TOS SCIENCE DESIGN FOR GROUND BEETLE ABUNDANCE AND DIVERSITY

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	10/10/2014	ECO-02008	Initial release
B	06/14/2018	ECO-05584	• Minor format and text edits • Corrected protocol names in reference documents, • Updated NEON TOS site numbers, • Added text from Ecosphere publication (Opportunities for Researchers section); D Hoekman, KE LeVan, et al. 2017. Design for Ground Beetle Abundance and Diversity Sampling within the National Ecological Observatory Network. Ecosphere. 8(4):e01744. DOI: 10.1002/ecs2.1744 • Updated DNA barcoding section to include project names, site-specific deviations from the design • update sampling schedule for beetles (MODIS)
C	04/06/2022	ECO-06790	• Revised logo • Update to reflect change in terminology from relocatable to gradient sites.

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1 DESCRIPTION

1.1 Purpose

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON subsystem components, linking NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. NEON strives to make measurements in ways that enable continental-scale science to address the Grand Challenges. Design Documents flow from questions and goals defined in the NEON Science Strategy document, and inform the more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and Calibration/Validation (CalVal) and maintenance plans.

1.2 Scope

This document defines the rationale and requirements for ground beetle abundance and diversity sampling in the NEON Science Design.

1.3 Acknowledgments

The design was reviewed by and refined with input from a technical working group consisting of researchers with relevant expertise. The ground beetle technical working group includes George Ball, Robert A. Browne, Robert Davidson, Terry Erwin, James LaBonte, Karen Ober, Barry Knisley, Jonathan Lundgren, David Maddison, Wendy Moore, Jari Niemelä, David Pearson, John Spence, Kip Will and Timothy Work. Tanya Chesney also contributed to this document.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD[01]	NEON.DOC.000001	NEON Observatory Design
AD[02]	NEON.DOC.000913	TOS Science Design for Spatial Sampling
AD[03]	NEON.DOC.004312	NEON Research Coordination Guidelines

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling

2.3 External References

N/A

2.4 Acronyms

All acronyms used in this document are defined in RD[01].

3 INTRODUCTION

3.1 Overview of the Observatory

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform for understanding and forecasting the impacts of climate change, land use change, and invasive species on ecology. NEON is designed to enable users, including scientists, planners and policy makers, educators, and the general public, to address the major areas in environmental sciences, known as the Grand Challenges (**Figure 1**). NEON infrastructure and data products are strategically aimed at those aspects of the Grand Challenges for which a coordinated national program of standardized observations and experiments is particularly effective. The open access approach to the Observatory's data and information products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth and understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01] and AD[02].

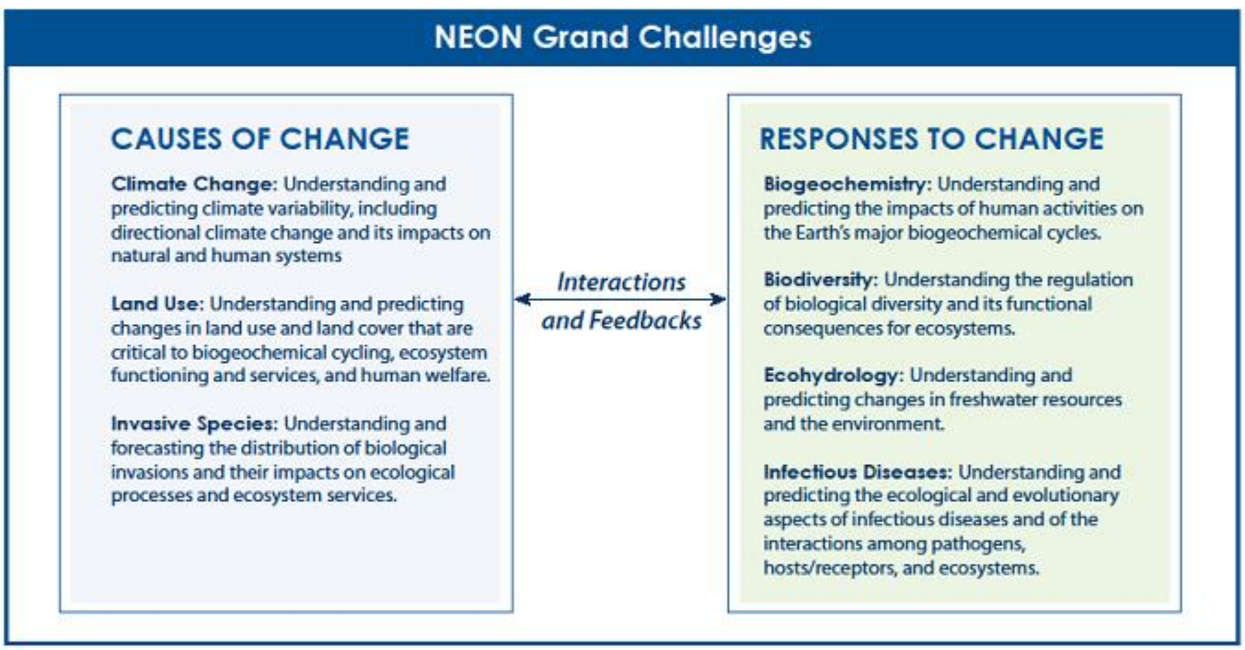

Figure 1. The seven Grand Challenges defined by the National Research Council (Committee on Grand Challenges in Environmental Sciences 2001).

3.2 Components of the Observatory

There are five components of the Observatory: the Airborne Observation Platform (AOP), Terrestrial Instrument System (TIS), Aquatic Observation System (AOS), Aquatic Instrument System (AIS), and Terrestrial Observation System (TOS). Collocation of measurements associated with each of these components will allow for linkage and comparison of data products. For example, remote sensing data provided by the Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. (2008) and Schimel et al. (2011).

3.3 The Terrestrial Observation System (TOS)

The NEON TOS will quantify the impacts of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa), infectious disease, soil, and nutrient fluxes across system interfaces (air, land, and water) (AD[01], AD[02]). The sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Plot and organism-scale measurements will also be coordinated with the larger-scale airborne measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.neonscience.org).

The standardization of protocols across all sites is key to the success of NEON (and its novelty) and must be maintained at all sites through time. Thus, although specific techniques may be required at some sites (e.g., due to different vegetation types), protocols have been developed to ensure data comparability. These details can also be found in individual design documents available through the NEON website (www.neonscience.org).

The TOS Science Designs define the scientific strategies leading to high-level sampling designs for NEON sentinel taxa, terrestrial biogeochemistry, and infectious disease, linking NEON Grand Challenges and science questions to specific measurements. The TOS Spatial Sampling Design document describes the sampling design that collocates observations of the components of the TOS (AD[02]). TOS Science Design documents were developed following input from the scientific community, including module-specific Technical Working Groups, and the National Science Foundation. Science Designs will be reviewed periodically to ensure that the data collected by NEON are those best suited to meet the requirements of the observatory (AD[01]), are (to the extent possible) consistent with standards used by the scientific community, and fit within the scope of NEON.

4 INTRODUCTION TO THE GROUND BEETLE ABUNDANCE AND DIVERSITY SAMPLING DESIGN

4.1 Background

4.1.1 Ground Beetles as a Sentinel Taxon

The Terrestrial Observation System (TOS) at NEON is charged with monitoring the responses of biodiversity and ecosystems to environmental change. While several different invertebrate groups were considered, a NEON design committee (AIBSnews 2007) selected ground beetles (Coleoptera: Carabidae) as a focal taxon for measurement.

Ground beetles have been extensively used as indicator species of arthropod biodiversity, environmental change (Rainio and Niemela 2003, Koivula 2011), land use (Vanbergen et al. 2005a) and land management (Work et al. 2008). The ground beetle family is large, widespread, and associated with the ground layer, rather than any particular plant community, ensuring that some ground beetles are present in virtually all terrestrial habitats over the entire extent of the observatory (Lövei and Sunderland 1996). The family is rich in species (over 40,000 species described globally, circa 3,000 species in NEON's spatial extent), abundant in individuals and well known taxonomically (Larochelle and Larivière 2003). Though ground beetles range in body size from less than a millimeter to several centimeters, they are straightforward to sample and identify (Kotze et al. 2011). For example, they have distinctive features such as a 5-5-5 tarsal formula, and a first abdominal segment completely separated by their rear legs, that facilitate their identification by non-experts (Forsythe 2000).

In addition to being widespread and well-known biologically, ground beetles are sensitive to environmental conditions and form well-defined richness gradients. Consequently, ground beetles are well suited as "sentinels" of arthropod biodiversity (Rainio and Niemela 2003, Koivula 2011). Their value to environmental science is evident in their status as model organisms for population biology, landscape ecology and conservation biology (Kotze et al. 2011). Ground beetles form an important component of terrestrial food webs and can influence terrestrial trophic structure. They are omnivorous, and most species are predators as both adults and larvae. In addition, they are common prey for small mammals, birds, reptiles, amphibians and other larger arthropods (Larochelle and Larivière 2003). Their role in food webs is recognized both in wildland and agricultural settings, where they contribute to biological control of pests (Kromp 1999). Ground beetles are prevalent in agricultural systems, where their community composition is affected by land management (Purvis and Fadl 2002, Legrand et al. 2011) and their diversity can influence weed seed predation (Gaines and Gratton 2010). In addition, ground beetles have been used to assess ecological effects of urbanization (Niemelä and Kotze 2009).

4.2 NEON's Contribution

Earth's environment is changing rapidly, and data collected at broad temporal and spatial scales will increase our ability to understand, forecast and manage our changing biosphere (Keller et al. 2008). NEON's ground beetle sampling will provide a cost effective and informative measure of biodiversity, environmental and land-use change. This work will add to the considerable base of ground beetle studies and enable us to characterize their response to ecosystem drivers quantified across the observatory (Vanbergen et al. 2005b, Work et al. 2008).

The need for and value of long-term and large scale research to understand our changing environment is well recognized and best practices include high species resolution taxonomy and well-characterized variability in the local environment (Work et al. 2008). NEON has been designed to meet these research needs and expectations. NEON will collect data for 30 years, much longer than most sampling efforts and the spatial extent of NEON includes Alaska, Hawaii, the continental US, and Puerto Rico. Taxonomic resolution will be to the species level and will be temporally stabilized using DNA barcoding and expert taxonomists. In addition, the sampling environment will be thoroughly characterized by a broad suite of biotic and abiotic measurements (Schimel et al. 2011).

NEON ground beetle sampling will improve our understanding of the distribution, ecology and evolution of ground beetles in both wildland and human-dominated landscapes at a very wide scale over several decades. NEON ground beetle data will have added value through active collocation at the plot and site level with other NEON data (e.g., plant productivity and diversity, rodent density, temperature, precipitation and hundreds of other NEON data products). NEON ground beetle data will be complementary to other ongoing projects to monitor particular groups of invertebrates (e.g., The Xerces Society for Invertebrate Conservation). NEON's archived samples will be available for loan, for example for gut analyses, isotopic measurements, symbiont surveys and genomic analyses of rapid evolution in response to environmental change. In addition, bycatch from ground beetle sampling will be available for loan and represents a valuable resource for the ecological community (Buchholz et al. 2011).

4.3 Purpose and Scope

This document defines the rationale and requirements for ground beetle (Coleoptera: Carabidae) abundance and diversity in the NEON Science Design. Details about protocols including educational materials to conduct protocols, quality assurance and quality control or calibration and validation procedures are addressed in protocol specific documents.

5 SAMPLING FRAMEWORK

5.1 Science Requirements

This science design is based on Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

5.2 Data Products

Execution of the protocols that stem from this science design procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. NEON will apply quality assurance and control algorithms on all data before posting to the portal, and will report associated error metrics (in the form of quality flags) with the data. These data and samples are used to create NEON data products, and are documented in the NEON Level 1, Level 2 and Level 3 Data Products Catalog (RD[03]). Data collected from NEON ground beetle pitfall trapping efforts will be freely available via the online portal (http://data.neonscience.org/home).

Ground beetle data will be reported at the trap, plot and site level. The following data, relevant to ground beetle trapping, will be available:

- Trapping report: the locations, times and dates of trap setting and collection at each site, and all associated field metadata.
- Abundance: the number of individuals of each species/sex combination collected in each sample.

Trapping reports will indicate when technicians collected traps and data will usually be available for 40 traps per site per collection bout (2013 to 2017 collections). In 2018, 30 traps were deployed per plot (i.e., all but the North trap). Missing records (i.e., fewer than 40 records per bout in 2013-2017) indicate a lower level of sampling effort for that bout. Reduced sampling effort may arise due to weather or logistical constraints. The content of traps are reported in the sorting data; in these data NEON provides the abundance and identity of vertebrate bycatch, the presence of invertebrate bycatch, and the abundance and identity of carabids. Trapping records without corresponding sorting records indicate empty traps (zeros) in the dataset. Metadata concerning trap condition are also available to aid the interpretation of these zeros.

5.2.1 Opportunities for researchers

Data generated from this design can be used along with other NEON data or combined with data collected during independent research. Below are a number of potential uses for NEON ground beetle data.

Activity vs. abundance

Although NEON is using pitfall traps to measure carabid abundance, there are a variety of alternative trapping techniques. Researchers interested in leveraging the distributed NEON network could undertake sampling at NEON sites using one or more supplementary methodologies. A comparison of the abundance and diversity metrics derived from these techniques (versus the values NEON already provides) would further clarify which species are overrepresented in NEON pitfalls. Even a short-term effort of a few bouts might allow researchers to adjust the raw abundance values provided by NEON in models of species relationships.

Competition and species interactions

The regular sampling of carabid beetles via pitfall traps will allow for analyses of patterns of species co-occurrence, which is the first step to understanding species relationships, niche sharing and competition (Niemela 1993). Data from 2014 (available at http://data.neonscience.org/home) reveal patterns that suggest both spatial (**Figure 2a**,**c**) and temporal (Figure 2b,d) partitioning of sites among carabid species. At the Smithsonian Institute of Conservation Biology (SCBI), NEON's core site in domain 2, two of the most abundant species are found in the same type of habitat (Figure 2a) but at different times of the year (**Figure 2b**). Other species occur together seasonally (Figure 2d), but do not share habitats (Figure 2c). Using NEON data, additional correlations in co-occurrence patterns between species with large ranges can be examined at broad geographic and temporal scales. Researchers interested in conducting experimental work at one or more NEON sites may also perform manipulative experiments (e.g., by altering variables of interest such as plant cover, nutrient availability, etc.) at locations adjacent to NEON plots. Experimental results can then be extrapolated over the larger network or examined in the context of multi-year sampling by NEON at particular sites.

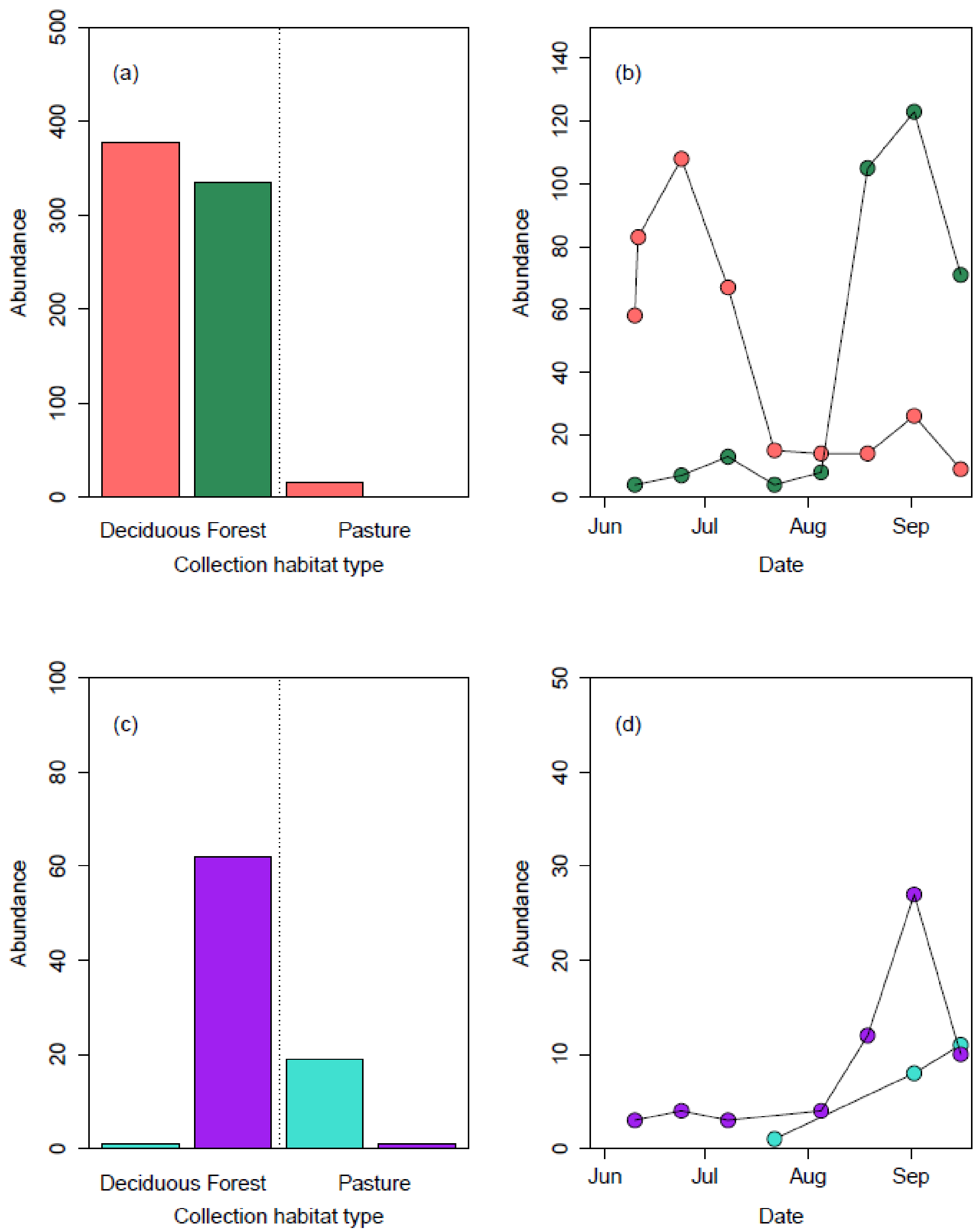

Figure 2. *Chlaenus aestivus* (red), *Pterostichus coracinus* (green), *Harpalus pensylvanicus* (blue) and *Pterostichus stygicus* (purple) abundance at SCBI over the 2014 field season. Panels show the total number of beetles per species captured (a,c) at all time points in 2014 per habitat type and (b,d) across all habitat types per collection event throughout the field season at the Smithsonian Conservation Biology Institute (SCBI), VA.

Land use and community shifts

Carabids are a useful indicator of land use change because ground beetle communities exhibit high levels of species turnover between habitat types (Do and Joo 2015), and community composition would change noticeably following land-user alteration. NEON carabid data collected in 2014 show that sites characterized by varying land use regimes supported ground beetle assemblages that differed greatly with respect to abundance, total richness (**Figure 3a**) and species composition. Species assemblages of carabid beetles also varied significantly between domains (PerMANOVA F = 30.04, r2 = 0.28, p = 0.001), sites (PerMANOVA F = 14.88, r2 = 0.09, p = 0.001) and habitat types (Figure 3b; PerMANOVA F = 4.29, r2 = 0.04, p = 0.001). This suggests that researchers interested in community response to habitat and land use change over time may use NEON data to relate carabid community composition to changes in the landscape. As abiotic factors at a site change (e.g., temperature or precipitation), relationships between community composition and those factors may also be examined.

Temporal occurrence and climate shifts

Because pitfall sampling will be conducted throughout the growing season, traps will capture the seasonal change of many ground beetle species. These data provide information about the relationship between shifting weather and ground beetle occurrence, which are valuable data for modelling how phenology might change with future climate alterations. Data generated by NEON in 2014 and collection data from the Global Biodiversity Information Facility (GBIF) suggest how this data set might be useful. For instance, *Cicindela punctulata* Olivier, 1790 is a tiger beetle with a large range throughout the continental United States (**Figure 4a**). Collection data from NEON and GBIF indicate that across its range, *C. punctulata* is most commonly collected in the middle of summer (**Figure 4b**). However, there is a predictable delay in the date that *C. punctulata* is first sighted based on the latitude of sampling (Figure 4c); a result that likely reflects the response of *C. punctulata* to a temperature gradient. In the data collected by NEON in 2014, the first sighting of *C. punctulata* is in line with what one would expect based on the GBIF records. Going forward, if first emergence of *C. punctulata* is influenced by temperature and if temperature shifts through time (e.g., it gets hotter at continental interiors or at northern latitudes), then phenological shifts by *C. punctulata* may be detected through occurrence records provided by NEON and in affected areas. The museum records and other information aggregated by databases like GBIF are haphazardly and inconsistently collected as a rule. Because NEON will sample sites consistently throughout its 30 years of operation, the seasonal occurrence data that NEON provides will be more complete across the continent for large ranging taxa. As a result, NEON may provide data showing shifts in seasonal occurrence of particular species through time, and enable scientists to examine the local, regional, or even continental factors that drive such shifts.

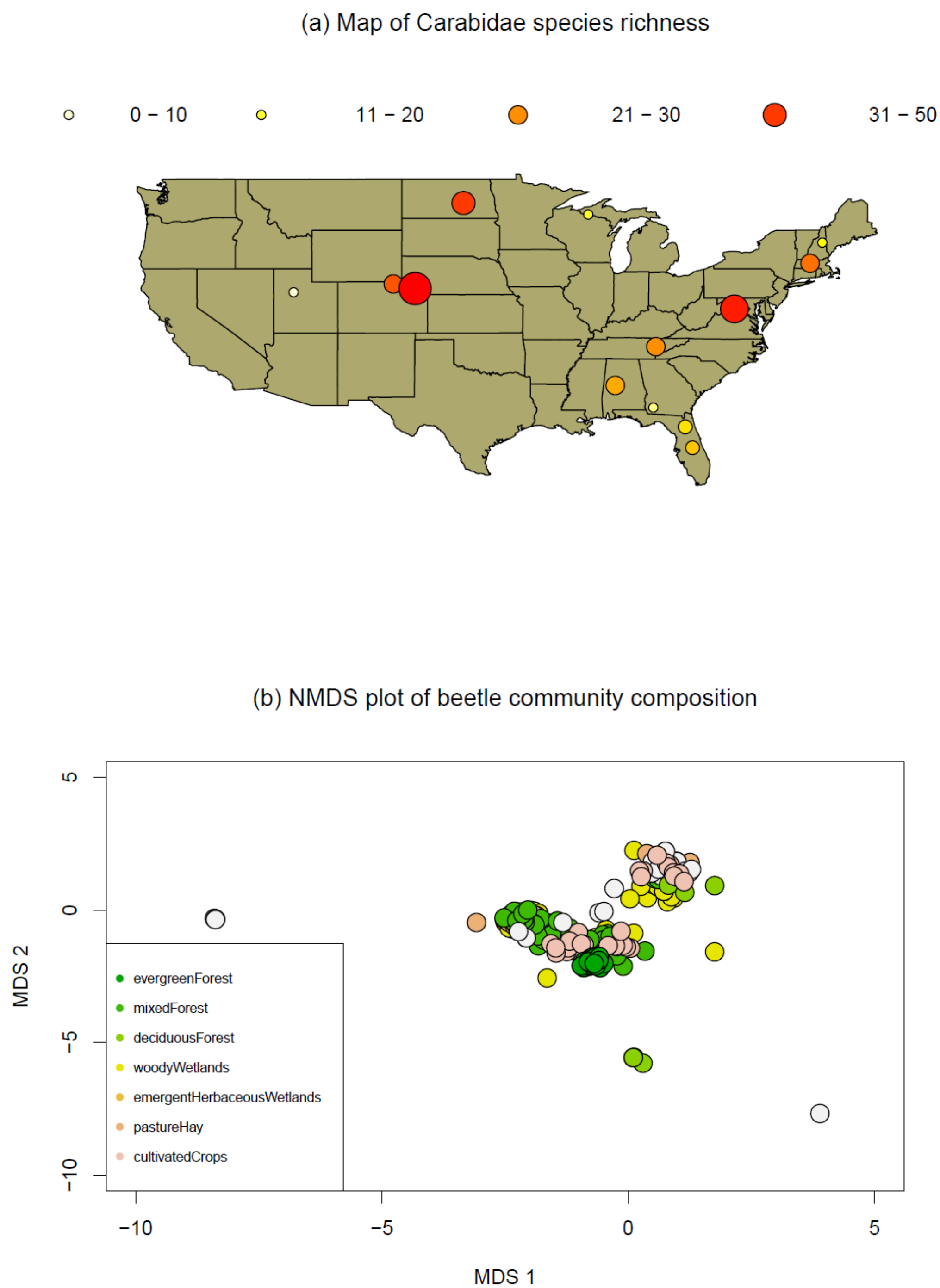

Figure 3. (a) Observed patterns of Carabidae richness within NEON domains. (b) Non-metric multidimensional scaling plots show differences in the carabid community at NEON sites in 2014. Ordination points are colored to differentiate NLCD class habitat types. Each point represents NMDS score received by a plot given the total carabid community observed at a plot in 2014 (N = 386 plots from 11 sites).

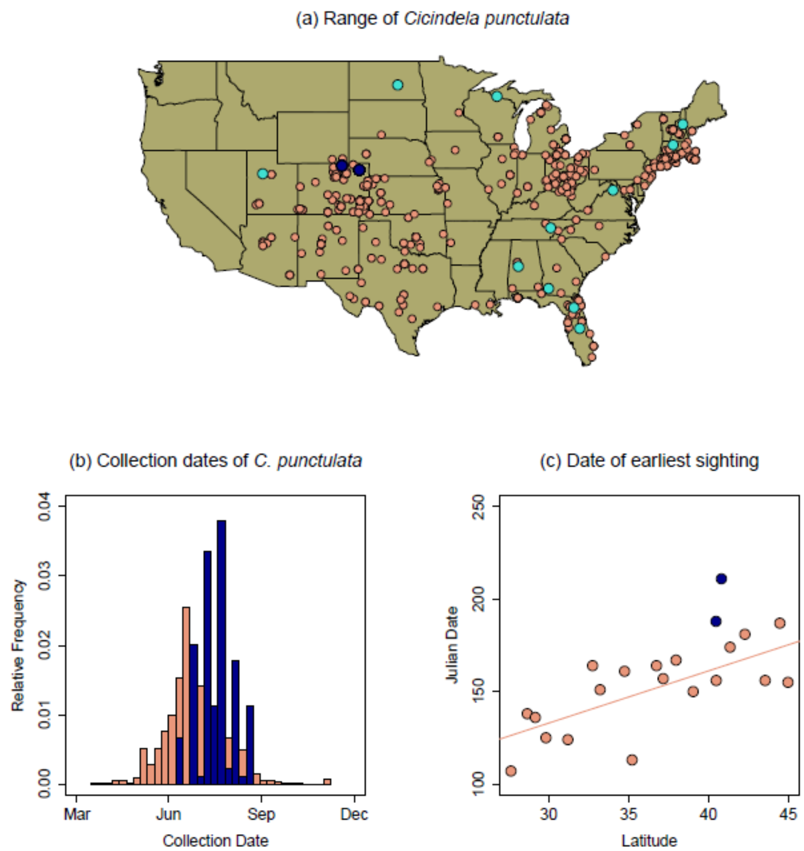

Figure 4. (a) The known range of *Cicindela punctulata* according to GBIF (peach circles). NEON sampled 13 locations in 2014 for carabid beetles (blue circles) and caught specimens of *C. punctulata* (dark blue circles) at two sites. (b) Dates on which *C. punctulata* were collected according to GBIF (peach bars) and NEON (blue bars). (c) The relationship between degree latitude and earliest date that *C. punctulata* is recorded from GBIF (peach circles). The earliest collection dates of *C. punctulata* at two NEON sites (blue circles) follows the same phenological pattern. Points are binned by degree latitude; portion of latitudinal range only considered if more than twenty records were found in that latitudinal zone.

Community-based analyses & trophic relationships

A valuable aspect of the NEON project is the co-location of ecological sampling—which generates knowledge about the abundance and diversity of selected species within plants, microbes, arthropods, mammals and birds—along with measures of abiotic conditions (e.g., precipitation, temperature, snow depth, dust inputs, etc.) and soil properties (e.g., soil moisture, organic content, bulk density). NEON will generate 30 years of co-located data that facilitate the study of changing species assemblages and interactions within and between trophic levels. One interesting insight may be elucidation of the relationships between the "green" food web (fueled by above ground plants) and the "brown" food web (i.e., the below ground dynamics driven by microbes, plant litter, and coarse downed wood).

5.3 Priorities and Challenges for Ground Beetle Abundance and Diversity Sampling

In keeping with NEON's broad mission, this design must be able to be deployed across a wide range of sites in a standardized way (e.g., methods, sampling frequencies) such that data are comparable across time and space. The design must be relatively uncomplicated so that it can be deployed consistently by disparate field crews over multiple years with minimal chance of alteration. An additional sampling priority to promote comparability and the characterization of relationships between disparate measurements is collocation with other measurements across the observatory. To this end, ground beetle sampling will occur at distributed plots within NEON sites, where other organismal and abiotic measurements are taken.

To promote utility to the scientific community, ground beetles will be sampled using pitfall traps, a standard and effective sampling method (Kromp 1999, Rainio and Niemela 2003, Kotze et al. 2011). This standardized, well established and widely used sampling method was selected to maximize comparability across time and between domains within the observatory and also to be comparable to external data sets. Pitfall trapping has been used for more than a century and is still the most commonly used method for ground beetle collection.

6 SAMPLING DESIGN FOR GROUND BEETLE ABUNDANCE AND DIVERSITY

NEON's ground beetle abundance and diversity sampling will target all members of the family Carabidae (ground beetles). Ground beetles are ubiquitous and can be found at all NEON sites.

Pitfall traps are a passive collection technique for estimating terrestrial invertebrate species richness and relative abundance and therefore provide a reliable measure of ground beetle abundance and diversity (Baars 1979). Pitfall traps collect a wide range of arthropod taxa, and are particularly effective in sampling mobile, surface-active taxa like ground beetles. Specifically, pitfall traps measure the density of activity in the area of a trap, or more simply "activity density," a measure that combines both arthropod abundance and movement.

Pitfalls will be deployed continuously during the entire growing season (length varies among sites) to encompass the activity of all ground beetle species. Pitfalls at both core and gradient sites will be sampled (emptied and re-set) every 2 weeks. Across the observatory and within each domain, core and gradient sites will be sampled with equal intensity. This strategy prioritizes even sampling across sites to maximize comparability of ground beetle data among all NEON sites.

Ground beetles in pitfall samples will be separated from bycatch and identified to species, thus providing an estimate of ground beetle abundance and biodiversity in each domain each year. NEON pitfall traps will collect large numbers of many common ground-dwelling arthropods in addition to ground beetles. The non-ground beetle arthropods are termed "bycatch" and although the identification of these additional taxa would be advantageous, as their inclusion would encompass additional taxonomic diversity and feeding guilds (e.g. herbivores, detritivores), their inclusion would considerably increase sampling costs in terms of processing time, analysis and storage space relative to the advantages of focusing on ground beetles. The bycatch will be archived and available from the NEON collections to other researchers for additional analyses.

6.1 Sampling Method

NEON uses a shallow, medium-sized cup trap, that has been shown to perform well in comparison to other sizes (Work et al. 2002). NEON's pitfall trap design consists of two 16 oz. deli containers (7 cm deep with an 11 cm diameter, 540 mL total volume) nestled within one another. The lower container ensures that the trap remains flush with the ground, maintains the integrity of the hole, and enables efficient collection and resetting of the trap. Holes drilled into the base of the lower container allow excess moisture to drain; this also prevents the upper container from floating. The upper container holds a fluid preservative that kills and safeguards beetles from degradation (a 1:1 mixture of water and propylene glycol, a non-toxic antifreeze). The contents of the upper container are collected during each sampling event. Depending on ambient temperatures at a site, technicians will replace the sample with 150 or 250 mL of preservative on a biweekly basis. A square plastic cover (20 x 20 cm) elevated 1.5 cm above the trap entrance protects the container from weather (e.g., dilution from rain, drying from sun) and prevents unintended bycatch of medium to large vertebrates.

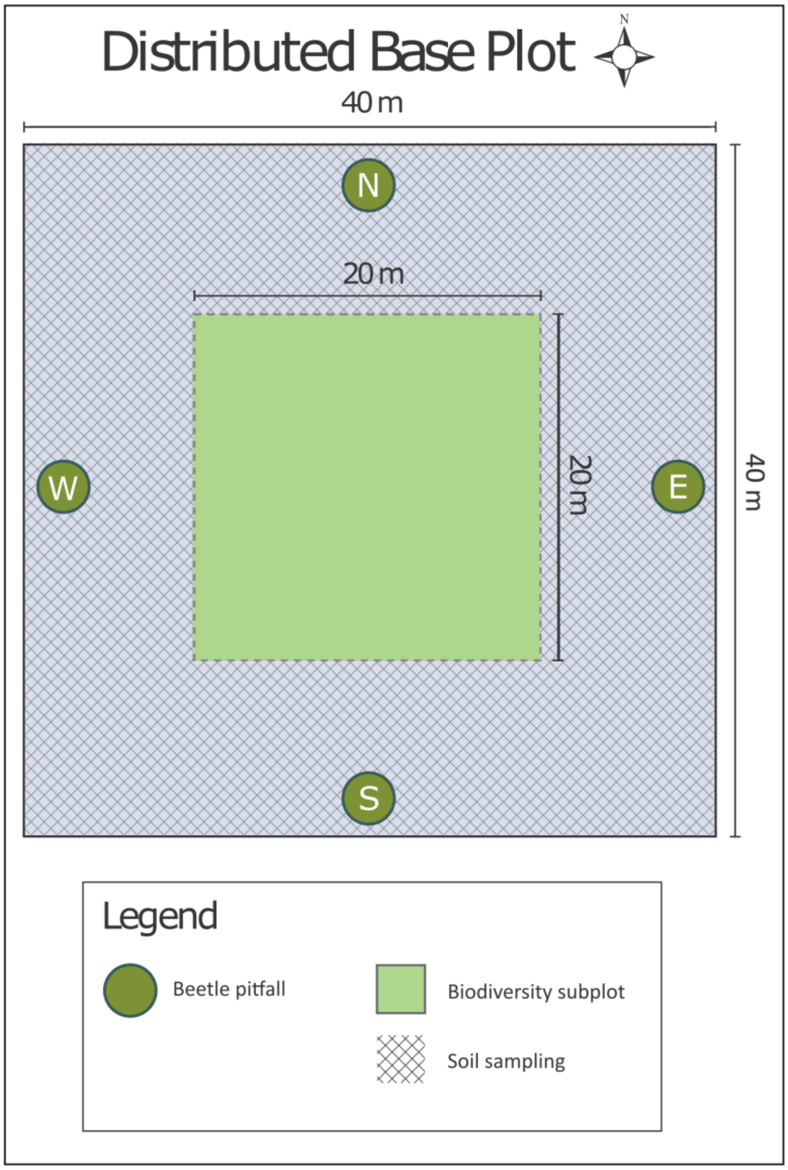

Figure 5. Standard beetle plot layout within a distributed plot. Four traps are placed at one of the cardinal directions (North, East, South, West) at each of 10 distributed base plots.

Pitfall trapping is the most common method for sampling ground-level arthropods (Kotze et al. 2011). Alternative methods exist for capturing ground beetles, including sticky traps, malaise traps, ultraviolet light traps, flight intercept traps, sweep netting, hand picking, point counts, fogging, quadrat sampling and litter washing (Rainio and Niemela 2003). However, pitfall trapping is overwhelmingly the most used method in a wide variety of environments as it is the easiest, cheapest and most reliable method to use over the long term.

The primary bias of pitfall trapping is that it confounds abundance and activity. Activity varies among species and can be affected by environmental or physiological factors that influence activity, e.g., temperature, humidity, seasonality and food availability (Purvis and Fadl 2002). Thus, it can not necessarily be inferred from pitfall data that species A is more abundant than species B in the environment simply because it is more abundant in pitfall samples. It may be that species A is more active and therefore more likely to be trapped. Because of this activity bias, larger beetles are more likely to be trapped than smaller beetles because they cover greater distances (Spence and Niemela 1994). Despite these known biases, pitfall trapping is the standard method for characterizing ground beetle communities (Rainio and Niemela 2003). Other potential disadvantages of pitfall trapping can be neutralized using specific techniques. For example, the NEON sampling design includes an odorless preservative to avoid attracting arthropods; pitfalls covers will be installed to prevent both flooding and desiccation; and rather than solitary pitfall traps, sets of up to 4 well-spaced traps will be deployed to overcome occasional trap losses or other problems with individual traps.

While pitfall traps may require slight modifications for deployment in specific locations, the sampling technique is versatile and can be utilized at all sites. Site-specific considerations include substrate, temperature, humidity and seasonal water cover. Substrate is important because sand content, soil moisture and vegetation cover all influence the relative ease of digging and servicing pitfall traps. For example, different vegetation types, such as sphagnum moss, grasses, or dense woody roots may require different tools and/or techniques for pitfall deployment and ensuring durability of pitfall holes. Temperature and humidity will affect how quickly water in the trap evaporates, effectively concentrating the preservative. Finally, seasonal ponds or stream flooding may limit the specific placement of pitfall trap locations within a site.

6.1.1 Pitfall Trap Prototyping

In an effort to test the broad applicability of pitfall trapping, NEON prototyped pitfall traps at a variety of different sites from 2009-2013. While testing different trap designs and components, ground beetles were collected at 16 sites with broad geographical coverage, including sites in Alabama, Colorado, Florida, Massachusetts, Michigan, New Hampshire, North Dakota, Tennessee, Utah and Virginia.

Several specific deployment and equipment issues were resolved during prototyping, including the critical importance of burying the cup flush with the ground and the tools necessary to achieve this in different soil types. Different cover designs were tested and it was determined that plywood is susceptible to rotting and splintering, so it has been replaced with composite plastic. The height of the cover was modified to reduce vertebrate bycatch. The propensity of covers to slide down nails and close the trap was overcome by adding plastic spacers to the trap design. The tendency for nails to rust in some environments was fixed by switching to plastic stakes. A variety of cup sizes were tested and smaller cups were deemed problematic because spiders built webs across the cup opening.

Prototyping efforts culminated in an abbreviated field season of ground beetle sampling in the summer of 2012 at three sites in Domain 03 (Jones Ecological Research Center, Ordway-Swisher Biological

Station and Disney Wilderness Preserve). At each site, up to 40 pitfall traps were deployed for a total of 5 weeks from the beginning of August through the first week of September (Table 1). On average between 2 and 5 ground beetles were captured per plot in a week of pitfall trapping. The abbreviated trapping season yielded a total of 413 ground beetles, representing 34 unique species (**Table 1**) and demonstrated the feasibility of the proposed methods to be deployed at the domain scale (Hoekman et al. 2013).

Table 1. Summary of ground beetle data from Domain 03 prototype, summer 2012.

	Total number of ground beetles collected	Number of plots sampled (1 week deployment)	Average number of ground beetles collected per plot per week	Average number of ground beetles collected per site per week	Number of ground beetle species recorded
Jones	91	60	2	15	9
Ordway	46	60	1	8	5
Disney	276	60	5	46	20
Domain 3 total	**413**	**180**	**2**	**69**	**34**

NEON plan: Each sampling bout will consist of 14 days of continuous pitfall trapping. Every two weeks, specimens trapped in pitfall cups will be collected and processed in the domain lab. Specifications for beetle trap construction and deployment are described in the TOS Protocol and Procedure: Ground Beetle Sampling (RD[04]).

6.1.2 Considerations for Vertebrate Bycatch

Within pitfall collections, bycatch is primarily composed of spiders and insects. Depending on their size, pitfall traps can be used to capture a wide range of ground-dwelling animals; e.g., larger pitfalls are used to survey amphibians, reptiles and small mammals (Bury and Corn 1987, Hobbs and James 1999). Small traps designed to capture invertebrates do occasionally capture small vertebrates, including frogs, salamanders, and shrews.

NEON has taken various steps to reduce vertebrate bycatch in our pitfall traps. First, the pitfalls are relatively small (11 cm diameter) and shallow (7 cm depth), thus only vertebrates about the size of large ground beetles are vulnerable to being captured. Recent studies suggest that shallower (7 cm) pitfalls are less likely to capture vertebrate bycatch than deeper (15 cm) pitfalls (Pendola and New 2007 and references therein). Larger vertebrates could easily exit the cup if they happened to fall in (but see cover below). The preservation fluid in the pitfalls (propylene glycol) is colorless and unscented, so the traps are not baited and do not attract ground beetles or bycatch. Each pitfall trap will be covered with a 20x20 cm hard plate, 1.5 cm above the lip of the trap. The cover will deny access to all but the smallest vertebrates.

6.1.3 Site-specific modifications

Effort was made to implement the protocol as described above at all sites within the observatory, without modification. However, exceptions were made for 3 sites on the basis of potential interactions between vertebrates and traps: GUAN in Puerto Rico, GRSM in Tennessee, and YELL in Montana. An additional deviation was made to suspend sampling at STER during peak carrion beetle activity (primarily the first two weeks of August), due to degradation of samples by carrion beetle consumption.

DOMAIN 04: GUAN

In Guanica forest (core site), significant concern presented regarding the potential capture of the endangered Puerto Rican crested toad (*Peltophryne lemur*). NEON proposed to the US Fish and Wildlife Service (USFWS) that a trial deployment of the beetle trap design be implemented. First, the two-cup, nested design as described above was deployed using a deeper trap cup (32 oz. deli cup) of the same lip circumference as the shallower traps. A larger amount of propylene glycol and water preserving fluid (500 mL; same concentration) and a dowel rod (1 inch diameter and 4.5 inches length) was placed into each cup. The dowel rod was proposed as a means to allow a captured Puerto Rican crested toad to survive a fall into the cup. Through close coordination with the Puerto Rican Department of Natural and Environmental Resources, some or all traps were temporarily inactivated during Puerto Rican crested toad breeding events and toadlet migrations.

In 2017, NEON provided two field seasons of data to the USFWS demonstrating that vertebrate bycatch numbers were similar between Guanica and other sites in the network, but that carabid numbers were severely depressed by the cup modifications. With these data in hand, the USFWS approved the use of the same shallow pitfall cup design used at all other sites in the network (without modification) for field season 2018 and future sampling.

DOMAIN 07: GRSM

At Great Smoky Mountain National Park (core site), concerns were raised regarding interactions between bears and pitfall traps. Moreover, park regulations specify that if bears interact with a trap, then the trap is required to be deactivated for one month (first offense) or the remainder of the field season (second offense). In consultation with the Park, NEON elected to implement the same cup trap design at Great Smoky Mountain National Park as is used elsewhere in the network, but with electrified fencing placed around each cup trap for the duration of the sampling season. At the end of each sampling season, the fencing is removed. This fencing is installed to prevent bears from interacting with the trap or eating trap contents. Due to the large gauge of the wire, the presence of the electric fencing is unlikely to negatively affect beetle collections over 30 years – although rates of vertebrate bycatch may be affected.

DOMAIN 10: STER

At Sterling (gradient site), sampling will be suspended during the seasonal pulse of carrion beetles (Silphidae *spp*.). Carrion beetles typically become super-abundant at this site in the first two weeks of August, at which time they represent more than half of the specimens within a trap cup and 250 mL of preservative is insufficient to collect the quantity of active individuals. During this time, carrion beetles consume pitfall trap contents – rendering the identification of collected material difficult to impossible and disrupting accurate assessment of diversity and abundance. Based on prior observations of carrion beetle seasonal abundance at the site, traps will be temporarily deactivated on the first scheduled August collection to avoid sample degradation. Deactivating pitfall traps entails: removal of the interior pitfall cup (such that only the exterior cup with holes in it remains), putting the deli cup lid on top of the interior pitfall cup, removing the PVC spacers, and closing the 20 x 20 cm pitfall cover down flush to the ground. Staff will deactivate traps for two full bouts during highest carrion beetle activity and redeploy traps for the remainder of the season following this hiatus.

DOMAIN 12: YELL

At Yellowstone (core site), concerns over vertebrate bycatch were raised by the National Park Service. As a result, a few modifications will be prototyped in 2018. First, a metal hardware cloth with 2.54 cm gauge will be installed over the entry of the trap cup (intended to reduce vertebrate bycatch). The second requirement concerns 'trap predation', which is any disturbance and/or excavation of the cup trap that appear to derive from an animal (e.g., a bear) trying to eat the contents of the trap. If three or more instances of trap predation are observed during collection (out of 40 total traps possible), the park liaison will be notified within 24 hours. Five or more instances of trap predation within a single collection bout will trigger temporary trap closures across the entire site for two bouts (28 days).

Trap contents will be evaluated at the close of the 2018 field season. If meshing does not reduce vertebrate captures and/or introduces biases in carabid captures, then the meshing will not be implemented in future seasons.

6.2 Sample Processing in the Lab

Specimens collected in pitfall traps will be sieved out of the propylene glycol and transferred into 95% ethanol. After 24 hours, the ethanol will be replaced to ensure low water content in the ethanol used for storage of the samples. Ground beetles in pitfall samples will be separated from non-target taxa (termed "bycatch") and identified to species, in order to provide an estimate of ground beetle abundance and species diversity in each site each year. Although all vertebrate bycatch will be identified and removed from samples within 24 hours of recovery from the field, invertebrates and carabids from pitfall samples will be sorted in the lab during the growing season or subsequent off-season. At that time, all ground beetle specimens will be identified to species or sorted to morphospecies by field staff.

Field staff will use a domain-specific voucher collection and available dichotomous keys to identify ground beetles. During NEON Construction, a voucher collection has been assembled for each site, and this collection will be supplemented throughout NEON Operations with additional specimens that represent morphological variation within and between morphospecies. A subset of staff-sorted carabids will be pinned. Field staff will pin at least 20 specimens from each site of carabids that are easily identified by trained staff, if available, and at least 100 specimens per site, as available, of carabids that are not easily identified or have only received morphospecies designations. A species of carabid is considered 'easily-identified' if:

1) at least 100 specimens of a species receive the same species-level taxonomic assignment from both NEON field staff and taxonomic experts, and

2) there is less than 5% mis-identification of that species by NEON field staff.

Specimens that are not pinned will be stored in 95% ethanol and archived.

From each domain each year, some ground beetles will be sent to external facilities for taxonomic verification. This subset will be selected in order to represent some individuals from every morphospecies; individuals that are rare or difficult to identify will be prioritized. Some beetles will be sent to expert taxonomists for identification and some ground beetles will be photographed and submitted for DNA barcoding, which has been shown to be effective for distinguishing ground beetle species (Raupach et al. 2010). These subsets may overlap and exact numbers will depend on the abundance and diversity of collected ground beetles and annual funding.

Non-target invertebrates

NEON pitfall traps will collect large numbers of common non-target, ground-dwelling arthropods as bycatch in addition to ground beetles. Although the identification of these additional taxa would be useful, as they encompass additional taxonomic and trophic diversity (e.g., herbivores, detritivores), their inclusion would considerably increase costs in terms of processing time, supplies (e.g., EtOH, jars), analysis, storage, and curation. For this reason, NEON will not provide counts or taxonomic identifications of non-target invertebrates sampled by pitfall traps. However, archived bycatch from NEON ground beetle pitfall traps with their associated metadata will be available from NEON collections for future processing and analyses by interested scientists.

Vertebrate bycatch

To date, the vast majority of NEON pitfall trap bycatch are arthropods. This is a result of active steps taken by NEON during the trap design and prototype phase that have reduced risk of vertebrate bycatch in pitfall traps. The effectiveness of these modifications is clear from the data themselves. In 2014, thirteen sites were sampled between 28 and 154 days for a total of 52,746 trap-nights. While invertebrate bycatch was collected in a third of recovered traps (1275/3759 traps), less than four percent of traps contained any vertebrate bycatch (135 traps). This low rate of capture (0.0033 vertebrates per trap-night) is comparable or lower than that reported by other carabid sampling studies that sought to minimize vertebrate bycatch (i.e., Pearce et al. 2005: 0.073 mammals per trap-night, Lange et al. 2011: 0.025 mammals per trap-night).

6.3 Taxonomic verifications and reference collections

Funding constraints require that the taxonomic identity of some ground beetle specimens will be solely determined by well-trained field staff rather than experts in carabid identification. Training materials and a comprehensive voucher collection will allow for accurate identifications in the majority of cases. However, NEON will mitigate potential errors in parataxonomist identifications by sending a subset of carabids annually to external facilities for secondary verification by expert taxonomists. This sample will comprise specimens that are representative of the perceived morphological variation in every morphospecies, although individuals that are rare or difficult to identify will make up the majority of the subset. Additionally, some beetles sent for secondary taxonomic review will be also photographed and their tissues submitted for DNA sequencing of the Folmer region of the CO1 gene (a.k.a. DNA barcode; Folmer et al. 1994, Hebert et al. 2003). This region is effective for use in the identification of most ground beetle species (Raupach et al. 2010), with only a few exceptions (Maddison 2008). Exact numbers of specimens expertly identified versus identified through DNA sequencing will depend on the abundance and diversity of collected ground beetles and annual funding (with up to 95 individuals sequenced per site per year). However, beetles that are rare, particularly difficult to identify, or poorly represented in previous collection events will be prioritized for DNA sequencing. DNA sequence data will supplement expert identifications and provide greater resolution in cases of poorly resolved taxonomy (e.g., the genus *Elaphropus*) or cryptic species (e.g., *Harpalus texanus* vs. *Harpalus pennsylvanicus*).

Identifications provided by experts or sequence data will improve the quality of staff-derived classifications in the future. Following that secondary identification, a subset of positively-identified beetles will be returned to the domain lab of origin and will enhance the voucher collections used by the field staff when making their initial taxonomic assessment. This will allow staff to compare newly acquired specimens to a growing collection of high quality vouchers, thereby ensuring increasing accuracy in the identification of new specimens through time. Furthermore, the combined use of expert identifications and sequencing will also improve the ability of the broader scientific community to make accurate identifications. As NEON accumulates and publishes sequence data on specimens that have also been identified by carabid experts, the quality and quantity of sequence information available for many carabid species will grow. Publically-available DNA reference sequences will aid in understanding the inter- and intra-specific variation within beetle populations, support accurate identification of carabid specimens by non-experts and reveal the presence of cryptic species. To date, the specimens for the NEON DNA sequence reference library were collected during field prototype campaigns or obtained from museum archives (Gibson et al. 2012). All assembled resources for each specimen - sequence data, photos, and other ecological information - can be publicly accessed online from the Barcode of Life Datasystem (BOLD, http://boldsystems.org/). Two BOLD projects have been established; a project for operational data (‘Ground beetle sequences DNA barcode’ or project code BETN) and a project for prototype data (‘NEON Prototype Beetle sequences DNA barcode’ or project code BETP). Future sequence data will continue to be posted on the BOLD repository throughout the life of the observatory.

6.3.1 Archiving

All specimens (including unsorted "bycatch") and any extracted DNA will be archived and available for use by the scientific community.

6.4 Spatial Distribution of Sampling

Each domain includes one to three terrestrial sites, a single "core" site in a wildland location where sampling will occur for the entire 30-year lifespan of the observatory, and up to two "gradient" sites that may periodically be reassigned (i.e., moved within the domain). At each NEON site, 10 distributed plots will be selected for pitfall sampling with 4 pitfall traps deployed per plot (40 traps per site). Plots will be distributed across up to three dominant vegetation types to best represent the different habitats present at each site while maintaining sufficient replication within each vegetation type. The number of plots per vegetation type will be proportional to the percent cover of that type at the site. This stratified-random approach will benefit the ground beetle sampling, because vegetation cover has been shown to be an important predictor of ground beetle community composition (Dufrene and Legendre 1997, Work et al. 2008). Therefore, the important ground beetle species present at a site are more likely to be encountered and recorded if sampling effort is spread across different plant communities.

At each distributed plot a pitfall trap will be placed in each cardinal direction 20 meters from the center of the plot. This level of replication will provide a sufficiently large sample to characterize the ground beetle community, including rare species, and is a greater or comparable sampling effort to other large-scale pitfall trapping schemes (Dufrene and Legendre 1997, Vanbergen et al. 2005b, Work et al. 2008, Brooks et al. 2012). Collectively, the 4 pitfall traps will represent the ground beetle community at the plot level, but traps will be far enough apart (30-40m) to represent independent samples of the beetle community at a site level (Digweed et al. 1995) to maximize site-level replication (N=40 between 2013 and 2017). As a budget saving measure, NEON deployed three traps per plot in 2018.

Long-term evaluation of within-plot replication

The carabid sampling program is intended to parameterize significant changes in abundance for particular species and changes in aggregate measures of diversity (e.g., richness, Shannon's diversity). Based on reviews of the literature described above, NEON deploys 40 traps to capture carabid diversity at all NEON sites. However, fluctuating budgets require re-evaluation of the carabid sampling design in order to preserve long-term sustainability of the carabid sampling program. Therefore, NEON will perform an analysis of sites in initial operations to optimize the carabid sampling program such that site-level metrics of diversity are appropriately estimated. Previous analyses of the NEON carabid sampling program have shown wide differences in species accumulation curves at different sites (see Figure 6; exemplar curves from 3 sites during NEON construction), suggesting that this data-driven approach will yield a sampling schedule and collection regime that optimizes the carabid sampling program.

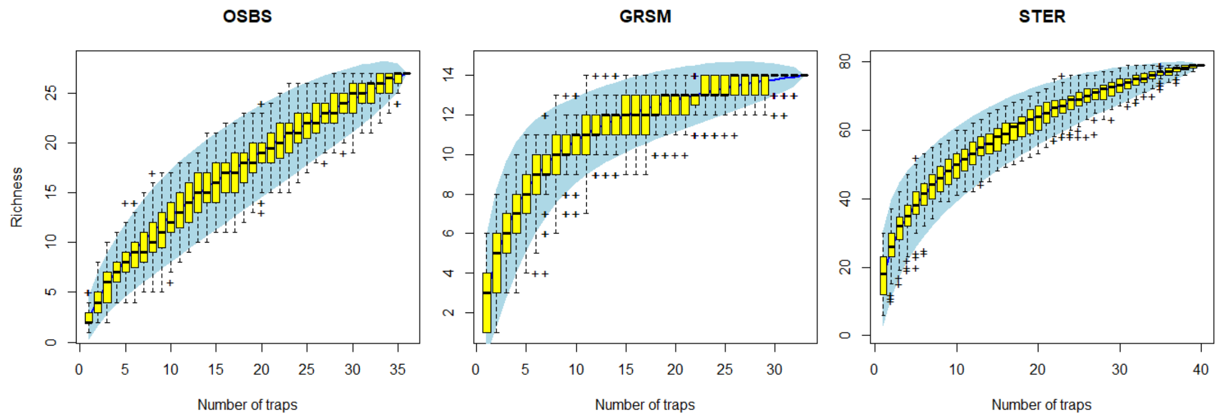

Figure 6. Species accumulation curves at 3 NEON sites: OSBS is Ordway-Swisher Biological Station in Florida, GRSM is Great Smoky National Park in Tennessee, and STER is Sterling in Colorado.

6.5 Temporal Distribution of Sampling

Sampling will take place every 14 days throughout the growing season, when ground beetles are active and likely to be caught in pitfall traps. Every two weeks, specimens collected in pitfall cups will be sieved out of the propylene glycol and transferred into 95% ethanol. After 24 hours, the ethanol will be replaced to ensure low water content in the ethanol used for storage of the sample. Pitfall samples may remain in 95% ethanol for several months after collection until the end of the field season, when the technicians have time to process them. During the field season or subsequent off season, pitfall samples will be sorted, separating ground beetles and bycatch.

Ground beetles display seasonal abundance and diversity patterns. The exact sampling dates will vary among domains based on the length of the growing season. The growing season may vary between years and change over longer time scales. NEON Science may align this threshold with collection of other NEON TOS data based on additional biologically relevant thresholds (e.g., degree days, vegetation index, phenological observation) and the season may be truncated when necessary based on logistical and budgetary constraints.

Adjustments to the Sampling Window

Prior to 2016, NEON beetle sampling was planned around a temperature threshold that would determine the start and end of the field season. From 2013 to 2015, pitfall trapping at a site began when the 10-day running average low temperature was >4°C and end when it was <4°C. However, challenges in scheduling appropriate staffing in the face of a dynamic and unpredictable timeline forced a re-examination of this straightforward temperature gauge. In an effort to constrain the sampling window (for logistical reasons) in an ecologically-informed manner, NEON science staff explored the use of habitat greening to create a more predictable framework for scheduling bouts.

For this effort, NEON staff used MODIS (Moderate Resolution Imaging Spectroradiometer) EVI phenology data from NASA records from the most recently available decade (2001-2009; 2009-MODIS-EVI. NASA EOSDIS Land Processes DAAC, USGS Earth Resources Observation and Science (EROS) Center, Sioux Falls, South Dakota (https://lpdaac.usgs.gov), accessed 2012). For each site, staff determined the Julian date of increasing green-up and the mid-point between decreasing greenness and minimum greenness. The average date of increasing green-up for each site is used as the suggested start for sampling at that site. The average mid-point date between decreasing greenness and minimum greenness for each site is used as the suggested end for sampling at that site. On average, these criteria yield 11 bouts per site (approx. 22 weeks of continuous sampling) at all sites except Domains 04 and 20. These domains feature tropical habitats with greening data suggestive that year-round sampling could be appropriate. In light of budget constraints, a 13-bout window (6 months) of continuous sampling at Domains 04 and 20 is used. Onset of sampling for the D04 window is timed with historical start of the rainy season in southwestern Puerto Rico. The D20 window occurs from April to September to avoid the Winter bird breeding season (recommendation based on site host restrictions).

In 2016, these MODIS-based threshold dates were included in the TOS Protocol and Procedure: Ground Beetle Sampling (RD[04]) as suggested start and end dates. However, field staff continue to monitor temperatures and delay deployment when the listed start date passes if temperatures remain persistently below 4 °C. Likewise, sampling may conclude earlier than the MODIS-based dates if temperatures fall persistently below the 4 °C threshold. As a safeguard against possible discrepancies between MODIS readings and on-the-ground greening and temperatures, domain staff notify the NEON Insect Ecologist if the timing suggested by temperature thresholds would result in initiating or completing sampling more than one month off from the MODIS-derived dates. This information may be used to fine tune future scheduling.

6.6 Logistics and Adaptability

6.6.1 Potential for Changing Physical Locations of Sampling Plots

Spatial patterns associated with ground beetle-related phenomena of interest are likely to change over the course of NEON's lifespan. These changes could include local patterns of distribution, abundance and diversity. A fully fixed plot design in which plot locations within sites do not change has a number of logistic and statistical conveniences but does not allow for spatial flexibility to accommodate/track such changes. One way to capture this anticipated variability is to sample at different distributed plots in different years.

After three years of data collection, we will evaluate the effectiveness of our sampling design in meeting the requirements for ground beetle sampling. At this time, we will consider a sampling with a partial replacement design. Following this design, the locations of a subset of ground beetle sampling plots would be fixed while the location of another subset of plots would be moved to other distributed plots. The number of plots to remain fixed vs. moved would be based on the covariance in ground beetle abundance and diversity between years (Skalski 1990). Higher inter-annual variability would result in a higher proportion of fixed plots. In contrast, lower inter-annual variability would result in a higher proportion of re-assigned plots; in effect, maximizing the amount of information at the site scale by redistributing sampling effort. Fixed plots serve to monitor trends while moved plots gather data from new locations within the area being sampled. Decisions about if and when to change the sampling design will be made in consultation with an expert review committee and the Observatory Director.

6.6.2 Supplemental Trapping Methods

While pitfall traps are the most efficient, versatile and widespread method for collecting ground beetles, their effectiveness at sampling the entire ground beetle community varies between different habitat types (e.g., pitfalls are particularly effective in grassland and boreal forest sites). For this reason, it may be prudent to supplement pitfall trapping with other collection methods at a subset of NEON sites. NEON encourages researchers to conduct supplemental sampling of ground beetles utilizing alternative methods; details on working with NEON can be found on the NEON website.

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