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The Plant Diversity Sampling Design for The National Ecological Observatory Network

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INTRODUCTION

Observations of plant diversity, a multifaceted concept that considers variation at multiple organizational levels in a defined space and time (Heywood 1995, Hubbell 2001, Stohlgren 2007), contributed to the origins of the theory and practice of ecology (Darwin 1859, Magurran and...
Study at multiple resolutions—genetic (Hinchliff et al. 2015), trait (Adler et al. 2014, Enquist et al. 2015), population (Clark et al. 2004, 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 process 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 interactions are sensitive to 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 such as climate change (Walther et al. 2002, Moritz and Agudo 2013, Ash et al. 2016), species invasions (Jeschke et al. 2014), and ecological heterogeneity (Stein et al. 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. 2004). Observation over decades could illuminate patterns of change and facilitate the iterative forecasting of future conditions (Clark and Gelfand 2006, Luo et al. 2011a, b). 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 National Ecological Observatory Network (NEON) will collect consistent and comparable ecological data across the continent for three decades. The Observatory is designed to 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 (Keller et al. 2008). Greater insights into ecological cause and effect relationships will be facilitated by integrating ongoing systematic observations of the drivers of change and ecological response at 47 sites throughout the continental United States, including Alaska, Hawaii, and Puerto Rico (Vitousek 1994, Keller et al. 2008, Luo et al. 2011a, b). The sites encompass wild spaces and cross a variety of gradients (e.g., land use, species invasion, nitrogen deposition) to address regional and continental-scale ecological questions. The NEON design (Keller et al. 2008, Schimel et al. 2011) and sampling of terrestrial organisms and soil (Kao et al. 2012, Thorpe et al. 2016) are described in greater detail elsewhere.

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

1. 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.
2. Enable society and the scientific community to use ecological information and forecasts to understand and effectively address critical ecological questions and issues.
3. Provide physical and information infrastructure to support research, education, and land management.

Traceable links between these high-level goals and the data the Observatory network produces provide a top-down framework for the development of this 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.

**Design Criteria**

Design criteria that guide the NEON design for sampling plant diversity include the following: (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 network. 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 yr (Table 1).

**Objectives of the plant diversity sampling**

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 Millsapau 2012):

1. Plant diversity objective: Observations are designed to clarify the causes and consequences of changes in spatial and temporal patterns of plant diversity.

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**Table 1.** Traceability between a subset of the high-level National Ecological Observatory Network (NEON) requirements that guide the cohesive design of the NEON across all science, engineering, education, and cyberinfrastructure and the plant diversity requirements that further define the context and parameters of the plant diversity design and resulting data products.

<table>
<thead>
<tr>
<th>Plant diversity design requirements</th>
<th>Select high-level NEON requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEON measurements of plant diversity will include observations of plant genomics, native and invasive plant species, and functional traits collocated with other NEON observations of bird, small mammal and fish species, soil, and atmosphere</td>
<td>NEON’s measurement strategy will include coordinated and co-located measurements of drivers of environmental change (physical and chemical climate, land use, and biological invaders) and biological responses (matter and energy fluxes, biomass and plant productivity, diversity and genomics of key organismal groups, infectious diseases and community, phenological and population indicators)</td>
</tr>
<tr>
<td>Observations of plant diversity will be collocated with local measures of ecosystem properties and within the same and other dominant landscape characteristics at the scale of the site</td>
<td>NEON’s spatial observing design will systematically sample national variability in ecological characteristics, using an a priori division of the nation to allow extrapolation from limited intensive sampling of core wildland sites back to the continental scale</td>
</tr>
<tr>
<td>Observations of plant diversity will facilitate spatial extrapolation that can be linked to remote sensing and integrated with other sources of plant diversity data across the continent</td>
<td>NEON will allow extrapolation from the Observatory’s local sites to the nation. NEON will integrate continental-scale data with site-based observations to facilitate extrapolation from the local measurements to the national Observatory</td>
</tr>
<tr>
<td>NEON will sample plant diversity with sufficient intensity to allow detection of changes over the life of the Observatory</td>
<td>NEON infrastructure and observing system signal-to-noise characteristics will characteristics will be designed to observe decadal-scale changes against a background of seasonal-to-interannual variability over a minimum 30-yr lifetime</td>
</tr>
<tr>
<td>Minimally, plant diversity will be observed each year to enable detection of annual rates of change</td>
<td>NEON observing strategies will be designed to support new and ongoing ecological forecasting programs, including requirements for state and parameter data, and a timely and regular data delivery schedule</td>
</tr>
<tr>
<td>NEON shall observe plant diversity with repeatable and standardized methods that will allow comparison within and across sites and through time</td>
<td>NEON measurements will be standardized and calibrated to allow comparison across sites and over time to enable understanding of ecological change in time and space. Calibration and standardization will also allow new sensors/measurements to be calibrated against the old</td>
</tr>
</tbody>
</table>

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2. 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 timescale to allow detection and quantification of most trends over the 30-yr time span of NEON (Schimel et al. 2011).

3. 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.

4. 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 (Barnett et al., in press).

5. Sampling frame: The spatial extent of NEON sites bounds the area available to sample plant species (Bonar et al. 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 km²) to wildland sites (e.g., Central Plains Experimental Range, 65 km²). 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.

6. 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 (Bonar et al. 2012).

The plant diversity design is informed by these requirements and objectives. The resulting data products, tissues, and vouchers result from specific design components that reflect the multi-faceted approach to observing plant diversity: gene expression and phylogenetic differences, species-level taxonomic classifications, functional characteristics, and remote sensing.

Collection of material for assessments of plant species genetic diversity

National Ecological Observatory Network 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 (see Elmendorf et al. 2016 for a description of NEON plant phenology sampling). 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 (Hinckley et al. 2016) measurements and subjected to biomass and productivity observations (C. L. Meier et al., in preparation) across NEON sites. In some cases, the 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 trade-offs between intra- and interspecific diversity.
(Gemeinholzer et al. 2010, Neves and Forrest 2011). Tissue from ten individuals of each the species selected for phenology sampling will be collected—from the phenology plot near the tower, Distributed Base Plots (see Thorpe et al. 2016), and, when necessary, 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 et al. 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.

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 following:

1. the multi-scale plot that facilitates repeatable observations at a variety of scales,
2. the spatial and temporal sample design to facilitate detection of trends within and across NEON sites, and
3. the data products and possible analyses.

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 et al. 1995), and shares scales of measurement as well as subplot and plot shapes with a standard proposed by Dengler (2009), but most closely emulates the approach developed by the Carolina Vegetation Survey (Peet et al. 1998). Plant taxonomic composition will be recorded in 20 × 20 m square plots comprised of four 10 × 10 m subplots containing nested 10- and 1-m² subplots (Fig. 1). 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:

1. The identity of each species according to naming conventions maintained by the U.S. Department of Agriculture, Natural Resources Conservation Service PLANTS Database (USDA, NRCS 2016) will be recorded in each subplot—eight 1 m², eight 10 m², and four 100 m². Because the NRCS database is dynamic, the date data were accessed will be included on the NEON data portal.
2. Estimates of taxon-specific abundance will be made with estimates of cover within the 1-m² subplots.
3. A detailed description of the collection, including all variables measured, the cover-abundance estimates, and treatment of growth forms, is available in the NEON protocol for sampling plant diversity (Barnett 2018).

Little exists in the way of a standardized protocol for sampling plant species diversity that is suitable for a continental-scale Observatory. Biodiversity Observatories in Africa developed a multi-scale plot that is very similar to the Whittaker plot (Shmida 1984) for sampling and scaling across diverse land cover (Jürgens et al. 2012). However, GEO BON (Scholes et al. 2017), for example, stresses the importance of documenting key variables, but does not go beyond discussion of a diversity of methods for in situ collection due to site-specific requirements (Pereira et al. 2017). A protocol typically reflects specific questions or objectives, and the various options have trade-offs (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 (Table 1). 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
A plot-based method is repeatable, allows collocation of protocols, and is capable of describing species–environment relationships (Stohlgren 2007). 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 U.S. states, Stohlgren et al. (1998, 1999, 2003) contributed to a general understanding of environmental plant species controls of invasion.

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, 400-m$^2$ plot design. Considerations and design trade-offs included the following:

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**Fig. 1.** 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 (Thorpe et al. 2016).
1. **Plot size.**—Larger plots detect a greater number of species at local, plot scales. Highly linear transects (Parker 1951) and point-intercept methods (Barbour et al. 1999) 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 area sampled (Gilliam 2007). 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 400 m$^2$, the NEON plot is large relative to transect and small-plot methods (Parker 1951, Daubenmire 1959), but smaller than both the 1000-m$^2$ plots commonly used to intensively document local species composition (Whittaker 1960, Stohlgren et al. 1995, Peet et al. 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.

2. **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 the composition and cover-abundance (direct estimates of cover, e.g., Walter et al. 2015) of herbaceous species at small 1-m$^2$ scales and capturing diversity of large-stature, well-spaced tree species at 100- and 400-m$^2$ scales (Peet et al. 1998, Gilliam 2007), 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 1-m$^2$ plots observed by the Nutrient Network (Adler et al. 2011, Dengler et al. 2011) are directly comparable to the NEON 1-m$^2$ sub-plots. In cases where scales of observation do not coincide, the multi-scale NEON data enable 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 U.S. 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…”

3. **Plot shape.**—Linear or rectangular methods for observing plant diversity tend to observe more species than circular or square plots of similar size. 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 20 × 50 m multi-scale plot (Shmida 1984) contained nested subplots that changed shape with scale, confounding
the perimeter-to-area ratio across scales and resulting species–area curves (Fig. 2). The Carolina Vegetation Project plot (Peet et al. 1998) partially resolved this issue by nesting square plots within square 100-m² modules, maintaining a square until the ten modules (2 × 5) result in the 1000-m² rectangular plot. The NEON plot, composed of four Carolina Vegetation Project modules (2 × 2), is square. It takes less time to sample than larger rectangles (Barnett and Stohlgren 2003, Stohlgren 2007), and integrates with other NEON protocols and data streams. For example, 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 multi-scale 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…”

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 (Barnett et al., in press). 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 (250–1400 m radius) the NEON tower (Tower Base Plots; Thorpe et al. 2016). These measures allow within-plot collocation with observations of vegetation biomass, productivity, structure, and soil (Hinckley et al. 2016) and nearby observations of plant phenology (Elmendorf et al. 2016). 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, Thorpe et al. 2016). 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 km²; Thorpe et al. 2016). 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 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 (see Barnett et al., in press). 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-yr 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)—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 of 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 address 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 require 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 United States 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 (Rosenzweig 1995, Gotelli et al. 2009), level of expertise, and effort (Gotelli and Colwell 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 (Fig. 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 (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 10 km$^2$ of deciduous forest and the same number of plots in 100 km$^2$ of evergreen forest. The design recognizes that disproportionate levels of plant diversity can be found in relatively rare vegetation types (Myers 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.”

**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 (Magurran et al. 2010). The multiple bouts required to detect these species are prohibitively expensive and not

Fig. 3. A biodiversity-based approach to standardizing effort may facilitate comparability of trends and patterns across sites (A) and across National Land Cover Database cover types within a site (B), 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 Talladega National Forest.
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.

Data and opportunities for analysis.—The data from the plot-based sampling will result in low-level NEON data products that will be available for download from the NEON data portal:

1. Quality-controlled data on the presence and cover of species at 1-m² subplots.
2. The presence of species at 10- and 100-m² subplots.
3. The complete species list in each 400-m² plot for each plot.
4. The nativity of species to the United States according to the U.S. Department of Agriculture PLANTS Database.
5. Additional cover of other features (e.g., rock, litter, wood, water) in each 1-m² subplot.

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, Cressie et al. 2009). Design-based inference requires data collected according to a probabilistic design (Bonar et al. 2012). Various modeling approaches might benefit from the collection of data according to an alternative stratification or gradient or a random sample, 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 (Barnett et al., in press).

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 C₃ and C₄ 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 S1, Cornelissen et al. 2003, Kattge et al. 2011). NEON will select a mix of dominant and rare species for phenology observations (Elmendorf et al. 2016) and foliar biogeochemistry (Hinckley et al. 2016), which will also be a subset of those species documented in the plant biomass, structure, 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 S1). 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.
**Sample design for airborne observations of plant species diversity and abundance**

The estimation of patterns of plant diversity from remote sensing information across large spatial extents is an active area of research (Asner and Youngsteadt 2012, Asner et al. 2012, Schimel et al. 2013). Approaches involve the direct detection of species by isolating unique hyperspectral signatures (Asner and Martin 2009, 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:

1. The NEON remote sensing platform will fly at each NEON site annually, producing LiDAR and hyperspectral products at resolutions <3 m (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.

2. 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 end-members of the hyperspectral data can also be identified by intersecting the spatially explicit, plot-based stem maps with high-resolution imagery (Asner and Youngsteadt 2012, Asner et al. 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 end-member bundles can be used to estimate species cover fractions on a per-pixel basis (Feret et al. 2008, Asner and Martin 2009).

3. 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 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 leverage 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 plants 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.

**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 and sent to museums or other facilities as part of the NEON Bioarchive program (Kao et al. 2012, Thorpe et al. 2016). 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 (Vellend et al. 2011, Kao et al. 2012).

**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 observations 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.

**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 the following:

1. 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.
2. How does plant diversity/species richness respond to short- and long-term climatic variability within and across spatial and ecological cover-type strata?
3. To what extent can aerial observations accurately measure (and predict) attributes of terrestrial ecosystems such as species richness or dominant cover type?
4. Are there detectable trends in diversity over time? Is variation across adjacent regions synchronized, that is, what are the spatio-temporal dynamics?
5. How synchronous are temporal variations among spatial scales? Do different scales show more or less noise or trend?
6. Are variations in diversity among years responsive to variations in summary climate characteristics (e.g., mean annual temperature, maximum temperature, mean annual precipitation, drought intensity)?
7. How stable is community productivity over time? Is there a link to fluctuations in diversity?
8. Can trends in species composition over time be linked to diversity and productivity dynamics?
9. Will warming increase plant diversity where plant growth is temperature limited, or will warming and reduced moisture availability result in decreased plant diversity?

**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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LITERATURE CITED


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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2603/full