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SPECIAL FEATURE: NEON DESIGN

The soil and plant biogeochemistry sampling design for The National Ecological Observatory Network

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Abstract. Human impacts on biogeochemical cycles are evident around the world, from changes to forest structure and function due to atmospheric deposition, to eutrophication of surface waters from agricultural effluent, and increasing concentrations of carbon dioxide (CO_2) in the atmosphere. The National Ecological Observatory Network (NEON) will contribute to understanding human effects on biogeochemical cycles from local to continental scales. The broad NEON biogeochemistry measurement design focuses on measuring atmospheric deposition of reactive mineral compounds and CO_2 fluxes, ecosystem carbon (C) and nutrient stocks, and surface water chemistry across 20 eco-climatic domains within the United States for 30 yr. Herein, we present the rationale and plan for the ground-based measurements of C and nutrients in soils and plants based on overarching or "high-level" requirements agreed upon by the National Science Foundation and NEON. The resulting design incorporates early recommendations by expert review teams, as well as recent input from the larger natural sciences community that went into the formation and interpretation of the requirements, respectively. NEON's efforts will focus on a suite of data streams that will enable end-users to study and predict changes to biogeochemical cycling and transfers within and across air, land, and water systems at regional to continental scales. At each NEON site, there will be an initial, one-time effort to survey soil properties to 1 m (including soil texture, bulk density, pH, baseline chemistry) and vegetation community structure and diversity. A sampling program will follow, focused on capturing long-term trends in soil C, nitrogen (N), and sulfur stocks, isotopic composition (of C and N), soil N transformation rates, phosphorus pools, and plant tissue chemistry and isotopic composition (of C and N). To this end, NEON will conduct extensive measurements of soils and plants within stratified random plots distributed across each site. The resulting data will be a new resource for members of the scientific community interested in addressing questions about long-term changes in continental-scale biogeochemical cycles, and is predicted to inspire further process-based research.

Key words: carbon cycling; continental scale; long-term data collection; network science; nutrient cycling; Special Feature: NEON Design.

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INTRODUCTION

Humans are changing the fundamental chemistry of ecological systems on Earth by altering the global biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P), 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 and deforestation, fundamental changes in the distribution and nature of freshwater resources, widespread land cover and land use change, and increasing industrial extraction and application of reactive elements (Vitousek et al. 1997, Weathers et al. 2013*a*). Many of these biogeochemical changes have resulted from industrialization involving technological advances, such as the widespread application of the Haber–Bosch process, which enabled industrial-scale conversion of atmospheric N_2 to nutritionally available ammonia, thereby increasing our ability to meet the agricultural needs of a growing human population (Galloway et al. 2008). However, such biogeochemical modifications have resulted in many unwanted consequences. The human signature on biogeochemical cycles can be seen in global redistribution of elements (e.g., P), nutrient imbalances, and ecological impacts at local to global scales: in eutrophic surface waters (e.g., Carpenter et al. 1998, Correll 1998, Baron et al. 2013), declines in health and shifts in the composition of forest species (e.g., Horsley et al. 2002, Thomas et al. 2010, Trumbore et al. 2015), higher incidences of some infectious diseases (e.g., McKenzie and Townsend 2007), and spread of invasive species (e.g., Vitousek and Walker 1989, Ashton et al. 2005, Lovett et al. 2006, Crowl et al. 2008). Examples of these responses can be found in most regions of the world.

Despite having documented the connection between anthropogenic perturbations to biogeochemical cycles and ecosystem effects across the globe, our knowledge is sparse concerning the linkages among elemental cycles (Schlesinger et al. 2011), the degree to which previously impacted ecosystems are recovering in response to policy changes (e.g., Driscoll et al. 2001, 2003, 2007), and how shifts in climate and hydrology interact with ecosystem and biogeochemical responses (e.g., Falkowski et al. 2000; Gruber and Galloway 2008). For example, we are uncertain about the ultimate fate of reactive N in terrestrial and aquatic systems (Galloway et al. 2008, Schlesinger 2008). In part, this uncertainty is a function of not fully knowing where N is transported in the landscape and across regions—sequestered in upland terrestrial sinks, or released and transported through aquatic and atmospheric systems—or how well we are measuring and constraining key processes, such as denitrification (Groffman et al. 2006, Houlton et al. 2015). There are still further unknowns regarding changes to biogeochemical cycles at regional to continental scales—the scales at which many policies are made. Observational and modeling studies at larger scales require coordinated, standardized, long-term data collection.

Spatially extensive, long-term data for some biogeochemical measurements are becoming increasingly available through network science efforts (Weathers et al. 2013*b*). These resources may provide some of the missing information needed to improve our understanding of ecosystem function and ability to predict the responses of ecosystems to future changes. Multiple examples highlight the value of long-term data to answer crucial scientific questions about environmental change. 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

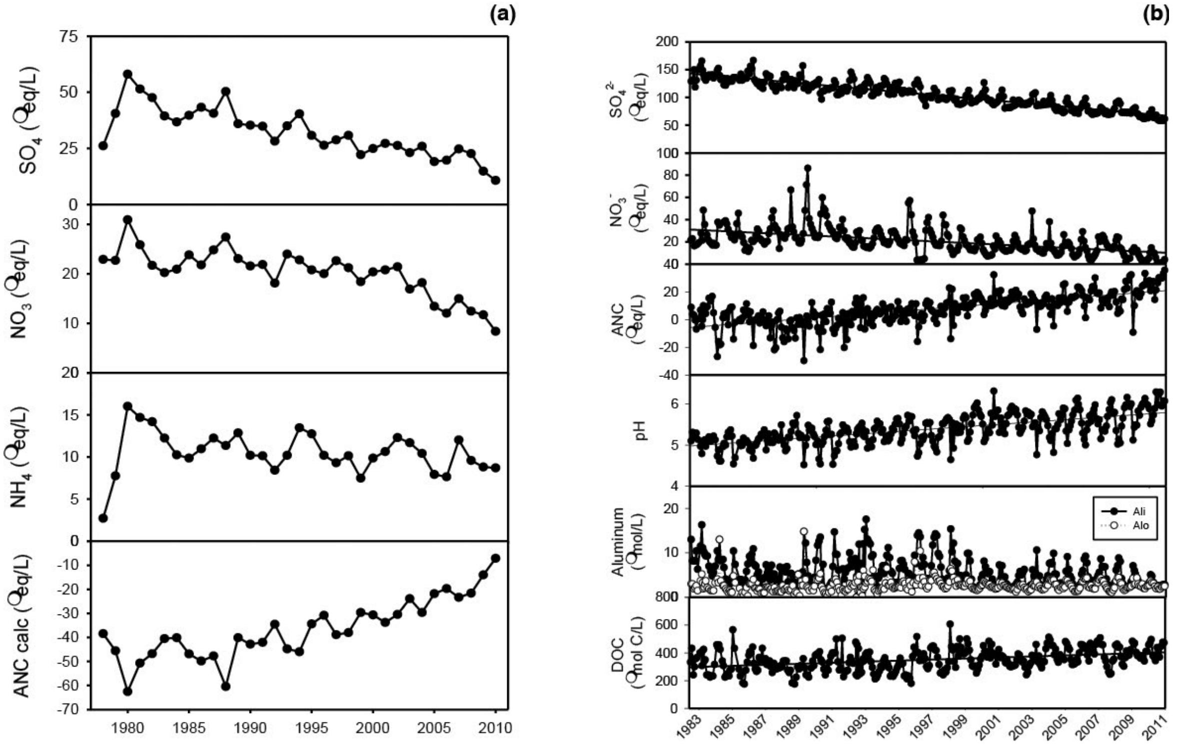

Fig. 1. Long-term chemistry records of (a) precipitation at Huntington Forest, NY, and (b) Big Moose Lake, Adirondack State Park, NY (Source: C. Driscoll, *unpublished data*; NADP Station NY20, http://nadp.sws.uiuc.edu/ and Adirondack Lakes Survey Corporation, http://www.adirondacklakessurvey.org/)

experimental research sites such as Coweeta and 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 United States. In addition, monitoring networks, such as the National Atmospheric Deposition Program (NADP, http://nadp.sws.uiuc.edu/), started in response to problems of environmental and ecological importance, including acid rain and mercury deposition.

Decades after the establishment of these study areas and monitoring stations, collection of long-term biogeochemical data has contributed to changes in policy to decrease negative impacts on ecosystems (Weathers and Lovett 1998, Greaver et al. 2012). For example, 30 yr of regional precipitation and lake chemistry observations in the Adirondack State Park (New York, U.S.) enabled documentation of a baseline pattern of the effects of acid rain, decline in sulfate concentrations following Clean Air Act legislation, and recovery during the late 1990s and 2000s (Driscoll et al. 2001, 2003, 2007, *unpublished data*) (Fig. 1). Other efforts have provided insight into ecosystem nutrient budgets using long-term observations (Peterjohn et al. 1996, Likens et al. 2002, Brookshire et al. 2011) and manipulation experiments of nutrient inputs, such as at Harvard Forest (Magill et al. 2004, Nadelhoffer et al. 2004) and the Catskill Mountains, NY (Lovett et al. 2013), land cover/land use change at Coweeta, Hubbard Brook, and H.J. Andrews research sites (Turner et al. 2003), and soil management at agricultural sites (Paul et al. 1997).

Recent funding of the National Ecological Observatory Network (NEON) provides the 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 synthesis across the network is often difficult. Further, NEON sites have been purposely selected to span a wide range of ecosystem types that compose the broader U.S. environment, in contrast to the more randomized site-selection process for LTER.

Previous overview papers (Field et al. 2006, Keller et al. 2008, Schimel et al. 2009, Schimel et al. 2011) outline the focus of the NEON strategy, informed by the 2001 and 2003 National Research Council (NRC) reports, which describe Grand Challenges in the earth and environmental sciences. In addition, they include ideas put forward during early discussions, workshops, and town halls at national scientific meetings focused on brainstorming plans for the first continental-scale ecological observatory (see a description of some NEON planning efforts at: http://ibrcs.aibs.org/core/index.html#). NEON is designed to provide the data that will be used to improve both our understanding of complex ecological systems and ability to forecast patterns of ecological change at local, regional, and continental scales using standardized and coordinated measurements of ecological taxa and environmental processes. These measurements will be made at 30-yr core sites ($n = 20$) and 5–10-yr relocatable sites ($n = 40$) across the United States To achieve this goal, NEON and NSF agreed upon a requirements framework, which guided the design of sampling strategies for each core science theme (e.g., biogeochemistry, biodiversity, ecohydrology, infectious disease) of NEON (Schimel et al. 2011).

This study provides an overview of the terrestrial biogeochemistry sampling design for NEON consistent with the overarching or "high-level" requirements outlined in Schimel et al. (2011). The high-level requirements state the general terms of how NEON will operate (e.g., "observing the causes and consequences of environmental change"), the themes on which it will focus (e.g., "biodiversity, biogeochemistry, ecohydrology [etc.]"), and the resources that it will provide to the public (e.g., "infrastructure…long-term, continental-scale information…resources"), which connect to its core mission (Schimel et al. 2011). We describe the motivation and rationale behind the suite of focal measurements, the development of the spatial and temporal sampling design, the general field sampling and analytical approaches, and the constraints on the scope of this design. We focus exclusively on describing NEON's measurements of terrestrial C and nutrient pools and soil N cycling. NEON's broader biogeochemistry measurements, described elsewhere, include atmospheric deposition and CO_2 fluxes at eddy covariance towers, and within-site ecosystem-functioning assessments, including ecosystem productivity, aboveground biomass estimates, ecosystem (gas and material) exchange, and the effect of terrestrial systems on productivity and chemistry of aquatic systems. 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 does climate affect ecological stoichiometry at the continental scale? and How do regional disturbances (e.g., wildfire and drought) affect coupled C, N, and water cycles? Standardized measurements of biogeochemical stocks and soil nutrient transformations will be made within the tower footprint ($n = 4–20$ 40 m × 40 m Tower plots) and a subset ($n = 6–16$ 40 m × 40 m Distributed plots) of the plots across the permitted area of each NEON site; Fig. 2 shows an example of the Domain 3 NEON core site, Ordway-Swisher Biological Station, Florida, including the eddy covariance tower, potential Distributed plot locations 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. Within plots, NEON will conduct sampling of biogeochemical stocks and soil N processes, microbial community composition and biomass, and vegetation (structure, biomass, tissue chemistry, and species inventories). Within sites, these measurements will be co-located with atmospheric nutrient deposition and meteorological data at the eddy covariance tower, and sample collections of ecological taxa (insects, birds, and small mammals) and vector-borne infectious diseases. Approximately 50% of NEON's terrestrial observation sites are co-located with an aquatics observation site, which will enable analysis of data across systems, in some locations.

DESIGN CRITERIA

There are four general design criteria underlying NEON's terrestrial biogeochemistry sampling program, which aim to satisfy the requirements of the program, including (1) the measurements will enable investigators to evaluate biogeochemical change across a diversity

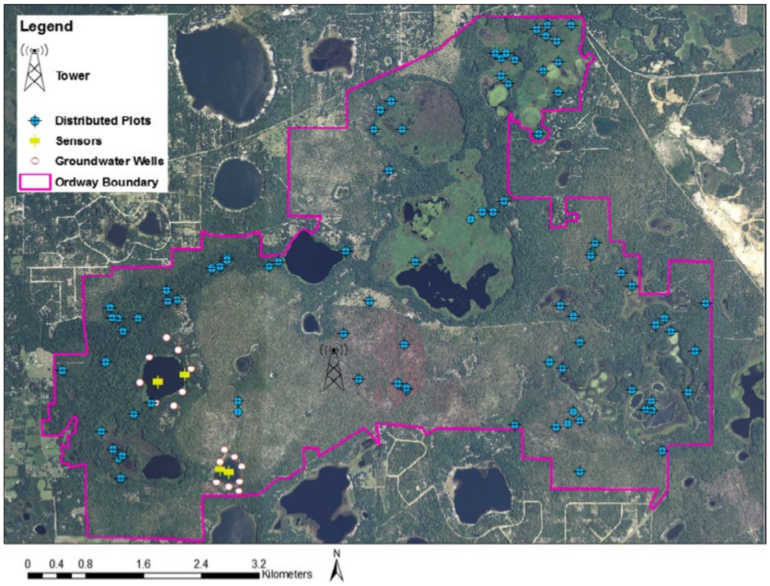

Fig. 2. Example of a NEON site, Ordway-Swisher Biological Station, Florida (Domain 3) with tower location, Distributed plots in dominant vegetation communities, and co-located aquatic observation stations.

of ecosystem types, and facilitate further research; (2) the spatial and temporal sampling design will provide sustained contributions to the natural sciences community over 30 yr; (3) the chosen methods and associated quality assurance/quality control (QA/QC) approaches will be consistent across sites and allow for comparisons across other network observatories; and (4) there will be an approach in place to reevaluate the measurements and sampling strategy over the lifetime of the observatory.

Measurement suite

NEON measurements include drivers of biogeochemical cycles (e.g., precipitation, temperature, parent material characteristics) and response variables (e.g., net nitrification and net N mineralization rates in soils, stable isotopic composition of bulk soils and plant tissues, surface water nutrient fluxes, and nutrient uptake by plants). A focus on these measurements is in direct response to the Observatory requirement: "NEON shall observe the causes and consequences of environmental change to establish the link between ecological cause and effect" (Schimel et al. 2011). The anticipated timescales of turnover or alteration of turnover for different components of biogeochemical cycles require different sampling protocols. While most factors can change abruptly if the surrounding environment is perturbed, the potential for short-term change is often at the level of biota—specifically, microbial communities and larger fauna of the soil and canopy. These organisms exhibit faster compositional turnover times and gene systems that potentially respond to local variations much more so than long-lived vegetation, for example. Soil physical properties (e.g., texture, mineralogy) can be thought of at the other extreme, as they can remain relatively constant for hundreds to thousands of years, in the absence of landslides, human activity, or other geomorphological disturbance.

Given differential response rates of ecosystem components, NEON's terrestrial biogeochemistry field sampling strategy could take one of several approaches. One approach would be to focus on characterizing spatial heterogeneity in C and nutrient stocks across each site, creating a long-term data set of element pool sizes in soils and plant tissues, and relying on research questions from the natural sciences community to initiate short-term process-based studies that would capture the mechanisms underlying patterns in the data.

Past research has shown that on the order of decades, unless drastic disturbance has occurred, total ecosystem C and nutrient stocks do not change dramatically (Magill et al. 2004). At the same time, heterogeneity in soil physical properties within sites may overwhelm the ability to discern subtle but important spatial and temporal patterns (e.g. Johnson 1995), thereby undermining one of the primary requirements of the NEON project. Another approach would be to focus on measuring transformation rates, such as soil CO_2 efflux, nitrification, and denitrification, which are likely to change in response to daily, seasonal, and climate change drivers (see Hopkins et al. 2014, Reichstein et al. 2014). Focusing exclusively on these measurements, however, would not capture the environmental drivers or resulting feedbacks, which are another requirement of NEON. Capturing spatial and temporal variability in process rates is also challenging from a financial and labor-investment perspective at the large scale of the NEON project.

Given these considerations, NEON's field sampling strategy will target a smaller suite of selected physical drivers, and some biogeochemical responses at optimal spatial extents and temporal frequencies. These key measurements include a baseline characterization of soil physical and chemical properties, regular measurements of soil and plant C and nutrient pools, and more frequent measurements of microbially mediated N transformations in soils. The sampling strategy relies on targeting minimal sampling frequencies necessary to capture long-term trends in the focal measurements and make cross-site comparisons. The goal is for the resulting long-term data set to constitute a useful contribution to ecological science because of its coordinated approach across large spatial and temporal extents. Next, we outline the priority measurements of biogeochemical drivers and responses. We discuss the spatial sampling strategies and temporal frequencies associated with these measurements following their brief descriptions below.

Drivers of biogeochemical cycles

NEON's high-priority measurements of controls on biogeochemical cycling include meteorological variables, atmospheric deposition, soil physical and chemical properties, and soil temperature and soil moisture. Meteorological variables and C and nutrient inputs will be measured at the eddy covariance tower location at each site, and are summarized in Fig. 3. Soil physical and chemical characteristics, as well as soil temperature and soil moisture measurements will be sampled in a subset of the 40 Distributed plots within each site to capture spatial heterogeneity. During a one-time site characterization effort in the initial sampling years, NEON will measure soil chemical and physical factors, including: soil texture (i.e., particle size distributions), soil color, soil aggregation, bulk density, porosity, organic horizon mass and layer thickness, total C and nutrient concentrations (N, P, S), exchangeable anions and cations, rooting and soil depths, and pH (Table 1). These attributes influence the distribution of resources in the soil matrix, including air space, shape the development of hydrological flow paths, which in turn control redox states and nutrient transport, and influence plant and microbial physiological activity and growth. Soil properties will be measured by soil genetic horizon (2 mm fraction) to a depth of 1 m.

Soil surveys with comparable measurements have been compiled at the continental scale by organizations such as the U.S. Department of Agriculture Natural Resources Conservation Service (NRCS), which created the STATSGO and SSURGO soil databases, yet these data are collected at coarser scales than the NEON design, and the suite of analyses are not consistent across all sample locations. For the NEON soil surveys, the first-order level sampling scheme would entail excavation and descriptions of multiple soil pits. At locations with permit restrictions, soil horizons will be described and sampled using soil cores. The contractors will follow NRCS protocols for field description and laboratory analysis of soils (see Burt 2004, Schoeneberger et al. 2012); adoption of these protocols will also enable cross comparison between NEON and NRCS sites.

In addition to basic soil characterization, soil water content and temperature are fundamental controls on biogeochemical processes, and must be measured throughout operation of the Observatory. Previous studies by several groups (e.g., Groffman et al. 2012) have documented the importance of measuring soil temperature and soil water content, both for the purposes of ex-

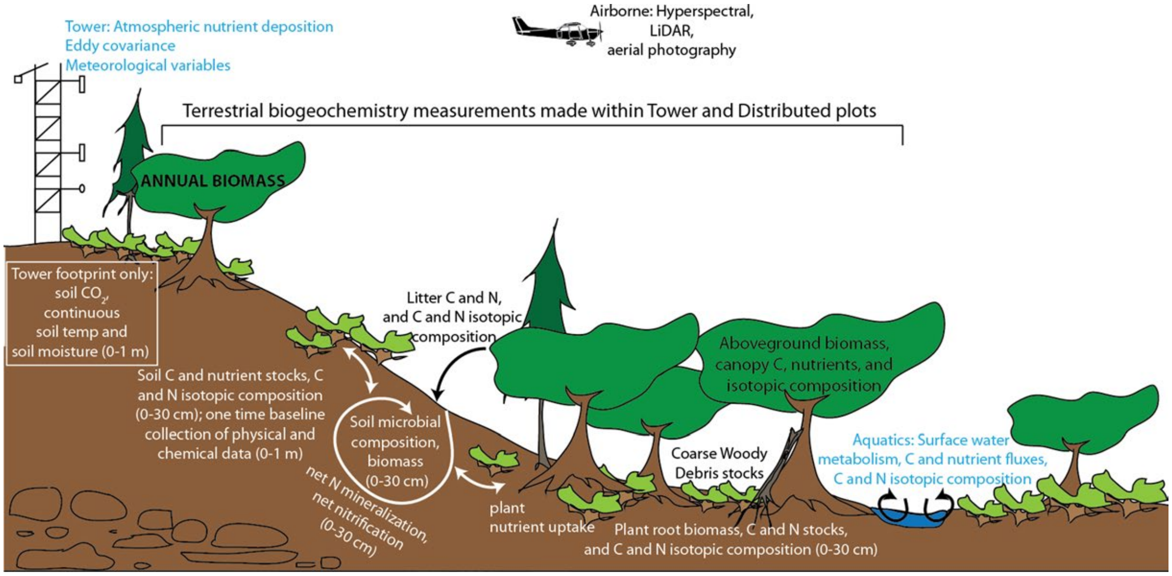

Fig. 3. NEON biogeochemistry measurements within a site, showing variables that are part of the terrestrial biogeochemistry design (Terrestrial Observation System, TOS), as well as those associated with the eddy covariance tower (NEON's Terrestrial Instrument System), above- and belowground plant biomass (TOS), microbial community (TOS), and ground and surface waters (NEON's Aquatic Observation and Instrument System).

plaining patterns in biogeochemical data, and for informing ecosystem models. Ideally, continuous measurements of soil temperature and moisture probes would be collected across NEON sites. However, funds for sensors are not included in the budget for NEON's spatially distributed terrestrial observations, so they will be collected manually at periodic sampling dates. Within five Tower plots, NEON will make continuous sensor-based measurements of soil temperature and soil water content.

Soil biogeochemistry

NEON's high-priority soil biogeochemical measurements include C and nutrient stocks, stable isotopic composition, and rates of N transformations. Measurements of the soil microbial community will be made on the same soil cores (Fig. 3). Throughout the lifetime of each NEON site, the proposed soil stock measurements include total C and major nutrient pools (N, P, and S), soil C and P fractions, pH (in calcium chloride and water), exchangeable anions and cations, and natural stable isotopes of C and N ($\delta^{13}C$ and $\delta^{15}N$) of bulk soil pools (Table 1). A focus on these constituents is consistent with other components of the NEON biogeochemistry design, including measurements of plant tissues, atmospheric deposition of mineral elements at the tower location, and the chemistry of surface waters. The resulting data will enable investigators to analyze ecosystem stoichiometry, and changes in some of the nutrient sources and processes that can alter these relationships. For example, following C:N ratios in soils over time is a useful indicator of microbially mediated transformations (e.g., Kaye and Hart 1997) and correlates with ecosystem losses of nitrate and dissolved organic carbon (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 the 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 investigate the origins and transformation processes of different nutrients (e.g.,

Table 1. Soil and plant biogeochemical measurements in Distributed plots at NEON sites.

Measurement(s)	Variable	Timing and frequency	Spatial extent (# reps or plots by site)
Soil characterization	Texture Bulk density Organic horizon mass Porosity Soil color Stone content Soil depth Rooting depth Total C, N, P, S Organic C Exchangeable cations and anions pH Fe, Al, Mn	Once during NEON Construction	10–40 locations (from within a chosen subset of Tower and Distributed plots)
Soil C and nutrient stocks; stable isotopes	Total C, N, P, S Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, H^{+}, Al^{3+}, NH_4^{+}) Anions (Cl^{-}, NO_3^{-}, SO_4^{2-}, PO_4^{3-}) C and P fractions $\delta^{13}C$, $\delta^{15}N$ pH	July-August window, every 10 yr	Replicates per plot (3) Tower plots (4) Distributed plots (6–11)
Soil inorganic N pools and transformations	Soil inorganic N Nitrification Net N mineralization	1–3 times within a sampling year (every 5 yr)	Replicates per plot (1) Tower plots (4) Distributed plots (6–11)
Soil temperature and water status	Soil temperature Soil moisture	All microbial and biogeochemical soil collections	Replicates per plot (3) Tower plots (4) Distributed plots (6–11)
Plant foliar tissues	Total C, N, P, S Chlorophyll Acid unhydrolyzable residue (Lignin) $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Timed with airborne data collection; every 5 yr	Individuals per plot (3, forested and mixed communities; 2 clip strips, herbaceous) Tower plots (4) Distributed plots (6–16)
Litterfall	Total C, N $\delta^{13}C$, $\delta^{15}N$	Every 5 yr	Replicates per plot (1–2) Tower plots (20–30)
Roots	Total C, N $\delta^{13}C$, $\delta^{15}N$	July-August; every 5 yr	Replicates per plot (1–2) Tower plots (20)

Gebauer et al. 1994, Robinson 2001, Pendall 2002), soil organic matter turnover rates and decomposition extent (e.g., Bernoux et al. 1998, Marín-Spiotta et al. 2009), and N loss pathways (Houlton and Bai 2009, Bai et al. 2012).

Rates of soil biogeochemical processes are expected to respond to changes in climate, influencing the amount and forms of C and nutrients that move among air, land, and water 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 (Emmett et al. 2004, Loik et al. 2004, Barnett et al. 2005, Hart 2006, Cable et al. 2008, Hopkins et al. 2014, Reichstein et al. 2014). NEON will provide insight into distributed soil biogeochemical process rates by periodically quantifying rates of net N mineralization and nitrification using field-incubated soil cores (Table 1) and measuring microbial community diversity and abundance. Over the broad spatial scale of NEON, public users of these linked data sets will be able to explore relationships between microbial groups and biogeochemical processes in terrestrial ecosystems.

Plant biogeochemistry

NEON will quantify variation in plant foliar tissue chemistry at the plot to site scale using a combination of ground- and airborne-based methods. Ground-based collections at a subset of Tower and Distributed plots will include sampling and analysis of total C and nutrient concentrations (N, P, S) in sun-lit foliage of dominant and co-dominant canopy species.

Stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of foliage, as well as other chemical characteristics (i.e., chlorophyll and lignin as measured by acid unhydrolyzable residue), and leaf mass per area will be measured (Table 1). These data will provide indicators of photosynthesis and nutrient uptake and storage in terrestrial ecosystems (Wright et al. 2004, Frank et al. 2015). NEON will sample vegetation (individuals) representing the dominant and/or co-dominant species in tall-stature communities; within each plot, priority will be placed on sampling georeferenced individuals for which structural data will be collected over the lifetime of NEON. In herbaceous, as well as mixed forest-grassland communities, georeferenced clip strips (i.e., aboveground biomass) will be harvested for determination of total dry weight per area and bulk chemical analysis.

In parallel with sun-lit foliar tissue sampling for chemistry, NEON will provide data to quantify above- and belowground biomass from additional measurements of plant structural properties, leaf area index, and root biomass. Litterfall will be measured in the years when sun-lit foliar tissue sampling occurs, but collections will take place throughout the year. Roots (by size class: <0.5, 0.5–1, 1–2, and 2–10 mm) and litterfall (by functional type: needles and leaves) will be analyzed for total C, total N, and the natural stable isotopes of C and N ($\delta^{13}C$ and $\delta^{15}N$).

Airborne observations, including hyperspectral, LiDAR, and high-resolution aerial photographs, will be collected across each NEON site every year. The ground-based plot-level assessments of plant chemical characteristics 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 photosynthetic pigments) at large spatial scales (see Ollinger et al. 2002, Asner and Vitousek 2005, Wang et al. 2007, 2010). 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 creating large-scale data surfaces for modeling efforts (i.e., scaling to the site level and beyond). Foliar chemistry data are needed to constrain leaf photosynthetic capacity in some ecosystem models. These data are available only for some AmeriFlux or Fluxnet tower sites (see Ollinger et al. 2008); hence, their collection at the NEON sites should be useful to those working to improve model representations.

Spatial and temporal sampling strategy

The terrestrial biogeochemistry measurements provide a link between the spatially constrained, intensive measurements of atmospheric deposition and CO_2 fluxes at the tower location and quantification of nutrient fluxes and metabolism in surface waters of downstream aquatic ecosystems (Fig. 3). In addition, they are critical to the calibration and validation of the airborne hyperspectral data collected at each site. 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 with these other data sets. To determine the spatial and temporal collection of samples at each site, we have developed an initial estimate of the intra- and inter-annual sampling frequencies required for the resulting data set to enhance ecological understanding at site to continental scales. This strategy follows the Observatory requirement that states, "NEON shall address ecological processes at the continental scale and the integration of local behavior to the continent, and shall observe transport processes that couple ecosystems across continental scales" (Schimel et al. 2011). Following the initial years of NEON data collection, the efforts of early data users will inform whether the current sampling approach is sufficient or should be revised to better meet the stated requirement and serve the community.

An important goal for NEON is to provide investigators with sufficient data to distinguish between spatial variability and temporal trends in ecological phenomena. The high-level requirement corresponding to this goal states, "NEON shall detect and quantify ecological responses to and interactions between climate, land use, and biological invasion, which play out over decades" (Schimel et al. 2011). For the priority measurements that we have identified in Table 1, the data collection falls into two categories of measurements: (1) initial site characterization to provide a baseline data set necessary to inform the design of ecosystem stock and process measurements and (2) operational sampling during the lifetime of NEON, which will be used for long-term trend analysis. We allocate NEON's terrestrial biogeochemistry measurements into these two categories to determine the minimum intra- and inter-annual sampling frequencies for initial data collection during operation of the Observatory.

The initial soil survey will occur once at each NEON site during construction of the Observatory. A team of NRCS soil scientists local to each NEON domain will be contracted to determine the number and location of soil pits/cores required to describe soil variability within the site; budgetary constraints cap the maximum number of sampling locations at 40. In addition to the soil survey, stem mapping of tree species, including diameter at breast height (dbh) and relative abundance, will occur as part of site characterization within the Tower and Distributed plots. These data will inform which individuals (i.e., dominant and co-dominant species) will 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 to assess their contribution to plot- and site-scale plant nutrient stocks.

Bulk soil C and nutrient stocks and stable isotopes will be the least temporally variable of the measurements in the terrestrial biogeochemistry design. NEON will make these measurements once every 10 yr; at relocatable sites, which are scheduled to move every 5–10 yr, sampling will only occur once. NEON will sample the top 30 cm of soil (as measured from the top of the organic layer, and at sites where the depth to bedrock is greater than 30 cm) and cores will be separated by organic (if present) and mineral horizons. At each sampling event, three random cores will be collected within four Tower and 6–11 Distributed plots per site; these 40 × 40 m plots will be sampled over the lifetime of the Observatory, and coring will occur outside of the center 20 × 20 m subplot where plant biodiversity will be assessed.

Greater inter-annual, as well as intra-annual sampling frequencies at each site are required to capture the dynamics of soil N transformation rates, which represent components of biogeochemical cycles that are expected to undergo short-term changes in response to various drivers. In four plots near the tower location and 4–6 plots distributed across the site, sampling will occur every 5 yr. Within each year, no more than three sampling events (field incubations of soil cores) will occur: one temporally coordinated incubation (all sites) and up to two targeted incubations capturing "hot moments" of biogeochemical importance. Examples of hot moments that NEON will target include: summer in California (Domain 17), when 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., Central Plains/Domain 10 and Northeast/Domain 1), and first rains of the growing season (or monsoon rains) in arid environments (e.g., Southwest/Domain 14). Sampling during these particular periods will cluster by groups of like domains. During all soil collection efforts, NEON plans to measure soil temperature and soil water content (at least three times per year).

Plant tissue sampling, including sun-lit foliage, litterfall, and roots, will occur at fixed 5-yr intervals at each site. Sampling of sun-lit foliage will coincide with flight campaigns by NEON's Airborne Observatory Platform (AOP), and occur in four Tower plots and 6–16 Distributed plots per site. In tall- and mixed-stature systems, three individuals that represent dominant and/or co-dominant canopy species will be sampled. In low- and mixed-stature systems, one to two 0.1 × 2 m clip strips per plot will be harvested, dependent upon the size of the plot. In addition, NEON will estimate the herbaceous percent cover of the plots to provide data necessary to scale foliar C and N measurements.

Collections of litterfall and root tissues for biomass and chemical analysis will occur within dominant vegetation communities of the Tower plots only, primarily to reduce collection and processing time. Thus, the spatial sampling design for biogeochemical analysis of these tissues will be one to two randomly located cores for root biomass (0–30 cm) and one to two litter baskets in each of 20 Tower plots. In both cases, the number of replicates and plots will be dependent on the plot size and vegetation stature of the system, respectively, and will have accompanying net primary productivity data. Soil cores for root sampling will be collected once per sampling year, and litterfall will be collected throughout the sampling year, but composited by functional group across the collection period for chemical and isotopic analysis.

Standard methodology and QA/QC of data

The third design criterion involves the choice of methods to provide consistency across NEON sites, and to align with those that have been implemented by other established observatory networks. The corresponding requirement states, "NEON measurements shall be standardized and calibrated to allow comparison across sites and over time to enable understanding of ecological change in time and space." Cross-calibration and standardization will also allow new sensors/measurements to be brought online (Schimel et al. 2011). Central to meeting the stated objectives is the establishment of standard operating protocols for the field, and identifying laboratory analysis procedures to be implemented by contracted, external facilities. For field sampling of soils, one of the primary challenges for standardization of protocols is to sample consistently across sites with different soil structural properties, features (e.g., soil horizonation, the presence of rocks or a hardpan), and depths. With respect to plant communities, differences in canopy structure, growth forms, rooting depths, life-history traits, phenology, and diversity affect the approach to sampling. This variability across sites affects how sun-lit canopy foliage is obtained, as well as the sizes of the Tower and Distributed plots required. With respect to sun-lit foliage sampling, we will use protocols designed for sampling different categories of sites (e.g., herbaceous, mixed, and tall-stature vegetation. With respect to plot sizes, NEON will begin operations at all sites with standard 40 × 40 m plots for biogeochemistry. Laboratory analyses of soil and plant material will be consistent with standard practices within the ecological and soil science communities, such as published methods by LTER and Soil Science Society of America.

At each NEON domain laboratory, field technicians will be responsible for initial preparation/processing of samples following field collections. Samples will be shipped from domain laboratories to a few external, contracted laboratories to complete physical and chemical analyses. Development of analytical contracts for all analyses is currently in progress. Contracted facilities will be required to comply with strict QA/QC and auditing protocols implemented by NEON's Calibration/Validation Laboratory, to ensure consistency and data quality among sites. The Calibration/Validation Laboratory will maintain a collection of standard materials to include with samples submitted for elemental and isotopic analysis, and periodically audit operations at contracted facilities. Ideally, the facilities contracted by NEON should be educational/training centers in addition to providing community analysis services.

During the lifetime of NEON, there will be some flexibility in the laboratory protocols to accommodate methodological and technological advances; however, every attempt will be made to compare any new method/instrumentation vs. previously established methods. NEON, in collaboration with the appropriate expert communities, will evaluate these methods as they become part of standard research practice, and adjust accordingly. For example, laser-based instrumentation is becoming more widespread in the measurement of stable isotope abundance. As these techniques replace older ones, NEON's protocols and associated documentation will be updated. This cross-calibration and revision of methods will ensure that measurement efforts benefit from technological advances (which may also reduce operating costs), as well as continuity in long-term data sets. Documentation of all data processing and calibration will be available with NEON data sets on the web portal (data.neoninc.org) and freely accessible to the public, per the Observatory requirement for free and open exchange of scientific information (Schimel et al. 2011).

Approach to sampling prioritization and optimization

The fourth design criterion is an approach for prioritizing and evaluating sampling efforts over the lifetime of NEON to optimize allocation of financial and labor resources. One of the strengths of NEON is that it is required to implement a standard suite of measurements and methodological approaches to allow for comparison across all sites (Schimel et al. 2011). Such a standardized approach to sampling across sites has not yet occurred at broad scales for terrestrial biogeochemistry. Generally, financial constraints drive limitations in the suite of elemental and isotopic constituents listed in Table 1 and identification of minimum inter- and intra-annual sampling frequencies for each measurement. Over the lifetime of NEON, it may be necessary to reallocate resources among the focal measurement areas to optimize the information content of the data sets. In particular, we anticipate that the larger community of ecosystem ecologists may desire more frequent measurements of inorganic nutrient pools (e.g., NO_3^-, NH_4^+) and nutrient transformations, or inclusion of throughfall, soil water, and trace gas measurements distributed across each site. We also recognize that there are other physical and chemical constituents that would be valuable to measure. With the understanding that the broader research community may want to conduct additional analyses, NEON will maintain an archive of the 10-yr bulk soil collections and all plant tissue samples (i.e., sun-lit foliage, roots, and litterfall). These resources will be available via a proposal process, and are one of the means by which the larger natural sciences community can conduct research that leverages or complements NEON. For example, NEON data streams and archived samples will be a useful resource to investigators developing proposals to do more detailed, process-based research.

As NEON's operational period begins, regular iteration with the larger natural sciences community will be critical to verify that the sampling and analysis design and NEON's scientific priorities are aligned, as well as to engage the community in thinking forward about NEON science. In particular, the terrestrial biogeochemistry component of NEON will benefit from regular workshops with the broader community to evaluate data streams and discuss the degree to which the sampling design is aligned with the rest of the field. As outlined in this document, many biogeochemical measurements are inherently subject to short-term changes and are spatially heterogeneous (Lovett et al. 2005); they also require manual rather than automated measurements, in most instances. Therefore, flexibility in the NEON design strategy will be critical to assure that personnel efforts and financial resources are used efficiently and effectively. Ideally, 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 1–3 yr during the lifetime of NEON. This approach will help to create the transformative data and community research experience that are central to the NEON mission.

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