seasonal, and among-year variation in food availability may drive the population dynamic of the communities in organic paddy fields (White 1993; Mark and Joern 2000).

The results in chapter 2 showed that the interaction between mud snail and loach effects on the community structure of aquatic and terrestrial arthropods. Density of mud snail and loach, and their interaction altered the bio-based nutrient concentration in term of N and C and indirectly influenced the abundance of terrestrial arthropods through the growth and nutrition of rice plant. This result also showed that there is an interaction between different feeding functional of aquatic species, and it's effects on their performance. Our laboratory experiments showed that the loach decreased the mortality of snails and their interaction was negatively correlated. It suggested that the behavior of loach in the inside of soil may interfere the snail which existed on the surface of the soil as a scraper. Although the relationship between the loach and the snail were negatively correlated, our comprehensive study indicates that each species of aquatic with different functional feeding give different positive impact on the community structure organic paddy fields.

Futhermore, in chapter 3 showed that soil organisms through the soil nutrient effect on the performance of herbivores in term of the rice grasshopper (*Oxya japonica*). Our study specifically relates the soil nutrition of organic and conventional fields to differences in foliar chemical content, which in turn influence the growth and performance of *O. japonica*. We found that the N concentration was higher in the soil and plants of conventional fields, while the C concentration and C/N ratio were higher in organic fields. However, the plants grown in high N might be more tolerant of insect damage, as reflected by the high rice plant biomass in conventional paddy fields, although the abundance of *O. japonica* was high. The *O. japonica* nymphs that fed on plants grown in conventional soil also had higher rates of growth and development. In contrast, the nymphs that fed on organic plants were slower to develop and consumed less plant tissue. This result suggested that organic paddy field soil with bio-based nutrient can control the population of herbivorous insects which potentially as a pest insect.

In addition the present study showed that the aquatic and terrestrial communities were influenced by the different histories of organic paddy fields. Organic farming is an advantageous method for aquatic organisms (Yamazaki et al. 2004). Long term of organic farming can greatly enhance the basal resources such as detritus and algae due to increasing organic input every growing season (Entry et al. 1997; Kandeler et al., 1999; Garcı´a-Gil et al., 2000). Long period of bio-based nutrient management in the organic fields could foster the abundance of aquatic organisms (Chinnadurai et al. 2014). Many studies have shown that aquatic macroinvertebrate distribution has been related to the basal resources in aquatic ecosystems (Pavluk et al., 2000). Our results showed that the abundance of scrapers, detritus, and algae were greater in 20 years of organic paddy fields, suggesting that the appearance of the scraper in paddy fields were affected by the long term histories of organic paddy fields. Present study resulted that the abundance of important species of aquatic predators in paddy fields such as dragonfly larvae and diving beetle, were increased in 20 years organic paddy fields. Dragonfly and diving beetle are the bio-indicator organisms for functional agrobiodiveristy in aquatic ecosystem of paddy fields (Tanaka et al. 2011). However, the results indicated that generally the abundance and community structure of terrestrial arthropods were no significant differences between the histories of organic paddy fields in all year. It's suggested that organic fields could control the excessive nutrient in plants and in turn decreases the population of terrestrial arthropods, although the organic paddy fields have long histories as organic ones. The present study suggests that long term of organic paddy fields enhance the availability of bio-based nutrient in the fields and in turn affects the community structure in aquatic ecosystem through increasing the abundance of macro-invertebrates and their interactions.

The present study suggests that long term of organic paddy fields would enhance the availability of bio-based nutrient in the fields which in turn affected the community structure in aquatic ecosystem through increasing the abundance of macro-invertebrates and their interactions. Moreover, the long term application of bio-based nutrients in organic paddy fields could control the population of terrestrial arthropods particularly the pest insects and increase the growth and production of rice plants. This study indicated that long cultivation of organic paddy fields would improve the biotic interactions within and between aquatic and terrestrial ecosystem, which might achieve sustainability of the paddy field system and rice production.

Summary

To understand the effects of bio-based nutrient on the growth of rice plants in relation to the abundance of aboveground arthropods in organic paddy fields with different histories of management, the community structures of aquatic organisms and aboveground arthropods were revealed in relation to the growth of rice plants in the organic fields of 5 and 20 years old. The broad aim of this study was to examine (1) how the different histories in term of the period as an organic paddy field influence on the community structure of aquatic communities and (2) how these effects result in terrestrial community structures through plant-mediated interactions. This study demonstrated that the period of paddy fields cultivated with organic methods affected the aquatic community structure, although there have been no effects on the terrestrial arthropod community structure. The results also confirmed that long term of the organic paddy field increased the nutrition concentration in detritus, and these may effect on the abundance of the aquatic macro-invertebrate and their interactions. However, nutrient concentration in the rice plant was not highly changed in the long term of organic paddy fields which had low effect on the abundance of terrestrial arthropods. In addition, the rice plants in 20 years organic paddy fields had greater productivity than those in 5 years organic paddy fields.

To clarify how the interaction of aquatic organisms influences on bio-based nutrient subsidies in organic paddy fields, which directly or indirectly influence on the abundance of terrestrial arthropods, factorial experiments were conducted in the field by modifying the densities of mud snails and loaches. The results showed that the densities of the mud snails and loaches, and their interactions may alter the bio-based nutrient concentration in term of N and C and indirectly influenced the abundance of terrestrial arthropods through the growth and nutrition of rice plant. The presence of loaches as an aquatic predator decreased the

abundance of other aquatic organisms such as dipteran larvae which in turn reduced the available prey for terrestrial predators such as spiders. In contrast, mud snails improved bio-based nutrient concentration, especially in the treatment with high density of mud snails, and it increased the abundance of emergenced insects and their terrestrial predators.

In addition, to reveal the effects of soil organisms on the abundance of aboveground arthropods, field researches were conducted in 5 and 20 years of organic paddy fields. The activity of soil organisms, nutrient availability and abundance of the arthropods were examined in this study. The results showed that subsidies of organic input in the organic paddy fields increased the microbial N immobilization, denitrification, and N availability. However, it did not affect significantly on the abundance of aboveground arthropods.

The present study suggests that long term of organic paddy fields would enhance the availability of bio-based nutrient in the fields which in turn affected the community structure in aquatic ecosystem through increasing the abundance of macro-invertebrates and their interactions. Moreover, the long term application of bio-based nutrients in organic paddy fields could control the population of terrestrial arthropods particularly the pest insects and increase the growth and production of rice plants. This study indicated that long cultivation of organic paddy fields would improve the biotic interactions within and between aquatic and terrestrial ecosystem, which might achieve sustainability of the paddy field system and rice production.

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