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SPECIAL FEATURE: NEON DESIGN

Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network

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Citation: Hoekman, D., K. E. LeVan, G. E. Ball, R. A. Browne, R. L. Davidson, T. L. Erwin, C. B. Knisley, J. R. LaBonte, J. Lundgren, D. R. Maddison, W. Moore, J. Niemelä, K. A. Ober, D. L. Pearson, J. R. Spence, K. Will, and T. Work. 2017. Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network. Ecosphere 8(4):e01744. 10.1002/ecs2.1744

Abstract. The National Ecological Observatory Network (NEON) will monitor ground beetle populations across a network of broadly distributed sites because beetles are prevalent in food webs, are sensitive to abiotic factors, and have an established role as indicator species of habitat and climatic shifts. We describe the design of ground beetle population sampling in the context of NEON's long-term, continental-scale monitoring program, emphasizing the sampling design, priorities, and collection methods. Freely available NEON ground beetle data and associated field and laboratory samples will increase scientific understanding of how biological communities are responding to land-use and climate change.

Key words: abundance; Carabidae; climate; diversity; global change; ground beetles; long-term monitoring; Special Feature: NEON Design.

Received 1 March 2016; revised 27 July 2016; accepted 28 September 2016; final version received 10 February 2017. Corresponding Editor: Eve-Lyn S. Hinckley.

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Introduction

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform designed to enhance understanding and forecasting of the ecological impacts of climate change, land-use change, and invasive species through the monitoring of biodiversity and ecosystem responses. A portion of the observatory is dedicated to data collection at terrestrial sites that are distributed across the United States and where standardized methods will be used for 30 yr to collect data and samples of the physical and biological environment (Kao et al. 2012, Thorpe et al. 2015). These open access data and samples will enable users (including scientists, planners and policy makers, educators, and the general public) to map, understand, and predict the effects of human activities on ecosystems and to understand and effectively address critical and geographically far-reaching ecological questions (NRC 2001, MEA 2005). National Ecological Observatory Network infrastructure and data are strategically aimed at those questions for which a coordinated national program of standardized observations is particularly effective. Detailed information on the overall NEON design can be found in the NEON Science Strategy document at www.neonscience.org.

National Ecological Observatory Network sampling will provide data at the temporal and spatial scales necessary to facilitate understanding, forecasts, and management of Earth's rapidly changing biosphere (Keller et al. 2008, Schimel et al. 2011, Schimel and Keller 2015). To this purpose, NEON data collection will occur at 47 sites throughout the continental United States, Alaska, Hawaii, and Puerto Rico. Sites are distributed within 20 ecoclimatic regions, termed domains (Hargrove and Hoffman 2004), that collectively span the range of climatic conditions and vegetative communities found within the NEON purview (Fig. 1). Each domain includes one core site (the location of which is fixed for 30 yr, ranging from 11 to 214 km^2 in size) and up to two relocatable sites (the location of which may be reassigned every 7–10 yr over the 30-yr life span of the observatory, ranging from 5 to 50 km^2 in size). Core sites are located in wildland areas to provide baseline measurements of the changing biotic and abiotic characteristics of associated domains. A broad array of measurements and samples will be collected at each site, with all components of NEON data collection (terrestrial, aquatic, airborne, and instrumental) being collocated in order to facilitate the linking of all NEON data in cross-disciplinary analyses. For example, measurements of the occurrences of organisms at plots will be coordinated with the coarser-scale airborne measurements (e.g., the timing of vegetative greening), which will provide a set of synergistic biological data at the regional scale (Kampe et al. 2011). Details of all NEON design elements and algorithms can be found in individual design documents available through the NEON website (www.neonscience.org).

The NEON Terrestrial Observation System (TOS) will quantify the effects of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa as well as causative agents of infectious disease) and biogeochemical cycling within air, land, and water systems (Kao et al. 2012, Thorpe et al. 2015). Sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions, by which occurrence data are extensively available and comparisons may be standardized across the continent. The TOS sampling design also captures spatial heterogeneity of organisms at each site by sampling sentinel taxa across major vegetation types, in order to facilitate inference at regional and continental scales through statistical or process-based modeling approaches (Fig. 2). Early in the conceptualization of NEON, a design committee (AIBSnews 2007) selected ground beetles (Coleoptera: Carabidae) as an ideal candidate sentinel taxon.

Here, we provide the rationale behind NEON's ground beetle abundance and diversity sampling. Ground beetle sampling protocols (available at www.neonscience.org) are based on this design, and therefore, understanding the priorities that underlie the design will inform the use of NEON data. National Ecological Observatory Network's ground beetle sampling will provide a cost-effective and informative measure of a biological response to environmental, climate, and land-use change. These decisions (e.g., frequency of sampling, number of plots per site) reflect trade-offs between (1) scientifically validated collection methods for ground beetles and the need

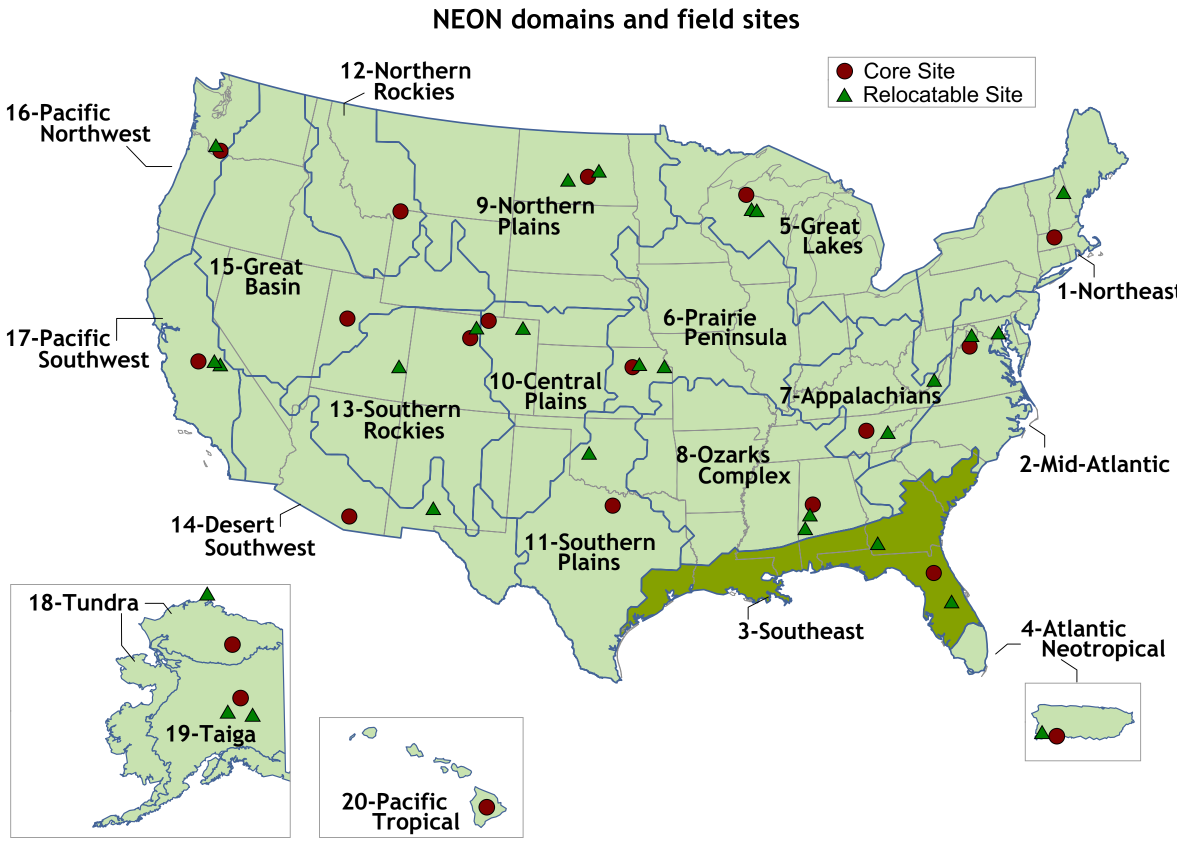

Fig. 1. Spatial hierarchy of National Ecological Observatory Network sampling scheme including 20 domains and 47 sites. A representative domain and its core (red circle) and relocatable (green triangle) sites are highlighted (see Fig. 2).

for robust data; (2) resource availability within the NEON project and local logistical constraints across field sites.

Ground Beetles as a Sentinel Taxon

The ground beetle family is species rich (over 40,000 species described globally, ~3000 species in NEON's spatial extent), abundant, well known taxonomically (Bousquet 2012), and straightforward to sample and identify (Kotze et al. 2011). In addition, ground beetles are widespread and occur across a diverse set of habitat types, ensuring that ground beetle populations are present in virtually all terrestrial habitats (Lövei and Sunderland 1996). As a group, ground beetles are also sensitive to environmental conditions and form well-defined richness gradients (e.g., North American latitudinal gradient). As a result of these characteristics, ground beetles have been extensively used as indicator species (or "sentinels") of arthropod biodiversity, environmental change (Rainio and Niemelä 2003, Koivula 2011), altered land use (Vanbergen et al. 2005), land management practices (Purvis and Fadl 2002, Legrand et al. 2011), and the effects of urbanization (Niemelä and Kotze 2009). 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).

Beyond the value of ground beetles, themselves, as sentinel taxa, they also form an important component of terrestrial food webs and can influence terrestrial trophic structure. Most species are omnivorous and influence lower trophic communities by preying on other arthropods (which most species do, as both adults and larvae) or on weed seeds (Gaines and Gratton 2010). As a

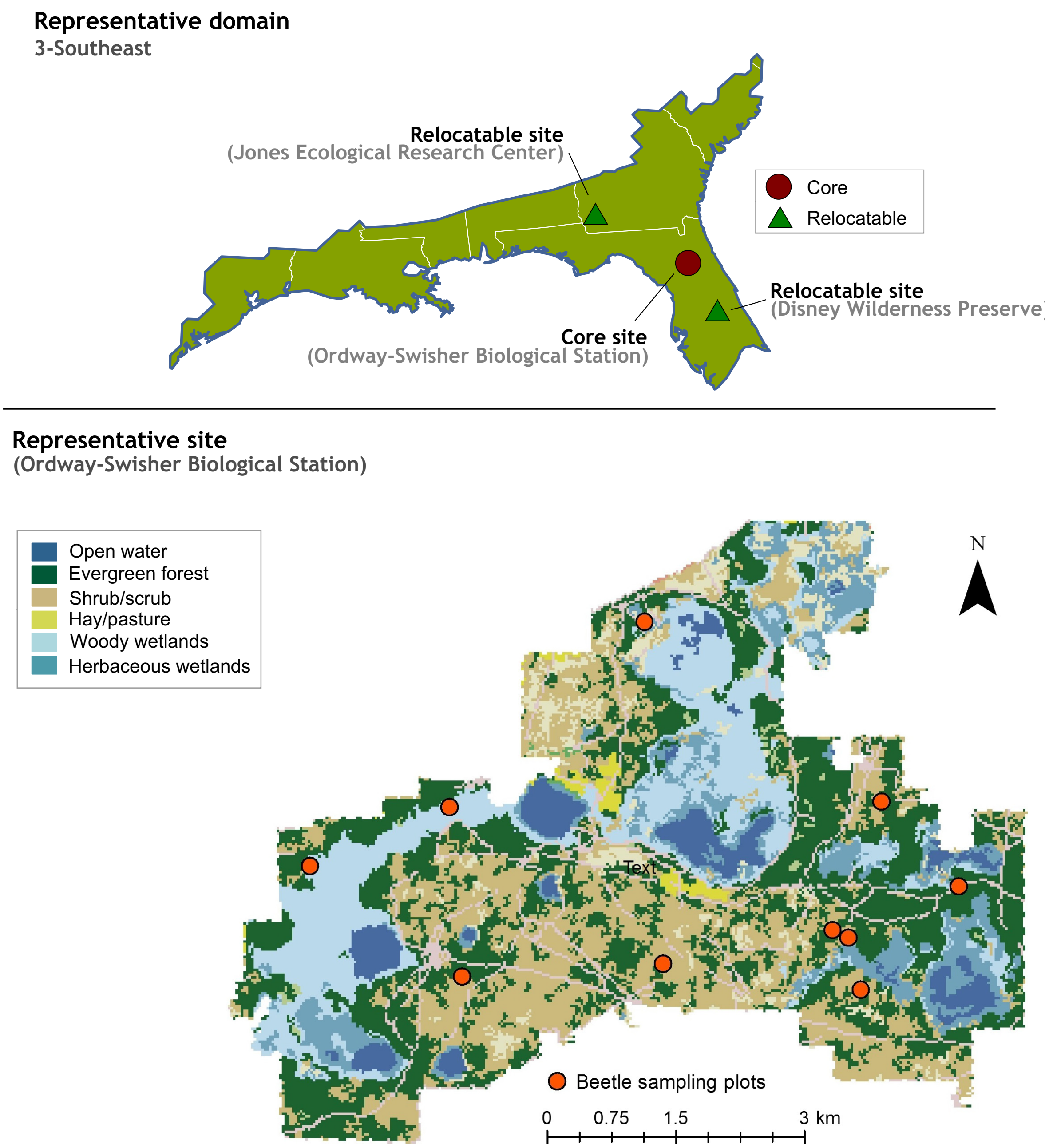

Fig. 2. A representative domain with its core and relocatable sites highlighted, as well as a representative site with potential sampling point locations spread across vegetation types.

result, ground beetles contribute to biological control of pests in both wildland and agricultural settings (Kromp 1999). With regard to higher trophic communities, ground beetles are common prey for small mammals, birds, reptiles, amphibians, and other larger arthropods (Larochelle and Larivière 2003).

General Sampling Design Framework

National Ecological Observatory Network's ground beetle abundance and diversity sampling will target all members of the family Carabidae (ground beetles). Sampling will follow standardized, well-established, and widely used sampling methods that were selected to maximize comparability of data across time, among sites, and with external sampling efforts. The following criteria were prioritized within NEON's ground beetle sampling design: (1) high efficacy across a range of sampling environments; (2) ability to be implemented in a standardized manner across numerous sites that cover a wide range of biotic and abiotic conditions; (3) relatively simple methodologies that can be performed consistently by disparate field crews and over multiple years, with minimal need for alteration; and (4) wide acceptance and use by the research community to increase the comparability of NEON data to data from historical sampling efforts.

At each site, ground beetle sampling will take place at plots, called "distributed" plots, where a number of other measurements are made by NEON. Such sampling collocation will facilitate comparisons of the abundance patterns of different organisms and allow analyses to incorporate site-specific environmental conditions. Measurements of plant diversity and biomass, soil chemistry, and microbial diversity will be collected from the same plot locations sampled for ground beetles. Other measurements (e.g., mosquito, small mammal, tick, and bird abundance and diversity) will be taken in the same vegetation types at each *site*. Coordinating observational and instrumented measurements is a key component of the NEON design.

Trapping Method

Pitfall traps

Ground beetles will be sampled using pitfall traps. Pitfall trapping is a passive collection technique for estimating terrestrial invertebrate species richness and relative abundance and therefore provides a robust 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, a measure that combines both arthropod abundance and movement. Pitfall trapping has been used for more than a century and is still the most commonly used, and effective, method for sampling ground-level arthropods (Kotze et al. 2011). Despite potential drawbacks associated with pitfall traps, this technique still enjoys widespread use within the scientific community largely because pitfall traps represent the simplest, cheapest, and most easily standardized method for long-term studies (Rainio and Niemelä 2003). For this reason, pitfall trapping was chosen over alternative methods for capturing ground beetles, such as sticky traps, malaise traps, ultraviolet light traps, flight intercept traps, sweep netting, hand picking, point counts, fogging, quadrat sampling, or litter washing (Rainio and Niemelä 2003).

All trapping methods contain inherent biases, but the primary bias of pitfall trapping may be a confounding of abundance and activity. Activity varies among species and can be affected by environmental or physiological factors such as temperature, humidity, seasonality, and food availability (Purvis and Fadl 2002). Thus, it cannot necessarily be inferred from pitfall data that species A is more abundant than species B simply because species A is more abundant in pitfall samples—species A may simply be more active and therefore is more likely to be trapped. Because of this activity bias, larger beetles are more likely to be trapped than smaller ones (Spence and Niemelä 1994). Users of NEON carabid data may have to account for these sampling differences before making inferences.

The final caveat associated with the usage of pitfalls is that they are a form of destructive sampling. Many invertebrate sampling techniques are destructive, but most laboratories do not collect data on the spatial and temporal scale proposed by NEON. Non-destructive sampling approaches were considered and ultimately rejected because of the need for consistent and accurate species identifications. One of the aims of the carabid sampling program is to provide diversity data from a diverse suite of species and subspecies (anticipated diversity: 234 genera; 2984 species; 446 subspecies). Field-based identifications of the quality required by models and on the scale of NEON would be literally impossible. However, NEON has attempted to mitigate the negative effects of destructive sampling through trap modifications and the restrictions on sampling within a site (e.g., just 40 traps to sample sites up to 214 km^2 in size).

Fig. 3. A photo of a National Ecological Observatory Network pitfall trap mid-installation. The final design includes two nested cups, flush at ground level. In this photo, the technician highlights the short PVC spacers that elevate the hard plastic cover 1.5 cm above the trap entrance.

Trap specifications

Over a lengthy prototype period, NEON minimized other potential disadvantages of pitfall trapping through specific trap design modifications. National Ecological Observatory Network's pitfall design uses two nested plastic cups (Fig. 3; 11 cm in diameter by 7 cm deep with 473 mL capacity); the outer cup has holes drilled in the bottom and allows for easy reset of the trap, while the inner cup is flush with the ground and collects the specimens. NEON uses a medium-sized trap that has been shown to perform well in comparison with other sizes (Work et al. 2002). Samples are collected biweekly by staff throughout the growing season, and a small amount of preservative fluid (ratio of 50% propylene glycol to 50% water; between 150 and 250 mL) is used to maintain the integrity of specimens between collections. NEON pitfall traps feature an opaque plastic cover that prevents both flooding and desiccation. The design of the pitfall also minimizes the capture of non-target vertebrate taxa; NEON pitfall traps use an odorless preservative, a relatively shallow cup, and a narrow entrance created by positioning the plastic cover 1.5 cm above the cup entrance. The limited ingress and shallow trap depth limits vertebrate capture, while the odorless preserving fluid guarantees that no taxa are actively attracted to the trap. Thus, this trap design best optimizes the capture of target taxa while reducing the risk of data loss or vertebrate impact.

Trap design prototyping

Pitfall trapping is a simple and reliable method of passively sampling ground-level arthropods (Kotze et al. 2011), and all pitfall traps rely on a buried cup maintained flush with the ground, frequently filled with some type of preservative fluid. However, variations on this theme can result in differences in the arthropod community sampled and levels of vertebrate bycatch observed (Pearce et al. 2005, Lange et al. 2011). As a result, substantial testing was performed to determine the optimal NEON pitfall trap design between 2009 and 2013. During this trial period, variables such as trap size, composition, and durability were assessed at 16 broadly distributed sites in Alabama, Colorado, Florida, Massachusetts, Michigan, New Hampshire, North Dakota, Tennessee, Utah, and Virginia. Trap dimensions were finalized after a variety of cup sizes were tested; small cups were deemed problematic because spiders built webs across the cup opening (thereby preventing ground beetles from entering traps), while larger cups presented issues with vertebrate bycatch. A variety of trap modifications to reduce vertebrate bycatch were considered, including funnels, mesh, and hard covers. The best design that balanced vertebrate exclusion with unobstructed capture of carabids featured the use of cover with a low clearance over the trap entrance. Through prototyping, trap durability was improved by replacing metal and wooden components with plastic alternatives. Plywood, initially planned for the pitfall trap cover material, was found to be overly susceptible to rotting and splintering. For similar reason, metal nails were replaced with plastic stakes that resist degradation (i.e., rust). Plastic spacers were also positioned under trap covers to prevent them from sliding down and closing traps. Finally, several specific deployment and equipment issues (e.g., the identification of special tools necessary for specific soil/root/rock substrates) were resolved.

The choice of preservative was also considered during the prototyping of traps. Although some studies entirely forgo the use of any preservative ("dry trapping"), these experiments generally target vertebrates and check traps on a daily basis (e.g., Fisher et al. 2002). Invertebrate studies that collect specimens over a few weeks require the use of a preservative to maintain the integrity of the samples; the preservative of choice is usually propylene glycol and sometimes diluted with water (e.g., Fork 2010, Hogg and Daane 2010). The popularity of propylene glycol is derived from several useful properties, including its evaporation resistance, low surface tension, and lack of toxicity to vertebrates. Probably due to the difference in surface tension, traps that use propylene glycol catch more diverse assemblages of invertebrates compared to water alone (Weeks and McIntyre 1997). In the prototyping process, we found that a 50:50 mixture of propylene glycol to water appropriately maintained fluid levels for the duration of the collection window while collecting carabids from a variety of size classes (body length: 2–35 mm).

Following the design modifications described above, prototyping efforts included an abbreviated field season of ground beetle sampling in the summer of 2012 at three sites in Domain 03 (Jones Ecological Research Center [JERC], Ordway-Swisher Biological Station [OSBS], and Disney Wilderness Preserve [DSNY]; Fig. 2). At each site, up to 40 pitfall traps were deployed for a total of 6 wk from the middle of July through the first week of September (Table 1), with collections of each trap occurring on a weekly basis. An average of two ground beetles were captured per plot in a week of pitfall trapping in Domain 03 (range: 0–27 individuals·plot^{-1}·wk^{-1} for the whole domain; 0–27 individuals·plot^{-1}·wk^{-1} at JERC; 0–5 individuals·plot^{-1}·wk^{-1} at OSBS; 0–27 individuals·plot^{-1}·wk^{-1} at DSNY). The abbreviated trapping season yielded a total of 413 ground beetles, representing 34 unique morphospecies or species (Table 1), and demonstrated the feasibility of the pitfall traps to be deployed at the domain scale (Hoekman et al. 2013).

Special considerations

While pitfall trap deployment may require slight modification in specific locations, the sampling technique is versatile and can be utilized at all sites. National Ecological Observatory Network's standard pitfall trapping design, summarized above, may be altered slightly to accommodate site-specific factors such as substrate, temperature, humidity, and seasonal water cover. For example, preservative levels within traps are occasionally altered from the standard due to site-specific temperature (higher temperatures lead to greater evaporation and thus the need for higher levels of preserving fluids in pitfall traps) and humidity (traps in more humid locations require lower levels of preserving fluid due to decreased evaporation). Likewise, sites with high levels of bear activity may use electrified fencing around the plot perimeter to prevent trap destruction. Permitting requirements and concern over special-status species (e.g., rare, threatened, or endangered species) may also require deviations in sampling regime, such as the checking of traps on a more frequent basis or the institution of temporary plot closures. Finally, the standard location of traps may have to be altered slightly due to localized challenges with standard implementation such as the appearance of seasonal water features (i.e., vernal pools or intermittent streams) or patchy soil substrates. Slight changes in sampling between sites may impact data comparability, but represent the best compromise between a strict application of a standardized protocol across the continent and acknowledged physical or regulatory variation between sites.

Table 1. Summary of ground beetle (GB) data from Domain 03 prototype sampling.

Locations	Total no. GB collected	No. plots sampled†	Avg. no. GB collected·plot^{-1}·wk^{-1}	Avg. no. GB collected·site^{-1}·wk^{-1}	No. GB species recorded
JERC	91	60	2	15	9
OSBS	46	60	1	8	5
DSNY	276	60	5	46	20
Domain 3 total	413	180	2	69	34

Note: Avg., average; JERC, Jones Ecological Research Center; OSBS, Ordway-Swisher Biological Station; DSNY, Disney Wilderness Preserve.

† One-week deployment.

Spatial Distribution of Sampling

Each domain includes three sites, a single "core" site in a wildland location where sampling will occur for the entire 30-yr life span of the observatory, and up to two "relocatable" sites that may periodically be reassigned (i.e., moved within the domain, estimated 7–10 yr per location).

Site- and plot-level sampling

At each NEON site, pitfall sampling will occur at 10 distributed plots with four pitfall traps deployed per plot (total of 40 traps per site). Plots are sampled as sets of four well-spaced traps (Fig. 4; minimum distance between traps within a plot is 28 ± 2 m), as opposed to just one per plot, to avoid data loss due to occasional trap loss or plot disturbance. 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 (Barnett et al., *unpublished manuscript*). The number of plots per vegetation type will be proportional to the percent cover of that type at the site. This stratified approach will benefit the ground beetle sampling because vegetation cover is an important predictor of ground beetle 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 the site's dominant vegetation types.

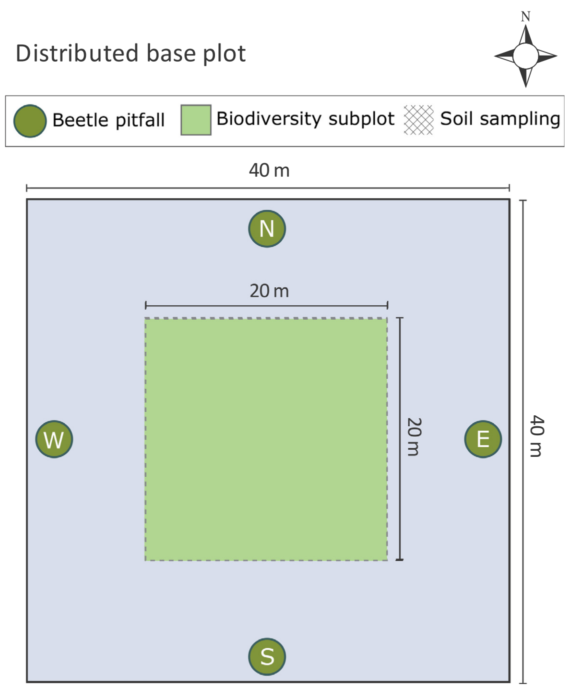

Fig. 4. A diagram of the arrangement of traps within each plot. Four traps (referred to by their cardinal orientation; N for north, etc.) are installed within each distributed base plot at least 25 m apart. This figure also emphasizes the collocation of beetle sampling with other protocols at the same plots.

The location of the pitfall traps within each distributed plot (a 40 m^2 square) will be the same for each site, with each trap positioned on the midpoint of a plot edge. Because each plot is cardinally oriented, these traps are referred to in the data by their cardinal direction for convenience (i.e., the "north" trap; Fig. 4), but the exact latitude and longitude of each trap will also be provided. This organization results in trap placement at least 25 m (but typically 28 m) from any other trap within the same plot. This level of trap replication will provide a sufficiently large sample to characterize the assemblage of ground beetles, including rare species, and is greater than or comparable to the sampling effort employed by other large-scale pitfall trapping schemes (Dufrene and Legendre 1997, Vanbergen et al. 2005, Work et al. 2008, Brooks et al. 2012). Collectively, individuals captured in the four pitfall traps will represent the ground beetle assemblage at the plot level and plots will be far enough apart to represent independent samples of the beetle community at a site level (Digweed et al. 1995).

National Ecological Observatory Network is primarily implementing a fully fixed plot design, in which plot locations within sites do not change. This design has a number of logistic and statistical conveniences. However, sampled plots will be evaluated biannually for efficacy. If any trap cannot be sampled for at least 50% of expected bouts over the evaluation period, beetle sampling may be reallocated to another distributed plot at the site. This will prevent reduced sampling effort due to habitat changes that are incompatible with beetle occurrence (i.e., if a marsh expands and the plot becomes aquatic, there will still be 10 viable plots sampled). Decisions about any changes to the overall beetle sampling design will be made by NEON staff in consultation with an expert review committee.

Temporal Distribution of Sampling

Ground beetles display seasonal abundance and diversity patterns. For this reason, and in order to optimize capture efficiency of seasonally active beetles, ground beetle sampling will occur during the season of greatest vegetative growth (the growing season). Sampling effort will be distributed evenly during the growing season and between core and relocatable sites. Continual sampling in this fashion will generate a time series of abundance and diversity data, and will maximize comparability of ground beetle data among all NEON sites.

Exact sampling dates will necessarily vary among sites and will be based on the length of the growing season as determined by the phenology of the plant community and temperature thresholds (where applicable). At all sites, plant phenology signals (e.g., new leaf production, senescence) will indicate the start and end of sampling. For sites located in northern latitudes, the season may be further restricted and pitfall trapping at a site will not occur when temperatures are below a minimum threshold (when the minimum temperature averaged over the previous 10 d is <4°C) because the activity of surface-dwelling insects is minimal at those colder temperatures. The growing season may also vary between years and change over longer time scales; as the growing season changes, ground beetle sampling window will be adjusted to fully capture activity throughout the growing season.

Sampling Bouts and Sample Collection

Pitfall traps will be deployed continuously throughout the entire growing season to encompass the activity of all ground beetle species at a site. At both core and relocatable sites, traps will be sampled (emptied and re-set) every 14 d. At collection, specimens from pitfall traps will immediately be sieved out of the propylene glycol–water preservative and transferred into 95% ethanol. After 24 h, the ethanol will be replaced to ensure low water content in the ethanol used for longer-term storage of the samples. Pitfall samples may remain in 95% ethanol until sampling is finished for the year, potentially several months after collection, if time does not allow for laboratory processing during the growing season.

Domain Laboratory Processing

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 h of recovery from the field, invertebrates and carabids from pitfall samples will be sorted in the laboratory 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 technicians. The technicians will use a domain-specific voucher collection and available dichotomous keys to identify ground beetles. During construction, a voucher collection will be assembled for each site and this collection will be supplemented with additional specimens that represent morphological variation within and between morphospecies. A subset of technician-sorted carabids will be pinned. Technicians will pin at least 20 specimens from each site, if available, of carabids that are easily identified by trained technicians 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 technicians and taxonomic experts and (2) there is <5% misidentification of that species by technicians. Specimens that are not pinned will be stored in 95% ethanol and archived. All archived materials (i.e., vertebrate bycatch, non-carabid invertebrates, and carabids) will be sent to established collections, the identity of which will be determined based on a future Request for Proposals.

Non-target invertebrates

National Ecological Observatory Network 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

Depending on their size, pitfall traps can capture a wide range of ground-dwelling animals. Larger pitfalls are sometimes used intentionally to survey amphibians, reptiles, and small mammals (Bury and Corn 1987, Hobbs and James 1999), but small traps that are designed to capture invertebrates do occasionally capture small vertebrates, including frogs, salamanders, and shrews. However, 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, 13 sites were sampled between 28 and 154 d 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 to 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).

Taxonomic Verifications and Reference Collections

Funding constraints require that the taxonomic identity of some ground beetle specimens will be solely determined by well-trained technicians 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 (aka 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 vs. identified through DNA sequencing will depend on the abundance and diversity of collected ground beetles and annual funding (with up to 96 individuals sequenced per site per year). Beetles that are rare, particularly difficult to identify, or poorly represented in previous collection events, however, 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 technician-derived classifications in the future. Following that secondary identification, a subset of positively identified beetles will be returned to the domain laboratory of origin and will enhance the voucher collections used by the technicians when making their initial taxonomic assessment. This positive feedback loop will allow technicians 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. The specimens for the NEON DNA sequence reference library, to date, 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 Data System (BOLD, http://www.barcodinglife.com/). Future sequence data will continue to be posted on the BOLD repository throughout the life of the observatory.

Data

Data collected from NEON ground beetle pitfall trapping efforts will be freely available via the online portal (http://data.neonscience.org/home). National Ecological Observatory Network 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.

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

1. Trapping report: the locations, times, and dates of trap setting and collection at each site, and all associated field metadata.
2. 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. Missing records (i.e., fewer than 40 records per bout) indicate a lower level of sampling effort for that bout. Reduced sampling effort may arise due to weather or logistical constraints. The contents 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.

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 (vs. 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 (Niemelä 1993). Data from 2014 (available at http://data.neonscience.org/home) reveal patterns that suggest both spatial (Fig. 5a, c) and temporal (Fig. 5b, d) partitioning of sites among carabid species. At the Smithsonian Institute of Conservation Biology, NEON's core site in Domain 2, two of the most abundant species are found in the same type of habitat (Fig. 5a) but at different times of the year (Fig. 5b). Other species occur together seasonally (Fig. 5d), but do not share habitats (Fig. 5c). 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) 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.

Land-use and community shifts

Carabids are a useful indicator of land-use change because ground beetle communities

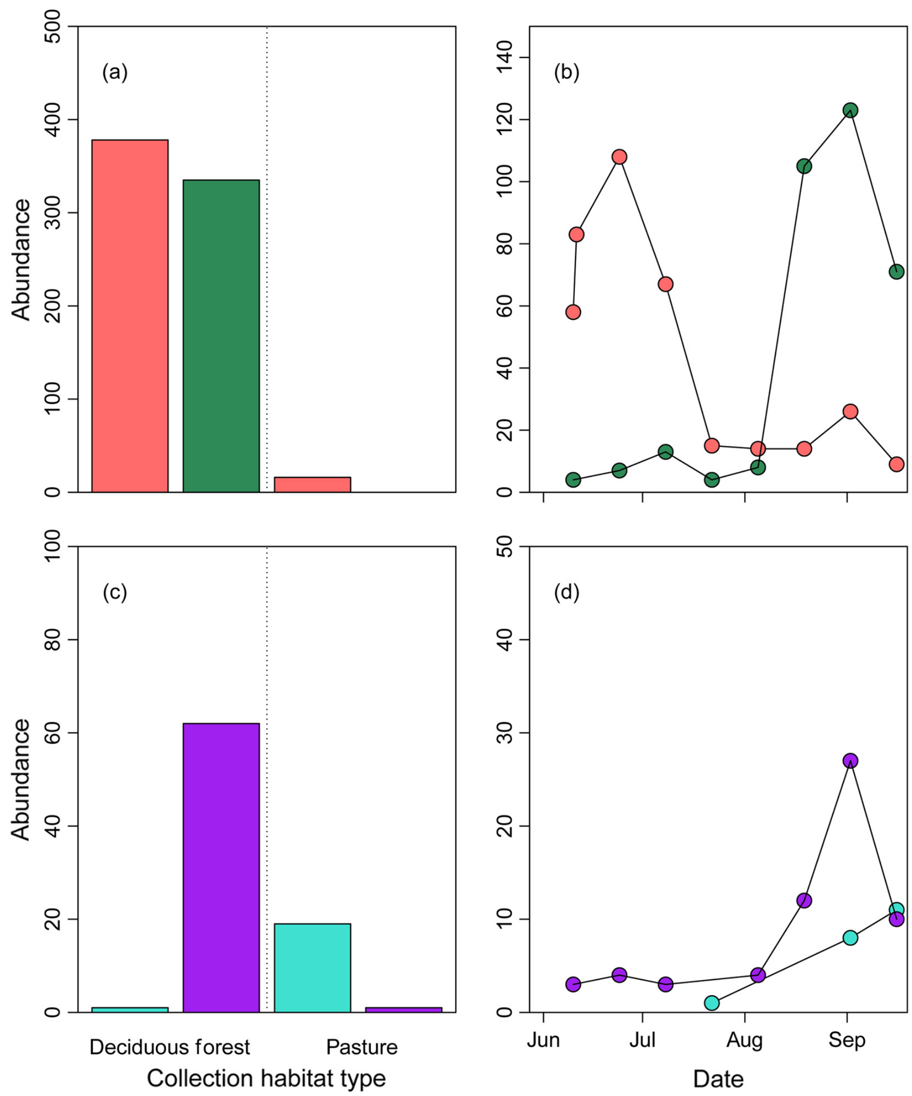

Fig. 5. The total number of *Chlaenus aestivus* Say, 1823 (red) and *Pterostichus coracinus* Newman, 1838 (green) captured (a) within different habitat types and (b) across all habitat types during collection events throughout the field season at the Smithsonian Conservation Biology Institute (SCBI), Virginia. The total number of *Harpalus pensylvanicus* DeGeer, 1774 (blue) and *Pterostichus stygicus* Say, 1823 (purple) captured (c) within different habitat types and (d) across all habitat types during collection events throughout the field season at SCBI.

exhibit high levels of species turnover between habitat types (Do and Joo 2015), and community composition would change noticeable following land-use alteration. National Ecological Observatory Network 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 (Fig. 6a), and species composition. Species assemblages of carabid beetles also varied significantly between domains (PERMANOVA $F = 30.04$, $r^2 = 0.28$, $P = 0.001$), sites (PERMANOVA $F = 14.88$, $r^2 = 0.09$, $P = 0.001$), and habitat types (Fig. 6b; PERMANOVA $F = 4.29$, $r^2 = 0.04$, $P = 0.001$). 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

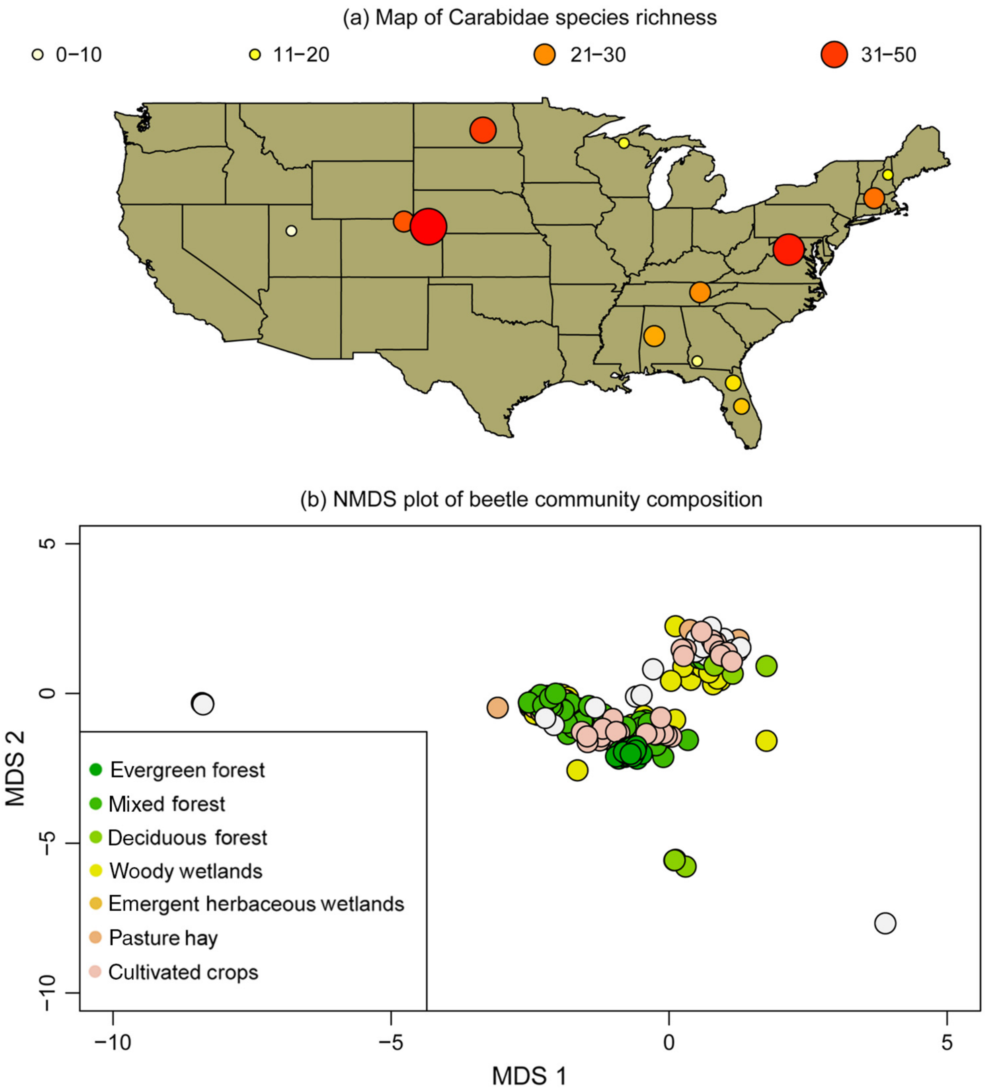

Fig. 6. (a) Observed patterns of Carabidae richness within National Ecological Observatory Network (NEON) domains. (b) Non-metric multidimensional scaling (NMDS) plots show differences in the carabid community at NEON sites in 2014. Ordination points are colored to differentiate National Land Cover Database (2001) class habitat types. Each point represents the NMDS score received by a plot given the total carabid community observed at a plot in 2014 (N = 386 plots from 11 sites).

in the landscape. As abiotic factors at a site (e.g., temperature or precipitation) change, 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 modeling 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 these data might be useful. For instance, *Cicindela punctulata* Olivier, 1790 is a

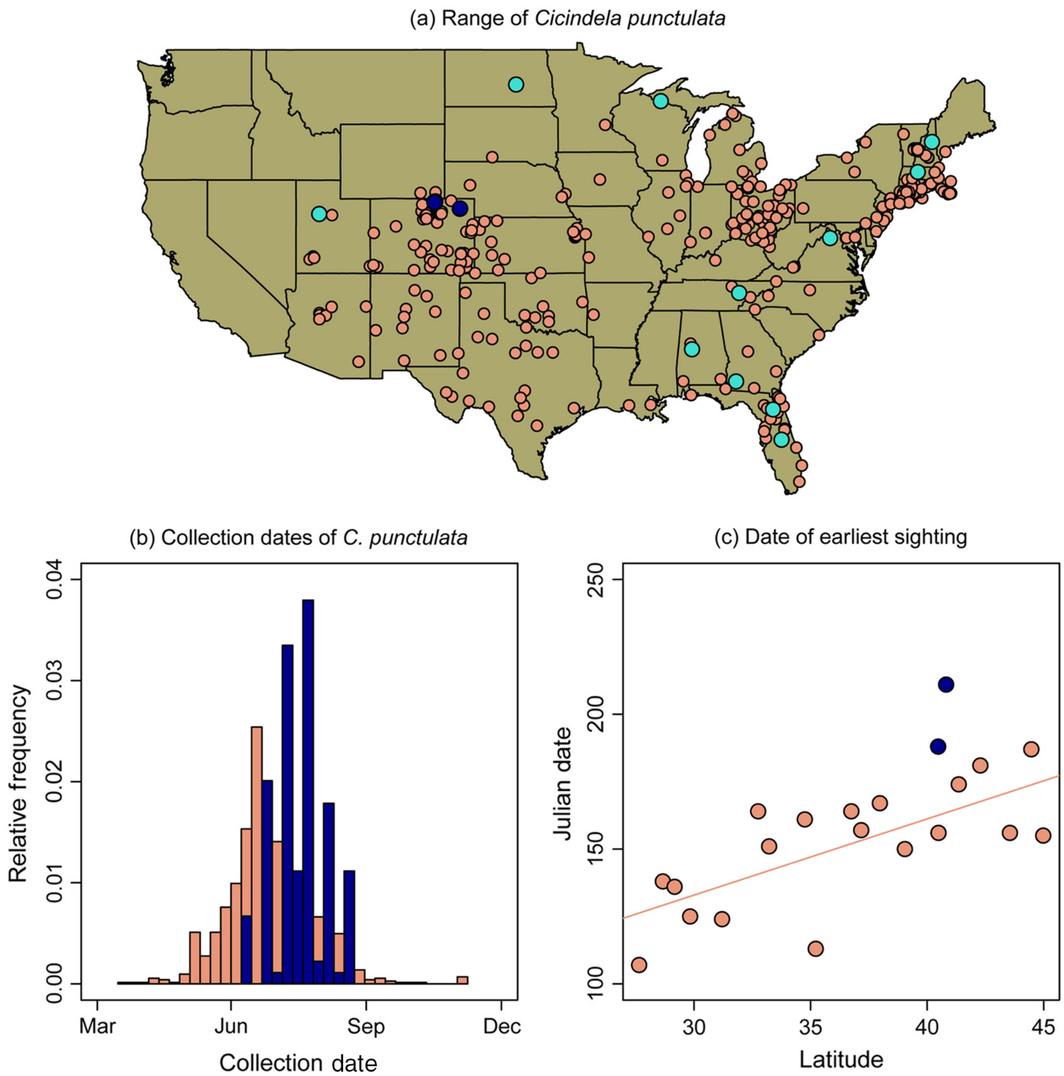

Fig. 7. (a) The known range of *Cicindela punctulata* according to Global Biodiversity Information Facility (GBIF; peach circles). National Ecological Observatory Network (NEON) sampled 13 locations in 2014 for carabid beetles (blue circles) and caught specimens of *C. punctulata* (dark blue circles) 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) follow the same phenological pattern. Points are binned by degree latitude; portion of latitudinal range was only considered if more than 20 records were found in that latitudinal zone.

tiger beetle with a large range throughout the continental United States (Fig. 7a). Collection data from NEON and GBIF indicate that across its range, *C. punctulata* is most commonly collected in the middle of summer (Fig. 7b). However, there is a predictable delay in the date that *C. punctulata* is first sighted based on the latitude of sampling (Fig. 7c), 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 yr 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.

Cross-taxa analyses

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) and soil properties (e.g., soil moisture, organic content, bulk density). National Ecological Observatory Network will generate 30 yr 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 aboveground plants) and the "brown" food web (i.e., the belowground dynamics driven by microbes, plant litter, and coarse downed wood).

Acknowledgments

The National Ecological Observatory Network is a project sponsored by the National Science Foundation and managed under cooperative agreement by NEON Inc. This material is based upon work supported by the National Science Foundation under Cooperative Service Agreement EF-1029808. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors and do not necessarily reflect the views of the National Science Foundation. Use of trade or product names does not imply endorsement by the U.S. Government. The authors acknowledge K. K. Blevins and C. M. Gibson for contributions to early drafts of the design as well as members of the Terrestrial Observation System group at NEON for formal and informal input during the iteration process. Comments from two anonymous reviewers improved the manuscript, and we are grateful to Dr. E.-L. S Hinckley for coordinating its review.

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