FY2024 PROGRESS REPORT OAK RIDGE NATIONAL LABORATORY'S TERRESTRIAL ECOSYSTEM SCIENCE — SCIENTIFIC FOCUS AREA

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ABSTRACT

Understanding fundamental responses and feedbacks of terrestrial ecosystems to climatic and atmospheric change is the aim of the Terrestrial Ecosystem Science Scientific Focus Area (TES SFA). The proposed research efforts of the ORNL TES SFA seek to provide answers to the following overarching question: How vulnerable to climate change are C stores of terrestrial ecosystems in eastern North America, and what are the implications for C-climate feedbacks? The TES SFA focuses on ecosystems subject to water, energy, and nutrient constraints whose impacts are highly uncertain in Earth system models. Our proposed science includes manipulations, multidisciplinary observations, database compilation, and fundamental process studies integrated and iterated with modeling activities at multiple scales. The dominant manipulation is the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment testing responses to multiple levels of warming at ambient and elevated CO₂ for a peatland ecosystem. Long-term observations of ecosystem function at an eddy covariance site in Missouri (MOFLUX) characterize ecosystem response to dominant hydrologic limitations. Research activities at SPRUCE and MOFLUX cover a spectrum of environmental drivers and complement each other. Process-level work occurs at smaller scales and aims to improve mechanistic representation of processes within terrestrial biosphere models. The TES SFA integrates experimental and observational studies with model building, parameter estimation, and data analytics to yield reliable model projections. This integrated model-experiment approach focuses on improving the land model (ELM) of DOE's Energy Exascale Earth System (E3SM) model and fosters enhanced, interactive, and mutually beneficial engagement between models and experiments.

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1.0 PROGRAM OVERVIEW

Terrestrial ecosystems store vast amounts of C and globally these C stores are increasing in C density as ecosystems remove CO₂ from the atmosphere via physiological C feedbacks (Friedlingstein et al. 2020). However, climate change is expected to alter net ecosystem exchange (NEE) across biomes and mobilize vulnerable C stores (IPCC 2022). Earth system models (ESMs) predict that as temperature and precipitation regimes change, climate–C feedbacks will overwhelm physiological–C feedbacks (IPCC 2021). Such climate impacts will make large terrestrial C stores vulnerable to loss in the form of greenhouse gas releases to the atmosphere, further exacerbating climate change. The mechanisms underlying climate-driven C losses remain uncertain, causing large inter-model variability in ESM ensemble simulations and strongly limiting Earth system predictability.

Temperature and water dynamics across space and time regulate processes at multiple scales from soil biogeochemical cycles to vegetation C uptake. Climate change is already increasing temperatures, shifting precipitation norms, and altering the timing, magnitude, and location of extreme events such that water availability and vapor pressure deficit (VPD) are increasingly driving ecosystem function. The water cycle is particularly important to future C–climate feedbacks because increased atmospheric demand and altered precipitation patterns could shift energy- and water-limited ecotones and will intensify climate extremes such as drought and fire (USGCRP 2018). Elevated CO₂, however, can ameliorate some effects of increased temperatures and reduced soil moisture. In the TES SFA, we target improved understanding of the C–climate feedback, focusing on mid-latitude temperate to boreal forest ecotones and ecosystems of eastern North America—systems that span wide gradients in moisture availability and temperature. Ongoing and proposed research efforts in the ORNL TES SFA seek to provide answers to the following overarching question:

How vulnerable to climate change are C stores of terrestrial ecosystems in eastern North America, and what are the implications for C-climate feedbacks?

The TES SFA focuses on ecosystems subject to water, energy, and nutrient constraints whose impacts are highly uncertain in ESM predictions. Key areas of uncertainty include the responses of cold, water-logged, high-C peatlands to a changing climate. At the other end of the spectrum, the responses of ecosystems on the ecotone between energy- and water-limited regions are also not well represented in models. In transition zones, plant species approach their biogeographical limits imposed by environmental stressors and/or interspecies competition, and they often experience extremely dynamic processes of mortality, natality, and growth (Gu et al. 2015, 2016a). Climate-driven increases in temperature or aridity can also drive increased atmospheric demand for water, which may exceed vegetation capacity for transport despite available soil water, leading to desiccation and reduced C uptake. Belowground-coupled C, water, and nutrient biogeochemical cycling are not well understood and impart significant control on aboveground ecosystem structural and functional responses to changing climate.

2.0 SCIENCE QUESTIONS, GOALS

We structure the TES SFA around the following theme-motivating questions that target key knowledge gaps and uncertainties in the climate and disturbance responses of boreal and temperate ecosystems:

- 1. By how much and which mechanisms will warming affect southern boreal peatland ecosystem productivity, C storage, and greenhouse gas fluxes? Can elevated CO₂ ameliorate the likely negative effects of warming?
- 2. How do water availability and water cycle extremes interact with climate change to regulate net ecosystem exchange and energy balance within temperate and boreal forests?
- 3. How does environmental change alter nutrient distribution and dynamics, and what are the implications for understanding and predicting ecosystem *C* fluxes?
- 4. How do temperature, water availability, and plant inputs affect soil C and microbial functions, and what are the implications for ecosystem C storage and greenhouse gas fluxes?
- **5.** Are the humid, high-C ecosystems of North America more vulnerable to changing climate and disturbance regimes than predicted by CMIP6? How does the collective knowledge gained from the TES SFA affect our understanding of the C feedbacks in the region?

Goals

This section provides an overview of the five TES SFA research themes goals and key science issues.

*Theme 1: Carbon Cycle Responses to Warming and Increased Atmospheric CO*₂ *Concentration*

The goal of Theme 1 is to provide mechanistic understanding and model improvements to understand the impacts of warming associated with climate change on peatland ecosystem C cycle and balance leading to uptake from or release of C to the atmosphere. We use consistent above- and belowground manipulations of the ecosystem climate and atmosphere to examine plausible future conditions that ecosystems will be exposed to under a warming climate with elevated CO_2 atmospheres. Our work will complete a decade of operation of the SPRUCE experiment (Hanson et al. 2017; https://mnspruce.ornl.gov/). This manipulation examines sustained temperature increases across a broad range of warming conditions ($+0^{\circ}C$ to $+9^{\circ}C$) with and without the addition of elevated CO_2 atmospheres (+500 ppm). Modeling efforts synthesize our experimental results over time and across spatial scales and provide the basis for a functional wetland land surface model applicable to the SPRUCE peatland and related wetland ecosystems.

Theme 2: Ecosystem, Water, Energy and C Processes under Compounding Climatic Stressors

The goal of Theme 2 is to develop predictive understanding of coupled ecosystem water, energy, and C-cycle processes that can be transferred directly to improving the performance of ecosystem and land surface models. Theme 2 also uses the understanding of complementary ecosystem processes (e.g., nutrient cycling, microbial activities) gained in other TES SFA themes to inform representations of land surface processes in climate models. Research will emphasize the SPRUCE and MOFLUX field sites in sensitive ecotones at the two ends of a forest ecosystem water availability spectrum. For energy processes, we propose to answer the long-term question of why state-of-the-art measurement techniques cannot close the land surface energy budget. Theme 2 will test the hypothesis that the transient energy storage in photophysical, photochemical, and biochemical reactions of photosynthesis, particularly the proton motive force (PMF; the electric and proton concentration gradients across the thylakoid membrane established by photosynthetic electron transport), are sufficiently large to affect leaf and land surface energy balance and temperature regimes. For C processes, Theme 2 will develop advanced, mechanism-based methods that partition observed net fluxes into contributions from different pathways across scales (gross primary production [GPP], autotrophic/heterotrophic above/belowground respiration, point to ecosystem scales) and innovative analysis approaches such as coupled photophysical, photochemical, and biochemical

(CPPB) modeling and machine learning (ML). For water processes, we will target uncertainties in limitation through the soil-plant-atmosphere continuum (SPAC), ranging from pore-level assessment of soil–root connectivity to ecosystem-level exchanges of water and C with the atmosphere.

Theme 3: Nutrient-C Feedbacks

The goal of Theme 3 is to quantify the role that nutrients play in modulating C cycle feedbacks. We use empirical data collection, database curation, and an array of field sites to improve the ability of nutrient-enabled ESMs to predict ecosystem response to climate change. To improve the representation of nutrient cycling in ESMs requires expanding our scientific scope beyond the boreal zone and the SPRUCE experiment. Comprehensive nutrient budgets and dynamics need to be quantified from a variety of ecosystem types so that a clear understanding of plant nutrient acquisition strategies and microbial nutrient use during decomposition can be attained. As theme's research develops in the coming years, we will expand observations at MOFLUX to include plant and soil nutrient dynamics, downslope leaching, erosion, and litter chemistry. At Morton Arboretum, we will do research to complement ongoing process measurements of above- and belowground plant phenology in monospecific forestry plots with measurements of resin-available N and P in soils. Lastly, we will support continued development and expansion of the global trait database FRED with an emphasis on data curation from underrepresented biomes underlain by organic soils (e.g., bogs) as well as fine-root traits that inform the representation of root nutrient acquisition in models across a variety of plant functional types (PFTs). Model refinement of nutrient acquisition, allocation algorithms, nutrient resorption for whole ecosystems, plants, and microbes will take place by linking ELM with TAM, enabling dynamic vegetation feedbacks with FATES, and testing process hypotheses in MAAT.

Theme 4: Soil Carbon Cycling and Microbial Processes

The goal of Theme 4 is to determine the influence of soil C cycling and microbial processes due to climate change impacts on soil temperature, moisture, and plant inputs. For at least a decade, the role of the microbial community in soil C storage and greenhouse gas emissions has been highly debated. Traditional first-order models remain unable to capture many nonlinear processes such as priming, changes in inputs, microbial community shifts, and acclimation of microbial physiology to climate changes. However, models that explicitly include microbial pools and functions retain artifacts of their structural configuration. These structures vary widely and encompass different representation of soil aggregation; partitioning of microbial community into bacteria, fungi, and archaea or various functional groups; simulating microbial life strategies, representation of microbial uptake and leaching of dissolved organic C (DOC); types of DOC; and whether to include enzymes explicitly. Consequently, different microbial-explicit models will produce different predictions of future soil C stores and greenhouse gas emissions. In this Theme, we seek to use new observations and the long-term data collection at MOFLUX within the multi-model environment of MAAT to determine optimal model configurations to best predict the future trajectory of soil C storage in the context of long-term climate changes and short-term climate events at the forest-prairie ecotone. A major outcome of this effort will be to discover the efficacy of including different structures, C pools, and parameters in microbial models and thereby provide appropriate and tested model configurations to the ELM community. At SPRUCE, we will take advantage of a wealth of existing microbial data, ongoing and new regular data collections from multiple decomposition experiments, higher frequency microbial biomass estimates using multiple methodologies, and new automated real-time greenhouse gas emission measurements (Theme 1) to understand and predict how different microbial functional

groups, such as bacteria, fungi, and archaea, contribute to observed high-frequency CH₄ and CO₂ emissions as a function of experimental treatments, seasons, and time.

Theme 5: Regional Integration and Extrapolation

The goal of Theme 5 is to implement our improved process knowledge in a regional modeling framework to quantify and understand the broader scale vulnerability of ecosystem C and C feedbacks of humid ecosystems of North America under climate change. The regional domain includes our core and secondary sites (SPRUCE, MOFLUX, MA, Walker Branch), covers key C stores over a large range of potentially vulnerable temperate and boreal ecosystems, and sits on what is currently the humid side of the strong continental gradient in aridity index. Integration and extrapolation activities leverage ELM developments in Themes 1-4 and integrate them to make multi-site and regional simulations. We also leverage highresolution observations to support vulnerability assessments where models may be missing key processes or structures. These regional simulations and analyses using improved process knowledge of peatlands and forest processes will be assessed against state-of-the-art C cycle climate models to assess how knowledge gained within the SFA changes our understanding of climate-C feedbacks and future projections. We will use advanced multi-site model calibration to estimate PFT parameters for ELM and FATES-enabled ELM across our study domain. We will develop high-resolution data sets to drive the model and run high-resolution simulations to help capture fine spatial variability and better represent nonlinearity in ecosystem responses to climate change. High-resolution observations and model data will be utilized to provide detailed quantification of model uncertainty and comprehensive assessment of ecosystem vulnerability across North America.

3.0 TES SFA PROGRAM STRUCTURE AND PERSONNEL

Responsibility for the TES SFA resides within DOE BER's Earth and Environmental Sciences Directorate and is aligned with associated and related activities of the Climate Change Science Institute (CCSI) at ORNL. The organization chart for the TES SFA is presented in **Fig. 1**. The TES SFA includes a science and management organization to guide and direct research activities. The TES SFA Leadership Team, comprised of the individuals listed in **Fig. 1**, provides advice on the yearly SFA plans and budgets, monitors progress, adjusts project plans as appropriate, directs informatics development efforts, and resolves issues in a timely manner.

Dr. Paul J. Hanson will transition away from his role as lead Co-Principal Investigator for the TES SFA at the end of 2024 due to his planned retirement. Dr. Melanie A. Mayes and Dr. Daniel M. Ricciuto take over as the co-PI for empirical and modeling-focused tasks, respectively, which are integrated across the TES SFA. Theme Leads and Task leads described in **Fig. 1** are given independent science and financial responsibility to achieve the goals of their respective Themes and Tasks. An Executive Committee consists of the project Co-PIs, leads from each Theme, data management, and a representative from the Early Career Community (Section 6).

The TES SFA is supported by 40 dedicated ORNL scientific and technical staff. Over 100 individuals from the USDA Forest Service, and various other collaborating universities and laboratories have participated in the SPRUCE and MOFLUX projects.



Fig. 1. Organizational chart for the TES SFA (2024 status).

The following proposed individual Theme and sub-task leads take responsibility for their respective initiatives.

Theme 1 Carbon Cycle Responses to Warming and Increased Atmospheric CO₂ Concentration

Task T1.l

Paul J. Hanson leads the management and operations of the SPRUCE project with direct support of the onsite Project Manager, Kyle Pearson. Kyle Pearson is located fulltime in Grand Rapids, Minnesota. He maintains SPRUCE operations with the support of a single full-time technical support person (Mark Guilliams). Misha Krassovski (Technical Professional systems engineer) designed and maintains site communication equipment, and he works with Kyle and Mark to sustain operations of the automated data acquisition system for SPRUCE (both environmental and biological monitoring systems). A coordinating panel consisting of the SPRUCE coordinating investigator (Hanson initially), the local USDA Forest Service contact (Kolka), the Theme and sub-task leaders for all SPRUCE activities, and members from the scientific community make up the experimental advisory panel. This group serves as the decision-making body for major SPRUCE experiment operational considerations and is the decision-making body for vetting requests for new research initiatives to be conducted at SPRUCE.

Task T1.2 Natalie Griffiths, Paul J. Hanson, Colleen Iversen, David Weston, Melanie Mayes, Steve Sebestyen, and postdoctoral research staff are splitting efforts in this area.

Paul Hanson will transition his lead on tree and shrub growth and vegetation phenology to Natalie A. Griffiths and Technical staff. David Weston leads characterization of growth and community dynamics of the diverse *Sphagnum* communities occupying the bog surface beneath the higher plants. Colleen Iversen, with technical assistance from technical personnel and John Latimer (subcontractor) follow belowground root and fungal growth. Jonathan Stelling is a post-doc based in Grand Rapids, Minnesota (supervised by David Weston and Melanie Mayes). Community compositional changes are being led by Brian Palik (USDA Forest Service) and Rebecca Montgomery (University of Minnesota). Work on hydrologic cycling is led by Steve Sebestyen (USDA Forest Service) and Natalie Griffiths (ORNL).

Task T1.3 Lianhong Gu, Jeff Warren, Andrew Richardson, and Kyle Pearson work on this task. Characterization of plant physiological responses are led by Jeff Warren, past and planned postdoctoral staff, and independently funded external collaborators. Through an external subcontract, Andrew Richardson interprets automated tree and shrub canopy phenology patterns assisted by Kyle Pearson who collects biweekly on-the-ground observations of changing vegetation conditions.

Task T1.4 Key modelers include Xiaoying Shi, Jiafu Mao, Xiaojuan Yang, Daniel Ricciuto, and Yaoping Wang at ORNL with external subcontracted efforts led by Xiaofeng Xu and Yiqi Luo. Dr. Shi will lead SPRUCEMIP with contributions from Dr. Ricciuto and Dr. Wang. Dr. Shi and Dr. Ricciuto will further improve the *Sphagnum* submodel as well. Dr. Luo will also work with SPRUCEMIP outputs to create a traceability framework. Dr. Mao will lead efforts to improve phenology and allocation modeling in ELM-SPRUCE along with Dr. Wang. Dr. Yang will work in improving the representation of isotopes in ELM-SPRUCE, as well as nutrient cycling. Dr. Xu and Dr. Ricciuto will further improve the representation of methane cycling to integrate functional genes for methanogenesis and methanotrophy. All personnel will be involved in working with empiricists in Theme 1 to validate and calibrate ELM-SPRUCE with past and proposed observations.

Theme 2 Ecosystem Water, Energy, and C Processes under Compounding Climatic Stressors

Jeff Warren (ORNL) leads plant hydraulic and water relations work along the SPAC, including neutron imaging of root-rhizosphere dynamics and collaborates with Luke McCormack (MA) on soil respiration at MA. Melanie Mayes (ORNL) leads soil C flux activities at MOFLUX. Jeff Wood (University of Missouri) leads onsite activities at MOFLUX, including analysis of ecosystem responses to drought. Lianhong Gu (ORNL) leads activities in measuring and analyzing fluxes of trace gases, water vapor, energy and SIF, and leads LeafWeb. David Kramer (Michigan State University) contributes to detailed investigation of PMF bioenergetics. David Weston leads investigations of microbial-plant interactions.

Theme 3 Nutrient Carbon Feedbacks

Verity Salmon leads the empirical measurements of N and P mineralization, vegetation nutrient stoichiometry, and the SPRUCE destructive N and P observations at the end of experimental manipulations. Verity Salmon will also work with Natalie Griffiths on measuring leaf litter nutrient concentration. Griffiths, along with Steve Sebestyen (USDA Forest Service) and Keith Oleheiser (ORNL) will lead efforts on measuring depth-specific nutrient concentration in porewater and lateral outflow, and C, N, P dynamics during *Sphagnum* decomposition. Luke McCormack will collect measurements of nutrient cycling and nutrient uptake at MA. Xiaojuan Yang will lead the efforts in integrating the measurements at SPRUCE and Morton Arboretum into ELM-SPRUCE to improve the model representation of nutrient cycling dynamics and nutrient uptake. Matt Craig and Melanie Mayes will lead the measurements of nutrient pools and fluxes at MOFLUX. Matt Craig, Melanie Mayes, and Anthony Walker will develop nutrientenabled MAAT framework and evaluate and improve MAAT and ELM-FATES using measurements at MOFLUX.

Luke McCormack and Colleen Iversen will further develop and expand FRED to include more fine root traits relevant to nutrient uptake and increase the global coverage.

Theme 4 Soil Carbon Cycling and Microbial Processes

Task T4.1 Chris Schadt and a new postdoc, along with SPRUCE technical staff, will complete the sampling and analysis of SPRUCE microbial communities. Natalie Griffiths and Randy Kolka lead the field-scale decomposition tasks, with assistance from Chris, Alyssa, and SPRUCE technical staff for analyses. Melanie Mayes will lead the SPRUCE incubation experiments, with assistance from Natalie, Chris, and SPRUCE technical staff. Xiaofeng Xu of San Diego State University will lead the microbial modeling portion.

Task T4.2 Jeff Wood at the University of Missouri will collect MOFLUX samples and ship them to Melanie Mayes for analyses by ORNL technical staff. Field-scale litter decomposition

experiments will be assembled by Matt Craig, Jeff Wood, and Melanie Mayes, with analyses of the microbial community will be performed by Chris Schadt and a new postdoc, along with SPRUCE technical staff. Matt Craig will lead the incubation experiments with assistance from Melanie Mayes and ORNL technical staff. Matt Craig and a new R&D staff hire will lead the MOFLUX soil C and microbial modeling activities using the MAAT framework.

Theme 5 Regional Integration and Extrapolation

Task T5.1 Daniel Ricciuto will lead model-data calibration efforts using the Offline Land Model Testbed (OLMT). Xiaoying Shi and Xiaojuan Yang will help to integrate the model developments from themes 1-4 into the site-level versions of ELM-SPRUCE and ELM-Peatlands to be used. Anthony Walker will help to test and develop FATES within the ELM-Peatlands framework.

Task T5.2 Anthony Walker will lead the regional modeling efforts and work with Daniel Ricciuto and Xiaoying Shi to perform the simulations. Deeksha Rastogi and Shih-Chieh Kao will assist in providing downscaled meteorological drivers for the high-resolution simulations. Dali Wang will provide other input datasets and the high-performance computing infrastructure to perform the high-resolution simulations.

Task T5.3 Key modelers include Jiafu Mao, Yaoping Wang, and Matthew Craig at ORNL with external subcontracted efforts led by Mingzhou Jin and Anping Chen. Dr. Mao will lead efforts to develop the CIVE and identify where and in which season the greatest vulnerability is expected along with Dr. Wang and Dr. Craig. Dr. Jin will compare the modeled and observed CIVE to quantify model uncertainties. Dr. Chen will apply the emergent constraint framework to reduce uncertainties in model-projected CIVE. All personnel will work together and be involved in working with modelers in T5.1 and T5.2, evaluating and constraining CIVE simulations across scales.

4. PERFORMANCE MILESTONES AND METRICS

This section represents a summary of TES SFA activities accomplished since the 2023 progress document data April 2023. The material is organized by research Theme. Following the description of progress for each TES SFA science task, a table of anticipated deliverables is provided with annotations regarding progress. Theme-specific publications and completed manuscripts are listed for each Theme and are redundant with summary material for publications and data bases in the two Appendices A and B.

4AI. REVIEW OF SCIENTIFIC PROGRESS BY THEME

Theme 1: Peatland C Cycle Responses to Warming and Elevated CO₂

Theme 1 uses a combination of field measurements and modeling experiments to address the following questions at SPRUCE: *By how much and by which mechanisms will warming affect southern boreal peatland ecosystem productivity, C storage, and greenhouse gas fluxes? Can elevated CO₂ ameliorate the likely negative effects of warming? Theme 1 measurements include all components of NPP above- and belowground, net exchange of CO₂ and CH₄ and their autotrophic and heterotrophic components, C flux via lateral outflow, physiological and hydraulic processes driving photosynthesis and respiration contributions to the C cycle, and SIF characterization of GPP. Also included are seasonal changes in phenology of, and long-term changes in, vegetation community composition, as well as warming-induced changes in peat C*

stocks measured by traditional C concentration bulk density assessments and interpretations of isotopic change with time.

T1.1 SPRUCE Operations

SPRUCE warming treatments ran full time throughout 2023 and we have now completed 8.5 years of effectively uninterrupted SPRUCE manipulations. Warming treatments were maintained day and night throughout the year with only minor interruptions. The eCO₂ exposures are applied only during daytime hours during the active growing season (April through November). Table 1 shows the achieved whole-ecosystem warming treatments and eCO₂ treatments for the 2023 calendar year. Treatment data are archived in Hanson et al. (2016D).

In 2023, the unique isotopic signatures of air in the added CO₂ treatments continued to be approximately -28 ∂ ‰ for ¹³C and -528±14 Δ ‰ for ¹⁴C. Through 8 full active seasons of eCO₂ exposures new tissue growth under eCO₂ has stabilized at new isotopic signatures commensurate with the experimental exposures to eCO₂. Tissue ¹³C and ¹⁴C signatures for *Sphagnum* and *Maianthemum* plants are different that for the taller plant species because they reincorporate respired forms of [CO₂] from the peat profile. The newest data for 2023 samples are still pending.

Plot #s	Target	_Mean Air	_Mean Soil	Ambient Daylight	Elevated	
	Temperature	Temperature	Temperature	Mean Growing	Daylight Mean	
	Differential	at +2 m	at -2 m	Season [CO ₂]* at	Growing Season	
				+2 m	$ CO_2 ^{**}$ at +2 m	
	(Delta °C)	(°C)	(°C)	ppm	ppm	
Plots 7 & 21	Ambient	6.6, 6.5	5.7, 6.5	425		
Plots 6 & 19	+0	8.1, 7.5	4.7,6.1	438	829	
Plots 11 & 20	+2.25	10.1, 10.3	7.2,7.3	434	826	
Plots 4 & 13	+4.5	12.5, 12.6	9.4, 9.4	427	824	
Plots 8 & 16	+6.75	14.8, 14.7	11.6, 11.6	434	919	
Plots 10 and 17	+9.0	16.9, 16.8	13.7, 13.5	442	902	

Table T1.1. Mean annual air and soil temperatures and CO₂ concentrations by SPRUCE plot for 2023. Plot numbers in red text correspond to elevated CO₂ treatments.

*For this 2023 enumeration of eCO₂ exposures the growing season runs from day of the year 116 through 317 to match the active season in the +9 °C treatment plots.

**Elevated CO₂ would approach 900 ppm in all plots if a specific active season definition were used for each warming treatment.

Task T1.2 - Changing C Balance and Peat Stocks under Warming and eCO2

Shrub-layer NPP and Tree Growth – After 8 years of warming and eCO_2 treatments the shrub community has shown a strong positive response to warming treatments with evidence for enhanced biomass accumulation under eCO_2 (Fig. 2). Coincident with these biomass changes, the shrub community composition has also changed with warming with a few minor species being eliminated under high warming levels (e.g., Maianthemum) and the expansion of Vaccinium angustifolium in the warmest treatments (Fig. 3). Evidence for a positive community response to warming has been present in the annual net primary production data every year (data not shown).



Fig. 2. Shrub layer standing dry mass of live and dead tissues after 8 years of SPRUCE treatments for ambient and elevated CO₂ (eCO₂) atmospheres.

Fig. 3. Shrub layer community composition after 8 years of manipulation based on dry matter contributions to standing dry mass.

Unlike the consistent response of shrub community growth through time. The annual aboveground growth (ANPP) for the tree species *Picea* and *Larix* showed variable responses over time (**Fig. 4**). In the early years of the study (2016 to 2019) tree growth showed a negative or minimal response to the warming treatments, but in recent years (2022 to 2023) tree growth appears to respond positively to the sustained warming treatments. We hypothesize that this change results from altered nutrient cycles that have released beneficial nutrients (e.g., N, P, and base cations) over time.



Fig. 4. Shrub layer community composition after 8 years of manipulation based on dry matter contributions to standing dry mass.

Sphagnum moss physiology, productivity, and community interactions – Previous studies within this project demonstrated that warming manipulations at the SPRUCE site alter the microbial communities associated with Sphagnum moss. These changes led to reduced nitrogen fixation (Carrell et al. 2019 Global Change Biology; Petro et al. 2023 Global Change Biology) but also enhanced stress tolerance in the moss itself (Carrell et al. 2022, New Phytologist). However, despite these microbial provided adaptations, Sphagnum cover declined sharply with increasing temperatures (Norby et al. 2019, Ecology Evolution). This decline was attributed to a combination of factors, including temperature and

water limitations, along with increased shade competition from shrubs (Norby et al. 2023, Ecology Evolution).

Moss Community Assessment: Consistent with past observations, current data confirms that Sphagnum cover remains near full in ambient temperature plots ($+0^{\circ}$ C warming). However, warming manipulations continue to result in moss cover loss. Notably, the current year's data suggests a potential interactive effect between elevated CO₂ and temperature (**Fig. 5**). This is evidenced by a steeper decline in moss cover, reaching near-complete loss, in the three highest temperature treatments with elevated CO₂.



Fig. 5. Percent Sphagnum moss cover across temperature treatments and year. All ambient aCO₂ enclosures are within top panel, while elevated eCO₂ are within lower panel.

Expanding Community Observations: This fiscal year, we broadened our investigation of the Sphagnum microbiome to include microbial Eukaryotes (protists) and viral interactions through collaboration with Dr. JP Gibert (Duke U.). Protists influence ecosystem function directly through photosynthesis and respiration, and indirectly via predation on bacteria and fungi. Furthermore, viruses can influence microbial community dynamics with strong interactions with warming. Two collaborative manuscripts are now published on this topic (Kilner et al. 2023, Wieczynski et al 2023).

Root growth – Using allometric equations derived from root voucher specimens and major axis regression, we estimated root mass from root length and diameters measured from minirhizotron images (2015-2021, predominantly in spring and summer). We then estimated root mass C as 45% of root mass and evaluated how this shifted for each plant functional type with our warming and CO₂ treatments, as well as microtopography (which we know mediates responses of root length to our manipulations). There is some evidence for a positive impact of warming on daily root C production ($F_{1, 40.7}$ = 3.6, P = 0.0644; **Fig. 6**), with trees providing the greatest amount of new root C of the three plant functional types that we examined ($F_{2, 873.5}$ = 9.5, P < 0.001). This effect was not meaningfully mediated by either CO₂ treatment or microtopography (P \gg 0.05 for both terms). Next steps are to systematically evaluate our allometric relationships and determine over which ranges of root diameter they remain valid.





 CO_2 and CH_4 efflux – Greenhouse gas (CO₂ and CH₄) efflux has been measured in 2023 and data fit to regression Q10 equations and evaluated across the experimental categories (**Fig. 7**). Methane emissions were positively correlated with temperature treatment, but due to water table dynamics were highest in the early season (May and June). CO₂ nighttime respiration was positively correlated with temperature treatment; however, extrapolated daily CO₂ efflux is lower in plots with eCO₂, driven by higher day-time CO₂ uptake values, outpacing nighttime respiration. This could be a detectable effect of CO₂ fertilization, increasing photosynthesis in elevated temperature treatments.



Fig. 7. CH₄ and CO2 flux measurements from 2023 in SPRUCE experiment. Top showing seasonal variability of CH₄ flux due to water table fluctuations. Bottom Q10 fit (base @ 15C = 2.81, Q coefficient = 2.73).

TOC concentrations and lateral outflow losses: Total organic carbon (TOC) concentrations in lateral outflow (i.e., stream flow) continue to be elevated in the warmest enclosures, likely due

to increased mineralization of peat and leaching of recently produced photosynthate by vegetation. Concentrations of other solutes, primarily cations and metals that form complexes with dissolved organic matter (i.e., Ca, Mg, Mn, Al, Fe), are also elevated in lateral outflow. Further, we are beginning to observe elevated total nitrogen and total phosphorus concentrations in outflow; this response was muted in previous years. While concentrations of many solutes are increasing with warming, stream flow has decreased, likely due to increased evapotranspiration. Overall, this has resulted in a decrease in solute mass fluxes from the peatland, including lower TOC flux with warming. This decreased TOC flux slightly counters the increased net C loss from the peatland with warming.

Bog Surface Elevation Change – Through 8 years of warming manipulations the warmest plots show the greatest loss of peat bog surface elevation approaching a loss of 16.5 cm of total elevation (**Fig. 8**). Observations in the field suggest that elevation loss is dominated by the loss of live Sphagnum cover and the reduction in acrotelm peat. Alexandra Hedgepeth is in the process of combining 5-year isotopic peat changes by depth to better define and explain these patterns.



Fig. 8. Change in the SPRUCE bog elevation over time by treatment plots where grey = ambient, blue = +0°C, green = +2.25°C, yellow = +4.5°C, orange = +6.75°C, and red = +9°C. The Y axis is the fractional change in absolute elevation for a base elevation of 412.526 m.

Task T1.3 - Changing Phenology and Physiological Processes

Phenology – Aboveground vegetation phenology patterns continue to follow those of prior treatment years but have been reevaluated and summarized by Schädel et al. (2023). Richardson et al. (2024) took a comprehensive look at multiple years of snow cover phenology under the SPRUCE treatments and showed how future warming, at levels consistent with IPCC projections, will result in transformative changes to the winter season in boreal peatlands, with impacts on how these ecosystems function, and how they impact the climate system.

Woody plant physiology and thermal acclimation of photosynthesis – The SPRUCE physiology team has recently focused on estimating woody plant respiration based on CO₂ efflux from branches and stems. We hypothesized that 1) woody respiration would acclimate to whole ecosystem warming, leading to lower basal respiration rates for a given temperature and buffering temperature impacts on net vegetation carbon uptake; 2) the Q10 temperature coefficient will decrease, leading to lower sensitivity to increasing temperatures; 3) projections of future climate change temperature are below the thermal threshold for respiratory damage; 4) inclusion of woody respiration will significantly impact net GPP in ELM-SPRUCE. To assess maximum ability for acclimation of woody respiration, we selected two SPRUCE plots that have the largest differences in temperatures: open-topped, enclosed Plot 17 that targets +9 °C, and unchambered Plot 21 that is ~1.5 °C below ambient. We collected respiration-temperature response curves for *P. mariana*, *L. laricina*, *C. calyculata*, *R. groenlandicum* and *V. angustifolium* from 15, 25, 35 and 45 °C, with a subset ramped up to higher temperatures (up to 60 °C) until their rates began to decline – indicating respiratory failure. Initial results suggest almost complete acclimation to temperature for temperature for *P. mariana*, *L. laricina*, *C. calyculata*, *R. groenlandicum*, but opposite responses for *V. angustifolium* (Fig. 9). The *Vaccinium* has been growing rapidly in the +9°C enclosures leading to very different morphology (large, long green stems, large leaves, excessive growth), and this result may be related to active growth respiration. Data were collected in late June 2023. To ensure there was no effect of late-spring phenology on results, we will repeat some measurements in August 2023, when all treatments are assumed fully mature. Acclimation results will be applied to ELM-SPRUCE to assess impact of inclusion of acclimation on net primary productivity.



Fig. 9. Woody respiration rates increase exponential with temperature, but under higher growth temperatures (+9°C) respiration rates acclimate by downregulation of respiration at a given temperature. Vaccinium, which has greatly increased vigor in the warm plots, responds differently.

Coupled measurements of SIF-EC: The SIF-EC system originally developed by TES SFA scientists and deployed at the SPRUCE site and later licensed to Campbell Scientific is now being used by multiple national and international projects (e.g., NSF Macrosystems Program, and Amazon FACE). Lianhong Gu was invited to give a talk on SIF measurements during the European Space Agency FLEX Fluorescence Workshop in Frascati, Italy in 2023. He is also invited by the project leaders of LEMONTREE (Land Ecosystem Models based on New Theory, obseRvations and ExperimEnts, <u>https://research.reading.ac.uk/lemontree/</u>) to attend a workshop on "Ecosystem processing of absorbed solar energy and its coupling to carbon fixation: towards a new synthesis" in Reading, UK in 2024. He will present the latest modeling and measurement results on SIF-GPP relationships.

<u>Task T1.4 - Representing Peatland Ecosystem Processes within Mechanistic and Landscape</u> <u>Wetland Models</u>

SPRUCE Multi-model Intercomparison Project (SPRUCEMIP) – Given the importance of accurately simulating peatland stored C response to climate change, it is important to assess how well the models perform, to evaluate our current understanding, and to improve the future

project. The SPRUCE Model Intercomparison Project (SPRUCEMIP) is initiated to quantify, within a unified intercomparison framework, the uncertainties in C cycle warming and elevated CO₂ responses for the peatland ecosystem. Which will provide the critical synthesis, benchmarking, evaluation, and feedback needed to improve the current peatland modeling predictive capabilities and future projections. Our assessment incorporated 11 distinct models, scrutinizing their predictions for net ecosystem carbon exchange (NEE), NPP, CH₄ fluxes, and their underlying components against a comprehensive on-site carbon cycle dataset. We found that a variety of trends and disparities in NEE, NPP, HR and CH₄ sensitivities to temperature across different models and observed values under the two distinct CO₂ conditions become apparent (**Fig. 10**). Yiqi Luo's group created a traceability analysis framework based on the SPRUCEMIP outputs, and the analysis framework provided valuable insights into the contributions of each component to the overall uncertainty, enhancing our understanding and improving the reliability of model prediction. The overall SPRUCEMIP paper are under development and will be finished in August 2024.



Fig. 10. The variance in carbon budget component sensitivity to temperature across different models as compared to observed values under two distinct scenarios: ambient and elevated CO₂ conditions.

Synchronized phenology and plant growth C allocation in ELM-SPRUCE – New processes are added to fine root growth, mortality, and nutrients uptake in ELM-SPRUCE. Fine root phenology is modeled independently from aboveground phenology as functions of soil temperature and water. The allocation ratio between leaf and fine root is modeled as a function of water table height. Nutrients uptake processes separately consider direct uptake by fine roots and indirect uptake via mycorrhizal fungi, as functions of fine root biomass, relative carbonnutrient status of the vegetation, and environmental conditions. The modified processes resulted in improved simulated sensitivity of vegetation growth to warming. A manuscript is in working to report those developments. *Mechanistic CH4 modeling* – The SDSU team has been working on further improvements and applications of the ELM-SPRUCE methane model over the past year. A manuscript detailing the mechanistic modeling of drought impacts on CH₄ cycling in the S1 bog is currently in preparation. Moreover, a modeling study was conducted to verify the feasibility of using genomic data to enhance model parameterization for CH₄ cycling simulations, and same modeling method is now being applied to the S1 bog. The goal is to integrate the in-situ CH₄ cycling and genomic information to fully understand how methanogens and methanotrophs respond to warming and elevated carbon dioxide, as well as their effects on CH₄ cycling under various climate scenarios.

Further development of the Sphagnum PFT submodel – To address the impacts of warming and drying on Sphagnum in the ELM-SPRUCE model, we are enhancing the model's water dynamics. This improvement involves integrating observed relationships between soil water content and water table depth. Additionally, we will utilize observations of moss hydration to refine the regulation of photosynthesis under drying conditions. This will provide a more accurate representation of Sphagnum responses to environmental changes, ultimately improving the predictive capability of the ELM-SPRUCE model.

Theme 1 Deliverable Progress

The SPRUCE project which dominates Theme 1 activities has now completed 8 years of whole-ecosystem warming manipulations. Science measurement and modeling tasks represent the dominant effort and deliverable progress is reflected in the following table.

Task	Year	Deliverable	Leads	Status
T1.2	2024	Paper: Net CO ₂ and CH ₄ flux in 2022–	Stelling, Mayes,	In Preparation
		2023 contrasting new automated vs. older manual methods.	Hanson, Krassovski	
T1.2	2024	Paper: 5-year peat change 2016-2020	Postdoc, Hanson	In Preparation
T1.3	2024	MODEX paper: Testing woody	Warren, Ricciuto	In Preparation
		physiological eCO ₂ and temperature		
		responses		
T1.1	2024	Paper on outflow C and nutrient	Griffiths	In Preparation
		concentration responses		
T1.1	2024	Paper on Sphagnum NEE, microbial food	Weston, Gibert	In Preparation
		webs, and N ₂ -fixation		
T1.4	2024	Mechanistic modeling the drought	Xu et al.	Near completion
		impacts on CH ₄ cycling in S1 bog		
Multiple	2024	International wetland meeting – what did	Mayes et al.	Completed
		we learn from SPRUCE and how do we		
		extrapolate it beyond SPRUCE?		
T1.4	2025	MODEX: Model intercomparison results	Shi, Ricciuto	Planned
		to be published		

Theme 1 Deliverables FY24 and FY25

Theme 1 Publications

- Denham SO, Barnes ML, Chang Q, Korolev M, Wood JD, Oishi AC, Shay KO, Stoy PC, Chen J, Novick KA (2023) The rate of canopy development modulates the link between the timing of spring leaf emergence and summer moisture. *Journal of Geophysical Research: Biogeosciences* 128:e2022JG007217. <u>https://doi.org/10.1029/2022JG007217</u>.
- Dusenge ME, Warren JM, Reich PB, Ward EJ, Murphy BK, Stefanski A, Villanueva R, Cruz M, McLennan DA, King AW, Montgomery RA, Hanson PJ, Way DA (2023) Boreal conifers maintain carbon uptake with warming despite failure to track optimal temperatures. *Nature Communications* 14:4667. <u>https://doi.org/10.1038/s41467-023-40248-3</u>.

- Kilner CL, Carrell AA, Wieczynski DJ, Votzke S, DeWitt K, Yammine A, Shaw J, Pelletier DA, Weston DJ, Gibert JP (2024) Temperature and CO₂ interactively drive shifts in the compositional and functional structure of peatland protist communities. *Global Change Biology*. 30(3):e17203.
- Ma S, Jiang L, Wilson RM, Chanton J, Niu S, Iversen CM, Malhotra A, Jiang J, Huang Y, Lu X, Shi Z, Tao F, Liang J, Ricciuto D, Hanson PJ, Luo Y (2023) Thermal acclimation of plant photosynthesis and autotrophic respiration in a northern peatland. *Environmental Research Climate* 2:025003. doi: 10.1088/2752-5295/acc67e
- Norby RJ, Baxter T, Živković T, Weston DJ (2023) Shading contributes to Sphagnum decline in response to warming. *Ecology and Evolution* 13:e10542.
- Norby RJ, Warren JM, Iversen CM, Childs J, Jawdy S, Walker AP (2022) Forest stand and canopy development unaltered by 12 years of CO₂ enrichment. *Tree Physiology* 42:428-440. https://doi.org/10.1093/treephys/tpab107
- Ofiti NOE, Altermatt M, Petibon F, Warren JM, Malhotra A, Hanson PJ, Wiesenberg GLB (2023) Warming and elevated CO₂ induced shifts in carbon partitioning and lipid composition within an ombrotrophic bog plant community. *Environmental and Experimental Botany* 206:105182. <u>https://doi.org/10.1016/j.envexpbot.2022.105182</u>.
- Ofiti NOE, Huguet A, Hanson PJ, Wiesenberg GLB (2024) Peatland warming influences the abundance and distribution of branched tetraether lipids: Implications for temperature reconstruction. *Science of the Total Environment* (in press), <u>https://doi.org/10.1016/j.scitotenv.2024.171666</u>
- Ofiti NOE, Schmidt MWI, Abiven S, Hanson PJ, Iversen CM, Wilson RM, Kostka JE, Wiesenberg GLB, Malhotra A (2023) Climate warming and elevated CO₂ alter peatland soil carbon sources and stability. *Nature Communications* 14:7533. https://doi.org/10.1038/s41467-023-43410-z
- Petro C, Carrell AA, Wilson RM, Duchesneau K, Noble-Kuchera S, Song T, Iversen CM, Childs, Schwaner G, Chanton JP, Norby RJ, Weston DJ, Kostka J (2023) Climate drivers alter nitrogen availability in surface peat and decouple N₂ fixation from CH₄ oxidation in the Sphagnum moss microbiome. *Global Change Biology* 29(11):3159-76.
- Richardson AD, Novick KA, David Basler D, Phillips JR, Krassovski MB, Warren JM, Sebestyen SD, Hanson PJ (2024) Experimental whole-ecosystem warming enables novel estimation of snow cover and depth sensitivities to temperature, and quantification of the snow-albedo feedback effect. *Journal of Geophysical Research Biogeosciences* 129:e2023JG007833. https://doi.org/10.1029/2023JG007833
- Schädel C, Seyednasrollah B, Hanson PJ, Hufkens K, Pearson KJ, Warren JM, Richardson AD (2023) Using long-term data from a whole ecosystem warming experiment to identify best spring and autumn phenology models. *Plant Environment Interactions* 4:188-200. doi:10.1002/pei3.10118
- Wieczynski DJ, Yoshimura KM, Denison ER, Geisen S, DeBruyn JM, Shaw AJ, Weston DJ, Pelletier DA, Wilhelm SW, Gibert JP (2023) Viral infections likely mediate microbial controls on ecosystem responses to global warming. *FEMS Microbiology Ecology* 99(3):fiad016.

Theme 2: Water, C, and Energy Processes under Compounding Climatic Stressors

MOFLUX site updates

In May 2024, a celebration and workshop marking the 20th anniversary of MOFLUX was held at the University of Missouri. The workshop served the dual functions of introducing the history of MOFLUX to SFA personnel, Lincoln University Missouri RENEW team, and other collaborators who will be doing work at the site in the coming years, and a planning meeting for future research activities. Two graduate students defended their MS theses that contributed to MOFLUX-related science. Ms. Grace Cochran found that (i) the forest is most responsive to rainfall when under moderate drought stress (manuscript in revision to resubmit to Agricultural and Forest Meteorology), and (ii) surface energy imbalance can be modeled using machine learning, with results implying biological processes may be important to solving the longstanding problem. Mr. Hunter Seubert quantified the temporal patterns of spatial variation of soil respiration and found that water status was an important determining factor, but that there were also factors beyond subsurface climate regulating spatial variation.

Science updates

Energy balance research – The Earth's surface layer (ESL) is where an overwhelming majority of life on this planet dwells. Human activities and climate dynamics have been fundamentally changing the energy processes of this layer with long-term consequences on the environment and livability in this vital layer. Yet, even our most advanced measurement technology – the eddy covariance (EC) approach – cannot close the energy budget of the ESL. This problem has been plaguing EC and earth system scientists for decades. It casts doubt on the data that have been used to validate Earth System Models and calls into question our understanding of ESL energy processes. Our latest research shows that energy transfer in the ESL is far more complex than has been treated so far by the EC and Earth system research communities. This complexity arises from the unappreciated coupling of mass, heat and mechanical (kinetic and potential) energy in the ESL which is an open thermodynamic system. The lack of understanding of this coupling has led to simplistic equating of heat energy with enthalpy exchange, formation of ill-conceived concepts and theories, and misguided measurements of turbulent heat flux. Starting from the first principles of physical fluid mechanics and thermodynamics, we systematically derived fundamental equations of heat transfer in the ESL with both mass and total energy conserved simultaneously. Over a homogenous Earth surface, the following set of 1D equation governs the coupled mass and total energy exchanges between the Earth surface and atmosphere:

$$\overline{NEE}_{IE} = \int_{0}^{z_{m}} \frac{\overline{\partial(\rho_{d}c_{vd} + \rho_{v}c_{vv})T}}{\partial t} dz + \left\{ \left(c_{pd}\bar{\rho}_{d} + c_{pv}\bar{\rho}_{v} \right) \overline{w'T'} + \left(c_{pd}\bar{D} + c_{pv}\bar{E} \right) \overline{T} + c_{pd}\overline{w'\rho_{d}'T'} + c_{pv}\overline{\rho_{d}'T'} + c_{pv}\overline{\rho_{v}'T'} \right\}_{z=z_{m}}$$

$$(1)$$

$$\overline{NEE}_{KE} = \frac{1}{2} \left\{ \int_{0}^{z_{m}} \frac{\overline{\partial [\rho(\boldsymbol{u}\cdot\boldsymbol{u})]}}{\partial t} dz + \left[\overline{\boldsymbol{u}\cdot\boldsymbol{u}}(\overline{D} + \overline{E}) - \frac{\left(\int_{0}^{z_{m}} \frac{\overline{\partial \rho_{d}}}{\partial t} dz + \overline{w'\rho'_{d}}\right) \overline{\rho'(\boldsymbol{u}\cdot\boldsymbol{u})'}}{\overline{\rho}_{d}} + \overline{\rho} \overline{w'(\boldsymbol{u}\cdot\boldsymbol{u})'} + \frac{1}{\overline{\rho}_{d}} \right\}$$

$$\left. \overline{w'\rho'(\boldsymbol{u}\cdot\boldsymbol{u})'} \right\|_{z=z_{\mathrm{m}}} \right\}.$$
(2)

$$\overline{NEE}_{PE} = g \left[\int_{0}^{z_{m}} z \frac{\overline{\partial \rho}}{\partial t} dz + (\overline{D} + \overline{E}) z |_{z=z_{m}} \right].$$
(3)

$$\overline{D} = -\int_0^{z_{\rm m}} \frac{\partial \rho_{\rm d}}{\partial t} dz.$$
⁽⁴⁾

$$\bar{E} = \overline{w'\rho_{\rm v}'} - \frac{\int_0^{z_{\rm m}} \frac{\partial \rho_{\rm d}}{\partial t} dz + w'\rho_{\rm d}'}{\bar{\rho}_{\rm d}} \bar{\rho}_{\rm v}.$$
(5)

$$\overline{NEE}_{\mathbf{v}} = \int_{0}^{z_{\mathbf{m}}} \frac{\overline{\partial \rho_{\mathbf{v}}}}{\partial t} dz + \left(\overline{w'\rho_{\mathbf{v}}'} - \frac{\int_{0}^{z_{\mathbf{m}}} \frac{\partial \rho_{\mathbf{d}}}{\partial t} dz + \overline{w'\rho_{\mathbf{d}}'}}{\overline{\rho}_{\mathbf{d}}} \overline{\rho}_{\mathbf{v}} \right) \bigg|_{z=z_{\mathbf{m}}}.$$
(6)

$$\overline{NEE}_{TE} = \overline{NEE}_{IE} + \overline{NEE}_{KE} + \overline{NEE}_{PE}.$$
(7)
$$\overline{NEE}_{SH} = \overline{NEE}_{TE} - c_{vv}\overline{NEE}_{v}\overline{T}_{s}.$$
(8)

Here NEE_{IE} , NEE_{KE} , NEE_{PE} , NEE_{TE} , and NEE_{SH} denote the net ecosystem exchanges (NEE) of internal energy, kinetic energy, potential energy, and total energy. NEE_v denotes the NEE of water vapor. D and E denote the dry air and water vapor fluxes across the EC instrument plane. c_{vd} and c_{pd} are the specific heat capacities of dry air at constant volume and at constant pressure, respectively, and c_{vv} and c_{pv} are the corresponding parts for water vapor. ρ is the total air density which is the sum of the density of dry air (ρ_d) and water vapor (ρ_v). u is the vector of wind

velocity and w is its vertical component. g is the gravitational acceleration constant, t is time, z is height, and z_m is the height of the EC measurement plane. T is air temperature and T_S is surface temperature. The horizontal bar represents mean whereas the prime represents fluctuations around the mean.

For comparison, the EC community has conventionally measured the sensible heat flux as follows:

$$\overline{NEE}_{SHconv} = \int_0^{z_m} \frac{\partial (\rho_d c_{pd} + \rho_v c_{pv})T}{\partial t} dz + (c_{pd} \bar{\rho}_d + c_{pv} \bar{\rho}_v) \overline{w'T'} \Big|_{z=z_m}.$$
(9)

Eq 9 shows that the conventional measurement of sensible heat flux is an attempt to measure the exchange of enthalpy. This conventional way violates the first law of thermodynamics for open systems for which heat energy does not equal to enthalpy exchange. We are currently still evaluating the difference between the new, which we believe is correct, and the convectional, which we believe is incorrect, sensible heat flux measurements. But we are confident that the new equations will substantially improve surface energy balance closure. Our advances improve the understanding of energy processes in the ESL and establish a solid theoretical ground for the revised EC approach for measuring heat transfer in the ESL and for testing ESMs. It also suggests that the modeling of sensible heat exchange in ESMs may have to be revised.

Ecosystem hydraulics – As part of theme 2 activities concerning plant-to-ecosystem responses to compounding stressors, we contributed to a recently accepted concept paper on "ecosystem hydraulics" and how water relations concepts from organ- and organism-scales can reveal the hydraulic constraints on the interaction of vegetation and climate and provide new mechanistic understanding and prediction of forest water use and productivity.

SPRUCE water relations – In order to understand differential tree hydraulic sensitivity to compounding heat and drought stressors, we are installing 16 research grade ultra-narrow FOV infrared radiometers (Apogee model SI-131-SS) to selected sap flow trees (two tamarack, two spruce) in the warmest four plots. The radiometers are focused on representative foliage to provide real-time high resolution foliar temperatures (± 0.2 °C). Data will be paired with sap flux density and periodic leaf water potential measurements to assess damaging or fatal thermal thresholds and linkages to transpiration and leaf water availability during heat events. Samantha Colunga, a recent MS student from UTRGV, received a competitive 2024 NNSA internship to focus on this analysis. We will also explore linkages to drone-based and Phenocam imagery.

New work on understanding soil water capillarity and availability to plants under drying conditions is focused on validation and analysis. In April, a subset of soil water sensors were removed from selected plots for assessment of actual saturated water content, field capacity and residual mass after drying. Data are being used improve the accuracy of the regression between sensor output (in mV) and actual water content to refine the soil water retention curve. The soil water dataset is further being examined and categorize periods of sensor failure due to low temperature, and hysteresis in soil wetting and drying regressions. Initial results indicate the subsequent analysis will greatly improve understanding of soil water availability in relation to water table depth in the hummock and hollows (e.g., **Fig. 11**).

Modeling the light reaction of photosynthesis – Genetically improving photosynthesis is a key strategy to boosting crop production to meet the rising demand for food and fuel by a rapidly growing global population in a warming climate. Many components of the photosynthetic apparatus have been targeted for genetic modification for improving photosynthesis. Successful translation of these modifications into increased plant productivity in fluctuating environments will depend on whether the electron transport chain (ETC) can support the increased electron transport rate without risking overreduction and photodamage. At present atmospheric conditions, the ETC appears suboptimal and will likely need to be modified to support proposed

photosynthetic improvements and to maintain energy balance. We derived photochemical equations to quantify the transport capacity and the corresponding reduction level based on the kinetics of redox reactions along the ETC. Using these theoretical equations and measurements from diverse C3/C4 species across environments, we identified several strategies that can simultaneously increase the transport capacity and decrease the reduction level of the ETC (**Fig. 12**). These strategies include increasing the abundances of reaction centers, cytochrome b6f complexes, and mobile electron carriers, improving their redox kinetics, and decreasing the fraction of secondary quinone-nonreducing photosystem II reaction centers. We also shed light on several previously unexplained experimental findings regarding the physiological impacts of the abundances of cytochrome b6f complex and plastoquinone. The model developed and the insights generated from it facilitate the development of sustainable photosynthetic systems for greater crop yields.



Fig. 11. Soil water content as a function of relative water table depth at SPRUCE.



Fig. 12. The two-dimensional state space formed by the fraction of open photosystem II (PSII) reaction centers (q) and the linear electron transport rate (J_{PSII}). $(q_{J'_{PSIImax}}, J'_{PSIImax})$ marks the intersection between a CO₂ response curve at a sub-saturating light level and the CO₂-saturated light response curve, representing a conditional

maximum *J*_{PSII} of a partially swollen thylakoid.

 $(q_{J_{PSIImax}}, J_{PSIImax})$ marks the intersection between a CO₂ response curve at a saturating light level and the CO₂-saturated light response curve, representing the intrinsic maximum *J*_{PSII} of a fully swollen thylakoid. Carboxylation limited by RuBP regeneration, Rubisco, and triose phosphate utilization (TPU) each occupies one of the three corners of the q - J_{PSII} state space as marked. From right to left, the electron transport chain (ETC) is increasingly reduced as indicated by the color gradient. In general, bioengineered photochemical optimization can improve the efficiency of the ETC by shifting the q - J_{PSII} state space towards the upper right corner as indicated by the arrow at the top of the diagram.

Leafweb – Leafweb has provided C4 photosynthesis data to the international C4 metaanalysis workgroup led by Prof. Dani Way of the Australian National University. Leafweb background processing code is being updated for the calculation of energy storage in thylakoids during photosynthesis to support energy balance research.

Collaborative activities

Theme 2 and 5 are collaborating on mentoring Patrick Neri, a PhD student from University of Arizona on modeling plant – microbial interactions on plant responses to stress. Theme 2 is providing site access facilitation to the lab of Prof. Alexandra Konings of Stanford University in conducting remote sensing of ecophysiology and to the Lincoln University of Missouri RENEW project to conduct hydrology measurements at the MOFLUX site.

Task	Year	Deliverable	Lead	Status
T2.2	2024	Publish papers on Testing the Missing Energy Hypothesis	Gu	On track
T2.3	2024	MOFLUX: Publish paper on long-term radial tree growth climate	Wood	In planning
		responses, and correlations with NPP when data overlap		
T2.4	2024	SPRUCE: Sap flow synthesis paper	Warren	Analysis
				underway
T2.4	2024	Neutron-based rhizosphere hydration dynamics – Paper	Warren	Completed
T2.4	2024	MOFLUX: Publish ecosystem scale hydraulics paper	Wood	On track
T2.2	2025	Model paper: Coupled photophysical, photochemical, and	Gu	On track
		biochemical processes		
T2.3	2025	MA: Publication of root, mycorrhizal and soil respiration dynamics	Warren/	In planning
		across diverse species	McCormack	
T2.3/	2025	MOFLUX: Develop ML model frameworks for analyzing flux and	Wood, Gu,	In planning
2.4		biometric time series for testing C and water sub-hypotheses	Mayes	
T2.4	2025	MOFLUX: Publish paper on soil respiration spatial variation	Wood	In planning
T2.4	2025	MOFLUX: Publish paper on influence of rainfall dynamics on daily	Wood	In planning
		cycle climate		

Theme 2 Deliverable status FY24 and FY25

Theme 2 Publications

- Brügger A, Bilheux HZ, Nelson G, Kiss A, Morris J, Connolly M, Long A, Tremsin A, Strzelec A, Anderson M, Agasie B, Finney C, Wissink M, Hubber M, Pellenq R, White C, Heuser B, Craft A, Harp A, Tan C, Morris K, Junghans A, Sevanto S, Warren JM, Florez FE, Biris A, Cekanova M, Kardjilov N, Schillinger B, Lin J, Frost M, Vogel S (2023) CUPI2D: Complex, Unique and Powerful Imaging Instrument for Dynamics. *Review of Scientific Instruments* 94(051301): 1-12. https://doi.org/10.1063/5.0131778
- Chen R, Liu L, Liu X, Liu Z, Gu L, Rascher U (2024) Improving estimates of sub-daily gross primary production from solar-induced chlorophyll fluorescence by accounting for light distribution within canopy. *Remote Sensing of Environment* 300:113919. <u>https://doi.org/10.1016/j.rse.2023.113919</u>.
- Chen R, Liu Z, Liu Z, Liu X, Kim J, Kim HS, Lee H, Wu G, Guo C, Gu L (2024) SIF-based GPP modeling for evergreen forests considering the seasonal variation in maximum photochemical efficiency. *Agricultural* and Forest Meteorology 344:109814. <u>https://doi.org/10.1016/j.agrformet.2023.109814</u>.
- Denham SO, Barnes ML, Chang Q, Korolev M, Wood JD, Oishi AC, Shay KO, Stoy PC, Chen J, Novick KA (2023) The rate of canopy development modulates the link between the timing of spring leaf emergence and summer moisture. *Journal of Geophysical Research: Biogeosciences*, 128:e2022JG007217. <u>https://doi.org/10.1029/2022JG007217.</u>
- Gu L (2023) Optimizing the electron transport chain to sustainably improve photosynthesis. *Plant Physiology* 193:2398-2412. <u>https://doi.org/10.1093/plphys/kiad490</u>.
- Gu L, Grodzinski B, Han J, Marie T, Zhang Y-J, Song YC, Sun Y (2023) An exploratory steady-state redox model of photosynthetic linear electron transport for use in complete modeling of photosynthesis for broad applications. *Plant, Cell and Environment* 46:1540-1561.
- Holtzman N, Wang Y, Wood JD, Frankenberg C, Konings AG (2023) Constraining plant hydraulics with microwave radiometry in a land surface model: Impacts of temporal resolution. *Water Resources Research*, 59(11):e2023WR035481, <u>https://doi.org/10.1029/2023WR035481</u>.

- Johs A, Quin S, Coats L, Davison B, Elkins J, Gu X, Morrell-Falvey J; O'Neill H, Warren J, Pierce E, Herwig K (2024) New Opportunities for Neutrons in Environmental and Biological Sciences. Frontiers in Environmental Science and Engineering 18:92. https://doi.org/10.1007/s11783-024-1852-z
- Liu X, Qiao Y, Zhou W, Dong W, Gu L (2023) Determinants of photochemical characteristics of the photosynthetic electron transport chain of maize. *Frontiers in Plant Science* 14:1279963. doi: 10.3389/fpls.2023.1279963
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Theme 3: Nutrient-C Feedbacks

As the Theme 3 research team collects field data, runs model simulations, and designs large and small scale experiments we are driven by one overarching research question: *How does environmental change alter nutrient distribution & dynamics, and what are the implications for understanding and predicting ecosystem C fluxes?* We broadly aim to improve coupling of the C and nutrient cycling in earth system models by probing the nutrient dynamics of organic matter decomposition at the SPRUCE experiment, quantifying nutrient acquisition and belowground dynamics at SPRUCE and the Morton Arboretum, and by working on an ecosystem nutrient budget and model at the MOFLUX site. In FY24, significant progress has been made by both empirical and modeling teams assigned to these tasks.

T3.1: Nutrient Dynamics of Organic Matter Decomposition

Empirical data collection within T3.1 has focused this year on completing sample analysis from a year-long soil incubation that characterized C, N and P cycling in rhizosphere soils following five years of field manipulations (deliverable due end of FY2024). A manuscript is in preparation and analysis thus far indicates significant decoupling of C, N and P cycling has taken place. Cumulative CO_2 mineralized in the lab decreased with field temperature and with depth (**Fig. 13**), indicating that with warming, a greater amount of quickly cycling, labile C was lost in the field. Cumulative NH₄ leached in the lab increased with field temperature (**Fig. 13**). This indicates that warmer plots have a large amount of quickly cycling N is readily available for release. Cumulative PO₄ losses tended to increase with eCO₂ but showed no impact of warming in the field (**Fig. 13C**). Collectively, these results show the manipulative treatments at SPRUCE have had differential impacts on N versus P, with the warmest plots potentially becoming more P limited under ambient CO₂ conditions. In addition to this soil incubation dataset, decomposition dynamics at SPRUCE have also been captured with a set of Sphagnum litterbags deployed at SPRUCE and collected during years one through six of the experiment. The modeling team has been working on nutrients and decomposition using this data long with litterbag data from other peatland sites in Europe

and Canada. The synthesized dataset will be analyzed and then used for model parameterization and evaluation.



Fig. 13. CO₂ release (A), leached N-NH₄ (B) and mineralized P from field collected but laboratory incubated SPRUCE peat samples.

T3.2 Nutrient Acquisition by Plants

At the SPRUCE site the first few years of experimental manipulations showed an exponential increase in nutrient availability (Iversen et al 2022) and increase in fine root productivity of shrubs using ingrowth core techniques (Malhotra 2020). As the experimental treatments at SPRUCE have continued, our team, in conjunction with Theme 1, have used minirhizotron images to quantify the location and rate of fine root production. In FY24, image analysis spanning 2015 through 2021 was completed. This data indicates fine root production of shrubs and non-woody plants (sedges & forbs) responds positively to warming but tree fine root production does not (**Fig. 14**, Weber et al. In prep). Elevated CO₂ alters the response of root production to warming in hummocks (increased for shrubs, decreased for non-woody plants) while having limited impact on root production in hollows. The depth of root production has been relatively insensitive to temperature and eCO₂: new roots of non-woody plants routinely have the deepest roots across all plots (30.8 cm, 95% CI: [22.6, 41.2]) while new tree and shrub roots are found mostly in the top 15 cm of the peat profile.



Fig. 14. Root production by monthly soil temperatures and SPRUCE ambient versus elevated CO₂ treatments.

In addition to measuring rates of fine root production, in FY24 the SPRUCE team quantified mycorrhizal root colonization of fine roots and characterized the microbial community on and roots using amplicon sequencing (Duchesneau et al 2024). Results showed that mycorrhizal colonization was greater for trees under eCO_2 but not warming. The relative abundance of the microbial community around tree and shrub roots was impacted by both warming and eCO_2 .

Empirical data on fine roots and their associated microbes will be instrumental in refining the representation of the fine root pool within ELM. Efforts are underway to bring in the three-pool fine root model TAM (transport and absorptive fine roots with mycorrhizal fungi) into ELM. New data structure needed has been added into the model and tested. Synthesis of relevant dataset is ongoing.

T3.3 Ecosystem N Responses to Hydroclimate Variability at MOFLUX

Work is underway to characterize the ecosystem N budget at the MOFLUX site. We have initiated quarterly samplings of soil N transformations to monitor intra- and interannual variation in soil N processes, with plans to supplement these regular measurements with additional measurements during and after drought events. We have additionally begun sampling aboveground litter and living tissue to determine soil-to-litter fluxes of N and P, as well as nutrient resorption. Finally, in coordination with Theme 4, a long-term root decomposition experiment was initiated in Fall 2022, and we initiated the first of several leaf litter decomposition experiments in Fall 2023. Samples are currently being processed as they are harvested to examine N and P release curves during decay.

We will use these data streams to test our emerging hypothesis that drought events intensify N losses from this ecosystem, by leading to an accumulation of mobile forms of N post-drought. Supporting this, data from a nearby NADP collection site reveals that nitrate concentrations in rainwater are greater in years with lower precipitation (P = 0.003; **Fig. 15A**) after accounting for a general decrease in nitrate concentrations over time (P < 0.001). Moreover, based on long-term tree inventory data collected by Jeff Wood and colleagues, we find evidence that drought-induced mortality, which has selectively affected oak species (Gu et al. 2015; Wood et al. 2018), has led to a reduction in ectomycorrhizal (ECM) dominance relative to arbuscular mycorrhizal (AM) dominance (**Fig. 15B**), which could drive greater N leaching losses over the long term (Phillips et al. 2013).



We continue work on the development of a generalizable soil decomposition model in the multiassumption architecture and testbed (MAAT). At present, Matt Craig and Anthony Walker are working on a scheme to generalize the representation of soil nutrient dynamics using the ELM soil model and CORPSE-N (Sulman et al. 2017) as a test case.

Task	Year	Deliverable	Lead	Status
T3.1	2024	Paper: SPRUCE surface peat incubation NP dynamics analysis	Salmon et al.	In Preparation
T3.1	2025	SPRUCE paper: ¹⁵ N isotope dilution for gross N mineralization and nitrification	Salmon et al.	Planned

Theme 3 Deliverables for FY2024 and FY2025

T3.2	2025	FRED Release of v4.0	Iversen, McCormack	Planned
Т3.3	2025	MOFLUX paper: MAAT Develop nutrient-explicit soil decomposition model	Craig, Walker	Planned

Theme 3 Publications/Manuscripts

Duchesneau K, Defrenne CE, Petro C, Malhotra A, Moore JA, Childs J, Hanson PJ, Iversen CM, Kostka JE (2024) Responses of vascular plant fine roots and associated microbial communities to whole-ecosystem warming and elevated CO₂ in northern peatlands. *New Phytologist* 242:1333-1347. <u>https://doi.org/10.1111/nph.19690</u>

Theme 4: Soil C Cycling and Microbial Processes

The governing question for this theme is: *How do temperature, water availability, and plant inputs affect soil C and microbial functions, and what are the implications for ecosystem C storage and greenhouse gas fluxes?*

Major activities, since February 2022, at SPRUCE involved continued preparation of metagenome samples from the 2022 sampling. A new addition for this cycle involves collection of three additional acrotelm-only core samples in May, July, and October. These samples are designed to better understand seasonal signatures and to better identify any changes in the microbial community, since the deep metagenomes are quite stable. The additional core samples will be analyzed using qPCR primers designed for better identification and dynamics of methanogens, acetogens, and methanotrophs. Analyses await the onboarding of a postdoc who will lead the analyses. Biannual measurements of cotton strip decomposition continue to quantify labile C decomposition. Year 5 collections of the peat decomposition ladders was performed in 2023, and a manuscript describing the results from the first 3 years of peat decomposition was published showing constant decomposition rates but exhibiting changes in the microbial community (Roth et al. 2023).

Efforts continued to support improved soil carbon modeling by including microbial processes. A modeling publication represented historical dynamics of GPP, NPP, heterotrophic and autotrophic respiration, microbial biomass C and N, and dissolved C and N, and soil organic C in the top 30 cm and 1 m of soils from 1901-2016 using the CLM-Microbe model (He et al. 2024). Lead author Liyuan He was a PhD student at the MSI San Diego State University, and her work was partially funded under the SFA. A long-term (2y) incubation dataset (Kluber et al. 2020D) from forest and grassland land covers at MOFLUX and three other upland soil sites were modeled using MEND (Wang et al. 2015). Modeling was used to understand the extent to which a common set of microbial parameters could be applied across the different sites, soil types, and land covers (Jian et al. 2024). Lead author Siyang Jian was a PhD student at the HBCU Tennessee State University, and his work was partially funded through the SFA. We found that key microbial parameters could be generalized at the soil series level (4 distinct soil series) but not land cover type (forest vs. grassland). The common set of parameters included processes controlling microbial growth and maintenance as well as extracellular enzyme production and turnover. This study demonstrates that future microbial model developments prioritize soil series type over plant functional types when implemented across various sites.

An additional effort focused on providing better accessibility of microbial data for use in microbial soil carbon cycling models. An existing dataset and preliminary manuscript were developed under the SFA a few years ago by comparing data from methods for quantifying microbial biomass including chloroform fumigation, PLFA, total DNA yield, and gene copy numbers by qPCR. An MS student was recruited from Jess Gutknecht's lab at the University of

Minnesota recently to complete the manuscript (now in review with coauthors) and a dataset (Buell et al. 2024D-a). Additionally, comparison of PLFA and chloroform fumigation methods from three SPRUCE peat coring events in 2021 and 2022 were included in the manuscript in preparation and are available in the following dataset (Buell et al. 2024D-b; Buell et al. 2024D-c), with additional DNA yield and qPCR datasets still under preparation.

In an external collaboration, we conducted a root decomposition microcosm experiment to see how decay rates and root-derived MAOM formation varies across species, mycorrhizal type, and root order (Biedler et al. 2023). A main finding was that the early stage of root decay was very important for both the formation of new soil C and the decomposition of pre-existing soil C. In the current phase of the TES SFA, we seek to better characterize the controls on the early phase of litter decomposition. In fall 2023, litterbags of 4 major species were deployed across the 5 MOFLUX transects, with collections occurring quarterly for one year and yearly thereafter. This experiment will be re-deployed yearly for 5 years to study interannual variation in early-and late-stage decay. Additionally, the fall 2022 root decomposition experiments (deployed at 15- and 40-cm depth) will be collected in fall 2024 and the need for additional experiments reevaluated at that time. New root ingrowth cores were deployed in spring 2024, with expected collections at 6 months and at 1 year. Root materials will be analyzed for chemistry and lignin content associated with Theme 3.

The results from quarterly soil sampling in the immediate tower footprint at MOFLUX that that began in 2017 and continued through 2023, with sampling for total, dissolved, and microbial C and N; pH; gravimetric water content; and texture, are being compiled into a dataset for publication. The quarterly sampling going forward will involve expanding the sampling locations to include all 5 transects, addition of C and N analyses of particulate organic matter and mineral associated organic matter; qPCR analyses for bacteria, fungi, and archaea; and in collaboration with Theme 3 analyses for available P, extractable NO₃ and NH₄, and net N mineralization and nitrification. The deliverable manuscript on trends and controls of autotrophic and heterotrophic respiration at MOFLUX is on hold due to the departure of the staff member leading the effort; it will be continued later in FY24 and into FY25.

As described in the Theme 3 updates, work on a generalizable soil decomposition model in the MAAT framework continues. We are leveraging previous work on the MAAT C-only models (Craig et al. 2021) to inform incubation forthcoming incubation experiments. Additionally, we are contributing to a review on soil C saturation (Georgiou et al. *in prep*) based on previous model comparison performed within MAAT. Further microbial modeling will integrate the measured microbial variables with modeling to address major questions: 1) how will soil microbial responses to elevated atmospheric CO₂ and warming will change the ecosystem structure and function? 2) how do soil microbes adapt to elevated CO₂ and warming?

Task	Year	Deliverables	Lead	Status
T4.1	2024	MODEX paper: trends and controls of autotrophic and heterotrophic respiration at MOFLUX 2017–2022		Dataset of quarterly soil sampling 2017-2022 is near completion. MODEX manuscript will require into FY25.
T4.1	2025	SPRUCE: Separating the effects of warming and drying of peat in lab incubations		In progress

Theme 4 Deliverables for FY2024 and FY2025

Theme 4 Publications/Manuscripts and Data Publications (D)

- Beidler KV, Benson MC, Craig ME, Oh Y, Phillips RP (2023) Effects of root litter traits on soil organic matter dynamics depend on decay stage and root branching order. *Soil Biology and Biochemistry* 180:109008. <u>https://doi.org/10.1016/j.soilbio.2023.109008</u>
- Buell ZW, Dabbs J, Steinweg JM, Phillips JR, Kluber LA, Yang ZK, Miller RM, Gutknecht JLM, Schadt CW, Mayes MA (2024Da). Interrelationships among methods of estimating microbial biomass across multiple soil orders and biomes: Supporting data. Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. <u>https://doi.org/10.25581/ornlsfa.031/2274949</u>.
- Buell Z, Phillips J, Ottinger S, Lowe K, Schadt CW, Mayes MA (2024Db) Chloroform fumigation extraction for microbial biomass and dissolved organic carbon from SPRUCE, 2021-2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.109/1998876.
- Buell Z, Felice M, Philips J, Ottinger S, Lowe K, Gutknecht JLM (2024Dc) SPRUCE Phospholipid Fatty Acid (PLFA) abundances, August 2021-June 2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.112/1998897
- He L, Rodrigues JLM, Mayes MA, Lai C-T, Lipson DA, Xu X (2024) Modeling microbial carbon fluxes and stocks in global soils from 1901 to 2016, Biogeosciences, 21:2313-2333, doi:10.5194/bg-21-2313-2024.
- Jian S, Li J, Wang G, Zhou J, Schadt CW, Mayes MA (2024) Generalizing microbial parameters in soil biogeochemical models: Insights from a multi-site incubation experiment. *Journal of Geophysical Research: Biogeosciences* 129:e2023JG007825. https://doi.org/10.1029/2023JG007825
- Kaur N, Ricciuto DM, Mayes MA, Tian H (2023) Response patterns of simulated corn yield and soil nitrous oxide emission to precipitation change. *Ecological Processes* doi:10.1186/s13717-023-00429-w.
- Roth S, Griffiths NA, Kolka RK, Oleheiser KC, Carrell AA, Klingman DM, Seibert A, Chanton JP, Hanson PJ, Schadt CW (2023) Elevated temperature alters microbial communities, but not decomposition rates, during 3 years of *in situ* peat decomposition. *mSystems* 8:e00337-23.
- Schoelmerich M, Ly L, West-Roberts J, Shi L-D, Shen C, Malvankar N, Taib N, Gribaldo S, Woodcroft B, Schadt C, Al-Shayeb B, Dai X, Mozsary C, Hickey S, He C, Beaulaurier J, Juul S, Sachdeva R, Banfield J (2024) Borg extrachromosomal elements of methane-oxidizing archaea have conserved and expressed genetic repertoires. *Nature Communications (In Press)*
- Singh S, Mayes MA, Kivlin SN, Jagadamma S (2023) How the Birch effect differs in mechanisms and magnitudes due to soil texture. *Soil Biology and Biochemistry* 179:108973 doi: 10.1016/j.soilbio.2023.108973.

Theme 5: Regional Integration and Extrapolation

Motivating question for Theme 5: Are the humid, high-C ecosystems of North America more vulnerable to changing climate and disturbance regimes than predicted by CMIP6? How does the collective knowledge gained from the TES-SFA affect our understanding of the C feedbacks in the region?

State-of-the-art projections of terrestrial C feedbacks to atmospheric CO₂ increase show an increase in C across the boreal and temperate region. This C increase is driven by a positive CO₂-fertilization feedback (~0.01 kgC m⁻² ppm⁻¹) with strong model agreement and a close to neutral C-climate feedback but with little model agreement. Low model agreement in the magnitude and sign of the C-climate feedback is indicative of the fact that many models do not represent the processes controlling C-climate feedbacks in as much mechanistic detail as the physiology of CO₂ fertilization. There are significant feedback processes missing in CMIP6 models, such as peat formation and loss, demographic biome shifts, and responses to extremes and disturbance.

To better predict these feedbacks, in Theme 5 we are developing a comprehensive scaling approach to integrate information from SPRUCE and MOFLUX with observations from other sites and high-resolution gridded datasets (**Fig. 16**). To complement this scaling approach, we are also developing the site-specific ELM-SPRUCE into a multi-scale ELM-Peatlands model (**Fig. 16**). The approach includes data synthesis to identify mechanistic controls and the transferability of findings across different peatland types and regions. Key drivers of variation in peatland feedbacks include meteorological conditions, vegetation type, microtopographic variation, and

hydrological characteristics. This scaling strategy also involves community engagement and international collaboration to enable broader application across varied peatland ecosystems.



Fig. 16. Relationships between ecological scale, physical land units, models and empirical observations.

Task 5.1 Multi-site Model Calibration & Evaluation of ELM-Peatland Wetland Types & PFTs

An initial version of ELM-Peatlands was created from ELM-SPRUCE to enable testing at additional peatland sites. Updates are continuing with model development tasks in Theme 1 and regional modeling capabilities under Task 5.2. We selected 20 globally distributed peatland sites for calibrating and evaluating ELM-Peatlands. These sites include raised bogs, open bogs, sphagnum-dominant bogs, poor fens, and rich fens, covering various climatic conditions and geographical locations. For example, sites like Delta Burns Bog and Le Forbonnet experience high annual precipitation, while sites like Western Peatland and Lompolojänkkä have lower precipitation levels. Through iteration with DOE and the project team on our scaling approach, we identified key axes of variation that will be used to select additional sites and generate initial site groupings for optimization.

The Offline Land Model Testbed (OLMT) is the primary tool for optimizing ELM-Peatlands parameters across sites. A recently refactored version of OLMT is currently being used to create surrogate models for individual sites, which will enable calibration of plant functional type (PFT) and peat parameters. We are developing a framework to analyze spatial patterns in optimized parameters from multiple sites and their uncertainties to determine the appropriateness of PFT and peatland categorizations for ELM-Peatlands and the necessity for additional types. Using the site-level surrogate models, we will optimize parameters for multi-site, multi-PFT calibration, allowing for efficient testing of different PFT and site combinations without new ELM ensemble runs. This multi-site approach is currently under development in OLMT.

Task 5.2 Evaluating Regional Carbon-Climate Feedbacks in ELM-Peatlands

In the early part of this Phase of the SFA, Task 5.2 is working to develop datasets and software infrastructure to run ELM and ELM-Peatlands at high-resolution over our focal domains. In the proposal we defined our domain as North American humid, non-permafrost, and non-coastal ecosystems. Since the proposal, we have refined our domains to two east-west

transects selected from our original modeling domain for manageability and inclusion of additional peatland sites. A northern transect that includes SPRUCE and most North American non-permafrost peatlands and a southern transect that includes MOFLUX and eastern deciduous forest. Both transects include the east-west aridity gradient and ecotone that may shift under climate change.

For both transects we have 4-km DAYMET meteorological forcing data from 1980 to 2014 downscaled to 3-hourly temporal resolution using the GSWP3 dataset. The total size of these data is 695 GB. The GSWP3 dataset ends in 2014 and appears unlikely to be updated, so under the SFA we will develop DAYMET temporal downscaling to use the ECMWF ERA5 meteorological dataset. Shifting to ERA5 will allow us to extend our high-resolution historical forcing to 2023 and will enable extension beyond 2023 as DAYMET and ERA5 are updated on a sub-annual basis. Once we have DAYMET ERA5 in place we will spatially downscale CMIP model output over our transects.

We are also developing the other input datasets required by ELM, surface and domain files. To facilitate this, development of the kiloCraft set of python scripts are supported by the SFA. KiloCraft allows for subsetting, regridding, reprojecting of ELM input data and checking these operations. These scripts are in active development and have been used to generate an initial set of high-resolution model input data for model testing.

Task 5.3 Quantification of Boreal and Temperate Ecosystem Vulnerabilities and Their Model Uncertainties

Existing vulnerability assessment frameworks often focus on single disaster events, failing to consider the complex interactions between multiple components of ecosystem vulnerability. To address these limitations, we have developed the Compound Indicators for Vulnerable Ecosystems (CIVE) framework. The CIVE framework follows the IPCC guidelines, integrating exposure, sensitivity, and adaptive capacity to calculate overall ecosystem vulnerability. We categorize natural disasters into four main types: heat-related, water-related, air-related, and cumulative climate-related disasters. For each category, we select relevant ecosystem variables to measure the impact, including vegetation and soil, water, carbon, and energy. The Exposure Index (EI) is calculated by determining the frequency and duration of disaster occurrences, categorized into short-term (days to weeks), medium-term (months to seasons), and long-term (years) durations. A dynamic weighting method is applied to derive the EI. Event windows are used to calculate the following metrics for selected variables:

- Response Time (RPT): The time it takes for a variable to start responding after an event occurs.
- Degree of Impact (DOI): The peak value of deviation caused by the hazards.
- Recovery Time (RCT): The period needed for a variable to return to its pre-hazard condition.
- Relative Resilience (RR): The system's recovery capability after a disturbance.

The Sensitivity Index (SI) is primarily derived from the RPT and the DOI, while the Capacity Index (CI) is calculated based on the RCT and RR. By combining these indices, we derive a single vulnerability value using GPP as an example in relation to each type of natural disaster, providing a comprehensive measure of ecosystem vulnerability. This approach enables a nuanced understanding of how different ecosystem variables and disaster types interact, informing more targeted and effective conservation and mitigation strategies. The relevant framework will be applied to regional ELM simulations produced by Task 5.2 to quantify model uncertainties and derive constrained model projections for boreal and temperate ecosystem vulnerabilities.

Task	Year	Deliverable	Lead	Status
T5.1	2025	Complete muti-site optimization framework in OLMT	Ricciuto	In Progress
T5.1	2025	Optimize PFT-level parameters for use in ELMv3-Peatlands	Ricciuto	Planned
T5.2	2025	Implement 4-column peatland regional modeling framework	Ricciuto	In Progress
T5.2	2025	Finish preparing high-resolution downscaled climate driver data	Walker	In Progress
T5.3	2025	MODEX paper: Global investigation of how soil C traits mediate ecosystem responses to multivariate environmental change	Craig	Planned
T5.3	2025	Development of Compound Indicators for Vulnerable Ecosystems (CIVE)	Mao	In Progress

Theme 5 Deliverables for FY2024 and FY2025

Theme 5 Publications

- Chen J, Liu Z, Mao J, Zhao T, Tu T, Cheng L, Dong C (2023) Co-regulation of water and energy in the spatial heterogeneity of drought resistance and resilience. *Environmental Research Letters*, 18:114007, 10.1088/1748-9326/acfccc.
- Foster K, Sun W, Shiga Y, Mao J, Michalak A (2024) Multiscale assessment of North American terrestrial carbon balance. *Biogeosciences*, 21:869-891. <u>https://doi.org/10.5194/bg-21-869-2024</u>.
- Hao Y, Mao J, Jin M, Wang Y, Tang R, Lee ZW (2024) Evaluating the effects of heatwave events on hydrological processes in the contiguous United States (2003–2022). *Journal of Hydrology*, 131368.
- Meng, F., A.J., Felton, J. Mao, N. Cong, W.K. Smith, C. Körner, ... & A. Chen (2024). "Consistent time allocation fraction to vegetation green-up versus senescence across northern ecosystems despite recent climate change." *Science Advances*, 10(23):eadn2487.
- Shi X, Wang Y, Mao J, Thornton PE, Ricciuto DM, Hoffman FM, Hao Y (2024) Quantifying the long-term changes of terrestrial water storage and their driving factors. *Journal of Hydrology* 635:131096.
- Tang, R., Jin, M., Mao, J., Ricciuto, D.M., Chen, A. and Zhang, Y., 2024. Quantifying wildfire drivers and predictability in boreal peatlands using a two-step error-correcting machine learning framework in TeFire v1. 0. Geoscientific Model Development, 17(4), pp.1525-1542.
- Wang D, Yuan F, Schwartz P, Ricciuto D, Thornton P, Kao K, Thornton M, Walker A, Cao Q, Gu Z (in press) Enable Kilometer-scale E3SM Land Simulation with User-defined Datasets and KiloCraft. Proceedings of 12th International Congress on Environmental Modeling and Software.
- Wang D, Yuan F, Schwartz P, Kao S, Thornton M, Ricciuto D, Thornton P, Reis S (2023) Data Toolkit for Ultrahigh-resolution ELM Data Partition and Generation. *Proceedings of 25th Congress on Modeling and Simulation*, pp 996-1003.
- Wang Y, Mao J, Brelsford CM, Ricciuto DM, Yuan F, Shi X, Rastogi D, Mayes MM, Kao SC, Warren JM, Griffiths NA (2024). Thermal, water, and land cover factors led to contrasting urban and rural vegetation resilience to extreme hot months. *PNAS nexus*, 3(4):147.
- Zhang L, Jiang F, He W, Wu M, Wang J, Ju W, Wang H, Zhang Y, Sitch S, Walker AP, Yue X, Feng S, Jia M, Chen JM (2023). A Robust Estimate of Continental-Scale Terrestrial Carbon Sinks Using GOSAT XCO2 Retrievals. *Geophysical Research Letters*, 50:e2023GL102815. <u>https://doi.org/10.1029/2023GL102815</u>

Promoting Inclusive and Equitable Research (PIER) Plan – Progress

The goal of the ORNL TES SFA PIER plan is to create and maintain an equitable, inclusive, encouraging, and supportive research environment through the following four objectives: (1) maintain a management structure and develop a Code of Conduct to support project equity, (2) include a diverse group of individuals in the project and provide career advancement opportunities, (3) develop strong mentoring skills and cultural awareness among the entire team, and (4) seek the involvement of people historically underrepresented in the research community in the project.

<u>Management structure and Code of Conduct</u> – The TES SFA is planning the exact management structure. We have implemented a new "All Hands" monthly meeting – and thus far have used this meeting venue to gather thoughts about MOFLUX and SPRUCE FY24 experimental plans, to discuss responses to BER's proposal review questions and the scaling whitepaper, and to discuss meeting frequency and content. We have a google drive that everyone can access, and notes from All Hands meetings and other meetings are gathered therein. We decided to expand the All-Hands meetings to 1.5 hours/month to accommodate regular science talks, and mentoring training (see below). The Theme Leads, PIs, and Data Management Leads have had a single "Executive Committee" meeting, but we are calling it a Theme Leads meeting, and the primary focus is to make budget decisions. The Early Career Community is not yet organized, but there are plans to work on it in FY24. The SFA has developed a draft Code of Conduct – led by an SFA postdoc – that is currently under review by the entire team.

<u>Project diversity and career advancement</u> – The SFA team conducted one staff and one postdoc interview thus far, with the outcomes still in internal review. Diverse interview panels were convened to interview applicants from diverse backgrounds and institutions. The SFA decided to fund a proposal from a postdoc on the team to implement monitoring of evaporation in the SPRUCE enclosures.

<u>Mentoring skills and cultural awareness</u> – We are preparing to implement mentoring training in the All-Hands meetings using science-based curricula from the Center for the Improvement of Mentored Experiences in Research (CIMER) (https://cimerproject.org/). CIMER is a partner in NSF's Inclusive Graduate Education Network (IGEN) alliance, and they have a curriculum developed specifically for the National Laboratories. Theme Leads Mayes and Salmon are currently Trained Facilitators in the CIMER curricula.

<u>Increasing the involvement of underrepresented groups</u> – Two publications in the FY24 accomplishments are associated with PhD students from MSI and HBCU institutions (He et al. 2023, Jian et al. 2024), that were partially funded in the past cycle of the SFA. The SFA hosted researchers from Central Michigan University who are interested in collaborations under an RDPP project. The TES SFA team also hosts a visit from Reaching a New Energy Sciences Workforce (RENEW) project at the HBCU Missouri Lincoln University in June. Lincoln students collect data and maintain instrumentation deployed to monitor energy, water, and carbon budgets using an experimental watershed approach involving a network of microclimate monitoring stations and a stream depth gauging station at the outlet of a watershed in the footprint of MOFLUX, and they participated in MOFLUX's 20th anniversary workshop at the University of Missouri and the field trip to MOFLUX.

TES SFA Data Systems, Management, and Archiving Update

The Data Management Team (DMT) has several important updates from FY24. Collectively, they have been:

- Updating the SPRUCE Measurements list by meeting with individual theme leads to confirm the status of current data collections and associated dataset submissions. These meetings will continue a regular basis to check progress and expand to include all the TES SFA data collections. This list will be extremely beneficial in checking off dataset submissions during the close-out of SPRUCE.
- Implementing the <u>ESS-DIVE Sample ID and Metadata Reporting Format</u> where 2017-2023 MOFLUX soil cores (see Theme 4) will be assigned International Generic Sample Numbers (<u>IGSN</u>) sample ids registered by System for Earth Sample Registration <u>(SESAR</u>). By assigning a IGSN unique identifier, metadata is created in a searchable database and accessible on a landing page giving a digital representation to what is or was a physical sample. We are in the planning and development stage to apply this methodology to the SPRUCE deep peat sampling this year. The IGSN ids will be included in associated project datasets following the ESS-DIVE reporting format protocols. Physical sample archiving is being implemented at ORNL with these samples involving the development of a storage laboratory containing -80 °C and -20 °C freezers, dry storage, and shipment preparation facilities.

- Developing a resources page containing guidance documents and templates available in one project accessible location for researchers preparing datasets to access these materials and easily start preparing datasets for submission including submissions directly to ESS-DIVE.
- Arranging to work with the Lincoln University (LU) RENEW students to discuss data management and more specifically, to start the process of archiving Lincoln's microclimate data from the MOFLUX site.
- Developing a publication workflow to remove time-consuming manual work in transferring digital records from OSTI to ESS-DIVE using API services from both repositories. Three datasets were published following this approach. An Additional five datasets have been identified for testing to meet new ESS-DIVE metadata and reporting format guidance and that work is in progress.
- Data acquisition and real time display of SPRUCE experimental plot monitoring data continue to be available as <u>real-time visual displays</u>.
- Implementing two ESS-DIVE Reporting Formats for all new incoming datasets: <u>ESS-DIVE</u> <u>Reporting Format for Comma-separated Values (CSV) File Structure</u> and <u>ESS-DIVE Reporting</u> <u>Format for File-level Metadata (FLMD)</u>

The project has a total of 175 public datasets with three project-only SPRUCE datasets (**Fig. 17**). The DMT continues to move forward in mirroring datasets to ESS-DIVE with a current total of 64. Since March 2023, the project has published 14 new datasets and updated 12 datasets. For a list of all datasets, see Appendix B.



Fig. 17. The total number of publicly available TES SFA datasets and the number of datasets mirrored at ESS-DIVE.

Affiliated TES SFA-Supported Publications

Staff supported by the TES SFA continue to collaborate and complete work funded by US DOE BER in prior fiscal years that may not explicitly be funded under Themes 1 through 5. The following listing shows additional manuscripts completed since April 2023 with TES SFA support that have not previously been noted in TES SFA reports.

- Barney M, Hopple AM, Gregory LL, Keller JK, Bridgham SD (2024) Anaerobic oxidation of methane mitigates net methane production and responds to long-term experimental warming in a northern bog. *Soil Biology and Biochemistry* 190:109316, <u>https://doi.org/10.1016/j.soilbio.2024.109316</u>
- Felice M, Blake CM, Sebestyen S, Gutknecht JLM (2024) Microbial abundances and carbon use under ambient temperature or experimental warming in a southern boreal peatland. *Biogeochemistry* (in press), https://doi.org/10. 1007/ s10533- 024- 01129-z.
- Keiser AD, Smith M, Bell S, Hofmockel KS (2019) Peatland microbial community response to altered climate tempered by nutrient availability. *Soil Biology and Biochemistry* 137:107561, https://doi.org/10.1016/j.soilbio.2019.107561

4AII. SCIENCE HIGHLIGHTS SINCE APRIL 2023

- ORNL TES SFA staff authored 50 papers that have been published or are in press/accepted status since April 2023.
- As of this report we have sustained SPRUCE warming by elevated CO₂ treatments through 8.5 annual cycles.
- Theme 1: Shrub communities show enhanced biomass accumulation with warming that is enhanced under eCO₂ and trees which originally showed negative responses to warming are now showing a similar pattern. Positive warming responses are hypothesized to result from nutrient availability changes.
- Theme 1: Newly available high temporal resolution data on the net flux of CO₂ and CH₄ from peatlands confirms important mechanistic connections to warming and water limiting reductions in CH₄ efflux.
- Theme 1: Richardson et al. (2024) took a comprehensive look at multiple years of snow cover phenology under the SPRUCE treatments and showed how future warming, at levels consistent with IPCC projections, will result in transformative changes to the winter season in boreal peatlands, with impacts on how these ecosystems function, and how they impact the climate system.
- Theme 2: The TES SFA celebrated 20 years of continuous research at the Missouri eddy flux site (MOFLUX) in May 2024. A workshop organized by Jeff Wood was held and attended by a combination of initial project participants (Gu, Pallardy, Hanson), current SFA researchers, new University of Missouri student participants, and collaborators from Lincoln University of Missouri and Sandford University.
- Theme 2: Lianhong Gu has produced a theoretical explanation for energy imbalance issues that have been a long-standing issue for eddy covariance analyses and will submit a manuscript for review in the near future.
- Theme 2: Using advances from previous TES SFA modeling studies of the light reactions of photosynthesis, Theme 2 published a paper on how the electron transport chain may be bioengineered to increase photosynthesis while simultaneously minimizing the risk of photodamage.
- Theme 3: A long-term soil incubation indicates that N and P are differentially impacted by warming and eCO2 treatments. Minirhizotron image analysis at SPRUCE indicates 1) shrub and non-woody fine root production responds positively to warming at SPRUCE and 2) eCO2 impacts production rates for these PFTs based on microtopography.
- Theme 4: Depth specific rates and mechanisms of peat decomposition across elevated temperatures were assessed for the first 3 years of treatment at SPRUCE (Roth et al. 2023). There was little effect of warming on peat decomposition, but microbial communities showed increases in diversity as well as alteration of patterns of their interaction networks with warming.
- PIER Plan: The TES SFA proposed PIER plan is being implemented with a combination of training events and detailed document protocol development.
- Data Management: The TES SFA has generated a total of 175 public datasets since its inception and continues to work with the ESS-DIVE program to make them available and consistent with community expectations.

4AIII. ANALYSIS OF PUBLICATIONS

Through senior and coauthored effort, TES SFA staff produced 50 publications and completed manuscripts since our last summary report. This total includes 43 published/in press/accepted journal articles, 1 in press, 2 proceedings paper and two ORNL Technical reports. This level of 1-year productivity of (44 y⁻¹) is similar to the average for prior years under the

TES SFA (40-46 y⁻¹) and includes 4 papers in the *Nature* family of journals and 4 papers in *Global Change Biology*. A TES SFA cumulative publication summary since 2015 is provided in **Appendix A** with the most recent publications from the current reporting period listed first. This listing duplicates the Task-specific summaries already provided.

Journal selection for publication of TES SFA work is at the discretion of the author team. Journals are selected to achieve maximum exposure of the research results for the science community. We focus on journals having high impact factors, but that is not necessarily the primary criteria for the selection of a journal for publication of a given research result. Highprofile journals (e.g., *Science, Nature* family of journals, *PNAS*) are pursued for the publication of results anticipated to be of general interest to a wide audience. We find that solid and wellpresented scientific results are well received and cited in all our chosen journals.

We also continue to place significant and sustained effort on the production of archived data sets based on TES SFA work. A complete and cumulative summary of TES SFA data sets is provided in **Appendix B**.

4B. FUTURE SCIENCE GOALS AND PLANS

Future science goals and plans for the TES SFA were detailed in the quadrennial review document submitted to DOE BER in April 2023. That material was reviewed and evaluated recently and is not repeated here for the sake of brevity of this report.

4C. NEW SCIENCE FOCUS AND IDENTIFIED KNOWLEDGE GAPS

The ORNL TES SFA research group reorganized our efforts into five Themes in the 2023 review to better reflect the key questions and mechanisms to be studied in FY2024 through FY2028. The details of these new goals and milestones are detailed in the "Research Plan" section of the April 2023 proposal (https://tes-

 $sfa.ornl.gov/sites/default/files/ORNL_TES_SFA_Proposal_2023.pdf).$

4D. COLLABORATIVE RESEARCH

We continue to encourage key external groups to develop complementary research tasks for the benefit of TES SFA research tasks. Support for the following independently funded research groups is provided through the use of SPRUCE leased office/lab facilities and access to the SPRUCE experimental site on the S1 Bog:

- Dr. Joel Kostka (Georgia Tech), Jeff Chanton (Florida State) and colleagues continue to obtain support for studies of microbial ecology at SPRUCE and we look forward to continuing to work with them.
- Dr. Andrew Richardson (Northern Arizona University) leads the TES SFA task on phenology under subcontract with ORNL.
- Drs. Brandy Toner and colleagues from the University of Minnesota, are examining mercury and sulfur dynamics in the SPRUCE experiment using funding provided through the USDA Forest Service.
- Dr. Karis McFarlane and colleagues at LLNL-CAMS provide measurement support for ¹⁴C isotopic composition of air, plant tissues and peat from the S1-Bog and SPRUCE experimental plots.
- Dr. Nancy Glenn (Boise State) is contracted through SPRUCE to provide ground-level LIDAR observations as a supplement to our destructive woody harvests and *Sphagnum* production estimates. Jake Graham is executing the onsite work.
- Dr. Yiqi Luo's group (Cornell) utilizes high-temporal-resolution, model-data iterative analyses to better define measured ecosystem responses with the intention of helping the research group apply measurement efforts to critical processes.

- Dr. Xiaofeng Xu (San Diego State University) continues work with the modeling group on improved biogeochemical cycling models for methane flux.
- Dr. Jalene M. LaMontagne (DePaul University) joined the SPRUCE group in 2017 to study mast seeding patterns in response to climate change.
- Dr. M. Luke McCormack (Morton Arboretum) makes observations across a phylogenetically and ecologically diverse suite of tree species from mature forestry plots at The Morton Arboretum, Lisle, IL to advance work on the ELM models.
- Dr. Al Kovaleski (University of Wisconsin) evaluates how cold hardiness changes in the tree and shrub species due to warming treatments, and the interaction with a longer growing season and warmer winters, and how different chilling models perform in predicting dormancy stage in woody perennials.
- Dr. Jessica Gutknecht (University of Minnesota) executes independent measures of microbial biomass using PLFA at SPRUCE for a number of years; and is now collaborating with Theme 4 on multiple measures of microbial biomass.
- Dr. Michael Gundale (SLU, Swedish University of Agricultural Sciences) has funding investigating SPRUCE feathermoss responses from outside sources.
- Dr. Colin McCarter (Nipissing University) is an unfunded collaborator studying changes in peat physical properties with climate change.

CITED REFERENCES (NOT IN THE TES SFA APPENDIX LISTS)

- IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2391 pp. doi:10.1017/9781009157896.
- IPCC (2022) Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Phillips RP, Brzostek E, Midgley MG (2013) The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* 199:41-51. https://doi.org/10.1111/nph.12221
- USGCRP (2018) Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Volume II: [Reidmiller, D.R., C.W. Avery, D.R. Easterling, K.E. Kunkel, K.L.M. Lewis, T.K. Maycock, and B.C. Stewart (eds.)]. U.S. Global Change Research Program, Washington, DC, USA, 1515 pp. doi: 10.7930/NCA4.2018.
5. STAFFING AND BUDGET SUMMARY

5A. FY2024 FUNDING ALLOCATION BY PROGRAM ELEMENT

Total expected available funding for ORNL's TES SFA in FY2024 included \$7,181K carryover from FY2023 (including \$2,849K for external commitments) and \$8,300K of new budget authorization. FY2024 spending to date is summarized in the following table.

data include prior year carryover amounts.					
Task	Cost Through 11 June 2024 (\$K)	Commitments Through 11 June 2024 (\$K)	Remaining Funds 11 June 2024 (\$K)		
Theme 1: SPRUCE, C Cycle, Warming,					
etc.					
T1: SPRUCE Operations	1,073	1,432	\$1,066		
T1: Science	753	174	438		
Theme 2: MOFLUX, Water Cycle					
T2: MOFLUX Operations	124	3	5		
T2: Science	645	174	516		
Theme 3: Nutrient Cycle Feedbacks	481	136	390		
Theme 4: Microbial Processes and Soil C	625	126	328		
Theme 5: Modeling and Science Extrapolations	1,004	256	944		
Students	-3	8	32		
Data Management	118	0	16		
SFA Technical and Professional Support	666	64	381		
Other	1	0	191		
TES SFA Reserve	0	0	971		
Decommissioning Reserve	0	0	1,941		

FY2024 Budget expenditures by TES SFA Program Element through	11 June	2024.	The
data include prior year carryover amounts.			

We are currently spending at rates consistent with the spending plans outlined in the April 2023 TES SFA renewal proposal budgets for FY2024 through FY2028. We anticipate total FY2024 carryover funds for FY2025 to include to be approximately \$3,000K for FY transition funding and SPRUCE decommissioning funds reserved for use in fiscal years 2026 through 2028.

\$5,487K

\$2,372K

\$7,219K

5B. FUNDING ALLOCATION TO EXTERNAL COLLABORATORS

SFA Totals

A variety of collaborations are maintained and funded by the TES SFA to provide necessary commodities, and disciplinary expertise and effort in areas critical to the completion of research tasks. In FY2024 we directly funded the following individuals or groups.

The University of Missouri (\$160K) is subcontracted to provide MOFLUX on site execution of the following measurements: stand-level eddy covariance, soil CO₂ efflux, belowground production via repeated minirhizotron image collections, stem allometric increment data, and litter basket net primary production. Since June 2016, J.D. Wood serves as Missouri site-PI of the MOFLUX site.

Yiqi Luo- Northern Arizona University (\$140K) – Dr. Luo's research group at Cornell has developed an ecological forecasting capability at SPRUCE. Using the TECO model as a demonstration, data assimilation capabilities are being developed and applied using SPRUCE observations, and forecasts were made for the 10 experimental plots using a range of future scenarios. A methane model was also included in TECO.

Xiaofeng Xu - San Diego State University (\$60K) - Dr. Xu had developed and tested a CH₄ modeling capability for application within ELM-SPRUCE modeling efforts. This work has contributed to multiple manuscripts. Work to refine and optimize the model with SPRUCE researchers continues.

M. Luke McCormack – The Morton Arboretum (\$75K) – The Morton Arboretum (and Dr. M. Luke McCormack) have been subcontracted to enable the evaluation of key eastern US species plant traits on site to better parameterize ELM model inputs.

RhizoSystems, LLC (\$38K) – The company who designed and built the automated minirhizotrons (AMRs) continue to be subcontracted for support and maintenance of these systems.

Interagency Agreement with the USDA Forest Service (\$40K per year) – This agreement allows Forest Service employees to help with the operation, planning and execution of the SPRUCE experimental infrastructure and science tasks. It also provides some coverage for the use of the USDA FS bunk house on the Marcell Experimental Forest.

Andrew Richardson – Northern Arizona University (\$98K forward funded from prior FY funds) – This contract allows Dr. Andrew Richardson's group to maintain the automated phenology observations and greenness calculations for all treatment and ambient plots on the SPRUCE site. Dr. Richardson also leads the phenology task for the SPRUCE project.

Nancy Glenn – Boise State University (\$97K) This contract provides twice annual terrestrial lidar scans of the SPRUCE experimental plots to help assess vegetation growth and microform elevation change (hummock and hollow distributions).

Karis McFarlane – Lawrence Livermore National Laboratory (\$76K) – We contract with LLNL to provide isotopic analyses (¹⁴C and ¹³C) for air (x5 events per year) and tissue analyses (x1 per year) to provide a record of the application and accumulation of unique isotopic tracers into the SPRUCE ecosystem. We are considering supplementing these funds to provide a more complete picture of isotope changes since the beginning of SPRUCE on more tissues and species as we approach the end of the SPRUCE project at the end of 2025.

Dr. David M. Kramer – Michigan State University (\$40K) provides modeling support for energy balance modeling within the ELM models.

Infrastructure subcontracts in support of the SPRUCE project in FY2020 include funds and funding for site maintenance (**Pokegama Electric \$55K**), electrical service (**Lake Country Power \$156K**), propane supply (**Lakes Gas Co. \$420K**), eCO₂ supply (**PRAXAIR Inc. \$124K**), fiber internet connections (**\$10K**), and leased space in Minnesota (**\$41K**). The amounts required for each of these operational contracts are reevaluated annually as actual usage rates and prices change.

Various subcontracted funds are also used to support ORNL Student participation programs.

5C. PERSONNEL ACTIONS AND PROCEDURES

<u>Staffing Changes</u> – Joshua Birkebak was hired as a technical professional to provide TES SFA Theme support. Sadly, our colleague of many years Joanne Childs suddenly passed away from illness during the preparation of this report. TES SFA staff will initially transfer her responsibilities to other existing research technicians and professionals as we consider new hiring plans and associated budget allocations.

<u>Anticipated Future Hires</u> – Looking ahead to FY2025, the TES SFA is in the process of hiring replacement postdoctoral fellows to sustain effort and to supplement full time staff positions and as the budget allows.

<u>Procedures for advancing new and developing investigators</u> - New TES SFA staff members are commonly first hired through postdoctoral research associate positions and their performance and contributions to task activities are tracked. Our postdocs are vetted for potential future roles as task leads and are hired as staff into leadership roles as appropriate for our needs.

Where identified disciplinary needs are established (and for which adequate funding is available) the TES SFA also has the capacity to hire established staff persons directly into a task leadership role. When a need for new staff is identified but funding is insufficient to initiate a new hire, ORNL internal funds may be requested through a strategic hire program to bring individuals on board. This internal program allows for a 1 to 2-year transitional period to enable the TES SFA group to establish an appropriate, stable, and fully funded position.

Within the TES SFA, task accomplishments and budget management are executed at an overarching level by the Principal Investigators with feedback from all Theme leads. Individual Theme leads are given the responsibility to track scientific progress and the responsibility for managing their fiscal resources within an annual cycle. Training to allow new staff to understand ORNL procedures, accounting systems, and managerial activities is available and provided when appropriate. Such training, in addition to one-on-one mentoring with established staff, provides developing staff with the information and skill sets required to transition into leadership roles. At the institutional level, ORNL has formal programs for mentoring high-potential early career staff, and we use informal mentoring at the personal level to ensure that staff with potential leadership qualities are identified and helped with career development.

5D. NATIONAL LABORATORY INVESTMENT IN THE PROGRAM IN FY2024

Concepts for the belowground warming technologies used for the SPRUCE Experiment (Task R1) were initiated with ORNL LDRD funds totaling \$480K in FY2008 and FY2009. In FY2014, ORNL provided the equivalent of \$1000K staff support from internal funds to allow completion of the SPRUCE warming aboveground infrastructure.

In FY2024 ORNL funds were provided to enable the development of long-term sample storage facilities in support of previous and ongoing TES SFA projects (e.g., Walker Branch, EBIS, SPRUCE, MOFLUX, etc.)

The Climate Change Science Institute brings together all ORNL Climate Change staff including members of the TES SFA to foster day-to-day interactions among modelers, experimentalists and data management specialists. The TES SFA is supported by world-class capabilities at ORNL. The National Leadership Computing Facility provides an open, unclassified resource that we will use to enable breakthrough discoveries in climate prediction. We continue to be engaged with neutron sciences through cutting-edge root and rhizosphere imaging research at the High Flux Isotope Reactor (HFIR) and active participating in science development teams for the future VENUS beamline at SNS and proposed beamlines at the potential Second Target Station. We work with the DOE BER data center ESS-DIVE as our primary permanent data repository (see also Appendix B). We also use other facilities at collaborating DOE National Laboratories. The Lawrence Livermore National Laboratory – Center for Accelerator Mass Spectrometry (LLNL-CAMS) provides large volume, high precision ¹⁴C measurements for ecosystem tracer studies. Pacific Northwest National Laboratory's Environmental Molecular Science Laboratory combines advanced instrumentation such as high-throughput mass spectrometry, advanced microscopy instruments, and NMR instruments with high performance computing.

5E. CAPITAL EQUIPMENT

Since the threshold amount of funds needed to define a capital expenditure is high, no ORNL TES SFA funds have been used to acquire capital equipment in FY2024. Funding for SPRUCE experimental infrastructure maintenance and development at the S1 Bog are not classified as capital expenditures but represent an analogous investment for the decadal duration of the experiment.

APPENDIX A: COMPLETE PUBLICATION LIST – ORNL TES SFA

Published, accepted and in review papers since the April 2023 Progress Report and Renewal proposal

- Abramoff RZ, Warren JM, Harris J, Ottinger S, Phillips JR, Garvey SM, Winbourne J, Reinmann A, Hutyra L, Allen DW, Mayes MA (2024) Shifts in belowground processes along a temperate forest edge. *Landscape Ecology* 39:100. https://doi.org/10.1007/s10980-024-01891-3
- Barreto C, Conceicão PH, de Lima EC, Stievano LC, Zeppelini D, Kolka RK, Hanson PJ, Lindo Z (2023) Large-scale experimental warming reduces soil faunal biodiversity through peatland drying. *Frontiers in Environmental Science* 11:1153683. doi:10.3389/fenvs.2023.1153683
- Beidler KV, Benson MC, Craig ME, Oh Y, Phillips RP (2023) Effects of root litter traits on soil organic matter dynamics depend on decay stage and root branching order. *Soil Biology and Biochemistry* 180:109008. <u>https://doi.org/10.1016/j.soilbio.2023.109008</u>
- 4. Brügger A, Bilheux HZ, Nelson G, Kiss A, Morris J, Connolly M, Long A, Tremsin A, Strzelec A, Anderson M, Agasie B, Finney C, Wissink M, Hubber M, Pellenq R, White C, Heuser B, Craft A, Harp A, Tan C, Morris K, Junghans A, Sevanto S, Warren JM, Florez FE, Biris A, Cekanova M, Kardjilov N, Schillinger B, Lin J, Frost M, Vogel S (2023) CUPI2D: Complex, Unique and Powerful Imaging Instrument for Dynamics. *Review of Scientific Instruments* 94:051301. <u>https://doi.org/10.1063/5.0131778</u>
- 5. Chen J, Liu Z, Mao J, Zhao T, Tu T, Cheng L, Dong C (2023) Co-regulation of water and energy in the spatial heterogeneity of drought resistance and resilience. *Environmental Research Letters*, 18:114007, 10.1088/1748-9326/acfccc.
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- Duchesneau K, Defrenne CE, Petro C, Malhotra A, Moore JAM, Childs J, Hanson PJ, Iversen CM, Kostka JE (2024) Responses of vascular plant fine roots and associated microbial communities to whole-ecosystem warming and elevated CO₂ in northern peatlands. *New Phytologist* 244:1333-1347. doi: 10.1111/nph.19690
- Dusenge ME, Warren JM, Reich PB, Ward EJ, Murphy BK, Stefanski A, Villanueva R, Cruz M, McLennan DA, King AW, Montgomery RA, Hanson PJ, Way DA (2023) Boreal conifers maintain carbon uptake with warming despite failure to track optimal temperatures. *Nature Communications* 14:4667. <u>https://doi.org/10.1038/s41467-023-40248-3</u>.
- 11. Foster K, Sun W, Shiga Y, Mao J, Michalak A (2024) Multiscale assessment of North American terrestrial carbon balance. *Biogeosciences*, 21:869-891. <u>https://doi.org/10.5194/bg-21-869-2024</u>.
- 12. Gu L (2023) Optimizing the electron transport chain to sustainably improve photosynthesis. *Plant Physiology* 193:2398-2412. <u>https://doi.org/10.1093/plphys/kiad490</u>.
- 13. Gu L, Grodzinski B, Han J, Marie T, Zhang Y-J, Song YC, Sun Y (2023) An exploratory steadystate redox model of photosynthetic linear electron transport for use in complete modeling of photosynthesis for broad applications. *Plant, Cell and Environment* 46:1540-1561.
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- He L, Rodrigues JLM, Mayes MA, Lai C-T, Lipson DA, Xu X (2024) Modeling microbial carbon fluxes and stocks in global soils from 1901 to 2016, Biogeosciences, 21:2313-2333. doi:10.5194/bg-21-2313-2024.
- Holtzman N, Wang Y, Wood JD, Frankenberg C, Konings AG (2023) Constraining plant hydraulics with microwave radiometry in a land surface model: Impacts of temporal resolution. *Water Resources Research*, 59:e2023WR035481, https://doi.org/10.1029/2023WR035481.
- Jian S, Li J, Wang G, Zhou J, Schadt CW, Mayes MA (2024) Generalizing microbial parameters in soil biogeochemical models: Insights from a multi-site incubation experiment. *Journal of Geophysical Research: Biogeosciences* 129:e2023JG007825. https://doi.org/10.1029/2023JG007825
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APPENDIX B: TES SFA DATA SETS

The TES SFA data products are served to the public with CC BY 4.0 data usage rights license and accessible on the SPRUCE (https://mnspruce.ornl.gov) and TES SFA (https://tes-sfa.ornl.gov) websites with some available at the ESS-DIVE repository (https://data.ess-dive.lbl.gov/data). Researchers are encouraged to publish the federally funded scientific data in a timely manner. While all metadata records are available to the public, researchers may request that some data collections be accessible only to the project team typically while awaiting associated manuscript publication. Data users should include the full data set citation with the DOI in the reference section of any published paper.

These datasets include regularly updated time-series of SPRUCE environmental data, peat analyses, modeling archives, code releases, results of laboratory incubations, links to genomic products at JGI, "supporting validation data" for specific publications (e.g., organic matter characterization), web-based tools (e.g., LeafWeb), historical Walker Branch data, literature compilations (e.g., FRED 3.0), and characterization of SPRUCE plots (e.g., elevation).

TES SFA Software:

- 1. MAAT v1.3.1 is now open source and is available at https://github.com/walkeranthonyp/MAAT.
- 2. IMACSS, the software that controls FAME, has been licensed to Campbell Scientific Inc.

Complete Data Set List (** denotes a new or upgraded data set from April 2023 to May 2024)

SPRUCE Public Data Sets (Through June 2024)

- **Barney M, Hopple AM, Gregory LL, Keller JK, Bridgham SD (2024D) SPRUCE Anaerobic oxidation of methane mitigates net methane production and responds to long-term experimental warming in a northern bog: Supporting data. Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/10.25581/spruce.115/2204381
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- ** Buell Z, Philips J, Ottinger S, Lowe K, Schadt CW, Mayes MA (2024D) SPRUCE: Chloroform Fumigation Extraction for Microbial Biomass and Dissolved Organic Carbon from SPRUCE, 2021-2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.109/1998876.
- **Buell Z, Felice M, Philips J, Ottinger S, Lowe K, Gutknecht JLM. (2024D) SPRUCE Phospholipid Fatty Acid (PLFA) Abundances, August 2021-June 2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.112/1998897
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- Finzi AF, Giasson MA, Gill AL (2016D) SPRUCE Autochamber CO₂ and CH₄ Flux Data for the SPRUCE Experimental Plots. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. <u>http://dx.doi.org/10.3334/CDIAC/SPRUCE.016</u>
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- 106. Zalman CA, Keller JK, Tfaily M, Kolton M, Pfeifer-Meister L, Wilson RM, Lin X, Chanton J, Kostka JE, Gill A, Finzi A, Hopple AM, Bohannan BJM, Bridgham SD (2018D) SPRUCE Small Differences in Ombrotrophy Control Regional-Scale Variation in Methane Cycling among Sphagnum-Dominated Peatlands: Supporting Data. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. https://doi.org/10.25581/spruce.043/1434643

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- 108. Krassovski MB, Hanson PJ (TBD) SPRUCE Experimental Plot Top View Aerial Photographs beginning June 2019. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. <u>https://doi.org/10.25581/spruce.076/1604061</u>
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- Kluber LA, Phillips JR, Wang G, Schadt CW, Mayes MA (2017D) Soil Respiration and Microbial Biomass from Soil Incubations with ¹³C Labeled Additions. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. <u>https://doi.org/10.3334/CDIAC/ornlsfa.010</u>
- 20. LeafWeb. LeafWeb is a TES SFA-funded web-based tool for the automated numerical analyses of leaf gas exchange measurements. LeafWeb is a Service-in-Exchange-for-Data-Sharing (SEEDS) Project. With the approval of the user, the data LeafWeb

receives are preserved and added to a global database of biochemical, physiological, and biophysical properties of single leaves to support studies of plant functions and terrestrial carbon cycle modeling. Access LeafWeb at <u>http://leafweb.ornl.gov/</u>.

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- 24. **Missouri Ozark Flux (MOFLUX) Measurement Data.** TES SFA-funded site characterization and flux measurement data, starting in 2004 and continuing, are archived by the AmeriFlux Program. Data and can be accessed at http://ameriflux.ornl.gov/fullsiteinfo.php?sid=64.
- 25. Mulholland PJ, Griffiths NA (2016D) Walker Branch Watershed: Hourly, Daily, and Annual Precipitation. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. <u>http://dx.doi.org/10.3334/CDIAC/ornlsfa.006</u>
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- 29. Mulholland PJ, Griffiths NA (2016D) Walker Branch Watershed: Daily Climate and Soil Temperature Data. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. <u>https://doi.org/10.3334/CDIAC/ornlsfa.008</u>
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- 31. Pallardy SG, Gu L, Wood JD, Hosman KP, Sun Y (2018D) Predawn Leaf Water Potential of Oak-Hickory Forest at Missouri Ozark (MOFLUX) Site: 2004-2020. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. <u>https://doi.org/10.3334/CDIAC/ornlsfa.004</u>
- 32. Pallardy SG, Gu L, Wood JD, Hosman KP, Hook LA (2019D) Vegetation Inventory of Oak-Hickory Forest at Missouri Ozark (MOFLUX) Site: 2004-2017. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. Access on-line at: <u>https://doi.org/10.25581/ornlsfa.016/1498529</u>
- 33. Shi X, Wang D (2014D) **GSOD Based Daily Global Mean Surface Temperature and Mean Sea** Level Air Pressure (1982-2011)", doi:10.15149/1130373.
- 34. **Tool for Evaluating Mesophyll Impact on Predicting Photosynthesis (<u>TEMIPP</u>).** TEMIPP is a Microsoft Excel spreadsheet-based tool used for demonstrating the impact of lacking an explicit representation of mesophyll diffusion in a photosynthetic model on the predicted response of

photosynthesis to the increase in CO₂ partial pressures. TEMIPP is provided as a supplement to the recent publication: Sun Y, Gu L, Dickinson RE, Norby RJ, Pallardy SG, Hoffman FM (2014) Impact of mesophyll diffusion on estimated global land CO₂ fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 15774–15779, doi: 10.1073/pnas.1418075111. Download TEMIPP at http://TES SFA.ornl.gov/node/80.

- 35. Walker Branch Watershed Long-Term Data Archive. Repository for TES SFA-funded data collections of long-term hydrology, stream ecology, chemistry, and biogeochemistry measurements and research. Data can be accessed at http://walkerbranch.ornl.gov/.
- 36. Warren JM, Iversen CM, Garten Jr CT, Norby RJ, Childs J, Brice D, Evans RM, Gu L, Thornton P, Weston DJ (2013D) PiTS-1: Carbon Partitioning in Loblolly Pine after ¹³C Labeling and Shade Treatments. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, Tennessee. <u>https://doi.org/10.3334/CDIAC/ornlsfa.001</u>.
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- Huntzinger DN, Schwalm CR, Wei Y, Cook RB, Michalak AM, Schaefer K, Jacobson AR, Arain MA, Ciais P, Fisher JB, Hayes DJ, Huang M, Huang S, Ito A, Jain AK, Lei H, Lu C, Maignan F, Mao J, Parazoo N, Peng C, Peng S, Poulter B, Ricciuto DM, Tian H, Shi X, Wang W, Zeng N, Zhao F, Zhu Q, Yang J, Tao B (2016D) NACP MsTMIP: Global 0.5-deg Terrestrial Biosphere Model Outputs (version 1) in Standard Format. ORNL DAAC, Oak Ridge, Tennessee. <u>https://doi.org/10.3334/ORNLDAAC/1225.</u>
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TES SFA Task 7 Data Sets:

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- 12. Andres RJ, Boden TA (2016D) Annual Fossil-Fuel CO₂ Emissions: Uncertainty of Emissions Gridded by One Degree Latitude by One Degree Longitude, 1751-2013. ORNL/CDIAC, electronic database. doi: 10.3334/CDIAC/ffe.AnnualUncertainty.2016.
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