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6 **Synergistic effects of primates and dung beetles on soil seed accumulation in snow**
7 **regions**

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17

18 **Abstract**

19 This study aimed to reveal the soil seed accumulation processes for endozoochorous
20 plants in the heavy-snowfall forests of Japan, where seed dispersal agents are few when
21 compared to tropical forests. We assessed (1) primary seed dispersal by Japanese
22 macaques (*Macaca fuscata*) by identifying dispersed seeds found in their feces, and (2)
23 secondary seed dispersal by dung beetles by using beads (as seeds mimics) of different
24 sizes, to quantify the frequency of seed burial and burial depths. We studied this
25 diplochorous system in different forest types (undisturbed beech forest, conifer
26 plantation, and secondary beech-oak forest) and during the spring and summer seasons.
27 The key findings were as follows: (1) macaques dispersed the seeds of 11 and 14 plant
28 species during spring and summer, respectively; (2) seeds dispersed by macaques in the
29 spring were smaller and twice as abundant than those dispersed in the summer; (3)
30 although no differences were observed in the amount of beads buried by beetles
31 between seasons, all bead sizes tended to be buried in deeper soil layers in the spring
32 than in the summer; and (4) the seed supply to the soil in undisturbed beech forest and
33 conifer plantation was greater than the one in secondary beech-oak forest. Similar to
34 what has been observed in tropical forests, seeds defecated by frugivorous mammals
35 can be successfully incorporated into the underground soil seed bank through a
36 diplochorous macaque-beetle system in temperate forests of deep snow regions.

37

38 **Key words** Biological interaction · Conifer · *Fagus crenata* · *Macaca fuscata* ·
39 Scarabaeinae Seed dispersal

40

41 **Introduction**

42 When investigating the demography of a plant, we tend to focus only on the shoot
43 system, the plant body that is aboveground, because of the constraints of our visibility.
44 However, some plants possess a much larger population as seeds in the soil seed bank
45 compared to the population of stems in an area (Cook 1980; Fenner 1985; Silvertown
46 and Charlesworth 2001). This difference becomes more pronounced for early
47 successional or ruderal plants, which usually have longer seed longevity than late
48 successional or mature forest species (Bossuyt and Hermy 2001; Decocq et al. 2004).
49 The abundance and species composition of soil seeds has a marked influence not only
50 on the dynamics of plant populations and communities, but also on the resilience of
51 ecosystems to natural catastrophes (Bakker et al. 1996; Hopfensperger 2007).

52 Formation of a soil seed bank for endozoochorous plant species requires primary
53 seed dispersal by vertebrates in order to escape the area of high seed mortality near the
54 parent plant, and may be further facilitated through secondary seed dispersal by other
55 animals that transport seeds to sites that are safe from seed predators (Vander Wall and
56 Longland 2004, 2005; Dalling 2005; Feer et al. 2013). Rodents and granivorous ants are
57 known to act as secondary seed dispersers because of their scatter-hoarding behavior,
58 but they are also recognized as seed predators (Forget 1996; Retana et al. 2004, Vander
59 Wall et al. 2005; Koike et al. 2012a). Other ant types do not eat the seeds, but they
60 collect only elaiosome-bearing seeds, in particular those that have been dispersed by
61 birds (Böhning-Gaese et al. 1999; Vander Wall et al. 2005). Dung beetles (Coleoptera:
62 Scarabaeinae), on the other hand, have been identified as being superior secondary seed
63 dispersers for mammal-defecated seeds. Dung beetles transport many seeds

64 underground where they are safe from seed predation; these insects are commonly rich
65 in biomass among decomposer organisms throughout temperate and tropical regions
66 (Andresen and Feer 2005; Nichols et al. 2008).

67 Dung beetle diversity and biomass have been shown to be highly sensitive to
68 changes in mammal fauna (resource [feces] providers) both in tropical biomes (Hanski
69 and Cambefort 1991; Nichols et al. 2009), and in temperate forests of heavy snowfall
70 regions (Enari et al. 2013). The latter type of ecosystem supports mammals in limited
71 abundance and species richness, resulting in corresponding limited biomass and
72 diversity of beetles when compared to those in warmer forests (Hanski and Cambefort
73 1991; Davis et al. 2002; Enari et al. 2011, 2013). These facts logically indicate that the
74 agents contributing to multistep seed dispersal systems, such as diplochory (Vander
75 Wall and Longland 2004, 2005) may be limited in regions that experience heavy snow,
76 which might lead to low functional redundancy for sustaining seed dynamic processes
77 for plant species dispersed by frugivorous mammals.

78 With the exception of a few studies conducted in temperate zones—grassland
79 (D'hondt et al. 2008) and forests with light snowfall (Koike et al. 2012b)—all of the
80 studies focusing on the ecology and behavior of dung beetles as part of a diplochorous
81 system have been conducted in the tropics (Andresen and Feer 2005; Nichols et al.
82 2008). As a result, we have little knowledge of the ecological processes involved in the
83 formation of a soil seed bank for plants with seeds dispersed through endozoochory in
84 ecosystems with deep snow and limited agents of seed dispersal. This study intended to
85 evaluate the diplochorous system consisting of primary seed dispersal by Japanese
86 macaques (*Macaca fuscata*) and secondary seed dispersal by dung beetles in the

87 Shirakami Mountains, which are located in one of the heaviest snowfall regions in the
88 world. The macaque is a primate species adapted for survival in cold and heavy snow
89 conditions (Enari 2014) and is regarded as both a key seed disperser (Otani 2010; Tsuji
90 et al. 2011) and resource provider for dung beetles (Enari et al. 2011, 2013). Compared
91 to fruiting phenology in the tropics, that in temperate forests drastically varies with the
92 the seasons, resulting in rapid fluctuations in fruit availability for animals (Hanya et al.
93 2013). Moreover, the pronounced changes in weather conditions among seasons results
94 in restriction of the active period of most dung beetles, especially in deep snow regions
95 where they appear mainly between June and September (Enari et al. 2011, 2013). The
96 exact duration of their activity period also depends strongly on the type of forest cover
97 (Enari et al. 2011). Thus, in this study we discuss the ecological features of the
98 diplochorous system in terms of the influences of seasonality and forest type, as well as
99 its net effect on the local ecosystem process.

100 **Methods**

101 **Study area**

102 We conducted the current study in the northeastern part of the Shirakami Mountains,
103 at the northern end of mainland Japan (central coordinates = 40°32'9"N, 140°14'43"E).
104 The study area has a cool-temperate climate with heavy snowfall from mid-November
105 to late March, with snow cover reaching depths of 2 m in the low-lying flatlands and >4
106 m in montane regions. Lingering snow is usually observed until mid-May, meaning that
107 the soil surface is covered by snow for half of the year. The southwestern side of the
108 study area falls within the Shirakami Mountains World Heritage Site (designated by
109 UNESCO), where undisturbed forests of beech (*Fagus crenata*) occur. In contrast,

110 anthropogenically disturbed broadleaf forests of beech and oak (*Quercus crispula*),
111 because of fuel wood productions, and conifer plantations (Japanese cedar, *Cryptomeria*
112 *japonica*) are irregularly distributed in the northeastern side of the study area. According
113 to the weather station at the Shirakami-sanchi World Heritage Conservation Center, the
114 mean air temperature and the total precipitation are $18.4 \pm 1.7^{\circ}\text{C}$ (SD) 92.8 ± 21.4 mm
115 in June, and $23.6 \pm 3.8^{\circ}\text{C}$ and 185.6 ± 67.5 mm in August, respectively (c.f., annual
116 mean, $10.0 \pm 1.3^{\circ}\text{C}$ and $1,679.8 \pm 262.3$ mm).

117 Japanese macaques are distributed throughout the study area. In 2008, individual
118 and troop densities in and around the study area were 5.2 individuals km^{-2} and 0.2 troop
119 km^{-2} , respectively (Enari and Sakamaki 2011). The area is inhabited by five
120 medium-sized or large frugivorous/omnivorous mammals other than macaques: *Martes*
121 *melampus*, *Mustela itatsi*, *Nyctereutes procyonoides*, *Meles anakuma*, and *Ursus*
122 *thibetanus*. Among these, *U. thibetanus* is widely known as an effective seed disperser
123 in terms of dispersal quantity and dispersal distance (Koike et al. 2008, 2011), but in
124 deep snow regions its feces are less attractive to dung beetles compared to macaque
125 feces (Enari et al. 2013).

126 Of the 152 dung beetle species that have been recorded for the Japanese
127 archipelago (Kawai et al. 2005) the authors have confirmed the presence of 15 species
128 in the Shirakami Mountains (Enari et al. 2011, 2013). Dung beetles are usually divided
129 into three functional groups according to nesting strategy and dung-relocation behavior:
130 dwellers, tunnellers, and rollers (Cambefort and Hanski 1991). Only the latter two
131 groups bury feces in tunnels underground (Estrada and Coates-Estrada 1991; Feer 1999).
132 In the Shirakami Mountains six tunneller species that use macaque feces are present

133 (*Onthophagus ater*, *O. nitidus*, *O. fodiens*, *O. atripennis*, *Phelotrupes auratus*, *P.*
134 *laevistriatus*), but no rollers that use macaque feces exist (Enari et al. 2011, 2013). The
135 abundance of most species is notably high in spring regardless of forest type (Enari et al.
136 2011).

137 **Primary seed dispersal by macaques**

138 To collect fresh feces we captured three adult female macaques from different
139 troops in the study area using box traps. All individuals were fitted with radio collars
140 (Advanced Telemetry Systems, Isanti, Minnesota, USA; weight = 120 g, approximately
141 1% of the average of the three females' body mass) and released. All manipulations
142 were in accordance with the Wildlife Protection and Hunting Law of Japan. We
143 followed the movement of each troop and collected fresh feces during spring (June–July
144 2007 and June–July 2008) and summer (August–September 2007). We washed each
145 sample with running water through a 0.5-mm mesh sieve to extract the seeds and
146 identified each seed species following Nakayama et al. (2006).

147 **Secondary seed dispersal by dung beetles**

148 During spring and summer of 2012 we evaluated secondary dispersal by dung
149 beetles of seeds in macaque feces, in three forest types where the seasonal compositions
150 of existing dung beetle assemblages had been previously investigated (Enari et al.
151 2011): undisturbed mature beech forests with a closed canopy (age of *ca.* 140 y), conifer
152 plantations with a closed canopy (*ca.* 40 y) and secondary beech-oak forests with an
153 open canopy (*ca.* 50 y). The soil type at all sites was brown forest soil. We measured
154 soil hardness, which may influence the ability of beetles to bury feces, 60 times at each
155 site using a Yamanaka soil hardness tester (Fujiwara Scientific Co., Ltd., Tokyo, Japan).

156 All measurements were taken on the same day. We found soil hardness values of $1.99 \pm$
157 3.37 (SD) kg cm^{-2} in undisturbed beech forests, 1.29 ± 2.19 kg cm^{-2} in conifer
158 plantations, and 1.60 ± 0.85 kg cm^{-2} in secondary beech-oak forests—there was no
159 significant difference among sites (One-way ANOVA, $F_{2, 177} = 1.29$, $P = 0.28$).

160 To assess seed burial by dung beetles we used fresh macaque feces collected by
161 following the troops, as mentioned previously. After removing dung beetles found on
162 the feces, we homogeneously mixed all of the collected feces and prepared 15-g fecal
163 piles, the mean weight of macaque feces in the Shirakami Mountains (Enari et al. 2011).
164 We then placed ellipsoidal plastic beads, used as seed mimics, of three sizes into each
165 fecal pile: 10 large beads (length [L] = 6.1 mm, thickness [T] = 5.9 mm), 15 medium
166 beads (L = 4.1 mm, T = 3.9 mm), and 30 small beads (L = 2.2 mm, T = 1.4 mm). The
167 size and abundance of beads used were chosen according to what is naturally found in
168 macaque feces in cool-temperate forests (Tsuji et al. 2011). Next, we set the
169 bead-containing fecal piles (with a total of 55 beads each) along a 40-m transect at 10-m
170 intervals (i.e., five fecal piles). One transect was used in each site, on two dates: June 22,
171 2012 and August 16, 2012. To protect the experimental fecal piles from natural
172 disturbances (such as rain and rodents), we covered each pile with a wire cage (30 cm \times
173 20 cm \times 20 cm; 1-cm mesh size) with a roof. The mesh size used allowed free passage
174 of all dung beetles observed in cool-temperate forests of Japan (Koike et al. 2012a).
175 After one month we collected soil samples within a radius of 10 cm from each fecal pile.
176 Samples were collected at 5 cm increments at depths between 0 and 30 cm, i.e., from
177 Layer 0 (0 – 5 cm depth) to Layer 5 (25 – 30 cm). The maximum depth was determined
178 according to Koike et al. (2012b), who reported that dung beetles can burrow to a depth

179 of up to 29 cm in temperate forests. Some beads might have been buried simply by
180 falling into natural soil crevices. Therefore, we considered only beads found in Layers 1
181 – 5 as having been buried through dung beetle activity. We recovered beads from every
182 soil sample using 1-, 3-, and 5-mm mesh sieves and compared the mean percentages of
183 beads buried in the different soil layers at each forest type and between seasons.

184 **Data analyses**

185 To identify areal and seasonal differences of primary seed dispersal by macaques,
186 we compared the frequency of macaque defecations found in the different forest types
187 by using Bonferroni z statistic (Neu et al. 1974) and the weight of macaque feces and
188 the abundance of seeds in the feces in spring vs. summer by using Mann–Whitney U
189 test. We also evaluated the abundance of seeds of different sizes in spring vs. summer
190 by using Welch's t test. We then quantified secondary seed dispersal by dung beetles on
191 the basis of the frequency of seed burial and burial depth. To analyze the frequency of
192 seed burial, we compared the percentage of beads buried by beetles (i.e., beads found in
193 layers 1 to 5) in the three forest types by using a significance test for multiple
194 comparisons of proportions (Ryan 1960). To reveal seasonal trends in burial depths of
195 beads with respect to bead size, we compared the abundance of beads buried in different
196 soil layers by using the Steel–Dwass multiple comparison test (Steel 1960). To assess
197 the validity of these multiple comparisons under the current sampling design, we
198 calculated the achieved statistical power (i.e., $1-\beta$ error probability) for each
199 significance test by using the post-hoc power analysis with effect size f ($\alpha = 0.05$;
200 Cohen 1988). We performed all these procedures by using R ver. 3.0.2 (R Core
201 Development team).

202 **Results**

203 **Seed dispersal by macaques**

204 We collected 88 fecal samples in spring, of which 18.2% were from beech forest;
205 22.7%, from conifer plantation; 34.1%, from secondary broadleaf forest; and the
206 remaining mainly from riverbeds. We collected 81 fecal samples in summer, of which
207 24.7% were from undisturbed beech forest; 16.0%, from conifer plantation; 30.9%,
208 from secondary broadleaf forest; and the remaining mostly from the edges of farmlands.
209 The frequency of macaque defecations in the different forest types showed no
210 significant difference during both spring (Bonferroni z statistic: n.s., $\chi^2 = 4.7$, $z = 2.1$)
211 and summer (n.s., $\chi^2 = 3.7$, $z = 2.1$). The mean weight of macaque feces was similar in
212 both seasons (spring: 12.8 ± 12.5 g, $N = 88$; summer: 12.4 ± 8.1 g, $N = 81$;
213 Mann–Whitney U test: $z = -1.42$, $P = 0.15$). While we did not observe a seasonal
214 difference in the percentages of seed occurrence in the feces (86.3% in the spring and
215 86.4% in the summer), mean seed abundance per feces was significantly different:
216 106.0 ± 215.9 seeds in the spring vs. 53.8 ± 102.4 in the summer (Mann–Whitney U
217 test: $z = 2.25$, $P = 0.02$).

218 In the feces collected during the spring, we found seeds of 11 plant species
219 (Appendix 1). Among the observed species (excluding those that we were unable to
220 identify to the species level) were two tall trees, five shrubs, and one liana. In the feces
221 collected during the summer we found 14 seed species. Excluding the three species that
222 were identified only up to family level, we found seeds from two tall trees, three shrubs,
223 and six lianas.

224 While most seeds found during spring were smaller than the size of the medium

225 beads (i.e., 4.1 mm), seeds found in the summer varied more in size (Table 1; Appendix
226 1). All seed species found in macaque feces in both seasons, with the exception of two
227 species, were shade-tolerant or early successional species (Forestry Development
228 Technological Institute 1985; Satake et al. 1993; Appendix 1) whose seeds are
229 commonly found in persistent soil seed banks (Nakagoshi 1985; Thompson 1992)
230 surviving >1 year (Mizui 1993).

231 **Secondary seed dispersal by dung beetles**

232 The disappearance of the fecal piles during the study period was 100% for every
233 experimental fecal pile, both in the spring and summer seasons. The percentages of
234 beads that we were able to recover were $90.7 \pm 10.6\%$ in the spring and $94.7 \pm 8.1\%$ in
235 the summer for large beads, $81.8 \pm 15.3\%$ in the spring and $85.8 \pm 13.7\%$ in the summer
236 for medium beads, and $72.9 \pm 18.7\%$ in the spring and $86.7 \pm 11.0\%$ in the summer for
237 small beads.

238 In total, dung beetles buried 28.5% and 39.4% of the experimental beads (based on
239 recovered beads) in soil layer >5-cm deep during spring and summer, respectively.
240 However, the percentage of beads buried by beetles showed different trends among
241 forest types depending on season (Table 2). In spring, bead burial was significantly
242 higher in undisturbed beech forests, regardless of bead size (Ryan's multiple
243 comparisons test; $P < 0.05$), followed by conifer plantations. In summer, bead burial in
244 undisturbed beech forests were not always the highest, while burial in conifer
245 plantations were constantly high for every bead size (Table 2). According to the
246 post-hoc power analysis, the value for every achieved statistical power was more than
247 0.8, which supports the validity of the current sampling design for these comparisons

248 (Cohen 1988).

249 The mean percentage of beads buried in the different soil layers varied seasonally
250 (Fig. 1). This evaluation led to the following findings: (1) regardless of forest type and
251 season, larger beads were rarely buried in deeper soil layers; (2) although dung beetles
252 tended to disperse beads in relatively shallow soil layers (mostly Layer 1) during the
253 summer, they could bury beads in deeper soil layers during the spring; and (3) the trend
254 of (2) became more obvious for medium and small beads in both undisturbed beech
255 forests and conifer plantations, which was supported by the Steel–Dwass multiple
256 comparison test. When showing the results of the power analysis, most values of the
257 achieved statistical power for large beads was less than 0.50, meaning a deficiency in
258 sample size or low effect size, which was inferred from the fact that most large beads
259 were found in Layer 0 (i.e., surface soil).

260 **Discussion**

261 **Soil seed accumulation process**

262 The current findings show that in our study region, a region with heavy snow and
263 limited seed dispersal agents, seeds defecated by macaques are highly likely to be
264 incorporated into the underground soil seed bank through secondary dispersal by dung
265 beetles. In fact, the percentage of beads buried by dung beetles in our study is similar to
266 values reported in several studies conducted in the tropics (range = 25–52%; see
267 Chapman 1989; Andresen and Feer 2005 and references therein). Further, the
268 percentage of seed burial by beetles we estimated is likely to be an underestimate of the
269 real value, given our conservative inclusion criterion (i.e., only beads found at depths >5
270 cm were treated as beads buried by dung beetles). On the other hand, it should also be

271 noted that our methods removed the influence of granivorous animals, and thus we are
272 presenting seed burial by dung beetles in the absence of seed removal by other biotic
273 factors. Seed removal by granivorous animals, in particular rodents, however, might
274 have a limited influence on seed burial by beetles in our study site, because large beetles
275 (*Phelotrupes* spp.), which have been shown to have a superior ability for burying seeds
276 in the temperate forests of Japan (Koike et al. 2012b), have the same daily rhythm as
277 macaques (i.e., diurnal; Enari et al. 2011), whereas most rodents are nocturnal. This
278 indicates that *Phelotrupes* spp. probably find and bury feces before the risk of seed
279 predation by rodents increases (Koike et al. 2012b).

280 Studies have repeatedly reported that the percentage of smaller seeds buried by
281 beetles is higher than that of larger seeds (Estrada and Coates-Estrada 1991; Shepherd
282 and Chapman 1998; Feer 1999; Andresen 1999, 2002; Andresen and Levey 2004;
283 Andresen and Feer 2005; Pouvelle et al. 2009; Feer et al. 2013). Moreover, several
284 studies conducted in the tropics demonstrated that seed burial depth is also inversely
285 correlated with seed size (Shepherd and Chapman 1998; Andresen 2002; Pouvelle et al.
286 2009; Feer et al. 2013). The current findings are consistent with those previous works.
287 Considering that the seeds dispersed by macaques in the spring are smaller but twice as
288 numerous as seeds dispersed in the summer (Table 1), the present findings suggest that
289 seed supply to the soil through the plant-macaque-beetle interaction might be higher in
290 the spring than in the summer.

291 Regarding the vertical soil layer distribution of buried seeds, there seems to be a
292 difference between tropical forests and the deep snow forests of Japan. While previous
293 studies in the tropics have shown that most seeds buried by dung beetles are found at

294 depths < 10 cm (Shepherd and Chapman 1998; Andresen 2001, 2002; Andresen and
295 Levey 2004), our study results show that beetles in the spring frequently buried seeds in
296 deeper soil layers (>10 cm) regardless of seed size (Fig. 1). Yet, this comparison should
297 be made with care, considering that seed sizes included in tropical studies tend to be
298 larger, on average, than those used in our study.

299 Besides the relatively small seed sizes used in our study, the observed burial depths
300 might also be partly explained by the seasonal abundance of beetles in this region,
301 where the timing of the occurrence of most beetles overlaps during a brief period, i.e.,
302 June (Enari et al. 2011, 2013). We also note that this seasonal prevalence is more
303 obvious for dweller species (Enari et al. 2011). Dwellers do not directly bury feces
304 underground but may change the shape and consistency of the feces, which might
305 promote dung and seed burial by tunnellers.

306 Although there was no remarkable difference in the percentage of bead burial
307 between spring and summer (Table 2), all bead sizes tended to be buried more shallowly
308 during the summer (Fig. 1). This, again, may be explainable in terms of the seasonal
309 abundance of different dung beetles species, in particular tunneller species, the size of
310 which is positively correlated with the size of the seeds that they can bury underground
311 (Feer 1999; Andresen 2002). In our study region, every tunnellers emerge in spring.
312 Among them, small tunnellers (*Onthophagus* spp.), which can bury seeds up to depths
313 of 10 cm (Koike et al. 2012b), are active in summer, while large tunnellers (*Phelotrupes*
314 spp.) with deep burial ability of up to 30 cm (Koike et al. 2012b) are less common in
315 summer, except in conifer plantations with closed canopy (Enari et al. 2011; Enari H
316 2014, unpublished data).

317 The consequences of burial for seed fate naturally vary with burial depth. Seeds
318 buried in deeper soil layers are least vulnerable to predation by rodents (Janzen 1982;
319 Estrada and Coates-Estrada 1991; Andresen 1999; Andresen and Levey 2004). Similary
320 one experiment conducted in temperate forests reported that seeds buried deeper than 2
321 cm are rarely detected by rodents (Koike et al. 2012b). Thus, it may be reasonable to
322 suppose that the burial activity of beetles in the summer still generates sufficient
323 benefits for seeds in terms of the avoidance of predation risk. On the other hand, burial
324 depth also influences seed longevity. Seed longevity in storage generally lengthens with
325 lower temperatures and moisture contents (Roberts 1972; Murdoch and Ellis 1992);
326 therefore, seeds buried deeper in the ground commonly have longer longevity (Toole
327 and Brown 1946; Dalling et al. 1998), although this physiological trait may vary with
328 species and habitat conditions. Unfortunately, there have been few empirical studies to
329 quantify the relationships between seed burial depth and longevity in temperate forests,
330 so it remains to be tested whether the storage environment of seeds buried in the
331 summer is suitable for long-term survival.

332 The current study did not directly focus on seed dispersal in autumn because it is an
333 inactive season for dung beetles. Although macaques still act as primary seed dispersers
334 during autumn (Otani 2010; Tsuji et al. 2011), dispersed seeds are probably under a
335 higher risk of predation. Such predation risk, however, might be diminished by the
336 presence of snowfall in late autumn. Given that macaque feces containing seeds of
337 autumnal fruits are preserved under snow cover until the next spring, those autumnal
338 feces possibly are buried underground by spring-emerging beetles (Enari H and
339 Sakamaki-Enari H 2013, unpublished data).

340 **Forest type differences in soil seed input**

341 The composition of dung beetle assemblages is highly sensitive to forest type and
342 habitat disturbance in the tropics (Vulinec 2000; Andresen 2003; Andresen and Feer
343 2005) and also in heavy snow regions (Enari et al. 2011). Our results suggest that soil
344 seed input varies with forest patches of different landscapes when dung beetle
345 assemblages change (Enari et al. 2011), even if those patches are geographically close
346 (Fig. 1; Table 2). Such local variations in seed supply could be driven by macaque
347 habitat use or its feces supply, which determines not only the primary seed input but
348 also the biomass of dung beetle serving as seed buriers. In fact, similar primate–beetle
349 chain effects have also been confirmed in the tropics (Vulinec et al. 2006; Pouvelle et al.
350 2009; Feer et al. 2013).

351 Not only the current vegetation, but also historical land use generally determines
352 the constituents of soil seeds in a subject area (Bossuyt and Hermy 2001). However,
353 such legacy effects tend to fade within 50 years after the formation of an existing stable
354 landscape because of the longevity of buried seeds (Bossuyt and Hermy 2001). This
355 means that the richness of soil seeds in climax forests observed in the heavy snow
356 regions—i.e., beech forests with few woody plants producing persistent soil
357 seeds—becomes depleted without a new seed supply (excluding beech) from outside the
358 forest patch. Our results (Fig. 1; Table 2) suggest that fresh seeds transported by
359 macaques (Appendix 1) constantly accumulate as soil seeds in climax forests.

360 Conifer plantations have often been dismissed in terms of biodiversity conservation
361 because of the monotone landscape they create (Hunter 1990). Moreover, considering
362 that approximately 40% of conifer plantations in Japan are >50 years old (according to

363 the public database from the Forestry Agency of Japan), a strong legacy effect on soil
364 seed accumulations can no longer be expected. The current experiments regarding
365 primary and secondary seed dispersals demonstrate that macaques provide stable soil
366 seed inputs even in conifer plantations, especially during the summer (Table 2; Fig. 1).
367 Unlike other large mammals in the region, macaques repeatedly use such plantations as
368 an alternative habitat, especially for their sleeping sites, throughout the year (Sakamaki
369 and Enari 2012; Enari and Sakamaki-Enari 2013, 2014). A study conducted in the
370 tropics shows a similar cascading phenomenon, referred to as the latrine effect
371 (Pouvelle et al. 2009). This study showed that forest areas used as sleeping sites by
372 primates (i.e., defecation areas) possess abundant seeds and high seed diversity (in
373 particular, small seeds belonging to pioneer tree species) in the soil. Unfortunately, the
374 details of the latrine effect are yet to be investigated in cool-temperate forests. However,
375 this effect might be a key ecosystem function of the processing-chain commensalism
376 beginning with macaques by supporting future generations of diverse organisms in
377 plantations.

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Figure legends

Fig. 1 Seasonal variation in the percentage (mean \pm SE) of bead buried in soil layers of different depths: Layer 1, 5-10 cm; Layer 2, 10-15 cm; Layer 3, 15-20 cm; Layer 4, 20-25cm; Layer 5, 25-30 cm. Asterisks above the bars show significant differences among layers according to the Steel-Dwass test, $P < 0.05$. The values between parentheses are achieved statistical power (i.e., $1 - \beta$ error probability), calculated by the post-hoc power analysis ($\alpha = 0.05$), and effect size f

Table 1 Differences in the length of seeds observed in Japanese macaque feces in the Shirakami Mountains, Japan

	Seeds	Large (> 4.1 mm) ^a	Small (< 4.1 mm)	Welch's <i>t</i> test
Spring (<i>N</i> = 88)	Abundance (/feces) ^b	25.5 ± 33.1	80.3 ± 219.3	<i>t</i> = -2.32, <i>P</i> = 0.02
	Number of species	3	> 8 ^c	NA
Summer (<i>N</i> = 81)	Abundance (/feces) ^b	19.5 ± 26.9	34.0 ± 103.7	<i>t</i> = -1.22, <i>P</i> = 0.22
	Number of species	> 7	> 7	NA

^aMean length of seeds according to Nakayama et al. (2006). Threshold value was determined based on the length of medium beads

^bMean ± standard deviation (SD)

^cThe symbol ">" denotes the class containing unidentified plant seeds

Table 2 Influence of forest type on bead burial (> 5 cm burial depth) by dung beetles during spring and summer, for large, medium and small beads used as seed mimics (see text for bead sizes). Forest types are: undisturbed beech forest (BF), conifer plantation (CP) and secondary beach-oak forest (SF).

		Large beads			Medium beads			Small beads		
		BF	CP	SF	BF	CP	SF	BF	CP	SF
Spring	% beads buried ^a	37.2 ^{+b}	13.3	10.4	55.8 ^{+b}	21.9	17.6	47.8 ^{+b}	25.0	25.0
	(1- β^c ; Effect size <i>f</i>)	(0.86; 0.99)			(1.00; 1.52)			(0.99; 1.37)		
Summer	% beads buried ^a	11.1	36.2 ^{+b}	4.0	61.0	45.2	19.4 ^{-b}	56.0	60.8	27.8 ^{-b}
	(1- β^c ; Effect size <i>f</i>)	(1.00; 1.91)			(1.00; 1.82)			(1.00; 5.42)		

^a The percentages of beads buried were calculated after excluding unrecovered beads

^b + and - signs represent significantly higher and lower, respectively, compared to the other two forest types, according to Ryan's multiple comparisons test ($P < 0.05$)

^c Achieved statistical power, calculated by the post-hoc power analysis ($\alpha = 0.05$)

Fig. 1

