

# TOS SCIENCE DESIGN FOR PLANT DIVERSITY

PREPARED BY	ORGANIZATION	DATE
David Barnett	FSU	08/31/2017
Peter B. Adler	Utah State University	08/31/2017
Benjamin R. Chemel	Eckerd College	08/31/2017
Paul A. Duffy	Neptune and Company	08/31/2017
Brian J. Enquist	University of Arizona	08/31/2017
James B. Grace	US Geological Survey	08/31/2017
Susan Harrison	University of California, Davis	08/31/2017
Robert K. Peet	University of North Carolina	08/31/2017
David S. Schimel	NASA Jet Propulsion Lab	08/31/2017
Thomas J. Stohlgren	Colorado State University	08/31/2017
MarkVellend	Université de Sherbrooke	08/31/2017

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	04/11/2022

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	СМ	04/11/2022

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and condusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.



# **Change Record**

REVISION	DATE	ECO #	<b>DESCRIPTION OF CHANGE</b>	
А	09/09/2014	ECO-02062	Initial release	
В	11/29/2017	ECO-05040 Updated descriptions of components of the design addition of future directions, and condensed text		
С	04/11/2022	ECO-06810	<ul> <li>Revised logo</li> <li>Update to reflect change in terminology from relocatable to gradient sites.</li> </ul>	



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## 1 DESCRIPTION

## 1.1 Purpose

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON subsystem components, linking NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continentalscale 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 (LO; 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 and the resulting plant tissue (AD[05]) and plant diversity (AD[08]) protocols that result in the plant presence data product (AD[03]).

## 1.3 Acknowledgments

The authors would like to thank Jim Clark, Deb Peters, Mark Whitten, Pam Soltis, Doug Soltis, Elena Azuaje, Rachel Krauss, David Gudex-Cross, Rebecca Hufft, and Geneva Chong for contributions to the design and this document. We would also like to thank the many members of the ecological community and the NEON Project staff who dedicated time and expertise to guide and review the NEON design.



## 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.000278	Tier 4 TOS Requirements Module	
AD[02]	NEON.DOC.000001	NEON Observatory Design	
AD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog	
AD[04]	NEON.DOC.000913	TOS Science Design Spatial Sampling Design	
AD[05]	NEON.DOC.000906	TOS Science Design for Terrestrial Biogeochemistry	
AD[06]	NEON.DOC.000907	TOS Science Design for Plant Phenology	
AD[07]	NEON.DOC.000914	TOS Science Design for Plant Biomass, Productivity, and Leaf Area	
		Index	
AD[08]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling	
AD[09]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment	
AD[10]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf	
		Mass per Area Measurements	
AD[11]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology	

#### 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



# 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].

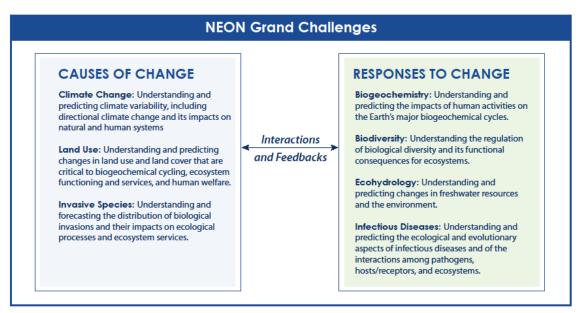


Figure 1. The seven Grand Challenges defined by the National Research Council (2001).



## 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.

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.

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. 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

Observations of plant diversity, a multi-faceted concept that considers variation at multiple organizational levels in a defined space and time (Heywood 1995, Hubbell 2001, Stohlgren 2007, Magurran 2013), contributed to the origins of the theory and practice of ecology (Darwin 1859, Magurran and McGill 2010). Study at multiple resolutions - genetic (Hinchliff et al. 2015), trait (Adler et al. 2014, Enquist et al. 2015), population (Clark et al. 2004, Clark et al. 2011), community (Vellend 2010, Clark et al. 2012), region (Zobel 1997, Huston 1999), and globe (Kreft and Jetz 2007) – has proved critical to understanding dynamic interactions of pattern and processes such as species interactions (Suttle et al. 2007, Adler et al. 2013), species-environment relationships (ter Braak 1987, Stohlgren et al. 1999), and the relationship of plant diversity to the structure and function of ecosystems (Diaz et al. 2004). These relationships are sensitive to environmental change. Disturbance, land use, trade and transportation, and changing climate result in the redistribution of species in novel environments (Lonsdale 1999), altered abundances (Knapp et al. 2002), and local extirpation (Sax and Gaines 2003). The examples are numerous and from all parts of the world, yet considerable uncertainty regarding the impact of accelerating changes in climate (Moritz and Agudo 2013, Walther et al. 2002, Ash 2016), ecological disturbance and heterogeneity (Stein et al. 2014), and species invasions (Jeschke 2014) on the status and trending patterns of plant diversity persists.

Disentangling uncertainty to further the understanding of changing patterns of plant diversity will benefit from new approaches to consistent and comparable ecological information (Keller et al. 2008, Collins 2016). Traditional funding cycles do not support long-term observations of ecological forcing factors and responses across large spatial extents (Magurran et al. 2010). Coupling the observations of plant diversity with the measurement of climate, atmosphere, and biogeochemistry across the United States would generate robust understanding of the drivers of plant diversity (Stohlgren et al. 1999, Peters et al. 2014). Observation over decades could illuminate patterns of change and facilitate the iterative forecasting of future conditions (Clark and Gelfand 2006, Yiqi et al. 2011). A network with this capacity faces challenges: 1) the methods must produce consistent and comparable data, yet be appropriate to the plant richness and structure observed in each unique ecological system; 2) the plant diversity data must be capable of integration with other data streams produced by the network as well as other sources of plant diversity data; and 3) the data must be informative and made freely available to the ecological community (Keller et al 2008).

The overarching NEON objectives provide additional context for the observations of plant diversity. The goal of NEON is to (Schimel 2011):

• Enable understanding and forecasting of the impacts of climate change, land use change, and invasive species on aspects of continental-scale ecology such as biodiversity, biogeochemistry, infectious diseases, and ecohydrology.



- Enable society and the scientific community to use ecological information and forecasts to understand and effectively address critical ecological questions and issues.
- Provide physical and information infrastructure to support research, education, and land management.

Traceable links between these high-level goals and the data the observatory produces provide a topdown framework for the development of the NEON plant diversity science design. The scope of the NEON mission is defined by the Grand Challenges in environmental science identified by the National Research Council (2001, 2003). High-level requirements synthesize the mission, Grand Challenges, and theoretical basis for measurements into formalized statements that describe the fundamental aspects and guiding architecture of the NEON strategy (Schimel et al. 2011). The plant diversity design is part of this requirements-driven hierarchical structure that provides both guidance and constraints for the plant diversity-specific criteria, requirements, design, and resulting data products.

## 4.2 NEON's Contribution

The NEON design will measure 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.

- <u>Observations across the continent:</u> With standardized protocols implemented by highly-trained field-technicians at sites across the United States, NEON will observe a consistent suite of plant diversity variables. In addition to facilitating a cohesive understanding of the trajectory and magnitude of trends 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 continent. Essential to scaling efforts is the generation of data directly comparable across NEON sites and to other large-scale collections 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).
- 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). The 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. 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 opport unity 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.

## 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, genetic tissue collection, 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 ~1000 m above the vegetation. The collection of material for genetic analyses and functional traits will target specific species measured select parameters (e.g., phenology and biogeochemistry).



#### 5 SAMPLING FRAMEWORK

A clear articulation of the objectives and scope of the plant diversity design will ensure an ongoing contribution of requirement-constrained and informative data to NEON (Cochran 1977, Lindenmayer and Likens 2009, Gitzen and Millspaugh 2012):

- **Plant diversity objective:** Observations are designed to clarify the causes and consequences of changes in spatial and temporal patterns of plant diversity.
- 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 tissue from a subset of species found at each site will be collected and made available for genetic analyses, plant species presence and abundance will be recorded in multi-scale vegetation plots, and functional traits will be assessed using a variety of protocols.
- **Population to be sampled:** The target will be the species in all but the most rare cover types (>5% of the site) within the extent of NEON sites. A statistically rigorous sample design provides a framework for sampling (AD[04]).
- Sampling frame: The spatial extent of NEON sites bounds the area available to sample plant species (Reynolds 2012). Most sites were defined by the location of the tower-based sensor measurements and the associated management or ownership boundary. NEON sites range in size from agriculture sites (e.g., Sterling, CO 3.2 km<sup>2</sup>) to wildland sites (e.g., Central Plains Experimental Range, 65 km<sup>2</sup>). 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 subset of the area was defined as a sampling frame to address large scale NEON science questions (Schimel et al. 2011) and other NEON measurements or atmosphere and soil.
- 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 of Observatory-produced 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).



NEON Doc. #: NEON.DOC.000912

#### 5.1 Science Requirements

Four design criteria that guide the NEON design for sampling plant diversity include 1) the observations must enable an understanding of changing patterns in plant diversity; 2) observations must be collocated with other NEON data; 3) the methods must be comparable through time, across NEON sites, and to other network or coordinated approaches to measuring plant diversity; and 4) the observations across space and time must be relevant for the life of the observatory. These criteria provide context for plant diversity-specific requirements that are also specifically linked to the high-level NEON requirements to ensure traceability and consistency across the continent for 30 years. This science design is based on these 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 (AD[01], AD[02]).

#### 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[03]).

#### 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 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 (Section 6.5) 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 (Kattage et al. 2011).



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.



NEON Doc. #: NEON.DOC.000912

#### 6 SAMPLING DESIGN FOR PLANT DIVERSITY

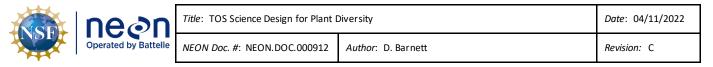
The design directs the observation of plant species presence, diversity, and abundance and the collection of plant material for genetic analyses. The plant diversity observations and resulting data products will be coordinated with plant phenology, structure, demographic and biogeochemical observations through the collocated sampling of individuals, species, and plots. Material for reference and genetic analysis from individuals and individuals of those species monitored for phenological transitions (AD[06], AD[11]) sites will be collected and available to the community (see section 6.1). Those same species – and individuals in some cases - and many more will be the subject of demographic and structure observations (AD[07], AD[12]), plant foliar chemistry and isotope analyses (AD[05], AD[10]), and plant diversity (presence and abundance). Many of the resulting data products 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 space-time ecological questions.

#### 6.1 Sample Design for Genetic Collections

NEON will collect and curate foliar material for analysis of genetic diversity. Plant tissue collections are integral to next generation phylogenetic and systematics studies (Soltis et al. 2013) including building morphological-genetic relationships (Hamrick and Godt 1996), identifying species (Kress et al. 2005), and providing a foundation for population genetics and phylogenetic studies (Drew et al. 2013). NEON will make available plant tissue from select plant species for analysis by the ecological community.

Material from a subset of species at each site will be collected; it is beyond the scope of the NEON effort to collect material on every species documented in plant diversity observations. To integrate NEON measures of vegetation, plant tissue will be collected from species specifically targeted for phenological observation (AD[06]). Initially the phenology effort will focus on three dominant species of different growth forms that are found near the NEON flux tower. After several years, twenty species representing a diversity of functional groups and relative abundances in the same area will be observed. Many of these species will also be targeted for foliar biogeochemistry (AD[05]) measurements, and subjected to biomass and productivity observations (AD[07]) across NEON sites. In some cases, the actual individual from which plant material is retrieved for the genetic archive will be the subject of the phenology, foliar biogeochemistry, and other vegetation protocols.

The plant tissue collection will balance tradeoffs between intra and interspecific diversity (Gemeinholzer et al. 2010, Neves and Forest 2011). Tissue from ten individuals of each species selected for phenology sampling will be collected – from the phenology plot near the tower and 'Distributed Base Plots' (AD[04]), and, when species are not found in plots, opportunistically across each site – every five years. Tissue will be flash-dried on silica in the field and archived at an external facility at -20 C (Neubig 2014). A minimum of one voucher for each population, from which tissue will also be collected and appropriately labeled, will be collected and archived. Under the assumption that rates of change will not



be sufficient to justify the cost of annual collections, tissue will be collected every five years and be made available through an archive facility for principle investigator-driven research.

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

Observations of plant species will be made within multi-scale plots at NEON sites. Documenting the composition and abundance of native and non-native plant species satisfies the requirements that "NEON's measurement strategy will include...biological invaders...and diversity of key organismal groups" and "NEON measurements of plant diversity will include...native and invasive plant species." Critical components of the design include:

- The multiscale plot that facilitates repeatable observations at a variety of scales
- The spatial and temporal sample design to facilitate detection of trends within and across NEON sites
- The data products and possible analyses

## 6.2.1 Plot Design

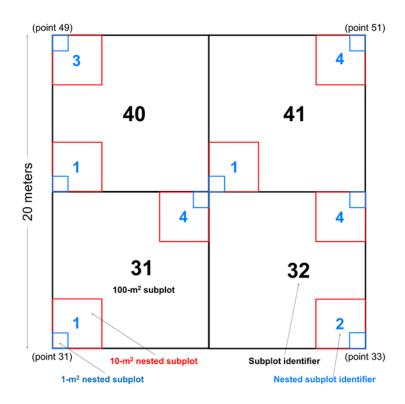
NEON will sample plant species with a multi-scale plot design that borrows from techniques pioneered by Whittaker (Shmida 1984), adopts modifications of his initial approach (Stohlgren 1995), but most closely emulates the approach developed by the Carolina Vegetation Survey (Peet et al. 1998). Plant taxonomic composition will be recorded in 20 x 20m square plots comprised of four 10m x 10m subplots containing nested 10m<sup>2</sup> and 1m<sup>2</sup> subplots (**Figure 2**). NEON field staff stationed at regional offices will be trained and calibrated annually at both local and observatory-wide trainings and by exchange across the Observatory. These botanists and plant technicians will make the following observations:

- The identity of each species according to naming conventions maintained by the US Department of Agriculture, Natural Resources Conservation Service PLANTS database (USDA, NRCS 2016) will be recorded in each subplot eight 1m<sup>2</sup>, eight 10m<sup>2</sup>, four 100-m<sup>2</sup>.
- Estimates of taxon-specific abundance will be made with estimates of cover within the 1-m<sup>2</sup> subplots.

A standardized protocol for sampling plant species diversity does not exist; a protocol typically reflects specific questions or objectives and the various options have tradeoffs (Stohlgren 2007). However, NEON plant diversity sampling must suit the countless questions directly related to NEON high-level questions that consumers of the data will ask. The requirements framework provides direction, NEON requirements specify, "...the co-location of data, detection of trends, and comparability through time and space" and resulting plant diversity-specific requirements state that sampling be "...implemented in a design that facilitates the intersection of plant diversity data and other ecological responses with factors that drive ecological pattern..." A plot-based method is repeatable, allows collocation of protocols, and is capable of describing species-environment relationships (Stohlgren 2007).

ine@n	Title: TOS Science Design for Plant I	Diversity	Date: 04/11/2022
Operated by Battelle	<i>NEON Doc. #</i> : NEON.DOC.000912	Author: D. Barnett	Revision: C

Plot-based efforts have demonstrated the capacity to compare dynamic species -environment relationships across time and space. In an effort to elucidate species -environment relationships, the large, multi-scale plot design borrows from a method Whittaker developed for gradient analysis and ordination techniques (Whittaker 1960). Revisiting some of Whittaker's plots, Damschen et al. (2010) documented changes in species abundance and richness. With many plots - a modified version of Whittaker's plot - across multiple US states, Stohlgren et al. (1998, 1999, 2003) contributed to a general understanding of environmental plant species controls of invasibility.



**Figure 2.** A schematic of the multi-scale plot for sampling plant species diversity and abundance (Peet et al. 1998). The plot is nested within a larger plot designed to accommodate other NEON protocols such as soils, beetles, and plant biomass and productivity; the corner points and associated subplot identifiers are numbered to correspond to this larger plot.

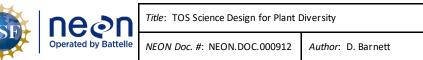
The method must be precise, accurate, and efficient to contribute to an understanding of changes in plant species composition through time (Stohlgren 2007). These attributes can be antithetical. Consideration of the advantages and disadvantages of available designs in the context of NEON requirements resulted in the selection of the square, 400m<sup>2</sup> plot design. Considerations included the following:

• Plot size. Larger plots detect a greater number of species at local, plot scales. Highly linear transects (Parker 1951) and point-intercept methods (Barbour 1987) tend to miss locally rare

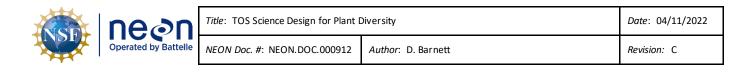


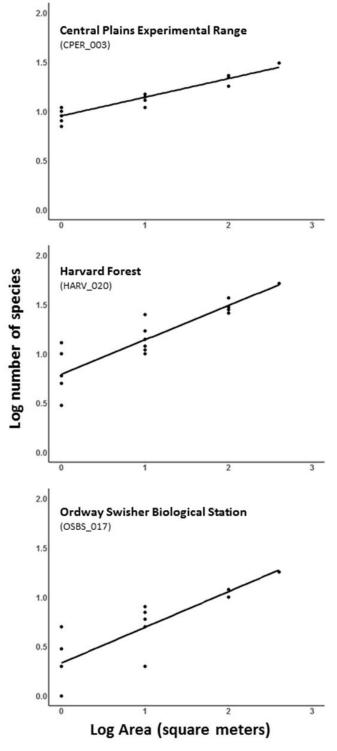
and highly aggregated species because they are vulnerable to spatial-autocorrelation, are biased toward broad-leaved species, and are only suitable for sampling understory or grass/herbaceous species (Stohlgren 2007). Small plots (Daubenmire 1959, Whittaker 1960) increase detection rates but tend to capture fewer species than large plots due to the small total are sampled. However, accurate and repeatable estimates of cover can be difficult in large plots, and recording all species across hundreds of square meters takes considerably more time than smaller plots (Stohlgren 2007). At 400m<sup>2</sup>, the NEON plot is large relative to transect and small-plot methods (Parker 1951, Daubenmire 1959), but smaller than both the 1000m<sup>2</sup> plots commonly used to intensively document local species composition (Stohlgren 1995, Peet 1998) and plots designed for mapping and tracking the location and identity of tree species (Condit 1996). The NEON plot represents a compromise designed to capture species across a diversity of systems with replication within each site.

Multi-scale sampling. A multi-scale plot provides a data-rich product, but each plot is time consuming. Observing and recording plant diversity at numerous subplots across multiple scales within a single plot requires more time than single-scale observations. The resources associated with this sampling time could be spent on a larger sample size or other components of NEON. However, the capacity of the design to meet NEON requirements justifies the expense. The multi-scale approach allows for a consistent, baseline plot size within and across sites that is well-suited for describing herbaceous species at small 1m<sup>2</sup> scales and capturing diversity of large-stature, well-spaced tree species at 100 and 400m<sup>2</sup> scales (Peet et al. 1998), satisfying the NEON requirement that measurements be "standardized and calibrated to allow comparison across sites and over time to enable understanding of ecological change in time and space." The multi-scale plot will enable detection of these spatio-temporal changes, addressing the requirement that NEON, "...establish the link between environmental cause and effect" by allowing description of patterns of within plot heterogeneity, species overlap, and the detection of trends at a variety of spatial scales where different forcing factors might operate. Finally, collecting data at a variety of scales facilitates comparison to other networks recording plant diversity data. Scales of observation coincide in some cases; the 1m<sup>2</sup> plots observed by the Nutrient Network (Adler et al. 2011, Dengler et al. 2011) are directly comparable to the NEON 1m<sup>2</sup> subplots. In cases where scales of observation don't coincide, the multi-scale NEON data enables the development of plot-specific species-area curves – a description of the relationship between area and number of species (Rosenzweig 1995) – that allow comparison to other methods (Stohlgren 2007). When other observations efforts also include multi-scale observations such as the US Forest Service Inventory and Analysis Program (Stolte 1997, Gray et al. 2012) and the National Park Service Inventory and Monitoring Program (Fancy and Bennetts 2012), plot-scale species accumulation curves offer an additional metric for the comparison of plant diversity (Fridley et al 2005, Stohlgren 2007). Comparability of data is essential to continental-scale ecology and addresses the requirement that, "NEON will allow extrapolation from the observatory's local sites to the nation..."

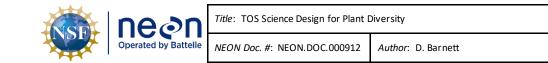


Plot shape. Linear or rectangular methods for observing plant diversity tend to observe more species than circular or square plots. The larger perimeter-to-area ratio of rectangles is likely to cross a larger environmental gradient and encounter different species that exploit that habitat diversity. Whittaker's 20m x 50m multi-scale plot contained nested subplots that changed shape with scale, confounding the perimeter-to-area ratio across scales and resulting species-area curves (Figure 3). The Carolina Vegetation Project plot (Peet) partially resolved this issue by nesting square plots within square 100m<sup>2</sup> 'modules,' maintaining a square until the six modules (2 x 5m) result in the 1000m<sup>2</sup> rectangular plot. The NEON plot – comprised of four Carolina Vegetation Project modules (2 x 2) is square. It takes less time to sample than larger rectangles (Barnett et al. 2003, Stohlgren 2007), and integrates with other NEON data streams. Additionally, the square plot and nested subplots will be more comparable to the square pixels of NEON's high-resolution remote sensing data. Pixels and plots may not align, but the multiscale diversity, vegetation structure, and foliar chemistry data collected will be better integrated when plots and pixels are the same shape (Carlson et al. 2007), satisfying the requirement that "NEON's measurement strategy will include coordinated and co-located measurements of drivers of environmental change and biological responses..."





**Figure 3.** Plot-specific species-area curves in log-log space (Rozenzweig 1995) provide a means to compare plot-specific species richness and heterogeneity.



#### 6.2.2 Spatial Sampling Design

The sample design – the mechanism for distributing plots across sites – addresses multiple design requirements and constraints. It must be sufficiently general to direct a diversity of organism and soil observations within sites from Puerto Rico to Alaska. The resulting data must support a diversity of questions and analytical approaches, and must facilitate integration of data across other NEON collections of biogeochemistry, atmosphere, and ecohydrology (Schimel et al. 2011) to address the high-level requirement of enabling the link between ecological cause and effect.

The unbiased sample associated with randomization (Cochran 1977, Thompson 2012) is the foundation of the NEON sample design (AD[04]). It eliminates the potential for bias and allows design-based inference of population parameters from points to the unsampled landscape with design-based estimators (Sarndal 1978, Stehman 2000). Model-based or gradient designs might better optimize for other approaches to inference, but data collected according to a random design can be assimilated into numerous model-based approaches (Cressie et al. 2009).

Plant diversity observations will be made at three of the 20-30 plots randomly placed near (250-1400m radius) the NEON tower ('Tower Base Plots', AD[04]). These measures allow within-plot collocation with observations of vegetation biomass, productivity, structure (AD[07), and soil (AD[05]) and nearby observations of plant phenology (AD[06]). The three plots also quantify the variability of plant diversity in the landscape reflected in sensor-based measures of soil and atmosphere to allow direct and rigorous quantification of how plant diversity might impact - or be impacted by - those physical and chemical dynamics (Kao et al. 2012). These plots satisfy the requirement that "NEON's measurement strategy will include coordinated and co-located measurements of drivers of environmental change..."

The majority of the plant species diversity sampling effort will focus on describing variation across the larger areas ('Distributed Base Plots' across sites ranging 5-214 km<sup>2</sup>, AD[04]). The capacity of observations to describe trends depends on space-time variation in the response as well as logistical and financial constraints that govern sample sizes. Previous studies (Stohlgren 2007) and early input from the ecological community resulted in initial baseline funding for a sample size of 30-40 plots that will be distributed across each site. Additional power analysis (Thompson et al. 2012) described sample sizes necessary for differentiating magnitude of trends between two sites as an initial case study. The test prescribed a sample size (about 20 plots/site depending on variability in space and time) robust to a variety of questions that might be asked of the data (AD[04]). The obligation to insure the data provide tangible contributions to elucidating the drivers of space-time trends (Legg and Nagy 2006, Keller et al. 2008, Schimel et al. 2011) and to address the requirement that, "NEON infrastructure and observing system signal-to-noise characteristics will be designed to observe decadal-scale changes against a background of seasonal-to-interannual variability over a minimum 30-year lifetime" resulted in several additional components of the sample design.

Stratification – the division of the landscape into non-overlapping subareas from which sample locations are identified (Cochran 1977, Johnson 2012) - increases sampling efficiency (Cochran 1977) and provides



a framework for describing the variability of landscape characteristics targeted by the NEON design. 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 that can be consistently applied across sites. Sampling within site strata as described by these cover classes promotes the description local landscape characteristics essential to the continental-scale observatory. NEON domains -a stratification of the continent – were derived from eco-climatic factors (Hargrove and Hoffman 2004) that contribute to large-scale patterns of vegetation. Within each domain, NEON sites are selected to represent the dominant vegetation type in the domain (Schimel et al. 2011). At each NEON site, the tower-based sensors were positioned to measure these dominant vegetation types. Placing plant diversity plots in the airshed of tower-based sensors will quantify relationships between state factors – variables that control characteristics of soil and ecosystems (Chapin et al. 2012, Clark et al. 2012, Sala et al. 2012) and ecological responses. Sampling these same dominant cover classes across the scale of the site will help quantify the variation in plant diversity across larger areas and facilitate extrapolation to larger scales (Urquhart et al. 1998), satisfying the requirement that "NEON shall addres ecological processes at the continental scale and the integration of local behavior to the continent...". Initial sampling will exclude the rarest NLCD cover types (< 5%) within each NEON site. Focusing available effort extends the guiding principle that the data must be meaningful in the context of NEON objectives. However, by excluding rare cover types, species and trends associated with a component of native and non-native flora will go undetected (Stohlgren et al. 1999). A continental-scale observatory targeting complete censuses of rare species would required many more plots and sites.

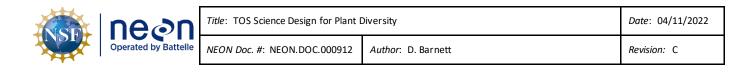
Comparability across strata and sites is crucial to enabling the function of the continental observatory: describing how variability in forcing factors at sites across the US drives different patterns in plant diversity or distributions of specific species within each site. However, comparison of diversity data is challenged by site size and environmental heterogeneity (Rozenzweig 1995, Gotelli et al. 2009), level of expertise, and effort (Gotelli et al. 2001, Chao et al. 2009). The design controls for these sources of variability with a diversity-based approach to sample intensity, attempting to sample to the inflection point of sample species-accumulation curves in each stratum targeted for sampling (Figure 3). Because the number of plots required to reach this inflection point is not known a priori, it is initially assumed that area can serve as a proxy for the heterogeneity (Kotliar and Wiens 1990, O'Neill et al. 1991, Pickett and Cadenasso 1995) that typically promotes diversity (Ricklefs 1977, Collins 1992, Harrison et al. 2003). Within each site, placing more plots in vegetation types with a greater footprint on the landscape is a means to that end and avoids, for example, a distribution of fifteen plots in 10km<sup>2</sup> of deciduous forest and the same number of plots in 100km<sup>2</sup> of evergreen forest. The design recognizes that disproportionate levels of plant diversity can be found in relatively rare vegetation types (Meyers 1990, Debinski et al. 1999, Stohlgren et al. 1999) by allocating samples proportionate to the square-root of the area of each stratum targeted for sampling. This results in a greater absolute number of plots in large cover types, but a larger number of plots in smaller cover types than would have resulted from an allocation directly proportional to the area of cover types. While this design will not be valid for all sites and vegetation cover types, the approach increases the likelihood of sampling beyond the steepest part of the species accumulation curve while protecting against reaching the plateau (i.e., oversampling). The

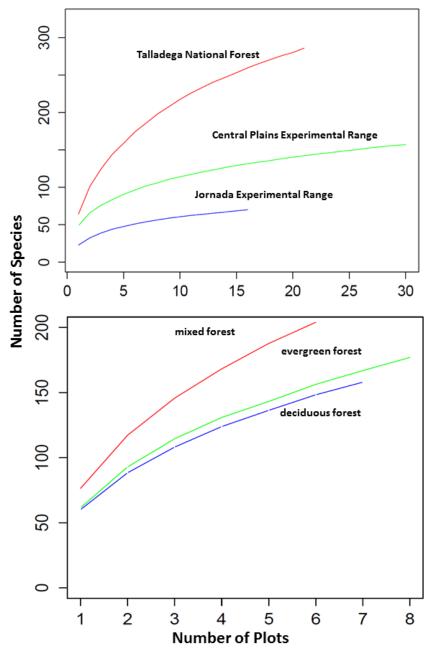


diversity-based standard satisfies the requirements that, "NEON measurements will be standardized and calibrated to allow comparison across sites and over time," to ultimately ensure that NEON, "...observe the causes and consequences of environmental change in order to establish the link between ecological cause and effect".

## 6.2.3 Temporal Sampling Design

The design must ensure that observations detect patterns of plant species diversity through time. The frequency and timing of sampling will be guided by the requirement to enable the documentation of annual rates of change that coincide with summaries of climate data and the requirement of documenting the dynamics of dominant cover types in the vicinity of the tower and across the site. Implementing the protocol at each plot at least one time each year will enable the quantification of annual rates of change. These sampling bouts will occur during a one to two month period when the majority of species flower or possess other parts conducive to identification. This period will generally be targeted by annual peaks in greenness as measured by MODIS, input from local ecologists, and by the flowering of those species observed as part of the NEON phenology observations. Ephemeral and cryptic species that do not flower or are not present during this sampling might be missed. The multiple bouts required to detect these species are prohibitively expensive and not warranted given the requirement to track dominant cover types and species. Some NEON sites experience multiple distinct phenological peaks populated by different species. For example, the Sonoran desert (represented by the NEON site at the Santa Rita Experimental Range) experiences a bimodal precipitation regime with rain in late winter and a monsoonal moisture pulse late in the summer. Plant species particularly adapted to the intersection of temperature and timing and amount of precipitation flower and can be identified during each event (Ogle and Reynolds 2004). Such sites may require more than one sampling event per year to adequately characterize annual plant species composition and abundance.





**Figure 4.** A biodiversity-based approach to standardizing effort may facilitate comparability of trends and patterns across sites (top) and across National Land Cover Database cover types within a site (bottom), as shown with data from NEON sampling efforts at the Talladega National Forest in Alabama. Species richness at sites and within cover types might be best compared at the inflection point on species-accumulation curves that may have been reached at the Jornada Experimental Range and the Central Plains Experimental Range with extant sample sizes, but remains elusive at the Talledega National Forest.



## 6.2.4 Data and opportunities for analysis

The data from the plot-based sampling will result in data products that will be available for download from the NEON data portal:

- Quality controlled data on the presence and cover of species at 1-m<sup>2</sup> subplots
- The presence of species at 10-m<sup>2</sup> and 100-m<sup>2</sup> subplots
- The complete species list in each 400-m<sup>2</sup> plot for each plot
- The nativity of species according to the US Department of Agriculture PLANTS Databases
- Additional cover of other features (e.g., rock, litter, wood, water) in each 1-m<sup>2</sup> subplot

# 6.3 Diversity of plant functional traits

Because the contribution to ecological processes can be redundant across plant species, plant traits may describe ecosystem function better than plant species identity and abundance (Loreau 2010). Functional traits – specific characteristics such as leaf size, seed size, and canopy height – drive processes such as net ecosystem exchange (Diaz and Cabido 1997, McGill et al. 2006). Further abstraction is obtained by grouping species with similar effects on ecosystem function – functional types or functional groups - such as evergreen shrubs and  $C_3$  and  $C_4$  grasses (Diaz and Cabido 1997, 2001, Hooper et al. 2005). The functional concept provides a framework for understanding the causes and consequences of changes in plant diversity by focusing on the mechanistic links between plants and ecosystem processes and environmental change (Diaz and Cabido 1997, Ustin and Gamon 2010, Chapin et al. 2012, Hooper et al. 2012).

This component of plant diversity is not a primary focus of NEON's collection efforts, but several protocols result in data products or subproducts that can be found in plant functional trait libraries (Appendix 1, Cornelissen et al. 2003, Kattge et al. 2011). NEON will select a mix of dominant and rare species for phenology (AD[06]) and foliar biogeochemistry (AD[10]), which will also be a subset of those species documented in the plant biomass, structure (AD[07]), and diversity protocols. The diversity of protocols collocated at sites, focused on specific species, and often measured from the same individuals provides a cohesive set of observations that could better describe how and why vegetation 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 (Appendix 1). Phenology will be measured many times throughout the growing season. Traits associated plant biomass and structure will be measured annually, and foliar biogeochemistry will be sampled every five years. Future efforts could collate these data and supplemental data into a functional trait library capable of furthering the NEON contribution to understanding the cause and consequences of ecological change.

#### 6.4 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). Approaches involve the direct detection of species by isolating 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 principal components of the hyperspectral imagery (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, several airborne and ground-based NEON data products will facilitate these efforts:

- The NEON remote sensing platform is planned to fly at each NEON site annually, producing LiDAR and hyperspectral products at resolutions less than 3m (Kampe et al. 2010). These data, particularly when combined with ground-based observations, are useful for mapping and extrapolating patterns of functional traits, functional groups, and multiple metrics of plant diversity.
- Dominant species will be mapped in plots near the tower and across NEON sites to track changes in biomass and calibrate the airborne observations. Species-specific endmembers of the hyperspectral data can also be identified by intersecting the spatially-explicit, plot-based stem maps with high-resolution imagery (Asner et al. 2012, Asner and Youngsteadt 2012). 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).
- Species richness, derived from either the mapped distributions of dominant species and/or the plot-based plant diversity observations can be spatially linked to the diversity of principal components of the hyperspectral imagery to calibrate estimates of diversity that can be extrapolated to the airborne footprint (Rocchini et al. 2011, Schimel et al. 2013).

Linking ground and airborne data facilitates the scaling of plant diversity data. In the absence of the airborne data, site-scale plant diversity metrics must be based on a diversity indices (Hill 1973), model-based approaches (Kreft and Jetz 2007), or design-based estimators (Thompson 2012). Calibrating and then directly mapping patterns of species distributions and diversity with airborne imagery leverages what is otherwise a ground-based sample from multiple plots to produce a census of diversity at NEON sites. Developed across time, these spatial representations have the potential to describe otherwise undetected and divergent patterns, rates of species turnover, and invasive plant species. The airborne data can also facilitate scaling the spatial extent of patterns of plant diversity as well. The plot data and airborne imagery might be linked with other fixed-wing and satellite remote sensing platforms to scale



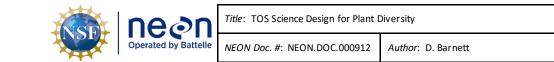
diversity data to even larger areas, furthering the NEON effort to understand and forecast ecological change at large scales.

## 6.5 Voucher Specimens

NEON will create a physical record of species recorded in plots and individuals sampled for the genetic archive. Herbarium specimens will be stored at NEON offices for training and validation purposes, as well as sent to museums or other facilities as part of the NEON bioarchive program (Kao et al. 2012). 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, Vellend et al. 2011).

## 6.6 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 with real data and feedback from the user community. 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 site-specific heterogeneity is sampled by the 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.



## 7 FUTURE DIRECTIONS

The plant diversity data that NEON will provide will allow the ecological community to ask numerous questions across spatial and temporal scales and disciplines. The consistent collection of plant diversity data linked with physical and chemical dynamics of atmosphere, biogeochemistry, ecohydrology, and various organisms, populations and communities will result in new and robust patterns and through time will facilitate the evaluation and development theory. Some examples of the questions that might be asked of the data include:

- How do patterns and rates of plant species invasion respond to changing climate and land use? Repeated, plot-based measurement of plant species over decades at sites exposed to divergent patterns of land use and climate trajectories will provide the opportunity to tease apart the relative importance and interactions of changing climate and land use on invasion and establishment of non-native plant species.
- How does plant diversity/species richness respond to short- and long-term climatic variability within and across spatial and ecological cover-type strata?
- To what extent can aerial observations accurately measure (and predict) attributes of terrestrial ecosystems such as species richness or dominant cover type?
- Are there detectable trends in diversity over time? Is variation across adjacent regions synchronized (i.e., what are the spatio-temporal dynamics)?
- How synchronous are temporal variations among spatial scales? Do different scales show more or less noise or trend?
- Do variations in diversity among years responsive to variations in summary climate characteristics (e.g., mean annual temperature, maximum temperature, mean annual precipitation, drought intensity)?
- How stable is community productivity over time? Is there a link to fluctuations in diversity?
- Can trends in species composition over time be linked to diversity and productivity dynamics?
- Will warming increase plant diversity where plant growth is temperature limited, or will warming and reduced moisture availability result in decreases plant diversity?

#### 7.1 Iteration of the Design

Collection of data provides the opportunity to test and evaluate the design. The comparability of species across sites based on the sample allocation, and the capacity of the data resulting to detect spatio-temporal trends will be evaluated during the first several years of collection. Additionally, feedback from the consumers of plant tissue collections and functional trait data, and from those integrating data with airborne and other NEON data streams will be considered as the NEON design is optimized. This iteration must occur with input from members of the ecological community: the data must be relevant to specific questions and analytical approaches and it must facilitate insights the next generation of ecologists will produce.



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**Appendix 1:** NEON will produce data products that are found in the TRY database (https://www.try-db.org) of plant functional traits. The traits listed below will either be produced by NEON or can be directly derived from NEON data products (e.g., leaf carbon/nitrogen ratio), but does not include plant functional traits that could be derived from NEON data products with additional data or linking to external databases (e.g., plant palatability, species occurrence range, elevation range).

TRY trait	NEON data product name	Frequency
Coarse woody debris (CWD) density	Coarse downed wood bulk density	
	sampling <sup>1</sup>	Every 5 years
Cone (strobilus) litter nitrogen (N) per		
cone litter dry mass	Litter chemical properties <sup>1</sup>	Annual
Crown (canopy) area per plant	Woody plant vegetation structure <sup>1</sup>	Annual
Crown (canopy) density <sup>1</sup>	Woody plant vegetation structure <sup>1</sup>	Annual
Crown (canopy) height (base to top)	Woody plant vegetation structure <sup>1</sup>	Annual
Crown (canopy) length: diameter along the		
longest axis	Woody plant vegetation structure <sup>1</sup>	Annual
Crown (canopy) width	Woody plant vegetation structure <sup>1</sup>	Annual
Fine root carbon (C) content per fine root		
dry mass	Root sampling tower plots	Every 5 years
Fine root carbon/nitrogen (C/N) ratio	Root sampling tower plots	Every 5 years
Fine root diameter	Root sampling tower plots	Every 5 years
Fine root dry mass per fine root fresh mass	Root sampling tower plots	Every 5 years
Fine root litter calcium (Ca) content per		
fine root litter dry mass	Root chemical properties	Every 5 years
Fine root litter magnesium (Mg) content		
per fine root litter dry mass	Root chemical properties	Every 5 years
Fine root litter nitrogen (N) content per		
fine root litter dry mass	Root chemical properties	Every 5 years
Fine root litter phosphorus (P) per fine		
root litter dry mass	Root chemical properties	Every 5 years
Fine root litter potassium (K) content per		
fine root litter dry mass	Root chemical properties	Every 5 years
Fine root litter sodium (Na) content per		
fine root litter dry mass	Root chemical properties	Every 5 years
Leaf area per leaf dry mass (specific leaf	Plant foliar physical and chemical	
area, SLA)	properties <sup>1</sup>	Every 5 years
	Plant foliar physical and chemical	
Leaf carbon (C) content per leaf dry mass	properties <sup>1</sup>	Every 5 years
Leaf carbon (C) isotope signature (delta	Diant falian atabla isatasa 1	
13C)	Plant foliar stable isotopes <sup>1</sup>	Every 5 years
Loof on the protocol (CAN) water	Plant foliar physical and chemical	
Leaf carbon/nitrogen (C/N) ratio	properties <sup>1</sup>	Every 5 years



	Plant foliar physical and chemical	
Leaf carbon/phosphorus (C/P) ratio	properties <sup>1</sup>	Every 5 years
	Plant foliar physical and chemical	
Leaf chlorophyll content per leaf dry mass	properties <sup>1</sup>	Every 5 years
	Plant foliar physical and chemical	LVELY 5 YEARS
Leaf dry mass	properties <sup>1</sup>	Every 5 years
	Plant foliar physical and chemical	LVELY 5 YEARS
Leaf fresh mass	properties <sup>1</sup>	Every 5 years
Leaf length	Plant canopy leaf mass per area <sup>1</sup>	Every 5 years
Leaf litter calcium (Ca) content per leaf		
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter carbon (C) content per leaf litter	Litter chemical properties	Annuar
dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter carbon/nitrogen (C/N) ratio (per		Annuar
species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter magnesium (Mg) content per		
leaf litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter nitrogen (N) content per leaf	Litter chemical properties	Annuar
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter phosphorus (P) content per leaf		Annuar
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter potassium (K) content per leaf		Annuar
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter sodium (Na) content per leaf		Annuar
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf litter sodium (Na) content per leaf		
litter dry mass (per species)	Litter chemical properties <sup>1</sup>	Annual
Leaf magnesium (Mg) content per leaf dry	Plant foliar physical and chemical	Annuar
mass (per species)	properties <sup>1</sup>	Every 5 years
Leaf nitrogen (N) content per leaf dry mass	Plant foliar physical and chemical	
(per species)	properties <sup>1</sup>	Every 5 years
Leaf nitrogen (N) isotope signature (delta		
15N) (per species)	Plant foliar stable isotopes <sup>1</sup>	Every 5 years
Leaf nitrogen/phosphorus (N/P) ratio (per	Plant foliar physical and chemical	
species)	properties <sup>1</sup>	Every 5 years
Leaf phosphorus (P) content per leaf dry	Plant foliar physical and chemical	
mass (per species)	properties <sup>1</sup>	Every 5 years
Litter calcium (Ca) content per litter dry		
mass	Litter chemical properties <sup>1</sup>	Annual
Litter carbon (C) content per litter dry		
mass	Litter chemical properties <sup>1</sup>	Annual
Litter carbon (C) isotope signature (delta		
13C)	Litter stable isotopes <sup>1</sup>	Annual
Litter carbon/nitrogen (C/N) ratio	Litter chemical properties <sup>1</sup>	Annual
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Litter magnesium (Mg) content per litter		
dry mass	Litter chemical properties <sup>1</sup>	Annual
Litter nitrogen (N) content per litter dry		
mass	Litter chemical properties <sup>1</sup>	Annual
Litter nitrogen (N) isotope signature (delta		
15N)	Litter stable isotopes <sup>1</sup>	Annual
Litter phosphorus (P) content per litter dry		
mass	Litter chemical properties <sup>1</sup>	Annual
Litter potassium (K) content per litter dry		
mass	Litter chemical properties <sup>1</sup>	Annual
Litter sulfur (S) content per litter dry mass	Litter chemical properties <sup>1</sup>	Annual
Plant growth form	Woody plant vegetation structure	Annual
Plant height	Woody plant vegetation structure <sup>1</sup>	Annual
Plant height growth relative to diameter		
growth	Woody plant vegetation structure <sup>1</sup>	Annual
Plant height versus plant width		
relationship	Woody plant vegetation structure <sup>1</sup>	Annual
Plant reproductive phenology timing	Plant phenology observations	
Plant vegetative phenology	Plant phenology observations	
Root aluminum (Al) content per root dry		
mass	Root sampling tower plots	Every 5 years
Root calcium (Ca) content per root dry		
mass	Root sampling tower plots	Every 5 years
Root carbon (C) content per root dry mass	Root sampling tower plots	Every 5 years
Root carbon (C) isotope signature (delta		
13C)	Root stable isotopes	Every 5 years
Root carbon/nitrogen (C/N) ratio	Root sampling tower plots	Every 5 years
Root dry mass per ground area	Root sampling tower plots	Every 5 years
Root nitrogen (N) content per root dry		
mass	Root sampling tower plots	Every 5 years
Root nitrogen (N) isotope signature (delta		
15N)	Root stable isotopes	Every 5 years
Root phosphorus (P) content per root dry		
mass	Root sampling tower plots	Every 5 years
Root sulfur (S) content per root dry mass	Root sampling tower plots	Every 5 years
Species status (nativity at growth location)	Plant presence and percent cover <sup>1</sup>	Annual

<sup>1</sup>Collected within multi-scale plot