Effects of grassland duration on butterfly communities and its relevance to grassland area

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Research Article

Keywords: temporal continuity of grassland, spatial continuity of grassland, conservation priority, grassland management, legacy effect , endangered species

Posted Date: March 13th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4056425/v1

Additional Declarations: No competing interests reported.

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Abstract

Semi-natural grasslands, renowned for their rich biodiversity, are experiencing global decline, necessitating urgent conservation prioritization. Recently, researches have established that the vegetation history significantly influences grassland biodiversity. This study focuses on three vegetation types in and around ski-run grasslands of central Japan: (i) ancient or old grasslands persisting for 300 to several millennia, (ii) new grasslands recently converted from forests 43 to 72 years ago, and adjacent forests serving as control vegetation type. We examined the effect of grassland duration, in relation to its spatial area, on butterfly communities. Transects of 5 × 20 m were established at six to seven sites for each vegetation type to survey butterfly communities. In 2017, plant communities were similarly surveyed along the same transects (Inoue, Yaida et al., 2021). Species composition, species count, and overall butterfly abundance were not affected by grassland duration. Nevertheless, the species composition was affected by the spatial area of old grasslands within a 100 m radius of each transect, with an increase in the number of rare grassland species in extensive old grasslands. *Minois dryas* and *Eurema mandarina* were identified as indicator species of extensive old grasslands (>8000 m^2 within 100 m radius of each transect), whereas no indicator species were detected in the other grassland sites. The abundances of *M. dryas*, *Brenthis daphne*, *Melitaea ambigua*, *Curetis acuta*, and *Zophoessa callipteris* were greater in areas where the areas of old grasslands within 100 m of each transect were greater. The previous study at these sites have shown that *Sanguisorba officinalis* (Rosaceae), the host plant of *B. daphne*, and *Lespedeza bicolor* (Fabaceae), the main host plant of *E. mandarina* and *C. acuta*, are indicators of old grasslands, explaining the dependency of these three butterfly species on old grasslands. Our findings suggest that extensive old grasslands should be accorded high conservation priority to preserve diversity of grassland butterflies.

Keywords

temporal continuity of grassland · spatial continuity of grassland · conservation priority · grassland management · legacy effect · endangered species

Introduction

Semi–natural, temperate grasslands, sustained through anthropogenic practices such as controlled burning, mowing, and grazing, are celebrated for their remarkable species diversity and great conservation significance (Akeroyd & Page, 2011; OECD, 2008; Wilson et al., 2012). However, they are undergoing global decline due to their conversion into farmland and urban developments, or through reforestation resulting from the abandonment of traditional management practices in Europe, as evidenced in various European nations including Britain (Ridding et al., 2015), Germany (Poschlod et al., 2005), Sweden (Cousins & Eriksson, 2008), and Estonia (Pärtel et al., 1999). This trend is similarly observed in Japan, where grasslands expanses are diminishing nationwide (Masui et al., 2017; Ogura, 2006; Ohta et al., 2021; Shoji & Yamamoto, 1995; Yamato & Hattori, 1999), with Sugadaira Highland in central Japan, witnessing approximately 92% conversion of grassland to forest over the past 100 years (Inoue, Okamoto, Kenta, 2021a).

Given the rapid decline in grasslands, it is urgent to identify and prioritize grasslands with high biodiversity for conservation. The vegetation history emerges as critical determinant in the formation and preservation of biodiversity–rich communities (Culbert et al., 2017; Honnay et al., 2017; Inoue, Yaida et al., 2021; Craioveanu et al., 2021). Grasslands and forests with a longstanding history are known to exhibit high plant diversity (Culbert et al., 2017; Yaida et al., 2019; Inoue, Yaida et al., 2021; Craioveanu et al., 2021). Moreover, the diversity and abundance of arbuscular mycorrhizal fungi are notably higher in grasslands aged between 12 to 20 years compared to newer grasslands (Honnay et al., 2017). Previous studies have also shown that bird community composition is affected by the duration of forests (Culbert et al., 2017) and increased diversity of butterflies has been observed in grasslands persisting for more than 30 years than newer grasslands found (Craioveanu et al., 2021). Oki et al. (2022) found decreasing diversity of butterflies that inhabit grassland or forest edges with increase of duration after abandonment of grassland management (i.e. duration of succession from grassland to forest).

The temporal effects of vegetation history also interact with spatial scale. Current plant diversity is strongly influenced by historical habitat connectivity (50–130 years before present; Lampinen et al., 2018; Lindborg & Eriksson, 2004). Both the temporal duration and spatial area of habitats positively influence the number of grassland plant species (Noda et al., 2022). Conversely, Uchida et al. (2016) did not detect the effects of the past and present grassland areas on diversity of plants, butterflies, and orthopterans.

Butterflies serve as pivotal indicators of ecosystem due to the variation in their habitat utilization across different life stages (van Swaay et al., 2010). They exhibit strong associations with habitat characteristics, including the diversity and abundance of plants (Maleque et al., 2009), vegetation

height (Dylewski et al., 2020), and availability of food plants (Piccini et al., 2022) and nectar plants (Kitahara et al. 2008). Furthermore, endangered species of butterflies depend on grassland habitats (Van Swaay & Warren, 1999, Ohwaki, 2018).

This study aims to elucidate the effect of grassland duration, and its relevance to spatial area, on butterfly communities, building upon our previous findings that underscored the positive effects of grassland duration on plant diversity (Inoue, Yaida et al., 2021) in the same study sites in old grasslands dated between 300 to 1000s years ago newer grasslands that established 52 to 70 years ago after deforestation, and adjacent forests, all situated in and around ski runs in central Japan. Our objectives were (1) to clarify the effect of grassland duration on species composition, abundance, and diversity of butterfly communities, (2) to explore the interplay between such effects of grassland duration and its spatial area, and (3) to examine how such effects on butterfly communities could be explained by plant 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. The Sugadaira Highland 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 formed and accumulated in grasslands (Shinji et al., 2002; Yamane, 1973). Surveys of old maps confirmed that almost the entire Sugadaira and Minenohara Highland was the single continuous grassland 300 years ago (Inoue, Okamoto, Kenta, 2021a). However, between 1937–1975, large parts of this region reverted to forests due to the plantation of *Larix kaempferi*, as well as natural succession to forests dominated by *Quercus crispula*, *Pinus densiflora*, and *Betula platyphylla*, due to the abandonment of grassland management. After that, skiing became popular in Japan around the 1960s–1970s and some parts of these forest plantations were then clear-cut and converted to ski runs of grasslands. We define the area covered with grasslands in 2010 that did not experience forestation as old grasslands, and those that were once forested in 1937–1975 as new grasslands. Finally, the area covered with forests in 2010, which originated from forestation in 1937–1975, was defined as forests, and areas adjacent to either old or new grasslands were chosen as forest study sites.

Grassland management practices, particularly mowing, were uniformly applied across old and new grasslands annually during the autumn season, from late August to mid-October, as part of extensive ski run management initiated in the 1960s - 1970s. Prior to this period, old grasslands were utilized as pasture and as meadows where people harvested grass for field fertilizer and livestock feed. Geographic Information System (GIS) mapping integrated topographic maps from 1912 and 1937 and aerial photographs from 1947, 1975, and 2010 (Geospatial Information Authority of Japan), to delineate the aforementioned three vegetation types (old grasslands, new grasslands, and forests). We chose seven old

grassland sites and six new grassland sites across various ski runs to minimize spatial biases, alongside six forest totes proximal to these grasslands (Fig. 1). These study sites were consistent with those utilized in our previous study of plant communities (Inoue, Yaida, et al., 2021), with exception of one forest site, which was lacked in this study.

Field investigations

At each study site, we set 5 × 20 m transects, ensuring a minimum 20 m buffer from the borders between grasslands and forests. This transect length was determined in consideration of the proximal adjacency of the three vegetation types around the study sites, resulting in relatively short transect in comparison to some of the other studies (Kitahara et al. 2008; Ramesh et al. 2010). Butterfly populations of all species were surveyed through transect walks, during which individual counts within a five m^3 area directly ahead of the observer were recorded over 12-minute intervals in both directions of the transect, conducted between 9:00 and 17:00. These surveys were repeated seven times under clear and windless weather between June and September of 2018, with scheduling adjustments to mitigate biases in visitation order and timing. The region's climatic conditions result in reduced lepidopteran activity outside the aforementioned survey period.

Environmental factors including vegetation height, slope, and elevation were previously documented (Inoue, Yaida et al., 2021) and were incorporated into the current study. Additionally, GIS data facilitated the quantification of the area of old and new grasslands surrounding each study site, calculated from the transect centroid within specified radii, ranging from 50 m to 1 km in 100 m increments.

Statistical analyses

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* package in *R* (Oksanen et al., 2020). We tested the effect of vegetation types on species composition by permutational, multivariate analysis of variance (PERMANOVA; Anderson, 2001; McArdle & Anderson, 2001), using the adonis function in the *vegan* package in *R*, with 10,000 permutations. To examine how grassland duration interacts with grassland area and other environmental factors, we fitted areas of old and combined grasslands within radiii ranging from 50 to 1 km, as well as vegetation height, slope, and elevation to NMDS plots by a permutation test using the *envfit* function in the *vegan* package in *R*. We found 100 m the most explaining the butterfly species composition, as explained later (Fig. 3), and used such spatial scale in the latter analyses. Spatial autocorrelation within species composition was evaluated through the Mantel test.

To examine the effects of vegetation types on the numbers of individuals and species, we constructed generalized linear mixed models (GLMMs) with a negative binomial distribution for numbers

of individuals, and with a Poisson distribution for species richness. We set study sites as a random effect. Three distinct models were formulated based on vegetation type: a null model, a two-vegetation-type model, and a three-vegetation-type model. Numbers of either (a) individuals, (b) species, (c) grassland species, or (d) grassland rare species, μ, was fitted to a linear predictor using either of the latter regression models:

$$\mu \sim \beta 0 + \beta 1\ V + 1|S$$

$$\mu \sim \beta 0 + \beta 1\ A + 1|S$$

, where β0 was an intercept, β1 was the estimator of regression coefficients of V (vegetation type) or A (area of old grassland from the centroid of each transect within a radius of 100 m), and 1|S was the random effect of study sites. In the null model, the numbers of species/individuals were assumed to be the same among vegetation types. In the two-vegetation-type model, the 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 likelihood ratio test.

Indicator species were detected using the *indval* function (INSPAN; Dufrêne & Legendre, 1997) in the *labdsv* package (Roberts, 2016) in *R*. In this indicator species analysis, study sites were categorized into three groups: large old grasslands (grasslands that have more than 8,000 m^2 old grasslands within 100 m of the transect centroid), other grasslands (grasslands that have less old grasslands), and forests. INSPAN 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. The resulting indicator values were tested by significance with a randomization test (9999 times).

Furthermore, permutational regression analysis was implemented, using our custom commands in *R*, to examine the effect of the dissimilarity of plant communities (Inoue, Yaida et al., 2021) and geographical distance on the dissimilarity of butterflies at each site. The same (Legendre et al., 1994; Lichstein, 2007; Nagamitsu et al., 2014) and similar (Kunin et al., 2009) methods have been used elsewhere. The 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 β0 was an intercept, β1and β2 were estimators of regression coefficients of P (dissimilarity of plant communities) and G (geographical distance), respectively. We used 10,000 matrix permutations 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.

Results

In total, 39 butterfly species (1058 individuals) were recorded. Initial analyses, disregarding the spatial area of grasslands surrounding each site, revealed divergent butterfly species compositions between grasslands and forests ($p < 0.001$, PERMANOVA, Fig. 2), with no significant difference between old and new grasslands ($p = 0.36$). However, upon incorporating the spatial scale of old grasslands, the area of old grassland within 100 m of each study site most affected butterfly species composition compared to other spatial scales (Fig. 3), and its effect was significant ($p < 0.05$, *envfit*, Fig. 4). Effects of other environmental factors (vegetation height, slope, elevation, and the area of the grasslands) on the species composition were not detected.

In analyses devoid of spatial considerations, the number of butterfly individuals (ΔAIC to null model = 15.4, $p < 0.001$, GLM and the likelihood ratio test, Table 1a) and species (ΔAIC to null model = 19.4, $p < 0.001$, Table 1b) were most explained by two-vegetation model, indicating more individuals (Fig. 5a) and species (Fig. 5b) in grasslands than in forests, with no statistical difference between old and new grasslands. When excluding data from forest sites and still not considering spatial aspect, the number of grassland species and that of grassland rare species were most explained by the null model (Table 1c) and two-vegetation model (ΔAIC to null model = 0.7, $p = 0.10$, Table 1d, Fig. 5d), respectively, indicating no difference in number of grassland species (Fig. 5c) between old and new grasslands, and marginally more grassland rare species (Fig. 5d) in old grasslands. With spatial aspect into account, the number of butterfly individuals was marginally affected by the combined area of both old and new grasslands within 100 m of each study site (ΔAIC to null model = 1.1, $p = 0.08$, Table 2a), showing marginally more individuals (Fig. 6a) where large grasslands exist; the number of species and that of grassland species were most explained by null model (Table 2b and 2c), showing no effects of grassland area (Fig. 6b and 6c); and the number of grassland rare species was significantly explained by the area of old grasslands within 100 m of each study site most (ΔAIC to null model = 2.5, $p < 0.05$, Table 2d), indicating more grassland rare species (Fig. 6d) in extensive old grasslands.

In the indicator species analysis (INSPAN), two, five, two, and four butterfly species were detected in extensive old grasslands, the combined grassland, forest, and any vegetation (specialist species), respectively, whereas no indicator species were detected in other grasslands (Table 3), which were not surrounded by extensive old grasslands. Numbers of individual butterflies exhibited both positive and negative relationship with the areas of the old (11 species) and the combined grasslands (6 species) (Table 3).

In permutational regression analysis, the dissimilarity of butterfly communities between study sites was not explained by dissimilarity of plant communities ($p = 0.45$) or by dissimilarity and geographical distance between study sites ($p = 0.37$).

Discussion

Abundance and species richness of butterflies were higher in grasslands than in forests (Table 1). Between old and new grasslands, in terms of whether the study sites themselves were old or new grasslands, butterfly species composition, abundance, species richness, and richness of grassland species did not differ (Fig. 2, Table 1, Fig. 5a-c), although the richness of grassland rare species was marginally higher in old grasslands. Notably, the area of old grasslands within a 100 m radius from the study sites emerged as critical factor affecting butterfly species composition (Fig. 3). The effects of grasslands duration have been reported in plants (Cousins & Eriksson, 2008; Honnay et al., 2017; Inoue et al., 2021b) and fungi (Honnay et al., 2017). In animal communities, Culbert et al. (2017) detected the significant and marginal effects of duration of forest and grassland, respectively, on bird communities, but they did not found the effects of duration of either forest or grassland on butterflies. Craioveanu et al. (2021) found increased butterfly diversity in grasslands that continued for > 30 years compared to newer grasslands, and so far as we are aware, this had been the only study that clearly found positive effects of grassland duration on animal communities. Our findings extend this effect of grassland duration, exceeding 300 years, on butterfly communities, when incorporating the spatial area of old grasslands.

The spatial scale of grasslands that affected butterfly communities in our study is relatively small compared to previous studies (more than 100s m, e.g. Öckinger et al. 2012; Bergman et al. 2018). Rossi and van Halder (2010) stated that short-scale effects may imply processes like mass effects and spillover, while large-scale effects involve metapopulation dynamics. In and around our study sites, grasslands and forests exist as intricate mosaics (Fig. 1) on a spatial scale of around 100 m. Mass effects and spillover in such spatial scales could possibly shape the butterfly communities there.

Contrasting with butterflies, plant species compositions in the same study area (Inoue, Yaida et al., 2021b) was determined by the historical status of the study sites themselves, rather than the surrounding spatial area of old grasslands. This discrepancy can be attributed to three factors: (i) the extensive requirements of plant resources, plant shoots for larvae and nectar for adults, resulting in butterflies' dependence on larger area of grasslands; (ii) the relatively brief lifespans of butterfly species necessitating expansive habitats for viable population maintenance; and (iii) the superior migratory capabilities of butterflies compared to plants. Lindborg & Eriksson (2004) suggest that insects are short-lived, highly mobile, and are less sensitive to landscape history compared to plants. 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. Plant species would be able to endure for

considerable periods even with non-viable populations, a situation known as "extinction debt" (Tilman et al., 1994).

Butterflies depend on plant shoots and nectar as food, and the diversity and composition of butterfly communities are generally affected by plant communities (Steffan-Dewenter & Tscharntke, 2000; Kitahara et al., 2008; Uchida & Ushimaru, 2014). Our study revealed no significant effects of slope and elevation—factors previously identified as non-influential to plant species composition in the study region (Inoue, Yaida et al. 2021), on species composition of butterflies. The species composition of butterflies was not affected by vegetation height nor plants species composition. The absence of such effects of plants on butterflies on butterfly suggests that butterfly communities may be responsive to plant communities across broader spatial scale.

At a broad spatial scale, the synergistic relationships between butterflies and their host plants provide profound insights into the effects of duration and area of grasslands on plant-butterfly interactions (Table 5). First, an alignment in habitat preferences is observed between butterflies and host plants; a mutual inclination towards old grasslands is evident among these biotic counterparts. Second, our study did not encounter any instances where a plant species exhibited a preference for old grasslands without a corresponding preference from its butterfly herbivores. These concurrences supports the hypothesis that grassland duration predominantly influences plant communities, subsequently affecting lepidopteran populations.

There is growing recognition that old-growth grasslands are important for the conservation of plant diversity (Nerlekar & Veldman, 2020). In this study, we show that old-growth grassland is also important for insects. In conclusion, we propose that the extensive old grasslands should have high conservation priority as unique biological communities with more grassland rare species. Old grasslands may exist as fragments but the total area of old grassland in a 100 m radius affected butterfly diversity, consistent with Riva & Fahrig (2023) that suggested even fragmented habitats have large conservation values. New grasslands are easily generated by human activity, such as the creation of parks, roads, and buildings, or the 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, extensive 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).

Acknowledgments The authors are grateful to the land owners of our study sites: “general foundation Nirei kai,” “Sugadaira Bokujo,” “Hatsunekan,” “Imai-kan” and “Jozan-kan,” as well as the management companies: “Sugadaira Pine Beak Ski,” “Sugadaira Ski House Co., Ltd.,” “Oku Davos Snow Park” and “HARE Sugadaira-Kogen Snow Resort,” for allowing our field surveys.

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Author contributions

TI and TK planned the study, TI performed the fieldwork, and TI and TK wrote the manuscript and approved it.

Funding

This work was supported by JSPS KAKENHI (JP17K07557 and 21J10385), by JSPS Young Scientist fellowship to TI, and by the Environment Research and Technology Development Fund (JPMEERF20234005) of the Environmental Restoration and Conservation Agency provided by the Ministry of the Environment of Japan.

Data Availability

The used data are hosted by the authors' institute and can be requested at the institute.

Declarations

Competing Interests: The authors declare no conflict of interest.

Ethical approval: Not applicable.

462

463 Table1. Using the whole data of old and new grasslands and forests, two vegetation-type models, which
464 discriminate between the whole grasslands and forests, were selected for the numbers of individuals (a) and
465 species (b), with grasslands having a higher number of those (Fig. 2, 3). The three vegetation-type model,
466 which discriminated between old grasslands (>300 years old), new grasslands (43–72 years old), and forests,
467 were not selected for (a) and (b). Using only the data of old and new grasslands, null model was selected
468 for the number of grassland species (c), and two vegetation-type model, which discriminate between old
469 and new grasslands, was selected for the number of rare grassland species (d). Bold letters indicate the best
470 models that had the lowest AIC. df indicates degrees of freedom. ΔAIC shows the difference in AIC
471 between the best model and the model accompanied by each ΔAIC value. * p-values were derived from the
472 likelihood ratio test and indicated the reliability of the best model relative to the model accompanied by
473 each p-value.
474

Model	df	AIC	ΔAIC	p
(a) Number of individuals				
Two vegetation-type model (grassland vs forest)	3	967.5	0.0	
Three vegetation model	4	969.5	2.0	0.92
Null model	2	982.9	15.4	< 0.001
(b) Number of species				
Two vegetation-type model (grassland vs forest)	3	521.8	0.0	
Three vegetation-type model	4	523.7	1.9	0.81
Null model	2	541.2	19.4	< 0.001

(c) Number of grassland species

Null model	2	410.1		
Two vegetation-type model (old grassland vs new grassland)	3	412.1	2.0	< 0.001

(d) Number of grassland rare species

Two vegetation-type model (old grassland vs new grassland)	3	266.8		
Null model	2	267.6	0.7	0.10

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Table 2. GLMM showed that the number of butterfly individuals (a) was higher in sites with larger combined grassland areas; that the number of species (b) and grassland species (c) was not explained by any type of grassland area; and that the number of grassland rare species (d) was higher in sites with larger old grassland area. Those grassland areas were calculated from the centroid of each site within a radius of 100 m.

Model	df	AIC	ΔAIC	p
(a) Number of individuals				
Combined grassland area	3	981.7		0.08
Null model	2	982.9	1.1	
Old grassland area	3	984.6	1.7	0.61
(b) Number of species				
Null model	2	396.1		
Old grassland area	3	397.9	1.9	0.73
Combined grassland area	3	398.0	2.0	0.91
(c) Number of grassland species				
Null model	2	326.8		
Old grassland area	3	327.1	0.3	0.19

Combined grassland area		328.8	2.0	0.98

(d) Number of grassland rare species

Old grassland area	3	254.4		< 0.05
Null model	2	257.0	2.5	
Combined grassland area	3	258.7	4.2	0.59

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Table 3 . Indicator species analysis (INSPAN) detected four, five, two, and two butterfly species in the whole group of all vegetation (any vegetation), combined grasslands (any type of grasslands), forests, and large old grasslands (grasslands that have more than 8,000 m^2 old grasslands within 100 m of the transect centroid), respectively, whereas no indicator species were detected in other grasslands (grasslands that have less than 8,000 m^2 old grasslands within 100 m of the transect centroid). Only shown for significant species ($p < 0.05$), except for species detected as indicators for "any vegetation", which means the presence of those species in any vegetation type.

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 nagiae	Combined grassland	100	0.001
Parnara guttata	Combined grassland	92	0.001
Brenthis daphne	Combined grassland	77	0.008
Ochlodes venatus	Combined grassland	77	0.013
Hesperia florinda	Combined 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 4. Linear models showed that populations (number of individuals) of 11 butterfly species were explained by the area of either old grassland or combined (both old and new) grassland. Five species including four rare species showed more population where old grassland areas were larger, while only one non-rare species had a larger population where the combined grassland area was larger. Those grassland areas were calculated from the centroid of each site within a radius of 100 m. AICs of the best models are shown in bold. Species are shown only when regression coefficients of the best models were significant ($p < 0.05$, likelihood-ratio test). Rareness shows the Japanese nation-wide endangered rank (EN or VU) of each shown species or whether such species is categorized as endangered in at least one prefecture.

	AIC			Coefficient		Rareness
Species	Old	Combined	Null model	Old	Combined	
Minois dryas	**90.77**	97.24	95.37	5.54	2.93	+
Brenthis daphne	**70.96**	74.45	75.67	2.61	6.46	VU
Melitaea ambigua	**58.84**	59.81	61.39	1.29	3.91	EN
Curetis acuta	**22.07**	26.26	27.86	0.44	1.08	-
Zophoessa callipteris	**4.06**	5.22	6.51	0.15	0.46	+
Damora sagana	**48.08**	51.30	51.01	-0.90	-1.87	+

Celastrina argiolus	44.27	**35.35**	45.77	0.63	3.43	-
Papilio dehaanii	23.50	**20.01**	22.16	-0.12	-0.92	+
Eurema mandarina	33.98	**32.74**	33.27	0.25	-1.13	-

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Table 5. Habitat preferences of interacting with old grassland butterflies and plants coincide or are smaller for butterflies. Habitat preferences of butterfly species examined by linear regression or indicator species analysis (INSPAN), shown in the "Detection method" columns, compared with that by host plants of each butterfly species based on INSPAN in Inoue, Yaida et al. (2021). Host plants of each butterfly species were taken from Shirouzu (2006). All butterfly species that showed a preference for large old grassland based on INSPAN (Table 3) or linear regression (Table 4) are shown. Note that the effect of grassland area was not detected in plants in Inoue, Yaida et al. (2021), in contrast to butterflies, and their habitat preferences were analyzed irrespective of habitat area. We found no cases in which a plant species preferred old grassland whilst its specific herbivorous butterflies did not. p values are derived from either linear regressions or INSPAN. n.s. in the interaction column indicates that habitat preference by those host plants was not statistically detected in Inoue, Yaida et al. (2021).

Butterfly				Host plant				Interaction
Species	Habitat preference	Detection method	p	Species	Habitat preference	Detection method	p	Coincident of preference between butterfly and host plant
Curetis acuta	Large old grassland	Regression	0.012	*Lespedeza bicolor*	Old grassland	INSPAN	0.003	Coincident
Brenthis daphne	Large old grassland	Regression	0.020	*Sanguisorba officinalis*	Old grassland	INSPAN	0.001	Coincident
Eurema mandarina	Large old grassland	INSPAN	0.011	*Lespedeza bicolor*	Old grassland	INSPAN	0.003	Coincident
Minois dryas	Large old grassland	INSPAN	0.023 0.021	*Miscanthus sinensis*	Combined grassland	INSPAN	0.006	Butterfly's preference is narrower

		Regression						
Zophoessa callipteris	Large old grassland	Regression	0.058	*Sasa senanensi*	Forest	INSPAN	0.156	n.s.
Melitaea ambigua	Large old grassland	Regression	0.055	*Veronicastrum japonicum*	Combined grassland	INSPAN	0.521	n.s.
Lycaena phlaeas	Large old grassland	Regression	0.148	*Rumex acetosa*	Forest	INSPAN	0.354	-

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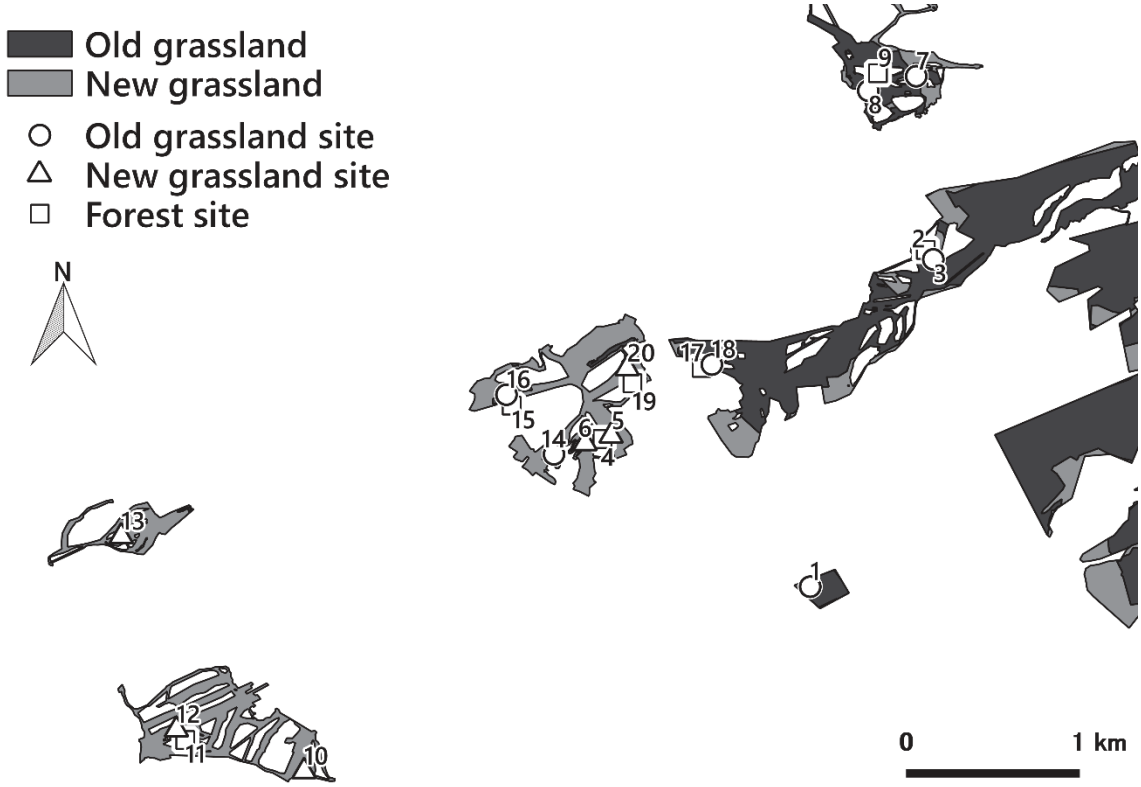

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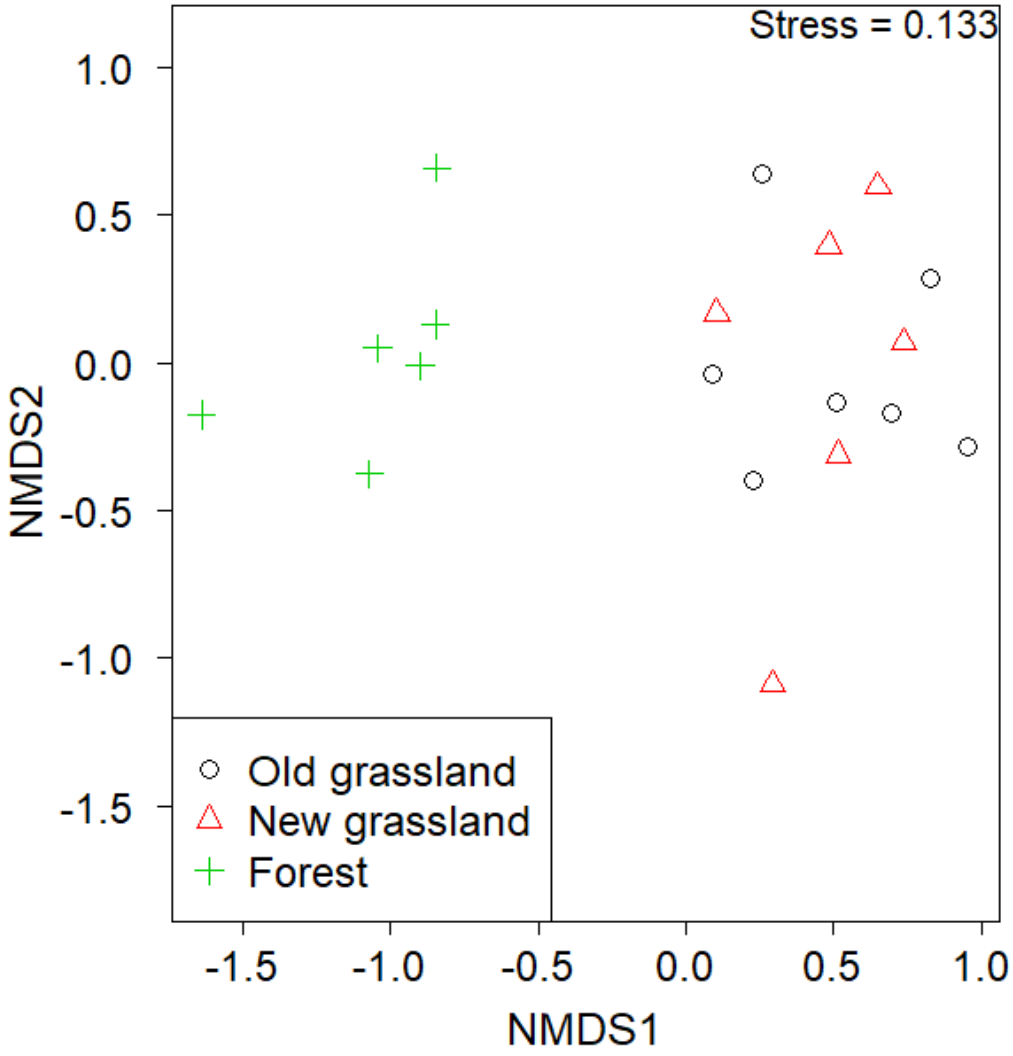
525 Fig. 1. Distribution of old (mostly >300 years old) and new (43–71 years old) grasslands in the Sugadaira
526 and Minenohara Highlands in central Japan. White space indicates lands other than grasslands, including
527 forests, arable fields, residential areas, and roads. Locations and vegetation of study sites are shown by
528 either circles (old grassland), triangles (new grassland), or squares (forest).



529 Fig. 2. Butterfly species composition differed between grasslands and forests (PERMANOVA $p < 0.001$),
530 but old and new grasslands were not significantly different ($p = 0.356$). Non-metric multidimensional
531 scaling (NMDS) using the Bray-Curtis index of dissimilarity of butterfly composition between study sites
532 across the three-vegetation types. Horizontal and vertical axes represent the first and second axes
533 extracted by NMDS, respectively.

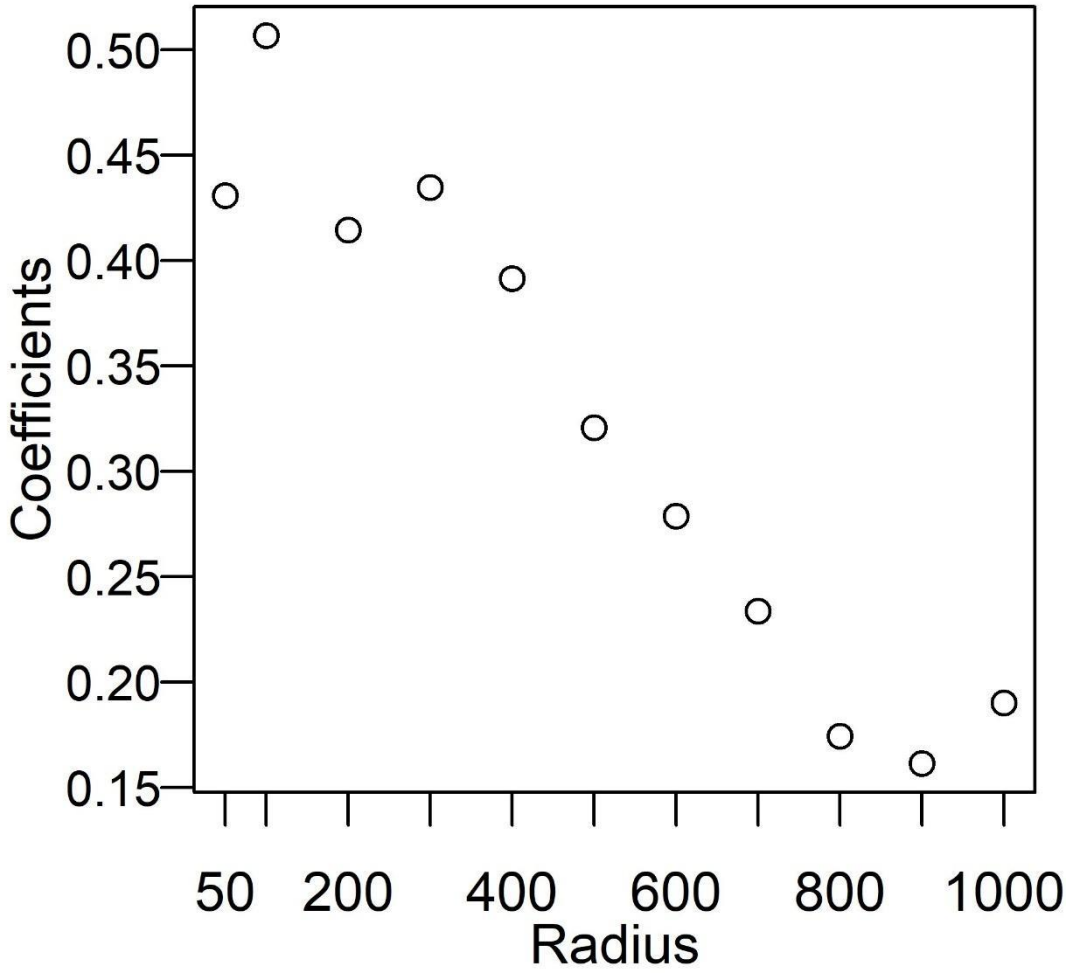
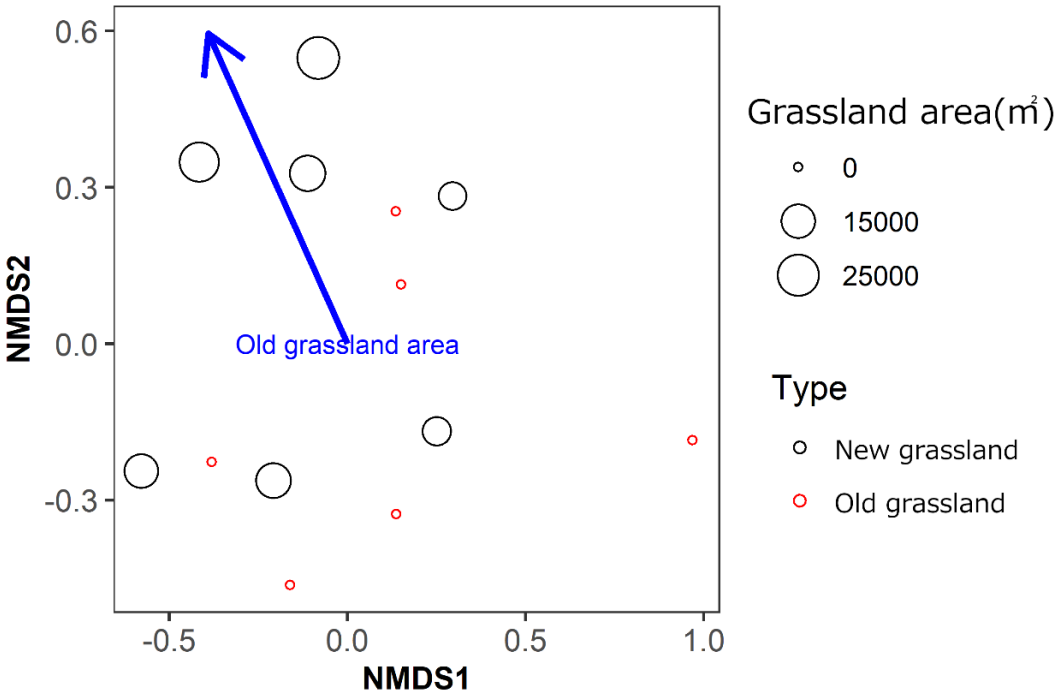


Fig. 3. Area of old grassland, from the centroid of each site within a radius of 100 m, most explains variation in butterfly species composition using Bray-Curtis dissimilarity, shown by the highest regression coefficient of *envfit*. We compared the effects of different spatial scales of old grasslands on the species composition, by using a range of radius from 50 to 1000 m.



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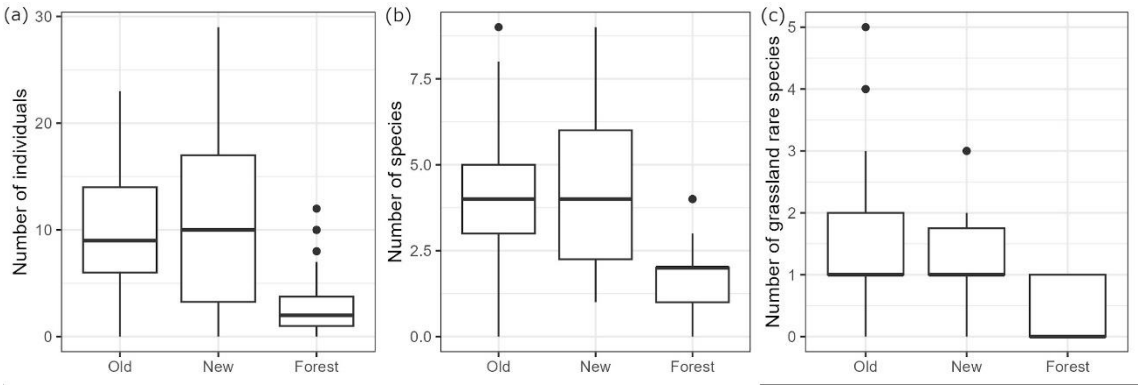
545 Fig. 4. Old grassland area within 100 m of each study site affected butterfly species composition. Non-
546 metric multidimensional scaling (NMDS) plot using the Bray-Curtis index of dissimilarity of butterfly
547 composition between study sites of grasslands. Horizontal and vertical axes represent the first and second
548 axes extracted by NMDS, respectively. The blue arrow indicates the effect of the old grassland area
549 within 100 m of each study site detected by *envfit* ($p < 0.05$), whereas effects of other environmental
550 factors (vegetation height, slope, elevation, and the area of the grasslands) were not detected.



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554 Fig. 5. More individual (a), species (b), and grassland rare species (c) of butterflies in grasslands than in
555 forests. Numbers of individual butterflies in old grasslands (Old), new grasslands (New), and forests (see
556 detail in the text). Differences between old and new grasslands were not statistically supported, with
557 grassland butterflies significantly more abundant than those in forests (Table 1a). Bars and boxes are
558 medians and quartiles, with ranges between minimum and maximum values. An outlier (values less than
559 the first quartile minus 1.5 times the interquartile range, IQR, or greater than the third quartile plus 1.5
560 times the IQR) is shown by a dot.
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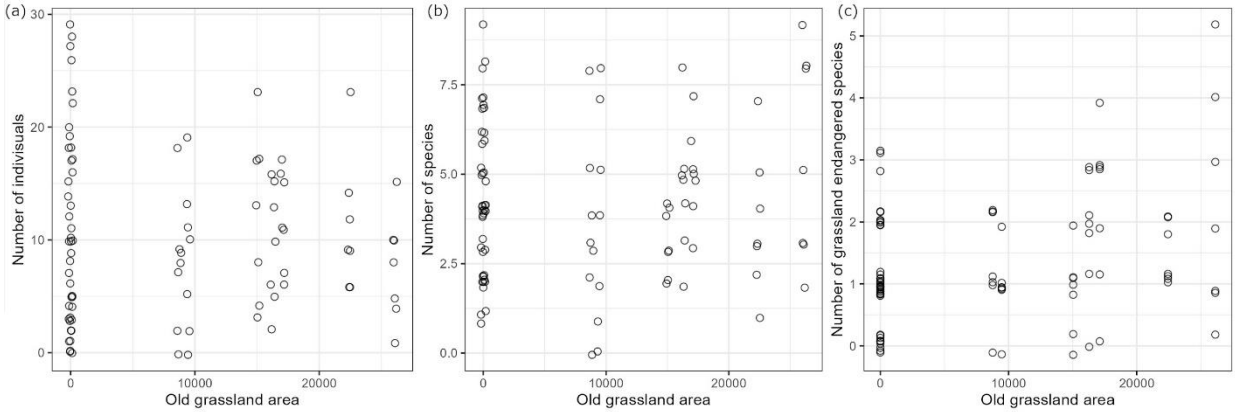

Fig. 6 The old grassland area within a 100 m radius around the study sites was used as an explanatory variable, and the number of (a)individuals, (b)species, and (c)rare grassland species was used as an objective variable. Larger old grassland area around the study sites indicates a greater number of rare grassland species of butterflies.

Supplementary Files

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- SupplementaryTables.xlsx