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Responses of scarab beetles to nutrient-enriched artificial treeholes

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Abstract

Treeholes are important microhabitats in forest ecosystems and support various invertebrates. The nesting activities of cavity-nesting birds continuously supply animal-derived resources, such as prey remains, feces, and carcasses, thereby substantially altering the internal resource environment of treeholes. However, the relative importance of animal-derived resource inputs and the environmental conditions associated with treeholes for scarab beetle abundance remains poorly understood. In this study, we conducted two artificial treehole experiments: a resource-input experiment manipulating the frequency of animal-derived resource inputs, and a height experiment examining whether environmental conditions associated with installation height influence the occurrence and abundance of treehole-dwelling scarab beetles. Continuous supplementation with animal-derived resources notably increased the abundance of *Protaetia brevitarsis* (Lewis, 1879). Canopy openness was positively associated with abundance; however, this relationship declined with continued resource input. *P. brevitarsis* also occurred more frequently in trees installed at increased heights. These results highlight the importance of both animal-derived resource inputs and environmental conditions in influencing the abundance of scarab beetles in treeholes, and demonstrate the applicability of artificial treeholes for studying treehole ecosystems.

1. Introduction

Treeholes are formed through the long-term decay processes of trees, including excavation by bird species and fungal infection, and provide a habitat for diverse animals (Cockle et al. 2012; Zheng et al. 2016). Consequently, treeholes are important in ecosystem functioning (TreMs; Goldingay 2009; O'Connell and Keppel 2016; Bütler et al. 2024; Ranius et al. 2024). Treeholes play an important role in maintaining community structure and species diversity in forest ecosystems. However, large, old trees suitable for treehole formation are exposed to multiple threats, including drought, pests, diseases, logging, land-use change, habitat fragmentation, and climate change, making their conservation an internationally important issue (Lindenmayer and Laurance 2017). The decline of these large old trees leads to a reduction in treeholes and is expected to limit the habitat availability for tree-dependent invertebrates. Therefore, the conservation of treehole environments is required to maintain forest ecosystem diversity.

Treeholes frequently accumulate various organic materials, such as decayed wood fragments, animal feces, and carcasses, which can function as nutrient-rich resource patches (Hanzelka et al. 2023). These resource environments strongly affect the occurrence and growth of detritivorous or organic matter-dependent invertebrates (Hanzelka et al. 2023). Scarabaeidae includes species whose larvae utilize decaying organic matter and inhabit treeholes or similar enclosed microhabitats (Jonsell 2012; Pilskog et al. 2016). Several of these species contribute to nutrient cycling by decomposing organic matter within treeholes and facilitating the occurrence and growth of other tree-associated insects, such as syrphid flies, by producing nutrient-rich substrates (Sánchez-Galván et al. 2014). Scarab larvae act as ecosystem engineers in treehole environments (Sánchez-Galván et al. 2014). Certain treehole-dwelling

scarab beetle species are threatened, and the decline and degradation of treehole resources are important conservation concerns for these species (Maurizi et al. 2017; Lindman et al. 2020). Therefore, the occurrence of treehole-dwelling scarab beetles is expected to represent the quality and quantity of treehole resources, as well as forest continuity, and may serve as an indicator of forest ecosystem conditions.

Animal-derived organic matter, such as nest materials, feces, and food remains, accumulates through the nesting activities of cavity-nesting birds and forms nutrient-rich resource environments within treeholes (Hanzelka et al. 2023). These resources can positively affect the occurrence and population density of certain scarab larvae (Ranius and Nilsson 1997; Nasu et al. 2011), whereas negative associations between cavity-nesting bird use and scarab beetle larval occurrence have been reported (Carpaneto et al. 2010), indicating inconsistent effects. In the treehole-dwelling scarab *Osmoderma barnabita* Motschulsky, 1845, non-woody resources, such as leaf litter supplied from outside treeholes, can play an important role in larval development (Landvik et al. 2016; Ranius et al. 2024), and females may also select oviposition sites based on suitable resource conditions for larval growth (Landvik et al. 2016). These variations in response to external resource inputs may be related to species-specific preferences for resources among scarab beetles (Ranius and Nilsson 1997; Carpaneto et al. 2010). In addition, the effects of resource input by cavity-nesting birds may vary depending on the physical environment of the tree holes. In other detritus-based enclosed ecosystems, resource input strongly influences community structure and abundance, whereas its effects are mediated by abiotic conditions, such as light environment, canopy openness, vertical position, and temperature (Petermann et al. 2015; Ptatscheck and Traunspurger 2015; Kratina et al. 2017). Therefore, the relative importance of resource availability and environmental conditions in determining treehole use by scarab beetles may vary across study systems and experimental settings, making it challenging to derive generalizable patterns.

Artificial treeholes provide experimental systems for investigating ecological processes associated with natural treeholes, while enabling increased control over habitat characteristics. Previous studies have used artificial treeholes to examine the effects of cavity characteristics, resource availability, and environmental conditions on tree-associated organisms, demonstrating their applicability in evaluating factors affecting the occurrence and abundance of treehole-dwelling invertebrates (Jansson et al. 2009; Hilszczański et al. 2014; Linde et al. 2025; Asano and Yoshida 2026). However, artificial treeholes may differ from natural treeholes in terms of the internal environmental conditions. For example, wooden bird nest boxes exhibit greater temperature fluctuations and lower humidity than those of natural treeholes (Maziarz et al. 2017; Sudyka et al. 2023). In addition, invertebrate communities in artificial treeholes constructed from wooden boxes do not necessarily correspond completely to those found in natural treeholes (Jansson et al. 2009). However, artificial treeholes provide a valuable experimental framework for investigating ecological processes because they enable the effects of resource availability and environmental conditions to be evaluated separately under field conditions (Asano and Yoshida 2026). This approach is consistent with the broader use of small, spatially constrained natural systems as model systems for ecological research (Srivastava et al. 2004).

In this study, we conducted two manipulative experiments using artificial treeholes to elucidate how animal-derived resource availability and installation environment affect their utilization by treehole-dwelling scarab beetles. First, we simulated the nutrient-rich environments formed by the nesting activities of cavity-nesting birds and examined how differences in the frequency of animal-derived resource inputs influenced their utilization by treehole-dwelling scarab beetles. Second, we manipulated the installation height of the artificial treeholes to examine the effect of environmental differences associated with the vertical position on their use by scarab beetles. We hypothesized that (1) repeated inputs of animal-derived resources would promote the occurrence and abundance of scarab beetles associated with cavity-nesting birds by creating nutrient-rich conditions similar to those observed in bird nests, and (2) habitat use would vary with installation height because the vertical position is associated with differences in the surrounding environmental conditions.

2. Materials and methods

2.1. Study sites

2.1.1. Resource-input experiment (Site A)

The experiment manipulating the input frequency of animal-derived resources (bird carcasses) was conducted at Site A within the Field Museum Tamakyuryo of the Tokyo University of Agriculture and Technology, located in the western part of Tokyo, Japan (35.63842° N, 139.37822° E) (Fig. 1). The mean annual precipitation and mean annual temperature are 1643.0 mm and 14.7°C, respectively (1991–2020 averages; Japan Meteorological Agency 2026). Site A comprised a forested area of approximately 49.5 ha, with an elevation ranging from 140 to 180 m. The terrain consists of a valley oriented from northwest to southeast flanked by two hills. The forest is dominated by broad-leaved stands primarily composed of *Quercus serrata* Murray and *Quercus acutissima* Carruth, with partially planted stands of *Cryptomeria japonica* (Thunb. ex L. f.) D. Don and *Chamaecyparis obtuse* (Siebold & Zucc.) Endl.

2.1.2 Height experiment (Site B)

The height manipulation experiment for the artificial treeholes was conducted at Site B within the Field Museum Karasawayama of the Tokyo University of Agriculture and Technology, located in Tochigi Prefecture, Japan (36.3582° N, 139.6005° E) (Fig. 1). The mean annual precipitation and mean annual temperature are 1258.0 mm and 14.5°C, respectively (1991–2020 averages; Japan Meteorological Agency 2026). Site B comprised a forested area of approximately 161.8 ha at elevations ranging from 90 to 200 m. The vegetation is dominated by plantations of *C. japonica* and *C. obtusa*, which account for approximately 50% of the area, whereas broadleaf forests occupy approximately 30% and are mainly composed of *Q. serrata*, *Quercus salicina* Blume, and *Castanopsis sieboldii* (Makino) Hatus. In this study, artificial treeholes were installed primarily in broadleaf forest stands.

2.2. Construction and experimental design of artificial treeholes

In this study, artificial treeholes were constructed using *C. japonica* wood. Each artificial treehole was equipped with a front opening to allow access by invertebrates, and a 2 cm mesh wire was attached to prevent entry by birds and mammals. The roof was designed to facilitate internal observation and resource input. Five drainage holes (5 mm in diameter) were installed at the bottom to prevent water accumulation. Inside each artificial treehole, 3 L of wood chips made from broadleaf tree material (Bio breeding mat for beetles; MARUKAN Co., Ltd.) were added to simulate a decayed woody substrate environment. The same substrate volume was previously used in artificial tree holes, from which multiple scarab beetle species have been recorded (Asano and Handa 2026).

The artificial treeholes used in the resource input experiment were rectangular boxes (12 mm thickness, 40 cm height × 21 cm width × 21 cm depth) with a circular entrance hole (8.5 cm diameter) on the front panel (Fig. 2a). They were installed approximately 1.5 m above the ground on tree trunks using a palm rope. To simulate the high-nutrient environment characteristics of bird nests, resource addition experiments were conducted using chicken chicks as an organic resource input (Fig. 2b). In 2024, a single chick was introduced at the start of the experiment (initial input), whereas in 2025, chicks were added at the beginning and multiple times during the experimental period (continuous input). In 2024, 35 artificial treeholes were installed in March, and wood chips (3 L) and a chicken chick (40.1 ± 3.0 g) were introduced on April 8. Subsequently, 34 units were retrieved on November 5, 2024, with one unit lost owing to branch fall damage. In 2025, 35 artificial treeholes were reinstalled at the same site on March 31, and wood chips (3 L) and a chicken chick (38.4 ± 2.8 g) were introduced. Additional resource inputs were conducted five times (May 28, June 27, August 6, September 2, and October 6), with one chick added to each artificial treehole at each occasion (mean mass per chick: 37.6 ± 2.3, 40.3 ± 2.5, 41.6 ± 4.1, 40.1 ± 3.6, and 38.8 ± 3.7 g, respectively). All artificial treeholes were retrieved on November 17, 2025.

The artificial treeholes used in the height manipulation experiment were smaller than those used in the resource-input experiment (25 cm height × 14.5 cm width × 14.5 cm depth), with a circular entrance hole of 7.5 cm diameter. These were fixed to tree trunks in broadleaf forest stands using palm rope at three height levels: 1.5, 3.0, and 4.5 m above ground. Each treatment consisted of 15 artificial treeholes (45 holes in total). All artificial treeholes received identical resource inputs to simulate high-nutrient bird nest-like conditions. On June 6–8, 2025, artificial treeholes were installed and filled with wood chips (3 L) and a chicken chick (39.2 ± 3.4 g). Additional chicks (40 ± 3.4 g) were added to each artificial treehole from July 18 to 19, 2025. All units were retrieved on September 14, 2025.

2.3. Sampling of scarab beetles in artificial treeholes

2.3.1. Resource-input experiment (Site A)

In 2024, 14 surveys were conducted in 35 artificial treeholes between April 8 and October 21, at approximately biweekly intervals. During each survey, the interior of each artificial treehole was visually inspected, and all invertebrates, including scarab beetles, were collected and recorded. One artificial treehole was damaged during the study period, and five additional units were not visually surveyed.

In 2025, sampling was conducted in conjunction with five chick addition events (May–October). All 35 artificial treeholes were inspected at each input event, and the presence of invertebrates, including scarab beetles, was recorded. Adult scarab beetles observed within artificial treeholes were identified to the species level, and their abundance was recorded. Other invertebrate taxa recorded in artificial treeholes have been reported in a separate study; therefore, only scarabaeid beetles were included in the present analysis.

After retrieval, the substrates inside the artificial treeholes were sorted, and scarab beetle larvae were collected and recorded. In 2024, 34 artificial treeholes were retrieved and subjected to substrate sorting, whereas in 2025, 35 artificial treeholes were retrieved and processed. The larvae were reared in the laboratory until adult emergence for species identification. All specimens were preserved and stored in a laboratory at Shiraume Gakuen College, Japan.

2.3.2. Height experiment (Site B)

In the height experiment conducted in 2025, invertebrates within artificial tree holes were examined only at the time of retrieval. On September 14, 2025, all artificial treeholes were retrieved, and the accumulated substrates were sorted to collect and record scarab beetle larvae and individuals at other developmental stages. For species identification, the collected larvae were reared in the laboratory until adult emergence.

2.4. Environmental factors

In this study, five environmental variables potentially affecting invertebrate communities within treeholes were considered: tree species richness, number of trees, total basal area, canopy openness, and diameter at breast height (DBH) of the trees in the artificial treehole. For trees within a 10 m radius of each artificial tree hole, all trees with a DBH > 10 cm were identified to the species level, and their girth at breast height (GBH) was recorded. The total basal area (TBA; cm^2) was calculated as the sum of the basal areas (BA) of all measured trees, with BA derived from the GBH measurements. In 2024, canopy openness was estimated using hemispherical photographs taken approximately 1.5 m in front of each artificial treehole using a fisheye lens. These images were analyzed using CanopOn version 2. In 2025, the same procedure was conducted from early September to mid-October, prior to leaf fall.

2.5. Statistical analysis

All scarab beetles recorded in this study were predominantly *Protaetia brevitarsis* (Lewis, 1879) in the resource input experiment and exclusively *P. brevitarsis* in the height experiment; therefore, the number of individuals of this species was used as the response variable in all subsequent analyses. Prior to the analyses, multicollinearity among the explanatory variables was assessed using the variance inflation factor (VIF), and all variables showed a VIF < 5. Model fit was evaluated using the DHARMa package, with simulation-based residual diagnostics used to assess overdispersion, zero inflation, and uniformity of residuals. Model selection was conducted using the Akaike's Information Criterion (AIC), and the model with the lowest AIC was selected as the final model. All statistical analyses were performed using

R version 4.5.1 (R Core Team 2025). For the resource-input experiment, generalized linear mixed models (GLMMs) were constructed with the resource-input treatment and surrounding environmental variables as fixed effects and artificial treehole ID as a random effect. A Poisson error distribution was initially assumed, and candidate models, including the interaction between the resource and input treatment and canopy openness, were compared. For the height experiment, generalized linear models (GLMs) were constructed with the installation height and surrounding environmental variables as fixed effects. We assumed a negative binomial error distribution.

3. Results

3.1. Resource-input experiment

Visual surveys recorded four scarabaeid beetle species within artificial treeholes. In 2024 (initial input), only a single adult individual of *Rhomborhina polita* Waterhouse, 1875 was observed across the 14 survey occasions. In contrast, in 2025 (continued input), the cocoons of three species were recorded during five surveys: *Glycyphana fulvistemma* Motschulsky, 1860 (n = 3), *Eucetonia roelofsi* Harold, 1880 (n = 3), and *Protaetia brevitarsis* (n = 6). Following substrate sorting and laboratory rearing, 159 individuals of the two scarabaeid species were recorded (Table 1). Most individuals were *P. brevitarsis*, with 10 individuals recorded in the initial input year (2024) and 145 individuals recorded in the continued input year (2025). In contrast, *E. roelofsi* was only recorded during the continued input year (n = 4).

To evaluate the effects of resource input treatments and environmental variables on the abundance of *P. brevitarsis*, a set of candidate models, including five environmental variables, was constructed, and the models were compared using AIC. The model that included the interaction between resource input treatment and canopy openness had the lowest AIC value (122.8) (Table 2). In this model, the continued input treatment (2025) exhibited a significant positive effect on *P. brevitarsis* abundance compared to that of the initial input treatment (2024) (estimate = 4.54, $p < 0.001$). Canopy openness also had a positive effect (estimate = 5.02, $p = 0.021$), whereas tree species richness had a negative effect (estimate = − 5.00, $p = 0.043$). The total basal area showed a positive but non-significant effect (estimate = 1.95, $p = 0.125$). Although not statistically significant, the interaction between resource-input treatment and canopy openness exhibited a negative tendency (estimate = − 3.12, $p = 0.062$), suggesting that the effect of canopy openness was reduced under the continued input treatment (Fig. 3).

3.2. Height experiment

A total of 184 individuals of *P. brevitarsis* were recorded following substrate sorting and laboratory rearing. Among the installation heights, the highest abundance was recorded at 4.5 m (113 individuals; mean ± SD = 7.5 ± 11.2 individuals), followed by 3.0 m (62 individuals; 4.1 ± 9.3 individuals), and 1.5 m (nine individuals; 0.6 ± 2.3 individuals).

To evaluate the effects of installation height and environmental variables on *P. brevitarsis* abundance, a set of candidate models, including six environmental variables, was constructed, and model selection

was conducted using the AIC. The intercept-only model exhibited the lowest AIC (140.0), although the model including installation height was also strongly supported (ΔAIC = 1.35) (Table 3). To further evaluate the effect of installation height, parameter estimates from the height-including model were examined, revealing that both 3 and 4.5 m installations showed higher *P. brevitarsis* abundance compared to that of the 1.5 m installation (3 m: estimate = 2.85, p = 0.098; 4.5 m: estimate = 3.45, p = 0.067) (Fig. 4). In contrast, canopy openness, DBH of the host tree, total basal area (TBA), tree species richness, and number of trees showed no evident effects on *P. brevitarsis* abundance.

4. Discussion

4.1. Characteristics of scarab beetles recorded in artificial tree hollows

In this study, four scarabaeid beetle species were recorded in artificial treeholes. Previous studies have shown that both *R. polita* and *E. roelofsi* complete their larval development within treeholes (Matsumoto et al. 2003; Seshima 2023); therefore, the occurrence of these species in the present study is consistent with their known association with treehole habitats. In addition, *P. brevitarsis*, the most abundant species observed in this study, has previously been reported in treeholes as well as in the nests of cavity-nesting birds (*Strix uralensis* Pallas, 1771) (Nasu et al. 2011; Koshiyama et al. 2019a). Additionally, larvae of *G. fulvistemma* utilize decaying wood and decomposed organic matter as food resources (Iijima and Takeuchi 2007). These observations suggest that all scarabaeid species observed in this study are likely associated with organic-matter-rich depositional environments formed within treeholes during their larval stage and can broadly be classified as saproxylic insects within a detritivorous group. These results indicate that the artificial treeholes effectively provided habitat conditions suitable for several saproxylic scarab beetles associated with organic matter-rich treehole environments.

Rhomborhina polita was represented by only a single adult individual during the study period, and no larval occurrence was confirmed. *Rhomborhina polita* has previously been identified in tree holes near the root collar (Matsumoto et al. 2003). Treeholes vary in internal moisture and temperature conditions depending on their height, and those at lower vertical positions tend to exhibit more stable microclimatic conditions with reduced seasonal temperature variations (Lindman et al. 2023; Ranius et al. 2024). In contrast, wooden bird-nest boxes typically exhibit greater temperature fluctuations and lower humidity than those of natural treeholes (Maziarz et al. 2017; Sudyka et al. 2023). These differences in microclimatic conditions may negatively affect the oviposition site selection and larval survival of this species. However, records of larval development sites for this species remain limited (Matsumoto et al. 2003; Sakai and Fujioka 2007), and the developmental ecology across the continuous microhabitat gradient from treeholes to root-associated soil remains poorly understood. The lack of larval records in both experiments suggests that the artificial treeholes used in this study may not have fully reproduced the habitat conditions required by *R. polita*. As this species has previously been recorded in tree holes

near the root collar, more stable microclimatic conditions associated with lower vertical positions may be important for habitat use.

Similar to *R. polita*, the occurrence of *E. roelofsi* and *G. fulvistemma* was also limited. These two species emerge as new adults from summer to autumn (Iijima and Takeuchi 2007; Sakai and Fujioka 2007). In this study, their occurrence was confirmed through the collection of cocoons observed incidentally on the surface during routine inspections; however, sampling and substrate sorting were conducted in early to mid-November at Site A and in mid-September at Site B, respectively, suggesting that many individuals may have already emerged at the time of sampling. Additionally, both species visit the flowers of herbaceous plants such as *Erigeron annuus* (L.) Pers. and *Cirsium japonicum* dendritic cells (DC) during the adult stage (Iijima and Takeuchi 2007; Sakai and Fujioka 2007), indicating that they may have utilized the surrounding vegetation and floral resources as part of their life history. Therefore, these resource-use traits may be influenced by conditions within treeholes as well as by the surrounding landscape context. However, the influence of floral resource availability on the distribution and occurrence of saproxylic beetles has not been sufficiently examined (Ranius et al. 2024), and remains an important topic for future research. The limited occurrence of these species in the present study suggests that the use of artificial treeholes depends on both internal habitat conditions and external resource availability.

4.2. Effects of animal-derived resources on *P. brevitarsis*

In this study, the number of *P. brevitarsis* individuals was notably higher in 2025, when animal-derived resources were continuously supplied, than in 2024, when resources were added only once at the beginning of the experiment. These findings indicate that repeated animal-derived resource input improves the suitability of treeholes as larval habitats and oviposition sites for *P. brevitarsis*. Organic materials, such as animal remains and food residues deposited by bird nesting activities in treeholes, can function as temporary high-nutrient resource patches, thereby supporting the development of invertebrate communities (Krištofík et al. 2003; Hanzelka et al. 2023). Additionally, bird nests used for longer periods tend to support higher abundances of saprophagous insects, likely because of continuous resource input and progressive decomposition of accumulated materials (Hamao and Nasu 2022). *Protaetia brevitarsis* larvae utilize humified organic matter and animal-derived materials (Koshiyama et al. 2019b). Furthermore, larvae have been collected from compost-like substrates to which both plant residues and animal-derived food waste such as fish skin and bones have been repeatedly added, indicating that this species readily exploits nutrient-rich substrates containing animal-derived organic matter (Koshiyama et al. 2019a). These observations suggest that *P. brevitarsis* responded strongly to the high nutrient resource patches formed through repeated inputs of chick carcasses in the present study. Furthermore, laboratory experiments have shown that larvae provided with both humus and animal tissues exhibit either faster development or increased adult body size compared to those fed only humus (Koshiyama et al. 2019b). In addition, in the saproxylic beetle *O. barnabita*, females may select oviposition sites based on resource conditions that are favorable for larval development (Landvik et al. 2016). Collectively, these findings suggest that repeated animal-derived

resource inputs improve the quality of treehole habitats for *P. brevitarsis* by increasing the availability of suitable larval resources and potentially influencing oviposition site selection.

Among environmental factors, canopy openness had a positive effect on *P. brevitarsis* abundance. Light conditions and associated microclimatic factors, such as temperature and humidity, influence species composition and abundance (Ranius et al. 2024). For example, an increased occurrence of the saproxylic beetle *Osmoderma eremita* (Scopoli, 1763) has been reported in treeholes located in open or semi-open habitats, suggesting a preference for sun-exposed conditions (Ranius and Nilsson 1997). Although this habitat preference has not been explicitly reported for *P. brevitarsis*, the present results suggest that this species is more likely to utilize tree holes in relatively open environments. However, in the present study, when animal-derived resources were continuously supplied until 2025, the effect of canopy openness declined. This suggests that, under conditions of abundant and continuous resource input, internal resource conditions within treeholes may become more important than the surrounding environmental conditions in determining habitat use. Notably, studies using water-filled and artificial treeholes have shown that differences in organic matter input strongly influence insect abundance and community composition, with resource availability acting as the primary driving factor (Gossner et al. 2015; Petermann and Gossner 2022). These results indicate that animal-derived resource inputs may alter the relative strength of environmental constraints on habitat selection in *P. brevitarsis*.

Tree species richness was negatively correlated with *P. brevitarsis* abundance. However, empirical studies that directly examine the effects of tree species diversity in surrounding forest stands on treehole-dwelling invertebrates are limited. Recent reviews have also highlighted that the key determinants of treehole-associated communities are typically treehole characteristics, availability of treehole resources, and landscape structure; however, tree species diversity has not been extensively investigated (Ranius et al. 2024). Therefore, the observed negative relationship was more likely to indicate the underlying stand structure or management history than the direct effect of tree species richness. For example, young or managed stands dominated by small-diameter trees have reduced the availability of natural treeholes (Zawadzka et al. 2016), and the tree species richness in the present study may have demonstrated these structural conditions. However, because natural treehole abundance and large tree distribution have not been directly quantified, the underlying mechanisms remain unclear. In addition, because adults of *P. brevitarsis* feed on tree sap and floral resources (Sakai and Fujioka 2007; Okajima and Araya 2012; Kishimoto-Yamada et al. 2017), larval habitat conditions as well as the availability of feeding resources associated with tree composition may influence habitat use. These results suggest that the negative relationship between tree species richness and *P. brevitarsis* abundance observed in this study may indicate differences in stand structure or habitat conditions rather than a direct effect of tree species richness.

4.3. Effects of treehole height on *P. brevitarsis*

In our height experiment, although the model including installation height was not selected as the best model, a consistent tendency was observed in which *P. brevitarsis* abundance was higher in artificial treeholes installed at 3.0 and 4.5 m compared to those at 1.5 m. This suggests that installation height

may influence the use of artificial treeholes by this species. Invertebrate communities in treeholes are not consistently structured by height alone, although vertical position influences species composition and species-specific distribution (Yoshida et al. 2018; Petermann and Gossner 2022). Furthermore, a recent review indicated that entrance height is an important environmental factor, although the direction and magnitude of its effects vary among species (Ranius et al. 2024). These results suggest that installation height may influence the use of artificial treeholes by *P. brevitarsis*, although the effect of height likely indicates associated environmental differences rather than vertical position alone.

The highest abundance of *P. brevitarsis* was observed at 4.5 m. In the resource input experiment, canopy openness positively affected *P. brevitarsis* abundance. Therefore, treeholes located at greater heights are likely to experience less shading from the surrounding vegetation, potentially resulting in more open microclimatic conditions. Therefore, changes in light availability and associated microclimatic conditions, such as temperature and humidity, rather than height, may have influenced habitat use by this species. Kaneko et al. (2026) captured adult *P. brevitarsis* predominantly in traps placed at higher positions, indicating that the species frequently uses the forest canopy or upper strata during its adult stage. In addition, larvae have been reported from tree holes, as well as from the nests of *Strix uralensis* and *Ciconia boyciana* Swinhoe, 1873 (Nasu et al. 2010, 2011). As these large arboreal cavity-associated birds use elevated nesting sites within trees (Shiraishi and Kitahara 2007 Nasu et al. 2010), these records suggest that *P. brevitarsis* can exploit resource patches located in the forest canopy. The higher abundance of *P. brevitarsis* in artificial treeholes installed at elevated heights observed in the present study is consistent with this ecological characteristic.

4.4. Limitations and future directions

Several limitations of this study should be considered when interpreting its results. First, the artificial treeholes used in this study could not fully reproduce the environmental conditions and biological communities associated with natural treeholes (Jansson et al. 2009; Maziarz et al. 2017; Sudyka et al. 2023). In particular, differences in internal microenvironmental conditions may influence organisms inhabiting artificial treeholes. Therefore, the responses observed in this study may not necessarily represent the responses of scarab beetles to natural treeholes.

Second, the resource-input experiment compared different resource-input regimes over two consecutive years, with the initial input in 2024 and continued input in 2025. Although the notable increase in *P. brevitarsis* abundance under continued resource input suggests an effect of repeated resource addition, the effects of resource input cannot be completely separated from the potential interannual variation. Future experiments using different resource input treatments within the same year may provide stronger evidence of the effects of resource input frequency.

Third, although four scarab beetle species were recorded, the statistical analyses focused on *P. brevitarsis*, which accounted for most of the individuals collected. Therefore, the effects of animal-derived resource input and installation height observed in the present study may not be generalizable to other scarab beetle species with different ecological requirements. Further studies involving multiple

scarab beetle species, as well as direct comparisons between artificial and natural treeholes, are necessary to determine the generality of these patterns.

5. Conclusions

This study showed that continuous input of animal-derived organic matter was associated with an increase in *P. brevitarsis* abundance, suggesting that this species responds strongly to high-nutrient environments. Several scarabaeid beetle species have been reported in bird nests, in addition to *P. brevitarsis* (Aiello 1987; Koshiyama et al. 2012; Choi et al. 2018; Zbyryt and Oleksa 2018), and the resource-related responses observed in this study may also occur in other treehole-associated scarabaeid beetles. In addition, increases in scarabaeid abundance associated with resource input may alter decomposition processes and humus quality within treeholes, potentially influencing the formation of communities in other treehole-dwelling invertebrates (Sánchez-Galván et al. 2014; Landvik et al. 2016). Future studies integrating the processes from resource input to beetle colonization and subsequent community assembly are necessary to advance our understanding of treehole ecosystem functioning.

Although the effect of installation height was not statistically significant, the observed tendency for higher abundance of *P. brevitarsis* in the elevated artificial treeholes provided evidence that this species may preferentially exploit arboreal resource patches. Together with the positive association between canopy openness and abundance observed in the resource input experiment, these findings suggest that the vertical position may influence the use of treeholes by *P. brevitarsis*. This study provides fundamental insights into how animal-derived resource inputs and environmental conditions influence the use of treeholes by scarabaeid beetles. Additionally, the developed experimental system facilitates the separation of resource and environmental effects that are challenging to distinguish in natural treeholes.

Declarations

Competing Interests

The authors declare no conflicts of interest.

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Author Contribution

RA collected and analysed the data, prepared the figures, and drafted the manuscript. TY supervised the study and contributed to the writing and revision of the manuscript. HK identified the insect specimens and contributed to the review and revision of the manuscript.

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Data availability

The datasets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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Tables

Table 1. Number of adults that emerged after the collection of substrates from artificial treeholes.

Species	Initial input (2024)		Continued input (2025)	
	Total	**Mean ± SD**	**Total**	**Mean ± SD**
Cetonia roelofsi	0	0 ± 0	4	0.11 ± 0.68
Protaetia brevitarsis	10	0.29 **±** 1.40	145	4.14 ± 9.20

Table 2. Effects of artificial treehole height on the abundance of *Protaetia brevitarsis*.

Variables	Estimate	Std. Error	z	p value
Main effects				
Intercept	-11.83	3.80	- 3.11	0.002
Continued	4.54	1.26	3.60	<0.001
Canopy openness	5.02	2.17	2.31	0.021
Tree species richness	-5.00	2.48	-2.02	0.043
TBA	1.95	1.27	1.53	0.125
Interaction effects				
Continued × Canopy openness	-3.12	1.67	-1.87	0.062

1. Results are based on a generalized linear mixed model (GLMM) with a Poisson error distribution and a log-link function. Artificial tree-hole ID was included as a random intercept to account for repeated measurements of experimental units. Model fit statistics were AIC = 122.9, BIC = 138.5, and −2 × log-likelihood = 108.9.

2. The main effects of resource-input condition and environmental variables represent effects under the reference condition (initial input treatment, 2024). The interaction term between resource-input condition and canopy openness represents the change in the effect of canopy openness under the continued input treatment (2025).

3. Continuous explanatory variables were standardized before analysis.

Table 3. Generalized linear mixed model results for the abundance of *Protaetia brevitarsis* in relation to resource-input treatment and environmental variables.

Variables	Estimate	Std. Error	z	p value
Intercept	-0.51	0.99	-0.52	0.604
Height 3 m	1.93	1.36	1.42	0.156
Height 4.5 m	2.53	1.36	1.86	0.063

1. Results are based on a generalized linear model (GLM) with a negative binomial error distribution (nbinom2) and a log-link function. Model fit statistics for the presented model were AIC = 146.1, BIC = 158.7, and −2 × log-likelihood = 132.1.

2. Artificial tree-hole height was treated as a categorical variable, with 1.5 m as the reference level. Parameter estimates for 3 m and 4.5 m represent differences compared with the reference level.

Figures

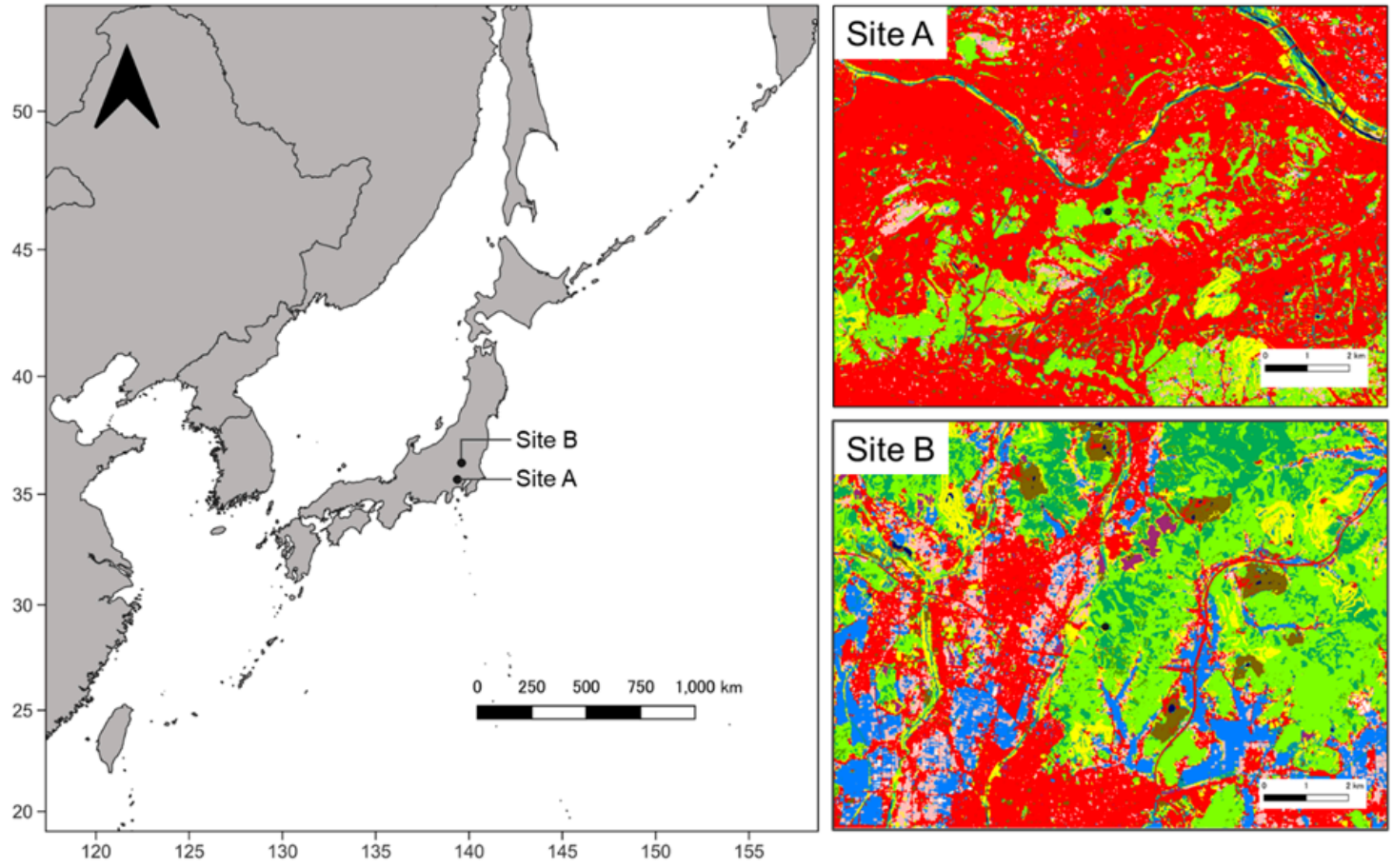


Figure 1

Location of the two study sites (Sites A and B). The left panel shows the locations of the study sites in Japan. The right panels show the surrounding land use and land cover based on the High-Resolution Land-Use and Land-Cover Map of Japan (JAXA EORC, 2024)

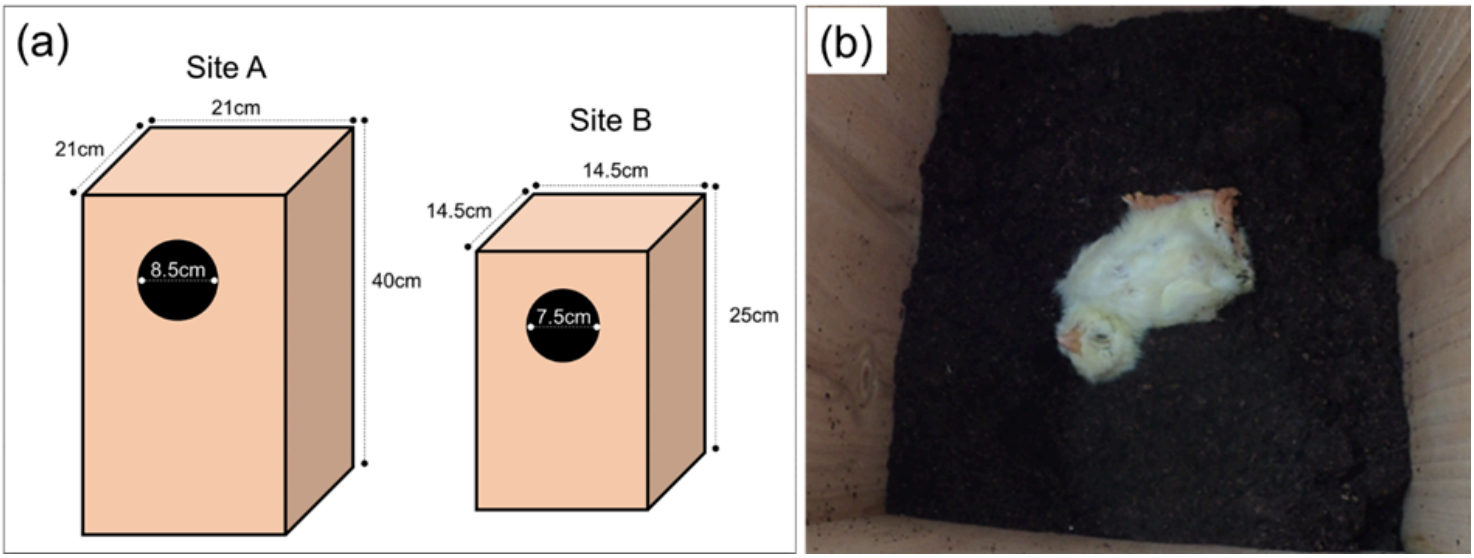


Figure 2

Artificial treeholes used in this study. (a) Sketch of the treehole structure, (b) inside view of the artificial treehole at the initial stage

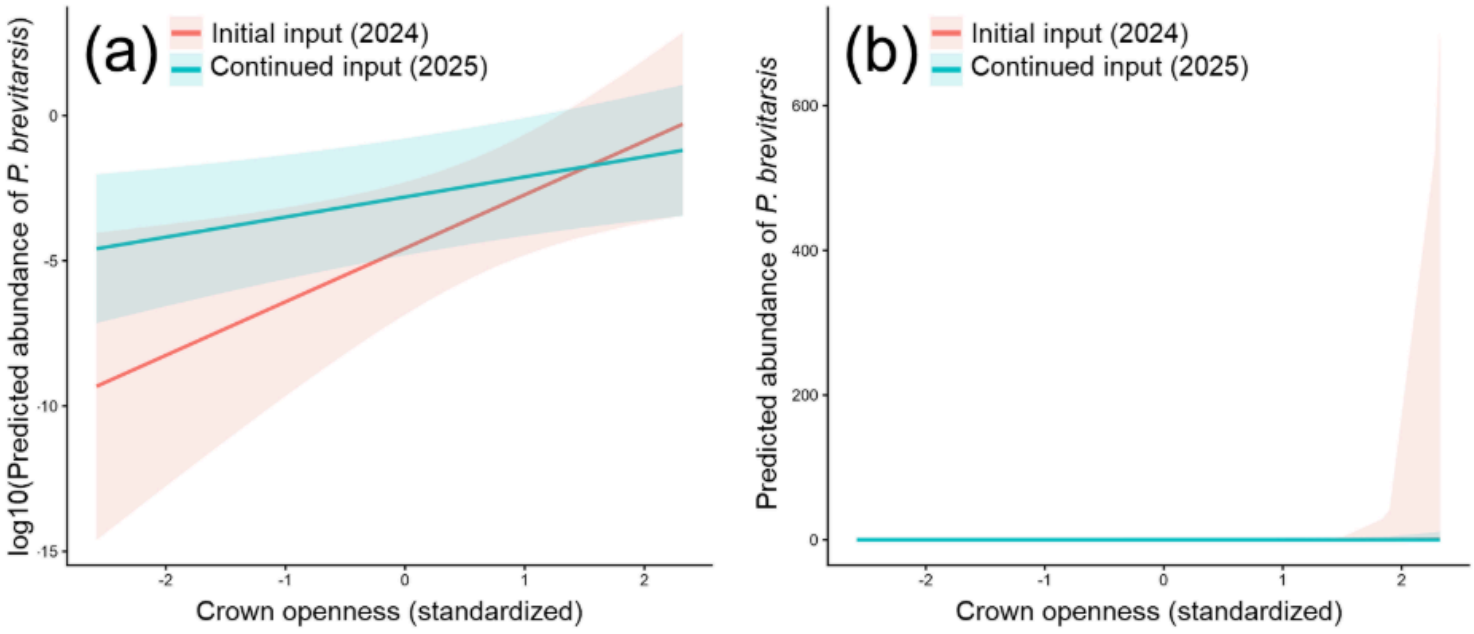


Figure 3

Year-specific relationships between canopy openness and the predicted abundance of *Protaetia brevitarsis*. Lines represent model predictions for the initial input year (2024) and the continued input

year (2025), with shaded areas indicating 95% confidence intervals. Panel (A) shows predictions on the log10 scale and panel (B) shows back-transformed predictions on the original abundance scale

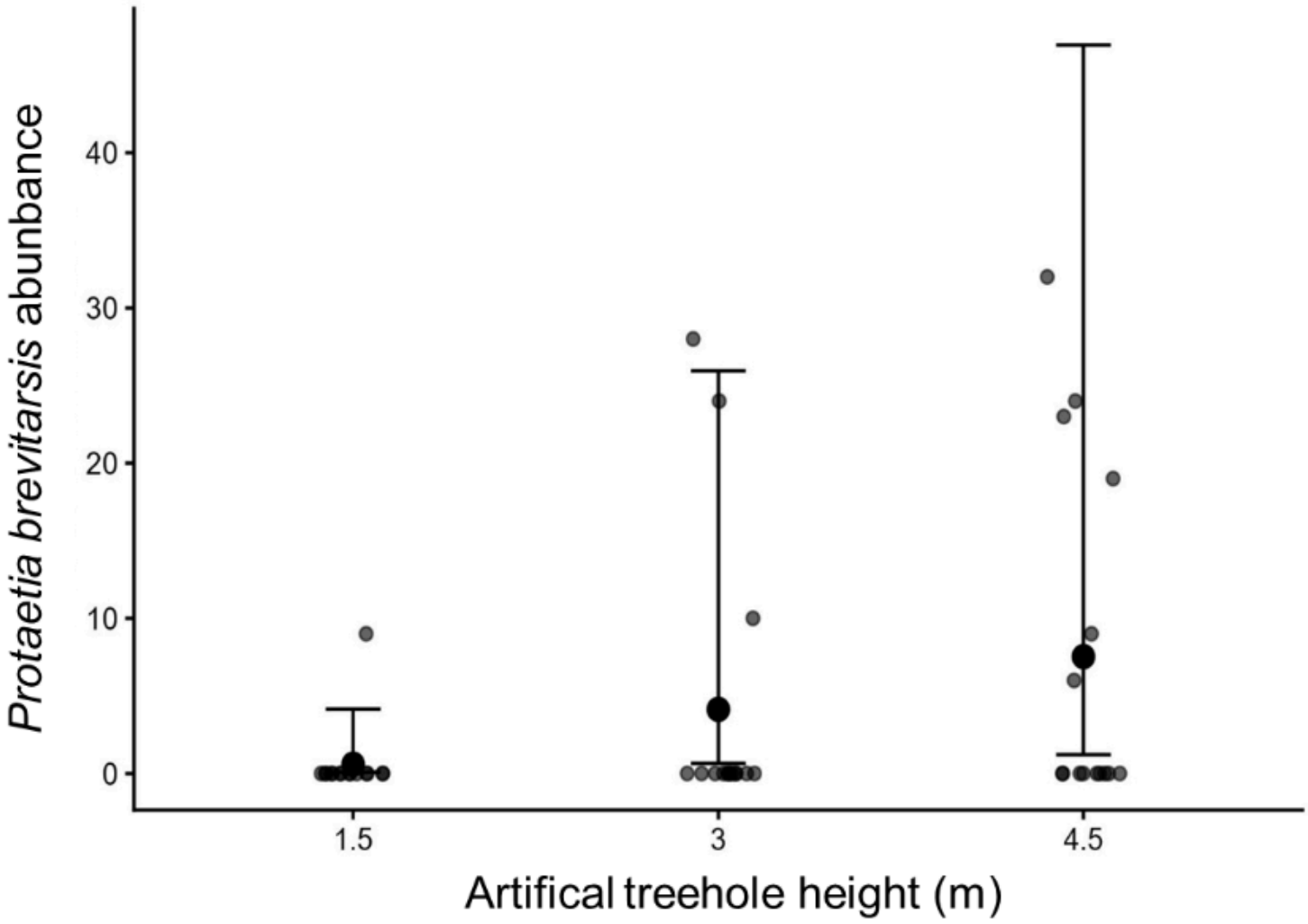


Figure 4

Predicted abundance of *Protaetia brevitarsis*across artificial treehole heights. Points indicate model predictions from the height-only negative binomial model, error bars indicate 95% confidence intervals, and gray points represent observed abundances