Effects of temporal and spatial continuity of grasslands on butterfly communities

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Abstract

Semi-natural grasslands have high biodiversity, but they are declining worldwide, and desperately need to be prioritized for conservation. Recently, numerous papers have concluded that vegetation history may influence biodiversity. We targeted three vegetation types in ski-run grasslands in central Japan: "old grasslands" that have been in existence for 300 to several thousand years, "new grasslands" that have been deforested for 43 to 72 years, and forests adjacent to those ski-runs. We established transects at six to seven sites for each vegetation type and surveyed butterfly communities visually. Plant communities were also surveyed along the same transects. Species composition, numbers of species, and total abundance of butterflies did not vary with grassland age; however, the area of old grasslands within 100 m of transects affected species composition. Minois dryas (Nymphalidae) and Eurema mandarina (Pieridae) were identified as indicator species of large, old grasslands (>8000 m2 within 100 m), whereas no indicator species were detected at other sites. Abundances of Minois dryas, Brenthis daphne (Nymphalidae), and Curetis acuta (Lycaenidae) were greater where old grassland areas occurred within 100 m of observed sites. Sanguisorba officinalis (Rosaceae), the host plant of B. daphne, and Lespedeza bicolor, the main host plant of E. mandarina and C. acuta, was identified as an indicator of old grassland, explaining the dependence of these three butterfly species on old grasslands. Previous studies have also shown that M. sinensis, the main host plant of M. dryas, is an indicator for the entire grassland, suggesting that the butterfly's distribution was restricted to a narrower area than that of host plants. Our results suggest that large, old grasslands should have high conservation priority to maintain grassland butterfly diversity.

Introduction

Semi–natural, temperate grasslands that are maintained by human management, involving fire, mowing, and grazing, have high species diversity and great conservation value (Akeroyd & Page, 2011; OECD, 2008; Wilson et al., 2012). However, they have been largely converted to farmland and residential areas, or changed to forests by abandonment of management in Europe, as in Britain (Ridding et al., 2015), Germany (Poschlod et al., 2005), Sweden (Cousins & Eriksson, 2008), Estonia (Pärtel et al., 1999). In Japan, grassland areas are declining throughout the country (Masui et al., 2017; Ogura, 2006; Ohta et al., 2021; Shoji & Yamamoto, 1995; Yamato & Hattori, 1999). In the Sugadaira Highland in central Japan, approximately 92% of the grassland area has changed to forest during the past 100 years (Inoue et al., 2021a). These declines have been caused by reduced artificial disturbances or reduced management for maintenance of grasslands, due to decreased grassland value (Takahashi et al., 2011).

Given the rapid decline in grasslands, it is urgent to identify grasslands that have high biodiversity / high conservation priority. One factor that may be relevant to formation and maintenance of biodiversity–rich communities is history of the vegetation (Culbert et al., 2017; Honnay et al., 2017). Present plant diversity is strongly influenced by past habitat connectivity (50–130 years before present) (Lampinen et al., 2018; Lindborg & Eriksson, 2004). Secondary-growth forests on previously cultivated lands or gardens have many plant species, including edible herbs (Koerner et al., 1997). High plant diversity occurs in grasslands and forests having long histories without disturbance (Cousins & Eriksson, 2008, Culbert et al., 2017; (Honnay et al., 2017; Kull & Zobel, 1991; Yaida et al., 2019; Inoue et al., 2021b). Diversity and abundance of arbuscular mycorrhizal fungi is higher in 12-20-year-old grasslands than in newer grasslands (Honnay et al., 2017). Bird community composition in forests is affected by temporal continuity of those forests (Culbert et al., 2017). Butterfly diversity is affected by past human disturbances, as in rainforests (Whitworth et al., 2016).

Such temporal effects of vegetation history also interact with spatial scale. Both the temporal continuity and spatial area of habitats positively influence the number of grassland plant species (Noda et al., 2022). The proportion of grassland-specialist butterfly species increases with increased size of the habitat patch (Brückmann et al., 2010), although habitat size did not affect grassland-specialist plant species in that study. Experimental fragmentation of grasslands also decreases butterfly diversity, but not of plants (Zschokke et al., 2000). Such differences in sensitivity to spatial scale of habitats between butterflies and plants can affect butterfly-plant interactions, when temporal and spatial scales of habitat continuity are considered together.

However, the effects of temporal continuity of grasslands (Inoue et al., 2021b), or the period during which grasslands continue without transitioning into other types of vegetation, and effects of spatial scale, have not been studied in animals. Butterflies have been used as ecosystem indicators because their habitat use changes during successive life stages (van Swaay et al., 2010). They have strong associations with habitat variables, including diversity and abundance of plants (Maleque et al., 2009). Additionally, many endangered butterflies depend on grassland as habitat (Van Swaay & Warren, 1999). Therefore, this study clarified effects of grassland temporal continuity on butterfly communities in order to identify grasslands with high conservation priority.

In this study, we focused on the effect of temporal continuity of grasslands on butterfly communities in an area where we previously reported positive effects on plant diversity (Inoue et al., 2021b), in order to understand such effects on plant-butterfly interactions. We

investigated butterfly communities in old grasslands (288–1000s years) and new grasslands (52–70 years after deforestation), both of which are managed as ski runs in the Sugadaira Highland in central Japan. Our objectives were (1) to clarify the effect of grassland temporal continuity on diversity and abundance of butterfly communities, (2) to examine whether community composition differs between old and new grasslands, (3) to examine effects of spatial distribution of old grassland on butterfly communities.

Methods

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 (36.51182–36.55727°N, 138.30424–138.35716°E; elevation 1,319–1,522 m, Fig. 1) in 2018. Surveys of old maps confirmed that almost the entire Sugadaira Highland was a large continuous grassland 300 years before present (bp) (Inoue et al., 2021b). The Sugadaira Highland of central Japan has semi–natural grasslands that have been maintained continuously over 4,000 years, as estimated by ^{14}C dating of the underlying Andosol (Yamanoi, 1996), which is specifically generated and accumulated in grasslands (Shinji et al., 2002; Yamane, 1973). However, during the last 100 years, large parts of this region have reverted to forests due to forest plantation, as well as natural succession. 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.

We classified the vegetation of study sites into three types: old grassland, new grassland, and forest. These three vegetation types were mapped using GIS (Inoue, Yaida, et al., 2021) integrating topographic maps from 1912 and 1937 and aerial photographs from 1947, 1975, and 2010 (Geospatial Information Authority of Japan). The area covered with grasslands throughout this period was defined as old grassland (at least 106 years old). The Sugadaira part of the study area (excluding study sites 7–9, which are in Minenohara, Fig. 1) were shown as grasslands in an old map published in 1722, indicating >300 years of existence as grassland (Inoue et al. 2021a). Although the Minenohara area was outside the area of the old map, both Sugadaira and Minenohara formed a single large grassland in 1910 (Inoue et al. 2021a) and there is no reason for the Minenohara sites not to have been grassland 300 years ago. Yamanoi (Yamanoi, 1996) suggested a >4200-year history for grassland in the study area, based upon radiocarbon dating of Andsol. Apart from those old grasslands, the grassland area (in 2010) that was forest until 43–71 years ago. All of these new grasslands were grasslands in 1910, but were later reforested or became reforested due to abandonment of grassland management, followed by natural succession. Those forests were subsequently cleared to create ski slopes between 1947 and 1975. The area covered with forests in 2010 was defined as forest and areas adjacent to either old or new grasslands were chosen as study sites. Canopies of those forests were dominated by *Quercus crispula* (Fagaceae) or planted *Larix kaempferi* (Pinaceae), with a dominant or sporadic understory of *Sasa senanensis* (Poaceae).

Field investigations

We investigated seven old grassland sites, six new grassland sites, and six forested sites. These were the same as in our previous study of plant communities (Inoue, Yaida, et al., 2021), except that there were seven forest sites in that study, one of which was excluded from this study. At each site, we set 5 × 20 m transects at least 20 m from borders between grasslands and forests. Individual numbers were recorded for all butterfly species by transect walks for 12 min in both directions (forward and backward) between 9:00 and 17:00. We repeated such butterfly transect surveys seven times when the weather was clear and windless between June and September in 2018.

As environmental factors, we used vegetation height, slope, and elevation as measured previously (Inoue et al., 2021b). Using GIS data, we also measured areas of old and new grasslands. Because some grassland sites were connected to other sites by a road or mountain top, we treated grassland areas within a radius of 50 m and between 100–1000 m in increments of 100 m.

Statistical analyses

To adjust for differences in numbers of butterflies among sites, numbers of species were rarefied based on the lowest number of individuals at any one site using the *rarefy* function in *vegan* library (Oksanen et al., 2018) in *R* version 3.5.0 (*R* Core Team, 2018). In subsequent analyses, the rarefied species number was used as species richness. To examine effects of vegetation types on numbers of individuals and species, we constructed generalized linear models (GLMs) with a negative binomial distribution for numbers of individuals, and with a Gaussian distribution for species richness. Three models were created based on vegetation type: a null model, a two-vegetation-type model, and a three-vegetation-type model. In the null model, numbers of species/individuals were assumed to be the same among vegetation types. In the two-vegetation-type model, numbers of species/individuals were assumed to differ between grasslands and forests. In the three-vegetation-type model, old grasslands, new grasslands, and forests were assumed to have different

numbers of species/individuals. The best model was selected based on the AIC and supported by the chi-square test, based upon likelihood ratio.

To reveal variation in butterfly species composition, we used ordination methods with non-metric, multidimensional scaling (NMDS). In NMDS, dissimilarity was calculated from all butterfly abundances with the Bray-Curtis index, using the *vegan* library in *R*. We tested the effect of grassland types (old or new) on species composition by permutational, multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001), using the *adonis* function in the *vegan* library in *R*, with 10,000 permutations. Environmental factors were fitted to NMDS plots by a permutation test using the *envfit* function in the *vegan* library in *R*. Furthermore, permutational regression analysis was implemented to examine the effect of dissimilarity of plant communities (Inoue et al., 2021b), and geographical distance on dissimilarity of butterflies at each site. Dissimilarity of butterfly communities, μ, was fitted to a linear predictor using a linear regression model:

$$\mu \sim \beta_0 + \beta_1 P + \beta_2 G$$

where β_0 was an intercept, $\beta_1$and β_2 were estimators of regression coefficients of P (dissimilarity of plant communities), and G (geographical distance), respectively. We used 10,000 matrix permutation to test the departure of regression coefficients from the null hypotheses of zero. We held the matrix for explanatory variables constant and permutated the objective variable matrix. *P*-value estimates were calculated from the number of permutations for which the absolute value of the regression coefficient exceeded the absolute value from the real data. This approach is similar to the Mantel test, but it used regression coefficients, rather than correlation coefficients, as statistics for testing the effect of each explanatory variable separately. The same (Legendre et al., 1994; Lichstein, 2007; Nagamitsu et al., 2014) and similar (Kunin et al., 2009) methods have been used elsewhere.

Indicator species were detected using the *indval* function (indicator value test or IndVal; Dufrêne & Legendre, 1997) in the *labdsv* library (Roberts, 2016) in *R*. In this indicator species analysis, study sites were categorized into three groups: large old grasslands (more than 8,000 m^2 within 100 m of the site center), other grasslands, and forests. The IndVal test was repeated for the three-vegetation-type case (large old grasslands, other grasslands, and forests). The two-vegetation-type case (grasslands and forests) and one-vegetation-type case (vegetation type not discriminated), as recommended by Dufrêne and Legendre (1997). Each species was then regarded as an indicator for the vegetation type that had the highest indicator value among vegetation types. Resulting indicator values were tested by significance with a randomization test (9999 times). We also constructed linear models to explain the number of individual butterflies of each species as the objective variable, by either the area (ha) of (i) old grassland or (ii) current grassland within a radius of 100 m from each study site, as the explanatory variable. Those linear models were then compared with each other as well as with null models based on *AIC*.

In order to examine what species characteristics affect preferences of butterfly species for large old grasslands, we constructed a linear model to explain regression of coefficients of the aforementioned linear model for the number of butterflies of each species as the objective variable and the area of old grassland as the explanatory variable, by three indicator species of butterfly: grassland-dependence (1 for dependent and 0 for not), univoltinism (1 for univoltine and 0 for not), and rarity (1 for those on the Red List if threatened in any Japanese prefecture and 0 for not). Grassland-dependence and univoltinism were defined as in Shirouzu (2006). Constructed models were evaluated based on AIC and likelihood-ratio test.

Results

In total, 39 butterfly species (1058 individuals) were recorded. The two-vegetation model was selected for both the number of individuals (ΔAIC to null model = 16.0, $p < 0.001$, GLM and the likelihood ratio test, Table 1, Fig. 2) and species (ΔAIC to null model = 10.5, $p < 0.001$, Fig. 3), indicating more individual butterflies and species in grasslands than in forests, with no statistical difference between old and new grasslands.

Grasslands and forests had different butterfly community compositions, ($p < 0.001$, PERMANOVA, Fig. 4), with no statistical difference between old and new grasslands ($p = 0.36$). However, when we incorporated the spatial scale of old grasslands, the area of old grassland within 100 m of each study site affected butterfly species composition ($p < 0.05$, envfit, Fig. 5). The effect of the area of old grasslands was the highest when the area was calculated using a distance of 100 m (Fig. 6). Effects of other environmental factors (vegetation height, slope, elevation, and the area of the grasslands) were not detected. In permutational regression analysis, dissimilarity of butterfly communities between study sites were not explained by dissimilarity of plant communities ($p = 0.45$) or by dissimilarity and geographical distance between study sites ($p = 0.37$).

In the indicator species analysis, two, five, two, and four butterfly species were detected in large, old grasslands, the entire grassland, forest, any vegetation (specialist species), respectively, whereas no indicator species were detected in new grasslands (Table 2). Numbers of individual butterflies were more or less explained by areas of the old (11 species) and entire area of grasslands (6 species) (Table 3).

Among examined species characteristics of butterflies, only grassland-specific species (Shirouzu, 2006) (ΔAIC = 1.13, p = 0.08, likelihood ratio test) tended to have high regression coefficients in the LM for the number of individual butterflies in each species relative to the area of old grasslands (Fig. 7). No species characteristics affected the regression coefficient for the number of individual butterflies in each species, based upon the entire area of grasslands.

Discussion

Butterfly abundance, species richness, and species composition in grasslands did not differ regardless of whether study sites were old or new grasslands; however, their species composition differed depending on the area of old grasslands (Fig. 6). In other words, unique butterfly communities existed in large, old grasslands. Regarding the spatial scale of old grasslands, grassland areas within 50 m – 1 km seemed to affect species composition; nonetheless, 100 m was the distance that best explained species composition (Fig. 6). So far as we are aware, this is the first report to show effects of temporal continuity of grasslands, which have been reported in plants (Cousins & Eriksson, 2008; Honnay et al., 2017, Inoue et al., 2021b) and fungi (Honnay et al., 2017), on animal communities, although bird communities are reportedly affected by temporal continuity of forests (Culbert et al., 2017) and the duration of succession from grasslands to forests (Oki et al., 2022).

In contrast to butterflies, which were affected by the area of old grasslands around them, plant species compositions in grasslands of the same study area (Inoue et al., 2021b) are best explained by whether study sites themselves are old or new grasslands. There are three possible explanations for why butterfly communities are affected by a larger spatial scale of old grasslands than plants. First, butterflies require large amounts of plant resources for their larvae to feed on plant shoots as well as for adults to feed on nectar, whereas plants utilize light and nutrients in reach of their leaves and roots. Second, lifetimes of all butterfly species are less than a year, and they require relatively extensive habitat for maintenance of minimum viable populations. In contrast, most plants species in those grasslands are perennial and even a single individual may sustain its species for a relatively long time. Third, butterflies have higher migration ability than plants, as discussed below.

The time-scale for persistence of effects of previous vegetation on present communities can be shorter for butterflies than for plants. In a study of plant communities in the same study area (Inoue et al. 2021b), species composition was more similar between new grasslands and forests than between old grasslands and forests, while this was not the case for butterfly communities in this study. Lindborg & Eriksson (2004) observed that insects are short-lived, highly mobile, and do not reflect landscape history, whereas plant populations may persist longer due to previously established perennials or via seed banks. Because butterflies depend on plant shoots and nectar as food, diversity and composition of butterfly communities are generally affected by plant communities (Steffan-Dewenter & Tscharntke, 2000; Kitahara et al., 2008; Uchida & Ushimaru, 2014). Also in our studies at Sugadaira, species richness of both butterflies and plants are highest in old grasslands, followed by new grasslands and forests, although the difference between old and new grasslands was statistically supported only for plants (Inoue et al. 2021b). Accordingly, the dominant effect of temporal grassland continuity on plants probably affects butterflies. Butterfly dependence on spatially larger habitats and their temporally quick response to reduction, fragmentation or degradation of old grasslands would first endanger grassland butterfly communities, rather than grassland plant communities, which would be able to sustain themselves for considerable periods, a situation known as "extinction debt" (Tilman et al., 1994). In fact, the proportion of grassland-dependent butterfly species on Japan's Red List (Ministry of the Environment Government of Japan, 2018, 2019) is higher than the proportion of plants, and their mean endangered rank is also higher.

Habitat preferences of butterflies and their host plants offer interesting insights into effects of temporal and spatial continuity of grasslands on plant-butterfly interactions (Table 4). First, habitat preferences of interacting butterflies and plants are basically coincident. When either butterflies or plants prefer old grassland, the interacting counterparts also do. Second, we found no cases in which a plant species preferred old grasslands, but its specific herbivorous butterflies did not. These two findings are consistent with the idea that temporal continuity of grasslands affects plants first, and then butterflies. Third, we found one case in which a butterfly (*Minois dryas*) prefers narrower habitat (old grassland) than the host plant (*Miscanthus sinensis*), which occurs in grasslands of any age. This may have happened because the abundance of *M. sinensis* is higher in old grasslands, as suggested by Yaida et al. (unpublished), even though the plant is still present in other grasslands, resulting in its status as an indicator of all grasslands (Inoue, 2021b). The latter authors investigated only presence/absence of plant species. Thus the present analysis is not inconsistent with the idea of indirect effects of old grasslands.

There is growing recognition that old growth grasslands are important for conservation of plant diversity (Nerlekar & Veldman, 2020). In this study, we show that old growth grassland is also important for inects. In conclusion, we propose that grasslands with long temporal continuity should have high conservation priority as unique biological communities. New grasslands are easily generated by human activity, such as creation of parks, roads, and buildings, or abandonment of arable lands. However, old grasslands are consistently declining, leading to declines in species that originally depended on such habitats, such as *Brenthis daphne* (vulnerable species in Japan), and *Melitaea ambigua* (endangered) (Ministry of the Environment Government of Japan, 2019). Once old-growth grasslands are converted to forests and then restored again as grasslands, recovery of their original biodiversity takes hundreds of years, making it practically impossible (Nerlekar & Veldman, 2020). Given the rapid decline and irreversibility of old grasslands, large old grasslands should be given top priority for biological conservation. Because a large part of old grasslands in Japan is semi-natural grassland that depends on human activity, such as pastures, hayfields and ski-runs, they should also be prioritized under international and national schemes, such as 30by30, which utilizes OECM (other effective area-based conservation measures) or habitats maintained by private organizations (Convention on biological diversity 2022).

Declarations

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Author contributions TI and TK planed the study, TI performed the field work, and TI and TK wrote the manuscript and approved it.

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Data Availability The used data are hosted by the authors' institutes and can be requested at the institutes.

Competing Interests The authors declare no conflict of interest.

Ethical approval Not applicable.

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Tables

Table1. Two vegetation-type models were selected, with grasslands and forests having different numbers of individuals (a) and species (b), and grasslands having a higher number of species and individuals than forests (Fig. 2, 3). The three-vegetation-type model discriminated forests, old grasslands (>300 years old) and new grasslands (43–72 years old). The two-vegetation-type model discriminated forests and grasslands (either old or new grasslands). The null model did 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 2. Four, five, two, and two butterfly species were detected in any vegetation, all grasslands, forests, and large old grasslands, respectively, whereas no indicator species were detected in new grasslands. 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 significant species ($p < 0.05$), except for species detected as indicators for "any vegetation", which means presence of those species in any vegetation types. Note that no indicator species were detected in new grasslands.

Butterfly 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	Entire grassland	100	0.001
Parnara guttata	Entire grassland	92	0.001
Brenthis daphne	Entire grassland	77	0.008
Ochlodes venatus	Entire grassland	77	0.013
Hesperia florinda	Entire 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 3. Butterfly populations of 11 and 6 species were more or less explained by old grassland area and entire grassland area, respectively. We constructed linear models to explain the number of individual butterflies of each species as the objective variable, by the area (ha) of either (i) old grasslands or (ii) all grasslands as the explanatory variable, which were then compared based on *AIC*. *AIC*s of the selected best models are shown in bold letters. Coefficients or effects of the selected explanatory variables (except null model) are shown.

	AIC			Coefficient	
Species	Old	Entire	Null model	Old	Old
Minois dryas	**90.77**	97.24	95.37	5.54	2.93
Brenthis daphne	**70.96**	74.45	75.67	2.61	6.46
Melitaea ambigua	**58.84**	59.81	61.39	1.29	3.91
Curetis acuta	**22.07**	26.26	27.86	0.44	1.08
Lycaena phlaeas	**35.19**	35.65	35.77	0.38	1.15
Zophoessa callipteris	**4.06**	5.22	6.51	0.15	0.46
Nymphalis vaualbum	**26.46**	27.65	26.53	-0.24	-0.54
Thymelicus sylvaticus	**26.46**	28.48	26.53	-0.24	0.13
Gonepteryx aspasia	**38.56**	40.18	38.81	-0.40	-0.73
Damora sagana	**48.08**	51.30	51.01	-0.90	-1.87
Speyeria aglaja	**70.10**	72.82	70.92	-1.51	-0.98
Celastrina argiolus	44.27	**35.35**	45.77	0.63	3.43
Argyreus hyperbius	19.87	**17.96**	18.42	-0.09	-0.63
Papilio dehaanii	23.50	**20.01**	22.16	-0.12	-0.92
Eurema mandarina	33.98	**32.74**	33.27	0.25	-1.13
Hesperia florinda	51.75	**49.54**	49.75	0.00	-2.01
Colias erate	96.60	**94.32**	95.05	-1.61	-12.64

Table 4. Habitat preferences of interacting old grassland butterflies and plants coincide or are smaller for butterflies. Habitat preferences of butterfly species examined by linear regression or indicator species analysis (IndVal), shown in "Detection method" columns, compared with that by host plants of each butterfly species based on IndVal in Inoue et al. (2021b). Host plants of each butterfly species were taken from Shirouzu (2006). All butterfly species that showed a preference for large old grassland based on IndVal (Table 2) or linear regression (Table 3) are shown. Note that the effect of grassland area was not detected in plants in Inoue et al. (2021b), in contrast to butterflies, and their habitat preferences were analyzed irrespective of habitat area. Butterfly habitat preference is either coincident with or narrower than that of host plant preference. We found no cases in which a plant species preferred old grassland, but its specific herbivorous butterflies did not. Statistical values showed the *ΔAIC* between the best model and the next best model for regression and *P*-value for Indval. n. s. indicates that habitat preference by those host plants was not statistically detected in Inoue et al. (2021b).

Butterfly				Host plant				Interaction
Species	Habitat preference	Detection method	Statistical value	Species	Habitat preference	Detection method	Statistical value	Coincident of preference between butterfly and host plant
Curetis acuta	Large old grassland	Regression	4.193	*Lespedeza bicolor*	Old grassland	IndVal	0.003	Coincident
Brenthis daphne	Large old grassland	Regression	3.491	*Sanguisorba officinalis*	Old grassland	IndVal	0.001	Coincident
Eurema mandarina	Large old grassland	IndVal	0.011	*Lespedeza bicolor*	Old grassland	IndVal	0.003	Coincident
Minois dryas	Large old grassland	Regression	4.600	*Miscanthus sinensis*	Entire grassland	IndVal	0.006	Butterfly's preference is narrower
Zophoessa callipteris	Large old grassland	Regression	1.152	*Sasa senanensi*	n. s.	-	-	-
Melitaea ambigua	Large old grassland	Regression	0.970	*Veronicastrum japonicum*	n. s.	-	-	-
Lycaena phlaeas	Large old grassland	Regression	0.461	*Rumex japonicus*	n. s.	-	-	-

Figures

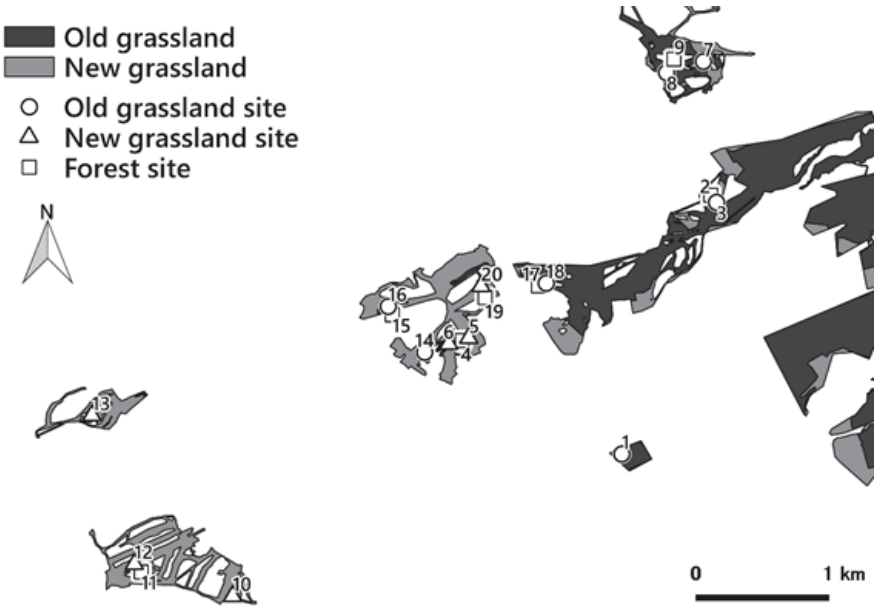

Figure 1

Distribution of old (mostly >300 years old) and new (43–71 years old) grasslands in the Sugadaira and Minenohara Highlands in central Japan. White space indicates lands other than grasslands, including forests, arable fields, residential area and roads. Locations and vegetation of study sites are shown by either circles (old grassland), triangles (new grassland) or squares (forest).

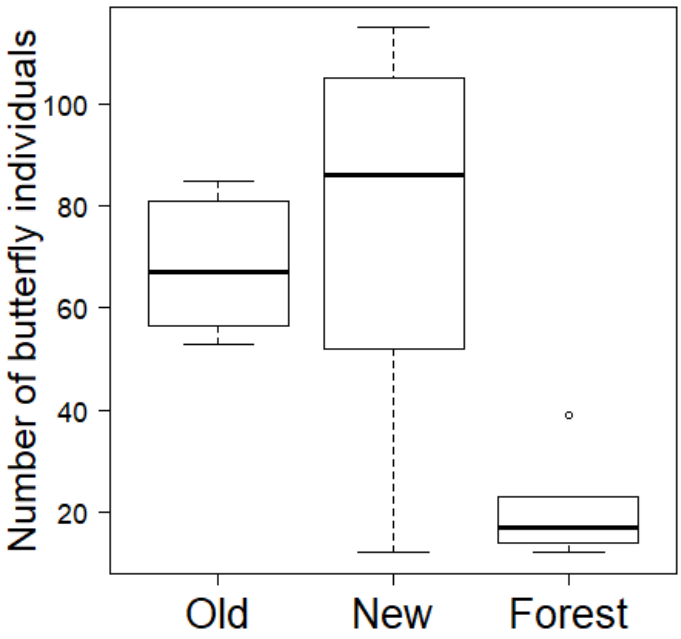

Figure 2

More individual butterflies in grasslands than in forests. Numbers of individual butterflies in old grasslands (Old), new grasslands (New), and forests (see detail in the text). Differences between old and new grasslands were not statistically supported, with grassland butterflies significantly more abundant than those in forests (Table 1a).. 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.

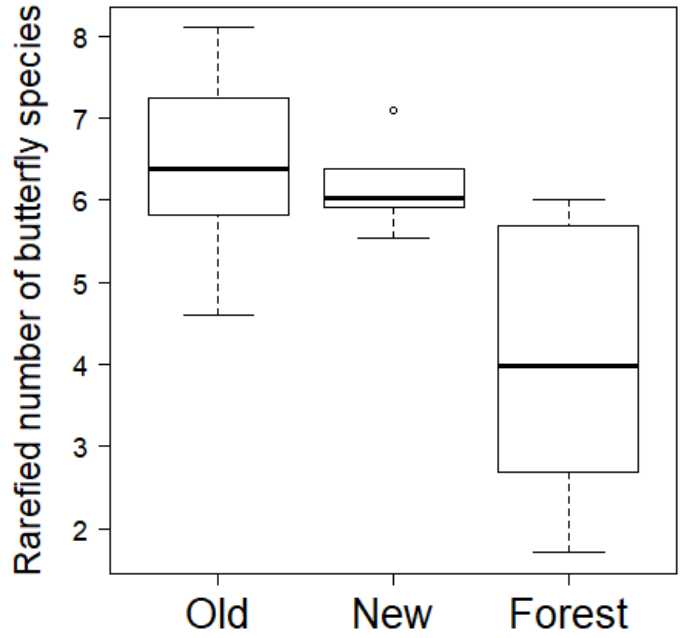

Figure 3

More butterfly species in grasslands than in forests. Rarefied numbers of butterfly species in old grasslands (Old), new grasslands (New), and forests. Differences between old and new grasslands were not statistically supported, with grassland butterflies significantly more abundant than those of forests (Table 1b). Bars and boxes are medians and quartiles, with ranges between minimum and maximum values. An outlier is shown by a dot.

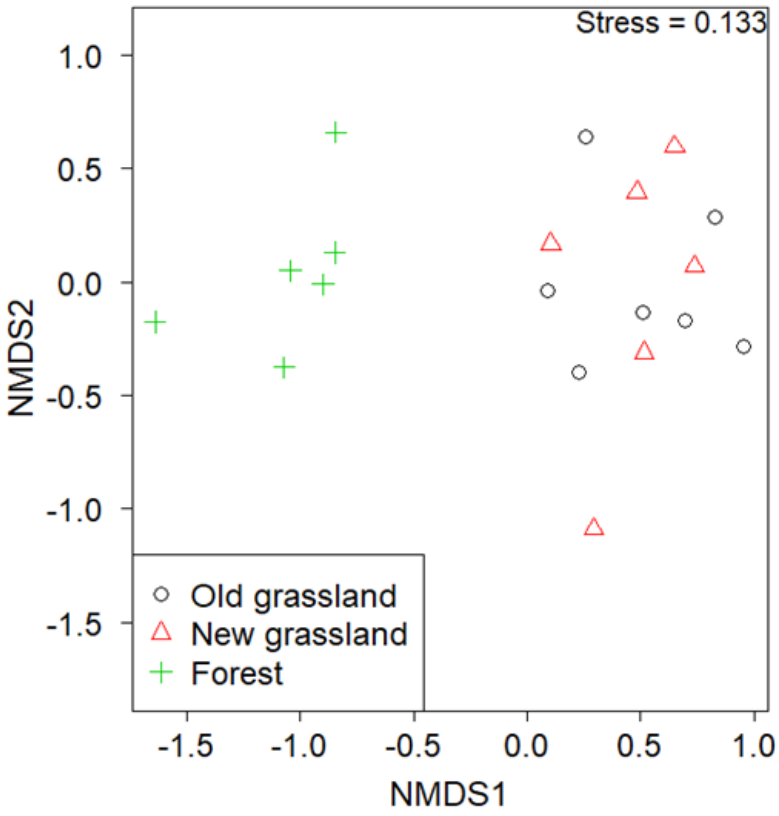

Figure 4

Butterfly species composition differed between grasslands and forest (PERMANOVA $p < 0.001$), but old and new grassland were not significantly different ($p = 0.356$). Non-metric multidimensional scaling (NMDS) using the Bray-Curtis index of dissimilarity of butterfly composition between study sites across the three-vegetation types. Horizontal and vertical axes represent the first and second axes extracted by NMDS, respectively.

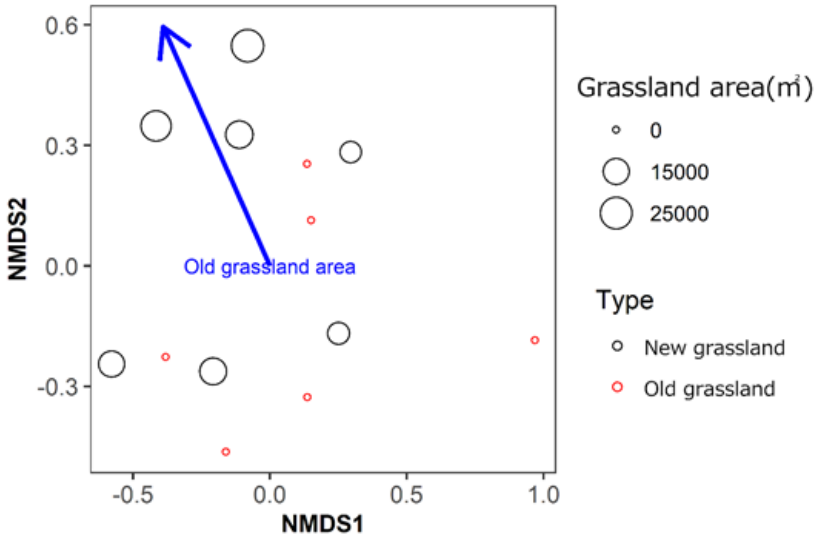

Figure 5

Old grassland area within 100 m of each study site affected butterfly species composition. Non-metric multidimensional scaling (NMDS) plot using the Bray-Curtis index of dissimilarity of butterfly composition between study sites of grasslands. Horizontal and vertical axes represent the first and second axes extracted by NMDS, respectively. The blue arrow indicates the effect of old grassland area within 100 m of each study site detected by envfit ($p < 0.05$), whereas effects of other environmental factors (vegetation height, slope, elevation, and the area of the grasslands) were not detected.

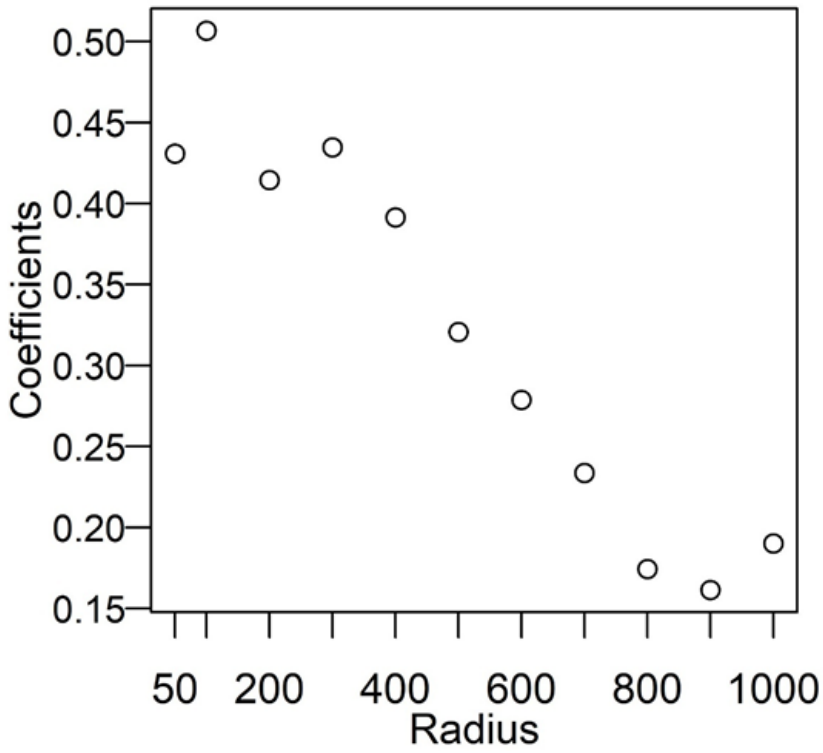

Figure 6

Grassland area within 100 m of the site explains most NMDS plot variation. Areas of old grassland within radius x m were fitted to the NMDS of the butterfly community (Fig. 5) using envfit. The relationship between the radius x and the coefficient of envfit. The radius x m of the explanatory variable is 50, from 100-1000 in increments of 100.

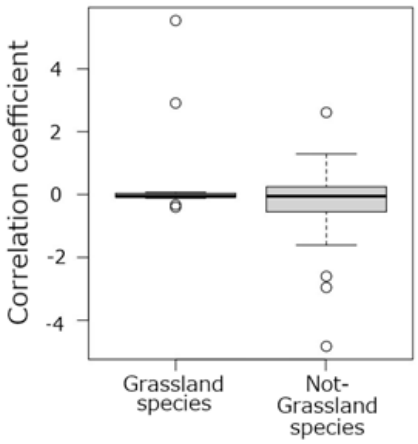

Figure 7

Grassland-specific butterfly species (Shirouzu, 2006) tend (p = 0.08) to have higher regression coefficients in the LM for the number of individual butterflies in each species, explained in relation to the area of old grassland.