Reintroduction of mowing on a steep slope increases species diversity of a grassland after abandonment

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Abstract

Semi-natural grasslands on steep slopes often show high plant species diversity, probably due to the water and nutrient limitations that prevent the growth of dominant species and decrease interspecific competition. Traditional management practices like mowing help to maintain species diversity, whereas land abandonment reduces diversity by increasing competition from dominant species and reducing seedling recruitment. The reintroduction of management can reverse species diversity declines, but suitable grassland restoration programs are scarce in Japan. To study the effect of short-term abandonment on seedling ecology, we monitored the vegetation of a Susogari grassland that had been abandoned for 3 years; the grassland occupies a steep slope (ca. 50°) on a hillside above paddy fields. We monitored the vegetation before abandonment, in the 3rd year of abandonment, and in the 1st and 2nd years after restoration of mowing management. Emergence and survival of seedlings was monitored for 18 months after reintroduction of management. We monitored 1183 seedlings of grassland species and non-target annuals in ten 1-m^2 plots. After mowing was reintroduced, most grassland species reappeared or increased in the first and second years. Few seedlings of perennial plants and no seedlings of annuals flowered. An exotic species, *Solidago altissima*, had a lower survival rate (10%) than grassland species (>30%), and all but two grassland species survived over the 18-month period. Our findings suggest that a steep slope acts as a strong filter that inhibits the establishment of non-target species while enhancing persistence of target grassland species.

1. Introduction

Semi-natural grasslands provide key areas for maintaining biodiversity in agricultural landscapes (Duelli and Obrist 2003; Smart et al. 2005). In these fragmented landscapes, remnant habitats such as field margins, ditches, stone walls, midfield islets, hedgerows, and ponds also harbor many grassland species (Cousins and Eriksson 2001; Marshall and Moonen 2002). Fragmented habitats usually have high rates of input of propagules of habitat generalist species from the surrounding area, which increases the probability of invasion by non-specialists, resulting in unique floristic compositions (Fischer and Stöcklin 1997; Boutin and Jobin 1998). The decline of semi-natural grassland areas and the species dependent on them increases the importance of surrogate habitats that can substitute, at least in part, for semi-natural grasslands (Duelli and Obrist 2003).

Remnant habitats such as midfield islets (Cousins and Eriksson 2001) and linear grasslands on steep slopes (Kitazawa and Ohsawa 2002) are associated with land that is not good for agriculture owing to poor abiotic conditions (i.e., steep ground or shallow soils). These remnant habitats can support high species richness. For instance, slope steepness often enhances the biodiversity of grassland species, which highlights the disproportionate conservation value of these sites and their importance as biodiversity hotspots (Luoto 2000; Pykälä et al. 2005; Bennie et al. 2006). Steep grasslands are also more resistant to invasion by competitive species than more level ones owing to nutrient limitation, water runoff, and soil disturbances (Pykälä et al. 2005; Bennie et al. 2006). These unusual topsoil conditions result in more stressed habitat conditions than in the surrounding areas (Loydi et al. 2013) and promote

seedling survival by providing a greater number of favorable microsites (Fowler 1988; Isselstein et al. 2002).

Abandonment of traditional agricultural practices has been a major threat to maintaining such remnant habitats in most marginal regions of Europe (Poschlod et al. 2005; Stoate et al. 2009) and in East Asia (Nakamura and Short 2001; Chen et al. 2021). Although successional patterns vary according to climate and landscape conditions (Prévosto et al. 2011; Pérez-Hernández and Gavilán 2021), most studies noted a decrease of specialist species in grasslands after abandonment (e.g., Rosenthal 2003; Török et al. 2008). This decline of specialists has motivated attempts to improve the diversity of typical grassland species in abandoned semi-natural grasslands (Prach 2003; Ruprecht 2006).

The reintroduction of former management regimes is a widely used restoration measure (Prach 2003; Ruprecht 2006). Both the availability and the persistence of target species are key aspects of grassland restoration success (Hutchings and Booth 1996a,b). First, the successful reconstitution of the vegetation community depends on seed availability in the soil seed bank and seed rain (Zobel 1997). If most floristic components have persisted in the vegetation or soil seed banks, then spontaneous restoration of the former vegetation is likely (Prach 2003). However, if viable seeds no longer exist, due to its being too long since abandonment, spontaneous restoration would be difficult (Mitchell et al. 1998; Rosef 2008). Seed banks usually contain the seeds of annuals and short-lived species, but characteristic grassland specialists are generally absent. Indeed, most grassland species have only transient or short-lived (<5 years) soil seed banks (Thompson et al. 1997). Thus, grassland specialists need to be actively introduced in most cases (Kiss et al. 2016).

In terms of the availability of seedlings for recruitment, the seedling stage is probably the most vulnerable part of the plant life cycle (Stebbins 1971; Fenner and Thompson 2005). Seedlings require specific conditions for successful establishment and subsequent growth (Grubb 1977), and they are more sensitive to environmental conditions and competition than mature plants (Turnbull et al. 2000). As a result, seedling mortality is exceptionally high, sometimes even reaching 100% (Silvertown and Dickie 1980; Heinken-Šmídová and Münzbergová 2012). In addition, the germination success of most species is reduced by competition with established plants (Fenner 1978). Thus, it is necessary to monitor individual seedlings for at least a year to determine their fate and whether plant communities are successfully recreated (Fenner 1978; Stampfli and Zeiter 1999).

In Japan, studies have shown that small grasslands on steep slopes (ca. 50°) are key elements for conserving regional floristic diversity in agricultural landscapes (Kitazawa and Ohsawa 2002; Okubo et al. 2005). Despite the fact that many such grasslands are threatened by land abandonment, relatively few restoration projects have been undertaken. Yamada (2008) reported that if a grassland had been unmanaged for more than 10 years, most grassland species did not become re-established, even if former management (mowing) was reintroduced. If the duration of abandonment is shorter, however, it is possible that the viable seed bank would be greater. If so, management reintroduction could lead to a higher probability of the restoration success (Metsoja et al. 2012). However, seeds and soils tend to run

off quickly on steep slopes, making it more difficult to restore the former grassland community. Indeed, Koyanagi et al. (2008) demonstrated that a steep grassland had only a small number of seeds of grassland species. Worse still, stressful conditions due to the steepness would inhibit the establishment of seedlings (Loydi et al. 2013). Thus, it remains unclear whether grasslands on steep slopes can be restored successfully even after a short period (<10 years) of abandonment.

This study was designed to evaluate the restoration of grassland vegetation on a steep slope restored after 3 years of abandonment. The restoration method was reintroduction of mechanical mowing. We monitored vegetation in three stages: pre-abandonment, during abandonment, and during restoration. We also monitored the fate of seedlings for 18 months after the reintroduction of mowing. We hypothesized that most propagules of the former plant community were still viable, and expected that spontaneous recovery of the former vegetation was likely. If the hypothesis is supported, seed runoff would not be too severe and would allow the restoration of the former vegetation community. We could then calculate the rates of seedling establishment and subsequent persistence, which would enable us to assess the influence of slope on the restoration outcome. By contrast, if the hypothesis is not supported, this would confirm that seedling recruitment, subsequent persistence, or both are bottlenecks for the restoration of the former vegetation community.

2. Materials And Methods

2.1 Study area

The study area is located in the Tama Hills, 30 km west of Tokyo (35°35'N, 139°25'E; Fig. 1). The mean annual precipitation at the nearby Hachioji meteorological station is 1540 mm. The mean annual temperature is 14.3°C, with a mean minimum of 3.2°C (January) and mean maximum of 26.1°C (August) (Japan Meteorological Agency 2021). Monthly precipitation in 2019 and 2020 is illustrated in Appendix 1; precipitation in January and February 2019 and August 2020 was less than the monthly average.

In the Tama Hills, hill ridges are about 30 m higher than the adjacent valley bottom. The valley bottoms are used as paddy fields, and ridges and hillsides are used for coppiced woodlands, a land use pattern that is a typical feature of Japanese agricultural landscapes. The valley bottoms have good water availability for rice culture, but the shade from adjacent ridges and their standing trees in the narrow valleys is disadvantageous. To improve light availability, standing vegetation has been periodically mown on the lower slopes (so-called Susogari grassland). In the study area, the lowermost slopes have traditionally been mown two or three times a year (Okubo et al. 2005; Yamada et al. 2005).

The study grassland is on a southeast-facing slope (S 45° E) at a mean angle of 52.6° ± 7.2° (SD), and the slope was mown in a band about 7 m wide. The woodland upslope of the grassland is dominated by the deciduous broad-leaved species *Quercus serrata*, *Quercus acutissima*, and *Castanea crenata*, formerly coppiced but left unmanaged for about 50 years. Rice culture in the valley bottom adjacent to the study plots was abandoned in 2016, when mowing also stopped.

2.2 Reintroduction of management

The study area lies inside the 33-ha Zushi-Onoji Historical and Environmental Conservation Area, which was proclaimed a Greenery Designated Conservation Area by the Tokyo Metropolis in 1978. The government entrusted the management to the residents, who established the privately run Machida Historic Environment Conservation Management Organization. Since 1997, the organization has managed the abandoned land as was previously done, aiming to restore the former agricultural landscapes in the area. The organization clear-cut 0.4 ha of the woodland in November 2017, when the slope below was covered with tall herbaceous vegetation. In October 2018, the slope was mown after having a gap of 3 years. Since then, the site was periodically mown (i.e., on 7 June 2019, 7 August 2019, 2 June 2020, and 5 August 2020) by the organization or some authors (Yamada S, Yoshida W and Iida M).

2.3 Monitoring

We monitored vegetation in 2008, a period under continuous management; in 2018, 3 years after the management was terminated; and in 2019 and 2020, the first and second years of management reintroduction. This grassland is organized into relatively small, discrete linear elements because of the abrupt slope angle; hence, we established ten 1-m × 1-m plots (quadrats) along a transect at intervals of 1 m. In each plot, we visually estimated the percentage cover of each species on 28 June 2008, 26 June 2018, 28 June 2019, and 2 June 2020. Small individuals with primary leaves were rarely able to be identified in 2008, so we did not survey such individuals in 2018, 2019, and 2020. In 2019 and 2020, several seedlings recorded in the seedling emergence survey (see below) were not recorded in the vegetation survey. In 2019, many seedlings and sprouts (vegetative regrowth) began to grow, both of which are assumed to affect subsequent vegetation development, so in June 2019, each perennial and woody species was recorded along with its growth stage (seedling or sprout). In addition, vegetation cover was visually estimated on 10 May 2019, 5 August 2019, 4 May 2020, and 7 August 2020 to reveal the relationship between vegetation cover and seedling survival.

Seedling emergence and survival was monitored once a month from May to October in 2019 and from April to October in 2020. To distinguish the contribution of germination and subsequent survival to the overall rate of establishment, allowing seedlings to become a permanent part of the vegetation, we marked every germinated individual with a numbered plastic tag. To assess seedling recruitment, we identified recruited seedlings as those that still had their primary leaves and were not recorded in a previous census. Individual seedlings were then monitored in subsequent censuses. We were unable to find about a third of the tags owing to severe soil disturbance at the site. Because we were unsure of whether the plants were still alive nearby without tags or had died, we defined them as disappeared and eliminated them from the calculation of survival rates. Flowering of individual plants, if any, was recorded.

Surface soil was sampled to a depth of 5 cm with a 4-cm-diameter core sampler on 25 December 2020. Sampling was conducted at four points where the vegetation survey was not done (see Fig. 1); the

center and four points equidistant from the center and each edge of the 1-m × 1-m unsurveyed points were sampled. The nitrate-N content in the surface soil was 0.5 mg/100 g, available P content was < 1.0 mg/100 g, and soil pH was 6.1, suggesting nutrient-poor conditions.

In early October 2020, light conditions were measured in the center of quadrats; a camera with a 180° hemispherical lens (Nikkor 8-mm f/2.8 fisheye lens) was positioned facing upward at 50 cm above the ground. Relative solar radiation was calculated in Gap Light Analyzer v. 2.0 software (Frazer et al. 1999), and the relative solar radiation was 66.6% ± 8.8%.

2.4 Analysis

Following Chibaken-shiryou-kenkyu-zaidan (2003), all species were classified according to their life histories: woody species; annual or biennial species (hereafter “annuals”); and perennial herb species, distinguished between small-statured plants (<30 cm) and larger perennials. Plant height classification was applied only to perennials, according to the reference above. Characteristic species representing grassland vegetation in Miscanthetea sinensis communities were defined as “typical grassland species” (Miyawaki 1994). Nomenclature is based on BG Plants (http://ylist.info).

About half the seedlings in the genus *Cirsium* were identifiedas either *C. oligophyllum* or *C. japonicum*, whereas other *Cirsium* seedlings died before identification. According to the flora list in the conservation area, unidentified *Cirsium* seedlings were likely to be either *C. oligophyllum* or *C. japonicum*; thus, the three categories were combined, and survival rates are given for *Cirsium* spp. The two *Cirsium* species represent semi-natural grasslands (characteristic species in Miscanthetea sinensis communities; Miyawaki 1994). We consider the establishment of *Cirsium* spp. to be informative for understanding the fate of typical grassland species. *Carex* spp. were assumed to be one of four taxa: *C. lenta* var. *lenta*, *C. lanceolata*, *C. leucochlora* var. *candolleana*, and *C. tristachya*. No seedlings could be identified to the species level, so all seedlings were combined and reported at the genus level.

Established vegetation communities in 2008, 2018, 2019, and 2020 were tested by principal component analysis (PCA) to confirm any differences in species composition among years. Presence/absence data were used for the PCA, which was conducted in PC-ORD for Windows v. 6 software (McCune and Mefford 1999).

We performed Student’s *t*-test and Holm’s *post hoc* tests to determine the significance of differences in the number of species. A *P*-value of <0.05 was considered significant. Statistical analyses were performed in R v. 2.13.1 software (R Core Team 2015).

3. Results

3.1 Established vegetation

The vegetation was characterized by different dominant species in different years. In 2008, *Houttuynia cordata* was dominant (Table 1). In 2018, *Miscanthus sinensis* was dominant at high frequency, followed

by *Pleioblastus chino*. No single species was dominant in 2019, and *M. sinensis* was again dominant in 2020. The numbers of species in total and representing each attribute are shown in Table 2. The total number of species was highest in 2008 and lowest in 2018 at a sampling scale of 10 m^2, but was similar in 2008 and 2020 at a scale of 1 m^2 (P = 0.145). The number of typical grassland species remained fairly constant over time at a scale of 10 m^2, but it declined significantly between 2008 and 2018 at a scale of 1 m^2 (P = 0.002) and then recovered (P = 0.280 for 2008 vs. 2019; P = 1.000 for 2008 vs. 2020). At both sampling scales, the number of small-statured species was significantly greater in 2008 than in 2019 and 2020 (1 m^2: P < 0.001 for 2008 vs. 2019; P = 0.032 for 2008 vs. 2020). Ferns showed a similar trend.

3.2 Difference in species composition between years

The results of the PCA illustrate the differences in species composition among years (Fig. 2). Plots sampled in 2008 cluster at the right of Axis 1, whereas plots sampled in 2018 are clustered at the lower left. Plots sampled in 2019 are generally higher on Axis 2 than those sampled in 2018. All but one plot sampled in 2020 was located to the left or upper left of those sampled in 2019. Three plots sampled in 2020 (i.e., P08, P09, and P10) were located closer to those in 2008 than to plots sampled in 2018 and 2019.

3.3 Seedling establishment

We observed 1183 seedlings in total over the 2-year survey. Among them, 63 were not identified and 58 were identified as either *Cirsium* spp. or *Carex* spp. Fifty-four species were recorded as seedlings, among which 17 were not recorded in the established vegetation in 2019 or 2020. All but 2 typical grassland species (*Imperata cylindrica* and *P. chino*) observed in established vegetation were recorded as seedlings (Appendix 2). In 2019, 615 seedlings in 46 species were recorded; the most abundant was *Thalictrum minus* var. *hypoleucum* (166), followed by *Veronica persica* (53) and *Youngia japonica* (36). In 2019, more perennial seedlings were recorded than annual seedlings. In 2020, 568 seedlings were observed; *Y. japonica* was the most abundant (271), followed by *V. persica* (67) and *T. minus* var. *hypoleucum* (46), and annual seedlings were more prevalent than perennial species.

The 1-year survival rate was >50% in *T. minus* var. *hypoleucum*, *Cirsium* ssp., and *Salvia japonica* and <20% in *Lysimachia clethroides* (Table 3). The two-season survival rate was markedly lower than the 1-year survival rate in *Solidago altissima*. We found a relatively small number of seedlings of 3 typical grassland species (*Gentiana scabra* var. *buergeri*, *Sanguisorba officinalis* and *Solidago virgaurea* subsp. *asiatica*; Table 3b and Appendix 2). Two seedlings of *G. scabra* var. *buergeri* and one seedling of *S. officinalis* and were recorded, and they died within a year. The timings of germination and mortality are shown in Figure 3. The germination rate of perennials was particularly high up to August 2019, whereas that of annuals was high in September 2019 and from July 2020 onward. The mortality rate was large in August 2019 and in April, September, and October 2020.

Eight individuals from six species flowered (Table 4).

4. Discussion

4.1 Restoration outcome

Three years of abandonment led to a distinct decline in the total number of species in established vegetation (Table 2). Numbers of annual species, small-statured species, and ferns also declined markedly. Declines in small species and annuals are common in the process of secondary succession (Rosenthal 2003; Pykälä 2004). The number of species of typical grassland plants was significantly smaller in 2019 than in 2018 at a sampling scale of 1 m^2, but was similar between years at a scale of 10 m^2. Thus, 3 years of abandonment led to a decline in the number of typical grassland individuals but did not lead to loss of most species at the community level. It usually takes more than a decade for most grassland species to disappear in the process of secondary succession (Kahmen et al. 2002; Rosenthal 2003; Török et al. 2008).

More importantly, reintroduction of mowing contributed to rapid increases in the number of typical grassland species at the 1-m^2 scale (Table 2). Accordingly, the frequencies of several typical grassland species were distinctly larger in 2019 or 2020 than in 2018 (Appendix 2). One reason for the fast recovery is obviously the persistence of soil seed banks. Soil seed banks of grassland species tend to survive for up to 5 years (Thompson et al. 1997), so 3-year-abandonment would be too short for the loss of viable seeds of most typical species. Seedling density was lower here than in grasslands in Europe (Stampfli and Zeiter 1999; Kladivová and Münzbergová 2016). However, it was larger than in a Japanese grassland, suggesting that density of the viable seeds is small in grassland on a steep slope (Koyanagi et al. 2008). One possible reason for the higher density of viable seeds here than in Japan would be the persistence of several tall grassland species in established vegetation in the abandoned phase. Yamada and Minami (2015) demonstrated that mowing twice a year significantly decreased seed production of *T. minus* var. *hypoleucum* relative to mowing once a year, and tall grassland species benefit from low-intensity mowing (e.g., biannual mowing, Bricca et al. 2020). Thus, as a restoration management practice, abandonment for a few years would be more advantageous for them to produce seeds than mowing twice a year, and would in turn increase the density of viable seeds. Interestingly, seedlings of the low-statured *Polygala japonica* and *Potentilla freyniana* had distinctly higher frequencies in 2020 than in 2018 (Appendix 2). Since their frequencies were limited during the abandoned phase (Appendix 2), little seed rain would be expected during that period, indicating that seeds persisted in the soil without running off for 3 years at the site.

Although the total numbers of species increased from 2019 to 2020 at a sampling scale of 10 m^2, the number in 2020 was still smaller than that in 2008 (Table 2). Numbers of small species and ferns in 2020 were also smaller than those in 2008, when most of these plants were observed at low frequencies (i.e., 0.1 or 0.2). An originally sparse population would lead to low probabilities of establishment of seedlings at the restored site. Abandonment of extensive land use practices causes a decline of short species more than of tall species (Rosenthal 2003; Pykälä 2004). Although several small grassland species did not decrease (i.e., *P. japonica* and *P. freyniana*), some other small species would be vulnerable to

abandonment. As the restored site has bright conditions, it may never be wet enough for several fern species, as suggested by Ghorbani et al. (2003). In addition, surveying ferns can be difficult, because it takes longer for tiny thalli and sori of several species to grow enough to identify via the anterior lobe phase. Thus, it remains unclear whether quick recovery is likely or not for ferns.

4.2 Seed recruitment and seedling survival

As expected, the 1-year seedling survival rate was greater than that in more level grasslands (Silvertown and Dickie 1980; Ryser 1993; Stampfli and Zeiter 1999). They were particularly high (>50%) in *T. minus* var. *hypoleucum* and *Cirsium* spp. (Table 3). Previous studies showed that the unusual topsoil conditions of steep slopes (nutrient limitation, water runoff, and soil disturbances) would limit the performance of dominant species (Pykälä et al. 2005; Bennie et al. 2006) and promote seedling survival (Fowler 1988; Isselstein et al. 2002).

The survival rates of several species were very low: the 1-y survival rate of *Lysimachia clethroides* was <20%, and the two-season survival rates of *S. altissima* and *M. sinensis* were <20% (Table 3). An important factor determining the behavior of seedlings is seed size (Gross 1984; Villar-Salvador et al. 2012). The seeds of *L. clethroides* and *S. altissima* are small. Species with the largest seeds have the lowest mortality, especially in gaps, as large seeds enable quick initial growth, which is an important characteristic for establishment. Another explanation determining the behavior of seedlings would be the difference in initial allocation of biomass between above- and belowground parts. Fast development of the root system makes the seedling less vulnerable to topsoil desiccation (Ryser 1993). A small root system was probably one of the causes of the high mortality. *Solidago altissima* and *M. sinensis* produce more biomass aboveground than that in its root system, while *T. minus* var. *hypoleucum* produces more biomass for their root systems than that aboveground (Yamada et al., unpublished data).

Monthly precipitation was unusually low in August 2020 (Appendix 1). Correspondingly, seedling mortality was greater on 9 September 2020 than on 5 August 2020 (Fig. 3). During summer, when the open gaps often dry up faster than the surrounding vegetation, seedlings may die owing to drought (Martinkova et al. 2009; Knappová et al. 2013). Frost heave and drought in winter would also increase the mortality of the winter annuals *Veronica persica* and *Cerastium glomeratum* (Ryser 1993; Stampfli and Zeiter 1999).

No annual plants flowered (Table 4). Most annuals observed here were nitrophilous species (i.e., favored by high nutrient content; Török et al. 2008) common in disturbed habitats. This grassland is characterized by a small number of annuals (Table 2; Yamada et al., 2005), and reproductive failure would have contributed to this low number. Limited nutrient availability of the steep grassland likely led to the reproductive failure of the perennials, as it takes more than 1 year for young plants to reach the size enabling survival and reproduction in a harsh environment (Špačková and Lepš, 2004).

4.3 Improving restoration of grasslands on steep slopes

Most typical grassland species did not reach the reproductive stage by the second year of management reintroduction, and it remains unclear whether seedlings of such species will successfully reproduce in the long run. If individuals are growing, however, their tolerance of harsh environmental factors (i.e., drought and frost) can become stronger over time. Nevertheless, vegetation cover was greater in 2020 than in 2019 (Appendix 3). The increased number of seedlings and steady growth of vegetative propagules increased the cover in the second year. Although interspecific competition is less likely owing to the site's harsh conditions, the frequency and timing of mowing should be determined carefully to prevent overgrowth by dominant species.

Many sprouts (vegetative regrowth) were observed in 2019, the first year of management reintroduction. For instance, more plots had sprouts of *P. freyniana*, *Barnardia japonica*, and *Sanguisorba officinalis* than in 2018 (Appendix 2). Although vegetative propagules have potentially shorter viability in soil than seeds, they can play an important role in restoration outcomes (Touzard et al. 2002; Matus et al. 2003). For instance, they can contribute to rapid seed production in restored sites. Further studies are needed of the role of propagules in successful restoration at recently abandoned sites.

The numbers of small species and ferns remained small until 2020, but these species were observed in other nearby habitats (Kitazawa and Ohsawa 2002). If suitable management can help safe sites to persist, migration of such species would be likely in the long run.

Microsite conditions vary over time at the study site. Locations in the PCA scatter plot were similar for three plots (P08, P09, and P10) in 2020 and 2008 (Fig. 2), indicating that the vegetation composition was reverting to the original state in several plots. But this was not the case in other plots. Grassland vegetation on steep slopes differs according to light conditions (Yamada et al. 2005), which changed with the clear-cutting of the upslope adjacent woodland in 2017. Although we did not measure relative light intensity in 2008, the absence of some species preferring bright conditions (i.e., *Cymbopogon tortilis* var. *goeringii* and *Thesium chinense*; Yamada et al. 2005) imply that the site was less bright in 2008 than in 2020 in P01–07. In 2020, P01–07 were significantly brighter than P08–10 (*t*-test, $P = 0.004$, data not shown), and this change in light conditions at the site would alter the vegetation composition from that in 2008 in P01–07. Woodland clear-cutting is a traditional agricultural management practice, and land managers should understand that vegetation in the grassland will not be stable but instead will change over time.

5. Conclusion

This study revealed the successful restoration of a grassland community on a steep slope (Susogari grassland). Three years of abandonment did not severely affect restoration, at least of most grassland species. Seed banks of most species did not diminish despite the high risk of soil runoff on the steep slope. Although several annuals and the invasive exotic perennial *S. altissima* appeared as seedlings, only a few were successful in producing seeds. By contrast, typical grassland species had relatively high survival rates in general. Thus, poor soil water and nutrient availability worked as strong filters to inhibit

the establishment of annuals and exotic species, contributing to the formation of the characteristic species composition in the grassland. Severe soil disturbance should affect mortality of the seedlings—indeed, we lost track of about a third of the seedlings and their tags in our survey—but species occurring in the original grassland vegetation benefitted from the steepness of the slope overall.

Declarations

Ethical Approval

Not applicable.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Susumu YAMADA, Wakana YOSHIDA, Minori IIDA and Yoshiko KITAGAQA. The first draft of the manuscript was written by Susumu YAMADA. Writing - review and editing was performed by Jonathan MITCHLEY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Datasets are included in submitted “related files”.

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Tables

Table 1 The major dominant species in each year

Year	Species	Frequency
2008	*Houttuynia cordata*	0.4
2018	*Miscanthus sinensis*	0.7
	Osmunda japonica	0.2
2019	–	–
2020	*Miscanthus sinensis*	0.3
	Pleioblastus chino	0.2

Dominant species were defined as those observed at least twice in 10 plots with cover of ≥20%. Frequency is the number of plots in which each species was dominant out of 10 plots.

Table 2 Changes in species richness according to species attributes

a) Species richness at a sampling scale of 10 m^2

	2008	2018	2019	2020
Total	69	36	47	59
Life history				
Annuals	6	0	2	5
Perennials	54	32	38	49
Woody species	9	4	7	5
Exotic species	2	0	1	2
Ferns	10	5	5	6
Typical grassland species	13	11	12	14
Small-stature perennials	9	1	1	5

b) Species richness at a sampling scale of 1 m^2

	2008	2018	2019	2020
Total	26.0 ± 5.1 a	10.6 ± 2.8 b	18.3 ± 3.0 c	23.4 ± 4.3 a
Life history				
Annuals	1.1 ± 1.2 a	0.0 ± 0.0 b	0.3 ± 0.7 ab	1.0 ± 1.1 ab
Perennials	21.7 ± 3.3 a	9.9 ± 3.0 b	14.9 ± 2.9 c	19.8 ± 3.8 a
Woody species	2.8 ± 1.8 b	0.7 ± 0.5 a	2.1 ± 1.0 b	2.4 ± 1.1 b
Exotic species	0.3 ± 0.5 ab	0.0 ± 0.0 b	0.2 ± 0.4 ab	0.6 ± 0.5 a
Ferns	4.0 ± 1.2 a	1.5 ± 0.5 b	2.0 ± 1.2 b	2.6 ± 1.0 b
Typical grassland species	6.9 ± 1.2 a	3.6 ± 1.7 b	5.5 ± 2.0 ab	6.9 ± 2.2 a
Small-stature perennials	3.1 ± 1.6 a	0.1 ± 0.3 c	0.4 ± 0.5 c	1.8 ± 1.5 b

Mean values ± SD are shown. Different letters indicate statistical differences (Student's *t*-test and Holm's post hoc tests; $P < 0.05$).

Table 3 Survival rates of nine major species and two genera

a)

Taxa	One-year survival rate	Two-season survival rate	One-season survival rate	
		By October 2020	By October 2019	By October 2020
Typical grassland species				
Thalictrum minus var. *hypoleucum*	0.571 (84/147)	0.375 (54/144)	0.634 (85/134)	0.367 (11/30)
Cirsium spp.	0.714 (20/28)	0.370 (10/27)	0.778 (21/27)	0.889 (16/18)
Euphorbia lasiocaula	0.571 (8/14)	0.308 (4/13)	0.571 (8/14)	0.375 (3/8)
Polygala japonica	1.000 (9/9)	0.667 (4/6)	1.000 (9/9)	-
Potentilla freyniana	0.667 (4/6)	0.500 (2/4)	0.800 (4/5)	0.857 (6/7)
Miscanthus sinensis	0.667 (6/9)	0.111 (1/9)	0.667 (6/9)	-
Other species				
Solidago altissima	0.531 (17/32)	0.172 (5/29)	0.806 (25/31)	0.429 (9/21)
Lysimachia japonica	0.067 (18/30)	0.522 (12/23)	0.782 (18/23)	0.650 (13/20)
Salvia japonica	0.583 (7/12)	0.500 (6/12)	0.700 (7/7)	0.875 (7/8)
Carex spp.	0.400 (4/10)	0.400 (4/10)	0.727 (8/11)	1.000 (1/1)
Ixeridium dentatum subsp. *dentatum*	0.444 (4/9)	0.222 (2/9)	1.000 (3/3)	0.000 (0/2)

One-year survival rate, survival of seedlings for 1 year after initial emergence; two-season survival rate, survival of seedlings at the end of the second year irrespective of the month of germination in the first year; one-season survival rate, survival of seedlings at the end of year of germination irrespective of month of germination

b) Number of species not listed in *a* and found as seedlings

Species	Number of species		Number of individuals	
	Survival	Death	Survival	Death
Typical grassland species	1	2	1	3
Others	6	7	14	23

Table 4 Number of seedlings with inflorescences and total number of seedlings

Species	Number of seedlings with inflorescences	Total number of seedlings
Annuals		
Pseudognaphalium affine	3	3
Youngia japonica	1	307
Picris hieracioides subsp. *japonica*	1	12
Perennials		
Polygala japonica	1	12
Ajuga decumbens	1	8
Carpesium glossophyllum	1	1

Figures

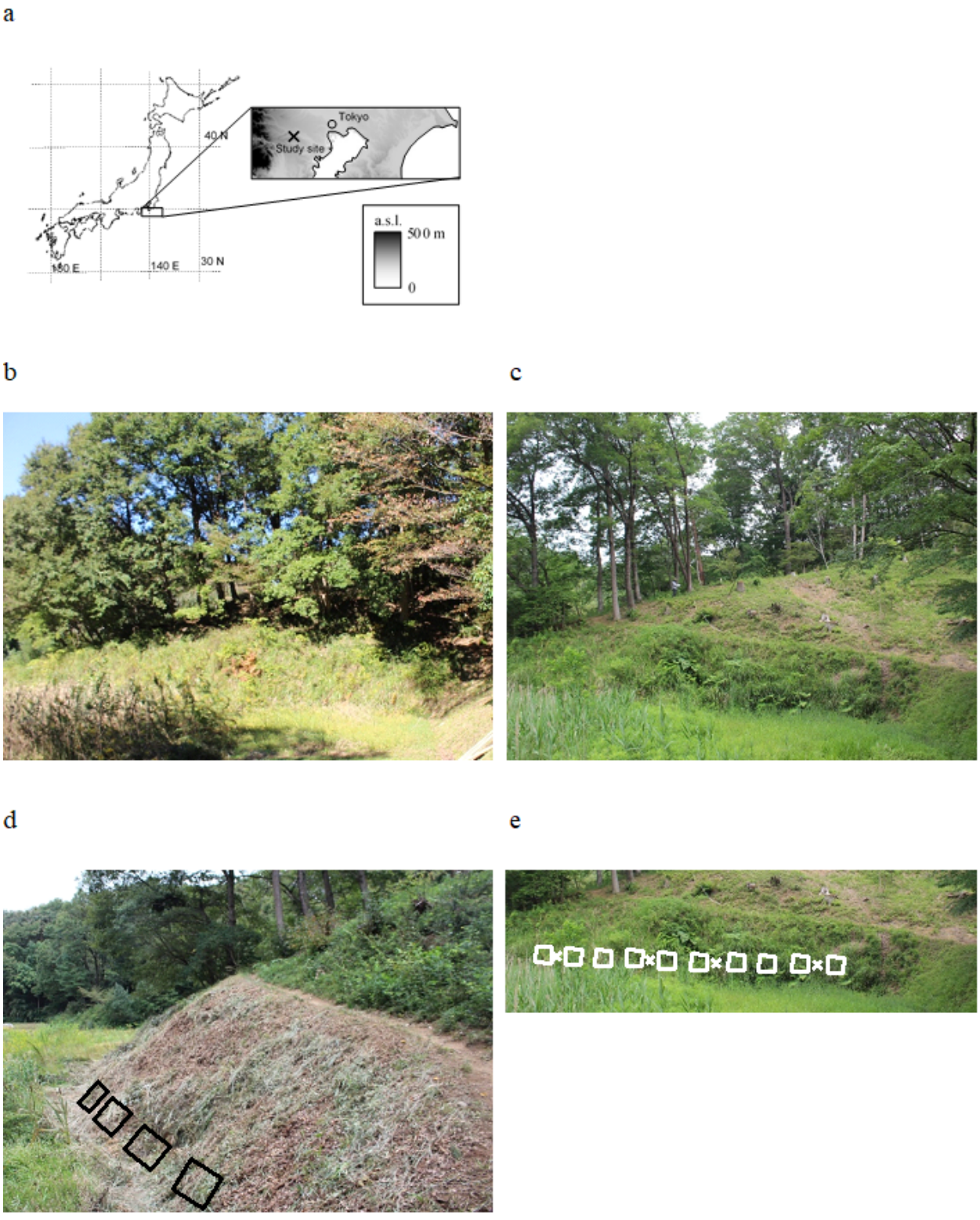


Figure 1

Description of the study area. (a) Location of the study site; (b) photo taken before the clear-cut of the upslope woodland (October 2017); (c) photo taken after the clear-cut of the upslope woodland and before the reintroduction of mowing (May 2018); (d) photo taken after the reintroduction of mowing (October 2018) and layout of □ seveval plots; (e) layout of □ the 10 plots and × soil sampling points (added to the photo taken in May 2018).

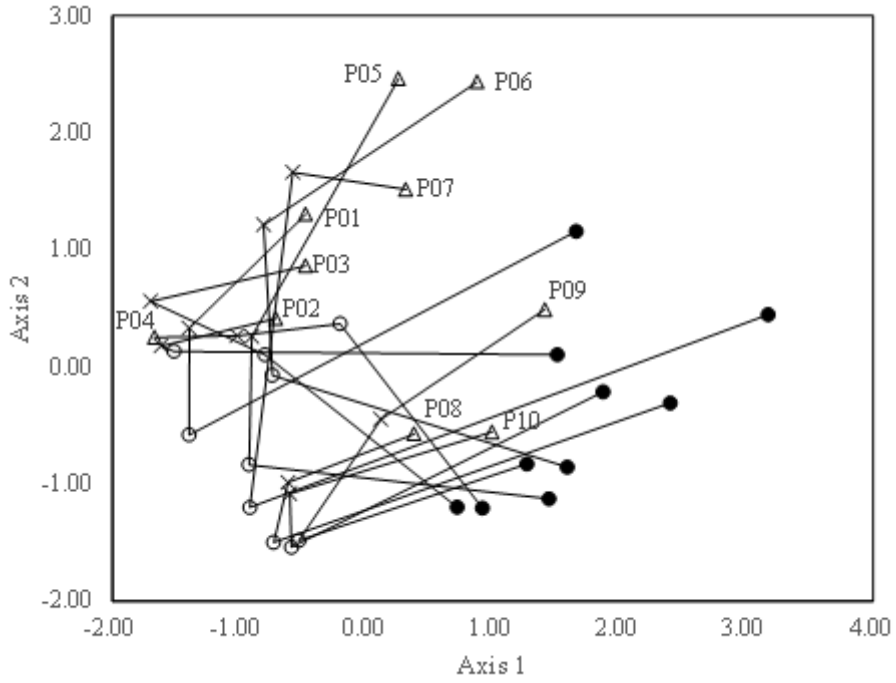
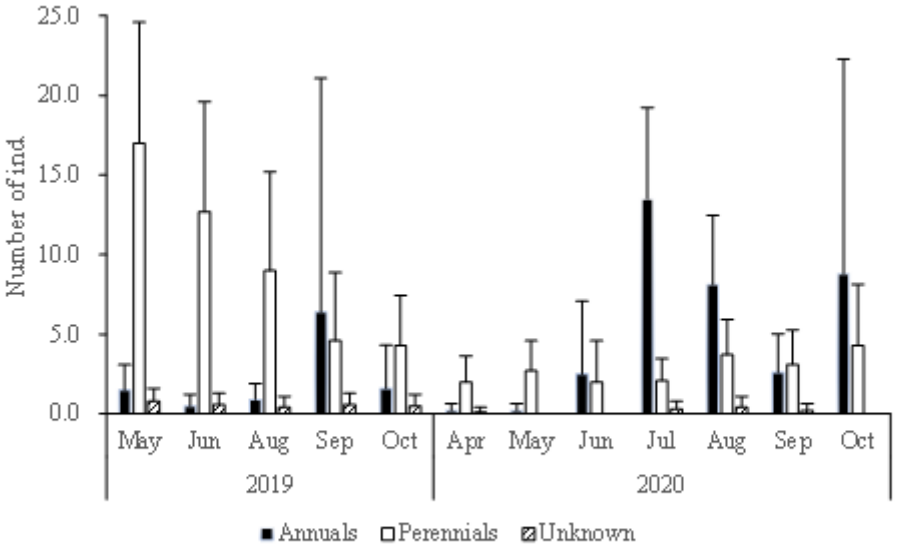
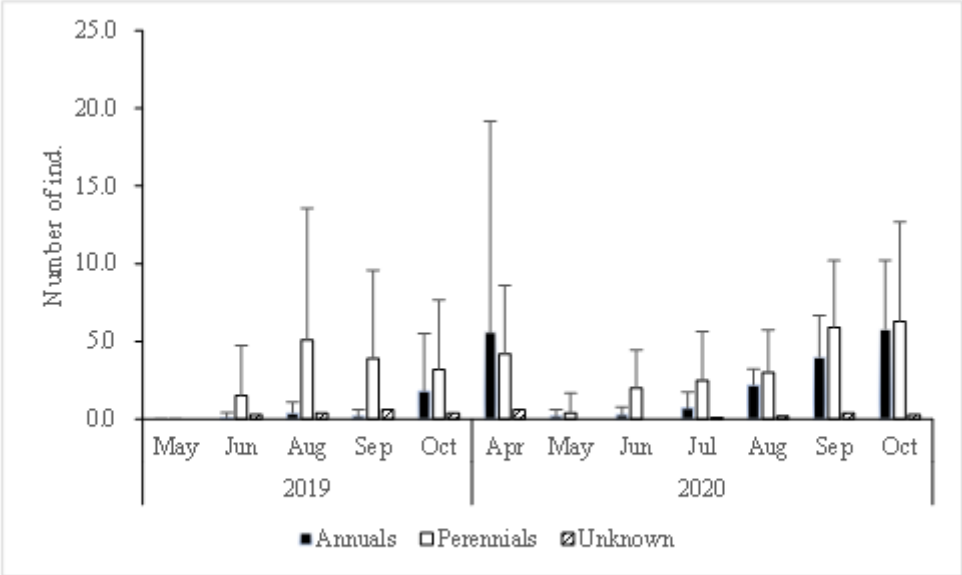


Figure 2

Scatter plots of the first two PCA axes in each survey period. ●, 2008; ●, 2018; ×, 2019; △, 2020. “P01–10”, IDs of plots.



a



b

Figure 3

(a) Emergence and (b) mortality of annuals and perennials in each period

Supplementary Files

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- AnagariAppendix1.docx

- AnagariAppendix2.docx
- AnagariAppendix3.docx