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ECOSPHERE

Deer overbrowsing on autumn-flowering plants causes bumblebee decline and impairs pollination service

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Abstract. Increased ungulate browsing has altered the composition of plant communities and food webs of forest ecosystems in many regions around the world. To evaluate the cascading impact of deer browsing on pollination and plant reproduction is critical to understand the roles of species interactions in maintaining plant populations and for conservation management. In this study, we investigated the relationships among floral resources of understory herbaceous plants, pollinator visitation, and fruit set of shrub species based on data accumulation over three years in six temperate deciduous forests with deer and without deer. We found that in deer browsed sites, the visitation rate of bumblebees had decreased due to severe reduction in the coverage of autumn-flowering herbaceous plants, while the effect varied between bumblebee species. On the other hand, other insect taxa showed no dependence on variation in autumn floral resources. The two genera of bumblebee-pollinated shrubs showed reduced fruit set due to severe decline in autumn-flowering herbaceous plants and bumblebee visitation (*Weigela*: −18.5% and *Rhododendron*: −21.9%). In contrast, the fruit set of shrubs pollinated by insects that did not show dependence on autumn floral resources were not negatively affected by deer browsing. Our results suggest that deer browsing have not only caused negative effects on herbaceous plants, but in addition have negative indirect effects on reproduction of woody plants through cascading effects of pollination linkages.

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INTRODUCTION

Recent human activities have caused changes in land use, livestock, and wildlife and ultimately lead to drastic increased ungulate populations worldwide (Garrott et al. 1993, Cote et al. 2004, Vazquez et al. 2010). Increased ungulate browsing has altered the composition of plant communities and decreased the diversity of herbaceous plants, including flowering plant species (Fuller and Gill 2001, Russell et al. 2001, Horsley et al. 2003, Yumoto and Matsuda 2006). The conse-quent degradation of forest floors is known to impact populations of both vertebrates and invertebrates, including birds, herbivorous insects and flower-visiting insects, and alters interactions among species (Vazquez and Simberloff 2004, Allombert et al. 2005, Gill and Fuller 2007). A recent study has also demonstrated that ungulate overabundance has become a key cause of exotic plant domination and indirectly depresses native plants (Kalisz et al. 2014). The cascading effects of ungulate browsing on interacting species may result in disruption of

mutualistic interaction webs (Rodriguez-Cabal et al. 2013).

Pollinators are essential for reproduction of wild plants (Ollerton et al. 2011). Experimental studies have shown that functionally diverse bee communities are needed to maintain plant communities that rely on pollination for high reproductive success (Albrecht et al. 2012, Fründ et al. 2013), while flowers are an essential food resource and regulate bee populations (Roulston and Goodell 2011). Recent large-scale declines in pollinator abundance and diversity have raised concerns about the maintenance of pollination services (Biesmeijer et al. 2006, Potts et al. 2010).

Reduction in floral resources by ungulate overabundance may affect the density of social bees especially bumblebees (Kato and Okuyama 2004, Vazquez and Simberloff 2004, Fujii 2010). Bumblebees are the most effective generalist pollinators for many entomophilous plant species in temperate forest plant communities (Heinrich 1976). They visit a wide array of flowers of trees, shrubs, and herbaceous plants in temperate forests from spring to autumn (Yumoto 1988, Inoue et al. 1990). Because bumblebees do not generally store large quantities of honey and pollen, they tend to be vulnerable to discontinuity in their food supply (Shelly et al. 1991, Williams and Christian 1991, Pelletier and McNeil 2003). Thus, decreases in floral resources due to deer browsing may result in severe habitat degradation for bumblebees, while other pollinating insects with more limited active season may be less affected. Consequently, the reproduction of plant species may be negatively affected by the reduction in bumblebee abundance. A recent study examining the pollination linkage in a cool temperate deciduous forest showed that floral resources of canopy trees affect reproduction in a spring ephemeral in the following year via bumblebee abundance (Inari et al. 2012). Regional and phenological variations in floral resources have also been suggested to influence the population dynamics of bumblebees, although the effects vary among bumblebee species. Our previous study also implied that the reproductive success and visitation rate of bumblebees in a bumblebee-pollinated rewardless orchid was extremely low, and fewer plants were flowering in late summer in the forest with heavily browsed area compared to forest without deer (Sakata et al. 2014). To evaluate the cascading impact of deer browsing on the pollination linkages among floral resources, pollinator community, and reproductive success of plant species would lead to a better understanding of the roles of indirect species interactions in maintaining the plant populations and forest ecosystems and of the likely consequences of the global issue of ungulate overabundance.

In temperate forests in Japan, trees and shrubs bloom sequentially from May to July and terminate before August. Beginning in late August, many herbaceous plants start to bloom and reach their flowering peaks in September (Inoue et al. 1990, Kato et al. 1990, Ushimaru et al. 2008). However, recent overabundance in deer (*Cervus nippon*) populations has drastically altered plant communities across Japan (Yumoto and Matsuda 2006, Sakaguchi et al. 2008). In the heavily deer-browsed area, the flowers are scarce after midsummer, and it has been suggested that the intense browsing has caused disappearance of autumn-blooming herbaceous plants (Kato and Okuyama 2004, Fujii 2010, Tamura 2010). The aim of this study was to elucidate how deer overbrowsing affects the pollination linkages among floral resources, pollinator communities, and reproductive success of flowering plant species. We hypothesized that the reduction in floral resources of understory herbaceous plants in autumn by deer browsing has caused decline in fruit set of shrubs via decline in bumblebee populations. The autumn-flowering herbaceous plants may be essential food resources for bumblebees because their demand would increase in autumn for colony development and queen production. To evaluate this prediction, we investigated the relationships among floral resources of understory herbaceous plants, pollinator visitation including bumblebees, and the fruit set of plants based on data accumulation over three years in six temperate deciduous forests with deer and without deer. We investigated not only bumblebee-pollinated plants but also plants pollinated by other insects to compare the pollination interactions among species with different functional relationships.

MATERIALS AND METHODS

Study sites

This study was conducted from 2012 to 2014 at six cool temperate deciduous forest sites in Japan; two sites with intensive deer browsing (AS: Ashiu forest, TZ: Tanzawa mountain) and four sites without deer browsing (SD: Sado forest station, NH: Kuchibuto mountain in Nihonmatsu, TR: Kaminakawa forest station in Tsuruoka, OG: Ogawa Forest Reserve; Fig. 1; Appendix: Table A1). The survey was conducted for varying durations among the different sites (range: one year to three years) since it was not possible to visit all six sites in the same season. All the sites are located in lowland deciduous forests with 600–800 in elevation. The dominant mature canopy trees are mixture of beech, conifers, deciduous oaks, and riparian trees such as *Aesculus turbinate* and *Pterocarya rhoifolia* (Y. Sakata, *unpublished data*). In sites AS and TZ, deer population density drastically increased in these sites in the 1990s and degradation of the forest floor by severe browsing has been reported (Tamura 2010, Sakaguchi et al. 2008). In contrast, deer are absent in sites SD and TR located by the Sea of Japan and sites OG and NH by the Pacific Ocean. Those sites are also deciduous forests but retain rich forest floor vegetation.

Vegetation survey

To investigate the differences in vegetation and abundance of autumn flowering herbs among the six sites, we set 20 random replicates of 2×2 m plots along the roadside forest floor in both ridges and valleys at all six sites and surveyed the vegetation in these plots in early September. Seed plants with more than 5% coverage were recorded at 5% intervals, and, those with less than 5% coverage were scored as 1% following the method used by Sakata et al. (2014). Autumn-flowering herbaceous plants were defined as plants that were not wind-pollinated and flowering at the time of the vegetation survey. Because most of the autumn-flowering herbaceous plants were perennials, we assumed that their population abundance would not change greatly over the years. Thus, although we studied pollinator visitation, pollination, and reproduction in multiple years in some sites, we estimated plant abundances only in the first year.

Pollinator visitation

We selected five genera of animal-pollinated hermaphroditic shrubs for this study because they are found in both deer-absent and deer-browsed sites, and the direct negative effects of browsing on plant reproduction, such as decrease in plant size, population size, and density, are expected to be smaller than those observed in herbaceous species. The five genera are *Weigela*, *Rhododendron*, *Menziesia*, *Viburnum*, and *Hydrangea*, which were commonly found in most sites (Appendix: Table A1). When the same species were not found among sites, we selected closely related species with similar flower morphology that belong to the same genus. We only compared sites that had the same abundance of flowering shrubs to avoid the effect of the relative abundance of the flowers on the behavior of the pollinators (Appendix: Table A1). Previous studies conducted in temperate forests in Japan have shown that bumblebees visit all these species, while the dependence on bumblebee pollination vary among species (Kato et al. 1990, 1993, Ushimaru et al. 2008, Sugiura 2012, Suzuki et al. 2014; S. Yoshida, *unpublished data*). Flower-visiting insects were observed for five individuals of each species per site on clear days in May, June, and July. Each flowering shrub individual was observed for 10 min in the morning and afternoon for two days (i.e., four observation periods of 10 min) and the numbers and types of visitors were recorded. Some insects were netted and kept as specimens to confirm the results obtained by observation. All insect specimens were pinned and their families, identified; some of the dominant visitors were identified at the species level. We also observed the specimens under a stereomicroscope to examine any attached pollen to confirm these flower visitors were pollinators. Pollinators were categorized as bumblebees, Apidae other than bumblebees, Halictidae, Andrenidae, other Hymenoptera, Syrphidae, Bombyliidae, Acroceridae, other Diptera, Lepidoptera, and Coleoptera.

Fruit set of shrubs

We selected 5–10 flowering individuals of each shrub species per site and tagged five shoots with blooming flowers on each shrub individual. The number of flowers was counted for marked shoots. For all of the shoots observed during
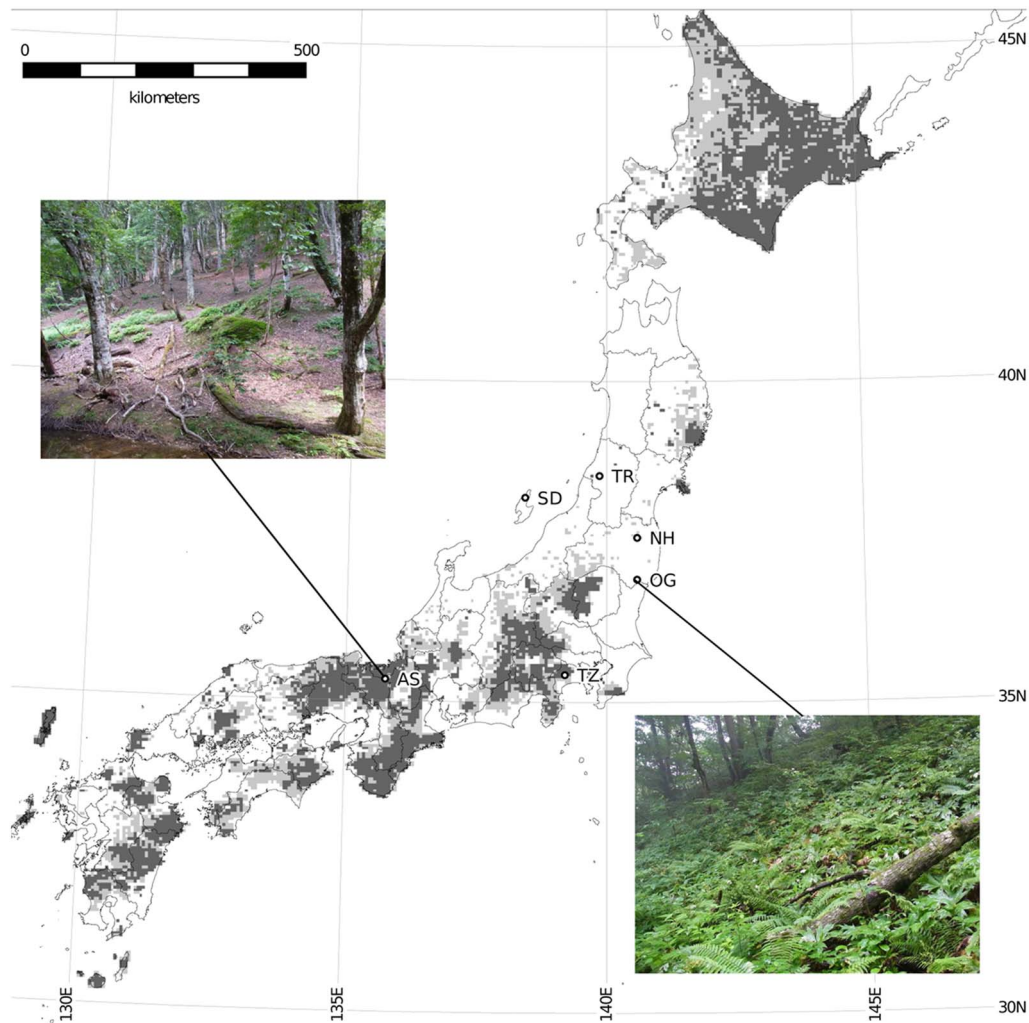
Fig. 1. Deer (*Cervus nippon*) distribution map and location of the six studying sites. The data source is digital data extracted from the Biodiversity Center of Japan, Ministry of Environment (deer distribution data, vector format data with 5×5 km mesh size.) Dark grey shows deer present in both 1978 and 2003, and light grey shows deer present in only 2003. See Appendix: Table A1 for abbreviations of sites. Photographs are landscape of understory vegetation in September in sites AS and OG.

the survey, the number of mature fruits was recorded and fruit set was calculated by dividing it by the number of flowers. We tried to sample as many individual shrubs in as many locations within each site as possible to minimize spatial autocorrelation among samples.

Statistical analyses

First, to test the effect of deer browsing on understory floral resources in autumn, a generalized linear mixed-effect model (GLMM) postu-

lating gamma error distribution was applied for abundance of autumn floral resources in each plot. The coverage of autumn-flowering herbaceous plants was set as response variable, and presence and absence of deer and latitude as explanatory variable. We included the classification of whether the site is located in the Sea of Japan side (AS, SD, TR) or Pacific side (TZ, OG, NH) to consider the effect of climate differences on floral abundance among the sites. The site was treated as a random factor. Setting the random

factor aimed to deal with unknown effects of climate and resource variations among sites in the study.

Second, to test the effect of understory floral resources in autumn on pollinator visitation, a GLMM postulating Poisson error distribution was applied for each insect taxon for all shrub species pooled together. In addition, we tested whether coverage of flower resources other than autumn-flowering herbaceous plants affect the pollinator visitation. For bumblebees, it was conducted for both the whole *Bombus* genus and for each of the *Bombus* species. The number of visits was set as response variable, and the averaged coverage per site, number of species of autumn-flowering herbaceous plants and other insect pollinated plants per site, and classification of whether the site is located in the Sea of Japan side or Pacific side as explanatory variables. The sampling year and site was treated as a random factor. Setting the random factor aimed to deal with unknown effects of climate variations and/or unmeasured floral resources over the years and sites in the study.

Third, to evaluate the effect of pollinators on the fruit set of the plants, a GLMM postulating Poisson error distribution was applied for each shrub genus. The number of fruits was set as response variable and the number of visits of each taxon (each species for *Bombus*) averaged within sites of each year and classification of whether the site is located in the Sea of Japan side or Pacific side of the site were set as explanatory variables. The natural logarithm of the number of flowers was entered into the model as an offset term to control for differing numbers of flowers in each shoot. Sampling year, site, identity of species, and individuals of each shrub were treated as random factors. We excluded taxa whose members rarely visited (other Hymenoptera, Acroceridae, and other Diptera). Setting the random factor aimed to deal with unknown effects of climate variations and/or unmeasured floral resources over the years and sites, and unmeasured genetic differences among species and individuals of shrubs in the study.

Fourth, to test whether cascading indirect effect of deer browsing on fruit set of shrubs exist (i.e., whether the fruit set of the shrubs differ between deer browsed and absent sites), we conducted GLMM postulating Poisson error distribution for each shrub genus. The number of fruits was set as response variable, the effect of deer and classification of whether the site is located in the Sea of Japan side or Pacific side of site as explanatory variables, and sampling year, sites, identity of species, and individual of each shrub as random factors. The natural logarithm of the number of flowers was entered into the model as an offset term to control for differing numbers of flowers in each shoot. Setting the random factor aimed to deal with unknown effects of climate variations and/or unmeasured floral resources over the years and sites, and unmeasured genetic differences among species and individuals of shrubs in the study. The analyses described here were conducted using lme4 package (Bates et al. 2011) of R 2.13.1 (R Core Team 2014). We selected the best model that had the lowest AIC value.

Finally, structural equation modeling (SEM) was used to model and quantify the direct and indirect effects of deer browsing on understory floral resources in autumn, pollinator visitation, and reproduction of the shrub species. SEM enables testing both direct and indirect effects when a factor can simultaneously be modeled as an outcome and as a predictor of other factors. Since our hypothesis requires an effect of deer on fruit set, the remaining comparisons between deer-browsed and deer-absent sites are reported only for the species that showed such effects in the fourth GLMM analysis. We built SEMs reflecting hypothesized relationships among variables, including indirect effects of deer browsing on the fruit set of shrubs via coverage of flowering herbs in autumn and pollinator visitation. In addition, because pollinator visitation may not be influenced by deer only via a decrease in flowering resources but also by additional effects of deer browsing not measured, we included the direct relationship between deer and pollinator visitation. We also included direct relationships between deer and fruit set of shrubs, and coverage of floral resources and fruit set of shrubs, because the fruit set of shrubs may be directly influenced by deer browsing or directly affected by the reduced coverage of floral resources. For pollinator visitation, we included only the pollinator taxa, which significantly influenced the fruit set ratio of each shrub genus in the third GLMM analysis.
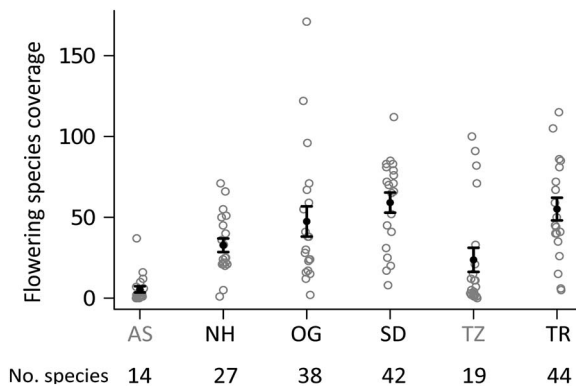
Fig. 2. Coverage and number of species of autumn flowering herbaceous plants (mean ± SE) in the six sites. Sites with gray letters are deer browsed sites, and sites with black letters are deer absent sites.

For each SEM, we fitted with robust maximum likelihood method using the R package lavaan (Rosseel 2012). The goodness fit of each model was evaluated with the chi-square test, and the comparative fit index (CFI): chi-square values higher than 0.05, and CFI higher than 0.90 indicate a good fit between the SEM model and observed data (Grace et al. 2012, Toyoda 2014). We deleted each path to examine its effect on the model fit, and paths were retained if their deletion caused lack-of-fit. The significance of each pathway was evaluated with a t-test computed on the unstandardized coefficients.

RESULTS

Neighboring vegetation and autumn-flowering herbaceous plants

The total number of plant species and families observed in each site were 49, 36 at AS, 75, 40 at NH, 83, 32 at OG, 102,47 at SD, 52, 36 at TZ, and 97, 43 at TR, respectively (Appendix: Table A2). The coverage and number of species of flowering herbaceous plants observed during the survey period at the deer browsed sites was much lower than that of the deer absent sites (Fig. 2). In the deer-browsed sites (AS and TZ), plants unpalatable to deer such as *Aconitum sanyoense* and *Boenninghausenia albiflora* were the only flowering species found during the survey in autumn, while in the deer-absent sites, many Asteraceae and Lamiaceae plants flowered (Appendix: Table A2). Deer presence had a

significant negative effect on the coverage of autumn-flowering herbaceous plants (Estimate: −1.29, SE: 0.22, Pr: <0.001, N = 120), while the effect of the sea side of the sites was not observed.

Pollinator visitation

A wide range of pollinators was observed for all species, while *Bombus* species and members of Andrenidae and Bombyliidae visited less frequently in deer-browsed sites (Appendix: Fig. A1). Plant coverage of autumn-flowering herbaceous plants had a significant positive effect on visits by *Bombus* species and Andrenidae (Table 1). For bumblebee species, a positive relationship was observed for *B. diversus* and *B. hypocrita* with respect to plant coverage of autumn-flowering herbaceous plants, whereas no such relationship was observed with *B. ardens*. On the other hand, negative relationship between plant coverage of other seed plants and pollinator visitation was only observed in Syrphidae. Significant effect of the sea side of the sites was observed on *B. diversus* and Andrenidae.

Visitation of *B. ardens* contributed to the fruit set of *Weigela*, *Rhododendron*, and *Menziesia* species, and P values of z-statistics for latter two species were higher than that for *Weigela* species, while *B. diversus* contributed to the fruit set of *Weigela* and *Rhododendron* (Appendix: Table A3). For *Weigela* species, visitation by members of Andrenidae also had a positive effect on the fruit set. In contrast, *Viburnum* species was negatively affected by Andrenidae and Syrphidae. Hydrangea species showed mixed effects among Apidae, Syrphidae, and Coleoptera though the effect of Syrphidae was marginal (estimate was not significantly different from zero; Appendix: Table A3).

Fruit set of shrubs

The fruit set of the five genera ranged from 0.25 to 0.87 (Fig. 3). The fruit set of Rhododendron species were higher in Japan Sea side, while it was higher in the Pacific Sea side for *Viburnum* species (Appendix: Table A3). Deer browsing had significant negative effects on fruit set in *Weigela* (Fig. 3a; Appendix: Table A4; 0.14–0.22 lower in deer browsed sites) and *Rhododendron* (Fig. 3b; Appendix: Table A4; 0.13–0.31 lower in deer browsed sites) species.

Table 1. Results of a generalized linear mixed model (GLMM) that predicts visit rate of eight taxa of insects and three species of bumblebees for all five shrubs genera pooled together.

Insect taxa	Coverage (autumn flowering herbs)				Coverage (other seed plants)				Sea side				Explained deviance (%)
	Est.	SE	z	Pr	Est.	SE	z	Pr	Est.	SE	z	Pr	
Bombus	0.05	0.008	−8.9	***	...	...	...	...	...	...	...	...	8.1
Apidae	...	...	...	...	...	...	...	...	...	...	...	...	...
Andrenidae	0.03	0.02	1.35	0.17	...	...	...	...	−2.06	1.63	−1.26	0.21	3.1
Halictidae	...	...	...	...	...	...	...	...	...	...	...	...	...
Syrphidae	...	...	...	...	−0.04	0.02	−2.56	*	...	...	...	...	2.2
Bombyliidae	...	...	...	...	...	...	...	...	...	...	...	...	...
Coleoptera	...	...	...	...	...	...	...	...	...	...	...	...	...
Lepidoptera	...	...	...	...	...	...	...	...	...	...	...	...	...
B. diversus	0.07	0.02	1.01	**	...	...	...	...	2.11	1.66	1.27	0.20	5.4
B. hypocrita	0.07	0.05	1.61	0.10	...	...	...	...	...	...	...	...	16.0
B. ardens	...	...	...	...	...	...	...	...	...	...	...	...	...

Notes: Coverage, coverage of the autumn-flowering herbs; Latitude, latitude of the sites. Ellipses indicate that variables were not selected in the best model. The sample size is $n = 1123$ (deer browsed sites: $n = 433$ and deer absent sites: $n = 690$). The significance of differences from zero was tested using z-statistics. Explained deviance is calculated as (null deviance − residual deviance)/null deviance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Conversely, *Viburnum* species showed increased fruit set in deer-browsed sites (Fig. 3d; Appendix: Table A4; 0.02–0.11 higher in deer browsed sites), whereas no significant differences were observed in the fruit sets of *Menziesia* and *Hydrangea* species among deer-browsed and deer-absent sites.

Structural equation model explaining among site variation in pollination and reproduction

For clarity, we report only the standardized path coefficients in the figures and present the unstandardized coefficients and standard errors in Appendix: Table A5. With *Weigela*, standardized path coefficients indicate that deer browsing had negative indirect effect on the visitation of *B. diversus* and positive indirect effect on *B. ardens* due to reduced plant coverage of autumn-flowering herbs (indirect path coefficient: *B. diversus*, $-0.97 \times 0.85 = -0.82$; *B. ardens*, $-0.97 \times -0.60 = 0.58$) and on the fruit set due to reduced plant coverage and *B. diversus* visitation (indirect path coefficient: $-0.97 \times 0.85 \times 0.29 = -0.24$; Fig. 4; Appendix: Table A5). On the other hand, a direct negative relationship was observed between deer browsing and visitation rates of Andrenidae, but not indirectly through autumn-flowering plant coverage. Deer browsing also had a direct negative effect on the fruit set, and plant coverage of autumn-flowering herbs had a direct positive effect on the fruit set.

With *Rhododendron* species, deer browsing had a negative indirect effect on the visitation of *B. diversus* and a positive indirect effect on *B. ardens* due to reduced plant coverage of autumn-flowering herbs (indirect path coefficient: *B. diversus*, $-0.86 \times 0.31 = -0.26$; *B. ardens*, $-0.86 \times -0.26 = 0.22$) and on the fruit set due to reduced plant coverage of autumn-flowering herbs and *B. diversus* and *B. ardens* visitation (indirect path coefficient: *B. diversus*, $-0.86 \times 0.36 \times 0.12 = -0.03$; *B. ardens*, $-0.86 \times -0.26 \times 0.15 = 0.03$; Fig. 4; Appendix: Table A5). A direct negative relationship was also observed between the presence of deer and visitation rate of bumblebees.

With *Viburnum* species, the deer browsing had a negative indirect effect on the visitation of Syrphidae due to reduced plant coverage of autumn-flowering herbs (indirect path coefficient: $-0.97 \times 1.19 = -1.15$) and a positive indirect effect on the fruit set due to reduced plant coverage of autumn-flowering herbs and Syrphidae visitation (indirect path coefficient: $-0.97 \times 1.36 \times -0.30 = 0.35$; Fig. 4; Appendix: Table A5). A direct effect of deer browsing was positive on the visitation of Syrphidae, and negative on Andrenidae. We excluded the direct path of deer browsing and plant coverage of autumn-flowering herbs on the fruit set since the goodness fit of the model greatly reduced when the path was included.
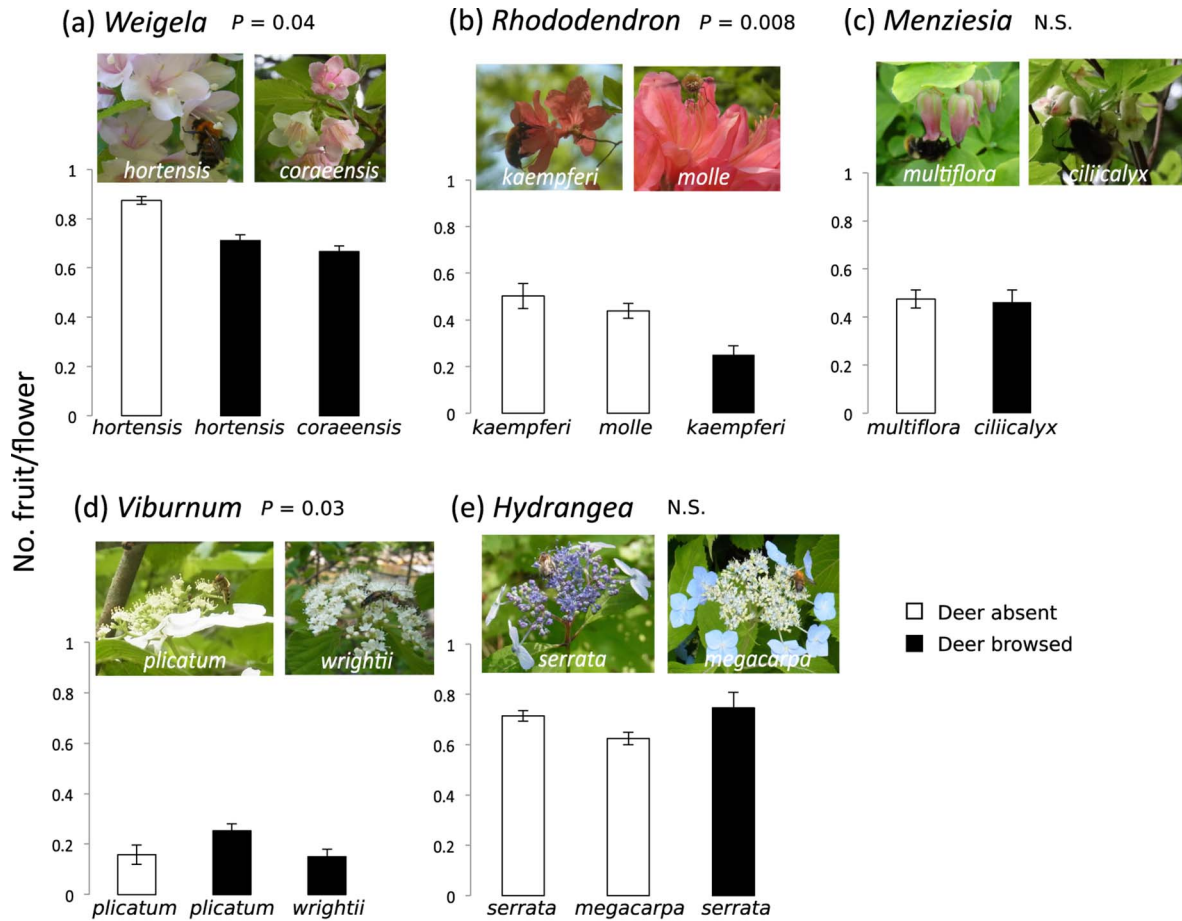
Fig. 3. Fruit sets for the five genera of shrubs between deer browsed and absent sites. Fruit sets are calculated as the number of fruits divided by the number of flowers, and shown separately between species within the genus. The significance of differences from zero was tested using z-statistics. See Appendix: Table A4 for the GLMM tables. Photographs are flowers of (a) *W. hortensis* visited by *B. diversus* in SD and *W. coraeensis* in TZ; (b) *R. kaempferi* visited by *B. diversus* in OG and *R. molle* visited by *Bombylius major* in SD; (c) *M. multiflora* visited by *B. ardens* in SD and *M. ciliicalyx* visited by *B. ardens* in AS; (d) *V. plicatum* visited by Sryphidae in AS and *V. wrightii* visited by Cerambycidae sp. in SD; (e) *H. serrata* visited by Andrenidae sp. in AS and *H. serrate* var. *megacarpa* visited by *B. diversus* in SD.

DISCUSSION

The present study suggests deer browsing affect the link between floral resources of autumn-flowering herbaceous plants, bumblebee abundance, and reproduction of spring- and summer-flowering shrubs. The vegetation survey revealed that flowering species coverage and number of species were extremely low in deer-browsed sites as compared to deer-absent sites. A previous study of insect and flower relationships conducted at site AS described 30 species of autumn-flowering herbaceous plants visited by *Bombus* species (Kato et al. 1990), but most of these species are rarely seen or have disappeared now (Appendix: Table A1). Thus, it is suggested that deer browsing has caused a large decrease in flowering resources in autumn.

The observation of pollinators revealed that the visitation rate of bumblebees was negatively affected by the reduction in autumn floral resources when data of all shrub genera were pooled together. While the insect taxa that contributed to the fruit set differed among
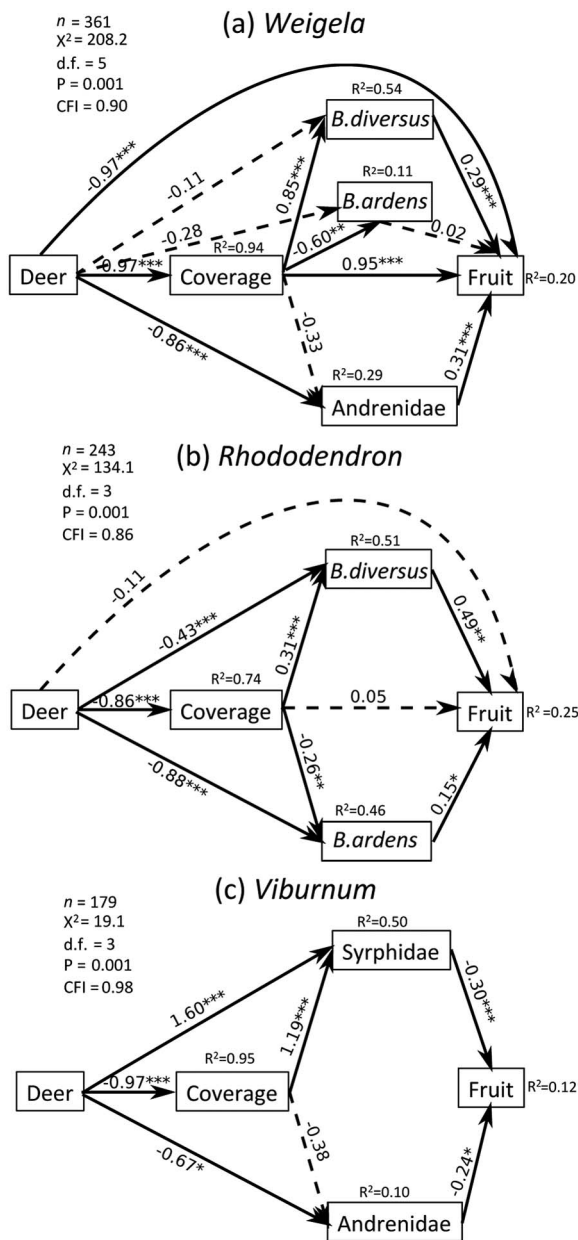
Fig. 4. Structural equation models (SEMs) representing direct and indirect effects of deer on pollination and reproduction of (a) *Weigela*, (b) *Rhododendron*, (c) *Viburnum* species. Values associated with arrows are the standardized path coefficients. Solid arrows indicate significant relationships ($P < 0.05$), and dashed arrows refer to non-significant paths. Significance of the path coefficients is indicated as follows; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Models were fitted with maximum likelihood method and overall goodness of fit estimated from chi-square statistic and comparative fit index (CFI).

genus of shrubs, the GLMM analyses suggest that the bumblebees have contributed to the pollination of the species of three genera, *Weigela*, *Rhododendron*, and *Menziesia*. Even when we considered the variations in environmental factors of sites and years, negative indirect effects of deer browsing were observed for *Weigela* and *Rhododendron*. However, the reduction in autumn floral resources affected differently among bumblebee species; *B. diversus* and *B. hypocrita* were positively affected by plant coverage of autumn-flowering herbaceous plants, while *B. ardens* was not. Because the worker bees of *B. diversus* and *B. hypocrita* are active through autumn, they may be particularly vulnerable to the decline in autumn-flowering herbaceous plants. On the other hand, the worker brood-rearing phase of *B. ardens* is in early summer, their population may be independent of autumn floral resources (Katayama 1996). Despite its reliance on bumblebee pollination (Kato et al. 1993), *Menziesia* species showed no indirect effects of deer browsing. This could be because this species has relatively shorter flower tubes compared to *Weigela* and *Rhododendron* species, and that it is mainly pollinated by the short-tongue *B. ardens*, so that deer browsing has negligible effects (Table 1; Appendix: Fig. A1). Together with the result of the SEM analysis, it is suggested that for both *Weigela* and *Rhododendron* species, the reduced fruit set in the deer browsed sites are partly caused by the decline in visitation rate of *B. diversus* that is negatively affected by the decline in autumn floral resources. However, because our observation was based on a limited period of time, we need longer observation to accurately quantify the activity of the pollinator and the importance of each pollinator on the fruit set of shrubs. The reason why the visitation of bumblebees was low in the deer browed sites may be due to the decline in bumblebee population in the forest sites. They may have moved to surrounding grasslands or human-disturbed habitats and changed their foraging behavior. Additionally we cannot deny other environmental factors such as agricultural intensification and effect of parasites that lead to abrupt declines in bumblebee population (Goulson et al. 2008).

On the other hand, insect taxa other than

bumblebees were not affected by the reduction in coverage of the autumn-floral resources, except Andrenidae (Table 1). This data appears to be consistent with the results of the previous study that showed solitary bees were less affected by isolation from natural habitats as compared to social bees including bumblebees (Gathmann and Tscharntke 2002, Williams et al. 2010).

The direct relationship observed between deer browsing and bumblebee visitation in the SEM analysis of *Rhododendron* indicates that the variation of the floral resources measured between deer-browsed and deer-absent sites did not entirely explain the effect of the deer browsing on bumblebee abundance. One possible mechanism for this is that deer trampling or browsing may have negatively affected not only the food resources but also the nesting environment. Bumblebees nest underground in cavities and often use abandoned rodent nests (Goulson et al. 2008). The scarcity of herbaceous plants on forest floors may result in fewer seeds and therefore less food for voles and mice (Byman 2011), leading to fewer nest sites for nesting bumblebee species. A direct effect of deer browsing was also detected in Andrenidae and Syrphidae. This suggests that although insect taxa other than bumblebees may be indifferent to the reduction in autumn floral resources, their nesting environment may be affected by deer browsing. Direct effect of deer and coverage on the fruit set was detected in *Weigela* but not in the other two genera. This may reflect direct deer browsing of *Weigela*, or facilitative effect of the understory vegetation via soil improvement.

For *Viburnum* species, only a negative effect of insect visitation (Syrphidae and Andrenidae) on the fruit set was detected. This result was unexpected since we could not postulate any reasons their visits would have negative effects on the fruit set, except that unmeasured factors that influenced the fruit set might be correlated with their visit rate. Although the visitation of Syrphidae was both directly and indirectly affected by deer browsing, they had opposite effects (i.e., positive direct effect and negative indirect effect), which lacks consistency. For *Hydrangea* species, no difference in fruit set between deer browsed and absent sites was observed (Appendix: Table A4; Fig. 3) and it was suggested that they were pollinated by insects not affected by deer browsing. Our results suggest that the fruit set of both *Hydrangea* and *Viburnum* are unlikely to be negatively influenced by deer browsing.

There are still unmeasured factors that may affect the results of the study system. First, because the same species was not available in all sites and we compared congeneric species, their difference in reproductive ecology may lead to biased measurements of the fruit set of the shrubs. We need further study comparing the same species among sites. Second, the magnitude of pollen limitation and resource limitation may mask the indirect effects of herbivore browsing on plant reproduction. Because, indirect effects of deer browsing on both self-compatible (*Rhododendron*) and self-incompatible (*Weigela*) species were detected in our study, it is be unlikely that the degree of self-compatibility is critical in explaining the variation in fruit set, while the amount of resources among shrub individuals may explain some of the variation in fruit set. Hand-pollination in each shrub at each site may contribute to evaluating the pollen limitation and pollination success of each species. Additionally, although our measures of reproductive performance focused only on the fruit set, the changes in species and visitation frequency of the pollinators may also affect the quality and genetic diversity of seeds. Further study is required to measure the quantity and quality of seeds. Previous study demonstrated that cattle can indirectly affected pollination and reproduction of herbaceous plants by virtue of their relative population density that decreases the relative proportion of conspecific pollen deposition rather than through their absolute density decreasing pollinator visitation (Vazquez and Simberloff 2004). Although in our system, the observed shrub species were abundant in deer-browsed sites, the disappearance of the seedlings indicates the population will be drastically decreased in the future. The indirect effects of deer browsing on plant reproduction may have a much larger impact than the results detected in our study.

Overall, our results showed difference in visitation frequency in pollinator taxa that strongly depended on the autumn floral resources, and that the difference in fruit set were only seen in shrub species that strongly depend

on pollination by those species. Among the many species interactions, the dominant interactions potentially represent "keystone interactions," and their persistence may be important for maintaining a much larger set of interactions (Vazquez et al. 2010, Rodriguez-Cabal et al. 2013). In fact, pollination by bumblebees might be biologically important for a lot of vegetation in cool temperate forests in Japan because they are essential pollinators not only for several shrubs but also for many trees and herbaceous plants throughout the year (Inoue et al. 1990, Inari et al. 2012). Although our data is not based on experiments that show a causal relationship of interactions but rather based on correlations of unplanned natural occurrences, our findings are consistent with the hypothesis that the reproductive success of bumblebee-pollinated plants is negatively affected by deer browsing. Manipulative experiments to illuminate the effects of different factors are critical to demonstrate the hypothesis. Our results encourage continuous monitoring and a focus on long-term trends in areas with increasing deer populations and to reform policies and management practices concerning these habitats to maintain ecosystem functions including pollination services.

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Supplemental Material

Ecological Archives

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