Effects, and Their Mechanisms, of Temporal and Special Continuity in Grasslands on Plants- and Butterflies- communities

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Abstract

　　Grasslands have been reported to be terrestrial ecosystems with high biodiversity. However, they are declining worldwide and in Japan. Therefore, there is an urgent need to conserve grasslands and to identify grasslands with high biodiversity that should be prioritized for conservation. In recent years, it has been reported that the history of grasslands influences their biological communities. In this doctoral thesis, I answer the questions of how the spatial and temporal scale of grassland history affects the plant-butterfly community and whether grassland history is a suitable indicator of grassland biodiversity and conservation priorities.

　　In Chapter 2, to evaluate whether the temporal continuity of grasslands promotes biodiversity, and thus can be an indicator of conservation priority, I studied vascular plant communities in old (300–1000s years) and new (52–70 years after deforestation) grasslands, as well as in forests, of Sugadaira Highland in central Japan. The number of plant species was highest in old grasslands, followed by new grasslands and forests. This pattern was much clearer in the number of grassland-dependent native and grassland-dependent endangered species, indicating the role of old grasslands as refugia for those species. The species composition differed between old and new grasslands. New grasslands had species compositions in between those of old grasslands and forests, suggesting that the plant community in new grasslands retains the influence of past forestation for more than 52 years after deforestation. Eleven indicator species were detected in old grasslands, but none in new grasslands, suggesting the uniqueness of the plant community in old grasslands. We conclude that the temporal continuity of grasslands increases plant diversity and can be an indicator of grasslands with high conservation priority.

　　In Chapter 3, to confirm the generality of this finding, I targeted three regions of central Japan (40 to 90 km apart from each other) including the previously-studied one. The number of grassland rare species and grassland native species were highest in the old grasslands. Species composition differed markedly between the old and new grassland, with the beta diversity amounting to as high as nearly half of that across the regions. Number of indicator species were 21 and four for the old and new grasslands, respectively, indicating that many plant species depend on the old grasslands. Among the indicator species of old grasslands, significantly larger proportion of species had underground stems. The detected strong effect of vegetation history across three regions clearly verified the high plant diversity and high conservation priority of grasslands with long temporal continuity.

　　In Chapter 4, I investigated whether grassland history also affects butterflies. Species composition, number of species, and total abundance of butterflies did not vary depending on whether the site was an old or new grasslands. However, the area of old grasslands within a 100 m radius of the site affected the species composition. When the sites were divided into large old grasslands (>8000 m^2 within a 100 m radius) and others, *Minois dryas* and *Eurema mandarina* were detected as indicator species in the large old grasslands, whereas no indicator were detected in the others. *Minois dryas*, *Brenthis daphne*, and *Curetis acuta* were more abundant as the area of old grassland increased. *Sanguisorba officinali*, the host plant of *B. daphne*, and Lespedeza bicolor, the main host plant of E. mandarina and C. acuta, depended on old grassland, explaining the dependence of these three butterfly species on old grasslands.

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However, *Miscanthus sinensis*, the main host plant of *M. dryas*, was an indicator for the entire grassland, suggesting that the presence of host plant alone cannot explain why *M. dryas* depends on the old grasslands. My results suggest that large old grasslands have high conservation priorities for maintaining grassland butterfly diversity.

In Chapter 5, migration among grasslands was investigated to gain a better understanding of spatial effects on butterflies. Three butterfly species, *Minois dryas* and *Brenthis daphne*, which differed in food grasses and habitat, plus *Plebejus argus*, which is found throughout the grasslands and has a large population, were surveyed for mark-recapture. Peak populations in 2021 were August 30 for *M. dryas*, August 2 for *B. daphne*, and July 12 for *P. argus*. No direct migration was recorded between study sites. Based on population size estimates, maximum abundance was 1560 individuals in *M. dryas*, 182 individuals in *B. daphne*, and 2550 individuals in *P. argus*. During this survey, no active migration between grasslands was observed for any of the three species. Therefore, the butterfly fauna in the vicinity of the sites is considered to reflect the surrounding environment, including the grassland history, as there was no active migration from the sites of occurrence.

The plant and butterfly communities varied with grassland history, with plants being more diverse in old grasslands, especially native and rare grassland species. Based on these findings, it can be suggested that sites with longer grassland continuity have unique and highly diverse communities and are a high conservation priority. Grassland history affected small scales for plants and broad scales for butterflies. The reasons for this may be related to differences in resource requirements and migratory capacities. For plants, it was found that species and perennial species with well-developed rhizomes were more common in old grasslands. It is thought that herbaceous species with these characteristics are suitable for semi-natural grasslands that are stable and subject to disturbance, and that the increase in the number of grassland species over the years since the grasslands were created has led to the formation of communities that are unique to old grasslands. Among butterflies, grassland species were found to be more abundant in old grasslands. It is thought that the formation of grassland plant communities and the communities of butterflies that prefer them have been formed. Based on these findings, the most effective way to conserve grassland butterfly-plant interaction systems would be to conserve large areas of old grassland.

Chapter 1. General introduction

Grasslands are terrestrial ecosystems with high biodiversity (Akeroyd & Page, 2011; J. B. Wilson et al., 2012). However, all over the world, natural grasslands have been lost through conversion to farmland, with 50% of the world's grasslands disappearing between 1700 and 1990 (Boakes et al., 2010; Goldewijk, 2001) . Semi-natural grasslands, where forestation has been prevented by burning, mowing, and grazing, and where agricultural use has been promoted, are widespread, especially in Europe and East Asia. Semi-natural grasslands are declining due to management abandonment and afforestation (Dengler & Tischew, 2018)。 These land use changes are one of the main causes of biodiversity loss (Gerstner et al., 2014; Sala et al., 2000).

In Japan, natural grasslands existed during the glacial period in a cold and dry climate and were inhabited by grassland species introduced from the continent (Suka, 2008) . Post-glacial warming and wetting decreased natural grasslands, but human activities have increased semi-natural grasslands, which have functioned as refugia for grassland plants and animals. Semi-natural grasslands have been used for agriculture, but after the 1950s, with the advent of chemical fertilizers and agricultural machinery, the demand for grassland-dependent livestock and manure decreased. This led to abandonment of management, forestation through afforestation, and conversion to other land uses. As a result, grasslands, which covered more than 13% of the country's land area in the 1900s, have been reduced to approximately 1% at the present time (Ogura, 2006). Due to the rapid decline of grasslands, grassland plants and animals are in danger of extinction (Kaneko et al., 2009; Nakamura, 2010).This decline in biodiversity due to inactive land use is considered by the Ministry of the Environment of Japan to be a "second crisis" of biodiversity (Ministry of the Environment of Japan, 2012).

Grassland management such as burning, mowing, and grazing is necessary to protect the biodiversity of semi-natural grasslands. In the past, grassland management was carried out with a great deal of effort for social and economic reasons, but now that the motivation for such grassland management has greatly decreased, it is not realistic to aim for the same level of grassland management for the same area as in the past. Therefore, it is necessary to identify grasslands that should be prioritized for grassland management to conserve biodiversity. As an indicator of grassland biodiversity, I focus on the vegetation history of grasslands. In recent years, it has been successively reported that the duration of grassland affects the current biological community. It has been reported that grasslands with longer duration have higher plant diversity (Cousins & Eriksson, 2002; Straubinger et al., 2022) , that duration affects the diversity and community of arbuscular mycorrhizal fungi (Honnay et al., 2017) , and that grasslands with longer duration endangered species have been reported (Yaida et al., 2019) . Investigating the impact of vegetation history on the biodiversity of current grassland plant species not only provides valuable insights for assigning conservation priorities, but also enhances our understanding of the processes by which biological communities are formed. However, few studies to date have investigated whether plant and animal community structure changes (e.g. Honnay et al., 2017). These temporal effects of vegetation history also interact with spatial scale. Both the temporal continuity and spatical area of habitats have positive effects on the number of grassland plants species(Noda et al., 2022).

Different effects from spatial factors have been reported for plants and butterflies. The proportion of grassland specialist butterfly species increases with increase of size of the habitat patch (Brückmann et al., 2010), although such habitat size did not affect plant specialist species in the same study. Experimental fragmentation   of habitat also shows decrease in diversity of butterflies but not of plants (Zschokke et al., 2000). Even the effects of grassland history may differ between plants and animals, depending on the spatial scale, but there are no studies on this.

The mechanisms by which grassland history influences biological communities are also unknown. Previous studies have shown that grassland specialist plants increase over time in grassland management (Waldén et al., 2017). The translocation of these grassland specialists over time is thought to create unique communities, but it is unclear what characteristics of grassland specialists make them suitable for the long-term continuity of grasslands. In this doctoral thesis, I discuss the effects of temporal continuity of grasslands on plant diversity and species composition in Sugadaira Highlands in Chapter 2, the effects of temporal continuity of grasslands on plant communities in the three regions in Chapter 3, the effects of temporal continuity of grasslands on butterfly communities in Chapter 4 Chapter 5 discusses the movement of butterflies between grasslands. Finally, a comprehensive discussion will be provided.

This doctoral thesis will address the questions of (A) the effects of the spatiotemporal scale of grassland history on biological communities and (B) what are the mechanisms by which grassland history affects biological communities and (C) whether grassland history is suitable as an indicator of diversity and conservation dominance. This will help to clarify the effects of the temporal and spatial continuity of grasslands on plant-butterfly interaction systems.

Chapter 2. The effects of temporal continuities of grasslands on the diversity and species composition
        of plants

2-1. Introduction

Grasslands may be natural, existing under natural climatic conditions and disturbance regimes, or they may be semi-natural, whereby they are maintained by artificial disturbances (Squires et al., 2018). Semi-natural temperate grasslands have high species diversity and conservation values (Akeroyd & Page, 2011; Organisation for Economic Co-operation and Development [OECD], 2008; Wilson, Peet, Dengler, & Pärtel, 2012). However, approximately half of the natural grasslands in the world have been converted to farmlands (Goldewijk, 2001), and semi-natural grasslands have also experienced a worldwide decline. In parts of Britain, 47% of semi-natural grasslands disappeared between 1960 and 2013 (Ridding et al., 2015). In Sweden, 38.3% of pasture declined from 1980 to 2003 (Food and Agriculture Organization of the United Nations [FAO], 2010). In Japan, although grasslands constituted 13% of land area 100 years ago, this area has gradually decreased and in the early 2000s grasslands made up just 1% of land area (Ogura, 2006). The decrease in grasslands in Japan is mainly attributed to the decline in the economic value of semi-natural grasslands and the abandonment of their management since 1950, followed by natural succession to forests (Nishiwaki, 1999; Ushimaru et al., 2018). This decline in semi-natural grassland area is one of the major causes of biological extinction in Japan (Ministry of the Environment of Japan, 2012, 2016).

Given the rapid decline in semi-natural grasslands due to ongoing social and economic issues, it's not practical to conserve all of the remaining grasslands. If it was possible to predict which grasslands harbour more biodiversity-rich communities compared to others, high conservation priority could be given to certain 'hotspot grasslands'. One factor that might be relevant to the formation and retention of biodiversity-rich communities is the history of the vegetation community. The effect of history on species richness and community assembly is one of the central issues in recent research in the field of community ecology. For example, present-day plant species diversity of European grasslands has been shown to be affected by habitat connectivity 50–100 years ago (Lampinen et al., 2018; Lindborg & Eriksson, 2004). In addition, current butterfly species richness in tropical rainforests has been shown to be affected by past disturbance (Whitworth et al., 2016), and bird community composition in a forest is affected by the maintenance of that forests temporal continuity (Culbert et al., 2017). Finally, the diversity of arbuscular mycorrhizal fungi has been shown to be higher in grasslands maintained over 12–20 years compared to those maintained for only ten years (Honnay et al., 2017). Thus, investigating the effect of vegetation history on current grassland plant species biodiversity will not only improve understanding of the process by which biological communities are formed, but also provide valuable knowledge for allocating conservation priority.

Semi-natural grasslands in the Sugadaira Highland of central Japan are believed to have been in a continuous state of existence since 4000 years ago, estimated by ^{14}C dating of the underlying Andosol (Yamanoi, 1996), which is known to be specifically generated by and accumulated in grasslands

(Yamane, 1973). Literary records also indicated that the almost entire area has been a grassland for 300 years (Inoue et al., 2021). Since the 1930s, parts of these grasslands have been maintained by mowing and used as ski runs, and during the last 100 years large parts of these have changed to forests due to forest plantation as well as natural succession triggered by the abandonment of human grassland management. When skiing became popular in Japan in the 1960s–1970s, some parts of these forest plantations were then clear-cut and converted to ski runs. However, overall approximately 92% of grassland area in this region has been converted to forests in the past 100 years. Recently, Yaida et al. (2019) reported that ski runs in this area functioned as refugia for grassland-specific endangered plants. In this study, I investigated plant communities in old grasslands (160–1000s years) and new grasslands (52–70 years after deforestation), both of which are managed as ski runs in the Sugadaira Highland. Although many studies have only focused on the effect of temporal continuity on species richness (Cousins & Eriksson, 2001, 2002; Gustavsson et al., 2007; Johansson et al., 2008), this study dealt with both species richness and composition. In addition, this study includes nearby forests. In order to examine the effect of temporal continuity of grasslands on plant communities and its relevance to conservation priority, this study addressed whether (i) plant diversity was higher in old grasslands compared to new ones; (ii) species composition differed between old and new grasslands; and (iii) the plant communities of new grasslands are still influenced by past forestation.

2-2. Methods
2-2-1. Study area and sites

Field surveys were conducted in ski run grasslands and adjacent secondary forests in the Sugadaira and Minenohara Highlands of central Japan (latitude 36.51182–36.55727°, longitude 138.30424–138.35716°, altitude 1319–1522 m) in 2017. In 2017, the mean annual temperature was 6.0 °C, with a mean temperature of the warmest and coldest month was 19.2 and - 6.1 °C in July and January, respectively, and the annual precipitation was 1061 mm (Sugadaira Montane Research Center, 2011).. Within this area, grasslands were identified and geographical information system (GIS) data were generated, using topographic maps from 1910 and 1937, and aerial photographs from 1947, 1975 and 2010. All of these resources are available from the Geospatial Information Authority of Japan (GIAJ, http://mapps.gsi.go.jp/maplibSearch.do#1). An old map (Sugadaira kaikon no zu) from 1855 was also used. In 1855 almost the entire study area, was semi-natural grasslands, probably maintained by pasturing, fire and mowing. Some grasslands have been consistently maintained since that time and have been used as ski runs since 1923. These grasslands, with a long history of the same vegetation (at least 162 years), were defined as old grasslands for this study. Other grasslands were afforested by plantation after 1910, then subsequently deforested between 1947 and 1965 to create ski runs. These grasslands were defined as new grasslands (52 to 70 years old). All ski run grasslands (new and old) are maintained by mowing in the autumn, a conventional form of management (Yaida et al., 2019).

2-2-2. Field investigation

Within the study area, seven old grasslands, six new grasslands, and seven forests were chosen so that study sites of each vegetation type were distributed as uniformly as possible (Figure 1). The forest sites were set just beside either old or new grassland sites and those forests included *Larix* plantation and *Quercus*-dominated ones. All grassland sites were in ski areas, except for one old grassland that was set in the University of Tsukuba's Sugadaira Research Station as a reference semi-natural grassland free from the effect of skiing. A 1 × 20 m transect was positioned in each study site at least 20 m away from borders between grasslands and forests. In each transect I recorded the presence of vascular plant species in July and September 2017. Several plants were classified into grassland-dependent native species according to Satake et al. (1981, 1982a, 1982b), and grassland-dependent endangered species by defining endangered species as those above the Near Threatened (NT) rank in the Red lists of at least one prefecture in Japan.

2-2-3. Statistical analyses

The numbers of total plant species, grassland-dependent native species, and grassland-dependent endangered species were compared among vegetation types, using generalized linear models (GLMs) with Poisson distributions and the log link, in the statistics software *R* ver. 3.5.0 (R Core Team, 2018). The following three models were constructed: the null model, the two vegetation model, and the three vegetation model. In the null model, the numbers of species were assumed the same among all vegetation types. In the two vegetation model, grasslands and forests were assumed to have different species numbers. And in the three vegetation model, old grasslands, new grasslands and forests were assumed to have different species numbers. Models were selected based on Akaike's information criterion (AIC) followed by the likelihood ratio test. To graphically ordinate the variation in plant species composition among the vegetation types, non-metric multidimensional scaling (NMDS) with the Jaccard index as the index of dissimilarity was conducted using the *vegan* library (Oksanen et al., 2018) in *R*. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001) was used to test the effect of grassland type (old or new) on the species composition. This analysis was implemented using the *adonis* function in the *vegan* library (Oksanen et al., 2018) in *R*, with 10,000 permutations. A Mantel test was conducted, using the *vegan* library in *R*, to detect whether there was spatial autocorrelation between the dissimilarity index and geographic distance for all pairs of grassland sites. Indicator species were detected for each of the three vegetation types using the *indval* function (IndVal test; see Dufrêne & Legendre, 1997) in the *labdsv* library (Roberts, 2016) in *R*. The IndVal test was repeated for the three-vegetation-type case (old grasslands, new grasslands and forests), two-vegetation-type case (grasslands and forests), and one-vegetation-type case (vegetation type not discriminated), as recommended by Dufrêne and Legendre (1997). For each species, the indicator value was compared across each vegetation type for each case. Each species was then regarded as an indicator for the vegetation type in which it had the highest indicator value.

2-3. Results

In total, 245 plant species were detected. For total plant species richness the three vegetation model was selected, indicating that each vegetation type effects plant diversity differently (Δ AIC to null model = 53.5, GLM and likelihood ratio test: $p < 0.001$, Table 1a). The number of plant species in each transect (Table S1) was the highest in old grasslands, followed by new grasslands and forests (Figure 2 (a)). The difference between old and new grasslands was supported for all vascular plants (Δ AIC to second model = 3.8, GLM and likelihood ratio test: $p < 0.05$, Figure 2 (a), Table 1a). Differences in the species richness of grassland-dependent native plants (Table S1) among vegetation types (Δ AIC to the second model = 20.1, GLM and likelihood ratio test: $p < 0.001$, Figure 2 (b), Table 1b) and that in grassland-dependent endangered plants (Δ AIC to the second model = 14.3, GLM and likelihood ratio test: $p < 0.001$, Figure 2 (c), Table 1c) were larger than that in species richness of all plant species.

Plant species compositions significantly differed between the forests and the whole grasslands (PERMANOVA: $p < 0.001$). Based on the only grasslands data then, it also differed between the old and the new grasslands ($p < 0.01$). Old grassland sites were narrowly distributed within the NMDS plot (Figure 3), showing that old grasslands have a specific vegetation community. New grassland sites were distributed between old grassland and forest sites, showing that the species composition is more similar between new grasslands and forests than between old grasslands and forests. The relationship between dissimilarity calculated by the Jaccard index and spatial distance is shown in Figure 4. Spatial autocorrelation in plant species composition was not detected (Mantel test, $p = 0.139$).

The IndVal test detected 11 indicator plant species for old grasslands, seven for grasslands (including both old and new), and two for forests (Table 2). However, no species was considered an indicator of new grasslands (Table 2).

2-4. Discussion

Compared with new grasslands, higher plant diversity was found in old grasslands (Figure 2 a), where the temporal continuity of grasslands was longer. These findings are in accordance with a previous study that reported a higher number of plant species in older grasslands (Gustavsson et al., 2007) compared to newly generated grasslands that were once used as arable lands. The current study also showed that old grasslands have a role as refugia for endangered species that depend on grasslands (Figure 2c). The finding that the diversity of grassland-dependent native species and grassland-dependent endangered species were clearly higher in the old grasslands (Figure 2b, c), is in agreeance with previous studies (Gustavsson et al., 2007; Johansson et al., 2008; Waldén, Öckinger, Winsa, & Lindborg, 2017) reporting richer grassland specialist species in old grasslands. Because the positive effect of the temporal continuity of a grassland on plant diversity has only been previously reported in Sweden (Gustavsson et al., 2007), the current study (in Japan) suggests the generality of this effect independent of other factors such as continent. In addition, the current findings suggest that plant diversity can be negatively affected by discontinuity of grassland history due to both cultivation (Gustavsson et al., 2007) and forestation (this study, both by afforestation and natural succession) . However, some previous studies (Cousins &

Eriksson, 2002; Culbert et al., 2017; Johansson et al., 2008) did not detect a difference in plant diversity between old and new grasslands. These studies might contain several sources of heterogeneity; such as regional heterogeneity due to a large study area (approximately 90 km) in Culbert et al. (2017), soil heterogeneity including wet, mesic, and dry soil in Cousins and Eriksson (2002), and management heterogeneity stemming from different levels of discontinuity in Johansson et al. (2008). Higher levels of heterogeneity increase the variance of species richness values even within the same grassland type; so that the effect of the temporal continuity of a grassland may be more difficult to detect. In contrast, this study, and that of Gustavsson et al. (2007), examined the effect within a narrow spatial-scale, using a simple binary comparison between continuity and discontinuity, and for only mesic or dry soil conditions.

This study confirmed that plant species composition differs between old and new grasslands (Figure 3). This finding concurs with a study by Honnay et al. (2017), which examined grasslands of different ages (8–20 years old). However, the current study covered a much longer time-scale, revealing that 52–70 years old grasslands had different plant communities from the older ones (160–1000s years old). In contrast to grassland sites, forest sites had a larger variation in species composition, shown in the NMDS plot (Figure 3). The Jaccard similarity index, which corresponds to the number of shared species between sites, was low between all forest sites. Also, a fewer number of indicator species were detected in forest sites (Table 2). Combined, these results suggest that plant communities vary considerably between forest sites, which can probably, at least partly, be explained by the fact that several different types of forests were included in this study (*Larix* plantation and *Quercus*-dominated forests with several types of forest floor). Despite such variation, the NMDS plot still showed that all of the forest sites formed a single cluster separated from both types of grasslands. However, the plant species composition of new grasslands and forests was more similar than that of old grasslands and forests (Figure 3), suggesting that new grasslands still harbor plant species that recruited in forests. Previous studies (Jonason, Bergman, Westerberg, & Milberg, 2016; Jonason et al., 2014) have reported that plant communities in grasslands generated after clearing forests are influenced by whether those forests once experienced grassland vegetation before forestation. Jonason et al. (2014) showed that grassland species remained as remnant populations after the afforestation. However, the difference in plant species composition between old and new grasslands found in this study (Figure 3) implies that even a single event of forestation eliminates some grassland plants. Although all of the new grasslands used to be semi-natural grasslands with a long history, many plant species, especially grassland-dependent ones, did not restore viable populations. Such irreversible effects of forestation found in this study have important implications for conserving grassland biodiversity.

Eleven indicator species were detected for the old grasslands, but none for the new grasslands (Table 2), suggesting that the old grasslands have a cluster of unique species as shown in Figure 3, whereas the new grasslands do not have particular characteristics in their species composition. This pattern can be explained by seed-dispersal limitation during the colonization of newly-generated grasslands (Bischoff, 2002; Rosenthal, 2006). Whilst plants with low seed-dispersal ability are most likely limited to old

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grasslands, those with high seed-dispersal ability could be distributed in both the old and new grasslands. This pattern was most clearly pronounced in *Adenophora triphylla* and *Sanguisorba officinalis* (indicator values of 100, Table 2): both of these species were present in all seven old grasslands but were absent in all the other sites (seven new grasslands and six forests). The high dependence of these two species on old grasslands can also be explained by their low seed-dispersal ability as they lack any organs specialized for seed dispersal (Numata et al., 1990; Numata & Asano, 1969). A study in a lowland plain in eastern Japan (Koyanagi et al., 2007) also detected these two species as indicator species for grasslands with high plant diversity, implying that those species-rich lowland grasslands may have long temporal continuity that has enabled the two species to recruit.

This study showed that old grasslands had higher plant species diversity, higher richness of grassland-dependent endangered plant species, and unique species compositions, clearly indicating that they are of high conservation priority. Although new grasslands are likely to be in a state of constant formation and disappearance, old grasslands are consistently decreasing because they are irreversibly lost due to the abandonment of grassland followed by forestation, leading endangered species to further decline. Ski runs in Japan are regularly maintained by mowing and include old grasslands with high conservation priority, thus this study further confirmed the important role of ski runs in Japan as refugia for grassland-specific endangered species (Yaida et al., 2019). Given the rapid and large-spatial-scale decline of semi-natural grasslands in Japan as well as across the world, it is not practical to conserve all grasslands. Thus, it is proposed that grasslands with long temporal continuity have high conservation priority and are where conservation effort should be concentrated.

Chapter 3. Strong effects of temporal continuity of grasslands on plant diversity and species composition over three regions.

3-1. Introduction

The effects of grassland history on species diversity of biological communities have been studied in European plants, arbuscular mycorrhizal fungi, butterflies, and birds (plants, Cousins & Eriksson, 2002; Kull & Zobel, 1991; arbuscular mycorrhizal fungi, Honnay et al., 2017; butterflies, Culbert et al., 2017; birds, Culbert et al., 2017）, and in East Asian plants (Noda et al., 2022). Positive effects have been found in plants, arbuscular mycorrhizal fungi, and no effects in butterflies and birds. However, the effect of grassland history on species composition of communities has been reported for plants in Belgium, where communities in old grasslands of at least 250 years differ from communities in new grasslands of 8-20 years old (Honnay et al., 2017), but not for other taxa or regions. Also, history effects have been conducted on histories and communities within specific regions (e.g Cousins & Eriksson, 2002; Gustavsson et al., 2007; Honnay et al., 2017) , with no studies covering multiple regions. Culbert et al. (2017) studied at a broad scale across prefectures, but did not consider the effects of distance between study sites. Therefore, the generality of the effect of vegetation history and its relative importance among the determinants of biological communities is not clear. Geographic distance is considered to be one of the key determinants of community variation (Nekola & White, 1999; Wang et al., 2015) . This is believed to be due to environmental differences with distance, different environments between distant communities acting as dispersal barriers, limited dispersal of organisms, and a combination of these factors (Soininen et al., 2007) . The importance of grassland history for these geographic distance effects has not been tested.

In Chapter 2, I clarified the effect of grassland history on vegetation in the Sugadaira Plateau. In this chapter, to verify that this effect is a general phenomenon regardless of region, we expanded the spatial scale of Chapter 2 and added two ski resort areas, Hakuba and Kirigamine, 40-90 km away from Sugadaira Kogen, to the study area. By doing so, I aim to (a) verify the generality of the effect of history, (b) verify how important historical factors are compared to geographical differences, and (c) elucidate the characteristics of species dependent on old-grasslands by increasing the number of plant species observed.

3-2. Materials and Methods
  3-2-1. Study area

Field surveys were conducted in 2017_2018 in the grasslands and adjacent secondary forests of three ski resort areas (Sugadaira, Hakuba, and Kirigamine) in Nagano Prefecture, Central Japan. The study site and data for Sugadaira are the same as in Chapter 2. using the same methodology as in Chapter 2, I traced vegetation changes from the 1910s to the present in and around the grasslands where the Hakuba and Kirigamine ski areas are located. 1910–1912, 1930–1937. Topographic maps and aerial photographs from 1946–1948, 1975, and 2010 were used to identify the meadows and geographic information system

data were generated by QGIS (QGIS Development Team, 2019) . All of these materials were obtained from the GSI website (http:// mapps.gsi.go.jp/maplibSearch.do#1). Historical data indicate that grasslands covered the area at least 300 years ago in Sugadaira (Inoue et al., 2021) , and dating of andosol indicates that grasslands existed in Sugadaira about 4000 years ago (Yamanoi, 1996) and in Kirigamine about 5000 years ago(Togashi et al., 2018) . In this study, we define old-grasslands as those that have never been forested since 1910, when relatively frequent records of grassland succession become available, and have persisted for at least 110 years. We also defined new grassland as areas of present grassland that contained a forested history within the same time period and were converted to grassland by deforestation. The grassland duration of new grasslands ranged from 45 to 89 years, which is discontinuously different from the duration in old grasslands.

3-2-2. Field investigation

In the study area, as in Chapter 2, vegetation was surveyed in 5 to 8 1 x 20 m plots (58 plots in total) for each vegetation type: old grassland, new grassland, and forest (Table 3, Figure 8). Two surveys were conducted in Hakuba in 2017 and in Kirigamine in 2018; the first survey was conducted from June to August and the second from September to October. The emerged plants were classified as grassland-dependent native species and grassland-dependent rare species based on ecological information from pictorial books (Satake et al., 1981, 1982b, 1982a) . Environmental factors were investigated concurrently with the botanical survey, and slope and vegetation height were recorded. Slopes were measured from the center of the transect to 5 m wide, and vegetation heights were measured at the center of the transect width at a total of five locations, 5 m apart, and averaged.

3-2-3. Statistical analyses

As in Chapter 2, the effects of vegetation type on the total number of plant species, grassland-dependent native species, and grassland-dependent rare species were analyzed with the statistical software *R* version 4.0.4 (R Core Team, 2021) using a generalized linear model (GLM) that assumed a Poisson distribution for the dependent variable. Model selection was based on the Akaike Information Index (AIC), and the reliability of the selected models was evaluated by likelihood ratio tests. To identify differences in plant communities among study sites, Jaccard's dissimilarity index was calculated from the presence and absence of plants using the *vegan* library (Oksanen et al., 2020), and coordinates were assigned to a two-dimensional plane using the nonmetric multidimensional scaling method (NMDS). From vascular plant data obtained in the grassland, we used PERMANOVA(Anderson, 2001; McArdle & Anderson, 2001) with the *adonis* function of the vegan package to determine differences in grassland history (old grassland vs. new grassland) and community composition by study area. differences in community composition by grassland history (old grassland vs. new grassland) and study regions. To identify plant species that characterize each vegetation type, an indicator species analysis (Indval, Dufrêne & Legendre, 1997) was performed using the *indval* function of the library *labdsv* (Roberts,

2019). In addition to the analytical methods used in Chapter 2, the following analyses were performed. Binomial tests were performed on the number of significant indicator species for old- and others obtained. In order to detect species characteristics specific to old grasslands, ecological information of plant species was collected(Numata et al., 1990; Numata & Asano, 1969; Numata & Yoshizawa, 1975). Among the ecological information on plant species, Raunkiaer's life form (Raunkiaer, 1934) was classified as annual or perennial, underground organ type was classified as underground stem developed or non-developed (Non-clonal growth monophyte), and dispersal organ type was classified as gravity/mechanical dispersal or wind/animal dispersal. Information of color of the flowers was also collected on the which was classified into whether they were purple (including blueish color), a characteristic that makes them a vector for hymenoptera (Wilson et al., 2004). A generalized linear models were constructed assuming a binomial distribution of the objective variable, and null model was compared with a model in which two variables, old grassland indicator species and other grasslands (new grassland and whole grassland) indicator species, were given as explanatory variables to determine what kind of species characteristics old grassland indicator species often have.

3-3. Results

In total, 487 vascular plant species were recorded. For total plant species richness, three vegetation model was selected by AIC, indicating that each vegetation type (old grassland, new grassland, and forest) had different number of species, although there was no statistical difference between three-type model and two-type model by likelihood ratio test (Table 4a). There were significant differences between the null model and both the three-type and two-type models ($p < 0.001$, likelihood test), with more species in grasslands than in forests (Figure 4a). For both grassland-dependent native species and grassland-dependent rare species, the two-type models were selected, by AIC and the likelihood ratio test ($p < 0.001$ for both, Table 4b)with more such species in old grasslands than in new grasslands (Figure 4bc).

Plant species in grassland sites differed between study regions (PERMANOVA, $p < 0.001$; Figure 7) and between old and new grasslands (PERMANOVA, $p < 0.001$; Figure 7). R^2 for regional difference and vegetation type (old vs. new grassland) are 0.122 and 0.053 (Table 5), which correspond to 0.349 and 0.230 in $|R|$, respectively, indicating comparable effect of vegetation type to regional difference. In the NMDS plot (Figure 7), new grassland sites were distributed between old grassland and forest sites, indicating relative similarity in species composition between new grasslands and forests. The IndVal test detected 15 and four indicator species for old and new grasslands, respectively (Table 6and 7). Indicator for old grasslands had higher proportion of species with well-developed underground stems ($p < 0.05$, Figure 8a), marginally higher proportion of perennial species ($p = 0.09$, Fig. 8b), and higher proportion of species with purple flower ($p < 0.01$, Fig. 8c).

3-4. Discussion

The number grassland-dependent native and rare species was particularly higher in the old grasslands (Fig. 4bc). This result is consistent with previous studies (Gustavsson et al., 2007; Johansson et al., 2008) that shows grasslands with longer duration have richer grassland-specialist plant species. It was also clear that species composition differed between old and new grasslands (Table 5). Th effect is consistent with Honnay et al. (2017) for Belgian plant community, however, the present study clearly suggest that such effect are common across plural regions. Whilst Honnay et al. (2017) compared grasslands of 8-20 years duration with those of at least 250 years, the present study compared rasslands of 45-89 years duration with those of at least 110 years. Inoue et al. (2021) suggest that 45-89 years duration is not sufficient for grassland to restore rich plant community, and the present study suggests that such temporal pattern is common between plural regions.

They also found that old grasslands have become refugia for endangered species dependent on grasslands (Figure 6c). Many species depend on old grasslands, which may have an important role in conserving biodiversity. In addition, new grasslands are located between old grasslands and new grasslands and are thought to retain the effects of having once been forests. It is known that in forests that were once open lands, plant diversity is higher due to the greater number of herbaceous species (Koerner et al., 1997) This indicates that herbaceous plants will survive for some time after forestation. In addition, some herbaceous plants form long-term seed banks (Koyanagi et al., 2011) . Based on these findings, it is possible that all new grasslands in Sugadaira in Chapter 2 were formerly grasslands, and that buried seeds from that time may promote the recovery of the original vegetation when the area is converted back to grassland. Also, in Hakuba and Kirigamine, some, but not all, of the former grassland forests have been converted back to grassland. However, as can be seen on the NMDS plot, there is little overlap between the extent of old grassland and new grassland in each area. It can be said that even if grasslands are generated from forests, grassland plant communities do not fully recover in the 45–89 year time period.

Geographic differences have been considered to be one of the factors determining communities (Nekola & White, 1999; Wang et al., 2015) . The three study regions were separated by a horizontal distance of 40–90 km and elevation of 4–990 m, making them geographically distinct habitats. In the NMDS plot (Figure 7), there was much overlap between Sugadaira and Kirigamine, but less overlap with Hakuba. The study sites in Hakuba were the lowest in elevation, and the lack of elevation overlap with the other two areas may have reflected this in the community composition. In sum, geographic differences were again a major factor in determining assemblages in this study. In contrast, the influence of history contributed nearly half as much as geographic differences (Table 6). This suggests that grassland duration is a community determinant comparable to some large (considerable) geographic variation.

Characteristics of indicator species were detected in old grasslands: species with well-developed underground stems and perennial plants. In a meta-analysis of many plant species, it is known that plants with longer life spans invest more in underground stems (Vico et al., 2016). In the r/K strategy concept (MacArthur & Wilson, 1967), both are characteristics of K-strategy species. In addition, K-strategy

species with longer life spans and higher subsurface investment may be favored in the grassland ecosystem if the ecosystem is subjected to a stable degree of disturbance over time that prevents forestation and maintains a suitable environment for herbaceous plant growth. The NMDS plots of old grasslands seemed to be highly concentrated in one location, and it is possible that over time, the creation of species communities with old grassland-dependent characteristics may be moving toward a plant species composition that could be described as a climax grassland. Regarding seed dispersal, there was no significant difference between species with long and short dispersal distances in this study ($p = 0.32$). It has been known that wind-dispersed and gravity-dispersed species increase in grasslands with longer management periods (Yaida et al., 2022) , and that Waller's ginseng and tree carrot, detected as strong indicators of old grasslands in Chapter 2, are gravity-dispersed species (Numata et al., 1990; Numata & Asano, 1969) , which is consistent with the fact that they are gravity-dispersed species. Because of the increase in the number of gravity-spread and wind-spread species with short and long spread distances, it is likely that no difference was detected in the analysis of this study, which was divided according to spread distance. Many of the indicator species in the old grassland had purple flowers (Figure 8c). Pollinators differ according to flower color (Bradshaw & Schemske, 2003). Purple flowers are known to be pollinated by hymenoptera (P. Wilson et al., 2004). This suggests that the hymenopteran community may change with the history of the grassland.

Chapter 4. The effects of temporal and spatial continuity of grasslands on butterfly communities.

4-1. Introduction

While previous chapters of this doctoral thesis have focused on plants, some previous studies have reported that vegetation history influences non-plant communities. Taxon diversity and abundance of arbuscular mycorrhizal fungi is higher in grasslands established 12–20 years ago than in grasslands established 8–11 years ago (Honnay et al., 2017). The composition of bird communities in forests was changed by the temporal continuity (Culbert et al., 2017). However, the influence of temporal continuity of grasslands had not been revealed on animals. When grassland plant communities are altered by the temporal continuity of grasslands, butterflies are among the animals most likely to be affected. Butterflies have different requirements for food and habitat at each stage of their life cycle (Van Swaay & Warren, 1999) . They are also strongly associated with habitat factors such as plant diversity and abundance (Maleque et al., 2009) . It can also be a predictor of the rate of decline of terrestrial insects (Thomas, 2005) . In addition, many endangered butterflies use grasslands as habitat (Van Swaay & Warren, 1999) and are of interest in conservation ecology.

In this chapter, I aimed to clarify the effects of temporal continuity of grasslands on butterfly communities within old grasslands (300 to several thousand years old) and new grasslands (52–70 years after deforestation) managed as ski runs in the Sugadaira Highlands, and to identify grasslands that are high conservation priorities. Understanding the impact of past vegetation on current butterfly communities can also answer ecological questions of conservation priorities and butterfly community formation processes. Here, I set four objectives: (1) to determine the effects of temporal continuity on butterfly diversity and abundance, (2) to determine whether community composition differs between old and new grasslands, (3) to determine the effects of spatial distribution of old grasslands on butterfly communities, (4) to identify butterfly characteristics associated with old grasslands.

4-2. Materials and Methods

4-2-1. Study area and Field investigation

As in Chapter 2, field surveys were conducted in ski run grasslands and adjacent secondary forests in the Sugadaira Highlands and Minenohara Highlands of the central Japan (latitude 36.51182–36.55727°, longitude 138.30424–138.35716°, elevation 1319–1522 m, Figure 1)in 2018. For each vegetation type (old grassland, new grassland, and forest), five to eight study plots were established in the same locations (58 in total) as in Chapter 2. However, one forest plot was not surveyed because the black hornet *Vespula flaviceps* was nesting there and it was not safe to survey. In each site, a 5 × 20 m transect was set up to include the vegetation study area in the center; one 12 minute transect walk was conducted and the number of individuals of all species were recorded; from June to September 2018, such butterfly transect surveys were conducted between 9:00 and 17:00, under sunny, windless conditions, on Seven surveys were conducted. The environmental factors used were vegetation height, slope, elevation. I also measured, using the GIS data, the area of old and new grasslands within a radius of 50m and between 100–1000 m in increments of 100 m.

4-2-2. Statistical analysis

To adjust for differences in butterfly abundance among study sites, species counts were rarefied to match the study site with the lowest observed abundance using the *rarefy* function in the *vegan* library (Oksanen et al., 2018) in the statistical software *R* version 3.5.0 (R Core Team, 2018). Throught the subsequent analyses, the number of species referred to the rarefied species number. To examine the effect of vegetation type on the number of individuals and species, generalized linear models (GLMs) were constructed assuming a negative binomial distribution for the number of individuals and a normal distribution for the number of species. The best model was selected based on the AIC and supported by a chi-square test based on the likelihood ratio. To determine the variation in butterfly species composition, butterflies were ordered using non-metric multidimensional scaling (NMDS) with the Bray-Curtis index; NMDS was calculated from the total butterfly abundance using *R vegan* library. The effect of grassland type (old/ new) on species composition was tested by permutational multivariate analysis of variance (PERMANOVA） (Anderson, 2001; McArdle & Anderson, 2001) using the *adonis* function of the *vegan* library in *R*, with 10,000 permutations. Environmental factors were fitted to NMDS plots by permutation tests using the *envfit* function of the vegan library in *R*. As the grassland area, the radius with the highest coefficient of determination in *envfit* was selected from old grasslands within 50 m and 100–1000 m radius (100 m each) from the center of the study site. Indicator species were detected using the *indval* function (IndVal（Indicator value）test; Dufrêne & Legendre, 1997) of the *R labdsv* library (Roberts, 2016). For this indicator species analysis, study sites were classified as broad old grassland, other grassland and forest by old grassland area within a radius R with the highest coefficient of determination in *envfit*. According to the method of Dufrêne and Legendre (1997), the IndVa test was repeated for three vegetation types (broad old grassland, other grassland, and forest), two vegetation types (grassland and forest), and one vegetation type (no vegetation type was discriminated). Each species was then considered as an indicator of the vegetation type with the highest index value among the vegetation types between repetitions. The index values obtained were tested for significance by a randomized test (9999 times). For each butterfly species, a linear model was constructed with the number of individuals as the objective variable and either old grassland area or current grassland area within a radius of R m as the explanatory variable, and its AIC was compared with the null model, and the model with the lower value was selected. Then, using the correlation coefficient between its abundance and old grassland area as an explanatory variable, and three species characteristics (grassland-dependent or not, univoltinism or not, rare or not (listed on the Red List of each prefecture as near threatened or more)) from *The Standard Butterflies in Japan* (Shirouzu, 2006) as objective variables, a generalized linear model was run assuming a binomial error distribution of the objective variables, and its AIC was compared with a null model, and likelihood ratio tests were conducted..

4-3. Results

In total, 39 species (1058 individuals) of butterflies were recorded. For both the number of individuals and species, two vegetation models were selected, indicating that there were more species and individuals in grasslands than in forests (individuals; ΔAIC to null model = 16.0, GLM and likelihood ratio test: $p < 0.001$, species; ΔAIC to null model = 10.5, GLM and likelihood ratio test: $p < 0.001$, Table 8, Figure 9). For butterfly community composition, grasslands and forests had different effects ($p < 0.001$, PERMANOVA, Figure 10). No difference in butterfly communities were detected between old grassland areas with a radius of 0 m from the study site center, meaning old grassland or new grassland ($p = 0.356$, PERMANOVA, Figure 11). On the other hand, old grassland area within a 100 m radius, where the coefficient of determination in *envfit* was the largest among several ranges, explained the variation in butterfly species composition ($p < 0.05$). Other environmental factors were not significant. In the indicator species analysis, two species were detected in large old grasslands (>8000 m^2 within a 100 m radius of the site center), five species in the entire grassland, two species in the forest, and four species in all vegetation, but not in other grasslands (Table 9). Four butterfly species were significantly correlated with the area of old grassland within a radius of 100 m. Three of them (*Minois dryas*, *Brenthis daphne*, *Curetis acuta*) showed a positive correlation, while *Damora sagana* showed a negative correlation (Table 10). One species of *Celastrina argiolus* had a significant positive correlation with the current grassland area (Table 10). Butterfly species characteristics affecting the correlation coefficient between old grassland area and population size tended to be influenced by whether the butterfly was a grassland species or not ($p = 0.08$), and population size tended to increase with old grassland area (Figure 12). No species characteristics were found to influence the correlation coefficient with current grassland area.

4-4. Discussion

Butterfly communities did not differ significantly between old and new grasslands. On the other hand, butterfly communities in sites with large old grassland within a 100 m radius were similar to each other and unique in that they were not found in the new grassland study sites. This result differ from that obtained for plants, where communities differed significantly between old or new grasslands. Two explanations can be offered for the larger spatial scales affecting community composition for butterflies than for plants: first, differences in resource requirements of butterflies and plants affect area effects. For plants, resources from grasslands are available to the range of their root. In addition, especially perennial plants, which compose the large part of the plant communities in my study regions, even a single individual is able to maintain their population for a certain period. Thus, for grassland plants, it is possible to survive even if the necessary resources are not present over a wide area.. However, butterflies require large amount of host plants for their larvae to grow to adult stage. And those adults also require many plant individuals to feed on nectar. In addition, butterflies are annual, so they require a quantity of resources each year to sustain their populations for reproduction. Therefore, butterflies would require large old grasslands rather than plants. Second possibility could be due to differences in

migratory ability. In general, butterflies have higher migration ability than plants. In this study, *Brenthis daphne*, which feeds on *Sanguisorba officinalis*, which depends on old grasslands, appeared as an indicator species for the whole grassland. This meant that the butterflies were detected in different locations than where they occurred. While butterflies that originate from old grasslands are able to fly freely from their origin, their numbers should decrease as they move away from their habitat. This analysis, may have   detected butterflies from a distance as an area effect. In order to identify the cause of the different spatial scale effects between plants and butterflies, it may be necessary to study communities of larva and pupa that identify the source grasslands of adult butterflies.

The number of butterfly species tended to be highest in old grasslands, followed by new grasslands and forests. This result differs from that of another study (Culbert et al., 2017) that found no change in the number of butterfly species with grassland duration. This previous study also examined plants in the same study site, and plants tended to decline in grasslands with longer histories ($p = 0.157$). In my study site, Sugadaira, the number of plant species was significantly higher in old grasslands, followed by new grasslands and forests, as shown in chapter 2. Previous studies (Steffan-Dewenter & Tscharntke, 2000; Uchida & Ushimaru, 2014) have shown that there is a positive correlation between the number of butterfly species and plant species at a given location. This suggests that the large number of plant species in the old grassland at Sugadaira may have had a positive effect on the number of butterfly species. However, Kitahara et al. (2008) showed that the number of nectar plant species is more important than the number of plant species for the distribution pattern of butterfly adults, and that plant species diversity is an indirect effect. The results for plants at Sugadaira only address the presence or absence of species, but if nectar source plants are as abundant as the overall plant population, they may have indirectly increased the butterfly population.

In comparison to that of plants, the results of IndVal gives interesting insights for understanding to the formation process of butterfly communities. There were three patterns between the distribution of butterflies and their host plants. First, the pattern of coincidence between the distribution of butterflies and host plants. In the case of *Eurema mandarina* and *Argynnis paphia*, the distribution of butterfly coincide with the distribution of their major host plants (Table 2, 10). This coincidence of plant and butterfly habitat could simply be due to the fact that there are more adult butterflies in areas with more host plants. Second, the distribution range of butterflies is smaller than that of their host plants. For example, although *Minois dryas* was an indicator butterfly of large old grasslands, its main host plant, *Miscanthus sinensis*, was an indicator of whole grasslands (irrespective of which old or new grasslands) (Table 2). It is possible that butterflies have a preference for certain vegetation types as well as the presence or absence of host plants. In the case of *M. dryas*, the amount of host plants may also be relevant. *Minois dryas* is said to be low-migratory (Hambler et al., 2004), and may have been detected as an old grassland indicator because they grow mainly in old grasslands that have rich *M. sinensis* and stays there. This inference is supported by the fact that *M. dryas* abundance was positively correlated with the old grassland area around the study sites. Third, the distribution of butterflies are wider than that of their host plants: while *B. daphne* was an indicator of the whole grassland, their host plant, *S. officinalis*, was

an indicator of the old grassland, and especially dependent on the old grasslands (Table 2). However, the abundance of *B. daphne* increased with the area of old grasslands (Table 11), suggesting that old grasslands are necessary for their occurrence; *Brenthis daphne* has a different dispersal pattern between males and females: females are more sedentary whilst males more migratory (Kitahara, 1996). Although this study did not distinguish between males and females, it is possible that the butterflies observed in the new grasslands were males migrating from the old grasslands to search for females.

For grassland species of butterflies, their abundance was found to increase as area of old grassland. It has been reported that butterfly diversity and abundance decrease with the number of years elapsed since grassland management was abandoned for grassland butterflies (Oki et al., 2022). The fact that plant communities in new grasslands are closer to forests than in old grasslands may be related to this. As in the case of forestation through vegetation succession, the residual forest influence in grassland conversion may be limiting the populations of grassland species.

In conclusion, this study proposes that grasslands with long temporal continuity have high conservation priority with unique biological communities. *Brenthis daphne*, which population size correlated with the area of old grasslands, indicating its dependence on old grasslands, is threatened as vulnerable species in Japan (Ministry of the Environment Government of Japan, 2020). Even if forested grasslands are converted to grasslands again, they would not provide suitable habitat for the *S. officinalis* that depends on old grasslands Wallace's meadow. In order to conserve this butterfly, which depends on old grasslands that are rarely newly emerged, it will be important to maintain the grasslands that exist today.

Chapter 5. Butterfly migration among grasslands

5-1. Introduction

Chapter 2 showed that grassland plant communities vary with grassland duration, and Chapter 3 showed that butterfly communities also vary with surrounding old grassland area. In order to understand the mechanisms by which butterflies are affected by grassland duration, it is necessary to determine the resource plant distribution and butterfly mobility. Butterflies have been treated as models for biological studies of dispersal (Van Kleunen et al., 2010) and long-distance migration has been observed in several species (Kanazawa et al., 2015; Miller et al., 2012; Stefanescu et al., 2013), among others many biologists have been interested in the migratory habits of butterflies. Butterfly migration had been revealed by methods such as label recapture and DNA analysis.

These have revealed that Butterfly migration is not a single pattern for each species, but varies according to environment (Nowicki et al., 2014; Roland et al., 2000), sex (Kalarus et al., 2013) and other factors. Migration distances vary between grasslands and forests, and butterflies are easily fragmented by forests (Nowicki et al., 2014; Roland et al., 2000). At Sugadaira, grassland fragmentation is occurring mainly due to forestation (Inoue et al., 2021), which may be fragmenting butterfly populations. If isolation made it difficult for butterflies depend on old grassland-specific resource plants to disperse to surrounding area, this would explain why butterfly communities are changing under the influence of environmental factors in grassland. On the other hand, *Brenthis daphne*, which feed on the old grassland-specific *Sanguisorba officinalis* (Chapter 2), were found throughout the grassland (Chapter 4). This deviation of host plant from　distribution range of butterflies could be explained by the high mobility of the *B. daphne* in this study site. Understanding whether some butterfly species are specific to old grasslands because they do not migrate and disperse far from resource plants, or whether they prefer old grasslands even though they are free-ranging, will help us understand how grassland plant communities form.

Therefore, in Chapter 5, I examined three grassland butterflies, *Minois dryas*, *Brenthis daphne*, and *Plebejus argus*, to determine the migratory dispersal, estimated population size, and seasonal extinction of occurrence for each species. Two of the species showed interesting discrepancies between the vegetation type preferred by the butterflies and the vegetation type preferred by their food plants: *Minois dryas* was detected in Chapter 3 as an indicator species for a large old grassland, while its main food plant, *Miscanthus sinensis*, was detected as an indicator species for the entire grassland. Therefore, *Minois dryas* may have a preference for old grassland or be restricted from moving from old grasslands to new grasslands; *B. daphne* was detected as an indicator species for the entire grassland, while its main food grass, *Sanguisorba officinalis*, was detected as an old grassland indicator species. *Brenthis daphne* abundance was positively correlated with old grassland area, and the extent of dispersal from the old grasslands where it occurs is of interest. *Plebejus argus* was included due to their large population size; *Minois dryas* is reported to be very low migratory, often within 10 m in grasslands (Kalarus & Nowicki, 2017). On the other hand, some studies have found migration of up to 4.4 km (Akeboshi et al., 2015).

Minois dryas inhabit two habitat types, dry grasslands and wet grassland and individuals in dry grasslands have been shown to have higher flight capabilities (Kalarus et al., 2013; Kalarus & Nowicki, 2017). The long-distance migration detected by Akeboshi et al. (2015) may be due to the fact that the study site was an dry grassland. *Brenthis daphne* has rarely been studied by mark-recapture. The dynamics of occurrence were surveyed by Kitahara (1996), but population estimates were not provided. Kitahara (1996) also reported a high proportion of males dispersing to the surrounding area in the early stages of occurence. A study of *P. argus* by the mark-recapture method reported that although there were transfers between habitats 600 m apart, new transfers were difficult to obtain when 1 to 3 km apart (Thomas & Harrison, 1992). In addition, their distribution was concentrated in areas where symbiotic ants were present. Furthermore, these studies did not examine multiple species of butterflies from distant taxa at the same location.

5-2. Materials and methods
5-2-1. Study area and Field investigation

The surveys were conducted in the Sugadaira Highlands of central Japan at 2-4 sites (latitude 36.523570-36.545518, longitude 138.331513-138.357224, elevation 1326-1470 m; Figure 13), ranging from 500 to 2500 m apart for each species. 1470 m; Figure 13). Three to six surveys were conducted between June and September 2021, at intervals of 1-21 days, walking for 2 hours per survey; the distance walked per survey ranged from 500 to 2600 m. The distance walked per survey ranged from 1.5 to 2.5 km. During the walk, butterflies of the target species were visually counted, all or part of them were captured with a capture net, numbered with unique ID number using an oil-based marker on the underside of the left hind wing, and released near the base of some herbaceous plant. If a marked individual was captured, its ID number was recorded.

5-2-2. Statistical analysis

Population size of the target species was estimated for each survey date using the Jolly-seber method (Jolly, 1965; Seber, 1965) from the data obtained from the mark-recapture surveys.

5-3. Results

Minois dryas were captured at 105, including 6 recaptures; they were surveyed beginning July 26, with a small peak on August 4, followed by a decline, then an increase again, with the largest peak on August 30. The estimated population size was 1560 (Figure 14a). *Brenthis daphne* were captured at 78, of which 11 were recaptures. The number of recaptures peaked on July 13, declined, and then peaked on August 2. The maximum estimated population size was 182 (Figure 14b); *Plebejus argus* were captured at 440, of which 28 were recaptures; it was surveyed from June 19, and captures were variable but unimodal, peaking in occurrence on July 12. The estimated population size was 2550 individuals (Fig. 14c); all three species were not observed moving between survey sites that were at least 500 m

apart. However, one movement of approximately 100 m was detected along the walking transect within the site for *B. daphne*.

5-4. Discussion

The total number of captures for the three species combined was 623, of which 0 individuals migrated more than 500 m and 1 individual migrated approximately 100 m. The proportion of individuals that migrated 100 m and 500 m for the three butterfly species studied was 1/623 or less. Looking at each species individually in detail, Minois dryas has very low mobility, with most of its intra-patch migration within 10 m (Kalarus & Nowicki, 2017). On the other hand, some studies have observed movements of up to 4.4 km (Akeboshi et al., 2015), an.initiatives, such as making the area a target area for companies and society to respond to TNFD and biodiversity offsets in the context of the trend of the TNFD and biodiversity offsets.

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Tables

Table 1 Model selection for GLMs regarding the effects of vegetation types on species richness of all (a), grassland-dependent native (b) and grassland-dependent endangered (c), vascular plants. Forests, old grasslands and new grasslands were discriminated for the three-vegetation model, and forests and grasslands for the two-vegetation model. All vegetation types were treated as the same for the null model. Bold indicates the best model. Delta AIC shows the difference in AIC between the best model and the others. *p*-Values are for the likelihood ratio test between the best model and each of the others. df indicates degrees of freedom

Model	df	AIC	ΔAIC	*p*
(a)				
Three vegetation model	3	238.3	0.0	
Two vegetation model	2	242.3	4.0	< 0.05
Null model	1	292.3	54.0	< 0.001
(b)				
Two grassland type model	2	95.1	0.0	
Null model	1	115.2	20.1	< 0.001
(c)				
Two grassland type model	2	98.4	0.0	
Null model	1	112.7	14.3	< 0.001

Table 2　Results of the indicator species analysis. Indicator values show the specificity of appearance in an indicator group (Dufrêne & Legendre, 1997). Only species with significant indicator values are shown (p < 0.05), except for those in the indicator group "All".

Species	Indicator group	Indicator value	p–value
Rubus subcrataegifolius	All	65	1.000
Celastrus orbiculatus	All	65	1.000
Solidago virgaurea	All	55	1.000
Convallaria majalis	All	50	1.000
Viola grypoceras	All	50	1.000
Artemisia　indica	Grassland	92	0.001
Miscanthus sinensis	Grassland	72	0.006
Potentilla freyniana	Grassland	72	0.003
Iris sanguinea	Grassland	69	0.007
Angelica pubescens	Grassland	69	0.008
Pteridium aquilinum	Grassland	65	0.018
Arenaria　lateriflora	Grassland	65	0.022
Toxicodendron orientale	Forest	57	0.005
Maackia amurensis	Forest	43	0.032
Toxicodendron trichocarpum	Forest	43	0.042
Adenophora　triphylla	Old grassland	100	0.001
Sanguisorba officinalis	Old grassland	100	0.001
Polygonatum odoratum	Old grassland	74	0.002
Cirsium spp.	Old grassland	72	0.001
Carex nervata	Old grassland	72	0.001
Lespedeza bicolor	Old grassland	68	0.003
Galium verum	Old grassland	62	0.007
Prunella vulgaris	Old grassland	58	0.016
Hosta　sieboldiana	Old grassland	57	0.019
Lysimachia clethroides	Old grassland	55	0.021
Ixeridium dentatum	Old grassland	50	0.040

Table 3 Location of the study area and number of sites. Old,New, and Forest indicates the number of sites in old grasslands, new grasslands, and forests, respectively.

Region	Latitude	Longitude	Elevation	Old	New	Forest
Sugadaira	N36.5118 - 36.5572	E138.3043° - 138.3571°	1319-1522 m	7	6	7
Hakuba	N36.5620 - 36.7828	E137.7948° - 137.8904°	790 - 1141 m	5	8	6
Kirigamine	N36.0689 - 36.1457	E138.1596° - 138.3032°	1375 - 1779 m	6	6	6

Table 4

Model selection for GLMs regarding the effects of vegetation types on species richness of all (a), grassland-dependent native (b) and grassland-dependent endangered (c), vascular plants. For the total plant species (a), the three-vegetation model (old grassland, new grassland, and forest) was selected, but there was no significant difference between it and the two-vegetation model and the two-vegetation model (grassland and forest) by likelihood-ratio test. For grassland–dependent and rare species (b, c), the two-vegetation model (old grassland and new grassland) was selected, with the old grassland having more species (Figure 2). All vegetation types were treated as the same for the null model. Bold indicates the best model. Delta AIC shows the difference in AIC between the best model and each of others. p-values are derived by the likelihood ratio test between the best model and each of the others. df indicates degrees of freedom.

Model	df	AIC	ΔAIC	*p*
(a) Species richness of all vascular plants				
Three-vegetation model	3	619.79	0.00	
Two-vegetation model	2	619.84	0.04	0.15
Null model	1	673.03	53.23	< 0.001
(b) Species richness of grassland-dependent native vascular plants				
Two-vegetation model	2	316.32	0.00	
Null model	1	347.57	31.25	< 0.001
(c) Species richness of grassland-dependent endangered vascular plants				
Two-vegetation model	2	303.15	0.00	
Null model	1	339.79	36.64	< 0.001

Table 5

Effects of region and vegetation history on grassland plant community composition by permutational multivariate analysis of variance (PERMANOVA) using the Jaccard dissimilarity index. My study area consists of three regions, which are 40 - 90 km apart from each other. Degrees of freedom, R2 values and significance levels by permutation tests were shown.

	Df	*R2*	*p*	
Region	2	0.122	< 0.001	***
Vegetation type	1	0.053	< 0.001	***

Table 6

Number of significant and non-significant indicator species in each vegetation category by IndVal. The number of significant indicator species was higher in old grassland than new grassland (binomial test p < 0.05). For Any vegetation, the *p*-values from the permutation test are all 1, so there are no significant species in this category.

	Significant	Non-significant	Total
Any vegetation	0	37	37
Any grassland	28	57	85
Forest	23	158	181
Old	15	66	81
New	4	99	103

Table 7

List of significant indicator species in each vegetation category by analysisIndVal. Only species with significant indicator values are shown ($p < 0.05$), except for those in the indicator group "Any vegetation" Indicator values (Dufrêne & Legendre, 1997) show the specificity of presence of each species in each vegetation type..

Name	Vegetation type	Indicator value	p
Sanguisorba officinalis	Old	72	< 0.001
Adenophora triphylla	Old	60	< 0.001
Arundinella hirta	Old	46	< 0.001
Galium verum	Old	43	< 0.001
Lespedeza bicolor	Old	50	< 0.001
Polygonatum odoratum	Old	45	< 0.001
Thalictrum minus	Old	34	< 0.001
Hosta sieboldiana	Old	36	< 0.01
Rubus parvifolius	Old	32	< 0.01
Convallaria majalis	Old	35	< 0.01
Geranium yesoense	Old	27	< 0.01
Patrinia scabiosifolia	Old	27	< 0.01
Lilium leichtlinii	Old	24	< 0.05
Carex nervata	Old	31	< 0.05
Tephroseris flammea	Old	21	< 0.05
Viola hirtipes	Old	24	< 0.05
Spodiopogon sibiricus	Old	24	< 0.05
Gentiana scabra	Old	24	< 0.05
Clinopodium chinense	Old	19	< 0.05
Cirsium oligophyllum	Old	17	< 0.05
Parnassia palustris	Old	17	< 0.05
Scabiosa japonica	Old	17	< 0.05
Weigela hortensis	New	29	< 0.05
Aquilegia buergeriana	New	26	< 0.05
Haloragis micrantha	New	20	< 0.05
Taraxacum officinale	New	22	< 0.05
Artemisia indica	Any grassland	90	< 0.001
Miscanthus sinensis	Any grassland	90	< 0.001
Potentilla freyniana	Any grassland	73	< 0.001
Ixeridium dentatum	Any grassland	59	< 0.001

Hypericum erectum	Any grassland	51	< 0.001
Aster microcephalus	Any grassland	49	< 0.001
Prunella vulgaris	Any grassland	41	< 0.01
Solidago virgaurea	Any grassland	51	< 0.01
Lysimachia japonica	Any grassland	39	< 0.01
Pteridium aquilinum	Any grassland	47	< 0.01
Potentilla fragarioides	Any grassland	33	< 0.01
Dactylis glomerata	Any grassland	36	< 0.01
Cirsium japonicum	Any grassland	33	< 0.01
Amphicarpaea bracteata	Any grassland	36	< 0.01
Erigeron strigosus	Any grassland	36	< 0.01
Oenothera biennis	Any grassland	36	< 0.01
Festuca ovina	Any grassland	31	< 0.05
Iris sanguinea	Any grassland	31	< 0.05
Eupatorium glehnii	Any grassland	36	< 0.05
Erigeron annuus	Any grassland	38	< 0.05
Lysimachia clethroides	Any grassland	40	< 0.05
Angelica pubescens	Any grassland	31	< 0.05
Plantago asiatica	Any grassland	26	< 0.05
Poa compressa	Any grassland	21	< 0.05
Phleum pratense	Any grassland	21	< 0.05
Luzula multiflora	Any grassland	21	< 0.05
Poa sphondylodes	Any grassland	21	< 0.05
Trifolium pratense	Any grassland	21	< 0.05
Veronicastrum japonicum	Any grassland	21	< 0.05
Poa pratensis	Any grassland	21	< 0.05
Cirsium sp.	Any grassland	26	< 0.05
Picris hieracioides	Any grassland	26	< 0.05
Euphorbia lasiocaula	Any grassland	23	< 0.05

Viola mandshurica	Any grassland	23	< 0.05
Acer rufinerve	Forest	53	< 0.001
Toxicodendron orientale	Forest	47	< 0.001
Toxicodendron trichocarpum	Forest	45	< 0.001
Corylus sieboldiana	Forest	40	< 0.001
Quercus crispula	Forest	48	< 0.001
Cornus controversa	Forest	37	< 0.001
Actinidia arguta	Forest	32	< 0.01
Euonymus oxyphyllus	Forest	26	< 0.01
Padus grayana	Forest	39	< 0.01
Cerasus maximowiczii	Forest	26	< 0.01
Chengiopanax sciadophylloides	Forest	26	< 0.01
Viburnum furcatum	Forest	29	< 0.01
Fraxinus lanuginosa	Forest	29	< 0.01
Lonicera gracilipes	Forest	21	< 0.01
Maianthemum dilatatum	Forest	21	< 0.05
Sambucus racemosa	Forest	24	< 0.05
Ligustrum tschonoskii	Forest	27	< 0.05
Acer amoenum	Forest	29	< 0.05
Dryopteris crassirhizoma	Forest	22	< 0.05
Viburnum wrightii	Forest	16	< 0.05
Dennstaedtia scabra	Forest	16	< 0.05
Pyrola asarifolia	Forest	16	< 0.05
Cryptomeria japonica	Forest	16	< 0.05
Lindera umbellata	Forest	16	< 0.05
Lindera praecox	Forest	16	< 0.05
Acer micranthum	Forest	16	< 0.05
Acer japonicum	Forest	16	< 0.05
Symplocos sawafutagi	Forest	19	< 0.05
Lindera obtusiloba	Forest	19	< 0.05
Celastrus orbiculatus	Any vegetation	52	1.000
Viola grypoceras	Any vegetation	48	1.000
Rubus subcrataegifolius	Any vegetation	45	1.000
Arenaria lateriflora	Any vegetation	40	1.000
Sasa senanensis	Any vegetation	38	1.000
Carex lanceolata	Any vegetation	36	1.000

Fallopia japonica	Any vegetation	29	1.000
Larix kaempferi	Any vegetation	28	1.000
Malus toringo	Any vegetation	28	1.000
Disporum smilacinum	Any vegetation	26	1.000
Rosa multiflora	Any vegetation	26	1.000
Betula platyphylla	Any vegetation	24	1.000
Euonymus sieboldianus	Any vegetation	24	1.000

Table 8

Model selection for GLMs regarding the effects of vegetation types on (a) abundance and (b) species richness of butterfly. The three-vegetation model discriminated forests, old grasslands (> 300 years old) and new grasslands (43–72 years old). Two-vegetation model discriminated forests and grasslands (either old or new grasslands). Null model does not discriminate any types of vegetation and all vegetation types were assumed to have the same effect. Bold letters indicate the best models that had the lowest *AIC*. df indicates degrees of freedom. *ΔAIC* shows the difference in AIC between the best model and the model accompanied by each *ΔAIC* value. * *p* values were derived from the likelihood ratio test and indicate the reliability of the best model relative to the model accompanied by each *p* value.

Model	df	AIC	ΔAIC	*p*
(a)				
Two vegetation model	3	173.2	0.0	
Three vegetation model	4	175.0	1.8	0.66
Null model	2	189.2	16.0	< 0.001
(b)				
Two vegetation model	3	64.1	0.0	
Three vegetation model	4	65.9	1.8	0.66
Null model	2	74.6	10.5	< 0.001

Table 9

Results of the indicator species analysis on butterfly. IndVal tests were performed for three vegetation types (large old grassland, other grassland, and forest), two vegetation types (grassland and forest), and one vegetation type (vegetation type not discernible). Only shown for species with significance ($p < 0.05$), except for species detected as indicators for "any vegetation" that means presence of those species in any vegetation types. Note that no indicator species were detected for new grassland.

Species	Vegetation group	Indicator value	p
Argynnis paphia	Any vegetation	95	1
Pieris dulcinea	Any vegetation	63	1
Papilio dehaanii	Any vegetation	32	1
Neptis sappho	Any vegetation	16	1
Fabriciana adippe	Grassland	100	0.001
Parnara guttata	Grassland	92	0.001
Brenthis daphne	Grassland	77	0.008
Ochlodes venatus	Grassland	77	0.013
Hesperia florinda	Grassland	62	0.036
Lethe diana	Forest	99	0.001
Neope niphonica	Forest	67	0.004
Minois dryas	Large old grassland	64	0.023
Eurema mandarina	Large old grassland	48	0.038

Table 10

Butterflies whose abundance were correlated with old or current grassland area. Three linear models with the number of butterflies of each species as the objective variable, and as explanatory variables, the area (ha) of old grassland around the study site or the area of current grassland or the null model were created. Best models were selected based on *AIC*, and butterflies for which the model with the area of old or current grassland were shown. Type is which grassland area was selected, old or current. Coefficient is regression coefficient estimated by linear regression. R^2 is the coefficient of determination. p is the result of testing whether the explanatory variable affects the objective variable.

Butterfly	type	Coefficient	R^2	p
Celastrina argiolus	current	3.43	0.615	0.001
Argyreus hyperbius	current	-0.63	0.172	0.158
Papilio dehaanii	current	-0.92	0.273	0.067
Eurema mandarina	current	-1.13	0.177	0.153
Hesperia florinda	current	-2.01	0.156	0.182
Colias erate	current	-12.64	0.189	0.137
Minois dryas	old	5.54	0.398	0.021
Brenthis daphne	old	2.61	0.403	0.020
Melitaea ambigua	old	1.29	0.296	0.055
Curetis acuta	old	0.44	0.451	0.012
Lycaena phlaeas	old	0.38	0.180	0.148
Zophoessa callipteris	old	0.15	0.290	0.058
Nymphalis vaualbum	old	-0.24	0.147	0.196
Thymelicus sylvaticus	old	-0.24	0.147	0.196
Gonepteryx aspasia	old	-0.40	0.159	0.177
Damora sagana	old	-0.90	0.315	0.046

| *Speyeria aglaja* | old | -1.51 | 0.195 | 0.131 |

Figures
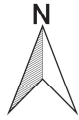
Figure 1 Distribution of old and new grasslands in the Sugadaira and Minenohara Highlands of central Japan. White space indicates other lands than grassland including forests, arable fields, residential are and roads. The vegetation type at each study site is indicated by the three symbols (circle = old grassland, triangle = new grassland and square = forest). The numbers indicate study sites.
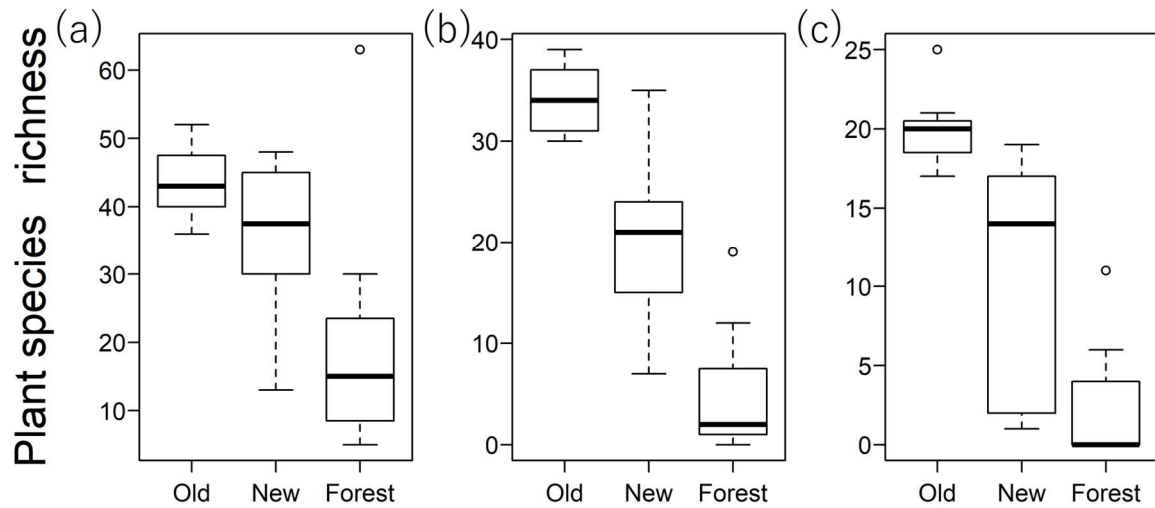
Figure 2 The number of (a) all, (b) grassland-dependent native and (c) grassland-dependent endangered plant species in each vegetation type. "Old" and "new" refer to old and new grasslands (see detail in the text). Bars and boxes are medians and quartiles, with ranges between minimum and maximum values. An outlier (values less than the first quartile minus 1.5 times the interquartile range [IQR] or greater than the third quartile plus 1.5 times the IQR) is shown by a dot.
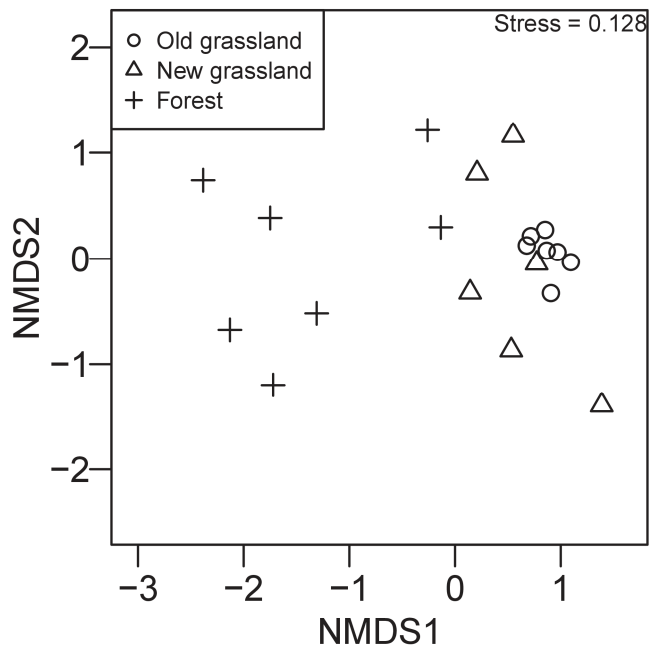
Figure 3 Nonmetric multidimensional scaling (NMDS) plot using the Jaccard index of dissimilarity of the plant composition of each study site across the three-vegetation types. Horizontal and vertical axes represent the first and the second axes extracted by NMDS, respectively.
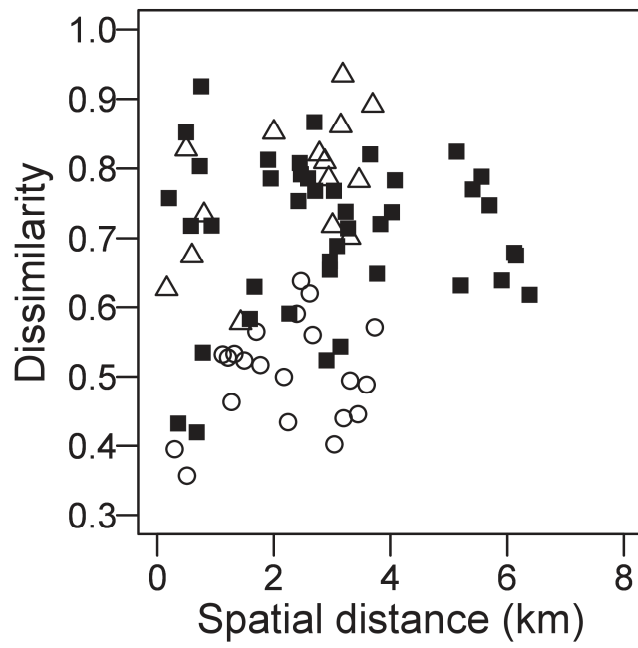
Figure 4 The relationship between the dissimilarity calculated by the Jaccard index and geographical distance for all pairs of grassland sites: old grassland versus old grassland (open circle), new grassland versus new grassland (open triangle) and old grassland versus new grassland (filled square)
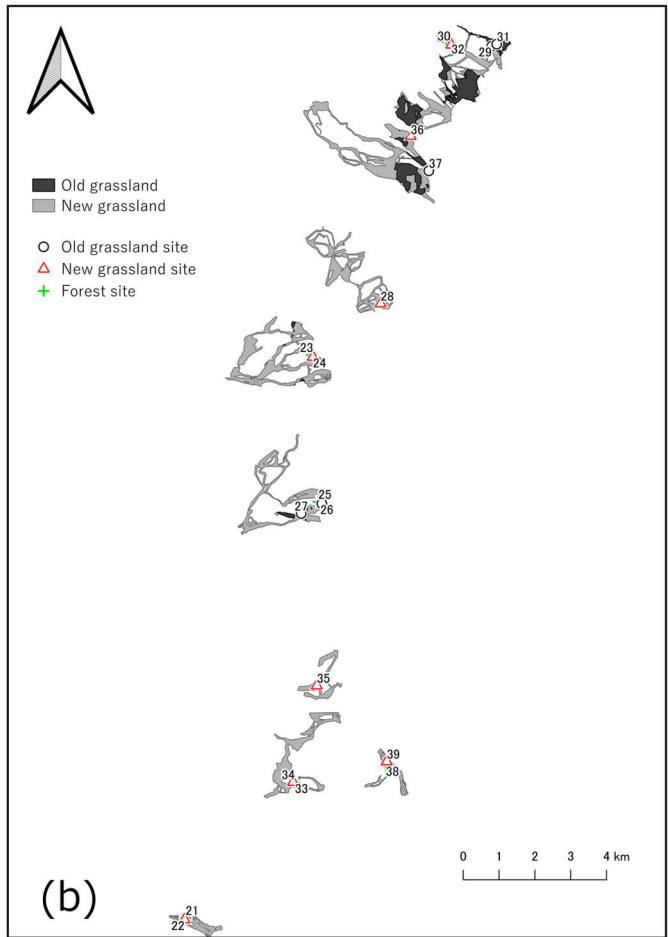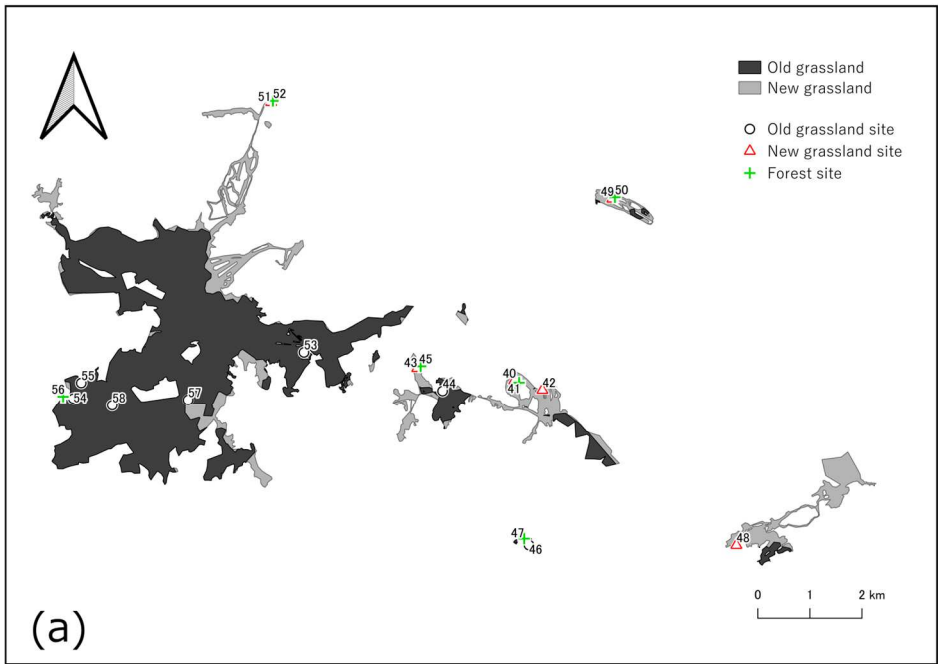
Figure 5 Grassland distribution in the study area of three regions, (a) Kirigamine, and (b) Hakuba. Sugadaira is already shown in Figure 1. Darker areas are old grasslands and lighter areas are new grasslands. White areas indicate vegetation other than grassland. The vegetation type at each study site is indicated by the three

symbols (Black circle = old grassland, red triangle = new grassland and green cross = forest). The numbers indicate study sites.
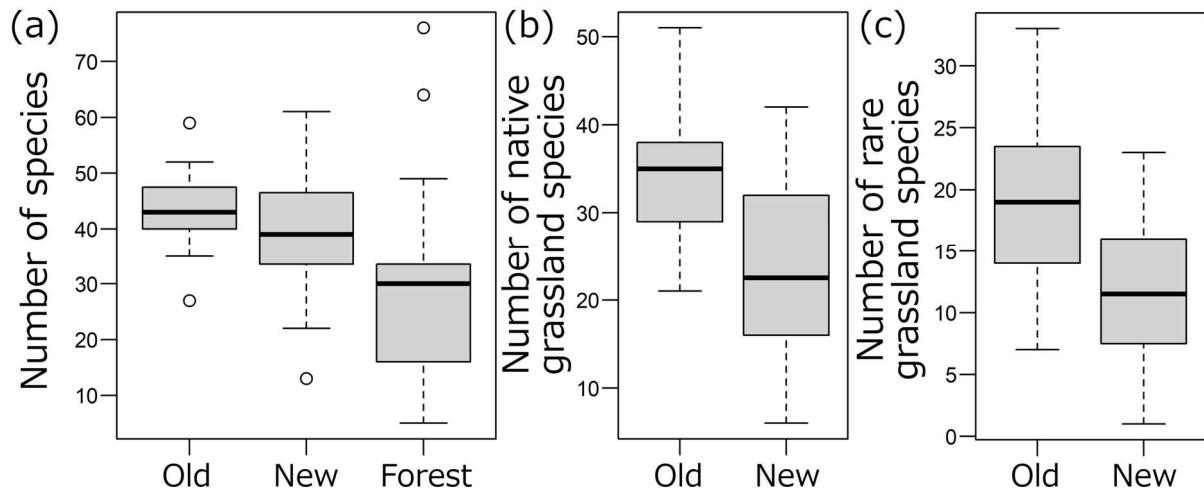
Figure 6 Number of all species (a) was higher in the old and new grassland (shown by Old and New in the horizontal axis) than forest (Table 4a). Number of grassland-dependent native (b) and endangered (c) species was higher in the old grassland than in the new (Table 4b, c, respctively). Bars and boxes are medians and quartiles, with ranges between minimum and maximum values. An outlier (values less than the first quartile minus 1.5 times the interquartile range (IQR) or greater than the third quartile plus 1.5 times the IQR) is shown by a dot.
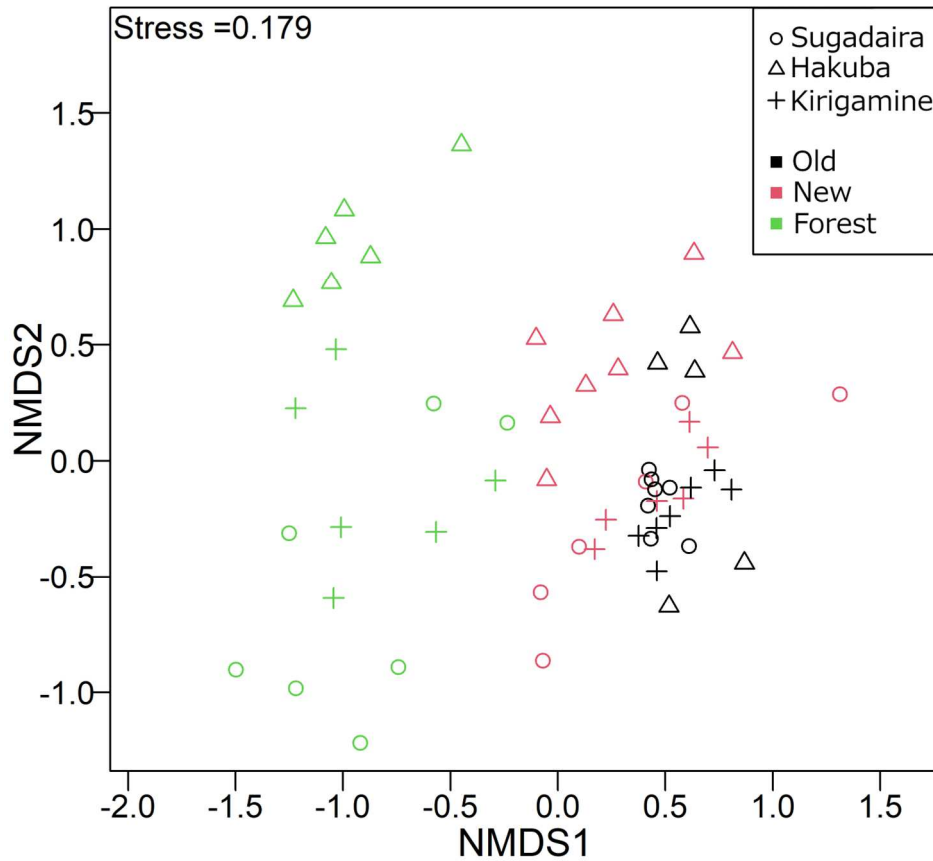
Figure 7 Nonmetric multidimensional scaling (NMDS) plot using the Jaccard index as dissimilarity of the plant species composition across each study site in the three vegetation types in three regions. Species composition differed between study regions and also between old and new grasslands (Table 5). The point symbol indicates each region: Sugadaira (open circle), Hakuba (open triangle), and Kirigamine (cross). The color of the point indicates the vegetation type: old grassland (black), new grassland (red), and forest(green). Horizontal and vertical axes represent the first and the second axes extracted by NMDS, respectively.

(a)

Indicator of old grassland

Indicator of other grassland

0.0 0.2 0.4 0.6 0.8 1.0
Proportion of species with developed underground stems

(b)

Indicator of old grassland

Indicator of other grassland

0.0 0.2 0.4 0.6 0.8 1.0
Proportion of perenial species

(c)

Indicator of old grassland

Indicator of other grassland

0.0 0.2 0.4 0.6 0.8 1.0
Proportion of purple flower species
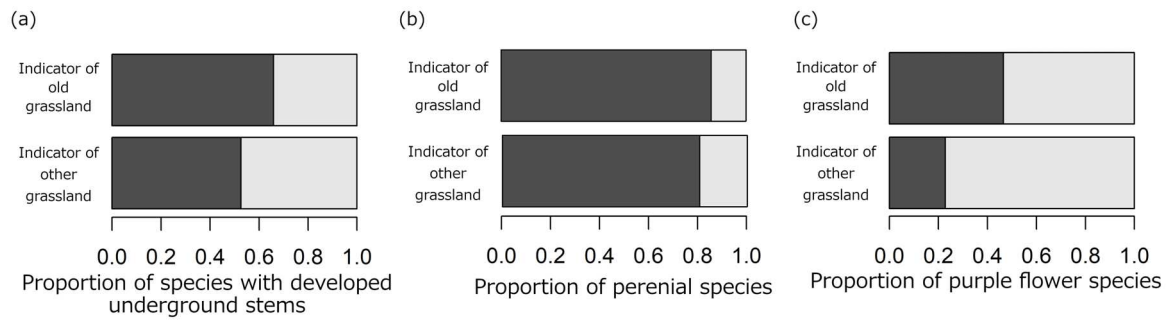
Figure 8 Characteristics of indicator species for old grassland and other grassland (indicator for new and any grassland). Indicator for old grassland had(a) higher proportion of species with developed underground stems ($p < 0.05$, linear logistic test), (b) marginally higher proportion of perennial species ($p = 0.09$), and (c) higher proportion of species with purple flowers ($p < 0.01$).
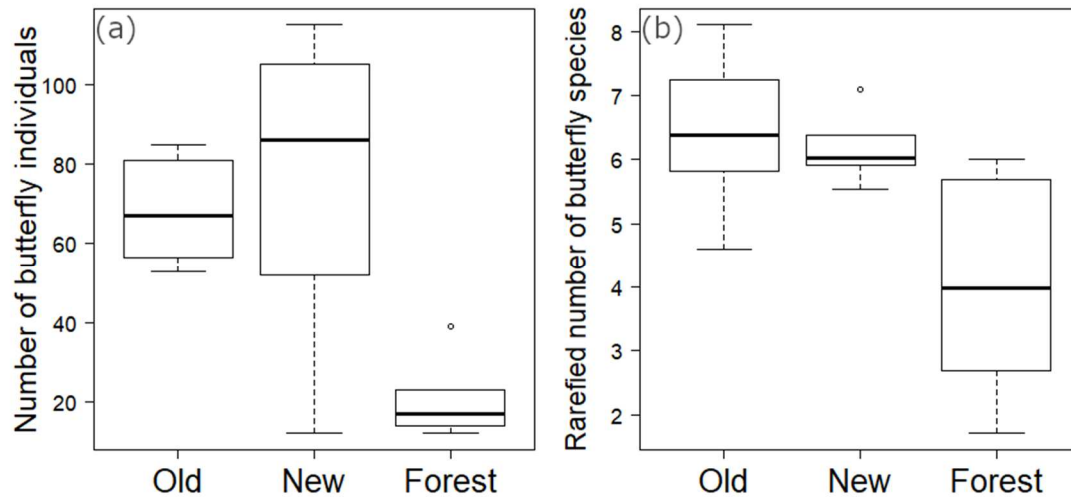
Figure 9 Number of butterfly (a)individuals and (b) species in each vegetation type in Sugadaira. The number of butterfly individuals tended to be larger in the order of new grassland, old grassland, and forest. The differences between old and new grasslands were not statistically supported, with grassland significantly more abundant than forest (Table 8b). "Old" and "new" refer to old and new grasslands (see detail in the text). Bars and boxes are medians and quartiles, with ranges between minimum and maximum values. An outlier (values less than the first quartile minus 1.5 times the interquartile range [IQR] or greater than the third quartile plus 1.5 times the IQR) is shown by a dot.
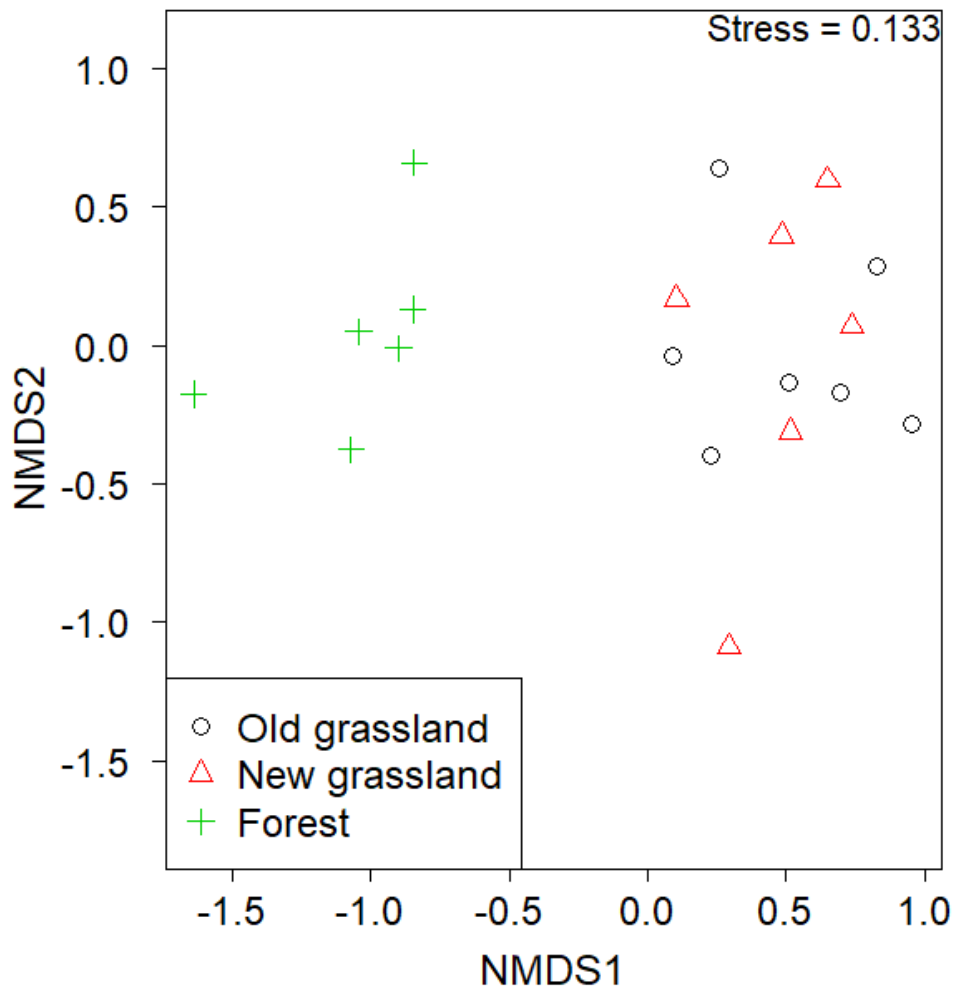
Figure 10 Nonmetric multidimensional scaling (NMDS) plot using the Bray-Curtis index of dissimilarity of the butterfly composition of each study site across the three-vegetation types. Butterfly species composition differed between grassland and forest (PERMANOVA p < 0.001). Old and new grassland communities were not statistically different (p = 0.356). Horizontal and vertical axes represent the first and the second axes extracted by NMDS, respectively.
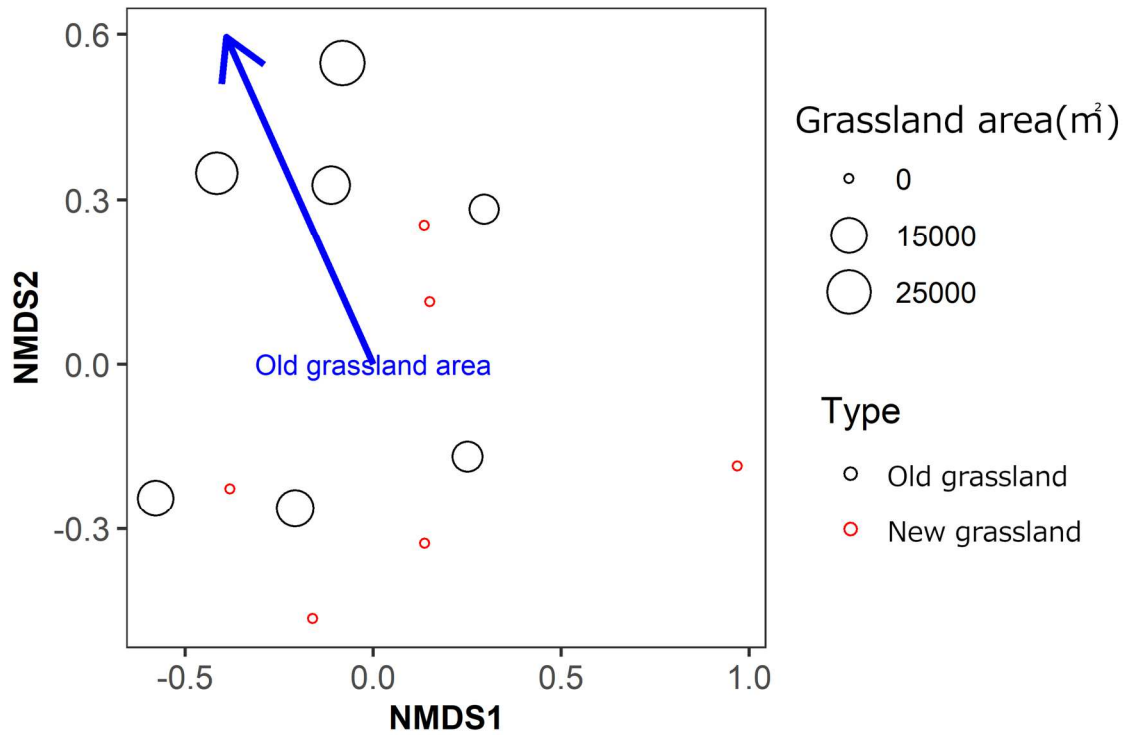
Figure 11 Nonmetric multidimensional scaling (NMDS) plot using the Bray-Curtis index of dissimilarity of the butterfly composition of each grassland site. The blue arrows indicate the results of envfit, where environmental factors (old grassland area and current grassland area within a 100 m radius of the study site, vegetation height, slope and elevation), of which only old grassland area explained the variation in community composition ($p < 0.05$). Horizontal and vertical axes represent the first and the second axes extracted by NMDS, respectively.
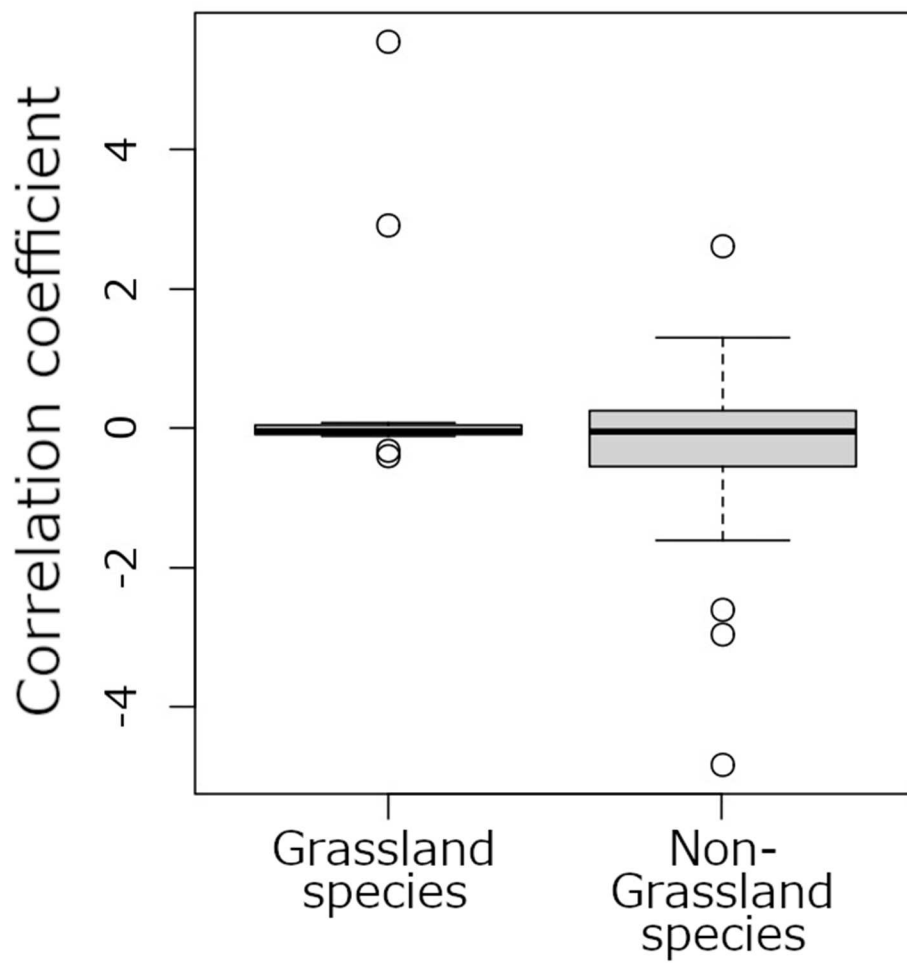
Figure 12 Correlation coefficients in the GLM between butterfly populations and old grassland area in relation to butterfly characteristics (grassland or not). Grassland butterflies tended to have higher correlation coefficients ($p = 0.08$).
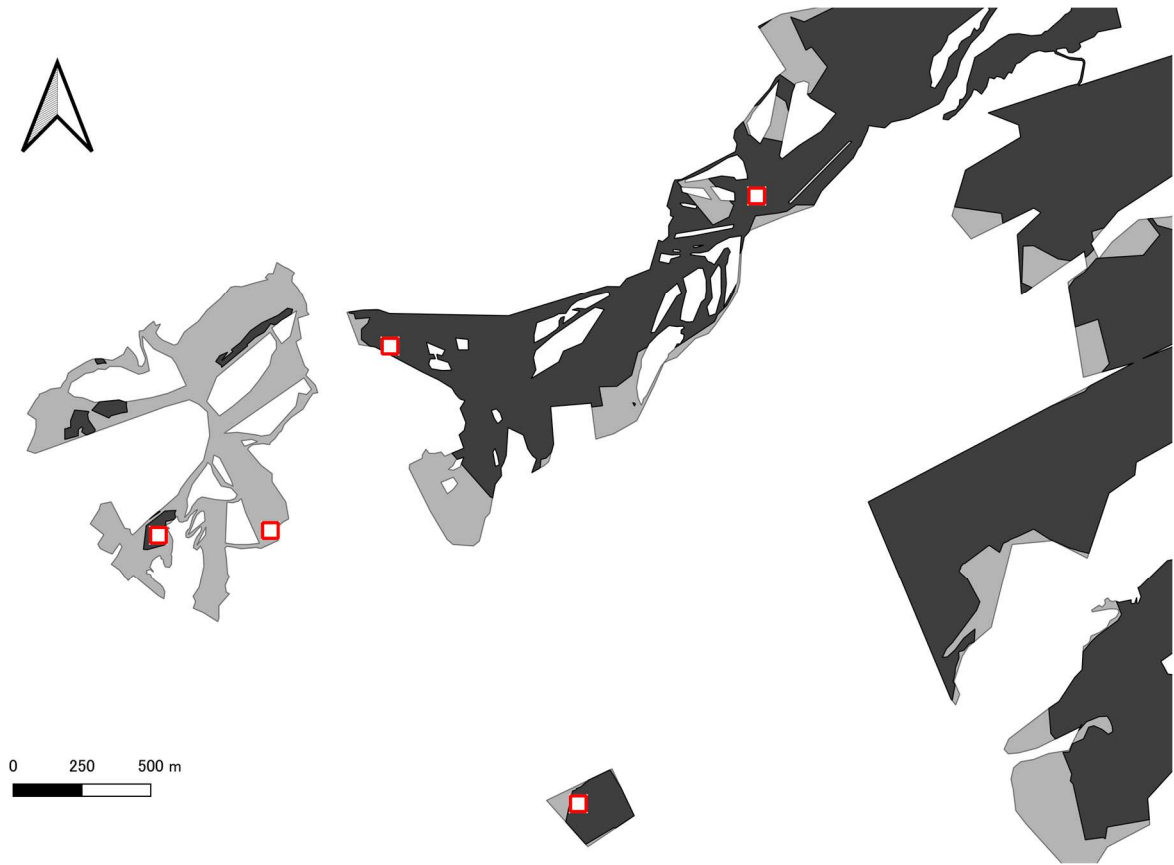
Figure 13 Study sites for mark-recapture. The survey was conducted at the location of the red square in the middle cutout. More than 500 m apart between survey sites.
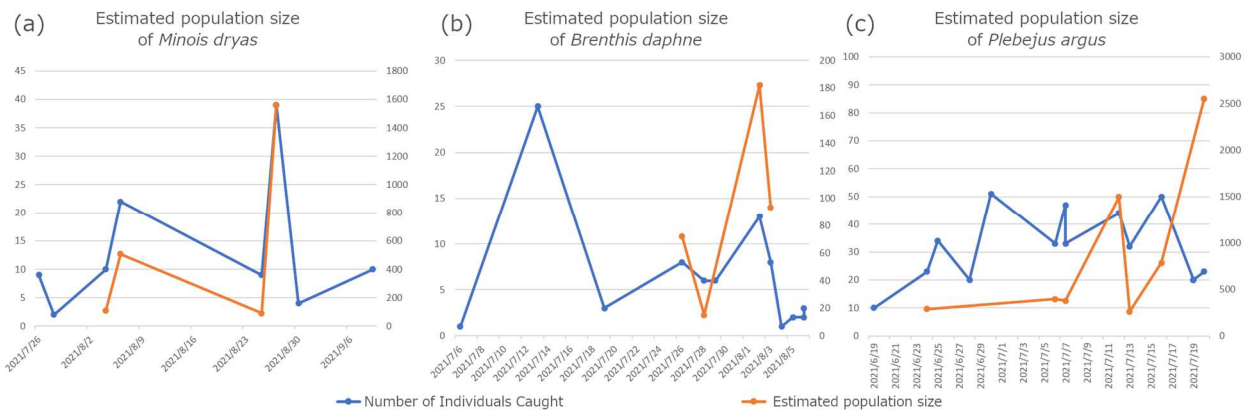
Figure 14　Results of mark-recaptures of (a) *Minois dryas*, (b) *Brenthis daphne*, and (c) *Plebejus argus*. The blue line indicates the number of individuals captured and corresponds to the left axis. The orange line indicates the estimated population size, corresponding to the right axis.