



Effects of dwarf-bamboo understory on tree seedling emergence and survival in a mixed-oak forest in northern Japan: a multi-site experimental study

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Abstract: A dense bamboo undergrowth and thick layer of its slowly decaying litter belong to the main factors inhibiting the establishment of woody species in forest stands of northern Japan. The successful regeneration often hinges on bamboo dieback after mass flowering or small-scale disturbances that remove the understory cover and create open sites suitable for seed germination and growth. We conducted a field experiment with repeated observations of emergence and survival of tree seedlings in undisturbed *Sasa senanensis* understory and experimental gaps simulating different disturbance events to determine mechanisms of bamboo interference with tree seedling regeneration. The experiments were repeated in four sites to test if seedling emergence was related to differences in site overstory composition and thus to differential seed input. Very few seedlings germinated under the *Sasa* layer and also few seedlings emerged in microsites where the *Sasa* above-ground biomass was removed but litter left intact. Only severe disturbance that exposed soil led to a significant increase in the number of seedlings. Smaller-seeded species with longer-distance seed dispersal such as *Betula* spp. and *Abies sachalinensis* were generally more abundant than larger-seeded species such as *Quercus mongolica* and *Acer mono*. The mechanism by which *Sasa* cover reduced seedling emergence was not uniform among tree species: emergence of smaller-seeded species – *Betula* spp, *Abies sachalinensis* and *Picea glehnii* – is reduced primarily by low levels of soil exposure, *Acer mono* emergence appears to be related to the reduced light level under *Sasa* cover, emergence of *Kalopanax pictus* and *Phellodendron amurense* is reduced by high litter production of *Sasa*, and *Quercus mongolica* suffered a high level of seed predation. The fact that seedlings of different species responded in different ways to particular treatments shows the importance of the regeneration niche for the maintenance of species diversity. The importance of bamboo understory as an ecological filter that shapes the future canopy composition and structure through differentially influencing tree species establishment is discussed.

Nomenclature: Ohwi (1965) for plants.

Abbreviations: RGR– Relative Growth Rate, RDA – Redundancy Analysis.

Introduction

Seedling establishment is often the critical step for the persistence of plant populations, and can thus influence species distribution and abundance (Grime 2001). Even trees are quite vulnerable as seedlings to various mortality factors, and their distribution relies on the probability of seedling survival during the early phases of establishment. Many factors may potentially inhibit the establishment of woody species such as lack of diaspores, seed predation, browsing, lack of water, high water-table, toxic substrata etc. (Dovciak et al. 2008).

The main cause of unsuccessful regeneration of woody species in many forests is competition with understory herbs and shrubs (Beier et al. 2005). A dense understory is developed in sparse old-growth forests where it intercepts much of

the remaining light and creates a deep shade on the forest floor (Nakashizuka 1987, Nambiar and Sands 1993). The understory plants may produce a great amount of litter, modify temperature, reduce soil water and nutrient content (Davis et al. 1998, 1999, Xiong and Nilsson 1999). Other inhibitory mechanisms such as production of allelopathic substances by understory plants have been reported (Nilsson and Zackrisson 1992). Consequently, seedling establishment of woody species in forests with dense understory frequently hinges on small-scale disturbances such as tree uprooting, animal trampling and grazing that remove the understory cover and create open sites suitable for the seed germination and growth.

In Hokkaido, northern Japan, vast areas of the central region were dominated by mixed-oak *Quercus mongolica* ssp. *crispula* forests (Ishikawa and Ito 1989). However, oak typi-

cally grows on fertile soils, which were extensively cleared for agriculture during the 20th century. Present-day examples of original *Quercus mongolica* stands are thus rare (Umeki and Kikuzawa 1999, Takahashi et al. 2003), and information on the regeneration is needed to determine suitable forest management and conservation strategies. During the second half of the 20th century, the regeneration ability of remaining forests has been further impacted by decrease in natural disturbance events (including fire suppression) and increases in selective logging of oak trees, often coupled with early occupation of clearings by dense bamboo grassland. Deforested areas, lacking exposed soils or decaying woody substrate suitable for seed germination, are being quickly overgrown by native dwarf bamboos that may block woody species regeneration for decades (Noguchi and Yoshida 2005).

Dwarf bamboos such as *Sasa borealis*, *S. senanensis* and *S. kurilensis* are common understory dominants in *Quercus mongolica* forests (Kolbek et al. 2003), especially in snow-rich upland areas where they form nearly continuous cover (1–2.5 m tall). Small numbers of tree seedlings and saplings are a characteristic feature of these forests and juveniles are often confined to raised microsites such as fallen logs, stumps, and mounds created by tree falls. Most information on bamboo understory interference with woody species regeneration comes from comparative studies of forest stands with different degrees of bamboo dominance (Umeki and Kikuzawa 1999, Abe et al. 2002, Taylor et al. 2004). These have consistently found higher density and diversity of saplings in openings with lower bamboo cover created after canopy disturbance or bamboo dieback after mass flowering.

However, more experimental studies are needed to determine whether a higher density of a particular species in disturbed patches (gaps) is a result of higher seed dispersal to gaps or higher germination probability and seedling survival, or lower seed mortality as caused by e.g., seed predation. There is a lack of studies that have simultaneously followed the fate of individual seedlings during the early regeneration phase in undisturbed vegetation and in natural or simulated disturbances to determine the stage in the life cycle to which disturbance is critical.

This study aims to describe and explain changes in emergence and survival of tree seedlings in experimental plots where the bamboo understory was undisturbed, partially or completely removed in stands representing close-to-natural mixed-oak forests in northern Hokkaido (Takahashi et al. 2003). Most natural disturbances and canopy gaps in these stands are due to fallen stems and branches and involve suppression or removal of dwarf-bamboo and litter layers as well as exposure of soil. The experimental treatments were designed to simulate these forms of disturbances. The first experiment took advantage of the exceptionally abundant masting year ensuring sufficient input of *Quercus mongolica* diaspores to test the effect of the *Sasa* bamboo stratum on survival of seedlings through three growing seasons. The second, community-oriented experiment tested the hypothe-

sis that the bamboo stratum of the forest understory differentially influences natural emergence and establishment of various tree species. The experiments were repeated in different sites to test if seedling emergence was related to differences in site overstory composition and thus to differential seed input.

Study site

The study was conducted in northern Hokkaido at the Uryu Experimental Forest, Moshiri Research Station, Hokkaido University, Japan (44°20' N, 22°15' E; altitude 380 m). The area has a sub-boreal climate with cool and snowy winter influenced by cold air masses from Siberia, and humid summer influenced by warm current of the Sea of Japan. According to climatic records from Moshiri Station (1932–1984), the area receives 1650 mm mean annual precipitation, most of it falls in winter. Snow cover is more than 2.5 m deep and usually lasts from November to May. The mean annual temperature is 3 °C (max. 33.7 °C, min –41.2 °C), mean monthly temperature in July and January was 17.9 and –12.1 °C, respectively.

The contemporary forest overstory in our study sites is dominated by *Quercus mongolica*, forming the main canopy between 20 and 30 m together with scattered *Abies sachalinensis* and *Betula ermanii* canopy trees (Table 1). The lower canopy between 10 to 20 m comprised many species with *Acer mono*, *Abies sachalinensis*, *Acer japonica* and *Tilia japonica* being the most abundant. Recent works in these stands (oak trees > 200 years old) have reported that recruitment rate during 16-yr period exceeded mortality for all the woody species except for *Quercus mongolica* that had no recruits (Takahashi et al. 2003). Similar oak declines have been reported from conifer-broad-leaved forests in northern Japan, where bamboo forms continuous understory cover (Abrams et al. 1999). In the long-term absence of disturbance, it is likely that the stands will become increasingly dominated by these later successional species, since they currently occur at higher densities in the lower tree and sapling stratum. Contrarywise, *Quercus mongolica* which is infrequent in the subcanopy and lower tree strata, is likely to decline over time.

Methods

Sasa removal experiment 1: emergence and survival of *Quercus mongolica* seedlings

The two types of experimental treatments were implemented within naturally-occurring clones of *Sasa senanensis*. In one treatment, *Sasa* was left undisturbed as a control (hereafter C) and in another it was removed from plots (hereafter SR). Treatments were implemented in 1 m² study plots and in a 0.5 m wide buffer zone around the perimeter of each study plot. In order to conserve an intact litter layer and soil horizons, each *Sasa* shoot was cut at the base and removed carefully from the plots. The experiment was started in the exceptionally abundant masting year of 1998/1999 to ensure

Table 1. Stand characteristics of four experimental sites in Moshiri Forest, Hokkaido, Japan. Shown are the numbers of stems > 1.3 m in four 0.16 ha plots, mean stem diameter at breast height (DBH), total basal area (BA in m²/ha) of each species, and the number of woody species.

Site	MJ			KJ			MP			SP		
	count	DBH	BA	count	DBH	BA	count	DBH	BA	count	DBH	BA
<i>Abies sachalinensis</i>	40	10.9	8.59	17	22.4	6.61	23	16.9	7.19	8	11.1	0.96
<i>Acanthopanax sciadophylloides</i>	-	-	-	-	-	-	8	13.9	1.13	4	17.3	0.85
<i>Acer japonica</i>	-	-	-	-	-	-	4	7.2	0.13	4	5.2	0.07
<i>Acer mono</i>	154	4.7	5.92	63	7.4	4.27	17	20.9	5.43	28	9.1	1.46
<i>Betula ermanii</i>	8	3.5	0.01	4	39.4	3.76	5	10.8	3.77	7	26.9	0.89
<i>Kalopanax pictus</i>	29	5.6	0.88	3	15.1	0.50	-	-	-	1	7.6	0.06
<i>Magnolia obovata</i>	3	2.9	0.02	-	-	-	-	-	-	13	6.2	0.42
<i>Phellodendron amurense</i>	2	3.9	0.03	-	-	-	-	-	-	1	20.8	0.88
<i>Picea glehnii</i>	-	-	-	-	-	-	-	-	-	1	61.4	2.6
<i>Prunus sergentii</i>	1	6.8	0.03	-	-	-	-	-	-	-	-	-
<i>Quercus mongolica</i>	17	24.6	18.5	3	23.6	1.95	17	47.6	17.8	26	52.3	38.1
<i>Sorbus commixta</i>	43	4.1	0.41	13	9.4	0.89	3	10.5	0.31	-	-	-
<i>Tilia japonica</i>	83	2.8	0.76	111	4.4	4.98	-	-	-	-	-	-
<i>Ulmus laciniata</i>	2	7.4	0.12	-	-	-	-	-	-	-	-	-
<i>Viburnum furcatum</i>	280	1.5	0.32	204	1.6	0.28	2	2.4	0.01	-	-	-
Total stem number	664			418			81			91		
Total basal area			35.3			23.2			35.8			46.3
Species number	12			8			8			9		

sufficient input of diaspores. The experiment began with massive emergence of oak seedlings after snowmelt in the spring of 1999 in two sites (MJ - Mt. Jinja compartment and MP - 407 compartment), each having 5 replicates with two *Sasa* treatments in one block (2 manipulations \times 5 replications \times 2 sites = 20 plots). New seedlings were marked individually, and their survival, height growth and cause of mortality were monitored through the three growing seasons until September 2001. Mortality was ascribed to summer wilting and herbivory (with distinction between seedling stems cut or roots cut).

Relative growth rates (RGR) in terms of height were calculated as $RGR = [\ln(y_{i+1}) - \ln(y_i)]/yr$, where y_{i+1} is final height, y_i is initial height, and yr is the length of the experiment in years. The survival data were analyzed by a Cox proportional hazard regression model which uses the time to death (weeks in our case) as a response variable. The analysis with censoring (seedlings which survived up to the end of the experiment had the censoring indicator status = 0) and a Weibull error distribution were used to test if the oak seedling survivorship was different between the *Sasa*-free and control plots, and if initial seedling height and the number of leaves did affect the time to death of a given seedling. We first fitted full analysis of covariance model with initial height \times treatment interaction term as a predictor and then proceeded with model simplification if interaction was not significant (Crawley 2002, p. 624).

Sasa removal experiment 2: natural recruitment of tree seedlings

Four forest stands (hereafter sites), in which the understory stratum was dominated by *Sasa senanensis*, were lo-

cated in Moshiri Forest. These sites are abbreviated and named in the text as follows: MJ (Mt. Jinja), KJ (Koichi - Mt. Jinja compartment), SP (Sawako - 407 compartments) and MP (Mitsuiishi - 407 compartments). These sites, located 1 to 3 km from each other, were chosen for similar understory composition, but they varied in overstory composition and density. These stands were partly influenced by selective logging and removal of mature oak trees, as evidenced by several tree stumps, in the second half of the 20th century, with gaps overgrown by *Sasa senanensis*.

In each of four Moshiri Forest sites, data on seedling emergence and survival were collected during the 2000 and 2001 growing seasons in 1 m² plots (with a 0.5 m wide buffer zone around the perimeter). We used a completely randomized block design with four types of experimental treatments simulating different intensity of undergrowth bamboo disturbance:

- (1) SR – *Sasa* removal. Above-ground biomass cut and removed, litter left in the plot. This simulated perturbation created by vertebrate trampling, *Sasa* dieback, or by fallen stems and branches, which remove the vegetation and exposes the forest ground, but leaves the litter layer and below-ground parts more or less intact.
- (2) SLR – above-ground *Sasa* biomass and litter removal. This simulated the disturbance created by e.g., light surface fire, heavy trampling or light landslides.
- (3) ES – exposed soil. Undergrowth vegetation including litter and below-ground rhizomes removed. This simulated large disturbance created by a fall of canopy tree.
- (4) C – control plot with *Sasa* present (with a 0.5-m-wide buffer zone around the perimeter),

The experimental treatments were implemented within the *Sasa* stands during summer 1999, each having 5 replicates, with four *Sasa* treatments in one block (4 manipulations \times 5 replications \times 4 sites = 80 plots grouped in 20 blocks). The five blocks were randomly located in each site within a 40 m \times 40 m plot (0.16 ha) where the overstory conditions were measured. The overstory was described by counting tree individuals $>$ 1.3 m tall, and measuring their stem diameter at breast height (DBH).

Seedling emergence was scored as the visibility of cotyledons or leaves above the ground. Seedlings were counted during the initial survey in June and new births at two or three more subsequent dates during the growing season. Recruitment of sympatric *Betula ermanii* and *Betula platyphylla* was recorded as *Betula* because the species are indistinguishable at emergence. In order to know the variation in *Sasa* understory, bamboos were collected from 20 1 m² SLR plots, sorted into leaves, shoots and standing dead, then oven-dried and weighed. Litter collected from the ground was oven-dried and pooled together with standing dead biomass. The fresh bamboo leaf blades were measured by LI-COR area meter (LI-COR 3100, Lincoln, Nebraska, USA) to calculate leaf area index (LAI).

Total community response (i.e., species composition of tree seedlings) to the experimental treatments was evaluated by constrained ordinations, redundancy analysis (RDA), using CANOCO for Windows package (ter Braak and Šmilauer 1998). We performed several RDA analyses in order to test for the effect of site, block, *Sasa* understory manipulation, year and their interactions on the species composition of tree seedlings. By combinations of explanatory variables and covariables, followed by an appropriate Monte Carlo test reflecting the hierarchical nature of the data (see Lepš and Šmilauer 2003 for appropriate permutation schemes in designed experiments) we constructed tests analogous to the testing of particular terms in ANOVA models. Both RDA with and without standardization by sample norm were used. RDA without standardization reflects both the differences in the number of seedlings and also the relative proportions of seedling species, while standardized RDA takes into account the proportions of seedling species only. The RDAs were carried out on the 2000 and 2001 dataset.

The numbers of seedlings of individual woody species were analyzed by repeated measures ANOVA on the log transformed values with experimental treatment as the fixed factor, site, and block nested within site as the random factors, and year the repeated measures factor. Significant effects of site and its interactions would show differences among sites. Significant effects of treatment and treatment by time interactions would show that there is a consistent effect of treatment, either constant over years (treatment), or variable over the years (treatment \times year interaction). Because site is a random factor (and accordingly the corresponding interactions with site are used as error mean square for treatment and its interactions), the effects can be generalized, also

outside the four experimental plots, irrespective of whether the effect of site or its interactions is significant or not.

The survivorship of seedling species that were abundant in 2000 was monitored until September 2001. Data from all the plots were pooled across sites ($n = 80$ plots) and a dependence of survival on bamboo manipulation was tested by the repeated measures ANOVA on the log transformed values and by analysis of deviance in S-Plus (McCullagh and Nelder 1989). For the latter case, the survival between 2000 and 2001 was modeled as a binomial variable, i.e., number of surviving seedlings from the number of all seedlings in the plot.

Results

Sasa removal experiment 1

A total of 429 *Quercus* seedlings were surveyed during the first census in 1999. Separate ANOVA performed on the data from the first year of the experiment did not reveal significant differences in the number of seedlings established in the *Sasa*-free plots and under the *Sasa* understory ($P > 0.05$). There were also non-significant differences in the number of established seedlings between two localities ($P > 0.05$). The survival analysis performed on the entire data set has shown that the seedlings survival from 1999 to 2001 differed significantly between the *Sasa* removal and control plots (analysis of deviance, $P = 0.0014$). Survival of *Quercus* seedlings was evidently inhibited by the compact dwarf-bamboo layer. The initial height and number of leaves were positively correlated ($r = 0.36$, $P < 0.001$), and had significant and positive influence on seedling survival ($P < 0.01$ in both cases); height \times treatment interaction term was nonsignificant ($P = 0.306$), indicating that the effect of initial plant size on survival was similar in the *Sasa*-free and control plots. The highest seedling mortality was registered during the first growing season (Fig. 1A), but the rate of mortality in *Sasa*-free plots (45.5%) was comparable with the rate of mortality under the *Sasa* understory (50.6%). After the second growing season the cumulative mortality reached 85.8% under the *Sasa* understory, whereas in the *Sasa*-free plots only 57.8%. At the end of the experiment, two growing seasons later the cumulative mortality reached 94.7% under the *Sasa* understory and 61% in the *Sasa*-free plots. The number of *Quercus* seedlings (mean \pm SE) left in the *Sasa*-free and control plots at the end of the experiment was respectively 15 ± 5.05 and 3 ± 0.98 seedlings. Overall, we identified the cause of mortality for 94.9% of the seedlings, the main cause being summer wilting in seedlings growing under the *Sasa* understory, whereas herbivory from rodents in seedlings in the *Sasa*-free manipulation (Fig. 1B). The presence of *Sasa* understory also significantly influenced the relative growth rates in terms of height increment of *Quercus* seedlings (Fig. 1C). Initial seedling height in early July 1999 (mean \pm SE) was 13.9 ± 1.3 and 14.1 ± 0.7 for *Sasa*-free and control plots (non-significant difference, $P = 0.65$), respectively. During the first growing season, RGR of *Quercus* seedlings was higher under the *Sasa* understory (seedling height at the end of season was $15.8 \pm$

0.42 and 18.1 ± 0.58 in the *Sasa*-free and control plots, $P = 0.001$), whereas during the two subsequent growing seasons RGR of oak seedlings was higher in the *Sasa*-free manipulation. Overall, taking the first and last height measurements, the relative growth rates from 1999-2001 was higher, albeit non-significantly, in seedlings growing in the *Sasa*-free plots than under *Sasa* cover.

Sasa removal experiment 2

Of the total number of tree species that germinated in experimental plots, 13 were sufficiently common for statistical analysis. They occurred in at least two different treatments in 2000 or in 2001. When both the 2000 and 2001 cohorts are combined, natural recruitment was dominated by *Betula*

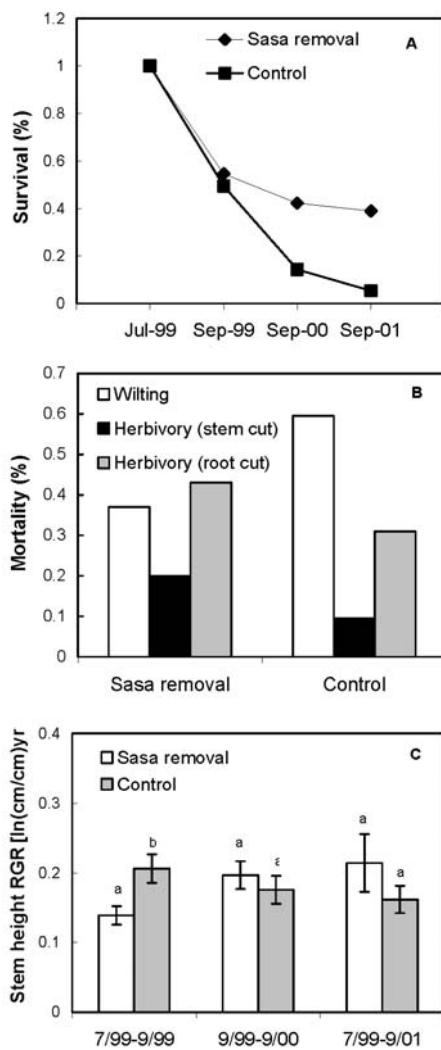


Figure 1. A. Total proportion of 1999 *Quercus mongolica* seedlings surviving through three growing seasons in the experimental plots with *Sasa* understory removed and present in Moshiri Forest (Hokkaido, Japan). B. Causes of mortality of *Quercus* seedlings. C. Variation in relative height growth rates (RGR) of *Quercus* seedlings. Histogram bars represent means \pm SE. Treatments with the same lowercase letters do not differ significantly ($P > 0.05$, Fisher LSD).

(39.3% of total seedling number), followed by *Abies sachalinensis* (25.1%), *Phellodendron amurense* (7.8%), *Acer mono* (7.5%) and *Kalopanax pictus* (7.2%). The other tree species had each less than 3% of total number of seedlings. The density of emergent seedlings varied by site (Table 2, A1). This was true for the total number of seedlings and the most common species. The highest number of seedlings was found in site KJ (37% of the total number), followed by MJ (30.1%), MP (17.5%) and SP (14.7%). This pattern seems at least partially related to differences in site overstory composition and structure (Table 1). Sites MP and SP, in which the canopy was dominated by large oak trees and the bamboo understory showed higher values for litter and aboveground shoot and leaf biomass, had the smaller seedling numbers compared to other two sites. Sites KJ and MJ, which had more diverse and stratified overstory and less-developed *Sasa* stratum, had more emergent seedlings. Fig. 2 displays results of the RDA test with the site as the only explanatory variable and with no covariables (permutation test consisted of randomly assigning blocks to sites, while keeping all plots belonging to a single block (split-plots) together). It shows that *Abies* emergence was smaller at sites KJ and SP. *Phellodendron* emergence was smaller at site MJ and SP. *Acer* and *Kalopanax* emergence was negligible at site MP and SP, while *Quercus* and *Picea* emergence was higher at sites SP and MP. This pattern appears related to the expected seed input based on abundance of given tree species in the overstory (Fig. 3). Sites KJ and SP had fewer overstory *Abies* in the stand, so little seed input was expected in these sites. In contrast, MJ, which has many mature *Abies* trees in the overstory, showed the highest emergence of *Abies* seedlings. *Acer mono* had the lowest recruitment in site SP presumably because of a small number of mature maple trees in the stand.

The amount of *Sasa* varied among sites, presumably in response to different site overstory conditions, but because of high variability in the data there were no statistically significant differences. Average biomass across all sites of *Sasa* stems, leaves and litter was 777.3 g/m^2 (min. 308.2 – max.

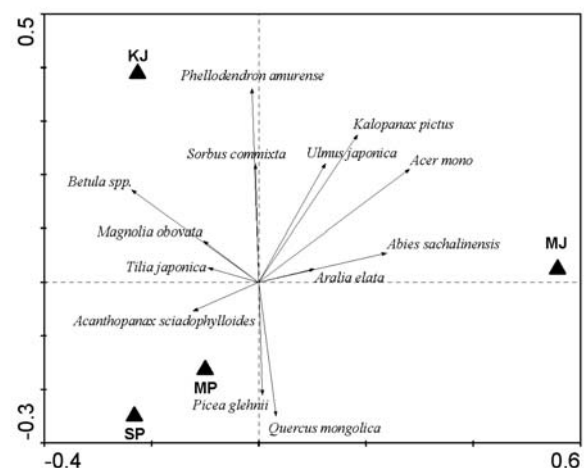


Figure 2. Result of RDA (A1 in Table 3) showing the distribution of seedling species at four sites in Moshiri Forest. Each centroid (triangle) represents a site.

1903.6 g/m²), 178.7 g/m² (87.3 - 356.1 g/m²), 641.7 g/m² (350.5 - 1300.3 g/m²), respectively. Mean *Sasa* LAI was 2.64 m²/m² (1.11 - 5.45 m²/m²).

The total number of seedlings differed significantly among treatments (Fig. 4). The highest number was found on exposed soil, followed by plots where *Sasa* and litter were removed but soil left intact. In 2000, there were nonsignificant differences in the total number of seedlings between these two microsites. There were, however, significantly more seedlings in the *Sasa*+litter removal in comparison with the *Sasa* removal, indicating a negative effect of litter layer on seedling survival.

The species composition of seedling bank differed among treatments. Both the non-standardized and standardized by sample norm RDA were significant by the Monte Carlo test (analyses A3 and A3st in Table 2), with more pronounced treatment effects found in non-standardized analy-

sis (Fig. 5). There are great differences in the eigenvalues corresponding to the first and second ordination axis (0.182 and 0.017). The much stronger first axis suggests a univariate trend in the data. In fact, the treatments form a gradient of disturbance intensity and we could expect that there will be prevailing unidirectional variation among them. The overall pattern is affected mainly by abundance differences between treatments with and without litter layer. The non-standardized RDA showed that species were much more common in the *Sasa*-free plots with litter removal or combined litter+rhizomes removal. However, a significant outcome of RDA standardized by sample norm shows that, when abundance differences among treatments are removed, species proportions are not constant, and seven of the species have higher proportions in plots with *Sasa* and litter (Fig. 6). It appears that the larger-seeded species such as *Quercus mongolica* and *Acer mono* tended to have higher emergence in plots with the untouched litter layer, whereas all of the

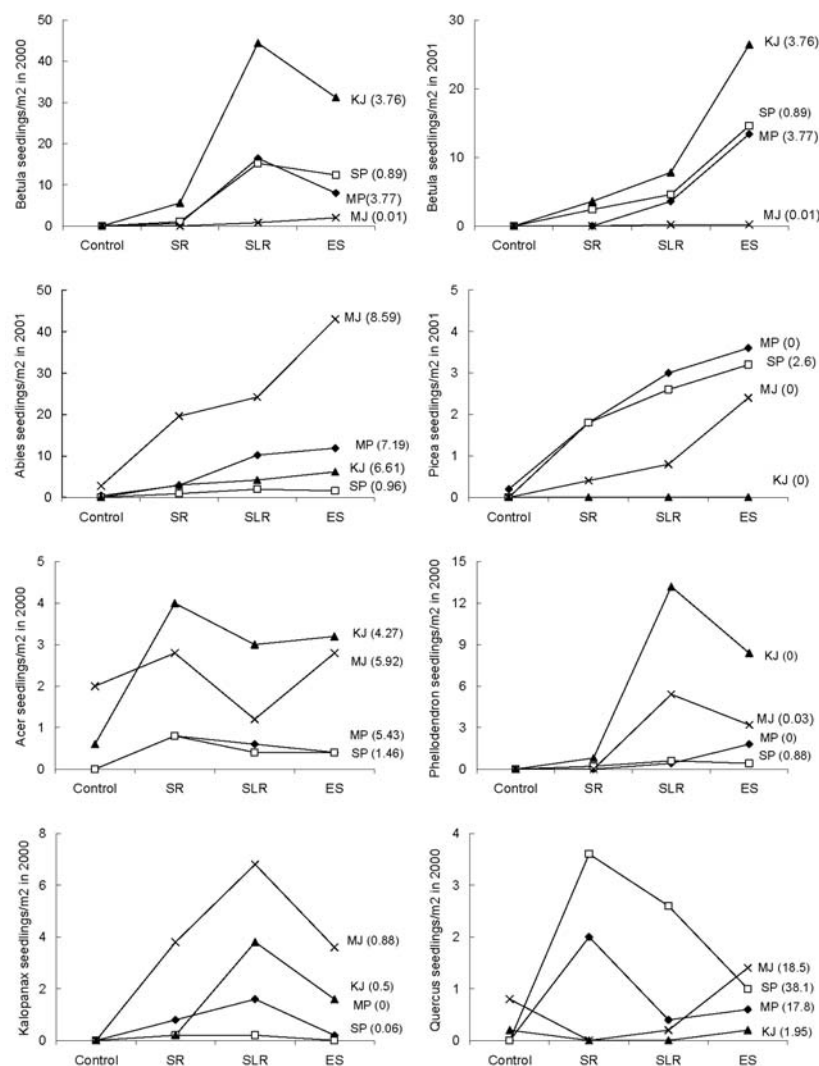


Figure 3. Mean density of emergent tree seedlings of selected woody species in four disturbance treatments (Control – undisturbed *Sasa* bamboo stand, SR – above-ground *Sasa* removal, SLR – above-ground *Sasa* and litter removal, ES – exposed soil) at four sites in Moshiri Forest (Hokkaido, Japan) in 2000 and 2001 seasons. Overstorey basal area (m²/ha) is in parentheses.

Table 2. Summary of results of all RDA analyses performed on seedling data. Both non-standardized and standardized (st) by sample norm RDA were used. Permutation (type of 499 permutations within plots used in Monte Carlo test) reflects the repeated measurement nature of the experiment and the split-plot design. In the column Permutation, we present: permutation on the whole-plot level, permutation on the split-plot level. In A3 to A5st, samples were only permuted within sites, so that between-site variation was excluded from the test. PID: plot identity. r: the species-environment correlation. Sum: sum of all canonical eigenvalues (i.e., explained variability). F all and P all: values of P and F respectively on all ordination axes.

Analysis	Env. Variable	Covariable	Permutation	r	sum	F all	P all
A1	Site (S)		freely, no	0.64	0.17	10.67	0.002
A2	Block (B)	S	freely, no	0.4	0.093	1.11	0.606
A3	Treatment (T)	Y, S	freely, no	0.83	0.202	21.65	0.002
A3st	T	Y, S	freely, no	0.79	0.127	11.23	0.002
A5	Year (Y)	T, S	no, freely	0.67	0.11	26.71	0.002
A5st	Y	T, S	no, freely	0.73	0.11	23.64	0.002
A6	Y*T		freely, freely	0.76	0.356	12.01	0.002
A6st	Y*T		freely, freely	0.75	0.284	8.605	0.002
A7	Y*T	PID, Y	freely, freely	0.76	0.044	5.42	0.002
A7st	Y*T	PID, Y	freely, freely	0.66	0.029	2.94	0.002

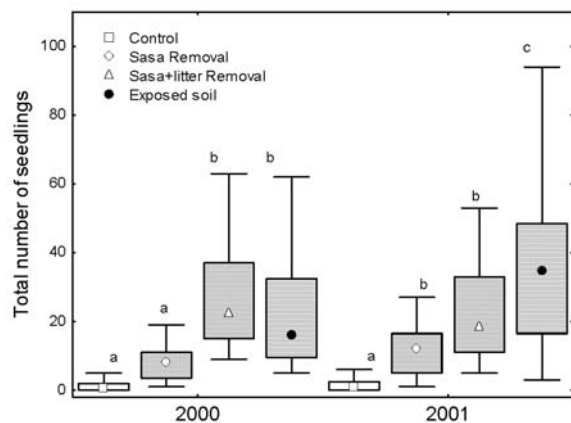


Figure 4. Box and whisker plots of the total number of seedlings in 1 × 1 m plots subjected to various treatments in two years. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 × interquartile range; upper quartile + 1.5 × interquartile range}). Treatments with the same lower-case letters do not differ significantly ($P > 0.05$, Fisher LSD).

smaller-seeded species (*Betula* spp, *Picea glehnii* and *Abies sachalinensis*) exhibited lower establishment in deep litter layer below *Sasa senanensis*.

The effect of understory manipulation on seedling species composition depended on the year. RDAs performed for each year separately, with four experimental treatments as the explanatory variables and sites as covariables, show that treatments in 2000 explained more variability (32.1%, $P = 0.001$) in seedling species composition than in 2001 (23.8%, $P = 0.001$), while the opposite was true for the effect of site, with 20.2% explained variability in 2000 and 28.6% in 2001. If the two years are considered separately, more seedlings emerged in 2001 than in 2000, but nonsignificantly.

There were significant year × treatment interactions, indicating that the effect of year and treatment is not additive. Fig. 6 shows the results of RDA test with the year × treatment interactions as the only explanatory variables and without covariables (corresponding to pooled main and interaction effects in ANOVA and providing the total explained variability, A6 in Table 2). The year × treatment interactions tested alone without main effect (with the year and plot identifier

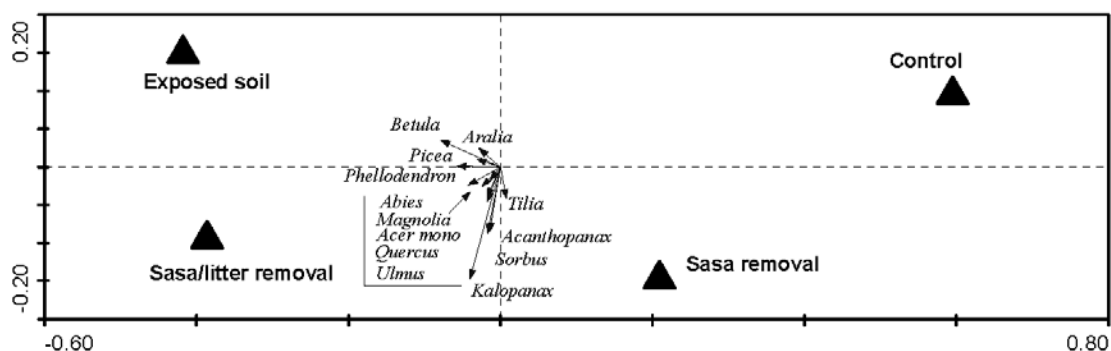


Figure 5. Result of nonstandardized RDA (A3) showing the distribution of seedling species in plots subjected to various treatments. The first and second eigenvalues are 0.182 and 0.017, respectively.

Table 3. Results of repeated measurement ANOVA (*P*-values) for the number of seedlings of ten woody species and the total number of seedlings (Total) at four sites in Moshiri Forest (Hokkaido, Japan). The ANOVA model included three main plot factors (Site, Treatment, and Block nested in Site), Site and Block being the random factors, and the repeated measures factor, Year. Factors in the error term are presented as the first letter of the factor name.

Effect	Df	Effect Error	df Error	<i>Betula</i>	<i>Quercus</i>	<i>Abies</i>	<i>Acer</i>	<i>Picea</i>	<i>Kalopanax</i>	<i>Phellodendron</i>	<i>Magnolia</i>	<i>Acanthopanax</i>	<i>Sorbus</i>	Total
Site (S)	3	B	16	0.000	0.017	0.000	0.002	0.095	0.001	0.000	0.094	0.074	0.218	0.004
Treatment (T)	3	S*T	9	0.002	0.206	0.000	0.043	0.014	0.003	0.028	0.131	0.133	0.115	0.000
Year (Y)	1	S*Y	3	0.007	0.594	0.036	0.420	0.068	0.784	0.041	0.142	0.468	0.564	0.526
S*T	9	B*T	48	0.000	0.001	0.008	0.780	0.136	0.022	0.000	0.510	0.275	0.015	0.029
S*Y	3	B*Y	16	0.609	0.495	0.000	0.001	0.144	0.012	0.139	0.076	0.014	0.073	0.007
T*Y	3	S*B*Y	9	0.025	0.908	0.000	0.248	0.010	0.402	0.006	0.088	0.709	0.677	0.026
S*T*Y	9	B*T*Y	48	0.000	0.137	0.065	0.823	0.157	0.005	0.281	0.923	0.008	0.275	0.319

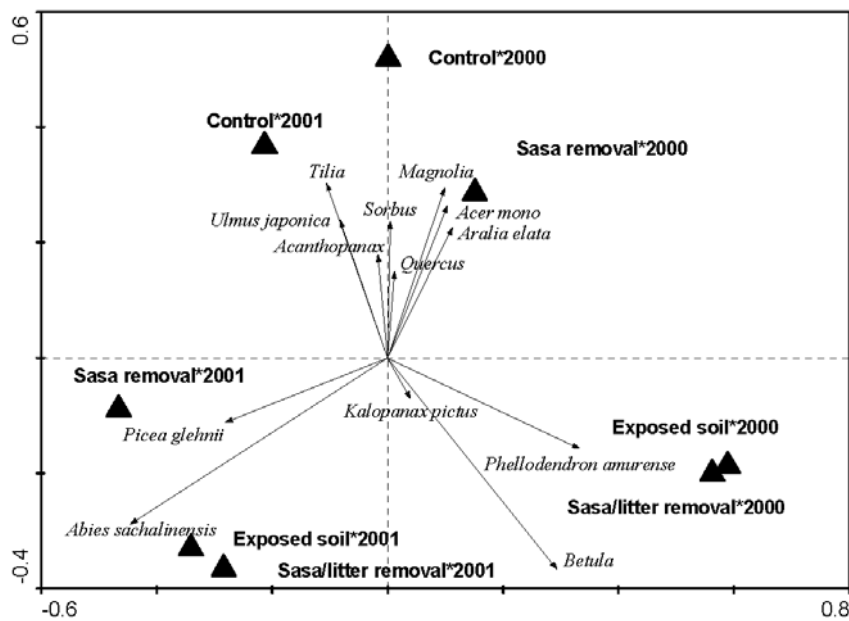


Figure 6. Result of Year × Treatment interaction analysis (A6st, standardized RDA), no covariables used. Results of standardized RDA show differences in the relative proportion among various seedling species rather than differences in seedling numbers. Each centroid (triangle) represents a treatment in a year. The first and second eigenvalues are 0.146 and 0.1, respectively.

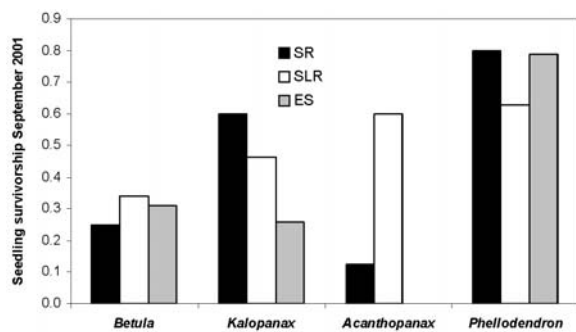


Figure 7. Mean proportion of 2000 seedling cohorts surviving in plots with the aboveground *Sasa* shoots removed (SR), the above-ground biomass plus litter removed (SLR), exposed soil (ES) until September 2001.

used as covariables), were significant in both non-standardized and standardized by sample norm RDA, indicating a shift in species composition between years. *Picea* and *Abies* were much more common in 2001 than in 2000 (Table 3), and their average emergence across all sites was higher on exposed soil. *Acer mono* seedlings were slightly more abundant in the *Sasa* removal plots with litter intact (Fig. 3). *Kalopanax* and *Phellodendron* emergence differed significantly among treatments in both 2000 and in 2001, with higher seedling numbers in the *Sasa*+litter removal treatment. *Quercus* emergence in both years did not show a consistent trend at different sites (significant site × treatment interaction), but averaged across all the sites, it was higher in the *Sasa* removal plots, albeit non-significantly. There was only a marginal effect of the *Sasa* understory manipulation on the emergence of *Acanthopanax* ($P = 0.133$) and *Sorbus* ($P = 0.115$) which consistently exhibited more seedlings in the *Sasa* removal or *Sasa*+litter removal plots. *Betula*

exhibited on average two times more seedlings in 2000 than in 2001, and its preference for microsites shifted between the years. While *Betula* in 2000 showed greater emergence in plots with the *Sasa*+litter removal (53%) rather than on exposed soil (38%), the opposite was true in 2001 with 71% of the seedlings emerging on exposed soil and 21% in the *Sasa*+litter removal plots.

The survivorship of naturally-regenerating tree-seedlings through the 2001 season in *Betula*, *Kalopanax* and *Phellodendron* was comparable between treatments, and neither the time × treatment in the repeated measures ANOVA, nor the direct testing for survival by analysis of deviance was significant (both $P > 0.05$; Fig. 7). This shows that the ratios of surviving seedlings in various treatments remained fairly constant. Only *Acanthopanax* exhibited significantly higher survival in SLR plots compared to other treatments (analysis of deviance, $P < 0.05$).

Discussion

This experimental study shows that nearly continuous cover of *Sasa senanensis* reaching 1.5 - 2 m in height represents the main barrier to tree regeneration, decreasing emergence of the majority of tree species found in the overstory. The seedling establishment was effectively prevented in the untreated control plots and in those where the litter was not removed, hence only severe disturbance that removed the dwarf-bamboo layer, including litter and below-ground rhizomes, led to a significant increase in the number of seedlings. However, the relative proportions of species among the seedlings differed between the microsites with different intensity of bamboo disturbance, indicating the segregation of regeneration niches among species.

Bamboo understory shapes the composition of tree seedling bank

In terms of absolute seedling numbers, natural recruitment in *Sasa*-free areas was dominated by *Betula*, followed by *Abies sachalinensis*, *Phellodendron amurense*, *Acer mono*, and *Kalopanax pictus* (cf. Yoshida et al. 2005). This order seems to reflect the different species life history traits, in particular the ability of seed dispersal. Smaller-seeded species with longer-distance seed dispersal (e.g., *Betula platyphylla* with seed weight of 0.257 mg) were generally more abundant than larger-seeded species (*Acer mono* - 166 mg; *Q. mongolica* - 2980 mg; Seiwa and Kikuzawa 1990, 1996). Moreover, the mechanism by which bamboo reduced emergence was not uniform among tree species: emergence of smaller-seeded species - *Betula*, *Abies* and *Picea* - was reduced primarily by low levels of soil exposure, *Acer mono* appeared to be related to reduced light levels under *Sasa* cover, *Kalopanax* and *Phellodendron* was reduced by high litter production of *Sasa*, and *Quercus* suffered a high level of seed predation. These results support the general hypothesis that plants of the forest understory represent an ecological filter that shapes the composition and organization of the seedling bank through differentially influencing tree species emer-

gence and establishment (George and Bazzaz 1999), thereby controlling the future canopy composition and spatial structure.

The bamboo stratum of the forest understory modifies the abiotic and biotic conditions of the forest floor in several ways. It influences soil nutrients (Tripathi et al. 2005) and moisture (Silva and Okumura 1996), produces a great amount of litter, and intercepts much of the remaining light (Konno 2001). Dwarf bamboos in Japanese beech forests reduce light levels transmitted through the overstory to 10-15% of full sun (Nakashizuka 1987). Under the dense *Sasa tsuboiana* stand relative light intensity was only 1% (Konno 2001). In the present study, the high LAI of *Sasa senanensis* understory with a maximum value of $5.5 \text{ m}^2 \text{ m}^{-2}$ beneath canopy gaps led to very low light levels for newly emergent seedlings and a small number of established seedlings compared to plots where the aboveground part of *Sasa* was removed. In a previous study, Takahashi et al. 2003 reported $2.8 \text{ m}^2 \text{ m}^{-2}$ mean *Sasa* LAI while we found $2.64 \text{ m}^2 \text{ m}^{-2}$ from the same study area.

Sasa also produced a great amount of litter, difficult to penetrate particularly for small-seeded species. Recently, it has been reported that *Sasa kurilensis* reduces the growth of *Betula ermanii* by decreasing soil water availability (Takahashi et al. 2003). Moreover, removal of *Sasa* resulted in increased nitrogen availability to trees (Tripathi et al. 2005). In the present experiment, removal of *Sasa senanensis* led to higher growth and survival of *Quercus* seedlings through increased resource (light, water) availability (cf. Matsuki et al. 2003). After the three growing seasons, the cumulative mortality reached 94.7% under the *Sasa* understory and 61% in the *Sasa*-free plots, the main cause being summer wilting and herbivory in seedlings growing under the *Sasa* understory, whereas herbivory from rodents in seedlings in the *Sasa*-free plots. This shows that understory interference with tree-seedling emergence and survival occurs not only directly through resource competition or micro-environmental modification, but may occur indirectly through the understory's influence on the behavior of seed predators. Wada (1993) and Iida (2004) likewise reported low densities of oak tree seedlings in dense bamboo patches in Japanese temperate forests resulting from the higher rodent densities and acorn removal rates below understory bamboo cover.

The effect of site on seedling emergence pattern

The density of emergent seedlings of each species varied also by site and was at least partially related to differences in site overstory composition and thus to differential seed input. Even *Betula* emergence increased with stand basal area of adult trees. *Betula* was not expected to show a clear relationship due to its ability of long-distance seed dispersal. *Betula* emergence pattern in New England deciduous forest was not related to basal area of *Betula*, but closely followed the pattern of soil exposure (George and Bazzaz 1999). Obviously, birch establishment is favoured when there is an exposed mineral seedbed and an abundant supply of seeds (Doležal et

al. 2004). In Hokkaido, *Betula ermanii* and *B. platyphylla* form often pioneer stands in large openings created after simultaneous death of *Sasa* or after scarification (complete removal of bamboo by bulldozer along with surface soil) which is one of the remediative measures to regenerate forest heavily invaded by bamboo (Yoshida et al. 2005). *Betula* possesses life-history traits to quickly occupy gaps, and bamboo may even amplify its success by reducing advanced regeneration (Taylor and Qin 1988). A large increase in the *Abies faxoniana* and *Betula utilis* seedling bank after bamboo dieback has been documented by Taylor et al. (2004). Similarly, *Betula* seedlings but also *Abies* and *Picea* seedlings increased after bamboo removal and were visibly concentrated in areas of soil exposure. Under dense *Sasa* understory with a deep litter layer, emergence of these species was nearly completely precluded in all sites regardless of expected seed input.

Importance of niche differentiation for species coexistence

Using our experimental approach followed by RDAs, we directly tested the regeneration niche hypothesis proposed by Grubb (1977), which suggests differential establishment of seedlings of various species in various microhabitats. In RDA, there is an important difference between the ecological implications of significant treatment effect in the standardized-by-samples and the non-standardized analyses. The former analysis tests the null hypothesis that there is no effect of the manipulation on seedlings. To reject this hypothesis, it is enough if the total number of seedlings differs between treatments, even if the proportion of individual seedling species remains constant. The latter analysis tests the null hypothesis that the relative proportions of species among the seedlings do not differ between the microsites. The test of the first hypothesis is usually more powerful, but the rejection of the second hypothesis is more ecologically interesting. A significant outcome of standardized-by-sample norm RDA shows that species proportions among microsites were not constant, and seven of the species have higher proportions in plots with *Sasa* and litter cover. The larger-seeded species such as *Quercus mongolica* and *Acer mono* tended to have higher emergence in plots with the untouched litter layer, whereas all of the smaller-seeded species (*Betula*, *Picea glehnii* and *Abies sachalinensis*) exhibited lower establishment in the deep litter layer and preferred exposed soil. The fact that seedlings of different species responded in different ways to particular treatments is a good argument for the importance of the regeneration niche (e.g., for maintenance of species diversity).

Sasa understory effect on long-term forest dynamics

The dense cover and longevity of *Sasa senanensis* bamboos made the continuous recruitment of trees difficult. Once established, bamboos spread vegetatively by belowground rhizomes, and cover the forest floor at high densities (Makita 1997). *Sasa senanensis* is monocarpic species and its simul-

taneous death after mass flowering can episodically provide large vacant space for tree regeneration. High post-flowering tree seedling establishment may influence forest structure and development over longer time scales and increase species diversity. However, synchronous flowering and dieback over large areas is a rare event (Janzen 1976). Hence, the long-term coexistence of many dominant species in the studied mixed-oak forests seems to be maintained by recurring disturbances rather than occasional large-scale *Sasa* dieback (Osawa 1992, Nakashizuka and Iida 1995). It is now increasingly accepted that *Quercus mongolica* forests in Hokkaido experienced periodic disturbances from fires, windstorms, or disturbances triggered by volcanic eruptions during the Holocene that facilitated long-term oak dominance (Iqarashi and Iqarashi 1998, Umeki and Kikuzawa 1999).

The periodic large-scale disturbances caused cyclic forest dynamics (Sano 1997) and were important in retarding the development of more shade-tolerant later successional tree species such as *Acer mono*, *Picea jezoensis* and *Abies sachalinensis* (Kubota 2000). Bamboos are resilient to small-scale disturbances and rapidly colonize small tree-fall gaps. This leads to more open stands but also to greater dominance of shade-tolerant species capable of recruiting under reduced light levels inside bamboo stands or tree islands that remain within "bamboo sea". The long absence of severe disturbance in the studied forests may explain recent invasion by later successional species (*Abies sachalinensis*, *Acer mono*, *A. japonica*) coupled with the lack of recruitment in oak and other light-demanding species. Our experimental data show that small-scale disturbances are more favorable for species with light seed (*Betula*) or shade-tolerant species (*Abies sachalinensis*, *Acer mono*) than dominant *Quercus mongolica*, which needs intense large-scale disturbances of main canopy together with bamboo dieback to successfully regenerate.

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