

SPRUCE Field Quantitative Stable Isotope Probing (qSIP) Data Set

Summary:

This data set provides the calculated excess atom fraction, amplicon sequencing counts, and clustering analysis results for the ^{18}O -labeled water incubation conducted at Spruce and Peatland Responses Under Changing Environments (SPRUCE).



Sampling Details:

Samples were collected from the following plots & treatments.

Table 1. Treatment temperature and CO₂ concentration

| Plot | Temperature Treatment | CO ₂ Treatment (500ppm) |
|------|-----------------------|------------------------------------|
| 6 | +0 | 0 |
| 19 | +0 | +500 |
| 20 | +2.25 | 0 |
| 11 | +2.25 | +500 |
| 13 | +4.5 | 0 |
| 4 | +4.5 | +500 |
| 8 | +6.75 | 0 |
| 17 | +9 | 0 |
| 10 | +9 | +500 |

Sponsor

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The SPRUCE experiment is a multi-year cooperative interaction among scientists of the [Oak Ridge National Laboratory](#) operated by UT-Battelle, LLC and the U.S. Forest Service, [Northern Research Station](#), [Marcell Experimental Forest](#).

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1. Data Set Overview:

This data set provides the excess atom fraction (EAF), 16S rRNA gene sequence abundances for individual bacterial and archaeal taxa and 16S rRNA gene copy numbers for each sample incubated with ^{18}O -labeled water in SPRUCE enclosures in August 2018, along with clustering analysis results.

Taxa have been organized into amplicon sequence variants (ASVs), which describe distinct taxonomic units.

2. Data Characteristics:

Spatial Coverage

Incubations took place at the 8.1-ha S1 bog forest site in northern Minnesota, 40 km north of Grand Rapids, in the USDA Forest Service Marcell Experimental Forest (MEF). These coordinates are the central location of the S1 bog.

Table 2. Site boundary latitude and longitude given in decimal degrees.

| Site (Region) | Westernmost Longitude | Easternmost Longitude | Northernmost Latitude | Southernmost Latitude | Elevation (meters amsl) | Geodetic Datum |
|-------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------------|----------------|
| S1 Bog, Marcell Experimental Forest | -93.48283 | -93.48283 | 47.50285 | 47.50285 | 418 | WGS84 |

Data File Descriptions:

There are six archived files encompassing data from the Field qSIP experiment.

1. “**SPRUCE_FqSIP_SampleMetadata.xlsx**” contains sample treatment metadata, see “Data Directory” tab for details and information on column headers.
2. “**SPRUCE_FqSIP_eafs_counts_long.xlsx**” contains calculated EAF values for each ASV, and corresponding 16S rRNA gene sequence read counts, see “Data Directory” tab for details and information on column headers.
3. “**SPRUCE_FqSIP_clusters.xlsx**” contains cluster designations for a subset of the total ASVs, according to three different clustering approaches, which summarize an ASV’s treatment response. See “Data Directory” tab for details and information on column headers.
4. “**SPRUCE_FqSIP_147_silva.fasta**” contains representative 16S rRNA gene sequences from the set of 144 ASVs of interest and sequences from three Archaeal ASVs (detected in the data, but not retained in the core or expanded pool of ASVs used for clustering analyses) that served as an outgroup during phylogenetic inference.

5. “**SPRUCE_FqSIP_147_ml_tree.tre**” phylogenetic relationships among aligned 16S rRNA gene sequences for 144 ASVs of interest inferred by maximum likelihood estimation
6. “**SPRUCE_FqSIP_147_bootstrap_ml_trees.tre**” 100 non-parametric bootstrapped maximum likelihood trees used to assess the phylogenetic conservation of treatment response across a range of plausible topologies.

Variable Naming Conventions – Naming conventions are described in the Data Dictionary in excel files.

Missing values – Missing values are represented by blank cells.

Data Dictionary:

See Data Dictionary in excel files for column header information.

3. Applications and Derivation:

To understand taxon-specific microbial growth responses to elevated temperature and CO₂, under simulated environmental change.

4. Data Acquisition, Materials, and Methods:

Site Description:

The site is the 8.1-ha S1 bog, a *Picea mariana* [black spruce] – Sphagnum spp. ombrotrophic bog forest in northern Minnesota, 40 km north of Grand Rapids, in the USDA Forest Service Marcell Experimental Forest (MEF). The S1 bog was harvested in successive strip cuts in 1969 and 1974 and the cut areas were allowed to naturally regenerate. The 1974 strips are characterized by medium density of 3-5 meter black spruce and larch trees with an open canopy. The 1969 harvest strips are characterized by a higher density of 3-5 meter black spruce and larch trees with a generally closed canopy.

Experimental Applications- Deep Peat Heating (DPH) and Whole Ecosystem Warming (WEW) + Elevated CO₂:

Deep Peat Heating (DPH) treatment was initiated on July 2014. This consisted of heating below ground at 2m depth to a target temperature above ambient air. Whole Ecosystem Warming (WEW) treatments were initiated in June 2015. WEW treatments were preceded by approximately one year of deep peat heating (DPH) only. Approximately 10 months after WEW was initiated at 5 warming levels (+0, +2.25, +4.5, +6.75 and +9 °C), elevated CO₂ treatments were initiated in June 2016, with treatment levels at +500 ppm above ambient in half the plots (Hanson et al. 2016). Sample incubation took place approximately four years after initiation of DPH, three years after the onset of WEW, and two years after initiation of elevated CO₂ treatments

Peat from just below the live sphagnum layer was collected on 08/13/2018 and placed in nylon mesh bags (41 micron, 30 % open area, Industrial Netting) that were buried in silica desiccant inside 125 mL autoclave sterile Nalgene bottles. New grade 48, 4-10 Mesh/Certified ACS grade desiccant was baked at 105 °C for 48 hours prior to use. Bottles were placed back in the enclosures under treatment conditions to dry for 2 days, with desiccant changed daily.

Dried samples were separated from silica desiccant and weighed into 50mL conical tubes then amended with either H_2^{18}O (treatment) or sterile nanopure water (control) to 91% gravimetric water content (GWC), which was the mean GWC prior to desiccation. To facilitate gas exchange and prevent moisture loss, tubes were capped with parafilm, and incubated in the treatment enclosures for 10 days beginning on 08/15/2018. Samples were snap frozen in the field on 08/25/2021, shipped overnight on dry ice, and stored at -80°C until DNA extraction. Sample DNA was extracted with DNeasy PowerMax Soil Kits (Qiagen, Hilden, Germany) according to manufacturer protocols. Ultracentrifugation was conducted to separate ^{18}O -enriched DNA based on buoyant density following the protocols used in Hayer et al. 2016 and Wang et al. 2021 followed by Illumina sequencing and qPCR of 16S rRNA genes. Excess atom fraction (EAF) values (a proxy for growth rate) were calculated for each taxon according to Hungate et al. 2015.



Figure 1. Left: Sample set up in 50mL conical tube. Right: In-situ incubation in SPRUCE enclosure.

Temperature data were pulled from soil probes ($n=3$) at 10cm depth in each enclosure, which collect readings in 30 minutes intervals. Measured temperature for each sample was calculated as the mean over 30 days prior to final sample harvest, thus capturing weather variability prior to incubation, during the 2 day drying period, and during the 10 day incubation.

Taxa were clustered by their EAF response pattern across temperatures under ambient or elevated CO₂ using three clustering approaches, and phylogenetic conservation of response traits were calculated as described in Bell et al. (2021).

5. References:

1. Hanson, P. J. *et al.* Attaining whole-ecosystem warming using air and deep-soil heating methods with an elevated CO₂ atmosphere. *Biogeosciences* **14**, 861–883 (2017).
2. Hayer, M. *et al.* Identification of growing bacteria during litter decomposition in freshwater through quantitative stable isotope probing. *Environ. Microbiol. Rep.* **8**, 975–982 (2016).
3. Wang, C. *et al.* The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization. *ISME* (2021) doi:10.1038/s41396-021-00959-1.
4. Hungate, B. A. *et al.* Quantitative microbial ecology through stable isotope probing. *Appl. Environ. Microbiol.* **81**, 7570–7581 (2015).
5. Bell, S.L. *et al.* ... (2021)

6. Data Access

This data is available through the Pacific Northwest National Laboratory's DataHub:
<https://data.pnnl.gov/>

All sequences have been deposited in Sequence Read Archive (SRA) under NCBI project ID PRJNA729971.

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