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Author(s)	Holle, Mukhlis Jamal Musa; Tsuyuzaki, Shiro
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The effects of shrub patch sizes on the colonization of pioneer plants on the volcano Mount Koma, northern Japan

Mukhlis Jamal Musa Holle^{1,2} & Shiro Tsuyuzaki¹

¹ Graduate School of Environmental Science, Hokkaido University, Sapporo Japan 060-0810

² Faculty of Biology, Gadjah Mada University, Sleman DI. Yogyakarta Indonesia 55281

Abstract

Vegetation recovery on Mount Koma, Hokkaido, Japan, has been slow after the catastrophic eruption in 1929, due to undeveloped soil and limited plant colonization. Nowadays, the seedling establishment is supported mostly by a nurse plant, *Salix reinii* forming shrub patches, facilitates the plant colonization. Although the effects of shrub patches should differ with patch sizes, the size effects have not been examined well. To examine the size effects, seed-sowing experiments were conducted on two common pioneer herbaceous species, *Miscanthus sinensis* and *Polygonum sachalinense*, in the field. The seed germination and seedling survival were monitored by the seeds sown into *S. reinii* patches (0.97 m² to 4.12 m² in area) for 4 months during snow-free periods. Microenvironments altered by the patches were measured. Lab-experiments were performed to characterize the seed germination and seedling growth.

Larger patches decreased light intensity and temperature more and increased litter and water content. The large patches promoted the seed germination of the two species. Interspecific interactions, examined by a seed mixture experiment, showed that the interaction increased the seed germination on *M. sinensis* and decreased that on *P. sachalinense*. On the lab-experiments at three temperatures (15, 20 and 25°C), *M. sinensis* seeds germinated more at higher temperatures and obtained higher seedlings biomass. *P. sachalinense* germinated the seeds more at 20°C and grew faster at lower temperatures. The total biomass of the two species was reduced by shade that intercepted 50% of light intensity. The seed germination and seedling growth of these two species became higher on litter with 2 cm in depth than on no litter. Soil water supported seed germination when the seeds of these two species were mixed while the water reduced the growth of *P. sachalinense* seedlings. Therefore, the dry soils were suitable for their growths. In all the treatments, *P. sachalinense* seedlings showed higher mortalities than *M. sinensis*.

In conclusion, the large patches facilitated more to the colonization of pioneer plants via seed germination and growth. Large patches acted as a nursery supporting the natural regeneration in the disturbed area by improving litter accumulation, maintaining soil water, reducing strong light and/or protecting from heat.

Keywords : facilitation, *Salix reinii*, shrub patch sizes, volcano, Mt. Koma, microenvironment

Introduction

Volcanic eruption is one of the major disturbances on ecosystems and often leads primary succession. In the early stages of succession after catastrophic disturbances represented by volcanic eruptions, facilitation (positive interspecific interaction) performed by nurse plants has been highlighted because the mechanisms should be applied for ecosystem conservation and restoration (Ren *et al.*, 2008). Nurse plants facilitate the establishments of colonizers by improving the environments that reduce inter-specific competition to nutrients and water and increase seed germination and seedling survival (Nunez *et al.*, 1999; Barrera & Smith, 2009).

Seedling stage is crucial to determine the fates in a life cycle. For example, two large perennials, *Petasites japonicus* and *Polygonum sachalinense*, show low seedling survivals on coarse volcanic ejecta on Mount Usu, northern Japan, soon after the eruptions (Tsuyuzaki & Haruki, 2008). Therefore, facilitation at seedling stages is a key to determine vegetation development through ameliorating microenvironments (Nunez *et al.*, 1999).

Nurse plants ameliorate both above-and below-ground microenvironments (Gomez-Aparicio *et al.* 2005), i.e., light intensity, temperature and litter accumulation on the above-ground and water and nutrients in the below-ground environments (Franco and Nobel, 1989; Ren *et al.*, 2008). They also have seed trap effects (Badano *et al.*, 2016) supporting high seedling emergence (Pueyo *et al.*, 2016). Shrubs often have facilitative effects by reducing interspecific competition and ameliorating microclimates and/or soils (Gomez-Aparicio, 2009; Butterfield and Briggs, 2011). On Mount Koma, northern Japan, a deciduous shrub, *Salix reinii*, has facilitative effects on colonizers by providing an ameliorated microclimate, increasing soil fertility and developing seedbank (Uesaka, 2004). The effects of shrub patches should be different with the sizes, although a few researches have been conducted. *Miscanthus sinensis* Andersson and *Polygonum sachalinense* Fr. Schmidt et Maxim. are common herbs in northern Japan (Tsuyuzaki & Hase, 2005) and develops their respective grasslands in northern Japan in the middle stages of succession (Tsuyuzaki 2005). These two herbs often established together. Therefore, the effects of patch sizes on the seed germination and seedling growth of cohabitants were examined by these two species. When these two species grow together, these two herb species should be interacted. The interaction was investigated by seed germination, seedling emergence and biomass allocation. In addition, these two herbs may accelerate the succession because they also have facilitative effects (Tsuyuzaki, 2005a; Barney *et al.*, 2006).

Therefore, the effects of patch sizes on seed germination and seedling growth of these two species were investigated. Various environmental factors are altered by shrub patches, i.e., temperature, light, litter and water. To understand what environmental factors affected the regeneration of *M. sinensis* and *P. sachalinense*, the effects of each environmental factor were separately investigated on the seed germination and seedling growth in lab-experiment. The objectives were detecting: 1) the size effects of *S. reinii* patches on the seed germination and seedling growth of *M. sinensis* and *P. sachalinense*; 2) the alteration of environments under different-sized *S. reinii* patches; and 3) the mechanisms of factors altered by the patches on the seed germination and seedling growth of *M. sinensis* and *P. sachalinense*.

59 **Materials and Methods**

60 *Study area*

61 Mount Koma is an active stratovolcano in the southern part of Hokkaido Island, Japan (42°04'N, 140°42'E, 1140 m elevation). The
62 climate is warm-cool temperate with 973 mm of annual precipitation in 2015 (Mori Climatological Observatory, 10 m elevation, 9
63 km from Mount Koma). From July to November in 2015, the mean annual temperature was 14.6°C (Japan Meteorological Agency,
64 2016). The last major eruption occurred in 1929 and the blast and debris avalanches completely destroyed the former vegetation.
65 The vegetation recovery is slow and has not been attained the climax (Tsuyuzaki & Hase, 2005).

66 *Field experiments*

67 All the field experiments were conducted on the southwestern slope between 750 m and 880 m elevations, where *S. reinii* patches
68 were dominant, during early summer to early autumn in 2015 and 2016. Shade by the tree canopy was weak, because of less trees.
69 In total, 20 *S. reinii* patches of which areas were 0.97 to 4.12 m² were selected for the survey. In addition, ten 1 m × 1 m plots were
70 established on the bareground, where the cover of *S. reinii* was less than 10%, as control. In July 2016 when the plants grew well,
71 seven and 23 soil samples were collected from baregrounds and patches ranging from 0.84 m² to 2.50 m², respectively, by a scoop.
72 The samples were collected down to approximately 5 cm after removing litter if accumulated. Each sample was stored in a plastic
73 bag until measured. The fresh weight of soil was measured soon after returning the laboratory. The samples were dried in an oven at
74 60°C for five days and weighted. Water content (%) was calculated as: (fresh weight - dry weight)/(dry weight) × 100. The
75 remainders of soils were used for greenhouse experiments to test if the patches have a seed trap effect. Litter thickness was measured
76 by selecting 5 random points inside of each patch, using a ruler and was calculated as the averaged thickness. Litter cover under the
77 patches was visually estimated. Litter was collected randomly from the patches in July 2016.

78 Temperature and light intensity were measured at one-hour intervals by data loggers (HOBO UA-002-XX, Onset,
79 Massachusetts, USA) underneath four *S. reinii* patches and on two baregrounds from July 22 to October 10 2015. Daily air
80 temperatures and sunshine hours recorded at Mori Climatological Observatory were also used. Relative light intensity (RLI) was
81 calculated by (the average of daily light intensity under patches) / (average of daily light intensity in bareground) × 100%.

82 The seeds of *M. sinensis* were donated by Snow Bland Seed Co. (Sapporo, Japan). The seeds were harvested from a *M.*
83 *sinensis* grassland near Sapporo in early autumn 2014. The seeds of *P. sachalinense* were collected from *P. sachalinense* grown on
84 Mount Koma in October 2014. The seeds were kept in an incubator at 5°C until use. Before sowing seeds for experiment,
85 germinating seeds in the incubator is conducted to check the seed viabilities. The seeds of *M. sinensis* and *P. sachalinense* were
86 sown to each patch and to each bareground in July 2015. Seedling emergence and death were monitored until November in 2005.
87 Cohabitant species were recorded in 19 randomly-established 2.5 m × 2.5 m plots. The number of aboveground shoots on each
88 species was counted in each patch or bareground.

89 *Greenhouse experiments*

90 To investigate the effects of seedling competition on the seed germination and seedling growth, the seeds of *M. sinensis* and *P.*
91 *sachalinense* were sown solely (hereafter, single treatment) or mixed (mixture) on vermiculite as the substrates in pots (5 cm in
92 diameter) on the greenhouse. A total of 20 seeds were sown to each plot. On the single treatment, 20 seeds on a given species were
93 sown to each pot. On the mixture treatment, 10 seeds of each species were sown to each pot. On each treatment, 10 replications
94 were used.

95 The seed-sown experiments were conducted on the seeds of *M. sinensis* and *P. sachalinense* in the greenhouse to detect the
96 effects of light intensity, litter and water supply on seed germination and seedling growth. Shade was developed by pots covered
97 with black sheer nets that reduced ca 50% of light intensity. Litter collected from Mount Koma was spread over vermiculite with 0,
98 1, 2 and 3 cm in depths. The litter consisted of *S. reinii* leaves. Water was sprinkled five times every day approximately 5 ml/pot
99 each time. On water supply experiments, water was sprinkled five times (wet treatment) or three times every day (dry).
100 Approximately 5 ml of water was supplied to each pot at each sprinkle.

101 The effects of temperatures on seed germination were examined at a cycle of 10°C/20°C (12 hr/12 hr), 15°C/25°C and 20°C
102 /30°C in incubators. Hereafter, these three treatments are called 15°C, 20°C and 25°C based on the average. Discontinuous light was
103 given to the seeds (12 hr dark/12 hr light). Seedlings were counted every day for first one week and every week thereafter until no
104 more seed germination occurred.

105 All the seedlings were harvested 70 days after the beginning of experiments except for 80 days under shade. The seedlings
106 were separated into the aboveground and belowground parts, dried at 60°C in an oven for five days and weighed.

107 Soil samples were collected from the field in the patch and in the bareground, by a soil sampler of which volume was 80 cm³.
108 Collected soils were spread on vermiculate in each pot. Water was sprinkled five times every day. After four months, seedlings
109 emerged from the seedbank were counted and identified.

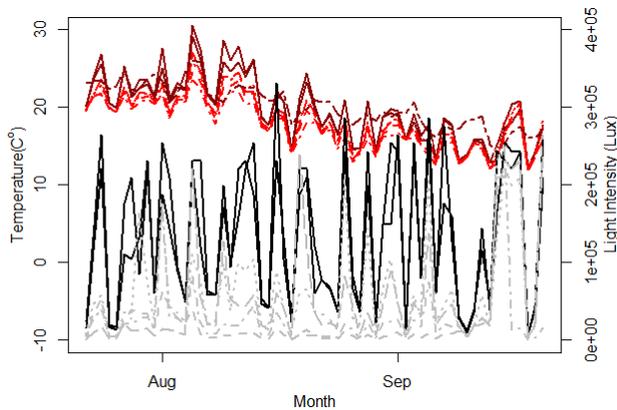
110 *Statistical analysis*

111 To examine the effects of patch size and seed mixture on the seed germination on each species in the field, generalized linear mixed-
112 effects model (GLMM) was used. The response variable was the number of germinated seeds and the explanatory variables were
113 patch size, time (September, October and November) and seed mixture. The random effect was patch and bareground codes. To test
114 the effects of environmental factors on the seedling biomass, generalized linear model (GLM) was used. On each of the two species,
115 seedling biomass was used as responsive variable and treatments (temperature, light intensity, litter thickness and tephra water

116 content) as explanatory variables. Repeated-measures ANOVA was used to compare the seed germination patterns in the lab-
117 experiments. All the statistical analyses were performed using R software (version 0.99.451).

118 Results

119 Alteration of microclimate by *S. reinii* patch



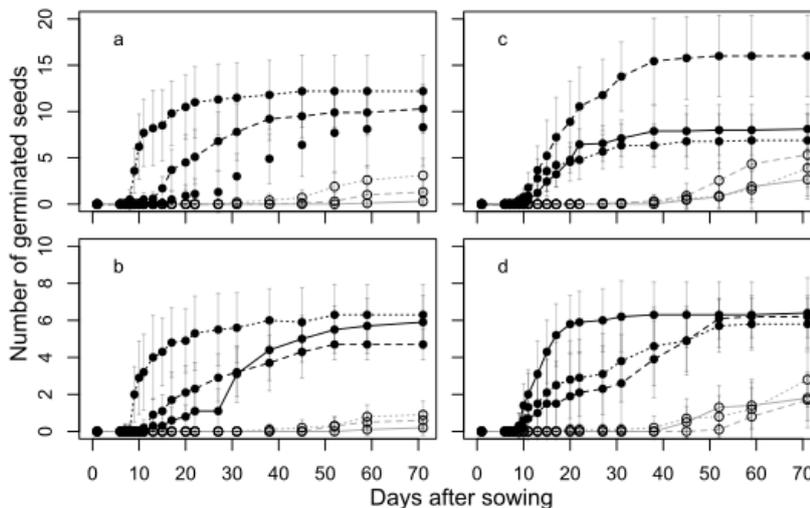
120 Figure 1. Mean daily temperature (reds) and light intensity (grayscale) measured on two baregrounds (darker-solid lines), four *S. reinii* patches (1.21 m² patch =
121 dashed line, 1.37 m² patch = dotted line, 1.99 m² patch = dotdash line, 4.12 m² patch = longdash line) and Mori Town Climate Observatory (twodash-dark
122 red line) from July 2015 to September 2015. The first day (day=1) is July 22 2015.

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124 *S. reinii* started the leaf flushing in late April and defoliated in late September in the two surveyed years. From mid-July to late
125 August, the temperatures were higher on baregrounds than on the patches (Fig. 1). Afterwards, the temperatures differences among
126 sites became narrower, due probably to the defoliation. The large patches reduced temperatures more than the small patches. The
127 highest temperature was recorded at 30.6°C on the bareground on August 5 2015 and the lowest one was at 11.9°C in the 1.2-m²
128 patch on September 18. The mean daily light intensity fluctuated with the sunshine hour (Fig. 1). The light intensity was correlated
129 to the temperatures on the bareground. Relative light intensity (RLI, %) was reduced with increasing patch size, with 19.4% in patch
130 with size 2.0 m² and 20.1% in 1.2 m² shrub. However, the temperatures lowered with increasing patch sizes.

131 The volumes of litter ranged from 2.02 cm³ to 4.9 cm³ in the patches, while litter was not accumulated on the baregrounds.
132 The large patch of which area was 2.5 m² showed the highest litter volume (8.4 cm³) and cover (2 m²). Litter was accumulated more
133 in larger patches, examined by the cover, thickness and amount (GLM, p<0.001). Water content ranged from 8.0% to 23.9% in July
134 2016 and was higher in the patches than in the bareground, 15 % and 11.72% respectively (GLM, p<0.01). Since the measurements
135 were conducted in summer, the high water content in the patches was derived mostly by the shade of *S. reinii* foliage.

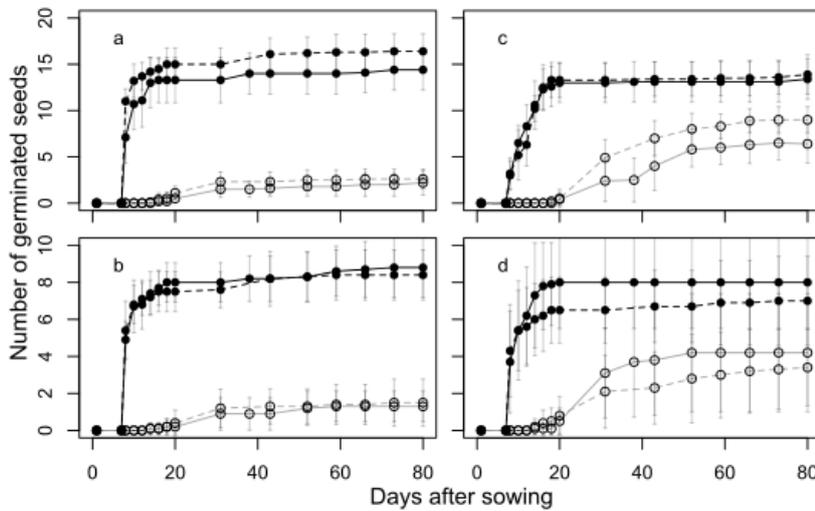
136 Effects of physical environments on seed germination

137 The seed germination started nine days after sowing seeds on *M. sinensis* and 11 days on *P. sachalinense* at 25°C (Fig. 3). The
138 germination delayed at 15°C on *M. sinensis*. The seeds of *M. sinensis*, independent of seed mixture, germinated most at the highest
139 temperature, 25°C (ANOVA, p<0.001). When the seeds were sown solely, *P. sachalinense* seeds germinated highest at 20°C
140 (p<0.001).
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Figure 3. Seed germination patterns at three different temperatures on (a) *M. sinensis* seeds sown solely, (b) *M. sinensis* seeds sown with *P. sachalinense* seeds, (c) *P. sachalinense* sown seeds solely, and (d) *P. sachalinense* seeds sown with *M. sinensis* seeds. Mean numbers of seedlings (symbols) are shown with standard deviations (error bars). Solid, dashed and dotted lines show 15°C, 20°C and 25°C. Black lines with filled circles and grey lines with circles indicate number of germinated seeds and number of dead seedlings, respectively.

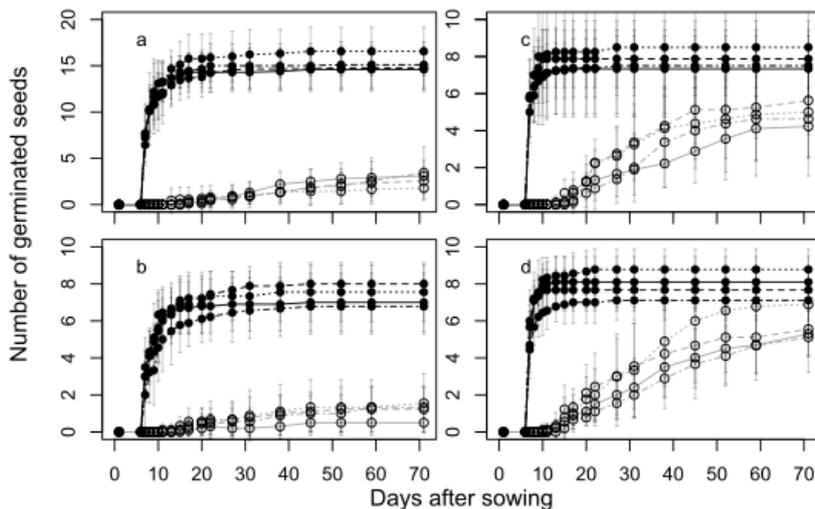


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Figure 4. The effects of shade on the seed germination of (a) *M. sinensis* seeds sown solely, (b) *M. sinensis* seeds sown with *P. sachalinense* seeds, (c) *P. sachalinense* sown solely, and (d) *P. sachalinense* sown with *M. sinensis* seeds. Mean numbers of seedlings (symbols) are shown with standard deviations (error bars). Solid and interrupted lines show un-shaded and shaded treatments, respectively. Black lines with filled circles and grey lines with circles indicate number of germinated seeds and number of dead seedlings, respectively.

Shade changed the seed germination patterns on both the species (ANOVA, $p < 0.001$) (Fig. 4). The seeds of *M. sinensis* and *P. sachalinense* grown solely germinated more under the shade, indicating that direct sunlight was not suitable for the seed germination for these two species. However, the shade increased the seedling mortalities except unchanged mortalities on *P. sachalinense* grown with *M. sinensis*. The seed germination patterns were altered by the seed mixture, i.e., no significant differences in germination between the shade and no shade for *M. sinensis* (ANOVA, $p < 0.001$) and lower germination under shade for *P. sachalinense* ($p < 0.001$). The mortalities of *P. sachalinense* were influenced by shade ($p < 0.001$). The mortalities of *P. sachalinense* were higher under shade than under no shade when *M. sinensis* seedlings were absent. In contrast, the mortalities were higher when *M. sinensis* seedlings were present under no shade.

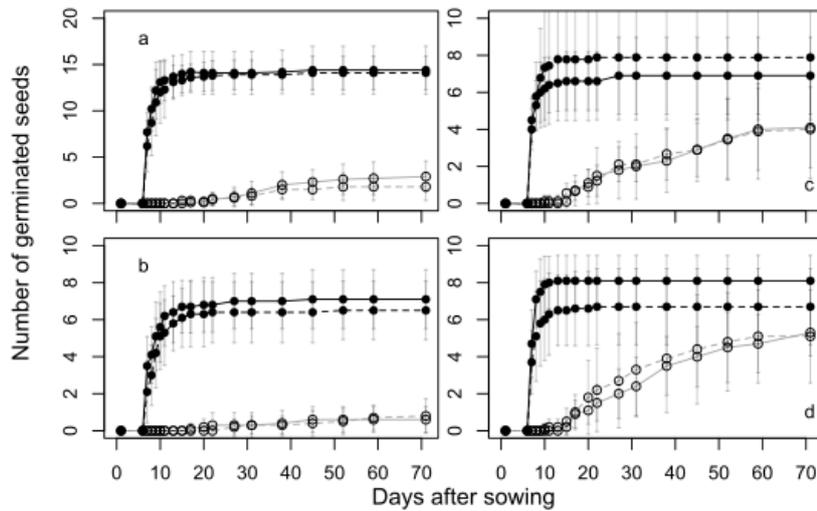
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Figure 5. The effects of litter thickness on seed germination of (a) *M. sinensis* seeds sown solely, (b) *M. sinensis* sown with *P. sachalinense* seeds, (c) *P. sachalinense* seeds sown solely, and (d) *P. sachalinense* sown with *M. sinensis* seeds. Mean numbers of seedlings (symbols) are shown with standard deviations (error bars). Solid, dashed, dotted and dashed-dotted lines show that the litter thickness was 0, 1, 2 and 3 cm. Black lines with filled circles and grey lines with circles indicate number of germinated seeds and number of dead seedlings, respectively.

On the litter thickness, the seed germination was highest on 2 cm in depth on both the species (ANOVA, $p < 0.001$) (Fig. 5) except 1 cm in depth on *M. sinensis* grown with *P. sachalinense* germinating earlier. Seed germination patterns differed between the dry and wet treatments on the two species (ANOVA, $p < 0.001$) (Fig. 6). *M. sinensis* and *P. sachalinense* had higher germination rates in the wet treatment but *P. sachalinense* grown solely germinated faster in the dry treatment.



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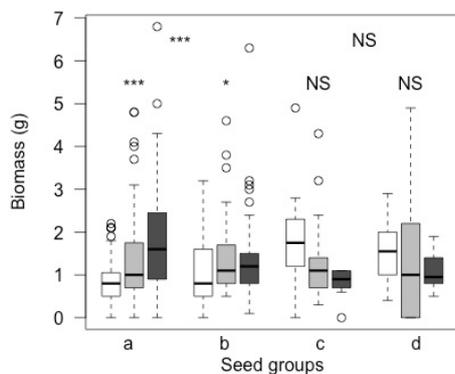
Figure 6. Water supply effects on seed germination of *M. sinensis* seeds sown solely (a), sown with *P. sachalinense* seeds (b), *P. sachalinense* seeds sown solely (c), and sown with *M. sinensis* seeds (d). Mean numbers of seedlings (symbols) are shown with standard deviations (error bars). Solid lines = wet treatment; interrupted lines = dry. Black lines with filled circles indicate number of germinated seeds and grey lines with circles indicate number of dead seedlings.

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Effects of physical environments on seedling growth

The seedling biomass of *M. sinensis* increased with increasing the temperatures with and without the seed mixture at 15°C to 25°C (GLM, $p < 0.05$) (Fig. 7). The responses of seedling growth to the temperature were weakened when the seeds were mixed ($p < 0.001$). *P. sachalinense* did not change the biomass among the examined temperatures, independent of the seed mixture ($p > 0.05$). These results showed the possibility that *M. sinensis* seedlings did not colonize in areas when the temperatures were low. *P. sachalinense* seedlings emerged on areas with a wide range of temperatures more than *M. sachalinense* seedlings. Intraspecific competition reduced the biomass of *M. sinensis* seedlings ($p < 0.001$) and did not affect the biomass of *P. sachalinense* ($p > 0.05$).

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Figure 7. The effects of temperatures on seedling biomass of (a) *M. sinensis* grown solely ($p < 0.001$), (b) *M. sinensis* grown with *P. sachalinense* ($p < 0.05$), (c) *P. sachalinense* grown solely (NS), and (d) *P. sachalinense* grown with *M. sinensis* (NS). Blank, grey and dark grey indicate 15°C, 20°C and 25°C, respectively.

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Shade reduced the total seedling biomass of *M. sinensis* with and without the seed mixture (GLM, $p < 0.01$) (Fig. 8). The reduction of total biomass was derived by the reduction of both above- and below-ground biomass when the seeds were sown solely ($p < 0.01$). However, the shade did not change the below-ground biomass when they grew with *P. sachalinense* ($p > 0.05$). Therefore, the changes in total biomass were induced more by changes in the above-ground biomass. *P. sachalinense* did not change the total biomass and above-ground biomass by shade with and without *M. sinensis* ($p > 0.05$), although the above-ground biomass was reduced greatly by shade when the seeds were sown with *M. sinensis* seeds ($p < 0.05$). The below-ground biomass of *P. sachalinense* differed between shade and un-shade treatments ($p < ***$), showing that this species maintained the biomass somehow by the allocation changes.

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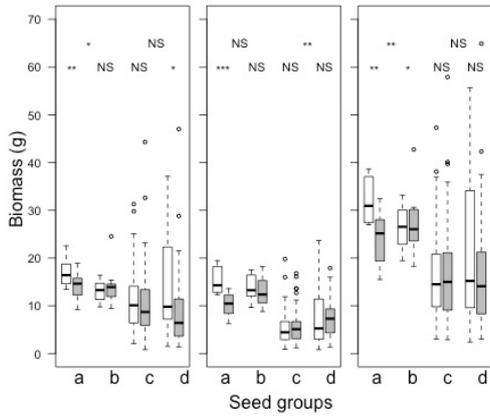


Figure 8. Box-whisker plots shown shade effects on the aboveground (left), belowground (middle) and total (right) biomass of (a) *M. sinensis* grown solely, (b) *M. sinensis* grown with *P. sachalinense*, (c) *P. sachalinense* grown solely, and (d) *P. sachalinense* grown with *M. sinensis*. White bars = without shade; grey bars = with shade. ***: significant at $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: Not significant, $p > 0.05$.

On *M. sinensis* seedlings, the biomass measured by the three parameters was not affected by the litter thickness ranging from 0 cm to 3 cm (GLM, $p > 0.05$) (Fig. 9). On *P. sachalinense* seedlings, when the seeds of the two species were mixed, the below-ground biomass was highest on litter with 2 cm deep ($p < 0.05$). The seedlings tended to be high biomass on 2 cm thick litter layer, although the biomass was not statistically different between litter thickness except the one case. Litter accumulation, as seen under *S. reinii* patches, was considered to be preferable for *P. sachalinense* seedlings.

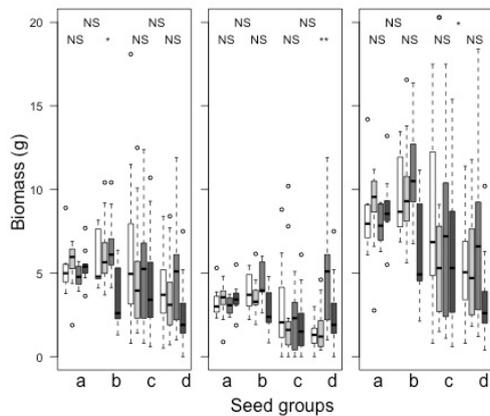


Figure 9. Box-whisker plots shown the effects of litter thickness on aboveground (left), belowground (middle) and total (right) biomass of (a) *M. sinensis* grown solely, (b) *M. sinensis* grown with *P. sachalinense*, (c) *P. sachalinense* grown solely, and (d) *P. sachalinense* grown with *M. sinensis*. Blank, light grey, grey and dark grey columns indicate litter thickness of 0, 1 2 and 3 cm. ***: significant at $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: Non-significant.

The effects of water on seedling growth.

The seedling biomass of the two species did not differ between the dry and wet treatments (GLM, $p > 0.05$) (Fig. 10), showing that the water did not restrict the seedling growths on the two species in a surveyed range of water supply. The wet treatment decreasing the growth of *P. sachalinense* seedlings showed that excess water inhibited the growth.

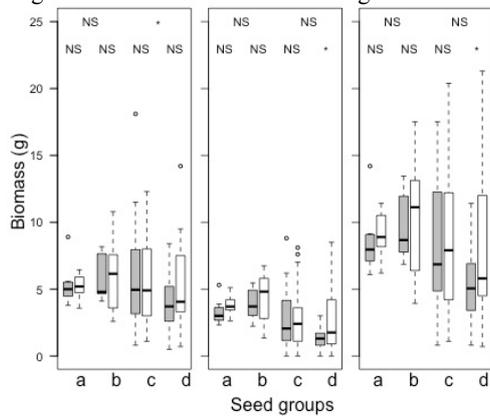


Figure 10. The effects of water supply on the aboveground (left), belowground (middle) and total (right) biomass of (a) *M. sinensis*, (b) *M. sinensis* mixture, (c) *P. sachalinense*, and (d) *P. sachalinense* mixture seedlings. Grey bars = wet treatment; white bars = dry. ***: significant at $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: Non-significant.

277 *Seedbank development*

278 Zero and five species emerged their seedlings on the soils collected from the baregrounds and patches, respectively. The five species
279 were: *Calamagrostis hakonensis* (2 seedlings), *Festuca rubra* (16), *Carex oxyandra* (7), *Erigeron acris* (2), and *Luzula capitata* (2).
280 The seedling densities were 0/m² on the six bareground soils, 3/m² on 0.87-m² patch, 1/m² on 1.11 m²-patch, 1/m² on 1.15-m² patch,
281 6/m² on 1.17-m² patch 1/m² on 1.29 m²-patch 2/m² on 1.36-m² patch 4/m² on 1.56-m² patch 3/m² on 1.86-m² patch 4/m² on 1.97-m²
282 patch 1/m² on 2.09-m² patch 1/m² on 2.11-m² patch and 2/m² on 2.5-m² patch. Therefore, the safe sites for seed germination were
283 the inside of patches.

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285 **Discussion**

286 *Patch size positively affects seed germination.*

287 On the seed-sown experiments conducted in the field, the seed germination rates averaged 3.2% on *M. sinensis* and 4.3% on *P.*
288 *sachalinense* in all the patches and plots. The seed germination rates increased with increasing patch sizes (GLMM, $p < 0.001$).
289 When the seeds of these two species were mixed and sown, *M. sinensis* increased the seed germination and *P. sachalinense*
290 decreased. The numbers of seedlings increased on the two species until September but decreased thereafter, due to death. The
291 seedling mortalities averaged 69.2% on *M. sinensis* and 64.4% on *P. sachalinense*. The patch sizes increased germination of both the
292 species ($p < 0.001$). *M. sinensis* seedlings survived more than *P. sachalinense* seedlings ($p < 0.001$), showing that *M. sinensis* was the
293 winner on the competition at the seedling stage.

294 The facilitation of *S. reinii* patches tended to be size-dependent, shown by increasing the seedling germination of the examined
295 two species with increasing the patch sizes. Facilitation by shrub patches is promoted by ameliorating microclimates and nutrients
296 (Gomez-Aparicio *et al.*, 2005) and capturing seeds (Franco & Nobel, 1989; Uesaka & Tsuyuzaki, 2004). However, these mechanisms
297 are not activated physically if the size is small (Hao *et al.*, 2016).

298 Since there was no litter on the bareground, the development of shrub patches was prerequisite to accumulate litter on the
299 mountain slope. Although small *S. reinii* shrubs were established on the baregrounds with less than 10% in cover, they did not
300 capture litter. The large patches retained more litter than the small ones. These results showed that the patch size was related to litter
301 accumulation.

302 The *S. reinii* patches had seed trap effects as well as in arid regions (Burrows, 1994; Aguiar & Sala, 1997; Hao, 2016),
303 although the shoot development of *M. sinensis* is restricted in the patches (Uesaka & Tsuyuzaki, 2004). The shrub patches affected
304 all the examined microenvironments. Low air temperature and solar radiation derived by the shade should protect water loss by
305 evapotranspiration. The seedling survivals of two shrubs, *Ulex parviflorus* (Fabaceae) and *Santolina canescens* (Asteraceae), are
306 supported by high soil moisture particularly in dry seasons in Sierra Nevada, Spain (Gomez-Aparicio *et al.*, 2005). Drought is often
307 observed in summer on Mount Koma (Uesaka & Tsuyuzaki, 2004). These results suggested that the soil moisture was critical for the
308 seedling emergence and improved by the shrub patches.

309

310 *Seed germination is affected by simulated physical environment under shrub patches*

311 In this experiment, all the physical environment treated to the seeds were simulating how shrub patches create specific properties of
312 microhabitats. Shrub patch altered microhabitat to several ways, such as lowering air temperature, reducing sunlight, accumulating
313 litter, and storing more water in the soil.

314 We found that the optimal temperatures for seed germination were higher on *M. sinensis* than on *P. sachalinense*. The
315 differences is likely to be related to their distributions, i.e., *P. sachalinense* has the southern limit in Honshu Island, Japan (Inoue *et al.*, 1992) and *M. sinensis* is widespread in eastern Asia (Hirata *et al.*, 2007). Furthermore, *P. sachalinense* grown with *M. sinensis*
316 had the highest seed germination rate at 15°C. This meant that the seed germination pattern was affected by biotic interactions. In
317 addition, *P. sachalinense* seeds showed the highest seed germination at the lowest temperature, 15°C, on the mixture treatment.
318 These results suggested that inter-specific interaction occurred even at the seed germination stage.

319 Shade by *S. reinii* patches increased the seed germination of the two herbs in both the field and laboratory, showing that the
320 direct sunshine inhibited the seed germination and weak shade was preferable for the seed germination. Direct sunshine often
321 induces seed dormancy (Hart, 1988) to avoid seedling death by excess transpiration. The *M. sinensis* seeds used in this experiment
322 did not require light for germination. However, the seed germination of *M. sinensis* is controversial; viz. the seed germination of *M.*
323 *sinensis* requires light (Hsu 1989) while light has no effect on the germination (Christian, 2012).

324 The seeds of two herbs germinated well on 1-2 cm deep litter but did not on 3-cm deep litter, showing that the optimal litter
325 thickness was present for the seed germination. Litter promotes seedling establishment in a certain range of thickness (Loydi *et al.*
326 2013) because of the water-holding capacity (Eckstein & Donath, 2005). Litter more than 3 cm thick was not appropriate for the
327 seed germination of the two examined species. The large amount of litter, more than 4 g of litter per pot, slows down seedling
328 emergence of grassland and woodland species (Donath & Eckstein 2008). Larger patches accumulated more litter on Mount Koma
329 and supported seedling emergence more, suggesting that the litter accumulation under the patches was not overabundance and
330 worked as safe sites for seed germination.

331 *M. sinensis* and *P. sachalinense* showed high germination rates in the wet treatment while the seed germination of them
332 occurred gradually in the dry treatment. Delayed seed germination is often observed in disturbed areas to avoid the seedling death
333

334 concurrently (Nomura & Tsuyuzaki, 2015). The delayed seed germination is likely to be advantageous on the bareground to avoid
 335 the concurrent seedling death, although only a few seedlings emerged on the baregrounds due probably to strong drought.
 336

337 *Physical environments are less affecting seedling growth*

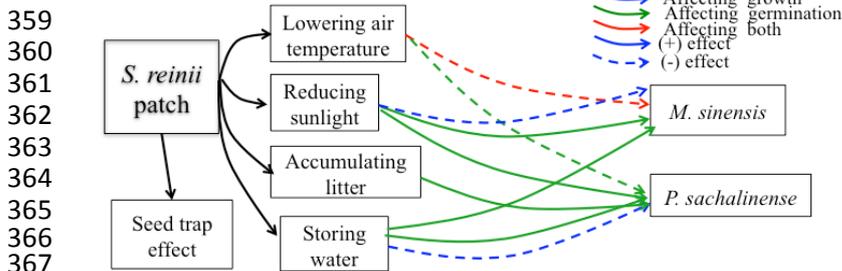
338 High temperature increased the growth of *M. sinensis* but did not affect the growth of *P. sachalinense* in the lab-experiments. The
 339 seedling growth of *M. sinensis* is fast at 20-30°C in a greenhouse experiment (Aso, 1976). Shade did not change the growth of *P.*
 340 *sachalinense* seedlings and reduced the growth of *M. sinensis* seedlings, showing that *P. sachalinense* were more shade-tolerant at
 341 the seedling stage.

342 *Polygonum aviculare* seeds were unable to germinate without cold stratification (Bradford, 2005). Chilling or cold
 343 stratification shifts the requirement of light for the seed germination from high to low light intensities. Since the seeds of *P.*
 344 *sachalinense* were cold-stratified before sowing, the seeds established even under the shade if the characteristics of *P. sachalinense*
 345 seeds were comparable to those of *P. aviculare*.

346 The litter thickness did not affect the seedling biomass on the two species when growing alone. However, the litter reduced the
 347 aboveground and total biomass of *M. sinensis* grown with *P. sachalinense*. These results suggested that, under the shrub patches, *P.*
 348 *sachalinense* should be the winner to *M. sinensis* on the competition. Litter influences the water status in the soil, blocking contact
 349 between roots and soils, etc. (Facelli & Pickett, 1991, Loydi *et al.*, 2013). *P. sachalinense* is dicot and develops large rhizomes
 350 (Inoue *et al.*, 1992), while *M. sinensis* is C₄ perennial monocot grass developing numerous fibrous roots (Clifton-Brown &
 351 Lewandowski, 2002). The root systems are totally different between these two species and seemed to be related to the effects of litter
 352 on their growths.

353 *M. sinensis* seedlings did not change the growth between the dry and wet treatments, showing that the growth adapted to a
 354 wide range of soil moisture. Interspecific competition did not change the growth of *M. sinensis* but reduced *P. sachalinense* growth.
 355 In dry seasons, litter retains water and therefore increases seedling emergence and growth (Loydi *et al.* 2013). The moderate amount
 356 of litter improved seedling establishment by storing enough water to break seed dormancy.
 357

358 **Conclusions**



368 Figure 11. Patch size effects on the seedlings of *M. sinensis* and *P. sachalinense* through altering the environmental factors. Dotted, dashed and solid lines
 369 indicate interactions that affect growth, germination, and both. Black and grey lines indicate the positive and negative effects, respectively, based on the
 370 statistical results.
 371

372 In conclusion, the patch effects of *Salix reinii* patches on the establishments of *Mischantus sinensis* and *Polygonum sachalinense*
 373 were: (1) the positive effects of *S. reinii* shrub patch size on seedling establishment were size-dependent, (2) *S. reinii* patches reduced
 374 air temperature and light intensity, increased litter amount and tephra moisture and trapped seeds, and (3) the patches providing
 375 moderate air temperature and high moisture in the tephra promoted the seed germination, and (4) litter accumulated by the patches
 376 supported the seedling growth. In addition, *M. sinensis* was the winner for the competition to *P. sachalinense* at the seedling stage.
 377 Not only at population level but also at landscape level, the size effects of facilitation should be focused more for the conservation
 378 and restoration of biodiversity.
 379

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 384

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494
495

496 **Author Contribution**

497 Mukhlsh Jamal Musa Holle collected the field and experimental data and analyzed the result. Tsuyuzaki Shiro helped supervise the
498 research and during research preparation. All authors discussed the results and contributed to the final manuscript.
499