The effects of shrub patch sizes on the colonization of pioneer plants on the volcano Mount Koma, northern Japan

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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.
Materials and Methods

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

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

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

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

Greenhouse experiments
To investigate the effects of seedling competition on the seed germination and seedling growth, the seeds of M. sinensis and P. sachalinense were sown solely (hereafter, single treatment) or mixed (mixture) on vermiculite as the substrates in pots (5 cm in 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 sown to each pot. On the mixture treatment, 10 seeds of each species were sown to each pot. On each treatment, 10 replications were used.

The seed-sown experiments were conducted on the seeds of M. sinensis and P. sachalinense in the greenhouse to detect the effects of light intensity, litter and water supply on seed germination and seedling growth. Shade was developed by pots covered with black sheer nets that reduced ca 50% of light intensity. Litter cover under the patches was visually estimated. Litter was collected randomly from the patches in July 2016.

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/30°C in incubators. Hereafter, these three treatments are called 15°C, 20°C and 25°C based on the average. Discontinuous light was 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 more seed germination occurred.

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

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

Statistical analysis
To examine the effects of patch size and seed mixture on the seed germination on each species in the field, generalized linear mixed-effects model (GLMM) was used. The response variable was the number of germinated seeds and the explanatory variables were patch size, time (September, October and November) and seed mixture. The random effect was patch and bareground codes. To test the effects of environmental factors on the seedling biomass, generalized linear model (GLM) was used. On each of the two species, seedling biomass was used as responsive variable and treatments (temperature, light intensity, litter thickness and tephra water
content) as explanatory variables. Repeated-measures ANOVA was used to compare the seed germination patterns in the lab-experiments. All the statistical analyses were performed using R software (version 0.99.451).

Results

Alteration of microclimate by S. reinii patch

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 = 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 red line) from July 2015 to September 2015. The first day (day=1) is July 22 2015.

S. reinii started the leaf flushing in late April and defoliated in late September in the two surveyed years. From mid-July to late August, the temperatures were higher on baregrounds than on the patches (Fig. 1). Afterwards, the temperatures differences among sites became narrower, due probably to the defoliation. The large patches reduced temperatures more than the small patches. The 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 patch on September 18. The mean daily light intensity fluctuated with the sunshine hour (Fig. 1). The light intensity was correlated to the temperatures on the bareground. Relative light intensity (RLI, %) was reduced with increasing patch size, with 19.4% in patch with size 2.0 m² and 20.1% in 1.2 m² shrub. However, the temperatures lowered with increasing patch sizes.

The volumes of litter ranged from 2.02 cm³ to 4.9 cm³ in the patches, while litter was not accumulated on the baregrounds. 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 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 2016 and was higher in the patches than in the bareground, 15% and 11.72% respectively (GLM, p<0.01). Since the measurements were conducted in summer, the high water content in the patches was derived mostly by the shade of S. reinii foliage.

Effects of physical environments on seed germination

The seed germination started nine days after sowing seeds on M. sinensis and 11 days on P. sachalinense at 25°C (Fig. 3). The germination delayed at 15°C on M. sinensis. The seeds of M. sinensis, independent of seed mixture, germinated most at the highest temperature, 25°C (ANOVA, p<0.001). When the seeds were sown solely, P. sachalinense seeds germinated highest at 20°C (p<0.001).
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 solely, and (d) *P. sachalinense* 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.

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.

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* 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.
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 width 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.

**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).

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.
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 belowground 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.
Seedbank development

Zero and five species emerged their seedlings on the soils collected from the baregrounds and patches, respectively. The five species were: Calamagrostis hakonensis (2 seedlings), Festuca rubra (16), Carex oxystachya (7), Erigeron acris (2), and Luzula capitata (2). 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, 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 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 the inside of patches.

Discussion

Patch size positively affects seed germination.

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

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

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

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

Seed germination is affected by simulated physical environment under shrub patches

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

We found that the optimal temperatures for seed germination were higher on M. sinensis than on P. sachalinense. The 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 had the highest seed germination rate at 15°C. This meant that the seed germination pattern was affected by biotic interactions. In addition, P. sachalinense seeds showed the highest seed germination at the lowest temperature, 15°C, on the mixture treatment. These results suggested that the soil moisture was critical for the seedling emergence and improved by the shrub patches.

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

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 thickness was present for the seed germination. Litter promotes seedling establishment in a certain range of thickness (Loydi et al. 2013) because of the water-holding capacity (Eckstein & Donath, 2005). Litter more than 3 cm thick was not appropriate for the seed germination of the two examined species. The large amount of litter, more than 4 g of litter per pot, slows down seedling emergence of grassland and woodland species (Donath & Eckstein 2008). Larger patches accumulated more litter on Mount Koma and supported seedling emergence more, suggesting that the litter accumulation under the patches was not overabundance and worked as safe sites for seed germination.

M. sinensis and P. sachalinense showed high germination rates in the wet treatment while the seed germination of them occurred gradually in the dry treatment. Delayed seed germination is often observed in disturbed areas to avoid the seedling death.
concurrently (Nomura & Tsuyuzaki, 2015). The delayed seed germination is likely to be advantageous on the bareground to avoid
the concurrent seedling death, although only a few seedlings emerged on the baregrounds due probably to strong drought.

**Physical environments are less affecting seedling growth**

High temperature increased the growth of *M. sinensis* but did not affect the growth of *P. sachalinense* in the lab-experiments. The
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.
sachalinense* seedlings and reduced the growth of *M. sinensis* seedlings, showing that *P. sachalinense* were more shade-tolerant at
the seedling stage.

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

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

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

**Conclusions**

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

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**References**


Author Contribution

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