

EFFECTS OF PLANT DIVERSITY ON BEETLE
ACTIVITY AND MOVEMENT

By

Camille Zwaan

Ecology and Evolutionary Biology, University of Colorado at Boulder

Defense Date: March 31, 2016

Thesis Advisor:

Dr. Michael Breed, Ecology and Evolutionary Biology

Defense Committee:

Dr. Michael Breed, Ecology and Evolutionary Biology

Dr. Pieter Johnson, Ecology and Evolutionary Biology

Dr. Peter Newton, Environmental Studies

Table of Contents

Abstract.....	2
Introduction.....	3
Background.....	6
Materials and Methods.....	12
Results.....	22
Discussion.....	28
Acknowledgements.....	34
References.....	35

Abstract

Plant diversity is declining due to anthropogenic land use change. Associated insects must respond to these altered environmental conditions; however, the effect of this decline on movement patterns of terrestrial arthropods is largely unknown. As top predators in arthropod communities, carabid beetles (Coleoptera: *Carabidae*) play a key role in ecosystem functioning and serve as biological pest control agents. Knowledge about carabid's movement such as dispersal patterns and food search behavior is crucial for understanding how this beetle perceives and responds to environmental factors. Experimental plant communities of a grassland biodiversity experiment were used to observe two common ground beetles, *Pterostichus melanarius* and *Harpalus rufipes*, on a microhabitat scale along a gradient of manipulated plant species richness and functional diversity. Fluorescent paint, which glows under ultraviolet light, facilitated observations of the nocturnal beetles. Beetle traces were recorded by hand and digitalized to analyze length travelled, dislocation, number of turns and step length. Additionally, pitfall trapping was used to assess recapture time. Results show that *P. melanarius* and *H. rufipes* respond to different aspects of vegetation—the lower the plant species richness, the more likely beetles will be moving. For *H. rufipes*, there were smaller scale movements in areas with higher functional diversity. Species and sex also played a role in determining movement patterns. Males travelled the furthest distance in both species. However, recapture rates differed by species with *P. melanarius* recaptured at a higher rate than *H. rufipes*. A novel observation method combined with traditional pitfall trapping gives a more complete picture of beetle movement and activity, furthering knowledge of beetle movement applicable to agricultural management plans.

Introduction

Insect populations respond to declining plant biodiversity caused by human land use change (Chaudhary et al., 2015). Decrease in plant biodiversity can reduce the number and diversity of associated insects (Haddad et al., 2009). This decrease shifts trophic structures; it is likely that generalist predators and omnivores are affected by these shifts due to a changing food supply (Hadaad et al., 2009; Scherber et al., 2010). A change in activity patterns and movement is also probable since food availability and vegetation structure influence movement behavior (Greenslade, 1964). However, insects' exact response to a decrease in plant diversity is widely unknown. This study investigates activity and movement patterns of two common ground beetles, *Pterostichus melanarius* and *Harpalus rufipes*, on a microhabitat scale along a gradient of manipulated plant species richness and functional diversity.

The study of movement is crucial to understand how an animal responds to its environment and helps explain processes such as foraging and dispersal of organisms (Jeltsch et al., 2013). The accumulation of individual behavior at a local level results in population patterns at a landscape level (Jopp & Reuter, 2005). In the past, animal observations have been restricted to small spatial-temporal scales on a local level (Jeltsch et al. 2013). With rapid technological advancements, the ability to collect high-resolution data over long periods of time, at small and landscape levels gives a more complete picture of animal movement processes (Jeltsch et al. 2013).

The emerging field of 'movement ecology' can provide valuable insights to biodiversity research by linking investigations on individual movements to population and community patterns (Jeltsch et al., 2013). Although movement ecology focuses primarily on individuals at a local scale and biodiversity research focuses on populations at a landscape level, it is the

individuals' movements that link different populations and help shape biodiversity (Jeltsch et al. 2013). A changing environment will likely elicit a response at the individual level which, in turn, affects larger population and community patterns.

Decreases in plant diversity are likely to result in structural and trophic environmental changes (Haddad et al., 2009). If there is greater plant diversity, there is higher diversity and abundance of arthropods (Haddad et al., 2009). Therefore varied, abundant food supplies may decrease the necessity of foraging movement. Furthermore, as mentioned above, vegetation structure directly affects movement. For example, Greenslade (1964) pointed out that tall grasslands impede movement more than land with bare ground. Additionally, with more vegetation structure, arthropods are able to move away from the horizontal plane of the ground into a third dimension (Greenslade, 1964). Even a small layer of litter can affect different species depending on if they move under, through, or on the surface of the litter (Greenslade, 1964).

I used a combination of direct observation and pitfall trap mark and recapture techniques to observe movement patterns of beetles along a gradient of manipulated plant diversity. Pitfall traps are containers buried in the ground so the rim is at the surface level; the beetles fall in and are unable to climb out (Lövei & Sunderland, 1996). Despite pitfall traps not being ideal for comparison among species (due to different species' likelihood of falling into traps), this method is the most widely used to study ground arthropods such as carabid beetles. Pitfall traps have been used to observe behavior in different habitat types (Chapman et al., 1999; den Boer, 1990; Fournier & Loreau, 2001). Direct observation via video recording has also been used to study carabid beetle preference at a habitat interface (Allema et al., 2014). However, no studies have looked at movement along a biodiversity gradient.

Understanding beetle movement has practical applications in agriculture by informing management plans that maximize arthropod diversity and ecosystem services. Since many carabids prey on agricultural pests, providing a habitat of more diverse and dense vegetation for these beetles can enhance pest control (Chapman et al., 1999). Recommendations include leaving field margins and strips of land between crops undisturbed (intercropping) to provide high quality habitat for beetles (Chapman et al., 1999). Chapman et al. (1999) found that at night, beetles move from dense vegetation to monocropped vegetation. This suggests that, at night, beetles will extend their foraging range to adjacent fields. Den Boer (1990) furthered the understanding of movement linked to dispersal by examining carabids' ability to disperse as a determining factor of beetle repopulation in the context of landscape fragmentation. Jopp and Reuter (2005) allowed a better understanding of population dynamics by modeling distribution patterns of dispersal based on local spatial resistance of the environment and different landscape elements. Finally, carabids are used as bioindicators of anthropogenic change. For example, by looking at their response to habitat fragmentation, insecticide use and different agricultural management practices (Basedow & Stork, 1990; Rainio & Niemela, 2003). Beetles could also be used as bioindicators for assessing biodiversity loss.

The "Trait-Based Biodiversity Experiment" is a component of the Jena Experiment that simulates biodiversity loss by creating an artificial gradient of plant diversity (plant species richness and functional diversity) (Roscher et al., 2004). As part of the Jena Experiment, my study combined a novel approach of direct observation and mark and recapture to explore activity and movement patterns of two model organisms of terrestrial arthropods. The carabid beetle species, *Pterostichus melanarius* and *Harpalus rufipes* were painted with fluorescent paint (which glows under ultraviolet light) and observed on a microhabitat scale at night. Beetles were

recaptured in pitfall traps and released daily back into the plot. Specifically, I investigated the questions: (1) Does plant diversity (plant species richness and functional diversity) affect beetle activity and movement? (2) Is there a difference in activity and movement between species studied and between sexes? I expected to see an increase in activity and longer, more directed movements as biodiversity decreases. Furthermore, looking to see if species or sex respond differently to decreasing plant diversity will give additional specificity about movement behavior.

Background

1. Study organisms

Carabids, members of the beetle family *Carabidae*, are commonly known as ground beetles. They are a cosmopolitan family of beetles with over 40,000 described species found in every habitat except in the Antarctic and waterless parts of the desert (Lövei & Sunderland, 1996). Most are black, brown or metallic and have long, slender cursorial legs and hardened forewings called elytra (Lövei & Sunderland, 1996). Carabids are herbivores, omnivores or carnivores that can provide ecosystem services by feeding on the seeds of weed species or consume crop pests and have therefore been subject to numerous studies (Lövei & Sunderland, 1996). The study organisms used in this experiment are *Pterostichus melanarius* (Illiger, 1798) and *Harpalus rufipes* (De Geer, 1774). The ability to differentiate sex in these organisms along with their large size, abundance, widespread distribution and inability to fly make these beetles excellent model organisms to study.

a. *Pterostichus melanarius*

Pterostichus melanarius is an abundant carabid beetle with a widespread distribution throughout Europe in gardens, grasslands, woodlands and agricultural fields (Luff, 2007). Its size ranges from 13 to 17 mm and its body and appendages are black (Luff, 2007). *P. melanarius* has deep stria-- parallel grooves in the elytra running anterior-posteriorly (Luff, 2007). The tarsals (the final segment of the leg furthest from the body) have clawed joints and bristles called ventral setae are present on its underside (Luff, 2007). In Coleoptera, the large, highly sclerotized segment (hardened by proteins) posterior to the head is called the pronotum. For *P. melanarius*, it is rounded on the sides and the rear angles have a small tooth (Luff, 2007). Wings are generally absent, which makes the beetle incapable of flight (Luff, 2007). *P. melanarius* is a nocturnal and mostly predatory generalist (Bohan et al., 2000). It generally breeds in autumn (Luff, 2007). Females (Figure 1) and males (Figure 2) are easy to differentiate. In females, all tarsals are the same size, whereas the last tarsal is smaller than the others in males, which allows the male to hold on to the female during copulation (Luff & Turner, 2007).



<http://www.thewcg.org.uk>

Figure 1. Female *Pterostichus melanarius*. Tarsals are the same size.



<http://www.thewcg.org.uk>

Figure 2. Male *Pterostichus melanarius*. Last tarsal is smaller than the others.

b. *Harpalus rufipes*

Harpalus rufipes is a common ground beetle usually found in dry, arable fields (Luff, 2007). Its size ranges from 11 to 16 mm (Luff, 2007). Its body is dark brown or black with short golden hair covering the elytra; legs and antennae are red-brown (Luff, 2007). The elytra has nine striae; the pronotum is rounded on the sides and straight on the posterior side forming sharp angles in the back; antennae are proportionally short with the first two antennal segments bare and the rest covered in fine and dense hairs (Luff, 2007). *H. rufipes* is nocturnal and omnivorous with a diet comprised half of prey and half of plant-derived resources (Luff, 2007). It breeds in summer and autumn (Luff and Turner 2007). Females (Figure 3) and males (Figure 4) can be differentiated by their tarsals; male tarsals are bigger and more robust compared to female tarsals that are more slender (Luff, 2007).



<http://www.thewcg.org.uk>

Figure 3. Female *Harpalus rufipes*.



<http://www.thewcg.org.uk>

Figure 4. Male *Harpalus rufipes*.

2. Pitfall trapping

Pitfall (Barber) traps are containers buried in the ground so the rim is at the surface level; the beetles fall in and are unable to climb out (Lövei & Sunderland, 1996). This technique is the most prevalent field method used to study ground-dwelling arthropods such as carabid beetles (Lövei & Sunderland, 1996). The trap is a passive catching method (as opposed to active where organisms are caught directly by a collector), which means that catches depend not only on population densities but also the organism's activity. Therefore pitfall trap data are imperfect for studying community patterns or making comparisons among orders or even species because different species may be more or less likely to fall into the trap (Lövei & Sunderland, 1996). Although pitfall trap data is widely used, factors influencing quantity and composition of catches are biased due to biological and environmental factors, the trap itself, and conditions of the experiment.

Biological and environmental factors that influence beetle activity (and thus catching efficiency) include size of organism, reproductive period, climate, vegetation, soil surface temperature, prey density and insecticide use (Greenslade, 1964; Lövei & Sunderland, 1996; Brose, 2003; Melbourne, 1999). For instance, studies show that abundance of larger specimens is overestimated when using pitfall traps (Lang, 2000; Hancock & Legg, 2012). This could be because larger species cover more ground and have lower chances of escaping from the pitfall trap. Melbourne (1999) and Greenslade (1964) investigated the influence of vegetation structure on pitfall trap catches. Melbourne (1999) measured ant catches in three types of habitat structure finding a large bias in pitfall trap captures depending on the habitat (Melbourne, 1999). Similarly, Greenslade (1964) discusses the difference between two carabid beetles: *Carabus arcensis* moves only near the surface of litter while *Pterostichus niger* is active on it but also

closer to the ground, explaining catch abundance differences for pitfall traps where vegetation was cleared around them (Greenslade, 1964).

Not only do external factors influence trap efficacy, but so does the trap itself. Physical attributes of the pitfall trap including size, material, shape, color, and absence or presence of lid also influence catch rate. Luff (1975) showed that small traps are better for catching small beetles and large ones are better for catching large beetles, but large traps are more efficient overall (Luff, 1975). Compared to plastic and metal traps, glass traps were most efficient in catching beetles and had best retaining efficiency (least escape) (Luff, 1975). Shape (for instance circular and rectangular traps) can also influence catches (Luff, 1975). A rectangular trap with straight edges is directional and will favor catches coming at it perpendicularly. On the other hand, for a circular trap, two comparable beetles will have the same probability of being caught regardless of their direction of movement (Luff, 1975). Traps can also be in the shape of a cup or funnel, and a cup-shaped trap has been shown to be more effective at catching organisms than a funnel-shaped one (Knapp & Ruzicka, 2012). Even color can influence trap rates, with carabids preferring white and yellow pitfalls traps compared to green and brown ones (Buchholtz et al., 2010).

Finally, conditions of the experiment will also vary catch efficacy of the traps. Schirmel et al. (2010) showed that experimental design influences trapping rates. They manipulated sampling interval and found that the majority of arthropods were caught more with shorter sampling intervals (Schirmel et al., 2010). For *Caribidae*, twice as many individuals were caught sampling monthly compared to weekly (Schirmel et al., 2010). Also, presence or absence of organisms or preserving fluid inside the trap could attract or repel beetles. For example, a beetle of the opposite sex and inadvertent catches of rotting small mammals, amphibians or slugs could

act as attractants, skewing the efficiency in trapping relative to other traps (Yi et al., 2012).

Sometimes, preserving fluids are also present in the traps and can act as attractants (propylene glycol) or deterrents (formaldehyde) (Knapp & Ruzicka, 2012).

Although an imperfect sampling method, pitfall trapping has remained prevalent due to its convenience and effectiveness in measuring activity density and can be useful for comparison if biases are consistent. Incorporating an experimental component to characterize bias or combining pitfall trap sampling with standardized area samples can give a more complete and accurate picture of beetle activity. However, direct observation (as opposed to passive catching) maximizes information about movement.

3. Direct observation methods

Small size, cryptic appearances and nocturnal behavior are some of the main problems faced when conducting insect observations. Using mark and recapture with pitfall trapping does not give high spatial and temporal resolution of information on movement. Advances in technology such as automatic video tracking systems and radio techniques have helped overcome this issue (Reynolds & Riley, 2002).

Digitizing data collection provides reliable and consistent gathering of information. Video has been used by Allema et al. (2014) to study movement behavior of carabid beetles to determine preference at a habitat interface. Noldus, Spink and Tegelenbosch (2002) developed an image processing system that automates behavioral observation and movement tracking to overcome limitations posed by older technologies (*i.e.* ability to track a single individual on a neutral background). In addition to visual techniques, radio techniques such as harmonic radar tagging enable researchers to obtain detailed movement patterns of organisms. Originally developed to locate avalanche victims, advances in technology have enabled the harmonic radar

tag's size and weight to be adapted for the study of insects. Harmonic radar works by attaching a small, lightweight tag to an individual. Radio waves hit the tag and it re-radiates a harmonic of the signal, which is then transmitted to a receiver. Broll et al. (2008) used this method to study population dispersal in carabid beetles in grasslands. However, this method does not allow for individual identification unless the organism is identified once it is found. This can be overcome by using radio telemetry. This technique uses different frequencies to identify individuals; however, transmitters can only be used on large insects and have a high potential for technical problems (Ricken & Raths, 1996).

Despite their efficacy, these techniques are often expensive, so alternate tracking methods must be considered. Small mammals present similar problems of being hard to see, especially at night. Lemen and Freeman (1985) developed a technique that uses fluorescent pigments to track small mammals. A captured mammal is covered with pigments then released. As it moves, pigment falls and leaves a trackable, fluorescent path observable under ultraviolet light. My study also uses fluorescent colors to mark beetles and observe their movement patterns under ultraviolet light at night. However, in my study the beetles are painted and then movement patterns are traced by hand.

Materials and Methods

I used the following methods to address the questions of whether plant diversity, species and sex affected beetle activity and movement.

1. Field site

The Jena Experiment (Figure 5) is a 10 ha field site located in the northern part of the city of Jena, Germany (Thuringia, Germany, 50°55' N, 11°35' E, 130 m above sea level). It is one of the

largest biodiversity experiments worldwide where researchers study the effects of biodiversity in experimental grassland communities. The goal of this large-scale project is to understand how biodiversity affects above-ground and below-ground consumers, as well as nutrient cycling (Roscher et al., 2004). The present study was conducted as part of the “Trait-Based Biodiversity Experiment”, from now on referred to as the TBE (Ebeling et al., 2014).



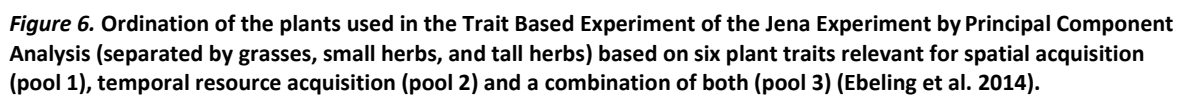
Figure 5: Aerial view of the Jena Experiment. Photo provided by Julia Tiede.

2. Experimental Design

a. *Trait Based Experiment*

Established in 2010, the trait-based biodiversity experiment (TBE) manipulates plant species richness (number of plants) and functional diversity of plant communities in order to disentangle the effects of taxonomical richness and niche space occupied by a plant community on ecosystem functioning (Ebeling et al., 2014). For the design of the TBE, 60 plant species from the main part of the Jena Experiment, 20 grasses and non-legume herbs were selected for traits associated with acquiring resources in space (plant height, leaf area, rooting depth and root length density) and in time (time of growth, flowering onset) to form a diversity gradient in resource-use characteristics (Ebeling et al. 2014). Legumes were excluded, as their presence is

The TBE was split into three blocks A, B and C (Figure 7). Block B was further split into two to avoid the area where tree shadows would fall and could potentially affect measurement.



To provide different diversity levels from pools in different blocks, diversity levels per pool and block A, B, C were selected randomly.

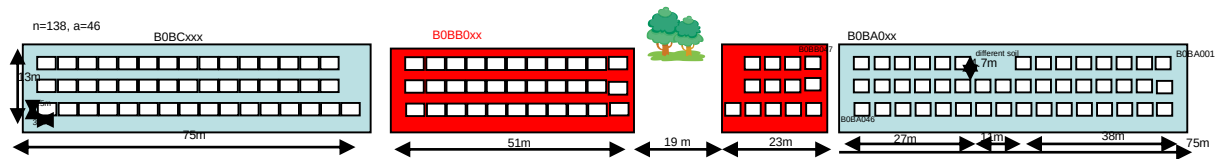


Figure 7. Representation of the three blocks of the Trait Based Experiment of the Jena Experiment. From left to right: C, B, A. Image provided by Julia Tiede.

b. Fenced area for beetles

In a strip (1 m x 3.5 m) in each plot of the TBE, fences were built to not allow beetle movement between plots (Figure 8). Transparent construction foil was wrapped around four wooden corner poles and sunken 15 cm into the soil with PVC panels. Three pitfall traps were dug to recapture beetles after their release into the plots.



Figure 8. Picture of beetle enclosures (1 m x 3.5 m) in the Trait Based Experiment. Photo provided by Julia Tiede.

c. Random sub-sample for nocturnal observations

After excluding 46 out of 138 plots where beetle observation would be impossible due to high-density vegetation, 30 plots were randomly selected for nocturnal observations. 10 monocultures (with a single plant species present), five plots containing two plant species, 5 plots with three plant species plots and 10 plots with four plant species each were randomly selected to increase replication and add weight on the ends of the species richness gradient for statistical purposes (Draper & Smith, 1998). In addition, three plots with eight plant species each were added for a total of 33 plots.

3. Collecting, care and marking of beetles

Beetles were sampled in the fields surrounding the experimental plot using pitfall traps from June 1st to July 28th, 2014. Traps were filled with moss, grass or leaves to prevent cannibalism once beetles were caught, and some also contained fruit or paper towels soaked in red wine to attract beetles. Traps were emptied every two to three days, and more frequently in the case of extreme weather (heavy rain or heat). The beetles were kept in plastic boxes with humid clay pebbles and moss and fed *ad libitum* with cat food (K-Classic Adult, Kaufland AG, Germany) and different seeds until their release into the plots.

Each beetle was individually marked on the elytron and pronotum using an engraving tool (Figure 9). Beetles assigned to the random subset plots for direct, nocturnal observations were painted on the elytron with one base coat of white paint marker and one topcoat of acrylic fluorescent paint. Beetles captured in pitfall traps were repainted if necessary. Beetles were color-coded according to species and sex. *P. melanarius* males were painted yellow and females

were painted white. *H. rufipes* males were painted orange and females were painted pink (Figure 10).



Figure 9. Markings on a *Pterostichus melanarius* individual. Photo by Julia Tiede.

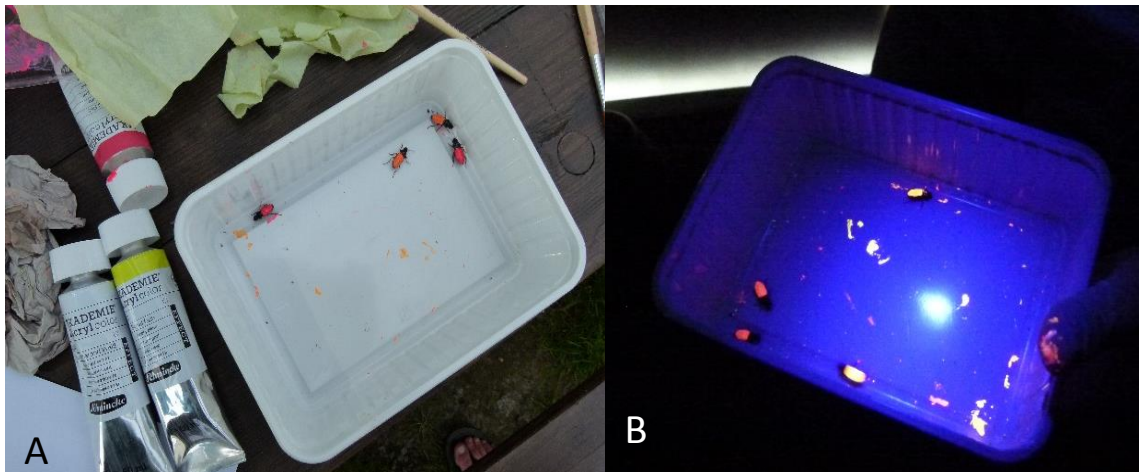


Figure 10. *Harpalus rufipes* under (A) daylight and (B) ultraviolet light. Photos by Camille Zwaan.

Engraving and painting did not affect beetle survival. I engraved and painted four *P. melanarius* (two males and two females) and four *H. rufipes* (two males and two females). These marked beetles, along with control beetles, were individually kept in plastic boxes (17 cm x 12.5 cm) with moist clay pebbles and moss (heated in a drying oven at 70°C and fed ad libitum with cat food; Figure 11). After one week, all painted beetles were alive and showed no different behavior than controls. In addition, recapture rates of painted beetles did not differ from marked but unpainted beetles during the four weeks of the experiment.

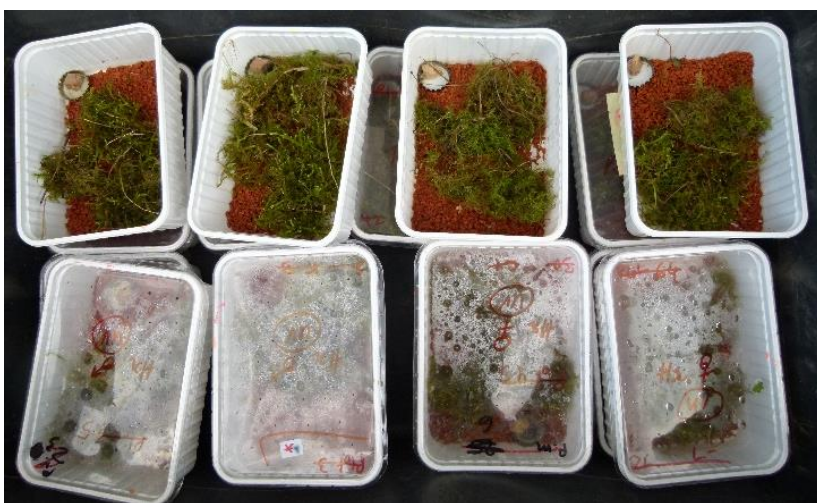


Figure 11. Experimental set up for impact of paint and marking on beetle mortality. Photos by Camille Zwaan.

The beetles' reaction to ultraviolet (UV) light was observed to ensure that UV light did not affect beetle behavior. Four beetles of each species (two males and two females) were kept in separate boxes (35 cm x 65 cm) with little plant structure and observed under low and UV light conditions. Beetles were placed in the boxes a few hours before observation began to allow them to adapt to their new surroundings. Beetles were then observed at dusk under low light conditions (enabling me to see the beetles without UV light). UV light was then shone into the box and initial reactions observed (Figure 12). This was repeated on three different days. The

projection of ultraviolet light into the box did not change the beetles' behavior. Individuals that were not moving continued to be still and beetles that were walking or mating continued doing so. If a strong ultraviolet light was shone from under 15 cm directly into the beetles' eyes, the beetles shifted out of the direct light and resumed their activities. However, during search and observations, light was never shone from that close.

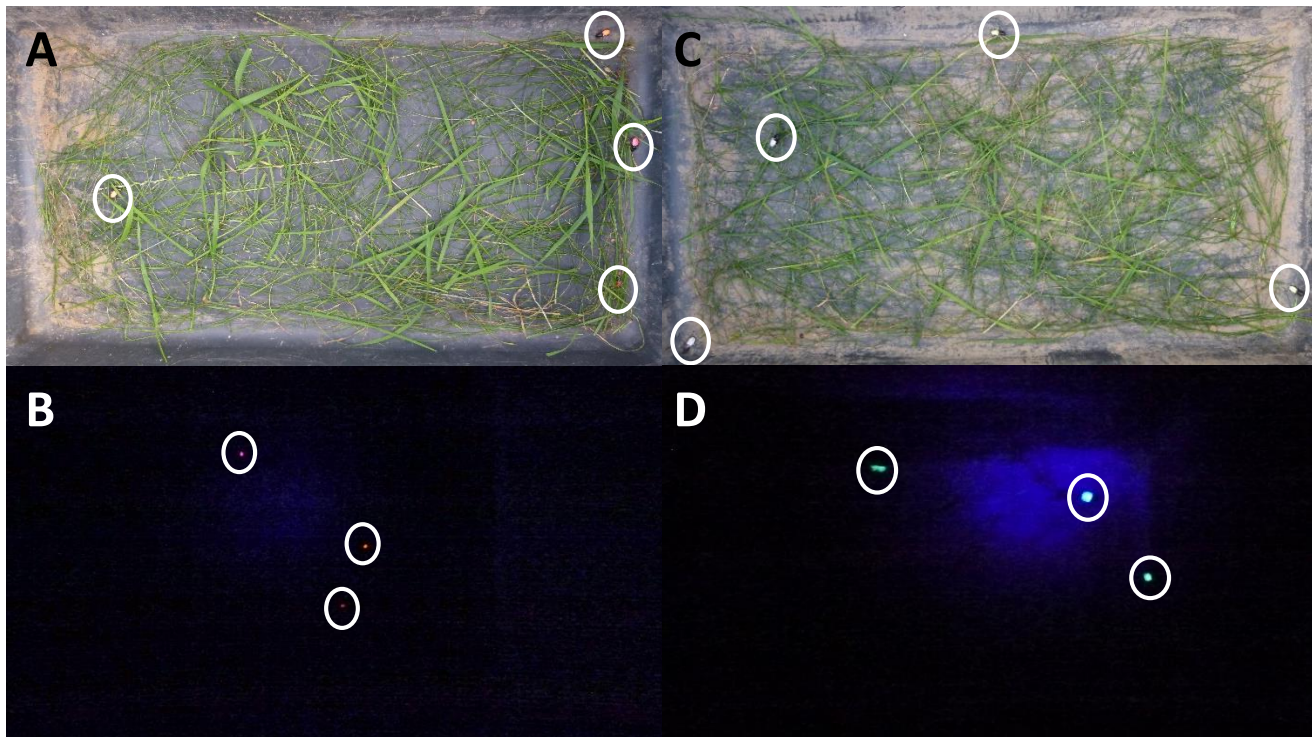


Figure 12. *Harpalus rufipes* in (A) daylight and (B) ultra-violet light. *Pterostichus melanaruis* in (C) daylight and (D) ultraviolet light. Photos by Camille Zwaan.

4. Indirect observation of activity: pitfall trapping

Pitfall traps were checked daily for recaptured beetles. The caught beetles were sampled for regurgitates and released back into the same plot. Since the beetles had enough time to recover until night, an effect of these procedures on the results is unlikely. The number of days it took for a beetle to be recaptured for the first time were recorded.

5. Direct observation: activity and movement paths

I used a Focal-animal Sampling method (observing one individual at a time for a determined length of time) in order to obtain detailed data about beetle movement paths (Altmann, 1974). I searched and observed marked beetles from August 4th to 14th, 2014 from 9 pm until midnight. Each plot was searched for five minutes ($\pm$ one minute depending on plot density). Once a beetle was found, it was observed for one minute and its path hand drawn (search time stopped during this observation period; Figure 13). Activity was assessed as non-moving if a beetle did not move during the entire observation period or moving otherwise.

From August 12th on, search time was decreased to 1-2 minutes based on plot density to maximize likelihood of finding beetles. Additional search time after 2 minutes generally did not result in finding additional beetles and fast cooling during the night as well as increase in humidity lowered my chances of seeing beetles. This change in observational methods, although undesirable, should not affect my analysis of the results, as what is being assessed is beetle activity, which does not depend on number of beetles found per plot.

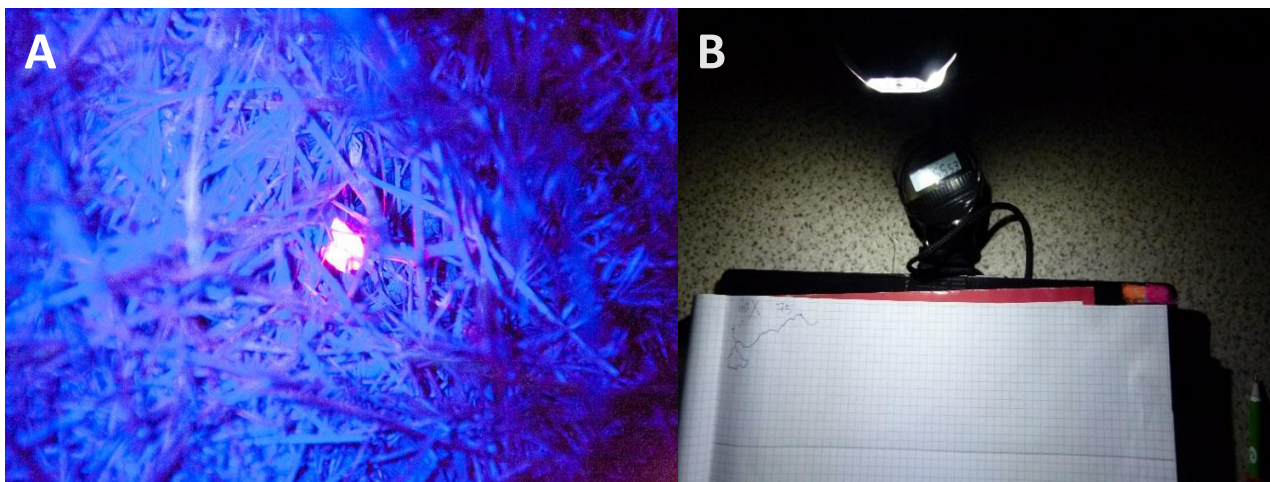


Figure 13. (A) Female *Harpalus rufipes* (pink) in a grass plot. (B) Path of male *Harpalus rufipes*. Photos by Camille Zwaan.

6. Trace analysis: digitizing movement paths

Hand drawn movement traces of individual beetles recorded in the field were scanned and the traces analyzed with JMicroVision (Version 1.2.7) (Figure 13). For each observation, the following data was analyzed:

- a) Path length [cm]: The length of the trace or the sum of all trace parts in the interior or along the edge. Proportion of trace in the interior was calculated by dividing it through the total trace length (trace length in interior / (trace in interior + trace along edge)).
- b) Dislocation [cm]: Direct displacement between the start point and end point of a trace.
- c) Average step length [cm]: The average step length was calculated by dividing the trace length by the number of steps separated by turns (trace length / (turns + 1)).
- d) Turns [N]: The number of turns defined by a directional change of more than 90°.

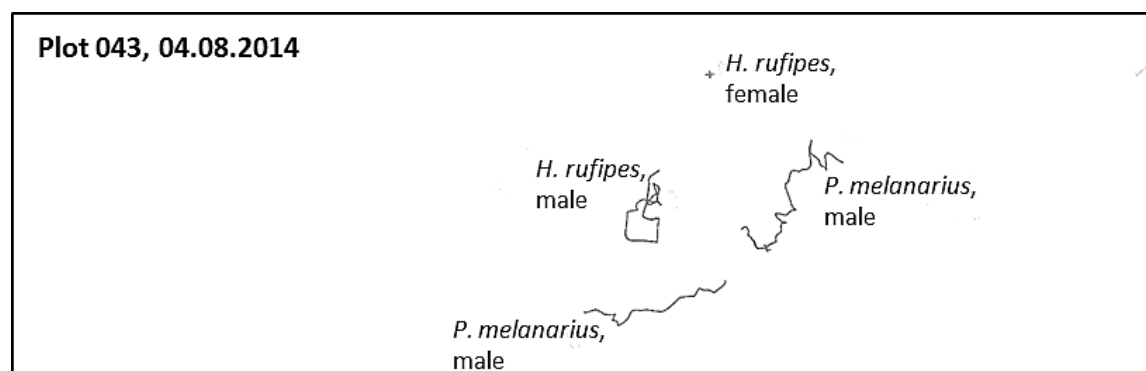


Figure 13. Digitized movement paths for Plot 042 on 04.08.2014.

7.

7. Statistical analysis

I used the data analysis software R Version 3.2.0 (2015-04-16) and the R package *lme4* (Bates et al., 2015) to perform generalized linear mixed models (GLMM) on response variables of observed activity and trace data. Significance level was set at 0.05. The goal of the analyses was to assess how treatment effects (e.g., plant species richness (PSR) and functional diversity

(FD)), beetle species, and the sex of the beetle influenced the response variables. I also included a species-by-sex interaction. However, because of the nested nature of the data in which beetles co-occurred within the same plot, I included a random effect of plot in all models. I did not include block as an additional random effect because preliminary analyses indicated it accounted for no additional variance beyond plot. The different response variables were modeled according to the appropriate distributions: activity was approximated using a binomial distribution (number moving vs. number not moving of those observed) and recapture as a Poisson distribution (number of days between recaptures). For trace data, number of turns was approximated as a Poisson distribution and data on length, dislocation and step length was log transformed to normal distributions. In cases where interaction between species and sex was significant, I subset the data to look at the difference in sex between species individually. If no interaction was found, I re-ran the model with no interaction between species and sex. Because PSR and FD were highly correlated (Pearson's $r = 0.79$), I ran separate analyses for each predictor variable to avoid problems of collinearity. Similar results were found with PSR and FD unless specified and statistics reported are for models run with PSR unless specified.

Results

1. Activity assessed via passive observation: pitfall trap recapture

a. Effects of plant diversity

Variation in plant diversity did not influence the time it took a beetle to fall into a pitfall trap. The generalized mixed model analysis showed that neither plant species richness (PSR) nor functional diversity (FD) explained variation in recapture time. Regardless of treatment, the average number of days for recapture was 4.6 ± 0.24 days ($n = 563$).

b. Effects of species and sex (Figure 14)

However, I detected significant effects of beetle species and sex (but no interaction between the two) explaining variation in recapture time. On average, *H. rufipes*'s recapture time (8.1 ± 0.47 days) was longer than *P. melanarius*'s (2.6 ± 0.21 days) (GLMM, species $z = -16.67$, $p < 0.001$, $n = 563$). Males were caught in a shorter amount of time on average (4.1 ± 0.30 days) than females (5.2 ± 0.41 days) (GLMM, sex species $z = -1.98$, $p = 0.048$, $n = 563$).

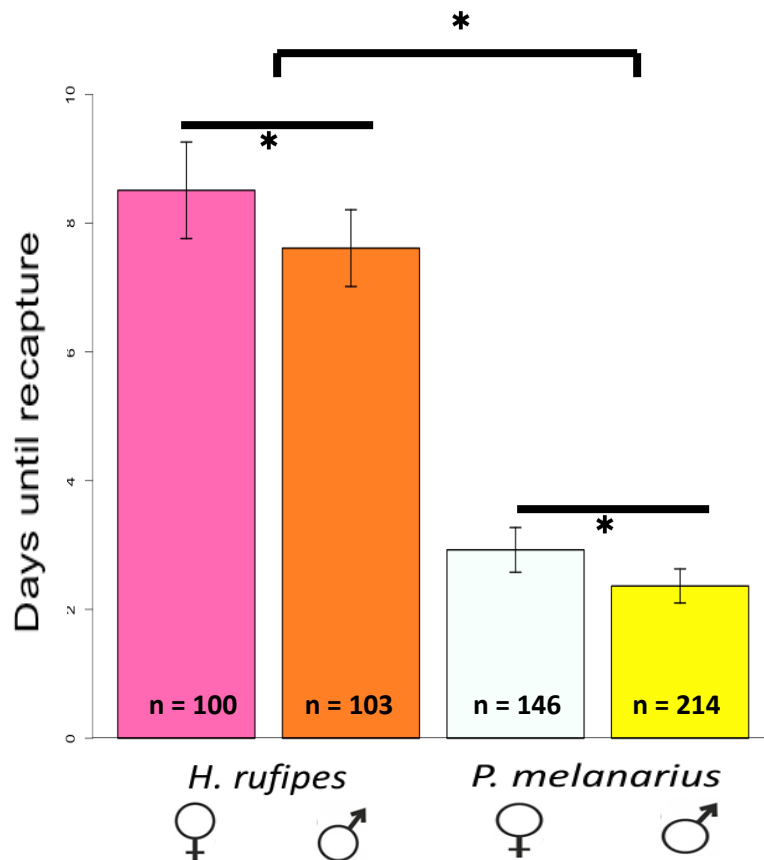


Figure 14. Bars represent mean number of days $\pm$ standard deviation until beetle was recaptured for *Harpalus rufipes* females (pink) and males (orange) and *Pterostichus melanarius* females (white) and males (yellow). Asterisks indicate significant differences as assessed by GLMM ($\alpha = 0.05$).

2. Activity assessed via direct observation: moving or not moving

a. Effects of plant diversity (Figure 15)

Beetles were significantly less likely to move in habitat with higher diversity than in habitat with lower diversity. Based on the generalized linear mixed model analysis, I found a negative main effect of PSR on the odds of beetles moving, such that with each additional plant species, there was a 0.8 ± 1.08 decrease in the odds that a beetle is moving (GLMM, PSR OR = 0.8 ± 1.08 , $z = -2.49$, $p = 0.013$). The same result was found if I used FD instead of PSR, with a 0.7 ± 1.13 decrease in odds of moving (GLMM, FD OR = 0.7 ± 1.13 , $z = -3.20$, $p = 0.001$). There was no significant effect of beetle species, sex, or their interaction.

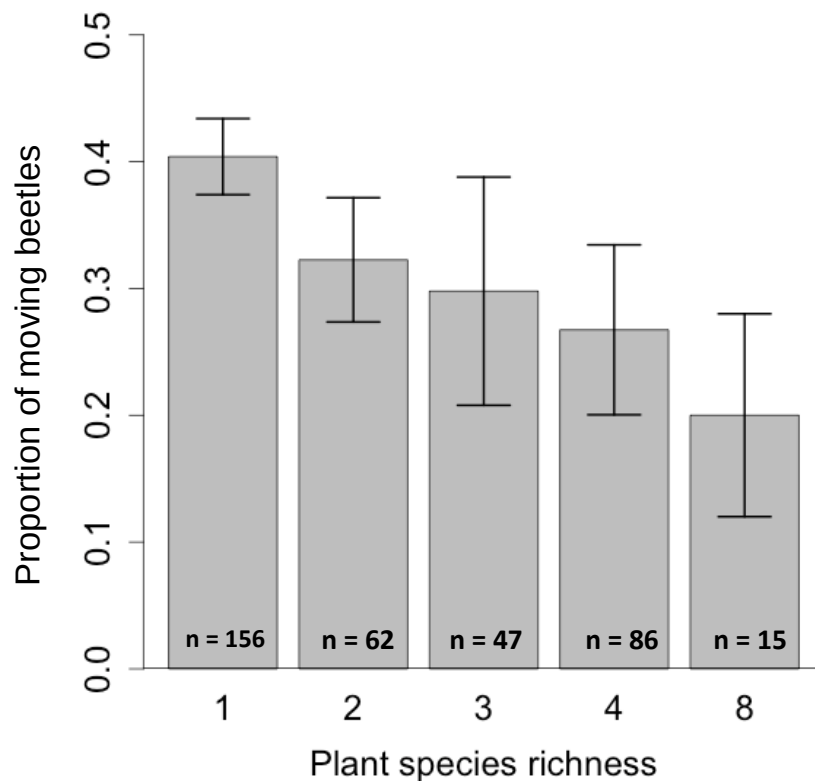


Figure 15. Bars represent proportion of moving beetles $\pm$ standard deviation as a function of plant species richness.

b. Effects of species and sex (Figure 16)

In addition to a main effect of PSR and FD, *H. rufipes* males were more likely to move than females, and for *P. melanarius* sex did not affect the likelihood of moving. The generalized linear mixed model analysis showed a significant interaction between species and sex in driving the odds that beetles moved (GLMM, $z = -2.83$, $p = 0.013$). For *H. rufipes*, males were 2.6 ± 1.41 times more likely to move than females (GLMM, $z = 2.79$, OR: 2.6 ± 1.41 , $p = 0.005$). For *P. melanarius*, no differences explained by sex were found.

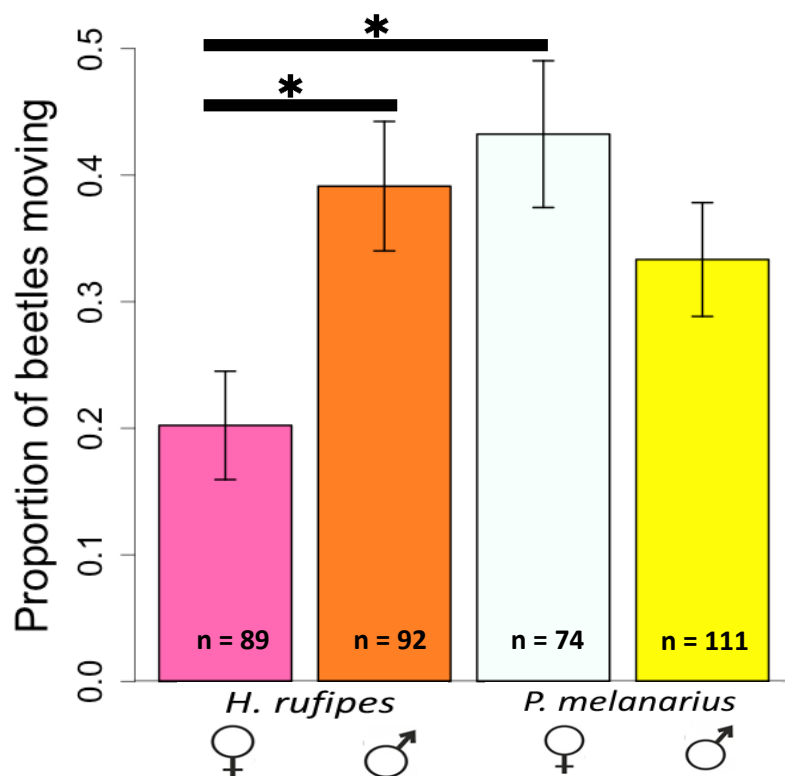


Figure 16. Bars represent observed proportion of beetles moving for *Harpalus rufipes* females (pink) and males (orange) and *Pterostichus melanarius* females (white) and males (yellow). Asterisks indicate significant differences as assessed by GLMM ($\alpha = 0.05$).

3. Movement traces assessed via direct observation

a. Effects of plant diversity (Figure 17)

Plant diversity had few effects on the movement pattern of beetles. I found no effects of PSR on length, dislocation, step length or number of turns while FD had a significant, negative effect only on step length (GLMM, FD $t = -2.40$, $df = 98.00$, $p = 0.018$). For instance, beetles at highest FD moved, on average, 16.7 cm per minute more than those in the lowest FD.

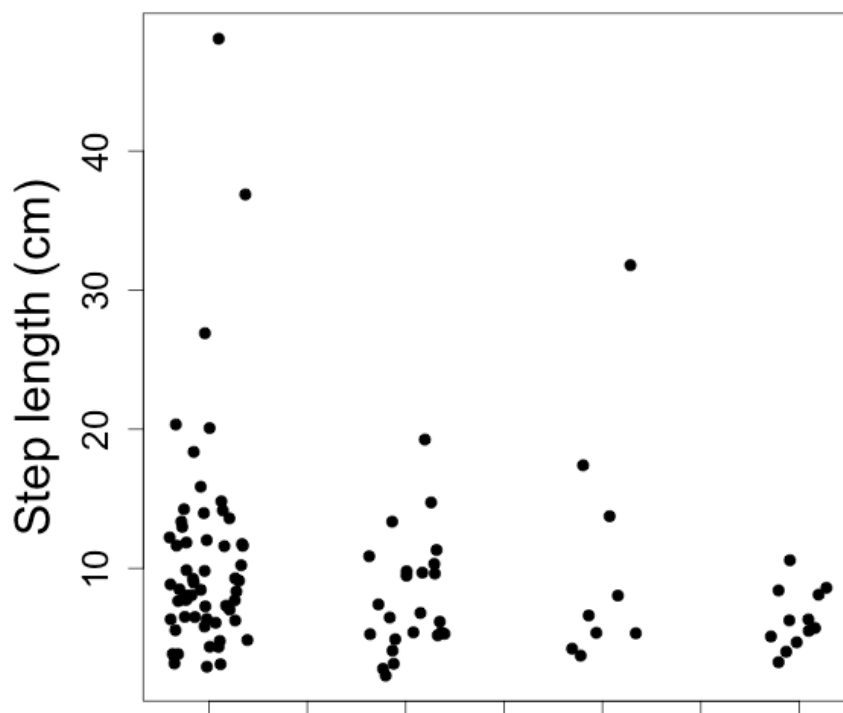


Figure 17. Scatter plot of step length in function of functional diversity.

b. Effects of species and sex (Figure 18)

I detected significant effects of beetle sex in explaining variation in path length and an interaction between sex and species in helping to account for number of turns. Thus, males' path length 1.46 ± 1.17 cm longer than those of females per minute (GLMM, $t = 2.44$, $df = 98.98$, $p = 0.017$), with no added effects of dislocation, step length or number of turns. For *H. rufipes*,

males have on average an additional 0.66 ± 0.18 cm turns per minute than females (GLMM, $z = 3.58$, $p < 0.001$), while sex had no effect on number of turns for *P. melanarius*. Species did not explain variation in path length, dislocation, or step length.

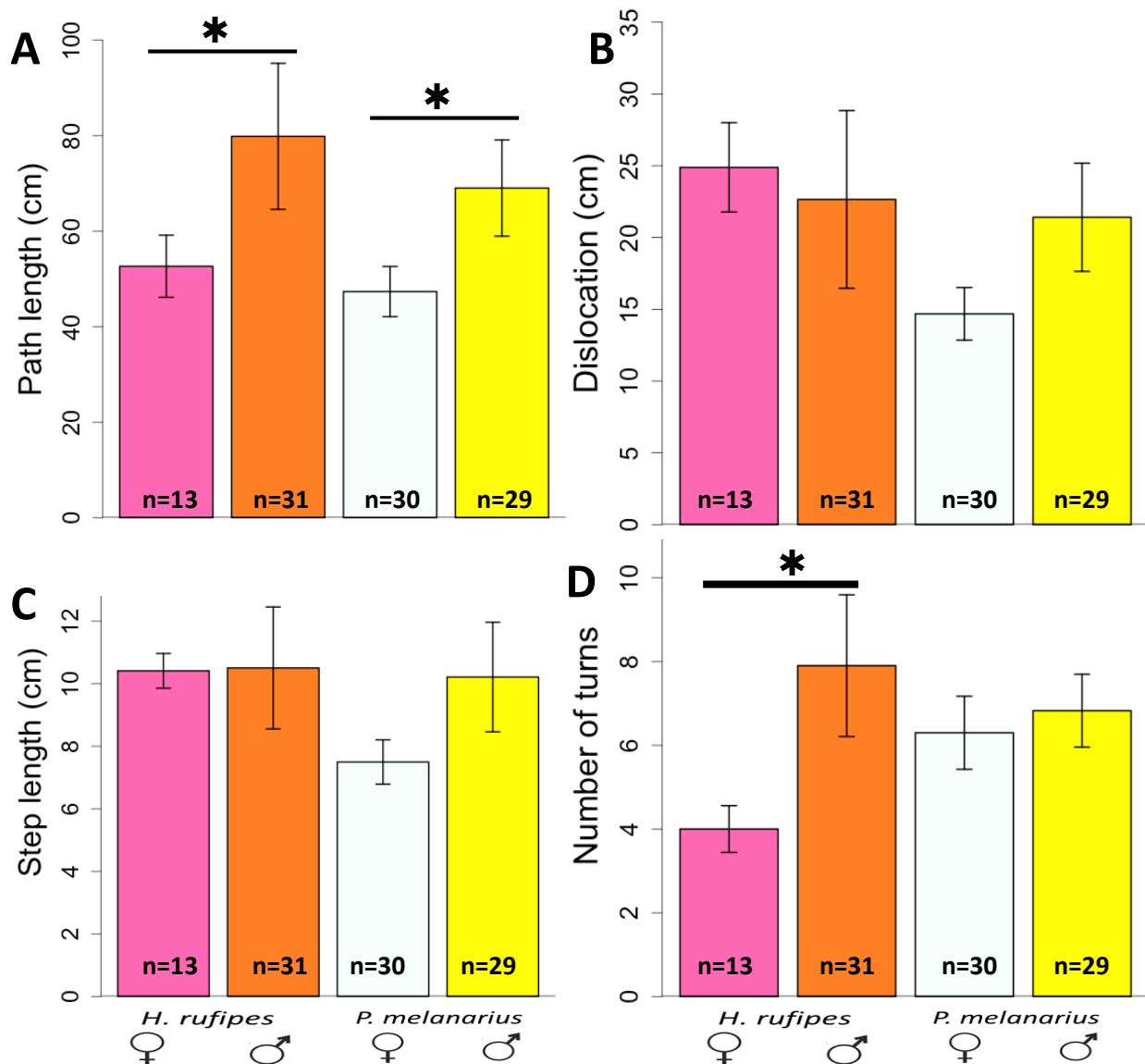


Figure 18. Bars represent means $\pm$ standard deviation of (A) path length, (B) dislocation, (C) step length and (D) number of turns of *Harpalus rufipes* females (pink) and males (orange) and *Pterostichus melanarius* females (white) and males (yellow). Asterisks indicate significant differences as assessed by GLM ($\alpha = 0.05$).

Discussion

This study assessed beetle activity and movement along a gradient of manipulated plant diversity (specifically plant species richness (PSR) and functional diversity (FD)). As initially expected, results suggest that beetle activity increased and movement behavior changed with decreasing plant diversity. Sex and species also played a determining role in activity and movement patterns. Direct observations revealed patterns that indirect observations via pitfall trapping did not, furthering the on-going debate on the efficacy of pitfall trapping.

1. Effect of plant diversity

Decreasing plant species richness and functional diversity promoted beetle activity for direct observations. These results support the idea that, with decreasing plant diversity, beetles will be more active in order to search for food or disperse to a more favorable habitat. A loss of plant diversity has a bottom up effect through food webs and decreases associated arthropod species richness (Haddad et al., 2009). Thus, less prey is available for beetles, increasing their hunger. Fournier and Loreau (2001) found that recapture rates in pitfall traps were lower for starved individuals suggesting higher activity with higher hunger levels. Therefore, a decrease in plant diversity leads to higher activity rates, and consequently, beetles may disperse to better quality habitats (*i.e.* higher plant diversity). In addition to the direct observations of beetle activity, recapturing marked beetles in pitfall traps indirectly assessed activity. Time until recapture was not affected by plant diversity. This could be due to other factors in the plots such as varying vegetation cover or structure.

For the analysis of beetle traces, results suggest that with decreasing FD, step length increased; however, step length did not vary with change in PSR, and no other responses (length,

dislocation or number of turns) were explained by a variation in either PSR or FD. An increase in step length suggests more directed walking, whereas a decrease in step length suggests a ‘meandering’ pattern. Benhamou (2014) calls these ways of walking searching modes, which are dependent on scale. Since natural resources tend to be aggregated, individuals can either exemplify ‘ranging search mode’, looking for patches of higher density of resources on a larger scale or ‘area-concentrated search mode’, looking for resources in a highly dense patch at a small scale (Benhamou, 2014). Thus, a longer step length would exemplify ‘ranging search mode’ while a shorter step length would represent ‘area-concentrated search mode’. Since there is higher step length with decreasing functional diversity, my results suggest that beetles could be exemplifying a ‘ranging search mode’, with more directed walking in order to cover more ground. The aim of this movement could be to find patches with higher prey availability, or even disperse to better quality habitats with higher plant diversity. Interestingly, no difference was seen in step length looking at PSR. This could be explained by the difference between plant diversity measures of PSR and FD. PSR represents the number of plants in a plot, while FD represents niche space covered by plant communities. Thus, resource availability could differ based on the different type of plant diversity. Alternatively, perhaps vegetation structure differs more drastically along the FD gradient than the PSR gradient. With denser vegetation, beetles would need a higher number of turns to avoid plant structure, explaining lower step length.

Policy implications

These findings have direct implications for applications in agriculture. Previous authors have recommended undisturbed field margins and intercropping (Chapman et al., 1999). My results suggest that these recommendations must be coupled with plant diversity, with particular attention paid to functional diversity (FD). If a farmer is not providing adequate habitat in field

margins or intercropped areas, beetles may disperse in search of better habitat (*i.e.* more diverse), no longer live in field margins, and no longer prey on crop pests. By focusing on FD, farmers can assure that a variety of niche space is covered. Since plant species richness (PSR) and FD are highly correlated, an increase in FD will also increase PSR.

2. Effect of species and sex

For activity assessed via pitfall trap recapture, *P. melanarius* was more active than *H. rufipes*. This difference could be due to physiological factors, but could also be explained by size with *P. melanarius* being larger than *H. rufipes*. Larger species cover more ground and are therefore more likely to fall into a trap. This supports findings by Lang (2000), Spence and Niemelä (1994) and Hancock and Legg (2012), that larger species were overrepresented in pitfall trap sampling. For sex, males were more active than females. This result contradicts my expectations because I expected females to be more active due to their larger size and physiology. Being larger, females should be more likely to fall into pitfall traps. Additionally, females produce eggs and therefore need to have a higher fat content than males (Luff, 1975). Thus, they could need more nutrition and search for prey more actively than males. Broll et al. (2008) found that *Carabus auratus* male beetles had a larger daily home range than females. Therefore, perhaps females are more active than what the pitfall trap data reflects because they are less likely to fall into the pitfall traps with more small-scale movements compared to males.

Contrary to the recapture data, observed activity was not affected by species. *H. rufipes* males had higher odds of moving than females while no significant difference explained by sex was observed for *P. melanarius*. Difference in breeding periods could account for this high difference in activity rates. *H. rufipes* breeds in the summer as well as autumn while *P. melanarius* breeds only in autumn (Luff, 1975). Since I sampled in the summer during *H.*

rufipes's breeding period, males' activity could be higher if male beetles are searching for females. The two observation methods' different temporal scales could also explain a higher activity for *H. rufipes* males. While a beetle was only observed for a minute, pitfall traps assessed activity for a whole twenty-four hour period. Also, the time interval of a few hours after sunset in which the observations were conducted could potentially bias the results if activity periods are sex-specific. Furthermore, the inclination for dispersal may be an additional factor affecting movement; however, dispersal processes are poorly understood (Clobert et al., 2008, Mabry et al., 2012). Moreover, in dispersal linked to habitat selection, leaving an area and settling in an area depend on phenotype (including sex) but also individual preference (Clobert et al., 2008; Stamps, 2006).

3. Methodology comparisons

Overall, different methods of observation identified different patterns in movement and activity. With recapture-based activity assessment, plant diversity explained no variation in recapture time. On the contrary, from direct observations, plant diversity and proportion of moving beetles were negatively correlated. Similarly to recapture data, movement traces via direct observation showed no effects of plant diversity on response variables except step length, which decreased with higher functional diversity. This is not what I expected; with increasing activity, longer path lengths are anticipated.

Using different observation techniques to observe differences between sex and species also resulted in disagreeing results. For recapture-based activity assessment, difference was found in both species and between sexes in each species. For activity assessed via direct observations, no overall sex and species differences were detected, only a difference in activity for sex in *H. rufipes*. Movement traces via direct observation resulted in a difference in path

length for sex, but no difference in species was observed. Specifically, *P. melanarius* males had longer path lengths than females, but a smaller proportion of males were observed active than females.

These discrepancies could be due to the fact that recapture-based and direct observation methods collect information from different spatial-temporal scales. Recapture through pitfall trapping gathers information from a whole plot over a longer period of time. On the other hand, direct observation gives a detailed, narrow view of beetle movement over a short time period. Observed activity also gives information about all of the beetles seen, thus I also know which beetles are inactive. This is not the case for recapture based activity measurements, where beetles could be missing from the plot for various reasons (inactivity, death, escape).

4. Future directions

Moving forward with this research, it would be interesting to take advantage of advancements in individual based tracking techniques such as automated video or harmonic radar tracking to observe individual movement patterns of ground beetles. This would allow for simultaneous sampling of all individuals, removing varying environmental factors for each observation as I had in human observation. I could also observe movement behavior over a larger time scale, which could help determine if activity is species or sex-biased at different times of day. Additionally, I would no longer need to exclude plots since observation ability would no longer be a problem. The whole plot could be used (3.5 m x 3.5 m) instead of a strip (1 m x 3.5 m), increasing the total area beetles can move. Also, beetles would be individually identifiable at all times. Movement patterns could then be paired with recapture times enabling a comparison between movement pattern and pitfall trap data. However, individual based tracking techniques

can be expensive. More affordable options could include comparing hand drawings to movement paths recorded via video tracking to assess accuracy of hand drawings and developing a technique allowing for individual identifications of beetles at night.

As this study was part of a larger biodiversity experiment, there will be large quantities of metadata available from other projects and researchers, which can be incorporated as explanatory variables in different models. Temperature has been positively correlated with activity rates; therefore, data on weather conditions such as humidity and temperature can help explain variation in activity and movement (Brunsting 1982; Hurlbert et al., 2008). Also, speculation about hunger as a driver of movement will be diminished with information on richness and abundance of available prey in specific plots. Finally, available vegetation data will include cover, leaf area index, biomass and vertical structure, further isolating the specific components affecting beetle movement.

To further disentangle the effects of vegetation structure and food availability, researchers at the Georg-August University in Göttingen, Germany are conducting a follow up study to my experiment. They are looking at effects of varying vegetation structure on movement patterns of ground beetles in the context of a mesocosm experiment with *H. rufipes*. Habitat with higher biomass and plant diversity may provide more prey items for carabids while also providing increased shelter for prey. Increased plant density may also indirectly affect beetle movement by providing shelter from beetle predators. This study could further knowledge of beetle dispersal and help interpret data on pitfall traps and predation events since hunger level and habitat structure are main drivers of variation in beetle movement patterns.

Acknowledgements

Many thanks to my committee members, Dr. Michael Breed, Dr. Pieter Johnson and Dr. Peter Newton for guidance, advice and valuable feedback throughout this process. Sincere appreciation goes to Dr. Pieter Johnson and Philip Pendergast for statistical advice. Special gratitude is extended to Julia Tiede for her mentorship, support, advice and friendship and Dr. Barbara Deming-Adams who encouraged me to take on the challenge of writing an Honors Thesis. Much appreciation is expressed to Andrea Feldmen and peer reviewers from her writing class as well as consultants from the CU Writing Center. Also, I am thankful for all my co-workers at the Jena Experiment and Dr. Deane Bowers, who advised me during the field work portion of this study. Finally, thanks to the Undergraduate Research Opportunity Program and the Georg-August University of Göttingen for funding.

References

- Allema, B., van der Werf, W., van Lenteren, J. C., Hemerik, L., & Rossing, W. A. (2014). Movement behaviour of the carabid beetle *Pterostichus melanarius* in crops and at a habitat interface explains patterns of population redistribution in the field. *PloS One*, 9(12), e115751.
- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, 49(3), 227–267.
- Basedow, T., & Stork, N. (1990). Effects of insecticides on Carabidae and the significance of these for agriculture and species number. *The role of ground beetles in ecological and environmental studies*, 115-125.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4.
- Benhamou, S. (2014). Of scales and stationarity in animal movements. *Ecology Letters*, 17(3), 261–272.
- Bianchi, F. J. J. A., Booij, C. J. H., & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1595), 1715-1727.
- Broll, A., Reike, H. P., & Mau, M. (2008). Pattern of activity of *Carabus auratus* (Coleoptera, Carabidae) in a grassland site – a harmonic radar study. *Faunistische Abhandlungen*, 26, 21-35.
- Brose, U. (2003). Bottom-up control of carabid beetle communities in early successional wetlands: mediated by vegetation structure or plant diversity? *Oecologia*, 135(3), 407–413.
- Brunsting, A. M. H. (1982). The locomotor activity of *Pterostichus oblongopuncta* Tus F.(Col., Carabidae). *Netherlands journal of zoology*, 33(2), 189-210.
- Buchholz, S., Jess, A. M., Hertenstein, F., & Schirmel, J. (2010). Effect of the colour of pitfall traps on their capture efficiency of carabid beetles (Coleoptera: Carabidae), spiders (Araneae) and other arthropods. *European Journal of Entomology*, 107(2), 277–280.
- Chapman, P. A., Armstrong, G., & McKinlay, R. G. (1999). Daily movements of *Pterostichus melanarius* between areas of contrasting vegetation density within crops. *Entomologia Experimentalis et Applicata*, 91(3), 479–482.
- Clobert, J., Le Galliard, J. F., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, 12(3), 197–209.

- Den Boer, P. J. (1990). Density limits and survival of local populations in 64 carabid species with different powers of dispersal. *Journal of Evolutionary Biology*, 3(1-2), 19–48.
- Draper, N. R., & Smith, H. (1998). Fitting a straight line by least squares. *Applied Regression Analysis, Third Edition*, 15–46.
- Ebeling, A., Pompe, S., Baade, J., Eisenhauer, N., Hillebrand, H., Proulx, R., ... Weisser, W. W. (2014). A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships. *Basic and Applied Ecology*, 15(3), 229–240.
- Fournier, E., & Loreau, M. (2002). Foraging activity of the carabid beetle *Pterostichus melanarius* Ill. in field margin habitats. *Agriculture, Ecosystems & Environment*, 89(3), 253–259.
- Greenslade, P. J. M. (1964). Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). *Journal of Animal Ecology*, 33(2), 301–310.
- Haddad, N. M., Crutsinger, G. M., Gross, K., Haarstad, J., Knops, J. M. H., & Tilman, D. (2009). Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters*, 12(10), 1029–1039.
- Hancock, M. H., & Legg, C. J. (2012). Pitfall trapping bias and arthropod body mass. *Insect Conservation and Diversity*, 5(4), 312–318.
- Hurlbert, A. H., Ballantyne, F., & Powell, S. (2008). Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecological Entomology*, 33(1), 144–154.
- Jeltsch, F., Bonte, D., Pe'er, G., Reineking, B., Leimgruber, P., Balkenhol, N., ... Bauer, S. (2013). Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. *Movement Ecology*, 1(1), 6.
- Jopp, F., & Reuter, H. (2005). Dispersal of carabid beetles—emergence of distribution patterns. *Ecological Modelling*, 186(4), 389–405.
- Knapp, M., & Ruzicka, J. (2012). The effect of pitfall trap construction and preservative on catch size, species richness and species composition of ground beetles (Coleoptera: Carabidae). *European Journal of Entomology*, 109(3), 419–426.
- Lang, A. (2000). The pitfalls of pitfalls: a comparison of pitfall trap catches and absolute density estimates of epigeal invertebrate predators in arable land. *Anzeiger Für Schädlingskunde = Journal of Pest Science*, 73(4), 99–106.
- Lemen, C. A., & Freeman, P. W. (1985). Tracking mammals with fluorescent pigments: a new technique. *Journal of Mammalogy*, 66(1), 134–136.
- Lövei, G. L., & Sunderland, K. D. (1996). Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology*, 41(1), 231–256.

- Luff, M. L. (1975). Some features influencing the efficiency of pitfall traps. *Oecologia*, 19(4), 345–357.
- Luff, M.L. (2007). *Handbooks for the identification of British insects*. St. Albans: Royal Entomological Society.
- Mabry, K. E., Shelley, E. L., Davis, K. E., Blumstein, D. T., & Van Vuren, D. H. (2013). Social mating system and sex-biased dispersal in mammals and birds: a phylogenetic analysis. *PLoS One*, 8(3), e57980.
- Melbourne, B. A. (1999). Bias in the effect of habitat structure on pitfall traps: an experimental evaluation. *Australian Journal of Ecology*, 24(3), 228–239.
- Niemelä, J., Haila, Y., Halme, E., Lahti, T., Pajunen, T., & Punttila, P. (1988). The distribution of carabid beetles in fragments of old coniferous taiga and adjacent managed forest. In *Annales Zoologici Fennici*, (pp. 107–119). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.
- Noldus, L. P. J. J., Spink, A. J., & Tegelenbosch, R. A. J. (2002). Computerised video tracking, movement analysis and behaviour recognition in insects. *Computers and Electronics in Agriculture*, 35(2–3), 201–227.
- Rainio, J., & Niemelä, J. (2003). Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity & Conservation*, 12(3), 487–506.
- Riecken, U., & Rath, U. (1996). Use of radio telemetry for studying dispersal and habitat use of *Carabus coriaceus* L. In *Annales Zoologici Fennici* (pp. 109–116). Finnish Zoological and Botanical Publishing Board.
- Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., ... Schulze, E. D. (2004). The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, 5(2), 107–121.
- Scherber, C., Eisenhauer, N., Weisser, W. W., Schmid, B., Voigt, W., Fischer, M., ... Tscharrntke, T. (2010). Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature*, 468(7323), 553–556.
- Schirmel, J., Lenze, S., Katzmann, D., & Buchholz, S. (2010). Capture efficiency of pitfall traps is highly affected by sampling interval. *Entomologia Experimentalis et Applicata*, 136(2), 206–210.
- Spence, J. R., & Niemelä, J. K. (1994). Sampling carabid assemblages with pitfall traps: the madness and the method. *The Canadian Entomologist*, 126(03), 881–894.
- Stamps, J. A. (2006). The silver spoon effect and habitat selection by natal dispersers. *Ecology Letters*, 9(11), 1179–1185.

- Yi, Z., Jinchao, F., Dayuan, X., Weiguo, S., & Axmacher, J. C. (2012). A comparison of terrestrial arthropod sampling methods. *Journal of Resources and Ecology*, 3(2), 174-182.