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Occurrence patterns of facilitation by shade along a water gradient are mediated by species traits

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Abstract

18 In disturbed habitats, shade often has facilitative effects on plants by ameliorating water
19 and thermal stresses. Facilitation by shade tends to increase as water availability decreases.
20 At the same time, several studies have suggested that facilitation by shade is not affected by
21 water status or collapses under extremely dry conditions. We hypothesized that traits of
22 beneficiary plants, specifically, the flexibility in the allocation of biomass between shoots
23 and roots, would mediate variation in the relationship between facilitation by shade and
24 water status. To test this hypothesis, we examined the responses of two bog species to shade
25 under various water conditions in a post-mined peatland. The seeds of *Rhynchospora alba*
26 and *Moliniopsis japonica* were sown under three water levels (dry: 53% peat water content,
27 wet: 77%, and control: 71%) \times two shading levels (50% shaded and unshaded). The
28 survival, biomass, and biomass allocation between the shoots and roots of the two species
29 were monitored for two years. Shade increased the survival and biomass of both species.
30 However, the facilitation of *R. alba* by shade was independent of water level, whereas the
31 strength of the facilitative effects on *M. japonica* increased as water content decreased. *R.*
32 *alba* preferentially allocated biomass to roots under dry conditions and was highly drought
33 tolerant. *M. japonica* did not alter the allocation of its biomass in response to either shade
34 or water level and was drought intolerant. Our results suggest that flexibility in biomass
35 allocation of beneficiary plants mediates occurrence patterns of facilitation by shade along
36 a water gradient. The facilitation of species with inflexible biomass allocation by shade
37 through the amelioration of water stress increases as water availability decreases, whereas
38 the facilitation of species with flexible biomass allocation is independent of water status.
39 Such species-specific facilitation would promote the coexistence of diverse species in a
40 community.

41 **Keywords:** Biomass allocation; Drought; Facilitation; Shade; Post-mined peatland.

42 **1. Introduction**

43 Shade provided by plant canopies regulates understory plant performance and therefore
44 plays a pivotal role in structuring plant communities (Hautier et al., 2009). In addition to
45 increasing the strength of competition through a reduction in light availability, canopy
46 shade facilitates understory plants by ameliorating their water and thermal stresses
47 (Callaway, 1995; Fulbright et al., 1995). Facilitation by shade is particularly important for
48 the establishment of plants in arid, alpine, and disturbed ecosystems characterized by
49 frequent droughts and high ground surface temperatures, and considerable research has
50 been conducted to understand occurrence patterns and their underlying mechanisms
51 (Tewksbury and Lloyd, 2001; Baumeister and Callaway, 2006; Armas et al., 2011). In
52 theory, facilitation by shade becomes stronger at higher levels of drought because the
53 importance of ameliorating water and thermal stresses increases as water availability
54 decreases (Holmgren et al., 1997). Many empirical studies have provided evidence that
55 supports the predictions of this theory (Tewksbury and Lloyd, 2001; Holzapfel et al., 2006;
56 Kikvidze et al., 2006; Seifan et al., 2010). However, contrasting results have also been
57 reported, where facilitation by shade has been found to occur independently of water
58 conditions (Sack and Grubb, 2002; Hastwell and Facelli, 2003; Sack, 2004) or to collapse
59 under extremely dry conditions (Kitzberger et al., 2000; Valladares and Pearcy, 2002;
60 Cavieres and Badano, 2009). Several factors are thought to explain the diverse relationship
61 between facilitation by shade and water condition: the indicators used to evaluate plant
62 performance, life-history stages, and the magnitude of shading (Holmgren and Scheffer,
63 2010; Martínez-Tillería et al., 2012). Nevertheless, the mechanisms underlying variation in
64 the relationship between facilitation by shade and water status are not yet fully understood.

65 Despite its potential importance in modifying the occurrence patterns of facilitation by
66 shade along a water gradient, species-specific adaptation of beneficiary plants to

67 environmental variation has been overlooked in previous studies. Plants respond to
68 environmental variation in various ways; one common way is for plants to alter the
69 allocation of their biomass between shoots and roots (Sultan, 2000). Under water-limited
70 conditions, increasing allocation to root mass is considered to be adaptive because
71 additional root mass improves water capture (Wilson, 1988; Bazzaz, 1996). Species that
72 preferentially allocate biomass to roots under drought are less affected by a decrease in
73 water, and thus, are likely to be tolerant of drought (Bazzaz, 1996). Drought-tolerant
74 species with the ability to flexibly alter biomass allocation require amelioration of water
75 stress by shade less than drought-intolerant species that do not have the ability to alter their
76 biomass allocation. Therefore, we predict that occurrence patterns of facilitation by shade
77 along a water gradient will differ between allocation-flexible and allocation-inflexible
78 species.

79 In this study, we investigated the survival and biomass gain of two bog species that
80 differ in the degree to which they can alter their biomass allocation under experimental
81 shade with various water content levels in a post-mined peatland. Peat mining is one of the
82 most destructive human disturbances in boreal wetlands, from which *Sphagnum* peat is
83 excavated, along with vegetation and litter (Tuittila et al., 2000; Lavoie et al., 2005). While
84 undisturbed *Sphagnum* peatlands do not desiccate, plant establishment in post-mined bare
85 ground is restricted because of exposure to frequent droughts and high temperatures caused
86 by direct sunlight (Campbell et al., 2002; Koyama and Tsuyuzaki, 2010). Therefore,
87 water-related facilitation by canopy shade would play an important role in plant
88 establishment in post-mined peatlands. At our study site, after *Rhynchospora alba* (L.) Vahl
89 (Cyperaceae, the earliest colonizer of bare ground) establishes, the survival of seedlings of
90 con- and hetero-specifics, including *Moliniopsis japonica* (Hack.) Hayata (Poaceae, later
91 dominant), becomes much higher than those grown under bare ground conditions,

92 suggesting that the shade of *R. alba* canopy facilitates their seedling establishment (Egawa
93 and Tsuyuzaki, 2011). Because *R. alba* is the earliest colonizer and expected to be better
94 adapted to high irradiance and water stress than *M. japonica*, *R. alba* is expected to allocate
95 its biomass more effectively under drought than *M. japonica*. We hypothesized that species
96 exhibiting effective biomass allocation under dry conditions by investing more in roots than
97 in shoots (*R. alba*) would be facilitated by shade independently of water conditions,
98 whereas species with less flexible allocation traits (*M. japonica*) would be facilitated by
99 interactions with water status through the amelioration of water stress by shade, and
100 consequently, facilitation would increase when water availability decreased.

101

102 **2. Materials and methods**

103 **2.1. Study site**

104 The study site, Sarobetsu Mire, is located in northern Hokkaido, Japan (45°06' N, 141°42' E,
105 7 m a.s.l.). The mire originally stretched 27 km from North to South and 5–8 km from West
106 to East, with an area of approximately 16,000 ha (Fujita et al., 2009). Between 1970 and
107 2003, *Sphagnum* peat was annually mined from the mire down to a depth of more than 3 m,
108 over an area of 2–22 ha, using a large suction-type peat rig (Nishimura et al., 2009). The
109 mining was conducted in a total area of more than 235 ha, and the former vegetation, litter,
110 and seed bank in the area were completely removed along with the peat. After the
111 extraction of high quality peat, residues were returned to the original locations. The
112 post-mined surface was covered with open water soon after mining and subsequently the
113 peat residue floated up and formed bare ground. Similar to other mined peatlands in
114 northern Europe and Canada, the post-mined bare ground at our study site is characterized
115 by harsh environments including frequent droughts, high ground surface temperatures, and
116 high winds, restricting plant establishment (Koyama and Tsuyuzaki, 2010). However, over

117 the course of more than 20–40 years, the bare ground is gradually replaced by *R. alba*
118 grassland, and eventually *M. japonica* grassland, usually within 10–20 years (Nishimura et
119 al., 2009).

120 The present study was conducted in 2010 and 2011. The mean annual temperature was
121 6.8 °C (minimum = –6.0 °C in February, and maximum = 21.9 °C in August) in 2010, and
122 5.6 °C (minimum = –7.6 °C in January, maximum = 20.8 °C in August) in 2011 (Japan
123 Meteorological Agency, 2011). The snow-free period is usually from mid-April to
124 mid-November. Total precipitation during the growing season from May to August was 492
125 mm in 2010 and 275 mm in 2011. Strong winds blow at the study site; the maximum wind
126 velocity during the snow-free period averaged 13.2 m/s with a peak gust of 27.1 m/s in
127 2010 and 12.4 m/s with a peak gust of 25.0 m/s in 2011. The seedlings of most species
128 emerge during late May and late June (Egawa and Tsuyuzaki, 2011), and the seeds are
129 dispersed in autumn until early November (Egawa et al., 2009).

130

131 **2.2. Seed sowing experiment**

132 **2.2.1. Species and seed sources**

133 Two perennial herbaceous species, *R. alba* and *M. japonica* were used for the seed sowing
134 experiment. *R. alba* has narrow, tubal leaves and stems and develops turf. *M. japonica* has
135 flat, long, tapering leaves and robust stems and forms dense tussocks. The height of mature
136 plants averaged 24 cm and 45 cm for *R. alba* and *M. japonica*, respectively (Egawa,
137 unpublished data). The seeds of the two species were collected from more than 50
138 individuals at the study site in autumn 2009 and were kept in paper bags at room
139 temperature until use. Before sowing, the seeds were cold stratified for one month at 2 °C in
140 the dark. The seed germinability of each species was assessed with 10 replicates of 50 seeds
141 placed on moistened filter paper (Whatman #1) in Petri dishes (90 mm in diameter) in an

142 incubator at 5 °C/25 °C (12 h/12 h) under 12-h of white fluorescent light (23 $\mu\text{mol}/\text{m}^2/\text{sec}$)
143 until no germination was recorded for more than two weeks. The seed germination rates
144 averaged 63.2% for *R. alba* and 70.8% for *M. japonica*, demonstrating that the seeds were
145 adequate for use in the seed sowing experiment.

146

147 **2.2.2 Experimental design**

148 On 18 May 2010, we established ninety-six 50 × 50 cm plots at 1-m intervals in a 16 ×
149 6-lattice rectangular frame on bare ground mined in 1972. Sixteen plots were randomly
150 assigned to each of six treatments in a factorial (three water levels) × (two shading levels)
151 design. Three water levels were created by manipulating the ground surface level: mounds
152 of up to 5-cm height, pits down to 5-cm depth, and unmanipulated controls. The ground
153 surface layer to a depth of 5 cm at all water levels was disturbed equally to ensure the
154 texture was uniform. Because the groundwater level averaged 10 ± 5 cm below the
155 unmanipulated surface during June and August (Nishimura et al., 2009), the mounds were
156 never saturated with ground water, but the pits were sometimes inundated. The
157 manipulation of water level sometimes alters microbial community structure and nutrient
158 conditions in peat surface layers in the long term (Jaatinen et al., 2007). However, the
159 period of our experiment was relatively short (two years) and the effects of the
160 manipulation of water level on peat nutrient conditions were likely to be small. Hereafter,
161 mounds and pits are termed dry and wet treatments, respectively. Two shading levels,
162 shaded and unshaded, were created using black sheer nets. Because sheer nets allow
163 shading levels to be manipulated accurately and to separate below-ground competition that
164 can mask aboveground effects of shade, the nets are widely introduced in experiments
165 exploring the mechanisms of facilitation by canopy shade (e.g., Seifan et al., 2010;
166 Semchenko et al., 2012). The rate of light transmission by the sheer nets was 50% of solar

167 radiation, which is equivalent to the shading rate of the *R. alba* canopy (Egawa and
168 Tsuyuzaki, 2011). Structures with their tops and sides covered by sheer nets (60 × 60 × 30
169 cm) were fixed at four corners with bamboo stakes and plastic strings at each shading plot.
170 The bottom of the sheer nets was placed 5 cm from the peat surface to allow wind and
171 water transfer. The plants did not touch the top or sides of the nets during the study period.
172 The sheer nets were expected to reduce the ground surface temperature by shading. In
173 addition, although our shading structures allowed the free movement of wind, the plants in
174 the nets most likely experienced reduced wind. Therefore, the shade treatment changed not
175 only light intensity but also several other aboveground environments in a way similar to
176 natural plant canopies, which are also known to reduce ground surface temperature and
177 wind speed.

178 To assess how the topographical manipulation altered water availability, volumetric
179 water content in the peat was monitored at a 1-cm depth for each water level at 1-h intervals
180 from 27 June to 23 September in 2010 using moisture sensors with 5-cm probes (ECH₂O
181 EC-5, Decagon Devices Inc., Pullman, Washington, USA). Light intensity and temperature
182 at the peat surface were measured in three shaded and unshaded plots, with no
183 topographical manipulation, at 1-h intervals from 4 June to 23 September 2010, by using
184 light/temperature data loggers (HOBO pendant logger MAN-UA-002-64, Onset Computer,
185 Bourne, Massachusetts, USA). The light data (lux) obtained were calibrated using
186 photosynthetically active radiation (PAR, $\mu\text{mol}/\text{m}^2/\text{sec}$).

187

188 **2.2.3. Seed sowing and sampling of plants**

189 We preliminary investigated seedling density under natural *R. alba* canopies at ten
190 randomly established 10 × 10 cm plots. Seedling density was 6.7 ± 0.6 (mean ± SE) per 100
191 cm^2 for *R. alba*, and 2.9 ± 0.4 for *M. japonica*. Based on this natural seedling density, we

192 set the number of seeds sown at eight per 100 cm² for *R. alba* and five for *M. japonica*.
193 This approach allowed us to increase samples size while keeping the low natural density to
194 minimize the risk of intraspecific competition. We sowed the seeds of both species in
195 monoculture to avoid interspecific competition that could affect the outcomes of the
196 experiment, although below-ground competition between *R. alba* and *M. japonica*
197 seedlings is weak due to differences in the root depth of seedlings (Egawa and Tsuyuzaki,
198 2011). Accordingly, the seeds of each species were sown under a factorial (three water
199 levels) × (two shading levels) design with eight replicates.

200 We used a 40 × 40 cm area in each plot for seed sowing, and 5 cm of the edges of the
201 50 × 50 cm plots were not used to avoid edge effects. We divided the 40 × 40 cm area in
202 each plot into sixteen 10 × 10 cm cells. On May 31, 2010, either eight *R. alba* or five *M.*
203 *japonica* seeds were sown at 3-cm intervals in each cell. The sown seeds were covered by
204 white sheer nets (10 × 10 cm) for the first two weeks to prevent scattering and
205 contamination of naturally dispersed seeds. The number of plants in each plot was counted
206 at 1-week intervals from June to September 2010 and at 1-month intervals from May to
207 August 2011. We randomly selected eight out of 16 cells in each plot, and excavated all
208 plants in the selected cells in mid-August 2010 (first summer). Plants in the remaining eight
209 cells in each plot were retained intact until the excavation during the same season in 2011
210 (second summer). Plants excavated in the first summer and the second summer were termed
211 first-year plants and second-year plants, respectively. The excavated plant samples were
212 kept in plastic bags and immediately transported to our laboratory. The plants were
213 separated into shoots, roots, and reproductive organs. Each sample was dried at 70 °C for 48
214 hours in an oven and weighed.

215

216 **2.3. Statistical analyses**

217 To explore differences in daily mean peat water content among the three water levels, a
218 non-parametric Friedman's repeated measures ANOVA followed by a Scheffe post-hoc test
219 was conducted because the variance was not homogenous. We used repeated measures
220 ANOVA to test differences in daily mean PAR and peat surface temperature between
221 shaded and unshaded plots. Differences in germination rates among treatments were tested
222 using a non-parametric Kruskal-Wallis test followed by a Steel-Dwass post-hoc test.

223 We investigated changes in plant survival, total biomass, and biomass allocation
224 between shoots and roots (termed shoot to root [shoot:root] ratio) in response to shade and
225 water treatments using generalized linear mixed models (GLMM). We established five
226 competing hypotheses assuming differential effects of shade and water on plants; plant
227 performances (survival, total biomass, and shoot:root ratio) were affected (1) by both shade
228 and water treatments, and the effects of shade varied depending on water level, (2) by both
229 shade and water treatments, but their effects were independent of each other, (3) by only
230 shade, (4) by only water, and (5) neither by shade nor water treatment. Our objective in
231 GLMM analyses was to explore which hypothesis was best supported by observations. For
232 plant survival models, we used a binomial error distribution with a logit-link function. The
233 response variable was the plant survival rate through to the second summer in a cell. We
234 only used survival data of plants on the cells that remained undisturbed until the second
235 summer. For the total biomass models, we applied a gamma distribution with a log-link
236 function. The response variable was total biomass of a plant, which is the summed biomass
237 including all shoots, roots, and reproductive organs. The biomass analysis was conducted
238 separately for the first-year and second-year plants of each species. For the shoot:root ratio
239 models, a Gaussian distribution with an identity-link function was applied. The response
240 variable was shoot:root ratio calculated by dividing dry shoot mass by dry root mass. As
241 well as the biomass analysis, the shoot:root ratio analysis was separately conducted for the

242 first-year and second-year plants of each species. In all GLMM analyses, full models
243 included the following fixed factors: shade with two levels (shaded and unshaded), water
244 with three levels (wet, dry, and control), and the number of conspecific plants in the cell
245 (termed plant density). Plant density was included to reduce any confounding effects of
246 intraspecific interactions on the outcomes of the experiment, although the low density we
247 set would have already minimized the intraspecific competition. The number of plants
248 germinating in the cell was used as plant density for survival, the first-year biomass, and
249 the first-year shoot:root ratio models, and the number of overwintering individuals was
250 used for the second-year biomass and shoot:root ratio models. Interaction terms between
251 each pairs of fixed factors were also included in the full models. Plots were included as a
252 random factor. We conducted stepwise model selection based on Akaike's Information
253 Criterion (AIC) to detect the best models. Model selection approaches are particularly
254 useful when several competing hypotheses are simultaneously confronted with data
255 (Johnson and Omland, 2004). This approach allowed us to identify the best supported
256 hypothesis regarding the effects of shade and water treatments on plant performance. All
257 analyses were performed using the R statistical package (version 2.12.1, R Development
258 Core Team, 2010).

259

260 **3. Results**

261 **3.1. Environmental conditions for each treatment**

262 The topographical manipulation significantly altered peat water content (Friedman's
263 repeated measures ANOVA, $\chi^2_2 = 163.1$, $P < 0.001$). Daily mean water content during June
264 and September 2010 was $52.5 \pm 0.9\%$ (mean \pm SE) on the mounds (dry) and $76.6 \pm 0.3\%$ in
265 the pits (wet), both of which were significantly different from the control ($70.4 \pm 0.7\%$)
266 (Scheffe post-hoc test, $P < 0.001$). The water content on the mounds was low and highly

267 variable depending on precipitation, whereas it was high and stable in the pits (Fig. 1). The
268 peat surface on the mounds was sometimes cracked because of severe drought, whereas the
269 surface of the pits was sometimes inundated. Shading significantly reduced the PAR on the
270 peat surface (repeated measures ANOVA, $F_{1,111} = 464.7$, $P < 0.001$; Fig. 2). The shading
271 treatment also significantly reduced peat surface temperature (repeated measures ANOVA,
272 $F_{1,111} = 275.0$, $P < 0.001$) which averaged 1.4 °C lower in the shaded plots than in the
273 unshaded plots (Fig. 2).

274

275 **3.2. Plant density and survival**

276 Seed germination for both *R. alba* and *M. japonica* began two weeks after sowing and was
277 completed within one month. The germination rates significantly differed among treatments
278 for both species (Kruskal-Wallis test, $\chi^2_5 = 118.6$ and $P < 0.001$ for *R. alba* and $\chi^2_5 = 17.3$ and
279 $P = 0.004$ for *M. japonica*). For both *R. alba* and *M. japonica*, the germination rate tended
280 to increase as water content increased (Table 1), indicating that initial plant density was
281 higher with greater levels of water content.

282 Two species showed different survival responses to the shade and water treatments
283 (Table 1). The GLMM showed that the survival of *R. alba* was enhanced by shade, but not
284 affected by water level (Table 2). This indicated that facilitation of *R. alba* survival by
285 shade occurred independently of water level. The GLMM also revealed that plant density
286 positively affected *R. alba* survival. In contrast to *R. alba*, shade facilitated the survival of
287 *M. japonica* by interacting with water level, and the facilitative effect of shade increased as
288 the water content decreased (Table 2). In the dry treatment, the survival rate through to the
289 second summer of *M. japonica* was less than 4% in unshaded plots and improved to 23.4%
290 in shaded plots (Table 1). Plant density did not affect *M. japonica* survival (Table 2),
291 indicating that the reduced survival under dry × unshaded condition resulted directly from

292 drought stress rather than from intraspecific competition for water.

293

294 **3.3. Total biomass**

295 The differences in total biomass among treatments were larger in *M. japonica* than in *R.*

296 *alba* in both the first and second years (Fig. 3). The biomass of first-year *R. alba*

297 individuals was affected by water level and plant density, while the effects of shade were

298 not detected (Table 2). Plant density positively affected the biomass of first-year *R. alba*,

299 and the positive effect of plant density increased as water content decreased. The biomass

300 of second-year *R. alba* individuals was increased by shade, water content, and plant density

301 (Table 2). The effects of shade and water on second-year *R. alba* were independent of each

302 other. The biomass of *R. alba* increased from the first year to the second year under all

303 treatments, although the 2-year biomass increment was greatest under wet conditions and

304 smallest under dry conditions (Fig. 3). More than 20 second-year *R. alba* individuals

305 reached the reproductive stage in the control and wet plots, and two individuals reached this

306 stage in dry plots. The biomass of first- and second-year *M. japonica* individuals was

307 determined by shade, water level, and their interaction. In both years, shade increased the

308 biomass of *M. japonica* more in dry conditions than in wet and control conditions (Table 2).

309 Plant density positively affected the biomass of *M. japonica* in both the first and second

310 years (Table 2), indicating that intraspecific interactions were facilitative throughout the

311 study period. The biomass of *M. japonica* individuals decreased from the first year to the

312 second year in the control and dry plots, particularly when shade was not provided (Fig. 3).

313 No *M. japonica* individuals reproduced over the two years of growth.

314 No damage by herbivores was observed to leaves and stems throughout the growing

315 season for both species, indicating that the effect of herbivores on plant biomass was

316 negligible.

317

318 **3.4. Shoot:root ratio**

319 Contrary to total biomass, differences in shoot:root ratio among treatments were larger in *R.*
320 *alba* than in *M. japonica*, particularly during the first year (Fig. 4). In the first year, *R. alba*
321 altered the shoot:root ratio depending on the degree of shade, water level, and their
322 interaction, as well as in response to plant density (Table 2). Shoot:root ratio of the
323 first-year *R. alba* decreased with a decrease in water content, suggesting that this species
324 invested more in roots than in shoots during drought. In addition, first-year *R. alba*
325 increased investments to shoots under shade, and shoot:root ratio was highest under wet ×
326 shaded condition (Fig. 4). During the second year, biomass allocation of *R. alba* was
327 determined only by the water level. During both years, *R. alba* individuals invested more in
328 roots than shoots when the water content decreased. *M. japonica* did not change the
329 shoot:root ratio in response to the degree of shade or water level during both the first and
330 second years (Table 2). This finding suggests that *M. japonica* is not able to flexibly alter its
331 biomass allocation between shoots and roots.

332

333 **4. Discussion**

334 The results of our experiment clearly demonstrated that the occurrence patterns of
335 facilitation by shade along a water gradient differed between species with different abilities
336 to flexibly alter their biomass allocation. Facilitation of *R. alba* that allocated more to root
337 mass as water availability decreased was independent of water status, while *M. japonica* did
338 not alter biomass allocation in response to drought and was facilitated by the interaction of
339 shade and water level. Semchenko et al. (2012) also reported that the occurrence patterns of
340 facilitation by shade differed between species with different biomass allocation traits. The
341 authors found that the growth of species that allocated more to roots in full compared with

342 50% daylight were least facilitated by shade in temperate grasslands. Although flexibility in
343 biomass allocation of beneficiary plants has not previously been recognized as a factor
344 dictating the occurrence patterns of facilitation by shade, such species-specific adaptations
345 to environmental variation has certainly played an important role in modifying facilitative
346 interactions in plant communities.

347 Facilitation of *R. alba* independent of water availability may be explained by two
348 possible mechanisms. On the one hand, shade ameliorated stress in *R. alba* proportionally
349 across water levels. This finding has been reported in several other studies (Baruch et al.,
350 2000; Sack and Grubb, 2002; Hastwell and Facelli, 2003; Sack, 2004). In our experiment,
351 shade reduced the ground surface temperature by 1.4 °C and may have reduced air
352 temperature. Reduced soil and air temperatures generally depress plant evapotranspiration
353 and contribute to the maintenance of leaf water potential (Valiente-Banuet and Ezcurra,
354 1991; Larcher, 2003), which can ultimately release plants from water stress. In the case of *R.*
355 *alba* that can adjust to various water conditions by altering its biomass allocation to roots,
356 the magnitude of water stress may not have differed among water levels, and the
357 importance of reduced temperatures by shade may have been nearly the same at all water
358 levels. On the other hand, facilitation of *R. alba* may not be related to the amelioration of
359 water stress. Instead, the shading nets might provide protection from wind. Plant canopies
360 often protect understory plants from wind (Smith et al., 2003; Baumeister and Callaway,
361 2006) because wind can physically damage plants and negatively affect their growth and
362 physiological performance (Ennos, 1997). At our study site, strong winds with gusts of up
363 to 27 m/s occurred during the study period (Japan Meteorological Agency, 2011). These
364 wind conditions likely caused more damage to *R. alba* with their narrow leaves and stems
365 than to *M. japonica* with robust shoots. Our shading nets should have reduced the impact of
366 wind on the plants in a manner similar to what may occur in natural plant canopies. Thus,

367 wind-related facilitation may have occurred independently of water level. In addition to
368 wind, plant canopies are known to protect plants from herbivores (García and Obeso, 2003;
369 Baraza et al., 2006). However, in our experiment, the effect of herbivores on the plants was
370 negligible for both *R. alba* and *M. japonica*, and therefore, protection from herbivores is a
371 factor that is unlikely to have contributed to the observed patterns of facilitation.

372 For *M. japonica*, the strength of facilitation by shade increased as water content
373 decreased, and this pattern was consistent with how theory predicts facilitation by shade to
374 occur along a water gradient (Holmgren et al., 1997). This result indicates that the most
375 important benefit provided by shade for *M. japonica* was improvement of plant water
376 relations. As we expected, *M. japonica* was drought-intolerant; when shade was not
377 provided, the survival rate of *M. japonica* was less than 4% in dry conditions, while in wet
378 conditions, the survival rate was greater than 60%. The drought-intolerance of *M. japonica*
379 is likely attributed to their poor ability to alter their biomass allocation. Because *M.*
380 *japonica* did not increase root mass under dry conditions, this species likely failed to
381 capture enough water and suffered from strong water stress. The temperatures reduced by
382 shade should have alleviated water stress for *M. japonica*, particularly in dry plots.

383 While our experiment was designed to assess the effects of shade in the absence of
384 below-ground competition, root competition between canopy and understory plants is
385 capable of modifying occurrence patterns of facilitation by shade under natural conditions.
386 For instance, canopy plants sometimes engage in competition for water with understory
387 plants, particularly in dry conditions (Valladares and Pearcy, 2002; Valladares et al., 2008).
388 The competition for water between canopy and understory plants in dry conditions can
389 reduce the benefits of canopy shade, and as a result, may mask the effects of aboveground
390 facilitation by shade during drought (Holmgren et al., 2012). Further studies are required to
391 quantify how below-ground competition between canopy and understory plants affects the

392 occurrence patterns of facilitation by shade along a water gradient, and how
393 species-specific adaptations of beneficiary plants modify these patterns.

394 In our experiment, positive interactions among understory plants were detected for
395 both species. In stressful ecosystems, positive density dependence in juvenile plants is often
396 observed, most likely because the risk of physical damages decreases with an increase in
397 neighboring plants (Anderson, 2009; Fajardo and McIntire, 2011). This mechanism likely
398 explains our results because strong winds that were capable of damaging plants occurred at
399 our study site. However, intraspecific interactions among understory plants may become
400 competitive beyond the timeframe of our experiment because the strength of competition
401 generally increases with the growth of plants (Weigelt et al., 2002). Competition among
402 understory plants may mask the facilitative effects by shade across all levels of water
403 content.

404

405 **5. Conclusion**

406 Our results emphasize that there is a strong relationship between the occurrence patterns of
407 facilitation by shade and the flexibility of biomass allocation of understory plants. The
408 facilitation of drought-intolerant species that cannot flexibly alter biomass allocation by
409 shade through the amelioration of water stress increases with a decrease in water
410 availability, whereas the facilitation of drought-tolerant species with flexible allocation
411 appears to be independent of water status. These species-specific facilitation patterns may
412 play an important role in permitting the coexistence of diverse species in a community.

413

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422

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535 **Table 1**

536 Germination rate and survival rate from germination to mid-August 2010 (the first summer), April 2011 (overwintering), and mid-August
 537 2011 (the second summer) under a factorial (three water levels) \times (two shading levels) design. Mean values are shown with SE. Different
 538 letters on germination rate indicate significant differences at $P < 0.05$ (Steel-Dwass post-hoc test). For results of survival analysis, see Table
 539 2.

Treatments	<i>Rhynchospora alba</i>				<i>Moliniopsis japonica</i>			
	Germination (%)	Survival (%) to			Germination (%)	Survival (%) to		
		mid-Aug 2010	April 2011	mid-Aug 2011		mid-Aug 2010	April 2011	mid-Aug 2011
Wet \times unshaded	66.9 \pm 4.6 ^{ab}	78.6 \pm 2.3	51.1 \pm 3.8	45.4 \pm 4.0	68.1 \pm 2.8 ^{abc}	85.1 \pm 2.0	62.0 \pm 4.2	61.2 \pm 4.2
Wet \times shaded	74.8 \pm 4.4 ^a	96.6 \pm 0.8	65.3 \pm 3.8	61.6 \pm 3.8	69.7 \pm 3.6 ^{ab}	90.5 \pm 1.7	73.5 \pm 3.6	73.0 \pm 3.7
Control \times unshaded	53.7 \pm 4.8 ^c	91.7 \pm 1.5	73.2 \pm 3.7	41.0 \pm 4.6	65.6 \pm 3.2 ^{abcd}	84.0 \pm 2.2	47.2 \pm 4.0	17.6 \pm 3.1
Control \times shaded	61.0 \pm 5.8 ^{bc}	96.5 \pm 1.0	70.5 \pm 4.4	57.6 \pm 4.9	60.3 \pm 5.9 ^{cd}	95.7 \pm 1.3	54.6 \pm 4.5	31.9 \pm 3.7
Dry \times unshaded	43.3 \pm 4.6 ^d	84.0 \pm 2.3	57.2 \pm 4.9	38.8 \pm 5.0	59.5 \pm 3.2 ^d	80.3 \pm 2.4	41.1 \pm 4.2	3.7 \pm 1.4
Dry \times shaded	37.8 \pm 5.9 ^d	96.1 \pm 1.2	85.4 \pm 3.2	69.6 \pm 4.6	65.5 \pm 4.1 ^{bcd}	96.5 \pm 1.0	64.5 \pm 3.9	23.4 \pm 3.8

540 **Table 2**

541 Effects of shade, water level, and plant density on the survival, total biomass, and shoot:root
 542 ratio of *Rhynchospora alba* and *Moliniopsis japonica* examined using GLMM. First year and
 543 second year indicate the age of the plants. The fixed factors in the best model based on the
 544 AIC are shown. Coefficients for shade, water level, interaction between shade and water, and
 545 interactions between plant density and shade and water were calculated with respect to the
 546 base levels shown by (0). “–” indicates a factor not selected in the best models.

Fixed factors	<i>Rhynchospora alba</i>					<i>Moliniopsis japonica</i>				
	Survival	Biomass		Shoot:root ratio		Survival	Biomass		Shoot:root ratio	
		First year	Second year	First year	Second year		First Year	Second Year	First year	Second year
Intercept	-0.893	1.746	2.866	6.369	6.623	-1.766	2.038	1.947	1.745	0.814
Shade		–			–				–	–
Unshaded	(0)		(0)	(0)		(0)	(0)	(0)		
Shaded	0.986		0.127	1.867		1.024	0.110	0.080		
Water level	–								–	–
Control		(0)	(0)	(0)	(0)	(0)	(0)	(0)		
Wet		0.435	0.109	2.612	0.052	2.293	0.253	0.447		
Dry		0.016	-0.298	-1.549	-1.667	-1.497	0.005	-0.247		
Plant density	0.106	0.044	0.081	0.213	–	–	0.027	0.092	–	–
Shade × Water level	–	–	–		–				–	–
Control × Shaded				(0)		(0)	(0)	(0)		
Wet × Shaded				1.690		-0.506	-0.173	0.065		
Dry × Shaded				0.417		0.936	0.187	0.952		
Water level × Plant density	–		–		–	–	–	–	–	–
Control × Plant density		(0)		(0)						
Wet × Plant density		-0.081		-0.369						
Dry × Plant density		0.005		0.136						
Shade × Plant density	–	–	–		–	–	–	–	–	–
Unshaded × Plant density				(0)						
Shaded × Plant density				-0.209						

547

548 **Legends of figures**

549 **Fig. 1.** Seasonal fluctuations in (A) water content in peat exposed to three topographical
550 levels (wet = pit, dry = mound, and control), and (B) precipitation from June to September
551 2010.

552
553 **Fig. 2.** Daily mean (A) photosynthetically active radiation (PAR; $\mu\text{mol}/\text{m}^2/\text{sec}$) and (B) peat
554 surface temperature ($^{\circ}\text{C}$) on shaded and unshaded plots. The bottom and top of each box show
555 the first and third quartile, respectively, and the bold lines in the middle indicate median values.
556 Bottom and top ends of horizontal lines represent the minimum and maximum values within
557 the normal data range from the first quartile minus $1.5 \times$ the interquartile distance (IQD) to the
558 third quartile plus $1.5 \times$ IQD, respectively. Outliers are indicated by open circles. Asterisks
559 represent significant differences between unshaded and shaded conditions at $P < 0.001$
560 (repeated measures ANOVA).

561
562 **Fig. 3.** Total biomass, the sum of the shoots, roots, and reproductive organs, of the first-year
563 and second-year plants of (A) *Rhynchospora alba* and (B) *Moliniopsis japonica* grown under a
564 factorial (three water levels) \times (two shading levels) design. The parenthetical number above
565 each column indicates the number of reproducing individuals. For an explanation of box plots,
566 see Fig. 2.

567
568 **Fig. 4.** Shoot:root ratio for the first-year and second-year plants of (A) *Rhynchospora alba*
569 and (B) *Moliniopsis japonica* grown under a factorial (three water levels) \times (two shading
570 levels) design. For an explanation of box plots, see Fig. 2.

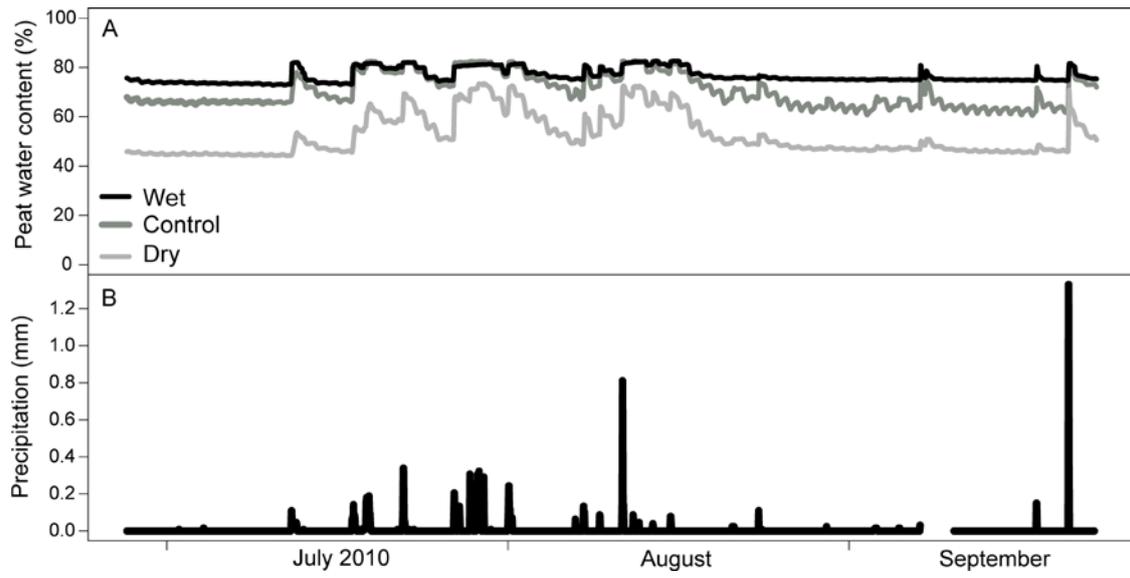


Fig. 1.

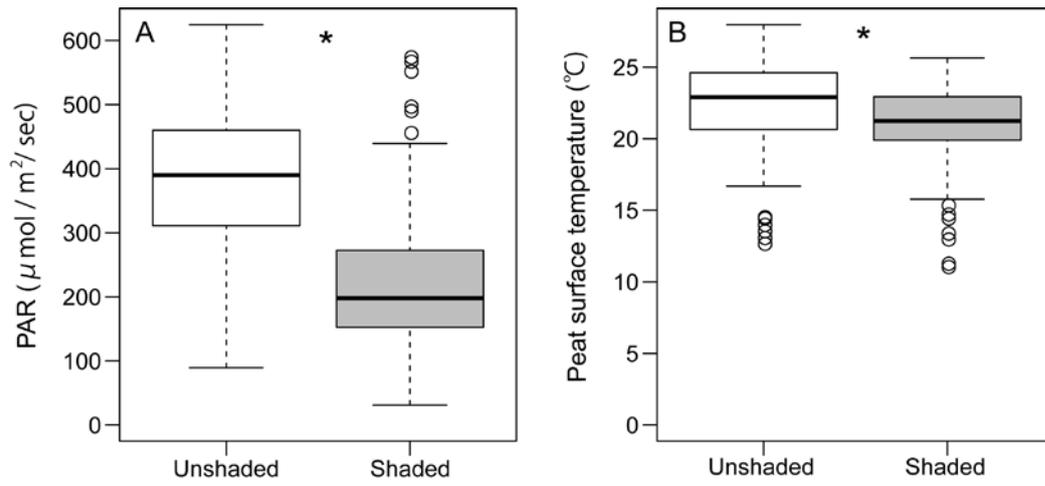


Fig. 2.

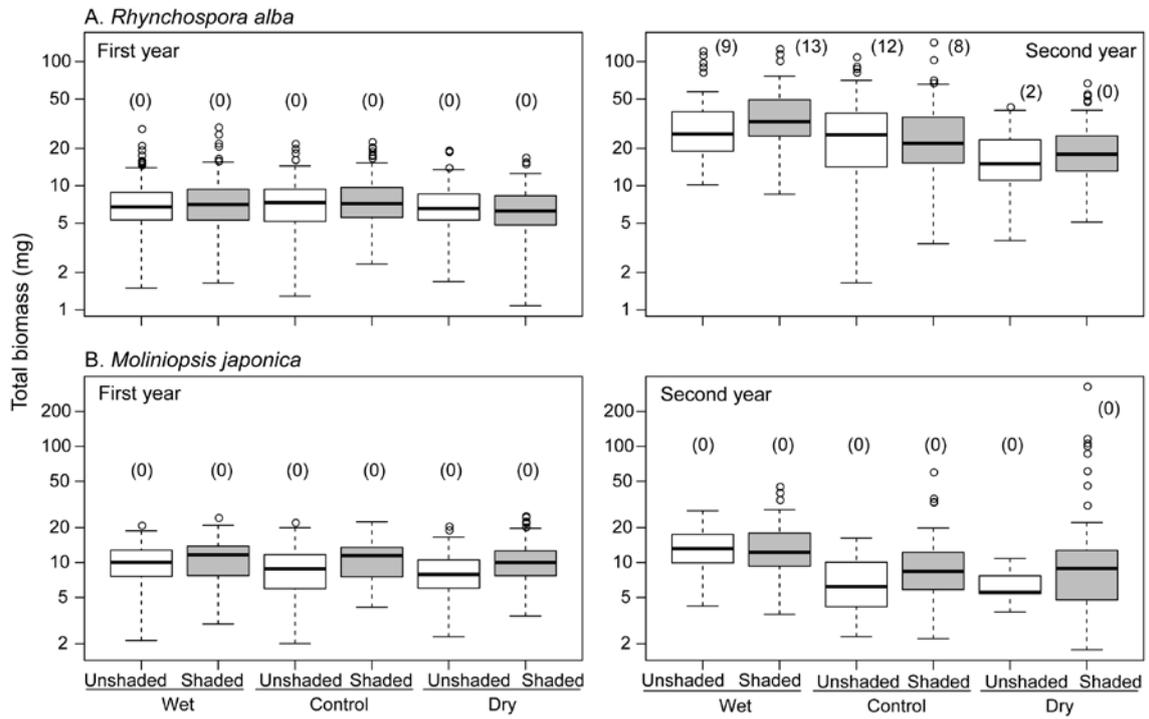


Fig. 3.

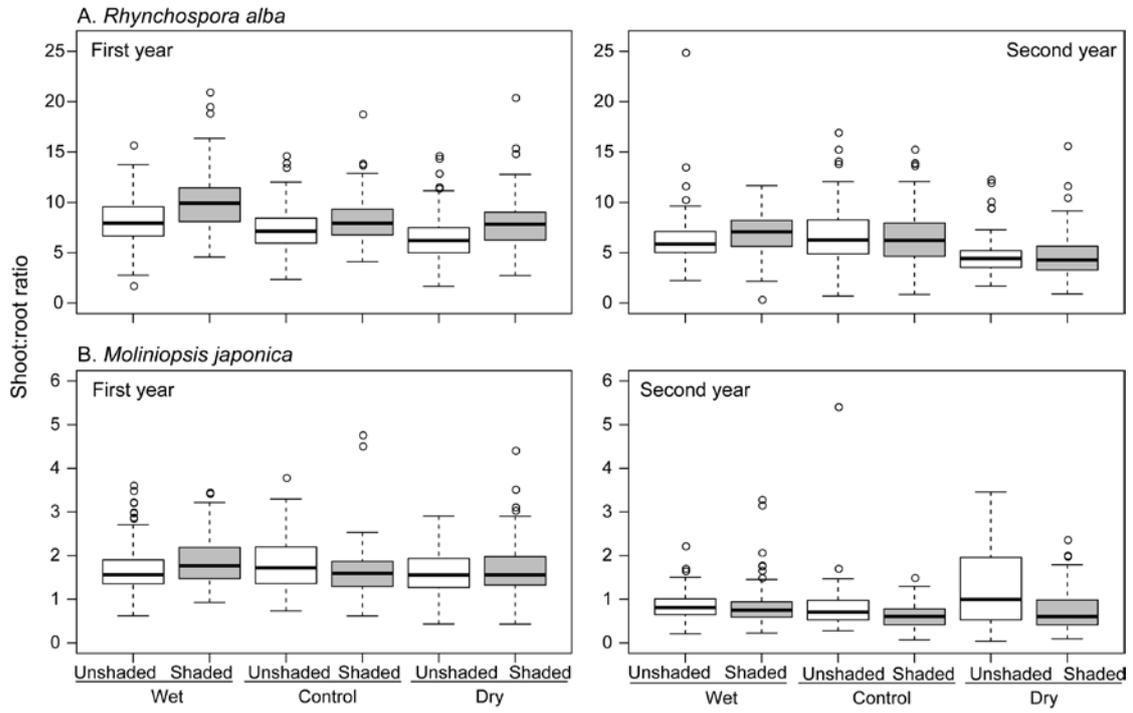


Fig. 4.