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Ecological divergence of burying beetles into the forest canopy

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Closely related species with overlapping geographic ranges encounter a significant challenge: they share many ecological traits and preferences but must partition resources to coexist. In Ontario, potentially eleven species of carrion beetles (Coleoptera: Silphidae) live together and require vertebrate carrion for reproduction. Their reliance on an ephemeral and uncommon resource that is unpredictable in space and time is thought to create intense intra- and interspecific competition. Evidence suggests that burying beetle species reduce competition by partitioning carrion for breeding across different habitats, temperatures, and seasons. Here, we test predictions of an alternative axis for partitioning carrion: vertical partitioning between the ground and forest canopy. We conducted a survey of carrion beetles from May to July 2016 at the Queen's University Biological Station across 50 randomly generated points using baited lethal traps at 0m and 6m. Ground traps yielded more species and individuals compared to those in the canopy, and the number of individuals and species caught increased through the season in both trap types. Ground and canopy traps were accurately distinguished by the presence or absence of three predictor species: ground traps contained more *Nicrophorus orbicollis* and *Necrophila americana*, while canopy traps contained more *Nicrophorus pustulatus*. Indeed, we trapped 253 *N. pustulatus* in the canopy, but only 60 on the ground; *N. pustulatus* was the most common species in the canopy, and the only species that was more common in the 6m traps than on the ground. *N. pustulatus* is thought to be rare across its geographic range, but our results suggest instead that *N. pustulatus* is uniquely common in canopy habitats, demonstrating a vertical partitioning of habitat and resources between *N. pustulatus* and other co-occurring burying beetles. Our results are consistent with *N. pustulatus* having diverged into canopy habitats as a strategy to coexist with closely related sympatric species when competing for similar resources. We still, however, do not know the traits that allow *N. pustulatus* to flourish in the canopy, exactly how *N. pustulatus* uses canopy resources for breeding, or the factors that restrict the expansion of other burying beetles

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Ecological divergence of burying beetles into the forest canopy

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ABSTRACT

Closely related species with overlapping geographic ranges encounter a significant challenge: they share many ecological traits and preferences but must partition resources to coexist. In Ontario, potentially eleven species of carrion beetles (Coleoptera: Silphidae) live together and require vertebrate carrion for reproduction. Their reliance on an ephemeral and uncommon resource that is unpredictable in space and time is thought to create intense intra- and interspecific competition. Evidence suggests that burying beetle species reduce competition by partitioning carrion for breeding across different habitats, temperatures, and seasons. Here, we test predictions of an alternative axis for partitioning carrion: vertical partitioning between the ground and forest canopy. We conducted a survey of carrion beetles from May to July 2016 at the Queen's University Biological Station across 50 randomly generated points using baited lethal traps at 0m and 6m. Ground traps yielded more species and individuals compared to those in the canopy, and the number of individuals and species caught increased through the season in both trap types. Ground and canopy traps were accurately distinguished by the presence or absence of three predictor species: ground traps contained more *Nicrophorus orbicollis* and *Necrophila americana*, while canopy traps contained more *Nicrophorus pustulatus*. Indeed, we trapped 253 *N. pustulatus* in the canopy, but only 60 on the ground; *N. pustulatus* was the most common species in the canopy, and the only species that was more common in the 6m traps than on the ground. *N. pustulatus* is thought to be rare across its geographic range, but our results suggest instead that *N. pustulatus* is uniquely common in canopy habitats, demonstrating a vertical partitioning of habitat and resources between *N. pustulatus* and other co-occurring burying beetles. Our results are consistent with *N. pustulatus* having diverged into canopy habitats as a strategy to coexist with closely related sympatric species when competing for similar resources. We still, however, do not know the traits that allow *N. pustulatus* to flourish in

the canopy, exactly how *N. pustulatus* uses canopy resources for breeding, or the factors that restrict the expansion of other burying beetles into this habitat.

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INTRODUCTION

The coexistence of closely related species presents an ecological challenge: they share many traits and preferences through recent common ancestry and often depend on similar resources, and yet are thought to require ecological partitioning to allow them to coexist. For this reason, burying beetles in the family Silphidae have been a focus of ecological studies of resource partitioning – they all require vertebrate carcasses for reproduction and these carcasses are thought to be limiting resources in their environment. Despite these similar requirements, many different Silphid species co-occur within communities, sometimes fighting over the same resources. Do these species partition carrion to coexist within communities, and, if so, how? Previous studies have suggested an answer to this question: burying beetles may coexist because they partition carrion based on habitat, timing of seasonal activity and breeding, timing of diel activity, and trade-offs between the ability to locate carcasses quickly versus competitive dominance (Anderson, 1982; Wilson, Knollenberg, & Fudge, 1984; Trumbo, 1990; Trumbo & Bloch, 1992; Benigner & Peck 1992; Lingafelter, 1995; Trumbo & Bloch, 2000; Trumbo & Bloch, 2002; Urbanski & Baraniak, 2015). These axes of ecological partitioning are plausible hypotheses to explain how different burying beetle species coexist, but the ecological distinctions of some species remain poorly known.

The ecology of one species in particular, *Nicrophorus pustulatus*, remains enigmatic. Historically, *N. pustulatus* was found to be widespread but rare across its range because few individuals were caught in ground traps typically set for burying beetles. However, the regular occurrence of *N. pustulatus* at lights (Anderson, 1982; Lingafelter, 1995) was difficult to explain. A later discovery of *N. pustulatus* feeding on gray ratsnake (*Pantherophis spiloides*) eggs (Blouin-Demers & Weatherhead, 2000; Keller & Heske, 2001) led some researchers to suggest that *N. pusutulatus* specialized on ratsnake eggs, or oviparous snake eggs in general, an idea supported by an observation of *N. pustulatus* larvae feeding on northern ringneck snake (*Diadophis punctas edwardsii*) eggs (Legros, Pratt & Beresford; 2010). However, the range of *N. pustulatus* extends beyond that of ratsnakes (LeGros & Beresford, 2010), and its occurrence in a failed Northern Saw-whet Owl (*Aegolius acadicus*) nest 8m high in the forest canopy in Connecticut, USA was inconsistent with *N. pustulatus* specializing on snake eggs (Philips, Root & DeSimone, 1983). The use of vertebrate carrion by *N. pustulatus* in captivity (Robertson, 1992; Trumbo, 1992; Rauter & Moore, 2002; Trumbo, 2007; Rauter & Rust, 2012) also suggested that this species might use vertebrate carrion in nature similar to other *Nicrophorus* species.

Further insect sampling suggested that *N. pustulatus* may be more common in the forest subcanopy and canopy, rather than on the ground where most *Nicrophorus* species breed and feed. *N. pustulatus* was first caught in the canopy in general insect traps (e.g. flight intercept traps; Ulyshen & Hanula, 2007). Following this work, burying beetle surveys using baited traps found *N. pustulatus* almost exclusively several metres above the ground (Ulyshen, Hanula & Horn, 2007; LeGros & Beresford, 2010). These canopy *N. pustulatus* would have gone undetected using ground-based surveys, such as carrion baited pit-fall traps (Su & Woods, 2001; Schroeder, Buddle & Saint-Germaine, 2009), that were typically used because burying beetles were thought to require ground soil for burying and reproduction. To date, *N. pustulatus* has been caught in baited elevated traps in only four studies. Ulyshen, Hanula and Horn (2007) discovered that *N. pustulatus* was more abundant in their canopy traps, where 33 individuals were found at 5m and 15m, and only 1 individuals was caught at 0.5m above the ground. LeGros and Beresford (2010) found *N. pustulatus* also preferred canopy habitats, where 6 individuals were caught in 6m traps and none at 2m and 4m. Lowe and Lauff (2012) investigated arboreal carrion use by suspending baited nest boxes 9-10m in the canopy, and found *N. pustulatus* on 6 occasions with no observations of reproductive behaviour, representing less than 4% of beetle encounters with carrion in their study. Dyer and Price (2013) collected perhaps the greatest numbers of *N. pustulatus*; they captured 106 individuals at 2.5m compared to 47 individuals at 0.5m in surveys in Maryland, USA. Overall, *N. pustulatus* has been repeatedly caught in elevated canopy and sub-canopy traps, but only rarely in large numbers.

Here, we test the hypothesis that *Nicrophorus pustulatus* is primarily found in the canopy, and that the burying beetle community differs in species occurrence and abundance between the ground and the canopy. To test these hypotheses, we employed paired, baited traps on the ground and 6m off the ground at our study site in southeastern Ontario, Canada and compared beetles trapped on the ground versus in the canopy. Because burying beetles typically breed on the ground (Anderson & Peck, 1985), we predicted that (1) traps on the ground would yield more species and individuals compared to those in the canopy, (2) ground and canopy traps would differ in their composition and abundance of species, and (3) canopy traps would collect a greater abundance of *Nicrophorus pustulatus* compared to ground traps.

METHODS

Study Species

The carrion beetle family, Silphidae, is comprised of two subfamilies: Silphinae and Nicrophorinae. Species in the subfamily Silphinae may avoid competition with Nicrophorinae species by using larger carcasses, whereas *Nicrophorus* prefer smaller carcasses that can be buried more easily (Anderson & Peck, 1985). Nicrophorinae, also known as the burying beetles, exhibit unique resource guarding and parental care behaviour. Burying beetles breed on small vertebrate carcasses and typically avoid competition with flies and other scavengers by burying or covering the carcass (Anderson & Peck, 1985). If more than one pair of adult beetles is present on the carcass, including pairs of different species, fighting typically ensues and continues until only one pair remains (Anderson & Peck, 1985). The winning pair then rears and cares for their offspring, using the carcass to feed their larvae. In southeastern Ontario, potentially twelve species of carrion beetles live closely together and compete for small vertebrate carrion. Carrion beetles have shown general patterns of spatial and temporal habitat partitioning. The seasonal timing of emergence and reproduction differ for some species of carrion beetles. Some emerge and begin breeding earlier in the spring (e.g. *Nicrophorus sayi*, *Oiceoptoma noveboracense* or *Oiceoptoma inequale*), or later in the summer (e.g. *N. tomentosus*), while most are active and reproductive in early- and mid-summer (Anderson & Peck, 1985; Scott, 1998). A number of species have shown preferences to certain habitats, such as marshes and bogs (e.g. *N. hebes*; Beninger & Peck, 1992; Sikes, Trumbo, & Peck, 2016), or primarily coniferous or mixed forest (e.g. *N. defodiens*; Anderson, 1982; Wilson, Knollenberg, & Fudge, 1984; Anderson & Peck, 1985). *N. marginatus* appears to specialize on carrion in extensive open field habitats (Anderson, 1982; Trumbo & Bloch, 2000), and was not collected in our study, likely because none of our traps were placed in suitable habitat. Other species of carrion beetles either prefer forested habitats or show no habitat preference (Anderson, 1982).

We included all species of carrion beetles from the family Silphidae that were caught in traps during our study. These species included six in the genus *Nicrophorus* (Nicrophorinae): *Nicrophorus orbicollis, N. pustulatus, N. tomentosus, N. sayi, N. defodiens, and N. hebes,* and four species from the subfamily Silphinae: *Necrophila americana, Necrodes surinamensis*, *Oiceoptoma inaequale, and O. noveboracense*.

Study Site

We collected burying beetles in baited lethal traps on the Queen's University Biology Station (QUBS, 44.5653, -76.322, 129m) properties near Elgin, Ontario, Canada during the reproductive period from early May until late July 2016. We set traps at 50 block-randomized points across QUBS properties that are the subject of long-term studies of diverse taxonomic groups (birds, plants, insects). These study points were originally chosen by randomly selecting GPS points that fell within the property boundaries, with the restriction that no point could fall within a body of water, and each point was at least 400m away from all other points. Our study site includes areas of regrowth forest dominated by Sugar Maple (*Acer saccharum*) and Ironwood (*Ostrya virginiana*), with some species of ash (*Fraximus* spp.), elm (*Ulmus* spp.), hickory (*Carya* spp.) and birch (*Betula* spp.), as well as Basswood (*Tilia americana*) (Martin, 1994). Other trapping locations at our study site include: areas of wet woodland composed mainly of Eastern White Cedar (*Thuja occidentalis*) and birch species, man-made conifer plantations, edges of small lakes and beaver ponds, forest edges, open fields that were once used for agricultural practices, or open rocky outcrops composed of Red Oak (*Quercus rubra*), Eastern White Pine (*Pinus strobus*), Red Juniper (*Juniperus virginia*), and a number of mosses,

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grasses, and lichen-covered rock (Martin, 1994). The canopy and vegetation at the study site is generally large, dense, and low, typically between 1.5-12m high (Martin, 1994).

Trapping Methods

At each trapping location, two concurrent traps were set and then collected after 7 days: a pitfall trap in the ground, and a trap of the same design suspended 6m above ground. We sampled each point twice: once in May/June, and once in July. We constructed our traps using plastic buckets approximately 35cm deep and 17cm in diameter. We filled the buckets with 6cm (depth) of saturated saline solution to kill and preserve the beetles. We covered the top of each trap with a 35cm^2 piece of chicken wire. We baited each trap with one chicken wing wrapped in cheesecloth, suspended from the middle of the chicken wire using steel craft wire. The bait was frozen until deployed in traps without any prior thawing or ripening and suspended so that it did not touch the edges of the trap or the saline preservative. We covered each trap with a 30cm^2 plywood board to prevent rainwater from entering. We secured each ground trap by placing large rocks from each site on top of the plywood board in an attempt to deter vertebrate scavengers from disrupting the traps. Canopy traps were hung 6m high in tree branches within 20m of the paired ground trap; the exact distance between the paired ground and canopy traps varied depending on the availability of soil for ground pitfall traps and trees for canopy traps (average = 4.8m between paired ground and canopy traps). Differences in the number of successful traps between the ground and canopy were caused by a greater disturbance of ground traps (N=18), likely by vertebrate scavengers stealing the bait and/or pulling the trap from the ground. Three traps were also omitted because of trap failure due to human error in deployment. A total of 34 traps, mostly in the canopy, were successfully deployed and were undisturbed by vertebrates but did not collect any carrion beetles. These traps were included in tests for differences in the number of beetles and number of species between ground and canopy traps, but were omitted from subsequent classification analyses because they provided no information on carrion beetle community composition.

Species and Sex Identification

Each beetle specimen was first identified as a Silphidae by their large size, possession of clavate or capitate 11-segmented antennae, prominent fore coxae, and elytra that were truncate, tricostate, or lacking costae. Once identified as a Silphidae, each specimen was identified to genus, species, and sex (Anderson & Peck, 1985) using the specific traits detailed below.

1. *Nicrophorus*

Nicrophorus species were distinguished from all other genera by their distinct elytra that are truncate in shape and black or very dark in colour with orange or red markings. *Nicrophorus tomentosus* was identified by their distinct pronotum with dense yellow pubescence. *N. sayi* was identified by the anterior orange spots on the elytra that curve around the humerus, and their curved metatibia. *N. orbicollis* have clubbed orange antennae and circular posterior spots. They also have characteristic, long elytral setae. *N. hebes* (previously *N. vespilloides*; Sikes, Trumbo & Peck, 2016) have black antennae and an orange base to their elytral epipleuron with a prebasal black spot. *N. defodiens* have black antennae and an elytral epipleuron base that is entirely black. *N. pusulatus* are perhaps the most distinct: mostly black with small elytral spots rather than bands. Their elytra are smooth without any setae. We sexed *Nicrophorus* using the shapes and pattern of abdominal segments: the last abdominal segment of females is usually squared off, while males have an additional abdominal segment that telescopes if the animal is gently squeezed on the sides.

2. *Necrophila*

Necrophila americana was identified by the distinct black disc surrounded by yellow on the pronotum. Females were identified by their prolonged or sharper elytral apices, whereas those of males are not prolonged and are rounder.

3. *Oiceoptoma*

Oiceoptoma inaequale were identified by their all black head, pronotum, and elytron. *O. noveboracense* were distinguished from *O. inaequale* by the orange-red margins of their pronotum and their brownish to black elytron. Females have prolonged or sharper elytral apices, whereas those of the males are not prolonged and are rounder.

4. *Necrodes*

Necrodes surinamensis have large eyes and a dark body with very prominent, raised elytral ridges. They have variable red-orange spots on the elytra. Males are distinct from females based on their leg morphology: expanded hind femora with large teeth and expanded foretarsi.

Statistical Analyses

Generalized Linear Models

We included all traps that caught carrion beetles, and traps that caught no beetles provided that they showed no evidence of disturbance from scavengers (i.e. the trap was intact with bait present). We omitted traps that caught no beetles if there was also evidence of trap disturbance.

To test our hypotheses that the number species and abundance of burying beetles was higher in ground versus canopy traps, we ran two generalized linear models with the number of species and total number of burying beetles as the response variables in two different models, and ground versus canopy trap and Julian date of trap retrieval as predictor variables in a saturated model. We checked for normality of predictors using Shapiro-Wilk tests, histograms, and residuals plots. We ran the full model for each response variable independently and examined its performance by plotting standardized residuals against fitted values and all predictors, by testing for linearity and homogeneity in the variance of residuals for each predictor using Bartlett's tests, and by testing if the distribution of residuals and predictors differed from normality using Shapiro-Wilk tests. We compared the performance of different models using the *dredge* command in the *MuMIn* package (version 1.15.6; Bartoń, 2016) to determine the model with best performance (maximum likelihood; lowest AICc). We present the results of our best-performing models in this paper (lowest AICc value).

Random Forest Models

We omitted traps from this and subsequent analyses if no beetles were caught in the traps, regardless of trap disturbance. We omitted these traps because traps with zero beetles provided no information on burying beetle community composition. To test our hypotheses that burying beetle community composition differed between the ground and the canopy, and that *N. pustulatus* was only prevalent in the canopy, we first took a machine learning approach. Specifically, we used Random Forest classification models in the *randomForest* package in R (Breiman, 2001; version 4.6-12; Breiman et al., 2015). Random Forest models combine many classification trees to identify which variables most accurately discriminate between groups (Cutler et al. 2007). Random forest is a powerful alternative to traditional parametric and semiparametric statistical methods for classification and discrimination because it makes no distributional assumptions about the data (Cutler et al., 2007), and can easily accommodate non-linear relationships that are common in nature (Friedl & Brodley, 1997). The Random Forest algorithm selects a random subset of the data (approximately 63%), and fits a classification tree to each subsample (Cutler et al., 2007). The accuracy of each classification tree is then assessed using the remaining (unselected or “out-of-the-bag”) portion of the data (Cutler et al., 2007). The out-of-the-bag data provide independent estimates of classification accuracy because they were not used to fit the classification tree (Cutler et al., 2007). Each classification tree uses only a small number of predictor variables at a time; we identified the optimal number of predictor variables for classification as the smallest number that yielded the lowest out-of-bag estimate of error rate. After many iterations of the model (10,000 in our case), the Random forest model provides an overall best classification error rate, an error rate specific to each group, the relative importance of each predictor variable for accurate classification, and other details such as the classification error rate for each individual data point (Breiman, 2001).

In our Random Forest models, we used trap height classification as the response (group) variable and the number of carrion beetles of each species collected at each survey point as the predictor variables. We calculated the classification accuracy as 1 – out-of-bag error rate and ran each model 10,000 times to obtain an average classification accuracy with 95% confidence intervals. We constructed variable importance plots to show the relative importance of all predictor variables for accurate classification in our model. We also used partial dependence plots to depict the effects of our most important predictor variables on the probability of correct classification (Cutler et al., 2007).

Binomial Generalized Linear Models

We also tested our hypothesis that the occurrence and abundance of different beetle species predicted ground versus canopy traps using a binomial generalized linear model (i.e. logistic regression) in R (version 3.3.1: R Core Team 2016). Trap (ground=0, canopy=1) was the response variable, and different species’ abundances were the predictor variables. We first ran a model with all species included separately, with no interaction terms, and checked the fit of the model using the *heatmap.fit* command in the R package *heatmapFit* (version 2.0.4; Esarey & Pierce, 2016). We then compared the performance of different models with all combinations of predictor variables to identify the model that performed best, as assessed by Akaike information criterion values, controlling for small sample size (AICc; lowest value indicating the best-performing model) using the *dredge* command in the *MuMI*n package (version 1.15.6; Bartoń, 2016). Perfect separation in our best-performing model led to inflated and inaccurate statistical results. Thus, we used Firth's penalized-likelihood logistic regression models to estimate coefficients and statistical results for reporting.

RESULTS

Ground and canopy traps differed in the total number of burying beetles caught per trap (Fig. 1) and the number of species caught per trap (Fig. 2). Ground traps caught significantly more beetles compared to canopy traps (glm, z=11.26, P<0.0001; Fig. 1), and the number of beetles caught in both ground and canopy traps increased with Julian date (glm, z=5.94, P<0.0001; Fig. 1). Ground traps also caught significantly more species of burying beetles compared to canopy traps (glm, z=8.23, P<0.0001; Fig. 2), and the number of species in both ground and canopy traps increased with Julian date (glm, z=7.10, P>0.0001; Fig. 2).

Ground and canopy traps also differed in the numbers and identities of species caught (Table 1, Figs. 3, 4). Our most accurate Random Forest model correctly classified trap location as ground or canopy 90.34% of the time [95% Confidence Intervals (CI): 90.23 - 90.45]. Model accuracy was similar for ground and canopy traps; ground traps were classified correctly 91.25% of the time (CI: 91.16 - 91.34; N=80 ground traps), while canopy traps were classified correctly 89.23% of the time (CI: 89.12 -89.34; N=65 canopy traps).

The presence and abundance of three species of carrion beetles were the most important predictors of trap height (Figs. 4-6). The presence of *Nicrophorus orbicollis* and, particularly, *Necrophila americana* were the best predictors of ground traps (Fig. 5), and the likelihood of a trap being on the ground increased with higher numbers of both species (Fig. 6). The presence, and increased numbers, of *Nicrophorus pustulatus* was the best predictor of canopy traps (Figs. 5, 6). The abundance of other species also helped to accurately classify trap height, but to a lesser extent (Figs. 4, 5). For example, our Random Forest classification model rerun with only *Necrophila americana*, *Nicrophorus pustulatus*, and *Nicrophorus orbicollis* correctly classified trap location as ground or canopy 87.59% of the time, while our model rerun with only

Necrophila americana and *Nicrophorus pustulatus* accurately classified trap location 86.21% of the time; the full model (all species included) had a classification accuracy of 90.34%.

Results from our binomial generalized linear models supported our Random Forest analysis. The presence and abundance of *Necrophila americana* and *Nicrophorus pustulatus* were the most statistically significant predictors of ground versus canopy traps in our best-performing model (lowest AICc; Table 2). The presence and abundance of other species of burying beetle were also significant predictors of ground versus canopy traps; however, the error associated with their effect sizes was larger (Table 2), suggesting that they were less consistent predictors. The coefficient estimates for *Nicrophorus pustulatus* were very different from all other species (Table 2), illustrating that *N. pustulatus* was uniquely common in the canopy and rare on the ground (*cf.* Fig. 4).

DISCUSSION

The abundance and occurrence of different burying beetle species differed between ground and canopy traps. Ground traps contained more burying beetles and more species of burying beetles than canopy traps (Figs. 1, 2). The number of beetles and number of species present increased as the season progressed for both ground and canopy traps, indicating that there is a greater abundance and greater species diversity later in the season (July) compared to early in the season (May) (Figs. 1, 2). Trap height classification of ground versus canopy traps differed with species present in the trap. Our most accurate Random Forest model for classifying trap height was very accurate (90.3% classification accuracy), which indicates that there were repeatable differences between ground and canopy trap heights in the species that were caught. Ground traps were accurately predicted by the number of either *Necrophila americana* or *Nicrophorus orbicollis* (Fig. 6 a, b), and greater abundances of either of these species indicated that the trap was more likely to be on the ground. Canopy traps were accurately predicted by the number of *Nicrophorus pustulatus*; the presence and increasing number of *N. pustulatus* in a trap was a strong predictor of canopy traps.

Our findings support previous studies that found greater abundances of *N. pustulatus* in elevated traps (Ulyshen & Hanula, 2007; Legros & Beresford, 2010). *N. pustulatus* was historically thought to be rare, but widely distributed, in eastern North America (Anderson & Peck, 1985), consistent with few individuals caught in previous studies (Anderson, 1982; Robertson, 1992; LeGros & Beresford, 2010; Brousseau, Cloutier & Hébert, 2010). Our study, however, suggests that *N. pustulatus* can be common, but only within the forest canopy. Ulyshen, Hanula, and Horn (2007) found 21 individuals of *N. pustulatus* in 15m canopy traps and progressively fewer at 5m (N=12) and on the ground (N=1), suggesting that *N. pustulatus* may be even more abundant at greater heights above our 6m canopy traps and may be a generalized canopy specialist across their range.

The only known breeding resource for *N. pustulatus* in nature is gray ratsnake eggs (Blouin-Demers & Weatherhead, 2000; Keller & Heske, 2001; Smith et al., 2007). However, the geographic range of *N. pustulatus* extends beyond the range of ratsnakes (Anderson & Peck, 1985; Smith et al., 2007; LeGros & Beresford, 2010; Brousseau, Cloutier & Hébert, 2010); thus, *N. pustulatus* must use other sources of food for breeding in some parts of its range. A single observation of *N. pustulatus* larvae feeding on northern ringneck snake eggs suggests that *N. pustulatus* may be able to use any oviparous snake eggs as food and are not limited to only the gray ratsnake (LeGros, Pratt & Beresford 2010). In our study, we successfully baited *N. pustulatus* into traps using chicken and in a laboratory setting, *N. pustulatus* will behave like a typical burying beetle and rear offspring on mice (Robertson, 1992; Trumbo, 1992; Rauter & Moore, 2002). Philips, Root and DeSimone (1983) discovered three adult *N. pustulatus* in a failed Northern Saw-whet Owl nest, supporting the idea that this species uses other food for breeding beyond snake eggs. In addition, a pair of *N. pustulatus* were observed and collected on dead Tree Swallow (*Tachycineta bicolor*) nestlings in a failed nest at the Queen's University Biological Station, in a nest box approximately 1m from the ground (unpublished data; A. Schizkoske, 2016). Further studies are needed to determine the typical food used for reproduction by *N. pustulatus*.

N. pustulatus may prefer canopy habitats to avoid intense competition for carrion on the ground (Ulyshen, Hanula & Horn, 2007) and to exploit important carrion resources in the canopy. Carrion in the canopy may include squirrels (Sciuridae), birds, and bats (Chiroptera) (LeGros & Beresford, 2010). In particular, nesting squirrels and birds are common in the canopy and frequently experience mortality (Ricklefs, 1969), providing a reliable resource during the peak breeding season (e.g., June at our study site for birds; Peck & James, 1987; Keast, 1990; Cadman et al., 2007). The breeding season of vertebrates in the canopy coincides with the emergence of *N. pustulatus* at our study site (Trumbo, 1990).

Why don't other *Nicrophorus* species use canopy habitat given the abundance of nesting vertebrates there? *Nicrophorus* beetles typically bury carcasses under soil or leaf litter to protect them from other competitors and assist in reproduction (e.g., insulation). This burying behaviour may not be possible in canopy habitats. The search for carrion in the canopy, including cavity searching, may also be more energetically costly and some *Nicrophorus* species may be unable to sustain flight for necessary periods or maneuver sufficiently to find carrion in this habitat. While we still do not understand the constraints on using canopy habitat, our data show that *N. pustulatus* are common in the canopy, and their use of the canopy suggests that vertical height is another important axis of resource partitioning among closely related species of burying beetles.

CONCLUSIONS

Most species of carrion beetles in our study, and elsewhere, typically use carrion resources located on the ground. *N. pustulatus* is an exception, primarily using carrion located in canopy habitats and only secondarily using carrion on the ground. Our findings illustrate a distinct vertical axis of resource partitioning in our carrion beetle community that may allow *N. pustulatus* to co-occur with other closely related species that all require the same limited resource for reproduction.

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Figure 1

Total number of beetles (log transformed) for ground traps (blue) and canopy traps (red) by date with 95% confidence intervals (grey).

The results of our generalized linear model show that the abundance of beetles was higher in ground traps compared to canopy traps, and that the abundance increased with Julian date for both ground and canopy traps.

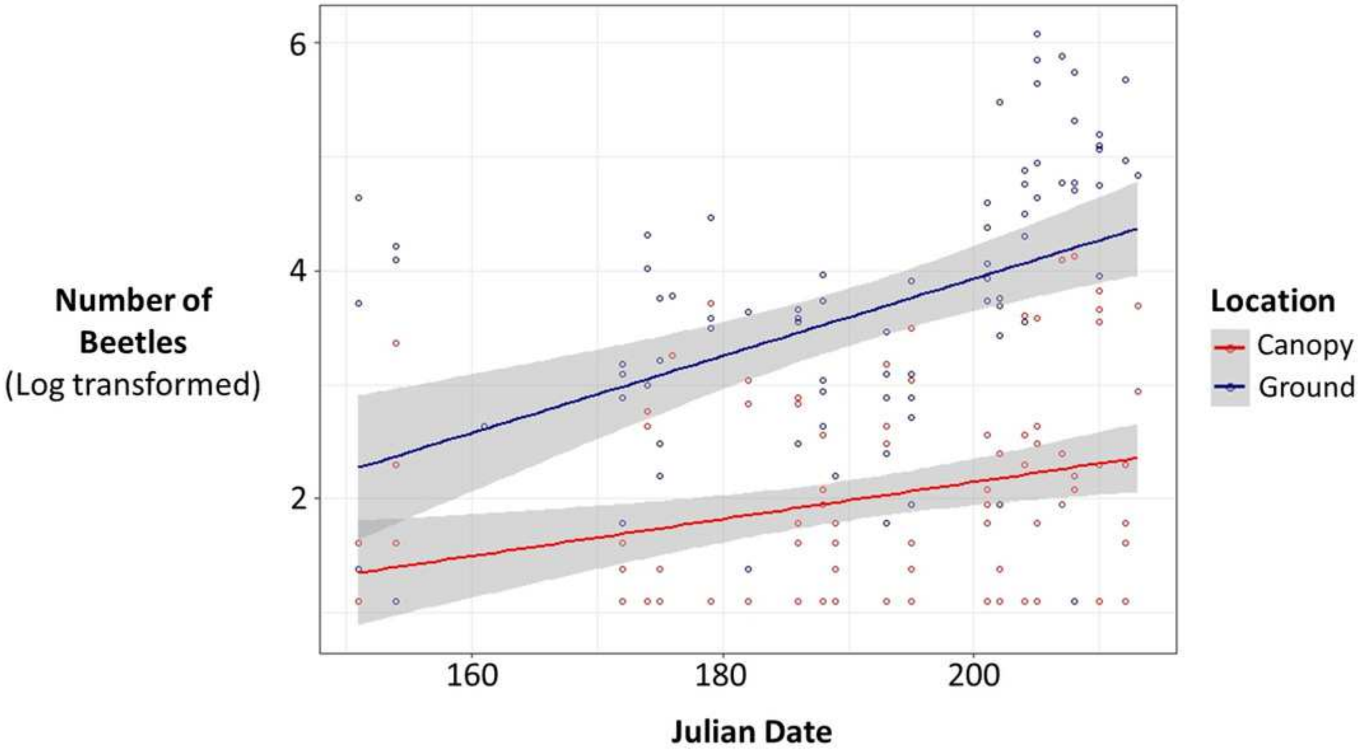

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Figure 2

Number of species of beetles in ground traps (blue) compared to canopy traps (red) by Julian date with 95% confidence intervals (grey).

The results of our generalized linear model show that ground traps contained more species than canopy traps, and the number of species caught increased with Julian date for both ground and canopy traps.

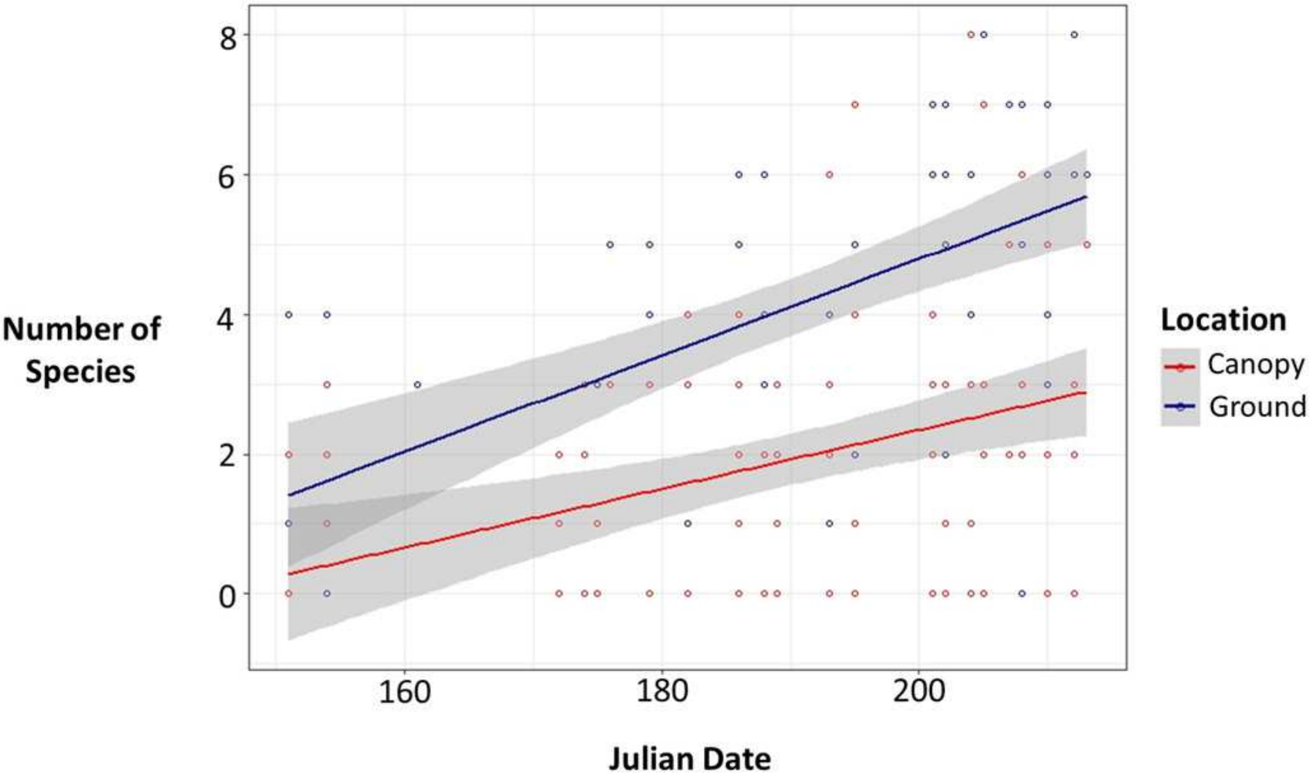

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Figure 3

Total number of beetles (log transformed) collected for each carrion beetle species in canopy traps (white) versus ground traps (grey).

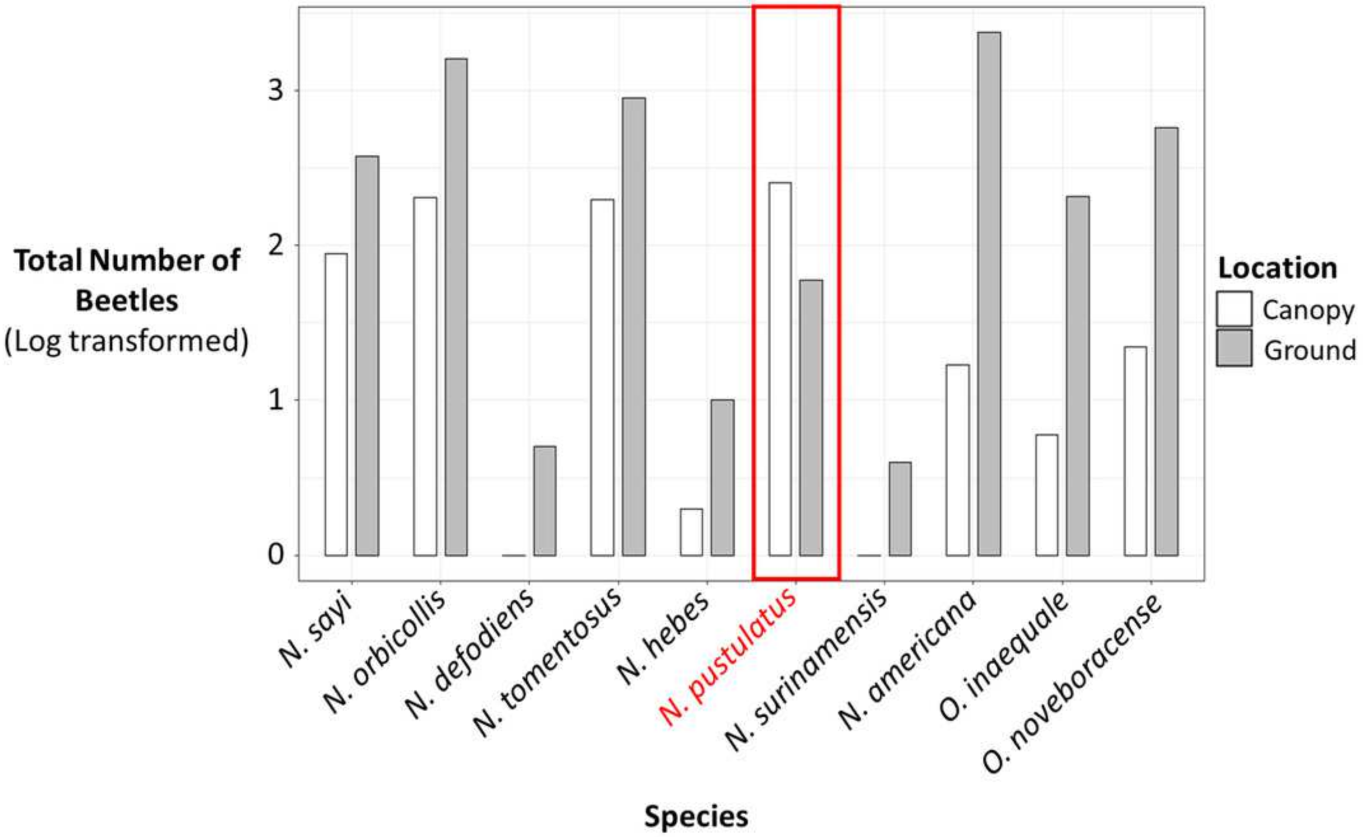

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Figure 4

Log (number of beetles per trap + 1) for each carrion beetle species collected in canopy traps (white) versus ground traps (grey).

The number of *N. pustulatus* per trap was higher in canopy traps compared to ground traps; all other burying beetle species were more common in ground traps. Boxplots show medians (thick lines), 25th and 75th percentiles (boxes), 1.5 times the interquartile range (whiskers), and outliers (points outside 1.5 times the interquartile range).

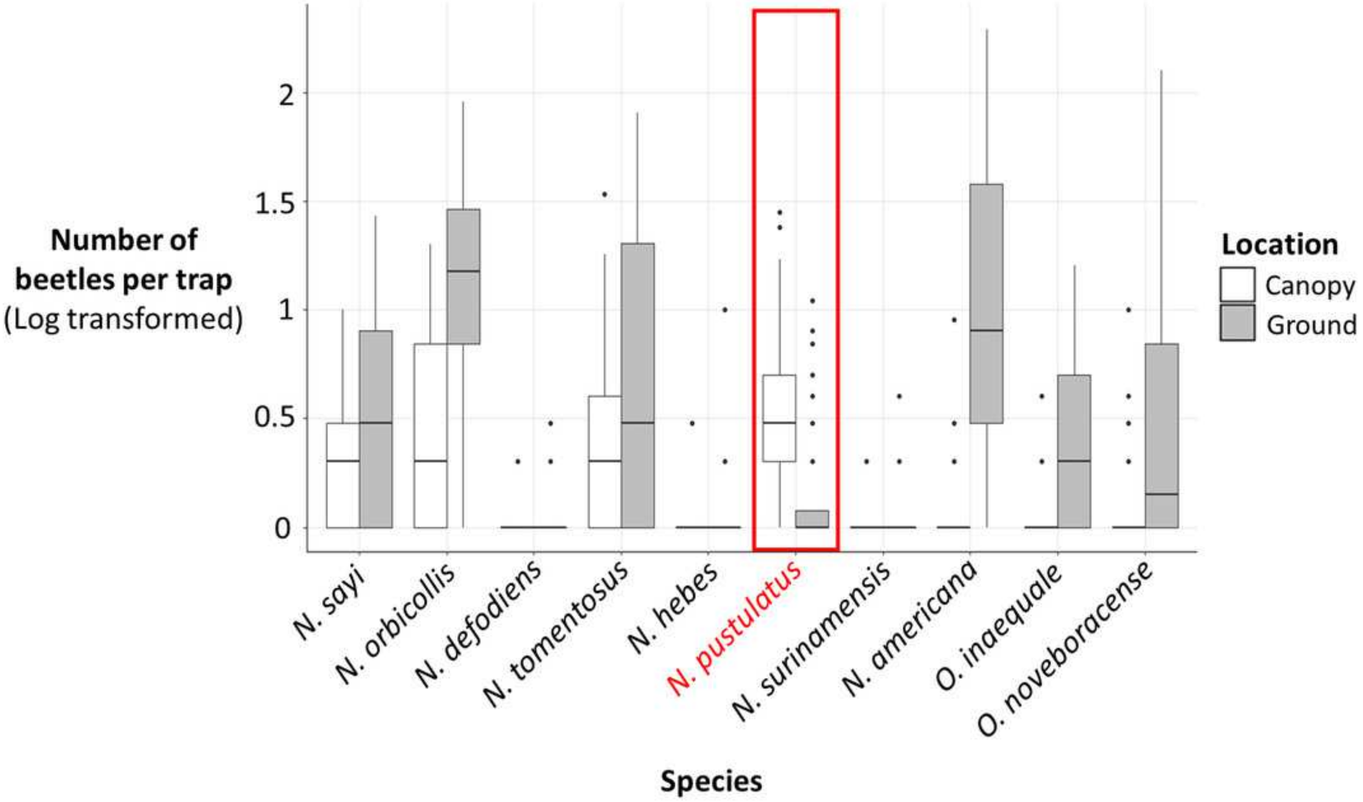

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Figure 5

Variable importance plots for classification of ground versus canopy traps.

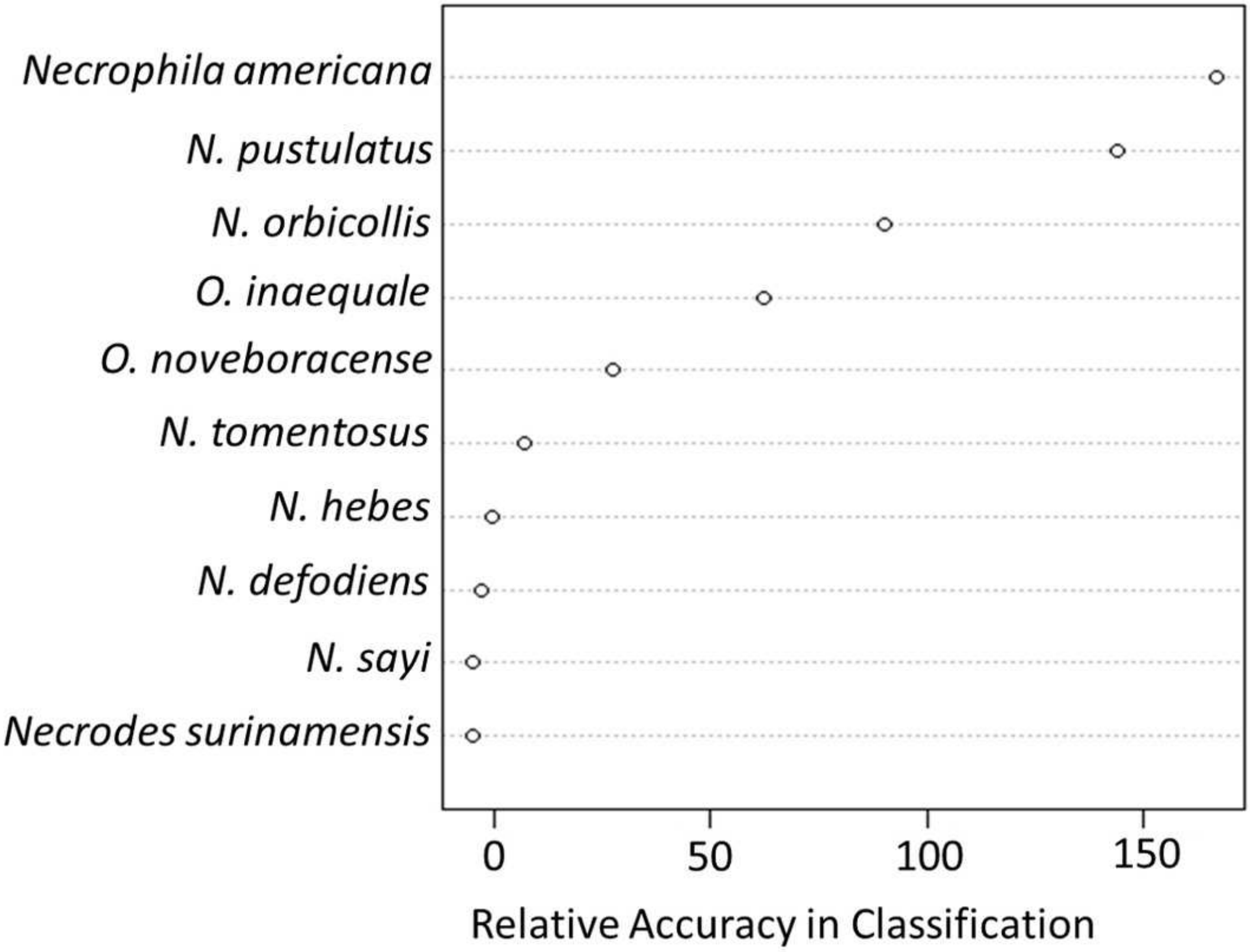
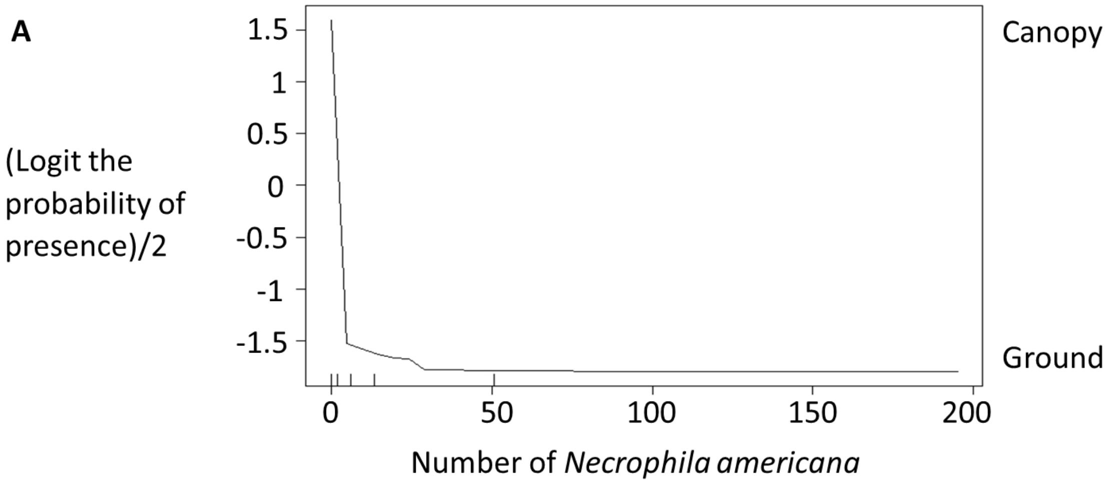
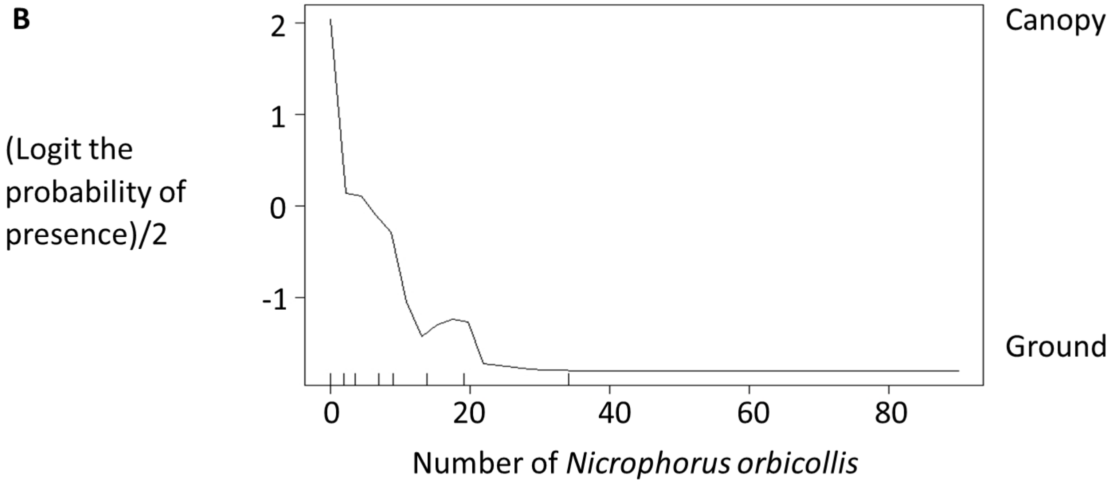


Figure 6(on next page)

Partial dependency plots show the marginal effects of each species' abundance on the probability that a trap was on the ground or in the canopy.

Traps with more *Necrophila americana* (A) and *Nicrophorus orbicollis* (B) were more likely to be ground traps; traps with more *Nicrophorus pustulatus* (C) were more likely to be canopy traps.


A
(Logit the probability of presence)/2
1.5
1
0.5
0
-0.5
-1
-1.5
Canopy
Ground
0
50
100
150
200
Number of *Necrophila americana*


B
(Logit the probability of presence)/2
2
1
0
-1
Canopy
Ground
0
20
40
60
80
Number of *Nicrophorus orbicollis*

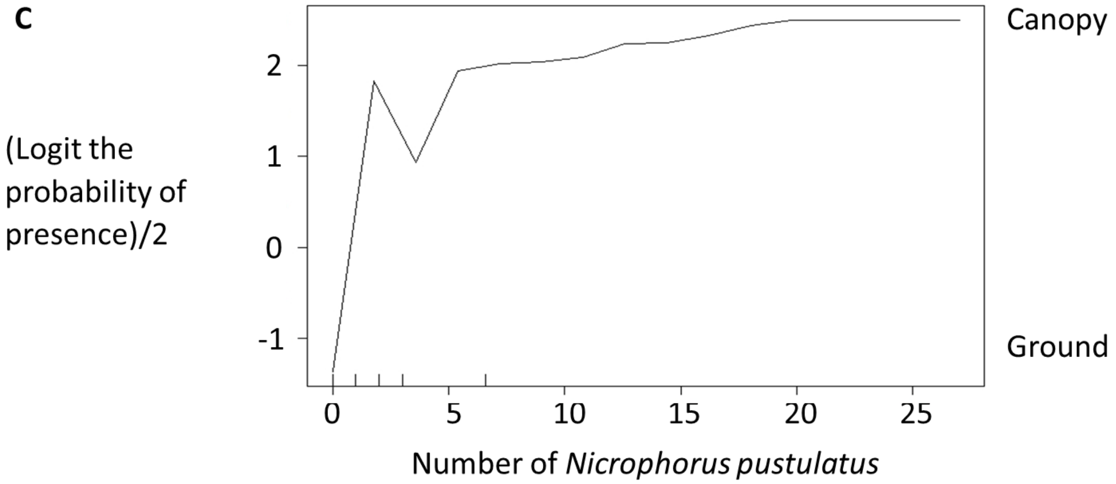

C
(Logit the probability of presence)/2
2
1
0
-1
Canopy
Ground
0
5
10
15
20
25
Number of *Nicrophorus pustulatus*

Table 1(on next page)

Summary of the results of our carrion beetle survey at the Queen’s University Biological Station (May-July 2016).

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Species	Total number of beetles by trap height		Number of traps with each species present	
	Ground (0m) N=80	Canopy (6m) N=65	Ground (0m) N=80	Canopy (6m) N=65
Nicrophorus orbicollis	1609	203	74	40
Nicrophorus tomentosus	902	198	47	33
Nicrophorus sayi	378	88	54	34
Nicrophorus pustulatus	60	253	20	50
Nicrophorus hebes	10	2	2	1
Nicrophorus defodiens	5	1	4	1
Necrophila americana	2361	17	63	8
Oiceoptoma noveboracense	574	22	40	9
Oiceoptoma inaequale	207	6	47	4
Necrodes surinamensis	4	1	2	1
Total	6110	791		
Total *Nicrophorus*	2964	745		

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Table 2(on next page)

Results of Firth’s penalized-likelihood logistic regression testing the hypothesis that the abundance of each carrion beetle species differed between ground and canopy traps (*N*=145 comparisons).

The model represents the best-performing logistic regression model (lowest AICc value), comparing models with all possible combinations of predictor variables.

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Firth's penalized-likelihood logistic regression[1]

Predictor variable	*Estimate*	*Lower 95% CI* [2]	*Upper 95% CI* [2]	*Chi-squared*	*p*
Intercept	0.551	-0.331	1.479	1.56	0.21
Necrophila americana	-0.912	-1.669	-0.476	19.39	<0.0001
Nicrophorus pustulatus	2.847	1.466	5.339	38.12	<0.0001
Nicrophorus orbicollis	-0.168	-0.364	0.005	3.45	0.06
Nicrophorus sayi	-0.191	-0.455	0.020	3.16	0.08
Nicrophorus tomentosus	0.393	0.169	0.941	9.03	0.003
Oiceoptoma noveboracense	-0.734	-2.023	-0.285	6.11	0.01

[1] implemented because of perfect separation

[2] CI = confidence interval