TOS SCIENCE DESIGN FOR TERRESTRIAL BIOGEOCHEMISTRY

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

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 (CalVal) and maintenance plans.

1.2 Scope

This document defines the rationale and requirements for Terrestrial Biogeochemistry in the NEON Science Design.

1.3 Acknowledgements

This document was written in collaboration with the Terrestrial Biogeochemistry Technical Working Group members, including: Gordon Bonan (NCAR), Gabriel Bowen (University of Utah), Benjamin Colman (Duke University), Paul Duffy (Neptune Consulting), Christine Goodale (Cornell University), Benjamin Houlton (University of California, Davis), Erika Marín-Spiotta (University of Wisconsin, Madison), Kiona Ogle (Arizona State University), Scott Ollinger (University of New Hampshire), Eldor Paul (Colorado State University), Peter Vitousek (Stanford University), Kathleen Weathers (Cary Institute of Ecosystem Studies), and David Williams (University of Wyoming).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.000001	NEON Observatory Design
AD[02]	NEON.DOC.001282	Introduction to the TOS Science Designs
AD[03]	NEON.DOC.000913	TOS Science Design for Spatial Sampling
AD[04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
AD[05]	NEON.DOC.000908	TOS Science Design for Microbial Diversity
AD[06]	NEON.DOC.000912	TOS Science Design for Plant Diversity
AD[07]	NEON.DOC.014048	Field and Lab Protocol: Soil Physical, Chemical, and Microbial Measurements
AD[08]	NEON.DOC.002212	Field and Lab Protocol for Measuring Soil Nitrogen Transformations
AD[09]	NEON.DOC.000914	TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index
AD[10]	NEON.DOC.001024	TOS Field and Lab Protocol for Canopy Foliage Chemistry and Leaf Mass per Area Measurements
AD[11]	NEON.DOC.014038	TOS Field and Lab Protocol: Core Sampling for Plant Belowground Biomass
AD[12]	NEON.DOC.001710	TOS Field and Lab Protocol for Litterfall and Fine Woody Debris
AD[13]	NEON.DOC.000907	TOS Science Design for Plant Phenology
AD[14]	NEON.DOC.000912	TOS Science Design for Plant Diversity
AD[15]	NEON.DOC.001241	NEON Algorithm Theoretical Basis Document: TOS Terrestrial Biogeochemistry of Soils and Plants – QA/QC of Raw Field and Lab Data and Chemical Composition Calculations
AD[16]	NEON.DOC.001242	NEON Algorithm Theoretical Basis Document: TOS Terrestrial Biogeochemistry – Stable Isotopes of Soils and Plants

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

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]	

2.4 Acronyms

Acronym	Definition
C	Carbon
^{12}C	Common stable isotope of carbon
^{13}C	Less common stable isotope of carbon
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
cm	Centimeter
CO_2	Carbon dioxide
EPA	Environmental Protection Agency
mm	Millimeter
g	Grams
h	Hours
K^+	Potassium
LTER	Long-term Ecological Research
LTM	Long-term Monitoring
m	Meter
M	Molar
MCMC	Markov Chain Monte Carlo
mg	Milligram
MH	Metropolis-Hastings
ml	Milliliter
mRNA	Messenger Ribonucleic Acid
N	Nitrogen
^{15}N	Less common stable isotope of nitrogen
^{14}N	Common stable isotope of nitrogen
NADP	National Atmospheric Deposition Program
NCSS	National Cooperative Soil Survey
NRCS	Natural Resources Conservation Service
PO_4^{3-}	Phosphate
P	Phosphorus
S	Sulfur
SO_4^{2-}	Sulfate
SSURGO	Soil Survey Geographic database
STATSGO	State Soil Geographic database
USDA	United States Department of Agriculture

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], AD[02].

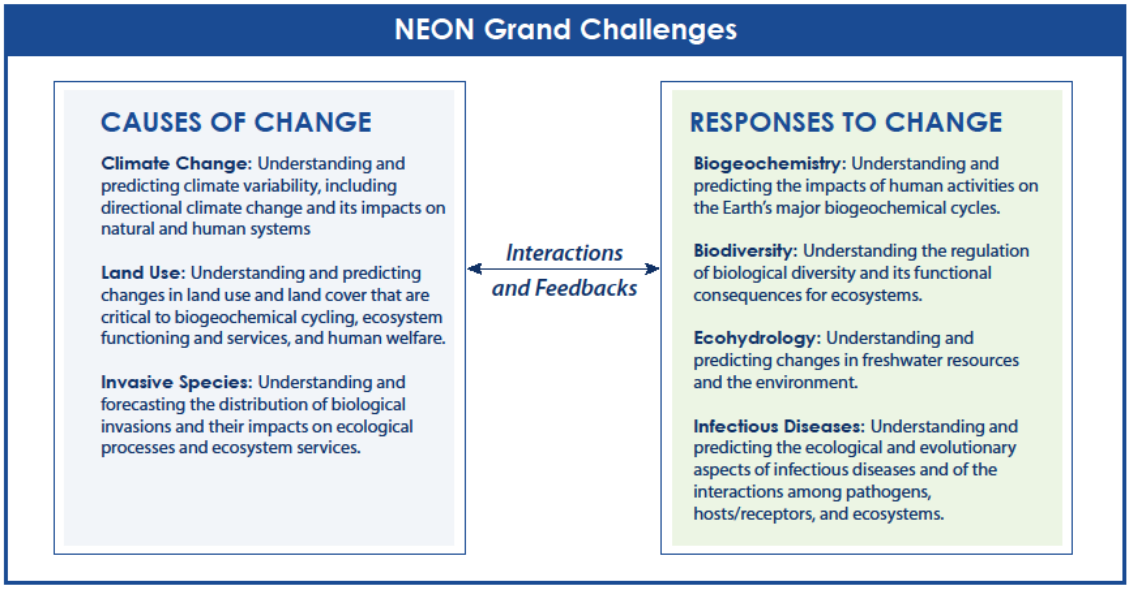


Figure 1. The seven Grand Challenges defined by the National Research Council (2001)

3.2 Components of the Observatory

There are five components of the Observatory, the Airborne Observation Platform (AOP), Terrestrial Instrument System (TIS), Aquatic Observation System (AOS), Aquatic Instrument System (AIS), and Terrestrial Observation System (TOS). Collocation of measurements associated with each of these components will allow for linkage and comparison of data products. For example, remote sensing data provided by the Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. 2008, Schimel et al. 2011.

3.3 The Terrestrial Observation System (TOS)

The NEON TOS will quantify the impacts of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa), infectious disease, soil, and nutrient fluxes across system interfaces (air, land, and water) (AD[01], AD[02]). The sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Plot and organism-scale measurements will also be coordinated with the larger-scale airborne measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.NEONinc.org).

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

The TOS Science Designs define the scientific strategies leading to high-level sampling designs for NEON sentinel taxa, terrestrial biogeochemistry, and infectious disease, linking NEON Grand Challenges and science questions to specific measurements (AD[02]). The TOS Spatial Sampling Design document describes the sampling design that collocates observations 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].

4 INTRODUCTION TO THE TERRESTRIAL BIOGEOCHEMISTRY SAMPLING DESIGN

4.1 Background

Humans are changing the fundamental chemistry of ecological systems on Earth by altering the global biogeochemical cycles of carbon (C), nitrogen (N), and other elements. These changes are driven by activities that include increasing emissions of carbon dioxide (CO_2) to the atmosphere from fossil fuel combustion, altering the distribution and nature of freshwater resources, changing land cover and land use, and increasing industrial extraction and application of reactive elements (Vitousek et al. 1997). Some of these impacts have been due to technological advances, such as the Haber-Bosch process, which enabled industrial-scale conversion of atmospheric N to nutritionally available ammonia, thereby increasing our ability to provide food to a growing human population. However, such modifications of biogeochemical processes have come with unintended consequences for the biosphere. The human signature on biogeochemical cycles can be seen in nutrient imbalances and ecological impacts at local to global scales: eutrophic surface waters (e.g., Carpenter et al. 1998, Correll 1998, Ryther and Dunstan 1971), declines in health and shifts in the composition of forest species (e.g., Horsley et al. 2002, Shortle et al. 1997), higher incidences of infectious diseases (e.g., MacKenzie and Townsend 2007), and spread of invasive species (e.g., Ashton et al. 2005, Crowl et al. 2008, Lovett et al. 2006, Vitousek and Walker 1989). Examples of these responses can be found in most regions of the world.

Despite having documented the connection between perturbations to biogeochemical cycles and ecosystem effects across the globe, our knowledge is sparser with respect to predicting changes in biogeochemical processes over large spatial extents using easily measured ecosystem parameters (e.g., Ollinger et al. 2002). Similarly, in only a few areas where intensive, regional studies have occurred, can one determine the degree to which previously impacted ecosystems are recovering in response to policy changes (e.g., Driscoll et al. 2007, 2003, 2001). In addition, there is a growing recognition that in order to understand the responses and feedbacks of ecosystems to global change, researchers must conduct integrated studies of the whole Earth system, including the interactions of climate, hydrology, and biogeochemical cycles (e.g., Gruber and Galloway 2008, Falkowski et al. 2000).

Ability to address these challenges will benefit greatly from spatially extensive, standardized collection of long-term observational data. In several cases, such investments have proven extremely valuable for tackling societally relevant problems. For example, long-term observations of increasing atmospheric CO_2 at Mauna Loa (the Keeling curve) have served both as evidence of human-induced climate change and inspiration for major research efforts. The establishment of experimental research sites such as Hubbard Brook in the 1950s and the Long-term Ecological Research (LTER) network in the 1980s created focal areas for long-term studies in the U.S. Additionally, monitoring networks, such as the National Atmospheric Deposition Program (NADP, http://nadp.sws.uiuc.edu/) and the EPA's Long-term Monitoring (LTM) of surface waters (Stoddard et al. 2003), started in response to problems of environmental and ecological importance, including acid rain and mercury deposition.

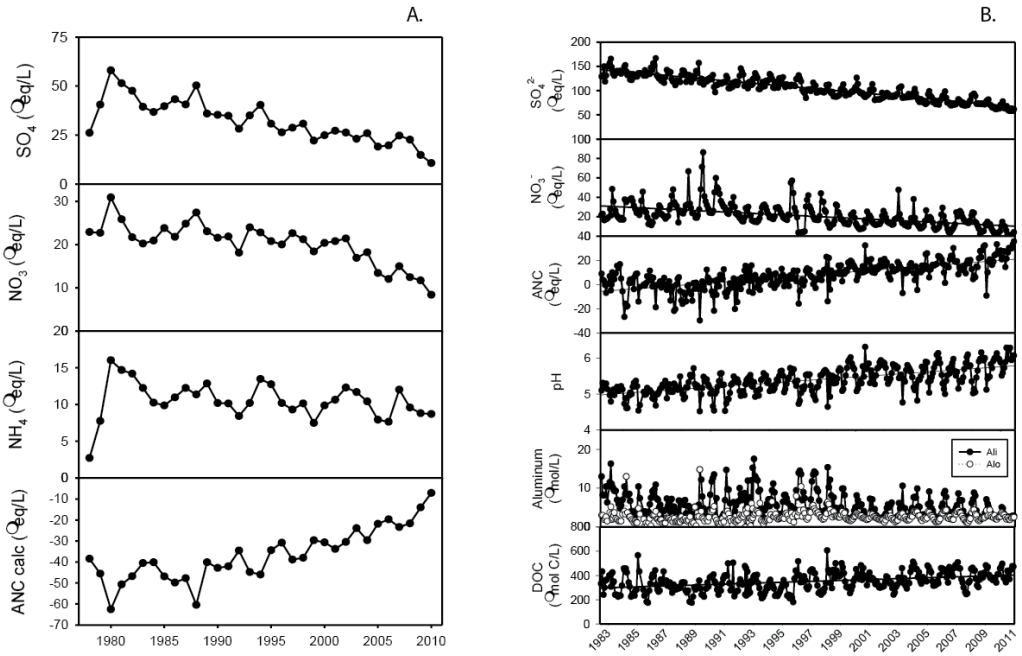


Figure 2. Long-term chemistry records of (A) precipitation at Huntington Forest, NY, and (B) Big Moose Lake, Adirondack State Park, NY (Source: C. Driscoll, in prep; NADP Station NY20, http://nadp.sws.uiuc.edu/ and Adirondack Lakes Survey Corporation, http://www.adirondacklakessurvey.org/).

Decades after the establishment of these study areas and monitoring stations, there are several examples of how collection of long-term biogeochemical data has prompted changes in policy to decrease negative impacts on ecosystems (e.g., Weathers and Lovett 1998, Lovett et al. 2007), and identified surprising ecosystem responses (e.g., Monteith et al. 2007). For example, 30 years of regional precipitation and lake chemistry observations in the Adirondack State Park (New York, U.S.) established a baseline pattern of the effects of acid rain deposition, decline in the concentrations of nitrate and sulfate in precipitation following Clean Air Act legislation, and recovery of lakes through the late 1990's and 2000's (e.g., Driscoll et al. 2007, 2003, 2001, unpublished data, Stoddard et al. 1999) (see Figure 2). These and related international monitoring networks detected unexpected increases in stream dissolved organic carbon (DOC) concentrations, a trend attributable to reductions in acid deposition through analysis of corresponding environmental data (Monteith et al. 2007). Other efforts have provided insight into ecosystem nutrient budgets using long-term observations (e.g., Likens et al. 2002) and the effects of nutrient enrichment on ecosystems using long-term experiments at Harvard Forest (e.g., Magill et al. 1997, 2004, Nadelhoffer et al. 2004) and land cover/land use change at Coweeta, Hubbard Brook, and H.J. Andrews research sites, as discussed in Turner et al. (2003).

Coupled to collection of spatially extensive, standardized, long-term observational data must be the assembly of interdisciplinary research teams to drive the advancement of process-based and predictive modeling frameworks (Hinckley et al. 2014). Models provide a means to challenge current knowledge and to guide future field investigations. Investment in such resources will provide a starting point to improve basic understanding of ecosystem biogeochemistry, as well as the ability to predict responses of ecosystems to future changes over large scales.

4.2 NEON's Contribution

Recent funding of the National Ecological Observatory Network (NEON) provides an opportunity to investigate ecological change at spatial and temporal scales that go well beyond those of the LTER and other long-term research sites, where data collection protocols, methods of analysis, and data storage are likely to be diverse and often incomparable. Previous overview papers (e.g., Field et al. 2006, Keller et al. 2008) outline the focus of the NEON strategy on addressing questions inspired by the 2001 and 2003 National Research Council (NRC) reports that outlined important next steps in earth and environmental sciences and highlighted seven Grand Challenge areas (NRC 2001, 2003). The guiding requirements for NEON follow directly from the seven Grand Challenge areas identified by the NRC and the NEON Science Strategy (Schimel et al. 2009), which more specifically summarizes network-wide approaches to sampling across space and time. At the highest level, NEON is designed to improve both understanding of complex ecological systems and the ability of researchers to forecast patterns of ecological change at local, regional, and continental scales using standardized and coordinated measurements of ecological taxa and environmental processes at 30-year core (n = 20) and 5 to 10-year relocatable locations (n = 40) across the U.S. Specific to the terrestrial biogeochemistry component of the observatory, NEON is designed to meet the high-level requirement of, "…further[ing] our understanding of the Earth's major biogeochemical cycles, evaluat[ing] how they are being perturbed by human activities, and determin[ing] how they might better be stabilized."

In order to construct NEON in an orderly and timely fashion, the project adopted a requirements-based framework to guide design of sampling strategies and infrastructure components. To understand the motivation behind the NEON Terrestrial Biogeochemistry design, it is useful to use the high-level requirements as a starting point. The requirements, summarized in Figure 3, emphasize four main themes: (1) promote an understanding of biogeochemical stocks and fluxes across air, land, and water systems, (2) use stable isotopes to infer biogeochemical and ecological processes, (3) colocate biogeochemical sampling with other NEON measurement platforms, and (4) measure constituents of interest primarily in plant tissues and soils. It should be noted that fulfilling these requirements relies also on measurement designs developed by the Terrestrial Instrument System (TIS) (i.e., associated with sensor-based tower measurements) and the Aquatic Observation and Instrument Systems (AOS and AIS) (i.e., groundwater and surface water measurements).

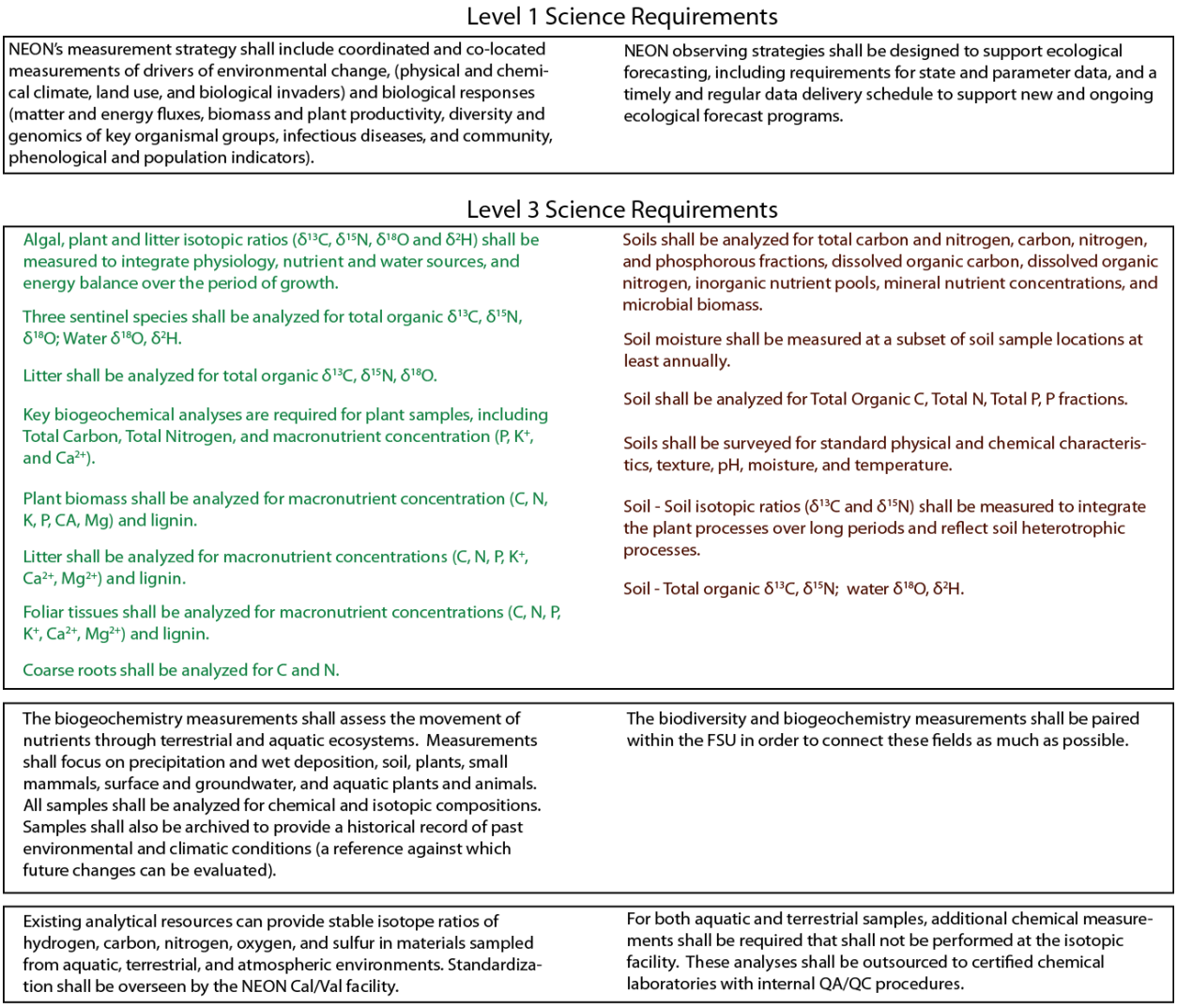


Figure 3. High-level requirements associated with the Terrestrial Biogeochemistry sampling design. Level 1 are overarching design goals, level 3 are requirements more specific to analyses and sampling approaches. Level 2 requirements are "systems requirements," which pertain more to infrastructure and sensor-based instrumentation; there are not any level 2 requirements associated with the Terrestrial Biogeochemistry sampling design.

4.3 Purpose and Scope

This document provides an overview of the terrestrial biogeochemistry sampling design for implementation at NEON sites. This document describes the motivation and rationale behind the suite of measurements identified to address the Grand Challenge questions and high-level requirements (Figure 3), the general field sampling and analytical approaches, and the development of the spatial and temporal sampling design. NEON's broader biogeochemistry measurements include nutrient deposition, C, energy, and water fluxes at tower (TIS) locations; C and nutrient fluxes in surface water sampling locations (AOS/AIS); terrestrial C and nutrient pools and select biogeochemical processes (TOS); and within-site assessments of ecosystem function, including ecosystem productivity

(TIS/TOS/AOP), biomass estimates (TOS/AOP), ecosystem (gas and material) exchange (TIS/TOS), and the effect of terrestrial systems on productivity and respiration in aquatic systems (TOS/AOS/AIS). Consistent with other sampling components within NEON, the goal of the terrestrial biogeochemistry design is to enable researchers to investigate a number of broad questions using observational and modeling approaches, such as how climate drivers impact ecological stoichiometry at the continental scale, and how regional disturbances (e.g., wildfire and drought) affect coupled C, N, and water cycles. Measurements of biogeochemical stocks and transformations will be made within the tower footprint and in distributed plots across the permitted area of each NEON site; Figure 4 shows an example of the Domain 3 NEON core site (Ordway-Swisher Biological Station, Florida), including the tower, distributed plots for terrestrial observations, and surface and groundwater sampling locations.

The terrestrial biogeochemistry measurements will be aligned temporally and spatially with those in adjacent atmospheric and aquatic systems within each site, as well as with other ecological measurements. Some measurements will be colocated at the plot-scale (i.e., sub-meter to meter), and others at that of the site (i.e., kilometer to tens of kilometers). Within tower and distributed plots, NEON will conduct sampling of biogeochemical stocks and soil processes, soil microbial communities, and vegetation (structure, biomass, species inventories, and chemistry of tissues). Within sites, these measurements will be colocated with nutrient deposition, meteorological data, C and water fluxes at the tower location, and sample collections of ecological taxa (insects, birds, and small mammals) and infectious diseases distributed across the sites. Approximately half of the NEON terrestrial observation sites are colocated with a NEON aquatic observation site in the same watershed. Colocation of terrestrial and aquatic observations will enable investigators to study C and nutrient transport across the terrestrial-aquatic interface, which is fundamental for measurements of ecological connectivity and quantifying lateral fluxes of biogeochemical cycles (Grimm et al. 2003).

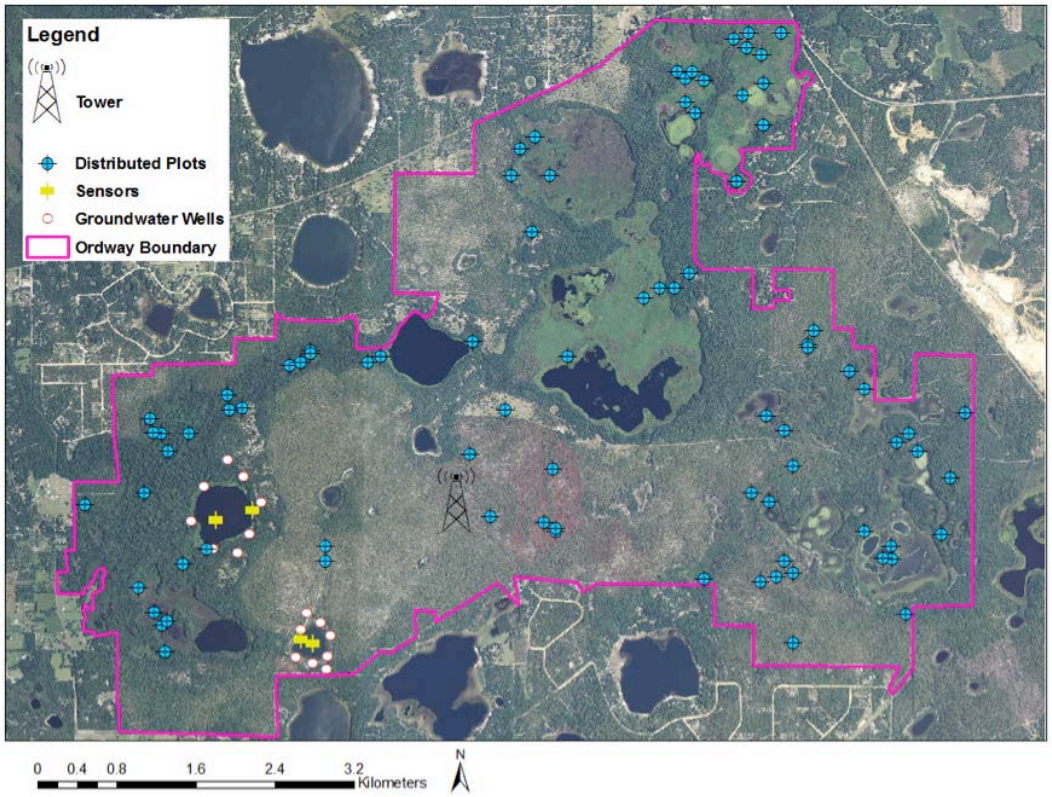


Figure 4. Example of a NEON site (Domain 3, Ordway-Swisher, Florida) with eddy covariance tower location, distributed plots, surface and groundwater sampling locations, and site boundary. Note: tower plots not shown.

5 SAMPLING FRAMEWORK

The NEON biogeochemistry sampling design focuses on providing data to address regional to continental scale questions regarding long-term changes to ecosystem nutrient stocks, process rates, major fluxes (measured at tower and aquatic sampling locations), and important feedbacks. There are four general design criteria underlying this program: (1) identification of measurements that will enable investigators to evaluate trends in biogeochemical cycles within a diverse array of ecosystem types distributed across NEON's spatial purview, and inspire further studies at sub-meter to continental scales; (2) determination of the spatial and temporal sampling design; (3) development of methodologies and QA/QC approaches that are consistent across sites and allow for comparisons with other network observatories; and (4) design of an approach to reevaluate the measurements and sampling strategy over the lifetime of the observatory. Discussion of these criteria is provided in the following sections.

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

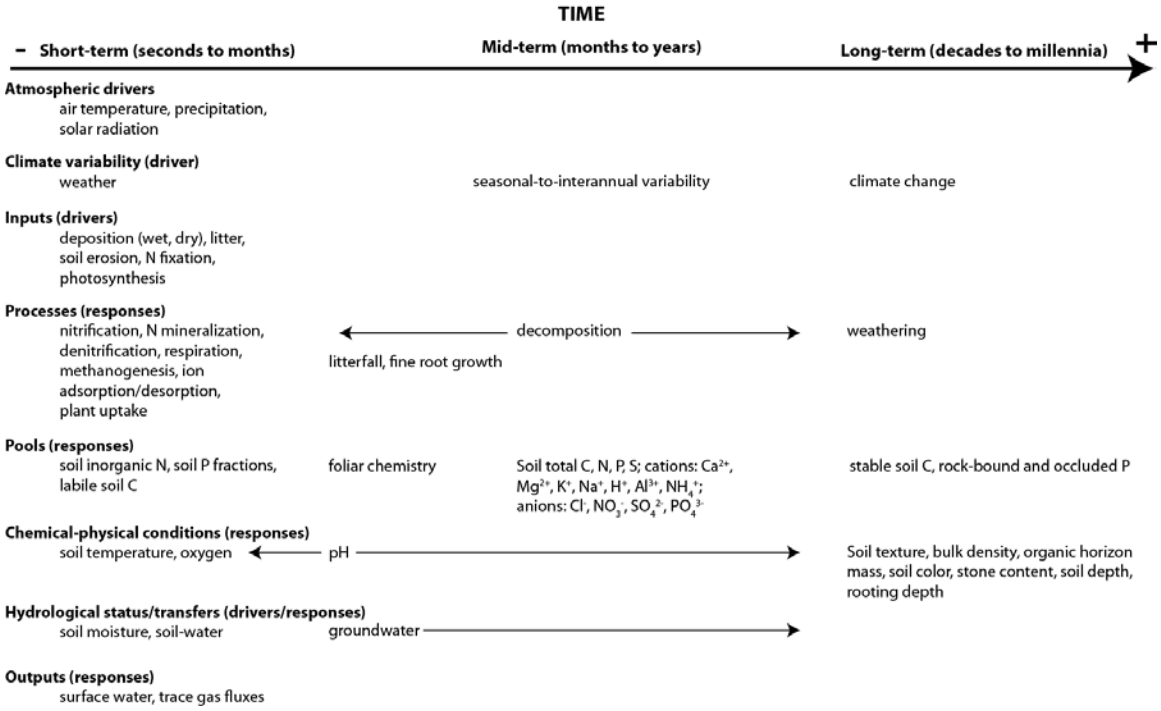
Execution of the protocols that stem from this science design procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (AD[04]).

5.3 Priorities and Challenges for Terrestrial Biogeochemistry

Components of biogeochemical cycles measured by NEON include drivers, such as precipitation, temperature, soil physical and chemical characteristics, and responses, such as biogeochemical transformation rates, C and nutrient fluxes, and nutrient uptake by plants and microbes. Drivers, responses, and the feedbacks between them can be viewed through the lens of anticipated timescales of change (see Table 1), which inform how they should be sampled over the lifetime of NEON. While most any factor can change abruptly at thresholds, the potential for change is highest at the level of soil biota (microbial communities and larger soil fauna), due to fast turnover times and gene systems that respond to local variations much more so than long-lived vegetation. Soil physical properties (e.g., texture, mineralogy) can be thought of at the other extreme, as they often remain relatively constant for hundreds or thousands of years, in the absence of disturbance. Thus, components of terrestrial biogeochemistry require different sampling strategies, depending upon the scale of measurement and expected spatio-temporal variability; with feedbacks and interactions that produce large-scale patterns that are relatively robust, for example, as the emergence of individual biomes.

NEON's terrestrial biogeochemistry sampling strategy could take one of several focuses within this framework. Emphasis could be placed entirely on characterizing spatial heterogeneity in C and nutrient stocks across each site, thereby creating a long-term dataset of storage in ecosystem components (soils and plant tissues). This design would rely heavily on collaborators from the larger natural sciences community to conduct short-term process-based studies that would capture the mechanisms underlying these patterns. However, past research has shown that on the order of decades, total ecosystem C and N stocks do not change dramatically (see Magill et al. 1997 compared with Nadelhoffer et al. 2004, Yanai et al. 2003) and heterogeneity in soil physical properties within sites may overwhelm the ability to discern important spatial and temporal patterns. Focusing only on changes in stocks, then, would not address the primary requirements of NEON (Figure 3), which include understanding dynamic phenomena within sites. Alternatively, NEON could focus on measuring transformation rates and important fluxes, including soil CO_2 efflux, nitrification, and denitrification, as well as soil water availability and chemistry, which are likely to change in response to climate and other drivers (see Barnett et al. 2005, Cable et al. 2008, Emmett et al. 2004, Hart 2006, Loik et al. 2004). However, focusing exclusively on these measurements would not capture the drivers or feedbacks affecting the results—again, not achieving the stated high-level requirements of NEON—and be extremely challenging from a resource (i.e., labor and financial) perspective.

Table 1. Anticipated timescales of change in components (drivers and responses) of biogeochemical cycles.



Given these considerations, and based on the framework provided in Figure 3 and Table 1, NEON will target key measurements of biogeochemical drivers, responses, and feedbacks at appropriate spatial extents and temporal frequencies. For the terrestrial biogeochemistry sampling design, these key measurements include: a one-time characterization of soil chemical and physical properties, and periodic sampling of soil and plant C and nutrient pools, C and N stable isotopes of soils and plant tissues, and net N mineralization and net nitrification rates in soils. Additional measurements at the tower and aquatic sampling locations will provide biogeochemical data across systems (air, land, and water). The goal of this approach is to provide a "research backbone" of data resources that will motivate further investigation by the natural sciences community, and provide insight into how ecosystem processes change through time. NEON will also endeavor to illuminate important feedbacks between the drivers and responses of interest. While it would be ideal to sample more of the processes and fluxes known to change over short timescales (i.e., those on the left-most column of Table 1) and in response to NEON's drivers of interest (i.e., climate, land cover/land use, and species composition) the NEON design will only incorporate a limited set of measurements that will grant investigators insight into biogeochemical cycling – net N mineralization and net nitrification. Parallel measurements at the tower location, including CO_2 fluxes, will provide further insight into short-term phenomena. NEON cannot (and should not) measure everything everywhere, and, thus, the sampling strategy for terrestrial biogeochemistry relies on targeting minimal sampling frequencies necessary to capture long-term trends in variables of interest (described below). This approach allows for creating a balance between meeting the scientific requirements of the design and understanding the logistical and financial constraints of conducting the field collections and sample processing. The resulting long-term dataset

will constitute an important contribution to ecological science, given NEON's coordinated approach across large spatial and temporal extents.

In general, the intensity of sampling efforts for soil and plant biogeochemistry should be proportional to the anticipated frequencies of change (and variability) for each measurement (Table 1). With the exception of soil N transformations, all other measurements within the terrestrial biogeochemistry design are on ecosystem components that are likely to have high spatial variability and low (i.e., 5-10-year temporal variability). Thus, measurements will be made of plant tissue chemistry (canopy foliar, litter, and roots) and soil extensively across each site in plots located within the tower airshed (i.e., within close proximity to the tower and within the dominant vegetation type) and distributed (i.e., within representative vegetation cover classes) across the site. The distributed plots (e.g., Figures 4 and 5) were specified as the foundation of the TOS Science Design for Spatial Sampling (see (AD[03])). The plan for the distributed plot locations was developed using a combination of classical statistical power analysis and spatially balanced sample allocation via the Reverse Randomized Quadrat Recursive Raster approach (RRQRR, Theobald et al. 2007) (see Figure 5). In general, a grid of potential, random plot locations is created for each permitted NEON site area, the area is stratified by vegetation community type, and then a stratified random list of plot locations is generated for the site. The data collected within the distributed plots are primarily aligned with the design goals associated with stand-alone TOS data products (e.g., all organismal sampling). Sampling within the tower plots supports the design goals associated with the entire TOS platform, but also provides a means to connect sensor-based measurements on and around the tower to the manual observations made across the site. All plots will be 40 m x 40 m. They will include a central 20 m x 20 m plot for plant biodiversity sampling, which will exclude soil sampling, due to its potential for disturbing the area. Thus, soil sampling will occur within the outer ring of this 400 m^2 area.

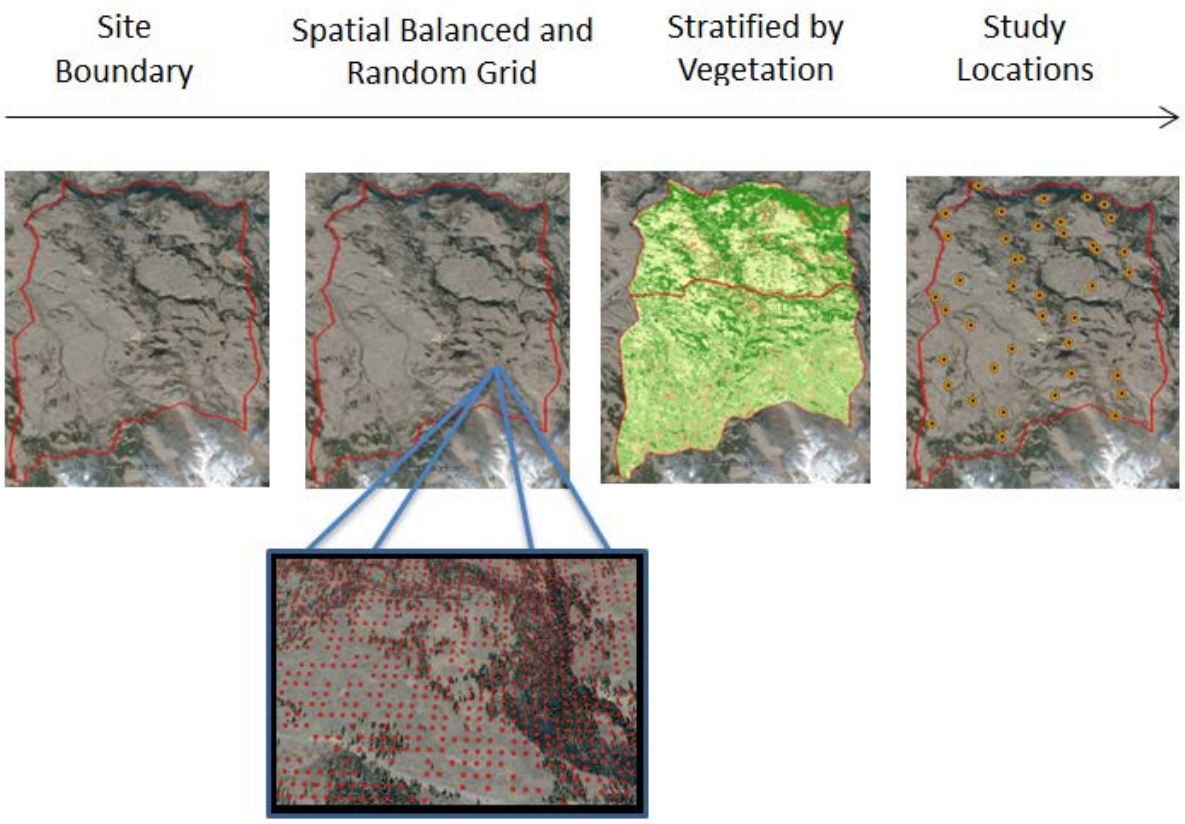


Figure 5. Steps in the RRQRR statistical framework to determine a stratified random distribution of plots across a NEON site.

An important goal for NEON is that it provides investigators with sufficient data to distinguish between variability and trends in ecological phenomena across space and time. In order to most effectively address this goal of enabling investigators to resolve complex patterns in space and time, two temporal strategies for sampling will be conducted that incorporate the considerations of temporal variability captured in Table 1. These two strategies include an initial site characterization to provide a baseline dataset necessary to inform the design of measurements and interpretation of data that fall under the second category, which is regular, periodic sampling during operations of the Observatory (i.e., the lifetime of NEON). Using the framework provided in Table 1, the expected timescales of change in drivers and response variables, the measurements described as part of the terrestrial biogeochemistry design fall into these two categories. The following section of this document describes the details of the methodologies, as well as the spatial and temporal sampling strategies for the terrestrial biogeochemistry sampling design.

6 SAMPLING DESIGN FOR TERRESTRIAL BIOGEOCHEMISTRY

6.1 Sampling Design for Terrestrial Biogeochemical Drivers

NEON's high-priority measurements of controls on biogeochemical cycling include climate variables (e.g., precipitation, air temperature, and solar radiation), precipitation chemistry, soil physical and chemical properties, and soil temperature and moisture. Climate variables and atmospheric deposition will be measured on the tower (part of the TIS) at the majority of sites. In brief, these datasets include: precipitation and dust deposition (amounts and a suite of chemical constituents), stable isotopes of water, C, and S in precipitation, CO_2 concentration profiles in air and soil (along the length of the tower, and in the top 1m of soil), energy fluxes, continuous soil temperature and moisture, and net ecosystem exchange. Measurements of soil physical and chemical characteristics, as well as distributed measurements of soil temperature and moisture fall under the purview of the Terrestrial Biogeochemistry sampling design, and will be made in distributed plots at each site in order to capture associated spatial variability. Prior to full operation of NEON in 2017, there will be a one-time soil characterization effort at each site. Sampling will include a suite of physical and chemical properties summarized in Table 2. These properties create the conditions promoting or suppressing soil microbial activity, affect air space in the soil matrix, influence plant physiological activity and growth, and shape the development of hydrological flow paths, which in turn control redox states and nutrient transport. In addition, they constitute a standard suite of measurements and laboratory analyses made during Natural Resources Conservation Service (NRCS) soil surveys, and, thus, will be comparable to other soil databases.

Although NRCS and other entities have conducted soil surveys with comparable measurements at the continental scale (e.g., STATSGO and SSURGO), the data are collected at coarser scales than the NEON design (i.e., kilometers versus meters), and the suite of analyses are not consistent across all sample locations. Measurement of soil physical and chemical characteristics by horizon to at least one meter depth (where bedrock is not less than 1 m from the soil surface) will provide data critical to interpreting shorter-term biogeochemical phenomena (e.g., N transformations), conducting process-based modeling efforts within sites, and broadly characterizing soil environments at the continental scale. Ideally, excavating and describing multiple soil pits would be done to characterize soils at each site (e.g., Vadeboncoeur et al. 2012). However, given the permitting restrictions at many NEON sites, soil pits will not be feasible everywhere; in restricted areas, soil cores will have to be collected, described, and analyzed instead. The one exception across all sites is within the tower footprint (located within the dominant vegetation type at each site) where one soil pit will be excavated, described, and sampled by NEON and NRCS scientists (total depth of 2m); the FIU team oversees this effort. For the distributed soil characterization effort, the number and location of soil pits or cores required to capture the spatial heterogeneity at each site shall be determined by a team of trained soil scientists contracted by NEON; a maximum of 40 locations will be sampled per site.

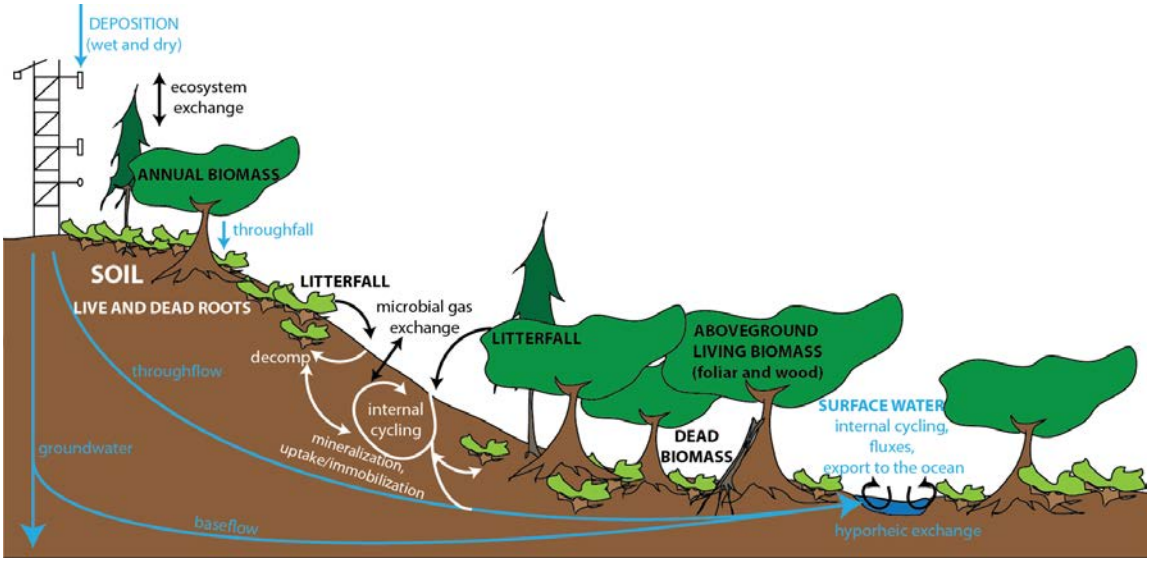


Figure 6. NEON biogeochemistry measurements within a site. Shown are variables that are part of the terrestrial biogeochemistry design (Terrestrial Observation System, TOS), as well as those associated with the tower (TIS), above- and belowground plant biomass (TOS), microbial community (TOS), and ground and surface waters (AOS/AIS). Subsurface hydrological flow paths that are important to constrain in biogeochemical budgets are shown, but will not be measured by NEON.

In addition to basic soil characterization data, soil water content and temperature are fundamental controls on biogeochemical processes, and must be measured as part of the baseline data framework. NEON will collect distributed measurements of soil temperature and water content across each site when soil cores are collected for C and nutrient stocks and stable isotope analyses, as well as net N mineralization and net nitrification rates (described below). Previous research by several groups (e.g., Groffman et al. 2012, Savage and Davidson 2001) has documented the importance of soil temperature and water content data, both for the purposes of observation in biogeochemical studies, and for informing models. While continuous data have become increasingly feasible to obtain due to greater affordability of instrumentation, the NEON budget does not include money for purchasing sensors and dataloggers to collect these measurements at multiple locations within each site. Within the tower footprint (i.e., the dominant vegetation type), automated instrumentation to measure soil water and temperature will be installed.

6.1.1 Sampling Methods

6.1.1.1 Soil Characterization

During the one-time soil characterization effort, field descriptions, soil sampling, and laboratory analyses will be done according to National Cooperative Soil Survey (NCSS) and Natural Resources Conservation Service (NRCS) protocols (see Burt, 2004; Schoeneberger et al., 2012). These approaches represent widely accepted and recognized standards for soil characterization and analysis, and, therefore, are appropriate choices for NEON. Table 2 shows the soil analyses that will be completed, and the methods that will be used. Contracted soil scientists local to each NEON domain will determine the locations to sample using their knowledge of soils at each site, as well as vegetation community and topographical information. Approximately 10-40 soil pit or coring locations will be chosen, based on the size and spatial heterogeneity of the site. (The upper limit of locations is constrained by available budget for this effort.) Field reports, including soil profile descriptions at each sampling location, a soil map, and recommendations for future NEON and community sampling efforts (e.g., areas requiring upland versus wetland sampling approaches) will be produced from the characterization work. In addition, physical and chemical analyses by soil horizon (listed in Table 2) will be made available to NEON data users via the online data portal, and soil samples by horizon will be archived according to NCSS protocols (air-dried, sieved to ≤ 2mm, and stored in a cool, dry location), see Section 6.1.1.3.

Table 2. Soil chemical and physical properties measured during the one-time characterization effort at each NEON site.

Analysis	Method[a]
Particle size distribution	Hydrometer method
Bulk density	By appropriate method; TBD based on sampling restrictions at site
pH	In water and $CaCl_2$
Total organic C; total N, S	Combustion; elemental analyzer
Inorganic C ($CaCO_3$)[b]	Acidification with HCl and measurement of evolved CO_2
Phosphorus	By appropriate method; Bray or Mehlich III, based on soil pH
Extractable cations and cation exchange capacity (CEC)	Ammonium acetate extraction
Aluminum[c]	Potassium chloride extraction
Electrical conductivity (EC)	Prediction and saturated paste
Cations and anions[d]	Saturated paste
Fe, Al, Mn, Si, P	Acid oxalate extract
Fe, Al, Mn	Citrate dithionite extract

[a] Method of analysis is described in brief. All laboratory analyses and QA/QC procedures will be completed according to the Soil Survey Laboratory Methods Manual (Online: http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/research/lab/guide/?cid=nrcs142p2_054247)

[b] Inorganic C will be measured only for samples containing $CaCO_3$

[c] Extractable Al will be measured only for samples with pH <5.5

[d] Saturated paste extracts will be collected for measurement of cations and anions for samples predicted to have measureable amounts by standard methods.

6.1.1.2 Soil Temperature and Water Content

Soil temperature and water content will be measured manually at each site during regular sampling of soils for C, nutrients, stable isotopes, and N processing rates (temporal frequencies described below). At each soil sampling location, soil temperature will be measured in the top 10 cm using a surface temperature probe (stainless steel with digital readout). Soil water content will be measured in the laboratory on subsamples of composite soil samples using the widely accepted thermogravimetric method (Topp and Ferré, 2002). Measurements of soil water content will be made on the top 30 cm of soil (see below), separated and analyzed by organic (if present) and mineral horizons. The procedures for field and laboratory components of this sampling are provided in Field and Lab Protocol: Soil Physical, Chemical, and Microbial Sampling (AD[07]).

6.1.1.3 Samples for Archiving

Subsamples of each soil horizon from soil profiles described and sampled at each NEON site will be archived for future analysis by members of the natural resources community. The contracted group performing the characterization work will archive one pint of soil (air-dried and sieved to ≤ 2 mm) for inclusion in the NRCS soil archive in Lincoln, NE. While access to these subsamples will not be managed by NEON, they will be accessible to the larger research community with permission from the NRCS.

Table 3. Archived samples from the soil characterization effort.

Sample	Processing	Storage conditions	Volume or mass stored	Number site^{-1} sampling year^{-1}[a]
Soil characterization	Air-dried, sieved to ≤ 2 mm	Ambient, dry cabinet	1 pint[b]	10-200

[a] Explanations for the expected ranges of samples generated are described above.
[b] As part of the soil characterization effort, one filled pint of soil per horizon will be archived, per NRCS standard operating procedures. Archived subsamples will be housed at the NRCS soil archive in Lincoln, NE.

6.1.2 Spatial Distribution of Sampling

The group contracted by NEON to conduct the field sampling and laboratory analyses for the one-time soil characterization effort will determine the spatial distribution of sampling locations of soil pits/cores. Based on the spatial heterogeneity (i.e., topography, expected variability of soil types, and vegetation communities) and size of each site, 10-40 sample locations will be chosen for descriptions and sampling of soils. These locations will be chosen to represent the range of soil types within each site. The spatial distribution of soil temperature and water content is described below in the section on Soil Biogeochemistry (Section 6.2).

6.1.3 Temporal Distribution of Sampling

During site characterization, a one-time soil survey will occur at each site. The physical and chemical analyses of soils by horizon (listed in Table 2), together with the field descriptions and interpretations will provide baseline soils data and inform the sampling strategies for regular, periodic measurements of soils by NEON. The temporal distribution of soil temperature and water content sampling is the same as that for soil C and nutrient pools and soil N transformations; it is described below in Section 6.2, Sampling Design for Soil Biogeochemistry.

6.2 Sampling Design for Soil Biogeochemistry

NEON's high-priority soil biogeochemical measurements include size of C and nutrient pools, stable isotopes of C and N, and targeted measurements of N transformation rates. Measurements of the soil microbial community are directly linked (i.e., made on subsamples from the same soil cores as those collected for soil C, nutrients, and stable isotopes) and are described in detail in TOS Science Design for Microbial Diversity (AD[05]). Throughout the lifetime of each observatory site, the soil stock measurements include total organic C and major nutrients (N, P, and S), exchangeable anions and cations, soil organic C fractions, and the stable isotopes of C and N by horizon (i.e., organic and mineral). A focus on these constituents is important, as they are major constraints on plant growth, influence microbial activity and redox reactions, and are indicative of ecosystem health. Measurement of these particular chemical constituents in soils is consistent with other components of the NEON biogeochemistry design, including measurements of plant tissues, ecosystem inputs at the tower location, and export in surface waters.

The resulting data will enable investigators to analyze ecosystem stoichiometry, and to infer the changes in sources and process rates that may explain patterns in the data. For example, following C:N ratios in soils over time is a useful indicator of microbially-mediated transformations (e.g., Kaye and Hart 1997) such as decomposition (Melillo et al. 1989), and correlate with ecosystem losses (Tietema and Beier 1995, Gundersen et al. 1998, Aitkenhead and McDowell 2000, Lovett et al. 2002). Data on total elemental stocks can also lend insight into identifying where nutrients are stored and released within a landscape and across different ecosystem types within NEON. By combining elemental concentration data with analyses of soil C and N stable isotopes, researchers will be able to address questions related to understanding the mechanisms underlying different nutrient sources (e.g., Phillips and Gregg 2003), soil organic matter turnover rates and decomposition extent (e.g., Marín-Spiotta et al. 2009, Bernoux et al. 1998), and integrated analyses of ecosystem processes (e.g., West et al. 2006, Robinson 2001) and loss pathways (Houlton and Bai 2009, Bai et al., 2012).

The rates of soil biogeochemical processes are expected to change in response to shifts in climate forcing, influencing the amount and forms of C and nutrients that move across systems (Gruber and Galloway 2008). Many previous studies from a wide range of disciplines within earth and environmental sciences have documented the short-term sensitivity of process rates to changing ecosystem drivers

(Barnett et al. 2005, Cable et al. 2008, Emmett et al. 2004, Hart 2006, Loik et al. 2004), as well as long-term controls (e.g., Barford et al. 2000, Richardson et al. 2007). While it would be extremely informative for NEON to quantify a large suite of biogeochemical process rates distributed across each site, including soil CO_2 efflux, nitrification and denitrification, net N and S mineralization, as well as inorganic nutrient pools, it is neither logistically nor financially feasible in the observatory context. Instead, NEON will provide insight into microbially mediated processing of N through periodic measurement of net N mineralization and net nitrification in soils. Net N mineralization is a measurement of the amount of inorganic N (ammonium and nitrate) released from organic material over time. Net nitrification is the measure of nitrate converted from ammonium over time. In both cases, these rates reflect net accumulation, so do not account for uptake and loss pathways within soils. Nitrogen process rates reflect the lability of substrate in soils, the activity of the microbial community, and the potential for N loss to the atmosphere (via denitrification) or export to aquatic ecosystems (e.g., as nitrate).

There will be many ways that these data can be analyzed by NEON data users, and, at the spatial extent of NEON, they will provide important insight into nutrient cycling in a variety of systems. Previous studies have found that more easily measurable ecosystem parameters, such as foliar N content and soil C:N ratios can be used to predict N processing rates (see Ollinger et al. 2002; Figures 6 and 7). NEON will be measuring these parameters as well, and will be able to provide data users with these predictive relationships over a larger suite of ecosystem types and spatial extent than has been done before.

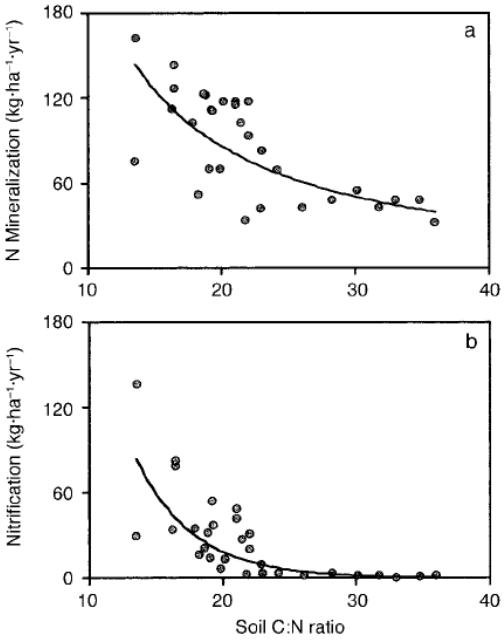


Figure 7. Soil (a) net N mineralization and (b) net nitrification in relation to soil C:N (top 10 cm). Figures are from Ollinger et al. (2002).

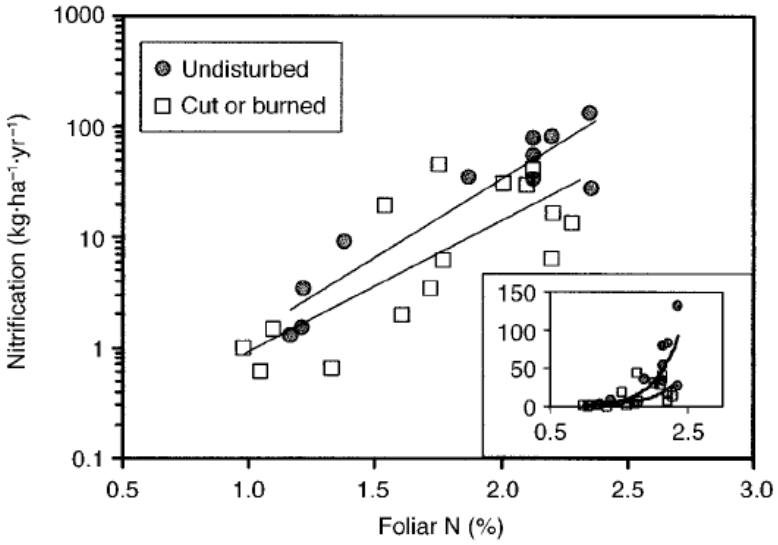


Figure 8. Net nitrification in relation to foliar N concentrations for disturbed and undisturbed forest stands. Inset shows the nonlinear response of nitrification. The trends are described by log(NO3) = 2.75(foliar N) – 2.96, (R2 = 0.81, P < 0.001) for undisturbed stands; log (NO3) = 2.94(foliar N) – 4.28, (R2 = 0.63, P < 0.001) for disturbed stands. Figure is from Ollinger et al. (2002).

Another potential use of these results is to compare them to information gleaned from microbial mRNA data to assess dominant biogeochemical processes in soils. Messenger RNA can grant insight into dominant pathways of microbial activity (e.g., nitrification, denitrification, sulfate reduction, methanogenesis). To date, this approach has been explored in marine systems (Stewart et al. 2012, Mou et al. 2011, Urich et al. 2008) but not soils. Comparison of information from direct measurements of biogeochemical transformations and extractions of mRNA (part of the microbial ecology component of the NEON design) may help to inform whether or not indirect assessments of cycling are possible.

6.2.1 Sampling Methods

6.2.1.1 Soil Carbon and Nutrient Pools

Soil cores for bulk chemical analysis will be collected manually in tower and distributed plots at each NEON site. NEON field domain staff will be trained in basic soil horizon identification (i.e., organic versus mineral soil horizons, where organic horizons are present) and handling and preliminary processing of soils for biogeochemical analyses. In general, the top 30 cm of the soil profile will be sampled for chemistry and N transformations. Where organic horizons are present, they will be separated from mineral soil, and then processed and analyzed independently. Details on the field sampling and laboratory processing of soils are provided in Field and Lab Protocol for Soil Physical, Chemical, and Microbial Measurements (AD[07]). For analyzing the chemistry of bulk soils, NEON will use analytical methods that are widely accepted in the soil science and ecosystem biogeochemistry communities (i.e., consistent with NRCS and SSSA standards) and are summarized in Table 3. All field and laboratory data

will be subject to QA/QC procedures detailed in NEON Algorithm Theoretical Basis Document: TOS Terrestrial Biogeochemistry of Soils and Plants – QA/QC of Raw Field and Lab Data and Chemical Composition Calculations (AD[15]) and NEON Algorithm Theoretical Basis Document: TOS Terrestrial Biogeochemistry – Stable Isotopes of Soils and Plants (AD[16]). All laboratories contracted with NEON for this work will need to demonstrate established QA/QC protocols and will be periodically audited by NEON Calibration/Validation personnel.

Table 4. Soil biogeochemical analyses and laboratory methods

Analysis	Method[a]
Total organic C; total N, S	Combustion; elemental analyzer
Phosphorus	By appropriate method; Bray or Mehlich III, based on soil pH
Soil organic C fractions	Method TBD 7/2014
Ammonium	Potassium chloride extraction, autoanalyzer
Nitrate	Potassium chloride extraction, autoanalyzer
$\delta^{13}C$, $\delta^{15}N$	Combustion; isotope ratio mass spec (e.g., Thermo Delta Plus XP IRMS)
pH	In water and $CaCl_2$

6.2.1.2 Soil Nitrogen Transformations

Soil cores for N transformations will be collected in tower and distributed plots and organic and mineral horizons will be separated (if an organic horizon is present) for analysis. Quantifying net N mineralization and nitrification rates is traditionally accomplished using soil incubations either in the field or laboratory (see Eno 1960, Binkley and Hart 1989). For field incubations, two soil cores are collected, one is transported to the laboratory for immediate extraction and analysis of ammonium and nitrate, while the other is put in a plastic bag (or other vessel, such as a PVC tube) and replaced in the soil borehole. Depending upon the investigator's study question and/or the ecosystem, the incubated core is removed one week to one or more months later and processed in the laboratory using the same process as the initial, paired soil core. To calculate net N mineralization, the final and initial masses of ammonium plus nitrate per unit dry soil (or nitrate only for net nitrification calculations) are then differenced, and a rate of production (usually per day) is reported by dividing the difference by the total incubation period. For laboratory incubations, one soil core is collected in the field and then subsampled for initial extraction of ammonium and nitrate and for incubation. The incubated soil is kept in the laboratory in conditions similar to the field and moisture is maintained according to an initial weight. At the end of the incubation period, a subsample of the core is extracted for ammonium and nitrate, the extractant is analyzed, and calculations are performed as for field incubated soil cores.

There are advantages and disadvantages to both field and lab incubation approaches. Field incubations can be done using a variety of approaches (e.g., buried bag, close-top core, resin). Generally, they maintain field temperature, but the water balance of the core can be influenced by the method. They are thought to provide better quality information than lab incubations (see discussion in Binkley and

Hart 1989), but they are more labor intensive because they require an individual to return to the sampling location to retrieve the soil core. In contrast, laboratory incubations are more streamlined in terms of the workflow (i.e., they do not require a return trip to the field site), but it is more difficult to maintain field conditions in the incubated soil core. Some studies have shown strong correlations between lab and field incubated soils for determination of N processing rates (e.g., Ollinger et al. 2002), making the more easily conducted lab incubation viable. However, other studies have found stark differences between the results of incubations under field and lab conditions (e.g., Johnson et al. 1980).

NEON requires standardized approaches in order to achieve the best degree of comparability among datasets from all sites. To this end, NEON will perform field incubations of soils for net N mineralization and net nitrification, not laboratory incubations. In addition, NEON will use standard laboratory processing and analysis of all soils: potassium chloride extraction of ammonium and nitrate at all sites. In the case of the extraction method, there is the potential in some locations for results to be influenced by using one standard approach. However, the alternative of using site-specific protocols initially does not permit data users to determine whether observed differences are due to methodology or real behavior. Once data are available and users can evaluate patterns at the broad spatial scale that NEON represents, they can also conduct follow-up studies to test whether or not methodological effects are present. Constraints at NEON sites may require some site-specific modification of field incubation techniques (e.g., buried bag, close-top core). The approaches for field sampling and laboratory processing of soils for N transformation rates are described in detail in Field and Lab Protocol for Measuring Soil Nitrogen Transformations (AD[08]).

6.2.1.3 Samples for Archiving

Subsamples of soil collected for analysis of soil C, nutrients, and stable isotopes will be archived for access by the larger natural sciences community. In the process of preparing soils for analysis of these constituents, two preparations will result. Due to the multitude of potential uses and expected demand of this archived resource, NEON will archive both preparations (see Table 5). In addition to these archived samples, frozen (i.e., -80°C) soil subsamples from soil microbial collections will be archived (these samples are splits from the soil cores collected for biogeochemical analyses).

Table 5. Archived samples from the soil biogeochemistry sampling effort

Sample	Processing	Storage conditions	Volume or mass stored	Number site^{-1} sampling year^{-1}[a]
Soil stocks and stable isotopes	1. Oven-dried (60°C, 48 hr), sieved to ≤ 2 mm, ground 2. Air-dried, sieved to ≤ 2 mm	Ambient, dry cabinet	1. Filled 20 ml vial 2. Remaining soil following subsampling for pH and soil moisture	30-120
Soil N transformations[b]	N/A	N/A	N/A	N/A

[a] Explanations for the expected ranges of samples generated are described above.
[b] Subsamples of soil collected for N transformation rates will not be archived.

6.2.2 Spatial Distribution of Sampling

Measurements of soil C, nutrient pools, and stable isotopes will be made in four tower plots and 6-16 distributed plots at each site. Four tower plots and a maximum of 6 distributed plots will be sampled for soil N transformation rates. The number of distributed plots will be determined by the degree of spatial heterogeneity in vegetation, soil types, and topography at each site. The upper limit of distributed plots for spatially heterogeneous sites is based on budgetary constraints for field labor and chemical analysis of samples. However, the upper limit should still provide sufficient information to discern important spatial patterns in phenomena of interest. This design will enable the NEON user community to analyze data in a variety of ways, including comparing variables of interest across vegetation types (e.g., soil C:N in the dominant compared with secondary vegetation communities), as well as employing geostatistical approaches. Within each plot, a center 20 m x 20 m subplot for plant biodiversity sampling will be maintained, and soil sampling will only occur outside of this area within the larger 40 m x 40 m plot. Collection of soil cores will be made at three random locations within plots during each collection event, for an upper limit of 60 sample locations for C, nutrient pools, and stable isotopes, and 30 sample locations for N transformation rates. Per sampling year (described below), 30-120 samples will be generated per site for analysis of C, nutrient pools, and stable isotopes (dependent on whether or not both organic and mineral horizons are present), and 90-180 samples for N transformations (this estimate includes multiple sampling events per year, described below). In the event that the number of soil samples for measuring N transformations is too great for the time budgeted by the Field Operations team, NEON will reduce the number of plots sampled, but preserve the temporal resolution (described below).

6.2.3 Temporal Distribution of Sampling

Soil C, nutrient stocks, and stable isotopes are not likely to be highly temporally variable at NEON sites. In other long-term projects, such as the chronic N addition study at Harvard Forest LTER, minimal changes in total N content and distribution were detected in soils after 10 years of elevated N deposition (see Magill et al. 1997 and Nadelhoffer et al. 2004). Thus, NEON will sample these constituents once every 10 years in the tower and distributed plots to capture long-term trends at each site. At relocatable sites, these measurements will be made once during initial operations and then once prior to decommissioning.

Greater inter-annual, as well as intra-annual sampling frequencies would be optimal to document the dynamics of soil N transformation rates, which are expected to undergo short-term changes in response to NEON's drivers of interest (see Table 1). However, these measurements are also very labor-intensive, and the NEON budget does not accommodate making them every year. To provide useful information on the intra-annual changes associated with N transformations, while compensating for the constraints of the project, NEON will sample soils to calculate rates of net N mineralization and net nitrification every 5 years, but within the sampling year, conduct soil core incubations three times. The three sampling periods will be during peak biomass at each site, as well as expected "hot moments" of biogeochemical importance to capture a range of temporal dynamics. These hot moments may differ by site. Examples include: summer in California, when surprisingly elevated rates of microbial N processing have been observed compared with the wet growing season (see Parker and Schimel 2011), snowmelt in locations where a seasonal snowpack develops (e.g., the Intermountain West and the Northeast), and first rains of the growing season (or monsoon rains) in arid environments (e.g., the Southwest). The initial 3 years of data collected in this manner will be analyzed and reviewed by a group of community experts and/or "NEON power users" in order to optimize the sampling strategy over time.

6.3 Sampling Design for Plant Biogeochemistry

NEON will quantify variation in plant tissue chemistry at plot to site scales using a combination of ground- and airborne-based methods. Ground-based collections will include sampling and analysis of total C and nutrient concentrations (N, P, S, Ca^{2+}, Mg^{2+}, K^{+}, and Al^{3+}) in sun-lit foliage of dominant and co-dominant canopy species, and C and N of litter and roots. Stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of these plant tissues, as well as other chemical characteristics (i.e., chlorophyll in canopy foliar and acid unhydrolyzable residue (chemical extraction of lignin) in canopy foliage and litter) will also be measured (see Table 4). These data will provide valuable information about nutrient uptake and storage in terrestrial ecosystems, both above- and belowground. In parallel with plant tissue sampling for chemistry, above- and belowground biomass estimates will be calculated from annual collection of plant species data, structural data, leaf area index, leaf mass per area, and collection of litterfall and root biomass every 5 years (see TOS Science Design for Plant Diversity (AD[06]) and TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index (AD[09])). These data are necessary for calculating

plant nutrient stocks (e.g., Pardo et al. 2005, Yanai et al. 2010) and estimating production rates, which drive biogeochemical models of the C cycle.

In 2012, NEON conducted a prototype effort in Domain 1 (Harvard Forest core site) and sampled canopy foliar tissues across a gradient of plant community structure (i.e., forested, mixed-stature, and disturbed/grassland). This gradient also included differences in plant groups (i.e., phylogenetic and photosynthetic pathways). One objective of the prototype was to determine whether sampling across a gradient of vegetation communities would yield differences in C, N, and stable isotopes of C and N in plant canopy tissues among these groupings. Figure 9 shows separation of plant groups by $\delta^{13}C$ values and N content, as well as C:N ratios. Data are separated according to phylogenetic groups, as well as photosynthetic pathways (i.e., the grasses included are C4). These data illustrate some of the patterns across vegetation communities that NEON data users will be able to explore within and among sites.

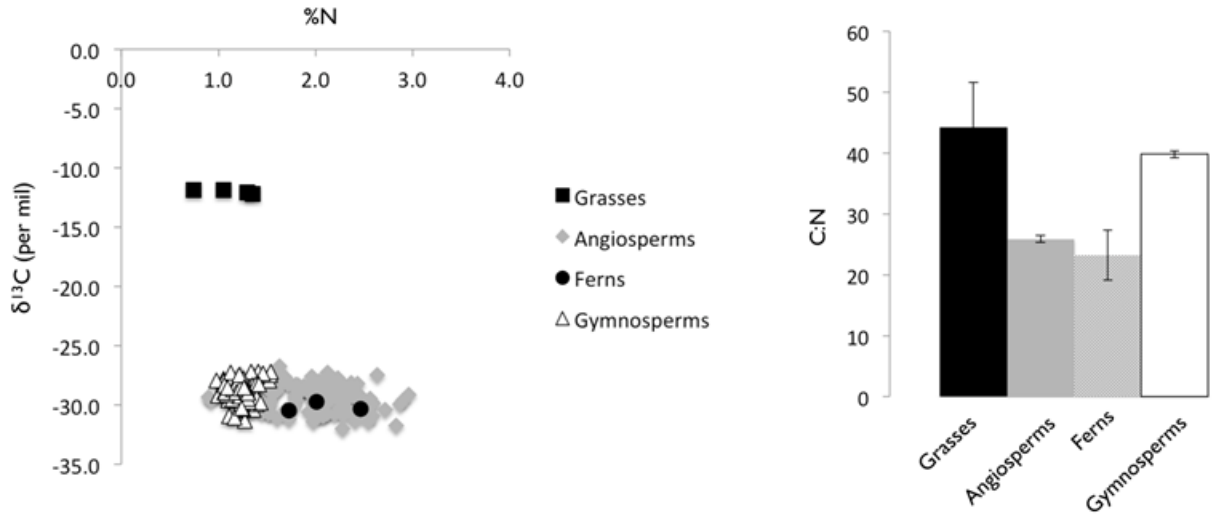


Figure 9. Results from the Domain 1 (Harvard Forest) NEON Prototype in 2012. (a) Percent N and δ13C of plant canopy foliar tissues by plant phylogenetic group (i.e., angiosperms, ferns, and gymnosperms) and photosynthetic pathway (i.e., C3 and C4), and (b) Mean (± 1 SE) C:N ratios across the same groups.

Airborne observations of plant canopy chemical and structural characteristics will be collected across each NEON core site every year. The ground-based plot-level assessments of plant chemical characteristics (described above) will be necessary to interpret the hyperspectral data. Other research groups have successfully integrated airborne remote sensing data with ground-based measurements of canopy nutrient concentrations to describe variation in the composition of some plant nutrients (especially N) and biochemical characteristics (especially pigments) at large spatial scales (Asner and Vitousek 2005, Ollinger et al. 2002, Wang et al. 2010, 2007). Relating the ground-based plant chemistry and structural measurements to the remote sensing data will rely heavily on algorithms developed by the airborne team and the research community. The integration of these data streams will be critical to create large-scale data surfaces for modeling efforts at regional to continental scales. Foliar chemistry data are needed to constrain leaf photosynthetic capacity in ecosystem models. Generally, these data

are unavailable for Ameriflux or Fluxnet tower sites. Thus, when model simulations are compared to tower sites, it is not always clear whether or not photosynthetic capacity is represented appropriately; data from NEON sites will help to constrain this and other ecosystem variables.

6.3.1 Sampling Methods

6.3.1.1 Plant Tissue Collection

Samples of sun-lit canopy foliar tissues will be collected in tower and distributed plots at NEON sites during peak biomass and coincident with data collection by the airborne team. At forested sites, NEON field personnel will obtain samples of dominant and/or co-dominant species using methods permissible at the site (e.g., shotgun, slingshot, tree climbers, or pole pruner). At grassland sites, samples (species bulked) will be obtained during aboveground biomass clip harvests (as described in TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index (AD[09]). At savanna and shrubland sites/areas, canopy samples will be collected using a combination of these approaches.

In forested and savanna/shrubland plant communities, field personnel will need to identify species and individuals for sampling. In order to calculate plant foliar N and other chemical constituents within each plot, dominant (e.g., > 50% of the plant community) and/or co-dominant species will be selected for sampling of leaf mass per area (LMA) and the suite of chemical constituents in Table 4. A total of three individuals will be sampled in each plot. Priority will be given to tagged individuals (dominant representatives of the plant canopy that are > 10 cm DBH, tagged for long-term measurements of structural characteristics). Second, to individuals sampled previously for canopy tissue, and finally to new individuals. A trained botanist on the NEON domain field staff will identify plant species present and inform those carrying out canopy foliar sampling. In savanna ecosystems (i.e., grasslands dotted with trees), trees within plots will be sampled, and clip harvests of herbaceous biomass will also be performed (see below). Further details on the field sampling of canopy foliar tissues are in TOS Field and Lab Protocol for Canopy Foliage Chemistry and Leaf Mass per Area Measurements (AD[10]).

In grasslands, field personnel will be given random coordinates for clip harvest strips. Each of three clip harvest strips per plot will be 0.1m x 2m, an aspect ratio chosen to maximize sampling of representative species in diverse communities (see TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index (AD[09])). Field personnel will cut and collect biomass, sort it to functional group, determine the dry weight per area, and submit a homogenized, ground subsample of each functional group for laboratory analysis (see Table 4).

Within plots located in the tower footprint, NEON will sample root biomass and litterfall to determine components of above- and belowground production. The approaches for sampling these tissues are described in the TOS Field and Lab Protocol: Core Sampling for Plant Belowground Biomass (AD[11]) and the TOS Field and Lab Protocol for Litterfall and Fine Woody Debris (AD[12]). Generally, they follow the approaches of the North American Carbon Program (see Hoover 2008), and, thus, data collected over a

similarly large spatial scale (i.e., continental) will be comparable across efforts. In the case of litterfall, NEON will obtain samples in traps (see discussion of spatial design below), traps within plots will be composited, material will be sorted by functional groups (e.g., conifers, deciduous), dried and weighed, and then a subsample will be ground for analysis of total C and N concentrations, stable isotopes of C and N, and lignin. Root biomass will be sampled in standard core volumes, roots sorted into four size classes (< 0.5, 0.5-1, 1-2, and 2-10 mm), and the same analyses of C, N, and stable isotopes will be performed on homogenized (dried, ground) subsamples. In cases where material is minimal, samples will be composited across classes for chemical and stable isotope analysis.

Table 6. Laboratory analyses and methods for plant tissues

Plant Tissue	Analysis	Method
Canopy foliar	Total organic C; total N, S	Combustion; elemental analyzer
	P, Ca^{2+}, K^{+}, Mg^{2+}	Nitric acid and hydrogen peroxide digestion; Inductively Coupled Plasma (ICP)
	Chlorophyll	Spectroscopy
	Lignin	Acid Unhydrolysable Residue (see Ryan et al. 1990 and McClaugherty et al. 1985)
	$\delta^{13}C$, $\delta^{15}N$	Combustion; isotope ratio mass spec (e.g., Thermo Delta Plus XP IRMS)
Roots	Total organic C; total N	Combustion; elemental analyzer
	$\delta^{13}C$, $\delta^{15}N$	Combustion; isotope ratio mass spec (e.g., Thermo Delta Plus XP IRMS)
Litter	Total organic C; total N	Combustion; elemental analyzer
	$\delta^{13}C$, $\delta^{15}N$	Combustion; isotope ratio mass spec (e.g., Thermo Delta Plus XP IRMS)
	Lignin	Acid Unhydrolysable Residue (see Ryan et al. 1990 and McClaugherty et al. 1985)

The primary decisions involved with choosing methods for sampling plant tissues for biogeochemistry were related to the design rather than to sample acquisition or laboratory techniques. Methods of collection for these types of plant tissues are straightforward (i.e., cut the sample and bag it), and the chosen laboratory techniques are general practice within the community. One decision of note related to the design was whether the sample unit is a georeferenced individual, or the plot. As part of the sampling designs for phenology and plant structure (see TOS Science Design for Plant Phenology (AD[13]) and TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index (AD[09])), NEON will tag and georeference some individuals within the site for long-term sampling. Providing chemical data at the scale of the individual may be useful to some data users, but imposes a fixed design from the initial years of the Observatory (i.e., individuals are tagged and chosen for sampling in year one), and does not allow for sampling species that may become a dominant part of the community over the course of 30 years. In the case of the latter, incorporating new individuals, if appropriate, will allow for

continued evaluation of the most representative species and individuals for canopy foliar chemistry sampling over the lifetime of the Observatory, and will more closely match the airborne data collection if the plant community changes over time. In all cases, tagged and new individuals sampled for chemistry will be georeferenced; tagged individuals will also have linked long-term phenological and structural measurements. Using this approach of incorporating georeferenced tagged and new individuals for sampling, users will be able to analyze NEON data at the scale of the individual, the plot, and the site.

A second important decision related to plant tissue sampling was whether to sort clip harvest and litter samples by species. The decision to sort grassland clip harvests and litter to functional groups was largely due to budgetary constraints for the field campaign. It will be impractical and financially unfeasible to sort and chemically analyze samples to the level of species in diverse grasslands. In addition, the time required to process litter samples for chemical analysis (i.e., homogenizing, grinding, and subsampling) would be too great if required at the level of individual species. NEON's approach to provide C, nutrient, and stable isotope data bulked across functional groups per area will likely provide sufficient detail for most data users, and meets the overall requirements of the NEON Project. Data on species diversity and abundance will be provided by NEON for each site, following the TOS Science Design for Plant Diversity (see (AD[14])), so if separation by species for chemical analyses of plant tissues is desired, the community will have the baseline information to support proposing further research.

Other design-related decisions are for aspects of the spatial distribution and temporal frequency of plant tissue sampling, which are described below.

6.3.1.2 Samples for Archiving

NEON will archive remaining plant material (oven-dried and ground) following subsampling for analysis of C, nutrients, and stable isotopes. In addition, whole leaf specimens from initial plant diversity assessments and stem-mapping efforts will be dried and archived in envelopes.

Table 7. Archived samples from the plant tissue collection efforts

Sample	Processing	Storage conditions	Volume or mass stored	Number site^{-1} sampling year^{-1}[a]
Canopy foliar tissues	Oven-dried (60°C, 48 hr), ground	Ambient, dry cabinet	Filled 20 ml vial	30-360
Litterfall	Oven-dried (60°C, 48 hr), ground	Ambient, dry cabinet	Filled 20 ml vial	20-50
Roots	Oven-dried (60°C, 48 hr), ground	Ambient, dry cabinet	Filled 20 ml vial	20-80

[a] Explanations for the expected ranges of samples generated are described above.

6.3.2 Spatial Distribution of Sampling

The spatial design for plant tissue collections differs based on the tissue. Sun-lit canopy foliar samples will be collected from 10-20 plots per site (i.e., four tower plots and 6-16 distributed plots), depending on the variability of the vegetation communities at each site. As described above, in forested and savanna/shrubland communities, species chosen for sampling will be representative of the community within the plot and individuals will be chosen for sampling from locations across the plot area. Per sampling event, 30-60 samples will be generated across the site. In grasslands and savannas, one random location for clip harvest strips will be identified within three of the four subplots, yielding 30-360 samples per site, dependent on the number of functional groups present. Collections of litter and root tissues for biomass and chemical analysis will be within the tower plots only. The reasons for this decision are that (1) root biomass measurements will be tied to data from minirhizotrons, which will be located near the tower only at each site, and (2) the time required for field technicians to collect and process these tissues for weights and chemical analysis requires that they be limited in their spatial extent to the tower plots/dominant vegetation community only. Thus, the spatial sampling design for biogeochemical analysis of these tissues will be two randomly located cores for root biomass in each of 10 tower plots, and four litter baskets in each of 10 tower plots. These collections will yield 20-80 samples of root tissues per collection event per site (depending upon whether or not samples are composited across size classes, defined above) and 20-50 litter samples per collection event per site, depending upon the number of functional groups present.

6.3.3 Temporal Distribution of Sampling

In addition to the soil survey, stem mapping and relative abundance of the plant species within the tower and distributed plots must be completed to determine the individuals (i.e., dominant and co-dominant species in forested and savanna/shrubland systems) that should be sampled for foliar chemistry. If functionally-important individuals (e.g., N-fixing species) are identified during preliminary stem mapping exercises, but are not dominant or co-dominant species, review of the measurement approach may be necessary to determine whether they should be sampled in order to quantify nutrient stocks. These one-time efforts to characterize the plant community are described in detail in TOS Science Design for Plant Diversity (AD[06]) and TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index (AD[09]).

Every 5 years, NEON will measure plant tissues (foliar, litter, and roots) in the tower and distributed plots. While the chemistry of foliar tissues, in particular, may change annually in response to climate drivers (e.g., rainfall, air temperature), disease, and other pressures, it is not logistically or financially feasible for NEON to sample at a finer temporal resolution. During sampling years, foliar and root samples will be collected once during peak biomass at each site. Litterfall traps will be deployed continuously and collected periodically (depending on the vegetation present) at each site. However, samples for chemical analysis of litterfall will be bulked across collections within a year (i.e., one composite sample for the year for each plot) and NEON will subsample and process litterfall collections for chemical analysis once every 5 years at each site.

6.4 Logistics and Adaptability

One of the strengths of NEON is that a standard suite of measurements with the same methodological approaches will be implemented at all sites. Therefore, a prioritization scheme does not limit the number of NEON sites at which samples will be collected, but identifies the measurements and priority analyses that should be conducted at all sites. The design targets minimum within-site sampling frequencies and key temporal periods of measurement necessary to provide a meaningful dataset. Sampling timing and frequency will be site-specific for some measurements, in order to measure responses to particular seasons or following important events. Sampling number will also be site-specific to capture spatial heterogeneity appropriately (ranges of expected plots are given in Section 6.4), and will largely be guided by the results of the initial soil characterization and plant community analysis/stem mapping efforts. Once this sampling design has been reviewed and implemented at NEON sites, initial data may indicate that there are pressing site- or region-specific measurements that should be added to address an important ecological issue or question, or other measurements should be removed; such modifications to the design approach—including changes in the priority of data types, sampling timing, sampling frequency, or spatial representation—may be incorporated once the initial design has been put in place across the observatory sites and upon review of the initial data streams by NEON personnel and community experts.

Generally, for the terrestrial biogeochemistry design, budgetary constraints drive limitations in the suite of analyses listed in Tables 3 and 4, the minimum inter- and intra-annual sampling frequencies for measurements of soil N transformations, in particular, and the ability to incorporate measurements of biogeochemical cycling into the design, more generally. Over the lifetime of the Observatory, it may be necessary to reallocate resources among the focal measurement areas within the terrestrial biogeochemistry design (or across NEON measurement platforms more broadly) to optimize the value and usability of the available data. In particular, it is likely that more frequent measurements of short-term biogeochemical transformations and fluxes (e.g., soil water and trace gases), which are not currently part of the design, may be necessary. These measurements are highly variable in space and time, as well as sensitive to small changes in environmental drivers. They are also a critical part of the science design, with respect to meeting the high-level NEON science requirements listed in Figure 3; the design described herein allows for the measurement of two key N cycling processes, in order to get some insight into short-term behavior at each site. In terms of detecting change over decadal time scales, and providing useful information to understand how ecosystems respond to global change drivers, these data will likely be a very valuable part of the terrestrial biogeochemistry design. As discussed above, the baseline characterization data, measurements of soil and plant tissue C and nutrient stocks and stable isotopes are important for providing a baseline dataset, and resources must be allocated to these efforts, at minimum. They will add value to the interpretation of several other NEON datasets, such as soil microbial community analysis, chemistry of surface waters, and hyperspectral data from the airborne observations, as well as many projects by NEON collaborators.

The first assessment of design feasibility will come from the NEON domain field personnel in charge of collecting and processing samples (with the exception of plant canopy foliar samples) for analysis by contracted laboratory facilities. Most sampling efforts for the terrestrial biogeochemistry design will require one week of dedicated field time (i.e., soil and plant tissue sampling) for two technicians to complete 1-3 sites, depending upon the complexity of each site. In addition to field time, domain personnel will also conduct sample processing (e.g., soil sieving, drying, subsampling) prior to sending samples to contracted laboratories. The most time-sensitive and lengthy post-field processing will be for soil N transformation rates. This procedure requires that technicians sieve soil cores, weigh subsamples, extract subsamples in 2M potassium chloride solution for 18-24 hr, and then filter the extractant for shipment to a contracted laboratory. Careful balancing of field time (i.e., number of soil cores collected per day) with the required post-field soil processing will be necessary to complete this protocol successfully. NEON's ability to make these measurements will require iteration between field personnel and staff scientists to determine an approach that is time-efficient and does not compromise the data.

Aside from logistical review of the design by NEON field crews, one of the tools that can be used to assess the initial sampling design is a statistical framework developed to aid in optimizing the sampling strategy across the observatory. The spatial and temporal components that define where and when soils and plant tissues will be sampled have implications for how the data can be analyzed on their own and in relationship to other datasets. However, it is useful to assess the adequacy of the sampling plans using both a classical statistical power analysis and analyses of simulated data within a Bayesian hierarchical data assimilation framework. The Bayesian hierarchical approach allows for the specification of both deterministic trend components and covariance structures with both spatial and temporal terms. In this approach, data are simulated in accordance with deterministic components and spatial-temporal covariance structures of interest. Statistical data assimilation is used to analyze the simulated data and estimate posterior distributions for parameters of interest (e.g., temporal trends). In reality, the number of samples and frequency of collections are driven by an understanding of baseline data needs (i.e., characterization), published data on inter-annual variability in C and nutrient stocks (i.e., periodic sampling of soils and plant tissues), published data on intra-annual variability in soil biogeochemical processes (i.e., soil N transformations), and budgetary constraints of the project. However, this statistical framework will provide a useful means to check the quality of information and to help justify an update the design, if resources permit. An example of a use case for the Bayesian statistical framework is given in Appendix 8.1.

As NEON operations begin, not only internal review by NEON science staff, but also regular iteration with the larger natural sciences community will be critical, in order to verify that the design and scientific priorities are aligned. In particular, the terrestrial biogeochemistry component of the observatory will benefit from regular workshops to evaluate data streams and discuss the degree to which NEON is providing useful resources to the rest of the field, including observational and modeling communities. As outlined in this document, biogeochemical transformations are inherently subject to

short-term changes and are very heterogeneous spatially and temporally; in most instances, they also require labor-intensive, manual measurements. Therefore, flexibility in the NEON design strategy will be critical to assure that personnel efforts and financial resources are not wasted or misdirected. Periodic evaluation of data streams, discussion of technological and methodological advances, and general iteration between NEON staff scientists and the ecosystem biogeochemistry community should occur every one to three years during the lifetime of the observatory. This approach will help to create the transformative data use and community research experience that is central to the NEON mission.

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APPENDIX A A STATISTICAL FRAMEWORK FOR EVALUATING NEON SAMPLING DESIGN

A.1 Introduction

The purpose of this appendix is to provide details regarding analyses performed in support of the specification of the terrestrial biogeochemistry sample design. NEON's high-level requirements provide guidance regarding the overarching science goals; however, the determination of measurements deemed relevant in the context of the design specification requires additional consideration. Flexibility within the design is required in order to accommodate unique aspects of ecological responses measured by each component of NEON. Computational analyses performed in support of the development of design constraints are intended to assess the sufficiency of the datasets that will be collected to accomplish the goals identified for specific components of NEON. The framework of computational analyses must also maintain the generality necessary to ensure relevance across numerous data uses considered.

At the most general level, the minimally sufficient sampling effort corresponds to the sample design specified by the plant biodiversity analysis to determine locations of distributed plots at NEON sites. These minimum sample sizes are characterized using a frequentist approach to a power analysis. This power analysis is based on the test of a difference between the slopes characterizing linear change through time at two locations. In this setting, repeat measurements through time are taken on the same sampling units within each group. With a minimum sample size of approximately 10 (depending on the specification of tolerable error levels, etc.), distributed sample locations were spatially located using the Reverse Randomized Quadrat Recursive Raster (RRQRR) method. Given this initial allocation, modifications to the terrestrial biogeochemistry sampling design can be made in accordance with the high-level requirements through the application of this simulation tool, which uses a Bayesian hierarchical modeling (BHM) framework for the analysis of simulated data. This analysis framework can also be applied to data products generated by multiple components of NEON.

The BHM framework provides the capability to depict complexity with respect to ecosystem processes and interactions (Cressie et al., 2009). This also presents a challenge in that the degree of generality, and hence relevance across a range of ecological responses, is inversely proportional to the level of complexity for a given model structure. Two general paths for the depiction of complex space-time dynamics using BHM are: 1) place a significant degree of effort into the characterization of these dynamics into the deterministic component of the process model, and 2) keep the deterministic component of the process model relatively simple and account for the space-time dynamics using more complex covariance functions. Wikle and Hooten (2010) provide a nice discussion of some issues related to the development of a modeling approach in this context.

The latter approach, sometimes called the second-moment approach (due to its relative focus on covariance structures), is the one selected as a basis for the quantification of sample design adequacy in the computational analyses presented here. This approach was chosen for two reasons. First, the

minimal assumptions associated with the process model component allow for a greater degree of generality; this feature is essential, due to the large number of response variables that will ultimately be considered. The intent is that this will allow for broader application of these analysis tools to different responses. More specific process models (i.e., the first approach) require that more complex models be built that describe specific dynamics of each individual response of interest. This also increases the degree of subjectivity associated with the model building and unnecessarily obfuscates the intent of the work presented here. To be clear, the development of complex process models is of fundamental importance when studying a specific system in detail; however, this approach does not align with the intent of this design characterization work as well as the second-moment approach. The second reason to implement the development of more complex space-time covariance functions that evolve from the second approach is that NEON will generate data products with a high degree of spatial and temporal coverage. The BHM framework allows for the iterative optimization of the data collection (for both NEON sampling and PI driven projects) through the application of statistical data assimilation to update the parameter distribution models. Together, these two objectives drive the selection and application of the latter approach.

Historical data play an important role in the work presented here. In general, subsequent application of these tools should be performed in concert with analyses of existing NEON data, both to inform the generation of synthetic data and provide context for analyses of data products. In this work, historical data are used to inform parameter values that specify various statistical properties of the simulated data. Thus, the simulated data provide a reasonably realistic depiction of the types of data products that will be generated by NEON. The data simulated in this work are analyzed in the BHM framework to characterize the impact of various design configurations on posterior distributions of parameters of interest. This approach allows for the quantitative assessment of various measures of sample adequacy under scenarios of interest, as determined by NEON's high-level science requirements.

A.2 Methods

Response Variable

The response variable of interest for this simulation study is peak annual CO_2 flux. The question of interest for this response is: Can a difference in the rate of change in peak annual CO_2 flux through time be detected between vegetation types? The proposed sampling that is of interest for characterization consists of a single annual sampling event where data are collected at each of the 40 locations specified by the RRQRR based sample locations. Data from the Harvard forest LTER (i.e., NEON Domain 1 core site) are used in this work (Figure 10) where the two largest vegetation types are deciduous and evergreen forest classes, which have 12 and 13 RRQRR sample locations, respectively. The simulation study focuses on quantifying the impact of varying sample frequencies (one, three and five years) of woil CO_2 fluxes on subsequent analyses performed on synthetic data generated from the RRQRR sample locations within each of these two vegetation classes.

Historical Data

In order to simulate data that are as realistic as possible, historical soil CO2 flux data collected from the Harvard Forest site were assembled (Figure 11). Metadata are available online (http://harvardforest.fas.harvard.edu:8080/exist/xquery/data.xq?id=hf194). Exploratory analyses of these historical data were performed and a subset of the data was assembled in order to provide the highest degree of consistency with the types of data that will be collected by NEON.

The first round of data reduction dealt with the exclusion of treatment plots; only data from control (i.e., not experimental treatment) plots were used. In order to minimize impacts that may be a consequence of chosen sample methods not articulated in the metadata, the selected dataset only includes measurements made by a single investigator using consistent methodology. The data associated with PI Davidson provide a ten-year record with good representation for the summer months when peak flux occurs, and uses manual collars to obtain measurements at the locations in Figure 11. Multiple measurements were taken within each month during the ten-year sampling period. Figure 12 shows the monthly distribution of CO_2 flux measurements for this dataset.

The historical data from Harvard Forest show that the largest monthly flux distributions are present in the months of July and August. The rest of this analysis is based on data available for the month of August. Within each month, multiple samples were taken across space and through time. With respect to time, samples were collected at approximately weekly intervals. Hence, using only the samples from the last sampling event for which data were collected in August further reduces the dataset by removing intra-monthly variability. Across space, samples were collected from multiple collars at multiple locations; this analysis excludes those with anomalously large or small values. The final historical data set had approximately 12 samples for each year, creating a subset of data with the corresponding variability that would be expected from deciduous and evergreen forest classes with 12 and 13 samples, respectively. Boxplots of the peak CO_2 flux distribution by year from the final working dataset of historical data are presented in Figure 13. Figure 14 shows boxplots for these same data by sampling location within each year, providing a sense of the spatial variability among sites. Figure 14 also depicts the relative differences in inter-annual variability among sampling locations through time.

Figure 15 shows the variability among collars within sampling locations. The collars within a sampling location do not have distinct spatial locations. This variability, thus, cannot be resolved with spatial covariance modeling; in this sense, the between collar variability within each site is analogous to the nugget in a variogram model. Exploratory analysis of the spatial correlation of the full historical dataset suggests that there is, at best, weak spatial correlation at the distances that will be encountered in the design locations. It is also worth noting that these exploratory spatial analyses are somewhat tenuous due to the relatively small sample sizes. Ultimately, the potential impact of a reduction in effective sample size as a function of the relatively shorter distances between the collars does not seem to be a critical issue. If the spatial correlation of these data were stronger at the scale of the collars, then this discrepancy in distances would need to be accommodated. Summary statistics for the final subset of

historical data are presented in Table 8. These data are used to specify values for parameters in the statistical model, which generates synthetic data for the simulation study.

A.3 Simulation Design

The primary focus of the work presented here is an assessment of the impact of sampling at one, three, and five year intervals. Hence, multiple realizations of annual peak flux were generated and analyzed at the one, three, and five year sampling frequency. The number of realizations was determined through iterative assessment of the stability of the results, in conjunction with the computational burden. For the annual sampling results, there were ten realizations, for the three year there were 100 realizations, and for the five year sampling there were 300 realizations. Each realization has 5000 samples from the posterior distributions generated via Markov Chain Monte Carlo (MCMC) methods.

Each realization in the simulation study is a time series of multivariate observations. The multivariate aspect of the data comes from the spatially explicit observations. Synthetic data are generated using a separate a process model and a data (measurement) model. The process model characterizes the underlying behavior of the system, incorporating spatial and temporal correlation as well as structural changes in the mean, due to other known or measured quantities. The measurement model then characterizes the variation and bias (if any) that relates the latent underlying process to the actual measurements. Separating the process and data model provides several advantages including better characterization of uncertainty associated with parameter estimates (Calder et al. 2003). Parameters are specified as fixed values for the generation of synthetic data. However, distributional models for parameters are specified as part of the BHM used to analyze the synthetic data (Table 9). Each of the model components is discussed in detail below.

A.4 Data Model

For this application, the measurement model is assumed to provide an unbiased measurement of the unobservable peak CO_2 flux with a fixed but unknown measurement variance, σ_{m}^2. Bias parameters can be readily accommodated in the data model if it is desirable to assess whether there is bias in the measurements (i.e., include the bias parameter and see if the data suggest that it is effectively no different from zero). Assuming measurement error to be normally distributed conditional on the unobserved latent process, Y, the data model can be written as:

$$\boldsymbol{Z}|\,\boldsymbol{Y},\sigma_m^2 \sim MVN(\mu(\boldsymbol{t}),\sigma_m^2\boldsymbol{I}) \qquad [1]$$

Where, Z is the vector of observations made on the underlying latent process Y,and $\mu(t)$ is the mean of the latent process, and t is the vector of times associated with the measurements, I is the identity matrix, and σ_{m} is a scalar that represents the overall magnitude of the measurement error in the same units as Z.

A.5 Process Model

The process model for the simulation of peak CO2 flux through time (i.e., t = 1, 2, …, 30) is specified as, $\mu(t)$, conditional on the parameters and is defined as,

$$\mu(\boldsymbol{t})|\, \sigma_p^2, SR, TR \sim MVN\left(0,\ \mathbf{C_p}\right) \qquad [2]$$

where C_p is the space-time covariance matrix defined below, σ_p^2 is the variance of the process, SR is the spatial range that specifies the decay rate of correlation for spatial distance (in meters) between measurement (d_{ij}) for a given point in time, and TR is the time range that specifies the decay rate of correlation for times (in years) between measurements ($|t_i - t_j|$) at a given location. A linear deterministic functional form was used to characterize the mean of the process model for the simulation of peak CO_2 flux through time (i.e. t = 1, 2, …, 30).

$$\mu(\boldsymbol{t}) \ = \ \beta_0 + \beta_1 t \qquad [3]$$

The mean component eqn [3] could also include covariate information, such as precipitation, but there is little difference in formulation if an additive linear increase in signal over time is the change of interest. Better process models using covariate information would, however, lead to a reduction in the magnitude of the variance associated with the process model. This deterministic component of the process model is considered to change uniformly across space for the work presented here. In practice, data from NEON will likely suggest a spatially varying mean for the process model. In this simulation study, the specification of a spatially varying mean surface would only serve to obfuscate the results.

A.6 Process Covariance

While the deterministic component of the process model changes uniformly as a function of space, the simulation design allows for the specification of a stationary, but separable spatio-temporal process covariance structure. In this sense, 'separable' implies that the covariance can be expressed as a product of two terms, each corresponding exclusively to a spatial and temporal component. This approach assumes that there will be spatial and temporal covariance in the response that is unaccounted for by the deterministic terms of the process model. The covariance matrix corresponding to this structure is shown in Eq [4].

$$C_{ij} = \sigma_p^2 \cdot \frac{\exp\left\{-\left(\frac{d_{ij}}{SR}\right)\Big/\left(\frac{|t_i - t_j|}{TR}+1\right)\right\}}{\left(\frac{|t_i - t_j|}{TR}+1\right)} \qquad [4]$$

It follows that the process equation for the underlying true ecological process of interest can be represented as,

$$Y_t = (\mu)\boldsymbol{t} + \ \mathbf{C_p} \qquad [5]$$

A.7 Parameter Models

There are parameters associated with both the data and process models. For the sake of clarity parameters are grouped accordingly, such that $\theta_t^z = \{\sigma_m\}$, $\theta_t^Y = \{\sigma_p, b_f, SR, TR\}$, $\theta_t = \{\theta_t^Z, \theta_t^Y\}$. For the generation of synthetic data, scalar values must be specified for the parameters. In order to analyze the synthetic data in the BHM framework, prior distributions are specified for each of the parameters. Prior distributions are assigned for each of the elements of θ_t (see Table 9). The parameters are assumed to be independent such that the joint distribution of the parameters is the product of the individual marginal distributions of each of the parameters in eqn [6].

$$P[\theta_t] = \prod_{i=1}^{n} P[\theta_i] \qquad [6]$$

Where n is the total number of parameters from both the data and process models.

For each of the sampling frequencies considered, distributions for parameters are updated by assimilating the current year's observations. This process begins at year 3 for all of the sampling frequencies considered; prior distributions for each of these parameters need to be specified at year 2. The year 2 priors for θ_t are informed based on values that are reasonably inferred from exploratory data analysis of the first two years data (Table 9).

A.8 Analysis of Synthetic Data from the Harvard Forest LTER (NEON Domain 1 Core Site)

The simulation framework is designed to provide a suite of tools that can be used to address the detection and quantification of trends in climatically driven ecological responses by bracketing the plausible ranges for flexible components of the design. In this application, each simulated times series contains realizations that correspond to annual sampling at distributed locations (Figure 19). At each time step of interest, simulated data are analyzed using a MCMC approach to update the prior distributions and estimate the posterior distributions of parameters. In this application, the distribution of the difference of the trend parameters for the different vegetation types is of primary interest. The intent is to mimic the analysis of data collected sequentially through time with the goal being to determine which functional form for the process model best characterizes the linkage between the ecological response of interest and the climate driver.

A.9 Computational Issues

Given the models for the data, process, and parameters, the focus of this exercise is on the posterior distribution of the process and parameters conditional on the observed data, which can be generally expressed as

$$p(Y, \theta_t | Z) \propto p(Z|Y, \theta_t) p(Y|\theta_t) p(\theta_t) \qquad [7]$$

where θ_t represents the collection of all parameters of interest at time t. This application evaluates the posterior distribution of the process and the parameters by conditioning on a functional form (eqn[2]) for the deterministic component of the process. The multivariate normal distribution form in eqn[1] and eqn[2] allows some simplification of the expression for the posterior distribution of the process and the parameters when each process is considered separately. The posterior of interest is expressed as

$$p(\theta_t|Z,Y) \propto p(Z|Y,\theta_t)p(\theta_t) \quad [8]$$

Samples from the posterior distributions for parameters in θ_t (eqn [6]) are obtained using a MCMC approach with a Metropolis-Hastings (MH) sampling algorithm from the MCMCpack library in R (Martin et al. 2008). The first update occurs at year three; therefore, priors for the parameter distributions are assigned at year two. Results are presented for years three and 18 for all three sampling frequencies. The final year of analysis for the sampling frequencies of one, three, and five years was 30, 30 and 28, respectively.

If samples from the posterior distribution for the process, conditional on the data and parameters, are desired, then they can be constructed post-hoc, by conditioning on the MCMC posterior samples for θ_t. The multivariate normal distributions for the process and measurement lead to a closed form for the process conditional on the parameters and data:

$$p(Y|\theta_t,Z) \sim MVN\left(V^*\left[V^{-1}\mu(t|\theta_t) + \frac{1}{\sigma^2}\boldsymbol{I}Z\right], V^*\right) \quad [9]$$

where

$$V^* = \left[V^{-1} + \frac{1}{\sigma^2}\boldsymbol{I}\right]^{-1} \quad [10]$$

The posterior predictive distribution for the process at a future time point, $t + i$, can be constructed in similar fashion by extending the mean $\mu(t|\theta_t)$ and covariance matrix V for the joint distribution through time $t + i$, and augmenting the I matrix with zeroes for times yet to be measured.

Due to the nature of the covariance structure of the posterior distributions and the potential high-dimensionality of the parameters (when including the process values Y), it is not a simple matter to summarize the posterior distributions succintly. Marginal distributions might be fit to some parameters, but a flexible multivariate model for capturing the potentially non-linear relationships between the parameters is generally not available. Thus, when new data become available, there is typically not a simple method of updating the posterior with the new data conditional on the previous results.

Consequently, for the analyses presented here, when synthetic data are assimilated at subsequent time steps, the model is expanded to accommodate the new data, and the MCMC is re-run from scratch (i.e., updating the year two priors). The dimensionality of the model can lead to slow computation as the data size increases. However, since the change in the parameters is likely to be relatively small as the sample size increases, this allows for previous MCMC runs to be potentially be used to construct starting values and Metropolis proposal distributions for increased efficiency of subsequent runs.

A.10 Results

Trend Parameter

The study question motivating this exercise is, Can a difference in the rate of change in peak annual CO2 flux through time be detected between vegetation types? The relative ability to detect this difference was assessed for sampling frequencies of one, three, and five years. For the simulation and analysis approach presented above, there were noticeable differences among the analyses of the different sampling frequencies with respect to the ability to detect a difference (in the rate of change in peak annual CO2 flux between vegetation types). Specifically, the certainty associated with the detection of the relative differences increased with sample frequency. These differences are represented in the posterior distributions of the trend parameters for the deciduous and evergreen sampling locations (Figures 16-18 and Table 10).

The measures of central tendency of the posterior distributions for the trend parameters are close to the specified values (Table 9) by year 18 (Table 10) across all sampling frequencies. There is little change in the mean of the posterior distributions between year 18 and the last year of updating (i.e., year 30 for the one and three year sampling frequencies and year 28 for the five year sampling frequency).

Between the updates at years three and 18, there is roughly an order of magnitude decrease in the standard deviation of the posterior distributions of the trend parameters for all sampling frequencies. The decrease is larger for the annual sampling frequency than it is for the three and five year sampling frequency scenarios. The standard deviation at year 30 is roughly 50% greater for the three year sampling frequency than that of the annual sampling. Although not an exact comparison of years, the standard deviation of the five year sampling frequency at year 28 is roughly 100% greater than that of the annual sampling frequency at year 30.

The posterior distribution of the difference between trend parameters for the deciduous and evergreen vegetation classes shows similar results (Figure 19 and Table 11), leading to a probability statement. Specifically, the probability that the difference in the trends is less than zero can be used as a measure of the ability of specific sampling scenario to detect a trend (Table 11). The year 18 results show a decrease in the probability that the difference is less than zero with increased sampling frequency. The probability corresponding to the three year sampling scenario is roughly four times greater than that of the annual sampling. Similarly, the probability corresponding to the five year sampling interval is over six times greater. By year 30, with the annual sampling frequency, there were no MCMC samples where the difference in trend corresponded to a value less than or equal to zero. For the three year sampling scenario, the probability of the trend being less than or equal to zero was approximately 8%. Interestingly, for the five year sampling frequency, the probability at year 28 is only 2%.

Other Parameters

For the annual sampling, the posterior distribution of the intercept parameter displays reduced variance with increasing time (Figure 11). For the three and five year sampling intervals, there is a negligible difference in the variance of the posterior distributions between year 18 and the final year of updating (Figures 12 and 13). The error parameters in the annual sampling case show slight shifts toward the values used to specify the simulated data; however, for the three and five year sampling cases, there is little noticeable movement away from the prior distributions.

A.11 Discussion

By simulating data based on characteristics gleaned from analyses of regional historical data in conjunction with the implementing spatial locations for the Harvard Forest site, a flexible framework for assessing various aspects of the sufficiency of the design is presented and tested. Specifically, these results demonstrate the impact of several sampling frequency scenarios on the ability to detect a difference in trend magnitudes of the peak CO2 flux between vegetation types. Differences between the sampling frequency scenarios are quantified and provide a means to assess the sufficiency of various sampling frequency scenarios. The difference in slopes considered here reflects the uncertainty associated with differences in the rates of change in the peak flux as a function of the different vegetation types through time. Subsequent analyses can easily be performed to assess the sensitivity of the design to varying trend magnitudes.

The parameters associated with the deterministic component of the process model (i.e., intercept and slope) are well informed (as measured by a reduction in the variance of the posterior distributions) by the annual sampling scenario. Specifically, the trend and intercept parameters for the annual sampling case demonstrate a consistent reduction in the variance of the posterior distributions with increased data collection through time. In contrast, the posterior distributions for the three and five years sample frequency scenarios demonstrate little change in the variance associated with the posterior distributions between year 18 and the final year of sampling. This result suggests there is negligible increase in the information content associated with these parameters that corresponds to the data collected between year 18 and final year of sampling.

The design does not appear to provide much useful information with respect to the spatial and temporal covariance structures considered here. In this context, it is worth noting that there was little evidence in the historical data to suggest anything beyond a weak temporal and spatial correlation structure should be imposed. It is possible that there was too much signal (deterministic) to noise (error) in these data to pick up the weak spatial and temporal covariance structure imposed. In addition, this situation makes the specification of the priors more influential on the final analyses since the data do not provide much information to make the posteriors meaningfully different from the priors. This finding may indicate that these terms should be eliminated from the model. In practice, for the sake of model building, that is a reasonable approach to take. For the purpose of this design characterization work, these analyses

present a useful baseline for comparison to subsequent scenario of design implementation to be considered.

There are several additional issues that should be considered when interpreting the results of this analysis. The first is that as NEON data are collected through time, there will be advances in the understanding of the system that correspond to more sophisticated process models. For example, there is variability associated with the date of occurrence of the peak flux. As models that account for additional complexity such as this are developed, less of the observed variability in the response will be accounted for by the covariance terms in both the process and data models. Ideally, the increased sophistication of the process models developed and implemented through time result in the non-independent component of the process covariance to be negligible. This is unlikely, so the utility of the more complex space-time covariance structure considered in this framework should persist.

Second, the consistency of the response between vegetation types for a given year (i.e. 'species uncertainty') is an interesting and important factor that may be worth considering in subsequent analyses. Essentially, this factor would address the question, For the climate observed in a given year, do we expect to see similar responses in peak annual CO2 flux between the vegetation types of interest? For example, in a hot and dry year, would one observe similar responses in peak flux for the different vegetation types of interest? In general, the capability to have the response be either similar or different between vegetation types will likely be useful for at least some situations. If the two vegetation types of interest are both primarily deciduous (e.g. 'deciduous open canopy' and 'deciduous closed canopy') then one might expect the responses to be quite similar. If however, the vegetation types under consideration are physiologically different, (e.g. 'conifer closed canopy' and 'deciduous closed canopy') then it may be of interest to have the responses be somewhat different within a given year. Functionally, for the case where there is between-vegetation type variability, separate time series realizations are generated for each vegetation type. This means there is a different sample taken from the process covariance for each vegetation type. If between-vegetation type variability is not desired in the model, then the realization from the process covariance is selected treating samples from both vegetation types as realizations from the same process. Then the data from each vegetation type are analyzed separately.

A.12 References

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A.13 Tables and Figures

Table 8. Summary statistics for peak CO2 flux estimates obtained from the reduced dataset with four sites and three collars within each site. A single sampling event was used for each year, therefore, there are 12 samples associated with each year.

Year	Min	1st Qu.	Median	Mean	3rd Qu.	Max	SD
1997	2.53	3.65	4.38	4.52	4.90	6.89	1.35
1998	4.49	5.95	6.51	7.44	7.87	15.52	2.92
1999	2.39	2.91	3.34	3.68	3.89	6.53	1.15
2000	2.74	4.55	5.54	5.61	6.16	8.85	1.82
2003	2.89	3.95	4.25	4.89	5.26	10.28	2.03
all years	2.39	3.72	4.66	5.23	6.23	15.52	2.28

Table 9. Parameter values used to generate synthetic data. Normal distributions are specified with a mean and standard deviation. Two values are specified for the β_trend parameter: deciduous = 0.05, and evergreen = 0.10. IGamma is the Inverse Gamma distribution as specified in the MCMCpack library (Martin et al. 2008) in R.

	β_0	β_{trend}	σ_m^2	σ_p^2	SR	TR
Value used in Simulation	0.47	Deciduous = 0.05, Evergreen= 0.10	0.47	0.5	500	0.05
Prior Distribution	Normal (5.3, 1)	Normal (0, 0.5)	Uniform (0.5, 5.0)	IGamma (5, 3)	IGamma (5, 1000)	IGamma (5, 2)

Table 10. Percentiles for the trend parameter posterior distributions for the 1, 3, and 5 year sampling frequencies of deciduous and evergreen.

Frequency	Vegetation	Year	Min.	1st	Median	Mean	3rd	Max.	SD
1yr	Deciduous	3	-1.000	-0.240	-0.046	-0.040	0.158	0.989	0.287
	Evergreen	3	-1.093	-0.255	-0.067	-0.069	0.119	0.791	0.276
	Deciduous	18	0.021	0.080	0.094	0.094	0.108	0.165	0.021
	Evergreen	18	-0.026	0.032	0.045	0.045	0.058	0.111	0.019
	Deciduous	30	0.067	0.093	0.099	0.099	0.106	0.136	0.010
	Evergreen	30	0.014	0.042	0.048	0.048	0.055	0.086	0.010
3yr	Deciduous	3	-1.164	-0.258	-0.065	-0.062	0.133	1.094	0.290
	Evergreen	3	-1.101	-0.294	-0.103	-0.100	0.093	1.152	0.286
	Deciduous	18	-0.035	0.074	0.096	0.096	0.118	0.227	0.033
	Evergreen	18	-0.093	0.024	0.043	0.042	0.062	0.185	0.029
	Deciduous	30	0.038	0.088	0.099	0.099	0.109	0.164	0.033
	Evergreen	30	-0.005	0.038	0.048	0.048	0.057	0.107	0.029
5yr	Deciduous	3	-1.181	-0.264	-0.068	-0.069	0.126	1.229	0.290
	Evergreen	3	-1.338	-0.287	-0.093	-0.092	0.101	1.100	0.288
	Deciduous	18	-0.068	0.073	0.097	0.097	0.121	0.248	0.036
	Evergreen	18	-0.103	0.022	0.045	0.045	0.068	0.189	0.034
	Deciduous	28	0.021	0.087	0.099	0.100	0.113	0.181	0.019
	Evergreen	28	-0.037	0.035	0.048	0.047	0.060	0.128	0.019

Table 11. Percentiles for the difference in the trend parameter posterior distributions for the 1, 3, and 5 year sampling frequencies. The realizations from the posterior distribution of the difference are also used to provide an estimate of the probability that the difference is greater than zero.

Frequency	Year	Min.	1st	Median	Mean	3rd	Max.	SD	Prob (diff<0)
1yr	3	-1.007	-0.157	0.026	0.030	0.209	1.091	0.278	0.467
	18	-0.026	0.035	0.050	0.049	0.064	0.126	0.022	0.020
	30	0.009	0.043	0.050	0.050	0.057	0.083	0.011	0.000
3yr	3	-1.155	-0.146	0.037	0.038	0.222	1.305	0.280	0.445
	18	-0.099	0.028	0.054	0.054	0.079	0.209	0.038	0.080
	30	-0.035	0.037	0.051	0.051	0.064	0.138	0.020	0.006
5yr	3	-1.407	-0.169	0.024	0.023	0.216	1.375	0.296	0.466
	18	-0.191	0.022	0.052	0.052	0.083	0.222	0.046	0.125
	28	-0.080	0.036	0.053	0.052	0.069	0.154	0.025	0.021

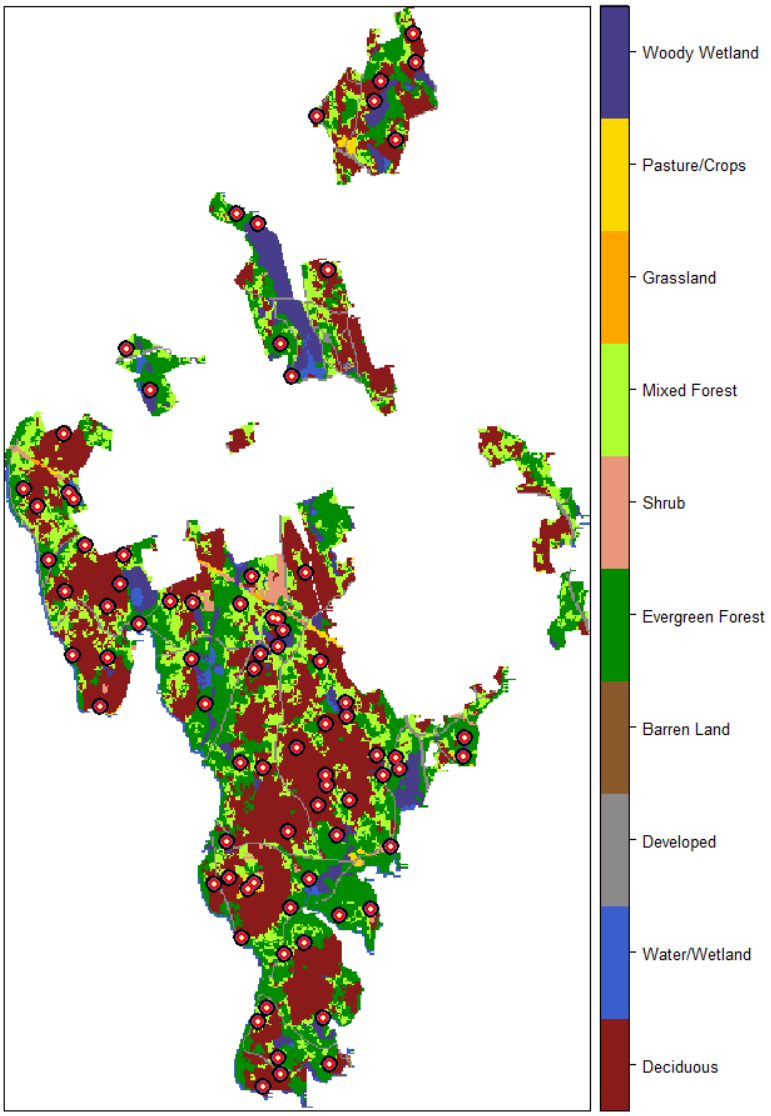

Figure 10. NLCD coverage of Harvard Forest Core Site within Domain 1. Distributed sample locations determined by the RRQRR design are shown for the vegetation types selected for sampling.

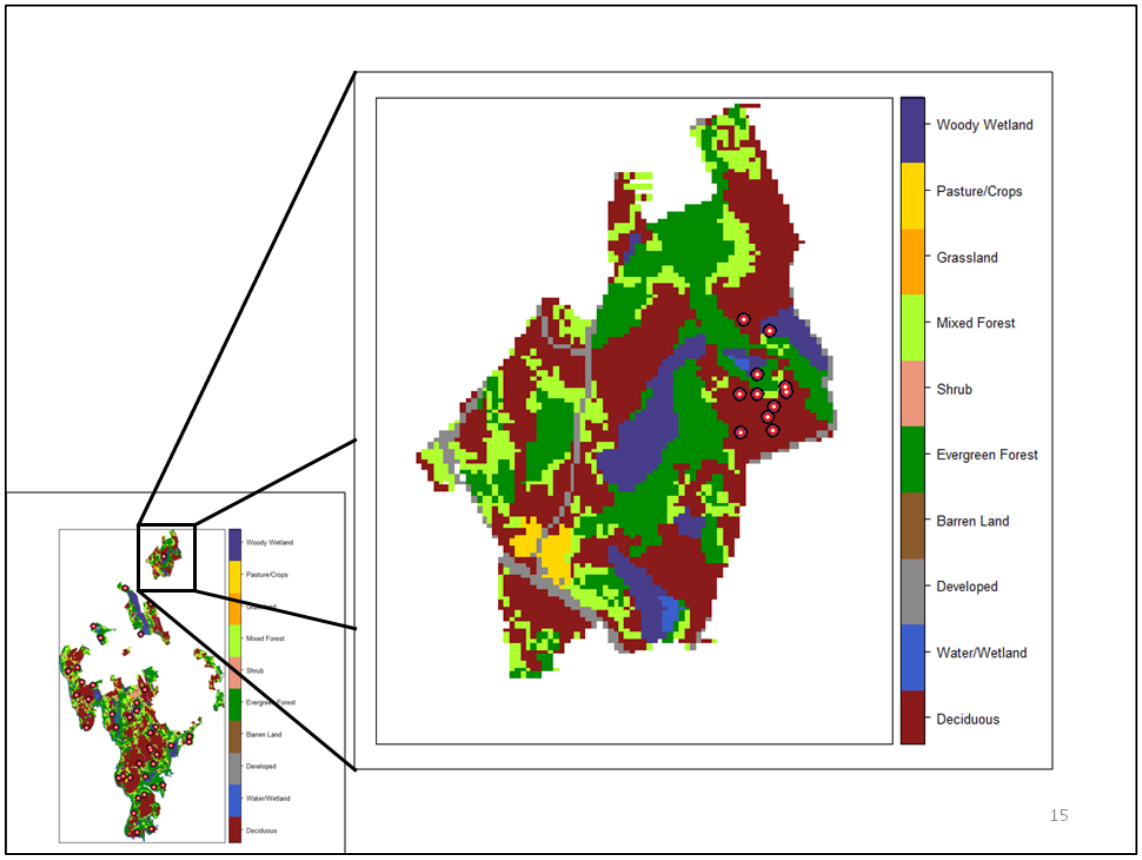

Figure 11. NLCD coverage of Harvard Forest Core Site within Domain 1. Sample locations are shown for the historical data used to inform the simulation study.

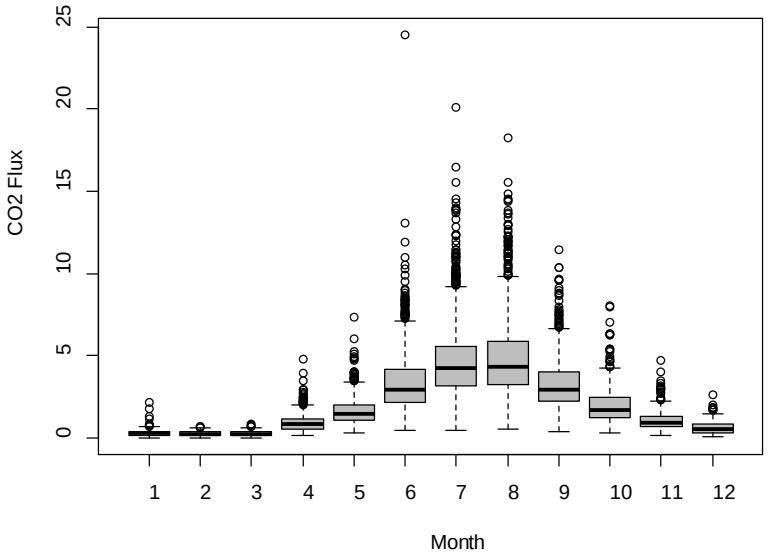

Figure 12. Boxplots of monthly distributions for CO2 flux from the control data, collected by PI Davidson using manual soil collars at the Harvard Forest. Metadata for the entire Harvard forest archive are available online (http://harvardforest.fas.harvard.edu:80http://harvardforest.fas.harvard.edu:8080/exist/xquery/data.xq?id=hf194). The grey boxes span the 25th and 75th percentile (i.e., the inter-quartile range, IQR). Medians are depicted with the black horizontal line within each box. Whiskers for reach boxplot extend out to the largest value less than the median +/- 1.5*IQR. Observations outside the median +/- 1.5*IQR are depicted as open circles.

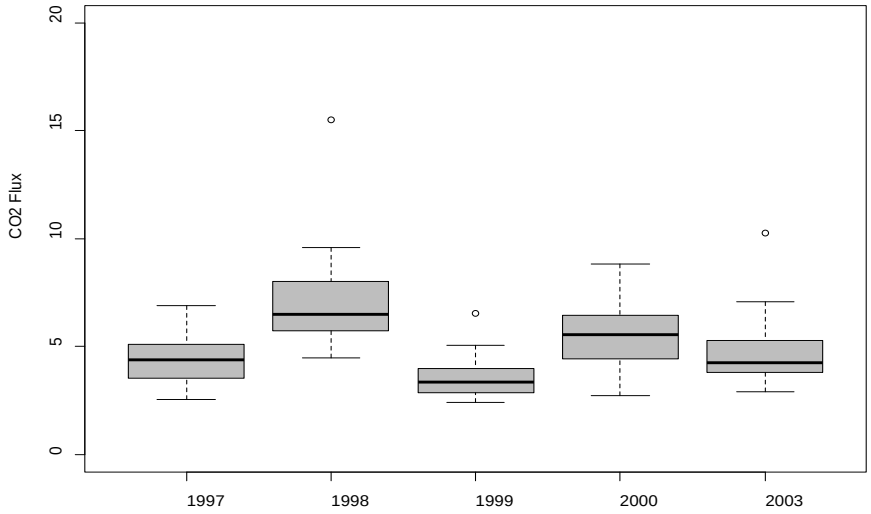

Figure 13. Boxplots of annual peak CO2 flux distributions for the final working dataset from the Harvard Forest. Each year has 12 samples arising from 4 locations with 3 collars each. Note that data are omitted for years 2001 and 2002 due to unequal sample sizes. The data for each year are taken from a single sampling event occurring towards the end of the month of August.

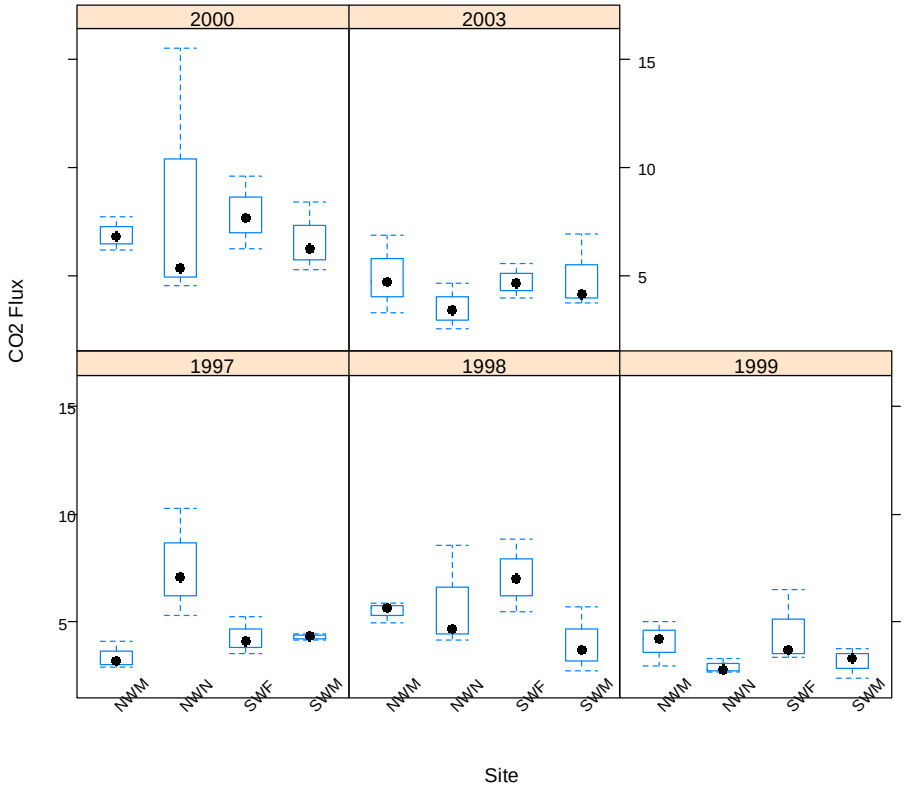

Figure 14. Boxplots of annual peak CO2 flux distributions by site within each year for the final working dataset from the Harvard Forest. There is some evidence that inter-annual variability varies among sites. For example, NWN appears to display greater inter-annual variability than NWM.

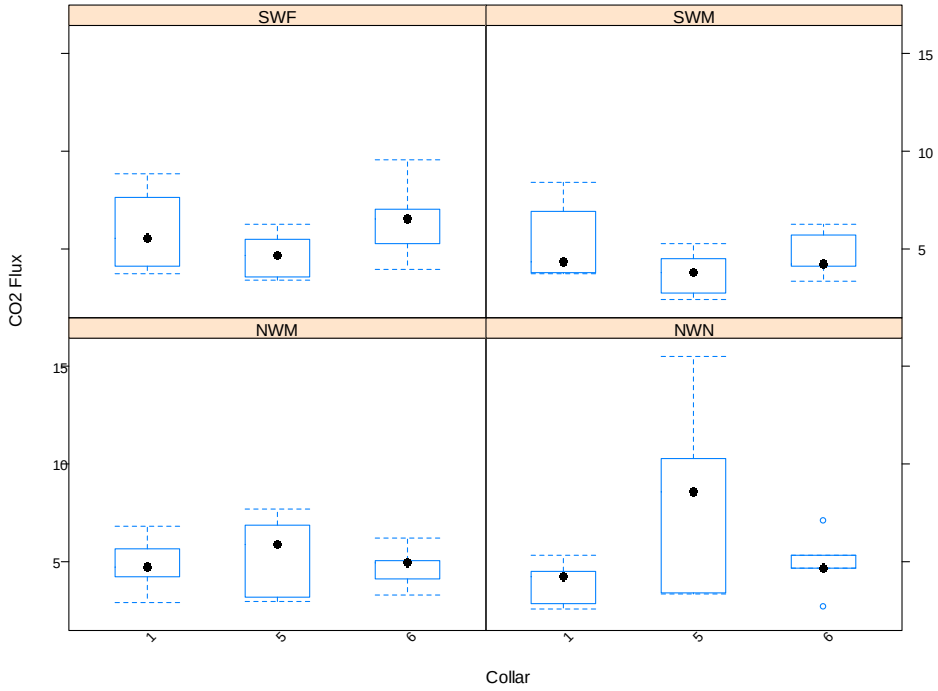

Figure 15. Boxplots of annual peak CO2 flux distributions by site within each year

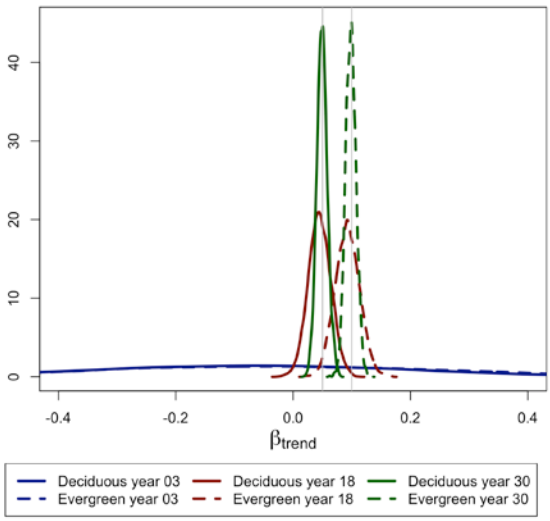

Figure 16. Posterior distributions for the trend parameters at years 3, 18, and 30 for both the deciduous and evergreen vegetation types with annual sampling.

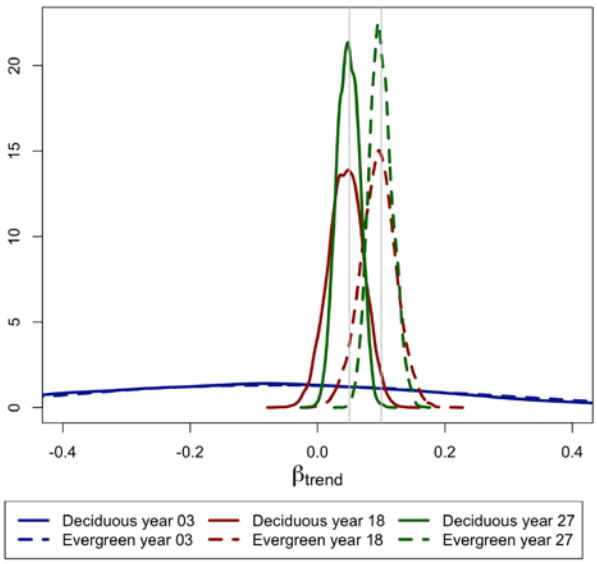

Figure 17. Posterior distributions for the trend parameters at years 3, 18, and 27 for both the deciduous and evergreen vegetation types with sampling every 3 years.

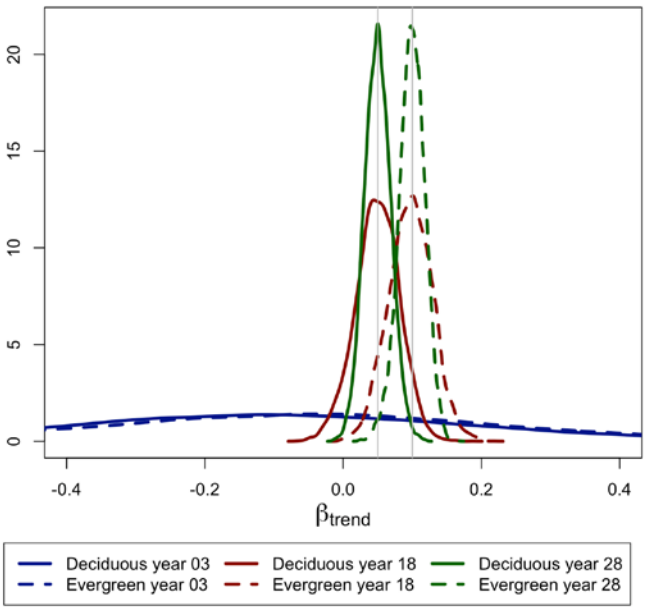

Figure 18. Posterior distributions for the trend parameters at years 3, 18, and 28 for both the deciduous and evergreen vegetation types with sampling every 5 years.

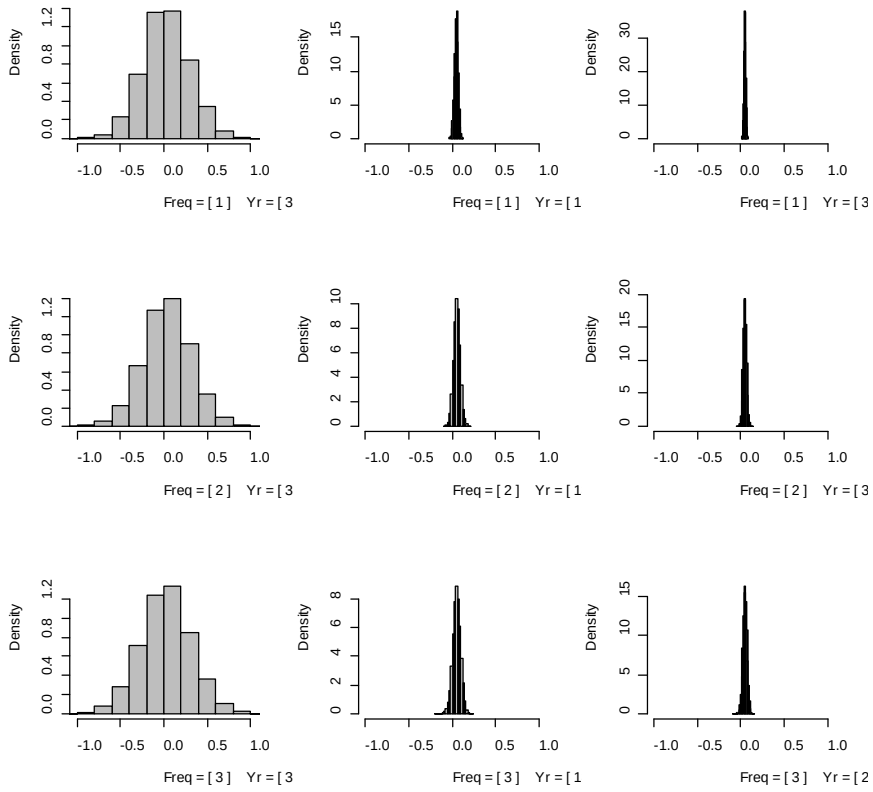

Figure 19. Histograms of the posterior distributions of the difference in the trends as a function of sampling frequency and year.

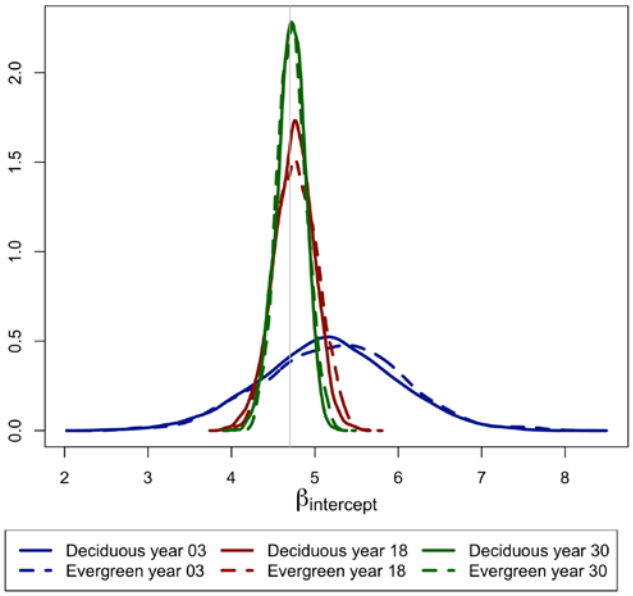

Figure 20. Posterior distributions for the intercept parameters at years 3, 18, and 28 for both the deciduous and evergreen vegetation types with sampling every year.

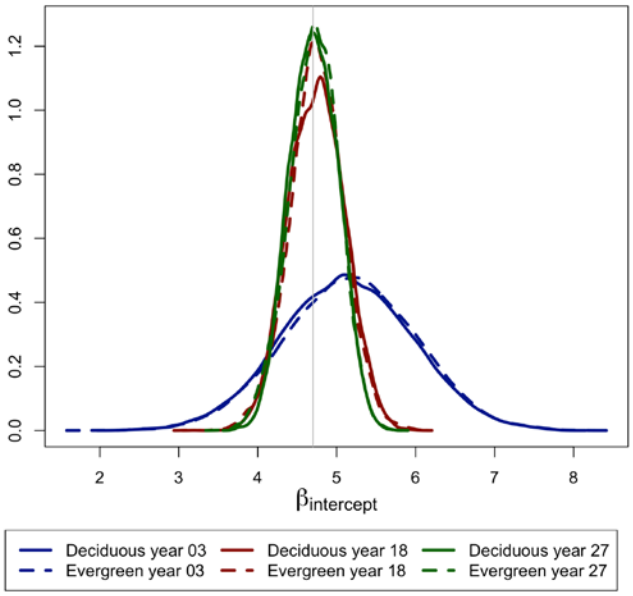

Figure 21. Posterior distributions for the intercept parameters at years 3, 18, and 28 for both the deciduous and evergreen vegetation types with sampling every 3 years.

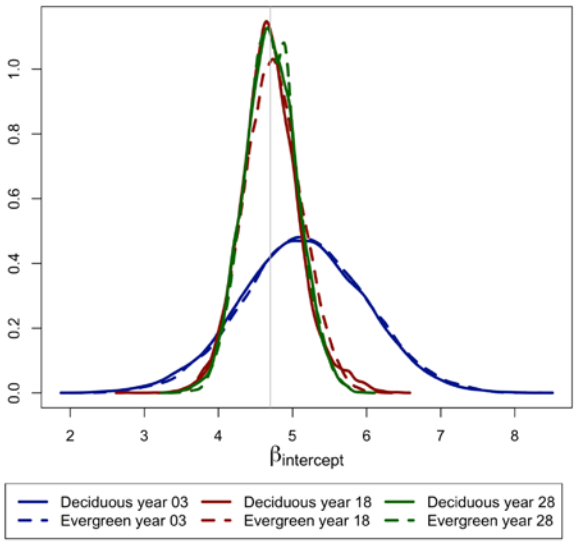

Figure 22. Posterior distributions for the intercept parameters at years 3, 18, and 28 for both the deciduous and evergreen vegetation types with sampling every 5 years.

A.14 Code Used for Simulations

```
#collection of functions used for simulating and analyzing data

spatio_temporal.post = function(theta.params,form,data.df,priors,post=TRUE,separable=NULL){
  n.obs = nrow(data.df)
  sites.sp = data.df[!duplicated(data.df[,c("x","y")]),]
  sites.n = nrow(sites.sp)
  sites.t = unique(data.df$t)
  t.n = length(sites.t)
  n.beta  = length(gregexpr("beta",form)[[1]])
  beta    = as.numeric(theta.params[1:n.beta])
  sigma.meas                                                                                    =
ifelse(is.na(as.numeric(theta.params[n.beta+1])),1,as.numeric(theta.params[n.beta+1]))
  sigma.process                                                                                 =
ifelse(is.na(as.numeric(theta.params[n.beta+2])),1,as.numeric(theta.params[n.beta+2]))
  h.range = as.numeric(theta.params[n.beta+3])   # spatial covariance range
  u.rho   = as.numeric(theta.params[n.beta+4])   # temporal AR1 coefficient
  if(separable==F){sp.time.int    = as.numeric(theta.params[n.beta+5])} # space-time interaction
coefficient

  priors = sum(unlist(lapply(1:length(theta.params),
              function(i,theta){
                x=theta[i];                                  dens=(eval(parse(text=priors[[i]])));
ifelse(is.na(dens),log(0.01),dens);
              },
              theta=theta.params)))
   if(priors==-Inf|priors=="NaN"){priors = -2e7}
   if( sigma.meas > 0 &  sigma.process > 0 &  sigma.process < 3 & h.range > 0 & h.range < 15000 &
u.rho > 0 &
                u.rho < 1 ){
       spdistmat = as.matrix( dist( data.df[,c("x","y")], diag=TRUE, upper=TRUE ) )
       tdistmat = as.matrix( dist( data.df[,"t"], diag=TRUE, upper=TRUE ) )
       nspdist = spdistmat / h.range
       ntdist = tdistmat / u.rho + 1
       if(separable==T){sp.time.int<-0}
       V.process = exp( -nspdist / ntdist^(sp.time.int/2) ) / ntdist
    V.meas<- diag(nrow(data.df))
    V.all<-    sigma.process*V.process + sigma.meas*V.meas
    y = data.df[,"response_1"]
    yhat = with(data.df,eval(parse(text=form)))
    loglik= dmvnorm(yhat,y,V.all,log=T)
        if(post){
      logpost = loglik + priors
    }else{
      logpost = loglik
    }
  } else {
    logpost = -1e5
  }
  if(logpost==-Inf|logpost=="NaN"){logpost = -3e5}
  logpost
}

##############################################################################################

model_choice.mcmc = function(mcmc.samples,separable=NULL){
  n.samples = nrow(mcmc.samples$params)
  w=rep(1/n.samples,n.samples)
  theta.mean = crossprod(w,mcmc.samples$params)
  df = length(theta.mean)
  theta = attr(mcmc.samples$data,"theta")
  theta.median =      apply(mcmc.samples$params,2,median)

  loglik.med                                                                                    =
spatio_temporal.post(theta.params=theta.median,form=mcmc.samples$form,separable=separable,
```

```
        data.df=mcmc.samples$data,priors=mcmc.samples$posteriors,post=T)

  loglik.mu                                                                                    =
spatio_temporal.post(theta.params=theta.mean,form=mcmc.samples$form,separable=separable,
        data.df=mcmc.samples$data,priors=mcmc.samples$posteriors,post=T)
  loglik.sum = 0
  lik.marg = 0
  post = numeric(n.samples)
  post = apply(mcmc.samples$params,1,spatio_temporal.post,mcmc.samples$form,separable=separable,
        data.df=mcmc.samples$data,priors=mcmc.samples$posteriors,post=T)
  center.post = mean(post)
  alpha = var(post)
  lmax = center.post + alpha
  lik.marg = sum(1/post)^(1)
  pD = -2*(center.post - loglik.mu)
  DIC = pD - (2*center.post)
  lik.marg = (lik.marg/n.samples)^(-1)
  AIC = -2*mcmc.samples$loglik + 2 * df
  BIC = -2*mcmc.samples$loglik + df * log(n.samples)
  list(AIC=list(AIC=AIC,df=df,loglik=mcmc.samples$loglik),
       DIC=list(DIC=DIC,pD.med=-2*(median(post)    -    loglik.med),dev.med=-2*loglik.med,med.dev=-
2*median(post),DIC.med = pD - 2*median(post),
                      mean.dev=-2*center.post,dev.mean=-2*loglik.mu,pD=pD),
       BIC=list(BIC=BIC,pD=pD,loglik=mcmc.samples$loglik,n=n.samples),
       lik_marg=list(lik.marg=lik.marg,alpha=alpha,lmax=lmax))
}

##############################################################################################

mcmc.samples.plot = function(obj,yr=1,separable=F){
#if(yr==5){obj$priors[]<-sub("log","",obj$priors[])}
obj$priors[]<-sub("log","",obj$priors[])
  params.df = stack(as.data.frame(do.call("cbind",lapply(obj$priors,
                  function(distr,n){
                    distr=gsub("d","r",distr)
                    distr=gsub(",log=TRUE","",distr)
                    distr=gsub("x","n",distr)
                              distr=gsub("renp","rexp",distr)
                    eval(parse(text=distr))
                  },n=20000))))

  params.df$type="prior"
  tmp=stack(as.data.frame(obj$params))
  tmp$type="posterior"
  params.df = rbind(params.df,tmp);rm(tmp)
  params.df$test = rep(NA,nrow(params.df))
  params.df$test[params.df$ind=="beta0"] = c("Beta 0")
  params.df$test[params.df$ind=="beta1"] = c("Beta 1")
  params.df$test[params.df$ind=="beta2"] = c("Beta 2")
  params.df$test[params.df$ind=="sigma.meas"] = c("Measurement Sigma")
  params.df$test[params.df$ind=="sigma.process"] = c("Process Sigma")
  params.df$test[params.df$ind=="spat.range"] = c("Spatial Range")
  params.df$test[params.df$ind=="time.range"] = c("Temporal Range")
  params.df$test[params.df$ind=="sp.time.int"] = c("Space Time Int")

  print(densityplot(~values|test,
                              group=type,adjust=2.0,
                              data=params.df,lwd=2,
                              panel = function(...) {
                              panel.fill(col = "#E1EBE3FF")
                              panel.densityplot(...)},
                              scales=list(x=list(relation="free"),y=list(relation="free")),
                              plot.points=F,main=paste("Year",yr),
                              auto.key=T,xlab="Parameter Values")
  )
}
```

```
#############################################################################################

spattemp.mcmc                                                                                 =
function(yr.begin=3,yr.end=3,data,force.samp=T,factr=1e10,separable=NULL,update.interval=5,
    formula=NULL,name="exp",mcmc=1000,burnin=1000,thin=1,tune=NULL,plotit=F){

  library(ramps);library(lattice);library(sp);
  library(MCMCpack);library(mvtnorm);library(SuppDists)
  base_name = paste(name,"_post_yr",sep="")

  saved.samples = ls(pat=base_name,envir=.GlobalEnv)
  remove.samples                =                saved.samples[saved.samples                %in%
paste(base_name,formatC(yr.begin:10,digits=1,flag=0),".mcmc",sep="")]
  theta = attr(data,"theta")
  sigma.meas = theta[["sigma.meas"]]
  sigma.process = theta[["sigma.process"]]
  if(is.null(formula)) {
    formula = attr(data,"formula")
    beta = theta[["beta"]]
    n.betas = length(beta)
  } else {
    n.betas = length(gregexpr("beta",formula)[[1]])
    beta                                                                                      =
c(rnorm(1,mean(data[data$t<5,"response_1"]),sqrt(var(data[data$t<5,"response_1"]))/3),runif(n.bet
as-1,-1,1.5))
  }
  param.names                              =                          c(paste("beta",0:(n.betas-
1),sep=""),"sigma.meas","sigma.process","spat.range","time.range","sp.time.int")

if(separable==T){
param.names<-param.names[-length(param.names)]
}

  n.params = length(param.names)
  if(is.null(tune)){
  tune = rep(1,n.params)
  } else if(length(tune)<n.params) {
  tune = rep(tune,n.params)
  }
  beta=c(4.7,1.)
  post_yr2.mcmc = list(
    theta.init = c(
      rnorm(1,beta[1],0.25), # beta0
      rep(0,length(beta)-1), # beta1
      0.5, # sigma.meas
      0.47, # sigma.process
      500, # spta.range
      0.05, # time.range
      0.01 # sp.time.int
    ),
    posteriors = as.list(
      c(paste("log(dnorm(x,5.3,1))",sep=""),  # beta0
        rep("log(dnorm(x,0,.5))",n.betas-1),  # beta1
        "log(dunif(x,0.01, 2.5))", # sigma.meas
        "log(dinvgamma(x,shape = 5, scale = 3))", # sigma.process
        "log(dinvgamma(x,shape = 3, scale = 1000))", # spat.range
        "log(dinvgamma(x,shape = 5, scale = 0.2))", # time.range
        "log(dinvgamma(x,shape = 10, scale = 2))") # sp.time.int
)
  )

if(separable==T){
post_yr2.mcmc[["posteriors"]] = post_yr2.mcmc[["posteriors"]][-n.params]
post_yr2.mcmc[["theta.init"]] = post_yr2.mcmc[["theta.init"]][-n.params]
}

  names(post_yr2.mcmc[["posteriors"]]) = param.names
```

```
  assign(paste(base_name,"02.mcmc",sep=""),post_yr2.mcmc)

year.vec<-seq(yr.begin,yr.end,update.interval)

for(yr in year.vec){
if(yr==3){update.interval=1}
    var.name = paste(base_name,formatC(yr,width=2,flag=0),".mcmc",sep="")
    var_prev.name = paste(base_name,formatC(yr-update.interval,width=2,flag=0),".mcmc",sep="")
    var_prev.name = ifelse(exists(var_prev.name),var_prev.name,
        ls(pat=base_name,envir=.GlobalEnv)[length(ls(pat=base_name,envir=.GlobalEnv))]
    )

data.update<-data[data$t%in%year.vec,]
data.update<-data.update[data.update$t<=yr,]
priors.update<-post_yr2.mcmc[["posteriors"]]
low.vec<-c(3,rep( -1, n.betas-1), .01, .01, 1, 0.01, 0.01)
up.vec<-c(7,rep( 1, n.betas-1), 4,  4, 10000, 1, 1)

if(separable==T){
low.vec<-low.vec[-n.params]
up.vec<-up.vec[-n.params]
}

    MLEs = optim(get(var_prev.name)[["theta.init"]],
        spatio_temporal.post,
separable=separable,
        form=formula,
        data.df=data.update,
        priors=priors.update,
        post=F,hessian=T,
        method = "L-BFGS-B",
        lower = low.vec,
        upper = up.vec,
        control=list(fnscale=-1,factr=factr,maxit=20)
    )

    if(is.null(get(var_prev.name)[["params"]])) {
      V.proposal = NULL
    } else {
      V.proposal = var(get(var_prev.name)[["params"]])

    }

    params = MCMCmetrop1R(spatio_temporal.post,
separable=separable,
        theta.init=get(var_prev.name)[["theta.init"]],
        form=formula,
        data.df=data.update,
        priors=priors.update,
        mcmc=mcmc, burnin=burnin, thin=thin, force.samp=force.samp,
        tune=tune,
        V = V.proposal,
        verbose=T,
        optim.method = "L-BFGS-B",
        optim.lower = low.vec,
        optim.upper = up.vec
    )

    dimnames(params) = list(NULL,param.names)
        posteriors = lapply(param.names,
        function(param,data){
        tmp = try( JohnsonFit(data[,param]) )
        if( inherits( tmp, "try-error" ) ){
        return( paste( "log(dnorm(",mean(data[,param]),",",sd(data[,param]),"))" ))
        } else {
        return( paste("dJohnson(x, list(",paste(tmp[-5],collapse=","),
        ",'",tmp,"'),log=TRUE)",sep="") )
```

```
        }},
        data=params)
    names(posteriors) = param.names
    mcmc.samples = list(
        form = formula,
        yr=yr,
        data = data.update,
        mles = MLEs$par,
        loglik = MLEs$value,
        theta.init = apply(params,2,median),
        params = params,
        posteriors = posteriors,
        priors = priors.update,
  prev.post = get(var_prev.name)[["posteriors"]]
    )

    mcmc.samples$model_choice = model_choice.mcmc(mcmc.samples,separable=separable)
    assign(var.name,mcmc.samples,envir=.GlobalEnv)
    if(plotit){
      win.graph()
      mcmc.samples.plot(obj=mcmc.samples,yr,separable=separable)
    }
  }
}

#############################################################################################

spattemp_sim.data = function(formula="beta[1] + beta[2] * t",data,
    n.reals=1,separable=F,
    beta=c(100,1.1),sigma.process=0.15,sigma.meas=0.1,
    h.range=0.01,u.rho=0.05,sp.time.int = 0.05){
  require(ramps)
  # generate the spatial covariance function
       spdistmat = as.matrix( dist( data[,c("x","y")], diag=TRUE, upper=TRUE ) )
       tdistmat = as.matrix( dist( data[,"t"], diag=TRUE, upper=TRUE ) )
       nspdist = spdistmat / h.range
       ntdist = tdistmat / u.rho + 1
       if(separable==T){ sp.time.int<-0}
       cormat = exp( -nspdist / ntdist^(sp.time.int/2) ) / ntdist
  V = sigma.process * cormat + sigma.meas * diag(nrow(data))
  # one check if the covariance matrix is valid (add other checks based on Gneiting 2002)
  cat(ifelse(min(eigen(V)$values)    >    -.Machine$double.eps,"Covariance    matrix    appears
valid","Covariance function not positive semi-definite"),"\n")
  # generate the response (need the mvtnorm library to generate mvnorm variates)
  responses              =              with(eval(parse(text=formula)),data=data)              +
t(mvtnorm::rmvnorm(n.reals,rep(0,nrow(data)),V))
  responses = as.data.frame(responses)
  names(responses) = paste("response_",1:ncol(responses),sep="")
  data = as.data.frame(cbind(responses,data))
list(beta=beta,sigma.process=sigma.process,sigma.meas=sigma.meas,h.range=h.range,u.rho=u.rho,sp.t
ime.int=sp.time.int)
       data$response_1[data$response_1<0]<-0
  return(data)
}

# Code used to generate realizations for this work
mcmcRdata = NULL
for(realization in realizations){
  for(sigProc in sigProcs){ # sigProc = sigProcs[1]
      combined_sites_lin.df                                                                =
spattemp_sim.data(formula="beta[1]+beta[2]*t",data=rbind(dataDeciduous,dataEvergreen),separable=T
,
                                          beta=c(beta0,    beta1[1]),    sigma.process=sigProc,
sigma.meas=sigmeas,
                                          h.range=spatrange,  u.rho=timerange,  sp.time.int  =
sptimeint)
      decid_sites_lin.df = combined_sites_lin.df[1:nrow(dataDeciduous),]
```

```
      assign(paste("decid_sites_",sigProc,"_",realization,".df",sep=""),decid_sites_lin.df)
      if(is_full_stoch){
        green_sites_lin.df                                                                    =
spattemp_sim.data(formula="beta[1]+beta[2]*t",data=dataEvergreen,separable=T,
                                          beta=c(beta0,      beta1[2]),      sigma.process=sigProc,
sigma.meas=sigmeas,
                                          h.range=spatrange,    u.rho=timerange,    sp.time.int    =
sptimeint)

      }else{
        green_sites_lin.df = decid_sites_lin.df
        green_sites_lin.df$response_1 = green_sites_lin.df$response_1 * 2
      }
      assign(paste("green_sites_",sigProc,"_","_",realization,".df",sep=""),green_sites_lin.df)
      for(interval in intervals){ # interval = intervals[2]
        for(vegType in vegTypes){
          cat("------ ",sigProc,interval,vegType," ------","\n")
          spattemp.try                                                                        =
try(spattemp.mcmc(yr.begin=3,yr.end=30,data=eval(parse(text=paste(vegType,"_sites_lin.df",sep=""
)),formula="beta[1]+beta[2]*t",
separable=T,
name=paste(vegType,"_",interval,"_",sigProc,sep=""),burnin=2000,mcmc=5000,thin=10,update.interval
=interval,tune=c(1,1,1,1,1,1),plotit=F))
          if(inherits(spattemp.try,"try-error")) break
          save.image(file=paste(host,"-",is_full_stoch,"-",interval,"-
r",realization,".Rdata",sep=""))
        }
        if(interval==5) {
          yrs = c("03","08","13","18","23","28")
        }else if(interval==3) {
          yrs = c("03","06","09","12","15","18","21","24","27","30")
        }else if(interval==1){
          yrs = c(paste("0",3:9,sep=""),10:30)
        }
        fileRdata       =       paste(host,"-",is_full_stoch,"-",interval,"-",min(realizations),"-
",max(realizations),".Rdata",sep="")
        for(yr in yrs){
          beta2Diffs2 = rbind(beta2Diffs2,c(realization,sigProc,interval,yr,

eval(parse(text=paste(vegTypes[1],"_",interval,"_",sigProc,"_post_yr",yr,".mcmc$params",sep="")))
[,2],

eval(parse(text=paste(vegTypes[2],"_",interval,"_",sigProc,"_post_yr",yr,".mcmc$params",sep="")))
[,2]
          ))

if(exists(paste(vegType,"_",interval,"_",sigProc,"_post_yr",yr,".mcmc$params",sep=""))){
            mcmcRdata = rbind(mcmcRdata,

get(paste(vegTypes[1],"_",interval,"_",sigProc,"_post_yr",yr,".mcmc$params",sep="")),

get(paste(vegTypes[2],"_",interval,"_",sigProc,"_post_yr",yr,".mcmc$params",sep="")))
          }
        } # yr
        save(mcmcRdata,file=fileRdata)
        write.csv(beta2Diffs2,paste("beta2Diffs2-",host,"-",is_full_stoch,"-",interval,"-
",min(realizations),"-",max(realizations),".csv",sep=""),row.names=FALSE)
      } # interval
  }
}
```