NEON USER GUIDE TO MICROBIAL COMMUNITY COMPOSITION (NEON.DP1.10081; NEON.DP1.20141; NEON.DP1.20086)

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1 DESCRIPTION

1.1 Purpose

This document provides an overview of the data included in this NEON Level 1 data product, the quality controlled product generated from raw Level 0 data, and associated metadata. In the NEON data products framework, the raw data collected in the field - for example, soil temperature from a single collection event - are considered the lowest level (Level 0). Raw data that have been quality checked via the steps detailed herein, as well as simple metrics that emerge from the raw data are considered Level 1 data products.

The text herein provides a discussion of measurement theory and implementation, data product provenance, quality assurance and control methods used, and approximations and/or assumptions made during L1 data creation.

1.2 Scope

This document describes the steps needed to generate the L1 data products for Microbial Community Composition and associated metadata measured on aquatic and terrestrial samples from input data. Three separate data products are described herein:

1. Soil Microbe Community Composition (NEON.DP1.10081)
2. Surface Water Microbe Community Composition (NEON.DP1.20141)
3. Benthic Microbe Community Composition (NEON.DP1.20086)

This document also provides details relevant to the publication of the data products via the NEON data portal, with additional details in the files, NEON Data Variables for Soil Microbe Community Composition (NEON.DP1.10081) (AD[05]), NEON Data Variables for Surface Water Microbe Community Composition (NEON.DP1.20141) (AD[06]), and NEON Data Variables for Benthic Microbe Community Composition (NEON.DP1.20086) (AD[07]), provided in the download package for each of these three data products.

This document describes the process for ingesting and performing automated quality assurance and control procedures on the laboratory data from samples generated by the following field sampling protocols: TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling (AD[10]) for upland soil samples; TOS Standard Operating Procedure: Wetland Soil Sampling (AD[11]) for wetland soil samples; or AOS Protocol and Procedure: Aquatic Microbial Sampling (AD[12]) for aquatic samples. The raw data that are processed as described in this document are detailed in the file, NEON Raw Data Validation for Microbial Community Composition (NEON.DP0.10081) (AD[04]), provided in the download package for this data product. Please note that raw data products (denoted by 'DP0') may not always have the same numbers (e.g., '10033') as the corresponding L1 data product.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Associated Documents

AD[01]	NEON.DOC.000001	NEON Observatory Design (NOD) Requirements
AD[02]	NEON.DOC.000913	TOS Science Design for Spatial Sampling
AD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
AD[04]	NEON.DP0.10081.001_dataValidation.csv	NEON Raw Data Validation for Microbial Community Composition (NEON.DP0.10081)
AD[05]	NEON.DP1.10081.001_variables.csv	NEON Data Variables for Soil Microbe Community Composition (NEON.DP1.10081)
AD[06]	NEON.DP1.20141.001_variables.csv	NEON Data Variables for Surface Water Microbe Community Composition (NEON.DP1.20141)
AD[07]	NEON.DP1.20086.001_variables.csv	NEON Data Variables for Benthic Microbe Community Composition (NEON.DP1.20086)
AD[08]	NEON.DOC.000908	TOS Science Design for Microbial Diversity
AD[09]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
AD[10]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
AD[11]	NEON.DOC.004130	TOS Standard Operating Procedure: Wetland Soil Sampling
AD[12]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
AD[13]	NEON.DOC.000008	NEON Acronym List
AD[14]	NEON.DOC.000243	NEON Glossary of Terms
AD[15]	OS_Generic _Transitions.pdf	NEON Algorithm Theoretical Basis Document: OS Generic Transitions
AD[16]		NEON's Ingest Conversion Language (NICL) specifications

2.2 Acronyms

rRNA	Ribosomal ribonucleic acid
16S	Small subunit of the rRNA gene
ITS	Internal transcribed spacer region of the small subunit of the rRNA gene

3 DATA PRODUCT DESCRIPTION

The Microbial Community Composition data products provide taxonomic data for bacteria, archaea, and fungi for soil and aquatic samples. NEON targets a region of the 16S ribosomal RNA gene to measure bacteria and archaea, and the internally- transcribed spacer (ITS) region of the ribosomal RNA gene to measure fungi. Data are generated using high- throughput technology that produces many thousands of sequence reads per sample (Armougom and Didier, 2009; Klindworth et al., 2013). The sequence data, which comprise the Microbe Marker Gene Sequences Data Products (NEON.DP1.10108; NEON.DP1.20280; NEON.DP1.20282), are used to generate taxon tables provided in this data product. The sampling plan implements the guidelines and requirements described in the Science Designs for TOS Terrestrial Microbial Diversity and Aquatic Sampling (AD[08] and AD[09]). Information on sample collection methods for each data product can be found in the field user guides:

- Soils: NEON User Guide to Soil Physical Properties, Distributed Periodic (NEON.DP1.10086)
- Surface water: NEON User Guide for Surface Water Microbe Cell Count (NEON.DP1.20138)
- Benthic habitats: NEON User Guide for Aquatic Benthic Microbe Collection (NEON.DP0.20270)

In general, samples are minimally processed in the field in order to reduce the introduction of microbial contaminants. After collection, samples are frozen in the field on dry ice and transported to ultra-low freezers at the NEON field laboratories. Samples are shipped to an analytical laboratory where DNA extraction, sample library preparation and DNA sequencing occur. The sequence data are quality-filtered and processed bioinformatically to generate taxon tables (Figure 1). Given the rapid pace of technological and bioinformatic development, the exact methods for sequence processing and taxon-calling may change over time.

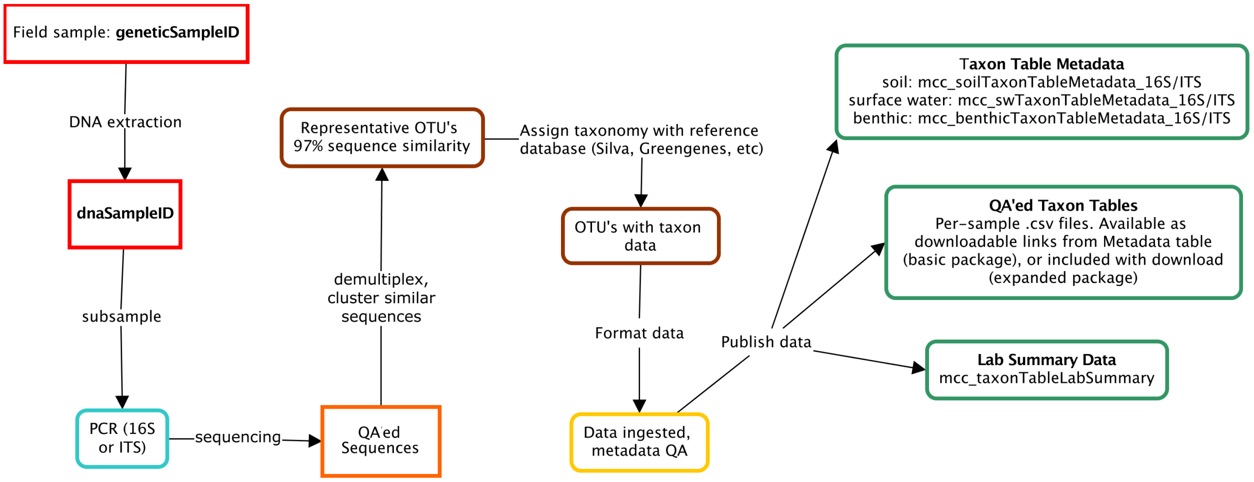

Figure 1: Diagram representing the microbial community composition data product workflow from sample collection to publication on the NEON Data Portal.

3.1 Spatial Sampling Design

Sampling for microbial community composition analysis is executed at all NEON sites, with data reported at the resolution of a single sampling location.

For soils, this equates to a randomly-assigned X,Y coordinate (± 0.5 meters) within a NEON plot. Ten plots are sampled at 3 randomly selected locations within each plot (Figure 2). In general, only the surface horizon is sampled to a maximum depth of 30cm, and horizons are broadly defined as either organic (O) or mineral (M).

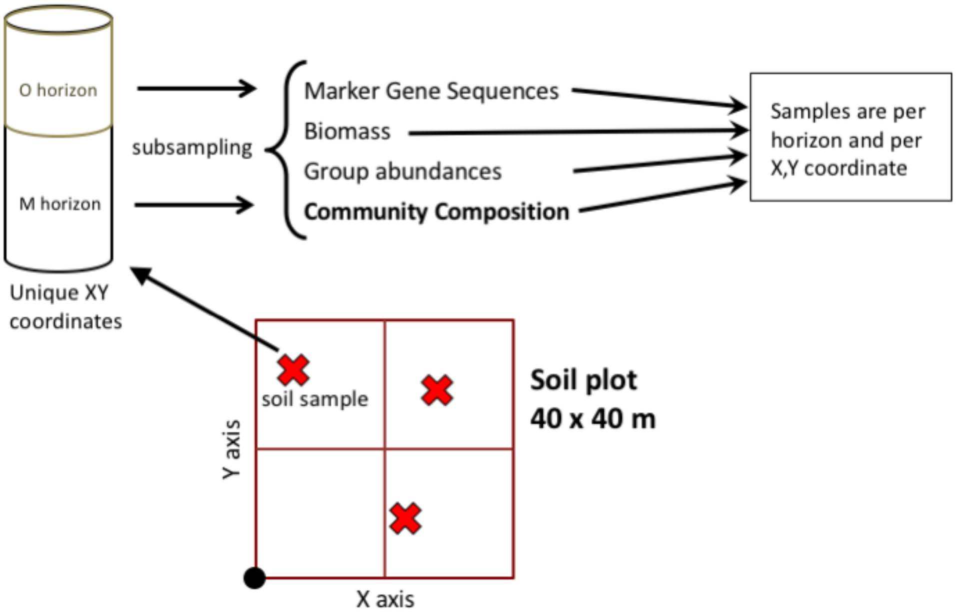

Figure 2: Overview of soil microbial field sampling, spatial design, and analysis workflow.

For aquatic surface water samples, this equates to the buoy sensor station and inlet/outlet locations within a lake, the buoy sensor station for large rivers, or the downstream sensor array for wadeable streams. For aquatic benthic samples, this equates to up to eight locations within a 1 km reach (Figure 3).

The spatial designs for the microbial community composition data products are described in more detail in the Data Product User Guides for Soil Physical Properties (NEON.DP1.10086), Aquatic Surface Water Cell Counts (NEON.DP1.20138), and Aquatic Benthic Microbe Collection (NEON.DP0.20270). For a description of the methods used in terrestrial plot selection, refer to the TOS Science Design for Spatial Sampling (AD[02]).

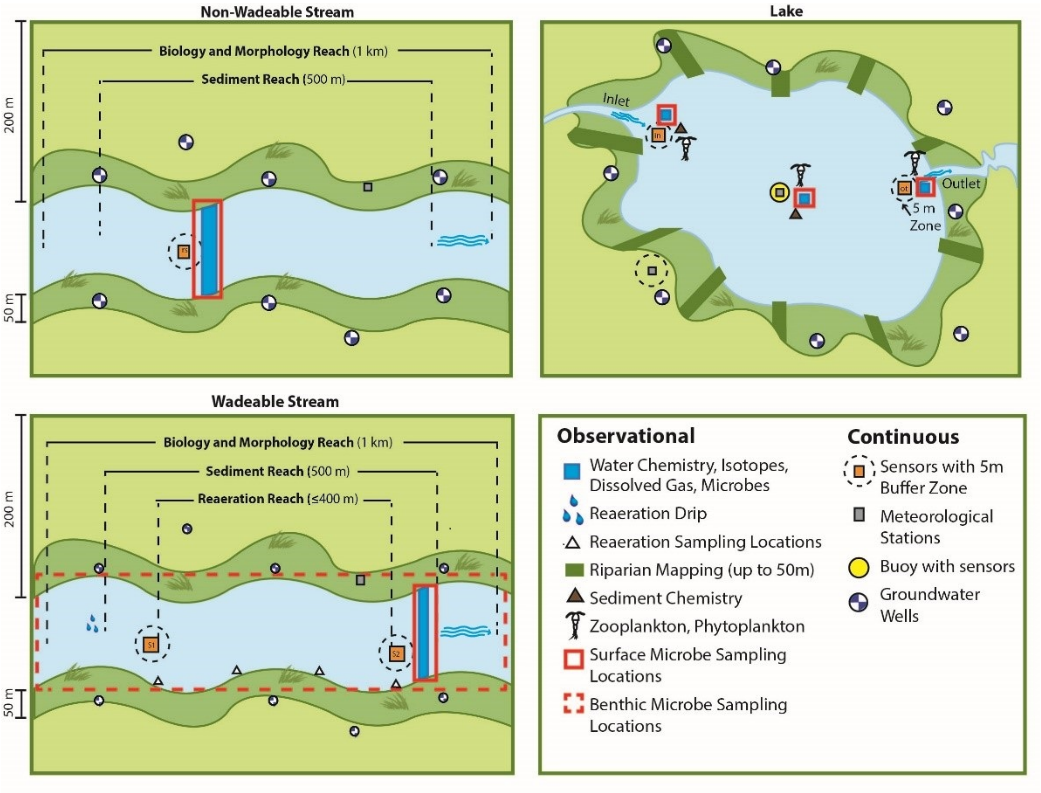

Figure 3: Generic NEON aquatic site layouts with microbial sampling locations highlighted in red boxes.

3.2 Temporal Sampling Design

For all samples, the temporal resolution is that of a single collection date. For a more detailed description of the temporal frequency of sampling and general field methods, refer to TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling (AD[10]) or AOS Protocol and Procedure: Aquatic Microbial Sampling (AD[12]) for soil and aquatic sampling protocols, respectively. Descriptions of the upstream field data products can be found in the Data Product User Guides for soil (NEON.DP1.10086), aquatic surface water (NEON.DP1.20138), and benthic (NEON.DP1.20270) field sampling.

3.3 Variables Reported

All variables reported from the analytical laboratory (L0 data) are listed in the file, NEON Raw Data Validation for Microbial Community Composition (NEON.DP0.10081) (AD[04]). All variables reported in the published data (L1 data) are also provided separately. For variables that are not present in the taxon tables, the variables are located in the following files:

- NEON Data Variables for Soil Microbe Community Composition (NEON.DP1.10081) (AD[05])
- NEON Data Variables for Surface Water Microbe Community Composition (NEON.DP1.20141) (AD[06])

- NEON Data Variables for Benthic Microbe Community Composition (NEON.DP1.20086) (AD[07])

For variables that are present in the taxon tables, the variables are listed in the Table below.

fieldName	Description	dataType	units
dnaSampleID	Identifier for DNA sample	string	NA
dnaSampleCode	Barcode of a DNA sample	string	NA
completeTaxonomy	Full taxonomic hierarchy for identified organism	string	NA
domain	The scientific name of the domain in which the taxon is classified	string	NA
kingdom	The scientific name of the kingdom in which the taxon is classified	string	NA
phylum	The scientific name of the phylum or division in which the taxon is classified	string	NA
class	The scientific name of the class in which the taxon is classified	string	NA
order	The scientific name of the order in which the taxon is classified	string	NA
family	The scientific name of the family in which the taxon is classified	string	NA
genus	The scientific name of the genus in which the taxon is classified	string	NA
species	The scientific name of the species in which the taxon is classified	string	NA
scientificName	Scientific name, or name of the lowest level taxonomic rank that can be determined	string	NA
individualCount	Number of individuals of the same type	unsigned integer	number

Field names have been standardized with Darwin Core terms (http://rs.tdwg.org/dwc/; accessed 16 February 2014), the Global Biodiversity Information Facility vocabularies (http://rs.gbif.org/vocabulary/gbif/; accessed 16 February 2014), and the VegCore data dictionary (https://projects.nceas.ucsb.edu/nceas/projects/bien/wiki/VegCore; accessed 16 February 2014).

To the extent possible, metadata names and terms are standardized according to the Genomics Standards Consortium, http://gensc.org/ (Kottmann et al., 2008; Yilmaz et al., 2011; Field et al., 2011). Efforts are also made to conform with the ENVO ontology (http://www.obofoundry.org/ontology/envo.html).

NEON TOS spatial data employs the World Geodetic System 1984 (WGS84) for its fundamental reference datum and GEOID09 for its reference gravitational ellipsoid. NEON Aquatic spatial data uses the Earth Graviational Model 96 (EGM96) for its reference graviational ellipsoid. Latitudes and longitudes are denoted in decimal notation to six decimal places, with longitudes indicated as negative west of the Greenwich meridian.

Some variables described in this document may be for NEON internal use only and will not appear in downloaded data.

3.4 Spatial Resolution and Extent

The finest resolution at which spatial data are reported is a single sampling location. For soils, this corresponds to a single X,Y coordinate location within a plot. For aquatics, this corresponds to a single station or habitat unit within a site.

3.4.1 Soils

sampleID (unique ID given to the individual soil sampling location and horizon) → **plotID** (ID of plot within site) → **siteID** (ID of NEON site) → **domainID** (ID of a NEON domain).

The spatial data are located in the data product Soil Physical Properties, distributed periodic (DP1.10086), in the table ***sls_soilCoreCollection***. The spatial data are measured at the plot *centroid*, and have an accuracy of ± 20 meters. A more precise measurement may be determined by calculating the offset from the plot centroid using the variables **coreCoordinateX** and **coreCoordinateY**. Refer to the User Guide for Soil Physical Properties, distributed periodic, for more information and instructions.

3.4.2 Aquatics

namedLocation (unique ID given to the location within a site) → **siteID** (ID of NEON site) → **domainID** (ID of a NEON domain).

The spatial data can be found in the following Data Products:

- Surface water samples: Surface water microbe cell count (DP1.20138), in the table ***amc_fieldSuperParent*** and ***amc_fieldGenetic***.
- Benthic samples: Benthic microbe marker gene sequences (DP1.20280), in the table ***amb_fieldParent***.

3.5 Temporal Resolution and Extent

The finest resolution at which temporal data are reported is the **collectDate**, the date and time of day when the sample was collected in the field.

The NEON Data Portal provides data in monthly files for query and download efficiency. Queries including any part of a month will return data from the entire month. Code to combine files across months is available here: https://github.com/NEONScience/NEON-utilities.

3.6 Associated Data Streams

This section describes the data products that are directly linked or closely related to the microbe community composition data products.

3.6.1 Soils

Soil data are derived from subsamples collected during soil biogeochemical and microbial sampling and include numerous related data products:

- Soil Physical Properties, distributed periodic (NEON.DP1.10086) - Field data, including soil moisture and pH, associated with a soil sample. These data are linked to the community composition data by the **geneticSampleID** in the table ***sls_soilCoreCollection***.
- Soil microbe marker gene sequences (NEON.DP1.10108): Microbial 16S and ITS sequence data. The **dnaSampleID** variable in the tables ***mmg_soilDnaExtraction, mmg_soilPcrAmplification***, and ***mmg_soilMarkerGeneSequencing*** can be used to link data in this product to soil community composition data.
- Soil microbe metagenome sequences (NEON.DP1.10107): Shotgun metagenomics sequences, typically derived from plot-level composited soil samples. The **genomicsPooledIDList** from the table ***sls_metagenomicsPooling*** contains the parent **sampleID**'s that can be linked to the **geneticSampleID**'s in the table ***sls_soilCoreCollection***.
- Soil microbe group abundances (NEON.DP1.10109): Bacteria/archaeal and fungal abundances as measured by quantitative PCR (qPCR). The **dnaSampleID** variable in the table ***mga_soilGroupAbundances*** may be used to link data in this product to the community composition data.
- Soil microbe biomass (NEON.DP1.10104): Microbial biomass as measured by PLFA. Use information in the Soil Physical Properties data product (NEON.DP1.10086, table ***sls_soilCoreCollection***) to obtain the **biomassID** corresponding to the **geneticSampleID**. The **geneticSampleID** links to the **geneticSampleID** in the Soil Marker Gene Sequences data product.
- Soil inorganic nitrogen pools and transformations (NEON.DP1.10080): Measurements derived by field incubations of soil cores or buried bags. Use the **sampleID** from table ***sls_soilCoreCollection*** to link data in this product to soil microbial community composition data.
- Soil chemical properties (Distributed periodic) (NEON.DP1.10078): Measurements of soil carbon, and nitrogen. As with soil inorganic nitrogen, the **sampleID** that generated the soil microbe community composition data can be used to link data.
- Soil stable isotopes (Distributed periodic) (NEON.DP1.10100): Measurements of soil carbon and nitrogen stable isotopes. As with soil inorganic nitrogen, the **sampleID** that generated the soil microbe community composition data can be used to link data.

3.6.2 Aquatics

Aquatic microbial community composition data are derived from samples collected in conjunction with other physical, chemical, and biological measurements. These include:

- Benthic (NEON.DP1.20280) and surface water (NEON.DP1.20282) Microbial 16S and ITS marker gene sequences. The variable ***dnaSampleID*** in the tables ***mmg_benthicDnaExtraction*** and ***mmg_swDnaExtraction*** can be used to link data to the aquatic microbial community composition data products.
- Surface water microbes: The field data are found in the Aquatic Cell Counts data product (NEON.DP1.20138). The variable **geneticSampleID** in the table ***amc_fieldCellCounts*** can be used to link these data to this data product.
- Benthic microbes: The field data are found in the Benthic microbe marker gene sequences data product (NEON.DP1.20280) and the metagenomics sequences (NEON.DP1.20279) data products. The variable **geneticSampleID** in the table ***amb_fieldParent*** can be used to link these data.
- Chemical properties of surface water (NEON.DP1.20093): Measurements of chemical constituents in water. The variable **parentSampleID** in the table ***swc_fieldSuperParent*** can be used to link data.
- Periphyton, seston and phytoplankton collection (NEON.DP1.20166): Field data associated with sample collection. These data are linked to the benthic community composition data by the field data table ***amb_fieldParent***, which is part of the Benthic microbe marker gene sequences data product (NEON.DP1.20280). The variable **parentSampleID** in the table ***alg_domainLabChemistry*** links to the **sampleID** in ***amb_fieldParent***.
- Periphyton, seston and phytoplankton chemical properties (NEON.DP1.20163): Measurements of chemical constituents in algal samples. The field **parentSampleID** in the table ***alg_domainLabChemistry*** links to the sampleID in the table ***amb_fieldParent***, which can then be linked to this data product by the **geneticSampleID**.
- Benthic (NEON.DP1.20277) and surface water (NEON.DP1.20278) microbe group abundances: Bacteria/archaeal and fungal abundances as measured by qPCR. The **geneticSampleID** in the tables ***mga_benthicGroupAbundances*** and ***mga_swGroupAbundances*** can be used to link data.

3.7 Product Instances

For soil samples, microbial community composition samples are collected at all terrestrial NEON sites. A maximum of 10 plots will be sampled at every site 1-3 times per year. Most years, the surface soil horizon (organic or mineral) will be collected, while once every 5 years during a coordinated microbes/biogeochemistry bout, up to 2 soil horizons will be collected during the peak greenness bout to a maximum depth of 30cm. For each soil horizon sampled, 3 unique locations are collected at each plot, for up to 6 samples per plot. Thus, there will be 30-120 product instances generated per site per year.

Aquatic samples are collected at all aquatic NEON sites. For surface water sampling, wadeable streams produce one sample up to 12 times per year, for a maximum of 12 product instances per site per year. Rivers produce up to 2 samples 6 times per year, for a maximum of 12 product instances per site per year. Lakes produce up to 4 samples 6 times per year, for a maximum of 24 product instances collected per site per year. Benthic microbial sampling occurs only at wadeable stream sites, where up to 8 samples are collected 3 times per year, for a maximum of 24 product instances per site per year.

3.8 Data Relationships

The following data relationships are based on merging data using human-readable sample identifiers. As NEON progresses in implementing barcodes as more robust unique identifiers for samples, these may also be used for joining records across tables. Each sample identifier described in the data relationships below also has an equivalent barcode field.

Duplicates and/or missing data may exist where protocol and/or data entry abberations have occurred; *users should check data carefully for anomalies before joining tables*.

3.8.1 Soils

The protocol dictates that each X,Y location sampled yields a unique **sampleID** per horizon per collectDate (day of year, local time) in the table ***sls_soilCoreCollection*** for the data product Soil Physical Properties, distributed periodic (NEON.DP1.10086). Every bout type that includes microbes (e.g. the variable **boutType** includes the string 'microbe') should sample for microbe community composition analysis. A record from ***sls_soilCoreCollection*** may have zero or one child records in tables ***mcc_soilTaxonTableMetadata_16S*** and ***mcc_soilTaxonTableMetadata_ITS*** of this data product. Additionally, each sample will be associated with 0-2 records in the table ***mcc_taxonTableLabSummary***, which describes the methods used to construct the taxon tables.

Each **geneticSampleID** is a subsample of the parent **sampleID** in the table ***sls_soilCoreCollection***, and is sent for DNA extraction. The DNA extraction laboratory data appear in the Soil Microbe Marker Gene Sequences (NEON.DP1.10108) data product, in table ***mmg_soilDnaExtraction***, and are linked by the **geneticSampleID**. There are one or more **dnaSampleID**s expected per **geneticSampleID**, depending on the number of DNA extractions that occur on a sample. Duplicate records for an individual **dnaSampleID** should not exist.

The tables ***mcc_soilTaxonTableMetadata_16S*** and ***mcc_soilTaxonTableMetadata_ITS*** are linked by the **dnaSampleID**. These tables include the per-sample metadata for each taxon table and target gene (16S for bacteria and archaea, ITS for fungi). Per-sample taxon tables are provided as downloadable .csv files using the link in the metadata table (basic) or are included in the download package (expanded).

Soil Physical Properties (NEON DP1.10086)

sls_soilCoreCollection.csv - > One record expected per **sampleID**. Generates samples used in Soil microbe marker gene sequences (NEON.DP1.10108), Soil microbe community composition (NEON.DP1.10081), Soil microbe group abundances (NEON.DP1.10109), and Soil microbe biomass (NEON.DP1.10104). Additionally, subsamples generated from soil sampleIDs are used in Soil Microbe Metagenome Sequences (NEON.DP1.10107), Soil inorganic nitrogen pools and transformations (NEON.DP1.10080), Soil chemical properties (NEON.DP1.10078) and Soil stable isotopes (NEON.DP1.10100).

Soil Microbe Marker Gene Sequences (NEON.DP1.10108)

mmg_soilDnaExtraction.csv -> One record expected per **dnaSampleID**. A geneticSampleID will represent one sample per plot/horizon/X,Y-coordinate combination and per collectDate (day of year, local time). Generally there will be only one DNA extraction per **geneticSampleID** but in some cases multiple extractions will be necessary.

Important Note: The DNA extraction table is generic: samples that may not be relevant to this data product may appear in the data table. To limit the DNA extraction dataset to those that are relevant to soil community composition, filter the records in the ***mmg_soilDnaExtraction*** table to include only those with a **dnaSampleID** that is also contained in the *mcc_soilTaxonTableMetadata* table.

Soil Microbe Community Composition (NEON.DP1.10081)

mcc_soilTaxonTableMetadata_16S.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_soilDnaExtraction*** table.

mcc_soilTaxonTableMetadata_ITS.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_soilDnaExtraction*** table.

mcc_taxonTableLabSummary.csv -> This table describes the laboratory methods used for tabulating the taxon tables, which each unique set of methods (**testMethod**) corresponding to a new record. Each record in this table will correspond with many records in *mcc_soilTaxonTableMetadata_16S.csv* and *mcc_soilTaxonTableMetadata_16S.csv*, and can be linked by the **testMethod**.

3.8.2 Aquatics

3.8.2.1 Surface water

The protocol dictates that each namedLocation sampled yields a unique **parentSampleID**, one sample per collectDate (day of year, local time) in the table ***amc_fieldSuperParent*** for the data product Surface Water Microbe Marker Gene Sequences (NEON.DP1.20282). Each **parentSampleID** may be subsampled into one **geneticSampleID** that is used for microbial analyses, and an archive sample, described in the table ***amc_fieldGenetic***. The **geneticSampleIDs** are sent for DNA extraction and correspond to the the **geneticSampleID** in ***mmg_swDnaExtraction***. There are one or more **dnaSampleIDs** expected per **geneticSampleID**, depending on the number of DNA extractions that occur on a sample. Duplicate records for an individual **dnaSampleID** should not exist.

A record from the field data may have zero or one child records in tables ***mcc_swTaxonTableMetadata_16S*** and ***mcc_swTaxonTableMetadata_ITS*** of this data product. Additionally, each sample will be associated with 0-2 records in the table ***mcc_taxonTableLabSummary***, which describes the methods used to construct the taxon tables.

The tables ***mcc_swTaxonTableMetadata_16S*** and ***mcc_swTaxonTableMetadata_ITS*** are linked by the **dnaSampleID**. These tables include the per-sample metadata for each taxon table and target gene (16S for bacteria and archaea, ITS for fungi). Per-sample taxon tables are provided as downloadable .csv files using the link in the metadata table (basic) or are included in the download package (expanded).

Surface Water Microbe Marker Gene Sequences (NEON.DP1.20282)

amc_fieldSuperParent.csv -> Field data associated with a surface water genetic sample. One record expected per namedLocation sampled and per collectDate generates a unique **parentSampleID**.

amc_fieldGenetic.csv -> One record expected per namedLocation and per collectDate. Record represents a subsample (geneticSampleID) of the field-collected samples (parentSampleID). Depending on the time of year, each record generates zero or one **geneticSampleID**, corresponding to the **geneticSampleID** in the table ***mmg_swDnaExtraction***.

mmg_swDnaExtraction.csv -> One record expected per **dnaSampleID**. Generally there will be only one DNA extraction per **geneticSampleID** but in some cases multiple extractions will be necessary. Duplicate records for an individual **dnaSampleID** should not exist.

Surface Water Microbe Community Composition (NEON.DP1.20141)

mcc_swTaxonTableMetadata_16S.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_swDnaExtraction*** table.

mcc_swTaxonTableMetadata_ITS.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_swDnaExtraction*** table.

mcc_taxonTableLabSummary.csv -> This table describes the laboratory methods used for tabulating the taxon tables, which each unique set of methods (**testMethod**) corresponding to a new record. Each record in this table will correspond with many records in *mcc_swTaxonTableMetadata_16S.csv* and *mcc_swTaxonTableMetadata_16S.csv*, and can be linked by the **testMethod**.

3.8.2.2 Benthic habitats

The protocol dictates that each namedLocation sampled yields a unique **sampleID**, one sample per collectDate (day of year, local time) in the table ***amb_fieldParent*** for the data product Benthic Microbe Marker Gene Sequences (NEON.DP1.20280). Each **sampleID** may be subsampled into one **geneticSampleID** that is used for microbial analyses, and an archive sample, described in the same table. The **geneticSampleID**s are sent for DNA extraction and correspond to the **geneticSampleID** in ***mmg_benthicDnaExtraction***. There are one or more **dnaSampleID**s expected per **geneticSampleID**, depending on the number of DNA extractions that occur on a sample. Duplicate records for an individual **dnaSampleID** should not exist.

A record from the field data may have zero or one child records in tables ***mcc_benthicTaxonTableMetadata_16S*** and ***mcc_benthicTaxonTableMetadata_ITS*** of this data product. Additionally, each sample will be associated with 0-2 records in the table ***mcc_taxonTableLabSummary***, which describes the methods used to construct the taxon tables.

The tables ***mcc_benthicTaxonTableMetadata_16S*** and ***mcc_benthicTaxonTableMetadata_ITS*** are linked by the **dnaSampleID**. These tables include the per-sample metadata for each taxon table and target gene (16S for bacteria and archaea, ITS for fungi). Per-sample taxon tables are provided as downloadable .csv files using the link in the metadata table (basic) or are included in the download package (expanded).

Benthic Microbe Marker Gene Sequences (NEON.DP1.20280)

amb_fieldParent.csv -> Field data associated with a benthic genetic sample. One record expected per namedLocation sampled and per collectDate generates a unique **sampleID**. Each record is a subsample (**geneticSampleID**) of the field-collected sample.

mmg_benthicDnaExtraction.csv -> DNA extraction data associated with a **geneticSampleID**. One record expected per namedLocation and per collectDate. Each unique DNA extraction from a **geneticSampleID** is given a **dnaSampleID**. Generally there will be only one DNA extraction per geneticSampleID but in some cases multiple extractions will be necessary.

Benthic Microbe Community Composition (NEON.DP1.20086)

mcc_benthicTaxonTableMetadata_16S.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_swDnaExtraction*** table.

mcc_benthicTaxonTableMetadata_ITS.csv -> One record is expected per **dnaSampleID**. Each record contains a **dnaSampleID** that corresponds to the **dnaSampleID** in the ***mmg_swDnaExtraction*** table.

mcc_taxonTableLabSummary.csv -> This table describes the laboratory methods used for tabulating the taxon tables, which each unique set of methods (**testMethod**) corresponding to a new record. Each record in this table will correspond with many records in *mcc_swTaxonTableMetadata_16S.csv* and *mcc_swTaxonTableMetadata_16S.csv*, and can be linked by the **testMethod**.

3.9 Special Considerations

3.9.1 Downloading Taxon Tables

Due to their large size, microbial taxon tables can be downloaded in two ways depending on whether the basic or expanded download package is selected.

- Basic Download: Taxon tables can be accessed from the web using the static URL links in the field **downloadFileUrl**, associated with each record in mcc_*taxonTableMetadata_16S|ITS.csv (* = soil, sw, or benthic). Pasting a URL into a web browser will automatically download the taxon table associated with a a **dnaSampleID**.
- Expanded Download: Per-sample taxon tables for all records in the desired spatial and temporal range are downloaded as a .zip file along with the data publication tables. Please note that:

a. Packaged files may be large and require longer to download.
b. To facilitate parsing the downloaded files, taxon tables downloaded in the expanded package are re-named to the value specified in mcc_*taxonTableMetata_16S|ITS **downloadFileName**.
c. File names specified in **downloadFileUrl** are no longer relevant.

For any download option, per-sample files should first be concatenated to ease analysis.

4 DATA QUALITY

4.1 Data Entry Constraint and Validation

Constraints are implemented during the process of ingest into the NEON database. The product-specific data constraint and validation requirements built into data entry applications and database ingest are described in the document NEON Raw Data Validation for Microbial Community Composition (NEON.DP0.10081), provided with every download of this data product. Contained within this file is a field named 'entryValidationRulesParser', which describes syntactically the validation rules for each field built into the data ingest validation. Data entry constraints are described in Nicl syntax in the validation file provided with every data download, and the Nicl language is described in NEON's Ingest Conversion Language (NICL) specifications (AD[16]).

Note: Data collected prior to 2017 were processed using a paper-based workflow that did not implement the full suite of quality control features associated with the interactive digital workflow.

4.2 Automated Data Processing Steps

4.2.1 Sequencing Data

Community composition data are derived from marker gene sequencing data, which must pass basic QA/QC checks, including meeting a minimum sequencing depth (e.g. number of sequences per sample), a maximum number of ambiguous base calls, and a minimum quality score. *Note:* The actual criteria may change over time as technology evolves and standards change. See the User Guides for marker gene sequencing data products (NEON.DP1.10108 for soil, NEON.DP1.20282 for surface water, NEON.DP1.20280 for benthic) for more details and documentation.

4.2.2 Community composition

After initial data QA/QC, sequence data are passed through a bioinformatics pipeline, where identical sequences are dereplicated and low-quality reads are filtered out. The sequence data that pass initial QA are then clustered and a representative sequence is selected from each cluster for taxonomic identification. The exact method for taxonomic identificaion will change over time and is defined in the download package metadata as well as the supporting documentation (e.g. SOP's and methods).

Following laboratory submission of metadata into the NEON automated data ingest process, the steps used to process the data through to publication on the NEON Data Portal are detailed in the NEON Algorithm Theoretical Basis Document: OS Generic Transitions (AD[15]).

4.3 Data Revision

All data are provisional until a numbered version is released; the first release of a static version of NEON data, annotated with a globally unique identifier, is planned to take place in 2020. During the provisional period, QA/QC is an active process, as opposed to a discrete activity performed once, and records are updated on a rolling basis as a result of scheduled tests or feedback from data users. The Change Log section of the data product readme, provided with every data download, contains a history of major known errors and revisions.

4.4 Quality Flagging

The **dataQF** field in each data record is a quality flag for known errors applying to the record. Please see the *Special Considerations* section of this document for a list of known errors that may be present in the data, and below for an explanation of **dataQF** codes specific to this product.

fieldName	value	definition
dataQF	legacyData	Data recorded using a paper-based workflow that did not implement the full suite of quality control features associated with the interactive digital workflow
dataQF	sequenceDepthBelowMinimum	Number of sequences associated with record was below the acceptance criterion for downstream analysis

4.5 Analytical Facility Data Quality

Bioinformatics analyses conducted on sequencing data conform to the current data quality standards used by practitioners. The data table ***mcc_taxonTableLabSummary*** (available in the expanded package) contains the bioinformatics methods and links to analysis code used to generate data, with each record describing the long-term methods and parameters used during the specified period of time. This table also provides the file names for the analysis code used by the lab to run the bioinformatics methods, which is not standardized for a particular computer language.

5 REFERENCES

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