TOS SCIENCE DESIGN FOR PLANT DIVERSITY

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Change Record

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1 DESCRIPTION

National Ecological Observatory Network (NEON) design documents are required to define the scientific strategy that links the NEON mission and high-level science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than those that limit their use to more local or ecosystem-specific questions. NEON strives to make measurements that enable continental-scale science to address the Grand Challenges. Design Documents flow from questions and goals defined in the NEON Science Strategy document (Schimel et al. 2011), and inform the more detailed procedures described in data product catalogues, algorithm specifications, and protocols.

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 TOS Science Design for Plant Diversity in the NEON Science Design.

1.3 Acknowledgments

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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 Spatial Sampling Design
AD[03]	NEON.DOC.000906	TOS Science Design for Terrestrial Biogeochemistry
AD[04]	NEON.DOC.000907	TOS Science Design for Plant Phenology
AD[05]	NEON.DOC.000914	TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index
AD[06]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling
AD[07]	NEON.DOC.001025	TOS Protocol and Procedure: Plot establishment
AD[08]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass per Area Measurements
AD[09]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
AD[10]	NEON.DOC.000987	TOS Protocol and Procedure: Vegetation Structure
AD[11]	NEON.DOC.014015	Fundamental Sentinel Unit Bioarchive Facility Design

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]		
RD [04]		

2.3 External References

External references contain information pertinent to this document, but are not NEON configuration-controlled. Examples include manuals, brochures, technical notes, and external websites.

ER [01]	
ER [02]	
ER [03]	

2.4 Acronyms

None given.

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], AD[02].

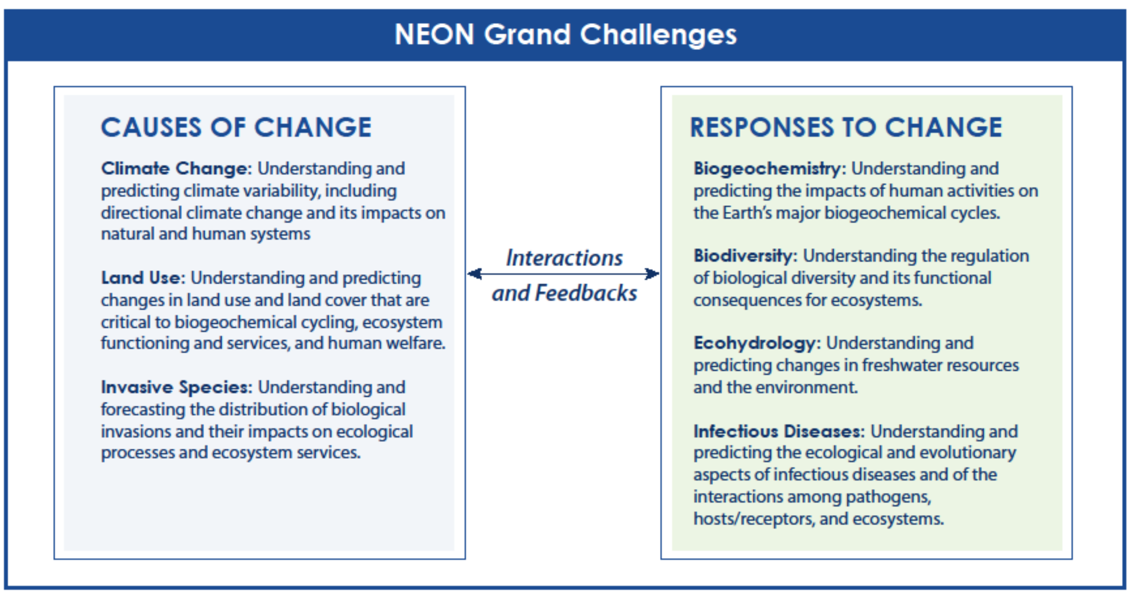

Figure 1. The seven Grand Challenges defined by the National Research Council (2001).

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, 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.NEONinc.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.NEONinc.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 (AD[02]). The TOS Spatial Sampling Design document describes the sampling design that collocates observations of the components of the TOS (AD[03]). TOS Science Design documents were developed following input from the scientific community, including module-specific Technical Working Groups, and the National Science Foundation (AD[02]). 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. Additional information on the development and review process can be found in AD[02].

4 INTRODUCTION TO THE TOS SCIENCE DESIGN FOR PLANT DIVERSITY

4.1 Background

Plant diversity is a multi-faceted concept (Stohlgren 2007). For the purposes of describing a suite of NEON plant observations, this document defines plant diversity to include gene expression and phylogenetic differences, and population, functional characteristics, and species level taxonomic classifications.

Observations of plant diversity have played a central role in the development of the theory and practice of ecology. Darwin documented the distribution of plant species assemblages in his backyard (Magurran and McGill 2010) prior to defining theories that described species interactions and species-environment relationships during subsequent global exploration (Darwin 1859). The science has evolved, furthering the understanding of the processes - mutation, drift, selection, dispersal, speciation, and extinction - that govern the interactions of species and species-environment relationships (Vellend 2010). Investigation of these processes and resulting patterns drive contemporary ecology. Understanding species fecundity, persistence, and distribution dominates population ecology (Clark et al. 2004). Community ecology focuses on the interactions of two or more species and the resulting impact on species composition in time and space (Vellend 2010). Other approaches to studying plant diversity focus on the importance of regional species pools, and the relationship between environmental factors and the distribution, occurrence, and abundance of species (Stohlgren 2007).

Plant diversity is sensitive to changes in climate (Ibanez et al. 2006, Magurran and Dornelas 2010), species invasion (Crall et al. 2006, Barnett et al. 2007), land use change, and disturbance (Dornelas 2010). Paleoecological records demonstrate the influence of shifting climate on species distributions (Wagner and Lyons 2010). Since natural selection is influenced by natural and anthropogenic-induced climate change, species not suited to emerging conditions will be forced to adapt or track change through a combination of dispersal and adaptation to novel conditions and interactions (Clark et al. 2012). Even without directional changes in climate, plant species composition and diversity will change as species invade native ecosystems and alter resource availability, species interactions, and disturbance regimes. Land use may drive the most pronounced changes. Disturbance to the structure of soil and species, changing disturbance regimes, and inputs to systems have direct and indirect impacts on plant diversity (Pickett and White 1985, Pickett et al. 1989). Collectively, many factors influence the direction and magnitude of changes in plant diversity including changes in genetic diversity, species composition and abundance, and distribution and interactions of other species in a complex environment.

Changes in plant species composition will have a reciprocal impact on ecosystem structure and processes such as the cycling of water, carbon, nitrogen, and phosphorous (Hooper and Vitousek 1998, Diaz et al. 2003). Both dominant species and unique species (e.g., nitrogen fixers or invasive species) dominate ecosystem function. The traits – phenotypic characteristics that influence species performance and/or ecosystem function (Grime 1973, Weiher 1999) - associated with these species, such as leaf nitrogen content and canopy height, are important to processes such as respiration that contribute to the structure and the functioning of ecosystems. The rarer species are generally thought to contribute less to the functioning of ecosystems. However, recognizing that systems simultaneously carry out multiple functions, recent field-based experiments provide evidence of the importance of species richness to functional diversity and ecosystem multifunctionality (Maestre et al. 2012). Similarly, a cross-continent network approach found that plant species richness at small scales may not be tightly linked to productivity (Adler et al. 2011). More data are needed to define the relative strength of diversity-ecosystem functional relationships, and to better understand how diversity effects documented in experiments scale to natural systems, across continents (Cardinale et al. 2012), and through time.

4.2 NEON's Contribution

The NEON design will measure many drivers and responses of ecological change through time, the variability in ecological trends at sites across the United States, and provide data that will allow researchers to tease apart the causes and consequences of these trends.

- Observations at the scale of the continent: With standardized protocols implemented by highly-trained field-technicians at sites across the United States, NEON will observe a consistent suite of plant diversity and composition variables. In addition to facilitating a cohesive understanding of the trajectory and magnitude of trends (e.g. changes in abundance of dominant species) at sites across the United States, the design will make a significant contribution to synergistic efforts to scale patterns observed at local scales to the extent of the continent. Generating data that is directly comparable across NEON sites and to other collections databases such as the US Forest Service Forest Inventory and Analysis observations, invasive plant species databases, extant vegetation plot databases (Peet et al. 2012), and other plant species (Stohlgren et al. 2005, Harrison et al. 2010) and plant functional databases (Kattge et al. 2011) will create opportunities to scale observations and predictions to regions and the continent.
- Long-term observations: The collection of long-term, consistent observations will inform temporal patterns and prediction of future trends (Stohlgren 2007). Analysis of the turnover of genes, individuals, species, and traits, at a variety of scales can provide insight into factors associated with species interactions, distributions, and relationships with abiotic factors. Emerging statistical techniques assimilate data from experiments and observations to infer temporal change and generate predictions that can be tested and improved over time (Read Hooten et al. 2003, Cressie and Wikle 2011). Their application to the NEON data streams will all allow insight to the factors associated with systematic, long-term change (Dornelas et al. 2013). That understanding can be challenged by the need to distinguish anthropogenic and natural drivers of change from stochastic influences, and the confounding interactions of spatial and temporal autocorrelation. Until now, the paucity of consistent, long-term observations has hampered predictions of plant species diversity in time and space.

- Integrated data collection: Coordinating the collection of plant diversity observations with other terrestrial, aquatic, ecosystem, and airborne measurements provides the opportunity to explicitly understand the causes and consequences of changes in plant diversity (Stohlgren et al. 2006). Co-locating consistent measures of the drivers of ecological change and a wide variety of the organisms and energy, carbon, and nutrient pools across a variety of substrates and environments, will expand a working understanding of ecological processes at scales important to the maintenance of the various components of plant diversity and ecosystem structure and function (Chapin et al. 1997, Luo et al. 2011).

Consistent, long-term observations across large spatial extents will provide a research opportunity not previously available using traditional ecological approaches. Many complex ecological processes and relationships manifest at time scales that exceed traditional funding cycles, and site-specific investigations often prove to be case studies with little power to contribute to a generalized understanding of factors and mechanisms that govern large-scale patterns.

Understanding why and how patterns of plant species richness vary in space and time has long been of interest to science (Darwin 1859, Gotelli et al. 2009). The relationship between the factors that control patterns of richness at global, regional, and local scales (Palmer 1994, Fridley et al. 2005), and how those controls change across differing scales of time and space (Cressie and Wikle 2011), helps explains aspects of the existing landscape. Predicting these shifts is challenged by uncertainty associated with the rate, extent, and influence of the drivers of change (Woodward and Kelly 2008), as well as uncertainty regarding differential ability of species to track change (Parmesan 2006), the reassembly of novel communities (Clark et al. 2011, Clark et al. 2012), and the models most appropriate for understanding and forecasting these patterns (Elith et al. 2010). NEON data will not resolve all of these questions in isolation, but it is becoming increasingly clear that spatio-temporal ecological patterns and processes are inextricably linked, and studying them simultaneously will greatly advance science (Fridley et al. 2007, White et al. 2010, Luo et al. 2011).

4.3 Purpose and Scope

The NEON design evolved from the need to answer scientific questions that relate pressing challenges in environmental science (National Research Council 2001), are relevant to large areas, and cannot be addressed with traditional ecological approaches (Schimel et al. 2011). These questions, in turn, defined a series of requirements and associated observations capable of detecting and quantifying the impact of disturbances and changing environments on plant diversity.

The design for observing plant diversity is constrained and guided by the high-level NEON requirements (Schimel et al. 2011). To support NEON's objectives, plant species composition, abundance, functional traits of select species, and material for genetic analysis will be observed, measured, collected, and archived for thirty years at sixty sites across the United States.

The data will be collected by plot-based sampling, by targeting selected species, and with remote sensing techniques. The remote sensing platform that includes hyperspectral imaging, light detection and range radar (LiDAR), and digital photography at sub-meter resolution will provide spatially continuous observations of species, functional groups and traits, and individuals from the 1000 m above the vegetation. The collection of material for genetic analyses and functional traits will target specific species measured for multiple aspects of the NEON design (e.g. phenology and biogeochemistry).

5 SAMPLING FRAMEWORK

NEON will enable understanding and forecasting of the impacts of climate change, land-use change, and invasive species on continental-scale ecology by providing infrastructure and consistent methodologies to support research and education. The traceable links between this high-level NEON mission statement and the raw and integrative plant diversity data products provide direction and constraints for the plant diversity design. The design is part of a hierarchical cascade, constrained by “upstream” requirements and the “downstream” need for plant biodiversity data products. These multiple objectives constrain the proposed design.

A well-defined sample design must articulate several quantitative and qualitative considerations (Cochran 1977, Lindenmayer and Likens 2009, Gitzen and Millspaugh 2012):

- **Plant diversity objective:** Observations are designed to inform the causes and consequences of changes in spatial and temporal patterns of plant diversity to support a continental-scale ecological observatory network.
- **Quantified sampling objectives:** Nominally, sampling must determine annual rates associated with the change of plant species composition, abundance, and richness at the spatial scale of a NEON site. Measurements are required to meet an overall uncertainty of 10-20% of the mean in the annual time scale to allow detection and quantification of most trends over the 30-year time span of NEON (Schimel et al. 2011).
- **Data to be collected:** Plant species presence and abundance will be recorded in multi-scale vegetation plots. Functional traits, demographic information, and material for genetic analysis will be sampled from targeted populations.
- **Population to be sampled:** A statistically rigorous sample design provides a framework for sampling plant species presence and abundance (Schimel et al. 2011). The target population is the extent of the NEON site.
- **Sampling frame:** The spatial extent of NEON sites bounds the area available to sampling plant species (Reynolds 2012). Typically sites were initially defined by the location of the tower-based sensor measurements and the terrestrial organismal site corresponds to associated management or ownership boundary. NEON sites range in size from small urban landscapes (e.g., domain 15 Murray 0.03 km^2) to wildland sites (e.g., domain 14, Jornada 780 km^2). In a few cases, the area available for sampling was too large to be reasonably sampled given budget and travel constraints. In these cases, primarily large national parks, a site was defined by a sample

frame according to the location of other NEON measurements and large scale NEON science questions (Schimel et al. 2011).

- **Intended Analyses:** Analyses will largely be carried out by the members of the ecological research community according to the specific questions they choose to ask using Observatory data. Plot-based sampling according to a probability-based design allows the use of variance estimators that allow inference to the unsampled population (Cochran 1977, Thompson 2012), allows for a variety of model-based approaches to inference, and avoids optimization for a particular organism or analysis (Reynolds 2012).

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. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (AD[04]).

5.3 Priorities and Challenges for Selection of Sampling Methods

Designing effective plant diversity field studies is challenged by taxonomy, detectability of species, multiple sources of error and bias, and the spatial and temporal scale of measurement. The design specifications address these challenges with specific methods, training, assessments, and metrics that will allow subsequent analysis to quantify error.

Taxonomy is incredibly diverse and not always well or consistently defined. Naming conventions that differ through time, regions, and by investigator result in a single plant species with multiple names, and those same names may have been applied to multiple plant species. NEON will maintain a standardized taxonomy by adhering to standardized lists (e.g. USDA PLANTS) and maintaining voucher specimens of the majority of the species observed during the life of the Observatory. Additionally, recruiting and retaining well-trained and qualified field botanists may be difficult, but investing in taxonomists and contributing to the training of the next generation of botanists will be essential to the implementation of plant diversity sampling throughout the life of the Observatory.

Data comparability is important to many aspects of a design intended to detect systematic change across time and over a large extent. To compare plant diversity within and across NEON sites, the design and data must allow for a standardization of sampling effort. Similarly, to scale the NEON effort beyond a comparison of isolated sites across the United States, the design must collect data that is comparable to other efforts. For example, plot-based sampling should allow comparison to other sources of plot data, and functional trait collections must meet minimum standards outlined by extant trait libraries.

Identifying and quantifying the sources of error and bias associated with the design is important to trend detection. Sources of error will include, but not be limited to the misidentification of species, bias associated with human interpretations of herbaceous species abundance, differing detection probabilities, instrument error, data processing and management errors, and inconsistencies in physical measurements such as diameter at breast height and measurements of leaf size. Error, especially in a long-term and large-scale observatory, cannot be avoided but should be managed by automated quality control algorithms. The impact of error can be estimated with calibration and validation procedures, and will be reduced through quality control and assurance measures, marking of plots and individuals that will be resampled, and a rigorous requirements framework that includes explicitly detailed protocols and a continental-scale program to train collectors of NEON data.

6 SAMPLING DESIGN FOR PLANT DIVERSITY

The design directs the observation of plant species presence, diversity, and abundance and the collection of plant material for genetic analyses. The plant diversity observations and resulting data products will be coordinated with plant phenology, structure, demographic and biogeochemical observations through the collocated sampling of individuals, species, and plots. Phenological transitions of individuals from multiple species will be observed (AD[05], AD[10]), material for reference and genetic analysis from these individuals and individuals of the same species from plots distributed across NEON sites will be collected and available to the community (see section 6.1). Demographic and structure characteristics of individual plant species will be monitored (AD[06], AD[11]), and plant material for foliar chemistry and isotope analyses will be collected and analyzed (AD[04], AD[09]). Additionally, many of these data products available capture functional traits of species (e.g. canopy height, leaf mass per area). The integration of these data products will provide the ecological community with a unique data set capable of addressing new questions and insight through space and time.

The primary plant diversity data products result from plot-based observations of plant species presence and abundance. These observations will describe the relative abundance of species, the density of woody plant species, species richness, and allow the derivation of various estimates of plant species diversity. Components of plant species diversity also will be observed with NEON's airborne observation platform (AOP). Imagery obtained by hyperspectral reflectance can provide proxies for plant diversity, and unique spectral signatures of species allows for a complete census of dominant species distributions at landscape (Schimel et al. 2013) scales.

6.1 Sample Design for Genetic Collections

NEON will collect and curate foliar material for analysis of genetic diversity (AD[12]). The collection of plant material for genetic analysis will allow sequencing of DNA barcode markers for species verification. Additionally, genetic analyses provide information useful to phylogenetic and taxonomic studies including building morphological-genetic relationships, identifying cryptic species, and providing a foundation for population genetics and phylogenetic studies. A collection facility at an existing university or museum will hold curated tissues that are flash dried and stored at -80 degrees Fahrenheit. The material will be available for principle investigator-driven research and questions.

Material from a subset of species at each site will be collected and stored; it is beyond the scope of the NEON effort to collect material on every species documented in plant biodiversity studies. To strengthen ties with other components of the NEON design, plant tissue will be collected from species targeted for phenological observation and foliar biogeochemistry measurements. The phenology effort will observe twenty species representing a diversity of functional groups and relative abundances in a localized area near the NEON flux tower. The biogeochemistry component of the NEON design will measure leaves from the three or four co-dominant species at distributed plots across the landscape.

The collection will balance tradeoffs between intra and interspecific diversity. Tissue from thirty to forty individuals of each species targeted for sampling will be collected. Material can be collected from each of the species to be observed as part of the phenology effort. Additionally, a sample will be collected from species sampled for biogeochemistry at each plot across the landscape.

6.2 Sample Design for Plot-Based Sampling of Plant Species Diversity and Abundance

A complete census of every species across each NEON site would be nearly impossible and a misguided use of resources. Plot-based sampling offers a repeatable way to sample the landscape. The sample design directs the distribution of plots across the local NEON site or sampling frame to bound plot-level observations.

6.2.1 Sampling Methods

6.2.1.1 Field Sampling

There is little in the way of a standardized protocol for measuring plant species diversity (Stohlgren 2007). A method that samples at multiple spatial scales allows for an improved understanding of processes operating at different spatial extents, addresses problems associated with detectability of species (especially at small scales), can facilitate an understanding of local and regional patterns of diversity, and provides data to validate airborne observations at multiple grain (i.e. pixel) sizes. Tradeoffs between plot size and number necessitate a choice of between understanding local and landscape-scale patterns (Barnett and Stohlgren 2003). Large plots capture a greater number of rare species at the plot scale, but do so at the expense of an improved understanding of variability across the landscape.

In addition to the efforts to standardize sampling effort (see "Sample allocation" above), comparability across NEON sites is crucial to furthering understanding at large spatial scales. Deploying the same plot in very different vegetation types may not be optimal with respect to cost savings and local information content, but a multi-scale approach allows for a consistent, baseline plot size, while also allowing the plot to be expanded in some ecosystems if necessary – capturing diversity of large-stature, well-spaced tree species for example. The plot should also provide data comparable to other networks recording plant diversity like the Nutrient Network (Adler et al. 2011) and the US Forest Service Inventory and Analysis Program (Stolte 1997). Additionally, plant diversity should be sampled in a way that maximizes the potential of the NEON remote sensing package. The area-based (pixel) information from these sensors suggests the use of a plot-based measurement versus frequency measures derived from point-sampling methods (Stohlgren 2007).

NEON will sample plant species with a multi-scale plot design developed by the Carolina Vegetation Project. NEON will sample a 20 x 20 m square plot comprised of four modules with nested subplots in each module (Figure 2, AD[07]) Information specific to NEON data products is collected as follows:

- Species composition, the species present, will be recorded in each subplot and across each module.
- Estimates of herbaceous abundance are made with ocular estimates of cover at the 1-m^2 and 10-m^2 subplots.
- The abundance of woody species will rely on protocols developed for characterizing plant productivity and structure (AD[10]). The location and size of each woody species will be recorded across each 10 x 10 m module. Abundance could be estimated by basal cover, canopy cover, or estimates of total biomass by individual, by species or by plot.

The co-location of NEON observations at the plot scale requires that plot shape and size determinations are not made in isolation. The plant productivity and biogeochemistry (soil and foliar) study designs favor modules of the Carolina Vegetation Project because size is easily increased, and the square module provides a more direct link to the airborne observations. While it will be difficult to directly associate the composition of herbaceous species at a small 1-m^2 subplot with an exact remote sensing pixel, this will be possible at the scale of a 10 x 10 m module. Furthermore, the characteristics of specific tree species mapped in plots will be directly related to imagery in an effort to calibrate LiDAR and develop spectral libraries of species.

Obtaining accurate estimates of species abundance is both difficult and critical to the design. For example, in many cases the composition of dominant species may not change over the life of the Observatory, but shifts in relative abundance of species and associated plant functional traits could have implications for ecosystem function. Woody plant species abundance will be calculated according to stem mapping protocols that will record basal area, canopy diameter, size and density. As currently designed, herbaceous species abundance will be recorded with ocular estimates of cover at subplots nested within the larger plot. Two points worthy of consideration emerge from this design:

1. If a metric other than cover is used, there will be two fundamentally different data types that will require the development of two different species abundance models.
2. An alternative to occular estimates of cover for estimating herbaceous plants is to use a point-intercept method for frequency data. In theory this method could resolve the disparate data type problem. Proponents of the intercept also argue that it is more repeatable in time and space. However, in practice it is difficult to make determinations about the intersection of canopy species, the method is biased toward broad-leaved herbaceous species, and it takes longer to implement than estimating abundance with ocular estimates.

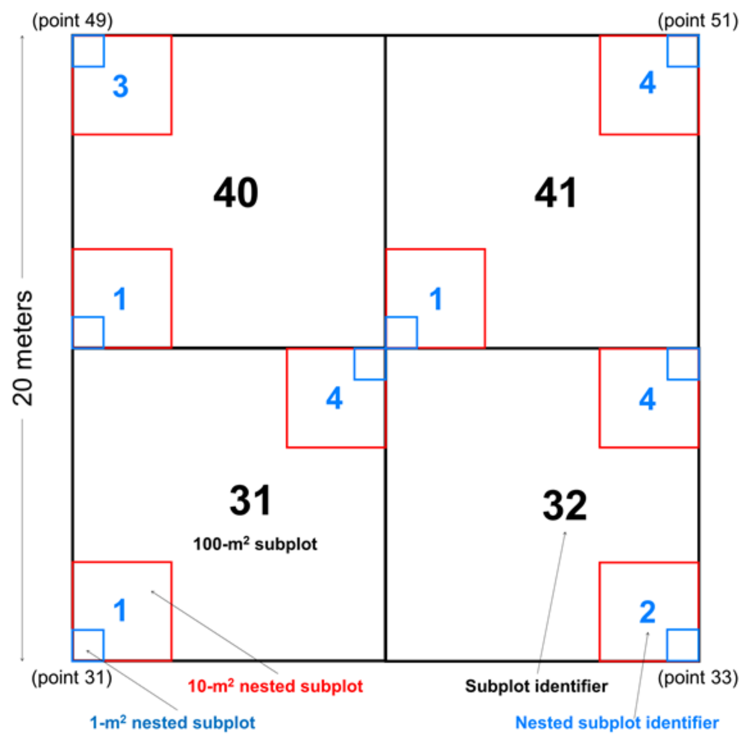

Figure 2. A schematic of the multi-scale plot for sampling plant species diversity and abundance.

6.2.1.2 Data Analysis

Analysis will largely be carried out by the ecological community. A goal is to collect data according to a design robust to a variety of estimation and modeling techniques (Sarndal 1978, Read Cressie et al. 2009). Design-based inference requires data collected according to a probabilistic design (Reynolds 2012). Various modeling approaches might benefit from the collection of data according to specific stratification or gradient, but most can also ingest data based on principles of randomization. The design-based estimators associated with the NEON design were developed but are discussed elsewhere (AD[03]).

6.2.1.3 Voucher Specimens

NEON will create a physical record of species recorded in plots. Herbarium specimens will be stored at NEON offices for training and validation purposes, as well as sent to museums or other storage facilities as part of the NEON bioarchive program (AD[12]). The archive provides a physical record of NEON taxonomic definitions and will support a variety of alternative research questions over the life of the Observatory (Kao et al. 2012).

6.2.2 Spatial Distribution of Sampling

The sample design for observations at local, site-specific scales must deliver data that optimally informs continental-scale ecology. Adopting the requirements framework provides traceability to elements of the continental sampling strategy and the high-level requirements that constrain the spatial observation at discrete landscapes across the continent. In addition to facilitating comparison across sites and at continental scales, the design is constrained by the need to co-locate terrestrial observations and facilitate the integration of data with other biological and physical measurements of the observatory (Schimel et al. 2011). Maintaining generality encourages iterative optimization of the sampling effort, while allowing it to remain robust to a range of questions and methods of analysis which the community may apply to NEON data products (see AD[03]).

Two principles guide the site-scale sampling design: randomization and robustness. Randomization at multiple levels of the design guards against the collection of data that are not representative of the populations of interest. The design must be robust in the sense that it is capable of performing under a diversity of conditions, and accommodate a variety of data types and questions (Olsen et al. 1999).

Randomization allows an unbiased description of the landscape (Thompson 2012), facilitates data integration, supports design-based inference (Sarndal 1978), and provides data that can be assimilated into numerous model-based approaches to inference and understanding. The design satisfies the constraints of randomization by sampling with a spatially-balanced framework known as the Reversed Random Quadrat-Recursive Raster (RRQRR; Theobald et al. 2007).

Stratification increases efficiency (Cochran 1977) and provides a framework for describing the variability of landscape characteristics targeted by the NEON design. Stratification according to the National Land Cover Database (Fry et al. 2011) provides a continuous land cover classification across the United States including Puerto Rico, Alaska, and Hawaii; this allows consistent and comparable stratificaiton across the diversity of NEON sampling frames. This stratification satifies multiple design requirements and objectives. Stratification is an integral part of the NEON design at multiple scales, and when applied to the terrestrial sample design, it provides consistency and ensures observations describe local landscape characteristics essential to the continental-scale observatory. NEON domains – essentially a stratification of the continent – were derived from eco-climatic factors that contribute to large-scale patterns of vegetation (Hargrove and Hoffman 2004). NEON sites are selected to represent the dominant vegetation type in the domain (Schimel et al. 2011). At each NEON site, tower-based sensors were positioned to measure these dominant vegetation types. The sensors measure ecosystem properties that drive ecological response (Chapin et al. 2012, Clark et al. 2012, Sala et al. 2012). Observing terrestrial biogeochemistry and organisms in this dominant vegetation type will quantify the relationship between state factors – variables that control characteristics of soil and ecosystems (Chapin et al. 2012) – and ecological response. Through time these observations will provide insight into the causes and consequences of change at NEON sites which, due to the scalable design, will further understanding at larger spatial scales.

Sample size determination ensures that NEON will contribute to ecological understanding over the life of the observatory. An overarching requirement of the design is that minimally sufficient data be collected within each stratum where samples are allocated. This ensures that the NEON effort will provide tangible contributions to conceptual models of the interactions between species and environmental drivers over the life of the observatory. Simply put, if plant diversity data will be collected in a given vegetation class, it is necessary to ensure that after thirty years, these data are sufficient to understand patterns and, ultimately, inform the NEON Grand Challenges (Legg and Nagy 2006). Quantitative sample size calculations are most often performed against the backdrop of a classical hypothesis test and corresponding power analysis. Given the diversity of questions the ecological community will ask of NEON data, an initial case – a test of the difference in the magnitude of trends between any two NEON sites – was considered to provide some guidance to sampling effort. Depending on the temporal correlation at each site, the variability associated with the response of interest, and the number of years required to detect a trend, a minimum sample size of ten is required in each stratum (AD[02], Figure 3).

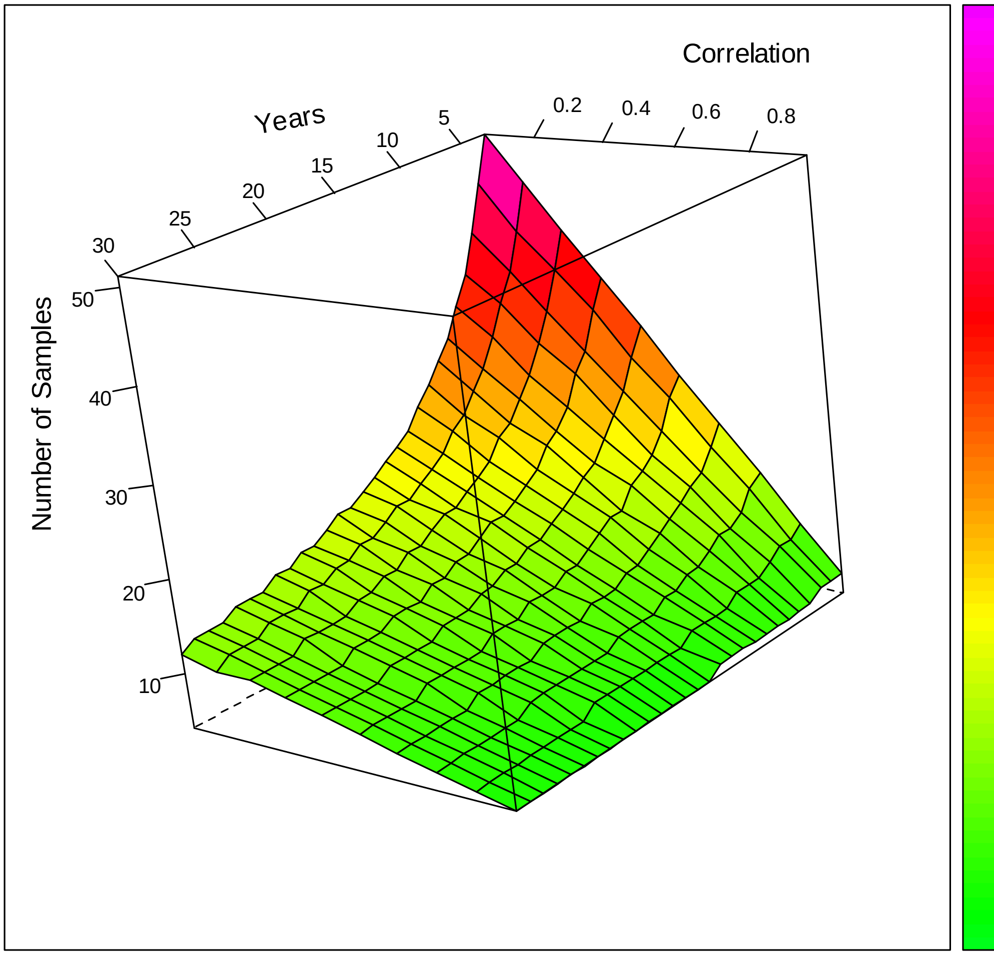

Figure 3. Minimum sample size as a function of sampling years and temporal correlation. Type I error is set at 0.1 and compound symmetric correlation structure is assumed.

Sample allocation must balance logistical constraints and science goals. Initial sampling will largely be limited to dominant cover types (greater than 5% of the frame) within each site boundary. This extends the guiding principle that if an ecological response is to be measured, the data must be meaningful in the context of NEON objectives. NEON sites, and the tower-based sensors, were selected to represent dominant vegetation types across the NEON domains. Plant diversity and other co-located terrestrial measurements will focus on quantifying variability of these types in an effort to better understand relationships between pattern and process at local scales, as well as to contribute to the description of biological patterns at larger scales (Urquhart et al. 1998).

Landscapes are patchy, and land cover provides one metric to describe that site-scale variability. Increasing the sample size in strata with greater variability standardizes the sampling effort and facilitates comparison. It also increases total sample size, which is costly. Science goals must be balanced by the expense of field-based observations. The plant biodiversity sampling approach, for example, will standardize by initially assuming area can serve as a proxy for variability. Within each site, placing more plots in vegetation types with a greater footprint on the landscape was a means to that end and avoided, for example, the distribution of twenty plots in 100-km^2 of deciduous forest and twenty plots in 1000-km^2 of evergreen forest. For plant biodiversity, an optimal design would standardize according to measured variance, observed species richness could direct sampling to the inflection of the species accumulation curve. The goal is to standardize sampling effort across each site, and in future design iterations optimize towards a diversity-based sampling intensity.

The design recognizes that disproportionate levels of plant diversity can be found in relatively rare vegetation types by adjusting the sample allocation according to the square-root of the area of each stratum. While this assumption will be less valid for some vegetation classes, it increases the likelihood of sampling beyond the steepest part of the species accumulation curve while protecting against hitting the plateau (i.e., oversampling).

6.2.3 Temporal Distribution of Sampling

To ensure comparability through time, annual efforts of plant diversity sampling must be adjusted to reflect interannual patterns of phenological variability. A sampling effort scheduled to take place at a specific date would likely reflect fluctuations in the response variable more indicative of response to patterns of weather fluctuating on short (e.g., a cool and wet spring) or mid-range time scales (e.g., Pacific Decadal Oscillation, El Nino, drought). The timing of sampling will be triggered by a subset of species and individuals being surveyed for trends in phenology near the NEON tower, a phenological camera mounted on the tower, or cues developed from the ratio of air to soil temperature as measured by the sensor array (Schimel et al. 2011). Several years of data from each of these streams of data are required to determine the most practical and logistically feasible approach. Additionally, in some NEON sites multiple blooms will be common. For example, the shortgrasse steppe of Colorado (represented by the NEON site at the Central Plains Experimental Range) supports a flush of growth and flowering in early spring and a second phenological peak when short bursts of moisture interrupt the late-summer dry season. Such sites may require more than one sampling occurrence per year to adequately characterize annual composition and abundance values.

6.2.4 Logistics and Adaptability

There is little precedent for an integrated ecological observatory at the scale of the United States over decades. Implementation of the design will be an iterative procedure. The first several years of data will test the design by confronting the assumptions, logic, and logistics used in the design and development phase. For example, the time required to sample and travel to plots will inform the accuracy of budgets to complete these tasks over the life of the Observatory. Similarly, species richness and composition data from plot sampling will describe spatial and temporal patterns of variability that can be used to re-evaluate sample size, timing, and frequency. Spatial patterns of variability will also be informed by the airborne observation platform that will also provide a novel, quantitative perspective on how well they are covered by the sample design. Optimization of the design in the first years of the Observatory will establish a system for the collection of local plant diversity data capable of informing understanding at the scale of the continent.

6.2.4.1 Diversity of Plant Functional Traits

The diversity of plant species and processes within an ecosystem challenges a clear understanding of the impact of changing biodiversity on ecosystem function (Loreau 2010). From a focus on what organisms do in a landscape, a construct has emerged that focuses on the functional classification of organisms and the way groups of organisms with similar functional traits interact with processes and the environment. Species are recognized in the context of traits that impact ecosystem function (Diaz and Cabido 1997, McGill et al. 2006) – functional traits - such as leaf size, seed size, and canopy height and structure. Further abstraction is obtained by grouping species with similar effects on ecosystem function or response to environment – functional types or functional groups - such as evergreen shrubs and nitrogen fixers that affect ecosystem function, and C_3 and C_4 grasses that respond to environmental conditions differently (Diaz and Cabido 1997, 2001, Hooper et al. 2005). Functional group diversity, and functional diversity – the composition and value of species-level functional traits – in a system, rather than species richness, determine ecosystem function (Diaz and Cabido 2001). Future NEON data products and small additions to the field collection efforts could provide the ecological community with a functional trait database capable of furthering the NEON contribution to understanding the cause and consequences of ecological change.

The functional concept provides a framework for understanding the relationship between changes in species and ecosystem function. Environmental change can result in the loss of species, change in relative abundance of species, or addition of species to an ecosystem. These alterations represent a change in the diversity of functional traits at the species level, and shifts in composition and diversity of functional types that have consequences for ecosystem function (Diaz and Cabido 1997, Hooper et al. 2012). Understanding the relationship between effect functional types and response functional types provides a mechanistic link that describes how species loss impacts ecosystem properties (Ustin and Gamon 2010, Chapin et al. 2012).

Which traits, which species, how many replicates of each species and at what landscape extent? Those are the primary design considerations. The investment in functional trait-related investigation over the last ten years has resulted in numerous standards and trait libraries. Observations will focus on those traits that directly respond to climate, land use, and invasive species or impacts on ecosystem function – the questions of interest to NEON. The Observatory has firm plans to measure many traits, but the list does not cover the entire suite of traits generally considered to be a minimum (Cornelissen et al. 2003, Diaz et al. 2004, Kattge et al. 2011). The NEON effort will not collect all of these traits, but will collect a subset of traits useful to interpreting ecological change through time.

An explicit standard for selecting species for observation does not exist. Many efforts focus on dominant species given their contribution to ecosystem structure and function (Grime 1998). NEON will select a mix of dominant and rare species according to observed relative abundance. The species targeted for measurement will be the same as those selected for the phenology, foliar biogeochemistry, and plant biomass observations. The design of these observations will independently capture a suite of plant functional traits. Organizing observations around species observed by other designs maximizes the efficiency of field and lab costs, and provides a cohesive set of observations that could better describe how and why plants and functional diversity are changing in response to a variety of forcing factors.

The frequency of sampling will vary by trait, but the complete suite of traits targeted for observation will be collected within the first five years of sampling at each site. Phenology will be measured many times throughout the growing season. Traits associated with the measurements targeted plant biomass and structure will be measured annually, and foliar biogeochemistry will be sampled approximately every five years.

6.3 Sample Design for Airborne Observations of Plant Species Diversity and Abundance

Relatively new techniques are being developed to estimate patterns of plant diversity from remote sensing information across large spatial extents (Asner et al. 2012, Asner and Youngsteadt 2012, Schimel et al. 2013). Principle approaches involve the direct detection of species by isolating species-specific unique hyperspectral signatures (Asner and Martin 2009, Read Kokaly et al. 2009), calibrating sensor returns and algorithm-derived estimates of ecosystem properties (foliar nitrogen, leaf area index, lignin content) with plot-based measures of diversity (Carlson et al. 2007), and relying on the principle components of the hyperspectral imagery (Read Rocchini et al. 2011, Schimel et al. 2013) as a proxy for plant species diversity.

While NEON will generally rely on the ecological community to derive plant diversity estimates from remote sensing data, a data product designed to map dominant plant species at each NEON core site by combining plot and remote sensing observations may be added. Mapping the distribution of dominant species would provide a spatially explicit representation of how drivers of change impact the distribution, abundance, and species-environment relationships at the site level. Additionally, improved estimates of aerial cover of dominant taxa and associated distributions of functional trait data will improve modeling efforts to describe the impact of changes to dominant species on ecosystem processes. Identifying the spectral signature associated with a particular species allows those species-specific endmembers to be isolated and mapped across the extent of a hyperspectral scene. Species-specific endmembers will be informed by intersecting the spatially-explicit, plot-based stem maps with high-resolution imagery. In the instance that the ratio of individual plant size to pixel grain does not result in a pure pixel, unmixing techniques based on species-specific endmember bundles can be used to estimate species cover fractions on a per pixel basis (Feret et al. 2008, Asner and Martin 2009).

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