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Ecological functions of ecotones from forest to paddy field in Japanese *satoyama* landscape

里山の森林から水田にかけての景観の推移帯が持つ生態学的機能

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Chapter I: Introduction

Satoyama is the traditional Japanese rural landscape that spreads between cities and mountains (Morimoto 2011). *Satoyama* is characterized by landscape mosaics consisting of agricultural and nonagricultural lands such as secondary forest, planted forest, grassland, and irrigation pond (Kadoya and Washitani 2011; MOE 2010), and high biodiversity is maintained through the many species that use multiple landscapes (Katoh et al. 2009). For example, the landscape mosaics of *satoyama*, where paddy fields and forests coexist in a short metric space, are convenient environment for frogs that repeat growth and reproduction between forests and paddy fields (Uruma et al 2012). In addition, the landscape mosaics in the *satoyama* seems to be a complex of independent landscape elements with different properties, but the connecting part of the landscape elements that compose *satoyama* landscape influence each other. The microenvironmental gradient formed on the transition zone of the landscape elements is a typical example. The connection part of the landscape elements (called an ecotone in this study) generally has the environmental gradient, and it makes possible for species that have different of niches to coexist depending on the degree of the gradient (Turner et al. 2004). In particular, the ecotone between forests and paddy fields would be an important habitat for plants, insects, birds, and mammals (Jiao et al. 2019). In those ecotone, forests and paddy fields are connected by paddy levees, however the ecological functions of the connecting parts of each landscape element are slightly different. In the forest adjacent to the agricultural land, a gradient of light environment is formed on the forest edge, and plant species with different light requirements can coexist (Chabrerie et al. 2013). Further, Lacasella et al. (2015) reported that the abundance and species richness of spiders and ground beetles in the ecotone from the forest to the grassland were highest at the forest edge. Those phenomena should be occurred because the environment inside and outside the forest influenced each other, and the intermediate environment was formed between the two landscape elements. On the other hand, the ecotone formed on the boundary of aquatic and terrestrial ecosystems has environmental gradient, and it functions as a habitat for various biological communities (Jiao et al. 2019; Kakudo 2009; Veen et al. 2013). This environmental gradient is confirmed remarkably on the aquatic side, and it can be due to the cause that differences in the properties of the terrestrial area (such as the shape and area of the waterside) alter the environment of the aquatic area. In particular, these effects may be large in the *satoyama* landscape where various shaped paddy fields and paddy levees are mixed in a mosaic pattern. Deepening the understanding of the relationship between the biological community and environmental gradient formed in the ecotone will be a clue to elucidate the mechanism by which *satoyama* maintains

high biodiversity. On the other hand, there are few examples of detailed researches on these factors, and the habitat function of *satoyama* ecotone remains a qualitative discussion.

Sado Island is a remote island with an area of approximately 855.25 km² located in the east of the Japan Island, and there are many *satoyama* landscapes (Fig. 1). On Sado Island, the introduction of conservation-oriented agricultural practices was recommended as part of the conservation of *Nipponia nippon*, the special natural monument of Japan, and the efforts such as reducing the pesticides and chemical fertilizers amount by more than 50% compared to the local practice and developing the paddy biotopes using fallow fields were being implemented (Niigata Sado City 2012, 2021; Uruma et al 2012). In such a farmland, much of the inorganic nutrients needed to grow crops should rely on organic matter decomposition functions driven by biological communities inhabiting at *satoyama* ecotone, and quantifying these functions is important for brush up the current efforts. In addition, the movement and excretion of biological communities maintain the nutrient cycle across various landscape elements, which may contribute to the nutrient cycle of *satoyama* basins (Jefferies 2000; Nakano et al. 1999; Natuhara 2013), and it might have the potential to maintain the high sustainability of *satoyama* ecosystems. On the other hand, in Japanese *satoyama*, large scale farm land consolidation and agricultural mechanization were carried out on many agricultural lands for the purpose of improving productivity since the 1960s, and decreased biodiversity has been confirmed because by the division of their habitat or the disappearance of the environmental gradient (Morimoto 2011). Further, in recent *satoyama*, there is concern that the functions of ecotone will decline by the wide use of pesticides or herbicides. Even on Sado Island, it can be confirmed that the *satoyama* which ecotone was divided by the farm land consolidation carried out after 1945 (Niigata Sado City 2012) or the paddy levees are managed by herbicide spraying in some areas. In recent years, there has been growing interest in the negative impacts of such anthropogenic disturbances on biodiversity and nutrient cycle of *satoyama*. However, there are few research that quantifying the effects of human activities around farmland on ecological functions of *satoyama* ecotone, and the existence of the biological communities inhabit the forest edges or paddy fields and the organic matter decomposition function driven by those communities remain unclear.

Therefore, the intention of this study was to investigate the effects of anthropogenic disturbance on ecological functions of *satoyama* ecotone focusing on the habitat function and organic matter decomposition function. In this study, we divided *satoyama* ecotone into three microtopography as follows: (1) the connection part between the forest and the paddy levee, (2) the paddy levee, and (3) the connection

part between the paddy levee and the paddy field (the transition zone between aquatic and terrestrial ecosystems), and we analyzed the effects of human activity on [1] microenvironmental factors, [2] biological communities, and [3] organic matter decomposition at each landscape element.

In the Chapter II, we focused on the difference in connectivity between forests and paddy levees, and investigated the effects of those factor on (1) environmental gradients, (2) soil animal communities, and (3) organic matter decomposition at *satoyama* ecotone. We predicted that as the connectivity between forests and paddy levees diminished, the environmental gradients formed at the *satoyama* ecotone would disappear, which should have a negative impact on the habitat function and organic matter decomposition function.

In the Chapter III, we quantified the impacts of agricultural disturbances on (1) environmental factors, (2) soil animal communities, and (3) organic matter decomposition at the paddy levee, which was the main landscape elements that form the *satoyama* ecotone. In this study, three paddy levee management methods were selected as agricultural disturbances: mowing (Control) and burning which are widely used as paddy levee management on Sado Island, and herbicide spraying which is widely used in modern agriculture in Japan. Anthropogenic disturbances on the paddy levees have negative impacts on their habitat function and organic matter decomposition function, and the influence were expected to increase in the order of mowing, burning, and herbicide spraying.

In the Chapter IV, we focused on the difference in the shape of the paddy field, and investigated the effects of those difference on (1) microenvironmental factors and (2) aquatic organism community in the paddy field. In this study, we conducted a hypothesis that the more complicated the shape of the paddy field, the larger the difference in the microenvironment in the water, and the more aquatic animals with different niches can live (the habitat function is enhanced).

In the Chapter V, under the hypothesis that the biomass of soil animal communities inhabiting paddy levees were supplied from the surrounding forests, we investigated the invasion route of soil animals communities (Oribatida and Collembola communities) at the paddy levees, and discussed whether those community could be a driver that promote the nutrient cycle across different landscape elements.

Through the above research, by quantifying the habitat function and organic matter decomposition function of *satoyama* ecotone, we aimed to elucidate the mechanism which the *satoyama* ecosystems maintain high biodiversity and sustainability.

Chapter II: Effects of connectivity between forests and paddy levees on soil animals

II-1 Introduction

Satoyama ecotones from the forests to the paddy levees have a microenvironmental gradient and enable the coexistence of various species that have different niches (Mizuno and Hashimoto 2013; Ohara and Ushimaru 2015; Turner et al. 2004; Uematsu et al. 2010). The environment of *satoyama* ecotone is greatly influenced by human activities. For example, the environment of the ecotone from forests to paddy levees is different between the area where traditional levee management is performed at undeveloped farmlands and modern levee management is performed at farmlands with infrastructure development. In the former, the ecological connectivity between the forest and the paddy levee exists, and the forest edge has vegetation structures such as mantle communities and grasslands where large agricultural machines cannot invade. Those ecotones allow the movement of biological communities between forests and paddy levees, and it would maintain the nutrient cycle around forest edges. Although, the forest edge structures are destroyed provided that large agricultural machines can approach the vegetation structures around forest edges. In the latter, the connection between the forest and the paddy levee has been physically lost by the installation of structures such as concrete waterway and farm road that divide the landscape elements. In those ecotones where the movement of biological communities were hindered by the division of forests and paddy levees, the aforementioned nutrient cycle may be lost. A detailed analysis of the effects of differences in forests and paddy levees connectivity on biological community and nutrient cycle would be a clue to understanding the mechanism of biodiversity decline in *satoyama*. However, few studies have quantitatively analyzed these effects.

Soil animals are one of the biological communities whose distribution patterns will change in response to differences in the connectivity between forests and paddy levees. Soil animals have low mobility and are sensitive to changes in the environment (Kaneko and Ito 2004; Ohkubo and Harada 2006). For this reason, it is expected that the soil animal communities inhabiting ecotone would change their distribution pattern in response to the changes in the environmental gradient caused by the division of forests and paddy levees or the disappearance of forest edge structures. In addition, the soil animal community promotes the organic matter decomposition in the soil and the nutrients circulation by feeding on litter or dead organisms, and has the role of supplying inorganic nutrients to the flora (Lavelle et al. 2006; Takeda 1994), hence it is an index of the nutrient cycle. On the other hand, there are many unclear parts about the behavior of soil animal

communities in *satoyama* ecotone.

Therefore, the intention of this study was to investigate the relationship between the difference in connectivity between forests and paddy levees and (1) environmental gradient, (2) changes in the composition of functional groups of soil animal communities, and (3) organic matter decomposition.

II-2 Materials and methods

II-2.1 Study site

The current study was conducted at the Iwakubi site (37°56'36.36"N, 138°29'23.35"E), Matsugasaki site (37°54'52.86"N, 138°28'38.92"E), and Agata site (38°3'28.75"N, 138°27'7.71"E), located on Sado Island, Niigata, Japan. The Iwakubi site has traditional paddy levee management, and approximately 460 terraced paddy fields that formed on the slopes of hills were registered as the Globally Important Agricultural Heritage (GIAHS). In the Iwakubi site, there are no artificial constructions that divide the forests and paddy levees in many of the forest edges, and most of them are composed of mantle communities or grasslands that consisting of shrubs, vines, and herbs near the forest edge. The Matsugasaki and Agata sites have modern paddy levee management, and farm land consolidation was completed in 1983 and 1980, respectively. In these two sites, forest edge structures such as mantle communities or grasslands were not confirmed at forest edges, and the division of ecotone by artificial constructions was observed between the forests and the paddy levees. In all study sites, the forest around the paddy fields consisted of broadleaved secondary forests (such as *Quercus serrata*, *Quercus crispula* Blume, *Carpinus tschonoskii* and *Quercus salicina*) and *Cryptomeria japonica* plantations, and moist litter was deposited on the surface of the forest floor. At the paddy levees of the Iwakubi site, the density of herbs covering the ground surface was low, and the ground surface was exposed at some paddy levees. In addition, moist litter were existed on the ground surface in these paddy levees. At the Matsugasaki and Agata sites, in many of the paddy levees, short grasses were densely grown on the ground surface in a crawling manner, and dry litter was present on top of them. In addition, the use of herbicides on the paddy levees was not confirmed at the Iwakubi, Matsugasaki, and Agata sites.

II-2.2 Sampling design

Twelve investigation points were selected, including six points at the Iwakubi site (selected from June to September 2016), three points each at the Matsugasaki and Agata sites (selected from April to June 2017).

Each investigation point was classified into the following three landscape types according to the degree of division of the ecotone (Table 1; Fig. 2). Landscape type I means the state of landscape elements gradually shift from the forests to the paddy levees because there are no artificial constructions that divide the ecotone and there are forest edge structures such as mantle communities or grasslands (Table 1; Fig. 2). Landscape type II means the state of only the connection between the forests and the paddy levees were maintained because there are no artificial constructions that divide the ecotone and there are no forest edge structures (Table 1; Fig. 2). Landscape type III means the state of the connection between the forests and the paddy levees were physically lost because the ecotone was divided by artificial constructions and there are no forest edge structures (Table 1; Fig. 2). A belt transect which straddles the paddy levee fields from the forest with 40-90 m was set up on each investigation point (Fig. 3). Moreover, five subplots (1 m square) were placed on each belt transect (two points at the forest, one point at the forest edge, and two points at the paddy levee) at 8-10 m intervals (Fig. 3). In landscape type I, a forest edge subplot was set up the point where the canopy of the forest was interrupted above the forest edge structure. In landscape type II and III, a forest edge subplot was set up the point where the canopy of the forest was interrupted. Further, when the distance between the forest edge and the paddy levee exceed 10 m because the artificial construction that divides the ecotone, a subplot of paddy levee 1 was placed at the point where those constructions were interrupted.

II-2.3 Microenvironmental factors

The soil moisture (vwc. %), soil hardness ($N\ cm^{-2}$), canopy openness (%), and litter amount ($g\ m^{-2}$) were measured in each subplot as microenvironmental factor.

The soil moisture was measured from the A0-L-layer to a depth of 7.5 cm in the ground using a TDR soil moisture meter (FIELDSCOUT TDR100, Soilmaxx Inc., Tokyo, Japan). Soil moisture was measured 48 h after rainfall. The measurement was carried out 7 times, and the average value was taken as the soil moisture content of each subplot. The soil moisture was measured on 7 October 2016 at the Iwakubi site and 5 October 2017 at the Matsugasaki and Agata sites.

Soil hardness was measured by piercing the Push-Cone soil hardness meter (DIK-5553, Daiki Rika Kogyo Co., Ltd., Saitama, Japan) vertically from the top of the A0-L-layer. Soil hardness was measured 48 h after rainfall. The measurement was carried out 7 times, and the average value was taken as the soil hardness of each subplot. The soil hardness was measured on 7 October 2016 at the Iwakubi site and 5

October 2017 at the Matsugasaki and Agata sites. The measured soil hardness was converted from indicator value (mm) to absolute value (N cm^{-2}) using the conversion formula of Yamanaka and Matsuo (1962).

To evaluate the light environment of each subplot, canopy openness was measured using the hemispherical photography (camera: COOLPIX 4500, lens: fisheye converter FC-E8, Nikon Corporation, Tokyo, Japan). The canopy openness was measured from 24-31 October 2016 at the Iwakubi site and 24-30 October 2017 at the Matsugasaki and Agata sites. The photographs were taken at a height of 30 cm from the ground surface of each subplot. The photographs were binarized to white in the open area and black in the shadow area using the hemispherical photograph analysis program CanopOn 2 (Takenaka 2009), and the canopy openness was measured.

Litter was collected concurrently with the investigations of soil animals. The litter was collected from the A0-L layer present in a 25 cm square frame placed in each subplot. The collected litter was dried at 60 °C for 48 h, and the dry weight was measured.

II-2.4 Soil animals

Soil animals were collected by two methods at each subplot: hand sorting and using Tullgren funnels. The targets of the investigation were limited to soil animal taxa that inhabit the underground or ground surface and are expected to be strongly affected by the artificial constructions which divide the ecotone. The investigation was conducted from 24-31 October 2016 at the Iwakubi site and 24-30 October 2017 at the Matsugasaki and Agata sites.

For hand sorting, a 25 cm square frame was set on the surface of each subplot of belt transect and the soil in the frame was sampled to a depth of 10 cm. To facilitate the collection of soil animals, the soil was sorted to the fine fraction using a 30 cm diameter sifter (1 cm mesh) and placed in a tray. Soil animals were collected for 40 min by hand sorting. Collected soil animals were stored in 80% ethanol solution.

The soil samples used for the Tullgren funnel were collected at 5 cm cube in each subplot. The collected soil samples were set in a Tullgren funnel, and soil animals were extracted for 48 h. The Tullgren funnel was made by installing a 13 cm diameter funnel at the lower stage of a 47 cm high metal rack with two stages. A 10 cm diameter net (3.9 mm mesh) was installed inside the funnel, and a soil sample was set on it. The light was irradiated using a 40 W incandescent bulb from a height of 15 cm above the soil sample. Soil animals that concentrated at the bottom of the funnel were captured by sample tubes containing 80% ethanol solution.

The collected soil animals were classified into 24 taxa based on Aoki (2015), and then categorized into six functional groups based on their eating habits as follows: predator that prey on other soil animals, decomposer that eat leaf litter and mineralize, shredder that eat leaf litter and crush, microbial predator that eat fungi or microbes, phytophagous that eat live plant tissue, and Omnivore that eat live animals and plants tissue or their litter (Table 2). Moreover, Collembola are categorized into three types according to the shape of the mouth: masticatory type, absorption type, and predatory type, masticatory type species account for 80-90% of their abundance in the forest ecosystem (Aoki 2015; Takeda 2002). However, diverse functional groups such as decomposer, microbial predator, and phytophagous coexist in the masticatory type Collembola (Aoki 2015; Shiraishi and Enami 2002; Takeda 2002), it is difficult to classify into each functional group. Therefore, “Collembola” was treated as one functional group. For the analysis of the functional group composition of soil animals, four functional groups (predator, decomposer, shredder, and Collembola) were used. Those functional groups appeared at each of the Iwakubi site, Matsugasaki site, and Agata site more than 100 individuals, and accounting for approximately 97% of the collected soil animals.

II-2.5 Organic matter decomposition

To evaluate the impact of the ecotone division, a decomposition investigation of organic matter was conducted by the Bait-Lamina test (Kratz 1998). This method used bait sticks with samples inserted at equal intervals, and it quantifies the organic matter decomposition rate by recording the number of holes where the sample has disappeared. It can measure the surface and underground organic matter decomposition rates simultaneously. Moreover, it is assumed that the Bait-Lamina test is more suitable for investigations on agricultural lands where anthropogenic disturbance is frequent because the investigation period is shorter (approximately 10-20 d) comparing with the litter-bag method.

Bait sticks were made by cutting 1 mm thick acrylic plates to 120 mm in length and 6 mm in width with 16 opening holes with a 1.5 mm diameter at 5 mm intervals from the edge of the stick to 10 mm (Fig. 4). In the holes of the bait stick, a paste that contained cellulose powder (BUBBLE STAR Co., Ltd., Kanagawa, Japan) and powder agar (Ina Food Industry Co., Ltd., Nagano, Japan) for the prevention of leaching was packed at a rate of 7:3 and dried for 24 h at 25 °C.

On 20 November 2018, bait sticks were installed at Iwakubi, Matsugasaki, and Agata sites. Ten bait sticks were installed at both ends of each belt transect and at forest edge (subplot of forest 1, forest edge,

and paddy levee 2). After the removal of the A0-L-layer of the soil, the bait stick was inserted perpendicular to the ground and the top hole touched the ground surface (F-layer). The bait stick was collected 10 d after setting, and the number of holes with a missing sample was recorded. In each belt transect, approximately 1-10 bait stick defects were confirmed which were presumed to be caused by small mammals, and those samples were deleted from the data analysis.

II-2.6 Statistical analyses

Using statistical software (KyPlot 5.0 Version: 5.0.0, KyensLab Inc., Tokyo, Japan), multiple comparisons (Tukey-Kramer) was performed to compare microenvironmental factors, soil animal abundance (predator, decomposer, shredder, and Collembola) between the landscape elements. Using the free software R ver. 3.3.2 (R Core Team 2016), the relationship between the soil animal communities and landscape structural factors or microenvironmental factors was calculated by nonmetric multidimensional scaling (NMDS). The analysis was performed by the R package *vegan* and *MASS* (Oksanen 2015). The dissimilarity of communities among subplots was calculated with the Bray-Curtis method. Regarding each landscape structural factor and microenvironmental factor experiment, 999 permutations were performed, and factors that showed significant probability ($P < 0.05$) were projected as vectors on the NMDS plane (Oksanen 2015). For soil animal data, the number of predator, decomposer, shredder, and Collembola in each subplot were used. For landscape structural factor data, the presence or absence of forest edge structure (1 if present, 0 if not present) and the artificial construction length (m) that divides the ecotone were used. For microenvironmental factor data, the soil moisture, soil hardness, canopy openness, and litter amount value in each subplot were used. The organic matter decomposition rate in each landscape type was compared by performing a Pearson chi-squared test among the forest, forest edge, and paddy levee. For the analysis, the function `chisq.test` in the free software R ver. 3.3.2 (R Core Team 2016) was used.

II-3 Results

II-3.1 microenvironmental factors

The soil moisture in the landscape type III (forest edge structure: not exist, ecotone fragmentation: exist) was significantly higher in the paddy levee subplots than in the forest and forest edge subplots (Fig. 5a, Tukey-Kramer, $P < 0.05$). On the other hand, the soil moisture in the landscape type I (forest edge structure: exist, ecotone fragmentation: not exist) was higher in the paddy levee subplots, but the variation for each

subplot was large, and there was no significant difference between the other landscape elements (Fig. 5a, Tukey-Kramer, not significant (n.s.)). In addition, the soil moisture in the landscape type II (forest edge structure: not exist, ecotone fragmentation: not exist) was higher in the forest 1 subplot than in the forest edge and paddy levee 2 subplots, but there was not significant difference (Fig. 5a, Tukey-Kramer, n.s.).

The soil hardness in the landscape type III was significantly higher in the paddy levee 2 subplot than in the forest subplots (Fig. 5b, Tukey-Kramer, $P < 0.05$). On the other hand, the soil hardness in the landscape types I and II were higher in the paddy levee subplots than in the other landscape elements, but there was not significant difference (Fig. 5b, Tukey-Kramer, n.s.). In the all landscape types, the soil hardness in the paddy levee subplots tended to have large variation than in the other landscape elements (Fig. 5b).

In the all landscape types, the canopy openness in the paddy levee subplots were higher than in the forest and forest edge subplots (Fig. 5c, Tukey-Kramer, $P < 0.05$).

The litter amount in the landscape type III was significantly higher in the forest 1 subplot than in the forest edge and paddy levee subplots (Fig. 5d, Tukey-Kramer, $P < 0.05$). On the other hand, in landscape types I and II, high litter amount values were observed in each of the forest edge and forest 2 subplots, but there were no significant difference between the other subplots (Fig. 5d, Tukey-Kramer, n.s.).

II-3.2 Soil animal communities

In this study, total of 7806 soil animals (1494 predators, 2813 decomposers, 838 shredders, and 2661 Collembolas) were collected.

In the all landscape types, the predator abundance was not significantly different between the all subplots (Fig. 6a, Tukey-Kramer, n.s.). However, in the landscape type III, the predator abundance in the paddy levee subplots were lower than in the forest subplots. In those subplots, the abundance of Araneae, Mesostigmata, and Prostigmata tended to be approximately 30-70% lower than in the forest subplots. In addition, the predator abundance in the landscape type I tended to have large variation in the paddy levee 1 subplot (Fig. 6a). In those subplots, *Carabidae* appeared intensively.

In the all landscape types, the decomposer abundance was not significantly different between the all subplots (Fig. 6b, Tukey-Kramer, n.s.). On the other hand, the decomposer abundance in the paddy levee subplots of the landscape type III tended to be approximately 60-75% higher than in the paddy levee subplots of other landscape types. Moreover, in the forest 2 subplot of landscape type I and paddy levee 2 subplot of landscape type III, there was a tendency for the variation of the decomposer abundance to be

large (Fig. 6b). In those subplots, the Oribatida, Oligochaeta, and *Enchytraeidae* appeared intensively.

The shredder abundance in the landscape type II was significantly higher in the forest 2 subplot than in the paddy levee subplots (Fig. 6c, Tukey-Kramer, $P < 0.05$). On the other hand, in other landscape types, there was no significant difference in the shredder abundance at any subplot (Fig. 6c, Tukey-Kramer, n.s.). In addition, the shredder abundance in the paddy levee subplots of landscape type III tended to be approximately 80-90% higher than in the paddy levee subplots of other landscape types. Moreover, in the paddy levee 2 subplot of landscape type III, there was a tendency for the variation of the shredder abundance to be large (Fig. 6c). In those subplots, Isopoda appeared intensively.

In the all landscape types, the Collembola abundance was not significantly different between the all subplots (Fig. 6d, Tukey-Kramer, n.s.). On the other hand, in the forest subplots of the landscape type III, *Isotomidae* appeared intensively in some subplots. Further, in the paddy levee subplots of the landscape type III, *Entomobryidae* appeared intensively in some subplots.

The NMDS analysis ordered the functional group composition of soil animal communities in each subplot and placed on the coordinate plane (Fig. 7). The NMDS stress value was 0.11. After 999 permutation tests, three factors were selected that could affect each functional group: the length of the artificial construction that divides the ecotone (Artificial construction length), soil moisture, and canopy openness (Fig. 7). On the coordinate plane, the soil moisture and canopy openness vectors were placed in the third quadrant while artificial construction length vector was placed in the fourth quadrant (Fig. 7). The set of subplots most dominated by predator was in the negative direction of the artificial construction length, soil moisture, and canopy openness vectors (Fig. 7). The set of subplots most dominated by decomposer was in the positive direction of the artificial construction length, soil moisture, and canopy openness vectors (Fig. 7). The set of subplots most dominated by shredder was in the positive direction of the artificial construction length vector (Fig. 7). The set of subplots most dominated by predator consisted of the forest and forest edge subplots in the landscape type I and the forest and paddy levee subplots in the landscape type II (Fig. 7). The set of subplots most dominated by decomposer included the forest, forest edge, and paddy levee subplots of all landscape types (Fig. 7). The set of subplots most dominated by shredder consisted of the forest subplot in the landscape type II and the forest and paddy levee subplots in the landscape type III (Fig. 7). The set of subplots most dominated by Collembola included all subplots except the forest subplots in the landscape type II and the forest edge subplots in landscape type III (Fig. 7).

II-3.3 Bait-Lamina test

The organic matter decomposition rate of landscape type I was significantly higher in the forest edge subplot than forest and paddy levee subplots (Table 3, Pearson chi-squared test, $P < 0.01$). The organic matter decomposition rate of landscape type II was not significantly different between the all subplots (Table 3, Pearson chi-squared test, n.s.). The organic matter decomposition rate of landscape type III was significantly higher in the paddy levee subplot than forest and forest edge subplots (Table 3, Pearson chi-squared test, $P < 0.05$).

II-4 Discussion

II-4.1 Effect of differences in landscape types on environmental gradients

In this study, soil moisture, soil hardness, and canopy openness tended to decrease from the paddy levee to the forest in most landscape types. Ohara and Ushimaru (2015) reported that the soil moisture and the amount of sunshine in the ecotone where the paddy levee and the forest were adjacent decrease significantly from the paddy levee to the forest edge, which was consistent with the results presented here. However, the soil moisture in the landscape type I varied widely between subplots on the paddy levee, and no significant difference was detected between the landscape elements. This result would be due to the fact that all of these study sites were terraced paddy fields formed in mountainous valleys (called a “yato” in Japanese), and that each paddy field has not been farm land consolidation. For example, in the fields formed on the yato, the soil moisture was higher than in the flat fields because the supply of groundwater from the surrounding forests (Arita and Kobayashi 1999). In this study as well, it is possible that the high water supply was maintained at the paddy levees in the landscape type I. Moreover, at the paddy levee in the landscape type I, waterways made by soil were used in all investigation points, and soil replacement or installation of modern drainage due to farm land consolidation were not done. At the paddy levees in the landscape type I, it is highly possible that the drainage capacity of each subplot was different due to the influence of their soil quality or location. As a result, the variation of soil moisture increased at the paddy levees in the landscape type I. In addition, in the landscape type II, although there was no significant difference, the soil moisture in the forest was higher than in the forest edge and the paddy levee, and the results were different from those reported by Ohara and Ushimaru (2015). Those results may be due to the combined action of the characteristics of the yato where landscape type II was located and the influence of litter which was abundant in the forest. At the forests in the landscape type II, it was predicted that a large

amount of litter was present on top of the water-rich soil which suppressed evapotranspiration from the ground surface.

The gradient of the litter amount was different for each landscape type, and the peak value tended to recede toward the inside of the forest as the division strength of ecotone increased. At the paddy levees in the landscape type III, all litter was derived from the herbs that grow on the paddy levees, because there are no vines that make up the forest edge structure or herbs that dominate the forest (Table A1). On the other hand, the constituent species of vegetation on the paddy levees in the landscape type I include not only *Equisetum arvense* or forbs that dominate the paddy levees but also *Polygonum thunbergii* or *Dioscorea japonica* that make up the forest edge structure and seedlings of *Carpinus tschonoskii* that dominate the forest edges or forests (Table A1). Perhaps the litter at those subplots was a mixture of herbaceous species that dominate the paddy levees and tree, vine, and herbaceous species that dominate the forest edges or the forests. In the landscape type I, it was suggested that the supply route of litter across different landscape elements was maintained and the amount of litter near the forest edge increased, because there was no division of the ecotone. In the landscape type II, it was confirmed that the amount of litter near the forest edge was low even though the connection between the forests and the paddy levees was maintained. In the landscape type II, since there are plant species (for example, *Dioscorea japonica*) that grow both in the forests and the paddy levees, it was predicted that the litter supply route might be slightly maintained (Table A1). However, at the forest edges in the landscape type II, the density of the shrub layer that constituted the forest edge structure was low, and it was assumed that the litter amount near the forest edges had been decrease.

II-4.2 Effect of differences in landscape types on soil animal communities

The functional group composition of soil animal community changed under the influence of landscape structural factors and microenvironmental factors. The results of the NMDS analysis showed that predator prefer the forests with low soil moisture and canopy openness. On the other hand, few subplots were dominated by predators at the paddy levees in the landscape type III, and it suggested that the artificial constructions which divide the ecotone have a negative impact on the predator that inhabit the paddy levees. The main taxa of predator such as Araneae and Mesostigmata use the litter or humus layer in the forest as living spaces (Aoki 2015; Bultman and Uetz 1982). Moreover, Haskell (2000) reported that the division of forests associated with road construction had a negative impact on the abundance of Araneae and Chilopoda.

Predator mainly inhabit the forest, and while the distribution can be expanded outside the forests if the connection between the forest and the farmland is maintained, it may be vulnerable to the division of the ecotone.

Decomposers and shredders include taxa that can inhabit a wide range of environments, and those functional groups dominated the paddy levees that separated from the surrounding forest by farm land consolidation. Oribatida that a major taxon of decomposers, is present in any soil containing organic matter, from forest soils to urban planting soils (Aoki 2015). In addition, Isopoda that a major taxon of shredder is widely distributed from coasts to forests (Kaneko 2007). Moreover, Riutta et al. (2012) reported that microenvironmental factors such as soil moisture did not affect the abundance of Isopoda and Diplopoda. On the other hand, at the paddy levees in the landscape type III, it was confirmed that the abundance of decomposer and shredder had large variation in each subplot, even though the difference in microenvironmental factors for each subplot was smaller than in the landscape type I. Those results may be because the predation pressure on the decomposer and shredder decreased as the predator population density decreased at the paddy levees. For example, Araneae that low abundance has been observed at the paddy levees in the landscape type III, contribute to population control by preying on other small arthropods (Kaneko 2007). In the landscape type III, the division of the ecotone can hinder the invasion of predators from the forest and form the paddy levee environment with different predator abundance at each subplot. As a result, the predation pressure on the decomposer and shredder differed from each subplot, and it is presumed that the difference in abundance of those functional groups increased.

Collembola maintained abundance in all landscape types. Ponge et al. (2003) reported that Collembola had different habitats and resistance to disturbance depending on the species, and the abundance was maintained by species that adapted to the environment not only in forests and grasslands but also in agricultural lands where pesticides or fertilizers were used. Moreover, Cole et al. (2008) reported that there were species in Collembola whose abundance increased due to the addition of intermediate physical soil disturbance. In the current study as well, it is assumed that no clear relationship was shown between the set of subplots most dominated by Collembola and landscape structural factors or microenvironmental factors, because Collembola that collected in each subplot would be composed of taxa that prefer different habitats or environments.

II-4.3 Effect of differences in landscape types on organic matter decomposition rate

The organic matter decomposition rate at the landscape type III was the highest on the paddy levee, which overlapped with the distribution of decomposer and shredder. Moreover, at one subplot, the presence of *Enchytraeidae* which feeding on samples was confirmed when bait sticks were collected (Fig. A1). It was suggested that these functional groups drive the nutrient cycle by decomposing litter at the paddy levees in the landscape type III. On the other hand, the organic matter decomposition rate at the landscape type I was highest at the forest edges where litter tended to be abundant, and no clear relationship was observed with each functional group of soil animals. It is presumed that this is because the nutrient cycle of the ecotone in the landscape type I was driven not only by the soil animal community but also by the soil microbial community. Fungi or bacteria in forest soil play a role in decomposing litter that crushed by large soil animals (Yamashita et al. 2013). Further, Zak et al. (2003) reported that increasing the diversity of plants in the forest increased the supply of litter, and promoted the decomposition activity by microbial community in the soil. It is assumed that the same phenomenon was observed in the results of this study, and to better generalize our findings, it is necessary to more detailed research.

Appendix 1 Vegetation of each belt transect

To clarify the main sources of litter in each subplot, the vegetation investigation was conducted from August 30 to September 3 and October 24-31, 2016 at the Iwakubi site and September 14-15 and 20-30, 2017 at the Matsugasaki and Agata sites. The investigation was conducted in the forest, forest edge, and paddy levee in each belt transect, and the coverage and natural height were measured. At the forest and forest edge, one quadrat (5 m square) was installed for investigating tree and herb species, and one quadrat (1 m square) was installed for investigating herbs species in the paddy levee. Table A1 showed the vegetation of each investigation site.

Appendix 2 *Enchytraeidae* feeding on the sample of bait stick

When collecting the bait stick, the existence of *Enchytraeidae* feeding on the sample was confirmed on the paddy levee of landscape type III (Fig. A1).

Chapter III: Impacts of agricultural disturbances on soil animals in terraced paddy field levees

III-1 Introduction

The paddy field landscape formed on the slopes of hills (called terraced paddy fields) has a linear seminatural grassland (paddy levee) on the adjacent surface of the forest and the paddy field. Paddy levees have various agricultural functions in rural landscapes, such as water retention, human or cattle footpaths, and cattle feed sources (Fukamachi et al. 2005). Additionally, paddy levees have various ecological functions. The most significant ecological function of the paddy levee is the formation of an ecotone that extends from paddy fields to forests. These ecotones have a gradient microenvironment and enable the coexistence of various species (Mizuno and Hashimoto 2013; Ohara and Ushimaru 2015; Uematsu et al. 2010). Furthermore, these biological communities should contribute to the nutrient supply from the paddy levee to the farmland. A typical example is organic matter decomposition by soil animals. Furukori et al. (2020) reported that stable soil animal communities occur in paddy levees and drive organic matter decomposition in the *satoyama* ecotone. In terraced paddy field landscapes, the inorganic nutrients generated by soil animal communities should dissolve in rainwater and flow into paddy fields because each paddy field is connected by a sloping paddy levee. Additionally, paddy levees account for approximately 20-30% of the total area of terraced paddy field landscapes (Mori et al. 2008; Tabata 1997; Uematsu and Ushimaru 2013) and the inorganic nutrients supplied from paddy levees can be an important resource for rice growth. However, in *satoyama*, seminatural grasslands existing on paddy levees have recently shown rapid decreases due to changes in farmland management methods and increases in abandoned cultivated land (Arita and Kobayashi 1999; Natuhara 2013; Yamada et al. 2005).

In traditional *satoyama*, the seminatural grasslands on paddy levees are maintained by regular mowing approximately four times a year (Seo and Kido 2000). However, to reduce the labor of regular mowing, herbicides have recently been widely used as a paddy levee management method (Matsumura et al. 2014). Additionally, paddy levee management in some areas is traditionally performed by burning (Matsumura et al. 2014), although this method has become less frequent in recent years. These trends in paddy levee management could possibly affect the ecology of the paddy levee. Kawano et al. (2009) reported that the habitat function of the paddy levee depends on its management method, and many rare herb species grow in areas managed by regular mowing or burning. However, more studies are required to understand the impact of burning and herbicide sprays on the ecology of paddy levees. In particular, most studies have

focused on the relationship between levee management methods and plant species diversity, whereas few have examined the soil animals or organic matter decomposition of paddy levees.

Soil animals are one of the biological communities that are deeply involved in the nutrient cycle at paddy levees. Soil animal communities increase the access of bacteria and fungi to litter by cutting and chewing litter and assisting in litter decomposition (Lakshmi et al. 2020; Yang et al. 2020). Moreover, soil animals promote the decomposition of organic matter by digesting litter or feeding on soil microbial communities, which increases their decomposition activity and the available nutrients in soil (Hasegawa et al. 2017). Thus, soil animal communities are essential in the early stages of litter decomposition. Nevertheless, few studies have quantified the organic matter decomposition function of soil animal communities in *satoyama* ecosystems because most of their roles consist of indirect effects mediated by soil microbial communities. Additionally, soil animals are known to be sensitive to environmental changes (Bardgett and Cook 1998; Ohkubo and Harada 2006), and these communities may change in response to differences in paddy levee management methods. For example, Melman et al. (2019) reported that tillage and crop residue management promote rapid changes in soil macrofauna abundance and community composition. Moreover, Wang et al. (2015) reported a negative correlation between the decrease in soil pH due to the use of inorganic or organic fertilizers on agricultural land and the abundance of soil microarthropods. On the other hand, when investigating actual farmland, the direct causal relationship between specific management methods and soil animal communities is often unclear because it is difficult to eliminate the effects of differences in soil quality and location (Mizuguti et al. 2011).

Therefore, the intention of this study was to investigate burning and herbicide disturbance effects on (1) microenvironmental factors, (2) soil animal communities, and (3) organic matter decomposition at paddy levees by field manipulation experiments.

III-2 Materials and methods

III-2.1 Study site

The current study was conducted at the Kisenjo experimental field of Niigata University, which is located on Sado Island, Niigata, Japan (38°00'51.93"N, 138°28'42.29"E). The experimental field was a terraced paddy field that was abandoned from 1972 to 2003 and converted to a wetland biotope after 2003. Since cultivation ceased in 1972, herbicides or other pesticides have not been applied in these areas. Each biotope had almost the same shape as the paddy fields and levees during rice cultivation. Each paddy levee was

maintained by mowing approximately one to three times per year since 2003. Each paddy levee consisted of a flat top and a slope connecting the lower paddy field. The uppermost paddy levees were not submerged because the overflowing water was drained by the narrow grooves installed at each paddy field. The vegetation of each paddy levee is composed of common herbaceous species on the paddy levees on Sado Island (such as *Artemisia indica*, *Houttuynia cordata*, *Miscanthus sinensis*, and *Persicaria thunbergii*) and some mountainous species (such as *Boehmeria tricuspis* and *Petasites japonicus*). The forest around the abandoned paddy fields consisted of secondary forests (such as *Pinus densiflora* and *Quercus serrata*) and *Cryptomeria japonica* plantations. The study site was within the distribution area of mesic brown forest soil, and there was a plowed layer of 10-30 cm in the place that was used as a terraced paddy field (Tsuji and Nakata 2006). The mean annual temperature and precipitation in the study area in 2019 were 14.6 °C and 1456.5 mm, respectively (JMA 2020).

III-2.2 Sampling design

Nine levees were randomly selected, and a belt transect with a length of 10 m and a width of 2 m was set up on each. Moreover, three subplots (1 m square) were placed in each belt transect at 5 m intervals. Each belt transect was established in the uppermost area of the paddy levees. Most of the belt transects were installed on paddy levees that were independent of each other, and if the paddy levees were adjacent, the distance between the belt transects was at least 10 m. All grasses on the paddy levees were cut to a height of 5 cm (24-28 May 2019) to equalize the initial environmental conditions of each belt transect (Fig. 8). Each belt transect was categorized into three experimental plots as follows: mowing only (“control plot” in this study), mowing and burning (“burning plot” in this study), and mowing and spraying herbicide (“herbicide plot” in this study). The experiments were performed three times (21-23 July 2019, 19-21 August 2019, and 9 September 2019) at each belt transect (Fig. 8).

The burning experiment was performed after mowing using an agricultural burner (Kusayaki GT-220, Shinfuji Burner Co., Ltd., Aichi, Japan). The flame temperature of this burner was 1100 °C, and its calorific value was 19.8 kW (17000 kcal h⁻¹). The experiment was performed for 80 min per belt transect (4 min m⁻²). JAEA (2011) investigated the temperature change of the ground surface of brown forest soil using an agricultural burner (Kusayaki GT-500, Shinfuji Burner Co., Ltd., Aichi, Japan), and it was observed that the temperature rose to approximately 500 °C after burning for 5 min. In addition, JAEA (2011) reported that a temperature rise to approximately 50 °C was observed at a depth of 3 cm in the soil after burning for

5 min; however, no remarkable changes in temperature were observed at a depth of 5 cm. In this study, a similar or slightly smaller temperature change was assumed.

The herbicide experiment was performed using Grass-Short, which consisted of 3.0% bispyribac-sodium ($C_{19}H_{17}N_4NaO_8$), 0.8% sodium borate, and 96.2% surfactant and water (KH026-06, Kumiai Chemical Industry Co., Ltd., Tokyo, Japan). Bispyribac-sodium is a selective, systemic postemergence herbicide used to control grasses and broad leaf weeds in paddy fields (Chirukuri and Atmakuru 2015). The herbicide liquid was made by diluting Grass-Short 100 times with water and spraying 2 L per belt transect ($100 L 10 a^{-1}$) after mowing the paddy levees.

The investigation was conducted three times: before the experiment (Fig. 8, 1-10 July 2019, “Before experiment” in this study), after the first experiment (Fig. 8, from 24 July to 4 August 2019, “Experiment once” in this study), and after the third experiment (Fig. 8, 19-30 September 2019, “Experiment thrice” in this study).

III-2.3 Microenvironmental factors

The soil moisture (vwc. %), litter amount ($g m^{-2}$), pH, and electrical conductivity (EC, $\mu S cm^{-1}$) were measured in each subplot as microenvironmental factors.

The soil moisture was measured from the A0-L-layer to a depth of 7.5 cm in the ground using a TDR soil moisture meter (FIELDSCOUT TDR100, Soilmaxx Inc., Tokyo, Japan). Soil moisture was measured 48 h after rainfall. The measurement was carried out 7 times, and the average value was taken as the soil moisture content of each subplot.

Litter was collected concurrently with the investigations of soil animals. The litter was collected from the A0-L layer present in a 25 cm square frame placed in each subplot. The collected litter was dried at 60 °C for 48 h, and the dry weight was measured.

The soil sample used for pH measurements was collected at 0-5 cm depth in each subplot using a 100 ml stainless sampling tube (DIK-1801, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). Those samples were collected from three spots in each subplot and bulked together. Each sample was air-dried at 20-30 °C for 48 h. The mixture of dry soil samples and distilled water at a ratio of 20 g:100 ml was allowed to stand at 20-30 °C for 1 h, and the pH value of the supernatant was measured using a digital pH meter (72788, Shinwa Rules Co., Ltd., Niigata, Japan).

The soil sample used for EC measurements was collected at 0-5 cm depth in each subplot using a 100 ml

stainless sampling tube (DIK-1801, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). Those samples were collected from three spots in each subplot and bulked together. Each sample was air-dried at 20-30 °C for 48 h. The mixture of dry soil samples and distilled water at a ratio of 20 g:100 ml was allowed to stand at 20-30 °C for 1 h, and the EC value of the supernatant was measured using a digital soil salinity meter (72799, Shinwa Rules Co., Ltd., Niigata, Japan).

III-2.4 Soil animals

Soil animals were collected by two methods at each subplot: hand sorting and using Tullgren funnels. The targets of the investigation were limited to soil animal taxa that inhabit the underground or ground surface and are expected to be strongly affected by burning and herbicide disturbance at paddy levees. The collected soil animals were classified into soil macrofauna and mesofauna based on Kaneko (2007) and Aoki (2015).

For hand sorting, a 25 cm square frame was set on the surface of each subplot and the soil in the frame was sampled to a depth of 10 cm. To facilitate the collection of soil animals, the soil was sorted to the fine fraction using a 30 cm diameter sifter (1 cm mesh) and placed in a tray. Soil animals were collected for 50 min by hand sorting. The collected soil animals were recorded with their taxa and abundance and then returned to each subplot to mitigate the impact of three investigations conducted in a short period.

The soil samples used for the Tullgren funnel were collected at 0-5 cm depths in each subplot using a 100 ml stainless sampling tube (DIK-1801, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). The collected soil samples were set in a Tullgren funnel, and soil animals were extracted for 48 h. The Tullgren funnel was made by installing a 13 cm diameter funnel at the lower stage of a 47 cm high metal rack with two stages. A 10 cm diameter net (3.9 mm mesh) was installed inside the funnel, and a soil sample was set on it. The light was irradiated using a 40 W incandescent bulb from a height of 15 cm above the soil sample. Soil animals that concentrated at the bottom of the funnel were captured by sample tubes containing 80% ethanol solution. The taxa and abundance of the collected soil animals were recorded and then stored in 80% ethanol solution.

III-2.5 Organic matter decomposition

To evaluate the effect of the experiments, a decomposition investigation of organic matter was conducted by the Bait-Lamina test (Kratz 1998).

Bait sticks were made by cutting 1 mm thick acrylic plates to 120 mm in length and 6 mm in width with 16 opening holes with a 1.5 mm diameter at 5 mm intervals from the edge of the stick to 10 mm (Fig. 4). In the holes of the bait stick, a paste that contained cellulose powder (BUBBLE STAR Co., Ltd., Kanagawa, Japan) and powder agar (Ina Food Industry Co., Ltd., Nagano, Japan) for the prevention of leaching was packed at a rate of 7:3 and dried for 24 h at 20-30 °C.

The bait sticks were installed at each belt transect (10 per subplot). After the removal of the A0-L-layer of the soil, the bait stick was inserted perpendicular to the ground and the top hole touched the ground surface (F-layer). The bait stick was collected 10 d after setting, and the number of holes with a missing sample was recorded.

III-2.6 Statistical analyses

Using statistical software (KyPlot 5.0 Version: 5.0.0, KyensLab Inc., Tokyo, Japan), one-way analysis of variance (one-way ANOVA) and Tukey-Kramer's HSD test (Tukey-Kramer) were performed to compare microenvironmental factors, soil animal abundance, and organic matter decomposition rates between each experimental plot. Using the free software R ver. 3.5.1 (R Core Team 2018), the relationship between the soil animal communities and microenvironmental factors or the experiments was calculated by NMDS. The analysis was performed by the R package *vegan* and *MASS* (Oksanen 2015). The dissimilarity of communities among subplots was calculated with the Bray-Curtis method. Regarding each microenvironmental factor and experiment, 999 permutations were performed, and factors that showed significant probability ($P < 0.05$) were projected as vectors on the NMDS plane (Oksanen 2015). For soil animal data, the number of taxa that appeared in more than one-third of all subplots (9 subplots or more) in at least one of the three investigations was used. For microenvironmental factor data, the soil moisture, litter amount, pH, and EC value in each subplot were used. For the experimental data, the number of burning and herbicide experiments was used.

III-3 Results

III-3.1 Microenvironmental factors

The soil moisture was not significantly different between all experimental plots before the experiment (Fig. 9a, Before experiment, one-way ANOVA, n.s.), after the first experiment (Fig. 9a, Experiment once, one-way ANOVA, n.s.), and 10 d after the third experiment (Fig. 9a, Experiment thrice, one-way ANOVA,

n.s.). Additionally, the soil moisture in all experimental plots tended to decrease gradually from early July to late September (Fig. 9a). The range of change was from approximately 20-40%.

The litter amount was not significantly different between all experimental plots before the experiment (Fig. 9b, Before experiment, one-way ANOVA, n.s.). However, the litter amount was significantly lower in the burning plot than in the control and herbicide plots after the first experiment (Fig. 9b, Experiment once, Tukey-Kramer, $P<0.05$). Furthermore, 10 d after the third experiment, the litter amount was significantly lower in the burning and herbicide plots than in the control plot (Fig. 9b, Experiment thrice, Tukey-Kramer, $P<0.05$).

The pH value was not significantly different between all experimental plots before the experiment (Fig. 9c, Before experiment, one-way ANOVA, n.s.), after the first experiment (Fig. 9c, Experiment once, one-way ANOVA, n.s.), and 10 d after the third experiment (Fig. 9c, Experiment thrice, one-way ANOVA, n.s.). Additionally, the pH value of all experimental plots tended to decrease slightly from early July to late September (Fig. 9c). The range of change was approximately 20%.

The EC value was not significantly different between all experimental plots before the experiment (Fig. 9d, Before experiment, one-way ANOVA, n.s.) and after the first experiment (Fig. 9d, Experiment once, one-way ANOVA, n.s.). However, the EC value 10 d after the third experiment was significantly higher in the herbicide plot than in the control plot (Fig. 9d, Experiment thrice, Tukey-Kramer, $P<0.05$).

III-3.2 Soil animal communities

In this study, 22 taxa and 7136 soil animals were collected through all investigations (Table 4).

The soil macrofauna abundance was not significantly different between all experimental plots before the experiment (Fig. 10a, Before experiment, one-way ANOVA, n.s.). After the first experiment, the lowest abundance was shown in the burning plot (Fig. 10a, Experiment once) and a significant difference was only observed between the burning and herbicide plots (Fig. 10a, Experiment once, Tukey-Kramer, $P<0.05$). The range of difference between the burning plot and herbicide or control plot was from approximately 65-70%. Additionally, the soil macrofauna abundance 10 d after the third experiment was significantly lower in the burning and herbicide plots than in the control plot (Fig. 10a, Experiment thrice, Tukey-Kramer, $P<0.05$).

The soil mesofauna abundance was not significantly different between all experimental plots before the experiment (Fig. 10b, Before experiment, one-way ANOVA, n.s.). After the first experiment, the abundance of soil mesofauna was significantly lower in the burning plot than in the control and herbicide plots (Fig.

10b, Experiment once, Tukey-Kramer, $P < 0.05$). However, the soil animal abundance 10 d after the third experiment was not significantly different between all experimental plots (Fig. 10b, Experiment thrice, one-way ANOVA, n.s.).

The NMDS analysis ordered the soil animal community in each subplot as different taxa, and the center of gravity of each taxon was placed on the coordinate plane (Fig. 11). The NMDS stress value was 0.24. After 999 permutation tests, six factors were selected that could affect each taxonomic group of the soil animal community: soil moisture, litter amount, pH, EC, burning experiment number, and herbicide experiment number (Fig. 11). On the coordinate plane, the EC vector, the number of burning experiments vector (burning vector), and the number of herbicide experiments vector (herbicide vector) were placed in the first quadrant while soil moisture, litter amount, and pH vectors were placed in the third quadrant (Fig. 11). Collembola, Oribatida, Prostigmata, Protura, and Symphyla were placed in the positive direction of the EC, burning, and herbicide vectors (Fig. 11), whereas Chilopoda and *Enchytraeidae* were placed in the positive direction of the soil moisture vector, Diplopoda and Isopoda were placed in the positive direction of the litter amount vector, and *Carabidae* was placed in the positive direction of the pH vector (Fig. 11). These results suggest that the burning and herbicide experiment had a negative effect on the abundance of soil macrofauna while but a relatively limited effect on soil mesofauna.

III-3.3 Bait-Lamina test

The organic matter decomposition rate was not significantly different between all experimental plots before the experiment (Fig. 12, Before experiment, one-way ANOVA, n.s.). After the first experiment, the highest organic matter decomposition rate was shown in the herbicide plot (Fig. 12, Experiment once) and significant differences were only observed between burning and herbicide plots (Fig. 12, Experiment once, Tukey-Kramer, $P < 0.05$). The range of difference between the herbicide plot and burning or control plot was from approximately 70-80%. On the other hand, the decomposition rate at 10 d after the third experiment was not significantly different between all experimental plots (Fig. 12, Experiment thrice, Tukey-Kramer, n.s.). Additionally, the decomposition rate of the herbicide plot tended to decrease by 44.48% from late July to late September (Fig. 12).

III-4 Discussion

III-4.1 Effects of burning and herbicide disturbances on the paddy levee environment

In this study, the microenvironmental factor that caused the largest change was the litter amount. Furthermore, the tendency of change was different for each experiment. In the burning plot, the litter amount remarkably decreased after the first experiment and remained low throughout the investigation period, which was possibly because the herbage existing on the paddy levee quickly disappeared in the burning experiment. Conversely, in the herbicide plot, a significant difference was not observed from the control plot after the first experiment and the litter amount remarkably decreased after the third experiment. These results suggest that the effects of the herbicide gradually appeared with repeated experiments. Bispyribac-sodium herbicide is adsorbed to soil approximately 4-8 h after spraying, and its dissipation requires approximately 30-50 d (Chirukuri and Atmakuru 2015). Therefore, in this study, just after the first experiment, herbicide spraying probably had no effect on the herbs at the paddy levee or the litter. Moreover, after the first experiment, since herbicide spraying was continued every approximately 20-30 d, the effect became remarkable after the third experiment.

In all experimental plots, the soil moisture and pH showed a gradual decrease with repeated experiments. Such a trend could be affected by the change in litter amount. Litter cover on the ground surface suppresses evaporation from the soil (Stavi 2020). In this study, it was presumed that the evapotranspiration of water from the ground surface increased due to the decrease in the amount of litter covering the paddy levee. On the other hand, the decrease in the pH value was likely related to litter decomposition by soil animal communities. Indeed, Limousin and Tessier (2007) reported that the decomposition of organic matter accumulated on the ground surface lowers the pH of soil at a depth of 0-5 cm.

The EC value did not show a significant difference between all experimental plots after the first experiment. Bera and Ghosh (2013) and Tata et al. (2015) reported that soil EC did not change before and after spraying bispyribac-sodium, which was consistent with the results presented here. On the other hand, the EC value was significantly higher in the herbicide plot than in the control plot after the third experiment. These results might be the result of repeated spraying of herbicide. Bispyribac-sodium has low volatility and is easily hydrolyzed (Tata et al. 2015; Watanabe et al. 2003), and its dissipation is faster in soils with higher organic carbon and an acidic pH (Chirukuri and Atmakuru 2015). Therefore, bispyribac-sodium that was repeatedly sprayed on the paddy levees could have been rapidly decomposed under the low pH environment and accumulated in the soil as an electrolyte. However, in this study, the chemical properties of the soil were not analyzed and the bispyribac-sodium results were only obtained at one study site. To better generalize our findings, it is necessary to expand the study site and perform a more detailed analysis

of the soil.

III-4.2 Effects of burning and herbicide disturbances on soil animal communities

The soil animal communities responded differently to burning. In soil macrofauna, the abundance tended to decrease just after the first experiment. In general, soil macrofauna are distributed in the soil from the litter layer (0 cm) to a depth of approximately 10 cm (Scheu and Poser 1996; Suárez et al. 2018; Tuf 2015). This soil layer may have been exposed to a rapid temperature rise due to the burning experiment. In addition, the disappearance of litter due to the burning experiment may decrease the abundance of soil macrofauna. Korobushkin et al. (2017) reported that fire-induced reductions in the amount of litter and damage to soil horizons have a negative impact on the soil macrofaunal community inhabiting the ground surface. As the authors also demonstrated that such effects continued for several years after the fire (Korobushkin et al. 2017), the negative impacts of burning experiments on soil macrofauna might persist over several years at our study site; thus, long-term monitoring is required. On the other hand, the abundance of soil mesofauna decreased just after the first experiment but was maintained at the same level as in the control plot at 10 d after the third experiment. The decrease in the abundance of mesofauna after the first experiment was probably due to the rise in soil temperature near the ground surface. N'Dri et al. (2017) reported that soil mite density decreased immediately after burning disturbance, which was consistent with the results presented here. While, 10 d after the burning experiment, the abundance of soil mesofauna was the same as that in the control plot, which may be because some taxa can move through gaps in the soil. Collembola, Oribatida, Prostigmata, and Symphyla maintained in the burning plot can move vertically through gaps in the soil (Aoki 2015; Krab et al. 2010; Urhan et al. 2008). Additionally, the vertical distribution of Collembola and Oribatida spans from the litter layer (0 cm) to a depth of approximately 95 cm in the soil (Jakšová et al. 2019; Rendoš et al. 2016). Soil mesofauna should be less affected by burning experiments than soil macrofauna because they are constantly moving through the soil gaps in response to changes in the surrounding environment.

The herbicide experiment also affected the abundance of soil macrofauna, which did not change significantly immediately after the first herbicide experiment but was significantly lower than the control plot at 10 d after the third experiment. This finding likely indicates a chain reaction of the herbicide experiments and a decrease in the litter amount. In the herbicide plot, mown litter covered the paddy levee after the first experiment. Therefore, Diplopoda, Isopoda, and Oligochaeta that use litter as a bait resource

or Chilopoda that live in the litter layer (Gerlach et al. 2014; Tuf 2015) maintained their abundance. However, these phenomena were temporary, and repeated herbicide spraying would likely gradually decrease the litter amount on the paddy levee, which would decrease the abundance of those taxa. Alternatively, the effects of herbicides on soil mesofauna seemed to be smaller than those on soil macrofauna. For example, Oribatida, whose abundance was maintained at the herbicide plot, is composed of many taxa adapting to a variety of environments, and their distribution ranges from natural forest soil to urban planting soil (Shimano 2018). Additionally, Collembola shows different resistance to chemicals for each species, and even in agricultural lands, where pesticides and chemical fertilizers are used, their abundance is maintained by species adapted to the environment (Ponge et al. 2003). In addition, Cole et al. (2008) reported that there are species in Collembola that increase their abundance when subjected to moderate physical soil disturbance. In this study, it was suggested that the short-term effect of herbicide spraying on the soil mesofauna was small.

III-4.3 Effects of burning and herbicide disturbances on organic matter decomposition

In this study, the organic matter decomposition rate increased just after the first experiment in the herbicide plot because of the enhanced decomposition by soil animals and soil microbial communities. In the herbicide plot, after the first experiment, the abundance of soil macrofauna increased and the amount of litter decomposition should have increased compared to that of the other experimental plots. Diplopoda, Isopoda, and Oligochaeta, which dominated immediately after the first herbicide experiment consume litter which is then crushed or decomposed (Frouz et al. 2014; Gerlach et al. 2014). In this study, the same phenomenon must have occurred in the herbicide plot. Additionally, underground fungi and bacteria decompose litter which is crushed by soil macrofauna (Yamashita et al. 2013). Furthermore, the activity of soil microbial communities increases during the decomposition of bispyribac-sodium (Ramalakshmi et al. 2017), and it may have promoted organic matter decomposition at herbicide sites. However, the organic matter decomposition rate in the herbicide plot decreased from late July to late September, which was consistent with the change in the abundance of soil macrofauna. This finding suggests that herbicide spraying temporarily increased the organic matter decomposition driven by soil animals at paddy levees, while the long-term use of weed killers negatively affected these functions.

Appendix 3 Impacts of agricultural disturbances on soil bacterial communities

To investigate the impact of burning and herbicide disturbances on soil bacterial communities, we analyzed soil bacterial deoxyribonucleic acid (DNA) using MiSeq (Illumina Inc., CA, USA). The soil sample was collected at 0-5 cm depth from the subplots at both ends of each belt transect using a 100 ml stainless sampling tube (DIK-1801, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). Those samples were collected from three spots in each subplot and bulked together. The soil samples were collected three times: before the experiment, after the first experiment, and after the third experiment (Fig. 8). Soil DNA was extracted from soil samples (0.5 g subplot⁻¹) collected from each subplot using ISOIL for Beads Beating (319-06201, Nippon Gene Co., Ltd., Tokyo, Japan). The concentration of the extracted soil DNA was measured using QuantiFluor[®] dsDNA System (Promega Corporation, WI, USA). The extracted soil DNA was diluted to an arbitrary magnification (50-3000 times), and 1st polymerase chain reaction (PCR) was performed targeting the 16S ribosomal ribonucleic acid (rRNA) gene for the soil bacterial community, using 515F/806R primer pairs with the adaptor sequence for the Nextera XT index kit (Illumina, San Diego, CA, USA). The 1st PCR was carried out in 25 µL volume using *TaKaRa Ex Taq*[®] hot start version (RR006A, Takara Bio Inc., Shiga, Japan). Cycling conditions were 2 min at 94 °C, followed by 30 cycles of 10 s at 98 °C, 30 s at 55 °C, and 20 s at 72 °C, and final elongation at 4 °C. The 1st PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Inc., CA, USA). Using the purified 1st PCR product as a template, 2nd PCR was performed to attach dual index and Illumina sequencing adaptors using Nextera XT Index kit v2 (Illumina Inc., CA, USA) and *TaKaRa Ex Taq*[®] hot start version (RR006A, Takara Bio Inc., Shiga, Japan). The 2nd PCR was carried out in 25 µL volume. Cycling conditions were 3 min at 95 °C, followed by 8 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, 5 min at 72 °C, and final elongation at 4 °C. Amplicons were purified using Agencourt AMPure XP (Beckman Coulter, Inc., CA, USA). The purified amplicons were quantified using QuantiFluor[®] dsDNA System (Promega Corporation, WI, USA) and were pooled in equimolar amounts in 4 nM. The mixed sample was paired-end sequenced on an Illumina MiSeq platform (Illumina Inc., CA, USA) at a read length of 2 by 300 bp using MiSeq Reagent Kit v3 (MS-102-3003, Illumina Inc., CA, USA). The amount of PhiX control added during the sequence was 20%. To analyze the sequence data, statistical software QIIME 2 version 2018.8 was used (Bolyen et al. 2018). Primer trimming, paired-end joining, quality filtering, chimera removal, and generating amplicon sequence variants (ASVs) were processed using the DADA2 algorithm (Callahan et al. 2016). The forward and reverse reads were truncated at 200 and 140 bp, respectively, correlating to a quality score of > 30. Singletons and doubletons were removed for further analysis. For taxa comparisons, the QIIME 2 q2-

feature-classifier plugin and the Naïve Bayes classifier trained on the Greengenes 16S rRNA gene database 13_8 (QIIME 2 development team 2018) were used. The sequences assigned to archaea were removed for further analysis. Soil bacterial sequences were rarefied at 20000 sequences each sample for alpha and beta diversity analyses. For the α -diversity, richness (number of ASVs) and Faith's phylogenetic diversity were calculated. The β -diversity of the soil bacterial community was calculated based on the weighted UniFrac distance, and each subplot was placed on the three-dimensional space by Principal Coordinate Analysis (PCoA). The soil bacterial functional genes involved in organic matter degradation were predicted using PICRUSt after importing the normalized soil bacterial operational taxonomic units (OTUs) table into PICRUSt in accordance with the KEGG database (Langille et al. 2013). The normalized soil bacterial OTU table was prepared from the ASV table using a closed-reference OTU picking method into QIIME 2 against the Greengenes reference database 13_5_otus (QIIME 2 development team 2018) at 97% identity. Using statistical software (KyPlot 6.0 Version: 6.0.2, KyensLab Inc., Tokyo, Japan), one-way ANOVA and Tukey-Kramer were performed to compare α -diversity and predicted copy number of soil bacterial functional genes.

In this study, a total of 1476161 sequences and 767 ASVs were obtained at the genus level. Fig. A2 showed the taxon composition of soil bacteria communities in each subplot at the phylum level. The soil bacterial community in each subplot was dominated by Proteobacteria, Acidobacteria, and Actinobacteria, with a relative dominance of 70.38%. The α -diversity of soil bacterial community was significantly lower in the control plot thrice and herbicide plot thrice than in the control plot once (Fig. A3, Tukey-Kramer, $P < 0.05$). In the Herbicide plot, the α -diversity of soil bacterial community tended to decrease gradually from early July to late September, however no significant difference was detected (Fig. A3, Tukey-Kramer, n.s.). As a result of the PCoA analysis, the composition of the soil bacterial community in each subplot had large variation even within the same experimental plots, and no regular changes in the soil bacterial community due to burning or herbicide experiments were observed (Fig. A4). Only in the burning plot, the difference in the diversity of the soil bacterial community tended to decrease after the first experiment (Fig. A4). Further, the functional genes involved in the carbon cycle in soil were not significantly different between all belt transects before the experiment (Fig. A5, Before experiment, one-way ANOVA, n.s.), after the first experiment (Fig. A5, Experiment once, one-way ANOVA, n.s.), and 10 d after the third experiment (Fig. A5, Experiment thrice, one-way ANOVA, n.s.), and stably existed in the soil on the paddy levees. Those results suggested that the soil bacterial communities inhabiting the paddy levees existed stably

without being affected by anthropogenic disturbances around the farmland. It would be due to the function of the soil bacterial community that the organic matter decomposition rate in the paddy levees where repeated the anthropogenic disturbances were maintained, albeit at a low level.

Chapter IV: Effects of inner levees formed in paddy fields on aquatic animal communities

IV-1 Introduction

The high biodiversity of *satoyama* is strongly contributed by the landscape mosaic structure of various environmental factors such as forests, paddy fields, rivers and grasslands (Jiao et al. 2019; Katoh et al. 2009). Paddy fields are the waterside environment that occupies the largest area in Japan (Kadono 2014), and have the function as the habitat for diverse biological communities (Natuhara 2013). “Database of Biota in Rice Paddies and Adjacent Areas” listed 6305 species of organisms (Ohtsuka 2020), and the biodiversity preservation function of paddy field is drawing attention. In general, it is known that the presence of an environmental gradient at the transition between aquatic and terrestrial ecosystems (called a water edge) and functions as a habitat for various organisms (Jiao et al. 2019; Kakudo 2009; Veen et al. 2013). In particular, in the terraced paddy field landscapes, the microenvironmental factors in the fields should become more complicated because they are composed of fields of uneven shape and have a higher water edge area per unit area than square field, and it may maintain the high biodiversity of *satoyama*.

On Sado Island, central Japan, it is recommended that wetland biotope or soil side ditch (called an “e” in Sado Island) are installed in the corner of paddy fields for conserving aquatic animals, which are the main prey resources of *Nipponia nippon* (Niigata Sado City 2021; Uruma et al. 2012), that listed as Endangered on the IUCN Red List 2018 (Kishimoto-Yamada and Minamiya 2020). “E” refers to waterways for warming the water supplied to the paddy fields, and also functions as a refugia for aquatic animals when the water in the rice field withers (Osada 2012). Wetland biotope or “e” are often installed in spaces where rice is not planted, and their shapes are diverse. It is expected that those differences will complicate the microenvironmental gradients or mosaics formed in the paddy fields, and they affect the aquatic animal communities. In recent years, in the paddy biotopes installed on Sado Island, the difference between those with abundant aquatic animals or not has become remarkable. However, there are many unclear points about the ecological factors cause those phenomena, and there are few research focusing on the effects of shape of the water edge or the ratio of the paddy field area to the water edge on biological communities in paddy fields.

Therefore, the intention of this study was to investigate effects of differences in water edge area (difference in length between paddy field and paddy levee boundary) on (1) microenvironmental factors and (2) aquatic animal communities in paddy biotopes by field manipulation experiments.

IV-2 Materials and methods

IV-2.1 Study site

The current study was conducted at the Koyakaiden experimental field, which is located on Sado Island, Niigata, Japan (38°02'19.17"N, 138°26'56.94"E). The experimental fields were paddy fields that was abandoned around 2000 and converted to a wetland biotope after 2017. The total area of these experimental fields was approximately 20 ha (including the area of the surrounding forest), and approximately 30 wetland biotopes using abandoned paddy fields existed (approximately 2 ha, some biotopes were managed as grasslands). The water used in the wetland biotope was supplied by the irrigation pond, which was approximately 1 km from the experimental field. Each biotope was maintained by annual tillage and 5-6 paddy levee mowing per year. The forests around the wetland biotopes consisted of secondary forests (such as *Quercus serrata* and *Aesculus turbinata* Blume), *Cryptomeria japonica* plantations, and bamboo forests (such as *Phyllostachys reticulata* and *Phyllostachys edulis*). The mean annual temperature and precipitation in the study area in 2020 were 14.8 °C and 1880.0 mm, respectively (JMA 2021).

IV-2.2 Sampling design

Three experimental plots (10 m square) were developed at the Koyakaiden experimental field from 23-24 June 2021. Ten inner levees that have different lengths were set up in each experimental plot and categorized into three experimental plots with different water edge areas (different the length of boundary between paddy field and paddy levee) as follows: control plot (Fig. 13, inner levee was not installed), 80 m plot (Fig. 13, inner levees with a length of 2 m were installed), and 120 m plot (Fig. 13, inner levees with a length of 4 m were installed). Moreover, twelve subplots (1 m square) were placed in the water edge and center in each biotope (Fig. 13). Water injection was done each experimental plot once per two weeks. Further, mowing on the paddy levee was performed once per month.

The investigation was conducted two times: one month after the biotope development (from 29 July to 1 August 2020, "One month later" in this study) and three months after the biotope development (21-23 September 2020, "Three months later" in this study).

IV-2.3 Microenvironmental factors

The water temperature (°C), water depth (cm), and litter amount (g) were measured in each subplot at

microenvironmental factors.

The water temperature was measured using a waterproof digital thermometer (CT-285WP, CUSTOM Corp., Tokyo, Japan). The measurement was performed at the bottom of the water in the center of each subplot.

Water depth was measured using a folding rule in the center of each subplot.

Litter was collected concurrently with the investigations of aquatic animals. The litter was scooped up 5 times in each subplot using the landing net (35 cm by 35 cm, 1 mm mesh). The collected litter was dried at 60 °C for 48 h, and the dry weight was measured.

IV-2.4 Aquatic communities

Aquatic animals were collected by hand sorting at each subplot. Using the landing net (35 cm by 35 cm, 1 mm mesh), scooped up the litter or bottom mud 5 times in each subplot, and the aquatic animals in the net were captured using tweezers. The collected aquatic animals were classified into family level based on Karita 2010, Kawai and Tanida 2018, Matsui and Seki 2008, Mitamura et al. 2017a, b, and Ozono et al. 2019, and stored in 80% ethanol solution.

IV-2.5 Statistical analyses

Using statistical software (KyPlot 6.0 Version: 6.0.2, KyensLab Inc., Tokyo, Japan), multiple comparisons (Tukey-Kramer) was performed to compare microenvironmental factors and aquatic animal abundance between the subplots. Using the free software R ver. 4.1.0 (R Core Team 2021), the relationship between the aquatic animal communities and water edge environmental factors or microenvironmental factors was calculated by NMDS. The analysis was performed by the R package *vegan* and *MASS* (Oksanen 2015). The dissimilarity of communities among subplots was calculated with the Bray-Curtis method. Regarding each water edge environmental factor and microenvironmental factor experiment, 999 permutations were performed, and factors that showed significant probability ($P < 0.05$) were projected as vectors on the NMDS plane (Oksanen 2015). For aquatic animal data, the number of taxa that appeared more than 100 individuals in each investigation was used. For water edge environmental factor data, the distance from the water edge (m), the surrounded by inner levees or not (1 if surrounded, 0 if not surrounded), and the adjacent to the flat water edge or not (1 if adjacent, 0 if not adjacent). For microenvironmental factor data, water temperature, water depth, and litter amount value in each subplot

were used.

IV-3 Results

IV-3.1 Microenvironmental factors

The water temperature was not significantly different between all experimental plots when the July investigation (Table 5, Tukey-Kramer, n.s.). On the other hands, the water temperature was significantly lower in the control water edge subplots than in the 80 m center subplots when the September investigation (Table 5, Tukey-Kramer, $P < 0.05$). However, in all experimental plots, no significant difference was detected between water edge and center subplots when the September investigation (Table 5, Tukey-Kramer, n.s.).

In the control and 80 m plot, the water depth was significantly shallower in the water edge subplots than in the center subplots through all investigations (Table 5, Tukey-Kramer, $P < 0.05$). In the 120 m plot, no significant difference was detected between water edge and center subplots through all investigation (Table 5, Tukey-Kramer, n.s.).

In the 80 m plot, the litter amount was significantly higher in the water edge subplots than in the center subplots through all investigations (Table 5, Tukey-Kramer, $P < 0.05$). However, in other experimental plots, no significant difference was detected between water edge and center subplots through all investigation (Table 5, Tukey-Kramer, n.s.).

IV-3.2 Aquatic animal communities

In this study, 25 taxa and 14565 aquatic animals were collected through all investigations (Table 6).

In the control plot, the aquatic animal abundance was significantly higher in the water edge subplots than in the center subplots through all investigations (Fig. 14, Tukey-Kramer, $P < 0.05$). The range of difference between the control water edge and control center subplots was from approximately 40-70%. In the 80 m plot, the aquatic animal abundance was significantly higher in the water edge subplots than in the center subplots when the September investigation (Fig. 14, Tukey-Kramer, $P < 0.05$). Further, although no significant difference was detected, those tendency was confirmed in the July investigation (Fig. 14, Tukey-Kramer, n.s.). In the 120 m plot, the aquatic animal abundance was not significantly different between water edge and center subplots when the July investigation (Fig. 14, Tukey-Kramer, n.s.). However, the aquatic animal abundance was significantly higher in the 120 m center subplots than in the 120 m water edge subplots when the September investigation (Fig. 14, Tukey-Kramer, $P < 0.05$). The range of difference

between the 120 m water edge and 120 m center subplots was 40.41%. Moreover, the aquatic animal abundance collected in the 120 m center subplots were highest through all investigations.

The NMDS analysis ordered the composition of aquatic animal communities in each subplot, and the subplots number and the center of gravity of each taxon was placed on the coordinate plane (Fig. 15, 16). The NMDS stress value was 0.16 when the July investigation (Fig. 15a, b). After 999 permutation tests, four factors were selected that could affect aquatic communities: the distance from the water edge (distance), the adjacent to the flat water edge or not (plane), water depth, and litter amount. On the coordinate plane, the water depth vector was placed in the positive direction of the first axis, the plane vector was placed in the negative direction of the first axis, the distance vector was placed in the positive direction of the second axis, and the litter amount vector was placed in the negative direction of the second axis (Fig. 15a, b). The control water edge and 80 m water edge subplots were placed in the third quadrant, and *Baetidae* and *Physinae* appeared intensively in those subplots (Table 6; Fig. 15a). The control center subplots were placed in the second quadrant, and *Baetidae* and *Chironomidae* appeared intensively in those subplots (Table 6; Fig. 15a). The 80 m center, 120 m water edge, and 120 m center subplots were placed across the first and fourth quadrants, and *Corixidae* and *Notonectidae* appeared intensively in those subplots (Table 6; Fig. 15a). In the July investigation, there was a tendency for the set of each subplot to have a large overlap (Fig. 15a). *Libellulidae* was placed in the positive direction of the plane vector (Fig. 15b). *Hylidae* was placed in the positive direction of the litter amount vector (Fig. 15b). In the September investigation, the NMDS stress value was 0.11 (Fig. 16a, b). After 999 permutation tests, five factors were selected that could affect aquatic communities: distance, the surrounded by inner levees or not (concave), plane, water temperature, and water depth. On the coordinate plane, the water depth vector was placed in the positive direction of the first axis, the plane vector was placed in the negative direction of the first axis, the distance and water temperature vectors were placed in the positive direction of the second axis, and the concave vector was placed in the negative direction of the second axis (Fig. 16a, b). The control water edge, 80 m water edge, and 120 m center subplots were placed across the second and third quadrant, and *Baetidae* and *Physinae* appeared intensively in those subplots (Table 6; Fig. 16a). The control center subplots were placed across the first and second quadrant, and *Baetidae* and *Platycnemididae* appeared intensively in those subplots (Table 6; Fig. 16a). The 80 m center subplots were placed across the first and fourth quadrant, and *Baetidae* and *Chironomidae* appeared intensively in those subplots (Table 6; Fig. 16a). The 120 m water edge subplots were placed across the third and fourth quadrant, and *Baetidae* and *Physinae* appeared intensively in those

subplots (Table 6; Fig. 16a). In the September investigation, there was no overlap in the set of each subplot (Fig. 16a). *Chironomidae* was placed in the positive direction of the water depth vector (Fig. 16b). *Baetidae* was placed in the positive direction of the water temperature and distance vectors (Fig. 16b). *Coenagrionidae* and *Physinae* were placed in the positive direction of the plane vector (Fig. 16b). *Culicidae*, *Dytiscidae*, and *Libellulidae* were placed in the positive direction of the concave vector (Fig. 16b). In addition, *Hydrophilidae* tended to distribute in the water edge of each experimental plot (Table 6; Fig. 16b).

IV-4 Discussion

IV-4.1 Aquatic communities that one month after the biotope development

In the July investigation, the aquatic animal abundance that collected in the control water edge subplots was highest, and *Baetidae* and *Physinae* were dominated in those subplots. In addition, regardless of the difference in the water edge area, the *Hylidae* and *Libellulidae* abundance tended to be higher in the water edge subplots than in the center subplots. In general, *Baetidae*, *Physinae*, and the frog tadpoles use litter (detritus) or the algae attached to them as main food resources (Fukuda et al. 2007; Hayashi et al. 2011; Takemon 1997). Moreover, Morikawa et al. (2013) reported that the litter supplied from around the water area created a hideout for dragonfly larva and had a positive effect on their abundance. The litter amount in the control water edge subplots tended to be higher than that in the control center subplots, which was probably the reason for the large abundance of *Baetidae*, *Hylidae*, *Physinae*, and *Libellulidae* that use them as food resources or habitats. On the other hand, in this study, there were taxa whose distribution pattern would have changed due to the influence of the inner levee installation. For example, *Physinae*, whose distribution was concentrated on the water edge of the control plot, should be one of them. The distribution of *Physinae* in the July investigation was concentrated in the control and 80 m plot water edge, while their population density was remarkably low at the 120 m plot. It was suggested that the inner levees became a barrier which hindered the movement of *Physinae* invading from the waterway. In addition, the *Corixidae* abundance in the 120 plot was higher than in other plots, and it may be a taxon whose population density has changed due to the influence of inner levee. Sychra et al. (2010) reported that some *Corixidae* preferred deep water area near the boundary between reed bed and open water. In the 120 m plot which has the largest area of water edge, the environment consistent with *Corixidae*'s preference may be maintained.

IV-4.2 Aquatic communities that three months after the biotope development

In the September investigation, the *Baetidae* and *Culicidae* abundance that appeared in the 120 m center subplots were higher than in other subplots, and the total aquatic animal abundance was the highest throughout all investigation. In addition, the *Dytiscidae* and *Hydrophilidae* abundance were maintained at the center subplots adjacent to the inner levee (120 m center subplots) to the same level as the water edge subplots. Sychra et al. (2010) reported that aquatic beetles were inhabitants of near the water edge. In this study as well, the installation of inner levees would have a positive effect on the abundance of those taxa. However, although the abundance of these aquatic beetles were maintained even at the water edge surrounded by inner levee, the largest abundance was observed in the flat water edge (Control water edge subplots). It was suggested that the water edge environment surrounded by inner levees was different from the flat water edge. To better generalize our findings, it is necessary to perform a more detailed analysis of the effect of water edge shape on the chemistry of paddy water such as dissolved oxygen, redox potential, pH, and EC and the effect of these changes on aquatic animal communities.

Chapter V: The invasion route of soil animals inhabit the paddy levees of *satoyama* ecotone

V-1 Introduction

In former Japan and present East Asian *satoyama*, the nutrients circulated through the litter compost supplied from the forests to the farmlands (Ichikawa et al. 2010) or excrement of grazing livestock at *satoyama* ecotone (Fig. A6), and their ecological sustainability were maintained. On the other hand, in recent Japanese *satoyama*, those nutrient cycle across different landscape elements are being lost because the spread of modern agriculture. However, even in modern *satoyama* ecotone, there would be hidden drivers that promote the nutrient cycle across the different landscape elements. For example, the soil animal communities that inhabit *satoyama* ecotone may be one of them. Soil animals inhabiting the farmlands were assumed to invade from surrounding landscapes (Kaneko et al. 2007). Auclerc et al. (2009) reported that Collembola communities inhabiting the boundary between forests and grasslands includes generalists which have no preference for habitat land use. Those soil animal communities can drive the nutrient cycle in *satoyama* ecotone through the movement of their biomass and the decomposition of litter. Although, there are few studies that quantitatively analyze these phenomena that may occur in *satoyama* ecotones.

Therefore, the purpose of this study was to clarify the invasion route of soil animal communities at paddy levees. The invasion route of the soil animal communities were investigated by the monitoring that the recovery process of soil animal abundance after burning experiment.

V-2 Materials and methods

V-2.1 Study site and sampling design

The current study was conducted at the Kisenjo experimental field of Niigata University, which is located on Sado Island, Niigata, Japan (38°00'51.93"N, 138°28'42.29"E). Five levees that adjacent to the surrounding forests were selected, and a belt transect with a length of 20 m and a width of 1 m was set up on each. Moreover, five subplots (1 m square) were placed in each belt transect at 5 m intervals as follows: forest edge, 5 m from the forest edge (5 m), 10 m from the forest edge (10 m), 15 m from the forest edge (15 m), and 20 m from the forest edge (20 m). Each belt transect was embedded with galvanized iron sheets (30 cm by 60 cm), except for the surface adjacent to the forests, to a depth of 15 cm (the joints of the sheets were reinforced with aluminum tape) to block the invasion soil animals from the surrounding paddy levees (Fig. 17).

The burning experiment was performed after mowing using an agricultural burner (Kusayaki GT-220, Shinfuji Burner Co., Ltd., Aichi, Japan). The flame temperature of this burner was 1100 °C, and its calorific value was 19.8 kW (17000 kcal h⁻¹). The burning experiment was conducted from 24-26 May 2019. The experiment was performed for 40 min per belt transect and repeated 4 times (the fourth burning experiment was carried out on 26 May 2019 in the all belt transects).

V-2.2 Soil animals

Soil samples before the burning experiment were collected on 13 and 21 May 2019. Soil samples after the burning experiment were collected 7 times: immediately after the burning experiment (26 May 2019), 7 d later (2 June 2019), 14 d later (9 June 2019), 28 d later (23 June 2019), 42d later (7 July 2019), 56 days later (21 July 2019), and 70 d later (4 August 2019).

Soil animals were collected by using Tullgren funnels (the size and mechanism were same as Chapter II and III) at each subplot. The soil samples that used for the Tullgren funnel were collected at 0-5 cm depths in each subplot using a 100 ml stainless sampling tube (DIK-1801, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). The collected soil samples were set in a Tullgren funnel, and soil animals were extracted for 48 h. The collected soil animals were classified into 19 taxa based on Aoki (2015), and stored in 80% ethanol. The targets of the investigation were limited to soil animal taxa that inhabit the underground or ground surface.

V-2.3 Statistical analyses

Using statistical software (KyPlot 5.0 Version: 5.0.0, KyensLab Inc., Tokyo, Japan), t-test was performed to compare soil animal abundance between before and after burning experiment. Further, using statistical software (KyPlot 5.0 Version: 5.0.0, KyensLab Inc., Tokyo, Japan), multiple comparisons (Tukey-Kramer) was performed to compare soil animal abundance in each subplot after burning experiment. For the statistical analysis, the abundance data of Oribatida and Collembola were used. Those two taxa accounted for approximately 83% of the total sample.

V-3 Results: Soil animal communities

In this study, 6119 Oribatida and 2528 Collembola were collected.

In the subplots that 5 m from the forest edge and 10 m from the forest edge, Oribatida abundance was

significantly lower after the burning experiment than before the burning experiment (Fig. 18, t-test, $P < 0.05$). In addition, Oribatida abundance tended to decrease after burning in other subplots (Fig. 18). The range of those changes were from approximately 50-80%. The fluctuation of Oribatida abundance for 70 d after the burning experiment tended to be different for each subplot (Fig. 19). In the forest edge subplots, the increase in Oribatida abundance immediately after the burning experiment was largest, and the abundance 14 d after burning recovered to 79.76% of that before burning (Fig. 19). On the other hand, in the subplots that 20 m from the forest edge, Oribatida abundance was continued to increase from 7 d later to 42 d later after burning. In the subplots that 20 m from the forest edge, the Oribatida abundance when 42 d after burning recovered to 83.83% of that before burning (Fig. 19). The Oribatida abundance for 70 d after the burning experiment tended to be higher in the subplots that 20 m from the forest edge, however no significant difference was detected between other subplots (Fig. 20, Tukey-Kramer, n.s.).

Collembola abundance was significantly lower after the burning experiment than before the burning experiment, except for subplots that 5 m from the forest edge (Fig. 21, t-test, $P < 0.05$). In addition, Collembola abundance tended to decrease after burning in the subplots that 5 m from the forest edge (Fig. 21). The range of those changes were from approximately 80-90%. Collembola abundance 70 d after the burning experiment tended to increase gradually until 56 d after burning (Fig. 22). These trends were more pronounced in the forest edge subplots. In those subplots, the increase in Collembola abundance after the burning experiment was largest, and the abundance after 42 d recovered to 60.73% of that before burning (Fig. 22). Collembola abundance immediately after burning was significantly higher in the forest edge subplots than in the subplots that 10 m from the forest edge (Fig 23, After burning, Tukey-Kramer, $P < 0.05$). Collembola abundance for 70 d after the burning experiment tended to be higher in the subplots that forest edge, however no significant difference was detected between other subplots (Fig. 23, Tukey-Kramer, n.s.).

V-4 Discussion: The invasion route of soil animal communities

In this study, we predicted that the soil animal community inhabiting the paddy levees would be composed of individuals which had invaded from the surrounding forests, and attempted to quantify their invasion amount. Since the Oribatida and Collembola abundance after burning experiment had the highest increase near the forest edge, and it was expected that some of the individuals collected from those subplots would have invaded by horizontal movement from the surrounding forests or outside the belt transect. However, in the subplots other than the forest edge, there was no significant difference in the Oribatida and

Collembola abundance after burning experiment between each subplot, and it was not possible to confirm a one-way increase in abundance from the forest edge to the paddy levee. In addition, there was no regularity in the fluctuation of Oribatida abundance in each subplot. The only tendency was for the Collembola abundance to increase gradually in all subplots. Those results may suggest that the Oribatida and Collembola within each belt transect repeat vertical movements as well as horizontal movements (see Chapter III for details). Recent previous studies have widely monitored the recovery process of the Oribatida and Collembola communities after a forest fire, but different considerations have been made regarding the sources of population biomass formed on the site after the fire. For example, Malmström (2012) reported that post-forest fire Collembola community was consisted surface species with high mobility. On the other hand, Huebner et al. (2012) reported that the post-forest fire Collembola community was consisted of small sized endogenic species. Moreover, Malmström (2010) reported that species composition of Oribatida and Collembola communities before and after high-intensity forest fires was different. The investigation of the Oribatida and Collembola community that inhabit not only the surface of paddy levees but also forests, the underground, and slopes of the terraced paddy field levees and comparing them with the community formed after burning will be possible to determine the source of biomass for the soil animal communities that inhabit the paddy levees of *satoyama* ecotone.

Chapter VI: Conclusion

In this study, we attempted to quantify the effects of anthropogenic disturbance on habitat function and organic matter decomposition function of *satoyama* ecotone, and the data obtained through the investigation contained many results that contrary to our hypothesis. For example, in chapter II, we predicted that the division of ecotone has a negative effect on environmental gradients, soil animal communities, and organic matter decomposition, and it was shown that the soil moisture and litter amount gradient disappear due to the division of forests and paddy levees. However, on the paddy levees divided from the forests, decomposer and shredder abundance and organic matter decomposition rate were increased because the reduced population density of predator. Further, in the field manipulation experiments conducted in Chapter III, the soil animal communities that inhabit the paddy levees were highly adaptable to anthropogenic disturbance, and it was confirmed that the soil macrofauna abundance and organic matter decomposition rate were temporarily increased immediately after the first herbicide experiment. In addition, it was observed that the population density of soil mesofauna communities on the paddy levee recovered rapidly after burning disturbance (Chapter III and V). Moreover, the functional genes of the soil bacterial community, which are deeply involved in litter decomposition at the paddy levees, existed stably in each experimental plot regardless of the type and frequency of anthropogenic disturbances (Chapter III). Those results would reflect the high stability and resilience of the *satoyama* ecosystems. In chapter II, the division of ecotone had a negative effect on the population density of predators inhabiting the paddy levees, although which triggered an increase in the population density of decomposer and shredder, and it should have had a positive effect on the organic matter decomposition function of the paddy levees. Further, the effect of herbicide spraying appeared as an increase in the litter amount, which would have had a positive effect on the population density of soil macrofauna and organic matter decomposition rate at the paddy levees. In addition, it should also be noted that in those study, the effects of anthropogenic disturbances at forests or paddy levees on biological communities tended to diminish as their body size got smaller. Soil mesofauna and bacteria with a small body size have a wide vertical distribution area in the soil and should be possible to avoid anthropogenic disturbances near the ground surface. Moreover, soil mesofauna, which can move through the gaps in the soil, may have quickly invaded the ground surface after anthropogenic disturbances and restored the population density. Perhaps those biological communities underpin the organic matter decomposition function of *satoyama* ecotone. The ecological functions of *satoyama* ecotone were protected by diverse biological communities with different functions, and it was suggested that those functions exert

the high stability and resilience against the single disturbance. On the other hand, in Chapter III, repeated spraying of herbicide has been shown to reduce soil macrofauna abundance and organic matter degradation rates, and it was suggesting that excessive anthropogenic disturbances have a negative impact on ecological functions of *satoyama* ecotone. In addition, it must be taken into account that many of the investigations conducted in this study were manipulation experiments conducted in a relatively short term. For example, the potential for the abundance of soil macrofauna to recover following their decline due to burning experiments remains unclear (Chapter III). Long-term monitoring of the effects of anthropogenic disturbances on *satoyama* ecotone will lead to comprehend the complete picture of the impact of human activity on *satoyama* ecosystems.

In this study, several phenomena that supports our hypothesis were confirmed. For example, in *satoyama* ecotone where forest edge structures such as mantle communities or grasslands exist, because the gentle environmental gradient of the litter amount was maintained near the forest edge, the organic matter decomposition rate increased (Chapter II). Further, in Chapter IV, it was confirmed that the population density of the aquatic animal community increase as the area of the water edge on the paddy field increased. In addition, the taxa of aquatic animals that dominate each subplot changed due to the difference in the shape of the water edge. Those results will be important as the quantitative data showing that the environmental gradients and landscape mosaics formed in *satoyama* ecotone contribute to maintain its high biodiversity and nutrient cycle. In addition, these phenomena are peculiar to *satoyama* ecotone maintained by traditional management methods, and it can be lost in the future due to the spread of modern agriculture. The task assigned to us is to analyze in detail the functions of the traditional *satoyama* ecotone and to show as quantitative data that the effect of anthropogenic disturbances on them. It is important that we continue our efforts to maintain or restore some of the functions of the *satoyama* ecosystems even in *satoyama* which modernized by technological progress.

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Table 1 Details of the study sites

Study site		Forest edge structure	Artificial construction (m)	Landscape types
Iwakubi	A	Grassland	0	Landscape type I
	B	Grassland	0	
	C	Mantle community	0	
	D	Mantle community	0	
	E	not exist	0	Landscape type II
	F	not exist	0	
Matsugasaki	G	not exist	40.82	Landscape type III
	H	not exist	37.27	
	I	not exist	5.74	
Agata	J	not exist	4.25	
	K	not exist	3.31	
	L	not exist	3.63	

“Artificial construction” indicates the length of the structure existing between the forest edge and paddy levee.

Table 2 Classification of functional groups

Functional groups	Taxonomic groups
Predator	Araneae, <i>Carabidae</i> , Chilopoda, Collembola, Mesostigmata, Opiliones, Prostigmata, <i>Pselaphinae</i> , Pseudoscorpionida
Decomposer	Amphipoda, Collembola, <i>Enchytraeidae</i> , Isoptera, Oligochaeta, Oribatida
Shredder	Diplopoda, Isopoda
Microbial predator	Astigmata, Collembola, Diplura, Protura, Symphyla
Phytophagous	Collembola, <i>Meloidae</i> , Gastropoda
Omnivore	Dermaptera, <i>Rhaphidophoridae</i>

References based on which soil animals were classified into functional groups (Aoki 2015; Inoue 2001; Kaneko et al. 2007; Kurosawa et al. 2006; Matsura 2009; Minagoshi 2005; Minagoshi 2017; Mizushima 2004; Sawada et al. 1999; Shiraishi and Enami 2002; Toyota 2004; Tsukiji 2011; Uchida and Kaneko 2004; Yokota 2002).

Table 3 Decomposition rate of each landscape type

Landscape type	Landscape	No. of collected sticks	Decomposition rate (No. of holes)	χ -squared	df	P-value
Landscape type I	Forest	39	4.01% (25)	12.71	2	P<0.01
	Forest edge	39	6.57% (41)			
	Paddy levee	38	2.47% (15)			
Landscape type II	Forest	16	6.64% (17)	3.05	2	n.s.
	Forest edge	20	4.06% (13)			
	Paddy levee	8	3.13% (4)			
Landscape type III	Forest	46	3.67% (27)	6.57	2	P<0.05
	Forest edge	58	3.88% (36)			
	Paddy levee	54	6.02% (52)			

(Pearson chi-squared test)

Table 4 Total abundance of each soil animal taxa

Soil animal taxa		Before experiment			Experiment once			Experiment thrice		
		Control	Burning	Herbicide	Control	Burning	Herbicide	Control	Burning	Herbicide
Macrofauna	Amphipoda	15	•	5	•	•	3	7	•	1
	Araneae	15	11	11	8	3	25	44	5	11
	<i>Carabidae</i>	13	13	8	20	6	8	2	•	•
	Chilopoda	86	74	49	62	21	39	47	13	7
	Dermaptera	1	1	•	•	•	1	1	2	1
	Diplopoda	9	5	11	28	4	18	39	5	7
	Gastropoda	7	1	•	1	•	3	1	•	•
	Isopoda	9	2	25	2	•	50	7	•	4
	Isoptera	40	3	•	7	•	5	•	•	•
	Oligochaeta	56	64	82	56	28	60	12	26	16
	Opiliones	1	•	•	•	•	•	1	•	•
<i>Pselaphinae</i>	1	2	3	•	1	1	1	•	•	
Mesofauna	Astigmata	4	85	19	1	•	1	5	7	•
	Collembola	308	197	226	127	32	132	164	105	110
	Diplura	•	2	2	13	1	3	9	3	6
	<i>Enchytraeidae</i>	36	33	38	24	20	72	30	4	2
	Mesostigmata	12	19	17	11	6	13	8	5	7
	Oribatida	356	749	415	271	110	264	330	294	239
	Prostigmata	70	109	84	55	19	31	44	52	41
	Protura	10	14	12	21	10	19	19	8	6
	Pseudoscorpionida	•	•	•	•	•	•	2	•	•
Symphyla	23	53	19	19	16	34	18	19	19	
Total abundance		1072	1437	1026	726	277	782	791	548	477

Table 5 Microenvironmental factors in each experimental plot

Season	Microenvironmental factor	Control (40 m plot)		80 m plot		120 m plot		P-value
		Water edge (n=6)	Center (n=6)	Water edge (n=6)	Center (n=6)	Water edge (n=6)	Center (n=6)	
One month later (July)	Water temperature (°C)	28.48±0.91	28.33±0.21	28.83±0.12	28.68±0.07	28.25±0.30	28.22±0.12	n.s.
	Water depth (cm)	11.77±2.46 ^c	19.17±2.41 ^a	14.42±2.83 ^{bc}	21.05±1.21 ^a	19.27±3.11 ^a	18.87±1.18 ^{ab}	P<0.05
	Litter amount (g)	7.18±5.43 ^{ab}	0.00 ^b	11.00±4.48 ^a	1.25±1.77 ^b	3.30±3.51 ^{ab}	8.80±8.19 ^{ab}	P<0.05
Three months later (September)	Water temperature (°C)	25.00±0.64 ^b	25.48±0.12 ^{ab}	25.42±0.22 ^{ab}	25.62±0.07 ^a	25.03±0.25 ^{ab}	25.13±0.18 ^{ab}	P<0.05
	Water depth (cm)	9.32±1.09 ^d	16.35±2.50 ^b	14.82±1.88 ^{bc}	25.42±2.32 ^a	14.25±4.18 ^{bc}	10.77±2.12 ^{cd}	P<0.05
	Litter amount (g)	3.18±2.53 ^b	0.70±1.02 ^b	32.58±18.36 ^a	3.60±3.91 ^b	12.62±8.68 ^b	3.60±1.85 ^b	P<0.05

The number in the table indicates the average value and the standard deviation. Different letters in the figure indicate significant differences (Tukey-Kramer, P<0.05).

Table 6 Total abundance of each aquatic animal taxa

Aquatic animal taxa	One month later (July)						Three months later (September)						Total abundance
	Control (40m plot)		80 m plot		120m plot		Control (40 m plot)		80 m plot		120 m plot		
	Water edge	Center	Water edge	Center	Water edge	Center	Water edge	Center	Water edge	Center	Water edge	Center	
<i>Baetidae</i>	413	158	267	143	160	104	251	488	202	193	261	970	3610
<i>Physinae</i>	740	77	279	16	1	•	630	151	538	22	221	280	2955
<i>Corixidae</i>	124	103	77	135	455	596	33	15	10	9	69	77	1703
<i>Notonectidae</i>	213	42	182	152	280	173	24	51	62	46	127	77	1429
<i>Chironomidae</i>	74	149	113	145	146	151	31	73	48	118	128	125	1301
<i>Platycnemididae</i>	5	•	•	•	•	•	379	216	66	18	95	153	932
<i>Coenagrionidae</i>	6	•	2	1	2	1	114	89	124	14	167	151	671
<i>Hydrophilidae</i>	12	7	10	2	9	4	217	8	51	1	102	112	535
<i>Culicidae</i>	1	•	7	•	6	8	37	9	19	4	97	293	481
<i>Libellulidae</i>	57	18	46	6	20	20	10	3	30	4	70	25	309
<i>Dytiscidae</i>	22	3	26	2	11	5	94	3	29	5	36	22	258
<i>Hylidae</i>	28	1	50	18	26	20	3	•	•	1	•	•	147
<i>Aeshnidae</i>	•	•	•	•	•	•	48	25	26	•	9	37	145
<i>Haliplidae</i>	6	•	1	•	3	3	4	•	3	•	1	2	23
<i>Cambaridae</i>	•	•	4	•	7	•	•	3	7	•	1	•	22
<i>Gerridae</i>	6	1	5	•	2	•	•	•	•	•	•	•	14
<i>Nepidae</i>	3	•	2	•	6	2	•	•	•	•	•	•	13
<i>Cobitidae</i>	•	•	•	•	2	1	•	•	1	•	•	•	4
<i>Gobiidae</i>	•	•	•	•	•	1	•	•	•	•	2	•	3
<i>Adrianichthyidae</i>	•	•	1	•	1	1	•	•	•	•	•	•	3
<i>Noteridae</i>	•	•	•	•	•	•	1	•	1	•	•	•	2
Trichoptera	•	•	•	1	•	•	•	•	•	•	•	1	2
<i>Hydrachnidae</i>	•	1	•	•	•	•	•	•	•	•	•	•	1
<i>Enchytraeidae</i>	•	•	•	1	•	•	•	•	•	•	•	•	1
<i>Pleidae</i>	•	•	•	•	•	•	•	•	•	•	•	1	1

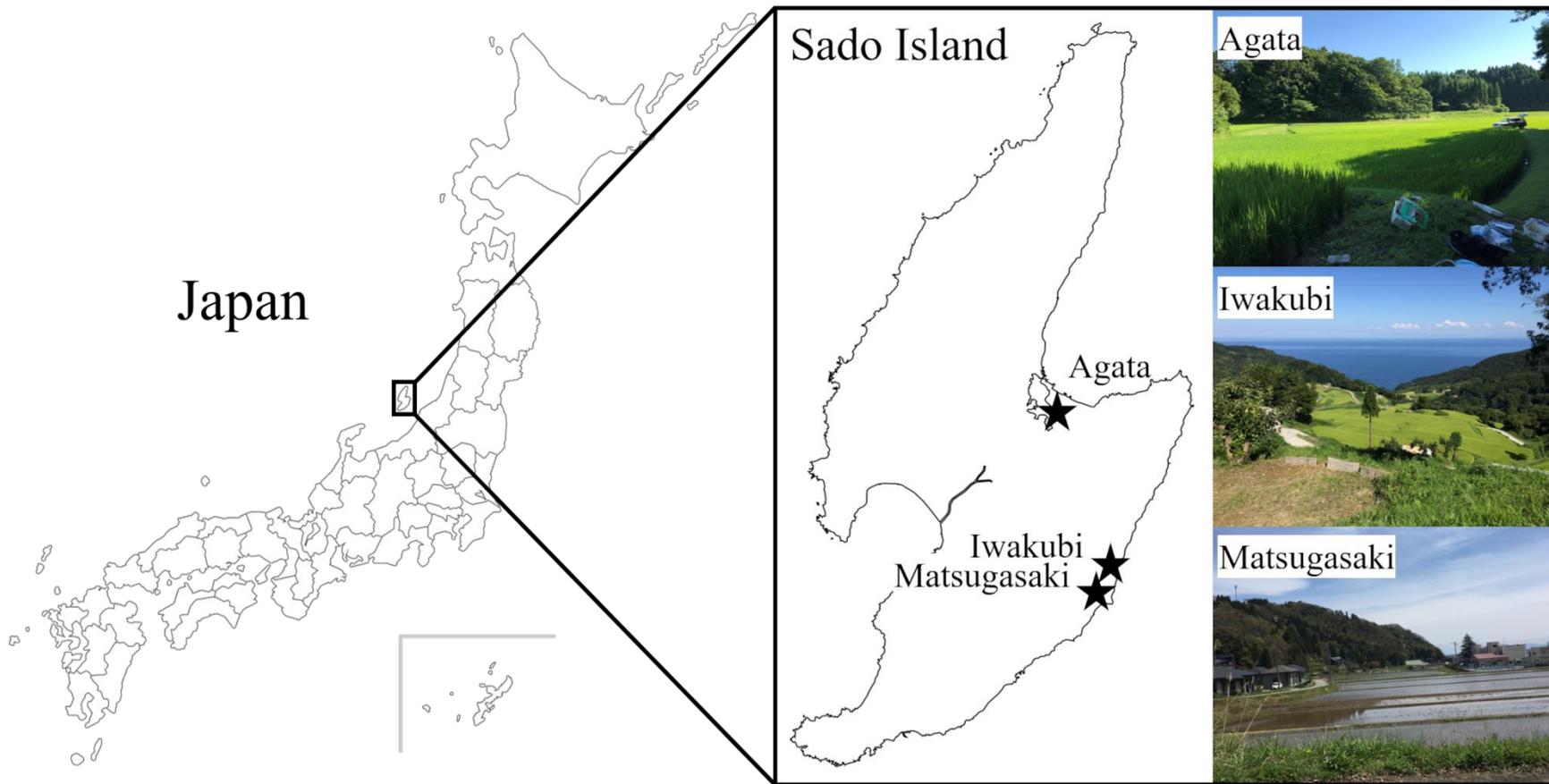
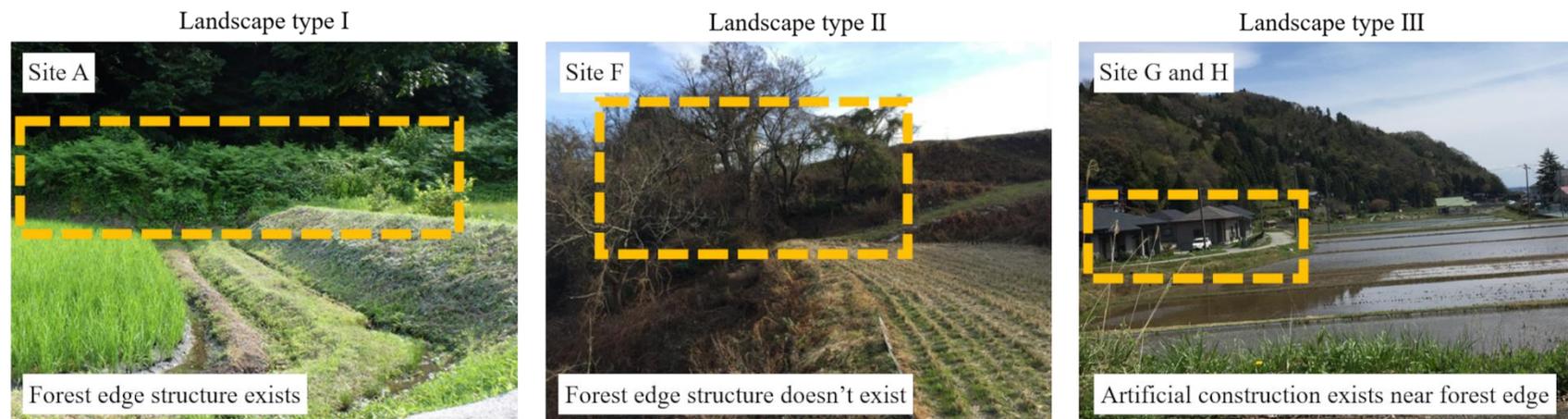


Fig. 1 The *satoyama* landscapes in Sado Island



In landscape type I, artificial construction doesn't exist, and forest edge structure exists. In landscape type II, artificial construction and forest edge structure don't exist. In landscape type III, artificial construction exists, and forest edge structure doesn't exist.

Fig. 2 Classification of the investigation points based on connectivity differences near the forest edge

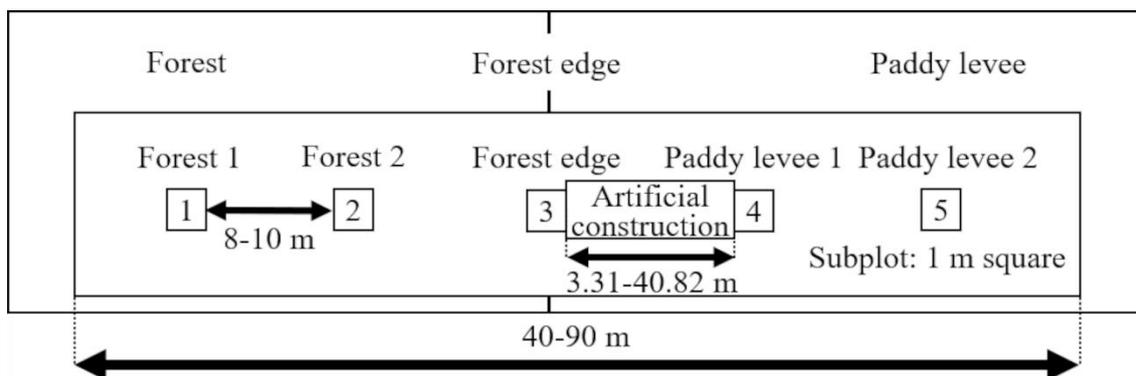


Fig. 3 Details of the belt transect in chapter II

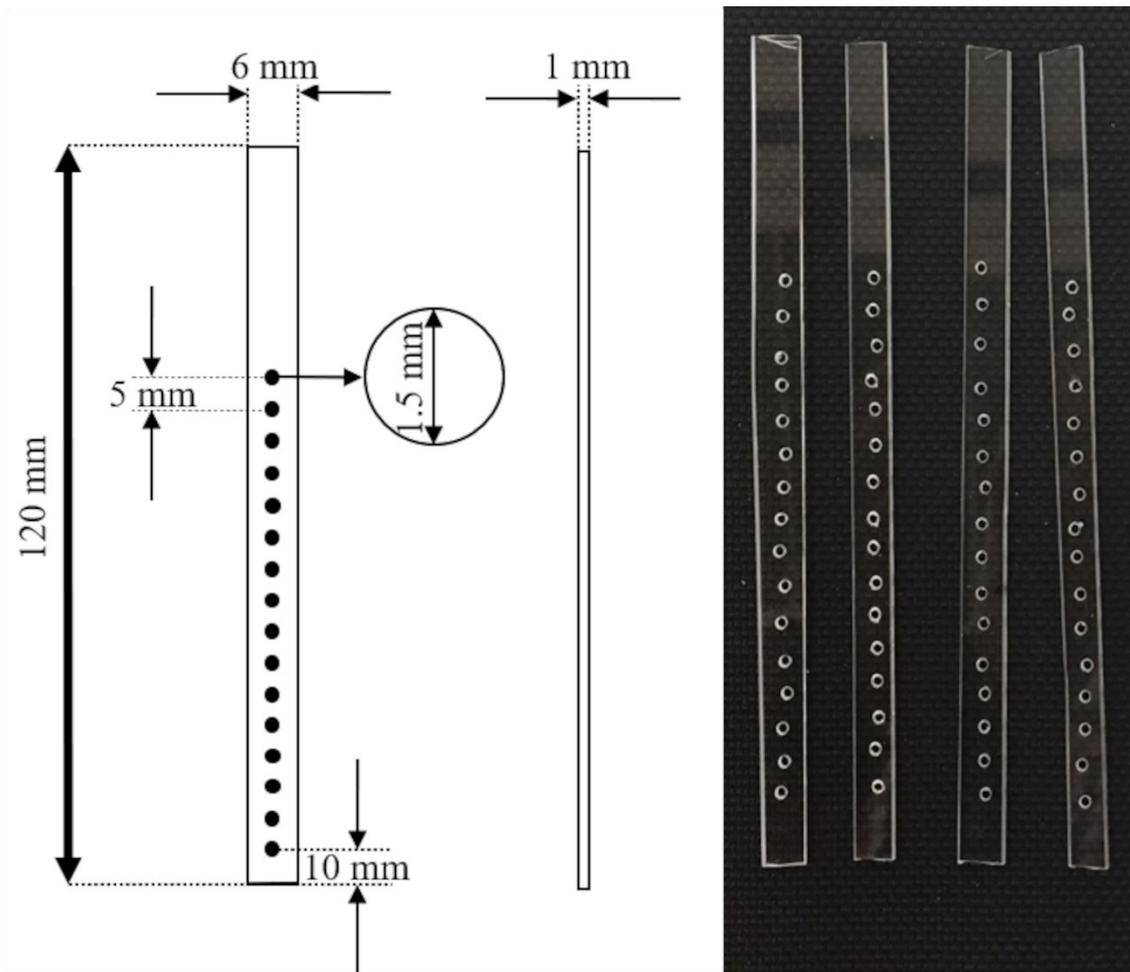
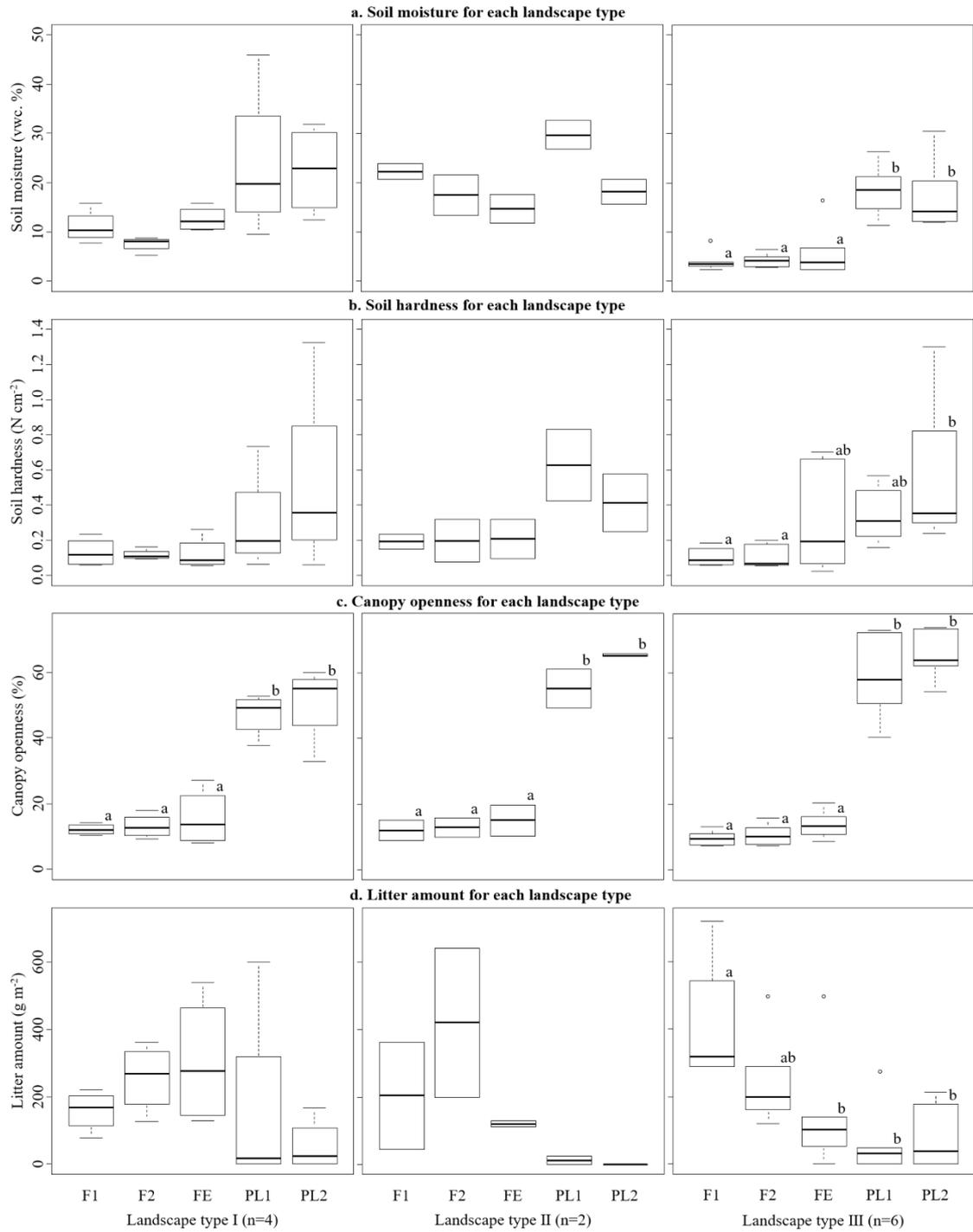
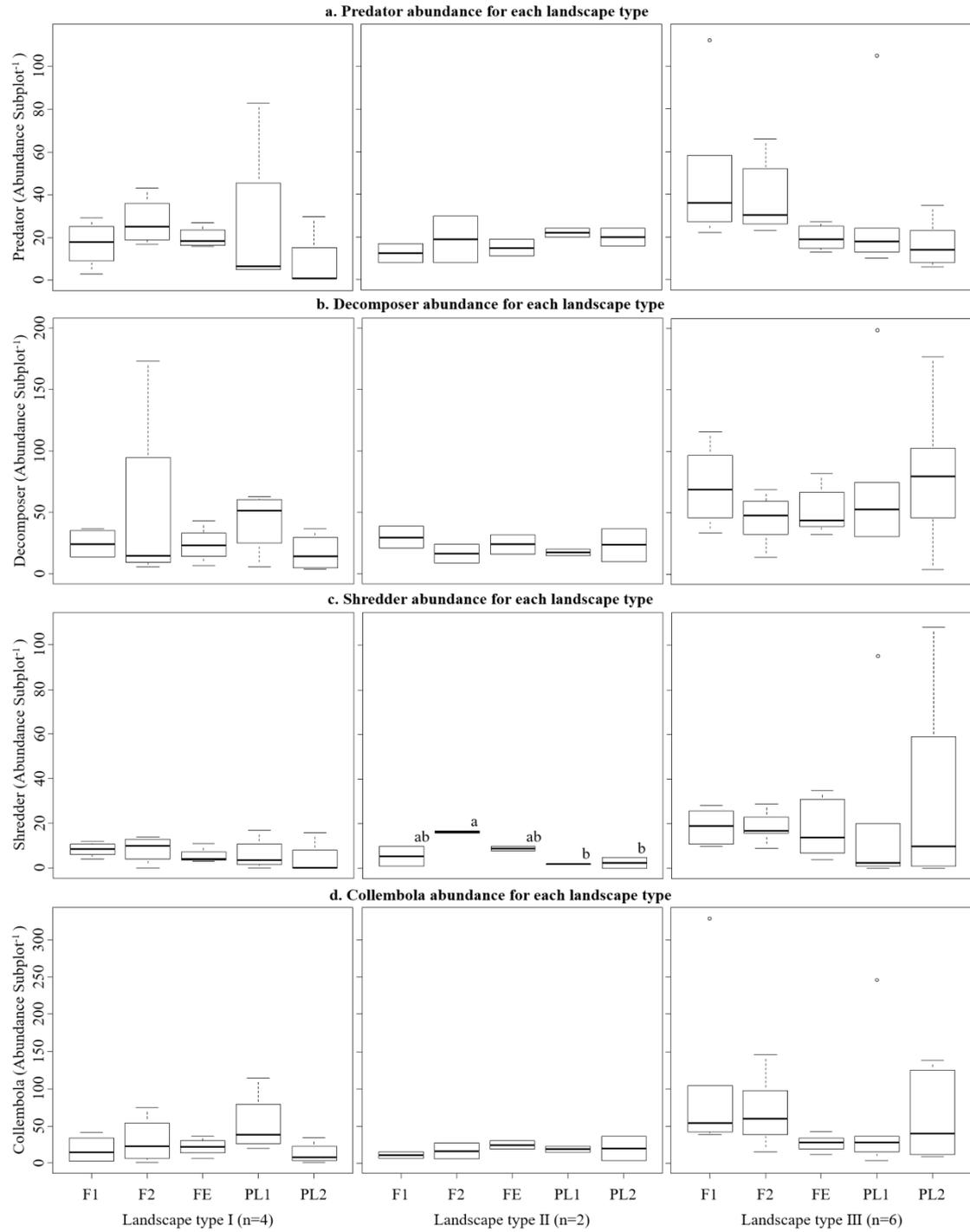


Fig. 4 The bait stick method



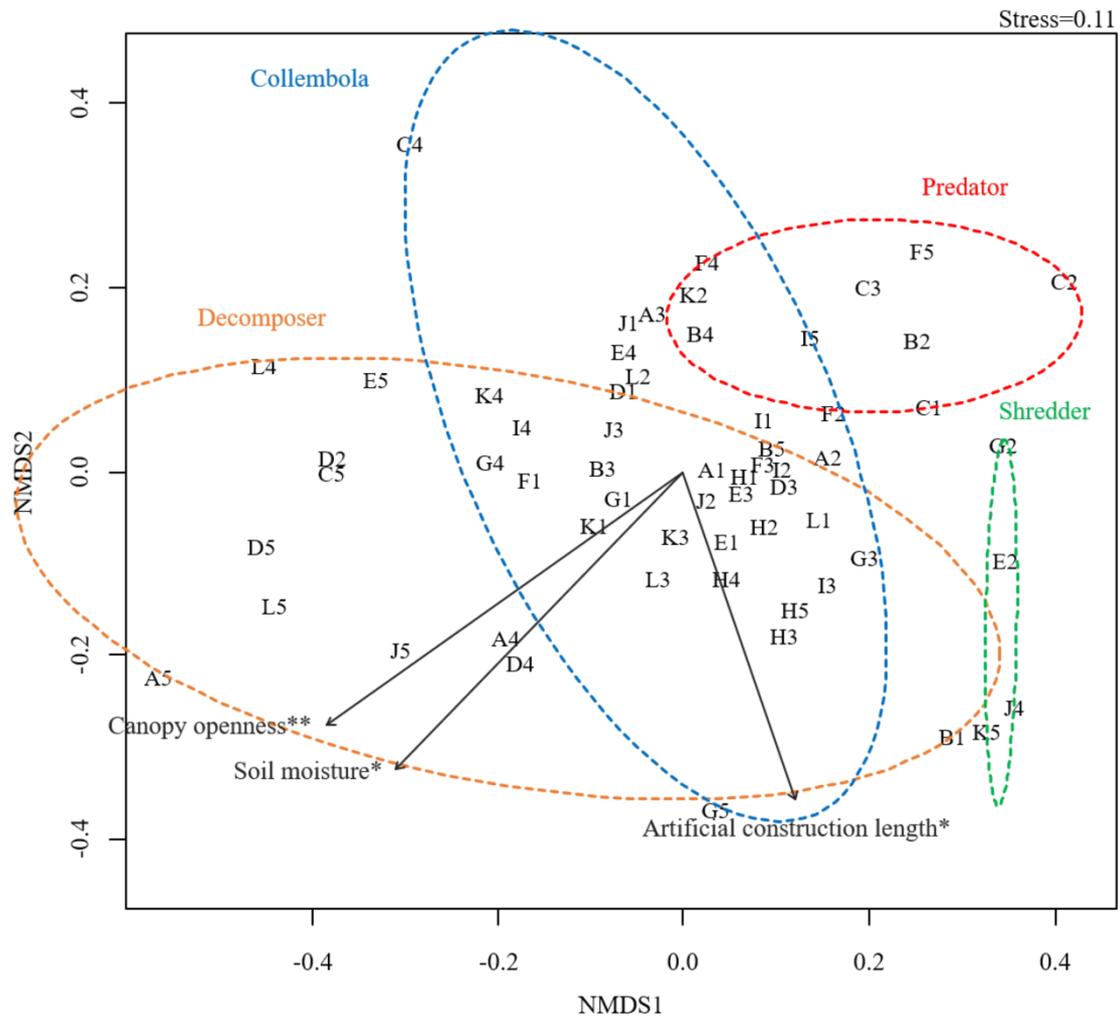
The line in the center of the box indicates the median value. The lower end of the box indicates the first quartile (25%). The upper end of the box indicates the third quartile (75%). The line under the box indicates the minimum value. The line above the box indicates the maximum value. White points outside the box indicate outliers. Different letters in the figure indicate significant differences (Tukey-Kramer, $P < 0.05$). F1 indicates subplot "Forest 1". F2 indicates subplot "Forest 2". FE indicates subplot "Forest edge". PL1 indicates subplot "Paddy levee 1". PL2 indicates subplot "Paddy levee 2".

Fig. 5 Microenvironmental factors among landscape elements



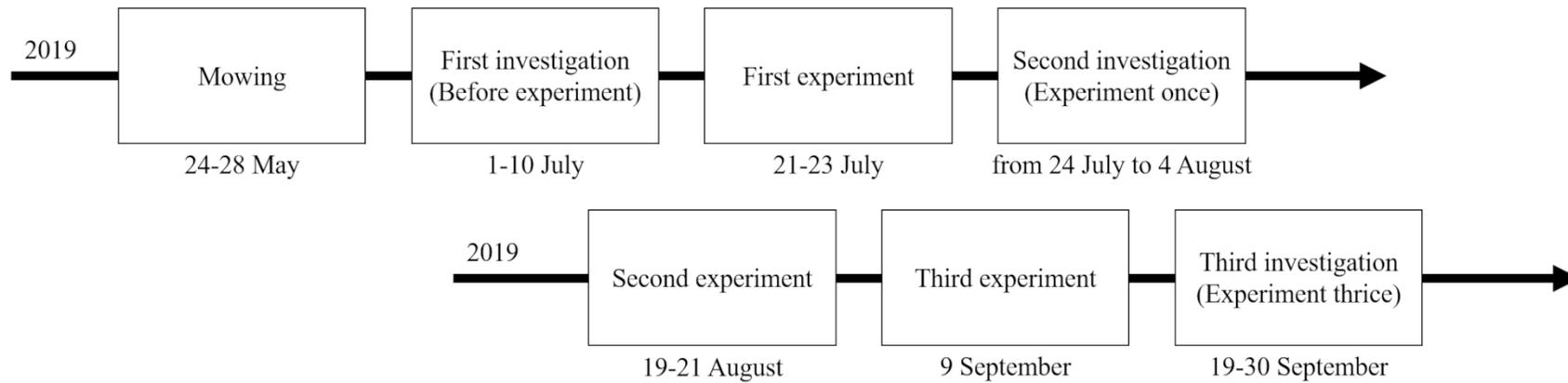
The line in the center of the box indicates the median value. The lower end of the box indicates the first quartile (25%). The upper end of the box indicates the third quartile (75%). The line under the box indicates the minimum value. The line above the box indicates the maximum value. White points outside the box indicate outliers. Different letters in the figure indicate significant differences (Tukey-Kramer, $P < 0.05$). F1 indicates subplot "Forest 1". F2 indicates subplot "Forest 2". FE indicates subplot "Forest edge". PL1 indicates subplot "Paddy levee 1". PL2 indicates subplot "Paddy levee 2".

Fig. 6 Abundance of functional groups among landscape elements



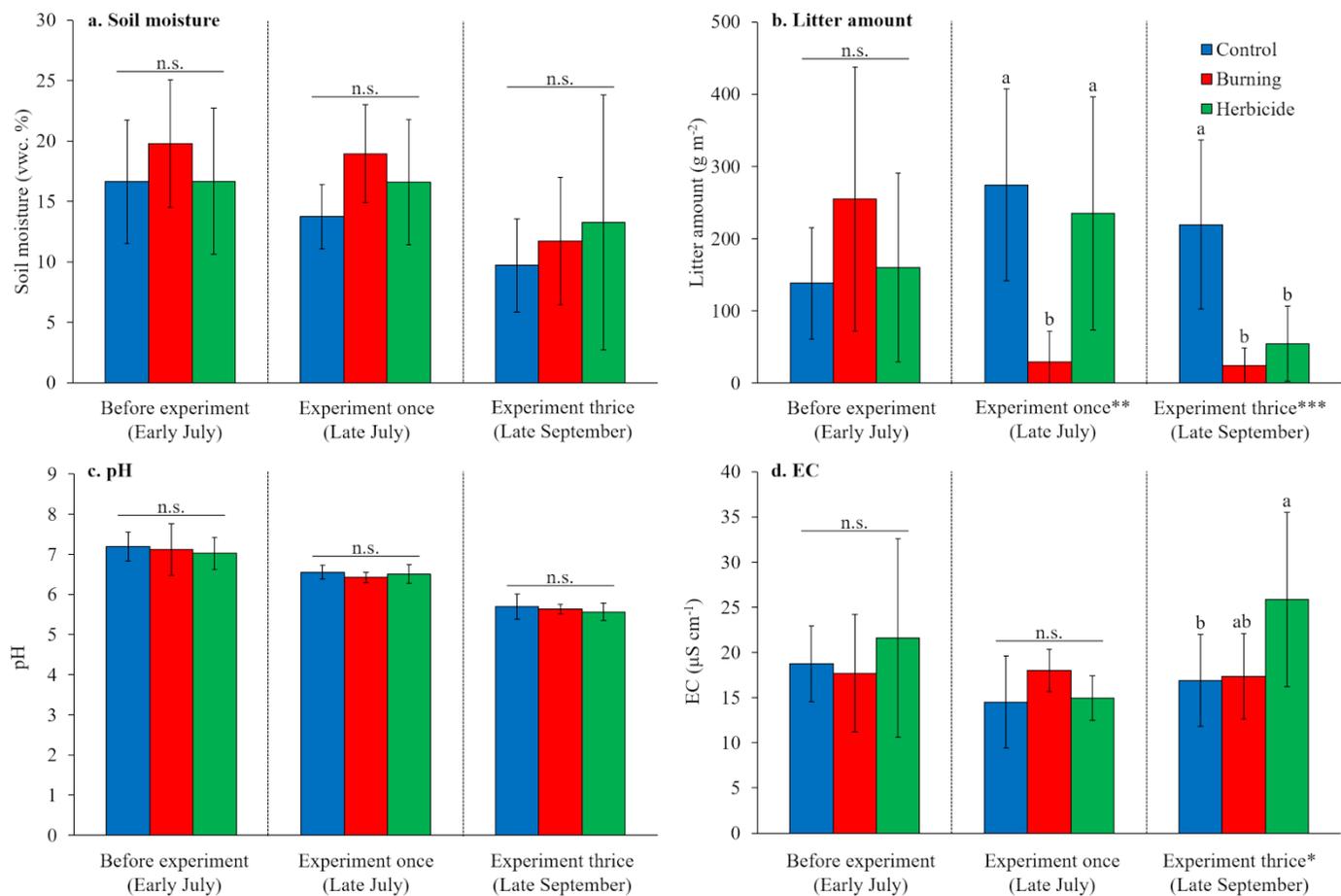
A, B, C, D indicate landscape type I. E, F indicate landscape type II. G, H, I, J, K, L indicate landscape type III. 1, 2 indicate the forest. 3 indicates the forest edge. 4, 5 indicate the paddy levee. In the subplot surrounded by the dotted line, each functional group is most dominant. * Significant probability at the 5% level. ** Significant probability at the 1% level.

Fig. 7 Relationship between soil animals and landscape structural factors or microenvironmental factors



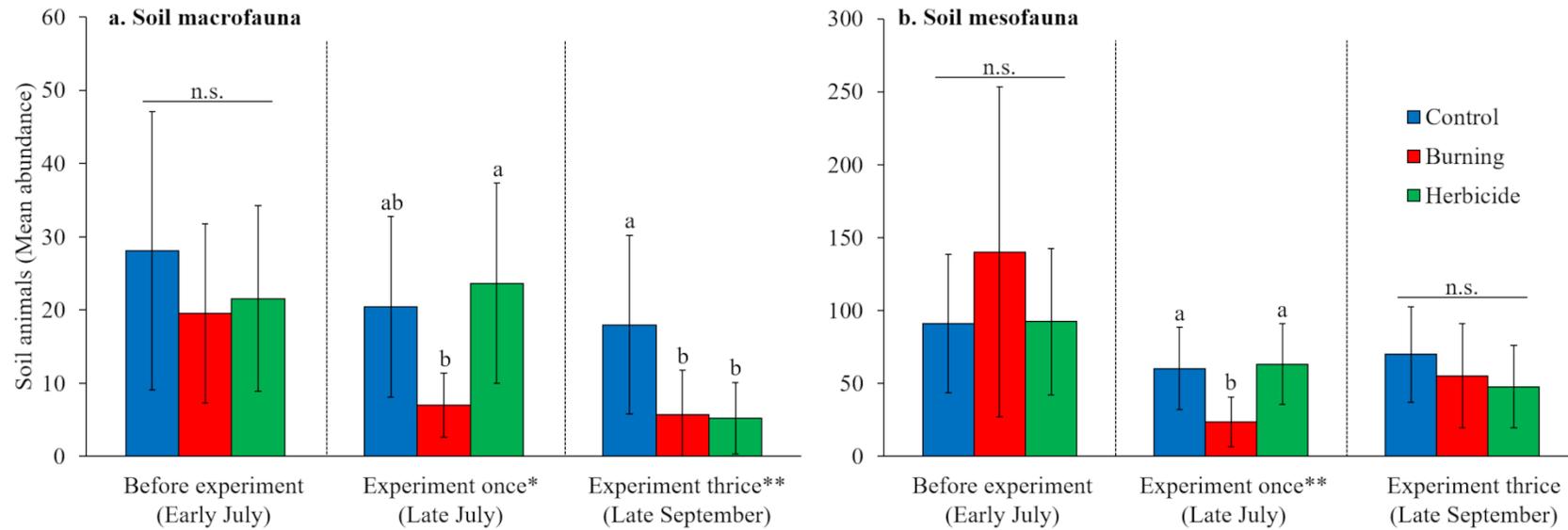
In the mowing carried out in May, the mown litter was removed from the surface of the paddy levee. In the mowing carried out from July to September, the mown litter was left on the surface of the paddy levee.

Fig. 8 Flowchart of the experiments and investigations



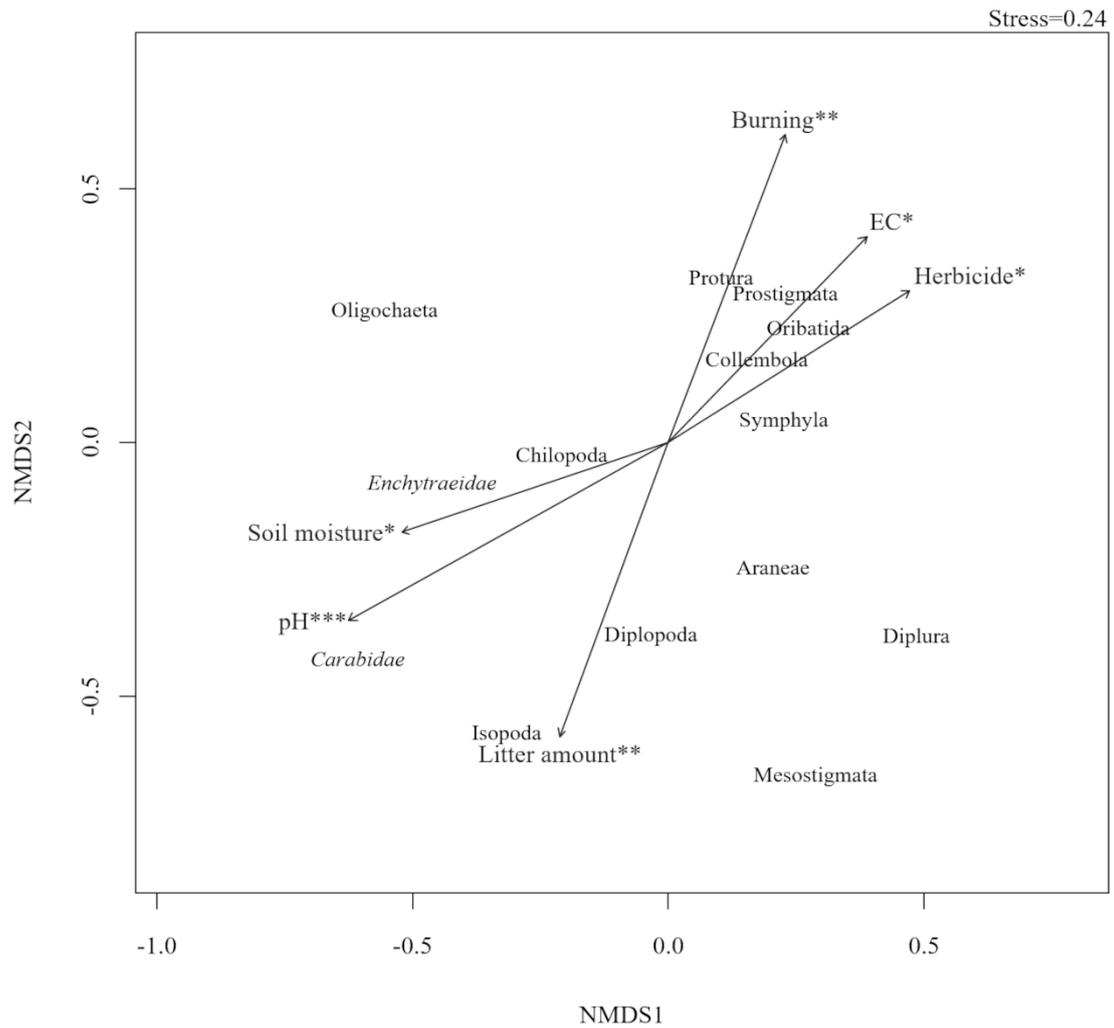
The graph indicates the average value. The bar indicates the standard deviation. One-way ANOVA and Tukey-Kramer were performed between each experimental plot (n=9) for every investigation (Before experiment, Experiment once, and Experiment thrice). * Significant difference at the 5% level (one-way ANOVA). ** Significant difference at the 1% level (one-way ANOVA). *** Significant difference at the 0.1% level (one-way ANOVA). The letter "n.s." above the graph denote no significant differences (one-way ANOVA). Different letters above the graph denote significant differences between the mean values (Tukey-Kramer, P<0.05).

Fig. 9 Microenvironmental factors in each experimental plot



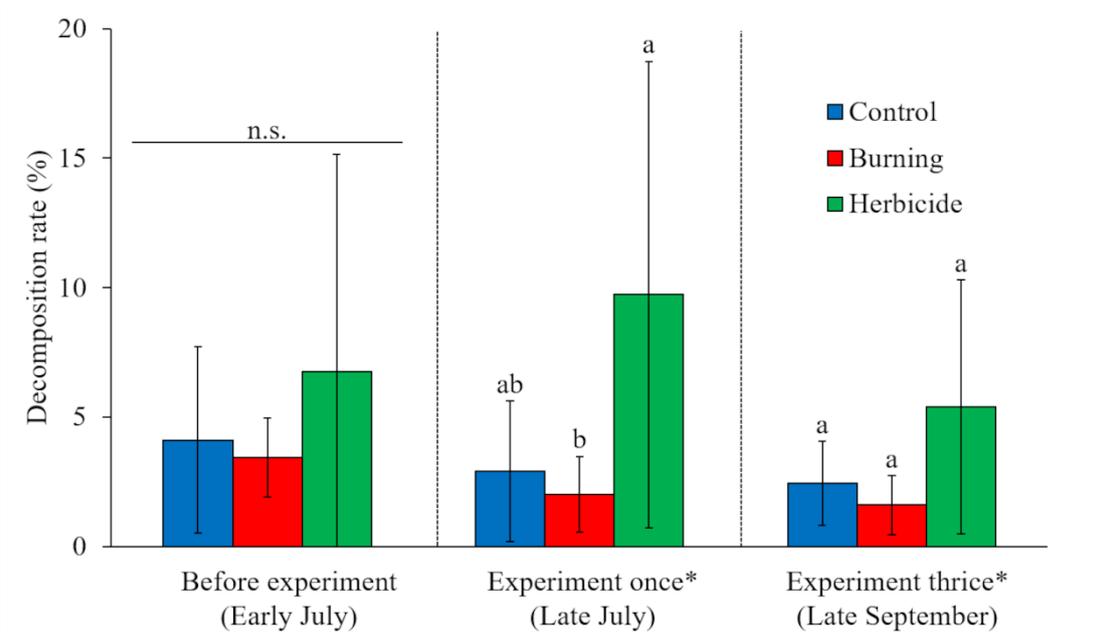
The graph indicates the average value. The bar indicates the standard deviation. One-way ANOVA and Tukey-Kramer were performed between each experimental plot (n=9) for every investigation (Before experiment, Experiment once, and Experiment thrice). * Significant difference at the 5% level (one-way ANOVA). ** Significant difference at the 1% level (one-way ANOVA). The letter “n.s.” above the graph denote no significant differences (one-way ANOVA). Different letters above the graph denote significant differences between the mean values (Tukey-Kramer, $P < 0.05$).

Fig. 10 Soil animal abundance in each experimental plot



* Significant probability at the 5% level. ** Significant probability at the 1% level. *** Significant probability at the 0.1% level.

Fig. 11 Relationship between soil animals and microenvironmental factors



The graph indicates the average value. The bar indicates the standard deviation. One-way ANOVA and Tukey-Kramer were performed between each experimental plot (n=9) for every investigation (Before experiment, Experiment once, and Experiment thrice). * Significant difference at the 5% level (one-way ANOVA). The letter “n.s.” above the graph denote no significant differences (one-way ANOVA). Different letters above the graph denote significant differences between the mean values (Tukey-Kramer, $P < 0.05$).

Fig. 12 Organic matter decomposition rate of each experimental plot

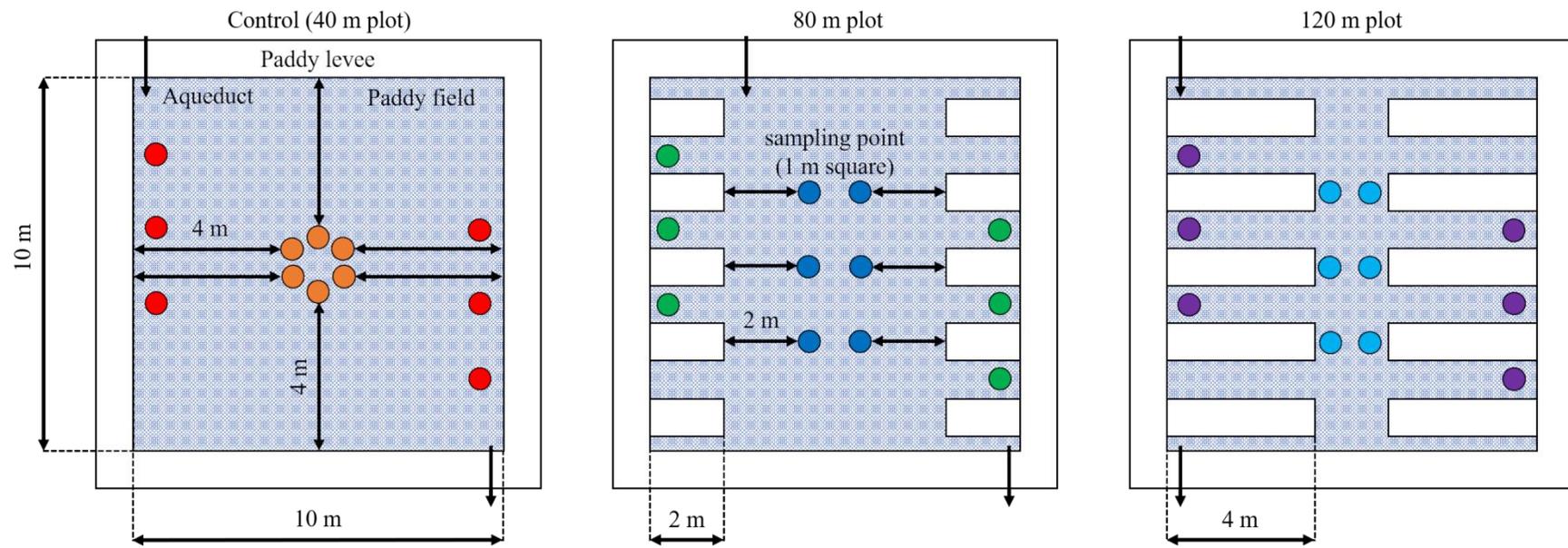
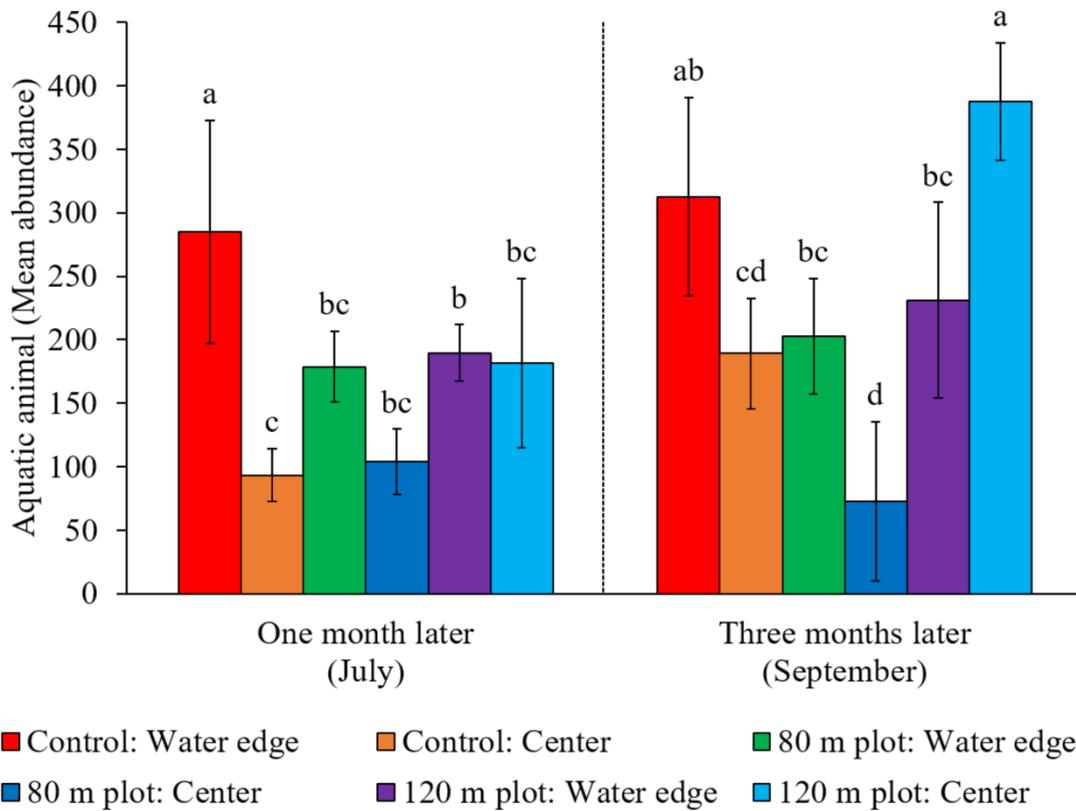


Fig. 13 Details of the experimental plots



The graph indicates the average value. The bar indicates the standard deviation. Tukey-Kramer was performed between each experimental plot (n=6) for every investigation (One month later and Three months later). Different letters above the graph denote significant differences between the mean values (Tukey-Kramer, $P < 0.05$)

Fig. 14 Aquatic animal abundance in each experimental plot

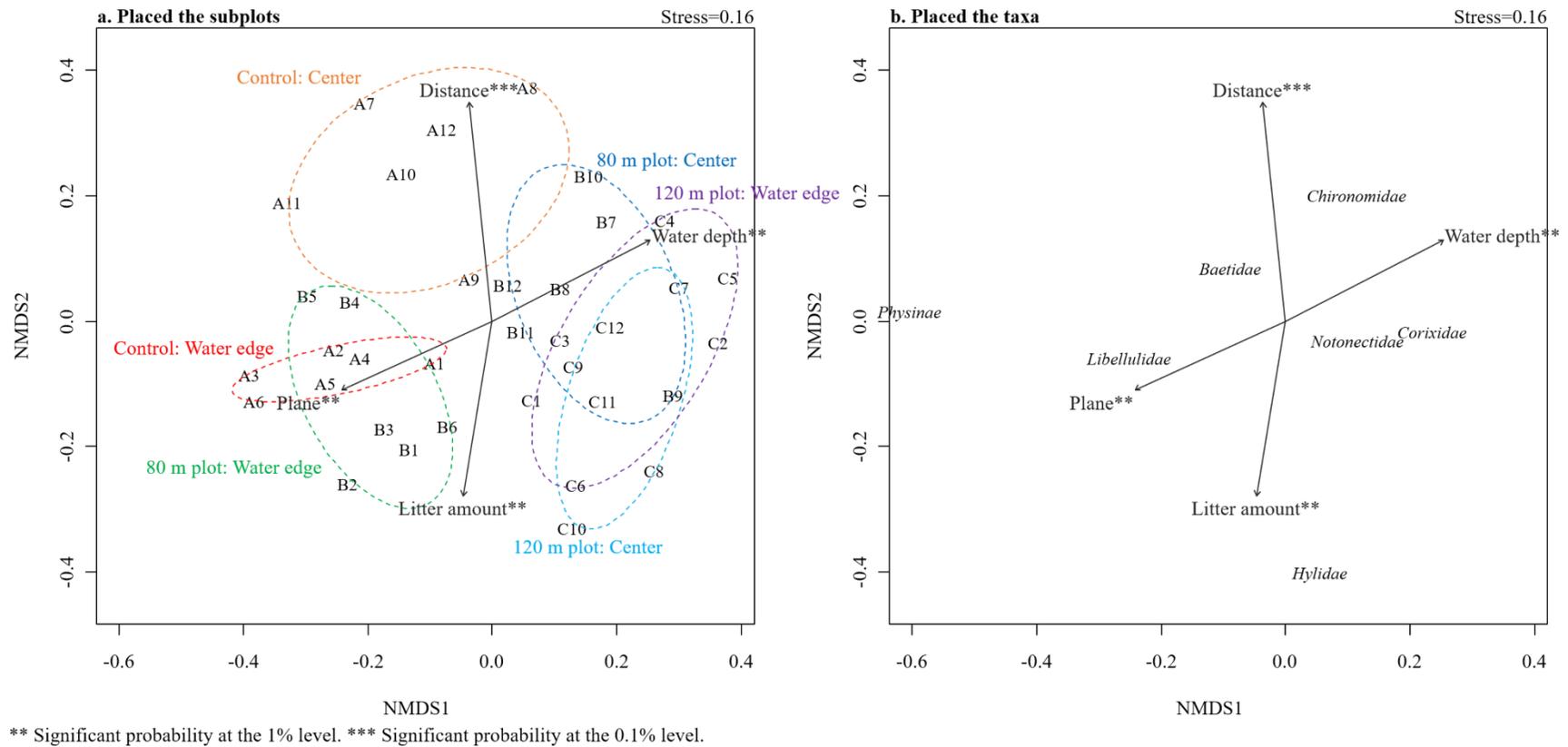
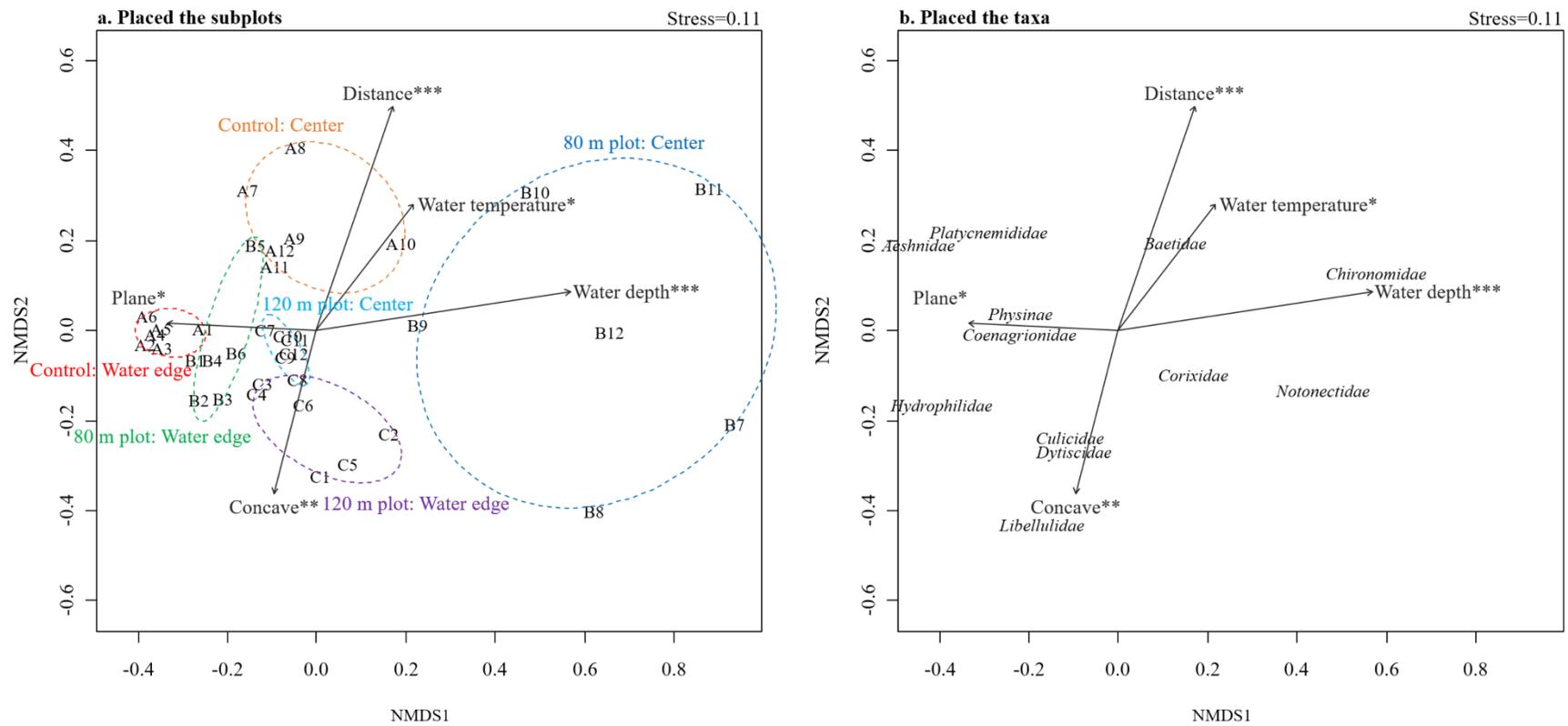


Fig. 15 Relationship between aquatic animals and water edge environmental factors in July investigation



* Significant probability at the 5% level. ** Significant probability at the 1% level. *** Significant probability at the 0.1% level.

Fig. 16 Relationship between aquatic animals and water edge environmental factors in September investigation

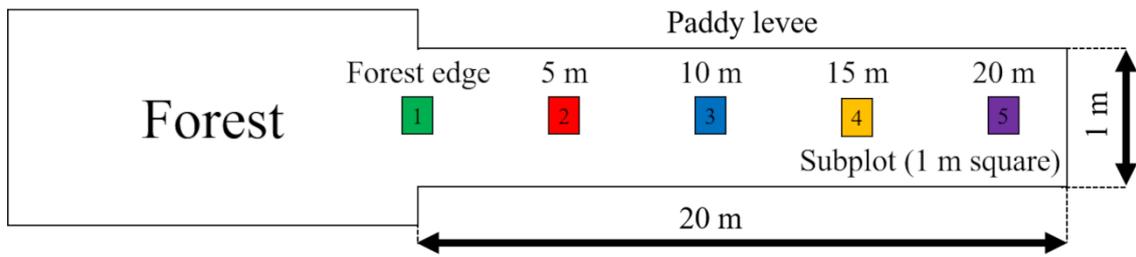


Fig. 17 Details of the belt transect in chapter V

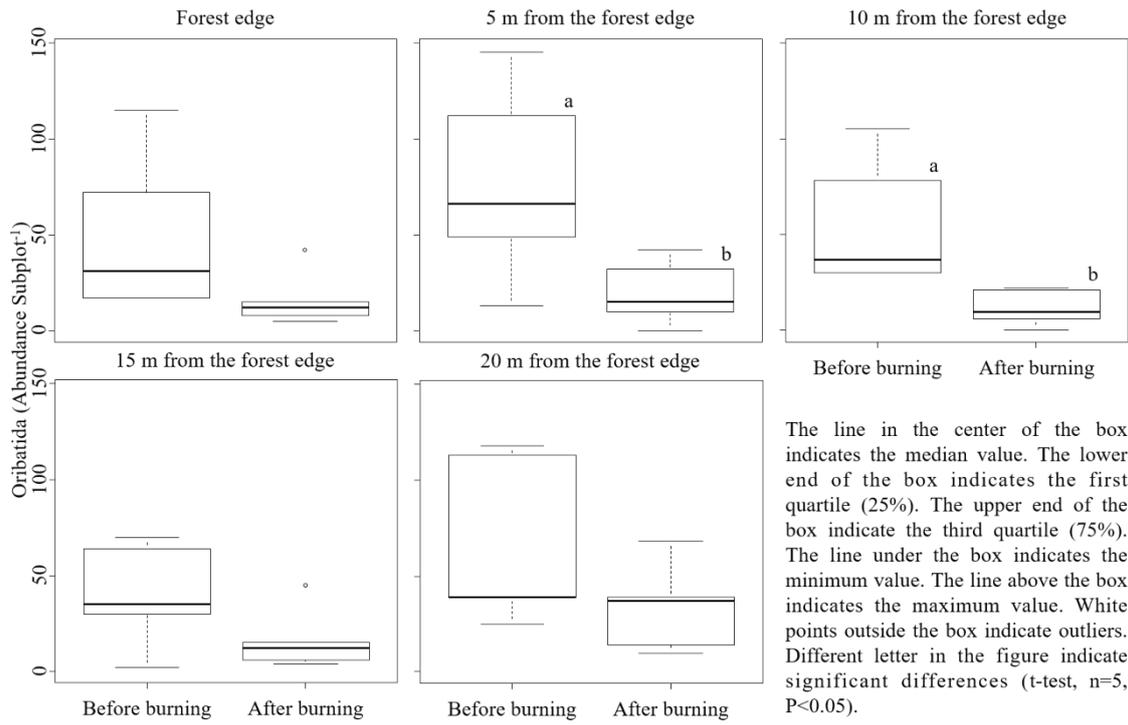


Fig. 18 Oribatida abundance before and after the burning experiments

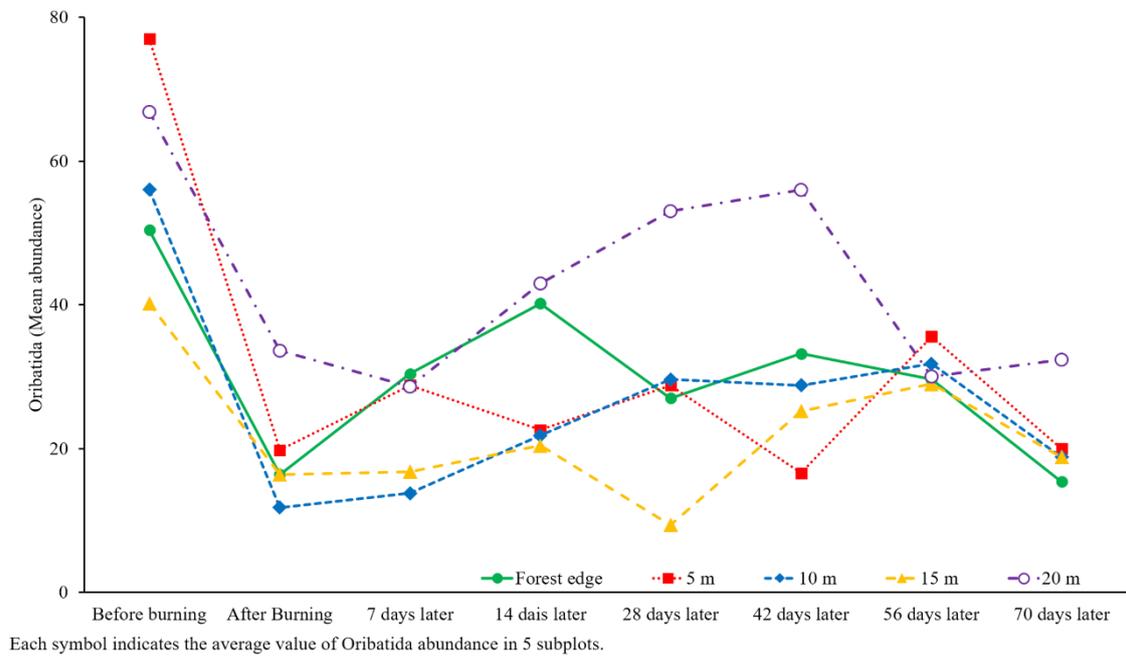
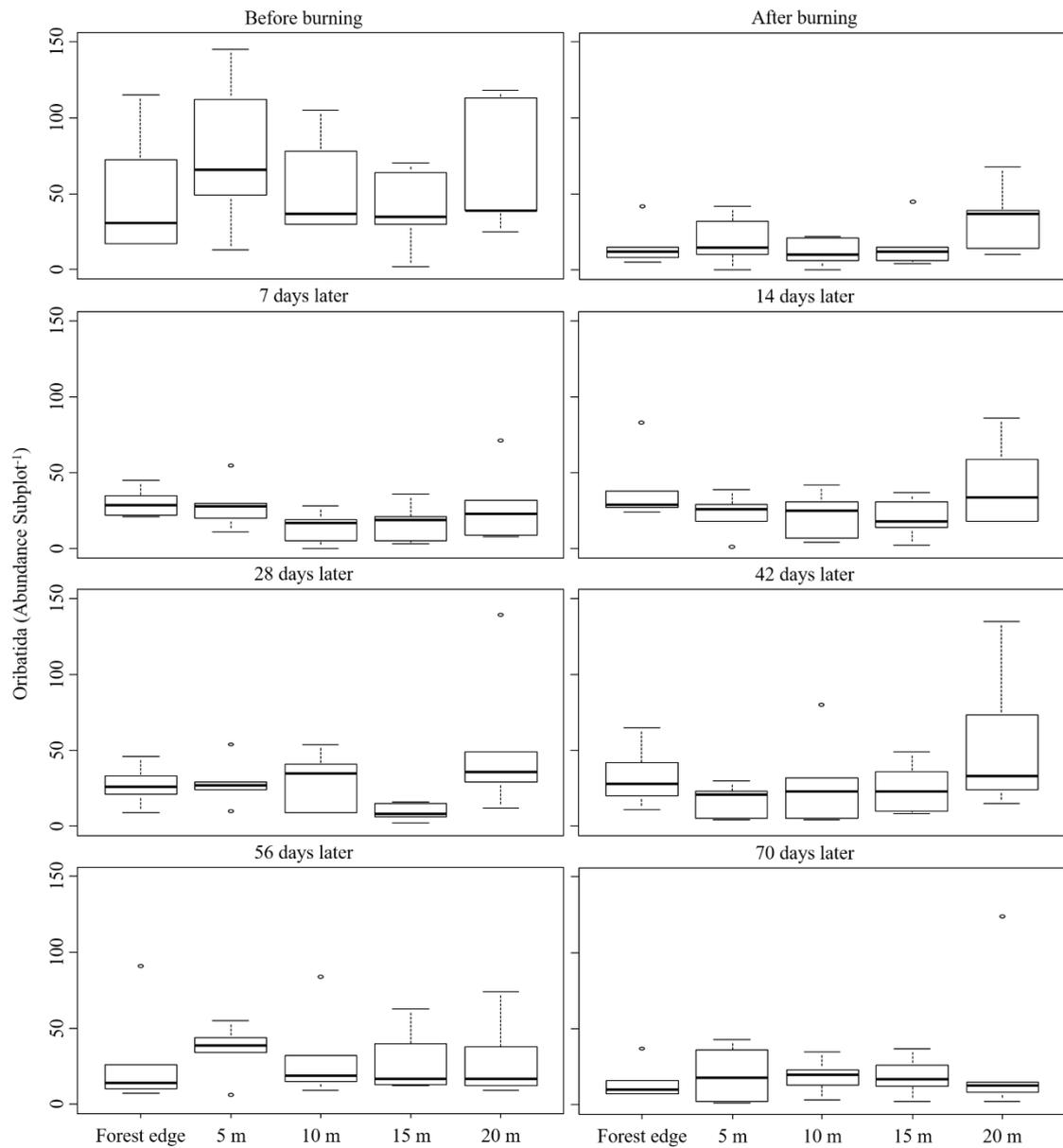


Fig. 19 Oribatida abundance fluctuation from the burning experiment to 70 d later



The line in the center of the box indicates the median value. The lower end of the box indicates the first quartile (25%). The upper end of the box indicate the third quartile (75%). The line under the box indicates the minimum value. The line above the box indicates the maximum value. White points outside the box indicate outliers. Oribatida abundance of each subplot was not significantly different in all investigations (Tukey-Kramer).

Fig. 20 Oribatida abundance from the burning experiment to 70 d later

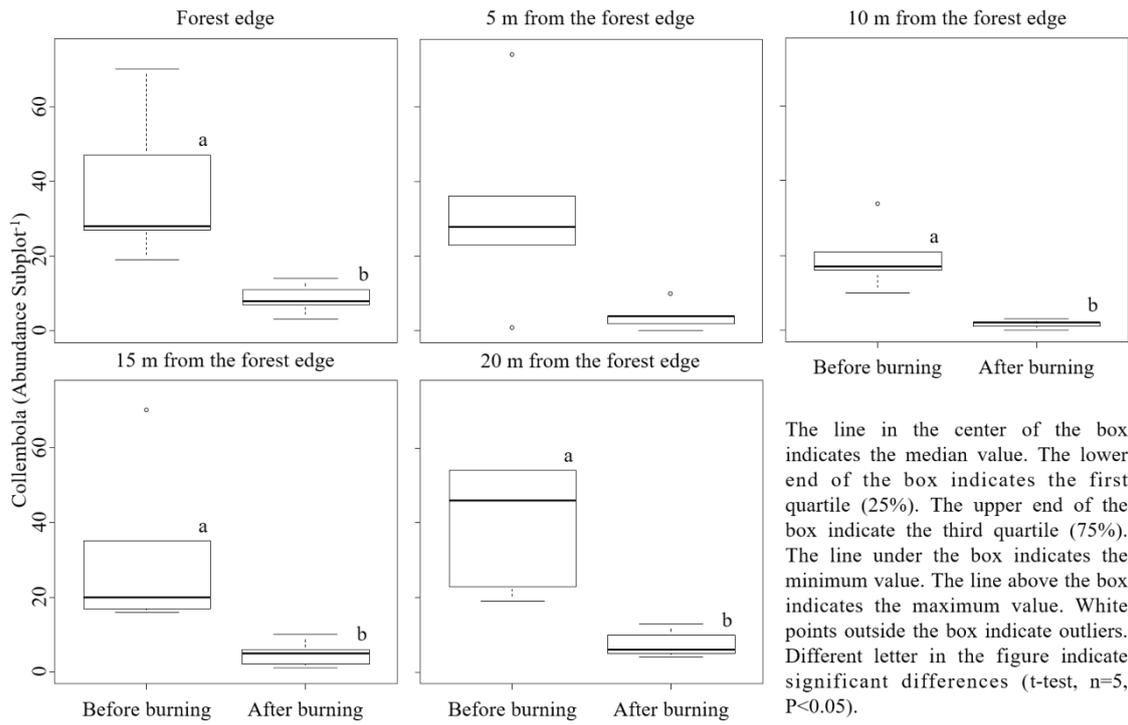


Fig. 21 Collembola abundance before and after the burning experiments

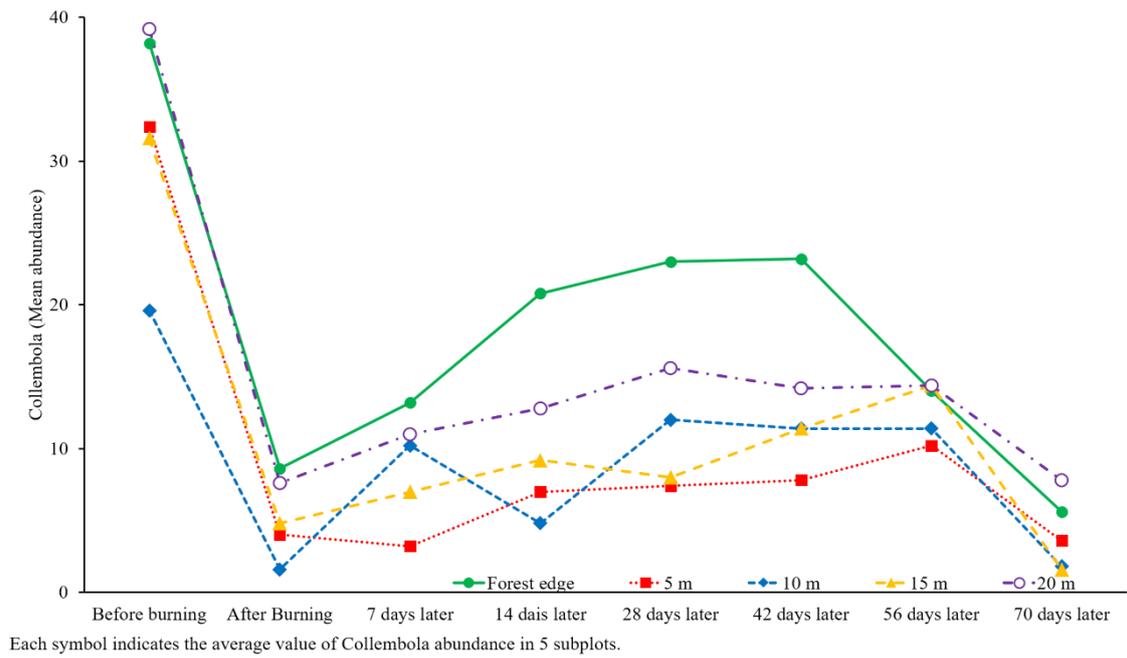
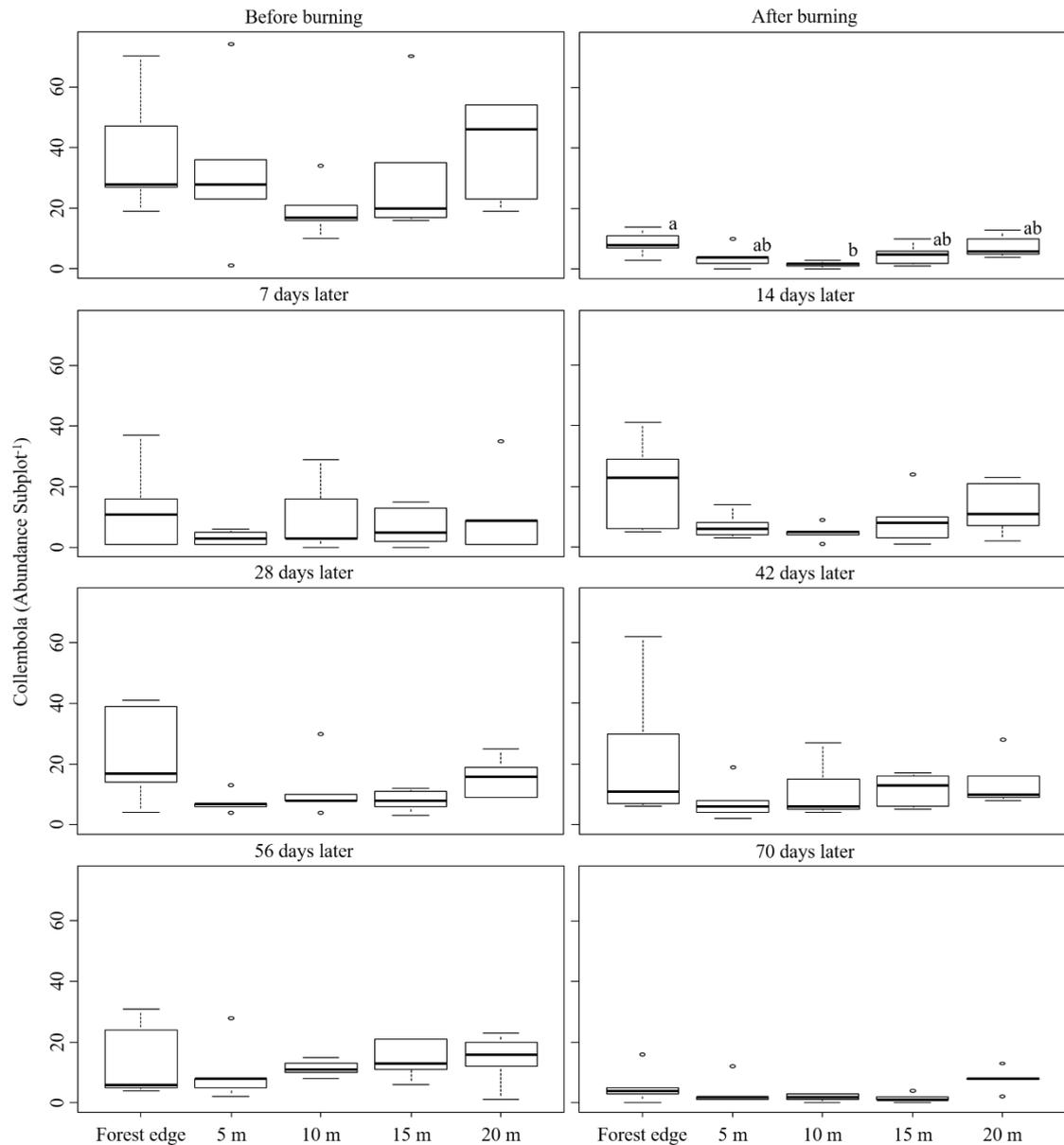


Fig. 22 Collembola abundance fluctuation from the burning experiment to 70 d later



The line in the center of the box indicates the median value. The lower end of the box indicates the first quartile (25%). The upper end of the box indicate the third quartile (75%). The line under the box indicates the minimum value. The line above the box indicates the maximum value. White points outside the box indicate outliers. Different letter in the figure indicate significant differences (Tukey-Kramer, $n=5$, $P<0.05$).

Fig. 23 Collembola abundance from the burning experiment to 70 d later

Table A1 Vegetation pattern in each belt transect

Plant species	Landscape type I												Landscape type II												Landscape type III																
	F				FE				PL				F			FE			PL			F				FE				PL											
	A	B	C	D	A	B	C	D	A	B	C	D	E	F	E	F	E	F	E	F	G	H	I	J	K	L	G	H	I	J	K	L	G	H	I	J	K	L			
<i>Carpinus tschanoskii</i>	30	20	.	.	.	10	.	.	80	.	80	20	10	30			
<i>Quercus serrata</i>			
<i>Cryptomeria japonica</i>			
<i>Quercus salicina</i>			
<i>Neolitsea sericea</i>			
<i>Acer amoenum</i> var. <i>matsumurae</i>	20	30	.	60	.	30	.	40	20			
<i>Fraxinus lanuginosa</i> f. <i>serrata</i>			
<i>Acer pictum</i>	.	20	+	10	.	10	30			
<i>Acer rufinerve</i>	.	20	.	10	.	10	.	20	10			
<i>Prunus grayana</i>	.	10	.	.	.	5	.	20	70			
<i>Juglans mandshurica</i> var. <i>sieboldiana</i>	.	.	70	.	.	40	40	40			
<i>Prunus</i> sp.	20	30	.	.	.	20	.	.	.	40			
<i>Quercus crispula</i> Blume	50	30	30	40			
<i>Camellia japonica</i>	60	50	10			
<i>Aesculus turbinata</i>	20	50			
<i>Magnolia obovata</i>	30	20			
<i>Kalopanax septemlobus</i>	50			
<i>Aucuba japonica</i> var. <i>borealis</i>	20	10	20	40	50	20	10	10	10	5	.	5	.	5	10	.	.	10			
<i>Lindera umbellata</i> var. <i>membranacea</i>	10	10	.	70	20	5	.	60			
<i>Cephalotaxus harringtonia</i> var. <i>nana</i>	10	20	40	.	30	20	30	20	5			
<i>Eurya japonica</i>	.	30	10	5	10	30	20	.	.	5	10	5			
<i>Viburnum dilatatum</i>	.	.	20	.	30	.	20	5	5	5	10	10			
<i>Corylus sieboldiana</i>	.	.	.	40	.	30	10	20	.	5	.	.	.	5	20	10			
<i>Rhus trichocarpa</i>	20	30	10	10	5	5	5			
<i>Camellia rusticana</i>	10	20	30	10	.	.	.	10	20	5			
<i>Pueraria montana</i> var. <i>lobata</i>	90	20	10	10	.	20	.	5				
<i>Akebia</i> × <i>pentaphylla</i>	.	40	.	10	.	40	50	70	50				
<i>Dioscorea japonica</i>	.	.	.	40	20	.	30	.	10	10				
<i>Paederia scandens</i>	70	10	5	5	.	+				
<i>Cayratia japonica</i>	50				
<i>Clematis apifolia</i>	90				
<i>Persicaria senticosa</i>	90				
<i>Equisetum arvense</i>	30	.	25	20	+	5	5	10	70	20	10	5
<i>Commelina communis</i>	.	5	+	10	.	10	.	.	.	5	5	5	10		
<i>Oxalis corniculata</i>	20	.	30	20	20	.	20	30	10
<i>Cyperaceae</i> sp.		
<i>Pteridophyta</i> sp.	5	.	.	.	10	30	.	.	5	10	.	.	20	.	40	.	20	10	30	5			
<i>Blechnaceae</i> sp.		
<i>Hydrocotyle</i> sp.		
<i>Polygonum thunbergii</i>	40	.	.	10	80	10		
<i>Galium odoratum</i>	.	.	5	.	.	.	10	80		
<i>Trifolium repens</i>	80	40		
<i>Persicaria filiformis</i>	30	30		
<i>Plantago major</i>		
<i>Pteridium aquilinum</i>	30	20		
<i>Desmodium podocarpum</i> subsp. <i>oxyphyllum</i>	50		
<i>Poaceae</i> sp.	10	5	5	30	30		
<i>Digitaria ciliaris</i>		
<i>Miscanthus sinensis</i>	10	50	
<i>Paspalum thunbergii</i> Kunth ex Steud.		
<i>Zoysia</i> sp.	70		
<i>Echinochloa oryzicola</i>		

F indicates subplot "Forest". FE indicates subplot "Forest edge". PL indicates subplot "Paddy levee". The number in the table indicates the coverage (%). The (+) in the table indicates the coverage was less than 1%. The table lists the plant species for which the total coverage at all study sites was 50% or more.

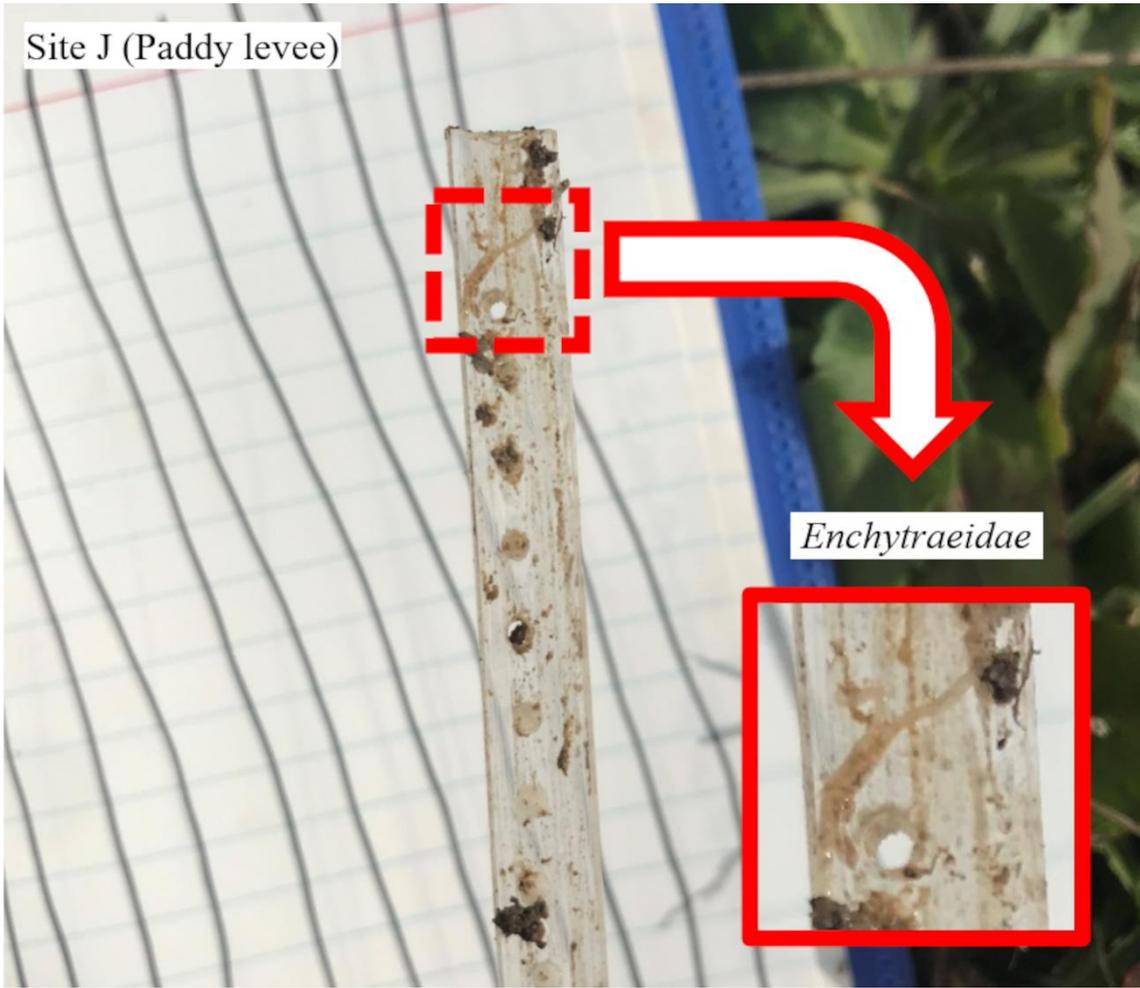


Fig. A1 *Enchytraeidae* eating samples placed on the bite stick

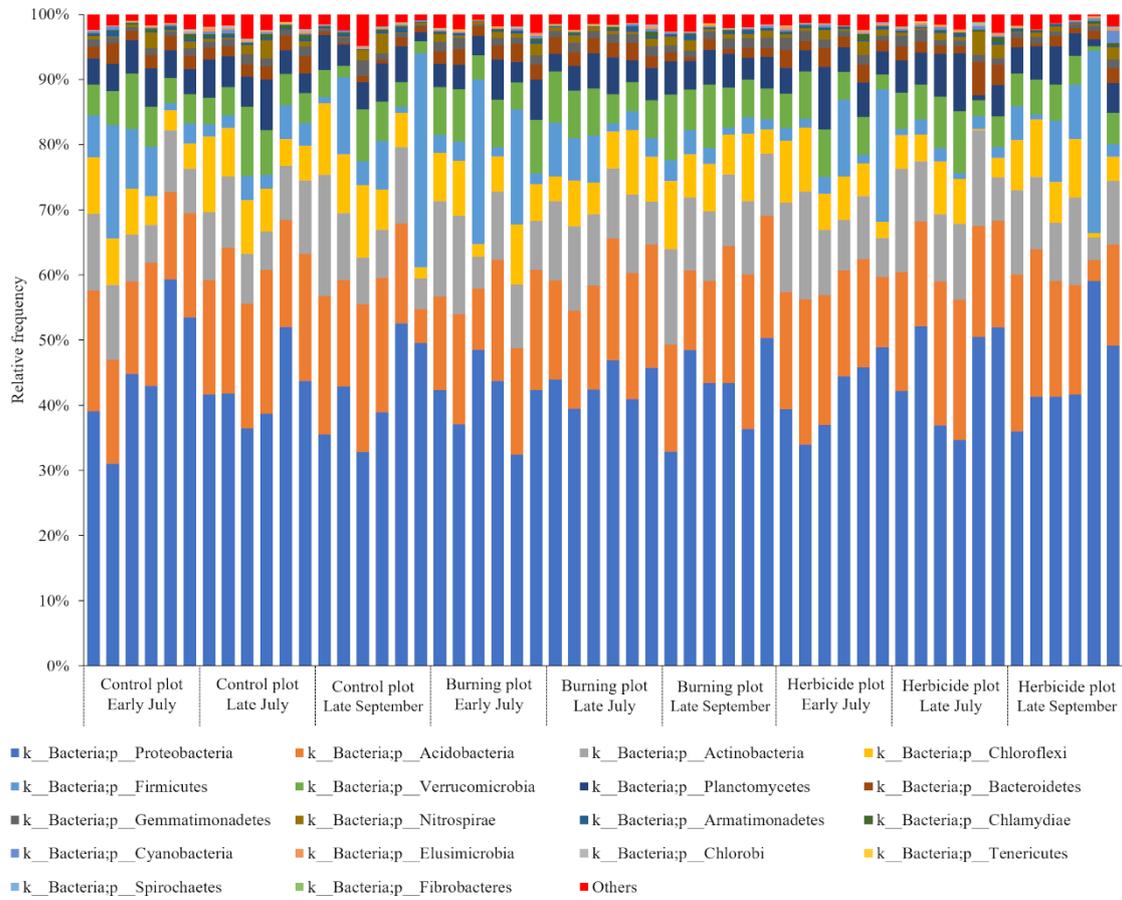
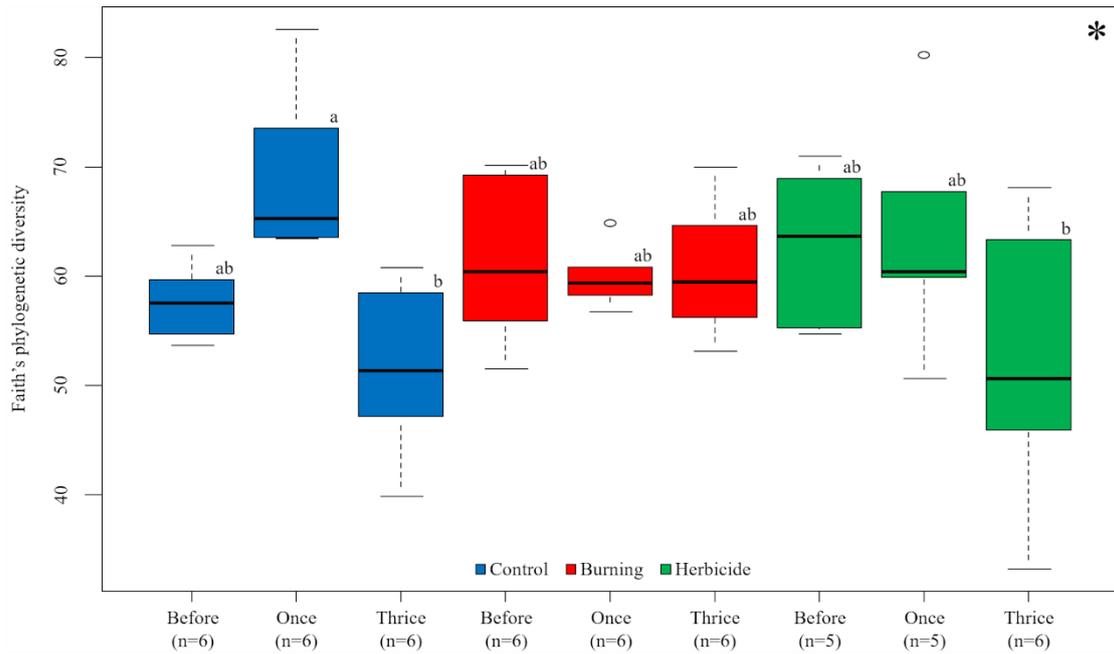
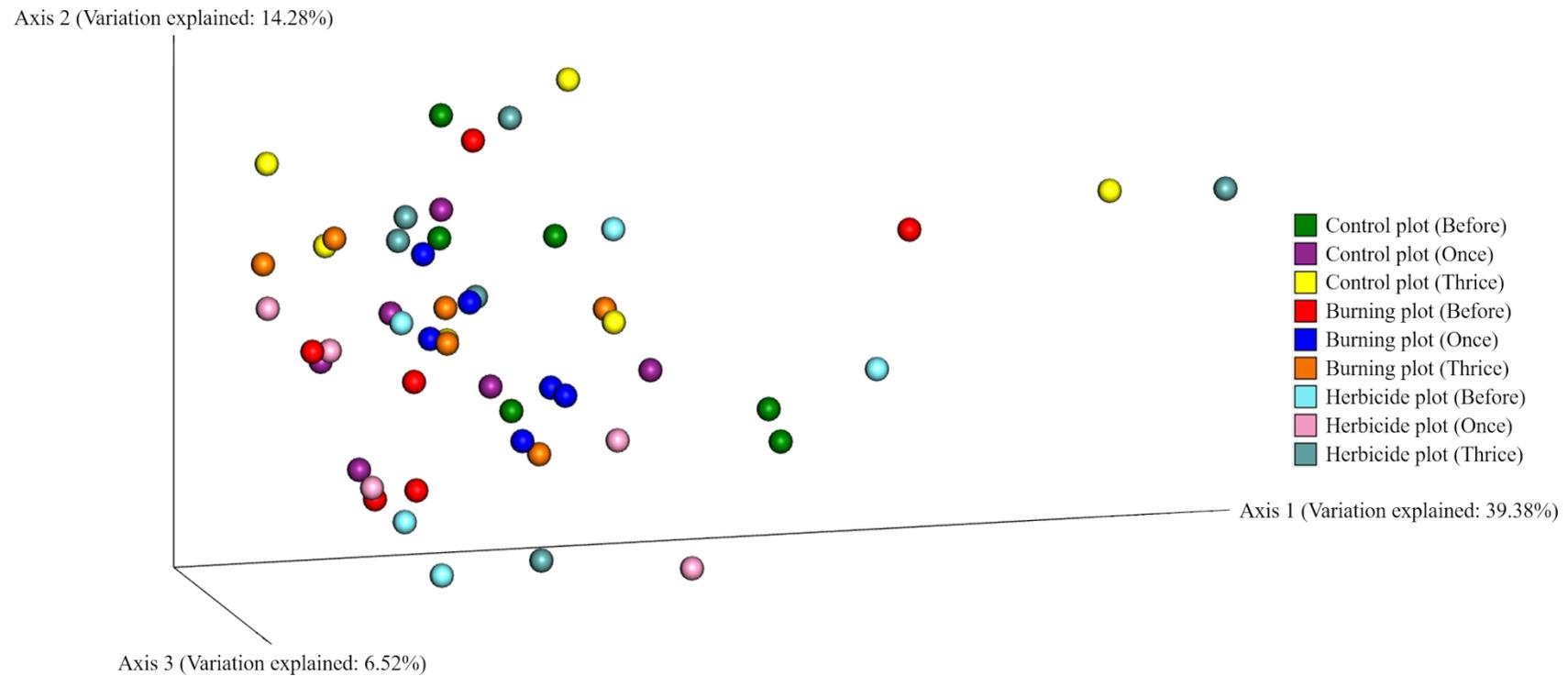


Fig. A2 Taxon composition of soil bacterial community



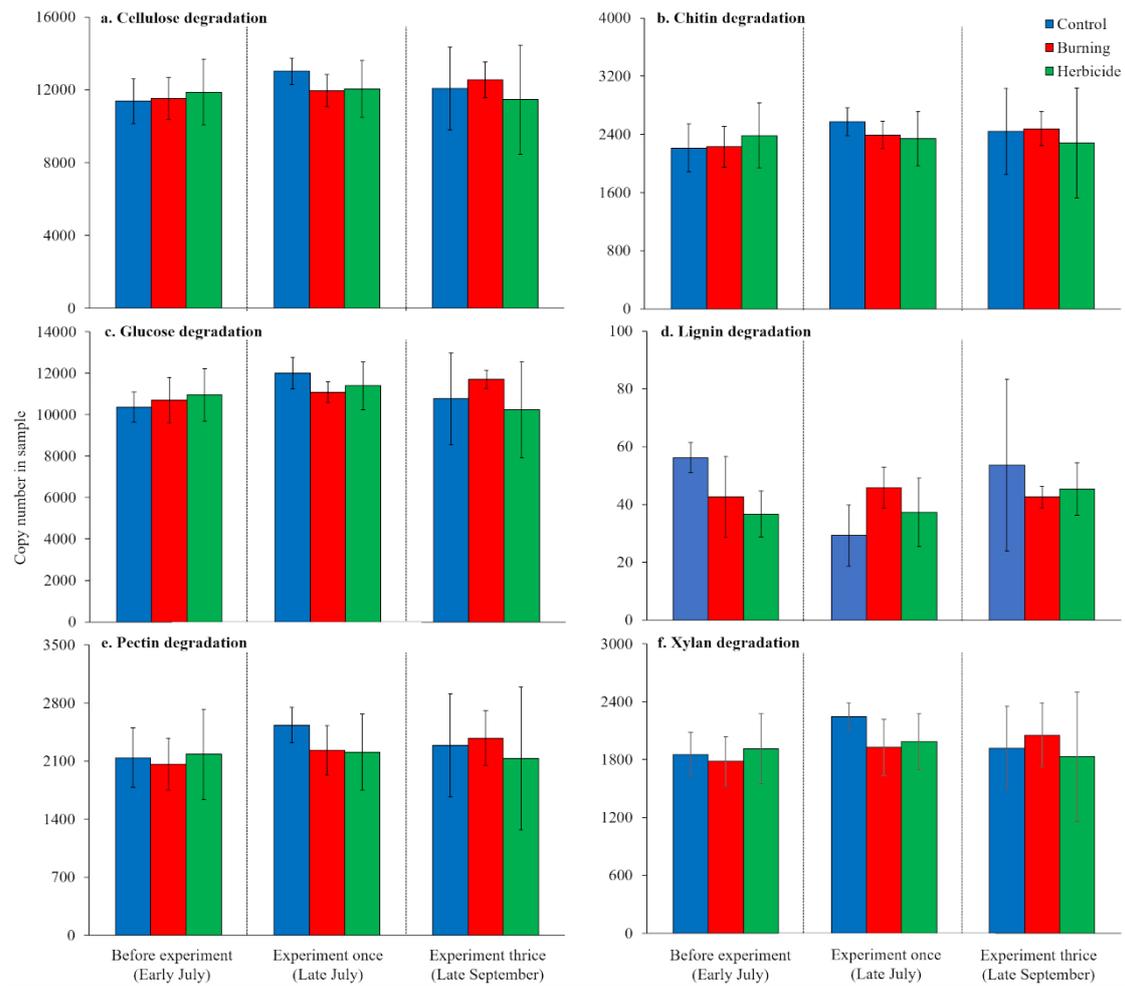
The line in the center of the box indicates the median value. The lower end of the box indicates the first quartile (25%). The upper end of the box indicates the third quartile (75%). The line under the box indicates the minimum value. The line above the box indicates the maximum value. White points outside the box indicate outliers. * Significant difference at the 5% level (one-way ANOVA). Different letters in the figure indicate significant differences (Tukey-Kramer, $P < 0.05$). Before indicates "before the experiment (Early July)". Once indicates "after the first experiment (Late July)". Thrice indicates "after the third experiment (Late September)".

Fig. A3 Faith's phylogenetic diversity (α -diversity) of soil bacterial community



The weighted UniFrac distance was used to calculate the β -diversity. Before indicates “before the experiment (Early July)”. Once indicates “after the first experiment (Late July)”. Thrice indicates “after the third experiment (Late September)”.

Fig. A4 β -diversity of soil bacterial community (PCoA)



The graph indicates the average value. The bar indicates the standard deviation. One-way ANOVA was performed between each experimental plot (n=3) for every investigation (Before experiment, Experiment once, and Experiment thrice). The copy number of all functional genes were not significantly different between all experimental plots (one-way ANOVA).

Fig. A5 Functional genes involved in the carbon cycle



Taken on June 22, 2017 in *satoyama*, northern Thailand.

Fig. A6 The herd of cattle grazing around the *satoyama* in northern Thailand