TOS SCIENCE DESIGN FOR PLANT BIOMASS, PRODUCTIVITY, AND LEAF AREA INDEX

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 DESCRIPTION

1.1 Purpose

The National Ecological Observatory Network (NEON) requires science design documents to define the scientific strategy leading to high-level protocols for NEON subsystem components, and to link NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. This document outlines NEON's sampling design for plant biomass, productivity, and leaf area index, and illustrates how the sampling design creates data products that allow NEON to respond to important ecological Grand Challenges (Figure 1)(National Research Council. 2001). Design documents flow from questions and goals defined in the NEON Science Strategy document (ER[01]), and inform the more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and Calibration/Validation and maintenance plans.

1.2 Scope

This document defines the rationale and requirements for sampling plant biomass, productivity, and leaf area index (LAI) in the NEON Science Design. More specifically, the sampling designs for above and below-ground vegetation components are defined, as well as how measurements will generate plant biomass, productivity, and LAI data products. Links between ground sampling and other NEON measurement platforms are explicitly defined, as are the criteria for standardizing measurements made across multiple disparate vegetation types. The document also describes the iterative sampling framework that will be employed to generate parameter estimates of similar uncertainty across disparate vegetation types. For each vegetation component, available methods and measurement choices are evaluated, spatial and temporal sampling strategies are explored, and relevant prototype data are presented and analyzed. From these inputs, a sampling design is selected and justified for each vegetation component, and the logistical, scientific, and budgetary implications of the selected design are articulated. In addition, components of the design are identified that may be adapted to changing budgets and labor availability, while still delivering required data products.

1.3 Acknowledgments

The design of the plant biomass, productivity, and LAI sampling for NEON described herein is the result of invaluable input from the Plant Productivity Technical Working Group, whose members include: Helene Muller-Landau, Tim Fahey, Richard Birdsey, Jim Lutz, Stefan Schnitzer, Michelle Mack, and Mark Friedl. The design also benefited immensely from ideas developed by the leaders of the NEON Tiger Team for plant biomass and productivity, as well as John Campbell, and Michael Keller.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD[01]	NEON.DOC.000001	NEON Observatory Design
AD[02]	NEON.DOC.001282	Introduction to the TOS Science Designs
AD[03]	NEON.DOC.000913	TOS Science Design for Spatial Sampling Design
AD[04]	NEON.DOC.000906	TOS Science Design for Terrestrial Biogeochemistry
AD[05]	NEON.DOC.000907	TOS Science Design for 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
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001484	Areas of mutual representativeness and exclusion around terrestrial infrastructure measurements

2.3 External References

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

ER [01]	Schimel, D, M Keller, S Berukoff, B Kao, H Loescher, H Powell, T Kampe, D Moore, W Gram (2011) Science strategy: Enabling continental-scale ecological forecasting.
ER [02]	
ER [03]	

2.4 Acronyms

Acronym	Definition
AGB	Above-ground biomass
AIS	[NEON] Aquatic Instrument System
ANPP	Annual net primary productivity
AOP	[NEON] Airborne Observation Platform
AOS	[NEON] Aquatic Observation System
BNPP	Below-ground annual net primary productivity
CAM	Crassulacean acid metabolism
CTFS	[Smithsonian] Center for Tropical Forest Studies

Acronym	Definition
CWD	Coarse woody debris
DBH	Diameter at breast height
ddh	Diameter at decimeter height
DHP	Digital hemispherical photo
DLPDS	Distance-limited perpendicular distance sampling
FIA	[United States Forest Service] Forest Inventory and Analysis
FRB	Fine root biomass
FRP	Fine root productivity
LAI	Leaf area index
LIDS	Line intersect distance sampling
LIS	Line intersect sampling
MODIS	Moderate resolution imaging spectroradiometer
NDVI	Normalized differential vegetation index
NEE	Net ecosystem exchange
NLCD	National land cover database
NPP	Net primary productivity
PDS	Perpendicular distance sampling
RRQRR	Reversed Randomized Quadrant-Recursive
RSE	Relative standard error
SOM	Soil organic matter
STRI	Smithsonian Tropical Research Institute
TC	Turnover coefficient
TIS	[NEON] Terrestrial Instrument System
TOS	[NEON] Terrestrial Observation System
VI	Vegetation index

3 INTRODUCTION

3.1 Overview of the Observatory

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 products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth, as well as understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01] and AD[02].

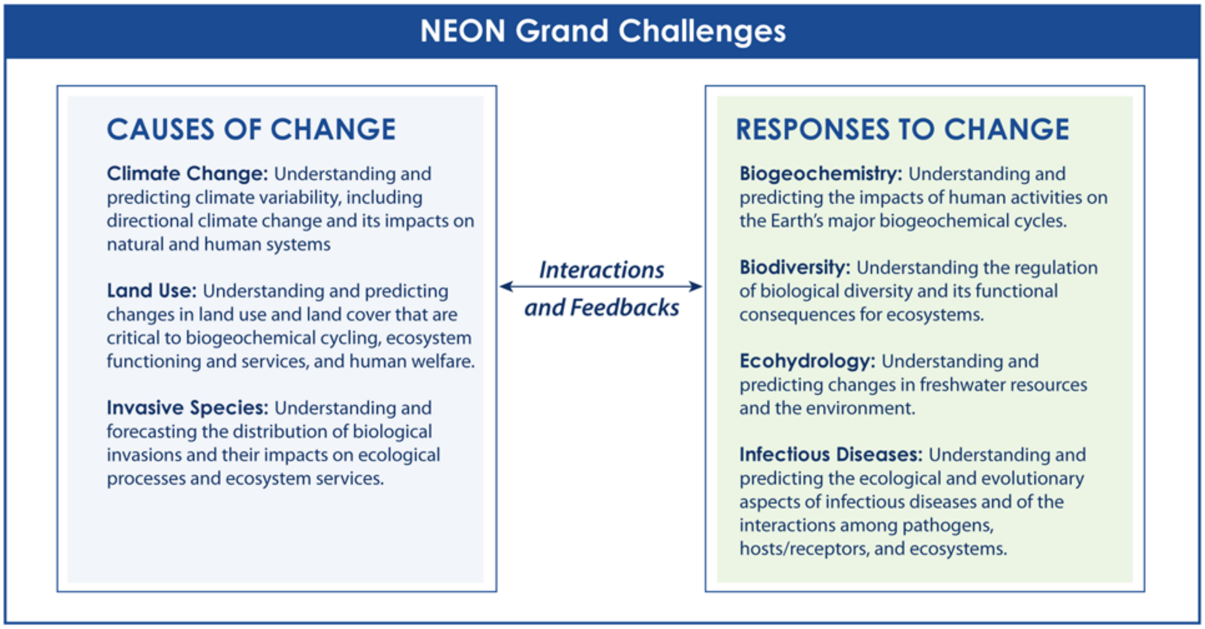

Figure 1. The seven ecological Grand Challenges defined by the National Research Council (National Research Council. 2001).

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 NEON AOP will link diversity and productivity data collected on individual plants and stands by the 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) and 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 TOS sampling foci are referred to as "modules" throughout this document. 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 AOP measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.NEONinc.org).

The standardization of protocols across all sites is key to the success of NEON (and its novelty) and must be maintained at all sites through time. Thus, although specific techniques may be required at some sites (e.g., due to different vegetation types), protocols have been developed to ensure data comparability. These details can also be found in individual design documents available through the NEON website (www.NEONinc.org).

The TOS Science Designs define the scientific strategies leading to high-level sampling designs for NEON sentinel taxa, terrestrial biogeochemistry, and infectious disease, linking NEON Grand Challenges and science questions to specific measurements (AD[02]). The TOS Spatial Sampling Design document describes the sampling design that collocates observations collected by each component of the TOS (AD[03]). TOS Science Design documents were developed following input from the scientific community, including module-specific Technical Working Groups, and the National Science Foundation (AD[02]). Science Designs will be reviewed periodically to ensure that data collection methods used 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 PLANT BIOMASS, PRODUCTIVITY, AND LEAF AREA INDEX SAMPLING DESIGN

4.1 Background

Humanity has strongly perturbed the global biogeochemical cycling of carbon (C) and nitrogen (N) throughout the past century, with one obvious consequence being ever-increasing concentrations of atmospheric carbon dioxide (CO_2) (Schlesinger 1997). Forecasting the effects of these perturbations remains difficult, however, because interactions between the atmosphere, terrestrial vegetation, and soils create feedbacks that may influence ecosystem C balance in opposing directions (Fan et al. 1998, Holland et al. 2000). Developing a better understanding of the spatial distribution and magnitude of above- and belowground plant biomass stocks and fluxes is critical to reducing uncertainty in large-scale models of the C cycle. Shifts in the balance between ecosystem-level net primary productivity (NPP) and heterotrophic respiration can have substantial effects on atmospheric CO_2 concentrations (Adair et al. 2008), and while patterns in the relative abundance of live plant biomass stocks are relatively well understood at large spatial scales, field-based estimates of above and belowground NPP based on a consistent sampling framework are more rare (Clark et al. 2001), particularly across a wide range of ecosystem types. Moreover, plant biomass stocks and fluxes are not static in space and time, and respond strongly to drivers like land-use change, atmospheric N deposition (Clark et al. 2001), changes in species composition (Brantley and Young 2007), and climate change (e.g. Cleveland et al. 2011). Long-term field-observations of multiple components of plant biomass and NPP (Figure 2) at site, regional, and continental scales are therefore essential for ecologists to predict how ecosystem C balance will respond to global change drivers.

Plot-based above and belowground plant biomass and NPP data are also an important complement to sensor-based measurements of ecosystem level biosphere/atmosphere C exchange – e.g. data derived from TIS flux towers. Parameters derived from day- and night-time eddy covariance data can be used to estimate net ecosystem exchange (NEE), and over time integrated NEE data provide an indication of whether a particular ecosystem has a positive or negative C balance. However, eddy covariance data cannot differentiate which vegetation components are accreting or losing C – i.e. woody tissues, foliage, reproductive tissues, etc. – or which species contribute most to observed NEE patterns. Repeated plot-based monitoring of annual above and belowground NPP can provide a relatively accurate and detailed picture of how vegetative C stocks and fluxes respond to various change drivers through time.

Although field-based estimates of biomass and NPP are extremely useful for mapping the spatial distribution of C at the regional and global scales (e.g. Saatchi et al. 2011), historically these data are difficult and expensive to obtain at high temporal frequency across large spatial scales. For example, the United States Forest Service operates an extensive Forest Inventory and Analysis (FIA) program that is the basis for most large-scale biomass estimates in the United States. The FIA program employs an inter-penetrating design that specifies annual measurement of plots within relatively large management units, with re-measurement of any given plot occurring on 5–10 y intervals (O'Connell et al. 2011).

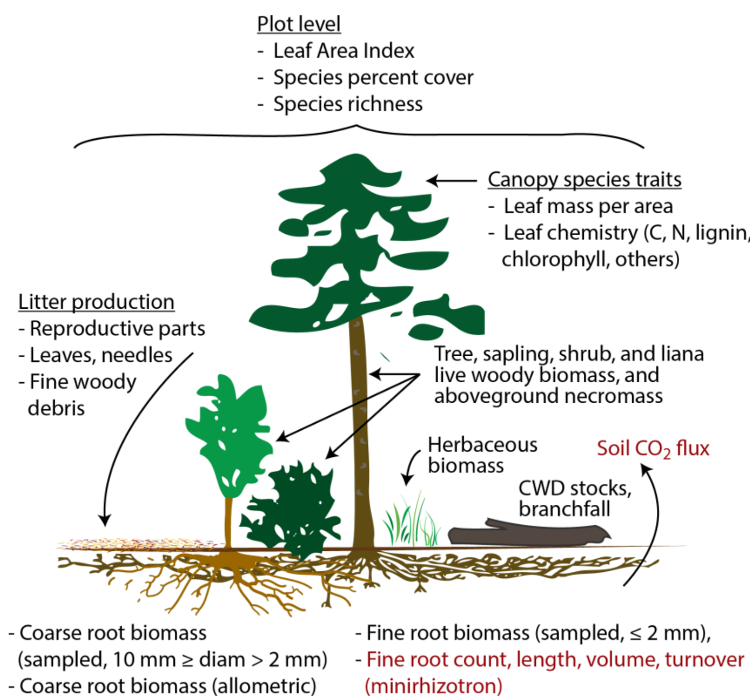

Figure 2. Plant biomass stocks, fluxes, species traits, and plot-level vegetation attributes that inform our understanding of terrestrial carbon cycling. Black text indicates TOS measurements, and red text indicates TIS measurements.

Because individual FIA plots are located within 3-mile diameter grid cells across the landscape, and because individual plots are re-measured relatively infrequently, there are spatial and temporal constraints associated with using FIA-based NPP estimates as inputs for modeling C cycling. To mitigate these problems, researchers commonly use high frequency LAI estimates derived from satellite remote-sensing instruments as an input variable for large-scale C flux models rather than NPP itself. For example, the Global Climate Observing System has identified LAI as an essential climate variable given its role in global carbon, energy, and water cycle models (GCOS 2006). Despite its importance as a key input variable for global C models, remote-sensing derived LAI estimates are infrequently validated in the field across a wide range of ecosystem types (but see Campbell et al. 1999, Baret et al. 2003, Canisius et al. 2010). As such, systematic, long-term field-validation of remote-sensing LAI data products is a persistent requirement for reducing uncertainty in the effects of global change drivers on ecosystem function.

To summarize, ongoing FIA monitoring efforts, satellite remote-sensing, and use of data from these and other sources within continental-scale models, forms the basis for our understanding of large-scale C-cycle dynamics in North America. However, model-based inferences would be improved with annual, continental-scale, plot-based inventory data, as well as systematic ground-validation of satellite-derived data products at the same scale.

4.2 NEON's Contribution

NEON's primary goals with respect to plant biomass and productivity sampling are to enable prediction of the effects of global change drivers on Earth's carbon, nutrient, and water cycles (see Grand Challenges in Figure 1), and to address several of the prominent, specific needs of the ecological and modeling communities (discussed above). To accomplish these goals, NEON has partitioned the continental United States plus Alaska, Hawaii, and Puerto Rico into twenty different eco-climatic domains (Figure 3). Within each domain, sampling will occur within wildland "Core" sites, selected to be broadly representative of the entire domain, and "Relocatable" sites that are selected to address broad science themes (i.e. land use change, climate impacts, invasive species, etc.). NEON sites are relatively small compared to domains, with approximately 80% of sites < 50 km^2.

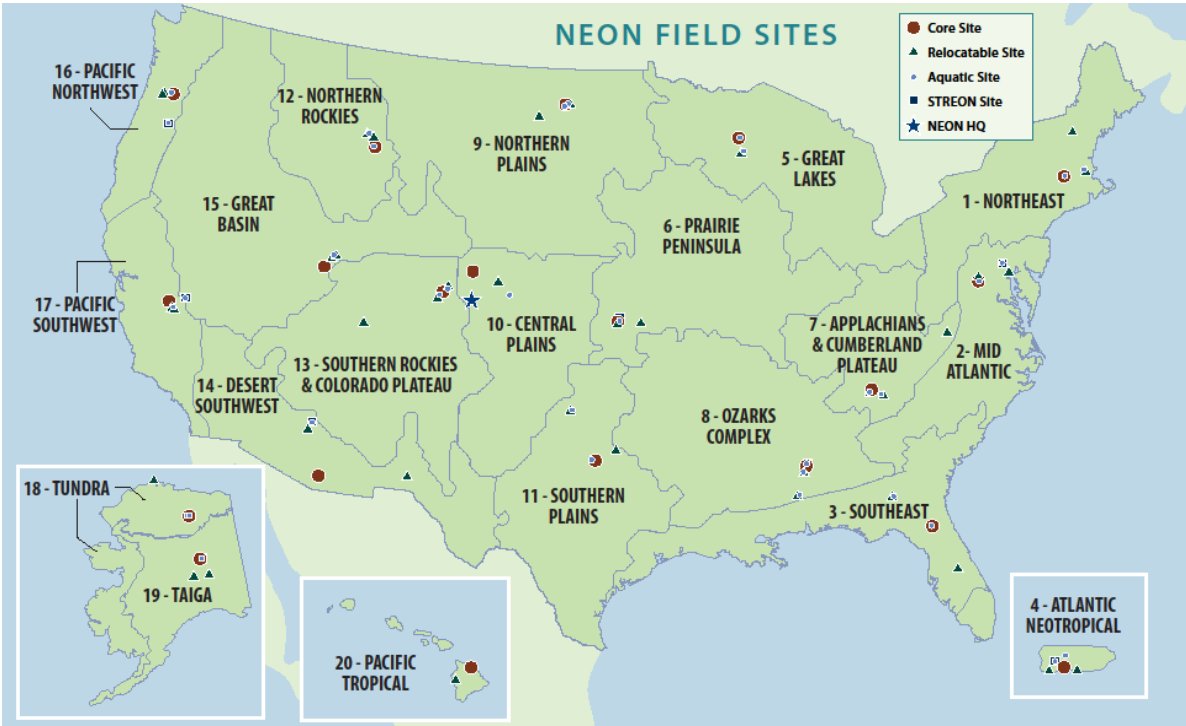

Figure 3. The twenty NEON domains, showing the location of core and relocatable sites.

There are three high-level components of the Observatory that are relevant to generating plant biomass, productivity, and LAI data products:

- **Airborne Observation Platform.** The AOP will use small aircraft outfitted with a hyperspectral imaging spectrometer, a waveform LiDAR, and a high-resolution camera to fly annually over all NEON sites (Kampe et al. 2010). These airborne instruments provide high-resolution datasets (approximately 1 m^2 pixels or smaller) on vegetation structure and canopy reflectance, the latter of which can be used to understand canopy chemical composition. The AOP is critical to NEON's ability to provide the ecological community with biomass, productivity, and LAI data products at scales larger than the plot.
- **Terrestrial Instrument System.** A flux tower will be constructed within the dominant vegetation at each site. Eddy-covariance sensors will generate NEE estimates over the lifetime of the site, and these data will indicate whether the dominant vegetation is a source or sink of C to the atmosphere, and how its rate of C gain/loss changes over time. Instrumented plots within the tower flux footprint, that are distinct from TOS plots described in detail in this document, will monitor soil temperature, soil moisture, soil respiration, and fine root turnover rates.
- **Terrestrial Observation System.** The TOS is a coordinated suite of plot and grid-based field sampling activities (**Figure 4**; AD[03]). For plant sampling, the salient components of the TOS include Distributed plots that are located throughout the site, and Tower plots that are concentrated within the TIS tower flux footprint; Tower plots are distinct from the instrumented TIS plots mentioned above. Both Distributed and Tower plots are established according to a spatially-balanced randomization algorithm (Theobald et al. 2007), and Distributed plots are

additionally stratified by National Land Cover Database (NLCD) vegetation type (Fry et al. 2011). A third set of Gradient Plots may be employed at a given site in order to sample important gradients in vegetation height, LAI, and canopy chemistry variables that are essential for forging links with AOP datasets. Gradient Plots will be placed non-randomly in order to span the full dynamic range of these variables, and these plots will only be employed when Distributed plots fail to span the full dynamic range of these variables.

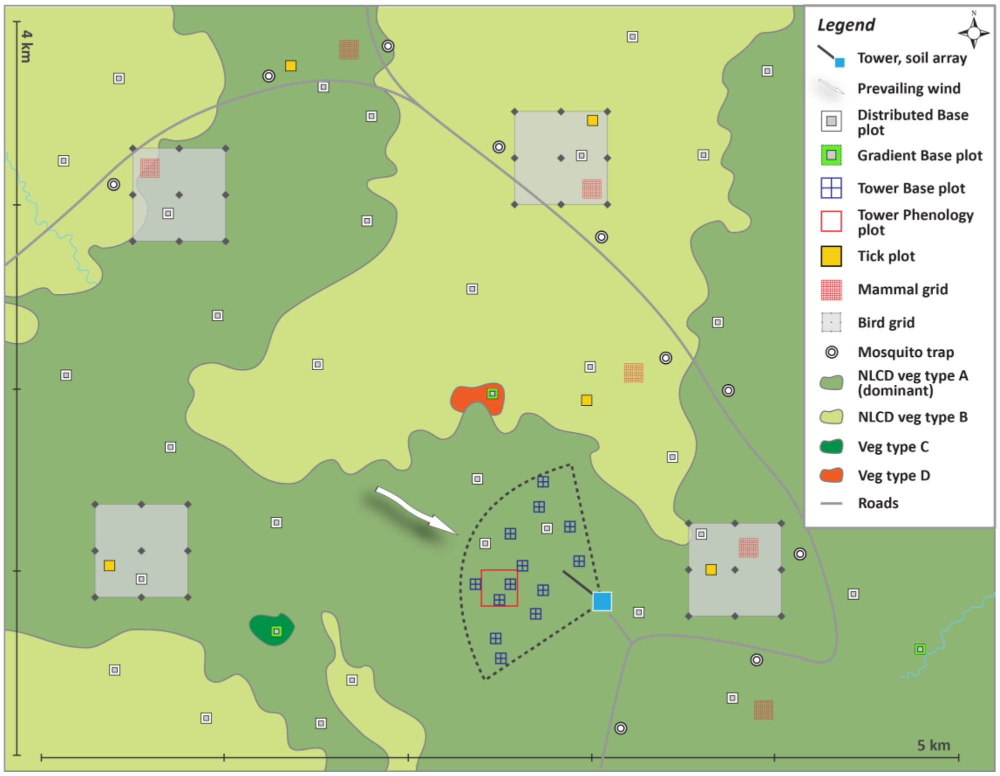

Figure 4. Schematic of terrestrial sampling at a NEON site. Plant biomass, productivity, and LAI sampling occurs in Distributed, Tower, and Gradient Plots.

Within each NEON site, the TOS Sampling Design for Plant Biomass, Productivity, and LAI will produce point and plot-level measurements that can be scaled up to landscape and regional levels. Upscaling will be possible because the sampling design for field measurements is tightly integrated with both the AOP and the TIS:

- **Integration of TOS and AOP.** One high-level component of the plant biomass and productivity sampling design is to measure aboveground biomass (AGB) stocks, vegetation structure, and LAI across the range of variability at each NEON site. The primary purpose of Distributed and Gradient plots is to enable collection of these data (Table 1 and Figure 4), and this sampling effort will support calibration and validation of AOP remote-sensing instruments. Field and remote-sensing data will then be integrated to produce site-level maps of these variables through time. Annual, site-level data for biomass and NPP can also be ingested into landscape-level models like the Community Land Model (Oleson et al. 2010).

- **Integration of TOS and TIS.** The Tower plot infrastructure at each site forms another key component of the plant biomass and productivity sampling design focused on measurement of above and belowground biomass and NPP within the same physical area from which TIS NEE estimates are derived (Table 1 and Figure 4). Aboveground plot-based measurements will include woody stem increment, herbaceous plant production, litter production, and LAI. Additionally, successive measurements of coarse woody debris will enable plot-based estimation of changes in this important C pool (Keller et al. 2004). Belowground plant biomass and production are significant and poorly constrained components of global C cycle models (but see Jackson et al. 1997), and as such, Tower plots will also support fine root biomass observations. Estimates of fine root standing stock (kg ha^{-1}) coupled with fine root turnover estimates (y^{-1}) from minirhizotrons installed in the TIS instrumented plots will allow estimation of annual belowground NPP within the dominant vegetation at each terrestrial NEON site. The coordinated, methodologically consistent, and long-term measurement of belowground fine root biomass and turnover rates at the continental scale will be a unique and important contribution to the field that will enable better understanding of the C cycle.

Taken together, implementation of the integrated NEON TOS, TIS, and AOP measurement platforms at 60 sites over 30 years represents a significant step forward in terms of predicting effects of global change drivers on plant biomass stocks and fluxes and C cycle dynamics.

Table 1. Spatial and temporal sampling strategy for plant biomass, productivity, and leaf area index across different vegetation components.

Vegetation component	Plot type	Plot number	Sampling events per year	Yearly sampling interval	Remarks
Belowground biomass*	Distributed	NA	NA	NA	Not sampled
	Tower	20-30†	1X per year	1y	Belowground productivity will be estimated at sites where minirhizotrons are installed.
Coarse woody debris (tally, bulk density*)	Distributed	20	1X per year	3y	Tally sampling only
	Tower	20-30†	1X per year	3y	Tally sampling every 3y; Bulk density 1X per site
Herbaceous biomass*	Distributed	≤ 20	1X per year	3y	Only clip plots with NLCD class ≠ Forest (Deciduous, Evergreen or Mixed)
	Tower	20-30†	1X-2X per year	1y	Mixed C3/C4 grasslands clipped 2X per year, other veg types clipped 1X per year; grazed sites clipped every 4 weeks
Leaf area index, total	Distributed	20	1X per year	5y	1 month sampling window overlapping AOP flight date
	Tower	3	Every 2 wks	1y	Sampling every 2 wks during growing season

Vegetation component	Plot type	Plot number	Sampling events per year	Yearly sampling interval	Remarks
Litterfall and fine woody debris*	Distributed	NA	NA	NA	Not sampled
	Tower	20-30†	Every 8 wks, minimum	1y	Increase frequency to every 2 wk sampling during senescence in deciduous forest
Mat-forming bryophytes*	Distributed	NA	NA	NA	Not sampled
	Tower	20-30†	1X per year	1y	Productivity sampling only; total biomass not sampled
Vegetation structure, woody stems	Distributed	≤ 20	1X per year	3y	Biomass estimated allometrically from structure data
	Tower	20-30†	1X per year	1y	

* Indicates that sampling method requires removal of material from site.

† In a given sampling year, all Tower plots are sampled; total Tower plot number varies by site between 20-30. Typically, forests are sampled with n=20 40m x 40m Tower plots, and smaller-stature vegetation is sampled with n=30 20m x 20m Tower plots.

5 SAMPLING FRAMEWORK

In the sections that follow, specific challenges are identified associated with sampling above and belowground plant biomass, productivity, and LAI within a framework defined by systems engineering requirements and data products. Specifically, Section 5.3 evaluates strategies for standardizing methodological, spatial, and temporal aspects of vegetation sampling across the Observatory. Subsections within Section 5.3 then provide detailed information about how the sampling designs for aboveground vegetation components, belowground biomass, and LAI were evaluated and selected.

5.1 Science Requirements

This Science Design addresses Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

5.2 Data products

Execution of the protocols that stem from this science design procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

5.3 Priorities and Challenges for Plant Biomass, Productivity, and LAI Sampling

A guiding principle for all of the NEON TOS Science Designs is the desire to create standardized sampling protocols and methods that are executed at the continental scale (Kao et al. 2012). For plant biomass and productivity, standardization presents unique problems associated with methodology, spatial design, and temporal design. Methodological standardization is problematic because there is no singular protocol suitable for sampling the diverse set of plant growth forms that are present at the continental scale. For example, clip-harvests are preferred for grasslands, but are difficult or non-sensical to perform with woody vegetation; similarly, vegetation structure measurements can be used to allometrically estimate the biomass of woody stems, but often do not provide relevant information about herbaceous biomass and productivity. From a spatial perspective, plot number, plot size, and plot spacing are equally difficult to standardize across multiple ecosystem types due to the varying physical stature and scale of individual plants that dominate different ecosystems. A relatively small number of plots on the order of 1 m^2 can produce adequate estimates of aboveground standing biomass, relative abundance, and species diversity in alpine tundra (Bowman and Seastedt 2001, Bowman et al. 2006). In contrast to systems dominated by herbaceous plants, biomass estimation in shrublands, forests, and savannahs requires much larger aggregate plot area, achieved either by increasing plot size, plot number, or both (Fahey and Knapp 2007). With respect to standardizing sampling temporally, the two main issues the design must address are: 1) creating robust annual estimates of plant biomass, productivity, and LAI across biomes with different phenologies; and 2) accounting for year-to-year differences in phenology within sites. Ignoring inter-biome and inter-annual within-site phenological differences has the potential to introduce unnecessary noise into annual estimates that are part of a long-term dataset.

In the subsections that follow, a framework is presented that addresses methodological, spatial, and temporal sampling challenges within the context of sampling plant biomass, productivity, and LAI across Distributed, Gradient, and Tower plots.

5.3.1 Vegetation Components

Because it is neither possible nor desirable to choose one standard method for sampling the biomass of all aboveground and belowground vegetation, NEON has defined a set of vegetation components that occur across the NEON domains, and adopted standard sampling protocols for these vegetation components. The 2008 NEON TIGER team identified the following unique types of vegetation for which standardized biomass and productivity sampling protocols will be implemented:

1. Woody plants (trees, saplings, shrubs, and lianas)
2. Herbaceous plants (graminoids, forbs, and some bryophytes)
3. Mat-forming bryophytes (e.g. *Sphagnum* spp.)
4. Leaf litter and fine woody debris
5. Coarse woody debris (CWD)
6. Coarse roots
7. Fine roots

The specific designs for these vegetation components are discussed in Section 6, but there are several conceptual issues relating to calculation of annual aboveground and belowground NPP that warrant further discussion here. For the *i* aboveground vegetation components (but excluding CWD), annual aboveground net primary productivity (ANPP) and belowground net primary productivity (BNPP) are calculated as the difference in AGB between times t_1 and t_2, where t_1 and t_2 have the units of years. However, for some of the *i* vegetation components, time intervals between sampling events may be less than a year, or multiple measurements made within the same year will be summed to estimate annual $ANPP_i$. At each site, aboveground $ANPP_{total}$ will then be calculated according to:

(1) $$ANPP_{total} = \sum ANPP_i$$

In practice, estimating $ANPP_i$ is often not as simple as calculating the difference between AGB from one year to another, even in common forest and grassland plant communities. In systems with woody-stemmed plants, $ANPP_{woody}$ can be calculated either by tracking biomass increment of individual stems using measurement intervals < 2 y, or $ANPP_{woody}$ can be calculated at the stand level using measurement intervals that can be > 2 y (Clark et al. 2001). NEON will estimate $ANPP_{woody}$ with the stand-level approach because it allows greater flexibility in the required sampling interval should the temporal sampling design need to be adapted to changes in budget, etc. Given the stand-level approach, $ANPP_{woody}$ is defined as:

(2) $$ANPP_{woody} = (\sum AGB_{t2} - \sum AGB_{t1}) + \sum AGB_{mortality} - (AGB_{min\,size\,ave} \times N_{new\,min\,size})$$

where $AGB_{mortality}$ = aboveground biomass of trees that died in the sampling interval + branchfall within the sampling interval, $AGB_{min\,size\,ave}$ = average aboveground biomass of trees above a minimum size cutoff, and $N_{new\,min\,size}$ = the number of new trees that now satisfy the minimum size requirement. *AGB* itself will be estimated from allometric equations that are either species-specific (Grier and Logan 1977, Taras and Clark III 1977, Ter-Mikaelian and Korzukhin 1997), region-specific (Gholz et al. 1979, Tritton and Hornbeck 1982, Schroeder et al. 1997), or continental/global in scale (Jenkins et al. 2003, Chave et al. 2005). When calculating woody-stem biomass at a given site, preference will be given to species-specific allometric equations developed from similar habitat types if available.

Ecosystems with significant herbaceous cover and/or significant grazing pressure present additional sampling challenges. In productive herbaceous systems with multiple biomass peaks in a given year, or in which production, senescence and decomposition occur simultaneously, a single biomass harvest per year will result in underestimation of ANPP (Sala and Austin 2000). Conversely, when multiple herbaceous biomass harvests are performed in a given year, unavoidable random errors are introduced that lead to overestimation of ANPP (Sala et al. 1988). Biondini et al. (1991) introduced a method for quantifying overestimation errors of this type, and NEON will correct ANPP estimates in grasslands using this approach in grasslands actively managed for grazing. Errors leading to underestimation of ANPP that occur when harvest number is less than the number of biomass peaks in a given year are more difficult to quantify.

When grazing by herbivores is significant, it is necessary to account for consumption of AGB and compensatory regrowth by plants (McNaughton et al. 1996). The standard method used to accomplish this is to sample AGB inside and outside of a number of portable grazing exclosures that are moved at regular intervals throughout the growing/grazing season (Knapp et al. 2007). Biomass estimates from both inside and outside the exclosures are then used to calculate annual *ANPP*:

(3) $$ANPP_{grazed} = \sum_{t=0}^{N}[AGB_{exclosure} - AGB_{outside}] + AGB_{final},$$

where exclosure biomass is harvested at the end of interval t_i, biomass outside exclosures is harvested at the beginning of interval t_i, and AGB_{final} is aboveground current-year biomass at the end of the year or growing season. At sites managed for grazing, NEON will employ a harvest interval of 4 weeks (Table 1), which will also allow accurate estimation of ANPP in grazed systems with more than one phenological biomass peak (e.g. grazed grasslands dominated by seasonally distinct C_3 and C_4 graminoid communities). Regardless of harvest interval and grazing management, clipped biomass will be sorted into functional groups at least once per growing season because functional traits influence how plants respond to various change drivers (e.g. Collatz et al. 1998). Clip-harvest sorting is described in detail in Section 6.2.

With respect to estimating annual belowground net primary productivity (BNPP), once per year measurements of coarse and fine root stocks is insufficient for calculating BNPP. BNPP is defined as the sum of coarse woody root production, fine root production, exudation and rhizodeposition, and root material lost to belowground herbivory. However, it is very difficult to reliably estimate exudation, rhizodeposition, and belowground herbivory. NEON will therefore only estimate coarse root and fine root production; the sampling designs for coarse roots and fine roots are presented in Sections 6.6 and 6.7, respectively.

Given the required use of multiple methods for assessing the biomass and productivity of these vegetation components, there are several aspects of the sampling for which standardization is important: 1) consistent sampling effort must be employed so that biomass of each vegetation component is estimated to within standardized uncertainty and repeatability limits; 2) biomass for all vegetation components must be reported with the same “growth increment mass per unit area” in order to facilitate calculation of NPP_{total} across all vegetation components; and 3) initial site characterization must be performed in order to determine which vegetation components are abundant enough at a site to warrant sampling (i.e. across all Tower plots, % cover for a given component is ≥ 10% of the total sampled area; % cover data will not be available for trees, so for this growth form, sampling will occur if at least one tree is present in > 10% of plots). The approach NEON will use to standardize sampling effort for plant biomass, productivity, and LAI across domains is discussed below.

5.3.2 Standardizing Sampling within NEON Plots

5.3.2.1 Sampling Distributed and Gradient Plots

The Distributed plots within each NEON site provide a physical basis for generating unbiased estimates of aboveground plant biomass and LAI at the site scale. These plots also link TOS biomass and biogeochemistry measurements with the AOP (AD[02], AD[03]), and provide a platform for generating numerous independent, co-located TOS data products (e.g. plant and beetle diversity). The NEON Distributed plots (40m x 40m in total size) feature a 20m x 20m (0.04 ha) central core that will be used for plant diversity, plant biomass, and LAI sampling, and this core area is further sub-divided into four 10m x 10m subplots (Figure 5). An additional annular area of 10m width surrounding this core will be used for co-located soil biogeochemistry and insect sampling, which gives the final dimensions of 40m x 40m (annular sampling area not shown in Figure 5). Distributed plots will not be utilized for belowground biomass sampling (Table 1).

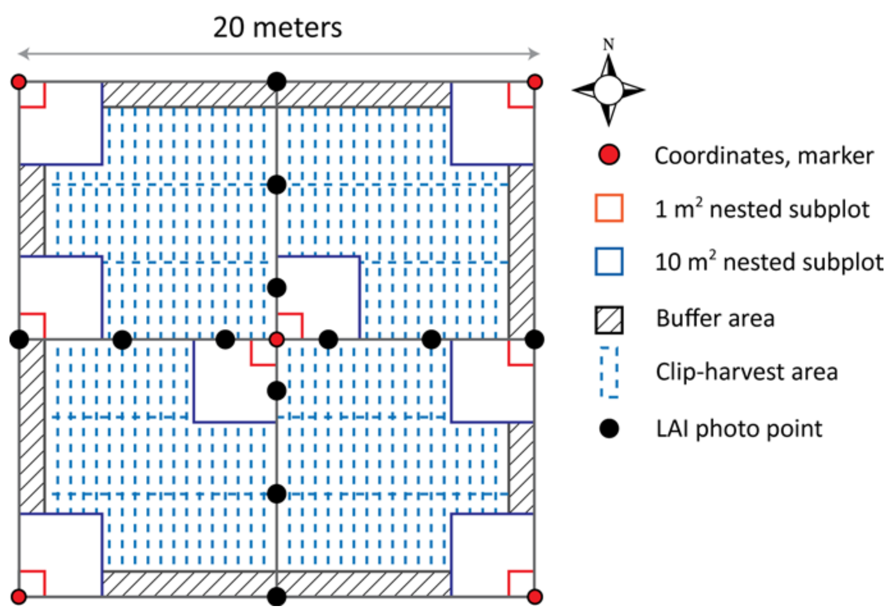

Figure 5. Distributed plot core sampling area, in which plant biodiversity, aboveground plant biomass, productivity, and LAI are sampled.

Within Distributed plots, subplots, and nested subplots are laid out according to a design modified from the North Carolina Vegetation Survey (Peet et al. 1998). Nested subplots are used to estimate percent cover and richness of herbaceous species, and depending on stem density, may be used for measurement of relatively small diameter woody stems (see Section 6.1 for details on woody vegetation). Herbaceous clip-harvesting is excluded from nested subplots in order to minimize the impact of clipping on plant biodiversity data products. Species richness will also be assessed in the clip-harvest area of the plot, and specific clip-harvest cells (Figure 5) will be rejected by field technicians if it is perceived that clipping will reduce observed plot-level species richness. That is, if an uncommon plant that would contribute to plot-level richness is growing in a potential clip-harvest cell, that cell will be rejected for clip-harvesting. The herbaceous clip-harvesting design is discussed in more detail in Section 6.2.

To maintain comparability with data collected from Distributed plots, Gradient plots will be the same size and shape as the Distributed plots. Only a small number of Gradient plots (if any) will be established at any given site because Gradient plots will only be established when vegetation height, LAI, and canopy chemistry data collected from Distributed plots fail to span the full dynamic range of these variables within AOP datasets collected at the site scale. The exact number of required Gradient plots will vary by site, but a maximum of n=5 Gradient plots will be sufficient to create AOP calibration/validation curves when data from these plots are combined with Distributed plot data.

5.3.2.2 Sampling Tower Plots

The purpose of TOS Tower plots is to enable consistent, field-based sampling of annual above and belowground NPP within the TIS tower footprint. Within this general framework, the goal is to sample the biomass of each *i* aboveground vegetation component such that the mean, μ_i, is estimated to within ± 10% and with a minimum of 90% confidence. Woody stem density, stature, and spatial heterogeneity will vary widely across biomes, and as such, the size and number of Tower plots must be scalable so that aboveground biomass uncertainty targets can be achieved while minimizing the total area sampled. Estimates of belowground coarse and fine root biomass will be more uncertain, due to the fractal distribution of root diameters in the soil (Taylor et al. 2013), and the relatively high allometric uncertainty associated with coarse root biomass estimation (Jenkins et al. 2003). Tower plot size and number will therefore be optimized on a per site basis using aboveground biomass data, rather than aboveground and belowground data.

5.3.2.3 Tower Plot Shape, Size, and Number

To maintain consistency with Distributed and Gradient plots, Tower plots are square, have a minimum size of 20m x 20m (0.04 ha), and a maximum size of 80m x 80m (0.64 ha). An example 40m x 40m Tower plot (0.16 ha) is shown in Figure 6. The maximum aggregate area that can be sampled across all Tower plots is approximately 4 hectares, and the minimum sampled area is 0.2 ha (n=5 20m x 20m plots). As an example, the area of the TIS tower footprint at the NEON D1 Harvard Forest core site is approximately 60-80 ha (depending on how the area of the flux footprint is calculated), which is relatively large compared to tower footprints at other sites. At this site, the aggregate Tower plot sampling area is initially 3.2 ha (n=20 40m x 40m plots), and therefore represents 4%–5.3% of the total tower footprint area. Maximum plot size and aggregate sampling area were chosen to adequately estimate μ_i to within approximately ± 10% of the mean with 90% confidence at sites with infrequent, very large trees (Keller et al. 2001), and the associated sampling effort is within the budgetary and logistical scope of Observatory field operations crews.

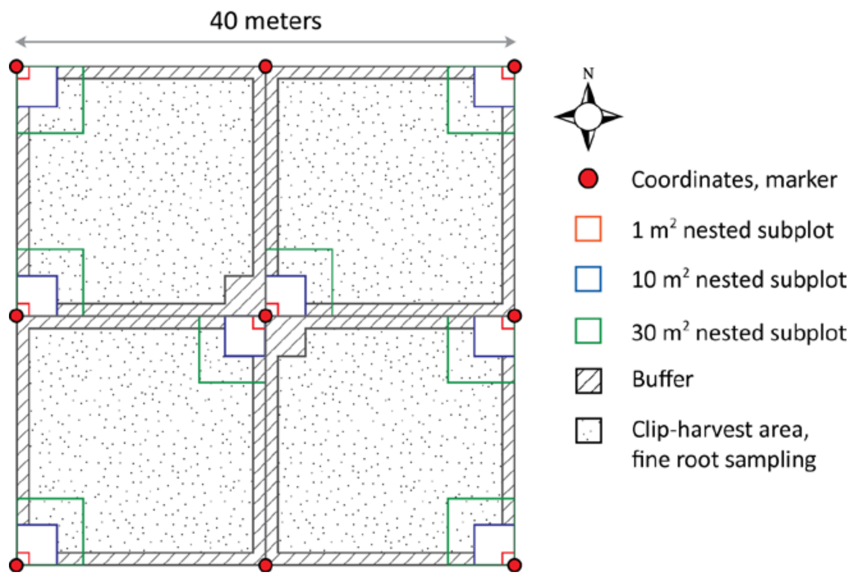

Figure 6. Example 40m x 40m Tower plot comprised of four 20m x 20m subplots. The clip-harvest area within each subplot is gridded into cells as shown in Figure 5, but cells are omitted here for clarity. Details of sampling activities that take place in the plot are described in Section 6.

The procedure NEON will use for quantifying the optimum size, number, and aggregate sampling area at a given site is iterative in nature, and will be informed either via data collected during NEON site characterization, or with pre-existing data where available and relevant. Ideal data for informing optimal Tower plot size and number at a given site are plot-level biomass values from the same plant community at the site, or from a similar nearby plant community. With respect to optimizing Tower plot size and number in forested communities, the Smithsonian Center for Tropical Forest Studies (CTFS) network of megaplots has collected spatially-explicit biometric and species ID data on thousands of woody stems within a number of very large plots (12 ha to 25 ha). Some of these datasets are available for optimizing Tower plot size and number (Figure 7A).

The spatially-explicit stem maps generated by CTFS are ideal for performing sampling simulations because the "true" biomass of a known, relatively large area can be subsampled iteratively with a specified plot size and number using a Monte Carlo approach. The distribution of mean aboveground biomass estimates (μ_{woody}) from the subsampling routine (Figure 7B; see Appendix A for R simulation code) can then be compared to the "true" μ_{woody} value, and it can be tested whether 90% of the subsampled μ_{woody} values fall within ± 10% of the "true" value. The procedure can be repeated with a variety of plot sizes and numbers in order to minimize the total area sampled while still meeting the desired uncertainty criteria (at least for woody stems). Furthermore, the underlying stem map can be manipulated to ascertain whether a specified plot size and number combination is adequate to detect expected levels of tree mortality (e.g. 1%, 2% stem removal, etc.); detecting tree mortality with minimal uncertainty is a critical component of reducing uncertainty in annual ANPP estimates in forested systems.

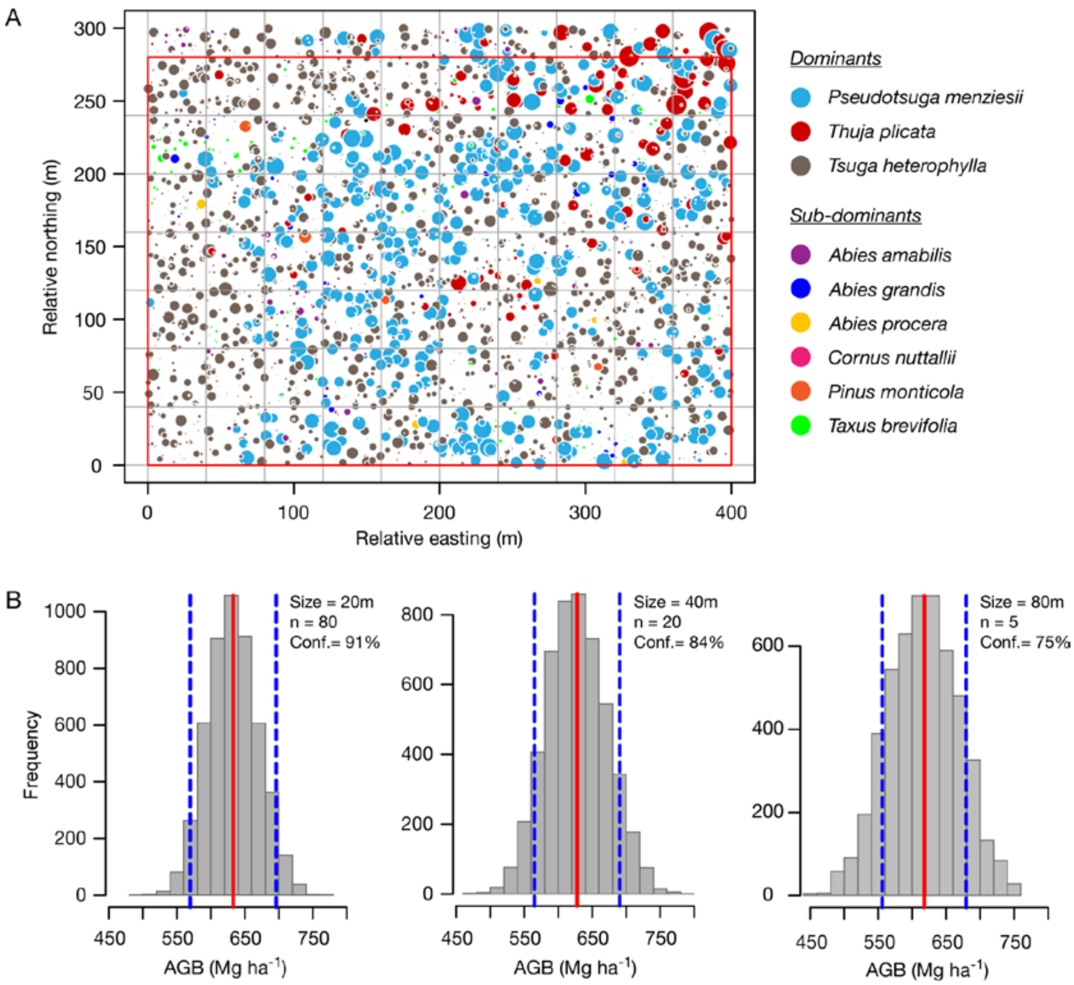

Figure 7. (A) Woody stem map from the Smithsonian CTFS Wind River megaplot in Washington state; symbol size is proportional to stem diameter, and symbol color indicates species. The red line shows the area defined for sampling simulation, and grey lines indicate potential 40m x 40m plots available for subsampling. (B) Distributions of aboveground biomass subsample means, given different plot and sample sizes. The total sampled area is constant for each simulation (3.2 ha), and each depicts n=5000 subsample iterations. Red lines indicate true AGB, dashed blue lines show true AGB ± 10%.

When existing plot-level data or stem maps are not available, the initial sample size will be n=30 20m x 20m plots for non-forested vegetation, and n=20 40m x 40m plots for forested vegetation. Shrub-dominated and savannah-type ecosystems will also initially be sampled with n=20 40m x 40m plots. These initial plot sizes and sample sizes are logistically feasible for field crews, and represent a large enough aggregate sampling area that data should be sufficient to optimize plot number and size in grasslands (Briggs and Knapp 1991) and forests (Keller et al. 2001). To test whether smaller sample sizes are adequate, Monte Carlo simulation techniques will be used to calculate μ_i, uncertainty in μ_i, and bootstrap confidence intervals for subsamples with *n* less than the initial sample size. If the initial sample size is not adequate to estimate μ_i to within ± 10% with 90% confidence, bootstrap techniques will be employed to estimate an adequate sample size, and field sampling efforts will be adjusted accordingly if resources permit.

5.3.2.4 Plot Establishment Criteria for Tower Plots

Once a Tower plot sample size *N* has been chosen, it is necessary to determine the spatial arrangement and spacing of plots in the field. To maintain consistency with Distributed plots, the location of Tower plots will also be specified according to a spatially balanced Reversed Randomized Quadrant-Recursive sampling algorithm (RRQRR; AD[03]; Theobald et al. 2007). However, in the case of Tower plots, NLCD vegetation type will not be used to stratify the tower footprint. Stratification will not be required, because in theory, the location of the tower has been selected so that the vegetation within the footprint is relatively homogeneous, and representative of the dominant vegetation at the site. Nonetheless, there are instances in which vegetation that is not representative of the dominant vegetation type may be found within the tower footprint – e.g. a small beaver pond near the edge of the footprint boundary at the D01 Harvard Forest core site, and an abrupt change in land management near the edge of the footprint boundary at the D03 Disney relocatable site. When plots are randomly placed within non-representative vegetation, the potential plot location will be rejected, and another random location will be substituted. The criterium for defining vegetation as non-representative is that the given vegetation type occupies ≤ 10% of the source area for the TIS flux measurements (RD[04]). Because the area of land that contributes 10% of the Tower flux signal changes as a function of distance from the Tower, a probability density function will be used to calculate 10% area thresholds as a function of distance on a site-by-site basis (RD[04]).

There are several additional constraints that influence placement of Tower plots within the tower footprint. First, 10m radius sampling exclusion zones have been defined around the tower foundation and the TIS instrumented soil plots so that TOS measurement activities will have minimal impact on instrumented measurements of albedo, soil moisture, soil temperature, and soil respiration (RD[04]). Second, similar to the method used to identify non-representative vegetation, a method for standardizing and minimizing the impact of TOS sampling activities on TIS CO_2 flux measurements across sites has been implemented. On a site-by-site basis, this method utilizes a probability density function to identify the portion of the tower footprint from which the majority of flux data are derived, and then estimates anticipated disturbance to that area from each TOS sampling activity, with TOS sampling activities weighted with respect to their impact on CO_2 measurements, as well as scientific co-location requirements (e.g. it is higher priority for NPP measurements to be located within the tower footprint than bird diversity measurements)(RD[04]). Third, Tower plots must avoid existing plot-based research infrastructure whenever possible. Finally, it is necessary to impose an additional constraint on Tower plot spacing because the RRQRR algorithm identifies potential random locations on a continuous 30m grid. Because Tower plots will be large enough to overlap several grid cells at some sites, thus leading to potential plot overlap, the RRQRR algorithm will be implemented such that edges of individual plots will be no closer to each other than 50% of the plot edge length. For example, edges of a given 40m × 40m Tower plot will be no closer than 20 m from another Tower plot of the same size.

5.3.2.5 Anticipated Tower Plot Challenges

On a per site basis, optimal sample size will differ among the *i* vegetation components. For example, it will likely require more plots to estimate woody stem mortality than are required to estimate aboveground standing woody biomass. Similarly, the number of sampling units (plots) required to estimate aboveground woody biomass will likely be different than the number of sampling units (clip-harvests) required to estimate herbaceous production. Ideally, sample size will be optimized for the most variable vegetation component, but logistical and budgetary constraints will impose limits. In the event that it is untenable to sample all vegetation components to the same level of uncertainty and with the same level of confidence, NEON will prioritize sampling total NPP to the same level of uncertainty across sites.

5.3.3 Belowground Vegetation Sampling

NEON will estimate coarse and fine root biomass within the dominant vegetation in the Tower footprint according to established techniques (vegetation dominance has been assessed either via Landfire, or the National Land Cover Database). Coarse root biomass and production will be estimated using published allometric relationships that depend on aboveground biometric variables as inputs – e.g. diameter at breast height (DBH) and species class.

To produce unbiased estimates of fine root biomass (FRB; g m^{-2} or kg ha^{-1}), NEON will employ a coring technique. In addition, at some sites the NEON TIS will install minirhizotrons within instrumented soil plots along an approximately 200 m long soil array transect extending outward from the NEON tower (Figure 4). The TIS minirhizotrons will produce fine root count, length and volume estimates, as well as turnover coefficients (TC, y^{-1}) at each site they are installed, and fine root production (FRP) will be calculated at these sites according to:

(4) $$FRP = FRB \times TC$$

The purpose of the TOS belowground biomass field sampling, therefore, is twofold: 1) To produce an independent, unbiased estimate of FRB; and 2) to integrate with TIS minirhizotron data such that a major component of annual BNPP, fine root production, may be calculated.

Fine root production will not be estimated in Distributed plots, but within the framework of the Tower plots, there are several aspects of sampling fine root standing stocks that must be addressed across sites:

1. Sample location and number
2. Variation in fine root production across diameter size classes
3. Between site differences in the distribution of fine root biomass with depth

The manner in which the sampling framework addresses these three challenges is discussed below.

5.3.3.1 Sample Number and Location

At a minimum, there will be one fine root biomass sample per 20m x 20m Tower plot per sampling bout, and a minimum of two randomly selected subplots selected for coring per bout for Tower plots that are 40m x 40m or larger (Figure 6). Each fine root biomass sample will consist of two pooled soil cores collected from a given clip-harvest "cell" (Figure 8). Given that distributions of fine root biomass in space are likely non-normal, (Taylor et al. 2013)uncertainty and confidence intervals for fine root biomass will be quantified using Monte-Carlo bootstrap techniques. In the unlikely event that fine root biomass can be estimated to within ± 10% of the mean with 90% confidence with a reduced sample size, sample size will be optimized using simulation techniques similar to those described for selecting the appropriate Tower plot sample size.

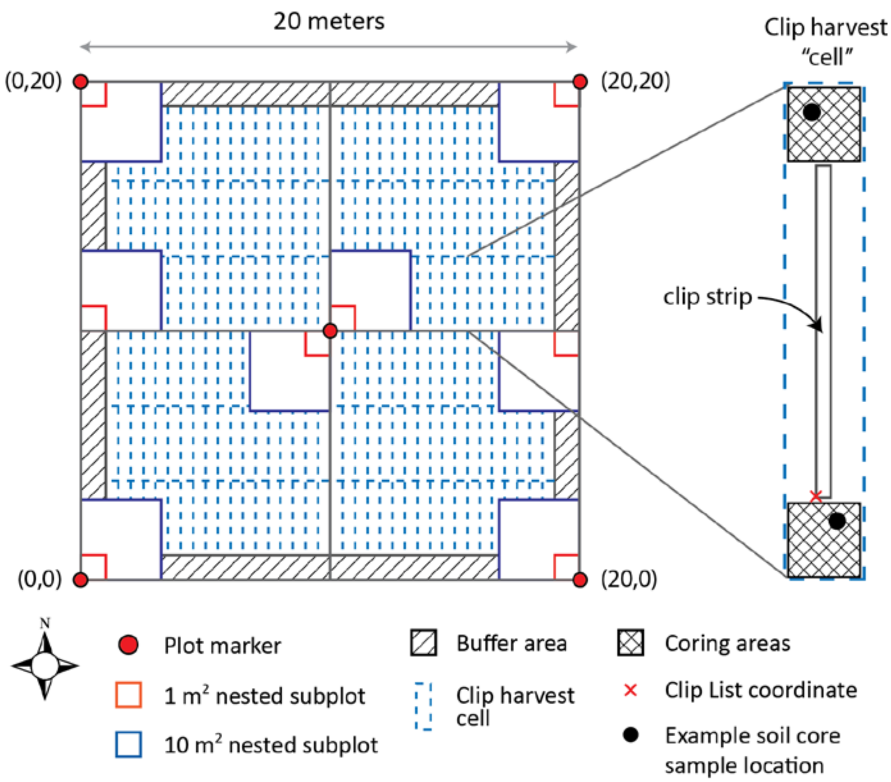

Figure 8. A 20m x 20m Tower plot showing the locations of 0.5m x 3m clip-harvest "cells" also used for belowground biomass soil core sampling (*left*). Within a clip-strip cell selected for soil sampling, two soil core samples are generated: one from the area to the North, and another from the South of the clip-strip (*right*).

With respect to location, fine root samples will be obtained from within the Tower plot clip-harvest "cells" shown in Figure 8 that are immediately adjacent to matched aboveground clip-harvests. Clip harvest cells are selected randomly from each subplot on an annual basis, so no single cell will ever be harvested and sampled for fine roots more than once. See Section 6.2 for additional details on randomly locating clip harvest cells.

5.3.3.2 Fine Root Production across Diameter Classes

Biomass production and turnover of fine roots depend heavily on diameter class (Tierney and Fahey 2007, Burton and Pregitzer 2008, Fan and Guo 2010). For example, in some systems, roots ≤ 0.5 mm diameter account for 60-69% of fine root production for the total pool of roots < 2 mm diameter (Steinaker and Wilson 2005). Nutrient concentration and turnover rates also differ across fine root size classes, with the smallest roots possessing higher N concentration and showing faster turnover rates compared to larger fine roots (Steinaker and Wilson 2005). Calculating one value of TC, and one value of FRB, for all fine roots less than a given diameter cutoff will therefore lead to systematic errors in FRP estimates, and estimates of fine root derived nutrient fluxes. Based on these considerations, NEON will reduce FRP uncertainty by calculating FRP with Equation 4 separately for biologically informative fine root diameter classes. See Section 6.7 for an evaluation of fine root biomass sampling methods that are suitable for achieving this goal.

5.3.3.3 Fine Root Biomass Distribution with Depth

In many studies, fine root biomass sampling is biased toward the surface soil (e.g. Cairns et al. 1997) due to the significant logistical difficulties involved with obtaining, transporting, storing, sieving, sorting, and weighing the large numbers of samples required to characterize root biomass along deep soil profiles (Berhongaray et al. 2013). While there is a greater proportion of root biomass near the surface compared to at depth at the global scale (Jackson et al. 1997), it is unclear what proportion of total fine root biomass remains unsampled when sampling depth limits are imposed. As such, an additional problem the NEON belowground biomass sampling framework must address arises due to the fact that standard FRB sampling depths will be applied across all sites, but the proportion of fine root biomass captured with a standard sampling depth will vary from site to site, thus adding uncertainty to estimates of FRP and fine root stocks.

To reduce uncertainty in belowground biomass stocks, and reduce uncertainty in the fine root biomass that is *not* routinely sampled at each site, NEON will excavate a soil pit at each site near the tower (soil pit dimensions are: 1.5m W x 2m L x 2m maximum D)(Figure 9). Realized depth may be less than 2m if bedrock or very large rocks are encountered before 2m depth is reached. Soil pits will be located systematically in the dominant vegetation type in which Tower plots are also established, and these pits will allow a spatially limited but important estimation of fine root biomass distribution with depth on a per site basis.

An obvious caveat associated with using fine root biomass depth distribution data from the soil pit at each site to inform estimates of total fine root production is that replication of depth sampling is poor (i.e. n=1 soil pit per site, with n=3 root profiles excavated per pit). While increased replication of soil pits at each site clearly would be desirable, there are several important points: 1) data from n=1 soil pits is highly preferable to no data; and 2) although root biomass is very heterogeneous in (X,Y) space, within the dominant vegetation at a site it is likely that the proportion of fine root biomass found at a given depth is comparatively more constrained. However, species-specific differences in vertical fine root distribution are documented (Mou et al. 1995), so it is unclear how broadly this assumption will be supported.

Figure 9. NEON soil pit excavated to 2 m depth at the D01 Harvard Forest core site in July 2012. Fine roots are sampled from three vertical profiles at the left, center, and right of the pit face. Additional soil samples are obtained for biogeochemical characterization of each identified profile, and for calibration of TIS soil moisture sensors.

5.3.4 Leaf Area Index Estimation

The LAI sampling framework includes two types of concurrent sampling, intended to enable detection of changes in LAI across space and through time. With respect to measurement of LAI across space, the strategy is to validate remote-sensing derived LAI estimates from the NEON AOP with ground-data collected from Distributed and Gradient Plots within a 1 month window overlapping the actual AOP flight date. The NEON AOP will fly each NEON site annually, and will use historical MODIS NDVI data from each site to time flights within ± 2 weeks of the average peak greenness date. Algorithms are available to estimate LAI from both discrete-return LiDAR and reflectance data (Cohen et al. 2003, Richardson et al. 2009, Ganguly et al. 2012), and Distributed and Gradient Plots (maximum n=20 total) will be selected such that ground-data span the full dynamic range of LAI observed within the remote-sensing datasets. Barring changes in algorithms used to process remote-sensing data, it is assumed that the relationship between LAI estimates derived from remote-sensing and LAI estimates from ground sampling will not change rapidly through time. As such, validation data from Distributed and Gradient plots will be collected every 5y throughout the lifetime of the NEON project, but not annually (Table 1). To improve prediction of LAI within sites using all available data, and to reduce uncertainty in LAI estimates, remote-sensing and ground-truth datasets will also be combined in the context of hierarchical Bayesian models (e.g. using methods presented in Finley et al. 2013).

The second component of the LAI sampling framework consists of temporally intensive LAI measurements that will be collected from a small subset of Tower plots (n=3). These data will complement the spatially extensive snapshot created from remote-sensing and ground validation LAI data. A time series LAI dataset from each site is particularly important because AOP flights may not occur exactly at peak greenness every year (e.g. due to compounding weather delays across sites in a given year). A time series dataset will therefore allow for improved estimation of maximum LAI per site per year within the dominant vegetation type.

5.3.5 Determining Timing of Sampling

The overarching goal for most NEON data products is to provide site-scale parameter estimates, as opposed to plot-scale estimates, based on standardized protocols on an annual time-step, although certain data products will have multi-year intervals (e.g. coarse woody debris volume; Table 1). The sampling framework must therefore provide a sensible means to standardize the onset date, frequency, and termination date of sampling across domains that differ widely in terms of the seasonality of biomass production. For example, in the savannahs of the D17 San Joaquin Experimental Range core site, biomass production of woody and herbaceous vegetation is maximal in the winter when precipitation is also at a maximum. In contrast, biomass production at the D18 Toolik Lake core site is controlled predominantly by temperature, and reaches a maximum during the summer. An additional level of complexity arises because timing parameters must be optimized for each *i* vegetation component on a per site basis. That is, even within a site, the onset, termination date, and sampling frequency, will not be the same for woody vegetation, herbaceous vegetation, litter production, etc.

One strategy for delineating appropriate sampling onset and termination dates on a per site basis is to use historical, remote-sensing land surface phenology datasets. For example, green-up and senescence dates can be estimated for each site using a 10 y average of various MODIS vegetation index (VI) data products plotted as a function of day of year (Zhang et al. 2003, Liang et al. 2011). Important sampling dates for each *i* vegetation component within a site can then be determined with reference to average site-wide green-up and senescence dates. An issue with this approach is that land surface phenology timecourses can deviate significantly from the average in any given year due to precipitation and/or temperature anomalies (Morisette et al. 2009). The consequence of such anomalies, given inflexible sampling windows derived from long-term averages of phenology data, is introduction of unnecessary noise into plant biomass and productivity datasets. For example, herbaceous plant productivity would be underestimated if a late, cold spring delayed the date of peak herbaceous biomass, and sampling windows were not moved to accommodate the delay.

Provided the limitations associated with relying solely on historical data to determine sampling windows for a given year, NEON will employ a hybrid approach informed both by historical remote-sensing data, as well as current-year empirical observations. Variability in historical MODIS-EVI phenology data will be used to provide likely windows for start- and end-of-growing season dates for each site, and current-year empirical phenology observations will be used to determine actual sampling start and stop dates within these windows. In Section 6, the application of this sample timing framework is discussed in more detail with reference to each vegetation component.

5.3.6 Potential Future Design Modifications

Optimization of the design will be iterative, with data generated by the design used to evaluate whether the desired precision of parameter estimates is achieved. Input from the community is desired to determine what statistical and modeling techniques are most appropriate for evaluating design performance. As described above, any changes will be reviewed to ensure: 1) The data collected are those best suited to meet the requirements of NEON (AD[01]); 2) are, to the extent possible, consistent with standards used by the scientific community; and 3) fit within the scope of NEON. Any significant changes to the design will be reviewed according to the plan described in AD[02].

Considering the anticipated 30-year lifetime of NEON, a significant challenge that NEON will face is how to introduce improvements in sampling methodology and equipment without disrupting the continuity of existing data product time series. There are at least two technologies that could significantly transform plant biomass data products in the coming years, and it will be important for any change in technology to be evaluated alongside existing methods for a period of time so that year-to-year comparability of Observatory data is maintained.

5.3.6.1 Ground-based LiDAR

Ground LiDAR can be used to map and measure numerous stem properties within plots, including generating volume estimates for individual woody stems, crown diameter, height, and other stem properties (Feliciano et al. 2011, Yao et al. 2011, Yang et al. 2013). Combined with wood density values (either from sampling or a database), biomass of individual stems could be estimated non-destructively and linked directly to parameters like canopy diameter or area that are measured via airborne remote sensing.

While ground LiDAR appears very promising, in the short-term it will be beneficial for NEON to collect stem data (DBH, height, etc.) in a manner consistent with the majority of existing datasets, and phase in the parallel use of ground-LiDAR as the hardware technology and data processing algorithms continue to be developed.

5.3.6.2 Ground-penetrating RADAR

Coarse roots represent a substantial, poorly constrained biomass pool in terrestrial ecosystems. The accurate detection and quantification of coarse root biomass is one of the more difficult mensuration problems in plant ecology. Coarse root biomass is typically estimated via allometric equations, and validation of these equations (and the development of region-specific new equations) is laborious and time consuming. The use of ground-penetrating RADAR may hold promise for better constraining the coarse root biomass pool (Butnor et al. 2008), and NEON will continue to assess this technology as it matures to see whether it should be incorporated into the plant biomass sampling design.

5.3.6.3 Analysis of Data Generated by the Design

To date, a Bayesian framework has been employed to assess adequacy of sample size for trend detection across environments with different underlying heterogeneity. Bayesian models hold considerable promise with respect to modeling cross-site data generated by the design presented here. However, the anticipated NEON user-community will have a major role to play when it comes to analyzing plant data collected at the continental scale.

6 SAMPLING DESIGN FOR PLANT BIOMASS, PRODUCTIVITY, AND LEAF AREA INDEX

The sampling design framework is intended to create data products that satisfy the NEON high-level requirements, and address the NRC Grand Challenges. Here, the following topics are discussed in detail for each vegetation biomass component or attribute that will be sampled (vegetation biomass components are organized by secondary headings below):

1. The definition of the measured vegetation biomass component or attribute, and an evaluation of the available approaches for measuring or estimating that component or attribute.
2. The selected method(s) for sampling the vegetation component or attribute
3. The selected spatial distribution of sampling
4. The selected temporal distribution of sampling, and
5. Logistics and adaptability associated with the selected sampling strategy

Analysis of applicable prototype data or equipment evaluation is brought to bear on these topics as warranted.

6.1 Sampling Design for Woody Vegetation

The measurement and mapping of woody stems is an important complement to data streams generated by the NEON AOP and TIS. These ground-collected data will validate LiDAR data used to map the structural complexity of vegetation, will enable mapping of plant biomass at the site scale, and in conjunction with carbon flux data, will facilitate understanding how biomass in different plant growth forms contributes to ecosystem level carbon flux.

The term 'woody vegetation' applies to any perennial plant that produces persistent lignified vascular tissue that remains aboveground throughout the dormant season (Van Buren et al. 2011, Jepson 2012). We also include ferns, cacti, and "other" perennial vegetation (e.g. yucca) in this group, because similar to woody stems, biomass of these plant growth forms is often estimated allometrically using vegetation structure data as inputs (e.g. Gholz et al. 1979). NEON will sample vegetation biomass and productivity across tree, sapling, shrub, liana, and other growth forms listed above, with varying methods and varying thresholds for inclusion in the sample depending on growth form. Below, we define trees, saplings/shrubs, and lianas, we evaluate approaches for estimating aboveground biomass (AGB) for all "woody" growth forms, and we select and justify an approach suitable for estimating AGB for each growth form within Observatory constraints. It is assumed that $ANPP_{woody}$ will be calculated using annual changes in AGB, as outlined in Section 5.3.1.

Trees

Definition: Self-supporting woody stems with diameter at breast height (DBH) ≥ 10 cm. Individuals are typically, but not always, species that are potentially canopy emergent.

Total tree biomass and annual productivity will be determined through allometric equations. Allometric estimation of biomass is standard practice, typically requiring inputs of DBH or volume, and sometimes height and wood density. The equation applied is determined by species or functional group for individual stems in plots. For the continental United States, the USFS FIA program estimates live and dead tree biomass with a component volume approach, with distinct parameters that convert volume to mass derived for 10 different eco-climatic regions of the conterminous U.S. (Smith et al. 2003).

Alternatively, the AGB of individual trees may be estimated from various stem or canopy measurements, though the most typical allometric input is either DBH or stem diameter measured at some alternate height. Because biomass is related to stem diameter via a power law, the linear form of numerous allometric equations becomes:

(5) $$ln(AGB) \sim \alpha + \beta * ln(diameter) + \varepsilon$$

Arithmetic AGB values are then obtained by exponential transformation of the natural log, which subsequently changes the distribution of the residuals. To account for this, some authors employ a correction factor (Baskerville 1972), although others do not (see Ter-Mikaelian and Korzukhin 1997 for examples).

Different allometric equations for estimating tree biomass are derived from multiple spatial scales, from that of the site (e.g. Grier and Logan 1977, Jenkins et al. 2004, Battles et al. 2008), the region (Schroeder et al. 1997), the continent (Jenkins et al. 2003, Smith et al. 2003), or even pan-tropical (Chave et al. 2005). Within the context of NEON, there are advantages and disadvantages to each type of equation. Site or region-specific equations for either individual tree species or groups of species (e.g. hardwoods vs. softwoods) are often the most accurate, but equations constructed using data collected across relatively small spatial scales may not be available for all species at all NEON sites. For U.S. studies, national-scale allometric equations from Jenkins et al. (2003) are frequently employed because a small number of equations can be used for most common species nationwide; however, at any given site, uncertainty may be increased relative to region × species-specific equations. Additionally, there are general equations for tropical forests developed across continents that rely either on stem-level DBH, height, and wood density inputs (Chave et al. 2005), or plot-level characteristics (Asner et al. 2012). An extensive wood density database is available (Flores and Coomes 2011), which could facilitate the use of general equations, but these equations have been developed in tropical regions, and it is unclear how they perform in ecosystems with very different vertical structure (e.g. boreal forest).

NEON is designed to facilitate scaling from the site to the continent; as such, equations which are broadly applicable may be desirable in this context. However, not all NEON data users will ask continental scale questions, and accurately characterizing a site within the constraints of a continental design argues for use of equations with the least amount of associated uncertainty. To that end, NEON will report biomass and uncertainty estimates as calculated from both the most geographically and/or species-specific equation available (these equations may be updated as newer ones become available), as well as reporting the 'Jenkins biomass estimate' (Jenkins et al. 2003). Biomass and uncertainty estimates will be reported in arithmetic units per stem (kg), and transformation correction factors will be supplied as appropriate so that NEON data users may apply corrections at their own discretion. Additionally, the input variables used to allometrically estimate biomass will be reported per individual (i.e. DBH and height). NEON will thereby enable researchers to estimate per stem biomass values using allometric equations other than those selected by NEON if they so choose.

Saplings and shrubs

Definition: Saplings and shrubs are defined as self-supporting woody stems with DBH < 10 cm and diameter at decimeter height (ddh) ≥ 1 cm. Woody stems with ddh < 1 cm are measured as part of the herbaceous plant sampling effort. Individuals may or may not be species that are canopy emergents. Shrubs (but not saplings) may be mapped as either points or polygons within plots, depending on the growth form.

Sapling biomass will be estimated using similar methods to those employed for trees, namely via the use of allometric equations that use key vegetation structure variables as inputs. However, in order to avoid estimation errors, sapling biomass will only be estimated with allometries constructed from trees with an appropriately small diameter class (Curtis 2008).

There are a variety of diameter and height cutoffs employed to define saplings (e.g. Lee et al. 2008). To speed assessment by technicians in the field, NEON will define saplings as self-supporting stems of potential canopy emergent or sub-canopy tree species with DBH < 10 cm. Due to high mortality, and occasionally high density, sapling individuals will not be mapped. Once individuals are recruited into the

DBH ≥ 10 cm size class, they will be identified to species, tagged, mapped, and measured according to the tree sampling strategy.

Consistently estimating biomass and ANPP across sites for shrub species will pose a significant challenge for NEON, due to the relative paucity of site × species-specific allometric equations in the literature. Although allometric equations have been developed for some common shrub species (Brown 1976, Smith and Brand 1983, Cleary et al. 2008, McGinnis et al. 2010), such equations likely do not exist for a substantial portion of shrub species. For those allometric equations that do exist, estimates of biomass are derived from input variables that tend to differ substantially from study to study. For example, percent cover (Chojnacky and Milton 2008), basal diameter (Brown 1976, Dahlin et al. 2011), crown volume (Cleary et al. 2008), and combinations of these variables (McGinnis et al. 2010) are used to estimate shrub biomass.

The NEON Operations budget is insufficient to develop site × species-specific allometric equations for shrubs using traditional harvest-based techniques. It is also unlikely that NEON would be able to consistently harvest shrubs across all NEON sites given diverse land-ownership. Shrub volume, individual shrub structural characteristics (e.g. basal diameter, canopy diameter, canopy volume, height), and plot-level % cover-by-species estimates will be more feasible for NEON to monitor, but do not allow direct calculation of biomass and ANPP. Wherever allometric equations exist, NEON will calculate shrub biomass estimates based on the most geographic and/or species-specific equations available. Where these equations do not exist for a given species, taxonomic relationships between species will be used to select an equation from the most closely related species for which an equation exists. However, estimating uncertainty when this approach is used will be very difficult.

Lianas

Definition: Lianas are defined as climbing plants with DBH ≥ 1 cm that germinate on the ground and produce either xylem-containing woody stems, or persistent, fibrous “sub-woody” stems (Gerwing et al. 2006). Lianas characteristically lose the ability to support themselves as they grow, and they reach the canopy via the aid of external mechanical support. Belowground connections among apparently “individual” stems can be complex, and it is therefore most straightforward to tally liana stems as those stems that are either independently climbing, or are in an early, self-supporting stage (Putz 1983).

Liana diversity is greatest in the tropics, and as such it is likely that a range of species may be encountered at the NEON sites in Hawaii, and possibly at sites in Puerto Rico. There are, however, a few species of lianas which are extremely common in the temperate, continental United States, and therefore may be encountered at a number of the NEON sites. These species include native *Vitis sp.,* *Toxicodendron sp*., *Parthenocissus quinquefolia*, and several important invasive species (e.g. *Hedera helix*, *Pueraria sp*., and *Lonicera japonica*, among others). Many temperate and tropical liana species appear to respond strongly and positively increasing concentrations of atmospheric CO_2, making this plant group a potentially sensitive responder to global change (Mohan et al. 2006, Schnitzer and Bongers 2011).

For liana sampling, NEON will broadly adhere to the guidelines published by Gerwing et al. (2006), and elaborated upon by Schnitzer et al. (2008). Biomass estimates for lianas are typically generated via allometric equations that use stem diameter, basal area, stem height, or some combination of these variables (Putz 1983, Gerwing et al. 2000, Gehring et al. 2004, Mohan et al. 2006). Allometric equations are species-specific in some cases (e.g. Mohan et al. 2006 for *T. radicans*), but due to the speciose nature of this plant group in the tropics, liana biomass estimates in tropical systems tend to rely on generalized allometric equations (Schnitzer et al. 2006). Similar to allometric estimation of tree biomass, liana biomass estimation may be made using generalized, regional, or species-specific allometries.

Consistent with the approach outlined for estimating tree biomass, NEON will calculate biomass estimates based on the most specific equation available, utilizing site and species-specific allometries wherever possible. However, identifying appropriate species-specific equations does present other challenges for NEON. For example, Mohan et al. (2006) derived their equation for *T. radicans* from individuals mostly < 70 cm in height, and it is not clear whether their equation will give reliable results for longer vines that ascend some distance above the ground, or for other species of *Toxicodendron* that live in the Western United States. Therefore, liana biomass will also be calculated using the generalized allometric equation presented in Schnitzer et al. (2006). This equation is derived from data pooled across five geographic regions, and is relatively robust with respect to prediction error across a wide geographic range (R^2=0.694, RSE = 1.02). The 'Schnitzer' generalized equation was developed based on measurement of many tropical species and it is not clear whether it is appropriate to use tropical liana allometries for estimation of liana biomass in temperate systems. That is, if the growth form of tropical lianas is significantly different from temperate lianas, unnecessary errors in biomass estimation could be introduced. As is the case with tree biomass allometric equations, the allometries used to estimate liana biomass may be updated as better equations become available, and inputs to allometric equations such as stem diameter will always be provided so that researchers may apply the allometry of their choosing.

6.1.1 Sampling Methods

6.1.1.1 Sampling Methods: Trees

Trees within NEON plots will be marked and tagged to facilitate consistent, repeatable measurements of stem diameter at the same measurement height from year to year. Individual stems will be marked according to site host preferences, but the preferred marking method is a numbered aluminum tag affixed with a nail 10 cm above the measurement location, combined with weather resistant paint or a lumber crayon to mark the measurement location itself.

Sloughing of loose bark, and growth of epiphytes such as moss and lichen can complicate attempts to collect high-quality, repeated stem measurements (Kloeppel et al. 2007). In order to ensure that sloughed bark does not cause apparent negative growth, loose bark will be removed prior to initial DBH measurements. Similarly, epiphytes will also be removed from the measurement location prior to recording data so that these plants do not inflate the recorded stem diameter.

Determining which trees to measure

Within Distributed and Tower plots, all individual stems with DBH ≥ 10 cm will be tagged, mapped, and measured for DBH, height, and species ID (the 10 cm diameter cutoff is similar to some STRI plots). For qualifying stems within Distributed plots, both maximum canopy diameter and the diameter orthogonal to the maximum will be measured (McGinnis et al. 2010).

Point of measurement, definition of breast height

If conventional FIA techniques are used to map and measure tree stems, DBH measurements should be made 4.5 ft (1.37 m) above the ground (Kloeppel et al. 2007, Curtis 2008). However, numerous studies in Canada, the UK, Australia, Europe, and the tropics have employed a 130 cm height aboveground for the point of DBH measurement for both trees and lianas (e.g. Chave et al. 2005, Gerwing et al. 2006). In order to be consistent with a large body of published literature from multiple countries, NEON will define breast height as 130 cm above the ground for trees measured in NEON plots. It is unlikely that measuring DBH 7 cm lower than the standard U.S. definition will influence allometric biomass estimates in a meaningful way. However, data from NEON sites that overlap with FIA plots (e.g. Bartlett Experimental Forest) may be assessed to test this assumption.

Point of measurement, additional considerations

There are several common situations that complicate determining where stem diameters should be measured. For stems growing on sloped ground, the uphill side of the tree should be used as the base measurement point. Leaning trees should be measured 130 cm along the stem, not 130 cm above the ground. Regardless of slope or stem angle, stems with anomalies including bulges, nodes, and damage at 130 cm along the stem should be measured 5 cm below the anomaly (Gerwing et al. 2006). Trees that split into multiple stems below 130 cm require additional stipulations; methods for dealing with split stems are adapted from those outlined for lianas in Schnitzer et al. (2008), but the guidelines are generally appropriate here:

1. If the tree splits between 40 cm and 130 cm, measure the diameter below the split, where the stem is regular again, and record the alternate measurement height.
2. If a tree splits within 40 cm of the roots, measure the diameter of the stem halfway between the ground and the split where the stem is regular, and record the alternate measurement height.
3. If the stem is deformed between the branch/split point and the ground, and it is not possible to make a single diameter measurement because the stem is not regular at any point between the branch/split and the ground (e.g. there is a high root collar), measure each qualifying bole 130 cm from the main rooting point, and tag and record data separately for each bole that meets the minimum diameter cutoff.
4. Exclude the tree from the census if there is a split within 130 cm of the roots, the main stem is not regular and cannot be measured, and none of the stem diameters beyond the branch/split is ≥ 10 cm DBH at 130 cm.

Species identification

Identification of measured stems will be carried out by either an external botanist capable of identifying locally encountered woody stems to species, or a trained NEON field technician.

Sampling tree mortality

Snags are defined as standing dead trees with an angle of lean of 45˚ or less from the vertical (Curtis 2008), and accounting for existing and newly generated snag biomass is very important for accurately estimating $ANPP_{woody}$ (Gower et al. 2001). Mortality of large trees is likely to be both spatially and temporally infrequent, averaging only 1%-2% of stems per year in mature forests (Kloeppel et al. 2007). Because ANPP is calculated on a per plot basis, it is therefore desirable for plot size to be large enough that within plot mortality can be estimated to a similar degree of uncertainty as biomass increment, leaf and branch production, etc. That said, the aggregate Tower plot area is constrained to ≤ 4 ha, and may be smaller than that at many sites due to permitting or other logistical constraints (e.g. existing research plots at the D01 Harvard Forest site). This means that uncertainty associated with mortality estimates may differ from other components of ANPP, but the contribution of that uncertainty to total ANPP estimates will be quantifiable. Dead and dying trees within plots will be mapped, tagged, and measured for DBH and height consistent with measurements for live trees.

Standing dead tree biomass will be accounted for differently from live tree biomass – specifically, reductions in wood density and changes in structure due to branch loss will be incorporated into the biomass estimate. Several methods for quantifying standing dead tree biomass are presented in Smith et al. (2003), and Domke et al. (2011). In general, NEON will calculate snag biomass by estimating foliage, bark, stem wood, and branch loss according to Smith et al. (2003). In the case of tall snags with broken tops, the top diameter will be estimated, though generating precision estimates can present problems (Harmon and Sexton 1996), and quantifying uncertainty in this parameter will be difficult.

Equipment considerations

For stem diameter measurements, the overarching goal of the sampling design is to detect annual changes in biomass increment over the course of the 30-year lifetime of the NEON project. One option that satisfies this goal is to use standard forestry DBH tapes at most sites because of their low cost, and because annual changes in stem diameter will likely be detectable with acceptable measurement uncertainty in most forest types. Another viable approach would be to measure the diameter of all trees within the Distributed and Tower plots using DBH tapes on a multi-year interval, but measure the diameter of a subset of stems annually with dendrometer bands (Clark et al. 2007). With this approach, the increase in measurement accuracy relative to DBH tapes comes with an increase in equipment costs, but a decrease in annual labor costs since only a subset of trees are measured in most years. However, permitting restrictions at some sites (e.g. those within National Parks) will make installation of dendrometer bands impossible, so for consistency and simplicity NEON will rely on annual stem diameter measurements with DBH tapes. To enable accurate, repeat measurements with DBH tapes, measurement locations on individual stems will be marked with aluminum nails and/or paint. Aluminum nails will be placed 10 cm above measurement point to avoid any effect of nails on tree growth; visual markers such as paint or lumber crayons will be placed directly on the measurement point.

To map individual stems and record height and canopy diameter data, NEON has evaluated laser rangefinder/clinometer/compass models from Häglof, LaserTech, and LaserAce, and units of this type should perform adequately if operated according to established guidelines (Blozan 2008). For height and canopy diameter measurements, airborne LiDAR-derived values may be more accurate, and less prone to user error than field-collected observations from the ground for canopy emergent individuals. However, ground-truth values will still be quite useful for understory stems, for stems growing on slopes, and for stems growing in areas with thick understory vegetation that prevents airborne LiDAR from accurately detecting the ground. Ground-collected canopy diameter measurements may also be more accurate than LiDAR derived estimates in dense, closed-canopy forests where detection of individual crowns is difficult.

6.1.1.2 Sampling Methods: Saplings and Shrubs

To obtain an appropriate sample size per plot, either the entire plot will be censused and measured, or nested subplots of 30 m^2, 10 m^2, or 1 m^2 will be selected from within the larger plot when stem density is high (Figure 5 and Figure 6; 30-40 individuals should be sampled per 400 m^2). To be consistent within plots and to enable tracking of individual measured stems from year to year, the same size measurement unit (e.g. the chosen nested subplot size) should be used for the entire plot, and the same size nested subplots within a given plot should be used from year to year. To determine the appropriate subplot size, the entire plot is visually surveyed and an assessment of stem density is performed for those sapling/shrub stems that meet the measurement criteria.

Species identification, tagging, and mapping

At some sites, individual shrubs will be easily identified and measured and will comprise the dominant canopy cover (e.g. Domain 15 Onaqui in the Great Basin) or understory. In contrast, some species of shrubs can be clonal or tend to occur in dense clusters such that it is difficult to consistently discern where individuals begin and end (e.g. tundra, chaparral). When individuals can be readily delineated, growth form is largely non-clonal, and the individuals in question form the dominant canopy cover visible to remote-sensing instruments, individuals will be identified to species, tagged, and mapped as points within both Distributed and Tower plots in order to build spatially-explicit links between ground-collected and remote-sensing datasets. In plots where shrubs are clumped or grow in thickets, thereby making mapping and tagging either impossible or very expensive and laborious, the perimeter of the shrub group will be mapped as a polygon, and the average height will be recorded in order to estimate volume of the shrub group. The species composition of the shrub group will also be recorded, and identification of species will be carried out by either a botanist, or by a trained NEON field technician.

Measurement considerations

The primary objective for sapling and shrub vegetation structure measurements is to provide input variables that may be combined with available allometric equations to estimate biomass. If allometric biomass equations do not exist for a given species x region combination, vegetation structure measurements will enable estimation of shrub/sapling volume. The majority of allometric equations developed for estimating shrub biomass rely on inputs of either stem diameter (either DBH or basal diameter) or canopy volume or diameter. Data collected from complex shrub groups are described in the paragraph above, and the following data will be collected when it is possible to discern individuals:

- *For individual shrubs with < 5 stems with qualifying diameter at breast height*: Measure the diameter of each qualifying stem, and select and mark the appropriate measurement height using the same guidelines employed for trees.
- *For individual shrubs with > 5 stems with qualifying diameter at breast height:* Measure the diameter at decimeter height (i.e. the diameter at 10 cm aboveground). Tag the largest stem

with a unique ID, and mark the diameter measurement location 10 cm aboveground. Mark and measure secondary qualifying stems 10 cm aboveground with paint or a lumber crayon.

- *For all shrubs:* Record height, maximum canopy diameter, diameter at ground level (this will differ from stem diameter for multi-stemmed individuals), and the geometric shape of the plant canopy – i.e. sphere, circular frustum, etc. Canopy volume will then be calculated per individual according to common geometric formulas (McGinnis et al. 2010).

Equipment considerations

Stem diameter will be measured either with a DBH tape, for stems ≥ 5 cm diameter at the measurement point, or with calipers for stems < 5 cm diameter at the measurement point. Height will be measured with a laser rangefinder/clinometer.

6.1.1.3 Sampling Methods: Lianas

Similar to trees, liana stem diameter will be measured at 130 cm above the rooting point. Due to the variety of complicated growth forms that lianas may adopt, NEON will follow established protocols in order to determine exactly where stem diameter should be measured on individual stems when it is not clear how to follow the simple “130 cm” guideline (Gerwing et al. 2006, Schnitzer et al. 2008)(Figure 10).

Figure 10. Liana measurement locations (red lines) for a variety of relatively standard (top panel) and non-standard (bottom panel) liana growth forms (redrawn with permission from Schnitzer et al. (2008).

Diameter cutoffs for measured stems

Gerwing et al. (2006) recommend that diameter cutoffs for lianas should be substantially smaller than that used for trees. This is because: 1) at any given diameter, lianas will be older than trees due to the fact that they tend to allocate less to woody stem increment growth than trees do; 2) at any given stem diameter, lianas tend to allocate more biomass to leaves than trees do – e.g. a 2 cm diameter liana has approximately as much leaf mass as a 10 cm diameter tree (Gerwing et al. 2000); and 3) at least in tropical systems, lianas reach the canopy at relatively small stem diameters compared to trees (Kurzel et al. 2006). Based on these considerations, NEON will adhere to the recommendations of Gerwing et al. (2006), and will implement a 1.0 cm diameter cutoff for liana inventories. Compared to a 2.0 cm cutoff, a 1.0 cm cutoff will provide better estimates of both liana species richness and abundance.

Ramets versus genets

Ramets may constitute a significant proportion of liana stem density and biomass and therefore should be sampled to accurately estimate the contribution of lianas to total forest biomass. Schnitzer et al. (2012) reported that more that 30% of the rooted lianas ≥ 1 cm diameter in the Barro Colorado island 50 hectare plot were clonal stems that were still attached to a central stem. According to Gerwing et al. (2006), separate individuals are defined as those that *appear* to be independently rooted, and are not obviously connected to another individual. "Apparent" genets may in fact be ramets connected belowground, but excavation to ascertain states of connectedness should be avoided. Individually-ascending stems that are connected below the measurement point as part of a clonal group should be measured according to Figure 10, and it should be noted in the dataset that these stems are part of a group.

Rooting location

All stems whose last rooting point before ascending into the canopy is located within the plot should be included in the census (Gerwing et al. 2006).

Cylindrical and non-cylindrical stems

Liana stems should be divided into two categories for measurement: cylindrical and markedly non-cylindrical; markedly non-cylindrical stems include flattened, triangular, ovoid stems, etc. (Gerwing et al. 2006). For cylindrical stems, the diameter will be recorded. Non-cylindrical stems will be measured at both the narrowest and widest points, and these data, along with the geometric mean of the two measurements will be recorded.

Equipment considerations

Liana stems should be marked and tagged with an ID number such that repeat measurements at the same stem position are possible. Unique ID tags may be affixed to stems location using aluminum wire and stems may be painted to mark the measurement location.

For roughly cylindrical stems, those with DBH ≥ 5 cm should be measured with a DBH tape, while those with DBH < 5 cm should be measured with calipers. Diameter measurements of non-cylindrical stems should be made with calipers.

6.1.1.4 Sampling Methods: Ferns

To estimate biomass for ferns, NEON will record the appropriate input variables required by allometric equations, namely: frond number, frond length, total plant height, stem basal diameter, or some combination of these variables (Gholz et al. 1979, Gonzalez et al. 2013). Measurements supporting allometric biomass estimation of ferns will be made within subplots or nested subplots (Figure 5 and Figure 6). Similar to biomass estimation for saplings, shrubs, and lianas, nested subplot size will be chosen such that the number of individual ferns measured is approximately 40 per 400 m^2.

6.1.1.5 Sampling Methods: Cacti

For pad-forming cactus species, it is possible to estimate individual cladode mass from width and length measurements for both current-year and older cladodes (Dougherty et al. 1996). NEON will therefore construct site-specific relationships between pad count, pad dimensions, and biomass for pad-forming cactus species. It is more difficult to estimate biomass for non-pad-forming barrel cacti, and NEON will ignore the biomass and productivity of these species. However, biomass can be estimated for some cholla-type cactus species using allometric equations and vegetation structure variables or volume as inputs (e.g. Búrquez et al. 2010). As such, NEON will estimate the biomass of those species with growth forms similar to those for which allometric equations exist, and will rely on field technicians to determine which cholla-type species should be assigned to a particular growth form.

Cacti or other CAM plants require considerably more drying time than do most forbs, up to 3 months according to USDA-ARS researchers at CPER (personal communication). Accordingly, the drying and weighing of these plants for the purposes of constructing allometric equations will occur outside of the anticipated clip harvest window so space in drying ovens is available.

6.1.2 Spatial Distribution of Sampling

Individuals classified as trees will be measured for vegetation structure in all Tower plots, provided at least one tree is present in > 10% of plots (Section 5.3.1). Smaller stature woody vegetation components, for which % cover can be estimated, will be measured for vegetation structure in Tower plots if % cover across all Tower plots is ≥ 10%. Vegetation structure measurements will be made in up to a maximum of 20 randomly selected Distributed plots, and the same guidelines employed in Tower plots will be used to determine whether the Vegetation Structure protocol will be implemented.

Vegetation structure data will be collected from woody stems with ddh ≥ 1 cm, although the types of per stem measurements and the size of the sampling area within the plot will vary depending on the growth form and plot level characteristics (i.e. stem density per growth form)(Table 2). For trees with DBH ≥ 10 cm, all stems will be measured and mapped throughout the entire plot regardless of density. For sapling/shrub and liana growth forms with DBH < 10 cm, as well as ferns, a nested subplot approach will be employed and the size of the measurement area will be independently optimized for saplings/shrubs, lianas, ferns, and cacti on a per plot basis, such that between 30-40 individuals per growth form per 400m^2 plot/subplot will be sampled. The NEON Distributed plots contain nested subplots of two sizes available for monitoring woody vegetation (1 m^2, 10 m^2; Figure 5), and Tower plots that are 40m x 40m or larger feature an additional 30 m^2 subplot (Figure 6); in all cases, the entire 400 m^2 plot/subplot may also be used for monitoring woody vegetation should stem density be sufficiently low. For all individual plots, once a subplot size has been chosen for a given growth form, that same subplot size will be used throughout the plot for that particular growth form. As an example, a 40m x 40m plot in a forested site with few large trees, a high density of understory regeneration, and moderate liana growth will have all trees with DBH ≥ 10 cm mapped and measured throughout the plot, liana measurement occurring in 30 m^2 subplots, and saplings with DBH < 10 cm measured in 10 m^2 subplots.

Table 2. Summary of nested subplot sampling strategy across woody growth forms and "other" plant types measured according to the vegetation structure protocol.

Growth form	DBH	ddh	Nested subplots	Target sample size
Trees	≥ 10 cm	NA	No	All individuals per 400 m^2 plot or subplot
Saplings/shrubs	< 10 cm	≥ 1 cm	Yes	30-40 individuals per 400 m^2 plot or subplot
Lianas	≥ 1 cm	NA	Yes	30-40 individuals per 400 m^2 plot or subplot
Ferns, cacti, and "other" plants	NA	NA	Yes	30-40 individuals per 400 m^2 plot or subplot

6.1.3 Temporal Distribution of Sampling

The priority for vegetation structure sampling is annual re-measurement of trees in the Tower plots; a subset of up to 20 Distributed plots will be surveyed every 3 years (Table 1) in order to map, tag, and measure individuals recruited into the miminum size class. However, Distributed plots may be re-measured less frequently as dictated by logistical and budgetary constraints (i.e. on a 5 y interval). Feasible interannual measurement intervals for woody stems in Distributed plots will be determined following data collection during the first three years of field operations, and before the NEON Observatory is commissioned into full operations in 2017. Gradient Plots will be surveyed and measured for vegetation structure every 3 years as well, as the primary purpose of Gradient Plots is to provide calibration/validation for remote-sensing datasets, and it is not expected that relationships between ground-collected and remotely-sensed datasets will change rapidly on an annual basis.

All woody vegetation components will be sampled concurrently and will ideally occur during the dormant season (Kloeppel et al. 2007). At temperate sites with deciduous vegetation where the growing season is defined primarily by temperature, vegetation structure measurements will occur either before or after the period of peak greenness, as identified using the MODIS-EVI phenology product; however, initial mapping and identification of woody stems may occur during the growing season when diagnostic leaf traits are present. In locations where the growing season is driven by seasonal precipitation rather than temperature, sampling will occur during the dry season. Low latitude sites with no distinct growing season may be sampled at any time of the year, in this case, sample timing may be dictated by logistics such as technician availability and schedule coordination with other NEON sampling modules.

Scheduling of annual sampling bouts, at least for the first year or two of operations, will be at the discretion of Domain managers rather than specified *a priori*, with the caveat that successive annual sampling bouts must be initiated within the same phenophase in which the previous year's bout occurred. Once a year or more of flux data are available, the sampling period may be adjusted based on CO_2 source/sink transition dates for each site.

6.1.4 Logistics and Adaptability

The number and size of Tower plots selected in the first year of sampling is based on vegetation structure (forested vs. non-forested) and an estimate of the maximum area (approximately 4 ha) that can feasibly be sampled in a given season. The sampling effort for woody vegetation structure will be evaluated for each site after it has been commissioned into Operations, and the sampling effort may be reduced in future years if a reduction is justified based on statistical analysis of the collected data.

In the event that the currently described sampling effort exceeds the capacity of field crews, Distributed plots will be sampled less frequently (every 5y), but the Distributed plot sample size will not be reduced from n=20 so that a biomass calibration curve can be constructed for use with AOP datasets.

The measurement methods selected may change as new technologies, tools or techniques become available. For example, ground-based LiDAR could be a good alternative to measuring shrub volume manually, as LiDAR estimates may have reduced uncertainty. Ground LiDAR technology is not currently accessible to NEON because of financial constraints but may become a reasonable option in the future.

6.2 Sampling Design for Herbaceous Plants

Definition: Plants in this vegetation component include non-woody forbs and graminoids, bryophytes that show distinct annual growth, certain low-stature vines (e.g. *Rubus ursinus*), as well as woody-stemmed plants with ddh < 1 cm (e.g. *Artemisia frigida*). In the text that follows, the word "herbaceous" can be taken to mean all of these growth forms unless specified otherwise.

The primary intent of the herbaceous plant sampling design is to produce unbiased estimates of herbaceous biomass and nutrient fluxes at the site level (using Distributed plots), and to produce unbiased estimates of biomass and ANPP within the NEON tower footprint (using Tower plots). In many instances, estimates of herbaceous biomass are a major component of herbaceous ANPP, but for some of the growth forms listed above the existence of persistent aboveground perennial tissues means that aboveground biomass is not equal to current year production (e.g. bryophytes, woody-stemmed sub-shrubs, etc.). When herbaceous plants, as defined here, are comprised of tissues produced in more than one year, NEON will prioritize estimation of herbaceous biomass in Distributed plots, and herbaceous ANPP in Tower plots.

Methodological considerations

For the majority of the herbaceous growth forms defined above, the most common method used to quantify herbaceous plant biomass is the clip harvest technique. For systems in which all aboveground herbaceous material is produced de novo on a yearly basis, clip-harvests are also directly related to herbaceous ANPP. The size and shape of clip-strips varies considerably across published studies, for example: 1) At the Domain 10 CPER site, the USDA-ARS program uses 0.5 m x 0.5 m harvest areas (0.25 m^2); 2) Briggs and Knapp (1991) used 0.2 m x 0.5 m clip harvests at Konza Prairie – a total area of 0.1 m^2 is a common size at this site; 3) at least one of the Cedar Cr. LTER experiments utilizes 0.1 m x 3 m strips, with clips from successive years occurring side-by-side within a larger 4 m x 4 m plot; and 4) The global Nutrient Network (NutNet) experiment employs two 0.1 m x 1 m clip strips for a total of 0.2 m^2 sample area. In general, longer and narrower strips are beneficial when biomass is locally patchy; shorter, square clip areas may suffice when biomass is distributed relatively homogenously across the sampling area.

Herbaceous biomass, and sometimes ANPP, may also be estimated allometrically by relating biomass/production to more easily measured input variables such as % cover, height, point-count hits, frond counts, etc. An additional advantage associated with relating herbaceous biomass to variables like height and % cover is that these inputs may also be derived from AOP datasets acquired at the site scale.

As such, it is possible that targeted, episodic clip harvests in Distributed and Gradient Plots could be coupled with AOP data to estimate total herbaceous biomass and productivity. However, coordinating the timing of AOP flights with respect to community phenology and peak biomass could be logistically difficult.

For herbaceous plants that produce all of their aboveground biomass in a given season, NEON will use the clip-harvest technique to estimate biomass and ANPP. When bryophytes and woody-stemmed sub-shrubs are clipped, care will be taken to clip current-year growth only; for these plants, clip-harvests will yield estimates of ANPP but not total biomass. Species-specific allometries will be used to estimate total biomass of ferns when equations are available.

The strategy for choosing clip-harvest locations for a given sample period within a growing season must also be standardized. Clip-harvest locations could be selected randomly within plots, selected in a stratified manner with respect to within-plot cover type, or selected systematically. Given that 1) the objective is to generate unbiased estimates of herbaceous biomass and ANPP on a per site per year basis, and 2) within-plot cover is not known *a priori* for either Distributed or Tower plots, it is logical to randomly choose clip-harvest locations within Distributed and Tower plots for each sampling period. Although there are disadvantages to this approach in some systems, i.e. increased uncertainty when herbaceous biomass is patchily distributed across plots, it is advantageous because the method can be consistently implemented across the approximately 3900 NEON plots in which clipping will occur, and when knowledge of per plot vegetation cover is minimal.

Biomass and productivity among functional groups

Reporting herbaceous biomass and productivity at the species level would provide the finest taxonomic granularity at each site with respect to changes in these variables through time. However, sorting speciose herbaceous communities to this taxonomic resolution requires significant personnel expertise and training, and will greatly increase the amount of time needed to sort clipped biomass. It will also be very difficult and expensive for NEON to quantify the uncertainty of species identification performed during sorting. Therefore, NEON will report biomass and productivity measurements in terms of functional groups rather than species. There are many ways to define functional groups (Lavorel et al. 1997), and the challenge for NEON is to select ecologically meaningful functional groups that are defined consistently across sites. Researchers at the SGS-LTER site sort aboveground herbaceous plant biomass into seven functional groups:

1. Cool season perennial graminoids
2. Cool season annual graminoids
3. Warm season perennial graminoids
4. Warm season annual graminoids
5. Forbs
6. Sub-shrubs, and
7. Previous years' litter

The Nutrient Network research group recommends sorting aboveground biomass into six different categories if time permits:

1. Bryophytes
2. Graminoid plants
3. Leguminous forbs
4. Non-leguminous forbs
5. Current year shrub and sub-shrub production, and
6. Previous years' litter

However, if time and/or available labor are scarce, NutNet guidelines call for sorting biomass into only three categories: bryophytes, vascular plants, and previous years' litter. There are advantages to using functional groups from both the SGS-LTER and NutNet protocols. This is because: 1) the distribution of warm versus cool-season grasses is expected to respond to increases in global temperature (Alward et al. 1999, Sandel and Dangremond 2012); and 2) the abundance of N-fixing, leguminous forbs is known to be affected by anthropogenic N deposition (Vitousek et al. 2002, Suding et al. 2005).

6.2.1 Sampling Methods

6.2.1.1 Sampling Methods: Clip-harvest

Clip-harvest location

Clip harvest locations within NEON Distributed and Tower plots will be selected *a priori*, and will be provided to field crews as an ordered list of random locations within individual plots/subplots that are acceptable for clip-harvesting (i.e. those locations that overlap 1 m^2 and 10 m^2 nested subplots reserved for repeated % cover measurements are omitted). Assuming up to 3 locations may be clipped per growing season, and that there will be 30 growing seasons, there will be up to 90 clip harvests performed per plot over NEON's lifetime. Once a clip-harvest area is accepted, the exact dimensions of the biomass removal area will be temporarily delineated using pre-marked cords and stakes. Technicians may reject clip locations if they are physically incapable of placing stakes in the ground at the specified location (e.g. a large diameter tree through the plot or presence of a fire ant hill). Given that some plots will almost certainly contain obstacles that will prevent some random locations from being used, the list of random clip strip locations per plot will exceed the number of anticipated harvests (Figure 5 shows 216 possible clip-strip locations in a standard 20m x 20m Distributed plot). This way, if the location assigned for a given round of sampling cannot be clip-harvested for logistical reasons (rocks, trees, etc.), technicians will simply move on to the next random location on the list.

Assessing ANPP in grazed ecosystems

Herbaceous productivity in ecosystems managed for grazing will be measured by placing a grazing exclosure over an additional random clip-harvest location per sampling period per plot (as per Knapp et al. 2007). Prototype grazing exclosures were constructed from 6-inch concrete remesh for the 2011 NEON Field Operations prototype at the D10 CPER site. Exclosures were cut from the remesh such that there was at least a 30 cm buffer from the edge of the exclosure to the edge of the protected clip-strip. Exclosures were then staked to the ground with ¼-inch diameter x 15-inch steel tent stakes to prevent movement by cattle. The design and materials of these exclosures performed acceptably, and the design can be modified to accommodate different vegetation heights as necessary (Figure 11).

Figure 11. Example of a grazing exclosure used at the Domain 10 CPER site.

Sorting to functional group

In order to align data collection categories with existing networks, and capture anticipated effects of global change drivers on the abundance of key functional traits, NEON will adopt functional categories from both NutNet and SGS-LTER protocols. Aboveground herbaceous biomass will be sorted into the following categories:

1. Bryophytes (those species that show distinct annual growth; non-mat-forming)
2. Cool season C_3 graminoids (as per Hattersley and Watson 1992)
3. Warm season C_4 graminoids
4. Leguminous forbs
5. Non-leguminous forbs
6. Current-year shrub and sub-shrub production (individuals with ddh < 1 cm), and
7. Previous years' litter

Successful implementation of sorting biomass into these groups depends heavily on hiring or training technicians capable of accurately identifying a large number of herbaceous plant species.

Following a relatively short 2-day training workshop focused on graminoid species ID, QA/QC results from the NEON 2011 CPER field operations prototype indicate that field technicians accurately sorted >95% of the harvested biomass into the SGS-LTER defined categories. Splitting the forbs group into leguminous and non-leguminous forbs requires technicians to reliably recognize legumes. This requirement will be included in the technician training program.

Sample processing

Clipped biomass will be sorted to functional group while still in the field, and stored in coolers chilled with re-usable cold packs or in a 4 ˚C refrigerator immediately following harvest. Clipped biomass will be re-checked for sorting accuracy within 24 hours of clipping, transported back to the laboratory in coolers as soon as possible following field collection, then dried for at least 48 h at 65 ˚C, and weighed to the nearest 0.01 g.

Aboveground perennial tissues

The aboveground component of perennial graminoid crowns will not be included in the clip-harvest biomass pool for two reasons. First, crowns are required to produce new leaf material, and their removal can substantially weaken the plant; grazers typically do not remove crown material (Milchunas and Lauenroth 1989). Second, because crowns are perennial structures that grow very slowly compared to leaves (Milchunas and Lauenroth 1992), crown biomass should not be incorporated into annual ANPP estimates. Similarly, aboveground woody components of shrubs and sub-shrubs not produced in the current year will not be clipped, as this biomass component either does not contribute to, or contributes only very marginally to ANPP.

Live aboveground bryophyte biomass

Aboveground bryophyte biomass is difficult to measure consistently so NEON will only report ANPP for this functional group, and ANPP will only be reported when it is possible for technicians to consistently distinguish current-year growth from older growth. All bryophyte species with determinate annual growth will be harvested according the clip-harvest strategy outlined here. All others will be harvested according to the strategy presented in the mat-forming bryophyte section of this document. Due to the highly hygroscopic nature of these plants, bryophyte biomass will be stored in desiccators following drying at 65 ˚C (Vitt 2007).

Herbaceous bioarchiving and tissue chemistry

NEON laboratory technicians will be responsible for drying, grinding, and shipping sub-samples of herbaceous tissues to external facilities for bioarchiving. Community-accessible sample archives will be maintained for at least the duration of the NEON project. In addition, when sampled Distributed plots are ≥ 50% herbaceous cover and are not classified as forest according to NLCD, NEON will analyze herbaceous clip-harvest samples for key chemical components as outlined in the Biogeochemistry Science Design (AD[04]).

6.2.2 Spatial Distribution of Sampling

Harvests of herbaceous plants will occur within randomly located clip-harvest strips in a randomly selected subset of Distributed plots (n=20), and in all Tower plots. For Distributed plots, clipping will only take place in those Distributed plots not classified as forest according to NLCD (i.e. Evergreen, Deciduous, or Mixed Forest types), and that are ≥ 50% herbaceous cover (Table 1). There will be one clip-strip per sampling period per 20m x 20m Distributed plot and one clip-strip per sampling period per 400 m^2 subplot for Tower plots. Clip-harvest strips are laid out as a series of North/South facing rectangles with dimensions of 0.1 m x 2 m. These strips exist within 0.5 m x 3 m "cells" that are numbered and systematically gridded out across the available sampling areas within the plot (e.g. Figure 5). The list of cell numbers is then randomized, and selection of strips from year to year proceeds down this randomized list. Prior to randomization, cells that overlap 1 m^2 and 10 m^2 nested subplots are omitted, ensuring that accepted clip-harvest strips are only placed *outside* the nested sub-plot components of the plot that are used for percent cover measurements. Due to logistical constraints, grazing exclosures will only be utilized in Tower plots, due to the inherent proximity of Tower plots to vehicle access, facilitating delivery and maintenance of exclosures.

6.2.3 Temporal Distribution of Sampling

The primary objective is to generate annual estimates of herbaceous biomass and productivity within the dominant vegetation type (i.e. within Tower plots). In the absence of managed grazing, herbaceous clip harvests will occur 1-2 times a year at each NEON Tower plot to capture peak growth for important C_3 and C_4 functional groups (Table 1). Sampling at selected Distributed plots will occur once every 3 years. Sampling onset dates and frequency will be dictated by local phenology and specified in the sampling protocol. A given sampling period will be concluded within 10-14 days of initiation, so that the plant community does not change appreciably during the time that all target plots are sampled. This guideline ensures that data collected across all plots within a given sampling campaign are comparable.

At sites actively managed for grazing, clip harvests will occur every 4 weeks in order to capture herbivore consumption and the plant compensatory re-growth response to grazing (Knapp et al. 2007). Grazing exclosures will be moved to a new random location on the same time interval. Because sampling and sorting clipped biomass requires significant time investment per sampling period (between 10-14 days), sorting is clearly incompatible with the frequency of harvests required to estimate herbivore consumption and plant compensatory regrowth. As such, only one "peak biomass" harvest per growing season will be sorted to functional group in grazed systems in which exclosures are employed.

6.2.4 Logistics and Adaptability

Herbaceous clip-harvests must be performed within Tower plots on an annual basis, and sampling these plots is the priority. Distributed plots that meet the criteria in Section 6.2.2 every 3 years, or one site per domain per year.

Additional possibilities for reducing labor and training requirements include the following non-mutually exclusive options: 1) sorting Tower plot clip-harvests to functional group every 3 years (i.e. one site per domain per year), and recording only total herbaceous ANPP and biomass in intervening years; and 2) eliminating functional group sorting entirely in either Distributed plots, Tower plots, or both, and only recording total herbaceous ANPP and biomass. Both of these options ensure that NEON continues to meet high-level requirements, but these options also reduce the utility of NEON herbaceous plant data with respect to the types of questions researchers may address, particularly with respect to plant functional group responses to global change drivers.

Finally, clip-harvest sample size may need to be increased in heterogeneous systems like deserts and savannahs, since random clip-harvest locations within a plot will not account for the patchy occurrence of herbaceous vegetation. The current design can accommodate increases in clip-harvest sample size by simply increasing the number of grid cells sampled per sample period per plot.

6.3 Sampling Design for Mat-forming Bryophytes

Definition: Bryophytes include all *Sphagnum* spp., as well as others common in Arctic tundra and boreal forest that may grow in peaty, wet mats and therefore require specialized techniques for estimating aboveground productivity. Such bryophytes are abundant at the Toolik Lake site (Walker et al. 2002), and are present at sites with boreal, alpine, and even tropical vegetation.

Where abundant, moss can significantly affect the balance between above and below ground C pools, and the overall estimate of ecosystem productivity and carbon use efficiency (Binkley and Graham 1981, Shaver and Chapin 1991, Street et al. 2012, Bona et al. 2013). Bryophyte productivity is therefore an essential element in NEONs vegetation sampling in sites with high cover of bryophytes namely, tundra and boreal forest systems.

When innate, clearly visible, markers of annual growth are present such as may be found on *Hylocomium splendens* and other feather mosses, bryophytes will be collected as part of the herbaceous vegetation clip harvest procedure. Innate markers include growth indicators such as frond spacing and branch patterns that clearly indicate a single season's growth (Vitt 2007)**.** In plots with greater than 20% cover of mat forming bryophytes on which annual growth is not easily determined, bryophyte ANPP will be measured according to annual growth using the design described in this section.

Productivity measurements can be achieved through a variety of methods including: ^{14}C labeling techniques (Aerts et al. 1992), cotton string markers, visual stain markers, and cranked wire measurements (Russell 1988, Glime 2007, Vitt 2007). Several of these methods measure growth in terms of length of bryophyte growth, however the relationship between length and biomass is not always consistent even for an individual species (Rincon and Grime 1989, Glime 2007) and therefore measurements require annual species x site calibration to be useful.

6.3.1 Sampling Methods

Current-year bryophyte production can be measured for a wide range of species via several distinct clip-harvest methods, but total biomass estimation is more difficult. The transition from live, actively-growing tissue to dead tissue is difficult to determine consistently as environmental conditions can turn green 'live' material brown before it is actually dead (Clymo 1970, Wielgolaski 1972, Vitt and Pakarinen 1977, Vitt 2007). Attempting to estimate standing aboveground bryophyte biomass would require a substantial increase in sampling effort; estimates would include unquantifiable uncertainty, may carry a much greater error estimate than biomass estimates for other vegetation components, and would necessitate an unacceptable level of destructive harvest resulting in adverse impacts to sensitive habitats. For these reasons, rather than sampling mat-forming bryophytes for total biomass, NEON will limit sampling of this vegetation component to total ANPP estimates via standard clip harvest techniques, removing only annual growth.

Mat-forming bryophyte ANPP will be estimated according to the clip harvest from net height method (Clymo 1970, Russell 1988). Nylon nets with 1 to 1.5 cm spaces cut to 20 cm x 20 cm squares (5 cm buffer around an interior square 10 cm on a side) will be anchored to sampling plots prior to snowfall the year before sampling is to occur. Throughout the growing season new vertical growth will occur above the level of the net; at the end of the season bryophytes will be clip harvested to the level of the net within the interior 10 cm x 10 cm square. Harvested samples will then be dried and weighed. ANPP for *Sphagnum* mosses calculated by this method are often done so with a correction for the mass of the capitulum; due to the indeterminate apical growth of mosses in this genus, plant material not produced during the sampling year may be carried up vertically along with the capitulum (Clymo 1970). A subset of plots (n=10) will be measured according to the capitulum correction outlined by Clymo (1970), and NEON will report both the uncorrected ANPP measurements and the capitulum correction factor for each site for each year.

Bryophytes often have a longer growing season than vascular plant counterparts (Street et al. 2012). In continental climes, mosses may begin growing before the ice melts. Nylon nets will be placed over bryophyte mats prior to snowfall at the end of the preceding growing season so that annual growth prior to snowmelt during the intended sampling season will be captured.

The net-clip-harvest method was selected because the other methods evaluated were not viable for NEON sampling due to the monetary expense, the necessary commitment of time, and the need to ensure consistency across NEON. All methods of measuring bryophyte productivity have advantages and disadvantages; the clip harvest to net height method is simple to implement, highly reproducible, and does not require species-specific calibration. Another benefit of using the net method is that, regardless of the topography, measurements will be made according to surface area of the bryophyte mat; no slope corrections will be necessary to express productivity per unit area. However, this method may underestimate productivity for bryophytes with lateral branching (Vitt 2007), and achieves lower levels of precision as bryophyte productivity declines (Clymo 1970).

Equipment considerations

Nets for clip harvest sampling will likely need to be fabricated specifically for NEON sampling. Nylon netting with appropriately sized 1 cm–1.5 cm mesh, such as that used for fishing, pond covers, sports netting, fruit bags, etc. is suitable and generally inexpensive. Bulk netting will be cut to 20 cm x 20 cm squares with a 10 cm x 10 cm square marked in the center to indicate the boundaries of the sample area. These nets will be anchored to the specified sample locations with standard garden staples or longer stakes if dictated by the vegetation. Anchors will be placed in the 5 cm buffer surrounding the central square such that they do not interfere with growth of material within the harvest square, and so that the square is snug against the bryophyte canopy so all new growth will occur through the grid cells. Minor manipulation of the mosses may be necessary to ensure that the net does not affect the growth pattern.

6.3.2 Spatial Distribution of Sampling

Bryophyte ANPP will be measured for all Tower plots that contain qualifying vegetation.

Sample size

At sites where mat-forming bryophytes occur, sampling effort will match that of the herbaceous plant clip harvest strategy. Harvest of annual growth will occur in one 10 cm x 10 cm (1 dm^2) sampling location for every 400 m^2 of plot. At sites where the smallest, 20 m x 20 m plots are employed, this means there will be one net harvest sample per plot per year.

Location of sample points

The location of bryophyte sampling nets will be dictated by the distribution of bryophyte mats within the plot. If total area of mat forming bryophytes represents less than 20% of the plot, clip harvest will not occur, as the impact of annual sampling on the plot over the life of NEON would be too great. In cases where the target vegetation occurs across the entire plot with 80%–100% coverage, harvest locations will be randomly assigned and may be collocated with herbaceous clip harvest strip locations to minimize traffic through the plot. When the % cover of mat-forming bryophytes is between 20%–80%, the sampling strategy will depend on the spatial arrangement of bryophyte mats. If cover is continuous in a portion of the plot, harvest points will be randomly assigned within the area; if cover is clumped in to distinct patches throughout the plot, patch area will be measured and mapped and assigned an ID (similar to the strategy for clumped shrubs). Patches will then be randomly selected for sampling. Whatever strategy is employed at a plot, no location will be harvested more than once during the lifetime of NEON.

6.3.3 Temporal Distribution of Sampling

If the total cover of bryophytes within the plots is great enough to warrant measurement of this vegetation (i.e. ≥ 10% cover averaged across all Tower plots), ANPP from bryophytes in Tower plots will be estimated annually. Distributed plots that contain bryophytes are not currently considered for regular sampling; however, if labor and funding is available, Distributed plots may be sampled episodically (i.e. every 3 years; one site per domain per year).

6.3.4 Logistics and Adaptability

The greatest concern in arctic tundra/boreal forest sites is mitigating lasting impacts of human traffic and destructive harvest to the site. If it becomes clear that the collection of annual growth from mat-forming bryophytes causes unintended trampling of sensitive habitats, the sampling interval may be increased to once every 3 years.

Maintaining the link between on the ground measurement of ANPP and carbon flux data collected from the tower instruments is essential to support scalability of the data and to address NEON grand challenges. If NEON is not able to conduct destructive harvest of bryophytes at all in these ecosystems, bryophyte mats or hummocks may be measured for changes in volume over time. However, the link between volume and biomass is not consistent between species, between sites, and possibly not even between years, so without annual calibration, NEON's ability to accurately characterize ANPP using volumetric estimates would be severely limited.

Over the 30-year life-span of NEON, a significant amount of foot traffic is anticipated in order to obtain samples from Tower plots. NEON will prescribe standard approach routes in order to protect the majority of vegetation from the majority of trampling, but this approach is not suitable for sampling highly-sensitive bryophyte mats. Because Tower plots will necessarily be located relatively close to a road, it may be logistically feasible for field technicians to mitigate impact by sampling bryophytes in these plots using ladders placed horizontally across the sample area, similar to the sampling strategy employed at Toolik Lake LTER, Cedar Cr LTER, etc. Monitoring of the impact to an arctic site will be concurrent with sampling activities, and on-going adjustments to the sampling strategy will occur as needed.

Finally, similar to clip-harvesting of herbaceous plants, net-clip sample size may need to be increased beyond n=1 sample per 400 m^2 plot/subplot if the distribution of ANPP for these plants is heterogeneous enough across the landscape that ANPP estimates cannot be made to within $\mu \pm 10\%$ with 90% confidence. The design can accommodate increases in sample size when % cover of these plants is relatively high by simply increasing the number of randomly selected bryophyte patches sampled per sample period per plot. However, when % cover of these plants is relatively low, the number of potential samples per year will be constrained by the desire to mitigate the sampling impact on the plots.

6.4 Sampling Design for Litterfall and Fine Woody Debris

Definition: Litterfall is defined as shed leaves and needles, reproductive parts (i.e. flowers, fruits, cones, seeds, etc.), and fine woody debris with butt-end diameter < 2 cm (modified from Clark et al. 2001, Bernier et al. 2008). Woody pieces with diameter ≥ 2 cm is considered coarse woody debris, and will be sampled according to the coarse woody debris sampling strategy outlined in this document.

Spatial arrangement and number of litter sampling units within plots

To measure litterfall and fine woody debris, NEON will employ two types of sampling units: 1) elevated, mesh litter traps; and 2) ground "traps". Bernier et al. (2008) recommend that 15-20 litter/ground trap pairs be used per roughly 1 km^2 of land-surface area. In terms of spatial arrangement, some authors indicate that litter traps should be randomly located within plots (Bernier et al. 2008), whereas others systematically place litter traps at the center of a larger plot, and then place the ground traps 2 m from the elevated trap, but at a random azimuth (Muller-Landau and Wright 2010).

Other factors to consider are the size and shape of elevated litter traps and ground traps. Elevated litter traps should be large enough such that the average size of abundant foliage and fine woody debris elements are easily intercepted by the trap. Ground traps are intended to intercept particularly large foliage elements that will not fit in elevated traps (e.g. palm fronds), and fine woody debris pieces that are too long to be sampled in elevated traps. Ground traps may also be used to estimate production of larger woody debris pieces (i.e. branchfall). Muller-Landau and Wright (2010) employ square elevated litter and ground traps that are both 0.5 m^2, for sampling litter and fine woody debris production in a tropical forest. However, due to the spatially heterogeneous production of fine woody debris, and particularly for larger pieces of fine woody debris, larger rectangular ground traps may be more appropriate (Muller-Landau, personal communication).

6.4.1 Sampling Methods

For both elevated and ground traps, only the portion of material that meets both the length and diameter criteria will be sampled (Muller-Landau and Wright 2010). Similar to the herbaceous clip-harvest procedure (described in this document), litter sampled from elevated traps will be sorted into functional groups following collection. NEON will adopt the following categories recommended by Bernier et al. (2008):

1. Leaves (broadleaf)
2. Leaves (needles)
3. Twigs/branches < 1 cm diameter
4. Woody material (e.g. cones, etc.)
5. Seeds
6. Flowers
7. Other (lichen, mosses, unidentifiable material, etc.)

Sorted litter will be analyzed for C, N, P, K, Mg, Ca, and other elemental "majors" via contracting with selected external laboratories (AD[04]). Litter collected from ground traps will be sorted into categories distinct from those used for elevated traps. Ground trap categories include:

1. Large leaves and fronds that will not reliably be intercepted by elevated traps – i.e. those ≥ 50 cm length
2. Woody debris with length > 50 cm, and diameter between 1–2 cm.
3. Pieces of intercepted woody debris with diameter between 2–10 cm are considered in the coarse woody debris section of this document.

To ensure the accuracy of annual litter production estimates, ground traps will be cleared of all relevant litter material following the annual sampling bout. Sorted litter from ground traps will be analyzed for the same chemical analytes as samples originating from elevated traps (AD[04]).

Laboratory processing

Following collection, litter will be transported back to the laboratory and dried at 65 ˚C until there is no weight loss between measurements made on two consecutive days (minimum 48 hrs). Litter will be sorted before drying to minimize production of litter fragments that are difficult to identify (Muller-Landau and Wright 2010). The woody portion of litter will be dried for an additional period at higher temperatures, 101-105 °C, to release bound water (Williamson and Wiemann 2010).

Equipment

Design of PVC litter traps will be adopted from STRI/CTFS. Non-oxidizable metal rods (e.g. aluminum, galvanized steel, or equivalent) will be used to hold elevated litter traps in place. The corners of ground traps will be marked with non-oxidizable metal or wooden stakes to facilitate precise re-measurement of the selected plot grid cell.

Laundry baskets are a frequently employed alternative to collecting litter in traps made from PVC (Bernier et al. 2008). The advantage to laundry baskets is that they are commercially available and inexpensive. However, NEON will adopt the PVC design used by STRI/CTFS because the length of legs on PVC traps can be adjusted on-site so that the intercept plane of the trap opening is always level, and the area of intercept is therefore kept consistent. It will be more difficult to ensure that laundry baskets are kept level on sloped ground.

6.4.2 Spatial Distribution of Sampling

Only Tower plots will be sampled for litterfall and fine woody debris, as sampling within Distributed plots is focused on estimating biomass stocks, rather than plant productivity. Consistent with existing protocols, NEON will establish at least one elevated litter trap and one paired ground trap per 400 m^2 plot/subplot in Tower plots with qualifying vegetation. Tower plots that do not qualify for litter sampling includes those Tower plots classified according to the NLCD as grassland herbaceous, sedge herbaceous, pasture hay, or cultivated crops. For Tower plots falling into all other NLCD vegetation classes, there are two spatial strategies for sampling litterfall and fine woody debris that depend on vegetation height, and the % cover of woody vegetation across all Tower plots at the site:

1) The % cover of woody vegetation ≥ 2 m height across all Tower plots is ≥ 50%. For systems with relatively high % woody vegetation cover, litter traps will be placed randomly within each 400 m^2 plot/subplot.
2) The % cover of woody vegetation ≥ 2 m height across all Tower plots is < 50%. For systems with relatively low % woody vegetation cover (e.g. mixed woodland or savannah ecosystems such as Domain 15 Onaqui or Domain 17 San Joaquin), randomly placed litter traps are unlikely to adequately capture litter dynamics from woody vegetation. In this case, NEON will target litter trap placement to areas of the plot with woody cover, and then use estimates of woody vegetation percent cover to scale litter production from the trap to the plot scale. Estimates of woody vegetation percent cover can be derived from remote-sensing imagery, or from initial site characterization work performed during plot establishment.

Elevated traps

Elevated mesh litterfall traps (70.7 cm x 70.7 cm; 0.5 m^2) will be placed at either random or targeted locations within each Tower plot as discussed above. These traps will reliably sample shed leaves, needles, reproductive parts, and fine woody debris with butt-end diameter < 1 cm and length < 50 cm. The selected position for elevated litterfall traps will remain constant from year to year.

Ground traps

Paired ground traps for collecting large leaves and fronds, and fine woody debris with butt-end diameter between 1 cm – 2 cm, and length > 50 cm, will be randomly located in plots at least 2 meters from elevated traps, consistent with Muller-Landau and Wright (2010). To avoid interfering with other sampling within the plot, the basic ground trap sampling unit will be one randomly selected 0.5 m x 3 m herbaceous clip harvest grid cell within the same plot module as the elevated trap. Ground traps will be cleared of all relevant litter one year prior to the onset of sampling so that any litter within the selected area can be assumed to be the result of annual production. Only portions of large fronds or long sections of fine woody debris that lie inside the ground traps will be sampled; these sample locations will not move from year to year and will be excluded from consideration as locations for herbaceous clip harvest.

6.4.3 Temporal Distribution of Sampling

The primary objective is to generate annual or per growing season estimates of litterfall and fine woody debris production within the dominant vegetation type (i.e. within Tower plots). All litter sampling will therefore be focused on estimating litterfall and fine woody debris production in the dominant vegetation within the Tower footprint in order to directly link these ground measurements to flux data from the NEON tower at each site.

Elevated traps

In predominantly deciduous systems with pronounced annual senescence, elevated litter traps will be sampled once in the spring to account for winter production of fine woody debris, followed by sampling every other week during the period of autumn senescence (Bernier et al. 2008).

Litterfall in coniferous forests (e.g. D10 Rocky Mountain Park and D16 Wind River), tropical evergreen broadleaf forests, or in xeric shrub systems (e.g. D14 Santa Rita and Jornada LTER) may be sampled with less frequency than deciduous broadleaf forests, but since there is no clear 'litterfall season,' sampling will occur year round. Sampling frequency at sites in Europe dominated by pine, spruce or fir ranged from 3-12 collections per year with the majority of sites sampled three times a year (once every four months) (Berg and Meentemeyer 2001). Búrquez et al. (1999) and Pavón et al. (2005) sampled litterfall in the arid desert systems in Mexico monthly. In systems dominated by plants that bear multi-year leaves or needles, NEON will therefore sample elevated traps throughout the year at least once every 8 weeks..

Estimates of deciduous litterfall will be calculated on a per annum basis, with all of the litter produced in a given year contributing toward the yearly estimate. Evergreen litterfall estimates from a given calendar year will not be strictly annual, due to the multi-year and somewhat variable lifespan of evergreen leaves and needles; however, the long-term average (n = at least 3 years) will be used to estimate per annum litter production in non-deciduous woody plant communities.

Ground traps

Ground traps will be measured once per year in Tower plots.

6.4.4 Logistics and Adaptability

Litterfall and fine woody debris production must be estimated within Tower plots on an annual basis. Within a year or growing season, Metcalfe et al. (2008) point out that litterfall collection efforts often have high levels of uncertainty and require greater sample size to accurately estimate annual production than other components of plant productivity. Additional traps may be installed at additional random locations per plot should variance of the litterfall estimate be unacceptably high, and if technician labor is available.

If dictated by logistical or financial constraints, the frequency of sampling elevated traps may be reduced at some sites. The current design of sampling litter every 8 weeks at evergreen or xeric sites may exceed what is necessary to capture annual litter production. However, litter traps left for too long in the field may be subject to granivory by small mammals, herbivory by insects, or increased decomposition and resulting loss of mass. Given these considerations, if it is necessary to sample traps with an interval greater than once every 8 weeks, NEON will flag data appropriately so that data users are aware of the sampling interval that was employed.

This design calls for sorting fresh litter into the specified functional groups if time permits. However, if it is logistically not feasible to sort fresh material, litter may be sorted after drying as time allows. However, sorting freshly collected litter is preferable because dry litter is easily fragmented and identifying small litter fragments to functional group will introduce uncertainty in sorting accuracy.

The sizes of elevated and ground litter traps have been chosen to either 1) be consistent with existing STRI/CTFS protocols or 2) fit within the existing NEON design for other sampling within the plot. If it is apparent that the volume of biomass collected from elevated and ground litter traps is to too great to efficiently dry and process given limitations on drying oven space in the NEON laboratory, trap size or number may be reduced if justified based on sample optimization analysis.

6.5 Sampling Design for Coarse Woody Debris

Definition: Coarse woody debris (CWD) is defined for the NEON observatory to be any fallen stem with diameter ≥ 2 cm at the point the CWD particle intersects the survey transect. Particles of CWD – i.e. logs – that meet this criterion are further divided into three different size classes according to diameter, as per Keller et al. (2004): 2–5 cm, 5–10 cm, and ≥ 10 cm. Logs ≥ 10 cm diameter must be ≥ 1.5 m length (Harmon and Sexton 1996), and logs between 2–10 cm diameter must be ≥ 50 cm length. Woody debris with diameter < 2 cm is considered fine woody debris and is sampled with litter traps and ground traps (see the sampling design for litter, in this document). Standing dead wood and woody debris – that is, snags with lean angle of 45˚ off of vertical or less, and dead branches attached to live trees – are accounted for along with standing live biomass (see Section 6.1).

6.5.1 Data Req'd to Estimate CWD Mass: Volume, Decay Class, Species Groups, Bulk Density

As part of a broader effort to estimate the size of important biomass pools at the site scale, NEON must estimate CWD mass. This is typically accomplished by measuring CWD volume with one sampling design, and coupling volume estimates with CWD bulk density by decay class by "species" values that are measured according to a separate design (Keller et al. 2004, Valentine et al. 2008). In addition to CWD volume, other parameters that may be of interest to the ecological community include frequency (count ha^{-1}) and aggregate length ($m\ ha^{-1}$). Below, the strengths and weaknesses of available methods for estimating these parameters within the context of NEON are described.

6.5.1.1 Volume Estimators for CWD

Line-intersect sampling (LIS) has traditionally been used to estimate CWD volume (Brown 1974). With the LIS method, pieces of CWD are encountered and tallied with probability proportional to length of the log. Unbiased estimates of CWD length are produced from simple tallies of pieces which intersect the sampling line, and when these data are combined with additional measurements of piece length and diameter, unbiased estimates of CWD frequency and volume are possible (de Vries 1986). Because LIS sampling tallies logs proportional to length and not volume, volume estimates derived with LIS tend to have greater error than corresponding frequency and length estimates made from the same sampling effort (Affleck 2010).

In order to optimize efficiency in terms of sampling time and precision of the CWD estimators, the ideal CWD sampling method for NEON is one that will tally logs with probability proportional to volume, and generate an unbiased estimate of volume with relatively high precision based solely on log tally number. At least three sampling methods have been introduced within the past 10 years that satisfy these criteria, and could be implemented by NEON:

1. Perpendicular distance sampling (PDS; Williams and Gove 2003)
2. Distance-limited perpendicular distance sampling (DLPDS; Gove et al. 2012); and
3. Line-intersect distance sampling (LIDS; Affleck 2008)

These three sampling methods are evaluated below with respect to ease of implementation in the field.

Perpendicular Distance Sampling

The PDS method is essentially a variable-radius plot method that generates an unbiased estimate of CWD volume solely on the basis of counts of included logs. Counts are multiplied by a volume factor (m^3 ha^{-1}) to estimate aggregate volume on an areal basis (Williams and Gove 2003). The volume factor is selected based on a knowledge of the maximum size of CWD likely to be encountered at a given site (Valentine et al. 2008). Limiting distances (D_L) are calculated for logs as a function of log cross-sectional area or diameter, and logs are counted if the distance D between the log and the sample point is less than D_L (Valentine et al. 2008). Look-up tables are employed in the field to quickly determine D_L at specific log diameters, and measurement of log diameters is only required for a log when its distance from the sample point is close to the limiting distance (Valentine et al. 2008). Unbiased estimates of log frequency can be generated if log length and cross-sectional area are also measured, and unbiased estimates of aggregate log length are possible with only the addition of log cross-sectional area measurement (Ducey et al. 2008). For QA/QC of the NEON CWD data product, technicians will be required to record cross-sectional area of tallied logs as a matter of standard operating procedure.

There are, however, a few notable theoretical and practical problems associated with PDS. The first is that the variance of the PDS frequency estimator can be very high, due to the fact that PDS is optimized for volume estimation (Williams and Gove 2003). However, given that CWD volume estimates have priority over frequency and length estimates in the NEON sampling framework, this issue may be of minor importance. The second problem with PDS is that D_L for logs with diameter > 60 cm can be > 100 m, depending on the chosen volume factor (Valentine et al. 2008). Moreover, large values of D_L are associated with detection errors in the field, particularly if visibility is limited (Ducey et al. 2013). Large limiting distances would also be associated with very long search times for qualifying logs. Nonetheless, using a metric of efficiency *E* that accounts for both the time required to sample an area, as well as the resulting sample variance, Ducey et al. (2013) show that *E* is significantly lower for PDS compared to LIS.

Distance-Limited Perpendicular Distance Sampling

The DLPDS method is similar in many respects to PDS, but the salient differentiating feature of DLPDS is that the search for tallied logs is constrained to within a user-selected maximum distance, D_{max} – e.g. 20 m (Gove et al. 2012). Reducing D_L to D_{max} limits search times, and improves detection of qualifying logs. Once D_{max} has been defined, a maximum cross-sectional area g_{max} is calculated, and the following sampling decisions arise (Ducey et al. 2013):

1. If log cross-sectional area $g \leq g_{max}$, then D_L for the log is determined as per standard PDS sampling, and the log is tallied if $D \leq D_L$
2. If $g > g_{max}$, then $D_L = D_{max}$, and the log is tallied only if $D < D_{max}$.

An additional requirement for case 2 is that *g* must be recorded in order to determine the log's contribution to CWD volume at the sampling point (Ducey et al. 2013). The DLPDS method provides an unbiased estimate of CWD volume. In addition to volume, aggregate length can also be estimated, in an unbiased way, as a simple function of the log tally (no log cross-sectional area required), and log frequency can be estimated if log length is also recorded (Gove et al. 2012). However, similar to PDS, the precision of frequency and length estimates made with the DLPDS technique are far worse than the precision of volume estimates (Gove et al. 2013). Ducey et al. (2013) determined that *E* for DLPDS volume estimates is generally comparable to or better than that of PDS, and *E* for DLPDS is always better than that for LIS.

The DLPDS method appears promising in terms of *E*, and implementation in the field is likely less error prone than PDS, due to the elimination of large limiting distances that must be searched for very large logs and the associated detection errors. However, detection errors are still likely because a 2-dimensional area must be searched for qualifying logs. Moreover, the DLPDS method has not been extensively field tested, and it is unclear how to systematically choose D_{max} and volume factors across the network of NEON sites.

Line-Intersect Distance Sampling

The LIDS technique for estimating CWD parameters is a probability proportional to volume method that, similar to LIS, involves counting logs intersected by a transect. The LIDS protocol supplies design-unbiased estimation of aggregate CWD volume via a simple tally, similar to the PDS method, but is less prone to detection errors in the field compared to both PDS and DLPDS because the search for logs included in the tally is directed along a line transect as opposed to a two-dimensional area (Affleck 2008). The LIDS approach differs from LIS in that transects do not have a fixed length. Instead, similar to PDS, the length of the transect is determined by a limiting distance, D_L, that is a function of the cross-sectional area of the largest logs encountered (Affleck 2008). In addition to aggregate volume, unbiased estimation of log frequency (count ha^{-1}) is possible when log length and cross-sectional area *g* are measured, and unbiased estimation of aggregate CWD length (m ha^{-1}) can be achieved when *g* is recorded for each tallied log (Affleck 2008). Similar to PDS and DLPDS, the precision of LIDS-derived estimates of frequency and aggregate length is worse than that for volume by approximately a factor of two (Affleck 2010).

With respect to application of transect-based methods in the field, both LIS and LIDS assume random azimuthal log orientation. One issue with LIDS is that estimator variance can increase significantly when logs are oriented non-randomly as a result of blow-down, landslides, chain-drag logging, etc. (de Vries 1986, Affleck 2008). In contrast, the PDS-type methods are not affected by orientation bias (Ducey et al. 2013). To compensate for orientation bias, multiple transects oriented at different angles – e.g. in a “Y” shape – can be employed at each sampling point (Affleck 2008, 2010). Using simulation tools, Affleck (2008) found that three LIDS transects arranged in a “Y”-shape generated similar coefficients of variation for volume estimates to the PDS method for both randomly and non-randomly oriented logs.

With respect to sampling efficiency *E*, Affleck (2010) compared LIS and LIDS in seven forest stands in Montana. He found that simultaneous estimation of frequency, aggregate length, and volume required more time with LIDS than with LIS, but that the gains in precision substantially offset the increase in sampling time in six out of seven forest stands. That is, LIDS was more efficient than LIS by 23% to 76% in six stands, and LIDS performed similarly to LIS in one stand (Affleck 2010).

Comparison of LIS, PDS, DLPDS, and LIDS

In general, CWD sampling methods derived from a probability proportional to volume theoretical basis appear to generate more or equally precise estimates of CWD volume compared to the traditional LIS technique, while simultaneously requiring less time to implement in the field. Compared to the PDS method, DLPDS is superior due to reduced log detection error rates in the field, particularly when visibility is poor due to understory vegetation or sloping, complex terrain. The LIDS protocol performs similarly to PDS in both a simulation and a field study (Affleck 2008, 2010), but there are no studies that compare DLPDS with LIDS in either a simulation or field environment. Considering all of the available data, both DLPDS and LIDS are suitable for implementation within the NEON framework, but it is likely that log detection error rates will be lower with the LIDS method compared to the DLPDS method.

6.5.1.2 CWD Decay Classes and Species Groups

There are two main questions with respect to tallying logs for CWD volume estimation: 1) how should decay classes be defined; and 2) how should species groups be defined?

The choice of decay classes will be consistent with those used for bulk density sampling, so the decay classes selected for bulk density estimation will also be used for grouping volume tallies. Grouping logs across decay classes by species is more complicated, due to the inherent difficulty of accurately identifying logs in an advanced state of decay to species. One option would be to use categories ranked according to decay-resistance: i.e. species would be classified as resistant, moderately-resistant, and non-resistant to decay, and could also be classified as "unknown" (modified from Harmon and Sexton 1996). However, this scheme requires distinguishing between members of the speciose *Pinus* and *Quercus* genera, since these genera have species in each category. Making these distinctions may pose accuracy problems at sites where species from both categories are present. Another option is to group logs into "hardwood" and "softwood" species groups (e.g. Valentine et al. 2008). The drawback to these simple categories is that, although assigning species to categories would likely be relatively accurate, there is considerable variation in decay resistance among species within group (Harmon and Sexton 1996).

6.5.1.3 Bulk Density Estimation

Bulk density of CWD logs is required in order to convert CWD volume estimates per unit area to biomass and C stock data products. In addition, bulk density values become smaller as log decomposition progresses (Harmon and Sexton 1996), so bulk density estimation across decay classes is required (Valentine et al. 2008).

There are two general methods described in the literature for sampling radial cross-sections for bulk density. Harmon and Sexton (1996) indicate that replicate plugs of heartwood, sapwood, and bark should be obtained from each radial cross-section, although obtaining plug samples of bark is likely not possible for many species. In addition, it seems that radial distance from the log center to the edge, as well as distances corresponding to transition from heartwood to softwood, and from softwood to bark would be required in order to correctly estimate the density of a log with no internal void volume. In contrast, Keller et al. (2004) removed bulk density plugs from cross-sectional log discs every 5 cm along one of eight randomly selected radii, and location of plug samples was not dictated by heartwood, sapwood, or bark.

6.5.2 Sampling Methods

CWD production

Production of coarse woody debris (i.e. branchfall) is an important component of ANPP (see Equation 2). However, CWD production cannot be estimated using the tally methods discussed above because observed log counts are influenced simultaneously by both production and decomposition, particularly if sampling intervals are multi-year. Production of CWD between 2-10 cm diameter will therefore carried out using the same 0.5m x 3m ground traps utilized for estimating fine woody debris production. Production of logs > 10 cm diameter is a process that typically begins with tree death, followed by a transition from standing to downed status. As such, production of CWD > 10 cm diameter is already accounted for during woody stem mortality surveys.

CWD volume and mass

NEON will adopt the LIDS method for estimating CWD volume, and more specifically, three LIDS transects arranged in a "Y" shape will be used per sample point in order to avoid potential problems with non-random log orientation. NEON technicians will identify logs to species when possible and will group tallied logs according to decay-resistance categories (i.e. resistant, moderately-resistant, non-resistant and unknown). Compared to simpler "hardwood" vs. "softwood" categories, there is a greater chance for classification errors with this approach, but the greater information content with respect to understanding C dynamics outweighs the risk.

For logs with diameter > 10 cm, NEON technicians will sample bulk density from radial cross-sections of logs, with equal sampling effort across decay classes; decay classes are defined according to USFS guidelines (Table 3).

Table 3. Decay classes of logs and their attributes, as defined by the USFS (from Valentine et al. 2008).

Class	Integrity	Texture
1	Sound, freshly fallen	Intact, no rot
2	Sound	Intact, sapwood partly soft
3	Heartwood sound, log supports its own weight	Sapwood can be pulled apart by hand, or is absent
4	Heartwood rotten, log does not support its own weight, but maintains shape; can be kicked apart, but breaking apart with hands is difficult	Soft, small, blocky pieces; a metal chaining pin can be pushed into heartwood
5	None; log does not retain shape and can be broken apart with hands; majority of log not incorporated into litter layer of soil	Soft, powdery when dry

Sampling effort will be stratified such that radial log sections are obtained with proportion inverse to the frequency of diameters encountered during volume sampling (as per Keller et al. 2004). That is, larger, less frequently encountered logs will be more likely to be sampled for bulk density, as these logs represent a disproportionately large component of total CWD mass.

Bulk density plugs will be sampled according to methods described by Keller et al. (2004). Plugs will be measured for volume in the lab, then oven-dried at 105 ˚C until constant mass in order to calculate plug density.

In addition to plug density, the void volume of cross-sectional discs will be estimated so that bulk density values at the log-scale are accurate (Harmon and Sexton 1996). Digital photographs of radial cross-sections will be used to quantify void volume, and the bulk density of a given radial section is then the product of average density multiplied by the proportion of total area that is not void (as per Keller et al. 2004).

For logs with diameter between 2–10 cm, bulk density will be estimated for representative samples by estimating the volume using total piece length and diameters of both piece ends. The entire piece will then be dried at 105 ˚C until constant weight, and labeled pieces may be cut into 10 cm lengths to facilitate drying.

A subset of dried bulk density plugs (from logs > 10 cm diameter) and piece sections (from logs 2–10 cm diameter) will be finely ground to at least 20 mesh so that concentrations of C, N, P, and elemental "majors" can be determined.

Equipment considerations

A laser rangefinder (LaserTech TruPulse 360B) will be used to measure distance relative to the plot centroid for each tallied log, and log diameter will be measured either with calipers, or a meter tape, depending on log diameter.

Plugs for bulk density determination will be extracted using plug-and-tenon cutters of known diameter. Void volume will be estimated using digital images collected with a DSLR camera and analyzed with Adobe Photoshop software or equivalent.

6.5.3 Spatial Distribution of Sampling

Sampling of coarse wood debris will occur at all Tower plots at forested sites and 20 Distributed plots per site within forested NLCD classes. To estimate CWD production and volume across the landscape, NEON technicians will establish 3 transects at pre-existing points associated with the Distributed and Tower plot centroids. These points are distributed according to a spatially-balanced, stratified random design (Theobald et al. 2007), and as such, measuring CWD at these points will produce an unbiased estimate of CWD parameters of interest at stand and regional scales.

At each point at which CWD volume will be measured, field technicians will tally logs along three LIDS transects that originate at the plot centroid, and are oriented such that there is a 120˚ azimuth between each transect – that is, the three transects roughly approximate a "Y" shape. While azimuthal spacing is constrained to 120˚, the azimuthal orientation of the three transects will be randomized on a per plot basis.

6.5.4 Temporal Distribution of Sampling

The production and loss of CWD are important components of the C cycle in forested ecosystems. Production is highly episodic and is mostly a function of tree mortality; decomposition is the dominant pathway by which CWD is lost from the system. Both production and loss processes for CWD are relatively infrequent and slow compared to production and loss of leaves and fine woody material (Harmon and Sexton 1996). As such, measurement of mortality and production will be annual, and as noted, will occur as part of measurement of woody vegetation structure (Section 6.1). Assessment of species x site-specific bulk density values will take place only once, either during initial site characterization or at the beginning of NEON operations. A one-time assessment of bulk density will be sufficient, as mean bulk density values by decay class per functional group are not expected to change appreciably through time.

Hoover (2008) recommends assessment of downed CWD volume on 5 year intervals, and Harmon and Sexton (1996) indicate that 2-5 year measurement frequencies are adequate. Given these guidelines, NEON field technicians will measure CWD volume every 3 years in both Distributed and Tower plots, or 1 site per domain per year (since there are 3 sites per NEON domain).

6.5.5 Logistics and Adaptability

CWD production

Because production of 2-10 cm diameter CWD is heterogeneous in space and time, sample size, sample area, or both may need to be increased in order to estimate CWD production to within ± 10% of the mean with 90% confidence. The number of randomly selected grid cells sampled per plot for CWD production can be increased, but increasing sample size in this manner may not be helpful if variation in CWD production occurs at spatial scales greater than that of the plot. Sample area can also be increased by combining two grid cells into a 0.5m x 6m ground plot (fine woody debris would still only be sampled in a 0.5m x 3m subsection). Once initial NEON data are collected, simulation analyses will be performed to determine the effects of increased sample size or area within plots.

CWD stocks

The Tower plots are the priority for CWD sampling. Because stocks of CWD are not dependent on season, sampling can occur at any time of the year that the plots are accessible and logs are readily identified and measured (i.e. not covered in snow), allowing for greater flexibility in scheduling sampling efforts based on technician availability.

In order to estimate CWD across the site, sampling must also occur in Distributed plots. If dictated by logistical and/or budgetary constraints, the following non-mutually-exclusive options are available to reduce sampling effort in Distributed plots: 1) measurement frequency may be reduced to once every 6 years; 2) a subset of n=20 plots may be selected, with sampled plots correponding to those used for calibration of AOP biomass estimates; and 3) CWD sampling may be eliminated entirely from Distributed plots.

6.6 Sampling Design for Coarse Roots

Definition: Following Burton and Pregitzer (2008), coarse roots are those with diameter > 10 mm.

6.6.1 Sampling Methods

Coarse root biomass estimation is typically accomplished via allometric equations that use DBH, and sometimes also height, as input variables (Burton and Pregitzer 2008). As with aboveground biomass estimation via allometry, the salient issue for coarse root biomass estimation is whether to: 1) attempt to use potentially more accurate regionally-derived allometries (e.g. Whittaker et al. 1974, Gholz et al. 1979, Grier et al. 1981, Omdal et al. 2001, Bond-Lamberty et al. 2002, King et al. 2007, Park et al. 2007, Vadeboncoeur et al. 2007), which may only be available for certain species at a site; or 2) use more general relationships between aboveground and belowground coarse root biomass derived from continental or global datasets (Cairns et al. 1997, Jenkins et al. 2003, Mokany et al. 2006).

Coarse root biomass will be estimated similarly to woody stem biomass. Namely, NEON will report coarse root biomass according to species x site-specific allometric equations, when these equations are available, as well as estimates from the continental-scale equations presented in Jenkins et al. (2003). When site x species-specific equations are not available, only coarse root biomass estimates using parameters from Jenkins et al. (2003) will be presented.

Coarse root production will be calculated as the difference in coarse root biomass between two timepoints, $AGB_{t2}–AGB_{t1}$, divided by $t_2–t_1$.

6.6.2 Spatial Distribution of Sampling

Allometric estimation of coarse root biomass will be possible using aboveground vegetation structure data collected from Distributed and Tower plots in which woody stems are present.

6.6.3 Temporal Distribution of Sampling

Because coarse root biomass and productivity estimates depend on aboveground vegetation structure parameters as inputs, the temporal distribution of sampling for this vegetation component is the same as that employed for woody vegetation (Section 6.1.3).

6.6.4 Logistics and Adaptability

Logistics and adaptability for coarse root biomass estimation are the same as those articulated in Section 6.1.4 for woody vegetation. Due to the paucity of site x species-specific allometric information, it is likely that it will not be possible to estimate μ to within ± 10% with 90% confidence. Because uncertainty in this case is not driven by the level of field sample replication, and is instead derived from the allometric equations, it will not be possible for NEON to address this problem within current logistic and budgetary constraints.

6.7 Sampling Design for Fine Root Biomass and Productivity

Definition: Following Burton and Pregitzer (2008), fine roots are those with diameter ≤ 10 mm.

There are numerous methods available for estimating fine root biomass and production, including coring, in-growth, isotope, minirhizotron, and model-based methods, as well as combinations of these approaches (Milchunas 2009). The NEON TIS will install minirhizotrons as part of the soil array near the NEON tower at a subset of sites (Figure 4), and at sites that receive minirhizotron installations, fine root turnover coefficients (TC, y^{-1}), frequency, and length will be calculated from minirhizotron images for each of the following diameter classes: < 0.5 mm, 0.5–1 mm, and 1–2 mm. Roots with 2–10 mm diameter will likely be encountered very infrequently, and roots in this size class do not contribute significantly to BNPP compared to the smaller size classes (Steinaker and Wilson 2005). Given the relationships in Equation 4 (Section 5.3.3), the NEON TOS sampling will focus on generating estimates of fine root biomass (FRB) in Tower plots, so that fine root production (FRP) can be estimated.

The most common and robust method to measure belowground standing stocks in both forest and grassland ecosystems is via relatively large diameter (5–10 cm) cores (Tierney and Fahey 2007, Burton and Pregitzer 2008). As such, NEON will use the soil coring technique to estimate belowground fine root biomass.

Root diameter classes

Fine root production is not equal across fine root diameter classes, with roots < 0.5 mm diameter accounting for several times more BNPP than roots between 0.5–2 mm diameter (Steinaker and Wilson 2005, Tierney and Fahey 2007). To account for differences in BNPP across fine root diameter classes, researchers typically sort roots within core samples into various size classes, and then calculate FRP separately for each class. Following Burton and Pregitzer (2008), NEON will sort roots within each core into < 0.5 mm, 0.5–1 mm, 1–2 mm, and 2–10 mm categories. Sampled roots > 2 mm diameter will contribute to belowground biomass estimation, but not estimation of BNPP, because minirhizotron data for roots > 2 mm diameter will likely be insufficient to estimate a TC for this size class with reasonable uncertainty.

Diameter and depth of cores

As noted above, 5–10 cm diameter cores are recommended for root biomass estimation. In a grassland ecosystem, Craine et al. (2003) used 5 cm diameter cores, and NEON has employed the same core diameter for root characterization work during site construction. NEON field technicians also sampled 66.5 mm ID (3-inch OD) x 50 cm length cores for fine root sampling as part of the 2011 Field Operations prototype at the Domain 10 CPER site. A core of 66.5 mm diameter x 50 cm length generates a sample of 2268 cm^3, and according to Taylor et al. (2013), this volume is sufficient to reliably include roots with diameter < 10 mm in each sample. However, NEON soil microbe and biogeochemistry sampling will employ 30 cm depth cores, so fine root cores will also be 30 cm depth in order to generate consistent data products across TOS sampling modules. As noted in Section 5, two cores per 400 m^2 plot or randomly selected subplot will be collected per sampling bout, and these two cores will be pooled for analysis. In order to simplify processing, cores will be extracted and treated as one sample, as opposed to sub-sampling cores by depth increment or horizon.

6.7.1 Sampling Methods

The standard operating procedure for processing soil cores is to sieve the cores to remove mineral particles from roots and soil organic matter (SOM), pick and sort the resulting root/SOM mixture to isolate roots within various size and live/dead classes, and then dry, weigh, and analyze the sorted root biomass (Burton and Pregitzer 2008). Methods for carrying out these steps are compared below, and the optimal method for NEON is identified.

Sieving techniques

During summer 2012, NEON staff scientists compared dry and wet sieving techniques with very sandy soil samples obtained from the Domain 3 Ordway-Swisher core site in North Florida. Sandy soils are arguably the easiest to sieve by either technique, and it was found that when using a 2 mm sieve, dry sieving required 6X-10X more time per sample than wet sieving. Based on these results, as well as literature recommendations (Tierney and Fahey 2007), NEON will process soil cores via the wet-sieving technique in order to separate roots/SOM from mineral particles.

Separating roots from SOM

Once soil cores are sieved to remove the mineral component of the core, it is necessary to separate roots from SOM. Both hand-picking and elutriators require significant time and equipment investments (Pierret et al. 2005, Pregitzer et al. 2008), and are not ideal with respect to NEON's labor and capital budgets. One option that saves time is for technicians to employ a length cutoff (e.g. 0.5–1 cm), and sort only those root fragments that are longer than the cutoff. However, length cutoffs can be difficult for multiple technicians to consistently implement (Koteen and Baldocchi 2013), and can lead to underestimation of fine root biomass by as much as 39% (Pregitzer et al. 2008). However, a randomization method has recently been developed that takes less time than exhaustive hand-picking and elutriation (Koteen and Baldocchi 2013). Briefly, cores are first wet sieved and manually sorted to isolate root fragments > 1 cm length. The resulting root/SOM mixture, termed the residual fraction, is then randomized by mixing with water in a beaker, and sub-samples of this mixture are then sorted into paired sub-sub-samples of root and SOM debris. The mean weight ratio of root:debris is then used to estimate the mass of root fragments < 1 cm in the dried residual fraction. Koteen & Baldocchi (2013) report that the randomization technique is accurate to within 3% of results obtained via exhaustive hand-picking, requires many hours less time per sample, and can be carried out with common, inexpensive laboratory equipment. Although the method is not widely used and has not been extensively tested, it appears the randomization technique saves considerable time compared to exhaustive picking, but is still not as rapid as employing a 1 cm length cutoff.

To satisfy the competing interests of reducing the time required to pick fine roots from SOM, while simultaneously reducing uncertainty in biomass estimates, NEON will adopt a hybrid approach for fine root biomass sampling. The majority of cores will be picked to a 1 cm length cutoff, but there will be a one time per site measurement of the proportion of total fine root biomass composed of fragments < 1 cm length using the method outlined by Koteen and Baldocchi (2013).

Biomass sorting

At the coarsest level, crowns, roots, corms, rhizomes, and bulbs must be separated from root material, as these tissues perform very different functions and have very different growth rates from roots (e.g. Milchunas and Lauenroth 1992). In addition, any root fragments with radial diameter > 10 mm will be discarded, due to the fact that these roots are "coarse" roots and their biomass will be estimated allometrically from stem DBH measurements. The remaining fine roots < 10 mm diameter will be sorted into the size classes described above.

In addition to sorting roots by size, NEON technicians will also attempt to distinguish between live and dead roots. Root color and structural integrity will be used to discriminate between live and dead roots, with dead roots being defined as those that are very dark or black, and/or brittle (Steinaker and Wilson 2005, Burton and Pregitzer 2008). It will not be possible to quantify the uncertainty associated with live versus dead sorting accuracy, as the required tissue staining techniques are beyond the scope of current staff time budgets, equipment budgets, and training capabilities.

Drying, processing, and analyses

Following sieving and sorting, roots will be dried at 65 ˚C for a minimum of 48 h or until constant weight. Dried roots will be ground with a Wiley Mill, and ground sub-samples will be analyzed for %C, %N, δ^{13}C, δ^{15}N, concentrations of elemental "majors", and ash content (AD[04]). All chemical analyses will be contracted with external facilities.

6.7.2 Spatial Distribution of Sampling

Soil cores for estimating fine root biomass will only be collected from Tower plots, and will be collocated with herbaceous clip-harvest "cells" in any given year. Within clip-harvest cells, cores will be extracted from the same physical space as the 0.1 m x 2 m clip-harvest if conditions allow (i.e. roots and rocks do not prevent sampling to the desired depth), or from the adjacent buffer area in the cell if obstacles are encountered. A similar approach is used at the Cedar Creek LTER site (Tilman 1982), where three cores are taken from the clip strips after aboveground biomass has been removed. At least n=2 cores per 400 m^2 Tower plot/subplot will be sampled per year. Given the potential range of Tower plot numbers and sizes at a given site, the estimated number of total cores per NEON tower footprint is therefore n=60-160. For grazed systems in which exclosures are utilized, cores will only be sampled from grazed locations.

6.7.3 Temporal Distribution of Sampling

Belowground fine root biomass will be measured every year at NEON sites. The exact date of belowground biomass sampling at a site in a given year will be guided by two considerations: 1) the date of aboveground clip harvest(s), and 2) seasonal variations in soil hardness. Ideally, belowground biomass sampling would either be contemporaneous with aboveground clip harvests for sites with significant herbaceous cover, or with maximum canopy biomass/leaf area for tree or shrub dominated systems. For example, researchers at the Cedar Creek LTER have sampled belowground cores from clip strips within 3 days of the aboveground harvest (e.g. Craine et al. 2003). Within the NEON framework, such a sampling approach would enable within-site understanding of temporal links (or lack thereof) between above- and belowground biomass and production. However, at some sites aboveground mid-summer peak biomass typically coincides with very low soil moisture, which makes belowground sampling very difficult when soils are rich in clay (e.g. the NEON Domain 10 CPER/SGS-LTER site, D. Milchunas personal communication). Moreover, at the Domain 10 CPER site aboveground biomass is not temporally linked with belowground biomass (Milchunas and Lauenroth 2001), so belowground sampling at sites like CPER is best targeted to periods of maximal soil moisture in the late spring/early summer when sampling is most feasible.

The timing of NEON belowground sampling will therefore be linked to the timing of aboveground peak biomass clip harvests or peak canopy biomass/LAI when possible, but will otherwise be timed to coincide with periods when soil moisture is at levels that facilitate sampling.

6.7.4 Logistics and Adaptability

Fine root biomass and production estimates are notoriously uncertain due to the spatially heterogeneous distribution of roots in the soil, and the massive time investment required to process the large number of core samples needed to minimize parameter uncertainty. The major challenges for fine root biomass sampling are therefore: 1) minimizing the time spent sieving, picking, and sorting samples; and 2) maximizing the number of samples processed (Berhongaray et al. 2013). Per site uncertainty associated with fine root biomass estimates is unknown *a priori*, and as such, it will be an iterative process to determine whether it is logistically feasible to adjust sample size such that fine root biomass can be estimated to within ± 10% of the mean with 90% confidence in a given sampling year.

Although it is NEON's goal to generate annual estimates of belowground fine root biomass on a per site basis, this sampling effort is particularly labor intensive compared to most other plant biomass and productivity parameters, as well as other TOS sampling modules (i.e. birds, mosquitoes, etc.). If it is not possible to sample 3 sites per domain per year with available field technician labor, fine root biomass sampling may be reduced to one site per year per domain (i.e. a 3 y sampling interval per site). This reduction in temporal sampling frequency will make detecting changes in fine root biomass through time more difficult, but will still allow a time series with a minimum of 4 points to be constructed for each relocatable site.

6.8 Sampling Design for Leaf Area Index

Definition: Leaf area index (LAI) is equal to the total one-sided leaf area per unit ground area. LAI is a useful proxy variable for numerous other variables of ecological interest, including plant biomass, plant productivity, forage quality, carbon balance, ecosystem energy flux, plant density, and the heterogeneity of plant cover.

As described in the sampling framework section, NEON will employ a two pronged approach for measuring LAI: 1) temporally intensive measurements at a small number of plots; and 2) spatially-extensive measurements at a large number of plots every 3 years (one site per domain per year).

Methodological considerations

One-sided leaf area index can be estimated using either "direct" or "indirect" techniques. Direct techniques rely on generating LAI estimates from labor-intensive destructive harvests, and then using allometric equations to calculate plot-level LAI values (Gower and Norman 1991, Chen et al. 1997). Indirect estimation of LAI depends on measuring canopy gap-fractions with an optical instrument, and then calculating LAI from the observed gap fraction, often with a correction factor to account for element clumping at needle, shoot, and canopy scales (Chen et al. 1997, Jonckheere et al. 2004, Weiss et al. 2004, Ryu et al. 2010).

Both direct and indirect LAI estimation methods present problems that require careful consideration. Although direct techniques are likely more accurate than indirect techniques, they require destructive harvests that are laborious, and require permits to destructively harvest a relatively large number of trees (e.g. n=10 per dominant species)(Gower and Norman 1991). At the continental scale of NEON, it is clear that destructive harvest permits will be impossible to obtain at sites situated within National Parks, as well as other sites, which makes consistent implementation of direct LAI estimation methods problematic. Indirect measurement of LAI is more rapid than direct estimation, and removes permitting obstacles that are significant at the scale of NEON. However, indirect techniques may underestimate true LAI values (Fassnacht et al. 1994), with underestimation becoming more severe at LAI values > 4 (Brantley and Young 2007). This means that indirect techniques will be particularly problematic in the tall, structurally complex forests of the Pacific Northwest (i.e. the Domain 16 Wind River site).

Plot layout

Various spatial configurations have been employed to estimate LAI, including uniform, random, and cyclic designs (Burrows et al. 2002). Burrows et al. (2002) indicate that the cyclic sampling design is preferable to the random design with respect to ease of point discovery and reducing travel time between points, and the cyclic design also maximizes the variance of information from plot to plot. For NEON's spatially-extensive LAI measurements, technicians will record LAI at points associated with the NEON Distributed plots, placed across the landscape according to a stratified random, spatially-balanced design (Theobald et al. 2007). Although this approach does not minimize travel time between plots, it will maximize co-location among data products at the plot scale, and will also generate an unbiased estimate of LAI at the site scale.

For temporally intensive LAI data collection, measurements will be made at three Tower plots that are closest to the NEON tower. Keeping the plots in as close proximity to each other as possible is important because it will ensure that all three plots can be measured in the approximately 1 hour of time during which crepuscular light occurs at the end of the day.

Measurement points within plots

In a field evaluation of eleven sampling schemes, in which different configurations of points were distributed within a 36m x 36m elemental sampling unit (ESU), it was empirically determined that cross-shaped and square-shaped arrangements of points produced LAI estimates with the lowest coefficients of variation (Majasalmi et al. 2012). The European VALERI project has also evaluated different point arrangements within plots within the context of validating satellite-derived LAI products (Baret et al. 2005). These authors found that squares, crosses, and various combinations of these shapes all described the variance within a 20m x 20m satellite data pixel equally well. Based on this result, the configuration of points (square versus cross) should be chosen with ease of accurate positioning and data collection in the field in mind (Garrigues et al. 2002). It should be noted, however, that the field of view of hemispherical, upward-facing optical instruments grows wider as a function of vegetation height, so as vegetation height increases, the degree of field-of-view overlap at the center of a cross or the corners of a square increases, and spatial-autocorrelation of the data increases. To mitigate these issues, the Canadian Centre for Remote Sensing (CCRS) LAI sampling guidelines stipulate that when average plot vegetation height is > 15 m, the LAI ESU should be increased to 40m x 40m (R. Fernandes, personal communication).

Comparison of LAI measurement systems

There are numerous specialized, commercial solutions available for the indirect measurement of LAI, and it is also commonplace to use digital cameras equipped with 180˚ hemispherical fisheye lenses (i.e. the DHP method). There are advantages and disadvantages to each of these equipment options and their associated methods, so NEON scientists evaluated the following systems against Observatory requirements: Decagon LP-80 AccuPAR, LAI-2200TC, Delta-T SunScan SS1, CID BioScience CI-110, and a Nikon D700 DHP system. The most important criteria when comparing these instruments were the ability to: 1) collect data in a consistent, repeatable manner from a variety of ecosystems with minimal changes to the required equipment (from short-stature grasslands to large-stature forests); 2) remotely conduct meaningful QA/QC analyses on Level 0 data products generated by the equipment; 3) train technicians to analyze the Level 0 data products produced by the instruments in an accurate and precise manner; and 4) create "best value" in terms of price and data quality.

6.8.1 Sampling Methods

NEON has elected to use an optically-based indirect DHP method to measure LAI, due to the fact that it will be possible to collect comparable data with this approach at all NEON sites. DHP systems can be used to collect LAI data in short-stature grasslands via downward-facing photos, and can efficiently capture both understory and canopy LAI in forests via upward and downward-facing photos. In herbaceous communities, use of wand-based systems can be difficult due to the fact that placing the wands on the ground can disturb the vegetation and alter the recorded LAI (He et al. 2007); cameras oriented to take downward-facing photos of herbaceous plants obviate this problem. Moreover, acquiring LAI data in forests with a DHP system does not require the measurement of incoming radiation above the canopy, as is required with some wands (e.g. the LiCOR LAI-2200). Another benefit of the DHP system compared to wand systems is that the field of view “seen” by the sensor is permanently recorded in a format that enables straightforward QA/QC prior to analysis – that is, images can be checked for focus, water droplets, lens fog, light conditions, etc, whereas the data logs produced by some wand systems cannot be easily checked for these sorts of issues remotely after data collection.

The use of a DHP system does present a few significant data collection problems within the context of NEON that must be mitigated. Compared to wand systems, DHP systems must be focused, and images must be properly exposed for accurate LAI analyses. These issues will be addressed via generation of an explicit standard operating procedure for use with selected cameras and software, as well as annual training refreshers.

Plot layout

NEON will collect DHP images from a cross-shaped arrangement of 12 sampling points super-imposed over the standard NEON 20m x 20m Distributed plot in order to measure LAI (Figure 5). When average plot vegetation height is > 15 m, the distance between points on the cross will be increased from 4 m to 8 m. At sites with very tall vegetation (e.g. D16, Wind River, canopy dominants are 60-70 meters tall) arrangement of sample points may need to be separated by more than 8 m; this will be assessed following the first season of data collection and may be adjusted in subsequent years. Revised sampling schemes may be informed by LiDAR data from AOP remote sensing flights.

Image analysis

There are few software packages available for analysis of LAI via DHP images, and based on the recommendation of CCRS researchers (R. Fernandes personal communication), NEON will adopt the CanEye software package developed by M. Weiss and colleagues at INRA (French National Agricultural Research Institute). Although CanEye has numerous desirable features – e.g. it is freely available, it is based on potentially customizable MatLab code, etc. – it is not widely used in a server-based environment such as that anticipated for NEON’s cyberinfrastructure. In addition, it is unclear whether clumping indices generated by the software might need to be evaluated on a site-by-site basis (Demarez et al. 2008).

Other ongoing software challenges that are not unique to CanEye include difficulties associated with standardizing analyses across different technicians with respect to pixel classification (plant vs. soil and plant vs. sky), and that technicians will require between 20–30 min/plot for image analysis (NEON unpublished observations).

Despite the shortcomings inherent to analysis of DHP images at the current time, DHP systems are a good choice for LAI analysis over the coming decade. In particular, images are readily archived and the NEON user-community can re-analyze images as needed should improved, more consistent software analysis options be developed in the future.

6.8.2 Spatial Distribution of Sampling

LAI will be measured in n=20 randomly selected Distributed plots, and n=3 Tower plots per site. Tower plots will be non-randomly chosen by NEON Field Operations to facilitate logistics of routine sampling. Up to n=5 non-randomly located Gradient Plots will be sampled if LAI values from Distributed plots fail to span the full dynamic range of LAI. The exact location of Gradient plots will be determined using AOP remote-sensing-derived maps of LAI.

6.8.3 Temporal Distribution of Sampling

The sampling start date for temporally-intensive LAI measurements at a given site will be based on the current-year phenology at that site. Phenology data will be generated both via the MODIS-EVI phenology product, as well as on-the-ground technician observations (AD[05]). To ensure that early-season LAI dynamics are adequately captured, data collection will begin in Tower plots when buds open and leaves/candles first become visible in the dominant plant species. Monitoring of LAI will continue every other week until the end of the growing season. A window for the end of the growing season is defined on a site-specific basis using long-term MODIS-EVI phenology data, and field technicians determine actual sampling stop dates in a given year when phenophases return to the off-season baseline.

LAI data from Distributed plots will enable ground-validation of AOP LAI algorithms; for the spatially extensive ground sampling effort, therefore, it is essential to collect LAI measurements close to the time that AOP remote-sensing data are collected at a given site. As part of the AOP/TOS prototype in 2010 at the Domain 3 Ordway-Swisher core site, LAI was measured every 10 m along 500 m length transects (n=8) at two different time points that were 2 weeks apart. Transects were all located within the dominant Sandhill vegetation type in which the NEON tower is also located. At this site, LAI did not differ significantly between the two timepoints (Figure 12) (Kampe et al. 2011). Based on these data, NEON will collect spatially-extensive ground LAI data at each site within a 1 month window that includes the actual AOP flight date. Validation datasets of this nature will be collected every 5 years per site.

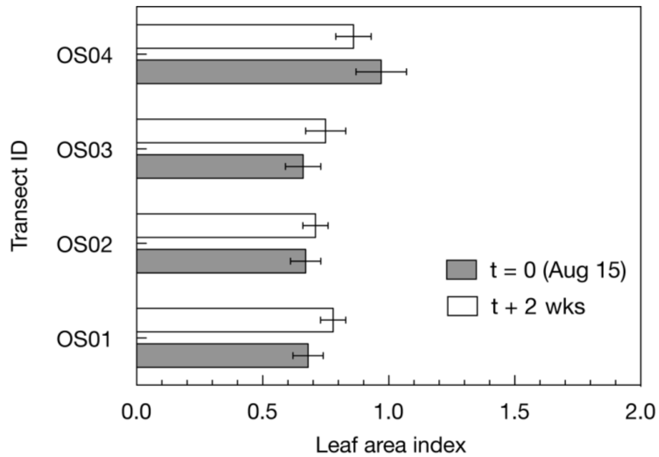

Figure 12. Leaf area index data collected with an LAI-2200 at the Domain 3 Ordway-Swisher core site, from the same four 500 m length transects at two different time points.

6.8.4 Optimization of LAI Sampling and Workflow

Following the collection of vegetation structure and LiDAR data at each site, NEON will evaluate the effectiveness of the proposed design in terms of the spacing and arrangement of photo points within a plot, the frequency of temporally intensive measurements and number of Distributed plots that are necessary to accurately calibrate AOP measurements. These aspects of the design may be adapted to optimize sampling of LAI on a site specific basis.

With respect to emerging technology, there are two aspects of the LAI sampling design that are clearly adaptable. First, NEON will continue to compare the methods and equipment discussed here against the costs and benefits of ground-based LiDAR. Ongoing collaborations with researchers at the Rochester Institute of Technology, Boston University, and University of Massachusetts (J. van Aardt, C. Schaaf, and others) suggest that LAI can be efficiently measured with dual wavelength ground-based LiDAR within the next 5-10 years, while simultaneously delivering a wealth of additional information about vegetation structure.

Second, the development of automated pixel classification algorithms that speed CanEye analyses is an area of active research (Duveiller and Defourny 2010). Duveiller and Defourny (2010) have shown that object-based image classification procedures can be used to accurately and efficiently separate leaves from soil in DHP images acquired over an agricultural maize canopy. However, at present there is not enough evidence that algorithms designed and tested within the context of relatively simple agricultural systems are sufficient to deal with the range of images that will be encountered across NEON. If a program with the demonstrated capacity to reliably distinguish leaves and needles from soil and sky becomes available, and NEON can implement algorithms to automate the process, technician analysis time per plot would be substantially reduced, and potential differences between technicians with respect to pixel classification would be eliminated.

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APPENDIX A R SAMPLNG SIMULATION CODE

```
###   Goal: Super-impose a grid of sub-plots of the specified size over a spatially-
explicit stem map. Randomly sample from this grid of subplots to calculate
aboveground biomass with a given subplot size and number.


##    Create function for sampling simulation. Define the following:
#     (1) plot.size = length of one side of the subsampling plot in meters;
#     (2) plot.num = number of subplots desired;
#     (3) iter.num = number of sampling iterations used to develop distribution of
#     AGB means associated with a given plot size and plot number; and
#     (4) input stem map data used for the sampling simulation
#     (5)   file="Jenkins_parameters.csv" must be in the current working directory

###   Define function for sample simulation

sampleSim = function(plotSize.m, plotNum, iterNum){

#     Calculate plot area in hectares
plotArea.ha = (plotSize.m^2)/10000

#     Calculate total sampled area in hectares
sampledArea.ha = plotArea.ha*plotNum



###   Read in user-supplied stem map data; it is necessary to prepare the stem map
with code in "Data_preparation.R" prior to sample simulation.
stemmap.df = read.csv(file.choose(), header=T)



###   Load table of Jenkins parameters to use for biomass estimation of individual
stems. Parameters come from Jenkins etal. 2003 Forest Science.
jpar.df = read.csv("Jenkins_parameters.csv", header=T)



###   Calculate biomass (kg) of each stem in stemmap.df with a "for" loop using the
appropriate Jenkins parameters
stemmap.df$agb.kg = NA
for (i in 1:nrow(stemmap.df)){

#     Retrieve correct Jenkins parameters for the stem based on "Jenkins_type" code
temp.b0 = jpar.df$b0[jpar.df$groupID==as.character(stemmap.df$Jenkins_type[i])]
temp.b1 = jpar.df$b1[jpar.df$groupID==as.character(stemmap.df$Jenkins_type[i])]

#     Using Jenkins biomass equation, calculate the biomass in kg for stem "i" using
stem "i" DBH value in "stemmap.df"
stemmap.df$agb.kg[i]  =  round(exp(temp.b0  +  temp.b1*log(stemmap.df$dbh.cm[i])),
digits=1)
```

```
#	Bracket for end of stem-mass "for" loop
}



###	Define the size of the spatially explicit stem map dataset in meters. Note the sizes calculated are not necessarily the area of the plot, but the area defined by the outer-most stems mapped within the plot. Calculated distances are rounded up to the nearest meter.

#	Length of E/W stem map boundary (m); E/W direction is defined as the "X" direction.
xDist.m = ceiling(max(stemmap.df$xdist) - min(stemmap.df$xdist))

#	Length of N/S stem map boundary (m); N/S direction is defined as the "Y" direction.
yDist.m = ceiling(max(stemmap.df$ydist) - min(stemmap.df$ydist))



###	Determine the number of columns and rows for the grid of subplots in the xDist.m and yDist.m directions based on the number of whole subplots that will fit within the stem map area, then calculate the total number of grid cells to create.

#	The 'trunc' function creates an integer by truncating the value of the argument toward zero.
nCol = trunc(xDist.m/plotSize.m)
nRow = trunc(yDist.m/plotSize.m)
nTotal = nCol*nRow


###	Create a three-column matrix to hold grid cell ID, and xDist.m and yDist.m associated with the SW corner of each grid cell:
#	Column 1 =	grid cell ID
#	Column 2 =	x-axis coordinate; corresponds to x-location of grid cell SW corner;
#			currently, code assumes cell locations are relative to SW corner of
#			stem map (SW corner = 0,0 position)
#	Column 3 =	y-axis coordinate; corresponds to y-location of grid cell SW corner

#	Create the matrix
grid.mat = matrix(data=NA, nrow=nTotal, ncol=3)
colnames(grid.mat) = c("cellID","Xcoord","Ycoord")

#	Create grid cell IDs and add to the matrix
gridID = seq(from=1, to=nTotal, by=1)
grid.mat[,1] = gridID

#	Create x-axis and y-axis coordinates for each grid cell and add to the matrix
```

```
tempX = seq(from=0, to=((nCol-1)*plotSize.m), by=plotSize.m)
grid.mat[,2] = rep(tempX, times=nRow)
tempY = seq(from=0, to=((nRow-1)*plotSize.m), by=plotSize.m)
grid.mat[,3] = rep(tempY, each=nCol)



### Calculate the "true" total biomass for the area in which the sampling simulation will occur (Mg ha-1), and the ± 10% biomass values. Filter stemmap.df so that "true" biomass is calculated based on the size of the sampling grid. Want the area being used for the sampling simulation to match the area being used to calculate "truth".

# Calculate the area of the sampling grid (ha)
gridArea.ha = nTotal*plotArea.ha


## Filter stemmap.df to select only those stems in the sampling grid, and calculate the total AGB of those stems

# Filter stemmap.df first in x-distance, then in y-distance
gridStem = stemmap.df[stemmap.df$xdist <= nCol*plotSize.m,]
gridStem = gridStem[gridStem$ydist <= nRow*plotSize.m,]

# Sum biomass values (kg) for all stems in gridStem, convert to Mg ha-1, and calculate ± 10% values
trueAGB.mgha = round((sum(gridStem$agb.kg)/1000)/gridArea.ha, digits=1)
trueAGB.mgha = append(trueAGB.mgha, c(0.9*trueAGB.mgha, 1.1*trueAGB.mgha))
names(trueAGB.mgha) = c("trueAGB","-10%","+10%")



### Use an if/else statement to determine whether plotNum > nTotal; if plotNum < nTotal, employ sample-iteration "for" loop to sample from "gridID" n=iterNum times

if (plotNum >= nTotal) {
print(paste("Total number of grid cells at the specified plotSize.m is",nTotal,"; please enter a value for plotNum <",nTotal), quote=FALSE)

} else {

cat(paste("The total number of grid cells at the specified plotSize.m =",nTotal,"\nThe number of grid cells subsampled at each iteration =",plotNum,"\nThe total sampled area across all plots at the specified plot size and plot number =",sampledArea.ha, "ha\n"))


## Plot "stemmap.df" and plot grid points over the top of the stem map.
plot(stemmap.df$xdist, stemmap.df$ydist, type="n", xlab="Relative easting (m)", ylab="Relative northing (m)", main="Stem map with grid cells (grey lines), and subsample boundary (blue lines);\nsymbol size ~ DBH", cex.main=0.9)
```

```
#     Add points with symbols sized according to DBH
symbols(stemmap.df$xdist, stemmap.df$ydist, circles=stemmap.df$dbh.cm, inches=0.1, add=TRUE)

#     Superimpose sampling grid over stem map; blue lines indicate boundary of superimposed sampling grid; symbol size indicates relative DBH of stems.
abline(v=grid.mat[,2], col=8)
abline(h=grid.mat[,3], col=8)
v1X = c(max(grid.mat[,2])+plotSize.m, max(grid.mat[,2])+plotSize.m)
v1Y = c(0, max(grid.mat[,3])+plotSize.m)
lines(v1X, v1Y, col=4)
v2X = c(0,0)
v2Y = c(0, max(grid.mat[,3])+plotSize.m)
lines(v2X, v2Y, col=4)
h1X = c(0, max(grid.mat[,2])+plotSize.m)
h1Y = c(max(grid.mat[,3])+plotSize.m, max(grid.mat[,3])+plotSize.m)
lines(h1X, h1Y, col=4)
h2X = c(0, max(grid.mat[,2])+plotSize.m)
h2Y = c(0,0)
lines(h2X, h2Y, col=4)


##    Create "agbIter" matrix. First column will hold mean AGB value in Mg ha-1 for each iteration of the sampling loop with a user defined plotSize.m, plotNumber, and iterNum; second column holds 0/1 flag value indicating whether mean AGB for a given iteration is within ± 10% of the true AGB.
agbIter = matrix(data=NA, nrow=iterNum, ncol=2)
colnames(agbIter) = c("AGB","Flag")



###   Sample-iteration "for" loop used to obtain distribution of biomass means according to user-specified plotSize.m and plotNum; loop iterates according to user-specified iterNum value.

for (i in 1:iterNum){

#     Create a random sample of n=plotNum subplots from the list of available grid cells, and sort according to increasing gridID number
plotRandom = sort(sample(gridID, size=plotNum, replace=F))


##    Use coordinates in grid.mat associated with randomly sampled grid cells (subplots) to filter stemmap.df dataset and select only those stems that fall within each grid cell. Calculate AGB (Mg ha-1) for stems that fall within the grid cell.

#     Create temporary vector used to hold the total AGB for each cell in plotRandom
plotRandom.agb = NA


###   Use a "for" loop to step through each element of the plotRandom vector, and calculate AGB for each grid cell in plotRandom.
```

```
for (k in 1:length(plotRandom)){

#	Isolate X and Y coordinates associated with random grid cell "k"
tempCell = grid.mat[plotRandom[k],]

#	Define "xdist" and "ydist" range within stemmap.df for grid cell "k" based on plotSize.m; filter first by "xdist" then by "ydist" to obtain a temporary matrix 
	containing only those stems that fall within the coordinates associated with grid cell "k".
tempStem = stemmap.df[which(stemmap.df$xdist >= tempCell[2] & stemmap.df$xdist < tempCell[2]+plotSize.m),]
tempStem = tempStem[which(tempStem$ydist >= tempCell[3] & tempStem$ydist < tempCell[3]+plotSize.m),]


##	Calculate biomass of all stems in tempStem, and store the total AGB for the grid cell in plotRandom.agb; use "if/else" for the case of no stems occurring within random grid cell "k"

if (nrow(tempStem) == 0){
#	AGB value for random cell "k" is zero if there are no stems in "tempStem"
plotRandom.agb[k] = 0

} else {

#	Calculate the biomass of all stems in tempStem (Mg ha-1)
plotRandom.agb[k] = (sum(tempStem$agb.kg)/1000)/plotArea.ha

#	Bracket for end of if/else statement
}

#	Bracket for end of plotRandom AGB "for" loop
}



###	Calculate mean AGB in Mg ha-1 for sampling iteration "i", and store in agbIter matrix
agbIter[i,1] = round(mean(plotRandom.agb), digits=1)

#	If/else statement to assign Flag value based on whether mean(plotRandom.agb) is within ± 10% of trueAGB.
if (mean(plotRandom.agb) >= trueAGB.mgha[2] && mean(plotRandom.agb) <= trueAGB.mgha[3]){

#	Assign "Flag" column to 1
agbIter[i,2] = 1

} else {

#	Assign "Flag" column to 0
agbIter[i,2] = 0
```

```
#       Bracket for end of "Flag" if/else statement
}

#       Bracket for end of sample-iteration "for" loop
}

#       Bracket for end of sample-iteration "else" statement
}



###     Summary output

##      Plot distribution of subsampled means, plot trueAGB.mgha ± 10%, calculate % of
iterations with mean within ± 10% of trueAGB.mgha

#       Plot distribution of subsampled means and trueAGB.mgha ± 10%
quartz()
hist(agbIter[,1], main="Distribution of AGB subsample means", xlab="AGB (Mg ha-1)")
abline(v=trueAGB.mgha[1], col=2, lwd=2)
abline(v=trueAGB.mgha[2], col=4, lty=2, lwd=2)
abline(v=trueAGB.mgha[3], col=4, lty=2, lwd=2)

#       Calculate and report %iterations with mean within ± 10% of trueAGB.mgha
inRange = round((sum(agbIter[,2])/iterNum)*100, digits=1)
cat(paste("The % of iterations with mean subsampled AGB within ± 10% of the true AGB
is",inRange,"%"))


##      Return list of function-generated results of interest to user
results = list(sampledAGB = agbIter, trueAGB = trueAGB.mgha, confidence = inRange,
sampledArea = sampledArea.ha)
return(results)


#       Bracket for end of function
}
```

Table 4. Parameters for functional groups used to allometrically estimate biomass of individual woody stems within the sampling simulation code above (from Jenkins et al. 2003). In the code, table values are stored in the "jpar.df" object.

groupID	species_group	b0	b1
aa	aspen_alder_cwood_willow	-2.2094	2.3867
mb	softmaple_birch	-1.9123	2.3651
mh	mixed_hardwood	-2.48	2.4835
mo	hardmaple_oak_hickory_beech	-2.0127	2.4342
cl	cedar_larch	-2.0336	2.2592
pm	douglas_fir	-2.2304	2.4435
tf	truefir_hemlock	-2.5384	2.4814
pi	pine_species	-2.5356	2.4349
sp	spruce_species	-2.0773	2.3323
wo	juniper_oak_mesquite	-0.7152	1.7029