



<i>Title:</i> TOS Science Design for Plant Phenology		<i>Date:</i> 04/11/2018
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## TOS SCIENCE DESIGN FOR PLANT PHENOLOGY

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<i>Title:</i> TOS Science Design for Plant Phenology		<i>Date:</i> 04/11/2018
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## Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	03/13/2015	ECO-02158	Initial release
B	04/11/2018	ECO-05459	Minor format and text edits. Updates to number of sites. Clarification of characterization and species selection.

<i>Title:</i> TOS Science Design for Plant Phenology		<i>Date:</i> 04/11/2018
<i>NEON Doc. #:</i> NEON.DOC.000907	<i>Author:</i> S. Elmendorf	<i>Revision:</i> B

**TABLE OF CONTENTS**

**1 DESCRIPTION.....1**

1.1 Purpose ..... 1

1.2 Scope..... 1

1.3 Acknowledgments..... 1

**2 RELATED DOCUMENTS AND ACRONYMS .....1**

2.1 Applicable Documents ..... 1

2.2 Reference Documents..... 2

2.3 External References ..... 2

2.4 Acronyms ..... 2

**3 INTRODUCTION .....3**

3.1 Overview of the Observatory..... 3

3.2 Components of the Observatory ..... 4

3.3 The Terrestrial Observation System (TOS)..... 4

**4 INTRODUCTION TO THE PHENOLOGY SAMPLING DESIGN.....5**

4.1 Background ..... 5

4.2 NEON’s Contribution..... 6

4.3 Purpose and Scope..... 7

**5 SAMPLING FRAMEWORK .....7**

5.1 Science Requirements..... 7

5.2 Data Products..... 7

5.3 Priorities and Challenges for Plant Phenology Sampling ..... 7

**6 SAMPLING DESIGN FOR PLANT PHENOLOGY.....8**

6.1 Sampling Methods ..... 9

6.1.1 Characterization..... 12

6.1.2 Phase I Sampling ..... 13

6.1.3 Phase II Sampling ..... 13

6.2 Spatial Distribution of Sampling..... 14

6.3 Temporal Distribution of Sampling ..... 14

6.4 Logistics and Adaptability ..... 16

<i>Title:</i> TOS Science Design for Plant Phenology		<i>Date:</i> 04/11/2018
<i>NEON Doc. #:</i> NEON.DOC.000907	<i>Author:</i> S. Elmendorf	<i>Revision:</i> B

6.4.1	Site-Specific Modifications.....	16
6.4.2	Incorporating New Technologies .....	17
6.4.3	Changes to Financial and/or Logistical Constraints .....	17
<b>7</b>	<b>REFERENCES .....</b>	<b>18</b>

**LIST OF TABLES AND FIGURES**

<b>Figure 1.</b>	The seven Grand Challenges defined by the National Research Council (2001).....	3
<b>Figure 2.</b>	Layout of phenology transect (teal square) with respect to the NEON Tower (cross shape), the instrument buffers (green area), the airshed (black lines) and the Tower Plant Productivity plots (small squares).....	11

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

**1 DESCRIPTION**

**1.1 Purpose**

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON subsystem components, linking NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. NEON strives to make measurements in ways that enable continental-scale science to address the Grand Challenges. Design Documents flow from questions and goals defined in the NEON Science Strategy document, and inform the more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and Calibration/Validation (CaVal) and maintenance plans.

**1.2 Scope**

This document defines the rationale and requirements for Plant Phenology in the NEON Science Design.

**1.3 Acknowledgments**

This document was written with input from the following coauthors:

- Ben Cook, NASA Goddard Institute for Space Studies
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- Susan Mazer, University of California, Santa Barbara
- Abe Miller-Rushing, Acadia National Park
- David Moore, University of Arizona
- Mark Schwartz, University of Wisconsin, Milwaukee
- Jake Weltzin, USA National Phenology Network

And updated for formatting and final release by Tanya Chesney.

**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
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Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

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.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
AD[05]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
AD[06]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
AD[07]	NEON.DOC.000914	TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index
AD[08]	NEON.DOC.001484	Areas of Mutual Representativeness and Exclusion around Terrestrial Infrastructure Measurements
AD[09]	NEON.DOC.003885	TOS Site Characterization Methods
AD[10]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling

## 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.001025	TOS Protocol and Procedure: Plot Establishment

## 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]	
ER [02]	
ER [03]	

## 2.4 Acronyms

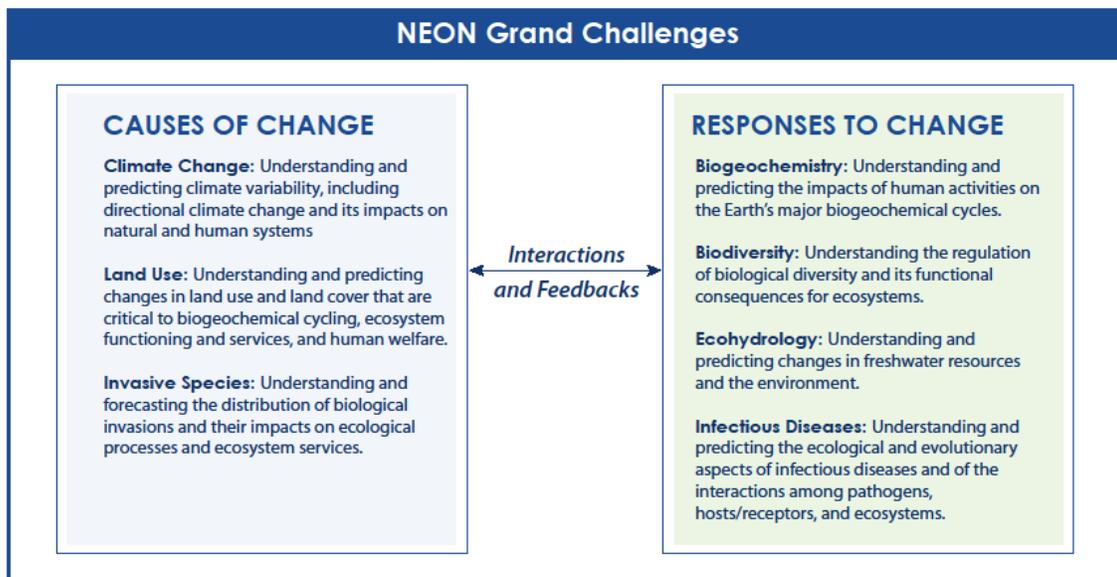
Acronym	Definition
NPN	National Phenology Network
PBB	Project Bud Burst
RCN	Research Coordination Network

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

### 3 INTRODUCTION

#### 3.1 Overview of the Observatory

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



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

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

### 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 allows for linkage and comparison of data products. For example, remote sensing data provided by the Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. (2008), Schimel et al.(2011).

### 3.3 The Terrestrial Observation System (TOS)

The NEON TOS will quantify the impacts of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa), infectious disease, soil, and nutrient fluxes across system interfaces (air, land, and water) (AD[01], AD[02]). The sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements 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 are 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](http://www.neonscience.org)).

The standardization of protocols across all sites is key to the success of NEON (and its novelty) and must be maintained at all sites through time. Thus, although specific techniques may be required at some sites (e.g., due to different vegetation types), protocols have been developed to ensure data comparability. These details can also be found in individual design documents available through the NEON website ([www.neonscience.org](http://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 of the components of the TOS (AD[03]). TOS Science Design documents were developed following input from the scientific community, including module-specific Technical Working Groups, and the National Science Foundation (AD[02]). Science Designs will be reviewed periodically to ensure that the data collected by NEON are those best suited to meet the requirements of the Observatory (AD[01]), are (to the extent possible) consistent with standards used by the scientific community, and fit within the scope of NEON. Additional information on the development and review process can be found in AD[02].

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

## 4 INTRODUCTION TO THE PHENOLOGY SAMPLING DESIGN

### 4.1 Background

Phenology is defined as the seasonal timing of life cycle events. The Intergovernmental Panel on Climate Change (IPCC) (Solomon et al. 2007) notes that phenology is one of the simplest processes for tracking changes in species' ecology in response to climate change. According to a recent synthesis, the onset of spring phenological events is advancing at a mean rate of 2.3 days per decade worldwide, likely due recent climate warming (Parmesan and Yohe 2003). Plants flower on average ~5 days earlier per °C increase in spring temperature (Wolkovich et al. 2012), so substantial changes in spring phenology are expected over the life of the Observatory.

In addition to being a variable that is sensitive to climate change, phenology is also a potentially important driver of ecological responses ranging from the demographic trajectories of individual taxa to biogeographical distributions to ecosystem processes. For example, species whose phenologies track climate variability tend to have improved growth, productivity, or reproductive success in contrast to those that do not (Cleland et al. 2012). On the other hand, phenologic advancement in response to warm spring temperatures followed by a late frost can have catastrophic effects on fruit and seed production and canopy development (Inouye 2008, Hufkens et al. 2012). In either case, a population's phenological sensitivity may be an early indicator of its demographic trajectory. These sensitivities may constrain broad-scale distribution patterns; phenology appears to be a key biological constraint in process-based species distributions models (Chuine 2010 and references therein).

Phenological shifts can, themselves, create feedbacks that alter species interactions and ecosystem processes. Differential sensitivities to phenological triggers can cause trophic mismatches between interacting organisms (Singer and Parmesan 2010, McKinney et al. 2012). The timing of leaf budburst and senescence can alter surface radiation, temperature, hydrology and carbon cycling (Bonan 2008, Richardson et al. 2010, Jeong et al. 2012, 2013). Phenological transitions may be triggered by a variety of cues, including chilling requirements, spring temperature, and daylight cues (Chuine 2000), but realistic parameterization of phenological models for many wild species has been limited due to the scarcity of relevant data (Jeong et al. 2012).

A number of techniques exist for monitoring and recording the phenological status and progress of plants, including *in situ* observations, modeling, eddy covariance towers, experiments, remote sensing, and digital photography (Cleland et al. 2007). However, formulating linkages between these different approaches to monitoring phenology, and scaling from individual-based monitoring (as implemented in citizen science programs, natural resource monitoring programs, and a variety of site-based long-term ecological studies), to larger scales is an active area of research (Morissette et al. 2008). By providing integrated suites of measurements on the seasonal progression of a diversity of taxa and ecosystem processes at intensively measured sites, NEON data will enable the scientific community to further

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

develop mechanistic linkages between the environmental drivers that affect plant phenology, as well as the functional consequences of changing phenology for a wide array of ecosystem types.

#### 4.2 NEON’s Contribution

NEON is poised to advance the field of phenology due to the combined contributions of the following attributes: 1) The monitoring of replicate individuals per species in order to quantify intraspecific variation in the timing of phenological events and its sensitivity to environmental conditions, and to increase the precision of estimates of the mean phenological trajectories at the population level; 2) Measurements of multiple species to characterize the range of phenological response patterns; 3) Accumulation of high quality, long-term, standardized measurements, recorded by professional field ecologists, across 20 major ecosystem types of the continental US; and 4) Collocation of plant phenological measurements with an extensive array of monitoring data from other sentinel taxa as well as meteorological, flux and ecosystem productivity data which may be used to understand linkages between climate, phenology, ecosystem processes and biodiversity. Elements of all of the above are currently being collected by a number of other programs (e.g. Ameriflux, NPN, LTER sites, National Parks) as well as multiple long term PI directed research projects, and both NEON and these allied projects and programs stand to benefit from this integration. For example, the collocation of multiple measurement systems at NEON sites may enable inference of ecosystem processes at an extensive network of spatially distributed sites where only *in situ* observations are feasible.

The phenological data collected by NEON will provide a rich dataset for informing continental-scale phenology over the lifetime of the Observatory, for forecasting future phenological shifts in response to anticipated anthropogenic changes, and for understanding the sensitivity of critical ecosystem processes to phenological change. Quantifying the range of phenological responses across a wide array of species and sites will aid in the development of more general phenological forcing models based on species and site characteristics, as well as understanding of their limitations in forecasting phenology where existing data are sparse. Bayesian hierarchical models are a promising avenue forward in community phenology forecasting (Ibáñez et al. 2010, Diez et al. 2012). To date such models have been limited either to sites with multiple species, or to single species observed over multiple sites; in contrast NEON will provide community-level data with observations on up to 20 species at 42 sites, using common protocols and in association with extensive meteorological information, in sites across the country.

By integrating ground-based observations with other North American phenological monitoring programs throughout the country (e.g., USA National Phenology Network), existing datasets (e.g. (Wolkovich et al. 2012), the PhenoCam network (<http://phenocam.sr.unh.edu/webcam/>), satellite imagery (e.g. US Remote Sensing Phenology, <http://phenology.cr.usgs.gov/>; MODIS 12Q2 product [https://lpdaac.usgs.gov/products/modis\\_products\\_table/mcd12q2](https://lpdaac.usgs.gov/products/modis_products_table/mcd12q2); MODIS for NACP <http://accweb.nascom.nasa.gov/>), and/or models such as (e.g. Spring Indices (Ault et al. 2011), GSI (Jolly et al. 2005), and a variety of chilling, thermal forcing and photoperiod models (Vitasse et al. 2011) *in situ* phenology observations made by NEON can contribute critical information to an annual ‘green

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

wave' (Schwartz 1998) projection over the continent. Integration of collocated NEON datasets ranging from *in situ* phenology, phenocam, LAI, productivity, eddy flux, along with sub-meter hyperspectral and LiDAR remote sensing data will be particularly valuable in determining both statistical and mechanistic linkages between the multiple components of seasonal cycles.

#### 4.3 Purpose and Scope

The purpose of sampling plant phenology is to capture inter-annual variation in the timing of phenological stages of plants. This document details the approach used to derive a scientifically rigorous, logistically feasible sampling design that meets the goals of NEON.

### 5 SAMPLING FRAMEWORK

#### 5.1 Science Requirements

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

#### 5.2 Data Products

Execution of the protocols that stem from this science design procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. The data resulting from the plant phenology protocols are used to create NEON data products, as outlined in the NEON Level 1, Level 2 and Level 3 Data Products Catalog (AD[04]).

#### 5.3 Priorities and Challenges for Plant Phenology Sampling

Two priorities were identified for NEON's plant phenological sampling: 1) capturing the mean and intraspecific variance of dominant species within each site 2) capturing a range of species-specific phenotypic responses that represent the community at each site. The first (dominant species' phenology) will enable linking phenological patterns observed above-ground to processes captured by other NEON measurement systems, such as ecosystem productivity and respiration, and carbon, water and nutrient cycling. It will also provide critical information on intraspecific variation in phenology patterns, which are poorly captured when monitoring efforts are limited to a census of one to several individuals/site. The second (community phenology), will inform questions regarding inter-specific variation in the timing and duration of phenological phases and their sensitivity to climate. It will provide a rich dataset across a diverse array of plant types (natives/exotics, overstory/understory, perennial/annual, deciduous/evergreen, herbaceous/woody, early and late-season, phylogenetic relatedness) that may permit generalization and predictions regarding the phenologies and sensitivities of other species in these functional groups that are not directly monitored. These priorities stem from those identified by the 2008 Tiger Team report (Davis et al. 2008), which emphasized the importance of

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

characterizing both ‘earliest dates’ and within-population variation in phenology. An increasing appreciation of the limitations of first appearance dates as a phenological estimator (Miller-Rushing et al. 2008, Moussus et al. 2010) since the publication of the Tiger Team reports suggested a shift in emphasis away from earliest dates as a focus of data products.

Due to resource limitations at NEON sites, compromises must be made between the number of species, the number of individuals per species to be monitored, the number of phenophases monitored, and the frequency of monitoring events; NEON cannot maximize the measurement of both inter- and intra-specific variation simultaneously. As a result, NEON will implement its phenology sampling in two phases in order to accomplish both inter- and intra-specific sampling goals. During Phase I, phenological sampling will concentrate on the intensive monitoring of 3 dominant species at each site. Phase II will consist of more limited sampling of up to 20 species/site. Phase I will last for the first 3 years of sampling following the Observatory transitioning to full Operations, after which sampling will transition to Phase II. The procedure for selection of individual species to be monitor at each site is described in section 6.1.1 below.

Both leaf and reproductive phenological events are sensitive to environmental change. However, quantifying with precision the dates of a large set of identifiable phenophases would require frequent sampling throughout the entirety of the growing season. Instead, NEON dynamically varies the sampling intensity in order to capture the phenology of key leaf transitions with greater precision, with coarser resolution sampling for flowering (see Section 6.5 below). A focus on canopy development was selected in order to facilitate linkages between *in situ* phenophase observations, digital hemispherical photos used to calculate leaf area index, tower-mounted phenocams and flux measurements originating from other tower-mounted instrumentation. . Where more precise estimates of flowering or fruiting events for specific taxa are of interest (e.g. to understand resources for particular species interactions) individual PIs may set up additional phenological monitoring in the area by leveraging the baseline phenology data collected by NEON as well as the meteorological measurements.

## 6 SAMPLING DESIGN FOR PLANT PHENOLOGY

NEON’s potential to link ground-based measurements, landscape greening metrics, and ecosystem processes is unique. However, NEON sites are relatively sparse, spatially, compared to continental citizen-science monitoring efforts such as the USA National Phenology Network ([www.usanpn.org](http://www.usanpn.org); hereafter NPN), Project BudBurst ([www.budburst.org](http://www.budburst.org), hereafter PBB) and affiliated national and regional monitoring networks. Using nationally standardized protocols and leveraging existing and ongoing efforts in other areas increases the potential for continental-scale analysis and forecasting by direct integration of NEON and other phenology data.

Plant phenology is typically quantified by noting the date of onset and the duration of particular phenophases, which may include both leaf and reproductive events. Without a common definition for specific phenophases, data interoperability becomes a limiting factor in continental-scale analyses, since

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

there is not a 1:1 mapping of phenophase definitions among all monitoring networks. The USA-NPN is the largest and most scientifically rigorous national phenological monitoring program in the U.S., developed with feedback from a large scientific community and natural resource managers. Consistent with NEON’s commitment to use existing nationally-accepted, vetted and standardized protocols wherever possible, and following the recommendation of the 2008 Tiger Team report, NEON employs USA-NPN phenophase definitions (Denny et al. 2014).

Additional advantages of NPN protocols for NEON include: (1) status- based monitoring; (2) repeated tracking of marked and georeferenced individuals or “patches,” rather than simply recording the date of ‘first events’ over unknown population sizes and (3) incorporation of both status and ‘intensity’ definitions for phenophases (Denny et al. 2014). Using status, rather than first event monitoring, is a departure from many historical phenology monitoring protocols, but has the advantage that events, such as leaf emergence in Mediterranean climates, or flowering in many desert species, that may occur multiple times during a single year can be captured. Status-monitoring also allows the explicit quantification of uncertainties in phenophase transition dates (which occur in continuous time) that are introduced by monitoring in discrete temporal bouts, as well as the duration of phenophases rather than just their date of onset. Monitoring marked individuals (or small patches for annuals and clonal plants) ensures that the phenology dates recorded are decoupled from changes in population size (Miller-Rushing et al. 2008). The protocols employed include ‘intensity’ metrics (e.g. % of leaves that are green vs. colored) along with phenophase start and end dates. By recording data that will allow the estimation of mean population start and end dates, as well as the intensity of each phenophase, these phenological data should provide better linkages to ecosystem function and landscape or remotely sensed phenologies than existing ‘first event’ phenology datasets, which quantify the phenological status of only the most extreme individuals.

In order to link phenology measurements to health and productivity, NEON will augment observations of leaf and reproductive phenology with annual status measurements on each individual/patch. These measurements include size (dbh, % cover, height, and canopy dimensions), disease status, health condition and structure. During annual measurement, plant tissues may also be collected for archive; these samples are available to the ecological community as part of NEON’s foliar tissue archive.

## 6.1 Sampling Methods

A 2012 NSF Research Coordination Network Report recommends tracking leaf phenology for dominant species in order to make linkages to remote sensing data. Such a strategy is also likely to allow the strongest inferences regarding the relationship between phenology and ecosystem processes, with the assumption that species contribute to ecosystem properties roughly in proportion to their relative abundance (Grime 1998). An additional priority is to characterize the community phenology of the site, which includes not only dominant species but subdominants and a range of functional groups and life history strategies. Such a strategy will both inform the range of phenological patterns occurring at a site, as well as predictive models of the sensitivities of particular species based on their traits.

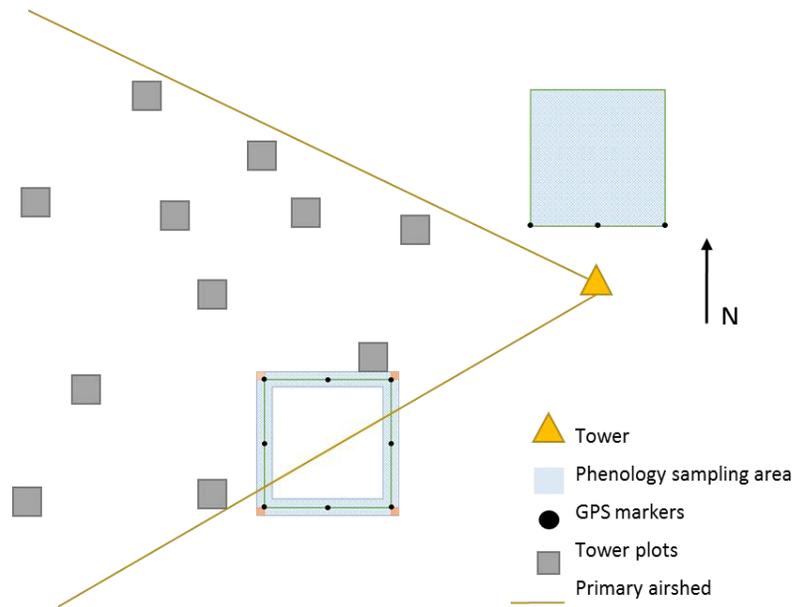
Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

The NSF RCN report (2012) recommends a minimum of 5-10 replicate individuals sampled for leaf phenology per site per species, with an ideal sampling intensity of 20-30 individuals. In the absence of existing data on intraspecific variance which might permit smaller sample sizes for particular species and sites, NEON is targeting the higher end of this range in order to quantify intraspecific variation in phenological timing (30 individuals/year) for three of the most dominant species in each site, during Phase I of phenology sampling. In Phase II (community phenology), a reduction in sampling intensity (reduced measurement frequency and fewer replicates within species) will occur coincident with a shift to sampling of a greater number of species, to better quantify the community phenology. Due to budgetary constraints, it is not possible to monitor the phenology of every species that exists within the tower fetch area at each site. Estimates based on preliminary budget allocation to plant phenology monitoring suggest that NEON should be able to monitor 5 individuals of approximately 20 species, up to 50x/year during the growing season at each terrestrial site. Therefore, NEON aims to continue to collect data on the three dominant species at each site during the ‘community phenology’ phase of the project, as well as an additional ~17 species at each site. The number of additional species to be sampled may be reduced as more accurate estimates of technician time per phenological observation at each site become available or if diversity of species at the site prevents selection of 20 distinct species.

In 2011, NEON conducted a prototype of the plant phenology sampling protocols at the Domain 10 Core site, Central Plains Experimental Range. Three species, *Atriplex canescens*, *Bouteloua gracilis* and *Bromus tectorum* were selected from the list of species common to the short grass prairie ecosystem. These species were selected 1) because they represent broad phylogenetic diversity, 2) are species with large geographic distributions and therefore are likely present at other NEON sites and 3) represent both native and non-native/invasive species. Thirty individuals/patches from each species were selected for sampling; these individuals/patches were scattered throughout three different sampling areas, none of which was located near the NEON Tower. Leaf and reproductive status of individuals was recorded weekly though phenophase intensity was not recorded. These initial efforts at implementing phenology sampling protocols were valuable more for assessing sampling methods than for the data collected. Changes to the design resulting from these efforts include (1) establishing a sampling transect from which to conduct phenology observations (2) focusing sampling to within the Tower airshed and (3) prioritizing characterization of the vegetation within the Tower airshed prior to selecting species. By restricting individual selection to those located along a 200 m x 200 m transect located near the NEON Tower sampling becomes more streamlined and efficient; traffic through other NEON plots and total travel time per sampling event is reduced by sticking to a single sampling transect. During phenology prototyping, one of the species selected for monitoring (*Bromus tectorum*), though regionally common, was not locally abundant; field crews were unable to achieve the intended sample size demonstrating that species selection needs to be based on a quantitative survey of the intended monitoring area (i.e. the Tower airshed); additionally, a site-specific survey of species abundance provide a ranking mechanism for selection of Phase II species (see section 6.1.1.1 for more details).

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

A common critique of many of the existing ground-phenology datasets is that observations are extremely limited in space and reported as points, whereas remote sensing data pixels from commonly used satellite products used to model phenology are ~250m (Schwartz and Hanes 2010). While some studies have found little spatial autocorrelation in plant phenologies within a homogeneous area (Schwartz et al. 2014), dispersion of monitored individuals throughout a larger area is important to capture the relevant (if any) spatial or genetic variation in plant phenology. To address these concerns, as well as those identified during prototype efforts, marked individuals are situated along a fixed, 800 meter square ‘loop’ transect (200 meters on a side) within the tower airshed (**Figure 2**). The tower airshed has been targeted for phenology sampling for both scientific and logistical reasons. First, to the extent possible, the Tower airshed is situated over a relatively homogeneous area in terms of vegetation type (AD[01]), so the intraspecific variation in phenology responses will, in general, be from individuals subjected to equivalent environmental conditions, including community composition. Second, environmental data collected by tower-mounted sensors will facilitate identification of drivers of observed phenological trends. Lastly, because of construction and infrastructure requirements, NEON Towers are located near roads and are more accessible than other areas within a site, placing the phenology transects near the Tower minimizes travel-time to and from the transect and facilitates sampling efficiencies.



**Figure 2.** Layout of phenology transect (teal square) with respect to the NEON Tower (cross shape), the instrument buffers (green area), the airshed (black lines) and the Tower Plant Productivity plots (small squares)

It is desirable to have at least a few individuals of the dominant (Phase I) species that are included in both the *in situ* observations and within view of tower-mounted phenocams, which are always north-

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

facing. This may occur without any additional effort (where the airshed is naturally to the north of the tower itself); in other locations, an additional three individuals of each of the dominant species that are visible from the phenocam located within a secondary phenology plot area also monitored. The tower footprint is always contained within the larger (~10x10km area) overflowed annually with NEON's airborne observation platform (AD[01], Kampe et al. 2010). Derived data on vegetation type and relative abundance from those remotely sensed data may enable scaling from individual species phenologies to landscape phenologies through relative abundance, using approaches outlined in Liang et al. (2011).

Implementation of the *in situ* plant phenology monitoring at each site occurs in three stages: Characterization, Phase I (dominant species phenology), and Phase II (community phenology). Refer to the Plant Phenology Protocol (AD[05]) for detailed information on how phenophases on marked individuals/patches are observed .

### 6.1.1 Characterization

Site characterization for phenology sampling consists of (1) conducting a quantitative survey of vegetation present in the Tower airshed following methods in the Vegetation Structure and Plant Diversity protocols (AD[06] and AD[10]) and (2) selection of species to monitor in both phases of phenology monitoring. Typically, characterization occurs once at a site, in advance of initiating terrestrial sampling. However, if a site experiences drastic change, such that the species composition and structure of the plant community no longer resembles the community surveyed for characterization, it may be necessary to re-survey the site.

Within the tower footprint, prior to operations, a trained botanist familiar with the flora at each site conducts a quantitative vegetation survey to quantify percent cover of herbaceous species in each of 8 - 1m<sup>2</sup> nested subplots per plot (according to AD[10]) and canopy area or basal area of woody species across the entire plot (according to methods in AD[06]. Site level species rankings are then determined by combining abundance values across growth forms and plot configurations. Abundance values from diversity field data include percent cover by species. Abundance values from field data included diameter at breast height (DBH), diameter at decimeter height (ddh) and canopy area from vegetation structure data and percent cover from plant diversity data. DBH and ddh are first converted to area at breast height (ABH) and area at decimeter height (adh). Then, values are summarized as total abundance per unit area for each plot (for woody vegetation) or subplot (for herbaceous species). Site averages and maximum values are calculated from these values. The ratio of the mean to the maximum value is used to determine species rankings for each abundance measure. The ratios are summed to provide overall rankings across abundance measures (see AD[09] for additional details on site characterization methods).

From the resulting species-abundance list, NEON selects three dominant species at each site for Phase I of phenology monitoring. The dominant species includes the two most abundant canopy species plus the single most abundant understory species for sites with greater than 50% canopy closure, and the

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

two most abundant understory species plus the single most dominant overstory species for sites with less than 50% canopy closure. At sites with no defined over overstory, e.g. grasslands, all three species are selected from the herbaceous community. Relative dominance is defined as the rank order of species-specific cover percentages in the airshed. Stratifying sample species by canopy and understory is desirable because understory and canopy species frequently occupy discrete temporal niches, with the understory species -- or in some cases individuals -- showing advanced phenology compared to canopy emergents (Richardson and O' Keefe 2009).

Additional community species to be sampled for Phase II will be selected from the community of the species present within the tower airshed according to the site-specific species abundance list, based on characterization data collection. Exceptions to the ordered selection process will be made to intentionally target state listed invasive species of concern, NPN target species and Project BudBurst (PBB) 10 most wanted species in regions of interest.

### 6.1.2 Phase I Sampling

For Phase I plant phenology sampling, NEON targets ~30 individuals (or small patches, in the case of annual or clonal species where delineation of individual ramets is problematic) of each of the three dominant species for phenology monitoring at evenly spaced locations around the 800m transect. For annual plants, locations may change slightly from year to year; an effort is made to retain the same locations interannually but adjustments may be made when plots do not contain any of the target species. Individuals selected are to be within 1-10m of the phenology transect and, where possible, span a range of life stages (e.g. include both canopy emergent and understory individuals). In addition, for sites where the tower phenocam does not include at least three individuals of the dominant species at each site, NEON aims to select and mark an additional 3 individuals of each dominant species within the phenocam view in order to make explicit linkages between phenocam greenness metrics and *in situ* phenophase observations. This additional sampling may not be achievable at all sites, depending on the availability of existing trails or boardwalks permit access to the relevant areas without causing undue disturbance.

### 6.1.3 Phase II Sampling

For Phase II plant phenology sampling, NEON will select additional species, up to 20 total, if site diversity allows. In order to target domain specific questions on invasive species, the regionally dominant invasive species will be selected as one of the 'community' species in cases where it is present along the sampling transect. NPN calibration taxa and PBB '10 most wanted' species will be similarly targeted. Individuals/patches to sample will be located along the phenology transect. Use of the ordered species list alongside the list of preferred species should ensure that a diversity of plant growth forms, invasives and natives are selected at sites where they are present in sufficient abundance, without any a priori definition of 'functional groups' (a concept which is not yet well understood for predicting phenology).

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

Individuals/patches monitored will be identified within 1-10m of the phenology transect. Technicians will aim to spread out sampling points for a given species along the transect, but (where possible) collocate individuals of different species at relatively few sampling points to increase sampling efficiencies. The locations of all individuals will be mapped.

## 6.2 Spatial Distribution of Sampling

The phenology transect at each site is oriented in the four cardinal directions. The minimum distance of the basal edge of the transect from the tower is site specific based on identified exclusion areas around Tower instrumentation (AD[08]). The exact location of the phenology transect are selected to facilitate inclusion of individuals located within tower plots for sampling (**Figure 2**).

In order to facilitate linkages between ground-based measurements, landscape greening metrics, NEON's most intensive measurements occur at the Tower and surrounding airshed. In addition to collocation with the instruments on the tower itself and soil array, this siting provides general collocation with the majority of the plant productivity plots and LAI measurements, which are concentrated in the tower airshed (AD[06], AD[07]). The targeting of phenological monitoring of plants in this area best leverages NEON's ability to contribute to an understanding of the correlates, causes and consequences of plant phenological change. NEON Core sites are selected be representative of the domain in terms of vegetation, soils/landforms, climate and ecosystem performance with the location of relocatable selected to address specific scientific questions often dealing with land use and connectivity (AD[01]). The placement of instrumented Towers within NEON sites is targeted in the dominant vegetation type at that site. This design allows for extrapolation from identified relationships between ecological drivers and responses made within the tower airshed to regional and continental trends (AD[01]).

An additional advantage of the concentrated placement of plants to be monitored in a relatively centralized location is that it reduces travel time during monitoring, allowing for greater sampling of species and individual plants. Thus while the monitoring of highly dispersed plants is desirable for some purposes, such as quantifying phenological variation across a localized environmental gradient, the number of observations that could be made over widely dispersed areas would be substantially reduced.

## 6.3 Temporal Distribution of Sampling

A standard sampling frequency for phenology has not been prescribed by the ecological community. Typically, sampling frequency varies by species, environment, and sampling objectives and resource limitations. The ideal frequency of sampling depends on analysis goals (e.g. fitting a thermal forcing model vs. long-term trend detection vs. quantifying intraspecific variation in phenology), as well as the degree of intraspecific and interannual variation in phenology. Expert scientific opinion, as contained in the 2012 NSF RCN report (2012), suggests a sampling interval of 2-4x/week to capture dominant species

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

phenologies. Miller-Rushing et al. (2008) recommend sampling every 2<sup>nd</sup> day to ensure a 97% chance of detecting a significant change in flowering date over 10 years of sampling, based on existing long-term flowering data collected in Massachusetts and Colorado. These recommendations assumed realistic anticipated rates of climate warming and interannual variability in temperature but a sensitivity of flowering date to temperature of 1 day/°C. A more recent synthesis of long-term phenology datasets worldwide (Wolkovich et al. 2012) suggests that flowering phenologies will, on average, shift at a rate of 5-6 days/°C). Therefore less frequent sampling may be adequate for many species for simple trend detection.

Following the RCN recommendations, the first three years of sampling dominant species (Phase I) phenology status aim to be recorded 3x/week during key transition periods. These data will be used to inform the sampling frequency necessary to characterize the mean (+/- 3 days S.E.) for leaf phenology transition dates for the 3 dominant species at the site in subsequent years. This target is based on a recent analysis by Jeong et al. (2012), which concluded that when observational error in estimating population mean transition days for key phenological events (e.g. budburst) are greater than +/- 3 days, parameterizing phenological forcing models is compromised. During Phase II, phenological observations will be reduced to 2x/week in order to accommodate sampling of a greater number of species.

During both Phase I and Phase II sampling, the most intensive (2-3x week), for sites with a single, definable pattern of seasonal activity per year phenological sampling only occurs during the active transition season for canopy development and senescence. The timing of canopy development periods at each site is not known a priori. In the absence of existing information to determine these transition dates at each NEON site, sampling dates are initially be determined from the analysis of local *in situ* measurements, where available, or remote sensing data when local knowledge is absent (see logistics and adaptability section 6.1.4, below). Sampling at intensive frequencies (2-3x/week) continue until the full canopy development or leaf greening has been achieved for all monitored individuals. During the intervening growing season, phenological observations of flowering occur biweekly; during the intervening dormant season no phenological observations occur. A second intensive sampling phase occurs coincident with senescence. In sites without a clear seasonality (e.g. Tropical), and/or multiple greenup periods per year, NEON will sample continuously with reduced frequency (1x/week, year round) for both phases of phenology sampling.

It is difficult to know a priori when to commence intensive sampling periods, since active periods vary both spatially across the continent, and interannually at each site. However, sampling efficiencies dictate that intercensus intervals vary dynamically in order to concentrate observations during periods of rapid change. Field crews at NEON sites will initiate seasonal sampling, at minimum, one week prior to onset of the earliest phenophase at each site. . This date is determined in collaboration with the site managers, using local information where available (such as at LTER sites where historical phenological data exists, or indicator plants at a nearby, lower elevation sites), phenology data from previous years at the site, or by historical MODIS data in sites where local information is not available to guide sampling.

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

Start of season metrics based on remote sensing data are typically biased early (White et al. 2009, Ganguly et al. 2010), so this should provide an ‘earliest’ outer bound on start of season. When one individual reaches the ‘trigger intensive sampling stage’, observation frequency increases to 3x (Phase I) or 2x (Phase II) weekly sampling.

- Trigger leaf-on intensive sampling stages are as follows:
  - Forb or Grass or Sedge: Initial growth
  - Drought-deciduous Tree/Shrub or Broadleaf Evergreen Tree/Shrub: Breaking leaf buds
  - Evergreen (non pine) Conifer/Deciduous Conifer: Breaking needle buds
  - Pine: Emerging needles

Intensive sampling stage ends when leaves/needles emerge and fill >50% of the canopy, then reduces to 1x/week until the canopy is >95% full with leaves. Sampling is then further reduced to once/two weeks to survey for open flowers.

Commencing three weeks before anticipated first date of senescence, based on local and/or MODIS data, sampling frequency increases to weekly. When one individual reaches the ‘trigger leaf off intensive sampling stage’, observation frequency will increase to or 2x/week sampling until all individuals show typical fall color, then dropping to 1x/week until all leaves are dropped/senescence is complete.

- Trigger leaf-off intensive sampling stage are as follows:
  - Graminoids/Forbs >5% dried or dead leaves
  - Deciduous Trees/Shrubs = 1 colored leaf
  - Evergreens = no leaf off intensive sampling stage

Time periods to commence intensive sampling phases on a site-specific basis may be modified as predictive accuracy increases (e.g. if it is possible to model start of season based on local temperature and moisture conditions with reasonably accuracy, and/or reliable local bio-indicators of start of season (e.g. phenocam greening, tower CO2 measurements) in order to increase sampling efficiencies. Thus we anticipate that the labor required for phenology sampling will be the greatest in the initial years of NEON and decline over time.

## 6.4 Logistics and Adaptability

### 6.4.1 Site-Specific Modifications

Modifications will need to be made for sites with plants that flower before leaf-out, sites with well-defined but extended growing seasons, sites with species with very different phenologies, sites with multiple growing seasons, and other scenarios that diverge from the typical temperate growing season. All site specific details including site-specific modifications, species selection and sampling windows are captured and tracked as part of the plant phenology sampling protocol (AD[05]).

Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

At a limited number of sites, where the tower airshed does not extend to the north of the tower, it is possible that the phenocam overlooks an area that differs in plant community composition than that found along the transect. In this case, if none of the species selected for phenology monitoring along the transect are present in the phenocam field of view, individuals that are dominant in the phenocam field of view are selected for monitoring and added to the list of species monitored along the transect.

#### 6.4.2 Incorporating New Technologies

Automated phenological monitoring using programmable, battery powered digital cameras has the potential to extend the spatial scale of automated sampling of plant phenology beyond the single phenocam situated on the instrument tower. However, the ability to derive accurate phenological information for a variety of taxa from these is still an active area of research (Crimmins and Crimmins 2008, Benton 2009). As these technologies evolve and costs decrease over the lifetime of the Observatory, it may become feasible to partially or wholly substitute automated measurements for technician observations. We anticipate that the initial years of calibration with dominant species will be extremely important for evaluating the potential to achieve automated monitoring of the species-specific phenophases.

As of this point in time, NEON does not plan to incorporate phenocams to monitor individuals along the phenology transect.

#### 6.4.3 Changes to Financial and/or Logistical Constraints

In the event the proposed sampling exceeds budgetary constraints, the technical working group recommends the following descope options (1): Sample relocatables on an every-other-year schedule, rather than annual sampling of phenology at all 3 sites; (2) At relocatables, sample only dominant species and/or species that are also sampled at the core site, skipping the more extensive community sampling. It is essential to retain the sampling frequency especially during the transition periods between phenophases and in order to detect changes over time a continuous record is best therefore, there is currently no descope recommendation for phenology sampling at the core site.

NEON is slated to sample for 30 years, and these are only a selection of the possible changes that may need to occur during the lifetime of the Observatory. Consultation with the NEON science staff and the technical working group should be made in order to ensure that changes to methodology, sample timing, frequency and allocation are consistent with NEON’s mission to provide high-quality, long-term data across the continent.

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Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
NEON Doc. #: NEON.DOC.000907	Author: S. Elmendorf	Revision: B

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Title: TOS Science Design for Plant Phenology		Date: 04/11/2018
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<i>Title:</i> TOS Science Design for Plant Phenology		<i>Date:</i> 04/11/2018
<i>NEON Doc. #:</i> NEON.DOC.000907	<i>Author:</i> S. Elmendorf	<i>Revision:</i> B

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