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Relationships between the developments of seedbank, standing vegetation and litter in a post-mined peatland

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Abstract To clarify seedbank developmental pattern and its determinants, we examined relationships between seedbank, standing vegetation and litter in a post-mined peatland on Sarobetsu Mire, northern Japan. In late fall 2006 and early summer 2007, peat was sampled from four vegetation types, and litter was also sampled from two of the four vegetation where thick litter was accumulated. Seed germination tests were performed by the samples in a glasshouse. The results were compared to the standing vegetation and litter accumulation. Seed density and species richness in seedbank gradually increased with increasing vegetation cover. Seeds in surface layer germinated more in fall than in summer. In vegetation with thick litter, small seeds, such as *Drosera rotundifolia* and *Gentiana triflora*, germinated more from peat than from litter in summer, probably due to the downward movement. Seed density in litter decreased from fall to the next summer. *Moliniopsis japonica* and *Rhynchospora alba* developed short-term persistent seedbank, whereas *D. rotundifolia* and *G. triflora* preserved long-term persistent seedbank in peat covered with thick litter. Seedbank development follows the development of the standing vegetation with litter. Litter derived from the standing vegetation is a prime determinant on the seedbank composition and development, by acting as seed trap and keeping seed dormancy.

Keywords: litter · post-mined peatland・seedbank・standing vegetation・revegetation

Introduction

Relationships between seedbank and standing vegetation vary spatially and temporally (Baskin and Baskin 1998, Leck 2003). For examples on species composition consistency between seedbank and the standing vegetation, the development of transient seedbank is determined by the seed supply from the vegetation in a European fen (van den Broek and Beltman 2006), and the patterns of vegetation distribution are regulated by seedbank composition along a stream channel in a freshwater tidal marsh (Parker and Leck 1985). In contrast to these studies, species composition in seedbank is not consistent with the standing vegetation in a lagoon wetland complex (Nicol et al. 2003), and dominant species in seedbank do not appear in the standing vegetation of an Appalachian *Sphagnum* bog (McGraw 1987). Seedbank formation patterns do not correspond with the standing vegetation following intensive disturbances (Thompson 2000). Since one cause is that the modes of seedbank development are different between species (Tu et al. 1998), species-specific seedbank strategy must be mentioned to clarify the roles of seedbank (Baskin and Baskin 1998).

In Sarobetsu Mire, northern Japan, *Sphagnum* peat had been mined for 33 years. The post-mined sites have an opportunity to investigate seedbank development patterns, because the former vegetation, seedbank and litter were completely removed from the ground surface. On the post-mined peatland, there are plural successional trajectories even in even-aged sites (Nishimura et al. in press). Therefore, we examined how the differentiation of the standing vegetation is related to the seedbank composition. Understanding the relationships between seedbank and standing vegetation may also help increasing resilience of ecosystems (Keddy 2000, Hopfensperger 2007).

Not only the standing vegetation but also litter accumulation determines seedbank development through modifying microenvironments (Weltzin et al. 2005, Kettenring et al. 2006). Litter is completely lacked after peat mining on post-mined peatlands by scrapping off the peat surface until plants re-establish. We hypothesized that litter accumulation primarily controls seedbank development, in particular, for persistent seedbank, in post-mined peatlands. We investigated that 1) detecting effects of litter and standing vegetation on seedbank development patterns, and 2) determining how the characteristics of seeds on each species are related to seedbank development.

Methods

Study site

The study area, Sarobetsu Mire, is distributed in northernmost Hokkaido, Japan (45°06'N, 141°42'E, 8 m a.s.l.). Peat formation in the mire started at Middle Jomon Era (4000-4500 years ago) when marine regression occurred (Ohira 1995). From 1970 to 2003, *Sphagnum*-peat was annually mined with 3-22 ha in area and more than 3 m in depth. Large pools were created as the mining progressed, because no drainages were constructed prior to peat extraction. Therefore, post-mined areas were covered with open water for a few years after peat mining. Mined peat particles were suspended in water during extraction, and were pumped to a processing plant to sieve and extract high-quality fibers for commercial use. The residues were returned to the original excavation site through an open channel. Peat derived from the floated residues gradually created the ground surface, starting from the cutting edge because of wind drift. Thereafter, the stable ground surface had been formed, and plant establishment has proceeded. However, distance from the cutting edge does not become a prime determinant on the pace of revegetation (Nishimura et al. in press), suggesting that vegetation recovery by vegetative reproduction was not intense in the earliest stages except in the vicinity of cutting edges.

Climate is categorized into a warm-cool temperate zone. In 2006, total precipitation was 1163 mm at Toyotomi Town, 6 km apart from the study site (SDMO 2006). Mean annual temperature was 6.6^{o}C with a minimum of -6.9^{o}C in January and maximum of 21.7^{o}C in August. Snow-free period is usually from May to October.

Vegetation survey and seedbank sampling

A cluster analysis of which vegetation data were obtained from the whole area of post-mined peatland confirms that five vegetation types are developed more than 25 years after peat mining (Nishimura et al. in press). Of these, we selected four vegetation types: 1) Bare ground (hereafter, abbreviated as BG) excavated in 1982, 2) *R. alba* sedgeland (RA) mined in 1972, 3) *M. japonica* grassland (MJ) mined in 1972, and 4) grassland dominated by three monocotyledonous species, *R. alba*, *M. japonica* and *P. australis*, mined in 1970.

To evaluate vegetation structures, an 8 m × 5 m site was set up in each vegetation type. The site was partitioned for setting up 40 60 cm × 60 cm plots that were separated by 40-cm buffer zones to each other. The four sites were more than 20 m apart from the peat-cutting edges to homogenize the effects of distance from the cutting edge and the resultant vegetative reproduction. Distance between the four sites ranged from 100 m to 400 m, except BG was 900 m far from the other three sites. Vegetation survey on each plot was carried out in mid-August 2006. Each plot was divided into 25 12 cm × 12 cm cells by a frame to measure plant cover accurately. On three dominant species, *R. alba*, *P. australis* and *M. japonica*, plant height, including flowering shoots if occurred, was measured by a steel tape with a scale at 1 mm interval. Litter thickness was measured in each plot by a ruler. Adjacent to the plots, the phenology of flowering and seed dispersal was recorded for species that were recorded from seedbank and/or standing vegetation during the years of 2006 and 2008.

To confirm seasonal changes in seedbank, peat samples were collected twice, i.e., in early November 2006 (fall) and middle July 2007 (summer). A steel soil tin (20 cm^2 in surface area, and 5 cm in depth) was used for sampling peat and litter. Sampling was conducted in four locations that were distributed on the directions to the four cardinal points 1 m separated from the margin of each site. Litter was none on BG, and was scanty on RA. Therefore, samples were collected only from peat layer on BG and RA. On MX and MJ where thick litter was accumulated, seedbank samples were collected from the peat and litter layers. Boundary between litter and peat layers was clearly distinguished by eyes. On each of four locations, 10 samples were collected from each layer within a 60 cm × 60 cm area. In each season, therefore, total numbers of samples were 40 in BG and RA, and 80 in MX and MJ. The total surface area was 800 cm^2 in each site. Based on the comparisons of the seedbank densities between summer and fall, we assigned transient, short-term persistent, and long-term persistent seedbank on each species (Thompson et al. 1997).

Seed germination experiment in glasshouse

To measure seed density in peat and litter samples, germination experiments were conducted in a glasshouse, the Center of Advanced Science and Technology (CAST), Hokkaido University. All peat and litter samples collected from each site were, respectively, homogenized within 48 hours after the sampling. A half of samples was sprayed less than 5 mm thick on trays filled with vermiculite immediately after the homogenization. The tray was made of transparent plastic with 16 cm × 23 cm in surface area and 6.5 cm in depth. Since seeds often require cold stratification for dormancy break (Baskin and Baskin 1998), another half was exposed at 3°C in a refrigerator with keeping high moisture wrapped by plastic bags in dark for one month. The seeds were sprayed on trays, as described above. Fine roots contained in peat samples were carefully removed before sowing. The trays were placed on tables, and were covered with white and sheer nets to prevent seed contamination from the outside. No artificial light was supplied, and temperature was maintained between 10°C and 35°C. Water was sprinkled 5-9 times within a day, depending on air temperature and moisture. The emergence of each seedling, which was marked by a numbered flag, was recorded every day for the first one month, and at every two days thereafter. The observation was continued until no seeds germinated for more than one month.

To confirm cold stratification requirements for the seed germination of *R. alba*, *M. japonica* and *P. australis*, germination tests were conducted in an incubator at 5°C/25°C (12 hr/12 hr) under a white discontinuous fluorescent light (12 hr /12 hr). All seeds were collected from living plants on the post-mined field in fall 2005. Cold stratification was performed in dark at 2°C for 2 and 8 weeks, and was compared to the control, i.e., seeds kept in a room temperature until use.

Seed mass

To estimate relationships between seed mass and vertical seedbank distribution, five species, *R. alba*, *M. japonica*, *Gentiana triflora* Pallas var. *japonica* (Kusnez.) Hara, and *Drosera rotundifolia* L., were selected for the measurements of seed mass because of the high frequencies in the seedbank and/or standing vegetation. Seeds were collected from living plants on the post-mined sites in fall 2006 and 2007, except *G. triflora* seeds due to intense granivorous damage. On each species, 50 seeds were dried at 70°C for 48 hours and weighed. Seeds included glumes, if available.

Statistical analysis

Plant cover, litter thickness and species richness per plot were compared between vegetation types by non-parametric analysis of variance (Friedman's ANOVA) and *post hoc* non-parametric Tukey-Kramer's honestly significant difference (HSD) test.

Similarities were calculated between the seedbank and standing vegetation and between seedbank in summer and fall. Two types of similarity were examined: qualitative Sørensen's index and quantitative Goodall's percentage similarity index (Tsuyuzaki 1991). Seeds in litter and peat layers were compiled on MX and MJ. For obtaining quantitative similarity, plant cover and seed number were transformed into relative percentage.

Unidentified species in the seedbank were not used.

Seed densities were compared between fall and summer samples in each vegetation type by generalized linear model (GLM) with the assumption of Poisson distribution. The effects of cold stratification on seed germination in each season were also examined by GLM with Poisson distribution.

Differences in the frequency and density of seeds were compared between vegetation types on five common species in the standing vegetation and/or seedbank (*R. alba*, *M. japonica*, *G. triflora* and *D. rotundifolia* and *P. australis*) by a hierarchical Bayesian model with zero-inflated Poisson regression, because of excess zeros. This model investigates two types of probability distributions separately, i.e., if presence of seeds in samples follows Bernoulli distribution, and if seed density in samples follows Poisson distribution (Pearce and Ferrier 2001). By the regression model, four explanatory variables (vegetation type, litter, summer season and cold stratification) were examined. The probability of presence of seeds on species k (P_k) was estimated by:

$$P_k = \frac{1}{1+\exp(-Z_k)}, \text{ and } Z_k = \beta_{0k} + \beta_{1k}V + \beta_{2k}L + \beta_{3k}S + \beta_{4k}C \qquad \text{[Eqn.1]},$$

where k indicates each of the five species coded as 1 to 5. Four explanatory variables, V, L, S and C, indicate vegetation type, litter, season and cold stratification, respectively. β_{0k} is the intercept of the equation on species k. Seed density on species k is assumed to follow the Poisson distribution of which mean is $\lambda_k \times Q_k$. λ_k is the expected value of seed density on species k when seeds are present, defined as:

$$\lambda_k = \exp(\beta_{5k} + \beta_{6k}S + \beta_{7k}L + \beta_{8k}S + \beta_{9k}C) \qquad \text{[Eqn.2]}$$

β_{ik} (i = 1 to 4 in Eqn. 1 and 5 to 9 in Eqn. 2) are the regression coefficients of each explanatory variable on species k. All coefficients were estimated by the posterior distribution of Gibbs sampling and a Markov Chain Monte Carlo algorithm (Clark 2005). When the peaks of the posterior distributions (median values) are close to 0, the variables are not significant to explain seed distribution. All statistical analyses were conducted with the statistical program R (ver. 2.6.0) (R Foundation for Statistical Computing, Vienna).

Results

Standing vegetation

From all the plots gathering to the two seasons, 20 vascular plant taxa and *Sphagnum* spp. were recorded (Table 1). Dominant species in the standing vegetation were three monocotyledonous perennials, *R. alba*, *M. japonica*, and *P. australis*. There were no annuals recorded (Fig. 1). The seed dispersal types were categorized into wind or gravity for most species. *P. australis* did not produce inflorescences until height was measured in mid-August, while *R. alba* and *M. japonica* did. Even though *P. australis* had not developed any inflorescences, the shoots were tallest of the three grass species, i.e., 77 cm in mean height. The heights of *M. japonica* and *R. alba* including flowering shoots averaged 49 cm and 28 cm, respectively.

Plant cover, litter thickness and species richness differed between the vegetation types (Table 1). BG had the lowest plant cover, no litter, and only one species (*R. alba*). On RA, plant cover was 54% consisting of 10 species. Peat surface, not being covered with any plant and litter, appeared patchily on RA. Plant cover and species richness were highest on MX and MJ, i.e., more than 60% in cover and 13 species. Litter completely covered the ground surface in both MX and MJ, and was thicker in MJ than in MX.

All recorded species started seed dispersal before seedbank sampling in fall (Fig. 1). Therefore, the samples contained seeds that dispersed in the present year. At the summer sampling, all species that were identified in the seedbank did not start seed dispersal, except for *Carex middendorfii*. *Eriophorum vaginatum*, *Andromeda palifolia* and *Hemerocallis middendorffii* dispersed seeds before the summer sampling (Fig. 1), although these species were not detected in the seedbank (Table 2). Seedling emergence ceased mostly for common species before the summer sampling. By comparisons between seedbank in fall and summer, therefore, we investigated whether seedbank was transient, short-term persistent, or long-term persistent.

Seedbank density and composition

In total, 485 seeds germinated in the glasshouse. From all samples collected in fall, 177 seeds germinated after cold stratification and 146 seeds in control. In lab-experiment, the seeds of *R. alba*, *M. japonica* and *P. australis* had more than 50% germination after cold but had less than 20% in control, indicating that the germination of these three species was promoted by cold treatment. However, there were no significant differences in numbers of germinated seeds collected from the filed between cold stratification and the control, suggesting that the seedbank in the filed had received cold stratification before the sampling in late fall. Numbers of seeds germinated in summer were 62 and 100 with and without cold stratification, respectively. The decrease in germination after cold was mostly derived from low germination on *D. rotundifolia*.

Seed density increased with increase in vegetation cover from BG to MX, and decreased in MJ in both the two seasons (Table 2), and was significantly higher in fall than in summer in all the vegetation types. Species richness in the seedbank was also highest in MX and MJ. Therefore, species richness and density in seedbank were roughly synchronized with the vegetation cover and richness in the standing vegetation. Qualitative similarities between seedbank and the standing vegetation conserved well, i.e., more than 60%, in fall and summer, except on BG where only one species established in the standing vegetation (Fig. 3), while quantitative similarity became higher in fall than in summer. These results implied that the seedbank composition, in particular, in fall was greatly influenced by seed dispersal in the present year. This also meant most species detected in summer, more or less, created persistent seedbank. Except for BG, qualitative and quantitative similarities between seedbank in summer and fall ranged from 44.4% to 66.7% and from 35.8% to 59.9%, respectively, showing that the persistent seedbank was developed well for specific species in the vegetated habitats.

On MX and MJ where litter accumulated on the peat surface, seed density in litter was

1366/m^2 on MX and 889/m^2 on MJ in fall, and declined to 139/m^2 on MX and to 89/m^2 on MJ in summer (Table 2). In contrast to litter, seed density in peat increased 1.44 and 2.15 times in MX and MJ, respectively, from fall to summer. These results indicated that seedbank was maintained more conservatively in peat than in litter when litter layer was developed. In peat layers on RA and BG where litter was little, seed density in peat was higher in fall than in summer. In total, therefore, seedbank densities seasonally fluctuated more in the surface layers, i.e., peat in BG and RA and litter in MX and MJ, than in the bottom layers, peat in MX and MJ.

Seed distribution and density of respective species

Five species were common in seedbank and/or standing vegetation: *G. triflora*, *D. rotundifolia*, *M. japonica*, *P. australis* and *R. alba* (Tables 1 and 2). On *R. alba* and *M. japonica*, seed densities in the seedbank were correspondent with the plant cover on the standing vegetation, in particular, in fall. On RA, *R. alba* had 38% in cover that was double of the second dominant species *P. australis* (Table 1). *R. alba* explained 93% of seedlings emerged from RA in fall (Table 2). On MJ where *M. japonica* was dominant, *M. japonica* accounted for 60% of germinated seeds in fall. As well as *M. japonica* and *R. alba*, the seeds of *G. triflora* were distributed more in vegetation where the own plants had high cover in the standing vegetation. *P. australis* emerged a few seedlings from seedbank in fall and zero in summer (Fig. 2), showing that *P. australis* did not form persistent seedbank. However, the seeds potentially had high germination ability, demonstrated by lab-experiment. *D. rotundifolia* germinated seeds mostly from seedbank in MX and MJ in both fall and summer, but the plant cover was zero of MJ (Table 1). In contrast to MJ, *D. rotundifolia* germinated less seeds from RA (Fig. 2) where the plant cover of *D. rotundifolia* was highest (Table 1).

Seedbank sizes on the five common species, except for *G. triflora*, were smaller in summer than in fall (Fig. 2). *R. alba* and *M. japonica*, two dominant species in the standing vegetation, germinated well in fall but less in summer, showing that these two species did not form long-term persistent seedbank. The effects of litter on seedbank differed between species (Table 3). *M. japonica* seeds accumulated more in litter layer than in peat layer in fall, but decreased greatly in the next summer, indicating that the seedbank was short-term persistent. On *D. rotundifolia*, more seeds germinated from peat layer in MX and MJ covered with thick litter in both fall and summer (Fig. 2). While, *D. rotundifolia* did not develop seedbank well in the peat of BG and RA where litter was less. The seedbank sizes of *D. rotundifolia* in the peat of MX and MJ were larger than in BG and RA (Table 3). These results showed that *D. rotundifolia* seeds formed a long-term persistent seedbank by utilizing litter cover. As well as *D. rotundifolia*, *G. triflora* seeds tended to germinate more in summer than in fall, and more from peat than from litter when thick litter overlaid.

Seed size

M. japonica seed was extremely heaviest of the five species, and averaged 1.21 mg with a range from 0.44 mg to 2.67 mg. *R. alba* produced secondary heavier seeds (mean = 0.75 mg, range = 0.29-1.01 mg), followed by *P. australis* (0.16 mg, 0.06-0.25 mg) and *D. rotundifolia* (0.01 mg, 0.00-0.02 mg).

Discussion

Relationships between seedbank and vegetation

On the post-mined peatland, seed density and species richness in seedbank increased with increasing vegetation cover. Increases in seed density and species richness in seedbank are promoted by new colonizers, and seedbank in well-developed vegetation does not often contribute to the development of the standing vegetation (Bekker et al. 2000, Walker and del Moral 2003). *M. japonica* and *R. alba*, dominant species in the standing vegetation, form short-term persistent seedbank, and disperse most seeds within vegetation where each of these species was dominant. Of these two species, the persistency of *R. alba* seedbank is probably flexible between habitats and/or regions (Poschlod 1995). Seed-dispersal distance decreases with increasing seed weight and with decreasing the height of flowering culms (Soons 2006). *M. japonica* produces the extremely largest seeds of the examined species, and *R. alba* produces the secondly largest seeds. In addition, *R. alba* and *M. japonica* develop short flowering culms. Therefore, the seed dispersal of the two species is likely to be restricted in the respective vegetation. Since the seedbank of dominant monocotyledonous species does not develop well in the external habitats, the seedbank function seems to be for population maintenance rather than population enlargement. Also, short seed-dispersal distance leads high similarity between seedbank and standing vegetation (Hopfensperger 2007).

P. australis lacks the seedbank in the post-mined peatland, as well as in a Canadian Delta marsh, and flourishes in such areas by vegetative reproduction (van der Valk 1981). *P. australis* seedbank develops in a freshwater tidal wetland where *P. australis* vegetation has not been distributed (Leck 2003), and the seedlings establish only in open habitats without macrophytes (Haslam 1972). *P. australis* populations should be maintained mostly by vegetative reproduction in Sarobetsu Mire. All species established on the post-mined peatland, as well as *P. australis*, utilize two reproductive strategies, sexual and vegetative regeneration, because of the destiny of perennials. However, of dominant monocotyledonous species, the priority of vegetative reproduction is particularly high for *P. australis*. *R. alba* and *M. japonica* certainly develop seedbank in the post-mined peatland.

D. rotundifolia develops a dense seedbank in peat beneath thick litter on MJ and MX, but does not show high plant cover in the standing vegetation, showing that the seedbank distribution is not correspondent with abundance in the standing vegetation. Litter often restricts seed germination by reducing light penetration and temperature fluctuation in the ground (Xiong and Nilson 1999). *D. rotundifolia* seeds collected form Sarobetsu Mire obtain the highest germination when light and cold treatments are given (Tsuyuzaki and Miyoshi in press). *D. anglica*, that intercrosses naturally with *D. rotundifolia*, requires light for seed germination (Baskin et al. 2001). In addition, the seed germination is increased by altering temperature (Crowder et al. 1990). Those results support that peat covered by litter is a key to promote the development of *D. rotundifolia* seedbank. On peat-mined bogs in the European Alps, *D. rotundifolia* seeds are accumulated more in greater depth (Poschlod 1995). The vertical movements of seeds should be easier in litter than in peat, in particular, for small seeds, and the mulch effects of litter, such as light interception and temperature stability, on seedbank may be comparable to peat.

G. triflora also makes a persistent seedbank in the post-mined peatland. *Gentiana* species tend to require long-term cold stratification for seed germination, e.g., six species require more than two months for breaking seed dormancy (Grime et al. 1981; Baskin and Baskin 1998). The duration of cold stratification in this study was one month that was enough to break the seed dormancy. Even though the reason that low seed density in fall seedbank is unclear, this species certainly develops seedbank in the peat layer beneath litter layer.

Effects of litter and peat on seedbank development

R. alba is generally an early colonizer in wetlands after disturbances (Poschlod 1995, Keddy 2000, Nishimura et al. in press). Litter negatively influences the plant growth of *R. alba* in a fen, i.e., *R. alba* dramatically increases in cover due probably to increase in light and soil temperature after artificial litter removal (Weltzin et al. 2005). Litter accumulation regulates not only plant growth but also the seedbank development on *R. alba*. In MX and MJ where thick litter is present, dispersed seeds are firstly captured by litter, which is shown by high seed germination from litter layer in fall. However, most seeds in litter are lost prior to the summer, probably because of germination and emigration from litter to peat. Litter seems to have seed-trap effects (Ghorbani et al. 2006) more than peat of which surface is flat in the post-mined peatland. In addition, seed germination occurs in the inside of litter layers even without contact to soil (Rotundo and Aguiar 2005).

Since *M. japonica* requires cold stratification for the seed germination and does not require light (Taylor et al. 2001), the seeds could germinate under thick burial after overwintering that gives cold stratification (Maas 1989). In addition, larger seeds represented by *M. japonica* germinated least from peat samples beneath litter. These results suggest that *M. japonica* seeds captured into litter germinate soon after snow-melt. In peat layer, seed densities increase from fall to the next summer in MX and MJ where thick litter is accumulated. The vertical movements of seeds occur, in particular, for finer seeds in various ecosystems, such as volcano (Tsuyuzaki 1989) and grass-shrub steppe (Rotundo and Aguiar 2005). *G. triflora* seed is 0.3 mm wide in average and 2.0 mm long (Ishikawa 1995) and *Gentiana amarelle* seed weighs 0.12 mg (Grime et al. 1981), showing that the size of *G. triflora* seed is located between *D. rotundifolia* and *P. australis*. In the post-mined peatland, *D. rotundifolia* and *G. triflora* that produce small seeds accumulate seeds more in peat than in litter. In the post-mined peatland, water supplied from melting-snow is likely to contribute to the movements of small seeds captured into litter toward peat soon after snowmelt.

Litter accumulation determines seedbank composition and promotes seedbank development on the post-mined peatland. In particular, litter is prerequisite to develop the seedbank of *D. rotundifolia* and *G. trifolia*, both of which characterize *Sphagnum* bog. In a lakeshore wetland in China, the seeds of a few species are accumulated more in deeper soils (Li et al. 2008). Thicker litter may promote seedbank development more. Furthermore, eight *Carex* species established in wetlands in Minnesota, USA, have species-specific responses to light (Kettenring et al. 2006) that is manipulated by litter distribution and thickness, and thus three-dimensional distribution patterns of litter should be mentioned to understand seedbank development patterns.

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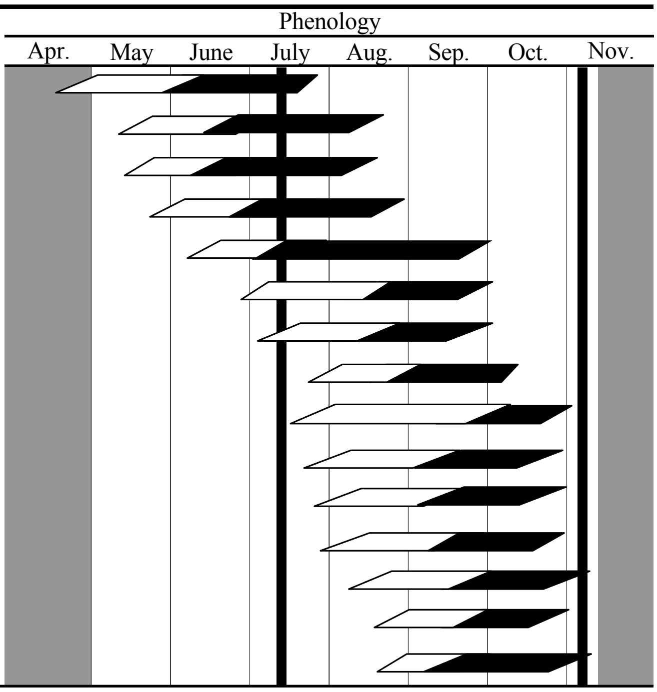
Species	Life form	Seed dispersal
Eriophorum vaginatum	Sedge	Wind
Carex middendorffii	Sedge	Gravity
Andromeda polifolia	Shrub	Wind
Hemerocallis middendorffii	Forb	Gravity
Vaccinium oxycoccus	Shrub	Animal
Drosera rotundifolia	Forb	Wind
Hosta rectifolia	Forb	Gravity/Wind
Rhynchospora alba	Grass	Gravity
Sanguisorba tenuifolia	Forb	Wind
Moliniopsis japonica	Grass	Gravity
Lobelia sessilifolia	Forb	Gravity/Wind
Altemisa montana	Forb	Wind
Solidago virgaurea	Forb	Wind
Gentiana triflora	Forb	Wind
Phragmites australis	Grass	Wind



Others, not evaluated (life form, seed dispersal type, phenology on flowering if it is known): *Empetrum nigrum* (shrub, animal, late April to middle May), *Helonipsis orientalis* (forb, gravity, middle May to middle June), *Rubus chamaedaphe* (forb, animal, middle May to middle June), *Ilex crenata* var. *paludosa* (shrub, animal), *Myrica gale* var. *tomentosa* (shrub, gravity, *Juncus tokubuchii* (rush, wind dispersal).

Fig. 1. Life forms, seed dispersal types, and phenology on flowering (open square) and seed dispersal (closed square) during 2006 and 2008 for species observed in the seedbank and/or standing vegetation. The names of varieties are omitted. Shaded columns indicate snow season. On life form, there are no annual species recorded. The thick vertical bars indicate the periods of seedbank sampling in summer (middle July) and fall (early November).

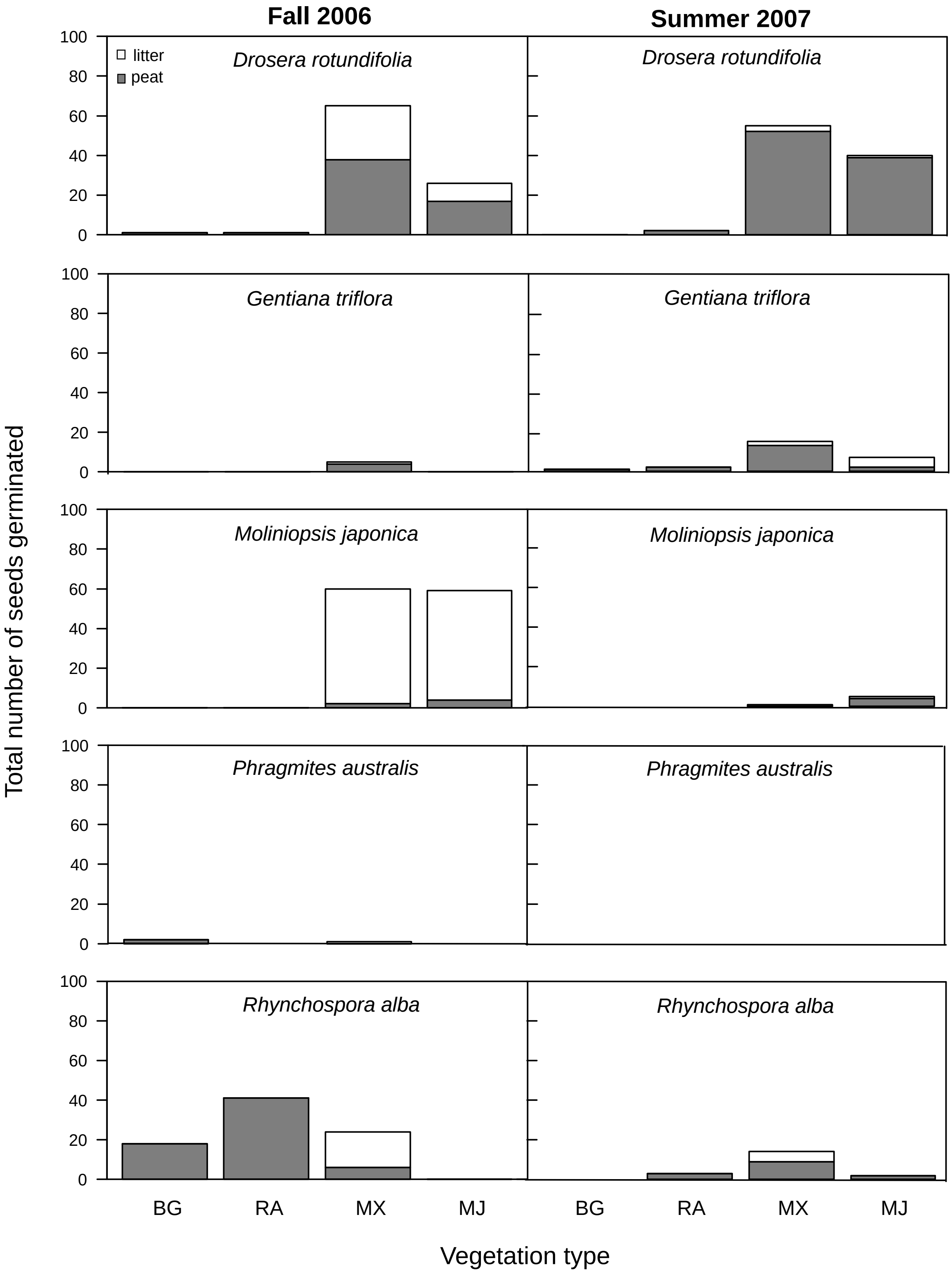


Fig. 2. Total number of seeds germinated from peat and litter collected from four vegetation types on post-mined peatland in fall 2006 and summer 2007. Forty 100-ml peat samples were collected from each of the four vegetation types in each season. Forty 100-ml litter samples were also collected from each of the two vegetation types, MX and MJ. Vegetation type: BG = bare ground, RA = *R. alba* sedgeland, MX = mixed grassland, and MJ = *M. japonica* grassland.

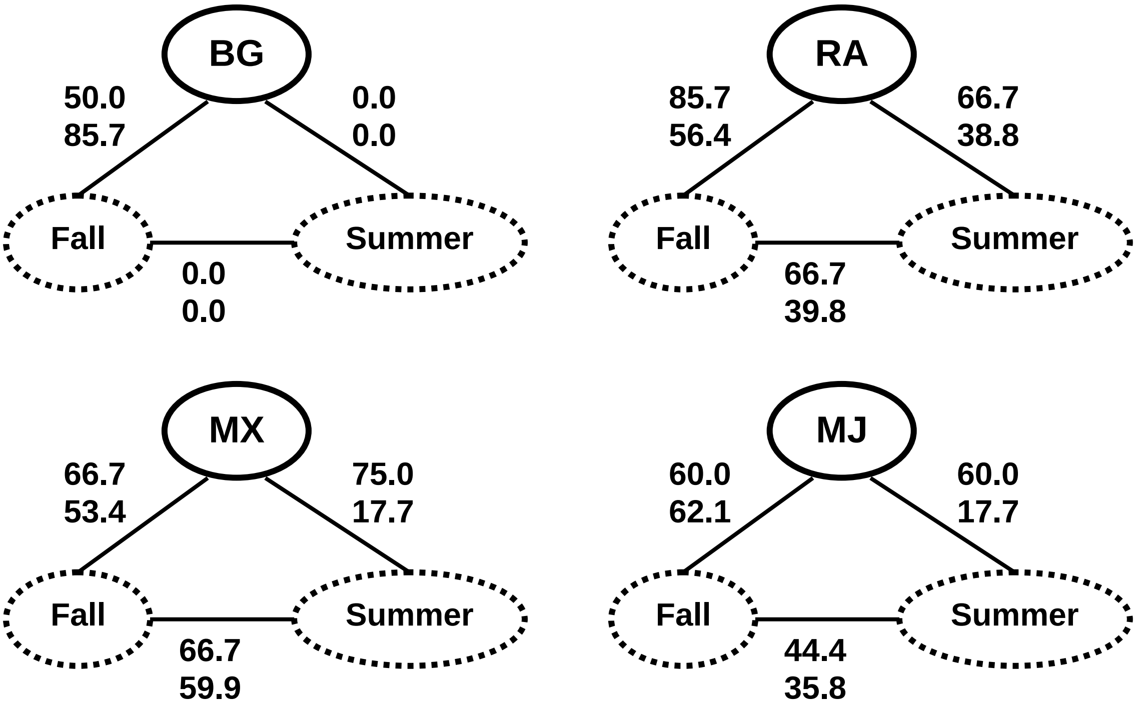


Fig. 3. Similarities between seedbank and standing vegetation in each season, and between seedbank in summer and fall. Solid ovals show standing vegetation and interrupted ovals show seedbank. Upper and lower numerals show qualitative and quantitative similarities, respectively. For abbreviations on vegetation types, refer to Fig. 2.

Table 1. Mean cover on each species (%), species richness, plant cover (%) and litter thickness (cm) in each vegetation type on post-mined peatland. The measurements were conducted in mid-August 2006. Mean is shown with standard deviation. Total species richness is also shown. In each vegetation type, 40 60 × 60 cm plots were measured. Different letters indicate significant differences in species richness, plant cover and litter thickness between vegetation types at $P < 0.05$ (Tukey-Kramer's HSD test). -: not recorded. Vegetation type: BG = bareground, RA = *Rhynchospora alba* sedgeland, MX = mixed grassland, and MJ = *Moliniopsis japonica* grassland.

	Vegetation type				Total
	BG	RA	MX	MJ	
Moliniopsis japonica (Hack.) Hayata	-	4.5±6.6	26.7±6.7	50.1±9.4	20.2±21.4
Rhynchospora alba (L.) Vahl	0.2±0.3	38.0±17.2	17.4±8.6	-	13.9±18.6
Phragmites australis W. Clayton	-	16.2±15.5	15.5±7.4	-	7.7±11.7
Carex middendorffii Fr. Schm.	-	0.5±1.7	0.3±0.9	23.3±9.9	6.2±11.3
Empetrum nigrum L. var. *japonica* K.Koch	-	-	4.6±5.5	-	1.1±3.2
Lobelia sessilifolia Lamb.	-	2.0±3.5	0.3±0.9	2.0±1.5	1.1±2.2
Hosta rectifolia Nakai	-	-	-	4.3±3.5	1.1±2.6
Vaccinium oxycoccus L.	-	2.7±3.7	0.6±1.2	0.6±0.7	1.0±2.2
Eriophorum vaginatum L.	-	3.2±8.0	0.6±1.5	-	0.9±4.3
Hemerocallis middendorffii Trautv. et Mey. var. *esculenta* (Koidz.) Ohwi	-	-	-	1.4±2.5	0.4±1.4
Andromeda polifolia L.	-	1.1±2.3	-	-	0.3±1.3
Drosera rotundifolia L.	-	0.9±0.8	0.2±0.3	-	0.3±0.6
Rubus chamaemorus L.	-	-	-	0.7±1.0	0.2±0.6
Sanguisorba tenuifolia Fisch. ex Link var. *alba* Trautv. et Mey.	-	-	-	0.7±1.7	0.2±0.9
Gentiana triflora Pallas var. *japonica* (Kusnez.) Hara	-	-	-	0.1±0.5	0.2±0.5
Solidago virgaurea L. subsp. *asiatica* Kitam.	-	0.2±0.5	0.4±0.7	0.1±0.4	0.1±0.5
Ilex crenata Thunb. var. *paludosa* (Nakai) Hara	-	-	-	0.3±0.5	0.1±0.3
Osmunda cinnamomea L.	-	-	-	0.1±0.8	0.0±0.4
Heloniopsis orientalis (Thunb.) C. Tanaka	-	-	0.1±0.5	-	0.0±0.4
Myrica gale L. var. *tomentosa* C. DC.	-	-	-	0.1±0.2	0.0±0.1
Sphagnum spp.	-	-	-	0.1±0.5	0.0±0.2
Total species richness	1	10	13	13	21
Species richness per plot	0.6±0.5^{a}	5.5±1.4^{b}	6.7±1.3^{c}	6.7±1.0^{c}	4.8±2.8
Plant cover (%)	0.2±0.3^{a}	54.3±13.3^{b}	62.3±8.8^{c}	67.9±9.0^{c}	46.1±28.7
Litter thickness (cm)	0.0±0.0^{a}	2.8±1.7^{b}	4.8±1.8^{c}	8.4±1.8^{d}	4.0±3.4

Table 2. Seed density (/m^2) in peat and litter layers on four vegetation types. The total surface area of collected samples was 800 cm^2 with 5 cm in depth on each vegetation types in each season. 'Control' means that samples were sprayed in glasshouse immediately after the sampling. 'Stratified' is that samples were stored at 3^{o}C for one month before sowing. Significant differences in seed densities between fall and summer are indicated by asterisks on each vegetation type ($P < 0.01$). Abbreviation of vegetation types, refer to Fig 1. From BG and RA, litter was not sampled.

		BG		RA		MX				MJ			
		Fall	Summer	Fall	Summer	Fall		Summer		Fall		Summer	
Species	Treatment	Peat	Peat	Peat	Peat	Peat	Litter	Peat	Litter	Peat	Litter	Peat	Litter
Artemisia montana	control									24			
	stratified												
Carex middendorffii	control											13	
	stratified											63	
Drosera rotundifolia	control					188	200	563	13	75	25	288	
	stratified	13		13	25	288	138	88	25	138	88	200	13
Gentiana triflora	control		13		13			88	25			13	25
	stratified				13	50	13	75				13	38
Hosta rectifolia	control												
	stratified			13							13		
Juncus tokubuchii	control						25						
	stratified										25		
Moliniopsis japonica	control						363			13	325	13	
	stratified					25	363		13	38	363	38	13
Phragmites australis	control	13											
	stratified	13					13						
Rhynchospora alba	control	138		275	13	25	38	63	13			13	
	stratified	88		238	25	50	188	50	50			13	
Solidago virgaurea	control			13						25			
	stratified												
Asteraceae sp. 1	control							13				13	
	stratified												
Others identified	control				13^{a}					13^{d}		13^{e}	
	stratified					13^{a} / 13^{c}							
Unidentified spp.	control		38				25				25		
	stratified		13								25	13	
Subtotal						652	1366	940	139	327	889	706	89
Total		265*	64	552*	102	2018*		1079		1216*		795	
Species richness		3	> 1	4	4	> 8		5		> 7		> 7	

Ohters: ^{a} = Asteraceae sp. 2. ^{b} = Poaceae sp. ^{c} = *Carex* sp. 1. ^{d} = *Ulmus japonica*. ^{e} = *Carex* sp. 2.

Table 3. Zero-inflated Poisson regression models for investigating determinants on seed distribution and seed density on five species. Vegetation, summer, litter and cold stratification are explanatory variables. Median values on the estimated posterior distribution of coefficients are shown with the confidence levels of 2.5% lower and 97.5% upper limits in parentheses. Abbreviations for vegetation types, refer to Fig 2. Asterisks show significantly different at $P < 0.05$.

	Species				
Variable	*D. rotundifolia*	*G. triflora*	*M. japonica*	*P. australis*	*R. alba*
Presence / absence of seeds (zero-inflated part)					
Intercept	-2.0 (-3.7, -0.4) *	-3.1 (-4.9, -1.4) *	-3.1 (-4.9, -1.5) *	-3.8 (-5.7, -2.1) *	-2.1 (-3.9, -0.8) *
Summer	-0.5 (-1.6, 0.7)	1.2 (-0.1, 2.5)	-3.2 (-4.7, -1.9) *	-1.8 (-3.7, 0.1)	-1.3 (-2.5, -0.1) *
Litter	-1.5 (-2.8, -0.3) *	-0.6 (-1.9, 0.7)	2.5 (1.2, 4.0) *	0.3 (-1.6, 2.2)	0.1 (-1.2, 1.3)
Cold stratification	0.3 (-1.9, 1.5)	0.1 (-1.2, 1.4)	0.5 (-0.6, 1.9)	0.2 (-1.3, 1.9)	0.6 (-0.6, 1.8)
BG	-1.8 (-4.1, 0.1)	-1.5 (-3.7, 0.4)	-1.9 (-5.0, 0.4)	0.7 (-1.5, 3.0)	0.6 (-1.0, 2.6)
RA	-0.8 (-2.5, 1.0)	-0.9 (-2.8, 1.0)	-2.0 (-5.4, 0.4)	-1.4 (-5.4, 1.0)	2.1 (0.5, 4.0) *
MX	2.7 (1.1, 4.6) *	1.0 (-0.5, 2.6)	1.2 (-0.4, 3.0)	-0.8 (-3.3, 1.4)	1.0 (-0.5, 2.9)
MJ	2.2 (0.7, 4.1) *	0.0 (-1.6, 1.6)	1.6 (-0.1, 3.3)	-1.9 (-5.4, 0.4)	-1.8 (-3.6, 0.1)
Seed density (Poisson part)					
Intercept	0.9 (-0.2, 2.0)	-0.3 (-1.6, 1.1)	0.0 (-1.1, 1.3)	-0.2 (-2.0, 1.0)	0.8 (-0.3, 1.9)
Summer	0.1 (-1.0, 1.1)	0.3 (-1.1, 1.5)	-0.9 (-2.1, 0.4)	-0.4 (-2.7, 2.1)	-0.8 (-2.0, 0.3)
Litter	-0.6 (-1.8, 0.8)	-0.4 (-1.7, 0.9)	1.1 (-0.3, 2.5)	-0.2 (-2.1, 1.5)	-0.1 (-1.4, 1.1)
Cold stratification	-0.3 (-1.2, 0.8)	0.1 (-1.1, 1.2)	0.1 (-0.9, 1.1)	-0.2 (-1.9, 1.4)	-0.1 (-1.1, 1.0)
BG	-0.1 (-0.6, 0.4)	0.0 (-0.6, 0.5)	0.0 (-0.6, 0.6)	0.0 (-0.6, 0.5)	0.0 (-0.5, 0.3)
RA	-0.2 (-0.7, 0.2)	0.0 (-0.6, 0.4)	0.0 (-0.6, 0.5)	0.0 (-0.6, 0.6)	0.1 (-0.2, 0.6)
MX	0.3 (0.0, 0.9) *	0.1 (-0.3, 0.6)	0.0 (-0.4, 0.4)	0.0 (-0.6, 0.5)	0.0 (-0.5, 0.4)
MJ	0.0 (-0.4, 0.4)	0.0 (-0.5, 0.5)	0.0 (-0.4, 0.4)	0.0 (-0.5, 0.5)	0.0 (-0.5, 0.4)