**The impact of tornado and salvage-logging on ground beetle (Coleoptera: Carabidae) taxonomic and functional diversity**

**Research objectives**

The goal of this study is to understand the impacts of combined natural and anthropogenic forest disturbance on ground dwelling arthropods. In 2012, a tornado event knocked over canopy trees in a mature deciduous forest. After logging occurred on part of the tornado-affected area, we were curious how these combined disturbances would affect the invertebrates living on the forest floor.

**Part A:** We aim to measure the activity-abundance, species richness, and diversity indices of ground beetles (Carabidae) in each forest management treatment. We predicted that initially after the windthrow and salvage-logging (2015), that species richness would increase in the windthrow and salvage-logged areas, driven by the immigration of open-habitat species. Furthermore we predicted that in 2015 this immigration would be more pronounced in the salvage-logged treatment relative to the windthrow, due to the more complete canopy opening. However, because the salvage-logged treatment has less woody debris and altered understory compared to the unsalvaged windthrow, we predicted that species richness in the salvaged treatment would be lower than that of the windthrow by 2022.

In addition to documenting general trends in carabid abundance and alpha-diversity, we wanted to understand if the community composition of the carabids differs between each forest management treatment. One major question was what happens to the “forest specialist” ground beetles when a tornado and salvage-logging occur? Are these forest-adapted species completely absent from the windthrow and/or salvaged areas, and do they move back into the disturbed regions after a decade has passed? Conversely, do “open-habitat” ground beetles invade into windthrow and salvaged treatments? Furthermore, do these open-habitat beetles remain after a decade, or have they already left or perished due to the regrowth of trees?

**Part B:** We want to further understand the biology of the carabids found at Powdermill, and to do this we are attempting to use a ecomorphological trait approach. An ecomorphological trait is a morphological trait that tends to be found in species adapted to a certain environmental condition (Fountain-Jones, Baker, and Jordan 2015). The traits we are interested in are primarily regarding locomotion, sensory capabilities, and their interaction. Ground beetle adults can be categorized by their locomotion habits. There are wedge-pushers, climbers, and surface walkers. The wedge-pushers may be approximately cylindrical in body shape and have a longer hind trochanter. They are usually better at burrowing into soil to escape a predator or to seek out prey. They may spend a large amount of time pushing through soil or leaf litter. The climbers have proportionally longer legs and antennae and have a flatter body shape. The surface walkers may have a more hump-shaped abdomen, short hind trochanters, and a body which is not at all dorsoventrally flattened. Sensory capabilities of ground beetles include touch and gustation through antennae, as well as vision through eyes. Because the main method of locomotion influences what kind of sensory abilities are most important for beetles, we want to assess these too. For example, protruding eyes may be important for climbing beetles, but may cause problems for burrowing beetles which must push through substrate. Extremely long antennae could similarly cause problems for burrowing beetles. Of course, sensory abilities likely relate heavily to diel activity patterns, but almost all the carabids we collected in 2022 seem to be predominately nocturnal (exception: *Notiophilus aenius*) (Larochelle and Larivière 2003). Sensory abilities are also likely related to shade preferences (“forest specialist” vs. “open habitat”), which is a comparison we will be able to make.

Our goal is to compare the mean trait values found in each forest management treatment, with the idea of understanding how environmental differences between treatments would favor carabid species with certain traits, or certain syndromes of traits. We also want to determine whether the functional diversity of carabids differs between forest management treatments, which could indicate a greater variety of habitat niches.

**Methods**

Study site

Research was conducted at Powdermill Nature Preserve (PNR) in Rector, Westmoreland County, Pennsylvania (include GPS coordinates for PNR here). This preserve was established as the field research station for the Carnegie Museum of Natural History in 1956 and is largely temperature deciduous forest. In June 2012, a tornado uprooted many canopy trees in two large areas of the forest, each about 120 × 480 m. These two areas are on north- or northwest-facing slopes, which were dominated by maple (*Acer* spp.), tuliptree (*Liriodendron tulipifera*), and black cherry (*Prunus serotina*) (Murphy et al. 2015), with an understory of predominately spicebush (*Lindera benzoin*) (Calinger et al. 2015).

From mid-summer through winter of 2013, half of each wind-disturbed area was salvage-logged using heavy machinery to remove the fallen and residual standing trees. In 2015, three transects were established across each section of forest impacted by the tornado (*n*=6 transects). Transects were established across the wind-disturbed and salvaged-logged disturbances that extended 50 m into the surrounding undisturbed forest on each side. Along each of these transects, four sites were established, each transect having a site in windthrow (*n*=6), salvaged (*n*=6), and surrounding undisturbed forest (*n*=12) wherein all data collection occurred.

Ground-dwelling invertebrate sampling

Ground-dwelling invertebrates were sampled using barrier pitfall traps in 2015 and 2022, representing three and ten years post-tornado. Barrier pitfall traps consisted of two pairs of plastic cups (each pair having an inner 500 mL cup and an outer 1 L cup) which were placed into the ground so that the lip of the cup was flush with the ground surface. The two pairs of cups were placed 1 m from each other, and garden edging (Suncast® eco edge) was placed between them to create a barrier. Cups were filled 4 cm high with propylene glycol (recreational vehicle and marine antifreeze, Peak Company Old World Industries, Clear Lake, Texas) with a few drops of detergent. Masonite board (100 cm2) was placed at 3 cm above each cup to prevent flooding from rain. Steel hardware cloth was secured over cups using 30 cm stakes to limit mammal disturbance.

Pitfall trap sampling was conducted continuously over the growing seasons in 2015 and 2022. Trap catch was collected every two weeks, and cups were refilled with propylene glycol. In 2015, pitfall traps were installed on May 27-28, and samples were collected on 9-10 June, 24-25 June, 8 July, 22 July, 5 August, and 17 August. In 2022, traps were installed on 1-2 June, and samples were collected on 15 June, 29 June, 13 July, 27 July, 11 August, 23 August, and 9 September. Trap catch was collected by pouring the sample through a fine mesh strainer and storing the contents in a specimen cup with 70% ethanol until sorting and identification.

Ground beetles (Carabidae) were identified to species using keys in Lindroth (1961), Bousquet (2010), Freitag (1969), Bousquet and Messer (2010), and Harden and Guarnieri (2017). Nomenclature followed Bousquet (2012). Species vouchers were deposited at the C. A. Triplehorn Insect Collection (OSUC), Museum of Biological Diversity, The Ohio State University, Columbus, Ohio where each specimen was given a unique identifier label (Table 1).

Morphological trait measurements

We selected nine morphological traits that are correlated with habitat preferences or species responses to the environment (Table 1) (Fountain-Jones, Baker, and Jordan 2015). These traits were *body length*, *antenna length*, *eye protrusion*, *eye length*, *pronotum width*, *abdomen width*, *rear leg length*, *rear trochanter length, and wing size*. Continuous traits were measured under a dissecting microscope using an ocular micrometer to the nearest 0.1 mm. For each species, traits were measured on up to six individuals, three males and three females if possible (Fountain-Jones, Baker, and Jordan 2015). These individuals were chosen in a way that attempted to encompass the intraspecific variation in body size observed for the species. To control for variation in beetle body size, relative measurements of all morphological traits were calculated as their ratio to body length for each individual (Ribera et al. 2001). Trait measurements were averaged across individuals to calculate species-specific means. In addition to the nine measured morphological traits, we utilized one functional trait from the literature: *habitat affinity*, which was coded as either “forest” or “open-habitat” (Larochelle and Larivière 2003). Species listed as having affinity for both forest and open habitat, or which were distinguished primarily by being hygrophilous, were marked as “open-habitat”.

Forest floor environment

Environmental variables on the forest floor were quantified to assess differences among windthrow, salvaged, and undisturbed forest. Percentage canopy openness was measured using a spherical crown densiometer directly above the pitfall traps to assess light availability on the forest floor. Canopy openness was measured on 9-10 June and 5 August in 2015, and on 1-2 June in 2022. Percentage cover of ground-level vegetation, leaf litter, bare ground, fine woody debris (<10 cm diameter at the large end), coarse woody debris (>= 10 cm diameter), and rocks were estimated in two randomly selected 1 m2 quadrats around each pitfall trap. Understory vegetation height (m) also was measured in the quadrats. Ground cover estimates were collected on 9 June, 7 July, and 5 August in 2015, and on [add dates] in 2022. Values from the two quadrats around each pitfall trap were averaged together for a site-level mean. Soil moisture was measured at three locations adjacent to each pitfall trap using a Dynamax Inc. (Houston, Texas) TH20 portable soil moisture meter with a Theta Probe ML2x sensor. Soil moisture measurements were taken biweekly when pitfall samples were collected. The three readings were averaged together for a single mean at each plot-date combination.

Table 2. Ecomorphological traits measured in this study.

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| **Trait** | **Calculation** | **Connection to habitat** |
| Body length | **a** | Shorter body length was found for ground beetles caught in wind-disturbed forests, relative to undisturbed forests (Sklodowski and Garbalinska 2011). Body length is correlated with many other morphological traits (Barton et al. 2011). |
| Antenna length | **b/a** | Tactile hunter species, which rely on sense of touch more than vision, tend to have longer antennae (Bauer and Kredler 1993). Longer antenna length relative to body length was found for ground beetles caught under a tree, versus in the open (Barton et al. 2011). |
| Eye protrusion | **(c – d)/a** | A greater eye protrusion was found in a tree-climbing ground beetle, and it may allow partial overlap in the frontal visual field. However, greater eye protrusion might prevent a ground beetle from moving through thick vegetation or soil (Talarico et al. 2007). |
| Eye length | **e/a** | Diurnal ground beetle species and/or those adapted to open environments tend to rely on vision for predator avoidance or prey detection (Talarico et al. 2007). |
| Pronotum width | **f/a** | A proportionally wider pronotum can be found in robust-bodied beetles, which tend to be found within open habitats (Barton et al. 2011). |
| Abdomen width | **g/a** | Similar pattern to pronotum width (Barton et al. 2011). |
| Rear leg length | **h/a** | Open habitats seem to favor ground beetle species with shorter legs relative to body length (Barton et al. 2011). |
| Rear trochanter length | **i/a** | The rear trochanter connects to the femur of the rear leg. It is longer, on average, in species that push themselves through soil and underneath leaf litter. It is shorter in species that walk or run above the surface of the substrate (Talarico et al. 2007). |
| Wing size | Marked as either macropterous (full-winged), brachypterous (reduced-wings), or dimorphic (some individuals having full wings while others having wing reduction) |  |

Statistical analysis

Because pitfall traps preferentially collect insects that are more active and mobile, the number of ground beetles caught in pitfalls is reported as activity-abundance rather than true abundance. Before analyzing activity-abundance, we first accounted for missing data. In 2022, mammals disturbed some of the pitfall traps, resulting in an occasional loss of trap catch. When the sample from one of the two sides of the barrier pitfall trap was lost, which occurred in 12 instances, we doubled the counts of species found at that plot during that interval. This resulted in an additional 13 beetles being added as a correction factor to the original count of 852 ground beetles in 2022 (3 beetles in forest, 8 in salvaged, 0 in windthrow). In 5 instances, which were all in the undisturbed forest plots, we lost both sides of the barrier pitfall sample. To correct for this, we standardized activity abundance. First, we summed the counts of ground beetles caught over the entire season. Then we divided the counts by the number of days each pitfall was operational (Sklodowski and Garbalinska 2011). This gave a measure of number of ground beetles caught per day at each plot.

We tested for differences in activity-abundance of ground beetles between windthrow, salvaged, and undisturbed forest using a linear mixed-effects model. Area (northeast area or southwest area) and transect nested within area were both included as random intercepts in the model to account for spatial structure. Residuals were tested for assumptions of normality and homogeneity of variances.

Taxonomic alpha-diversity measures

To verify if our sampling effort was sufficient to make estimates of species richness, we used species accumulation curves (Chao and Chiu 2016). We created species accumulation curves using the rarefaction method, which accumulates individuals rather than sites. This was implemented using the *specaccum* function in the R package ‘vegan’ (Oksanen, J. et al. 2024; R Core Team 2024).

To investigate the alpha-diversity at the plot level, we calculated measures of species richness, Shannon diversity, Simpson diversity, and Simpson evenness using the package “HillR” in R (Li 2018). Shannon diversity was calculated using Hill diversity with Hill number q=1. This form of Shannon diversity has a minimum of 0 and a maximum value of the species richness. Simpson diversity (Inverse Simpson Index) was calculated using the Hill diversity with Hill number q=2. Simpson evenness was calculated as the Inverse Simpson Index divided by the species richness. This metric measures the degree to which species have similar abundances.

To estimate the number of undetected species and thus estimate the true species richness of ground beetles, we used an asymptotic approach (Chao and Chiu 2016). We calculated the Chao1 estimator, which is a nonparametric estimator that gives a lower bound on the true species richness. This estimator incorporates the number of singletons and doubletons to estimate the number of undetected species and was implemented using the function “ChaoSpecies” using the R package “SpadeR” (Chao et al. 2016).

Taxonomic beta-diversity measures

Investigating beta-diversity answers the question: does the community composition of ground beetles differ between the wind-disturbed, salvaged, and undisturbed forest? Variation in community composition could be predominately *between* forest management treatments, or it could be predominately *within* each forest management treatment. We tested these possibilities using the Permutation-based Multivariate Analysis of Variance (PerMANOVA) and the Analysis of Multivariate Homogeneity of Group Dispersions methods, respectively. To implement these methods, we first calculated the distance in species-space between all pairwise combinations of our 24 plots. This was implemented using Bray-Curtis Dissimilarity with the *vegdist* function in the R package ‘vegan’ (Oksanen, J. et al. 2024). Because the inter-plot variability in total ground beetle catch was relatively low (σ / μ = 0.49), we did not perform any relativization prior to computing the distance matrix (McCune, Grace, and Urban 2002). PerMANOVA was conducted using the *adonis2* function in ‘vegan’ with 999 permutations. Beta-dispersion was implemented using the *betadisper* function in ‘vegan’. An Analysis of Variance (ANOVA) test was performed to test for differences in beta dispersion, and this was followed by pairwise tests using Tukey’s Honest significant difference test in the ‘stats’ package (R Core Team 2024).

The community composition of ground beetles was visualized using nonmetric multidimensional scaling (NMDS) using the ‘metaMDS’ function in ‘vegan’. We used a maximum of 500 random starts and a two-dimensional visualization. To verify a successful ordination, the Bray-Curtis dissimilarity between plots was graphed against the distance in ordination space using the *stressplot* function.

Idea: I’m also interested in which beetle species have positive spatial autocorrelation and which have negative spatial autocorrelation. For this I can use something called “Global Moran’s I”. Also, I think I should use presence/absence at each site rather than abundance.

Activity-abundance patterns of common species

For the 2022 collections, we individually analyzed any species of carabid where more than 30 individuals were collected. We tested for differences in activity-abundance between forest management treatments.

Community-weighted mean traits

Functional alpha-diversity

Functional beta-diversity

**Results**