

## NEON terrestrial field observations: designing continental-scale, standardized sampling

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**Abstract.** Rapid changes in climate and land use and the resulting shifts in species distributions and ecosystem functions have motivated the development of the National Ecological Observatory Network (NEON). Integrating across spatial scales from ground sampling to remote sensing, NEON will provide data for users to address ecological responses to changes in climate, land use, and species invasion across the United States for at least 30 years. Although NEON remote sensing and tower sensor elements are relatively well known, the biological measurements are not. This manuscript describes NEON terrestrial sampling, which targets organisms across a range of generation and turnover times, and a hierarchy of measurable biological states. Measurements encompass species diversity, abundance, phenology, demography, infectious disease, ecohydrology, and biogeochemistry. The continental-scale sampling requires collection of comparable and calibrated data using transparent methods. Data will be publicly available in a variety of formats and suitable for integration with other long-term efforts. NEON will provide users with the data necessary to address large-scale questions, challenge current ecological paradigms, and forecast ecological change.

**Key words:** abundance; biodiversity; biogeochemistry; demography; disease; ecohydrology; long-term monitoring; National Ecological Observatory Network; open-access data; phenology.

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## INTRODUCTION

Climate plays an important role in changing biogeochemistry, species distributions, and human health (Singh 2010, Thomas 2010, Finzi et al. 2011). Land management, land-use change, and habitat fragmentation are also important drivers shaping species distributions and biogeochemical cycles (Foster et al. 2003). Ecological forecasting and forward-thinking management decisions require knowledge of the factors underlying biogeographic patterns (e.g., Wiens 2011). A growing number of ecological challenges, both theoretical and practical, require knowledge over large areas (Peters et al. 2008). These challenges span the field, from understanding the complex organismal and community interactions governing the spread of invasive species, to the biogeochemical and ecohydrological controls over nitrogen transport from terrestrial ecosystems through rivers to oceanic “dead zones”. While site-based science is required to probe processes underlying such large-scale dynamics, networks of consistent measurements, together with studies of transport and mobility of organisms, matter, and energy are likewise required to integrate those process studies together.

A continental framework is required to enable data assimilation and develop models across large spatial and temporal scales, allowing scientists and decision-makers to address the relationships between these changing drivers and ecological responses (Schimel 2011). While experimental approaches remain critical to understanding fine-scale processes and mechanisms, strategic observations are needed to generate and test hypotheses (Carpenter 2003, Lindenmayer and Likens 2010, Sagarin and Pauchard 2010). It is clear that the next steps to understand large-scale ecological questions require long-term standardized sampling across a broad range of

habitats. Cohesive sampling is necessary to develop and verify novel theoretical models that more accurately predict continental-scale implications of local-scale sampling efforts. The success of 21st century ecology depends upon integration of long-term studies, short-term process-based research, and broad-scale observations (Peters 2010).

The National Ecological Observatory Network (NEON) is being constructed to address the need for new approaches to study the biosphere in the current era (Keller et al. 2008). Large-scale research can advance our understanding of declining global biodiversity not only by providing additional information on their causes, but also by addressing their ecological consequences (Butchart et al. 2010). Long-term observations are required to detect changes due to decadal processes such as climate shifts or the natural turnover of long-lived organisms in addition to capturing important episodic events, such as major storms or disease outbreaks. The long-term observational data gathered across the Long Term Ecological Research (LTER) network provide the impetus to establish cross-site experimental studies of the impacts of climate change, nutrient loading, loss of biodiversity, and invasive species on ecosystem functioning and species dynamics (Michaels and Powers 2011). NEON will join such existing networks in synthesizing long-term data, but NEON is unique in providing a standardized, coordinated sampling strategy across scales and focal taxa. The overarching scientific goal of NEON is to enable understanding and forecasting of the impacts that changes in climate, land use, and species invasions have on broad-scale ecosystem function.

To accomplish this goal, NEON must develop a rigorous modeling framework capable of extrapolating observations from individual sites to regional and continental scales. While much



Fig. 1. Plant productivity data will be collected by NEON technicians at all 60 sites to calculate aboveground biomass estimates, parse productivity data from the fixed instrument towers into vegetation components (trees, shrubs, etc.), and also serve as ground-validation data for the airborne remote sensing. In this image, field technicians are mapping and measuring dominant canopy trees (*Pinus palustris* and *Quercus laevis*) at the Ordway-Swisher core site in North Florida. (Photo by David Barnett.)

of the focus of the construction of NEON has been on the development of sensors and remote sensing, once NEON is operational, the terrestrial field sampling and subsequent taxonomic and laboratory analyses will provide the site based observations and be one of the largest components of NEON. NEON's terrestrial sampling relies on two strategies to collect ground-level data at individual sites. The first is a tactical selection of ecosystem components (e.g., litter, soil, and plants) and taxa with turnover and generation times ranging from hours to decades. The second strategy represents a hierarchy of measurable biological states and processes encompassing diversity (including genetic diversity), abundance, phenology, demography, infectious disease prevalence, ecohydrology, and biogeochemistry. Both ecosystem components and organisms must be analyzed

to capture the temporal dynamics of ecological drivers and responses. To make extrapolations from the individual site to larger scales, NEON will employ three other platforms: (1) an automated, fixed instrument system; (2) an aquatic sampling regime (including experimentation); and (3) an airborne remote sensing system. The integration of these platforms is essential to interpreting and utilizing NEON data. For example, ground data are required to parse productivity data from the fixed instrument towers into vegetation components (trees, shrubs, etc.) and also serve as ground-validation data for the airborne remote sensing (Fig. 1). To take advantage of existing data, the successful NEON design also requires strong collaboration with facilities and study sites that have been partially sampled, extensively sampled, or where gridded information is available.

The implicit value of the NEON terrestrial sampling is the integration of biodiversity and ecosystem studies (Fischer et al. 2010). It consists of several components: a suite of methodologies that can be employed across sites that vary at the continental scale, a field crew trained in NEON standardized field protocols, quality assurance procedures for data collection and sample processing, outsourced laboratory analyses, standardized equipment, handheld instrumentation, and back-end data architecture to assist in data collection at all sites. The terrestrial sampling design is created to bridge scales from individual organisms to populations, and from a single day to multiple decades. It will allow researchers to detect suspected and unknown aspects of ecological change in order to support continental-scale ecological forecasting and management. It will measure significant aspects of populations that are not easily measured with fixed instruments, and it will provide long-term datasets critical to understanding ecological dynamics. One of the challenges facing the terrestrial sampling design is the development and implementation of a scalable, comparable, and standardized framework that satisfies the multiple constraints that vary among sites. Field sampling at this scale is rare and the design is complex due to the requirement that data meet the criteria of the observatory (i.e., long-term collection with consistent methodology, calibration, open-access, and fully documented protocols and data processing).

The NEON terrestrial sampling design will contribute to our understanding of ecosystem responses to drivers of environmental change through extensive measurements of small mammal populations, ground beetle and mosquito abundances and diversity, soil microbial community structure and function, soil physical and biogeochemical parameters, disease vectors, and plant biodiversity, phenology, and productivity within and throughout NEON's 20 designated ecoregions (Keller et al. 2008). NEON will operate in three sites within each ecoregion. Within each ecoregion, one site is a fixed, wildland site and the remaining two sites are not fixed (relocatable) and are generally focused on areas under active management addressing land-use and land-management gradients. This allows for an adaptive design with the ability to

include additional sites over the course of the observatory.

Successful long-term ecological monitoring requires an adaptive design (Lindenmayer and Likens 2010). NEON is implementing this design strategy using successive evaluation cycles for the program as a whole and explicitly through modifications of data products (simple and complex analyses of raw data). The success of observational approaches such as those described here requires keen foresight to anticipate the science questions that these data might address in the decades to come. To optimize the scope of work that would provide useful information to test ecological hypotheses both now and in the future, NEON narrowed its focus to particular areas and taxa. Here we present the design decisions approved by the National Science Foundation during the Major Research Equipment and Facilities Construction review process for the NEON terrestrial measurements in preparation for NEON construction which began in 2012.

## NEON TERRESTRIAL MEASUREMENT SUITES

NEON addresses several key science areas identified in the 2001 National Research Council report "Grand Challenges in the Environmental Sciences". Every NEON measurement is traceable to one or more questions that panels of active researchers considered important during NEON design reviews (see examples in Box 1). All of the measurements should respond to the conceptual model underlying the NEON design: Drivers  $\leftarrow$  Feedback  $\rightarrow$  Response (Keller et al 2008). The following measurement suites are included in the NEON terrestrial sampling: biodiversity and invasive species, biogeochemistry, infectious disease, and phenology.

### *Biodiversity and invasive species*

As the climate shifts, many terrestrial species are exhibiting altered distributions in latitude or elevation at rates that are two and three times faster than previously reported (Chen et al. 2011). In contrast, other species' distributions are not keeping pace with the shifting climate (Zhu et al. 2012). Since species interactions can mediate ecosystem responses, disparate responses to the pressure of changing climate can cause co-

occurring and dependent species to disassociate, and introductions can further compromise diversity by altering disturbance regimes and competition. NEON will allow the linking of ecosystem, climatic, and biotic components to understand and accurately forecast future effects of climate change on biodiversity. A primary set of biodiversity measurements will be conducted to track changes in both the composition and abundance of focal taxa, and the introduction or increase in abundance of invasive species; the latter of which was estimated to cause more than \$120 B in damages and losses in the U.S. annually (Pimentel et al. 2005). Standardized sampling units will be distributed throughout the extent of designated areas within all NEON sites, scaled appropriately for the habitats and taxa sampled (see Box 2 for temporal and spatial resolution of each sampling method). These temporally and spatially explicit soil microbe, arthropod, plant, bird, and small mammal measurements, along with observations from airborne and satellite remote sensing, will allow NEON to provide the necessary data to improve models of species invasions and changes in biodiversity.

#### *Biogeochemistry*

The movement and forms of carbon (C) and nutrients (e.g., nitrogen (N) and phosphorus (P)) into and out of soils and surface waters provide a baseline of information on the overall health and function of terrestrial and aquatic ecosystems (Likens and Bormann 1974). Changes to biogeochemical cycling may lead to positive feedbacks, altered biodiversity, or decreased ecosystem resilience (e.g., Chapin et al. 2002). The NEON biogeochemistry field sampling will complement tower-based eddy covariance and nutrient deposition data, and will focus on the movement of C, N, and P through ecosystems, providing long-term records of nutrient cycling and organic matter accumulation throughout the 20 NEON ecoclimatic regions. Within each site, we will collocate C and nutrient elemental and isotopic measurements of soils, plant tissues, and microbes to provide an integrated dataset that can both inform more detailed field efforts by collaborating researchers, and ecosystem model development, such as the Community Land Model (e.g., Lawrence et al. 2011). Additionally, plant productivity measurements, including

stocks and fluxes of live and dead plant biomass, will quantify net primary production and respiratory losses. Intensive field measurements will be concentrated in the footprint of the fixed instrument towers to provide both a spatial and temporal component to Net Ecosystem Productivity modeling efforts, but less intensive sampling will occur more extensively across each site.

#### *Infectious disease*

Recent decades have been marked by a dramatic increase in the number of emerging and re-emerging infectious diseases, many of which are zoonotic and have the potential to profoundly impact human health (Daszak et al. 2000, Jones et al. 2008). Changes in land- and resource-use practices, global patterns of trade and travel, species interactions, ecosystem functions, and climatic conditions at multiple spatial scales have and will continue to modulate disease dynamics and intersystem linkages (Patz and Confalonieri 2005). In an attempt to characterize these epidemiological changes, NEON will sample pathogens and vector species associated with a variety of mosquito-, tick-, and rodent-borne diseases. The observatory's infectious disease program is predicated on a vector-based approach that will use general methods in an attempt to maximize the taxonomic, ecological, and geographic breadth of sampling. Data on seasonal and interannual variation in vector abundance and infection prevalence, both fundamental aspects of pathogen transmission, will be collected. The NEON design will allow these measures to be linked to the suite of environmental and organismal data collected within the observatory across multiple sites and decades, providing novel insights into the drivers of disease dynamics. These insights will enhance current understanding of disease epidemiology in natural systems, inform disease management strategies employed by public agencies, and spur additional research on the ecology and evolution of host/pathogen interactions.

#### *Phenology*

Phenology is one of the most sensitive and easily observed indicators of biotic response to climate variability (Menzel et al. 2006). Changes in the phenology of plants and animals are consistent with global warming predictions and

**Box 1****Questions that could be addressed using NEON terrestrial sampling**

A special edition of *Frontiers in Ecology and the Environment* (Volume 6, Issue 5, June 2008) provides additional questions one could address with a continental-scale ecological observatory. Two NEON data use cases are included: (1) Soil microbial data and (2) Leaf-area index (LAI) data.

- How do insect communities respond to invasive plant species?
- How is the phenology of breeding bird activity expected to shift in response to climate change?
- How will small mammal demography and disease prevalence change as a function of climate, productivity and insect abundance?
- What will the distribution and abundance of mosquito disease vectors look like in future decades?
- As climate and land-use change alter species distributions, where will competent West Nile Virus vectors and bird hosts begin to overlap or decouple?
- How does the presence of specific microbial functional genes relate to the rates of belowground biogeochemical processes?
- Does changing plant phenology impact the population dynamics of birds, small mammals, and insects?
- How will land-use change interact with invasive species and disease outbreaks to change the distribution, abundance, and life cycle of particular species?
- How do variations in climate affect biodiversity and invasion of pristine and managed ecosystems?
- How do patterns of human population distribution, land use, and water use alter plant biodiversity and ecosystem function?
- What are the impacts of biodiversity changes on ecosystem functions and services?

**Example 1: Informing global change predictions using soil microbial data**

Government leaders rely on predictive models to determine the potential effects of greenhouse gas emissions on society and the environment. However, uncertainty related to terrestrial biogeochemical cycling yields variations that can impact these predictions and political decisions. Gaps in our understanding of the terrestrial carbon (C) cycle have led to predictions that vary by 200 ppm in the projected amount of atmospheric CO<sub>2</sub> and a global temperature uncertainty of 1.5°C (Friedlingstein et al. 2006, Meir et al. 2006).

Much of this uncertainty is likely related to our incomplete understanding of the role of soil microbial biodiversity in terrestrial C cycling. The presence or absence of ecologically distinct groups of soil microorganisms can be more important to predicting ecosystem level nutrient cycling rates than the overall abundance of soil microorganisms. Shifts in fungal:bacterial abundance in soils can result in different extracellular enzyme activities and rates of C mineralization (e.g., Carney et al. 2007). Functionally dividing soil microorganisms into ecologically distinct oligotrophic and copiotrophic classifications can more accurately explain C mineralization rates than soil C quality and decomposition rates alone (Fierer et al. 2007). However, the recent rapid advances in the ability to detect soil microbial biodiversity and function (i.e., high throughput sequencing, metagenomics, metatranscriptomics) have not yet been coordinated with current models of the global C cycle (Chapin et al. 2009). Similarly, all coupled climate models presented in the 2007 Intergovernmental Panel on Climate Change report assume that decomposition is a first-order decay process proportional to the size of the soil carbon pool (Solomon et al. 2007, Todd-Brown et al. 2012). None of these models allows for changes in soil microbial function, despite several studies that indicate that soil bacterial and

**Box 1. Continued.**

fungus functioning will undergo short- and long-term shifts under warmer climate scenarios with higher atmospheric CO<sub>2</sub> (e.g., Carney et al. 2007, Allison and Martiny 2008, Allison et al. 2010, Allison 2012, Cheng et al. 2012).

Including a better assessment of the role of microbial biodiversity and activity in different soil types, and accurately estimating parameters that include shifting microbial function is crucial for realistic climate predictive tools. NEON will provide seasonal and long-term data necessary to determine the soil microbial role in CO<sub>2</sub> production at the continental scale. NEON will coordinate measurements of CO<sub>2</sub> production from eddy flux towers and soil properties at each of the core NEON locations with multiple soil microbial measurements at the ground level. By examining decomposition rates, soil C mineralization, and several levels of microbial biodiversity including: (1) fungal:bacterial ratios using phospholipid fatty acid analysis, (2) oligotrophic:copiotrophic microbial estimates using high throughput sequencing approaches, and (3) metagenomic assessments of heterotrophic and autotrophic capabilities, NEON will provide robust, coordinated datasets to inform, parameterize and validate terrestrial C and predictive global change models (Fig. 2).

**Example 2: Integrating ground and remote-sensing data with models to understand regional effects of drought**

Leaf area index (LAI) is a useful proxy variable for numerous other variables of ecological interest, including plant biomass, plant productivity, forage quality, carbon balance, ecosystem energy flux, plant density, and the heterogeneity of plant cover. LAI is also used widely as a key input variable to models that seek to predict ecological processes such as carbon cycling (Bonan 1993). Regional to continental scale estimates of LAI are typically derived from satellite data, but calibration of satellite data with aircraft and ground-collected data is rare. By leveraging NEON's aircraft and ground-collected estimates of LAI, it will be possible to rigorously develop calibrated, ground-truthed estimates of LAI at the continental scale.

In 2010, NEON measured leaf area index (LAI) at the Domain 3 Ordway-Swisher core site in North Florida using ground-based (Fig. 3A) and airborne remote-sensing techniques (Fig. 3B). Integration of these two types of data streams results in ground-validated maps of LAI at the site scale, and an iterative approach to optimizing the location of ground measurements will help to reduce uncertainty in LAI estimates derived from airborne remote-sensing data. Within the broader context of the network of NEON sites that span the country, annual site-based estimates of LAI can be incorporated into modeling frameworks (e.g., the NCAR CLM) that will enable a better understanding of regional changes in LAI and carbon flux from year to year. The combination of NEON ground and remote-sensing LAI data, such as those shown here, will therefore provide a framework for quantifying regional effects of drought—such as that experienced in the U.S. in 2012—on ecosystem carbon uptake and loss.

linked to local and regional climate change (Parmesan 2006). Forces such as temperature, water and nutrient availability, and the timing and duration of pest infestations and disease outbreaks all affect phenological phenomena. The NEON phenology measurements will record the seasonal progression of critical biological processes and the timing of ecological events by monitoring both focal plant and mosquito

species. The focus on these two groups is due to their relative ease of sampling and their sensitivity to climate change.

**FOCAL TAXA**

To address these grand challenge areas, a NEON design committee selected target organisms for terrestrial observations that represent

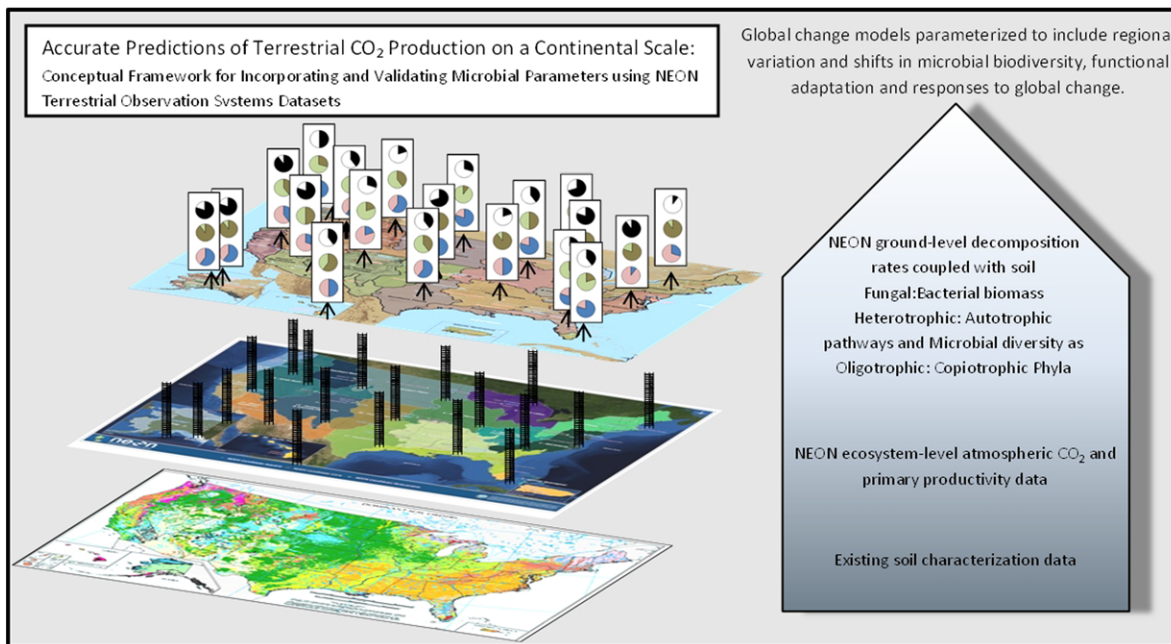


Fig. 2. Conceptual framework for incorporating and validating microbial parameters using NEON terrestrial observation systems datasets. (Illustration by Kathryn Docherty.)

differing generation times, phylogenetic complexities, life histories, and responses to community and environmental changes (AIBSnews 2007). These organisms are widespread in geographical distribution, generally have high population turnover rates, and are thought to be sentinels of change in the environment (see Box 3 for information on focal taxa).

IMPLEMENTATION

Over the past four years, NEON staff scientists, with input from working groups and the external scientific community (<http://www.neoninc.org/about/workinggroups>), have been developing protocols to address these selected measurement suites and taxa. Although many of the specific methods have been used previously, there is no precedent for the scale or degree of integration that the NEON protocols must achieve in order to coordinate standardized sampling across habitats and disciplines. The sampling design should enable spatially and temporally relevant analyses of all measurements, while consistently sampling across measurement suites.

The spatial extent of each terrestrial sampling

site varies from 10 km<sup>2</sup> to 315 km<sup>2</sup> with the primary objective of describing biological dynamics throughout all sites among dominant vegetation and soil types. Although the NEON sites will coordinate with existing monitoring programs whenever possible, the standardization of the sampling design across all sites is critical to the success of NEON and must be maintained across the observatory. A good statistical design is also crucial to the success of ecological monitoring (Lindenmayer and Likens 2010) and NEON has utilized a variety of analyses to develop appropriate sample sizes and strategies. The first version of the sampling design is available ([http://www.neoninc.org/sites/default/files/FSU\\_overview\\_Box3\\_site\\_methods.pdf](http://www.neoninc.org/sites/default/files/FSU_overview_Box3_site_methods.pdf)), but development of this design over the coming years will be an iterative process that will involve external input, analyses of existing and future data variability and error, and modifications based on operational logistics.

The terrestrial sampling will be performed by teams of trained local field crews that are supervised by local scientists specializing in various flora and fauna, and employ protocols that are nationally standardized and quality



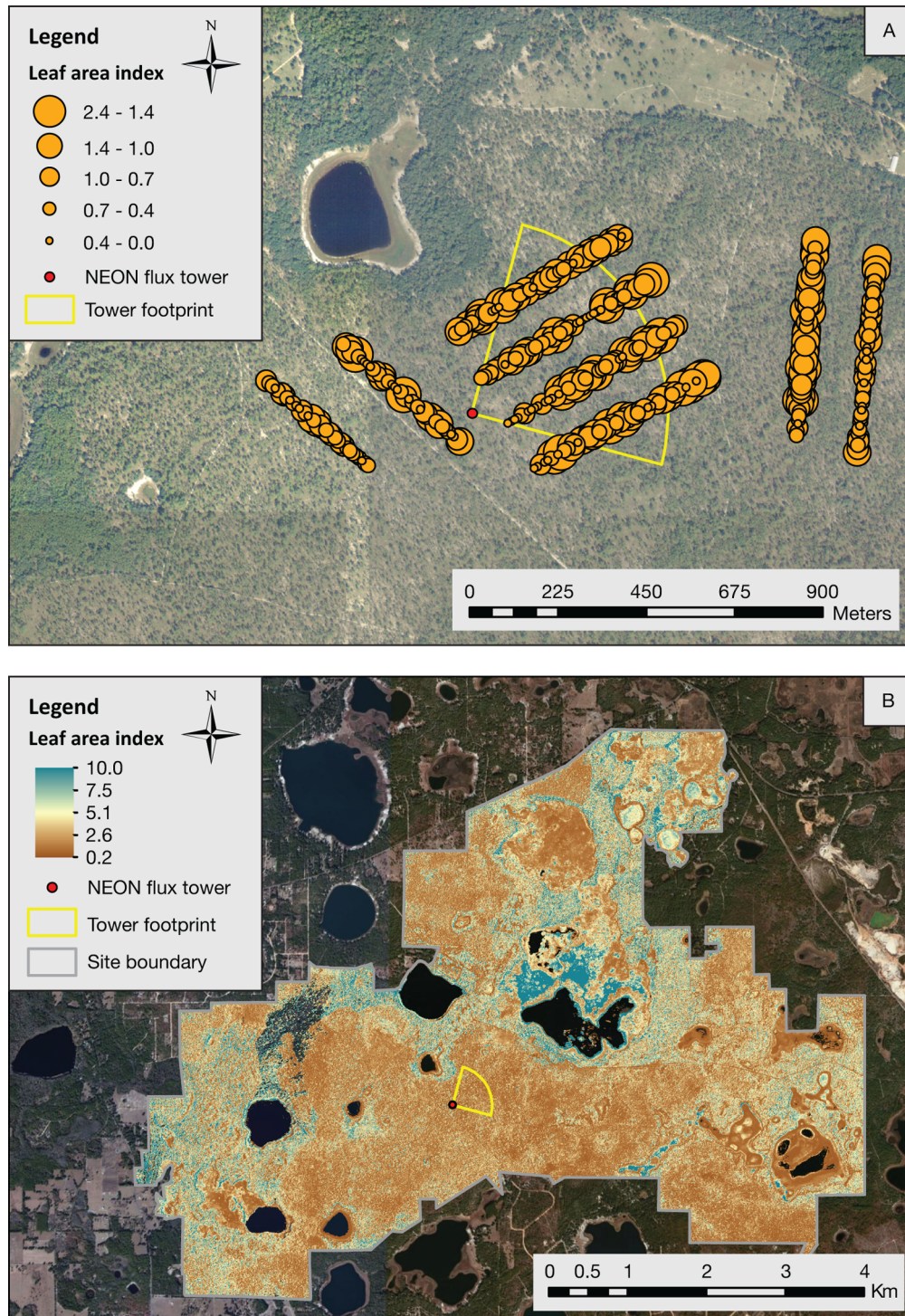


Fig. 3. (A) Leaf area index data collected on the ground with a LAI-2200 instrument within dominant Long-Leaf Pine and Turkey Oak “Sandhill” vegetation at the NEON Domain 3 Ordway-Swisher core site. (B) Leaf area index map for the entire NEON Domain 3 Ordway-Swisher site generated from airborne remote-sensing data.

**Box 2*****Conceptual diagram of sample and data pipeline***

The goal of the observatory is to provide the community with access to the data in both raw and processed formats once quality control is performed. In Fig. 4, light blue arrows indicate samples and dark blue arrows indicate data.

(A) Field samples are collected by trained NEON field crews and subcontractors.

(B) Once samples are collected in the field, additional sample processing (e.g., plant biomass, insect sorting, soil sieving) occurs in one of the 20 NEON Domain field labs. Samples will be shipped using appropriate storage methods (e.g., dry, frozen, preserved in ethanol) from the field labs.

(C) Samples are shipped to external facilities for additional analyses, including genetic, chemical, disease, isotopic, and taxonomic analyses. The following facilities are critical to NEON's goal of delivering data products of known provenance to the ecological community:

- A NEON audit laboratory will maintain quality control for the contract facilities.
- Genetic and genomic analysis facilities are necessary for characterizing the diversity, abundance and functional capabilities of microbes (bacteria, archaea, and fungi) in soils through massively parallel genetic, metagenomic, and other meta-omic sequencing. In addition, genetic facilities will be used for sequencing the DNA barcode marker for verification of a subset of the sentinel organisms. With this method we also gain additional value for phylogenetic and taxonomic studies including building morphological-genetic relationships, identifying cryptic species, and providing a foundation for population genetics and phylogenetic studies (see Gibson et al. 2012).
- A chemical analysis facility is necessary to quantify spatial and temporal variation in collected soil, litter, foliar, and woody tissues. Monitoring carbon and major nutrient (N, P, K, Ca, Mg) biogeochemistry (both totals and labile forms) will be the focus of this facility.
- Disease analysis facilities are necessary to improve our understanding of how the prevalence of infectious agents changes through time within ecosystems.
- An isotopic analysis facility is necessary to measure isotopic ratios of animal and plant tissues, as well as soils. From these data, scientists can glean information on integrated ecological processes in space and time, such as the origin and movement of crucial elements (e.g., N) and substances (e.g., dust) that directly impact ecosystem structure and function, as well as infer significant physiological and ecological processes on the landscape.
- Expert taxonomists are critical for verifying a representative subset of parataxonomist's identifications, as well as identifying taxa that parataxonomists are unable to resolve from the available resources at each of the Domain field labs.

(D) All vouchers and research samples will be curated at externally-contracted collection facilities. The NEON Collection Facilities will hold a curated collection of tissues, whole organisms, genomic extracts, soils, and processed samples from analytical measurements (e.g., chemical, disease, genetic) that can be requested by the community for external research purposes. These collections will be housed in existing universities, museums, and similar institutions, each of which will be amenable to a standard agreement regarding sample, specimen, and information management. Samples and specimens collected during regular, annual sampling will provide a reference collection for future studies of biological change. Replicate samples will be stored in a manner that protects against major loss in the event of a catastrophe and allows for destructive analysis of samples. NEON specimens will be stored at partner facilities but will be centrally cataloged in a publicly-available database and available by request. Digital voucher collections (e.g., photographs of live organisms) will also be utilized as

**Box 2. Continued.**

an archive method that does not require removal of sensitive or rare organisms. The collected samples will provide a resource for researchers to identify organisms, test for the presence of pathogens in archived blood and tissue samples, and perform new isotopic, biogeochemical, and genetic analyses on soil samples as new technologies emerge.

(E) All of NEON’s data (including high-level data products, low-level quality-controlled data, and raw data) as well as sampling protocols, analytical facilities used, and collections in external facilities will be available through the NEON web portal. The data products published by NEON will include raw measurement data and scientific data products from calibrated measurements (including the associated calibration data) through high-level products that combine multiple measurements and may involve considerable assumptions and interpretation (<http://www.neoninc.org/documents/513>).

(F) The NEON samples and data will be public and accessible by everyone equally. In addition to using NEON data to perform statistical analyses, teach classes, and write papers, the user community will be able to request samples from the NEON Collection to perform additional analyses and provide data and models back to the NEON portal for others to view and use. The metadata associated with each NEON datum will also allow the community to propose additional research at NEON collection sites that enhance the data being collected and answer other PI-driven questions (site access and sampling permission is through site landowners and managers, not through NEON). For more information on the development of the NEON web portal, please see [www.neoninc.org](http://www.neoninc.org).

controlled (see Box 2 for end-to-end flow of data and samples). In developing the design, NEON scientists faced challenges such as (1) how to standardize sampling across different biomes to get interpretable and comparable data, and (2)

how best to concentrate sampling to maximize efficiency and use of resources. The protocols continue to be developed and will be modified with both community input and lessons learned from prototyping activities during the design

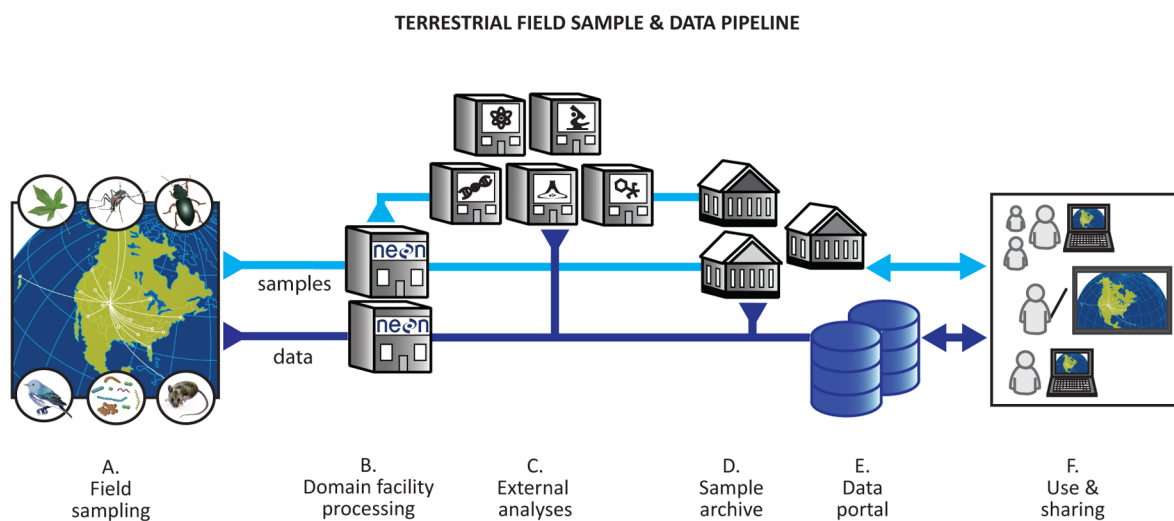


Fig. 4. Schematic of the terrestrial field sample and data pipeline. (Illustration by Cara Gibson.)

**Box 3*****Focal Taxa***

*Soil microbial communities.*—NEON's comprehensive monitoring of ecosystem functions includes measuring shifts in biodiversity, abundance, and activity of the soil microorganisms that catalyze transformations of all macronutrients and drive biogeochemical cycling. Spatially and temporally explicit analyses of soil bacterial, archaeal, and fungal communities will be assessed at all NEON sites, providing local- to regional-scale mapping and monitoring of soil microbial community structure and function at genetic and genomic levels, with archival samples for future analyses. The effects of human impacts such as altered land use, nitrogen additions, and elevated atmospheric CO<sub>2</sub> can cause long-term shifts in soil microbial community structure (e.g., Buckley and Schmidt 2001, Treseder 2008, Dunbar et al. 2012), but current knowledge of resilience and functional redundancy in microbial communities limits predictions of ecosystem responses to losses or additions of species and functional groups. Thus, microbial responses to global change are often oversimplified when incorporated in global change models; however physiological and community-level responses of microorganisms to global change may prove crucially important in the accurate prediction of future climate scenarios (Schimel 2000, Allison et al. 2010). NEON's sampling design, in combination with other massive sequencing projects (e.g., Terragenome Project [<http://www.terragenome.org/>], Earth Microbiome Project [<http://www.earthmicrobiome.org/>]), will provide complementary regional microbial biodiversity and activity data and MIMARKS metadata (Yilmaz et al. 2011) that can be used to inform global change models with microbial parameters.

*Mosquitoes.*—Mosquito (Diptera: Culicidae) sampling will allow analysis of community composition, abundance, phenology, and disease prevalence. Mosquito populations are already exhibiting changes in response to changing climates. Phenological shifts can lengthen disease transmission seasons, which, in addition to distribution changes, can dramatically alter disease spread among wildlife and human populations (Kilpatrick 2011). Currently, range expansions and invasions of non-native species are occurring (e.g., *Aedes albopictus*, the Asian tiger mosquito, is spreading across North America) and are expected to increase with warming climates in temperate latitudes (Bradshaw et al. 2004). Distribution and phenological changes can impact the spread of mosquito-borne diseases within and between wildlife and human populations (e.g., Pascual et al. 2006).

*Ground beetles.*—Ground beetles (Coleoptera: Carabidae) will be sampled at all sites to examine species richness and abundance and these data will address species invasions and biodiversity. Ground beetles have been studied by generations of researchers and their taxonomy, evolutionary history, distribution, ecology, life history, and adaptations are well understood. Ground beetles are generally predators as both adults and larvae, and have top down influences on trophic structure. Additionally, they are common prey for small mammals, birds, reptiles, amphibians, and other larger arthropods and so form an important link in ecosystem food webs (Lövei and Sunderland 1996). Given these factors, their straightforward collection, and their sensitivity to local community differences, ground beetles have been used extensively as indicator taxa (Rainio and Niemela 2003, Kotze et al. 2011, Koivula 2011). Longitudinal studies of ground beetle collections will enable inferences about invertebrate diversity under varying climate and land-use regimes.

*Small mammals.*—Small mammal studies have played a key role throughout the history and development of the field of ecology, particularly in the subdisciplines of behavioral, population, and community ecology (Stapp 2010). From NEON's perspective, species-specific demography and population sizes, prevalence of diseases important to public health, species richness, and

**Box 3. Continued.**

relative abundances can be monitored simultaneously and ultimately linked to land-use and climate changes, and therefore provide useful metrics of responses in biodiversity to these and other drivers. Small mammals as primary and secondary consumers interact significantly with plants and ground invertebrates, and generally represent size classes, life histories, and home range sizes that are distinct from the other NEON taxa. Moreover, they are abundant in virtually all ecosystems, unlike other vertebrates, from harsh deserts to arctic and alpine tundra (Merritt 2010). NEON will use mark-recapture methods to assess the dynamics of diversity and disease across time and space.

*Birds.*—Breeding landbirds were chosen for biodiversity measurements, because breeding birds (1) are generally conspicuous in the landscape either visually or aurally, and therefore most species are able to be observed more easily than most vertebrates using a single passive method (Hutto and Young 2002); (2) utilize the landscape at a larger scale than other NEON taxa, potentially allowing for more rapid responses to change; (3) are primary or secondary consumers; (4) can be indicators of highly-functioning food webs (Hechinger and Lafferty 2005); (5) are both affected by and act as a reservoir for some zoonotic pathogens (Claas et al. 1998, LaDeau et al. 2007); (6) are known to respond strongly to land-use change (Luther et al. 2008). Moreover, the long history of data collection at the national scale (e.g., the North American Breeding Bird Survey, BBS) allows for the integration of NEON sampling into a larger dataset to examine long-term trends (e.g., Bart et al. 1995). NEON will use a more intensive survey method to complement the BBS data to track changes in biodiversity among habitat and land-use types through time.

*Plants.*—The dominant vegetation types at each site will be monitored for changes in biodiversity, invasive species abundance, productivity, and phenology. The vegetation sampling design is guided by three general strategies, each of which employs a unique set of plots. At the spatial scale of the site, biodiversity, invasive species abundance, and vegetation structure will be measured at least annually using plot locations chosen according to a stratified random sampling design. Within the dominant vegetation of the NEON tower footprint, NPP will be apportioned into functional components (e.g., woody production, litter, herbaceous and bryophyte production, fine root production, etc.) via measurements made within randomly located Ecosystem Productivity plots. Finally, a set of validation plots distributed across each NEON site will be measured episodically (approximately every 5 y) to provide critical links between ground measurements and NEON's spectrophotometric and LiDAR remote sensing instruments; the locations of these plots will be chosen nonrandomly in order to span the full dynamic range of key variables also measured via remote sensing (e.g., vegetation structure and leaf area index). To monitor phenology at each site, at least three species per site will be selected with guidance from the community and from the USA National Phenology Network ([www.usanpn.org](http://www.usanpn.org)). The selected species will vary in ecological distribution, life history, and abundance.

and construction periods (2009-2017). All methods, raw data, and processed data will be made available to the public in a timely manner. At each step along the workflow, quality control measures will be put in place to quantify collection error and flag problematic data. All data from the field will be quality checked by

NEON staff scientists prior to public data release. Some field observations (e.g., species abundance and phenology) will be rapidly available to the community following quality control. For other data, some limited, post-field sample processing will occur in the 20 NEON field laboratories (Box 2, B) before samples are sent to analytical

facilities in order to process data for public release. Off-site analyses will be conducted at a limited number of qualified and experienced facilities in order to achieve economies of scale and comparability in measurements (Box 2, C). The NEON audit lab will quality control for data integrity and calibration, which are important for the long-term utility of these data (Lindenmayer and Likens 2010).

## DISCUSSION

Continental-scale observational approaches allow researchers to examine large-scale ecological problems that otherwise cannot be addressed with finer-scale, experimental approaches. NEON's terrestrial field observations will provide the necessary data to understand ecological responses to the grand challenges of biodiversity, invasive species, biogeochemistry, infectious disease, land-use change, and climate change. The design will provide consistent methodology, calibration, open access, long-term collection, and fully documented protocols and data processing.

It is clear that long-term data are required to answer many ecological questions. Data from long-term research at LTER sites are revealing the impacts of climate change on biotic communities and ecosystem functioning (e.g., Craine et al. 2011, Eisenhauer et al. 2012). In addition to studying ecological questions over long periods, many questions require data from a broad range of spatial scales, which can influence the form of the relationship between species density and productivity and the relationship between exotic and native species richness (Gross et al. 2000, Fridley et al. 2007).

NEON's high-level designs have been developed over the past few years and NEON staff continue to develop detailed sampling designs and protocols. As NEON moves into construction and begins working at sites across the country, input from regional experts will be required to ensure appropriate site-specific procedures that follow the higher-level NEON design. Successful monitoring requires not only a good design, but also integration with external partners and dynamic internal leadership (Lindenmayer and Likens 2010). NEON requires well-developed partnerships to maintain appropriate continental-scale data and analyses, in-

cluding those with USDA, NASA, USGS, CDC, and state public health departments. To be successful, NEON must also build and retain strong leadership and scientific staff to produce quality long-term data both during the construction period and throughout operation of the observatory.

Once the Observatory is operational, frequent use of its data will be a metric of success. An important goal of this effort is that the generation of high- and low-level data products and an efficient data system that will facilitate access by the community. Participation and input from the research community on both the broad sampling designs and site-specific protocols will enhance the NEON design, and thus the data available to the research community. To face 21st century environmental challenges, the Observatory and the larger community of scientists must unite to collect data and to develop models that address multidisciplinary questions and allow management of ecosystems at spatial scales that are unprecedented.

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