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Seed survival for three decades under thick tephra

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Abstract

Seed longevity *in situ* is a prerequisite for understanding the life histories and community dynamics of species, although long-term longevity under thick tephra has not been documented because of a lack of opportunity and/or awareness. The seed bank for this study was estimated by both germination and flotation tests. Seeds of 17 species have survived with high density, having been buried under thick tephra for 30 years, since the 1977–1978 eruptions on Mount Usu, Hokkaido Island, northern Japan. The total seed density was $>1000/\mathrm{m}^2$. *Rumex obtusifolius* was the most common seed-bank species for 30 years, but decreased in density between 20 and 30 years. More seeds of *Hypericum erectum* occurred in deeper soil. The total seed density decreased gradually for 30 years, but *H. erectum* and *Juncus effusus* did not decline. Native seeds tended to be viable longer than exotic seeds. These results suggest that small, native seeds tend to survive longer with deep burial, while the more numerous weedy, exotic seeds located at the soil surface declined faster. The seed bank provides long-term monitoring of seed survival under natural conditions, and could be used to detect genetic changes.

Keywords: *Hypericum erectum*, *Juncus effusus*, Mount Usu, *Rumex obtusifolius*, seed longevity, spatial heterogeneity, tephra, volcano

Introduction

Long-term seed longevity has not been documented from seeds buried under thick tephra because of the lack of suitable sites and/or awareness. There is a demand for further seed-bank studies of species in poorly investigated habitats (Holzel and Otte, 2004). There have been numerous studies to detect seed longevity using experimental seed burial tests (Leck *et al.*, 1989; Baskin and Baskin, 1998); however, most have been conducted under *ex situ* conditions, including seeds collected from fields and placed into milk bottles for 120 years, which would experience altered soil moisture and related factors (Telewski and Zeevaart, 2002). Even under natural conditions, the seeds of *Luzula parviflora* and *Carex bigelowii* that survived in soil for 100–400 years did so in a permafrost zone (McGraw *et al.*, 1991). A seed burial experiment has suggested that seed-bank longevity is poorly estimated by published databases (Saatkamp *et al.*, 2009) and that conditions for extreme longevity appear to be unusual, e.g. the low temperatures experienced in permafrost. Tandem accelerator mass spectrometry improves the measurement of seed age in soil, but has a several-year measurement error (Moriuchi *et al.*, 2000). Therefore, exact evaluation of seed banks buried under natural conditions provides more realistic data (Whittaker *et al.*, 1995).

The seed bank buried under thick tephra has previously been monitored at 10-year intervals, i.e. 10 and 20 years after the 1977–1978 eruptions, on Mount Usu, northern Japan (Tsuyuzaki, 1991; Tsuyuzaki and Goto, 2001). This seed bank had been well conserved, because predators were few, the movements of seeds by erosion and animal carriers rare, and contamination from the vegetation did not occur due to thick tephra. Therefore, the Mount Usu seed bank provides the opportunity for long-term monitoring of the survival and persistence of seeds under natural conditions, and this paper presents the seed-bank status 30 years after the eruption. Furthermore, seed densities between 20 and 30 years after the eruptions can be compared to detect significant changes in the seed bank. If the seed bank survives with sufficient seed numbers, it could be used to determine genetic changes under natural preservation.

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Materials and methods

Study site

Mount Usu is located on Hokkaido Island (42°32′N, 140°50′E) and is one of the most active volcanoes in Japan. The volcano is composed of two peaks, O-Usu (727 m) and Ko-Usu (609 m), enclosed by a caldera rim and crater basin. Before the 1977–1978 eruptions, the vegetation was dominated by broad-leaved forests, consisting mostly of *Populus maximowiczii* and *Betula platyphylla* var. *japonica* and meadows sown with *Dactylis glomerata* and *Trifolium repens*. Due to the deposits of thick tephra, mostly consisting of ash and pumice, during 1977 and 1978, the vegetation was completely destroyed on the summit. Soon after the eruptions, where the tephra was thin, vegetation recovery began as the result of vegetative reproduction, seeds in the former topsoil, seed immigration and artificial seeding (Tsuyuzaki, 2009). Volcanic eruptions occurred on the foot of this mountain in 2000, but dispersed only a trace of tephra in the study site, i.e. the crater basin.

Sampling

On 19 September 2008, 30 years after the eruptions, the seed bank was monitored again by excavating to the level of the former topsoil at three sites in the crater basin. One hundred 100-cm^3 topsoil samples were collected from each site. To avoid contamination from fresh seeds owing to the movements of tephra, gullies and adjacent areas were not selected. Sites were more than 20 m from each other. When the former topsoil was exposed, moisture (v/v) and temperature were measured by time-domain reflectometry (Hydrosense, Campbell Scientific, Logan, Utah, USA) with a 12-cm probe and a portable thermometer (Digimulti, D611, Takara, Yokohama, Japan), respectively. Three replicates were measured and averaged for each site. After that, a 50 cm × 50 cm quadrat was set up in each site. The quadrat was divided into 10 cm × 10 cm subquadrats. Two soil samples were collected from the upper layer (0–5 cm deep) in each subquadrat, and then two soil samples from the lower layer (5–10 cm). Each soil sample was collected with a stainless-steel can (20 cm^2 in surface area and 5 cm in depth). In each layer, the two samples were collected from upper-left and lower-right corners of each subquadrat. One sample was used to test germination and another was taken for the flotation test.

Seed-bank measurements

To measure species composition and seed density, two methods were used: a germination method (GM) and a flotation method (FM). The GM was conducted in a greenhouse on the university campus within 24 h after the soil collections. The soils were spread over vermiculite in a layer < 5 mm thick (except for large volcanic particles contained therein) in a container (22 cm × 15 cm in surface area, 10 cm in depth). The observations continued for 5 months until no more germination was observed. For FM samples, we used a centrifuged flotation method (Tsuyuzaki, 1994), as follows. The soil samples were agitated with 50% K_2CO_3 flotation solution (1.54 g cm^{-3}). The mixture was centrifuged ($\sim 4000\,g$) for 3 min, and the floating organic debris was decanted and filtered through two layers of miracloth (Calbiochem, California, USA). The seeds were rinsed with distilled water and kept in a refrigerator at 4°C until used. They were identified by morphological traits using voucher seed collections. The viability of seeds extracted by the flotation test was estimated from their firmness and intact appearance, using a seed-crushing technique, i.e. if seeds crushed by a needle and/or sectioned by a razor under a stereomicroscope were not juicy and/or became brown, they were considered to have died.

Seed densities for 10 and 20 years are shown with those for 30 years for purposes of rough comparison, although an exact comparison is not possible because only FM was performed in 1987 in six 50 cm × 50 cm × 10 cm topsoil blocks, each separated into upper and lower layers.

Statistical analysis

Species-accumulation curves were obtained in each site measured by GM and FM, to compare differences in species richness between sites (Ugland *et al.*, 2003). Potential total species richness in each plot was extrapolated by a bootstrap estimate, based on the proportion of subquadrats containing each species detected by GM and FM (Colwell and Coddington, 1994). Seeds in upper and lower layers were summed and used for obtaining the curves and potential total species richness. To estimate spatial heterogeneity in the seed bank, the vertical distributions of seed density and species richness were estimated by generalized linear mixed-effects model (GLMM) with the assumption of Poisson distribution of number of seeds in samples. In the model, the response variable is seed density in the samples of the lower layer; explanatory variable is that of upper layer over the lower layer, and sample code was assigned as a random effect. The two seed extraction methods, GM and FM, were examined separately. To investigate the horizontal heterogeneity of seed density, Moran's *I* was evaluated in each upper layer of the three sites. The weighted neighbour matrix for Moran's *I* was constructed by the assumption that the closest subquadrats were scored as 1 and the others 0. To determine the difference of detection sensitivity between GM and FM, the GLMM was applied to the total number of seeds.

To inspect the changes in seed density and frequency with time, data in the previous paper (Tsuyuzaki and Goto, 2001) collected 20 years after the 1977–1978 eruptions were compared with data obtained in this study by hurdle model, because of excess zeros in the samples. The model is constructed by combining two models, count and zero-hurdle models. The zero-hurdle model investigates the binomial distribution of presence and absence of seeds in the samples, and then the count model estimates the number of seeds after the corrections for overdispersion of zero seeds. To estimate the changes in species richness and number of seeds in the samples, three explanatory variables – year, seed extraction method and layer – were adopted. The seed-bank estimation procedures were the same between the 2 years, and the collection sites 20 years after the eruptions were close to the sites for this study. Seed volume was calculated assuming spheroid shapes in 1998 or 2008, when the seeds were extracted by FM. All statistical analyses were made with the statistical package R 2.10.1 (R Development Core Team, 2009).

Results

The tephra burial depths were 85, 130 and 160 cm in the three sites. With increasing burial depths, moisture increased from 11.3 to 34.3% and temperature decreased from 12.4°C to 11.6°C. The surfaces of the former topsoil were clearly defined. Neither visible animals, including ants, nor erosion were observed below 50 cm in the tephra, indicating that seeds produced by the standing vegetation were unlikely to have percolated down into the former topsoil. Therefore, the seed bank had been conserved without external contamination and influence by seed carriers, predators and erosion.

In total, 17 seed plant taxa were detected from the soil samples 30 years after the eruptions (Table 1). All species are certainly long-lived seeds under thick tephra. The number of seeds extracted by flotation method was significantly correlated with the number of seeds germinated by germination test (GLMM, $P < 0.001$), showing that the two methods were comparable, but FM had higher seed recovery than GM. Species richness ranged from 7 to 11 in the sites. Species-accumulation curves indicated that species richness did not peak by 25 100-cm^3 samples in all the sites. However, the potential total species richness was only 0.6–2.1 higher than the measured values, showing that the samples extracted 83–93% of total species. The total averaged seed density was 1215 per m^2. Five species were non-native; of these *Rumex obtusifolius* accounted for one-third of total seeds, followed by *Juncus effusus* var. *decipiens* and *Hypericum erectum*, both native species. Species showed a wide array of habitat preferences: open forest (*Betula platyphylla* var. *japonica* and *Aralia cordata*), grassland (*Carex oxyandra* and all exotics) and wet sites (*Ranunculus repens* and *J. effusus* var. *decipiens*). However, grassland species were most common. Only one annual and one woody species were detected.

Seed density and species richness in the upper layer did not predict those in the lower layer in both the GM and FM (GLMM, non-significant in all the cases), indicating that the seeds were heterogeneously distributed vertically. As well as the vertical distribution, the horizontal distribution was highly heterogeneous, i.e. Moran's *I* showed that three of six examined coefficients were not significant. In total, therefore, the distribution of seeds was highly heterogeneous along vertical and horizontal directions. Seed density in the upper layer (0–5 cm deep from the topsoil surface) was twice than that in the lower layer (5–10 cm deep), but there were more *H. erectum* seeds in the lower (192 per m^2) than in upper layer (40 per m^2).

From 20 to 30 years after the eruptions, species richness decreased based on the count model but increased according to the zero-hurdle model (Table 2). These results implied that the frequency of seeds in the samples increased but the density decreased. Total seeds also decreased over time. Since presence or absence in the samples became the same between species richness and total seeds, the results on the zero-hurdle model were the same for both. Of the three dominant species, *R. obtusifolius* decreased in density, but did not change in frequency. In contrast, *H. erectum* increased in density, and *J. effusus* in frequency. The upper layer contained more seeds and species in both years.

Seed distributions were spatially heterogeneous and seed-bank estimation procedures were different between years. Therefore, the interpretation of temporal changes of seed densities should be made with caution (Fig. 1). The seed density of *R. obtusifolius* decreased with time, in particular, from 1998 to 2008, while the density of the exotic *Poa pratensis* changed little. In contrast, the native species *J. effusus*, *H. erectum*, *Epilobium cephalostigma* and *Geum macrophyllum*, did not decrease in seed density.

Discussion

Since no seeds were incorporated below the tephra due to its thickness and the lack of any carriers, accurate seed longevity could be estimated under

Table 1. Seed density (per m^2) in the former-topsoil seed-bank on Mount Usu, northern Japan, after the 1977–1978 eruptions. The mean is shown with standard error. Life form: A, annual; P, perennial; and T, tree. Methods: GM, germination; FM, flotation. Asterisks in the Life form column indicate non-native plants to Japan

Year of seed collection		2008				1998[a]				1987[b]		
		GM		FM		GM		FM		FM		
	Life form	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Seed volume (mm^3)
Juncus effusus L. var. *decipiens* Buchen.	P	373 ± 138	17 ± 14	203 ± 81	33 ± 20	85 ± 34	16 ± 12		6 ± 6			0.03 ± 0.01
Rumex obtusifolius L.	P*	160 ± 27	20 ± 9	547 ± 68	107 ± 25	659 ± 129	180 ± 46	1055 ± 164	467 ± 172	1231 ± 512	202 ± 72	1.04 ± 0.03
Hypericum erectum Thunb.	P	53 ± 14	123 ± 26	27 ± 18	137 ± 40		41 ± 18		66 ± 25			0.04 ± 0.01
Epilobium cephalostigma Hausskin	P	17 ± 9	3 ± 3			6 ± 6						
Poa pratensis L.	P*	17 ± 9	3 ± 3	3 ± 3	3 ± 3	55 ± 21	8 ± 8	43 ± 18	16 ± 17	7 ± 8	2 ± 2	0.11 ± 0.02
Geum macrophyllum Willd. var. *sachalinense* (Koidz.) Hara	P	17 ± 7		100 ± 25	10 ± 6	24 ± 12		12 ± 12		133 ± 93	6 ± 4	2.75 ± 0.23
Carex oxyandra (Franch. et Savat.) Kudo	P	7 ± 5	37 ± 11	40 ± 15	57 ± 16	49 ± 27	16 ± 12	177 ± 54	57 ± 27			0.66 ± 0.02
Ranunculus repens L.	P	3 ± 3		37 ± 11	7 ± 5	6 ± 6		6 ± 6		2 ± 1		1.36 ± 0.17
Luzula capitata (Miq.) Miq.	P	3 ± 3	20 ± 8	13 ± 11			8 ± 8					0.26 ± 0.04
Rumex acetosella L.	P*	3 ± 3						6 ± 6		5 ± 3	1 ± 1	0.45 ± 0.07
Viola grypoceras A. Gray	P		13 ± 7	73 ± 17	73 ± 16	24 ± 15	8 ± 8	61 ± 22	49 ± 19	1 ± 1	5 ± 2	0.53 ± 0.06
Trifolium repens L.	P*			23 ± 9	23 ± 11	24 ± 15	8 ± 8	49 ± 21		40 ± 20	22 ± 15	0.42 ± 0.03
Betula platyphylla Sukatchev var. *japonica* (Miq.) Hara	T			7 ± 5				91 ± 30	16 ± 12			0.28 ± 0.04
Chenopodium album L.	A*				7 ± 5					4 ± 3	6 ± 4	0.35 ± 0.03
Aralia cordata Thunb.	P				3 ± 3	18 ± 14	25 ± 18	18 ± 14	8 ± 8			0.68 ± 0.05
Hydrocotyle ramiflora Maxim.	P					6 ± 6	8 ± 8	61 ± 29				0.09 ± 0.01
Taraxacum officinale Weber	P*					6 ± 6	8 ± 8					
Cerastium fontanum Baumg.	A						82 ± 59	37 ± 17		3 ± 3	4 ± 4	0.16 ± 0.02
Polygonum sachalinense Fr. Schm.	P									95 ± 59	18 ± 8	
Poa annua L.	A									39 ± 16	6 ± 3	
Plantago camtschatica Cham.	P									21 ± 16	3 ± 4	
Polygonum longisetum Bruijn	A									17 ± 7	37 ± 36	
Hydrangea paniculata Sieb. et Zucc.	T									7 ± 6	41 ± 37	
Sambucus racemosa L. subsp. *kamtschatica* (E.L. Wolf) Hulten	T									3 ± 2	5 ± 4	
Polygonum aviculare L.	A									1 ± 1	1 ± 1	
Others[c]		6 ± 5				24 ± 12		18 ± 14		8 ± 5	4 ± 3	
Unidentified spp.						12 ± 9	16 ± 12	963 ± 263	402 ± 169	66 ± 39	11 ± 6	
Total		653 ± 138	243 ± 36	1073 ± 109	460 ± 59	994 ± 145	434 ± 104	2598 ± 429	1082 ± 305	1683 ± 561	373 ± 62	

[a] Re-calculated from Tsuyuzaki and Goto (2001). There were four unidentified species by GM and 13 by FM. The seed-bank estimation procedures used in 1998 and 2008 are described in the text.

[b] Re-calculated from Tsuyuzaki (1991). There were seven unidentified species. Six 50 cm × 50 cm × 5 cm blocks were excavated from the upper and lower layers in 1987 and the seed bank was estimated by flotation test, and a few voucher seeds were used for the seed germination test for identification if unidentified by seed morphology.

[c] Others: *Alopecrus aequalis* Sobol. (Annual, 8 per m^2 in the upper layer of 1987 by FM), *Lotus corniculatus* L. var. *japonicus* Regel (Perennial, 4 per m^2 in the lower layer of 1987 by FM), *Erigeron annuus* (L.) Pers. (Annual, 6 per m^2 in the upper layer of 1998 by GM), *Eragrostis multicaulis* Steud. (Annual, 6 per m^2 in the upper layer of 1998 by GM), *Sagina japonica* (Sw.) Ohwi (Annual, 6 per m^2 in the upper layer of 1998 by GM), *Youngia japonica* (L.) DC. (Annual, 8 per m^2 in the lower layer of 1998 by GM), *Carex* sp. (Perennial, 18 per m^2 in the upper layer of 1998 by FM), *Labitae* sp. (3 per m^2 in the upper layer of 2008 by GM), and *Celastrus orbiculatus* Thunb. (Vine, 3 per m^2 in the upper layer of 2008 by GM).

Table 2. Changes in seed density per 100-cm^3 sample on total and three dominant species from 20 to 30 years after the 1977–1978 eruptions of Mount Usu, northern Japan, examined by the hurdle model. The data for 20 years after the eruptions were obtained from Tsuyuzaki and Goto (2001). Years are compared from 20 to 30 years after the eruptions, methods from flotation method (FM) to germination method (GM), and layers from Upper to Lower

	Model	Intercept	Year	Method	Layer
Species richness	Count	+2.197 **	−0.060 **	−0.772 **	−0.398 *
	Zero-hurdle	−0.536 NS	+0.040 **	−0.226 NS	−0.693 *
Total seeds	Count	+2.646 **	−0.035 **	−0.668 **	−0.631 **
	Zero-hurdle	−0.536 NS	+0.040 **	−0.226 NS	−0.693 *
Rumex obtusifolius	Count	+3.265 **	−0.099 **	−0.464 **	−0.413 *
	Zero-hurdle	+2.377 **	−0.137 **	+0.310 NS	−1.079 **
Hypericum erectum	Count	−21.842 NS	+0.966 NS	−8.421 NS	+1.234 NS
	Zero-hurdle	−7.410 **	+0.114 **	+1.939 **	+1.085 **
Juncus effusus	Count	−12.100 NS	+0.178 **	+8.710 NS	−1.254 NS
	Zero-hurdle	−3.065 **	+0.001 NS	+1.187 *	−1.366 *

** Significant at $P < 0.001$; * $P < 0.01$; NS, not significant.

natural conditions. Seed densities averaged 2056, 2555 and 1215 per m^2, 10, 20 and 30 years after the eruptions. The former topsoil showed no dryness, no light, low temperature and narrow temperature fluctuation, due to thick burial (Tsuyuzaki, 1991). In particular, temperature under the tephra was measured at 1-h intervals from 23 September to 26 October 1988, and the fluctuations expressed by standard deviation were less than 0.23°C at 50 cm depth from the ground surface and less than 0.17°C at 100 cm depth. Soil nutrients may affect seed longevity in the short term but not in the long term, i.e. more than 2 years (Bekker *et al.*, 1998a). Seed longevity is not influenced by soil types, but is influenced by soil water potential and temperature (Long *et al.*, 2009). These results suggest that the temperature and soil moisture were adequate for maintaining viability under tephra. These trends were already detected in the seed bank 20 years after the eruptions (Tsuyuzaki and Goto, 2001). However, the temporal changes in the seed densities of dominant species suggest that the survival patterns differ between species.

Grassland species were common and there were few forest-floor species, although the vegetation before the eruptions was of forests and meadows. The weedy grassland species *R. obtusifolius* was the most numerous species for 30 years, and the seed densities were 1433, 1180 and 417 per m^2 10, 20 and 30 years after the eruptions. Thus density gradually decreased between 10 and 20 years and more abruptly between 20 and 30 years, and was the major cause of the decline in total seed density. Although *R. obtusifolius* seedlings do not emerge under thick burial more than 8 cm deep, seed dormancy is lost once a temperature of 20°C is experienced for a few days (Benvenuti *et al.*, 2001). In addition, earthworms promote the vertical transport of seeds but do not favour *R. obtusifolius* seeds (Zaller and Saxler, 2007), suggesting that the seeds of *R. obtusifolius* accumulated near the ground surface before the eruptions. The seeds of weedy, grassland species, represented by *R. obtusifolius*, were predominant before the eruptions, and thus were frequent in the seed bank. However, the survival of weedy species decreased faster than that of native species. In particular, the seeds of *H. erectum* and *J. effusus* showed little decrease in seed densities. The seed-bank longevity is closely related to phylogenetic relatedness, including life forms, life-history traits and seed sizes (Probert *et al.*, 2009), and may

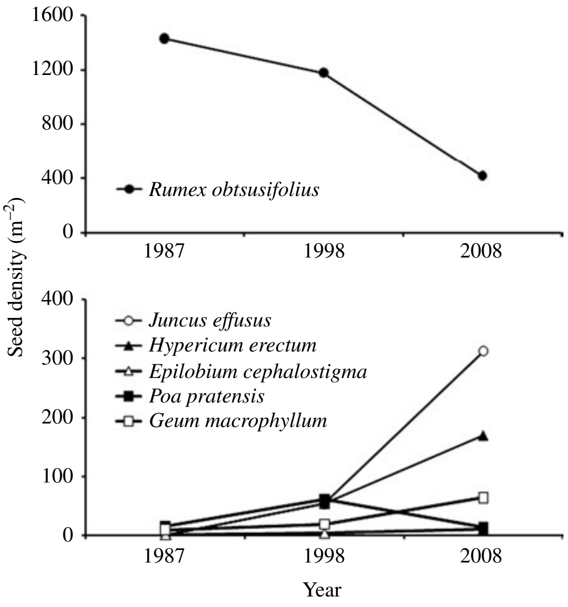

Figure 1. Fluctuations of seed density of six common species for three decades in the former topsoil under tephra. The averaged density is calculated based on the sum of the upper and lower layers. The average of germination and flotation methods are shown in 1998 and 2008; only flotation was employed in 1987.

be explained by such evolutionary characteristics. For examples, there are several species of *Hypericum* and *Juncus*, both of which are perennials and produce small seeds, developing long-lived seed-banks (Thompson *et al.*, 1997). Dr Beal's seed burial experiment showed that *Rumex crispus* survived for 80 years (Telewski and Zeevaart, 2002).

The seeds of the second most numerous species in 2008, *H. erectum*, were distributed more in the lower layer, and this trend did not change between 20 and 30 years after the eruptions. *H. erectum* produces small seeds, i.e. 0.04 mm^3 (Ishikawa-Goto and Tsuyuzaki, 2004) and 0.033 mg (Tsuyuzaki and Miyoshi, 2009). Small, rounded seeds are easily moved to the lower layer (Thompson *et al.*, 2001) and contribute to vertical heterogeneity of the seed bank (Bekker *et al.*, 1998b).

The third dominant species, *J. effusus*, also produces small seeds, i.e. 0.033 mg (Tsuyuzaki and Miyoshi, 2009). These seeds were captured more in the upper layer, but the density did not differ between the two layers, showing that the vertical movements of seeds occurred locally. The seeds of *J. effusus* were dominant in a seed bank of a former lake in Sweden (Skoglund and Hytteborn, 1990), and their age was estimated at more than 100 years (Jerling, 1983). The conditions around seeds under tephra may be comparable to the former lake, because of low temperature with high moisture.

Determining seed longevity in nature contributes to nature conservation, because species with long-lived seeds have lower local extinction rates (Stöcklin and Fischer, 1999). Biological invasion has recently been serious in various ecosystems, and is often promoted by seed-bank development by the small seeds of weedy species (Guo, 2003). Not only native species but also non-native ones were common in the seed bank of Mount Usu, indicating that the seed bank provides an opportunity for the long-term monitoring of native and non-native seed-bank populations under natural conditions. The seed bank under tephra allows long-term monitoring of seed survival, and could also permit genetic changes over 30 years or more to be detected.

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