

NEON TERRESTRIAL OBSERVATION SYSTEM: SPATIAL AND TEMPORAL SAMPLING STRATEGY

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1 DESCRIPTION

1.1 Purpose

NEON's Terrestrial Observation System (TOS) is comprised of the observational measurements of a diversity of taxa and soils at NEON's 47 terrestrial sites (AD[01]). The purpose of this document is to provide a cross-protocol summary of the spatial and temporal sampling strategies of the TOS. The summary is intended to facilitate understanding of the complete TOS spatial layout at a site, the distribution of sampling through time, both intra- and inter-annually, and the linkages among protocols. This information is expected to be useful for both the NEON end users as well as NEON Field Operations.

1.2 Scope

The information contained herein applies to the NEON TOS only. It is not intended to provide explicit instruction or guidance for protocol execution, but, rather, provide a convenient summary of all TOS activities across space and time. The scientific rationale underpinning all aspects of the TOS sampling design can be found in the TOS Science Design documents (RD[04] – RD[14]), and the detailed protocols executed by NEON field staff are found in the TOS Protocol and Procedure documents (RD[15] – RD[29]).



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

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

Ref #	NEON Doc Number	NEON Document Name
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.000906	TOS Science Design for Terrestrial Biogeochemistry
RD[05]	NEON.DOC.000907	TOS Science Design for Plant Phenology
RD[06]	NEON.DOC.000908	TOS Science Design for Terrestrial Microbial Diversity
RD[07]	NEON.DOC.000909	TOS Science Design for Ground Beetle Abundance and Diversity
RD[08]	NEON.DOC.000910	TOS Science Design for Mosquito Abundance, Diversity and Phenology
RD[09]	NEON.DOC.000911	TOS Science Design for Vectors and Pathogens
RD[10]	NEON.DOC.000912	TOS Science Design for Plant Diversity
RD[11]	NEON.DOC.000913	TOS Science Design for Spatial Sampling
RD[12]	NEON.DOC.000914	TOS Science Design for Plant Biomass and Productivity
RD[13]	NEON.DOC.000915	TOS Science Design for Small Mammal Abundance and Diversity
RD[14]	NEON.DOC.000916	TOS Science Design for Breeding Bird Abundance and Diversity
RD[15]	NEON.DOC.000481	TOS Protocol and Procedure: Small Mammal Sampling
RD[16]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[17]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Sampling
RD[18]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[19]	NEON.DOC.001711	TOS Protocol and Procedure: Coarse Downed Wood
RD[20]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[21]	NEON.DOC.014038	TOS Protocol and Procedure: Plant Belowground Biomass Sampling
RD[22]	NEON.DOC.014039	TOS Protocol and Procedure: Measurement of Leaf Area Index
RD[23]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
RD[24]	NEON.DOC.014041	TOS Protocol and Procedure: Breeding Landbird Abundance and
		Diversity
RD[25]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling
RD[26]	NEON.DOC.014045	TOS Protocol and Procedure: Tick and Tick-Borne Pathogen Sampling
RD[27]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Physical, Chemical, and Microbial
		Measurements
RD[28]	NEON.DOC.014049	TOS Protocol and Procedure: Mosquito Sampling
RD[29]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling



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2.3 Acronyms

Acronym	Definition
AOP	Airborne Observation Platform
AOS	Aquatic Observation System
NEP	Net Ecosystem Productivity
NLCD	National Land Cover Database
NPP	Net Primary Productivity
TIS	Terrestrial Instrument System
TOS	Terrestrial Observation System

3 INTRODUCTION TO THE TERRESTRIAL OBSERVATION SYSTEM (TOS)

NEON's Terrestrial Observation System (TOS) is one of five measurement subsystems of the Observatory. The TOS is comprised of the observational measurements of a diversity of taxa and soils at NEON's 47 terrestrial sites distributed throughout 20 ecoclimatic domains (AD[01], Kao et al. 2012, Thorpe et al. 2016). The NEON TOS is designed to quantify the impacts of climate change, land use, and biological invasions on terrestrial ecosystems by sampling key groups of organisms (sentinel taxa), pathogens, and biogeochemical constituents (Kao et al. 2012). The sentinel taxa are indicators of change in the environment and were selected to include organisms with varying life spans and generation times and that are relatively abundant and widespread to allow for standardized comparisons across the continent. These taxa also play important roles in ecosystem function and are key contributors to other measurements of interest (e.g., productivity and/or infectious disease).

Sampling of organisms and soil conducted by the NEON TOS captures a number of different aspects of terrestrial ecology, including biodiversity and phenology of native and invasive species, biomass and productivity, stoichiometry, genomics, and pathogen prevalence. Not all of these aspects are captured for every taxon: NEON requires an economical strategy because many of the biological observations remain labor intensive and expensive to obtain. More specifically, the NEON TOS monitors:

- Biodiversity of breeding landbirds, small mammals, vascular plants, mosquitoes, ground beetles, and soil microbiota (e.g., Hoekman et al. 2016, Hoekman et al. 2017).
- The timing and duration of phenological events in plant and mosquito communities (Elmendorf et al. 2016, Hoekman et al. 2016).
- Biomass, productivity, and biogeochemistry via collocated measurements of plant structure, biomass production and stocks, soil and plant biogeochemistry, and microbial diversity, abundance, and biomass (e.g., Hinckley et al. 2016).
- Vectors and pathogens to provide data on specific mosquito- (e.g., West Nile virus, dengue viruses), tick- (e.g., *Borrelia burgdorferi*, etiological agent of Lyme disease) and rodent-borne pathogens (e.g., hantaviruses) (Springer et al. 2016).

Finally, the TOS biorepository includes organism and soil samples collected and stored to enable future research by the NEON end-user community (Thorpe et al. 2016).

Table 1 summarizes TOS sampling with respect to plot number (spatial sampling) and sampling interval (temporal sampling). Sections 4 and 5 that follow provide greater insight into the spatial and temporal framework that organizes TOS sampling and that integrates the TOS with the AOP, AOS, and TIS measurement platforms.



Table 1. Summary of NEON TOS spatial and temporal sampling strategies by plot type and plot subtype.

			Plot	Sampling
Plot Type	Plot Sub-Type	Protocol	Number	Interval (y)
Distributed	Base plot	Canopy Foliar Chemistry	10-16	5
Distributed	Base plot	Coarse Downed Wood	20 (max)	5
Distributed	Base plot	Ground Beetle Sampling	10	annual
Distributed	Base plot	Herbaceous Biomass and Productivity	20 (max)	5
Distributed	Base plot	Leaf Area Index	20	5
Distributed	Base plot	Plant Diversity	30 (max)	annual
Distributed	Base plot	Soil physical, chemical, and microbial	6	annual or
		measurements		every 5 y*
Distributed	Base plot	Vegetation Structure	20 (max)	5
Distributed	Bird grid	BreedingLandbird Abundance and Diversity	$5 - 10^{\Psi}$	annual $^{\Omega}$
Distributed	Mammal grid	Small Mammal Sampling	3 - 8	annual
Distributed	Mosquito point	Mosquito Sampling	10	annual
Distributed	Tick plot	Tick and Tick-borne Pathogen Sampling	6	annual
Tower	Base plot	Plant Belowground Biomass	20 or 30	5
Tower	Base plot	Canopy Foliar Chemistry	4	5
Tower	Base plot	Coarse Downed Wood	20 or 30	5
Tower	Base plot	Herbaceous Biomass and Productivity	20 or 30	annual
Tower	Base plot	Leaf Area Index	3	annual
Tower	Base plot	Litterfall	20 or 30	annual
Tower	Base plot	Plant Diversity	3	annual
Tower	Base plot	Soil physical, chemical, and microbial	4	annual or
		measurements		every 5 y*
Tower	Base plot	Vegetation Structure	20 or 30	annual or
				every 5 y†
Tower	phenology	Plant Phenology	1	annual
	loop‡			
Tower	phenocam‡	Plant Phenology	1	annual

* Soil microbial community composition is sampled annually; soil chemistry, microbial biomass, and N-mineralization are sampled every 5 y.

 $^{\Psi}$ Breeding landbirds are sampled in grids at large sites; otherwise they are sampled at up to 25 Distributed Base Plots.

 $^{\Omega}$ Breeding landbirds are only sampled every 3 years in Hawaii, due to permitting restrictions.

⁺ Mesic sites with relatively fast growth-increment are sampled annually at a subset of plots, and all plots are sampled every 5 years; at relatively slow growth-increment sites all plots are sampled every 5 years.

[‡] Phenology loops are established adjacent to Distributed plots rather than Tower plots at D10 RMNP, D12 YELL, and D20 PUUM sites due to boundary and site host restrictions, and there is no phenocam plot at D20 PUUM.



4 THE NEON TOS SPATIAL SAMPLING STRATEGY

4.1 Introduction

Many of NEON's biological measurements will enable inference at regional and continental scales utilizing statistical or process-based models. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Within each NEON terrestrial site, TOS sampling is allocated at two different sampling scales: 1) the site scale, and 2) the scale of the 'airshed' – i.e., the comparatively smaller land-surface area within the site that is the source of the flux data collected by the NEON TIS tower (**Figure 1**).

At the site scale, the TOS has developed a site-level spatially-balanced, stratified-random sampling design that can be applied consistently across NEON domains (RD[11], Theobald et al. 2007). According to this design, plots are allocated across the landscape in proportion to the area of extant NLCD cover types (RD[11], Fry et al. 2011). With plot numbers proportional to area, many of the specific TOS sampling efforts are collocated with each other to allow comparison of data streams from multiple sampling efforts. Sampling has been optimized to ensure efficient and effective sampling within the budgeted scope of NEON. For example, soil nitrogen mineralization incubations are performed on subsamples from the same parent soil samples from which microbial community composition and microbial biomass are measured. TOS data collected at the site scale originate from Distributed plots (**Table 1**, and see RD[11] for additional spatial design information).

Within the tower airshed, a spatially-balanced randomized sampling design is employed. Although the tower plots are not stratified by NLCD class, most tower airsheds encompass only one NLCD class. Similar to site scale sampling, TOS sampling efforts within the tower airshed are collocated with each other to spatially integrate data streams from multiple sampling efforts. TOS data collected within the tower airshed originate from Tower plots (**Table 1**).

Sections below provide detailed information about TOS spatial designs, Distributed and Tower plot types, and how TOS sampling is collocated within and across plots. For complete sampling information by protocol see references in Section 2.2.







4.2 Unbiased Parameter Estimates

4.2.1 Distributed Plots and Site-Level Parameter Estimates

Distributed plots are established according to a stratified-random and spatially balanced design in an effort to collect data from organisms and soils from locations distributed throughout the dominant NLCD vegetation types at each terrestrial NEON site (RD[11], **Figure 1**). The sampling frame for each site is the boundary that encompasses the area NEON is permitted to sample and the area covered by NLCD cover types that comprise ≥ 5% of the permitted land surface area. NLCD cover types that are < 5% of total land surface area are not sampled by the TOS.

For most TOS data products generated from Distributed plots, sampling is allocated across NLCD cover types proportional to NLCD area. This design allows end-users to calculate the site-level mean for parameters of interest using simple averages for most data products, and unbiased parameter estimates can be made without explicitly accounting for NLCD area (RD[11]). Plant Diversity sampling is unique among the TOS protocols in that sampling is allocated proportional to the square root of NLCD area, rather than directly proportional to area. To calculate unbiased, site-level parameter values derived from TOS Plant Diversity sampling data, end-users must explicitly account for the area of NLCD vegetation types in order to properly weight plot-level data when scaling up to the site.

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Distributed plots are established either as 'base' plots that support multiple TOS protocols, or as protocol-specific point and grid locations that are adjacent to base plots. A maximum of 30 Distributed base plots is established at a site, and some sites may have fewer for logistical reasons (e.g., small site area). Plant Diversity sampling occurs in all Distributed base plots, and collocation of plant and soil sampling occurs in a subset of these plots to maximize the scientific value of these data streams. Ground beetle sampling takes place within a subset of Distributed base plots, and sampling locations adjacent to a subset of the Distributed base plots to maximize the scientific sampling locations adjacent to a subset of the Distributed base plots that also support beetle sampling (**Figure 1**, **Table 1**). There are additional constraints on mosquito and small mammal sampling locations which must be relatively close to roads for logistical reasons.

Most TOS protocols only utilize a subset of Distributed base plots, as noted above. When a subset of plots is needed for a particular TOS protocol, the subset is selected from each NLCD class according to a spatially balanced algorithm. In this manner, subsets of plots also generate unbiased, site-level parameter estimates.

Distributed Base Plots

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Distributed base plots are square multiscale plots designed to support multiple TOS protocols, and these plots inherit many of the features of 'Peet' plots designed for the North Carolina Vegetation Survey (Peet et al. 1998) (**Figure 2**). High-resolution spatial data are collected at the plot centroid and all plot corners. At most sites, the inner southwest corner and the plot centroid are marked with permanent 'primary' aluminum monument markers. Other corners are marked with a 'secondary' PVC marker when permitted (secondary marker material may vary by site).



Figure 2. (A) Plot layout for a 40m x 40m Distributed base plot. The inner 20m x 20m core is surrounded by a 10 m 'high-impact' sampling area that is used for soil microbial and soil biogeochemical sampling. The four points at the cardinal directions on the perimeter (double circle) represent the four pitfall traps placed at a subset of plots for Ground Beetle sampling; three of the four trap locations per plot are typically used for beetle sampling (RD[29]). Light grey numbers are 10m x 10m subplotIDs, and blackitalic numbers are nested subplotIDs. (B) Distributed base plot with pointIDs corresponding to those for which high-resolution TOS Spatial Data are provided. Same plot shown in (A) but sampling area detail removed for clarity.

Distributed base plots are 40m x 40m, and feature a 20m x 20m 'core' sampling area surrounded by a 10m 'high-impact' sampling area (**Figure 2**). The high-impact area is used for collection of soil biogeochemistry and soil microbe samples, and may also be available to NEON end-users who wish to collocate externally funded research with NEON measurements at the plot scale. Ground beetle traps are installed around the perimeter of the plot. The 20m x 20m core supports collocated plant diversity, herbaceous clip-harvest, leaf area index (LAI), vegetation structure, and plant canopy foliar chemistry sampling. Nested subplots are utilized for multi-scale plant diversity sampling and for standardizing woody vegetation structure sampling effort (see RD[16] and RD[25] for details).

The Distributed base plot core is gridded into 3m x 0.5m 'cells' to ensure that herbaceous biomass clipharvest sampling occurs at unbiased, representative locations within each base plot (**Figure 3**). Cells are randomized and a new random cell is sampled for each herbaceous clip-harvest bout. Cells that overlap 10 m² and smaller nested subplots are omitted from herbaceous clip-harvest sampling in order to preserve the integrity of plant diversity data from these sampling locations.



Figure 3. The 20m x 20m 'core' of a Distributed base plot, gridded into 3m x 0.5m sampling 'cells' that support TOS Herbaceous Biomass clip-harvest sampling within 2m x 0.1m clip-strips. Cells that overlap 10 m² and smaller nested subplots are not clip-harvested; for clarity, only 10 m² nested subplots are shown.

Distributed base plots also anchor site-level transect-based tallies of coarse downed wood (CDW) particle volume (RD[19]). Three randomly oriented CDW transects radiate outward from the centroid in a 'Y' shape (as described in Affleck 2008, Affleck 2010), and transects typically extend well outside plot boundaries (up to 200 m). Transect origins are 3 m from the actual centroid to ensure that plant diversity measurements from nested subplots near the centroid are not compromised. Coarse Downed Wood bulk density samples may be collected from within the entire 40m x 40m area, and may also be collected outside plots given site host permission.

Distributed Bird Grids

Bird grids (5-10 grids per site) are 500m x 500m and consist of 9 points separated by 250m and arranged in a square (**Figure 4**). Bird grid centers are allocated proportional to the NLCD cover types within the site sampling boundary, with a minimum of 50% of the grid falling within the target NLCD cover type. Where possible, Bird grids are collocated with Distributed base plots by placing the Bird grid center (**Figure 4**, point B2) in close proximity to the center of the base plot. At smaller sites, a single point count is done at the south-west corner of the Distributed base plot (point 21; **Figure 2B**) and grids are not established. Bird grids may also be collocated with Mammal grids, because Bird and Mammal grids are ideally collocated with the same Distributed base plots whenever possible. Similarly, a subset of Bird grids is collocated with Tick plots. See RD[14] and RD[24] for more detail.

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Figure 4. Design of the bird point count grid, consisting of 9 points separated by a minimum of 250 m. The center of the grid is ideally offset from a Distributed base plot (green circle), where plants, soils, microbes, and insects may also be sampled.

Distributed Mammal Grids

Mammal grids (3–8 per site) are 90m x 90m, with 100 trapping locations separated by 10m (**Figure 5**). Mammal grids are allocated proportional to the NLCD cover types within the site sampling boundary, with a minimum of 50% of the Grid falling within the target NLCD cover type. Due to the equipment and time required to complete sampling, the center of these grids (trap location E5) is not more than 300m from roads that can be accessed by NEON field staff. Where possible, these grids are collocated with Distributed base plots by placing them a specified distance (150m +/- 50m) and random direction from the center of the base plot. When fewer than 6 Distributed base plots are within 300m of roads, the Mammal grid centers are placed at a random azimuth and specified distance (150m +/- 50m) from the next available sample location from the ordered plot list that are within 300m of roads (see RD[13] and RD[15] for details). Mammal grids may also be collocated with Bird grids and Tick plots because Mammal grids, Bird grids, and Tick plots are ideally collocated with the same Distributed base plots whenever possible. See RD[13] and RD[15] for more detail.

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Figure 5. Schematic of small mammal trapping grids. One Sherman trap is placed at each point in the grid. Where permitted, a permanent marker is placed at coordinate E5, indicated by the red star.

Distributed Mosquito Points

Mosquito points (10 per site) are the points at which CO₂ traps are established. Mosquito points are allocated proportional to the NLCD cover types within the site sampling boundary. Due to the frequency of sampling and temporal sampling constraints, Mosquito points are typically located within 45m of roads accessible to sampling by NEON field staff, with a standard minimum distance of 5m from the road (see **Figure 1**). Due to the required proximity to roads, Mosquito points are often not collocated with other TOS Distributed base plots and grids. See RD[08], RD[28], and Hoekman et al. (2016) for more detail.

Distributed Tick Plots

Tick plots (6 per site) are 40m x 40m plots that are collocated with Distributed Base Plots (see **Figure 1**) and are allocated proportional to the NLCD cover types within the site sampling boundary. To reduce the probability that the sampling activities associated with Distributed base plots impact tick diversity and distribution (e.g., technicians inadvertently attracting or redistributing ticks), the Tick plot center is offset from the base plot center according to a specified distance (150m +/- 15m) and a randomly chosen direction. Tick sampling occurs by dragging a large cloth around the perimeter of the plot for a total sampling distance of 160 m (see RD[09], RD[26], and Springer et al. 2016 for more detail).

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4.2.2 Tower Plots and Parameter Estimates within the Tower Airshed

TOS Tower plots are established either as relatively small 'base' plots that support multiple TOS protocols, or as relatively large plots around the perimeter of which plant phenology observations are recorded (**Figure 6**).



Figure 6. Schematic illustrating two types of Tower Plots: Tower base plots (grey squares) and Tower phenology plots (large blue squares). Tower phenology plots consist of a primary loop (lower left) and a phenoCam plot (top right). The primary Tower airshed is indicated by the two yellow vectors that originate from the Tower (yellow triangle). The sampling area on the phenology loop is 10 meters to either side of the 200 m x 200 m perimeter, and the sampling area in the phenocam plot is a 200 m x 200 m area extending North from the southern border of the plot. Image not drawn to scale.

The locations of Tower base plots are determined according to a spatially balanced, random design (RD[11]). This sampling design ensures that end-users may readily calculate mean values of parameters of interest using simple averages. Measurements in Tower base plots are focused primarily on aboveand below-ground plant productivity measurements, and a small subset of Tower plots also supports soil and plant biogeochemistry, soil microbial community diversity and function, and plant diversity sampling (3 or 4 plots maximum, **Table 1**).

Tower Base Plots

NEON has established two Tower base plot configurations dependent on vegetation type:

- 1. Short-stature grassland and shrubland ecosystems: 20m x 20m plots, n=30 (max), maximum total sampled area = 1.2 hectares.
- Tall-stature forest and savannah ecosystems: 40m x 40m plots, n=20 (max), two 20m x 20m subplots per plot randomly selected for measurement, maximum total sampled area = 1.6 hectares.

Short-stature Grassland and Shrubland Ecosystems

At sites dominated by short-stature vegetation, a subset of plots (n=4) are identical to Distributed base plots and consist of a 20m x 20m 'core' surrounded by a high-impact area used for collocated soil biogeochemical and microbial sampling (**Figure 2**). Plant Diversity sampling takes place in 3 of these 4 plots in order to spatially collocate multiple TOS data products at the plot scale. The remainder of the Tower plots at short-stature sites lack the high-impact sampling area and are identical to the Distributed base plot 20m x 20m core (up to n=26, **Figure 7**); these plots support collocated above- and belowground plant biomass, productivity, and plant biogeochemistry measurements only. Each 10m x 10m subplot contains nested subplots that may be utilized to standardize the vegetation structure sampling effort for relatively small woody individuals (see RD[12] and RD[16] for more detail).



Figure 7. Plot layout for 20m x 20m Tower base plots. The plot lacks the 10m 'high-impact' sampling area that is used for soil microbial and biogeochemical sampling. Light grey numbers are 10m x 10m subplotIDs, and black italic numbers are nested subplotIDs. Plant sampling cells (3m x 0.5m) exist as in Figure 3 and are omitted here for clarity.

Within Tower base plots, above-ground herbaceous biomass and below-ground fine root biomass sampling are further collocated at the scale of the sampling 'cell' (**Figure 8**). Sampling cells are also used to randomly locate both elevated and ground litterfall sampling traps (RD[12] and RD[18]), but litterfall traps are collocated with other data streams at the level of the plot, rather than the sampling cell.



Figure 8. A 20m x 20m Tower base plot showing 3m x 0.5m sampling cells used for below-ground fine root biomass, herbaceous biomass, and litterfall sampling. Cells that overlap 10 m² nested subplots are not sampled, and all other nested subplots are omitted for clarity. Belowground fine root biomass soil sampling is collocated with herbaceous biomass clip-harvesting in the same sampling cells when possible.

Tall-stature Forest and Savannah Ecosystems

At sites dominated by tall-stature vegetation, all Tower plots are 40m x 40m and are comprised of four 20m x 20m subplots. Within each plot, two of the four 20m x 20m subplots are randomly selected for sampling (see RD[12] for more detail). Each subplot contains nested subplots that may be utilized to standardize the vegetation structure sampling effort for relatively small woody individuals (**Figure 9**, see RD[12] and RD[16] for more detail).

Tower plots in tall-stature vegetation are gridded into sampling cells similar to plots in short-stature vegetation (**Figure 9**). Sampling cells are used to collocate above-ground herbaceous biomass clipharvests and below-ground fine root biomass sampling. Litterfall traps are also randomly located using sampling cells.



Figure 9. Plot layout for a 40m x 40m Tower base plot. Within each plot, two of the four 20m x 20m subplots are randomly selected for sampling. Light grey numbers are 20m x 20m subplotIDs, and black italic numbers are nested subplotIDs. Plant sampling cells (dashed blue rectangles) support multiple plant biomass and productivity protocols and are omitted from 3 of the 4 subplots for clarity. Plant diversity and soil sampling areas are not depicted here (see Figures 2 and 10).

Similar to short-stature ecosystems, a subset of 40m x 40m Tower base plots support collocated soil biogeochemical and microbial sampling (n=4). Soil samples are collected from the area of the plot that would be the 'high-impact' sampling area in a typical Distributed base plot (**Figure 2**). Plant Diversity sampling takes place in 3 of these 4 plots in order to spatially collocate multiple TOS data products at the plot scale. In these 3 plots, Plant Diversity measurements occur in a 20m x 20m 'core' in order to maintain consistency with Plant Diversity data collected from Distributed base plots (Figure 10).



Figure 10. Plot layout for a subset of 40m x 40m Tower base plots that support Plant Diversity sampling. Note that a standard 20m x 20m Distributed base plot 'core' is superimposed over the centroid; this configuration allows for standardized Plant Diversity sampling to occur in a randomly selected subset of 40m x 40m Tower Plots.

Tower Phenology Plots

The majority of plant phenology observations are made on individuals located around an opportunistically placed 200m x 200m loop established adjacent to the NEON tower (**Figure 6**). Because the towers themselves are typically located within the dominant NLCD vegetation type at NEON sites, the phenology design produces phenophase and phenophase intensity data relevant to the dominant site vegetation. The sampling area on the phenology loop is 10 meters to either side of the perimeter. The exact placement of the loop is adjusted such that the number of mapped and tagged trees within Tower base plots that are also observed for phenology is maximized. See RD[05], RD[23], and Hoekman et al. (2016) for more detail.

A second 200m x 200m 'phenocam plot' is also established for plant phenology observations in the event that the mid-point of the loop's southern edge is not centered within the north-facing view of the tower's phenocam.

4.3 Spatial Collocation of TOS Sampling

Numerous TOS measurements are collocated across multiple spatial scales, as indicated in the previous sections. Here, we summarize TOS data products that are spatially collocated and indicate the scale at which collocation occurs (**Table 2**). Collocated plots of different types (e.g., Distributed base plot vs. mammal grid vs. tick plot) have the same plot identifier (plotID) in each data product, even if the plots are offset from one another. For each plot, the `appMods` field in downloadable TOS spatial data



indicates which protocols are collocated at the plot scale (protocols are identified in the `appMods` field according to 3-letter codes defined in the 'TOS Protocol' column in **Table 2** below).

 Table 2. Summary of spatial collocation scales for TOS protocols.

		Collocation	
TOS Protocol	Collocated Protocols	Scale	Collocation Details
	Herbaceous Biomass and Productivity	Sampling cell	BBC soil samples are collected North and South of each HBP clip-harvest.
Belowground Biomass roots (BBC)	Canopy Foliar Chemistry; Coarse Downed Wood; Leaf Area Index; Litterfall; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	Tower base plots only. Leaf Area Index is collocated with BBC in 3 Tower base plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which roots are sampled; sample size varies with site.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
BreedingLandbird Abundance and Diversity (BRD)	Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Mosquito Abundance, Diversity, and Phenology; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure; Small Mammals; Tick and Tick-borne Pathogens	Site	Distributed birdgrids are collocated with Distributed base plots, Distributed mammal grids, Distributed mosquito points, and Distributed tick plots, where possible.
Canopy Foliar Chemistry (CFC)	Belowground Biomass roots; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall;	Plot	BBC and LTR collocation occurs only in Tower plots.



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		Collocation	
TOS Protocol	Collocated Protocols	Scale	Collocation Details
	Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure		
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which CFC is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Coarse Downed Wood (CDW)	Belowground Biomass roots; Canopy Foliar Chemistry; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	BBC and LTR collocation occurs only in Tower plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which CDW is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Ground Beetle Abundance and Diversity (BET)	Canopy Foliar Chemistry; Coarse Downed Wood; Herbaceous Biomass and Productivity; Leaf Area Index; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	
	BreedingLandbird Abundance and Diversity; Litterfall; Mosquito Abundance, Diversity, and Phenology; Small Mammals;	Site	



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		Collocation	
TOS Protocol	Collocated Protocols	Scale	Collocation Details
	Tick and Tick-borne Pathogens		
Herbaceous Biomass and Productivity (HBP)	Belowground Biomass roots	Sampling cell	In Tower base plots BBC sampling occurs North and South of the HBP clip strip. No BBC and no collocation in Distributed base plots.
	Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Leaf Area Index; Litterfall Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	LTR collocation occursonly in Tower plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which HBP is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Leaf Area Index (DHP)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Litterfall; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	The 3-letter DHP code = Digital Hemispherical Photo, the method used to record LAI. In Tower base plots, collocation occurs in 3 plots selected by Field Operations; downloadable spatial data indicate that all Tower plots are <i>available for selection</i> . LTR collocation occurs only in Tower plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which DHP is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Litterfall (LTR)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood:	Plot	LTR implemented in Tower base plots only. CFC, DHP, and DIV are collocated with LTR in 3 plots (not



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		Collocation	
TOS Protocol	Collocated Protocols	Scale	Collocation Details
	Herbaceous Biomass and Productivity; Leaf Area Index; Plant Diversity; Soil physical, chemical, and microbial measurements; Vegetation Structure		necessarily the same 3 plots). SME is collocated in 4 plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which LTR is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Mosquito Abundance, Diversity, and Phenology (MOS)	Belowground Biomass roots; BreedingLandbird Abundance and Diversity; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Small Mammals; Soil physical, chemical, and microbial measurements; Tick and Tick-borne Pathogens; Vegetation Structure	Site	Distributed mosquito points are collocated with Distributed base plots, Distributed bird grids, Distributed mammal grids, and Distributed tick plots, where possible.
Plant Diversity (DIV)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Soil physical, chemical, and microbial measurements; Vegetation Structure	Plot	BBC and LTR collocation occurs only in Tower plots.
	PlantPhenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which DIV is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology;	Site	



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TOS Protocol	Collocated Protocols	Scale	Collocation Details
	Small Mammals; Tick and Tick-borne Pathogens		
Plant Phenology (PHE)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Soil physical, chemical, and microbial measurements; Vegetation Structure	Tower airshed	PHE plots are adjacent to Tower base plots that support the listed protocols.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Small Mammal Sampling (MAM)	Belowground Biomass roots; BreedingLandbird Abundance and Diversity Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Mosquito Abundance, Diversity, and Phenology; Soil physical, chemical, and microbial measurements; Tick and Tick-borne Pathogens; Vegetation Structure	Site	Distributed mammal grids are collocated with Distributed base plots, Distributed bird grids, Distributed mosquito points, and Distributed tick plots where possible.
Soil physical, chemical, and microbial measurements (SME)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Vegetation Structure	Plot	BBC and LTR collocation occurs only in Tower plots.
	Plant Phenology	Tower airshed	PHE plots are adjacent to a subset of Tower base plots in which SME is sampled.





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TOS Protocol	Collocated Protocols	Scale	Collocation Details
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	
Tick and Tick- borne Pathogens (TCK)	Belowground Biomass roots; BreedingLandbird Abundance and Diversity; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Mosquito Abundance, Diversity, and Phenology; Small Mammal Sampling; Soil physical, chemical, and microbial measurements; Vegetation Structure	Site	Distributed tick plots are collocated with Distributed base plots, Distributed bird grids, and Distributed mammal grids.
Vegetation Structure (VST)	Belowground Biomass roots; Canopy Foliar Chemistry; Coarse Downed Wood; Ground Beetle Abundance and Diversity; Herbaceous Biomass and Productivity; Leaf Area Index; Litterfall; Soil physical, chemical, and microbial measurements	Plot	BBC and LTR collocation occurs only in Tower plots. PHE plots are adjacent to a
	Plant Phenology	Tower airshed	subset of Tower base plots in which VST is sampled.
	BreedingLandbird Abundance and Diversity; Mosquito Abundance, Diversity, and Phenology; Small Mammals; Tick and Tick-borne Pathogens	Site	

4.4 Integration with the Airborne Observation Platform (AOP)

4.4.1 Distributed Plots

Data from TOS Distributed base plots can be used to produce stand-alone site-level unbiased parameter estimates (see Section 4.2.1). In addition, because Distributed base plots are established in a spatially-representative manner across all NLCD vegetation types ≥ 5% area, multiple data products from these plots are an important resource for calibrating and validating remote-sensing datasets collected by the NEON AOP. **Table 3** lists TOS protocols and data products with explicit design links to AOP data products.

	TOS Data Product	Related AOP Data	
Protocol Name (Number)	(Number)	Product(s) (Number)	Design Link
Canopy Foliage Sampling (NEON.DOC.001024)	Plant foliar physical and chemical properties (DP1.10026.001) Plant canopy leaf mass per area (DP1.10048.001) Plant foliar stable isotopes (DP1.10053.001)	Spectrometer Orthorectified Surface Directional Reflectance (DP3.30006.001); Canopy Nitrogen (DP3.30018.001); Canopy Water Content (DP3.30019.001); Canopy Xanthophyll Cycle (DP3.30020.001); Canopy Lignin (DP3.30022.001)	Foliar chemistry data are georeferenced for linking with pixels within AOP data. Woody individuals are tagged for repeat sampling when possible. Plot-based foliar chemistry data can calibrate analogous remote-sensing metrics derived from spectrometer reflectance data.
Leaf Area Index (NEON.DOC.014039)	Digital hemispheric photos of Plot Vegetation (DP1.10017.001)	LAI – Spectrometer (DP3.30012.001)	Plot-scale LAI can be derived from upward- and downward- facing photos and used to validate remote-sensingLAI data. Georeference data for plot centroid links with AOP pixels.
Herbaceous Biomass (NEON.DOC.014037)	Herbaceousclip harvest (DP1.10023.001)	Total Biomass Map – Spectrometer (DP3.30016.001)	Herbaceous Biomass data are georeferenced within the plot for linking with AOP pixels. Plot-based data can calibrate AOP data in systems dominated by herbaceous plants.
Plant Diversity Sampling (NEON.DOC.014042)	Plant presence and percent cover (DP1.10058.001)	Spectrometer Orthorectified Surface Directional Reflectance (DP3.30006.001); High-resolution Orthorectified camera imagery mosaic (DP3.30010.001);	Diversity metrics derived from plot-scale and within-plot plant presence and abundance data may be correlated with vegetation indices derived from hyperspectral spectrometer data.

Table 3. TOS protocols and associated data products with explicit design links to AOP data products.

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Protocol Name (Number)	TOS Data Product (Number)	Related AOP Data Product(s) (Number)	Design Link
		Vegetation Indices – Spectrometer (DP3.30026.001)	
Vegetation Structure (NEON.DOC.000987)	Non-herbaceous perennial vegetation structure (DP1.10045.001) Woody plant vegetation structure (DP1.10098.001)	Ecosystem Structure (DP3.30015.001); Total Biomass Map – Spectrometer (DP3.30016.001); Vegetation Indices – Spectrometer (DP3.30026.001)	Height data collected in the field may validate AOP LiDAR using georeferenced trees. Plot-level and individual-level biomass data derived from DBH data can be used to calibrate biomass maps. Derived plot-level composition and density data may inform AOP vegetation indices.

4.4.2 Gradient Plots

At some sites, Distributed plots may fail to fully capture site-level gradients in vegetation structure, leaf area index, or plant canopy chemistry. In order to span the same dynamic range of analogous variables in AOP remote-sensing datasets, Gradient plots may be established in Operations using a targeted, nonrandom approach informed by NEON's AOP remote-sensing data. Example Gradient plots that target areas of the landscape not associated with dominant NLCD vegetation types are shown in **Figure 1**. These plots are identical to Distributed plots in configuration and layout (**Figure 2**) and may include subplots for sampling plant diversity, soil and plant biogeochemistry, and soil microbiota.

4.5 Integration with the Aquatic Observation System (AOS)

A subset of TOS sites is spatially integrated with the NEON AOS at the watershed scale (**Table 4**). Links between the TOS and AOS subsystems are predominantly related to the biogeochemical cycling of carbon and nitrogen. Measurements help quantify pools and fluxes within litter and soils, and export and loss via groundwater and streams.

Domain	Domain Name	AOS Site ID	AOS Site Name	TOS Site ID	TOS Site Name	State
01	Northeast	НОРВ	Hop Brook	HARV	Harvard Forest	MA
02	Mid-Atlantic	POSE	Posey Creek	SCBI	Smithsonian Conservation Biology Institute	VA
		BARC	Barco Lake		Ordway-Swisher Biological	C1
02	Southoast	SUGG	Suggs Lake	0363	Station	FL
05	Southeast	FLNT	Flint River	JERC	Jones Environmental Research Center	GA

Table 4. NEON Aquatic Observation System (AOS) sites that are collocated with terrestrial sites at the watershed scale, modified from Cawley et al. (in prep).



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Demein	Demain Norma	AOS		TOS	TOC Site Norma	State
Domain	Domain Name	Site ID	AUSSILE Name	Site ID	TUS Site Name	State
		CRAM	Crampton Lake		University of Notre Dame	
05	Great Lakes	LIRO	Little Rock Lake	UNDE	Environmental Research	WI
		2			Center	
06	Prairie Penincula	KING	Kings Creek	KONA,	Konza Prairie Biological	ĸs
00	FT diffe F etilitisula		Kings cieek	KONZ	Station	K5
			Malker Branch (stream)		Oak Ridge National	
07	Appalachians	WALK	walker Branch (Stream)	UKINL	Laboratory	TN
		LECO	LeConte Creek	GRSM	Great Smoky Mountain NP	
		MAYF	Mayfield Creek	TALL	Talladega National Forest	
08	Ozarks Complex	BLWA	Black Warrior River	DELA	Dead Lake	AL
		TOMB	Lower Tombigbee River	LENO	Lenoir Landing	
		PRPO	Prairie Pothole (lake)	WOOD	Woodworth	
09	Northern Plains		Ducinia Lalva	DOFC	Dakota Coteau Field	ND
		PKLA	Prairie Lake	DCFS	School	
12	Northern Rockies	BLDE	Blacktail Deer Creek	YELL	Yellowstone NP	WY
13	Southern Rockies	COMO	Como Creek	NIWO	Niwot Ridge LTER	CO
10					Wind River Experimental	
16 Pacific Northwest		WARI	Martha Creek	WREF	Forest	WA
17	Pacific Southwest	TECR	Teakettle Creek	TEAK	Lower Teakettle	CA
18	Tundra	OKSR	OksrukuyikCreek	TOOL	Toolik LTER	AK
19	Taiga	CARI	Caribou Creek	BONA	Caribou-Poker Research Watershed	AK

TOS and AOS data products that may be integrated to understand key terrestrial/aquatic linkages are listed in

Table 5. Inputs of reactive atmospheric nitrogen are also quantified by the TIS (see Section 4.6 below).

Table 5. AOS and TOS data products that enable understanding biogeochemical links between terrestrial and aquatic ecosystems.

Subsystem	Data product name (Number)
	Aquatic plant bryophyte chemical properties (DP1.20063)
	Chemical properties of groundwater (DP1.20092)
	Chemical properties of surface water (DP1.20093)
AOS	Periphyton, seston, and phytoplankton chemical properties (DP1.20163)
	Sediment chemical properties (DP1.20194)
	Stable isotope concentrations in surface waters (DP1.20206)
	Stable isotope concentrations in groundwater (DP1.20276)
	Soil chemical properties, distributed initial characterization (DP1.10008)
	Plant foliar physical and chemical properties (DP1.10026)
	Litter chemical properties (DP1.10031)
TOS	Litterfall and fine woody debris sampling (DP1.10033)
	Soil physical properties, distributed initial characterization (DP1.10047)
	Plant canopy leaf mass per area (DP1.10048)
	Plant foliar stable isotopes (DP1.10053)

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Subsystem	Data product name (Number)		
	Root sampling (megapit) (DP1.10066)		
	Root sampling tower plots (DP1.10067)		
	Soil chemical properties (Distributed periodic) (DP1.10078)		
	Soil inorganic nitrogen pools and transformations (DP1.10080)		
	Soil physical properties (Distributed periodic) (DP1.10086)		
	Root stable isotopes (DP1.10099)		
	Soil stable isotopes (Distributed periodic) (DP1.10100)		
	Litter stable isotopes (DP1.10101)		
	Root chemical properties (DP1.10102)		
	Soil microbe biomass (DP1.10104)		

4.6 Integration with the Terrestrial Instrument System (TIS)

One of the primary goals of TOS Tower plot sampling is to enable calculation of net primary productivity (NPP) such that end-users may compare NPP estimates with Net Ecosystem Productivity (NEP) estimates derived from Tower flux data. To accomplish this, Tower plots are placed in the 90% flux area of the primary and secondary airsheds of each NEON tower (secondary airsheds are utilized where they exist) (**Figure 1, Figure 6**). Tower plots therefore provide a direct link between NEON's Terrestrial Observation System and Terrestrial Instrument System platforms. If it is not possible to place the requisite number of plots within the airshed(s), Tower base plots are also established outside the airsheds but not further from the tower than the length of the vector defining the extent of the primary airshed, and only if vegetation remains consistent with that inside the airshed. Due to the relatively small area of a typical tower airshed and the sensitivity of the sensor measurements to human disturbance, only TOS measurements that provide critical linkages to sensor-based data streams are collected from Tower Plots (**Table 6**).

Phenology plots represent another important link between the TOS and TIS subsystems. Phenology plots are established within the TIS tower airshed in order to create links between on-the-ground TOS phenophase intensity measurements collected from individual plants and phenology time series images produced by the phenocam mounted on the TIS tower (**Figure 6, Table 6**).

Finally, atmospheric deposition and soil sensor data collected by the TIS represent an important biogeochemical link between atmospheric and terrestrial systems. Combined with AOS water chemistry measurements and TOS biogeochemistry data, NEON subsystems provide key fundamental data for understanding biogeochemical inputs, terrestrial biomass stocks and production, and export (**Table 6**).

Table 6. Complementary TIS and TOS data products focused on biogeochemistry, phenology, and biomass and productivity parameters.

Link Type	Subsystem	Data product name (Number)
Biogeochemistry	TIS	Precipitation (DP1.00006)
		Wet deposition chemical analysis (DP1.00013)
		Stable isotope concentrations in precipitation (DP1.00038)
		Soil water and salinity (DP1.00094)

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Link Type	Subsystem	Data product name (Number)			
		Soil physical properties (Megapit) (DP1.00096)			
		Soil chemical properties (Megapit) (DP1.00097)			
		Soil chemical properties, distributed initial characterization (DP1.10008)			
		Plant foliar physical and chemical properties (DP1.10026)			
		Litter chemical properties (DP1.10031)			
		Litterfall and fine woody debris sampling (DP1.10033)			
		Soil physical properties, distributed initial characterization (DP1.10047)			
		Plant canopy leaf mass per area (DP1.10048)			
		Plant foliar stable isotopes (DP1.10053)			
		Root sampling (megapit) (DP1.10066)			
	TOS	Root sampling tower plots (DP1.10067)			
		Soil chemical properties (Distributed periodic) (DP1.10078)			
		Soil inorganic nitrogen pools and transformations (DP1.10080)			
		Soil physical properties (Distributed periodic)(DP1.10086)			
		Root stable isotopes (DP1.10099)			
		Soil stable isotopes (Distributed periodic) (DP1.10100)			
		Litter stable isotopes (DP1.10101)			
		Root chemical properties (DP1.10102)			
		Soil microbe biomass (DP1.10104)			
Dhanalagy	TIS	Phenologyimages (DP1.00033)			
Phenology	TOS	Plant phenology observations (DP1.10055)			
	TIS	Ecosystem Exchange; Tower - NEE, GEE, ER (DP4.00067)			
		Coarse downed wood log survey (DP1.10010)			
		Coarse downed wood bulk density sampling (DP1.10014)			
Biomass and Productivity		Herbaceousclip harvest (DP1.10023)			
	тоѕ	Litterfall and fine woody debris sampling (DP1.10033)			
		Non-herbaceous perennial vegetation structure (DP1.10045)			
		Root sampling (megapit) (DP1.10066)			
		Root sampling tower plots (DP1.10067)			
		Woody plant vegetation structure (DP1.10098)			



5 THE NEON TOS TEMPORAL SAMPLING STRATEGY

5.1 Multi-year Temporal Sampling Strategy

In general, TOS protocols that quantify organisms or populations with relatively fast turnover times are implemented annually, and often with multiple bouts per year (e.g., beetles, mosquitoes, small mammals, plant diversity). Protocols quantifying organisms or substrates that change relatively slowly are scheduled on 5-year intervals (**Table 1**). TOS protocols implemented on a 5-year interval are temporally coordinated in order to maximize the scientific potential of TOS data products. For protocols with 5 y measurement intervals,

Table 7 illustrates the scheduling strategy for two groups of biomass, productivity, biogeochemistry, and diversity protocols (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 field staff. These protocols are implemented predominantly in the same Distributed Plots (i.e., the protocols are spatially collocated). Tower Plots are utilized for belowground fine root biomass sampling (BGB) and litter biogeochemistry sampling (LTR-bgc).

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

Table 7, 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. 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 7). 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 7 are therefore implemented at every site.

Table 7. Coordination of 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.

	Interval		Plot	Year						
Protocol*	(y)	Plot Type	Number	1	2	3	4	5	6	7
BGB	5	tower	20 or 30†	Х					Х	

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	Interval		Plot	Year						
Protocol*	(y)	Plot Type	Number	1	2	3	4	5	6	7
CFC	5	both	16-20	Х					Х	
DIV	1	distributed	30	Х	Х	Х	Х	Х	Х	Х
LAI	5	distributed	20	Х					Х	
LTR-bgc	5	tower	20 or 30†	Х					Х	
NTR	5	both	10	Х					Х	
SLS-bgc	5	both	10	Х					Х	
SLS-mb	5	both	10	Х					Х	
CDW	5	distributed	20		Х					Х
HBP	5	distributed	20		Х					Х
VST	5	distributed	20		Х					Х
HBP	1	tower	20 or 30†	Х	Х	Х	Х	Х	Х	Х
LAI	1	tower	3	Х	Х	Х	Х	Х	Х	Х
LTR	1	tower	20 or 30†	Х	Х	Х	Х	Х	Х	Х
VST	1	tower	5-10‡	Х	Х	Х	Х	Х	Х	Х
CDW	5	tower	20 or 30†				Х			
VST	5	tower	20 or 30†					Х		

* Protocol codes and definitions: BGB = Belowground Biomass of fine root sampling; CFC = Canopy Foliar
 Chemistry sampling; DIV = Plant Diversity sampling; LAI = Leaf Area Index sampling; LTR-bgc = Litterfall
 biogeochemistry analysis; NTR = soil nitrogen mineralization incubation; SLS-bgc = Soil biogeochemistry analysis;
 SLS-mb = Soil microbial biomass analysis (PLFA); CDW = Coarse Downed Wood sampling; HBP = Herbaceous
 Biomass and Productivity sampling; VST = Vegetation Structure sampling; LTR = Litterfall sampling (no chemistry).

+ 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 RD[12] for VST fast/slow growth increment classification by site.

The group of 'Year 1' protocols focused on biogeochemistry (**Table 7**, grey cells) is coordinated further with the airborne remote-sensing flight schedule. The AOP does not fly every site every year and coordinating remote-sensing flights with this group of biogeochemistry protocols ensures a set of synergistic data products at the site scale that maximizes scientific utility of both TOS and AOP data products for the end-user community. Numerous links between the TOS and AOP described in Section 4.4 are only possible when data from both subsystems are collected within the same year.

5.2 Within-year Temporal Sampling Strategy

Within a sampling season, it is very important that TOS protocols are implemented at phenologically consistent times across sites in order for cross-site data to be comparable. For example, biological activity in a Mediterranean system such as that at the San Joaquin site (D17 SJER) has very different timing compared to a north temperate hardwood forest such as Harvard Forest (D01 HARV). Sampling these two different sites on the same calendar date could lead to erroneous conclusions about site differences. To determine phenologically consistent sampling dates across widely different ecosystems, the TOS utilizes MODIS-EVI phenology data for the majority of sites (Didan 2015), and more specifically, per site averages from the most recent 10 years that are updated every 5 years (e.g., **Figure 11**).



Figure 11. MODIS-EVI timecourse data for the Domain 14 Santa Rita Experimental Range site (D14 SRER). Data are from the years 2005-2014. There is an initial minor green-up early in the year centered around day 80, and another major green-up associated with the summer monsoon centered around day 240.

At sites where MODIS-EVI data are informative, the key parameters that guide TOS sampling are the greenness increase date, the peak greenness date, the greenness decrease date, and the date at which greenness returns to a minimum. Sites like the Domain 14 Santa Rita Experimental Range in the Sonoran Desert have two greenness cycles; when sites have two cycles, dates from each cycle may be used for a given protocol (**Figure 11**). MODIS-EVI dates themselves may not correspond directly to desired sampling times, but it is typically possible to determine per protocol optimal sampling times in relation to these dates. There are 3 high-level groups of TOS protocols that utilize these dates in different ways:

- Multiple bouts per year, bouts scheduled at regular intervals.
- Multiple bouts per year, bouts timed to seasonally important dates.
- Single bout per year, timed to seasonally important date.

For protocols with multiple bouts per year and regularly scheduled sampling intervals, the greenness increase date is typically associated with the onset of sampling (**Table 8**). However, because there is often considerable variability in the onset of biological activity from year to year, within-year temperature data may also be used in conjunction with average MODIS-EVI data to ensure that sampling is phenologically consistent across sites (e.g., mosquito sampling onset is fine-tuned in this manner).

Schedule Type	TOS Protocols	MODIS-EVI cue	
Multiple bouts per year, bouts at regular intervals*	Ground Beetle Sampling	Greenness Increase (sampling start) Greenness Decrease (sampling	
	Mosquito Sampling ⁺		
	Tick and Tick-Borne Pathogen Sampling		
	Small Mammal Sampling		
	Measurement of Leaf Area Index (Tower plots)	end)	
Multiple bouts per	Litterfall and Fine Woody Debris Sampling	Greenness Decrease	
year, bouts timed	Plant Phenology Sampling	Greenness Increase,	

Table 8. TOS protocol scheduling strategy within a sampling season.

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to seasonally		Greenness Decrease
important dates	Soil Physical, Chemical, and Microbial Measurements	Greenness Increase,
		Peak Greenness,
		Greenness Decrease
	BreedingLandbird Abundance and Diversity Sampling	
Cinalo hautaar	Canopy Foliage Sampling	Book Groopposs
Single bout per year, timed to seasonally important date	Measurement of Leaf Area Index (Distributed plots)	Peak dieenness
	Plant Diversity Sampling	
	Measurement of Herbaceous Biomass	
	Plant Belowground Biomass Sampling	Greenness Decrease
	Measurement of Vegetation Structure	

* Sampling onset is associated with greenness increase but sampling intervals are not the same across the listed protocols so temporal coordination at a site is limited to sampling start date.

⁺ Greenness Increase is broadly used for scheduling mosquito sampling onset but actual sampling onset within a given sampling season is determined by within-season temperature data.

The Litterfall, Plant Phenology, and Soils protocols represent a second category of TOS protocols with multiple bouts that target specific transition periods throughout the sampling season (**Table 8**). The Litterfall protocol shifts to intensive sampling during deciduous senescence periods at qualifying sites. Litterfall intensive sampling schedules are broadly timed according to MODIS-EVI greenness decrease dates, and are further informed on a yearly basis by on-the-ground observation. Similarly, the Plant Phenology schedule incorporates intensive sampling during periods of leaf-out and senescence, which are broadly correlated with greenness increase dates and greenness decrease dates, respectively, and are refined by on-the-ground observation. The scheduling of TOS soil sampling for microbial community composition, microbial biomass, and soil nitrogen transformation rates bears special mention because sampling is tightly coordinated through time. Microbial community composition, microbial biomass, and soil nitrogen transformation rates bears special mention because sampling is tightly coordinated through time. Microbial community composition, microbial biomass, and soil nitrogen transformation rates bears special mention because sampling is tightly coordinated through time. Microbial community composition, microbial biomass, and soil nitrogen transformation rates are also used for estimating N-transformation rates. Because microbial communities turn over quickly and N-transformation rates are dynamic over relatively short timescales, temporal and spatial coordination of these measurements ensures maximum synergy among TOS soil data products.

The last group of TOS protocols to consider are those typically implemented once during a given sampling season (**Table 1**). Protocols in this group that are timed to peak greenness are de facto coordinated with NEON AOP remote-sensing flights, due to the fact that the AOP also schedules flights using a related MODIS-NDVI peak greenness metric (Section 4.4). Aboveground Herbaceous Biomass and Plant Belowground Biomass sampling are both linked to site-specific greenness decrease dates in order to generate above- and below-ground biomass estimates from the same time of year (root and clip samples are spatially collocated within plots as well). However, these two protocols may be temporally decoupled if soil hardness prevents collecting root samples when aboveground biomass is maximal.

A few TOS sites show a relatively invariant MODIS-EVI signal throughout the year (i.e., D04 and D20 neotropical sites). At these sites, precipitation data are used to define wet/dry periods, and local knowledge

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is employed to determine the optimal time of year for sampling different taxa/soils with respect to the wet/dry season (**Figure 12**). Sampling in future years is maintained at a consistent annual interval from selected dates so that consistent time intervals are captured in the data products.



Figure 12. Precipitation data from the Puerto Rico Ensenada weather station near the Domain 04 Guanica Forest site (D04 GUAN). Data are from 1980 to 2015.



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