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Cover Photograph: Armadillidium vulgare with brood in her marsupium, from forests of Eastern University, Saint Davids, PA.

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Tracking Ground Arthropod Diversity in Urban Forests: Lessons Learned From a New Undergraduate Long-Term Project

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Abstract - The greater Philadelphia area contains a mosaic of forest fragments that can be used to track the health of urban ecosystems in educational settings. Here, we used ground and leaf litter dwelling arthropods as ecological indicators to assess the diversity of leaf litter communities in an old growth forest and a secondary regrowth forest. In this preliminary study that we conducted with a small team of undergraduate students, we found that the old growth forest samples contained more abundant and diverse arthropods than the secondary growth samples. We assert that increased arthropod diversity in the old growth forest is due to age and density of the foliage of the canopy. Conversely, the reduced diversity of arthropods in the secondary regrowth forest is likely due to clearing of the site almost 50 years ago, which have allowed early successional tree species to form monocultures that house fewer arthropod taxa. While our study shows that ground and leaf litter arthropod diversity in forest fragments in St. Davids, Pennsylvania can be sensitive to human disturbance, we need to improve the timing, frequency, spatial scale, and taxonomic resolution of our sampling. In this brief communication, we reflect on our initial results, and describe future directions for this long-term ecological research project that we designed for our undergraduate biology courses.

Introduction

Human activity puts pressure on natural areas within urban ecosystems. Some of these activities are severe enough to disturb intact natural habitats and change communities (Alberti and Marzluff 2004). On the other hand, some habitats in urban areas are intentionally protected from disturbance from human activities, so that their composition, ecological processes, and ecosystem services can be preserved or restored. It is therefore necessary to examine ecological components of these altered habitats to understand composition, function, and value of these changing urban landscapes. Specifically, taxonomic abundance and biodiversity of guilds can be tracked and compared so the status of habitats with different histories of human-mediated disturbance can be assessed (Vačkář et al. 2012). One common type of human-mediated disturbance in urban forests is land-use change, such as clearing, which consequently results in secondary succession that can affect forest ecosystems via trophic downgrading (Nytch et al. 2023). Specifically, removal of trees, grading, or sediment deposition can alter understory communities, which can be true across trophic levels (Woodcock et al. 2013). In previously cleared areas that are undergoing passive restoration, monitoring different components of the forest ecosystem can assess changes in ecosystem health of the habitat.

One method of tracking health of disturbed forests is by monitoring abundance and diversity of ecological indicators, such as ground-dwelling arthropods (Menta and Remelli 2020). Arthropod populations and communities can be particularly sensitive to changing

[1] Biology Department, Eastern University, 308 McInnis Hall, 1300 Eagle Road, St. Davids, PA 19087-3696. [*]Corresponding author: brian.alfaro@eastern.edu, (610) 225-5564

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landscape conditions, such as those in urban ecosystems (Kotze et al. 2020). While some arthropod taxa have advantageous innovations for urban environments, other groups decline in urbanized areas due to loss of natural habitats. In many cases, this can directly affect the abundance and distribution of organisms that are important for maintaining soil health (Parker et al. 2023). To monitor the local effects of landscape change to an ecosystem or community, an ongoing data set that describe arthropod communities can be used as ecological indicators (Carvalho et al. 2020).

Here, we describe an ongoing study designed to train undergraduates to conduct field work to track the effect of land-use change on arthropods in the understory of a mixed-deciduous forest in the suburban areas of the greater Philadelphia area (PA, USA). Specifically, we compared ground-dwelling arthropods in deciduous forest leaf litter between a minimally disturbed, old growth forest, and a nearby secondary succession forest, recovering from a major disturbance. Our field team of undergraduate students conducted this study with the following questions:

1) Can we detect enough variability in taxonomic diversity and composition of arthropods within and between 2 sets of 50 m transect lines in an old growth forest versus a secondary regrowth forest? Addressing this question, even in a small pilot study, is critical for two reasons. First, we intend to standardize an arthropod sampling protocol for undergraduate students for educational training in field ecology, biostatistics, and natural history. Second, we need to identify a minimum sampling unit for long-term monitoring of arthropod composition and habitat change in our study areas on campus. Quantifying and describing the frequency distribution of abundance and diversity estimates even in a limited survey will be important information for scaling the sampling effort in future field seasons.

2) Because our 2 sites are near each other, there is the possibility that the 2 forest floors share some of the same types of arthropods. We therefore asked: will our old growth and secondary growth sampling sites have contrasting or similar composition and diversity of ground-dwelling arthropods? We expected that the leaf litter from the old growth forest would have higher taxonomic diversity for ground-dwelling arthropods than the leaf litter from the forest floor of the young, secondary forest.

Materials and Methods

To answer our questions, we quantified and described the abundance and diversity of ground-dwelling arthropods in the understory leaf litter of 2 adjacent, mixed deciduous forest stands in greater Philadelphia, Pennsylvania (USA).

Study area

We sampled ground-dwelling arthropods in forest leaf litter among 2 sites in St. Davids (Delaware County, Pennsylvania), which is in the Delaware Valley, in late fall (November) of 2022. The 2 forest stands are within a 226 ha area that were naturally eastern deciduous forests. Our campus and study areas are within the administrative boundaries of metropolitan Philadelphia, and 24 km from the downtown financial district. Once occupied by the Lenape peoples, the Delaware Valley have been logged and developed by European settlers since the 1600s, and have been growing in population since the establishment of Philadelphia as a city. Still, there are old growth and secondary forest fragments that are intact in the valley. This area receives 1052 mm of rain per year, with an average of 121 days of rainfall annually. In the last 10 years, Saint Davids has had an average minimum temperature of -3°C (in January) and an average maximum temperature of 30°C (in July).

One sampling site, situated in Cabrini University (40.0552° N, 75.3740° W), is adjacent to a campus trail, and has a mature forest canopy. This old growth site (10 ha) is proximate to a 2-lane road (Eagle Road). An ongoing tree survey started in 1974 in the old growth stand has recorded more than 30 tree species that include Liriodendron spp. (Tulip Poplar), *Fagus grandifolia* Ehrh. (American Beech), *Acer rubrum* L. (Red Maple), and several common oak species: *Quercus velutina* Lam. (Black Oak), *Quercus rubra* L. (Northern Red Oak), and *Quercus alba* L (White Oak). Others less common trees in this site were *Carya glabra* Miller (Hickory), *Cornus sanguinea* L. (Common Dogwood), Betula spp. (Birch), *Prunus serotina* Ehrh. (American black cherry), *Nyssa sylvatica* Marshall (Tupelo/Sourgum), *Acer negundo* L. (Box Elder), *Quercus montana* Willd. (Chestnut Oak), and a naturalized Japanese tree, *Cercidiphyllum japonicum* Siebold & Zucc. (Katsura). The old growth stand also contains a small population of Rhododendron maximum L. in the understory.

The second area, located in Eastern University (40.0512° N, 75.3713° W), is a disturbed secondary habitat (3 ha) that was cleared and then covered in sediment deposit from dredged lakebed material in 1974 (Fig. 1). The disturbed forest area is part of an ongoing long-term study on northeastern mixed-deciduous forest succession (Fig. 1). The young forest contained Box Elder and *Juglans nigra* L. (Black Walnut) in 1992, but over time the Box Elders were increasingly crowded out by *Salix nigra* marshall (Black Willow). In the last decade, Red Maple has sprouted in the site, as well as hickory seedlings that likely were dispersed by small mammals. *Viburnum plicatum* Thunb. (Japanese Snowball Bush) from temperate Eurasia, an ornamental shrub in the U.S., is also currently present in the disturbed stand. The secondary forest stand is primarily composed of trees that are less than 50 years old.

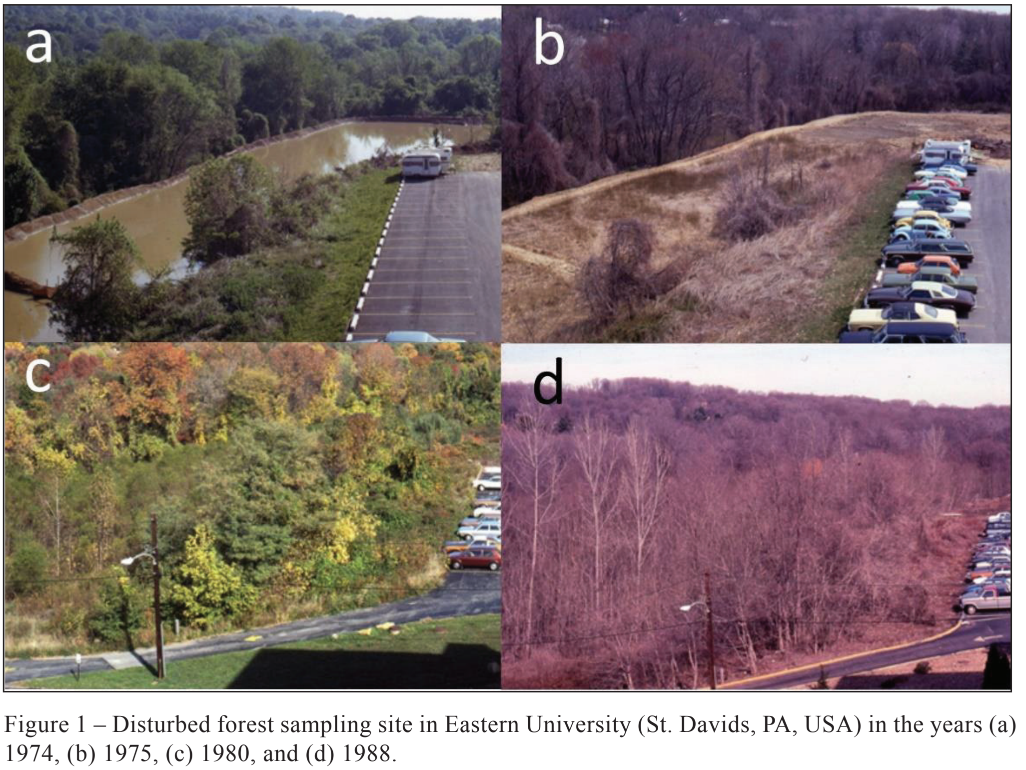

Figure 1 – Disturbed forest sampling site in Eastern University (St. Davids, PA, USA) in the years (a) 1974, (b) 1975, (c) 1980, and (d) 1988.

Sampling and arthropod processing

In each site, we delineated a 50 m transect, and then sampled from 10–13 random locations per transect (*n* = 23); we collected leaf litter with a minimum distance of 5 m between samples. We bagged approximately 2 L in volume per sample of leaf litter that was within the boundaries of a 0.25 m^2 quadrat, and stored this sample in a dark cabinet until the arthropods were collected. To collect ground-dwelling arthropods from each leaf litter sample, we implemented the Berlese funnel method. To set up the collection, we placed funnels with a 4–6 mm mesh hardware cloth disc into mason jars that contained soapy water. We put the funnel setup under fluorescent lights for 48 hours, and then stored the arthropod specimens in 75% ethanol until processing. To sort and identify the sampled arthropods, we used recognizable taxonomic units so that the undergraduate students can rapidly assess the specimens. Recognizable taxonomic units, sometimes called RTUs, are categories that can be used if technicians are expected to receive a day or less of training in taxonomic sorting and species identification (Oliver and Beattie 1993). The major recognizable taxonomic units we monitored were Coleoptera (beetles), Formicidae (ants), Oniscidea (terrestrial isopods – designated Isopoda for this study), Oribatida (orbatid mites), Collembola (springtails), and Chilopoda (centipedes). This sampling effort was part of Eastern University's Biology 309L (Ecology Lab) course in the Biology Department, wherein one series of modules trained undergraduate students on ground arthropod sampling and identification.

Analysis

We counted the number of individuals per recognizable taxonomic unit for each sample point for each site so we can quantitatively survey and compare the taxa present in both sites. We know that there was a possibility of non-random sampling because of the limited sampling areas, so we used the Brillouin diversity index (Boyle et al. 1990) instead of Shannon-Weaver index. To quantify how dissimilar the composition of the sampling points and the 2 sites were, we measured Bray-Curtis dissimilarity between sampling points tallied in old growth and secondary growth sampling points (Ricotta and Podani 2017). We used the package *vegan* in the R statistical software (Oksanen et al. 2023, R Core Team 2023) to calculate the Brillouin *H'* and Bray-Curtis dissimilarity values for each sampling point. To analyze variability of recognizable taxonomic unit diversity among the 2 sampling sites, we tested possible differences in mean taxonomic diversity of ground arthropods between the old growth and secondary regrowth sampling sites via Welch's t-test for *H'* (alpha diversity) and for Bray-Curtis dissimilarity (beta diversity).

Results

The forest floor in the old growth stand had the most arthropod specimens (396) compared to the secondary regrowth stand (133). We did not find any Coleoptera in the secondary regrowth stand, but beetles were abundant in the old growth forest (Table 1). With respect to recognizable taxonomic units, the old growth forest floor was more diverse than the secondary regrowth forest; this result approached significance (*d.f.* = 16, $p = 0.09$, Figure 2a). Ground arthropod composition among sampling points in the old growth forest were more dissimilar than sampling points in the secondary regrowth site, this result is not statistically significant (*d.f.* = 17, $p = 0.25$, Figure 2b).

Table 1 – Mean (standard deviation) of individuals counted per sample for each recognizable taxonomic unit for ground-dwelling arthropods in old growth (396 specimens, n = 13 sampling points) and secondary regrowth (133 specimens, n = 10 sampling points) forest sites, St. Davids, PA, (USA). Araneae (1 specimen) not shown.

Recognizable taxonomic unit	Old growth forest	Secondary growth forest
Coleoptera	6 (7.3)	0 (0)
Acari	7 (8.2)	5 (3.8)
Formicidae	2 (2.0)	2 (3.7)
Collembola	9 (12.0)	6 (4.6)
Isopoda	4 (5.9)	1 (0.9)
Chilopoda	1 (0.3)	1 (0.3)

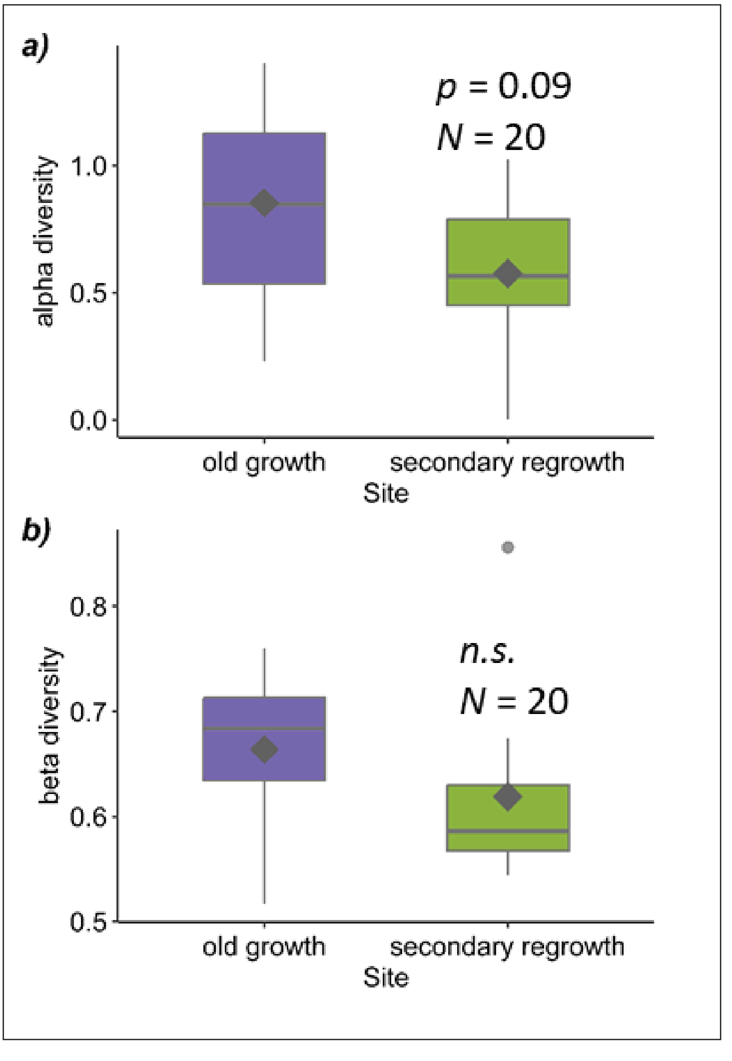

Figure 2 – Box plots for (a) alpha diversity of old growth (purple, n = 10 sampling points) and secondary regrowth (green, n = 10 sampling points) forest floors in St. Davids, PA, (USA). Box plots for (b) beta diversity of old growth (purple, n = 10 sampling points) and secondary regrowth (green, n = 10 sampling points) forest floors in St. Davids, PA, (USA).

Discussion

After analyzing our initial data, we recognize that our sampling strategy needs to be modified with respect to scale, timing, and taxonomic resolution. Further, we reframed our initial three questions into one new question that we can test in the future: Will differences in habitat and environmental features in disturbed versus intact forest floors translate into differentiation of arthropod abundance and diversity? To test this in the future, we sought to perform ongoing and expanded sampling efforts, based on our initial approach and new recommendations, to confirm if potential patterns are consistent or changing over time. We outline lessons we learned, and future directions we take, for this long-term study.

Even though our sampling points were limited to 2 transects, we detected enough variability in taxonomic diversity of ground arthropods in our old growth and secondary regrowth forest sampling sites. To answer our first question, we can use the methods and sampling design in this study as a standardized arthropod sampling protocol for undergraduate students for educational training in field ecology, biostatistics, and natural history. We can use abundance and diversity estimates of ground arthropods to describe forest floor fauna in future field seasons. Still, we can improve the resolution of our diversity and abundance estimates by increasing the taxonomic resolution of our arthropod specimens.

While we found variation in arthropod abundance and diversity with our limited sampling size, we realized that our original method is inefficient. Instead of collecting a volume of 2 L of leaf litter, we can sample from a 25 x 25 cm area to standardize our sample collection. This standardized collection method will yield absolute density of ground arthropods (individuals/m^2) that can be comparable to other studies. Furthermore, we intend to sample more plots from each forest type for replication. This can potentially provide multiple samples per student that each one can analyze for each semester. Compared to studies that include sampling in warmer periods of the year (e.g., Blair et al. 1994, Myers and Marshal 2021), we did not collect a lot of individuals, and it is likely that the relative abundance of some taxa were misrepresented. We therefore cannot make conclusions about community structure with our current data. The sensible approach in the future is to sample in warmer months when more arthropods will be active.

In future sampling, we intend to target potential indicator species. Specifically, we can use litter dwelling spiders and ground spiders as potential indicator species for forest leaf litter community, as there is strong support in the literature for long term studies using spiders as indicators of ecosystem health (Argañaraz et al. 2020). While one of our principal investigators work mostly in spider and scorpion identifications, we have connections to research museums (i.e. University of New Mexico and California Academy of Sciences) that could help with potential identification questions. We can also further refine this study by including sensitive species, such as beetles and ants (Carvalho et al. 2020). Our taxonomist for the project is also developing a non-insect Arthropods course for future academic years, which will improve the ability of potential students in sample identification. Having an arthropods field and lab course would also enable us to have quarterly sampling with students who are better versed in taxa of interest. Our department is also in the early stages of developing a reference collection for research and teaching. Nonetheless, we recognize that this is a baseline study to establish proof of concept. Our future plan is to take all arthropod specimens to family level and focus on the indicator taxa above to genus or species level.

We found that isopods were abundant within our sampling points in the old growth forest, but the authors and their students have seen them in the secondary growth forest during warm periods. While isopods are not our focal species, it would be a relevant research project for a senior thesis student or summer research student to explore whether isopods are beneficial or not to the habitats in question. The main author is an invasion biologist, and there are intriguing questions outside the scope of this project that we can answer with isopods. For example, we can examine if potential variation body size of non-native isopods in disturbed versus intact forests are due to differences in forest ground arthropod diversity and abundance, and habitat characteristics.

While we detected mites in all of the sites, we know that this taxon will be the most difficult for us to identify to the family level without extensive reference to a dichotomous key (i.e. Dindal 1991). While we do have contact with mite specialists who could assist with specimen identification, we have developed eDNA and specimen DNA extraction and PCR protocols that can work on field-collected mite samples (A. Martinez, Eastern University, Saint Davids, PA, unpubl. data; M. Weeks, Eastern University, Saint Davids, PA, unpubl. data). In addition to microscopy, we can identify mites to genus and species using the ITS2 rDNA barcoding gene (Ben-David et al. 2007). We can potentially refine this study by standardizing and scaling up our sampling protocols to enable us to have a long-term dataset tracking the impacts of urbanization and development on arthropod soil/litter taxa.

To answer our second question, the old growth and secondary growth sampling sites have contrasting abundance and diversity of ground-dwelling arthropods. The sampled leaf litter from the old growth understory contained more arthropods than the disturbed secondary forest. Overall, the old growth forest differed in arthropod abundance and diversity from the secondary growth forest, and this could be due to the differences in site conditions of the 2 forest floors. However, we collected leaf litter in November, when daily temperatures have started to drop to freezing conditions, which could be a reason why we found fewer arthropods in the secondary regrowth forest and the old growth forest (Fitzgerald et al. 2021). As we mentioned, we will launch a new arthropods field and lab course that would allow us to sample forest sites quarterly with trained students. This would let us collect a better representation of arthropod abundance and diversity by including field work in the warmer periods of the year (late summer/early fall).

In this study, we did not differentiate between trophic levels in assessing species diversity, and there are possible issues with this approach. Specifically, there can be sampling artefact from chilopods vs. mites, as mites are typically higher in abundance than centipedes in a given area (e.g., Napierała et al. 2015). In future work, with taxonomic refining to genus or species level, we plan to have more statistically sound and informative groupings of taxa and useful species diversity indices. We can reduce this artefact by performing rarefaction in our entire study area, and by using corrected richness and diversity indices, such as Chao1 (Chao and Chiu 2016) and the Brillouin index that we used in this study. For comparative analysis among sites, we can perform nonmetric multidimensional scaling analysis and PERMANOVA in the future (e.g., Argañaraz et al. 2020). We may use the Ecology class to gross sort the samples, and then the Entomology or non-insect Arthropods class to take the specimens down to lower taxonomic levels. Our summer researchers and thesis students can lead data extraction from field and lab sheets, data management, and statistical analysis of both arthropod, environmental, and leaf litter data.

One reason that the secondary regrowth understory is less diverse in arthropods is because the site is still recovering from the forest clearing of 1974. That is, the disturbed site likely does not have enough plant biomass and detritus on the forest floor to support a more diverse arthropod community (Schaffers et al. 2008). The secondary regrowth site currently contains mostly black willow and box elder. This near monoculture of the secondary forest site can be a factor in reduced ground arthropod diversity, as there is evidence that plant diversity can promote arthropod diversity (Dinnage et al. 2012). Nonetheless, we interpret this as 2 forest understories potentially having enough differences in forest composition to form differentiated arthropod composition between the 2 sites.

In previous field courses, students at Eastern University have observed distinct soil characteristics in the old growth versus regrowth forest sites. Old growth soils are shallow with well-defined horizons due to metamorphic rock parent material and nearby bedrock. In contrast, regrowth forest soils are deeper and potentially enriched by well-aged goose feces deposited from initial dredged material. The soil in the regrowth forest lack clear horizons in the deeper layer. These differences in soil characteristics, when combined with forest age and vegetation composition, may have resulted in enough habitat differences to affect ground-arthropod composition (Melliger et al. 2018).

While the studies that describe changes in soil biota after deposition of lakebed dredging are lacking, there are studies that describe community response of ground-dwelling arthropods to changes in forest composition and clearing practices. For example, deforestation can lead to a decrease in Coleoptera compared to natural forests (Gunnarsson et al. 2004, Wang et al. 2020), but this can be complicated by timing of sampling and habitat type. We saw differences in abundance and diversity of arthropods between the old growth and secondary growth forest sites, and there is potential for this pattern to be consistent in similar forest types in our study area. However, in addition to sampling size, one limitation of our current methods is that we cannot make direct associations of leaf litter or forest floor characteristics to arthropods. This is because we did not measure variables that describe forest leaf litter characteristics to be able to quantify forest floor differences, and possible association of forest floor features with ground arthropod diversity. Therefore, to explain possible differences among the 2 types of forest communities, we intend to include data collection of habitat variables. In particular, we intend to collect soil samples in each sampling point, and then analyze soil nutrient content for each sample. We can collect leaf litter data, such as litter depth, dry leaf mass of leaf litter collected, and species richness/diversity of leaf litter sample to explicitly describe and quantify leaf litter. We also intend to include soil variables, in particular soil temperature, soil moisture, and nutrient content, as well as weather data (amount of precipitation before sampling, and ambient temperature), that can affect our forest sampling sites.

In the years to come, local governments of urban areas can potentially mandate long-term biodiversity studies to monitor ecosystem health (Rall et al. 2015). Consequently, cities will need workers that can analyze the ever-increasing local and global biodiversity data sets. It is therefore important to establish long-term ecological research plots in urban areas at different scales for biodiversity sampling. Having long-term monitoring plots can allow tracking of ecosystem health of fragmented habitats interspersed in cities and suburban areas over time and space (Fa and Luiselli 2023). Additionally, the next generation of scientists and professionals can use these sites for hands-on training in field, lab, and data work for science-driven urban land management.

By conducting this potentially long-term study, we showed that our campus at Eastern University in St. Davids, PA (USA) has the potential to serve as a local observatory for natural heritage data to track how eastern mixed-deciduous forests behave in urban regions. More importantly, subsequent annual sampling efforts can benefit students, as this can provide a training opportunity for learning field sampling design, forest ecology, arthropod identification, and ecological data analysis.

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