TOS SCIENCE DESIGN FOR PLANT BIOMASS AND PRODUCTIVITY

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	11/05/2014	ECO-02362	Initial release
B	10/17/2018	ECO-05809	• Minor format and text edits throughout document; updated figures and text to reflect current site numbers. • Section 5: Updated Tower Plot section to reflect actual plot sizes established as well as random subplot selection strategy. • Section 5: Strategies for optimization of Tower Plot number. • Section 6.3: Removed net-clip bryophyte productivity sampling for mat-forming bryophytes; biomass stocks for these plants now estimated as part of Herbaceous Clip sampling. • Section 6.1: Added survey method for determining implementation of VST and LTR protocols. • Section 6.5: Added data-driven approaches to select CDW transect length and F-value, and to determine when CDW bulk density sampling is complete. • Section 6: Added specific optimization strategies for HBP, LTR, VST sampling. • Section 6.1: Added time-phased development of dendrometer band sampling for woody increment growth to VST section. • Section 6.9: Added scheduling information for suite of plant productivity protocols and integration with other TOS protocols. • Appendix B: Added table listing plant biomass and productivity sampling tables by site.

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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. 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 and productivity in the NEON Science Design.

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, Alan Knapp, Christopher Gough, Dafeng Hui, Kenneth Anyomi, 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. We thank Tanya Chesney for her thorough copy-editing and formatting of the document.

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
AD[06]	NEON.DOC.000912	TOS Science Design for Plant Diversity
AD[07]	NEON.DOC.000908	TOS Science Design for Terrestrial Microbial Diversity

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.002652	NEON Level 1, Level 2, and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001484	Areas of mutual representativeness and exclusion around terrestrial infrastructure measurements
RD[05]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[06]	NEON.DOC.001709	TOS Protocol and Procedure: Bryophyte Productivity

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.

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 (renamed to ForestGEO)
CDW	Coarse downed wood
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

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform for understanding and forecasting the impacts of climate change, land use change, and invasive species on ecological systems. NEON is designed to enable users, including scientists, planners and policy makers, educators, and the general public, to address the major areas in environmental sciences, known as the Grand Challenges (Figure 1). NEON infrastructure and data products are strategically aimed at those aspects of the Grand Challenges for which a coordinated national program of standardized observations and experiments is particularly effective. The open access approach to the Observatory's data and information products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth and understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01] and AD[02].

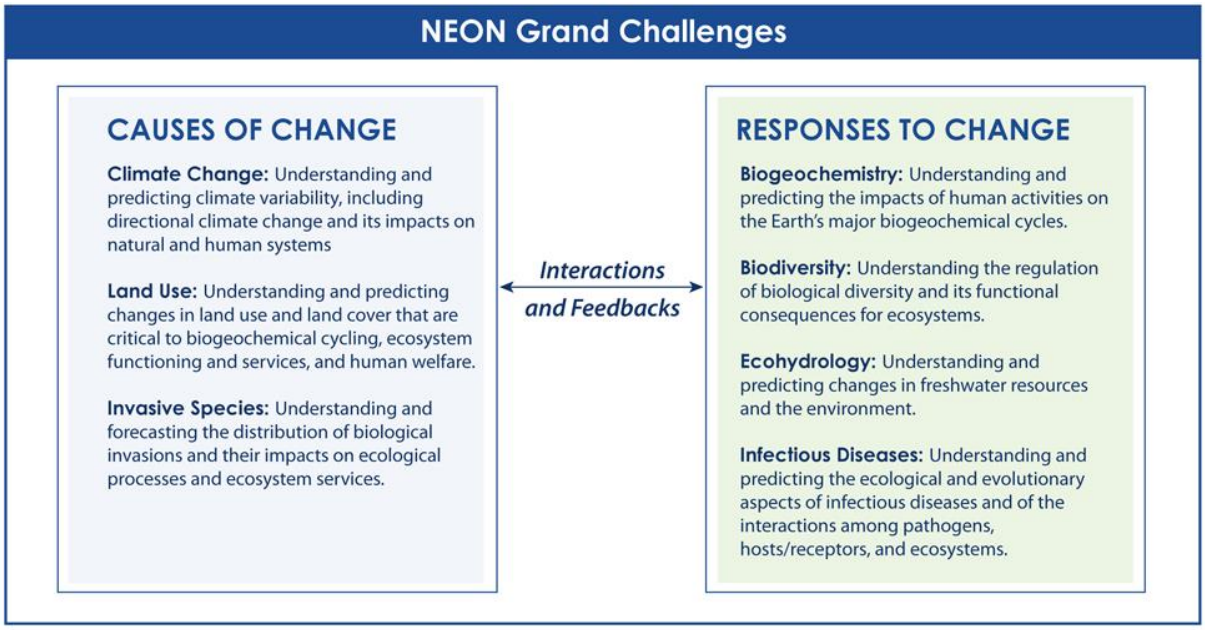

Figure 1. The seven ecological Grand Challenges defined by the National Research Council (National Research Council. 2001).

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 Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by

instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. (2008) 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 sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Plot and organism-scale measurements will also be coordinated with the larger-scale airborne measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.neonscience.org).

The standardization of protocols across all sites is key to the success of NEON 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.neonscience.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 discipline-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 AND PRODUCTIVITY 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 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). 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 biomass and NPP can provide a relatively accurate and detailed picture of how vegetative C stocks and fluxes respond to various change drivers through time.

Field-based estimates of biomass and NPP are extremely useful for mapping the spatial distribution of C at regional and global scales (e.g. Saatchi et al. 2011). However, historically these data have been 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).

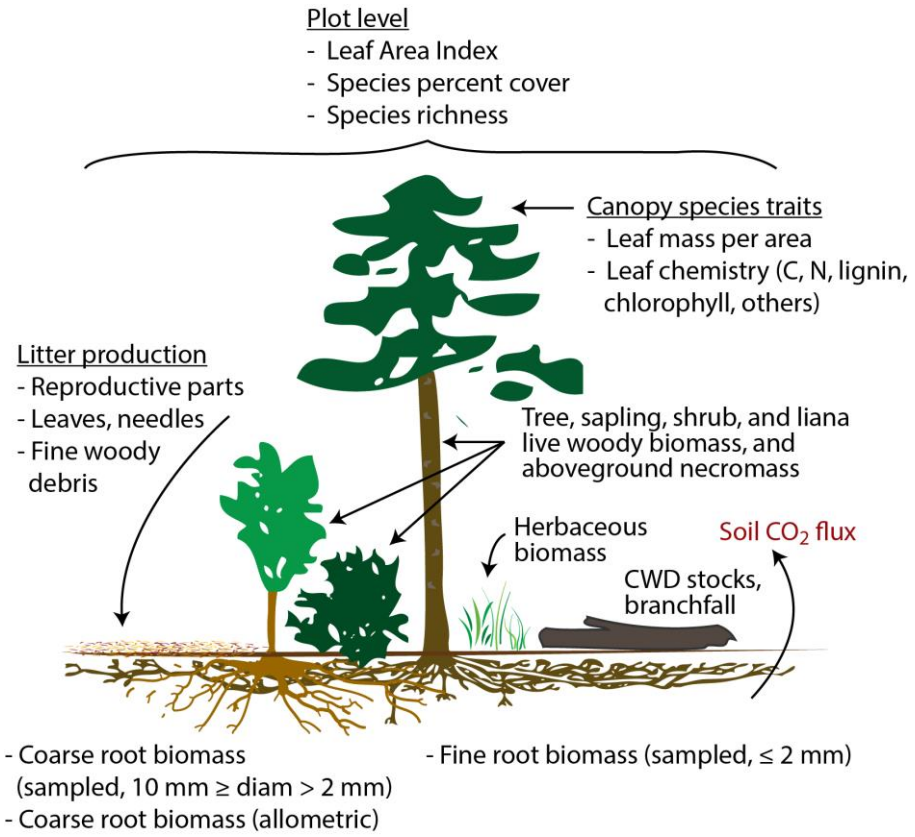

Figure 2. Plant biomass stocks, fluxes, species traits, and plot-level vegetation attributes that inform our understanding of terrestrial carbon cycling. Black text indicates TOS measurements, and red text indicates TIS measurements.

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 Leaf Area Index (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 and see Kao et al. 2012). To accomplish these goals, NEON has partitioned the continental United States plus Alaska, Hawaii, and Puerto Rico into twenty different eco-climatic domains. Within each domain, sampling will occur within wildland sites, selected to be broadly representative of the entire domain, and additional 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.

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 employs small aircraft outfitted with a hyperspectral imaging spectrometer, a waveform LiDAR, and a high-resolution camera to fly annually over most 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. Combined with plot-based ground data, the AOP is critical to enabling end-users to quantify biomass, productivity, and LAI at the site scale.
- **Terrestrial Instrument System.** A flux tower is constructed within the dominant vegetation at each site. Eddy-covariance sensors can be used to 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, and soil respiration.
- **Terrestrial Observation System.** The TOS is a coordinated suite of plot and grid-based field sampling activities (**Figure 3**; 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 (AD[03], 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. If funding is available and establishing Gradient Plots is warranted by the data, these plots will be placed non-randomly in order to capture rare vegetation types not sampled by standard Distributed Plots. These plots will only be employed when Distributed Plots fail to span the full dynamic range of the vegetation height, LAI, and/or canopy chemistry.

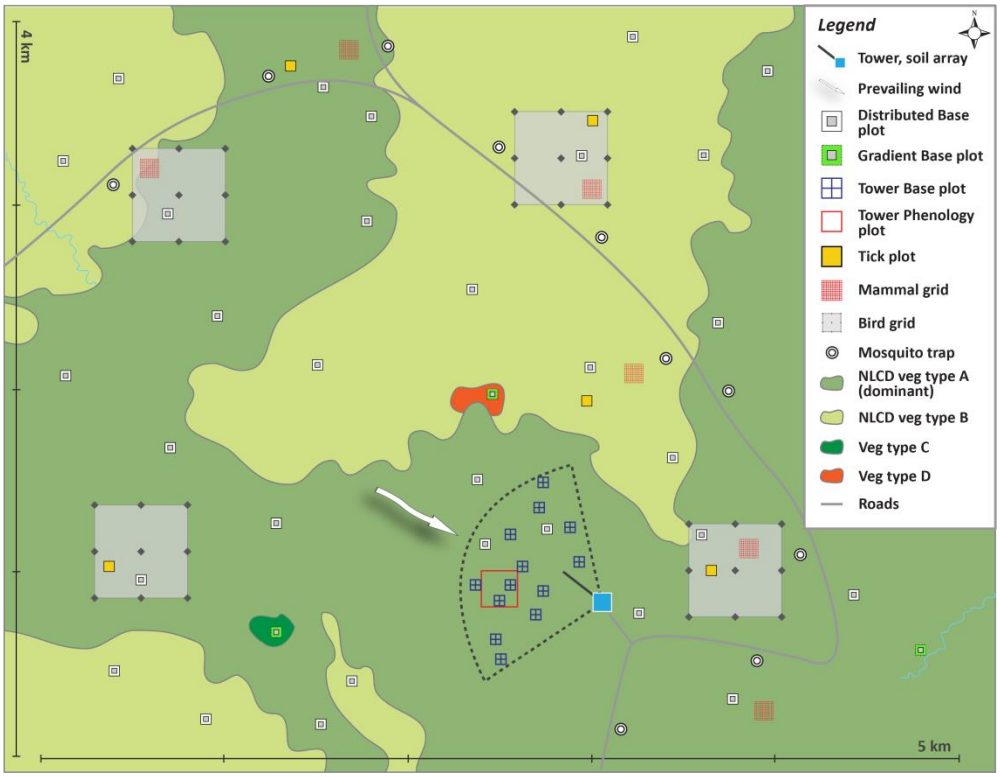

Figure 3. Schematic of terrestrial sampling at a NEON site. Plant biomass, productivity, and LAI sampling occurs in Distributed and Tower Plots.

Within each NEON site, the TOS Sampling Design for Plant Biomass and Productivity 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 3). Data from these plots will support calibration and validation of AOP remote-sensing instruments, and field and remote-sensing data can then be integrated by NEON end-users 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 3). Aboveground plot-based measurements 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). Tower Plots also support sampling for fine root biomass stocks because belowground plant biomass and production are significant and poorly constrained components of global C cycle models (but see Jackson et al. 1997). NEON estimates of fine root standing stock (kg ha^{-1}), coupled with fine root turnover estimates (y^{-1}) from external PI-driven research, will allow calculation of belowground NPP within the dominant vegetation at each terrestrial NEON site. The coordinated, methodologically consistent, and long-term measurement of belowground fine root biomass 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 47 sites over 30 years represents a significant step forward in terms of predicting effects of global change drivers on plant biomass stocks, fluxes, and C-cycle dynamics.

Table 1. Spatial and temporal sampling strategy for plant biomass and productivity 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	5 y	Belowground productivity can be estimated using external root turnover data.
Coarse downed wood (tally, bulk density*)	Distributed	20	1X per year	5 y	Tally sampling only
	Tower	20-30†	1X per year	5 y	Tally sampling every 5 y; Bulk density 2X per site, events separated by 5-6 y
Herbaceous biomass*	Distributed	≤ 20	1X per year	5 y	Only clip plots with NLCD class ≠ Forest (Deciduous, Evergreen or Mixed)
	Tower	20-30†	1X-2X per year	1 y	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	5 y	1 month sampling window overlapping AOP flight date

Vegetation component	Plot type	Plot number	Sampling events per year	Yearly sampling interval	Remarks
	Tower	3	Every 2 wks	1 y	Sampling every 2 wks during growing season
Litterfall and fine woody debris*	Distributed	NA	NA	NA	Not sampled
	Tower	20-30†	Every 8 wks, minimum	1 y	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	1 y	Productivity sampling only; total biomass not sampled
Vegetation structure, woody stems	Distributed	≤ 20	1X per year	5 y	Cold/dry sites measured on a 5 y interval
	Tower	20-30†	1X per year	1 y, 5 y	

* 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.

‡ Recommended for elimination by Plant Biomass and Productivity TWG in May 2018. See Section 6.3 for additional information.

4.3 Purpose and Scope

This document outlines NEON's sampling design for plant biomass and productivity, 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).

More specifically, the sampling designs for above and below-ground vegetation components are defined, including spatial, temporal, and methodological factors that are necessary for interpreting plant biomass and productivity data products. 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. We also describe the criteria for standardizing measurements made across multiple disparate vegetation types, as well as links between terrestrial biomass and productivity sampling and other NEON measurement platforms.

Finally, this document describes the iterative sampling framework that will be employed to generate parameter estimates of similar uncertainty across disparate vegetation types. Components of the design are identified that may be adapted to changing budgets and labor availability while still delivering high-quality data products.

5 SAMPLING FRAMEWORK

In the sections that follow, we identify specific challenges associated with sampling above and belowground plant biomass and productivity within a systems engineering and data product framework. Specifically, Section 5.3 evaluates strategies for standardizing methodological, spatial, and temporal aspects of vegetation sampling across the Observatory. Subsections within Section 5.3 provide detailed information about how the sampling designs for aboveground and belowground vegetation components 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 generates raw data and/or samples that satisfy NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON 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 temporal standardization, the two main issues the design must address are: 1) creating robust annual estimates of plant biomass and productivity across ecosystems with different phenologies; and 2) accounting for year-to-year differences in phenology within sites. Ignoring inter-ecosystem 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 and productivity across different NEON plot types.

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, we have defined a set of vegetation components that occur across the NEON domains, and have 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 should 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 downed wood (CDW)
6. Coarse roots
7. Fine roots

The TIGER team also recommended that NEON collect Leaf Area Index (LAI) data, as LAI is a critical input variable for modeling the global C-cycle.

The specific designs for these vegetation components and LAI 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 CDW), 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 collect woody stem data within the stand-level paradigm 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 can be estimated by end-users 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).

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 collect herbaceous productivity data at sites actively managed for grazing such that overestimation errors can be quantified by end-users. 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 an initial 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). Harvest intervals are evaluated on a per site basis following collection of a minimum of 3

years of data to determine whether consumption can be detected with a 4-week interval. The sampling interval may be extended if warranted by the data. Following harvest, 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 0.

The NEON TOS will also collect key belowground data that are necessary inputs for estimation of annual belowground net primary productivity (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 fine root turnover rates, exudation, rhizodeposition, and belowground herbivory. NEON will therefore only sample fine root biomass stocks, and provide vegetation structure data that end-users can use to allometrically estimate coarse root biomass. 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 the vegetation components defined above, 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.
    a. In Tower Plots, NEON collected percent cover data for herbaceous plants, small-stature woody plants, and bryophytes during site characterization. Basal area of trees with DBH ≥ 10 cm was also be recorded. If average % cover is ≥ 10% of the total sampled area for either herbaceous or small-stature woody vegetation then sampling is implemented. Percent cover data are not available for trees so for this growth form sampling occurs if at least one tree is present in > 10% of plots. For the bryophyte growth form the % cover threshold is 20% due to the sensitive nature of these plants and the desire to mitigate long-term impact to the plot vegetation.
    b. In Distributed Plots, sampling for vegetation components is determined on a per plot basis rather than assessing aggregate cover across all plots as for Tower Plots. Compared to Tower Plots, this difference in strategy is warranted because Distributed Plots often are established in different NLCD vegetation types whereas Tower Plots typically are established in only one NLCD vegetation type.

5.3.2 Standardizing Sampling within NEON Plots

5.3.2.1 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 abundance, composition, and diversity). A maximum of n=20 Distributed Plots are selected per site for plant productivity and biomass sampling. Plots are chosen according to a spatially-balanced stratified-random Reversed Randomized Quadrant-Recursive sampling algorithm (RRQRR; AD[03]; Theobald et al. 2007).

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 biomass, LAI, foliar chemistry, and plant diversity sampling. This core area is further sub-divided into four 10m x 10m subplots (Figure 4). 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 4). Distributed Plots will not be utilized for belowground biomass or litterfall sampling (Table 1).

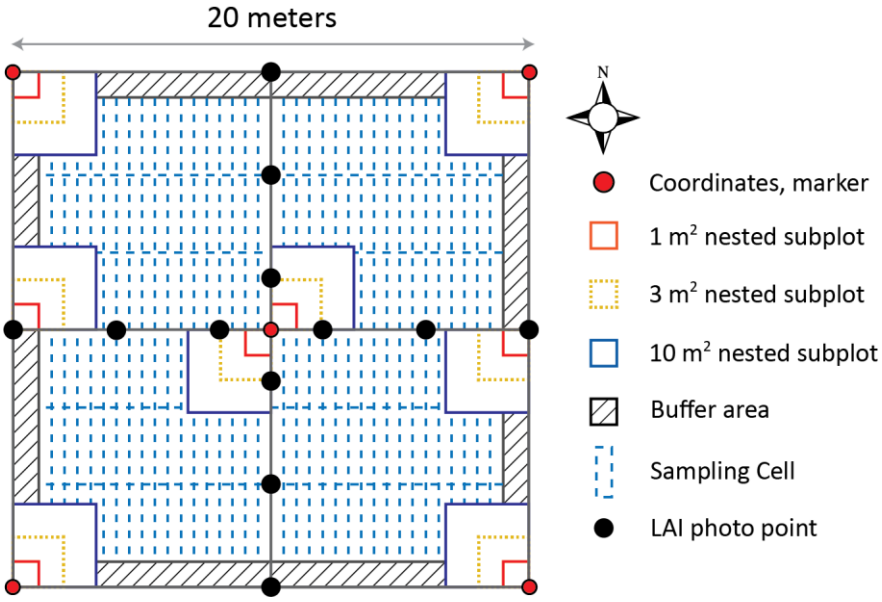

Figure 4. Distributed Plot core sampling area in which aboveground plant biomass, productivity, LAI, foliar chemistry, and plant diversity are sampled. Distributed Plots also support 25 m^2 nested subplots that are omitted from the diagram for clarity.

Within Distributed Plots, the subplots and nested subplots are laid out according to a design modified from the North Carolina Vegetation Survey (Peet et al. 1998). Smaller nested subplots are used to estimate percent cover and richness of herbaceous species (1 m^2 and 10 m^2 sizes), and depending on stem density, all nested subplot sizes may be used to standardize the sampling effort for relatively small

diameter woody stems (see Section 0 for details on woody vegetation). A grid of 3m x 0.5m Sampling Cells within the plot supports randomized, unbiased collection of herbaceous biomass clip-harvest and herbaceous canopy foliar chemistry samples. Herbaceous biomass clip-harvesting is excluded from 10 m^2 and smaller nested subplots in order to minimize the impact of clipping on plant biodiversity data products. Species richness is also assessed in the gridded sample cell area of the plot, and specific sampling cells (Figure 4) 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 0.

Gradient Plots are the same size and shape as Distributed Plots. Gradient Plots are 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. Data from Gradient Plots will be combined with Distributed Plot data to create calibration/validation curves for AOP datasets. The exact number of Gradient Plots will vary by site, but a maximum of n=5 Gradient Plots will be established per site due to budget constraints. Gradient Plots were not installed at NEON sites during the initial plot establishment effort, and may be installed in future years if warranted by the data and if sufficient budget is available.

5.3.2.2 Tower Plots

The purpose of TOS Tower Plots is to support consistent, field-based sampling to enable end-users to calculate annual ANPP within the TIS tower footprint. Tower Plots also support belowground fine root biomass sampling. When paired with fine root turnover rates from PI-driven research, the NEON fine root biomass data product can be used to calculate BNPP. Within this general framework, the goal is to sample the biomass of each *i* aboveground vegetation component such that a 20% change in year-to-year production can be detected with 80% power and alpha of 0.05. Woody stem density, stature, and spatial heterogeneity all vary widely across ecosystems, and as such, the size and number of Tower Plots should be scalable so that power analysis targets can be achieved while minimizing the total area sampled. Estimates of belowground coarse and fine root biomass are 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 are therefore determined 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 and have a minimum size of 20m x 20m (0.04 ha). Prototype work at the NEON Domain 01 Harvard Forest core site (HARV) and the Domain 03 Ordway-Swisher core site (OSBS) suggested that approximately 3 ha total Tower Plot area represents the logistical and financial upper limit for forested sites. Given a 3 hectare

maximum total area, sampling simulations using Smithsonian Megaplot data from the Wind River Experimental Forest site were performed to assess the impact of Tower Plot number and size on the precision of aboveground woody biomass estimates (**Figure 5**). This site is also the NEON Domain 16 core site. Sampling simulation code is provided in 0.

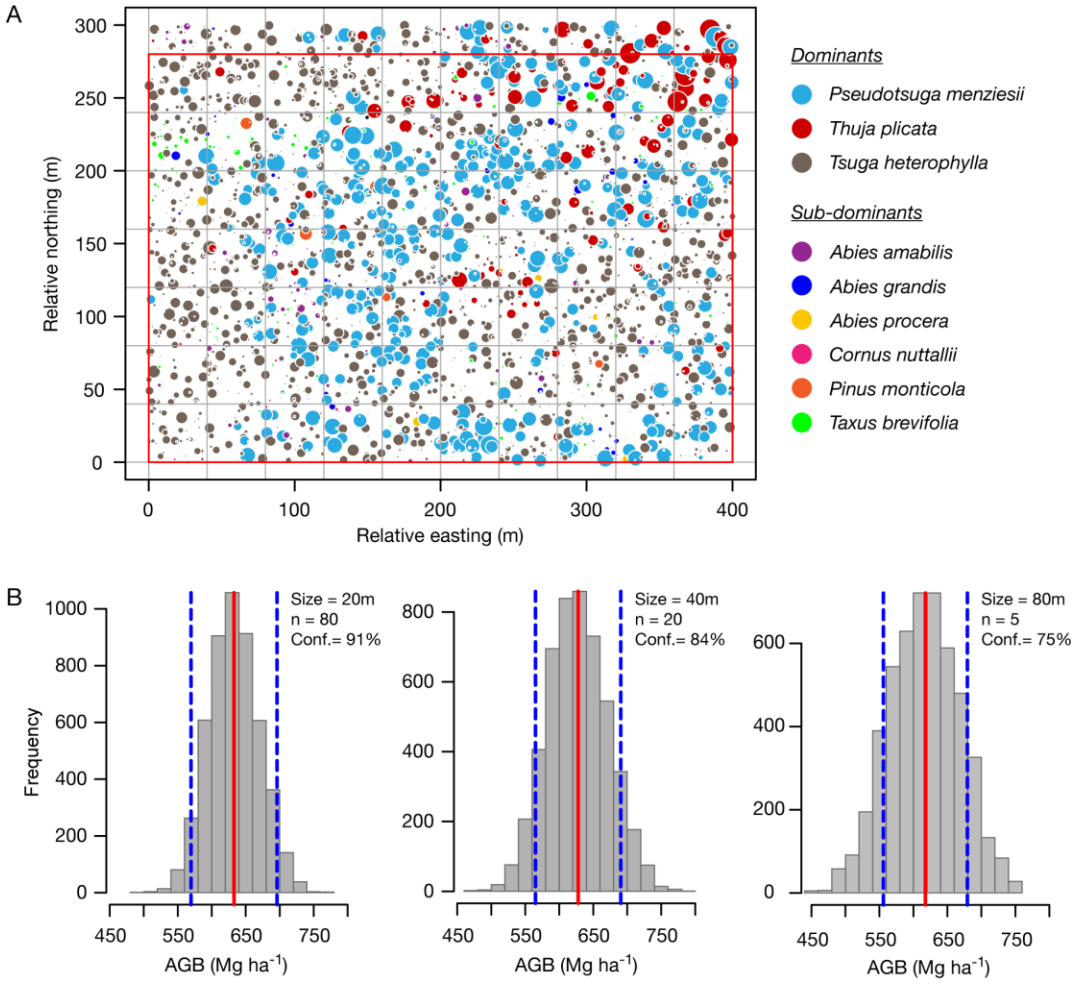

Figure 5. (A) Woody stem map from the Smithsonian Wind River megaplot in Washington state (NEON Domain 16 core site); symbol size is proportional to stem diameter, and symbol color indicates species. The red line shows the area defined for sampling simulation, and grey lines indicate potential 40m x 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%.

The Wind River sampling simulations revealed that greater numbers of smaller plots are more likely to result in AGB estimates within ± 10% of the known true AGB value compared to fewer, larger plots (**Figure 5B**). It was also assumed that smaller 20m x 20m plots may not adequately sample AGB and productivity compared to larger 40m x 40m plots when biomass is patchily distributed (e.g., savannah

ecosystems). Based on these results and considerations, NEON initially established two Tower Plot configurations during the project construction phase:

1. **Short-stature grassland and shrubland ecosystems**: 20m x 20m plots, n=30 (max), maximum total sampled area = 1.2 hectares.
2. **Tall-stature forest and savannah ecosystems**: 40m x 40m plots, n=20 (max), maximum total sampled area = 3.2 hectares (**Figure 6**).

Sample sizes for each plot type are maxima because property boundaries and small permitted areas at a subset of sites prevents establishing the desired number of plots.

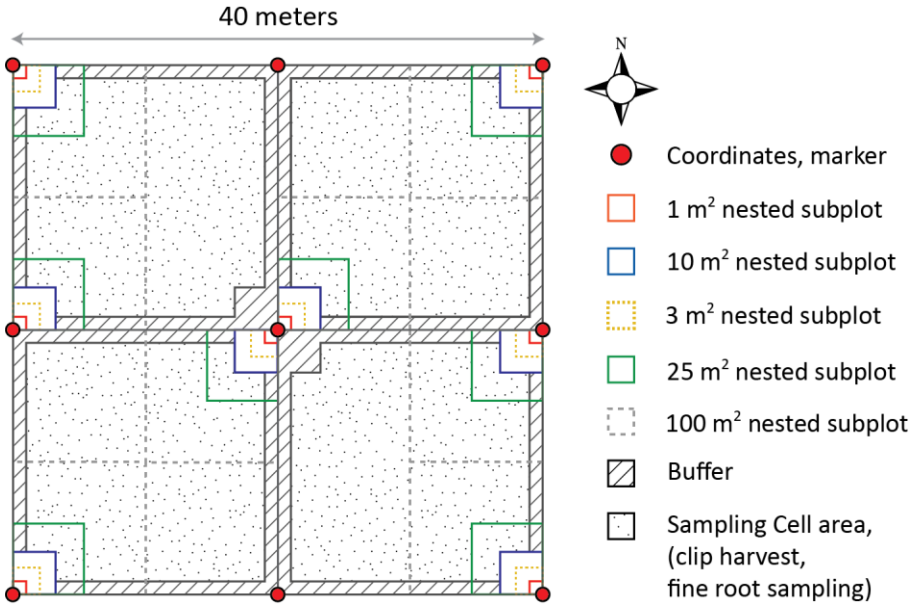

Figure 6. Example 40m x 40m Tower Plot comprised of four 20m x 20m subplots. The Sampling Cell area within each subplot is gridded into cells as shown in Figure 4, but cells are omitted here for clarity. Details of sampling activities that take place in the plot are described in Section 6.

Following initial plot establishment and implementation of the TOS Protocol and Procedure: Measurement of Vegetation Structure (RD[05]) at a subset of sites, it was evident that NEON did not have sufficient resources to complete annual sampling within 40m x 40m Tower Plots without sacrificing other important components of TOS sampling. To reduce the sampling effort by 50% at tall-stature sites, two of the four 20m x 20m subplots were randomly selected for sampling on a plot-by-plot basis. Random selection of subplots was chosen instead of decommissioning 50% of plots for several reasons: 1) Simulation results suggest that greater plot numbers are more likely to accurately capture AGB and ANPP (**Figure 5**); 2) Randomly selected subplots better enable quantifying the scale of spatial autocorrelation among plots and subplots compared to larger plots spaced further apart. Spatial auto-correlation is an important determinant of effective sample size (Griffith 2005); and 3) Random selection

of subplots does not require resources to decommission previously established plots. The Tower Plot configurations sampled during NEON Operations are therefore:

1. **Short-stature grassland and shrubland ecosystems**: as above.
2. **Tall-stature forest and savannah ecosystems**: 40m x 40m plots, n=20 (max), two randomly selected 20m x 20m subplots, maximum total sampled area = 1.6 hectares.

A minimum of n=5 Tower Plots are always established, resulting in a minimum sampled area of 0.2 ha (n=5 20m x 20m plots). To place the total Tower Plot area into the context of the size of the land area that informs the TIS flux data, the area of the TIS tower footprint at the NEON Domain 01 Harvard Forest core site is approximately 60-80 ha (depending on how the area of the flux footprint is calculated). At this site, the aggregate Tower Plot sampling area is 1.6 ha (n=20 40m x 40m plots, two randomly chosen subplots per plot), and represents 2%–2.7% of the total tower footprint area. 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 further optimize plot number and size in grasslands (Briggs and Knapp 1991) and forests (Keller et al. 2001).

As described above, optimization power analyses will be performed with a minimum of 3 years data to determine whether Tower Plot sampling at each site is adequate to detect a 20% change in productivity from year-to-year with 80% power (alpha = 0.05). Each component of productivity will be analyzed separately (i.e., litterfall, herbaceous production, woody increment, etc.), and total sampled area may be reduced for a given protocol in the event that a site is oversampled with respect to power analysis criteria. One end result of optimization is that the full suite of TOS biomass and productivity protocols may not be implemented in all Tower Plots at a site. In the event that power analyses reveal undersampling is consistently occurring at a site, NEON Science will make a formal recommendation to increase sampling effort, and 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 is also specified according to the spatially balanced RRQRR sampling algorithm (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. In theory, stratification is not required because 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 is 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 Vegetation Components and Unequal Sample Sizes

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 increment 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 collect data that enable coarse and fine root biomass estimation 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

can 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 collect and process soil samples. Combined with PI-generated fine root turnover coefficients (TC, y^{-1}), NEON data users can estimate fine root production (FRP) according to:

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

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 Fine Root Sample Number and Location

There will be two soil samples collected for fine root biomass per 20m x 20m Tower plot or subplot per sampling bout (**Figure 7**). Combined with the two standard NEON Tower Plot configurations, the belowground biomass sampling effort depends on the dominant vegetation at a site:

1. **Short-stature grassland and shrubland ecosystems**: 2 soil samples per 'cell' x 1 cell per plot x 30 plots per bout (max) = 60 soil samples per bout (max)
2. **Tall-stature forest and savannah ecosystems**: 2 soil samples per 'sampling cell' x 1 sampling cell per subplot x 2 subplots per plot x 20 plots per bout (max) = 80 soil samples per bout (max).

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 may be quantified using Monte-Carlo bootstrap techniques. Power analyses may also be employed to determine whether the sampling design is capable of detecting a 20% change in fine root biomass from year-to-year with 80% power (alpha = 0.05). Linear mixed-effects models will be constructed to understand variance partitioning within fine root biomass data. Within 'cell' or within-plot replication may be adjusted in a standardized manner at the Observatory scale depending on the outcome of these analyses.

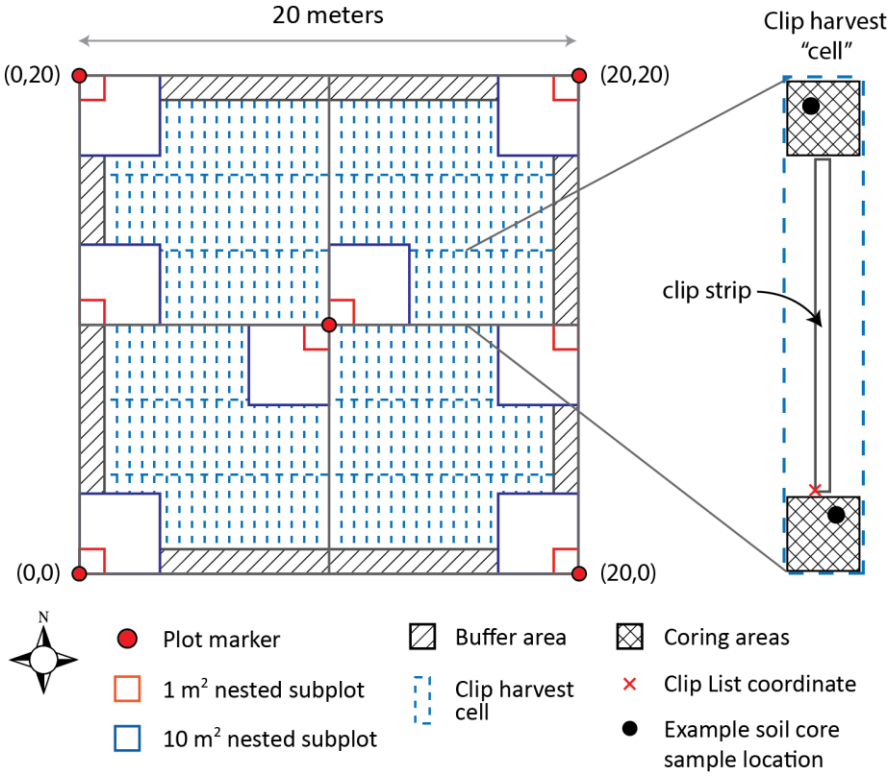

Figure 7. A 20m x 20m Tower plot showing the locations of 0.5m x 3m sampling "cells" also used for belowground biomass soil sampling (*left*). Within a sampling cell selected for soil sampling, two soil samples are collected: one from the area to the North, and another from the South of the clip-strip (*right*). Note that 3 m^2 and 25 m^2 nested subplot sizes are omitted from the diagram for clarity.

With respect to location, fine root samples are collected within the Tower plot sampling "cells" shown in Figure 7. Soil samples are collected immediately adjacent to matched aboveground clip-harvests. Sampling 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 0 for additional details on randomly locating sampling 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 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 sort fine roots into

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

Fine root biomass sampling is biased toward the surface soil in many studies (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. However, 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 excavated a soil pit at each site near the tower (soil pit dimensions are: 1.5m W x 2m L x 2m maximum D) (Figure 8). Realized depth were less than 2m if bedrock or very large rocks were encountered before 2m depth was reached. Soil pits were located systematically in the dominant vegetation type in which Tower plots are also established, and these pits allowed a spatially limited but important estimation of fine root biomass distribution with depth on a per site basis.

An obvious caveat associated with fine root biomass depth distribution data from the soil pit at each site is that replication of depth sampling is poor (i.e., n=1 soil pit per site, with n=3 root profiles excavated per pit). Increased replication of soil pits at each site clearly would be desirable. Nonetheless, NEON soil pit data represent an important contribution with respect to quantifying belowground fine root biomass stocks since continental-scale datasets at > 1 m sampling depths are rare.

Figure 8. 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 Plots within a 1 month window overlapping the actual AOP flight date. The NEON AOP will fly most NEON sites annually, and MODIS-EVI phenology data from each site are used to time flights within the average peak greenness window. Distributed Plots (maximum n=20 total) are selected according to a spatially-balanced stratified random design (Theobald et al. 2007). In the event that TOS LAI datasets fail to span the full dynamic range of LAI observed within AOP remote-sensing datasets, Gradient Plots may be established and sampled within key missed vegetation types. 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 and reduce uncertainty of LAI estimates using all available NEON data, NEON end-users may combine

remote-sensing and ground-truth datasets using 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-collected 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 and will complement NEON plant phenology datasets.

5.3.5 Determining Timing of Sampling

The overarching goal for most NEON data products is to provide annual, site-scale parameter estimates based on standardized protocols. Certain data products will have multi-year intervals rather than annual intervals (e.g. coarse woody debris volume; Table 1). The plant biomass and productivity 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, herbaceous biomass production 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 ability to detect changes in biomass and/or productivity is achieved. 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 faces 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.

6 SAMPLING DESIGN FOR PLANT BIOMASS AND PRODUCTIVITY

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 mensuration.
2. The selected method(s) for sampling
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. A final subsection summarizes how the multiple plant biomass and productivity sampling efforts may be scheduled through time at a model NEON site in order to maximize linkages with other TOS protocols and to generate robust time series data (Section 6.9). Sampling for each vegetation component by site is summarized in APPENDIX B.

6.1 Woody Vegetation Sampling Design

Mapping woody stems and collecting longitudinal measurements of structure (DBH, height, etc.) 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 collect vegetation structure data 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 species that are canopy emergent and attain heights greater than 4 – 5 meters.

Total tree biomass and annual productivity is typically determined by end-users via 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). NEON will report DBH, height, and crown dimensions on a per individual basis because it is unclear which equations NEON data users will employ to estimate biomass from vegetation structure parameters. NEON data will thereby enable researchers to estimate per stem biomass values using a range of allometric equations.

Small trees, saplings, shrubs, and small shrubs

There are a variety of diameter and height cutoffs employed to define and differentiate small trees, saplings, and shrubs (e.g. Lee et al. 2008). To speed assessment by field staff, NEON will define small trees and saplings as self-supporting stems of potential canopy emergent or sub-canopy tree species with DBH < 10 cm (see specific definitions below). Shrubs and small shrubs are those self-supporting woody individuals with DBH < 10 cm that typically do not reach the canopy in forested systems, or that constitute the entire canopy when trees are absent. NEON generally follows USDA Plants classifications to determine whether individuals are small trees or saplings versus shrubs or small shrubs.

Definitions:

- *Small trees*: Smaller self-supporting individuals with the potential to grow into a tree; also includes individuals of species that never attain tree stature at maturity (as defined above; e.g., *Picea mariana*, *Acer pensylvanicum*). Meets the criteria: 1 cm ≤ DBH < 10 cm, height typically less than 4 – 5 meters.
- *Saplings:* The smallest category of self-supporting individual with the potential to grow into a tree. DBH < 1 cm or height < BH, and diameter at decimeter height (ddh) ≥ 1 cm. Individuals may or may not be species that are canopy emergents. Woody stems with ddh < 1 cm are measured as part of the herbaceous plant sampling effort.
- *Shrubs:* Self-supporting woody individuals, often with multiple emergent stems. Diameter of at least one stem at breast height meets the criteria 1 cm ≤ DBH < 10 cm. Usually does not exceed 4 – 5 meters maximum height at maturity. Uncommonly, DBH can be ≥ 10 cm (e.g., *Rhododendron spp*.)
- *Small shrubs*: Self-supporting woody individuals, often with multiple emergent stems; includes typical 'subshrub' species that may never exceed 130 cm height, as well as individuals that will mature into the shrub growth form. The ddh is ≥ 1 cm for at least one stem.

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

Collecting vegetation structure data that allow consistent estimation of shrub and small shrub biomass and ANPP across sites is a significant challenge 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 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. NEON also cannot consistently harvest shrubs across all NEON sites given permitting constraints. Shrub volume, individual shrub structural characteristics (e.g. basal diameter, DBH, canopy diameter, canopy volume, height), and plot-level % cover-by-species estimates are more feasible for NEON to monitor. For shrubs and small shrubs, NEON will collect measurements similar to Lutz et al. (2014).

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 liana abundance and species diversity are highest at the NEON sites in Puerto Rico and Hawaii. However, there are a few species of lianas which are extremely common in the temperate, continental United States, and therefore may be encountered at a number of 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 to 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).

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, end-users may then estimate liana biomass using generalized, regional, or species-specific allometries. For liana sampling, NEON will collect DBH data in accordance with the guidelines published by Gerwing et al. (2006), and elaborated upon by Schnitzer et al. (2008).

6.1.1 Sampling Methods

6.1.1.1 Sampling Methods: Trees

Trees within NEON plots are tagged and marked at the correct measurement height to facilitate consistent, repeatable measurements of stem diameter at the same measurement location from year to year. Individual stems are marked according to site host preferences, and the preferred marking method is a numbered aluminum tag affixed with an aluminum nail 10 cm above the measurement location. Weather resistant paint or a lumber crayon is used 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, obviously loose bark is removed prior to initial DBH measurements. Similarly, epiphytes are also 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 within the sampling area 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 Smithsonian Megaplot datasets. For trees within Distributed plots, both maximum crown diameter and the diameter orthogonal to the maximum are measured (McGinnis et al. 2010). Combined with mapping data, crown dimensions will help link ground datasets with airborne remote-sensing datasets.

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 defines

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. To deal with these situations, NEON follows established guidelines adapted from the U.S. Forest Service Forest Inventory and Analysis National Core Field Guide (U.S. Forest Service 2012). Common bole irregularities and associated measurement strategies are shown in **Figure 9**.

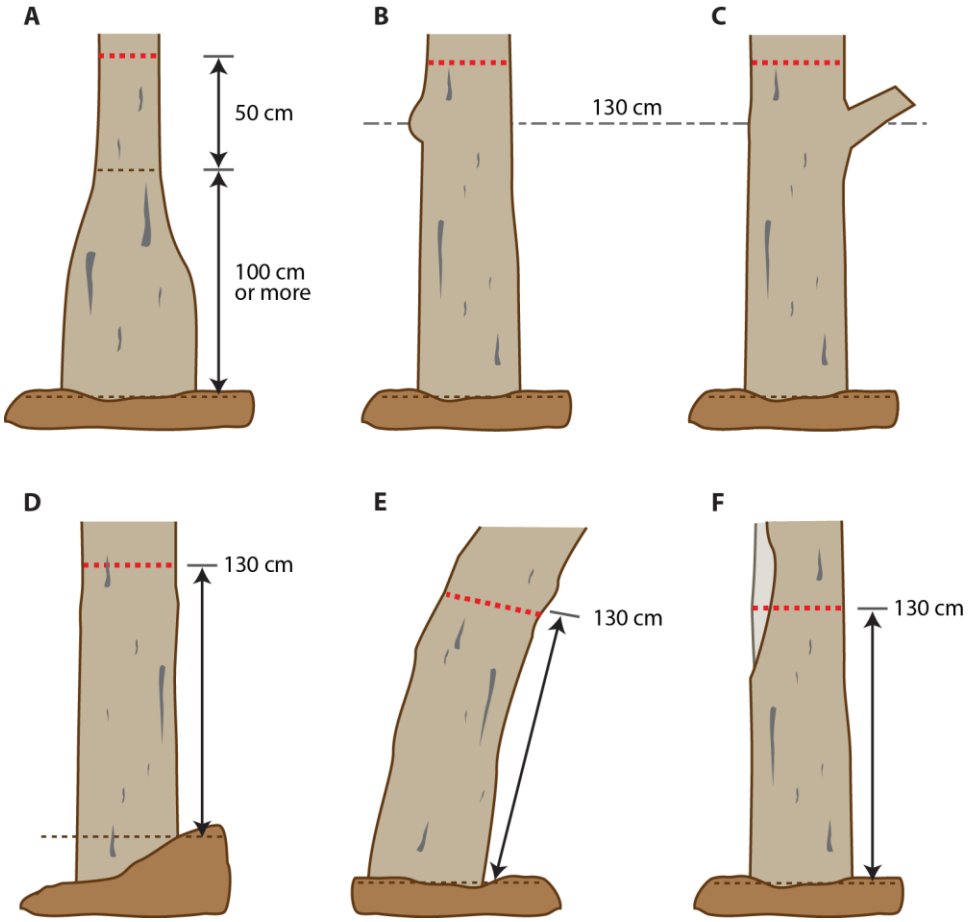

Figure 9. Common bole irregularities and associated measurement strategies; adapted from U.S. Forest Service FIA National Core Field Guide (U.S. Forest Service 2012). Red, dashed lines indicate the desired measurement location. (A) Pronounced thickening near the base: Measurement height is 50 cm above point at which swelling returns to normal; (B) and (C) Irregularities at breast height: Measurement height moved above the irregularity to location where bole is normal; (D) Sloped ground: Measurement height determined from uphill side of bole; (E) Leaning tree: Measurement height determined along underside of lean; (F) Missing wood or bark: Measure DBH for wood and bark that are present.

Trees that fork into multiple stems below 130 cm require additional guidance. Strategies for measuring forked stems are summarized in **Figure 10**.

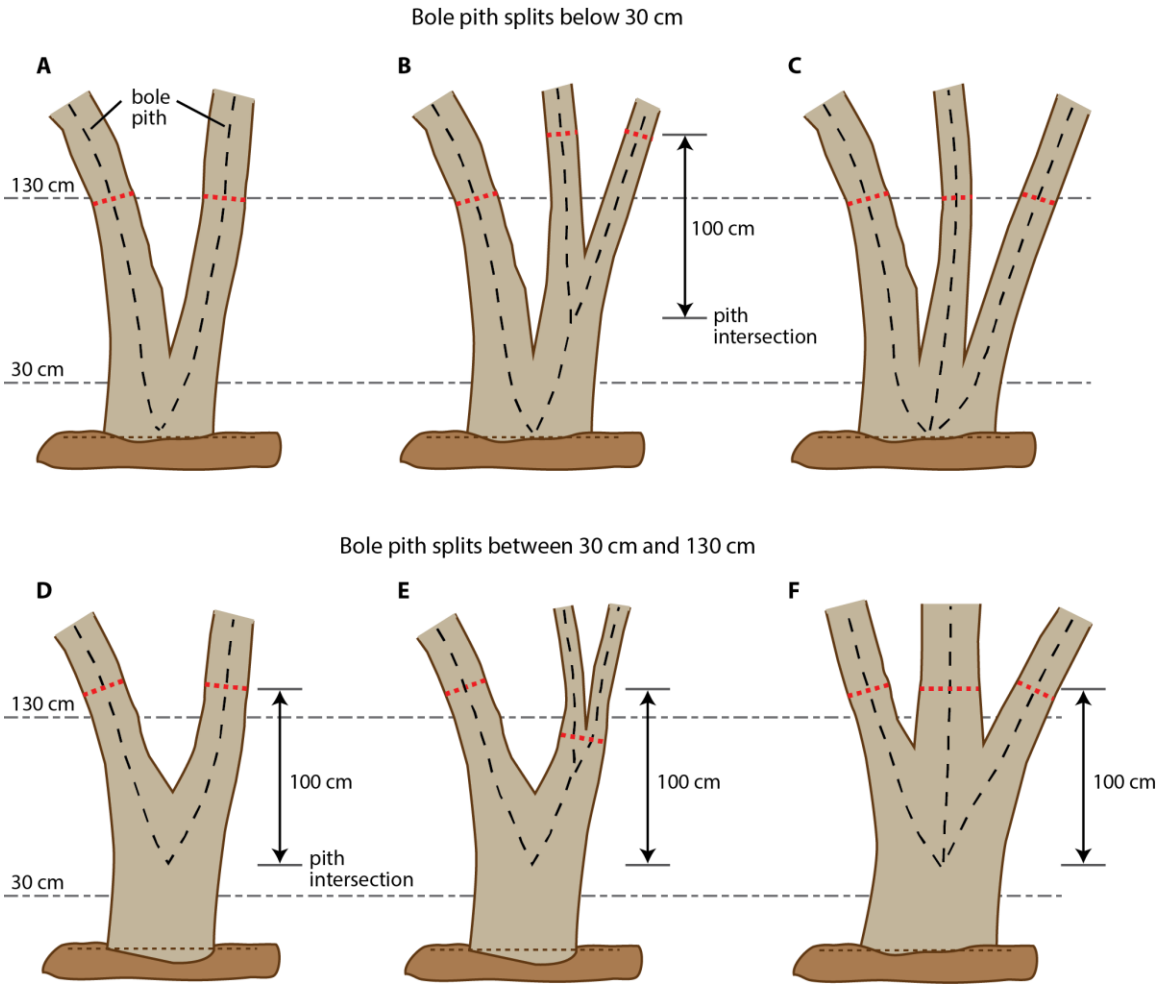

Figure 10. Stem diameter measurement strategies for forked, multi-bole individuals; adapted from U.S. Forest Service FIA National Core Field Guide (U.S. Forest Service 2012). Red, dashed lines indicate the desired measurement location; black, dashed lines indicate bole piths. (A) Simple split: Piths intersect ≤ 30 cm above the ground, stem diameter measured at 130 cm; (B) Compound split: Secondary splits are measured 100 cm above secondary pith intersection point; (C) Multiple boles: Stem diameter measured 130 cm along the stem for each bole; (D) Elevated simple split: Forks originating between 30 – 130 cm are measured 100 cm above the pith intersection point; (E) Elevated compound split: left fork measured 100 cm above pith intersection similar to (B), right fork measured below secondary pith intersection; (F) Elevated multiple boles: Each fork originates from same pith intersection point and is measured 100 cm above intersection.

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 ecologist.

Sampling tree mortality

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. However, the aggregate Tower plot area is constrained to ≤ 1.6 ha, and is smaller than that at some 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 snags and dying trees within plots will be mapped, tagged, and measured for DBH and height consistent with measurements for live trees. Snags are defined as standing dead trees with an angle of lean of 45˚ or less from the vertical (Curtis 2008). NEON will provide data dead or dying trees such that end-users can estimate standing dead tree biomass. Several methods for quantifying standing dead tree biomass are presented in Smith et al. (2003), and Domke et al. (2011). In general, NEON will enable calculating 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, NEON will measure or estimate the top break diameter, though generating precision estimates can present problems (Harmon and Sexton 1996), and quantifying uncertainty in this parameter will be difficult.

Equipment considerations

The overarching goal of the sampling design is to enable annual estimation of biomass increment over the course of the 30-year lifetime of the NEON project. One method that satisfies this goal is to measure all trees during each bout using standard forestry DBH tapes. DBH tapes are relatively inexpensive and changes in stem diameter will likely be detectable over NEON measurement intervals with acceptable uncertainty in most forest types. Another viable approach is to measure the diameter of all trees within NEON 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.

NEON has adopted an annual stem diameter measurement strategy with DBH tapes to ensure consistency across sites. To enable accurate, repeat measurements with DBH tapes, measurement locations on individual stems are marked with aluminum nails and/or paint. Aluminum nails are placed 10 cm above the measurement location to avoid any effect of nails on tree growth. Visual markers such as paint or lumber crayons are placed directly on the measurement location. Although NEON collects

DBH data with tapes, annual measurement of a subset of stems with dendrometer bands followed by measuring all trees over longer intervals has the potential to save considerable labor. NEON will therefore evaluate dendrometer band equipment and labor costs during the initial Operations period and may adopt a measurement strategy similar to Clark et al. (2007) in consultation with the Plant Biomass and Productivity Technical Working Group if warranted.

To map individual stems and record height and crown diameter data, NEON has evaluated laser rangefinder/clinometer/compass models from Häglof, LaserTech, and LaserAce. Units of this type should perform adequately if operated according to established guidelines (Blozan 2008). For height and crown diameter measurements of canopy emergent individuals, airborne LiDAR-derived values may be more accurate than field-collected observations from the ground. However, ground-collected data are valuable because crown detection algorithms are relatively immature and are an area of active research. Ground-collected crown-diameter measurements may ultimately be more accurate than LiDAR derived estimates in dense, closed-canopy forests where detection of individual crowns is difficult. Ground-collected data also provide insight for stems growing on slopes, and for stems growing in areas with thick crown or understory vegetation that prevents airborne LiDAR from accurately detecting the ground.

6.1.1.2 Sampling Methods: Small Trees, Saplings, Shrubs, and Small Shrubs

To obtain an appropriate sample size per plot, either the entire plot will be censused when vegetation is sparse, or nested subplots will be selected from within the larger plot when stem density is high (**Figure 4** and **Figure 6**). Nested subplot size is selected on a per plot basis in order to standardize the sampling effort such that a minimum of 20 individuals are sampled per 400 m^2. To be consistent within plots and to enable tracking of individuals from year to year, the same nested subplot size should be used for the entire plot, and the same size nested subplot size 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 small woody stems that meet the measurement criteria. Nested subplot size may be changed for a plot in consultation with NEON Science staff if vegetation within the plot experiences a significant and persistent state change (e.g., fire, bush-hogging, logging, etc.).

Species identification, tagging, and mapping

Small trees and saplings are generally tagged, measured, and identified to species. Small trees may be mapped if larger trees are absent from the plot, and saplings are never mapped. 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. When shrub or small shrub 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 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). In plots where shrubs are clumped or grow in thickets, thereby making mapping and tagging of individuals either impossible or very expensive and laborious, the perimeter of the shrub group is mapped as a polygon, and the average height is recorded in order to estimate volume of the shrub group. The species composition of the shrub group and percent live and dead for each species is also recorded, and identification of species is carried out by either a botanist, or by a trained NEON field technician.

Measurement considerations

The primary objective for small tree, sapling, shrub and small shrub vegetation structure measurements is to provide input variables that may be combined with available allometric equations to estimate biomass. Measurements of DBH and/or ddh are therefore a priority for small trees and saplings since allometric equations often rely on these input variables. 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. Based on existing shrub allometries (e.g., Lutz et al. 2014), NEON collects the following data from individual shrubs:

- *Shrubs with DBH ≥ 1 cm:* For each emergent bole connected to a common root system with DBH ≥ 1 cm, measure DBH on the thickest fork and also measure ddh (i.e., basal area). Additionally, record height, crown dimensions, and geometric crown shape to enable calculation of crown volume (McGinnis et al. 2010). Emergent boles with DBH < 1 cm or total stem length < 130 cm are not measured.
- *Small shrubs with DBH < 1 cm or total stem length < 130 cm:* For each emergent stem connected to a common root system, measure ddh (i.e., basal area). Emergent stems with ddh < 1 cm are not measured. Height, crown dimensions, and geometric crown shape are recorded as above for larger shrubs.

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 and crown dimensions will be measured with a laser rangefinder/clinometer or a graduated measuring stick.

6.1.1.3 Sampling Methods: Lianas

Similar to trees, liana stem diameter is measured 130 cm above the rooting point. Due to the variety of complicated growth forms that lianas may adopt, NEON follows established protocols 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 11).

Figure 11. 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).

Liana Measurement Diameter Cutoff

Gerwing et al. (2006) recommend that the stem diameter measurement threshold 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 adheres to the recommendations of Gerwing et al. (2006), and employs a 1.0 cm diameter cutoff for liana measurement. Compared to a 2.0 cm cutoff, a 1.0 cm cutoff provides 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 11, 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 are divided into two categories for measurement: cylindrical and markedly non-cylindrical. Non -cylindrical stems include flattened, triangular, ovoid stems, etc. (Gerwing et al. 2006). The diameter is recorded for cylindrical stems. Non-cylindrical stems are measured at both the narrowest and widest points, and the geometric mean of the two measurements is recorded.

Equipment considerations

Liana stems are 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 using aluminum wire and stems may be painted or marked with lumber crayon to indicate 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 records 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 are made within entire plots or within two randomly selected subplots (**Figure 4** and **Figure 6Error! Reference source not found.**). Similar to biomass estimation for saplings, shrubs, and lianas, nested subplots may be employed to standardize the sampling effort for ferns across plots. Nested subplot size is chosen such that the number of individual ferns measured is a minimum of 20 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 counts of both current-year and older cladodes (USDA personal communication). NEON therefore tallies cladodes produced in the current sampling year, as well as older cladodes in order to enable end-user estimation of cladode biomass and annual production. Constructing site-specific relationships between pad count and biomass for pad-forming cactus species is beyond the scope of NEON and can be taken up by the external user community.

Biomass can be estimated for some cholla-type cactus species using allometric equations that rely on stem diameter or crown dimension data as inputs (e.g. Búrquez et al. 2010). As such, NEON enables biomass estimation for species with cholla-like growth forms and trained field ecologists determine which individuals and species are assigned to a cholla-type growth form.

6.1.2 Spatial Sampling Guidelines

Presence and percent cover of qualifying vegetation is used to determine whether NEON collects vegetation structure data in Distributed and/or Tower Plots. Vegetation structure measurements are made in up to a maximum of 20 Distributed Plots selected according to a spatially-balanced, stratified random algorithm (Theobald et al. 2007). In addition, all Tower Plots may be utilized for vegetation structure measurement. Because Distributed and Tower Plots are established at different spatial scales, rules for implementing the vegetation structure protocol depend on plot type.

- Distributed Plots:
    - Assessed for vegetation structure measurement on a plot-by-plot basis.
    - Vegetation structure data are collected if % cover of qualifying vegetation is ≥ 10% or the plot contains at least one tree (Section 5.3.1).
    - If a visual assessment of cover indicates < 25% qualifying vegetation AND the NLCD class is not equal to forest, a quantitative survey is performed to determine whether the 10% threshold is met.
- Tower Plots:
    - Assessed for vegetation structure measurement by averaging cover across all Tower Plots.
    - Vegetation structure data are collected if average % cover of qualifying vegetation is ≥ 10% or ≥ 10% of plots contain at least one tree.
    - If a visual assessment of cover indicates < 25% qualifying vegetation averaged across all Tower Plots, a quantitative survey is performed to determine whether the average 10% threshold is met.
- All Plots:
    - When individual Distributed Plots or all Tower Plots do not qualify for data collection, a quantitative survey is performed every 5 years if qualifying vegetation is present.

Vegetation structure data are collected from woody stems with ddh ≥ 1 cm, and the size of the sampling area within the plot may vary depending on the growth form and plot level characteristics (i.e. stem density per growth form)(Table 2). The size of the nested subplots is independently optimized per growth form or growth form group on a per plot basis. The target sampling effort is a minimum of 20 individuals per growth form per 400m^2.

The NEON Distributed plots contain nested subplots of four sizes available for monitoring woody vegetation (1 m^2, 3 m^2, 10 m^2, and 25 m^2 ; **Figure 4**). Tower plots that are 40m x 40m or larger feature an additional 100 m^2 subplot (**Figure 6**). The entire 400 m^2 plot/subplot may be used for monitoring any of the growth forms listed above should stem density be sufficiently low. Once a nested subplot size has been chosen for a given growth form, that same nested 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 could be sampled with the following strategy:

- All trees with DBH ≥ 10 cm mapped and measured throughout the plot,
- Liana measurement occurring in 30 m^2 subplots, and
- Small trees and saplings with DBH < 10 cm and ddh ≥ 1 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
Small trees, saplings, shrubs, small shrubs*	< 10 cm	≥ 1 cm	Yes	Minimum of 20 individuals per 400 m^2 plot or subplot
Lianas	≥ 1 cm	NA	Yes	Minimum of 20 individuals per 400 m^2 plot or subplot
Ferns, cacti, and 'other' plants**	NA	species dependent	Yes	Minimum of 20 individuals per 400 m^2 plot or subplot

* Sum of individuals across all four growth forms (i.e., small trees + saplings + shrubs + small shrubs).
** Sum of individuals across all 'other' growth forms.

Because nested subplots may be used to standardize the sampling effort, and because the nested subplot size may vary from plot to plot, it is critical for end-users to refer to per plot sampling metadata when scaling up woody biomass and 'other' non-woody perennial biomass to the plot scale.

6.1.3 Temporal Sampling Guidelines

Vegetation structure data are collected from a subset of up to 20 Distributed Plots every 5 years (Table 1). Gradient Plots are also sampled every 5 years should they be established at a site. The purpose of vegetation structure measurements in Distributed and Gradient Plots is a) to enable plot-based estimates of site-level biomass and vegetation structural complexity, and b) to integrate with the NEON AOP hyperspectral and LiDAR datasets and provide critical calibration and validation data. The 5-year measurement interval is appropriate because it is not expected that relationships between ground-collected and remotely-sensed datasets will change rapidly. Furthermore, the NEON AOP flies each site 3 out of 4 years, on average, and thus site-level parameters informed by Distributed Plot measurements can be generated more frequently than every 5 years.

Tower Plot measurement intervals depend on site conditions. Due to measurement limitations associated with DBH tapes when growth rates are slow, vegetation structure data are collected with

different temporal sampling strategies for more mesic sites with relatively high growth rates compared to 'cold/dry' sites with relatively low growth rates:

- ***High growth rate sites***: 5 y measurement interval for all Tower Plots, and a spatially-balanced subset of the Tower Plots are selected for annual measurement (same plots measured every year). The subset sample size is determined on a per site basis to ensure that growth increment measured from the subset can be used to model annual growth increment in all Tower Plots within 20% (alpha = 0.05) of observed growth increment in existing initial Operations datasets.
- ***Low growth rate sites***: 5 y measurement interval for all Tower Plots.

In addition, years in which Distributed Plots and all Tower Plots are measured are staggered through time such that vegetation structure data are collected from a given site on a 2-3 year interval (Section 6.9). In Distributed Plots, Vegetation Structure sampling is also coordinated with Herbaceous Biomass sampling and Coarse Downed Wood tally such that all three vegetation components are measured in these plots in the same year at a given site (see Section 6.9).

All woody vegetation components are sampled concurrently, and measurement occurs primarily 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 are scheduled after the period of peak greenness, as identified using the MODIS-EVI phenology product. Measurements begin in a given year once 50% of the canopy has entered the senescence phenophase. However, mapping and identification of woody stems may occur at any time 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 occurs during the dry season. Low latitude sites with no distinct growing season may be measured for vegetation structure at any time of the year. Once a measurement period is chosen the same time period is scheduled in future years (± 2 weeks). 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 may be adjusted once 3 or more years of NEON TIS flux data are available for a site. Flux data will enable scheduling the onset of vegetation structure sampling to the time of year when the CO_2 source/sink transition occurs for each site.

6.1.4 Logistics and Adaptability

The number and size of Tower Plots initially selected is based on NLCD vegetation type (forested vs. non-forested) and an estimate of the maximum area that can feasibly be sampled in a given season (approximately 1.6 ha). The sampling effort for woody vegetation structure will be evaluated for each site after it has been commissioned into Operations, with the primary sampling goal being the ability to detect a 20% change in aboveground woody biomass from year-to-year with 80% power (alpha = 0.05).

Sampling effort may be reduced in future years if a reduction is justified based on statistical analysis of the collected data.

The selected methods for measuring vegetation structure may change in response to funding fluctuations, end-user and/or Technical Working Group (TWG) feedback, or emergence of new technologies, tools or techniques. For example, the Plant Biomass and Productivity TWG has recommended that annual DBH measurements in a subset of Tower Plots be implemented at all NEON sites, as opposed to only acquiring annual DBH data from mesic sites as currently designed. In response to the TWG recommendation, NEON will assess the costs and benefits of employing dendrometer bands to accomplish annual measurement at cold/dry sites. Changes of this type are implemented Observatory-wide after a prototype period at a limited number of sites and thorough evaluation of impacts to data quality and the operating budget.

Additionally, 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 Herbaceous Biomass and Productivity Sampling Design

Definition: Plants in this vegetation component include non-woody forbs and graminoids, bryophytes, certain vines that are not measured as part of the woody vegetation component (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 prioritizes estimation of total herbaceous biomass in Distributed Plots, and current-year herbaceous production from these plants 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 0.5m x 0.5m harvest areas (0.25 m^2, personal communication);
2. Briggs and Knapp (1991) used 0.2m x 0.5m 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 0.1m x 3m strips, with clips from successive years occurring side-by-side within a larger 4m x 4m plot (Tilman 1982); and
4. The global Nutrient Network (NutNet) experiment employs two 0.1m x 1m 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 selected the clip-harvest technique to estimate biomass and ANPP. When woody-stemmed sub-shrubs are clipped, care is taken to clip current-year growth only; for these plants, clip-harvests will yield estimates of ANPP but not total biomass.

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 2000 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 provides 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 greatly increases the amount of time needed to sort clipped biomass. It is also very difficult and expensive for NEON to quantify the uncertainty of species identification performed during sorting. Therefore, NEON reports biomass and productivity measurements with respect to functional groups rather than species. There are many ways to define functional groups (Lavorel et al. 1997), and the challenge is to define ecologically meaningful functional groups that are utilized consistently across NEON sites. Two examples from the literature revealed broad convergence in functional group definitions. Researchers at the Short-Grass Steppe (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 are selected *a priori*, and are 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 nested subplots smaller than 10 m^2 and that are reserved for repeated plant diversity % cover measurements are omitted). Assuming up to 2 locations may be clipped per growing season, and that there will be 30 growing seasons, there are a maximum of 60 clip harvests performed per plot over NEON's lifetime. Once a clip-harvest area is accepted, the exact dimensions of the biomass removal area are temporarily delineated using pre-marked cords and stakes or measuring sticks and flags. 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 exceeds the number of anticipated harvests (Figure 4 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 simply move on to the next random location on the list.

Assessing ANPP in grazed ecosystems

Herbaceous productivity in ecosystems managed for grazing is quantified by using small, portable grazing exclosures to estimate the biomass of herbaceous plants consumed by livestock. Grazing

exclosures are placed over an additional random clip-harvest location per sampling bout per plot and height is adjusted by site to ensure that the tallest plants are protected from livestock herbivory (as per Knapp et al. 2007)(**Figure 12**). Grazing exclosures may be constructed from from 6-inch concrete remesh fastened with hog-rings, 6-inch mesh panels fastened to conduit frames, or equivalent construction. Exclosures are sufficiently large such that there is at least a 30 cm buffer from the edge of the exclosure to the edge of the protected clip-strip. Exclosures are staked to the ground with ¼-inch diameter x 15-inch steel tent stakes or equivalent to prevent movement by cattle and bison.

Figure 12

Figure 12. Example of a grazing exclosure used at the Domain 10 CPER site.

Sorting to functional group

In order to align NEON herbaceous functional groups with existing networks and capture anticipated effects of global change drivers on the abundance of key functional traits, NEON has adopted functional groups from both NutNet and LTER protocols. Aboveground herbaceous biomass is therefore sorted into the following categories:

1. Bryophytes
2. Cool season C_3 graminoids (as per Hattersley and Watson 1992)
3. Warm season C_4 graminoids
4. Nitrogen-fixing plants
5. Forbs (non-N-fixing)
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 indicated that field staff 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 is included in the technician training program.

Sample processing

For most clip-harvest bouts, clipped biomass is sorted to functional group while still in the field, and is stored in coolers chilled with re-usable cold packs immediately following harvest. Clipped biomass is re-checked for sorting accuracy by a field botanist lead within 24 hours of clipping, and is transported back to the laboratory in coolers as soon as possible following field collection. Clip-harvests are then dried for a minimum of 48 h at 65 ˚C, and weighed to the nearest 0.01 g.

At grazed sites only the peak biomass bout is sorted to functional group (see Section 6.2.3 for temporal sampling details). Other bouts are not sorted to functional group but are sorted to remove previous years' litter. For bouts not sorted to functional group, subsampling may be employed to speed litter removal, and the size of the subsample is determined analytically on a per site basis via a sorting experiment (10%, 25%, 50% subsample size, etc.).

Aboveground perennial tissues

The aboveground component of perennial graminoid crowns is not 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 are not clipped, as this biomass component either does not contribute to, or contributes only very marginally to ANPP.

Live aboveground bryophyte biomass

NEON focuses on clipping live aboveground bryophyte biomass stocks when these plants are present within a randomly selected clip strip. Aboveground bryophyte biomass is difficult to measure 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). Common challenges include:

1. Difficulty separating live from dead bryophyte biomass when conditions are dry, and
2. The tendency for bryophytes to grow in layers that form a continuum between live plants and the soil organic horizon.

To increase consistency within and among bryophyte clip harvests, NEON works with community experts to develop site-specific training materials focused on clipping and sorting bryophyte biomass. Unlike other functional groups defined here, clipped bryophyte biomass is focused on live total stocks rather than current-year production. Additional site-specific studies by end-users are required to convert bryophyte biomass data into productivity estimates.

Due to the highly hygroscopic nature of these plants, bryophyte biomass is weighed immediately following drying at 65 ˚C, or is placed in a desiccator immediately following drying and prior to weighing (Vitt 2007).

6.2.2 Spatial Distribution of Sampling

Harvests of herbaceous plants occur within stratified-random, spatially-balanced subset of Distributed Plots (n=20 maximum) and in all Tower Plots. For Distributed Plots, clipping only takes place in those Distributed Plots not classified as forest according to NLCD and that are visually estimated to be ≥ 50% herbaceous cover (Table 1). That is, no clipping occurs in Evergreen, Deciduous, or Mixed Forest NLCD types, or in non-forested plots with herbaceous cover < 50%. Within each plot, clipping takes place within randomly located 0.5 m x 3 m 'sampling cells' that are numbered and systematically gridded out across the available sampling areas within the plot (e.g. Figure **4**). Clip 'strips' are then sampled within selected cells, and are laid out as a series of North/South facing rectangles with dimensions of 0.1 m x 2 m. The list of cell numbers is randomized, and selection of strips from year to year proceeds down this randomized list. Prior to randomization, cells that overlap 10 m^2 and smaller 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 collocated plant diversity measurements. For sites that are not managed for grazing, 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 plot or subplot for Tower Plots.

At sites managed for grazing, exclosures are utilized in Tower Plots only as these plots are focused on generating productivity estimates within the dominant vegetation at the site. One exclosure is sampled per bout per 400 m^2 plot or subplot. Exclosures are randomly placed using the list of randomized sampling cells and new random locations are exclosed for each sampling bout.

6.2.3 Temporal Distribution of Sampling

In the Tower Plots, the primary objective is to generate annual estimates of herbaceous biomass and productivity within the dominant vegetation type. In the absence of managed grazing, herbaceous clip harvests typically occur once per year; sites that show seasonally distinct biomass peaks for important C_3 and C_4 functional groups are sampled twice per growing season (Table 1). Sampling onset occurs after the average peak green date in order to capture peak biomass, as defined by MODIS-EVI. A given sampling bout is 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, Tower Plot clip harvests initially 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 are 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.

Distributed Plot sampling occurs once every 5 years per site. Similar to Tower Plots, sampling onset dates at each site are chosen to occur after the period of peak green, as defined by MODIS-EVI. Each sampling bout is similarly concluded within 10-14 days of initiation. Herbaceous biomass Distributed Plot sampling is conducted in the same year as Coarse Downed Wood tally and Vegetation Structure measurement in these same plots (see Section 6.9).

6.2.4 Logistics and Adaptability

Sampling intervals at grazed sites are initially every 4 weeks, and a data-driven approach is employed to optimize sampling intervals on a per site basis. If it is not possible to detect consumption with a 4 week interval following a minimum of 3 years of data collection, an 8 week interval is then scheduled. Should consumption not be detected with an 8-week interval, clip harvests are scheduled either 1 or 2 times per year as for sites not managed for grazing (described above). For the latter scenario, exclosures enable quantifying relative grazing intensity and standing stock biomass rather than productivity. It is anticipated that longer sampling intervals are required at grazed sites that are relatively dry and low-nutrient status where compensatory responses to herbivory may be minimal (e.g., D10 CPER, D13 MOAB, D14 SRER, etc.).

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 5 years and recording only total herbaceous ANPP and biomass in intervening years; 2) reducing and/or consolidating

functional groups to reduce labor required for sorting; and 3) eliminating functional group sorting entirely in either Distributed Plots, Tower Plots, or both, and only recording total herbaceous ANPP and biomass. 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.

6.3 Bryophyte Productivity Sampling Design

Definition: Bryophytes quantified by this sampling design component 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, bryophytes 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 a potentially important component of NEONs vegetation sampling at sites with high cover of bryophytes: namely, tundra and boreal forest systems. Bryophyte ANPP should be measured according to the design described in this section for plots that meet the following criteria:

- **Distributed Plots**: Mat-forming bryophytes ≥ 20% cover (assessed on a per plot basis).
- **Tower Plots**: Mat-forming bryophytes ≥ 20% cover averaged across all plots.

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 clip-harvest methods. Following review of available methods, NEON selected the net-clip method to estimate ANPP of mat-forming bryophytes (Clymo 1970, Russell 1988). Nylon nets with 1 to 1.5 cm mesh cut to 20 cm x 20 cm squares (5 cm buffer around an interior square 10 cm on a side) are anchored to sampling plots prior to snowfall the year before sampling is to occur. Throughout the growing season new vertical growth occurs above the level of the net. At the end of the season bryophytes are clip harvested to the level of the net within the interior 10 cm x 10 cm square. Harvested samples are then 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) are 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 are 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 is 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, the need for customized equipment not commercially available, and the fact that multiple assumptions and conversions are required to convert recorded data to ANPP. 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 need to be fabricated specifically for NEON sampling. Nylon or metal 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 is 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 are anchored to the specified sample locations with standard garden staples or longer stakes if dictated by the vegetation. Anchors are 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 vertical growth occurs 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 is measured at sites where the average percent cover of mat-forming bryophytes is ≥ 20% across all Tower Plots. Bryophyte productivity is not measured in Distributed Plots, as these plots are focused on biomass/standing stocks rather than productivity.

Sample size

At sites where mat-forming bryophytes occur, sampling effort matches that of the herbaceous plant clip harvest strategy. For every 400 m^2 of plot area, annual growth is clipped from five 10cm x 10cm nets situated within a single sampling cell. Clipped bryophytes are pooled across the 5 nets to produce one sample per 400 m^2. At sites where the smallest, 20 m x 20 m plots are employed, this means there is be one net harvest sample per plot per year.

Location of sample points

The location of bryophyte sampling nets is 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. Harvest locations are randomly assigned according to randomized lists of 'cells', as described above for the Herbaceous Biomass sampling design. In this manner, 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. ≥ 20% cover averaged across all Tower plots), ANPP from bryophytes in Tower Plots is estimated annually. Distributed Plots that contain bryophytes are not currently considered for bryophyte productivity sampling.

6.3.4 Logistics and Adaptability

Direct measures of bryophyte productivity are difficult to obtain with a consistent method across sites. The net-clip method selected here depends on several assumptions that must be met in order to generate high-quality data at a given site:

- Vascular plant overstory is sparse or absent.
- Microtopography is minor or absent.
- Bryophyte growth is predominantly vertical rather than lateral.
- Bryophyte growth rates are high enough to be detected over a one year period.

A rapid-response TOS Sampling Technical Working Group (TWG) was formed in 2017 to advise NEON about how to optimize TOS sampling and effectively respond to decreases in available budget while maintaining data quality. The TWG was comprised of external community members familiar with NEON sampling. As part of that exercise, the TOS Sampling TWG recommended discontinuing bryophyte productivity sampling on a site-by-site basis unless NEON could demonstrate the ability to meet each of the four assumptions listed above.

Following implementation of the Bryophyte Productivity protocol (RD[06]) over the 2016/2017 sampling season at all 6 NEON sites with qualifying bryophyte cover, the TOS Plant Biomass and Productivity TWG assessed evidence collected by NEON field staff and evaluated the assumptions listed above. Members of this TWG are also drawn from the external community, and the TWG considered additional feedback from bryophyte experts with experience quantifying bryophyte productivity. The evidence indicated that none of the 6 NEON sites sampled for bryophyte productivity met the assumptions of the net-clip method. The Plant Biomass and Productivity TWG therefore concluded that NEON should discontinue bryophyte productivity sampling and should focus on quantifying bryophyte standing stocks as part of

the Herbaceous Biomass sampling design (see Section 6.2). Bryophyte clip-harvest was discontinued in 2018 following presentation of TWG recommendations to the STEAC (NEON's Science, Technology, and Education Advisory Committee), and after concurrence from the NSF.

6.4 Litterfall and Fine Woody Debris Sampling Design

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 particles with diameter ≥ 2 cm are considered coarse downed wood, and are sampled according to the coarse downed wood sampling design outlined in this document (Section 6.5).

Spatial arrangement and number of litter sampling units within plots

To measure litterfall and fine woody debris, NEON employs 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 associated with the respective trap type is collected (Muller-Landau and Wright 2010). Similar to the Herbaceous Biomass sampling design (Section 6.2), litter sampled from elevated traps is sorted into functional groups following collection. NEON has adapted 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.)

8. Mixed

The mixed category is utilized because NEON has limited sorting to 1 hour per trap per bout; residual litter fragments comprised of multiple functional groups remaining after 1 hour are categorized as 'mixed.' Following sorting and drying, litter is analyzed for lignin, C, N, P, K, Mg, Ca, other elemental "majors", and ^{13}C and ^{15}N isotopes via contracting with selected external laboratories (AD[04]).

Litter collected from ground traps is 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 ≥ 2 cm are discarded. Particles of this size are sampled according to the Coarse Downed Wood sampling design (Section 6.5).

To ensure the accuracy of annual litter production estimates, ground traps are cleared of all relevant litter material following the annual sampling bout. Sorted litter from ground traps is not analyzed for chemical composition.

Laboratory processing

Following collection, litter is 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 is sorted before drying to minimize production of litter fragments that are difficult to identify (Muller-Landau and Wright 2010). The woody portion of litter is dried at 105 °C for a minimum of 48 h to release bound water (Williamson and Wiemann 2010).

Equipment

Design of NEON PVC elevated litter traps was adopted from the Smithsonian Tropical Research Institute, Forest Global Earth Observatory (STRI ForestGEO). Non-oxidizable metal rods (e.g. aluminum, galvanized steel, or equivalent) are used to anchor elevated litter traps in place. The corners of ground traps are 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 has adopted the PVC design used by STRI ForestGEO 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 is more difficult to ensure that laundry baskets are kept level on sloped ground.

6.4.2 Spatial Distribution of Sampling

Only Tower Plots are 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 establishes 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 are placed randomly within each 400 m^2 plot/subplot using randomized sampling 'cell' lists (as described in Section 6.2).

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 targets litter trap placement to areas of the plot with woody cover. Estimates of woody vegetation percent cover are then used to scale litter production from the trap to the plot scale. End-users may derive estimates of woody vegetation percent cover from remote-sensing imagery or from initial site characterization work performed during plot establishment.

Elevated traps

Elevated litterfall traps (70.7 cm x 70.7 cm; 0.5 m^2) are placed at either random or targeted locations within each Tower Plot as discussed above. These traps reliably sample shed leaves, needles, reproductive parts, and fine woody debris with butt-end diameter < 1 cm and length < 50 cm. Once the position is selected, the location of elevated litterfall traps remains constant from year to year.

Ground traps

Paired ground traps are 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 is one randomly selected 0.5 m x 3 m grid cell within the same 400 m^2 plot/subplot as the elevated trap. Ground traps are cleared of all qualifying 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 are sampled. Once selected, ground trap locations do not move from year to year and are 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 is therefore 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 are 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) may be sampled with less frequency than deciduous broadleaf forests, but since there is no clear 'litterfall season,' sampling occurs 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 once every 4 weeks.

Ground traps

Ground traps are sampled once per year in the spring to account for winter production of fine woody material.

6.4.4 Logistics and Adaptability

The primary sampling goal is to enable NEON end-users to calculate litterfall and fine woody debris production on an annual basis. A secondary goal is to parse production into the functional groups defined above, due to the fact that decomposition rate constants, C content, and N content are known to vary significantly across these groups (Bernier et al. 2008). Finally, the litter sampling design ideally should detect year-to-year changes in litter production of 20% or greater. Given these goals, implementation of the litterfall sampling design may be optimized by adjusting spatial sampling parameters, temporal sampling parameters, and/or litter sorting guidelines.

Spatial sampling optimization. Metcalfe et al. (2008) emphasize that litterfall collection efforts often generate high levels of uncertainty and require greater sample size to accurately estimate annual production compared to other components of plant productivity. Two analytical approaches will be used to evaluate the efficacy of the spatial sampling design:

1. A variance partitioning analysis will be used to determine the relative importance of within-plot versus among-plot sampling effort. The initial design established one trap per 400 m^2 plot/subplot, leading to 2 traps per plot within 40m x 40m Tower Plots (two subplots per plot are randomly selected for sampling, see Section 5.3.2.3). This analysis will indicate whether within-plot sampling explains significant variability in litter production on a site-by-site basis. If within-plot litterfall sampling explains relatively little variability in total production for most sites, the Plant Biomass and Productivity TWG will evaluate whether this sampling component should be eliminated. The decision to maintain or discontinue within-plot litterfall sampling will be applied to all NEON sites with 40m x 40m Tower Plots in order to keep the data product standardized across sites.
2. A power analysis will be performed on a site-by-site basis to evaluate whether the current sampling design is 80% likely to detect a 20% change in total litterfall production from year-to-year (alpha = 0.05). In the event that established plot and trap numbers do have sufficient power to detect a 20% change in litter production, additional power analyses will be performed to determine the minimum plot number required to detect said change, and traps will be removed from 'excess' plots in a spatially-balanced manner in order to meet the target sample size.

Temporal sampling optimization. If dictated by logistical or financial constraints, the elevated trap sampling interval may be increased at some sites. The current design of sampling litter every 4 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, increased decomposition and resulting loss of mass, or loss of mass due to wind or large animal/livestock disturbance. Given these considerations, NEON will collect litter from traps with a maximum 8-week sampling interval and this decision will be evaluated by the Plant Biomass and Productivity TWG only if funds are insufficient to maintain sampling on a 4-week interval.

Litter sorting optimization. The current design calls for sorting fresh litter into the specified functional groups prior to oven drying. 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.

Because litter sorting represents the largest labor component with respect to generating data from traps, NEON will evaluate the efficacy of sorting a subset of traps for every bout rather than sorting every trap. Subsample sizes are selected on a per site basis to ensure that subsample derived estimates of leaf, needle, and woody material production are within ± 10% of the mean for the full sample with 95% confidence. Litter from unsorted traps is reported as 'mixed.'

6.5 Coarse Downed Wood Sampling Design

Definition: Coarse downed wood (CDW) is defined as any fallen woody particle with diameter ≥ 2 cm at the point the particle intersects the survey transect. Particles of CDW – 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 of any size class must also be ≥ 1 m length (Harmon and Sexton 1996). Woody particles with diameter < 2 cm is considered fine woody debris and is sampled with litter traps and ground traps (see Section 6.4). Standing dead wood – 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 0).

6.5.1 CDW Mass Estimation: Volume, Decay Class, Species Groups, and Bulk Density Data

NEON collects data that enable estimating CDW mass as part of a broader effort to estimate the size of important biomass pools at the site scale. CDW mass estimates are typically dependent on measuring CDW volume with one sampling design, then coupling volume estimates with CDW bulk density values that are measured according to a separate design (Keller et al. 2004, Valentine et al. 2008). In addition to CDW volume, other parameters that may be of interest to the ecological community include frequency (count ha^{-1}) and aggregate length (m ha^{-1}). In the sections below, we compare the advantages and disadvantages of methods commonly used to estimate these parameters.

6.5.1.1 Volume Estimators for CDW

Line-intersect sampling (LIS) has traditionally been used to estimate CDW volume (Brown 1974). With the LIS method, particles of CDW are encountered and tallied with probability proportional to length of the log. Unbiased estimates of CDW length are produced from simple tallies of particles that intersect the sampling line. When tallied particles are additionally measured for length and diameter, unbiased estimates of CDW 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 CDW estimators, the ideal CDW 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 15 years that satisfy these criteria:

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 a variable-radius plot method that generates an unbiased estimate of CDW 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 CDW 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).

There are a few notable theoretical and practical problems associated with PDS with respect to implementation at NEON sites. 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 CDW volume estimates have priority over frequency and length estimates in the NEON sampling framework, this issue is 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). Large values of D_L are associated with detection errors in the field because exhaustively searching a circle with a radius > 100 m is difficult in complex terrain and/or when if visibility is limited (Ducey et al. 2013). Large limiting distances are also 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 CDW volume at the sampling point (Ducey et al. 2013). The DLPDS method provides an unbiased estimate of CDW 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 CDW 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 CDW 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 CDW 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, CDW sampling methods derived from a probability proportional to volume theoretical basis appear to generate more or equally precise estimates of CDW 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 CDW Decay Classes and Species Groups

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

The choice of decay classes for volume estimation must be consistent with those used for bulk density sampling. 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 is to use categories ranked according to decay-resistance: i.e., species are 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 can 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 CDW logs is required in order to convert CDW 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 several methods described in the literature for generating bulk density estimates from radial cross-sections collected from logs. 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, sampling along the radial distance from the log center to the edge is important for capturing within-log variation in bulk density, and it is also important to adequately sample the transition from heartwood to softwood, and from softwood to bark in order to correctly estimate the density of a log with no internal void volume. To account for internal void volumes and discontinuous portions of decay in different categories, an additional strategy is to collect representative wedges from a cross-sectional disk (Harmon, personal communication). Wedge subsamples are created with volume proportional to decay class within the cross-sectional disk.

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

CDW production

Production of coarse downed wood (i.e. branchfall) is an important component of ANPP (see Equation 2). However, CDW 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 CDW between 2-10 cm diameter is 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 CDW > 10 cm diameter is already accounted for during woody stem mortality surveys.

CDW volume and mass

NEON has adopted the LIDS method for estimating CDW volume, and more specifically, three LIDS transects arranged in a “Y” shape are used per sample point in order to avoid potential problems with non-random log orientation. Initial transect lengths are determined on a site-by-site basis by analyzing the largest likely log diameter from woody vegetation structure site characterization data and by selecting an F-value that results in a maximum transect length of 200 meters. Following the initial bout of tally sampling, F-values and transect lengths are optimized such that 7-9 particles are tallied per plot across all three transects (Affleck 2010).

NEON technicians identify logs to species when possible and group tallied logs into ‘unidentified hardwood’ and ‘unidentified softwood’ categories when identification to taxonomic family cannot be achieved. Compared to categorizing all logs 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 (Harmon, personal communication).

NEON technicians sample bulk density from radial cross-sections of logs using the wedge approach, with equal sampling effort across decay classes; decay classes are defined according to USFS guidelines (Table 3). Disk cross-sections are measured for diameter, height, and fresh weight, and wedges are collected in order to calculate a representative fresh weight to dry weight ratio in the lab (or complete cross-sectional disks are collected when logs are small)(Harmon and Sexton 1996). Bulk density wedge subsamples or disks are then oven-dried at 105 ˚C until constant mass in order to calculate bulk density.

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

Bulk density sampling effort is also 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 are targeted for bulk density sampling, as these logs represent a disproportionately large component of total CDW mass.

In order to ensure that bulk density sampling is focused on the most abundant CDW particles at a given site, NEON assigns each tallied particle to a unique decay class x size category x taxonID combination (DST). DSTs are then ranked by abundance at each site according to tally number across all plots. Starting with the most abundant DSTs, bulk density samples are collected according to rank abundance from those DSTs that comprise up to 80% of the cumulative tally. The remaining 20% of the cumulative tally is made up of DSTs tallied relatively infrequently and is not sampled for bulk density.

Equipment considerations

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

For bulk density determination, cross-sectional disks are cut from downed logs with either a chainsaw or a cross-cut bucksaw. Wedge subsamples are generated using a hatchet. Disks and wedge subsamples are weighed in the field using spring-scales.

6.5.3 Spatial Distribution of Sampling

Tallying of coarse downed wood with LIDS occurs at all Tower Plots at forested sites and up to 20 Distributed Plots per site within forested NLCD classes. To estimate CDW production and volume across the landscape, NEON technicians 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 CDW at these points produces an unbiased estimate of CDW parameters of interest at stand and regional scales (e.g., volume, frequency, length, etc.).

At each point at which CDW volume is tallied, field technicians 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 is randomized on a per plot basis.

Bulk density sampling may take place within NEON plots but is not constrained to NEON plot boundaries (with site host permission). Logs that intersect LIDS transects are not sampled for bulk density, as collecting a bulk density sample from a log shortens the natural length of the log and affects log-level data collected during the tally effort.

6.5.4 Temporal Distribution of Sampling

The production and loss of CDW 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 CDW is lost from the system. Both production and loss processes for CDW 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 is annual, and as noted, occurs as part of measurement of woody vegetation structure (see Section 0). Assessment of species x site-specific bulk density values is scheduled twice per site, the first time during initial site characterization or at the beginning of NEON operations. The second CDW bulk density bout is scheduled 5 years after the first bout, and large logs sampled in the first bout are targeted for repeat sampling in the second bout. When coupled with repeat sampling of individual logs, two bulk density bouts per site enable assessment of the speed with which logs progress through decay classes.

Hoover (2008) recommends assessment of downed CDW 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 measure CDW volume at a given site every 5 years in both Distributed and Tower

Plots, and sampling is staggered through time by plot type such that CDW is implemented at a given site every 2-3 years (see Section 6.9 for a detailed protocol scheduling example).

6.5.5 Logistics and Adaptability

CDW production

Because production of 2-10 cm diameter CDW is heterogeneous in space and time, sample size, sample area, or both may need to be increased in order to detect changes in CDW production from year-to-year of 20% or less. The number of randomly selected grid cells sampled per plot for CDW production can be increased, but increasing sample size in this manner may not be helpful if variation in CDW 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.

CDW stocks

Sampling CDW stocks is not dependent on season due to the slow turnover times of downed logs. Tallying and bulk density sample collection can therefore 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).

6.6 Coarse Roots Sampling Design

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).

NEON enables end-user estimation of coarse root biomass similarly to woody stem biomass. Namely, NEON collects DBH and height data as part of the woody vegetation sampling design (see Section 6.1). End-users may estimate coarse root production 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.

6.7 Fine Root Biomass Sampling Design

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). Given the relationships in Equation 4 (Section 5.3.3), the NEON TOS sampling focuses on generating estimates of fine root biomass (FRB) in Tower Plots. End-users may independently calculate fine root turnover coefficients in order to estimate fine root production (FRP) at NEON sites.

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 or monolith samples (Tierney and Fahey 2007, Burton and Pregitzer 2008). As such, NEON will use the soil coring technique to estimate belowground fine root biomass when possible. In the event that rocks or large roots prevent collecting core samples, monoliths may be collected with a soil knife instead.

Root diameter classes

Fine root production is not equal across fine root diameter classes. Roots < 0.5 mm diameter account 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, when sampling for fine root biomass researchers typically sort roots into various size classes and then calculate FRP separately for each class. Following Burton and Pregitzer (2008), NEON sorts roots within each soil sample into < 0.5 mm, 0.5–1 mm, 1–2 mm, and 2–10 mm categories.

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 employs 30 cm depth cores, so fine root sampling will also be to 30 cm depth in order to generate consistent data across multiple TOS sampling designs. As noted in Section 5, two soil samples per 400 m^2 plot or randomly selected subplot are collected per sampling bout. Roots from each sample are weighed separately, and within size categories, roots from these two soil samples are pooled for chemical analysis. In order to simplify processing, sampled soils are not separated by depth increment or horizon.

6.7.1 Sampling Methods

The standard operating procedure for processing soil samples is sieving to remove mineral particles from roots and soil organic matter (SOM), picking and sorting the resulting root/SOM mixture to isolate roots within various size and live/dead classes, followed by drying, weighing, and chemical analysis of 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 03 Ordway-Swisher (OSBS) 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 processes soil samples via the wet-sieving technique in order to separate roots/SOM from mineral particles. Dry sieving is employed at some desert sites with extremely fine, dry soils that also have fine, brittle roots.

Separating roots from SOM

Once soil samples are sieved to remove the mineral component of the soil, 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, soil samples 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 has adopted a hybrid approach for fine

root biomass sampling. The majority of soil samples are picked to a 1 cm length cutoff, and a spatially-balanced subset of soil samples are subjected to dilution sampling in order to measure 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 are discarded, due to the fact that these roots are "coarse" roots and their biomass can be estimated allometrically from stem DBH measurements. The remaining fine roots < 10 mm diameter are sorted into the size classes described above.

In addition to sorting roots by size, NEON technicians also attempt to distinguish between live and dead roots. Root color and structural integrity are 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 is not 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. However, total fine root biomass (live + dead) is sampled with high confidence.

Drying, processing, and analyses

Following sieving and sorting, roots are dried at 65 ˚C for a minimum of 48 h or until constant weight. Dried roots are ground with a Wiley Mill, and ground sub-samples are analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ (AD[04]). All chemical analyses are contracted with external facilities.

6.7.2 Spatial Distribution of Sampling

Soil samples for estimating fine root biomass are only collected from Tower Plots, and are collocated with herbaceous clip-harvest "cells" in any given year. Within clip-harvest cells, soil samples are collected to the north and south of the 0.1 m x 2 m clip-harvest strip if conditions allow (i.e. roots and rocks do not prevent sampling to the desired depth). 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 soil samples per 400 m^2 Tower Plot/subplot will be sampled per bout. Given the two different Tower Plot establishment schemes (see Section 5.3.2.3), the maximum number of total soil samples per bout is n=60 or n=80. For grazed systems in which exclosures are utilized, soil samples are only collected from grazed locations.

6.7.3 Temporal Distribution of Sampling

Belowground fine root biomass is sampled every 5 years at NEON sites. The exact date of belowground biomass sampling at a site in a given year is guided by two considerations: 1) the date of aboveground clip harvest(s), and 2) seasonal variations in soil hardness. Ideally, belowground biomass sampling is either 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 is therefore linked to the timing of aboveground peak biomass clip harvests or peak canopy biomass/LAI when possible, but is otherwise 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 is an iterative process to determine whether it is logistically feasible to adjust sample size such that detecting a 20% year-to-year change in fine root biomass is possible.

6.8 Leaf Area Index Sampling Design

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 employs a two pronged approach for measuring LAI: 1) temporally intensive measurements at a small number of Tower Plots; and 2) spatially-extensive measurements at a large number of Distributed Plots.

Methodological considerations

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 are 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 are particularly problematic in the tall, structurally complex forests of the Pacific Northwest (i.e., the Domain 16 Wind River site).

Plot layout across the landscape

Various plot 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 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 does maximize co-

location among data products at the plot scale, and also generates an unbiased estimate of LAI at the site scale.

For temporally intensive LAI data collection, measurements are made at three Tower Plots that are chosen to minimize access and travel times from the nearest road. 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. In the event that multiple NLCD vegetation types exist within the NEON Tower footprint, plots are selected from the dominant vegetation type.

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). However, it should be noted 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 Digital Hemispherical Photo (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 DSLR 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 raw data generated by the equipment;

3) Train technicians to analyze the raw data produced by the instruments in an accurate and repeatable manner; and

4) Create “best value” in terms of price and data quality.

6.8.1 Sampling Methods

NEON selected an optically-based indirect DHP method to measure LAI, due to the fact that it is possible to collect comparable data with this method at all NEON sites. DHP systems can 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). In contrast, 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 have been 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 collects 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 4). 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

NEON serves DHPs to end-users via the Data Portal and does not analyze DHPs for LAI values. End-users are free to develop image analysis algorithms or employ freely-available software packages to generate LAI from NEON collected DHPs.

Despite the difficulties inherent to DHP analysis, 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 new software analysis options be developed by the community.

6.8.2 Spatial Distribution of Sampling

LAI is measured in a spatially-balanced stratified-random subset of n=20 Distributed Plots, and in n=3 Tower Plots per site. Tower Plots are 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 and NEON has sufficient resources for Gradient Plot establishment. The exact location of Gradient plots will be determined using AOP-derived site-scale 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 collected from Distributed Plots is designed to enable ground-validation of AOP LAI algorithms. For the spatially extensive ground sampling effort, it is therefore 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 03 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 13) (Kampe et al. 2011). Based on these data, NEON collects spatially-extensive ground LAI data at each site within a 1 month window that includes the actual AOP flight date. Distributed Plot datasets of this nature are collected every 5 years per site.

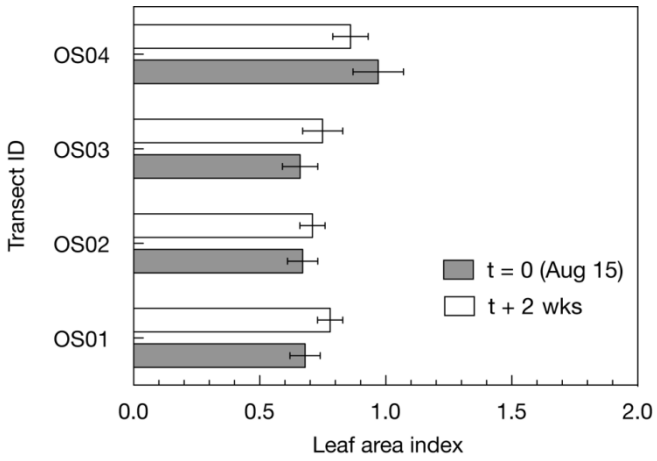

Figure 13. Leaf area index data collected with an LAI-2200 at the Domain 03 Ordway-Swisher core site, from the same four 500 m length transects at two different time points.

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 image 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 an algorithm 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, it will be possible to serve LAI estimates directly via the NEON Data Portal.

6.9 Scheduling Integrated Plant Biomass and Productivity Sampling

Protocols derived from this Plant Biomass and Productivity Science Design document are integrated in space and time with other TOS plant and soil sampling designs in order to maximize the scientific potential of the data products (AD[04], AD[06], AD[07]).

Table 4 illustrates that two groups of biomass, productivity, biogeochemistry, and diversity protocols are implemented every 5 years (grey shaded cells). The two groups are staggered through time to enable balanced sampling effort across funding years at the Observatory scale and to enhance knowledge retention among staff. These protocols are implemented predominantly in the same Distributed Plots (i.e., the protocols are spatially collocated) and Tower Plots are utilized for plant belowground biomass sampling and litter chemistry sampling.

Tower Plots support annual sampling for a subset of protocols, and annual sampling of Vegetation Structure within a subset of plots at sites with relatively fast growth (

Table 4, brown cells). Tower Plots also support sampling CDW every 5 years, and sampling all plots for Vegetation Structure (VST) every 5 years (

Table 4, orange cells). Sites with relatively small annual woody growth increment are not currently measured annually for VST within a subset of plots; Tower Plots at these 'slow-increment' sites are all measured every 5 years (**Table 6**). NEON is investigating whether available budget can support installation of dendrometer bands and annual measurement of VST within a subset of Tower Plots at slow-increment sites.

For CDW and VST, Distributed Plots and all Tower Plots are sampled every 5 years, and sampling by plot type is staggered through time (**Table 4**). Temporal staggering ensures that CDW and VST data are generated from a site every 2-3 years.

Site vegetation must meet criteria defined in TOS Science Design documents in order for a protocol to be implemented at a given site. Not all protocols listed in

Table 4 are therefore implemented at every site. See APPENDIX B for a list of Plant Biomass and Productivity protocol implementation by site.

Table 4. Integration of NEON TOS plant and soil sampling protocols through time. Years 1 through 7 are shown to illustrate the temporal grouping of protocols, and the pattern repeats beyond year 7.

Protocol Name	Interval (y)	Plot Type	Plot Number	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
Plant Belowground Biomass	5	tower	20 or 30†	X					X	
Canopy Foliar Chemistry	5	both	16-20	X					X	
Plant Diversity	1	distributed	30	X	X	X	X	X	X	X
Leaf Area Index	5	distributed	20	X					X	
Litterfall (chemistry)	5	tower	20 or 30†	X					X	
Soil nitrogen mineralization	5	both	10	X					X	
Soil chemistry	5	both	10	X					X	
Soil microbial biomass	5	both	10	X					X	
Coarse Downed Wood	5	distributed	20		X					X
Herbaceous Biomass clip	5	distributed	20		X					X
Vegetation Structure	5	distributed	20		X					X
Herbaceous Biomass clip	1	tower	20 or 30†	X	X	X	X	X	X	X
Leaf Area Index	1	tower	3	X	X	X	X	X	X	X
Litterfall	1	tower	20 or 30†	X	X	X	X	X	X	X
Vegetation Structure	1	tower	5-10‡	X	X	X	X	X	X	X
Coarse Downed Wood	5	tower	20 or 30†				X			
Vegetation Structure	5	tower	20 or 30†					X		

† All Tower Plots are sampled; total number of Tower Plots established at sites is either 20 or 30.

‡ A spatially-balanced subset of Tower Plots are selected for annual VST sampling at sites with relatively fast woody growth increment. See Table 6 for VST fast/slow growth increment classification by site.

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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 5. 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

APPENDIX B SUMMARY OF BIOMASS AND PRODUCTIVITY SAMPLING BY SITE

Table 6. Implementation of NEON TOS plant biomass and productivity sampling protocols by site. A 'Y' indicates protocol implementation and 'N' indicates not implemented. For the **Vegetation Structure** column, shaded cells indicate slow growth-increment sites that are measured every 5 years only and do not currently support annual measurement.

Domain: Site	Vegetation Structure	Herbaceous Clip Harvest	Litterfall	Coarse Downed Wood	Fine Root Biomass	Leaf Area Index
D01: BART	Y	Y	Y	Y	Y	Y
D01: HARV	Y	Y	Y	Y	Y	Y
D02: BLAN	Y	Y	Y	Y	Y	Y
D02: SCBI	Y	Y	Y	Y	Y	Y
D02: SERC	Y	Y	Y	Y	Y	Y
D03: DSNY	Y	Y	N	Y	Y	Y
D03: JERC	Y	Y	Y	Y	Y	Y
D03: OSBS	Y	Y	Y	Y	Y	Y
D04: GUAN	Y	Y	Y	Y	Y	Y
D04: LAJA	Y	Y	N	Y	Y	Y
D05: STEI	Y	Y	Y	Y	Y	Y
D05: TREE	Y	Y	Y	Y	Y	Y
D05: UNDE	Y	Y	Y	Y	Y	Y
D06: KONA	N	Y	N	N	Y	Y
D06: KONZ	Y	Y	Y	Y	Y	Y
D06: UKFS	Y	Y	Y	Y	Y	Y
D07: GRSM	Y	Y	Y	Y	Y	Y
D07: MLBS	Y	Y	Y	Y	Y	Y
D07: ORNL	Y	Y	Y	Y	Y	Y
D08: DELA	Y	Y	Y	Y	Y	Y
D08: LENO	Y	Y	Y	Y	Y	Y
D08: TALL	Y	Y	Y	Y	Y	Y
D09: DCFS	N	Y	N	N	Y	Y
D09: NOGP	N	Y	N	N	Y	Y
D09: WOOD	N	Y	N	N	Y	Y
D10: CPER	Y	Y	N	N	Y	Y
D10: STER	N	Y	N	N	Y	Y
D10: RMNP	Y	Y	Y	Y	Y	Y
D11: CLBJ	Y	Y	Y	Y	Y	Y
D11: OAES	N	Y	N	N	Y	Y
D12: YELL	Y	Y	Y	Y	Y	Y
D13: MOAB	Y	Y	N	N	Y	Y
D13: NIWO	Y	Y	Y	Y	Y	Y
D14: JORN	Y	Y	N	N	Y	Y
D14: SRER	Y	Y	Y	N	Y	Y
D15: ONAQ	Y	Y	N	N	Y	Y

Domain: Site	Vegetation Structure	Herbaceous Clip Harvest	Litterfall	Coarse Downed Wood	Fine Root Biomass	Leaf Area Index
D16: ABBY	Y	Y	Y	Y	Y	Y
D16: WREF	Y	Y	Y	Y	Y	Y
D17: SJER	Y	Y	Y	Y	Y	Y
D17: SOAP	Y	Y	Y	Y	Y	Y
D17: TEAK	Y	Y	Y	Y	Y	Y
D18: BARR	N	Y	N	N	Y	Y
D18: TOOL	N	Y	N	N	Y	Y
D19: BONA	Y	Y	Y	Y	Y	Y
D19: DEJU	Y	Y	Y	Y	Y	Y
D19: HEAL	Y	Y	Y	N	Y	Y
D20: PUUM	Y	Y	Y	Y	Y	Y