DISENTANGLING THE EFFECTS OF MICROCLIMATE ON VERTICAL AND HORIZONTAL STRATIFICATION OF BARK-BORING BEETLES IN SOUTHEASTERN U.S. DECIDUOUS FORESTS

by

THOMAS N. SHEEHAN

(Under the Direction of Michael D. Ulyshen and Joseph V. McHugh)

ABSTRACT

Bark and wood boring beetles include some of the most economically important forest insects, especially those that are introduced to novel environments. A proper understanding of species distribution is critical for detection and management. In this study we placed traps at three heights above ground at the edge and on the interior of two forests targeting two beetle guilds: wood/phloem feeding beetles and ambrosia beetles. We recorded temperature, humidity, and canopy cover for each trap. We found species richness to increase with height for wood/phloem feeding beetles and decrease with height for ambrosia beetles. We also found the combination of height and placement to significantly affect abundance for both guilds. Our results indicate that to capture the full diversity of these guilds, traps must be placed at multiple heights and at both the edge and interior of forests.

INDEX WORDS: Vertical stratification, woodborers, bark beetles, microclimate, diurnal, nocturnal, trap placement, monitor and detection

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DEDICATION

I would like to dedicate this to my parents Tim and Karen Sheehan, as well as my sisters Katie and Caroline, and brother Kevin for all their support and the opportunities they have provided me to be where I am. I would also like to dedicate this to all the dogs in my life for their emotional support. Lastly I would like to dedicate this to the insects (and other arthropods) that gave their lives for this research to be conducted, I can only hope they did not die in vain.

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INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Invasive species are one of the most important factors of human caused global change. The introduction of new species negatively affects ecosystems in many ways such as through competition, predation, habitat structure alteration, niche displacement, trophic cascades, etc. (Mooney and Cleland 2001; Walsh et al. 2016). Introduced species are the second most common threat to biodiversity, only behind habitat loss (Bellard et al. 2016; Wilcove et al. 1998). Increased human activity—trade and travel in particular—has further exacerbated these issues.

Beyond the ecological effects, certain species cause significant economic damage as well. Some estimates claim non-native species cost the United States almost $120 billion every year (Pimentel et al. 2005). In addition to crops, livestock, fisheries, and other resources, non-native species threaten forests, a valuable source of many commodities (timber, paper, recreation, etc.). At least 450 non-native forest insects have become established in the U.S. (Aukema et al. 2011). The subfamily Scolytinae alone has 58 non-native species known to be established in the continental U.S. (Haack and Rabaglia 2013). Non-native forest insects arrive from many sources, but the two most important are from the trade of live plants for horticulture and the use of wood packing materials, such as pallets and crates (Haack 2006; Lovett et al. 2016).

1.2 Feeding Guilds

Some of the most important forest insects include beetles that bore into trees. These can be separated into two general feeding guilds: fungal feeders and wood/phloem feeders. Fungal feeders consist of members in the curculionid subfamilies Scolytinae and Platypodinae as well as the small family of ship-timber beetles (Coleoptera: Lymexylidae). These “ambrosia beetles” create galleries inside trees in which they cultivate a symbiotic fungus for food. This relationship between beetle and fungus has evolved independently numerous times between multiple clades of both beetles and fungi (Hulcr and Stelinski 2017). These beetles feed on the fungal symbiont, not the tree directly.

Wood/phloem feeders consist of longhorned beetles (Coleoptera: Cerambycidae) and jewel beetles (Coleoptera: Buprestidae), as well as the remaining members of Scolytinae. These beetles generally bore into dead or dying trees, but can also attack healthy trees. They bore into trees and create galleries, where they feed on the wood/phloem directly. Although many have associated bacterial and fungal symbionts that can negatively impact trees, these beetles do not feed upon the fungus. While adults of Scolytinae primarily feed only on the host tree (Anderson 2002), adults of Cerambycidae and Buprestidae exhibit diverse feeding patterns such as eating pollen and flowers, foliage, bark, or nothing at all during the adult stage (Bellamy and Nelson 2002; Turnbow and Thomas 2002).

Most native wood-boring beetles are extremely beneficial to their ecosystems by providing many services such as decomposition and nutrient cycling, as well as food web dynamics and supporting general biodiversity (Cobb et al. 2010; Müller et al. 2008). In contrast, many non-native beetles attack healthy trees, such as *Agrilus planipennis* Fairmare the emerald ash borer (Poland and McCullough 2006). By competing with these native beetles and altering their environment, non-native beetles can cause further ecological damage. Non-native beetles can also cause concern for sustainable forestry by negatively affecting “biological diversity, forest health and productivity, water and soil quality, the carbon cycle, and socioeconomic values" (Chornesky et al. 2005). Native beetles can be pests as well, such as the southern pine beetle *Dendroctonus frontalis* Zimmerman, which is also an example of a bark beetle in the subfamily Scolytinae that is not an ambrosia beetle.

Some particular species cause tremendous damage. The non-native redbay ambrosia beetle *Xyleborus glabratus* Eichoff and its symbiotic fungus cause laurel wilt disease, responsible for over 90% of tree mortality for redbay trees *Persea borbonia* (L.) Spreng. in the southeastern U.S. (Fraedrich et al. 2008; Spiegel and Leege 2013). The non-native emerald ash borer *A. planipennis* causes billions of dollars in damages, as well as thousands of human deaths from respiratory/cardiovascular illnesses (Donovan et al. 2013; Haight et al. 2009; Herms and McCullough 2014). Nowak et al. (2001) estimated the Asian longhorned beetle *Anoplophora glabripennis* (Motchulsky) could cause maximum potential damage of $669 billion in the U.S.

1.3 Distribution Patterns

Proper detection methods are crucial for the management of potentially problematic non-native beetle introductions. The U.S. Forest Service established the Early Detection and Rapid Response Program (EDRR) in 2001, which found five new, non-native species of bark and ambrosia beetles in its first five years (Rabaglia et al. 2008). A successful management program requires sophisticated trapping techniques and an understanding of distribution patterns for the focal taxa.

To have efficient trapping methods, we must understand how species are distributed throughout the forest. For example, a study of *A. planipennis* collected significantly more specimens from traps at the mid-canopy (13 meters above ground) than at ground level (Francese et al. 2008). One can use this knowledge when targeting *A. planipennis* by including traps in the mid-canopy. With limited resources and sampling efforts, educated trap placement is key.

A tremendous amount of evidence supports the vertical stratification of insect communities in forests, although general patterns are not always clear. These patterns can be seen from numerous studies in both tropical forests with arthropods in general (Basset et al. 2003; Sebek et al. 2017) and beetles (Davis et al. 2011; Grimbacher and Stork 2007; Stork et al. 2016), as well as in temperate forests with arthropods in general (Ulyshen 2011), beetles (Holdsworth et al. 2016; Maguire et al. 2014; Weiss et al. 2016), flies (Maguire et al. 2014; Stireman et al. 2012; Weiss et al. 2016), and bees and wasps (Sobek et al. 2009). More specifically, a notable amount of research supports differences in distribution relative to canopy height for members of Cerambycidae, Buprestidae, and Scolytinae, as well as between the two feeding guilds, although often with conflicting results.

Members of Buprestidae generally exhibit higher abundance and species richness in the canopy compared at ground level, as well as at the forest edge compared to interior (Wermelinger et al. 2007, Francese et al. 2008); some studies, however, show no significant differences (Ulyshen and Hanula 2007).

Members of Scolytinae generally show preferences for the ground level compared to the canopy (Ulyshen and Hanula 2007) but some studies also show no significant differences (Wermelinger et al. 2007, Leksono et al. 2005).

Conflicting patterns have been reported for Cerambycidae, with some studies showing higher abundance/species richness at the ground level (Wermelinger et al. 2007) and other studies showing higher abundance/species richness in the canopy (Ulyshen and Hanula 2007). Vance et al. (2003) found higher abundance at ground level, but no significant difference for species richness; they did still find 11 species unique to canopy traps and 11 species unique to ground traps. Even when a group exhibits no significant differences in species richness or abundance, community composition can still differ.

1.4 Purpose of Present Study

Few studies have examined species distribution by feeding guild. Ulyshen and Sheehan (2017) found wood/phloem feeding beetle abundance and species richness to increase with height and for ambrosia beetle abundance and species richness to decrease with height.

Many possible factors could explain these differences in distribution aside from height itself. We predict that microclimatic factors—such as temperature, humidity, and canopy cover—are the driving forces behind distribution differences, rather than height alone. This hypothesis—although poorly studied for these specific groups—has a reasonable amount of support. For example, temperature and humidity can affect insect activity, dispersal, development, flight patterns, adhesion to substrate, etc. (Drury et al. 2016; Heepe et al. 2016; Jaworski and Hilszczański 2013; Zhang et al. 2008). One study found *Phoracantha semipunctata* F. (Coleoptera: Cerambycidae) to have lower reproductive success with higher moisture content in the bark of their host tree (Hanks et al. 1999). Sunlight and canopy openness also influence insect distribution (Jonsell et al. 1998; Gossner 2009) as well as plant diversity (Aavik et al. 2008). In particular, many species of Buprestidae and Cerambycidae exhibit preferences for either sun or shade, especially considering the wood substrates in which the larvae grow (Vodka et al. 2009; Vodka and Cizek 2013; Lindhe et al. 2005; Buse et al. 2007).

Vertical stratification of microclimatic variables—temperature, vapor pressure deficit, and incident light—becomes less pronounced at the edge of forests compared to the interior of forests (Didham and Ewers 2014). One can expect the edge of a forest to be more vertically uniform in terms of microclimatic factors compared to the interior since the interior forest floor is adjacent to the forest in all directions. Vodka and Cizek (2013) found differences in beetle distribution to be more significant between edge/interior gradients than canopy/floor gradients. Gossner (2009) found light intensity to be an important factor for both vertical and horizontal distribution of the insect suborder Heteroptera. Although life history differs between Heteroptera and the focal taxa, one can expect parallels for factors that affect distribution even between unrelated phytophagous insects.

When examining taxa that have a crucial symbiotic relationship (e.g. ambrosia beetles of Scolytinae), one must consider the needs of the symbiont. Research on vertical stratification of fungi in forests is severely limited, but Unterseher and Tal (2006) found microclimatic differences between upper canopy and forest floor that reflected fungal diversity. This study examined free-living fungi, but it can still hopefully lend some ideas to general fungal distribution patterns. When looking at the symbiotic relationship (both the beetle and the fungus), we may find certain patterns due to requirements from either. Weiss et al. (2016) found significantly higher fungus-feeding beetle species richness and abundance at ground level than the canopy. Perhaps this is due to requirements of the symbiotic fungus to remain in dark, cool, and humid areas, such as the interior ground level of forests.

Although many have studied the vertical distribution of woodboring beetles, few—if any—have also examined these microclimatic variables associated with height. For this study, we looked at beetles that belong to two different feeding guilds at three different heights above ground at both the edge and interior of the forest, while recording three key microclimatic variables: temperature, humidity, and canopy cover for each trap. Although this study primarily targets the native beetle fauna, we expect distribution patterns to remain consistent among native/non-native species in the same feeding guild/taxa.

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CHAPTER 2

DISENTANGLING THE EFFECTS OF MICROCLIMATE ON VERTICAL AND HORIZONATAL STRATIFICATION OF BARK-BORING BEETLES IN SOUTHEASTERN U.S. DECIDUOUS FORESTS

2.1 Introduction

Non-native insects have the potential to cause great damage to the environments they recently inhabit. They threaten biodiversity while also causing significant economic losses—billions of dollars every year (Bellard et al. 2016; Pimentel et al. 2005). Over 450 non-native insects have become established in forests, many quite detrimental (Aukema et al. 2011). Forests provide many valuable natural resources such as timber, as well as numerous ecosystem services such as carbon sequestration (Bonan 2008). Monitoring programs to detect recently arrived non-native insects require an understanding of distribution patterns. Some important taxa in forests—such as longhorned beetles (Coleoptera: Cerambycidae), jewel beetles (Coleoptera: Buprestidae), and bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) exhibit differences in distribution relative to height above ground, as well as between the edge and interior of forests (Bouget et al. 2011; Vodka et al. 2009; Vodka and Cizek 2013).

These taxa can also be separated into two separate feeding guilds: 1) the ambrosia beetles which include certain lineages of Scolytinae, as well as the closely related subfamily Platypodinae and 2) the wood/phloem feeding beetles which include members of Cerambycidae, Buprestidae, and the remaining members of Scolytinae. Both feeding guilds bore into trees and create galleries where their young develop. Whereas ambrosia beetles cultivate a symbiotic fungus for food, wood/phloem feeding beetles consume the tree directly, instead of a fungus.

Although patterns are difficult to determine and some studies show conflicting results, general conclusions can still be drawn. From a combination of three studies, Ulyshen and Sheehan (2017) found wood and phloem feeding beetle abundance and species richness to increase with height, while also finding ambrosia beetle abundance and species richness to decrease with height. These patterns may be explained by microclimatic factors associated with different heights. For example, one can expect sunlight and temperature to increase with height while also expecting humidity to decrease. Perhaps ambrosia beetles prefer locations with higher humidity for their fungal symbiont (i.e. lower heights). We may also expect ambrosia beetles to prefer the interior of forests compared to the forest edges for the same reason. For this study we will trap beetles from both guilds at different heights, as well as the edge and interior of forests. We will also record microclimatic data (temperature, relative humidity, canopy cover) for each trap location.

2.2 Methods

2.2.1 Study Sites

This study took place in two separate mixed hardwood forests in Athens, Clarke County, Georgia: Whitehall Forest—a ~840 acre experimental forest managed by the University of Georgia's Warnell School of Forestry and Natural Resources—and Tallassee Forest—a ~310 acre tract of undeveloped land managed by the Athens-Clarke County government. They are separated by approximately 10 miles, but the Middle Oconee River runs through both.

Whitehall Forest mainly consists of secondary mixed ~60-70 year old southern hardwood and pine forest (King et al. 2013; Nowakowski and Maerz 2009). It is an experimental forest and has numerous research facilities located on site. It is closed to the public, but sees relatively heavy use for research, instruction, and land management.

In contrast, Tallassee Forest receives much less disturbance, which has resulted in a tremendous amount of diversity. One survey found over 137 species of plants including giant cane *Arundinaria gigantean* (Walter) Muhlenberg—an important but rapidly disappearing plant in the Southeast (Porter 2014). It also recorded 58 species of butterflies, 63 species of birds, 13 families of aquatic invertebrates, and 22 species of amphibians and reptiles, many of which are rare or in decline (Porter 2014). Much of Tallassee has consisted of old growth hardwood forest for the past 75 years and includes seven of the Georgia Department of Natural Resources High Priority Habitats, as well as the largest American holly *Ilex opaca* Aiton forest in Georgia (Porter 2014).

2.2.2 Experimental Design

Each site had multiple factors of interest. There were two different trap placements of forest edge and forest interior (at least 50 m from the edge). Each placement had three height variations of 15 meters above ground, 5 meters above ground, and 0 meters above ground. Each combination of factors had five replicates for a total of 60 traps (Table 1). For the traps 15 meters above ground, we used a combination of a Big Shot throw-line launcher (SherrillTree Inc., Greensboro, NC, USA)—a slingshot mounted on a 2.4 m fiberglass pole—and a Big Launcher rope gun (SherrillTree Inc., Greensboro, NC, USA) to launch rope to the appropriate height. We selected sturdy branches that were clear enough underneath for ease of raising and lowering of traps, indiscriminate of tree species. Selecting all the same species of tree would have been ideal, but deemed impractical considering the limited number of satisfactory branches.

2.2.3 Trap Design

We constructed flight intercept traps using two intersecting sheets of Plexiglas 30.5 cm x 20.3 cm placed above a single lindgren funnel, attached with metal wire (Figure 1). The very bottom of each lindgren funnel was removed to prevent larger specimens from escaping. We applied sprayable Teflon to the funnel prior to the collecting season, which has been shown to increase trap yield of Cerambycidae (Allison et al. 2011; Allison et al. 2014; Allison and Redak 2017; Graham et al. 2010). We used propylene glycol—with a drop of soap to break surface tension—as both the killing and preserving agent, changed once every 2 weeks. Several studies have found that wet collection cups far outperform dry cups (de Groot and Nott 2003; Miller and Duerr 2007; Morewood et al. 2002). We attached one low release ethanol lure from Synergy Semiochemicals Corp., Burnaby, British Columbia, Canada (Item #3344) to each trap directly above the Plexiglas and replaced once every eight weeks, as recommended by the manufacturer. On June 7th we attached a colored card to each Plexiglas frame to attract pollinators (notably bees) for a separate study; there were four different colors: blue, yellow, white, and neon yellow (Figure 1).

2.2.4 Data Collection

Each trap had a datalogger (iButton Hygrochron Temperature/Humidity Logger) to record temperature and humidity once every two hours for the duration of the sampling period. To record gap fraction data (canopy cover), we took a hemispherical photo of the canopy for each trap location then analyzed with WinSCANOPY software to get gap fraction output data. For the trap locations at 5 m and 15 m we used an improvised cage to pull the camera up to the proper height (Figure 2). We took pictures of Whitehall Forest on June 6th, 2016 and Tallassee Forest on August 4th, 2016 after leaf expansion.

Specimens were collected approximately once every two weeks from March 15th-September 28th, 2016. For one month—between May 22nd and June 21st—specimens were collected twice a day, at sunrise and sunset, to gather data on the diurnal versus nocturnal activity for these beetles. All specimens were identified to species, with five exceptions that were identified to genus: *Hypothenemus, Pityophthorus, Pseudopityophthorus, Trischidias,* and *Thysanoes*, all in the subfamily Scolytinae. Members of Scolytinae were identified to genus/species using Wood (1982); Cognato et al. (2015); Rabaglia et al. (2006). Members of Cerambycidae were identified to species using Lingafelter (2008). Members of Buprestidae were identified to species using Harpootlian and Bellamy (2014). Fourteen specimens were too damaged to identify and were not included in any analysis. Voucher specimens will be deposited in the Georgia Museum of Natural History. Members of Scolytinae were placed in either guild using Kirkendall et al. (2015) and Wood (1982). Two species of *Hypothenemus* feed on fungus: *Hypothenemus concolor* and *Hypothenemus curtipennis* (Beaver 1986; Hulcr and Stelinski 2017). Although *Hypothenemus* specimens were only identified to genus, both of these species are fairly distinctive (Vega et al. 2015) and neither have been reported in the U.S.; thus we placed *Hypothenemus* spp. in the wood/phloem guild, as reported in Atkinson et al. (1988) and Wood (1982).

2.2.5 Data Analysis

We consider as fixed effects: height (0 m, 5 m, 15 m), placement (edge vs. interior), site (Whitehall Forest vs. Tallassee Forest), guild, and all interaction effects. We consider site as a fixed effect due to their differences: Whitehall Forest is a managed research forest whereas Tallassee Forest is an unmanaged tract of land with less disturbance.

We performed an ANOVA in SAS and limited the dataset to traps and sampling periods that yielded complete data (May 10th-August 2nd), which reduced the dataset to 89 species and 3334 specimens. All abundance measures were log transformed. We also performed the Tukey-Kramer test in SAS to compare means for the microclimatic variables, as well as abundance and species richness for the combination of height and placement and between sites.

With this SAS dataset, we performed sample-based rarefaction in EstimateS using the Mao Tau estimator, Equation 17 in Colwell et al. (2012). We used the classic formula for Chao1 and Chao2, as recommended by the program, instead of the bias-corrected formula. All other settings remained default. We plotted separate rarefaction curves for guild and placement with samples on the x-axis to demonstrate expected number of species for similar trapping efforts. We also plotted rarefaction with individual abundance on the x-axis due to large differences in abundance among different heights.

Similar to the dataset in SAS, we limited the PC-ORD analysis to traps and sampling periods to May 10th-August 2nd, but we then further limited it to only include species that were present in at least three samples, which resulted in a matrix with 46 species. Data were then relativized by species maximum. We performed indicator species analysis using PC-ORD and the methods of Dufrêne and Legendre (1997) to determine individual species associations for trap height, placement/height combination, and site. We also performed nonmetric multidimensional scaling to examine beetle composition for different trap heights using the Bray-Curtis distance measure.

For the day/night analysis we sampled from Whitehall Forest twice a day at sunrise and sunset from May 22nd until June 21st. Specimens collected between sunrise and sunset were classified as belonging to the day sample whereas those collected between sunset and sunrise were classified as the night samples. We used the mixed procedure of SAS to determine how time of day affected captures of the two guilds. The models consisted of placement, height, time of day and all interaction terms as fixed effects and trap location (i.e., the 30 trap locations) as a random effect. Response variables tested in this model were the abundance and richness of both guilds separately as well as temperature and relative humidity. We also used the mixed procedure of SAS to specifically determine if the relationships between beetle captures (abundance and richness for each guild separately) and trap height differed between the night and day samples. The model used to conduct this comparison of slopes included guild, time of day and trap height as fixed effects and the same location term as described above as the random effect. We also performed indicator species analysis in PC-ORD.

2.3 Results

A total of 141 species and 10,057 specimens were collected. Of these, 111 species and 2055 specimens belong to the wood/phloem feeding guild in the families Cerambycidae, Buprestidae, and Curculionidae (Scolytinae); 30 species and 8002 specimens belong to the ambrosia feeding guild in the families Curculionidae (Scolytinae, Platypodinae) and Lymexylidae. Due to variables such as weather, a total of 9 traps broke during their 2 week collecting period, resulting in missing data. An additional 9 traps spilled during their 2 week collecting period, resulting in incomplete data. Limiting the datasets to those with complete data yielded 89 species and 3334 specimens (Table 2).

2.3.1 Abiotic factors

We found significant positive correlation for our traps ($n = 52$) between height and gap fraction ($r = 0.5726$, $p < 0.0001$), height and temperature ($r = 0.4706$, $p = 0.0004$), as well as temperature and gap fraction ($r = 0.5592$, $p < 0.0001$). We found significant negative correlation for our traps ($n = 52$) between relative humidity and height ($r = 0.6444$, $p < 0.0001$), relative humidity and gap fraction ($r = -0.5617$, $p < 0.0001$), as well as relative humidity and temperature ($r = -0.8671$, $p < 0.0001$) (Table 3). The interaction of height and placement was not significant for temperature or relative humidity, but both height and placement independently had a significant effect on temperature ($F(2,40) = 8.95$, $p = 0.0006$), ($F(1,40) = 13.06$, $p = 0.0008$), relative humidity ($F(2,40) = 20.16$, $p < 0.0001$), ($F(1,40) = 6.43$, $p = 0.0152$), and gap fraction ($F(2,40) = 23.15$, $p < 0.0001$), ($F(1,40) = 31.17$, $p < 0.0001$) respectively (Table 4). The combination of height and placement did significantly affect gap fraction ($F(2,40) = 6.21$, $p = 0.0045$) (Table 4). Least square means demonstrated that the edge 15 m traps had significantly higher mean gap fraction than any other trap location (Figure 3). Mean temperature and mean relative humidity had more complex trap differences. For both temperature and relative humidity, there were no significant differences between edge and interior for any of the particular heights, but there were differences between the heights. For relative humidity, both edge and interior 15 m traps had significantly lower means than the 0 m traps, but the 5 m traps were not significantly different from any height. For temperature, none of the interior traps were significantly different by height, but the edge 15 m traps had a significantly higher mean temperature than the 0 m traps, with the 5 m traps not being significantly different from either (Figure 3).

2.3.2 Abundance

Our results indicate that abundance significantly decreases with height for the ambrosia guild ($F(2,39) = 12.69$, $p < 0.0001$), but height has no significant effect on abundance for the wood/phloem guild ($F(2,39) = 0.01$, $p = 0.9930$) (Table 5; Figure 4). The interaction between height and placement, however, had a significant effect on abundance for both the ambrosia guild ($F(2,39) = 4.37$, $p = 0.0372$) and the wood/phloem guild ($F(2,39) = 3.44$, $p = 0.0422$). Gap fraction also had a significant effect on wood/phloem guild abundance ($F(1,39) = 4.16$, $p = 0.0482$) as well as site ($F(1,39) = 14.81$, $p = 0.0004$), with more wood/phloem feeding beetles present at Tallassee Forest than Whitehall Forest.

Only gap fraction had a significant effect on abundance of Buprestidae ($F(1,39) = 12.46$, $p = 0.0011$), while site ($F(1,39) = 17.50$, $p = 0.0002$) and height ($F(1,39) = 4.69$, $p = 0.0149$) had a significant effect on abundance of Cerambycidae (Table 6). Height ($F(2,39) = 13.82$, $p < 0.0001$) and the interaction of height and placement ($F(2,39)$, $p = 0.0192$) had a significant effect on Curculionidae (Table 6). As for individual subfamilies, Cerambycinae (Cerambycidae) abundance was significantly affected by site ($F(1,39) = 17.70$, $p = 0.0001$), height ($F(2,39) = 6.97$, $p = 0.0026$), and the interaction of site and placement ($F(1,39) = 6.02$, $p = 0.0187$) (Table 7). Lamiinae (Cerambycidae) and Lepturinae (Cerambycidae) abundances were not significantly associated with any factor. Scolytinae (Curculionidae) abundance was significantly associated with height ($F(2,39) = 14.03$, $p < 0.0001$), the interaction of height and site ($F(2,39) = 3.24$, $p = 0.0497$), and the interaction of height and placement ($F(2,39) = 4.39$, $p = 0.0190$) (Table 7).

2.3.3 Species Richness

Similar to abundance, our results indicate that species richness significantly decreases with height for the ambrosia guild ($F(2,39) = 25.08$, $p < 0.0001$), but species richness also significantly varies among height for the wood/phloem feeding guild ($F(2,39) = 3.58$, $p = 0.0374$) (Table 8; Figure 5). Wood/phloem feeder richness significantly increased with gap fraction ($F(1,39) = 8.59$, $p = 0.0056$). Wood/phloem feeder richness was also significantly affected by site ($F(1,39) = 12.91$, $p = 0.0009$), with more wood/phloem feeding species present at Tallassee Forest than Whitehall Forest (Table 8; Figure 6). There was no significant interaction between height and placement for the species richness of either the ambrosia guild (*F*(2,39) = 0.11, *p* = 0.8950) or the wood/phloem guild (*F*(2,39) = 1.00, *p* = 0.3767).

Only gap fraction had a significant effect on species richness of Buprestidae (*F*(1,39) = 10.70, p = 0.0022), while site (*F*(1,39) = 19.80, *p* < 0.0001) and height (*F*(2,39) = 4.71, *p* = 0.0147) had a significant effect on species richness of Cerambycidae (Table 9). Height (*F*(2,39) = 25.87, *p* <0.0001) and site (*F*(1,39) = 4.94, *p* = 0.0321) had a significant effect on species richness of Curculionidae (Table 9). As for individual subfamilies, Cerambycinae (Cerambycidae) species richness had a significant effect from site (*F*(1,39) = 23.00, *p* < 0.0001), height (*F*(2,39) = 5.17, *p* = 0.0102), and the interaction of site and placement (*F*(1,39) = 7.63, *p* = 0.0087) (Table 10). Lamiinae (Cerambycidae) species richness had a significant effect from placement (F(1,39) = 4.35, *p* = 0.0436), but Lepturinae (Cerambycidae) species richness had no significant effects acting on it. Scolytinae (Curculionidae) species richness was significantly affected by site (*F*(1,39) = 6.77, *p* = 0.0131) and height (F(2,39) = 32.03, *p* <0.0001) (Table 10).

Many of the rarefaction curves did not reach an asymptote, implying that sampling is incomplete for particular trap locations (Figure 7). Still, it is clear that lower traps collected more species of ambrosia beetles and higher traps collected more species of wood/phloem feeding beetles, although there is a fair amount of overlap with the error bars.

2.3.4 Indicator Species

Six beetle species were significantly associated with Tallassee Forest, with three in the ambrosia guild and three in the wood/phloem guild; one ambrosia beetle was significantly associated with Whitehall Forest (Table 11).

Eight species were significantly associated with the 0 m traps and eight species were significantly associated with the 15 m traps—no species were significantly associated with the 5 m traps (Table 11). All but two of the species associated with the 0 m traps were in the ambrosia feeding guild, while all but one of the species associated with the 15 m traps were in the wood/phloem guild.

Thirteen species were significantly associated with certain height and placement combinations: three ambrosia species for edge 0 m, one ambrosia species for edge 5 m, three wood/phloem species for edge 15 m, three ambrosia species and one wood/phloem species for interior 0 m, and two wood/phloem species for interior 15 m (Table 11; Figure 8). No species were significantly associated with interior 5 m traps (Table 11).

2.3.5 Community Composition

Non-metric multidimensional scaling recommended a 3-dimensional solution with a final stress of 18.46. The R^2 values were 0.412, 0.142, and 0.140 for the three axes respectively. Axis 1 values vary significantly with site ($S(1,39) = 39.41$, $p < 0.0001$), height ($S(2,39) = 90.98$, $p < 0.0001$), and gap fraction ($S(1,39) = 7.05$, $p = 0.0114$) (Table 12). There was also a significant interaction of site and height ($S(2,39) = 3.36$, $p = 0.0450$), and interaction of height and placement ($S(2,39) = 6.50$, $p = 0.0037$). Axis 2 values varied significantly only with height ($S(2,39) = 3.92$, $p = 0.0281$) (Table 12). As can be seen by the 2-dimensional depiction using axes 1 and 2, the traps at 0 m above ground are different in composition to the traps at 15 m above ground, with traps at 5 m above ground intermediate between both groups, although all three differ significantly (Figure 9). Ambrosia richness appeared to increase with relative humidity, associated with the 0 m traps, whereas wood/phloem richness appeared to increase with both gap fraction and temperature, associated with the 15 m traps. Axis 1 average means broken down by placement and height are depicted in Figure 10, showing that the 0 m edge and interior traps do not differ, nor do the 15 m edge and interior traps, but the 5 m traps do significantly differ in community composition, with the interior 5 m traps representing a unique community.

2.3.6 Day/Night Comparison

From performing an ANOVA, we found time of day to significantly affect ambrosia abundance ($F(1,48) = 13.58$, $p = 0.0006$), ambrosia richness ($F(1,48) = 29.29$, $p < 0.0001$), wood/phloem abundance ($F(1,48) = 12.74$, $p = 0.0008$), and wood/phloem richness ($F(1,48) = 12.93$, $p = 0.0008$) (Table 13). We found a significant interaction effect of height and time of day on ambrosia abundance ($F(2,48) = 5.39$, $p = 0.0077$), but no significant interaction on ambrosia richness ($F(2,48) = 0.79$, $p = 0.4602$), wood/phloem abundance ($F(2,48) = 0.96$, $p = 0.3905$), or wood/phloem richness ($F(2,48) = 1.94$, $p = 0.1541$) (Table 13). We found a significant interaction effect of placement and time of day on wood/phloem abundance ($F(1,48) = 5.69$, $p = 0.0210$) and wood/phloem richness ($F(1,48) = 4.90$, $p = 0.0316$), but no significant interaction on ambrosia abundance ($F(1,48) = 2.02$, $p = 0.1613$) or ambrosia richness ($F(1,48) = 0.57$, $p = 0.4538$) (Table 13).

We found time of day to significantly affect temperature ($F(1,40) = 957.33$, $p < 0.0001$) and relative humidity ($F(1,40) = 748.50$, $p < 0.0001$). We found no significant interaction effect of height and time of day on temperature ($F(2,40) = 2.85$, $p = 0.0699$) or relative humidity ($F(2,40) = 1.64$, $p = 0.2060$) (Table 14).

We found a significant difference in slope between day and night for both total abundance ($t(112) = 2.82$, $p = 0.0056$) and ambrosia abundance ($t(112) = 3.52$, $p = 0.0006$), but not for wood/phloem abundance ($t(112) = -0.29$, $p = 0.7696$) (Figure 11). We found no significant difference in slope between day and night for species richness of any of the guilds:

total (*t*(112) = -0.91, *p* = 0.3651), ambrosia (*t*(112) = -0.92, *p* = 0.3588), and wood/phloem (*t*(112) = -0.17, *p* = 0.8659).

Nine species were significantly associated with either day or night. Two members of Cerambycidae—*Ecyrus dasycerus* (Say) and *Anelaphus villosus* (LeConte)—were significantly associated with night; one member of Cerambycidae *Neoclytus scutellaris* (Olivier) and six members of Scolytinae—*Ambrosiodmus obliquus* (LeConte), *Cnestus mutilatus* (Blandford), *Dryoxylon onoharaense* (Murayama), *Hypothenemus* spp. (Westwood), *Xyleborinus saxeseni* (Ratzeburg), and *Xylosandrus germanus* (Blandford)—were significantly associated with day.

2.4 Discussion

In this study we measured the biodiversity of two separate feeding guilds of beetles (wood/phloem feeding and ambrosia feeding) at varying heights and placements of edge or interior of the forest. We included the key microclimatic variables of temperature, relative humidity, and gap fraction in our model to determine their effect on the distribution of beetles belonging to these two guilds.

Our results indicate that species richness is significantly affected by height for the wood/phloem feeding guild, but decreases with height for the ambrosia feeding guild. The combination of height and placement did not significantly affect species richness for either guild. Abundance of ambrosia beetles significantly decreased with height, but abundance of wood/phloem feeding beetles did not significantly change with height. We found the combination of height and placement to significantly affect abundance for both feeding guilds, however.

The distribution of these taxa is complex and many studies show conflicting results: some that support our results, and some that do not. Many studies also focus on particular species instead of broader feeding guilds as we have. Some studies have found species richness to increase with height for wood/phloem feeders (Ulyshen and Sheehan 2017), but others have found no difference (Vance et al. 2003), and some have found species richness to decrease with height for wood/phloem feeding taxa (Dodds 2014).

Similar to species richness, some studies have also found abundance to increase with height for wood/phloem feeders (Ulyshen and Sheehan 2017), while others have found abundance to decrease with height for wood/phloem feeding taxa (Oshawa and Shimokawa 2011; Vance et al. 2003). Albert et al. (2012) found the great capricorn beetle *Cerambyx cerdo* L. (Coleoptera: Cerambycidae) abundance to decrease with height. One of the largest beetles in Europe, *C. cerdo* can reach up to 55 mm in length, which may explain this pattern. In contrast, the highly problematic emerald ash borer *A. planipennis* (Coleoptera: Buprestidae) is caught much more often in traps in the canopy compared to ground level (Francese et al. 2008). Graham et al. (2012) found no significant effect of height on wood/phloem species richness or abundance, but they did capture 21 species exclusive to one of two heights.

Reding et al. (2010) collected significantly more of the ambrosia beetles *Xylosandrus crassiusculus* and *Xylosandrus germanus* at traps 0.5 m and 1.7 m above ground than at 3 m above ground. Although they sampled a smaller height range than our study, it still supports the understanding that ambrosia beetles prefer lower heights. Other studies have also found ambrosia beetle abundance to decrease with height, such as the platypodine *Platyus quervicorusi* (Igeta et al. 2004) and the scolytine *Xyleborus glabratus* (Brar et al. 2012). Igeta et al. (2004) also found a strong association with the forest edge compared to the interior for *P. quervicorusi*.

We did not find placement to significantly affect abundance or species richness for either guild, but the combination of height and placement was significant for abundance of both guilds.

Vodka and Cizek (2013) found the difference of edge and interior to be a more important factor in beetle diversity than height, although this included many other families of saproxylic beetles. Although we did not find placement alone to have a significant effect, the significant effect of combination of height and placement on abundance for both guilds indicates the importance of sampling from both multiple heights and at the edge/interior of forests.

Of the seven indicator species for height belonging to the wood/phloem guild, all but *Prionus imbricornis* (L.) were significantly associated with 15 m traps (Table 11). Similarly, of the eight indicator species for height belonging to the ambrosia guild, all but *Cnestus mutilatus* (Blandford) were significantly associated with 0 m traps (Table 11).

These two exceptions can possibly be explained by important biological history. *Prionus imbricornis* was significantly associated with 0 m traps most likely because it is a root borer, often targeting the roots of grapevine, pear, and herbaceous plants (Beutenmuller 1896). It is also large in size, with adults reaching approximately 25 mm in length (Lingafelter 2008). In contrast, the association of *C. mutilatus* with 15 m may be explained because it targets stems with relatively small diameter (Kajimura and Hijii 1992; Schiefer and Bright 2004). In a study of host plants, Stone et al. (2007) found no successful attacks under 0.5 m above ground by *C. mutilatus*. Although they found a mean height of attack of about 2 m, they only used saplings that presumably did not reach 15 m in height (Stone et al. 2007). Perhaps there is a different explanation however, as there should still be twigs in the understory.

Wermelinger et al. (2007) found members of Cerambycidae to generally prefer open land, but also found some species to contradict this pattern and prefer the forest interior. General patterns can aid management decisions regarding where to place traps for detecting novel beetles; if targeting a particular species, however, exceptions may confound these patterns. Many studies demonstrate the heliophilia of Buprestidae, which supports our findings that abundance and species richness increase with gap fraction (Lindhe et al. 2004; Nitzu et al. 2016; Wermelinger et al. 2007; Widerberg et al. 2012).

Across both feeding guilds, six indicator species were significantly associated with Tallassee Forest, while only the non-native ambrosia beetle *Euwallacea interjectus* (Blandford) was associated with Whitehall Forest (Table 11). Although there was no significant difference between sites for the ambrosia guild, Tallassee Forest had significantly higher species richness and abundance for the wood/phloem guild compared to Whitehall Forest. This further supports the understanding that Tallassee Forest has a high amount of biodiversity in Athens, GA. This study also yielded six individuals of the giant stag beetle *Lucanus elaphus* Fabricius from 15 m edge traps at Tallassee Forest while none were collected at Whitehall Forest (Ulyshen et al. 2017).

Bouget et al. (2011) found conflicting results for abundance and species richness by height, mainly from differences in forest composition. Although we sampled in mixed hardwood forests (with relatively nearby pine stands), sampling in forests with different tree species compositions may result in different patterns.

Time of day had a significant effect on both temperature and relative humidity, which is to be expected. More importantly however, our results suggest that the relationship between temperature and height, relative humidity and height, temperature and placement, and relative humidity and placement do not differ between night and day (Table 14). Thus, we can reasonably assume that conclusions drawn regarding microclimate and height are consistent between night and day. Time of day had a significant effect on ambrosia abundance, ambrosia species richness, wood/phloem abundance, and wood/phloem species richness. Because the day slope was significantly steeper than the night slope, this suggests that the effect of height on ambrosia abundance is stronger during the day than at night.

Although studies examining the diurnal/nocturnal habits are limited, many species in both guilds do exhibit either diurnal or nocturnal habits (Linsley 1959; Seybold et al. 2012; Waqa-Sakiti 2009). Of the two indicator species for night, both belonged to Cerambycidae, while all but one of the indicator species for day belonged to Scolytinae; both indicator species for night belonged to the wood/phloem guild, while all but two of the indicator species for day belonged to the ambrosia guild (Table 11).

We accounted for the important microclimatic variables temperature, relative humidity, and gap fraction, but there are still numerous others that could influence distribution. Some abiotic factors that could further influence distribution are wind speed, wind direction, rainfall, barometric pressure, cardinal direction of gap exposure, etc. For example, both temperature and wind speed affected flight activity of *Orthotomicus erosus* (Wollaston) and *Pityogenes calcaratus* Eichhoff in a pine plantation in Israel (Mendel et al. 1991). The interactions of all these variables further complicate the issue (Pawson et al. 2017). Chen and Seybold (2014) found temperature, light intensity, wind speed, and barometric pressure to affect flight activity of the wood-phloem feeding scolytine *Pityophthorus juglandis* both individually as well as in combination. In addition to affecting flight activity, other abiotic factors may affect distribution in other ways, such as through altering pheromone plumes. Östrand and Anderbrant (2003) found recapture rates of male pine sawflies *Neodiprion sertifer* Geoffroy to increase with wind speed when using pheromone baited traps.

Although determining distribution patterns for multiple taxa and feeding guilds is complex, our study still allows us to draw certain conclusions. Our results support the understanding that height significantly affects wood/phloem feeding beetles species richness, while ambrosia beetle species richness decreases with height. We also found the combination of height and placement to significantly affect abundance for both feeding guilds.

In general, sampling from multiple heights as well as at both the edge and interior of a forest gives a more complete picture of diversity. Some species are only associated with particular heights, only the edge/interior of a forest, and even a combination of both. Without sampling all of these, problematic species may be missed from detection efforts.

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Table 1. Experimental design. Plcmt=Placement; Int=Interior

Site: Tallassee Forest					
Plcmt: Edge	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m
Plcmt: Int	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m
Site: Whitehall Forest					
Plcmt: Edge	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m
Plcmt: Int	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m	0m 5m 15m

Table 2. List of species in taxonomic order included for main analysis with count data for each height and placement combination, as well as site. Int = Interior; TF = Tallassee Forest, WH = Whitehall forest; W/P = Wood/phloem guild; A = Ambrosia guild; †=non-native species

		Guild	Edge 0m TF/WH	Edge 5m TF/WH	Edge 15m TF/WH	Int. 0m TF/WH	Int. 5m TF/WH	Int. 15m TF/WH	Total TF/WH
Buprestidae	**Agrilinae**								
	Agrilus bilineatus (Weber 1801)	W/P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Agrilus carpini Knull 1923	W/P	0/0	0/0	0/0	0/0	1/0	0/0	1/0
	Agrilus subrobustus† Saunders 1873	W/P	0/0	0/0	0/0	0/1	0/0	0/0	0/1
	Brachys ovatus (Weber 1801)	W/P	0/0	0/0	0/1	0/0	0/0	2/0	2/1
	Buprestinae								
	Actenodes acornis (Say 1833)	W/P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Anthaxia dichroa Bílý 1991	W/P	0/0	0/2	1/0	0/0	0/0	0/0	1/2
	Chyrsobothris rugosiceps Melsheimer 1845	W/P	0/1	0/0	3/1	0/0	1/0	0/0	4/2
	Chyrsobothris sexsignata Say 1839	W/P	0/0	1/0	0/0	0/0	0/0	0/0	1/0
	Chrysochroinae								
	Dicerca lurida (Fabricius 1775)	W/P	0/0	0/0	0/0	0/0	0/0	0/1	0/1
	Polycestinae								
	Acmaeodera tubulus (Fabricius 1801)	W/P	0/0	1/0	1/1	0/0	0/0	1/0	3/1
	Cerambycinae								
	Anelaphus moestus	W/	0/0	0/0	0/1	0/0	0/0	0/0	0/1

Cerambycidae	(LeConte 1854)	P							
	Anelaphus parallelus (Newman 1840)	W/ P	0/0	1/0	3/0	0/0	1/0	0/0	5/0
	Anelaphus villosus (Fabricius 1792)	W/ P	2/0	13/2	18/0	3/0	6/5	7/9	49/ 16
	Clytus marginicollis Laporte & Gory 1835	W/ P	1/0	1/0	1/0	4/1	1/0	0/0	8/1
	Cyrtophorus verrucosus (Olivier 1795)	W/ P	0/0	0/0	0/0	0/0	1/0	1/0	2/0
	Eburia quadrigeminata (Say 1826)	W/ P	0/0	1/0	6/1	0/0	0/0	4/3	11/4
	Elaphidion mucronatum (Say 1824)	W/ P	1/0	7/1	3/1	3/3	2/3	1/0	17/8
	Enaphalodes atomarius (Drury 1773)	W/ P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Euderces picipes (Fabricius 1787)	W/ P	2/0	3/0	5/0	1/0	1/0	1/2	13/2
	Euderces pini (Olivier 1795)	W/ P	0/0	1/0	0/0	0/0	0/0	0/0	1/0
	Euderces reichi reichi LeConte 1873	W/ P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Heterachthes quadrimaculatus Newman 1840	W/ P	0/0	0/0	0/0	0/0	0/1	1/0	1/1
	Molorchus bimaculatus Say 1824	W/ P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Neoclytus acuminatus (Fabricius 1775)	W/ P	1/1	0/1	0/0	0/1	0/2	0/0	1/5
	Neoclytus mucronatus (Fabricius 1775)	W/ P	0/0	0/0	0/0	0/0	1/0	0/0	1/0
	Neoclytus scutellaris (Olivier 1790)	W/ P	0/0	1/4	4/4	1/0	0/0	5/10	11/ 18
	Obrium maculatum (Olivier 1795)	W/ P	1/2	3/0	2/0	0/0	0/0	0/2	6/4
	Parelaphidion aspersum (Haldeman 1847)	W/ P	0/0	0/0	1/0	0/0	0/0	2/0	3/0
	Parelaphidion incertum (Newman 1840)	W/ P	0/0	0/0	5/0	0/0	0/0	5/1	10/1
	Psyrassa pertenuis (Casey 1924)	W/ P	0/0	1/0	2/1	0/0	0/0	0/0	3/1
	Xylotrechus colonus (Fabricius 1775)	W/ P	0/0	0/0	0/0	3/0	0/0	0/0	3/0
	Disteniinae								
	Distenia undata (Fabricius 1775)	W/ P	1/0	2/0	4/0	0/0	2/0	3/0	12/0

	Lamiinae								
	Aegomorphus modestus (Gyllenhal 1817)	W/P	0/0	0/2	0/0	0/0	0/0	1/2	1/4
	Doraschema cinereum (Olivier 1795)	W/P	0/0	1/0	0/0	0/0	0/0	0/0	1/0
	Ecyrus dasycerus (Say 1827)	W/P	0/0	0/1	0/0	1/1	1/0	1/0	3/2
	Eupogonius pauper LeConte 1852	W/P	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Goes debilis LeConte 1852	W/P	0/0	0/0	0/1	0/0	0/0	0/0	0/1
	Goes tigrinus (DeGeer 1775)	W/P	0/1	0/0	0/0	0/0	0/0	0/0	0/1
	Hyperplatys aspersa (Say 1824)	W/P	0/0	0/0	0/0	0/0	0/0	1/0	1/0
	Hyperplatys maculata Haldeman 1847	W/P	1/0	0/0	0/0	0/0	1/0	0/0	2/0
	Lepturges confluens (Haldeman 1847)	W/P	0/0	0/0	2/0	0/0	0/0	1/1	3/1
	Saperda discoidea Fabricius 1798	W/P	0/0	0/0	0/0	0/0	0/0	0/2	0/2
	Saperda lateralis Fabricius 1775	W/P	0/0	5/0	0/0	0/0	1/1	0/0	6/1
	Urgleptes facetus (Say 1826)	W/P	0/0	0/0	0/0	0/0	1/0	0/0	1/0
	Urographis fasciatus (DeGeer 1775)	W/P	0/0	0/0	0/0	2/1	0/0	0/0	2/1
	Lepturinae								
	Analeptura lineola (Say 1824)	W/P	0/0	0/0	0/0	0/0	1/0	1/1	2/1
	Bellamira scalaris (Say 1826)	W/P	0/0	0/0	0/0	0/0	1/0	0/0	1/0
	Brachyleptura vagans (Olivier 1795)	W/P	0/0	0/0	0/1	0/0	0/0	0/0	0/1
	Metacmaeops vittata (Swederus 1787)	W/P	0/0	3/0	0/0	1/0	0/0	0/0	4/0
	Stenocerus cylindricollis (Say 1824)	W/P	0/0	0/0	2/2	0/0	0/0	0/1	2/3
	Strangalia luteicornis (Fabricius 1775)	W/P	6/2	2/1	2/0	4/0	6/2	3/2	23/7
	Strangalia bicolor (Swederus 1787)	W/P	0/0	1/1	0/0	0/0	0/0	0/0	1/1
	Strangalia famelica famelica Newman 1841	W/P	0/0	0/1	0/0	0/0	0/0	1/0	1/1
	Strangalia famelica	W/	0/0	0/0	0/1	0/0	0/0	0/0	0/1

	solitaria Haldeman 1847	P						
	Typocerus velutinus (Olivier 1795)	W/ P	0/0	1/1	0/1	0/0	0/0	0/0	1/2
	Typocerus zebra (Olivier 1795)	W/ P	0/0	0/2	0/0	0/0	0/0	0/0	0/2
	Prioninae							
	Orthosoma brunneum (Forster 1771)	W/ P	1/2	0/0	0/0	0/1	0/1	0/0	1/4
	Prionus imbricornis (L. 1767)	W/ P	0/2	0/0	0/0	0/4	0/0	0/0	0/6
	Sphenostethus taslei (Buquet 1841)	W/ P	0/0	0/0	1/0	0/0	0/0	1/0	2/0
Curculionidae	**Platypodinae**							
	Euplatypus compositus (Say 1823)	A	1/0	3/0	0/1	0/0	0/0	0/0	4/1
	Oxoplatypus quadridentatus (Olivier 1795)	A	0/0	1/0	0/0	0/0	0/0	0/0	1/0
	Scolytinae							
	Bothrosternina							
	Cnesinus strigicollis LeConte 1868	W/ P	3/1	0/3	0/0	1/0	0/1	0/0	4/5
	Corthylina							
	Corthylus punctatissimus (Zimmerman 1868)	A	0/0	0/0	0/0	0/2	0/0	0/0	0/2
	Gnathotrichus materiarius (Fitch 1858)	A	0/0	0/0	0/0	0/0	0/0	1/0	1/0
	Monarthrum mali (Fitch 1855)	A	2/1	2/2	3/0	3/0	4/0	1/1	15/4
	Cryphalina							
	Hypothenemus spp. Westwood 1836	W/ P	16/ 13	4/8	0/3	21/ 16	3/2	4/3	48/ 45
	Ipina							
	Ips avulsus (Eichoff 1868)	W/ P	0/0	0/0	0/1	0/0	0/0	0/0	0/1
	Micracidina							
	Hylocurus rudis (LeConte 1876)	W/ P	0/0	0/2	1/1	0/0	2/1	0/0	3/4
	Micracisella nanula (LeConte 1876)	W/ P	0/0	2/0	0/0	0/1	0/0	0/0	2/1
	Pityophthorina							
	Pseudopityophthorus sp. Swaine 1918	W/ P	0/1	0/0	1/0	0/0	0/0	0/0	1/1
	Scolytina							

	Scolytus multistriatus† (Marsham 1802)	W/P	0/1	0/5	0/0	0/0	0/0	0/0	0/6
	Xyleborina								
	Ambrosiodmus obliquus† (LeConte 1878)	A	3/1	0/1	0/1	9/16	6/10	3/4	21/33
	Ambrosiodmus rubricollis† (Eichoff 1875)	A	0/4	0/0	0/0	1/1	1/0	0/1	2/6
	Ambrosiophilus atratus† (Eichoff 1875)	A	0/3	0/0	0/0	0/0	0/0	0/0	0/3
	Cnestus mutilatus† (Blandford 1894)	A	12/20	230/146	247/77	8/1	49/11	127/49	673/304
	Cyclorhipidion bodoanum† (Reitter 1923)	A	0/2	2/3	2/1	5/11	1/1	3/4	13/22
	Cyclorhipidion sp. ind. † (Hoebeke? 2017?)	A	0/0	0/0	1/0	0/0	0/0	0/0	1/0
	Dryoxylon onoharaense† (Murayama 1933)	A	10/11	1/2	4/1	32/19	8/6	4/0	59/39
	Euwallacea interjectus† (Blandford 1894)	A	0/3	0/1	0/0	3/6	0/5	0/0	3/15
	Xyleborinus gracilis (Eichoff 1868)	A	0/0	0/1	0/1	0/0	0/0	0/0	0/2
	Xyleborinus saxeseni† (Ratzeburg 1837)	A	20/26	3/18	3/3	40/52	10/11	1/7	77/117
	Xyleborus affinis Eichoff 1868	A	0/1	0/0	0/0	1/0	0/0	0/0	1/1
	Xyleborus bispinatus† Eichoff 1868	A	21/30	0/0	0/1	23/15	1/0	0/0	45/46
	Xyleborus celsus Eichoff 1868	A	0/0	2/1	1/1	0/0	0/0	0/1	3/3
	Xyleborus impressus Eichoff 1868	A	12/28	1/4	0/0	18/26	0/0	0/0	31/58
	Xyleborus viduus Eichoff 1868	A	0/0	0/0	0/0	0/0	0/1	0/0	0/1
	Xyleborus xylographus (Say 1826)	A	0/2	0/0	0/0	1/0	0/0	0/0	1/2
	Xylosandrus crassiusculus† Motschulsky 1866)	A	137/259	33/83	4/7	188/250	30/ 90	7/26	399/715
	Xylosandrus germanus† (Blandford 1894)	A	27/ 34	0/0	0/0	27/ 52	1/2	0/1	55/89
	Grand Total (Abundance)		281/452	333/299	339/116	404/481	145/156	194/134	1696/1638
	Grand Total (Number of species)		22/26	31/27	35/26	26/22	29/ 19	29/23	73/65

Table 3. Correlation of height and microclimatic factors.
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Height	Gap Fraction	Temperature	Relative Humidity
Height	-	0.57256***	0.47063**	-0.64436***
Gap Fraction	-	-	0.55919***	-0.56169***
Temperature	-	-	-	-0.86712***
Relative Humidity	-	-	-	-

Table 4. ANOVA results of height and microclimatic factors. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); ToD = Time of Day; Temp. = Temperature, RH = Relative Humidity
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Temp. F Value	RH F Value	Gap Fraction F Value
Site	1/40	0.94	0.28	0.79
Plcmt	1/40	13.06**	6.43*	31.17***
Site x Plcmt	1/40	0.65	0.13	0.01
Height	2/40	8.95**	20.16***	23.15***
Site x Height	2/40	3.93*	0.73	0.45
Plcmt x Height	2/40	0.69	0.08	6.21**
Site x Height x Plcmt	2/40	1.75	0.85	0.26

Table 5. ANOVA results of abundance by feeding guild; note abundance data was log transformed. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Total Abund. F Value	Ambrosia Abund. F Value	Wood/Phloem Abund. F Value
Site	1/39	0.95	0.20	14.81**
Plcmt	1/39	0.98	1.65	0.79
Site x Plcmt	1/39	0.58	0.37	0.59
Height	2/39	10.91**	12.69***	0.01
Site x Height	2/39	3.58*	3.58*	0.81
Plcmt x Height	2/39	4.49*	4.37*	3.44*
Site x Height x Plcmt	2/39	0.25	0.34	0.46
Gap Fraction	1/39	0.49	0.18	4.16*

Table 6. ANOVA results of abundance by family; note abundance data was log transformed. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Buprestidae Abund. F Value	Cerambycidae Abund. F Value	Curculionidae Abund. F Value
Site	1/39	0.19	17.50**	0.20
Plcmt	1/39	1.09	0.86	1.87
Site x Plcmt	1/39	0.56	1.80	0.29
Height	2/39	0.67	4.69*	13.82***
Site x Height	2/39	1.08	0.77	3.16
Plcmt x Height	2/39	0.62	1.43	4.38*
Site x Height x Plcmt	2/39	0.81	0.43	0.22
Gap Fraction	1/39	12.46**	1.62	0.16

Table 7. ANOVA results of abundance by subfamily; note abundance data was log transformed. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Cerambycinae Abund. F Value	Lamiinae Abund. F Value	Lepturinae Abund. F Value	Scolytinae Abund. F Value
Site	1/39	17.70**	0.95	1.84	0.20
Plcmt	1/39	0.71	2.51	0.02	1.74
Site x Plcmt	1/39	6.02*	0.01	1.18	0.29
Height	2/39	6.97**	0.83	1.61	14.03***
Site x Height	2/39	0.10	0.70	1.56	3.24*
Plcmt x Height	2/39	0.93	1.52	0.16	4.39*
Site x Height x Plcmt	2/39	1.44	0.30	0.37	0.21
Gap Fraction	1/39	0.18	1.43	0.83	0.19

Table 8. ANOVA results of species richness by feeding guild. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior)
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Total Richness F Value	Ambrosia Richness F Value	Wood/Phloem Richness F Value
Site	1/39	3.30	2.75	12.91**
Plcmt	1/39	0.73	0.22	0.69
Site x Plcmt	1/39	0.06	0.64	0.78
Height	2/39	1.94	25.08***	3.58*
Site x Height	2/39	1.63	0.56	1.48
Plcmt x Height	2/39	0.34	0.11	1.00
Site x Height x Plcmt	2/39	0.31	0.26	0.26
Gap Fraction	1/39	3.12	0.63	8.59**

Table 9. ANOVA results of species richness by family. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Buprestidae Richness F Value	Cerambycidae Richness F Value	Curculionidae Richness F Value
Site	1/39	0.40	19.80***	4.94*
Plcmt	1/39	0.66	1.04	0.00
Site x Plcmt	1/39	0.20	2.70	1.80
Height	2/39	0.70	4.71*	25.87***
Site x Height	2/39	0.98	2.32	0.03
Plcmt x Height	2/39	0.38	0.55	0.01
Site x Height x Plcmt	2/39	0.58	0.32	0.09
Gap Fraction	1/39	10.70**	3.67	0.06

Table 10. ANOVA results of species richness by subfamily. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Cerambycinae Richness F Value	Lamiinae Richness F Value	Lepturinae Richness F Value	Scolytinae Richness F Value
Site	1/39	23.00***	0.53	1.63	6.77*
Plcmt	1/39	0.05	4.35*	0.00	0.23
Site x Plcmt	1/39	7.63**	0.00	1.98	2.61***
Height	2/39	5.17*	0.75	2.81	32.03
Site x Height	2/39	1.90	0.69	1.35	0.06
Plcmt x Height	2/39	0.28	1.26	0.44	0.04
Site x Height x Plcmt	2/39	1.17	1.00	0.26	0.37
Gap Fraction	1/39	1.34	0.95	1.37	0.01

Table 11. Indicator species for height, placement and height interaction, site, and time of day. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior; W/P = Wood/Phloem feeding guild; Ambr = Ambrosia feeding guild; IV = Indicator value
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001 †=non-native species**

Species	Guild	Height (m)	IV	Plcmt x Height (m)	IV	Site	IV	Day/ Night	IV
Buprestidae									
Brachys ovatus (Weber 1801)	W/P	15	18.8 *	-	-	-	-	-	-
Chrysobothris rugosiceps Melsheimer 1845	W/P	-	-	Edge 15	34.0 *	-	-	-	-
Cerambycidae									
Anelaphus villosus (LeConte 1854)	W/P	15	40.7 *	-	-	T	55.1 **	Night	24.9 *
Distenia undata (Fabricius 1775)	W/P	-	-	-	-	T	34.6 **	-	-
Eburia quadrigeminata (Say 1826)	W/P	15	52.7 **	Int 15	29.4 *	-	-	-	-
Ecyrus dasycerus (Say 1827)	W/P	-	-	-	-	-	-	Night	23.3 *
Euderces picipes (Fabricius 1787)	W/P	-	-	-	-	T	43.3 **	-	-
Lepturges confluens (Haldeman 1847)	W/P	15	25.0 **	-	-	-	-	-	-

Neoclytus scutellaris (Olivier 1790)	W/P	15	45.3 **	Int 15	33.2 **	-	-	Day	23.1 *
Parelaphidion incertum (Newman 1840)	W/P	15	37.5 **	-	-	-	-	-	-
Prionus imbricornis (L. 1767)	W/P	0	26.3 **	-	-	-	-	-	-
Psyrassa pertenuis (Casey 1924)	W/P	-	-	Edge 15	28.9 *	-	-	-	-
Stenocorus cylindricollis (Say 1824)	W/P	15	25.0 **	Edge 15	30.0 *	-	-	-	-
Strangalia luteicornis (Fabricius 1775)	W/P	-	-	-	-	T	35.4 *	-	-
Curculionidae: Scolytinae									
Ambrosiodmus obliquus† (LeConte 1878)	Ambr	-	-	-	-	-	-	Day	27.3 **
Cnestus mutilatus† (Blandford 1894)	Ambr	15	52.9 **	Edge 5	35.9 *	T	60.9 *	Day	65.2 **
Dryoxylon onoharaense† (Murayama 1933)	Ambr	0	67.1 **	Int 0	47.9 **	-	-	Day	25.4 *
Euwallacea interjectus† (Blandford 1894)	Ambr	-	-	-	-	WH	32.1	-	-
Hypothenemus spp. (Westwood 1836)	W/P	0	61.0 **	Int 0	32.9 **	-	-	Day	53.3 **
Monarthrum mali (Fitch 1855)	Ambr	-	-	-	-	T	36.4 *	-	-
Xyleborinus saxeseni† (Ratzeburg 1837)	Ambr	0	68.5 **	Int 0	43.8 **	-	-	Day	61.3 **
Xyleborus bispinatus† Eichoff 1868	Ambr	0	82.1 **	Edge 0	45.4 **	-	-	-	-
Xyleborus impressus Eichoff 1868	Ambr	0	88.8 **	Edge 0	47.3 **	-	-	-	-
Xylosandrus crassiusculus† (Motschulsky 1866)	Ambr	0	72.5 **	Edge 0	36.3 **	-	-	-	-
Xylosandrus germanus† (Blandford 1894)	Ambr	0	91.8 **	Int 0	46.8 **	-	-	Day	36.0 *

Table 12. ANOVA of NMDS axes. Site = Forest (Whitehall vs. Tallassee); Plcmt = Placement (Edge vs. Interior); Int = Interior
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Axis 1 F Value	Axis 2 F Value	Axis 3 F Value
Site	1/39	39.41***	0.52	2.03
Plcmt	1/39	0.04	3.14	0.38
Site x Plcmt	1/39	0.60	1.70	1.45
Height	2/39	90.98***	3.92*	2.81
Site x Height	2/39	3.36*	0.21	1.77
Plcmt x Height	2/39	6.50**	0.54	1.58
Site x Height x Plcmt	2/39	0.13	0.03	0.64
Gap Fraction	1/39	7.05*	0.74	1.14

Table 13. ANOVA results of time of day on abundance/species richness for both guilds; ToD = Time of Day; Plcmt = Placement
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Ambr Abund. F Value	Ambr. Rich. F Value	W/P Abund. F Value	W/P Rich. F Value
Plcmt	1/48	0.09	0.58	0.03	0.03
Height	2/48	21.97***	15.02***	4.98*	4.40*
ToD	1/48	13.58**	29.29***	12.74**	12.93**
Plcmt x Height	2/48	0.54	0.29	2.42	2.61
Plcmt x ToD	1/48	2.02	0.57	5.69*	4.90*
Height x ToD	2/48	5.39**	0.79	0.96	1.94
Plcmt x Height x ToD	2/48	1.06	0.11	0.62	0.46

Table 14. ANOVA results of time of day on microclimatic factors; ToD = Time of Day; Plcmt = Placement; Temp. = temperature; RH = relative humidity
*** p-value <0.05 **p-value <0.01 *** p-value <0.0001**

	Num/ Denom DF	Temp. F Value	RH F Value
Plcmt	1/40	3.77	1.77
Height	2/40	11.13**	11.37**
ToD	1/40	957.33***	748.50***
Plcmt x Height	2/40	2.97	1.88
Plcmt x ToD	1/40	2.26	1.03
Height x ToD	2/40	2.85	1.64
Plcmt x Height x ToD	2/40	0.18	0.59

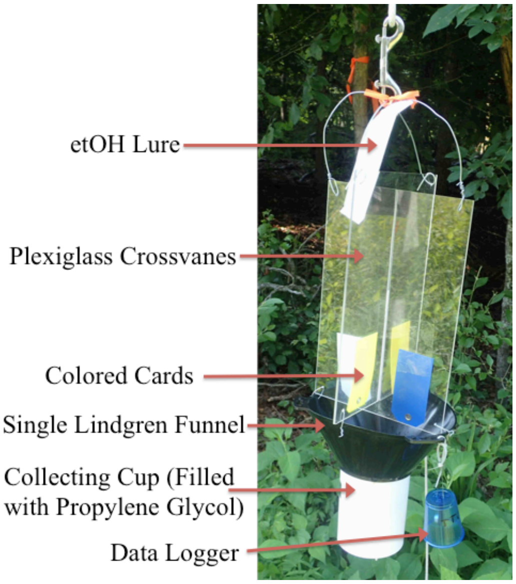

Figure 1. Design of flight intercept trap. 2 sheets of plexiglass placed above a single lindgren funnel with bottom removed and sprayed with Teflon. Killing/preserving agent: propylgene glycol, changed every 2 weeks. Baited with slow release ethanol lure, colored cards to attract pollinators.

Figure 2. Camera rig for hemispherical photos of canopy. Constructed using PVC pipes, hardware cloth, and heavy duty fishing line.

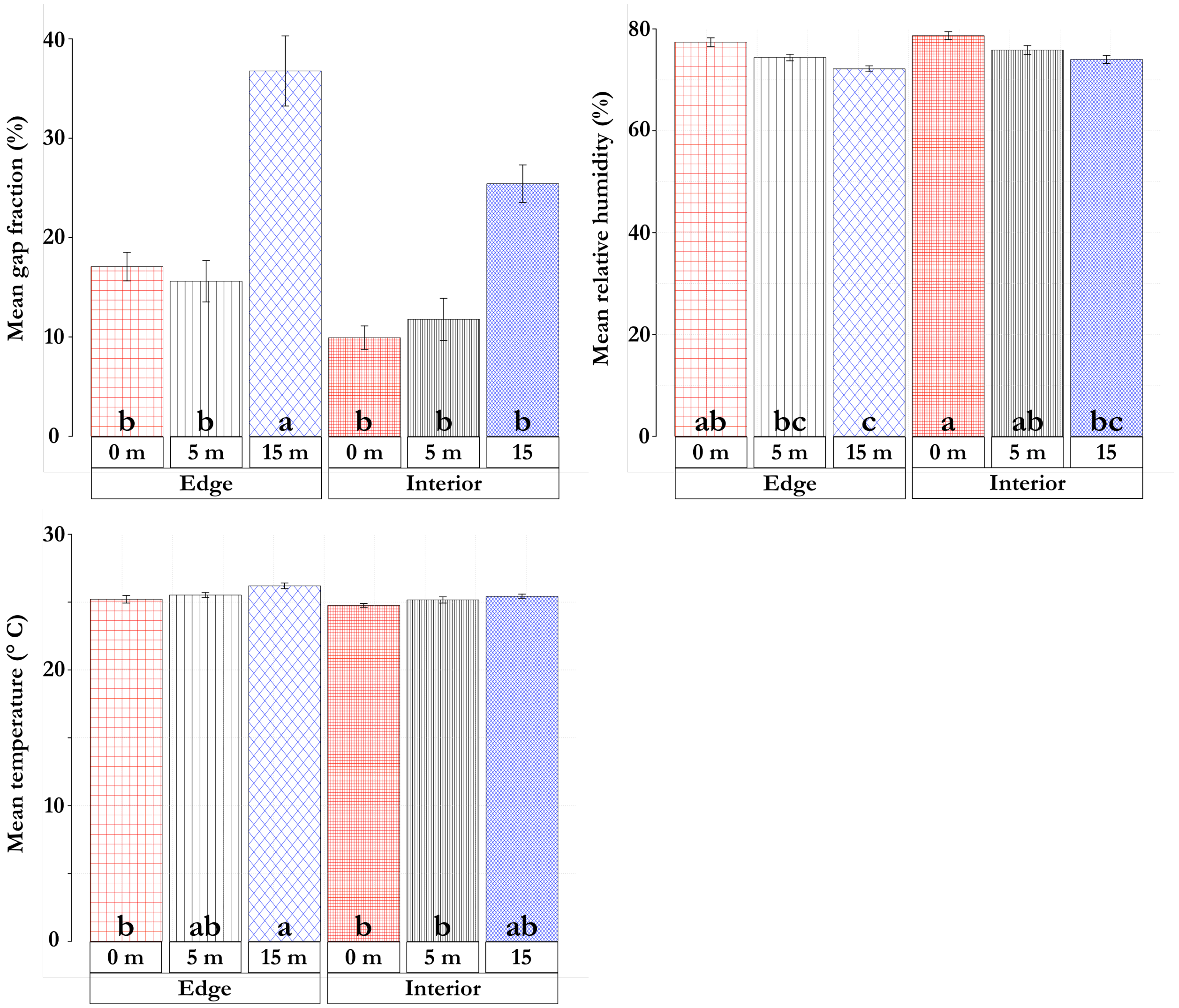

Figure 3. Mean relative humidity, temperature, and gap fraction by height and placement +/- standard error. Tukey's test was used for comparison of means.

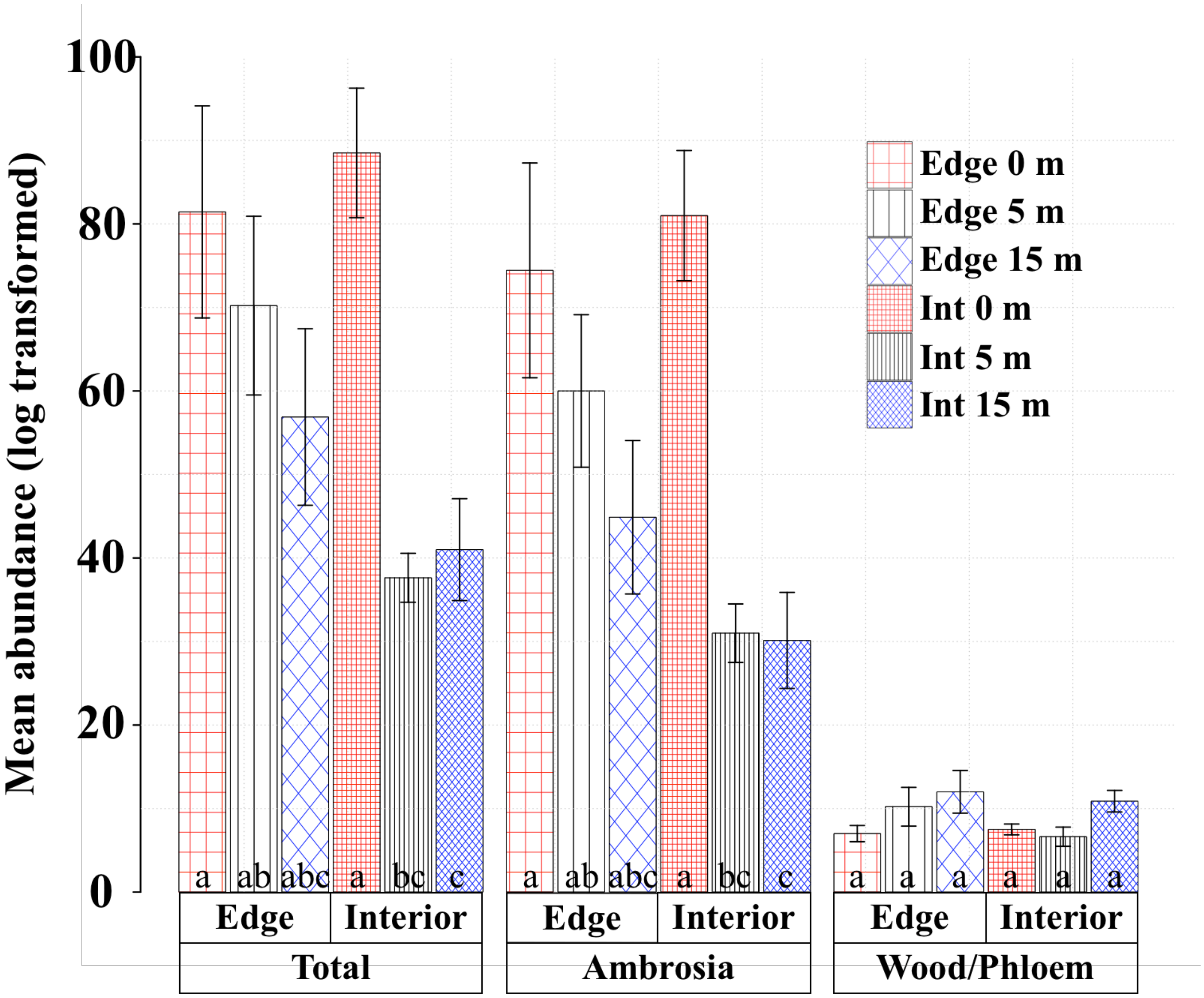

Figure 4. Mean abundance +/- se for height x placement interaction, separated by guild +/- standard error. Note abundance data was log transformed. Int = Interior

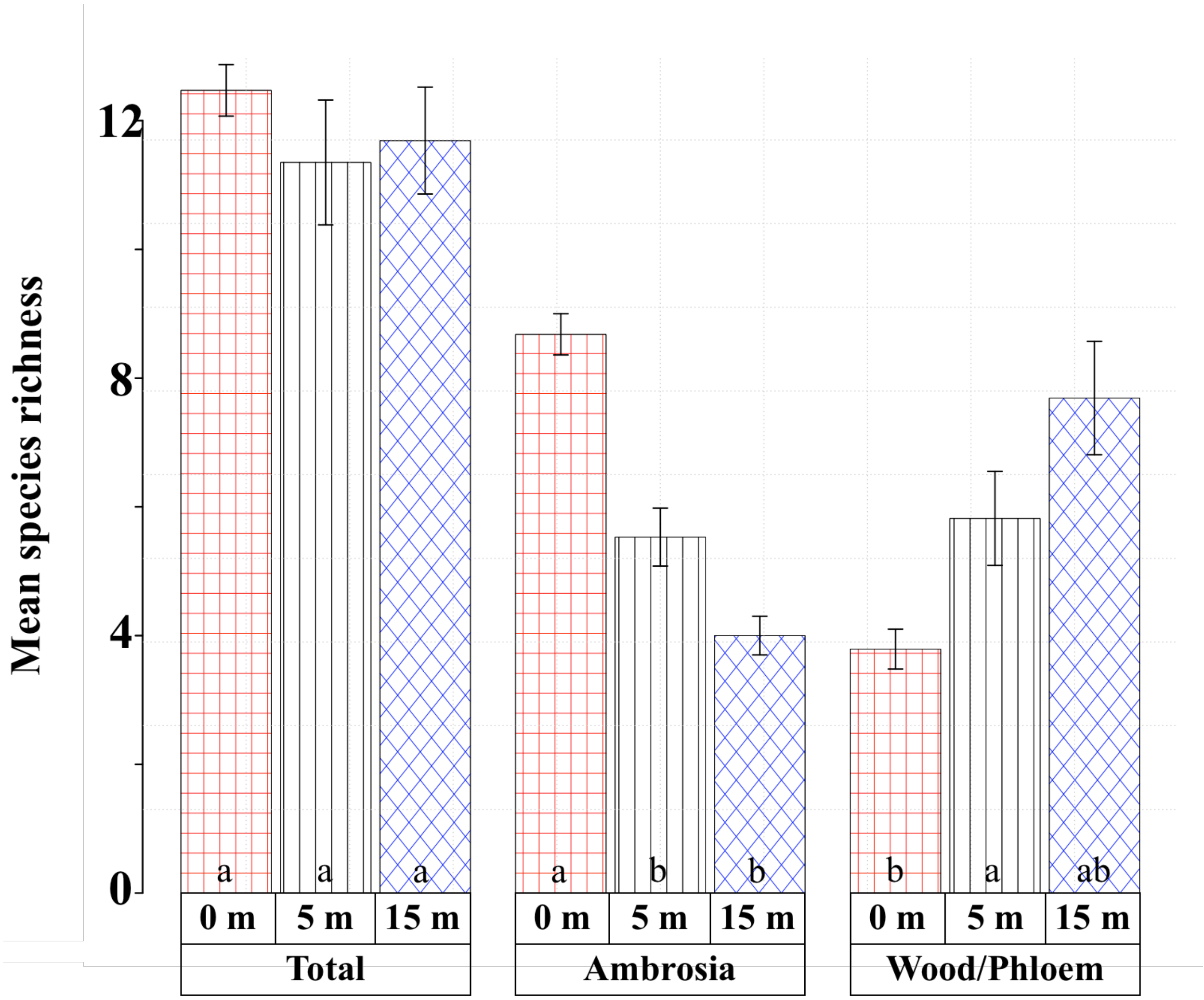

Figure 5. Mean species richness +/- se by height, separated by guild +/- standard error.

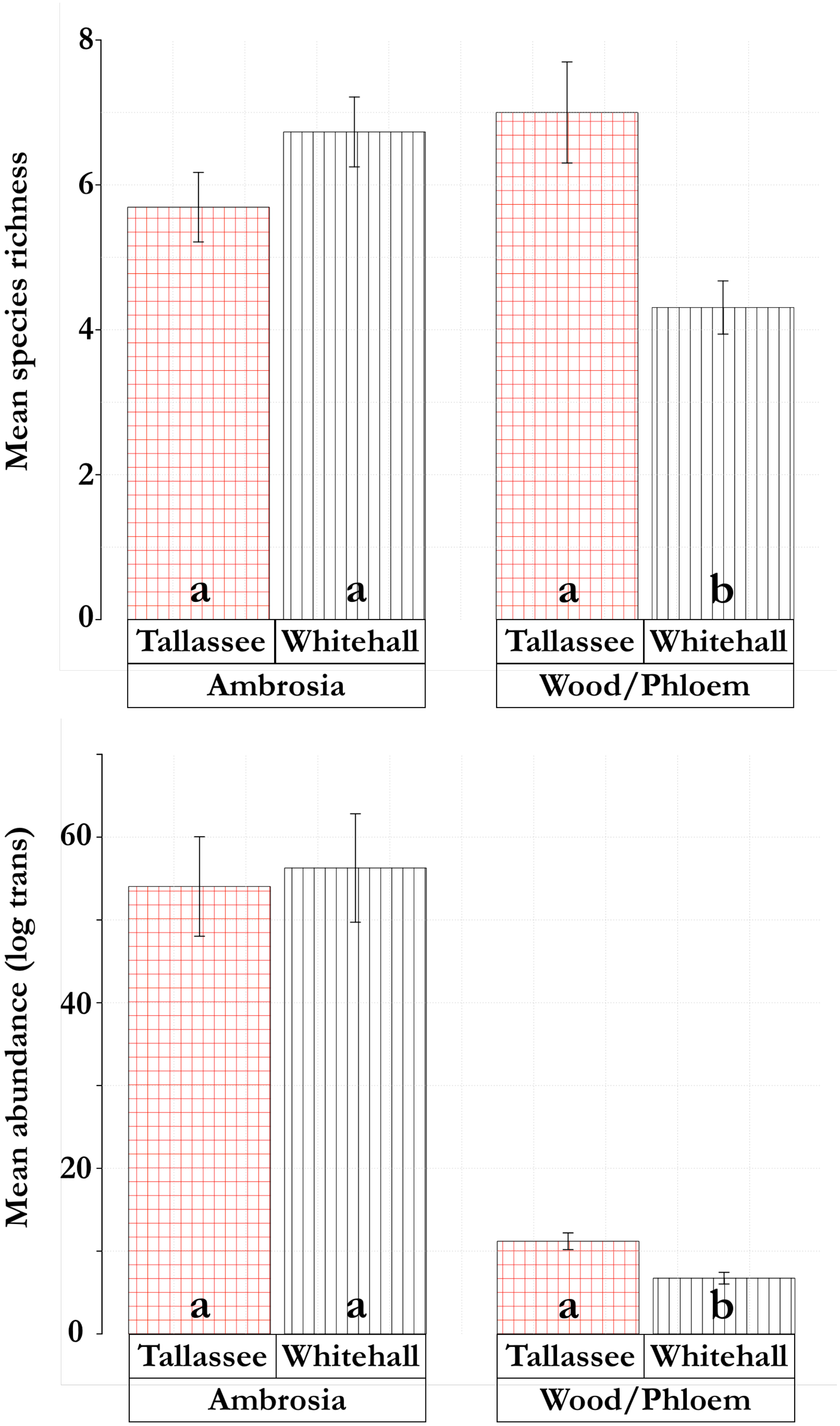

Figure 6. Mean species richness and abundance (log transformed) by site and guild +/- standard error.

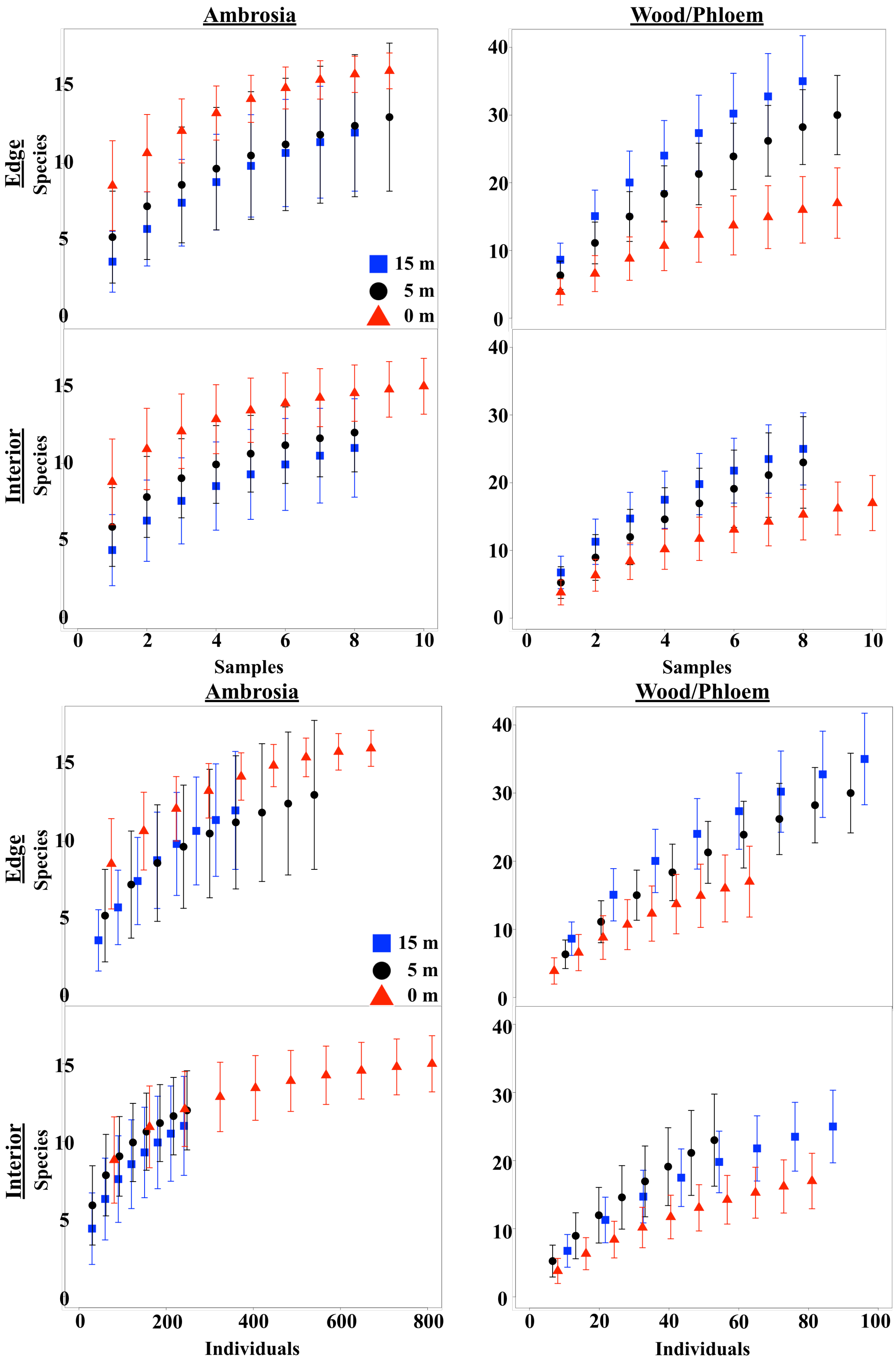

Figure 7. Sample based rarefaction using estimated number of species (S_{est}) with 95% confidence intervals. Scaled by both samples and individuals.

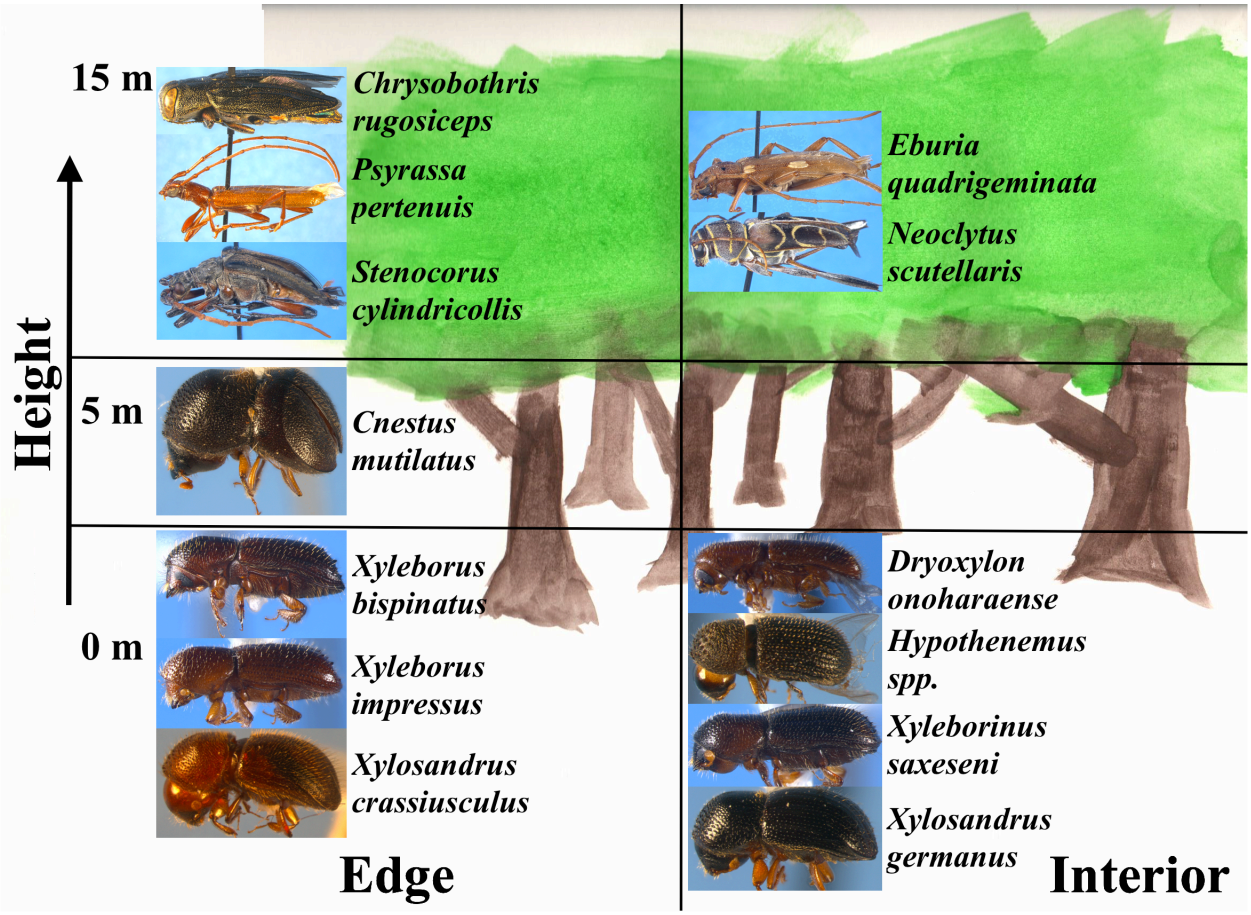

Figure 8. Indicator species for different height x placement combinations.

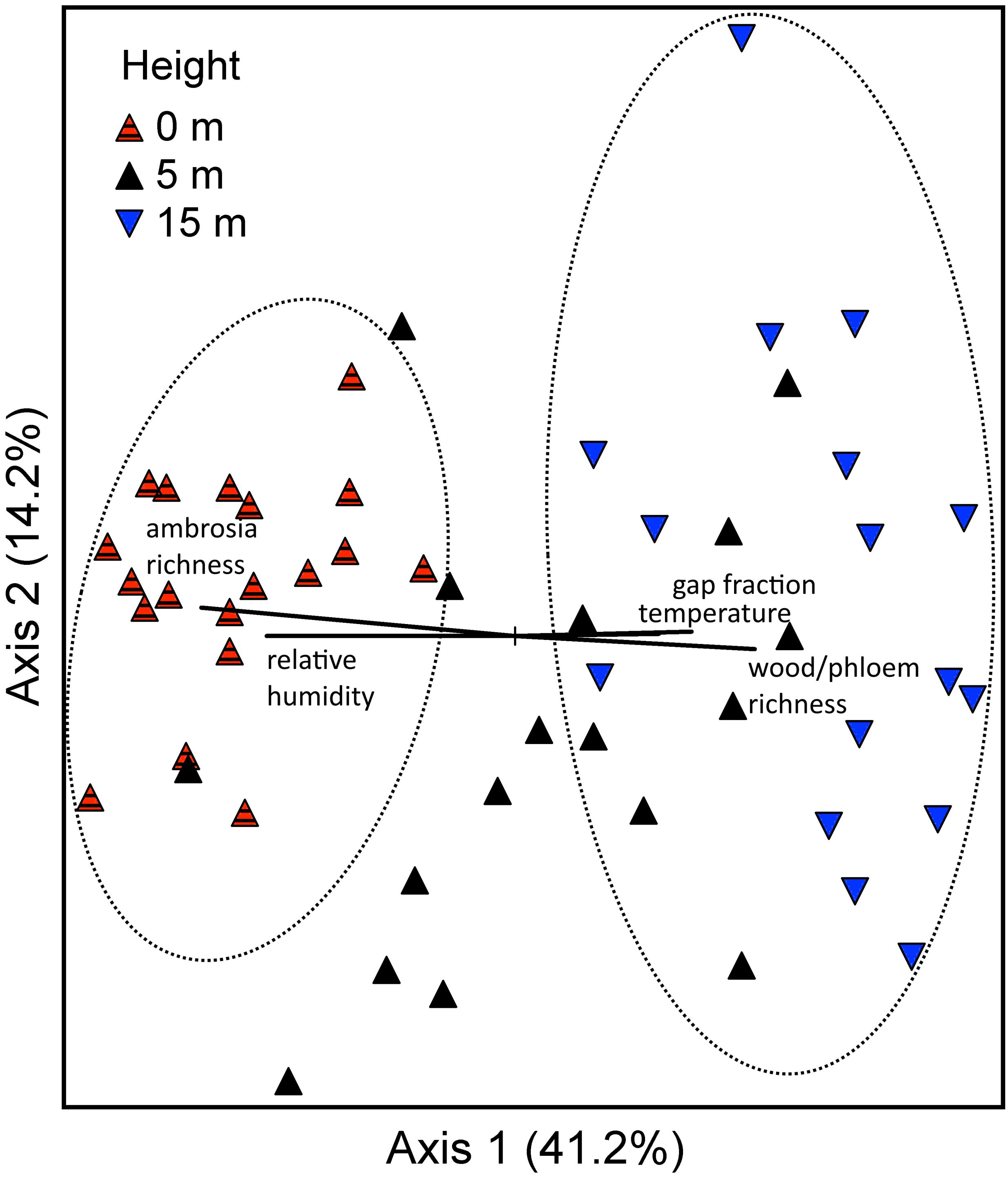

Figure 9. Non-metric multidimensional scaling ordination of community composition by height. Depicted by the different triangles, 0 m traps are associated with higher ambrosia richness and relative humidity; the 15 m traps are associated with higher wood/phloem richness, gap fraction, and temperature; the 5 m traps represent an intermediate, although statistically significant, difference.

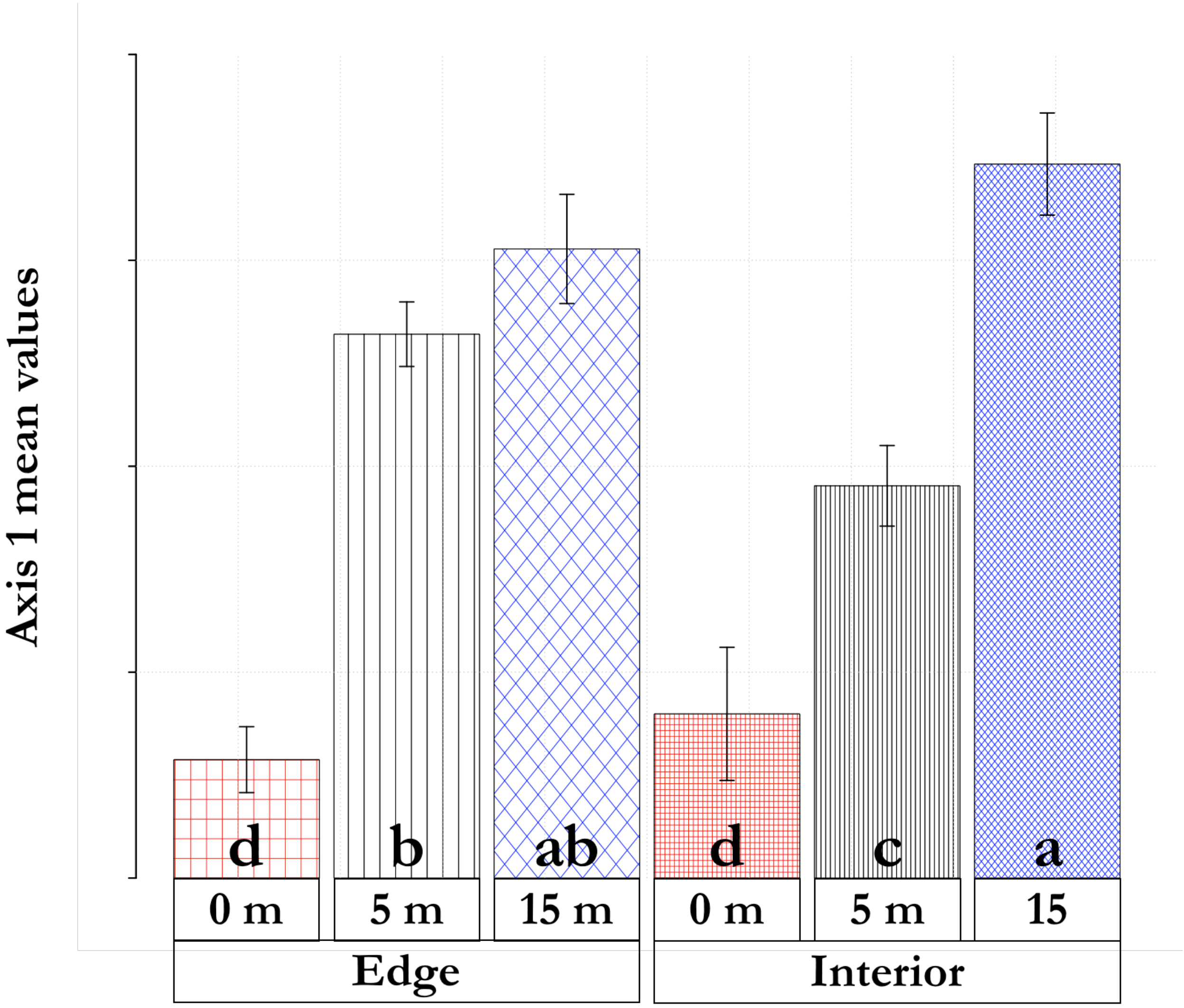

Figure 10. Graph of axis 1 mean values from NMDS plot +/- standard error.

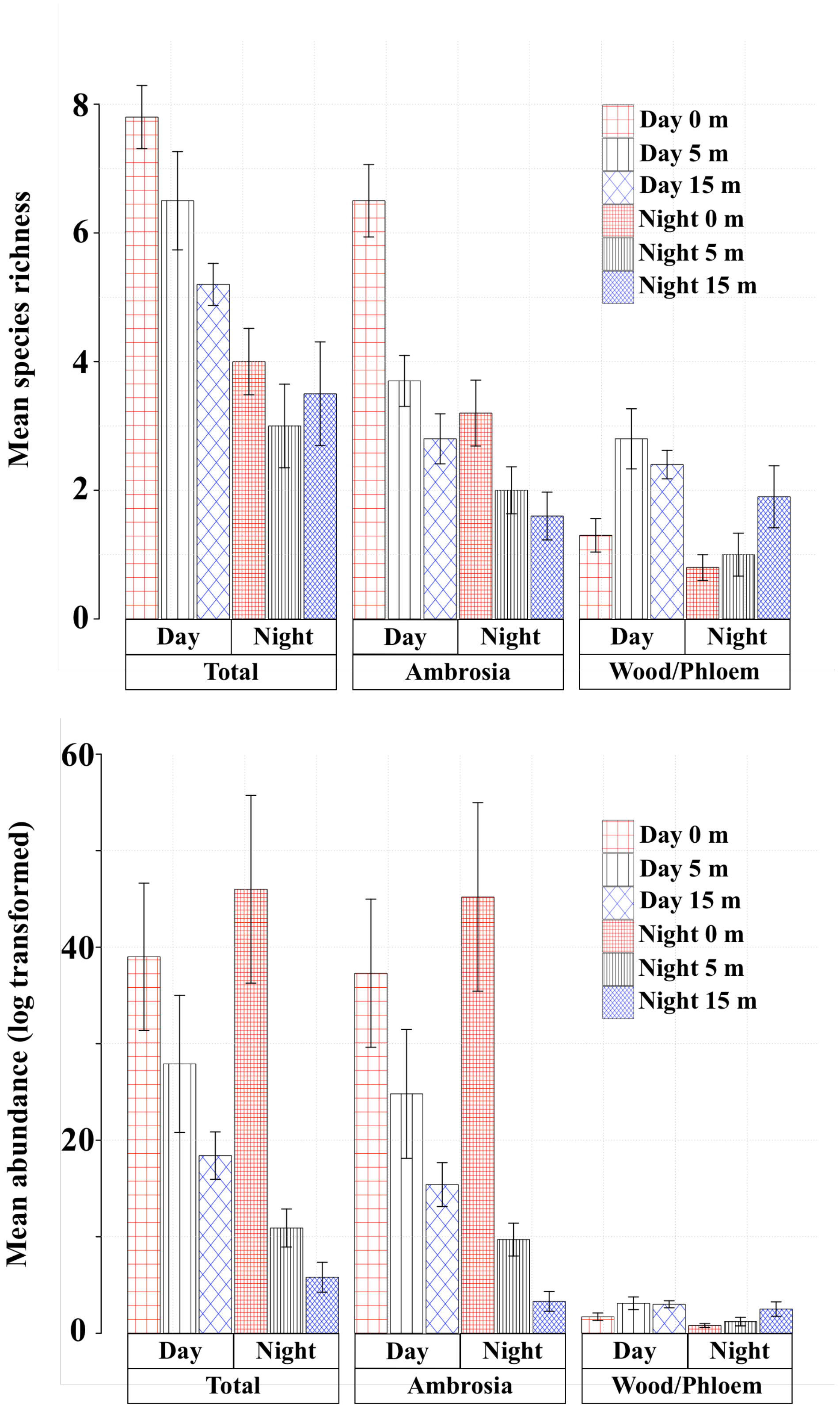

Figure 11. Mean species richness and abundance (log transformed) separated by time of day and by guild +/- standard error.